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Original research article

Prenatal exposure to polycyclic aromatic hydrocarbons and growth parameters

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Abstract

Background and objectives: It has long been known that airborne polycyclic aromatic hydrocarbons (PAHs) can negatively affect pregnancy and birth outcomes, such as birth weight, fetal development, and placental growth factors. However, similar studies yield divergent results. Our goal was to estimate the amount of monohydroxylated PAH (OH-PAH) metabolites in the urine of pregnant women/mothers and their newborns in relation to birth outcomes, such as placenta weight, Apgar 5', and the growth parameters of children up to the age of two.

Methods: Two cohorts of children born in 2013 and 2014 during the summer and winter seasons in the Czech Republic in the cities Karviná ($N = 144$) and České Budějovice ($N = 198$), which differ significantly in the level of air pollution, were studied. PAH exposure was assessed by the concentration of benzo[a]pyrene (B[a]P) in the air and the concentration of 11 OH-PAH metabolites in the urine of newborns and mothers. Growth parameters and birth outcomes were obtained from medical questionnaires after birth and from pediatric questionnaires during the following 24 months of the child's life.

Results: Concentrations of B[a]P were significantly higher in Karviná ($p < 0.001$). OH-PAH metabolites were significantly higher in the mothers' as well as in the newborns' urine in Karviná and during the winter season. Neonatal length was shorter in newborns in Karviná ($p < 0.001$), but this difference evened out during the next 3 to 24 months. Compared to České Budějovice, newborns in Karviná showed significantly lower weight gain between birth and three months after delivery. The OH-PAH metabolites in mothers' or newborns' urine did not affect birth weight. The presence of seven OH-PAH (top 25% of values of concentrations higher than the median) metabolites in the newborns' urine is associated with decreased length of newborn. Nine OH-PAH metabolites decreased placenta weight, which was the most significant, while seven OH-PAH metabolites decreased Apgar 5'.

Conclusion: We have shown a possible connection between higher concentration of OH-PAH metabolites in newborns' urine and decreased length, head circumference, placenta weight, and Apgar 5', but not birth weight.

Keywords: Birth length; Birth weight; Growth parameters; Head circumference; Monohydroxylated PAH metabolites; Placenta weight; Polycyclic aromatic hydrocarbons

Highlights:

- Two cohorts from two regions with different levels of air pollution were studied.
- The effect of prenatal exposure on growth parameters was studied up to the age of 2.
- The concentration of B[a]P in the air and OH-PAH metabolites in urine was assessed.
- Birth weight at delivery did not differ between newborns from either region.
- OH-PAH metabolites decreased placenta weight, length of newborns, and Apgar 5'.

Introduction

Air pollution poses a serious threat to human health. Respirable particulate matter of aerodynamic diameter ≤2.5 μm ($PM_{2.5}$), a significant constituent of polluted air, is being intensively studied along with the carcinogenic polycyclic aromatic hydrocarbons (PAHs) bound to it, including *e.g.*, benzo[a]pyrene (B[a]P), the most known human carcinogen used as a surrogate for other carcinogenic PAHs. Owing to their small size,

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$PM_{2.5}$ particles can penetrate into the human body and subsequently the bloodstream via the airways. Hence compared to larger particles they represent a significant health risk (Russell and Brunekreef, 2009).

Most PAHs are emitted into the air by anthropogenic sources, such as biomass burning, metal production, and coal and gas combustion (Abdel-Shafy and Manson, 2016; Lhotka et al., 2019). Central and Eastern Europe faces deterioration of the air quality due to PAHs (Air Quality in Europe, 2018). The limit for mean annual concentration of BaP (benzo-a-pyrene is one type of PAHs) in PM10 is set to 1 ng/m^3 (Directive 2008/50/EC, 2008). In the Czech Republic, BaP concentrations exceed the limit value regularly; specifically, the yearly mean concentration of BaP exceeded 17.4% of the area of the Czech Republic with a value of 1.96 ng/m^3, and it is estimated that 54.5% of the Czech population was exposed to amounts of BaP exceeding the limit (CHMI, 2013). The situation slightly improved in 2014, when 10.7% of the area of the Czech Republic exceeded the BaP limit and the yearly mean concentration of 1.98 ng/m^3 with estimated exposure of 51.1% of the population (CHMI, 2014b). The immission limit of PM_{10} and $PM_{2.5}$ is set at 40 μg/m^3 and 20 μg/m^3 (valid from 2020, earlier it was 25 μg/m^3), respectively (Directive 201/2012Sb., 2012). This limit was exceeded in 0.7% of the area of the Czech Republic in 2013 for PM10, exposing 4.8% of the population. The area with excess limits was exclusively in the Ostrava-Karviná-Frýdek-Místek agglomeration (CHMI, 2013). In 2014, the limit was exceeded in 0.45% of the area, exposing 2.2% of the Czech population (CHMI, 2014b). In 2013, the limit of $PM_{2.5}$ was exceeded in an area of 2.4% of the Czech Republic, exposing 9.6% of the population, and in 2014, 1.8% of the area and 8.6% of the population (CHMI, 2013, 2014b).

In this study, two regions of the Czech Republic with different levels of industrial production and related air pollution were evaluated. České Budějovice is located in the South-West part of the Czech Republic, surrounded by several lakes and the Šumava mountains from the west. The area of České Budějovice is known for its clean environment with its levels of air pollution (five years mean level (2013–2017) of BaP was 0.35 ng/m^3, PM_{10} 17 μg/m^3, and $PM_{2.5}$ 13 μg/m^3 (CHMI, 2018). Meanwhile, Karviná is located in the North-East part of the Czech Republic. It is surrounded by the large rural agglomeration of Ostrava, Frýdek-Místek, and Český Těšín in the Czech Republic, and Bialsko-Biala in Poland. The whole area is well known due to its rich history of mining and metal processing industry with its related air pollution. Five years (2013–2017) mean level of BaP in Karviná was 3.4 ng/m^3, PM_{10} 39.2 μg/m^3, and $PM_{2.5}$ 30.5 μg/m^3 (CHMI, 2018).

PAHs are produced by the incomplete combustion of organic matter. They are widely spread in the environment (WHO, 2010) and some of them have genotoxic (Binková and Šrám, 2004; Topinka et al., 2000), mutagenic, carcinogenic (IARC, 2012), and embryotoxic activities (Binková et al., 1999). PAHs can significantly affect birth outcomes. The first paper on this topic was published by Perera et al. in 1998 using new methods in molecular epidemiology. The impact of PAH exposure was determined as PAH-DNA adducts in cord blood leucocytes in Poland (Krakow and Limanowa). Newborns with PAH-DNA adducts above the median had significantly decreased birth length, weight, and head circumference. Dejmek et al. (2000) observed the impact of prenatal inhalation exposure to PAHs as the increase of intrauterine growth restriction (IUGR). The effects of PAHs on fetal development and growth may be explained by PAH penetration into the placenta and different fetal tissues (Milani et al., 2005; Neufeld et al., 1999; Smith, 1999) and by the direct interference with placental growth factors (Chadda et al., 2004; Detmar et al., 2008).

The impact of the increased concentration of PAHs exposure on birth outcomes is usually determined as (1) airborne PAHs, (2) PAH-DNA adducts in cord blood, or (3) monohydroxylated PAH (OH-PAH) concentration in urine.

Studies determining the airborne effect of PAHs on birth weight are only available in the USA (Choi et al., 2006), and Poland (Jedrychowski et al., 2017). In addition, the effect on fetal growth (intrauterine growth retardation, IUGR) has been studied in the Czech Republic by Dejmek et al. (2000).

Yang et al. (2020) published a meta-analysis of 11 studies about the association between the prenatal exposure to PAH and birth weight. They concluded that there is no significant relationship between prenatal exposure to PAHs and birth weight. They proposed that further studies are still needed.

Therefore, the aim of this study was to evaluate prenatal PAH exposure, expressed by eleven OH-PAH metabolites in the urine of mothers and their newborns from two localities with significantly different level of air pollution. We hypothesize that PAH exposures may affect placental weight, Apgar 5', and growth parameters from birth to 24-month-old children.

Materials and methods

We studied the impact of prenatal PAHs exposure on newborns from two locations in the Czech Republic: newborns and their mothers who lived in Karviná and České Budějovice. The criteria for inclusion mainly included non-smoking mothers and long-term residency within the locality. The České Budějovice group was selected as a control group, since the level of air pollution in this locality is long-term significantly lower than in Karviná, which is one of the most polluted cities in the Czech Republic (Fig. 1). Sampling of biological material (urine) was performed in both locations in two periods: summer 2013 (low air pollutant levels) and winter 2014 (high levels of air pollution).

Subjects

The subjects of the study were newborns born in České Budějovice Hospital, Department of Obstetrics and Department of Neonatology, and in the Karviná Hospital, Department of Obstetrics and Department of Neonatology. The study was approved by the Ethics Committees of both hospitals and the Institute of Experimental Medicine CAS in Prague (June 21, 2012). All mothers signed a written consent.

The heating in the homes of mothers was usually central heating or gas; open fireplaces were not used in any house. The samples were collected from normal deliveries (38–41 weeks) of non-smoking mothers and their newborns in the summer and winter seasons. The samples included venous blood and urine from 99 mothers (summer) and 100 mothers (winter) in České Budějovice, a locality with relatively clean air; and 70 mothers (summer) and 73 mothers (winter) in Karviná, a locality with high air pollution. Furthermore, cord blood and urine were collected from 99 newborns (summer) and 100 newborns (winter) in České Budějovice and 71 newborns (summer) and 74 newborns (winter) in Karviná (in both seasons there was 1 set of twins).

Each mother filled in a questionnaire about her age, BMI, current residency, eating habits, active smoking during and/or before pregnancy, gestational age, date of child birth, delivery type, child's birth weight and gender (summary in Table 1 and Suppl. Table 1).

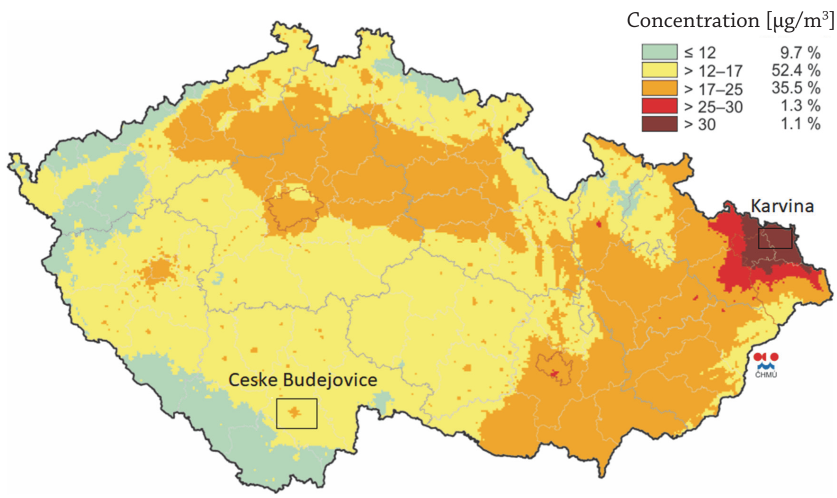

Fig. 1. Five-year average of annual average concentrations of PM2.5, 2010–2014 (CHMI, 2014a)

Air sampling and analysis of selected air pollutants

$PM_{2.5}$ and B[a]P was collected using a High Volume (HiVol) 3000 Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) to monitor the difference in air pollution levels between both localities during the two sampling seasons. Sampling was conducted for 24 h each day during childbirth in both seasons. The sampling was conducted as previously described (Topinka et al. 2011). Detailed information on air sampling, extraction of organic complex mixtures (EOM) from the filters, and chemical analysis of B[a]P is provided by Topinka et al. (2011).

Time frames related to pregnancy months are estimated by the known delivery date and gestation age confirmed by medical personnel. Air pollution concentration of $PM_{2.5}$ and B[a]P in these air frames was individually calculated for every mother-child pair from the public data of CHMI (online data: air quality information), related to both localities.

Information about the children

Paediatricians were asked to fill out a questionnaire, which included the child's medical records, containing information about weight, length, and head circumference at birth, and then at 3 months, 6 months, 18 months, and 24 months of age.

Collection and analysis of urine samples

Urine samples were collected into 50 ml tubes (Greiner Bio-one) and stored at −20 °C until transported to the Institute of Experimental Medicine. Aliquots (1–2 ml) of urine were frozen at −80 °C until analysis.

A total of 575 samples (287 urines from mothers and 288 urines from their children) were obtained. Only 531 samples had sufficient volume for all analyses, including creatinine determination, so the final results were reported for 531 samples.

OH-PAHs were analyzed in urine. OH-PAH metabolites were determined based on Urbancova et al. (2016) as 1-OH-naphthalene (1-OH-NAP), 2-OH-naphthalene (2-OH-NAP), 2-OH-fluorene (2-OH-FLUO), 1-OH-phenantrene (1-OH-PHEN), 2-OH-phenantrene (2-OH-PHEN), 3-OH-phenantrene (3-OH-PHEN), 4-OH-phenantrene (4-OH-PHEN), 9-OH-phenantrene (9-OH-PHEN), chrysen-6-ol (6-OH-CHRY), and 3-OH-benzo[a]pyrene (3-OH-BaP) in the set of 531 urine samples. The samples were measured using a validated and accredited analytical method in accordance with CSN EN ISO/IEC 17025:2018 and CSN EN ISO 9001:2016. To control the analytical procedure, every 20th sample was measured in parallel and standard reference material (SRM) 3673 (urine of a non-smoker) was measured with every batch of samples.

APGAR 5'

The APGAR score was determined by a paediatrician five minutes after delivery. This evaluates the newborn pulse rate, muscle tonicity, respiratory functions, skin color, and reflex. The rating scale is defined as 7–10 as reassuring, a score of 4–6 as moderately abnormal, and a score of 0–3 as low.

Statistics methods

For the statistical analysis, Statistica software (version 7.0, StatSoft, Dell, Tulsa, USA) and SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA) were used. Comparison between groups of mothers and children was performed using the Mann–Whitney *U*-test. Groups were defined by locality (České Budějovice and Karviná) and study period (summer 2013 – August–September, and winter 2014 – January–April).

The percentage for categorical parameters of characteristics of the studied population is calculated as the common mean of 0/1 status values, as it represents the percentage of this category in the study population. Logistic regression was used to estimate the multivariate models combining both continuous and dichotomous variables. Logistical regression was also useful for simple transformed parameters that do not have a sufficiently normal distribution.

Simple Spearman's rank correlation coefficient was used to verify the expected relations between children's birth parameters and placenta weight. The presented values are Spearman R with significance marker. The deviation is represented by standard deviation (SD).

To estimate the relationship between the continuous variables characterizing children's growth and the assigned air pollution exposure, simple regression on log transformed values due to the defect of normal distribution of original values was used. As representation of the estimated relations, standard normalized beta coefficient is presented with a marker of statistical significance: positive values indicate coincidence, negative values indicate contradictions.

Results

During the sampling periods, the mean concentrations of $PM_{2.5}$ in summer were 21.54 ± 11.78 μg/m^3 in Karviná, and 12.14 ± 7.23 μg/m^3 in České Budějovice, and in winter 55.35 ± 38.74 μg/m^3 and 26.39 ± 16.85 μg/m^3, respectively. Concentrations of B[a]P in summer were 1.31 ± 1.26 ng/m^3 in Karviná, and 0.44 ± 0.63 ng/m^3 in České Budějovice, in winter 5.15 ± 5.47 ng/m^3 and 1.43 ± 1.37 ng/m^3, respectively.

The concentrations of OH-PAHs in the urine of mothers and newborns were previously determined by Urbancova et al. (2016).

The general characteristics of the pregnancy parameters are shown in Table 1. Characteristics of lifestyle parameters are summarized in Suppl. Table 1.

Mothers in Karviná were younger than mothers in České Budějovice ($p < 0.01$). In Karviná, more mothers had primary and low secondary education ($p < 0.01$); overall, 33.1% mothers were single vs. 20.1% in České Budějovice. During pregnancy, more mothers were employed in České Budějovice ($p < 0.01$), which reflects the general differences in the unemployment rate in these regions. More mothers smoked before pregnancy in Karviná ($p < 0.01$), but no differences were observed during pregnancy. Alcohol consumption by mothers between those two regions did not differ before or during pregnancy. There were no differences in the type of delivery by vaginal, Cesarean section, or forceps, but in Karviná vacuum extraction was more frequent ($p < 0.05$).

Growth parameters, such as head circumference, length, weight, Apgar 5', and placenta weight are listed in Table 2, and separately for summer and winter in Suppl. Table 2. The length of the children was shorter at birth in Karviná vs. České Budějovice in both seasons. In České Budějovice in winter the length was shorter at 6, 18, and 24 months compared to summer ($p < 0.05$). Apgar score 5' was lower in České Budějovice in both seasons. The placenta weight was significantly lower in Karviná in both seasons compared to České Budějovice, and significantly lower in Karviná in winter compared to Karviná in summer ($p < 0.001$).

OH-PAH metabolites for mothers and children in both seasons are listed in Table 3 and Suppl. Table 3 for summer and winter. OH-PAH metabolites were significantly higher in mothers' as well as in the newborns' urine in Karviná. As concentrations of 6-OH-CHRY and 3-OH-BaP were under the detection limit, they are not mentioned in the tables. OH-PAH metabolites were higher in mothers' urine vs. newborns' urine in both localities and both seasons. Comparing summer vs. winter seasons, concentrations of OH-PAH metabolites were higher in both localities in winter sampling.

In Table 4 for both localities and Suppl. Table 4 separately for summer and winter, the impact of mothers' and children's urine OH-PAH metabolites on growth parameters is shown. The top 25% of concentrations contain the subsequently listed metabolites in mothers' urine: 1-OH-NAP decreased head circumference; 2-OH-FLUO decreased Apgar 5', and 1-OH-PYR decreased placenta weight in České Budějovice; 9-OH-PHEN decreased placenta weight in Karviná.

The effect of OH-PAH metabolites was more pronounced in children: head circumference was decreased by 2-OH-PHEN; length was decreased by 2-OH-FLUO, 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, and all OH-PAH; placenta weight was decreased by 2-OH-FLUO, 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, 9-OH-PHEN, 1-OH-PYR, and all OH-PAH; Apgar 5' was decreased by 2-OH-FLUO, 1-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, and all OH-PAH. Specifically, in Karviná, head circumference was decreased by 1-OH-NAP, 1-OH-PHEN; placenta weight was decreased by 4-OH-PHEN, 9-OH-PHEN, and all OH-PAH; Apgar 5' was decreased by 2-OH-NAP.

Table 5 shows the environmental pollution exposure from the 1st month of pregnancy to 24th month after delivery. Concentrations of $PM_{2.5}$ as well as B[a]P throughout this period were significantly higher in Karviná compared to České Budějovice ($p < 0.001$).

Placenta weight analysis showed that placenta weight significantly affected birth weight, birth length, and birth head circumference (Table 6). These results suggest a very significant role of placenta weight for fetus development.

Table 1. General overview of pregnancy parameters

Characteristic			Total		České Budějovice		Karviná	
			N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
Maternal age		years	343	31.3 ± 4.6	198	32.3 ± 4.2***	145	29.9 ± 4.7***
Maternal height		cm	337	167.6 ± 6.5	195	168.4 ± 6.3**	142	166.5 ± 6.7**
Maternal weight		kg	336	66.9 ± 13.5	195	67.4 ± 13.5	141	66.2 ± 13.6
Delivery type	Vaginal	%	344	62.5	199	64.8	145	59.3
	Cesarean section	%	344	33.1	199	32.7	145	33.8
	Forceps	%	344	0.6	199	1.0	145	0.0
	Vacuum extraction	%	344	2.6	199	0.5	145	5.5

Note: Mann–Whitney *U*-test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Logistic regression $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.001$.

Table 2. Children's growth parameters

				Total		České Budějovice		Karviná	
				N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
Head circumference	Absolute	at birth	cm	328	34.5 ± 1.4	197	34.6 ± 1.4	131	34.5 ± 1.3
		3 months	cm	284	40.3 ± 1.3	171	40.4 ± 1.4	113	40.2 ± 1.2
		6 months	cm	285	43.2 ± 1.4	170	43.3 ± 1.5	115	43.1 ± 1.3
		18 months	cm	284	47.7 ± 1.6	171	47.8 ± 1.6	113	47.5 ± 1.5
		24 months	cm	244	48.8 ± 1.5	138	49.0 ± 1.6	106	48.7 ± 1.4
	Gain	birth–3 m	cm	278	5.8 ± 1.5	171	5.8 ± 1.6	107	5.7 ± 1.2
		3–6 m	cm	282	3.0 ± 1.0	170	3.0 ± 1.1	112	3.0 ± 0.8
		6–18 m	cm	282	4.4 ± 1.1	170	4.5 ± 1.1	112	4.4 ± 1.0
		18–24 m	cm	242	1.2 ± 0.7	137	1.2 ± 0.8	105	1.2 ± 0.7
Length	Absolute	at birth	cm	336	49.6 ± 2.0	192	50.0 ± 1.7***	144	49.2 ± 2.2***
		3 months	cm	277	61.7 ± 2.6	167	61.9 ± 2.5	110	61.5 ± 2.6
		6 months	cm	281	68.3 ± 2.8	170	68.5 ± 2.7	111	68.1 ± 2.8
		18 months	cm	287	82.9 ± 3.5	171	82.9 ± 3.3	116	83.0 ± 3.8
		24 months	cm	247	88.8 ± 3.8	141	88.7 ± 3.6	106	89.0 ± 4.0
	Gain	birth–3 m	cm	272	12.0 ± 2.3	162	11.8 ± 2.1	110	12.2 ± 2.5
		3–6 m	cm	275	6.6 ± 2.0	167	6.6 ± 2.1	108	6.6 ± 1.8
		6–18 m	cm	280	14.7 ± 3.1	170	14.4 ± 3.2	110	15.0 ± 3.0
		18–24 m	cm	245	5.9 ± 2.5	140	5.9 ± 2.3	105	5.9 ± 2.6
Weight	Absolute	at birth	g	342	3429 ± 444	198	3465 ± 442	144	3378 ± 443
		3 months	g	261	5991 ± 740	163	5948 ± 696	98	6063 ± 808
		6 months	g	260	7644 ± 920	162	7570 ± 844	98	7767 ± 1026
		18 months	g	260	11263 ± 1517	160	11213 ± 1389	100	11342 ± 1707
		24 months	g	218	12822 ± 1791	129	12774 ± 1707	89	12893 ± 1914
	Gain	birth–3 m	g	261	2548 ± 636	163	2478 ± 604**	98	2665 ± 673**
		3–6 m	g	259	1666 ± 554	162	1626 ± 545	97	1733 ± 564
		6–18 m	g	256	3622 ± 1032	160	3648 ± 929	96	3580 ± 1189
		18–24 m	g	215	1577 ± 857	127	1580 ± 878	88	1573 ± 830
Apgar 5'				311	9.8 ± 0.5	182	9.7 ± 0.6***	129	10.0 ± 0.1***
Placenta weight			g	333	554 ± 131	191	577 ± 125***	142	522 ± 132***

Note: Mann–Whitney *U*-test compared by region * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and by period + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$.

Table 3. Mother's and children's urine OH-PAH metabolites at birth (ng/g creatinine)

Subject	OH-PAH	Total		České Budějovice		Karviná	
		N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
Mothers	3-OH-BaP	283	450.0 ± 0.0	169	450.0 ± 0.0	114	450.0 ± 0.0
	6-OH-CHRY	283	5.0 ± 0.0	169	5.0 ± 0.0	114	5.0 ± 0.0
	2-OH-FLUO	283	512.2 ± 587.8	169	468.8 ± 489.7	114	576.6 ± 706.1
	1-OH-NAP	283	992.3 ± 2221.8	169	802.2 ± 1514.7	114	1274.1 ± 2962.0
	2-OH-NAP	283	6788.6 ± 5446.4	169	5962.7 ± 4335.7**	114	8013.0 ± 6599.0**
	1-OH-PHEN	283	613.5 ± 627.4	169	440.0 ± 411.3***	114	870.7 ± 786.8***
	2-OH-PHEN	282	273.7 ± 296.4	169	242.9 ± 276.9***	113	319.9 ± 319.2***
	3-OH-PHEN	283	111.9 ± 118.1	169	97.7 ± 89.4	114	133.0 ± 148.9
	4-OH-PHEN	283	428.3 ± 942.6	169	351.1 ± 817.2**	114	542.7 ± 1096.6**
	9-OH-PHEN	283	662.5 ± 1373.8	169	550.1 ± 875.1***	114	829.1 ± 1877.3***
	1-OH-PYR	283	245.4 ± 172.3	169	223.7 ± 173.0***	114	277.7 ± 166.9***
	All-OH-PAH	283	10623.5 ± 8335.3	169	9137.0 ± 6393.5***	114	12827.2 ± 10218.1***
	3-OH-BaP	284	450.0 ± 0.0	169	450.0 ± 0.0	115	450.0 ± 0.0
Children	6-OH-CHRY	284	5.0 ± 0.0	169	5.0 ± 0.0	115	5.0 ± 0.0
	2-OH-FLUO	284	190.7 ± 199.4	169	110.4 ± 111.1***	115	308.8 ± 238.5***
	1-OH-NAP	284	372.8 ± 481.9	169	117.5 ± 227.7***	115	747.9 ± 511.3***
	2-OH-NAP	284	4013.6 ± 3483.3	169	3042.3 ± 2649.7***	115	5440.9 ± 4039.7***
	1-OH-PHEN	284	430.0 ± 539.2	169	145.9 ± 153.2***	115	847.4 ± 626.0***
	2-OH-PHEN	284	256.9 ± 289.4	169	100.6 ± 100.5***	115	486.5 ± 321.9***
	3-OH-PHEN	284	46.6 ± 55.4	169	18.5 ± 20.6***	115	87.9 ± 64.0***
	4-OH-PHEN	284	117.6 ± 284.3	169	78.1 ± 175.5**	115	175.7 ± 386.7**
	9-OH-PHEN	284	990.5 ± 1926.2	169	434.8 ± 691.1***	115	1807.2 ± 2715.9***
	1-OH-PYR	284	79.3 ± 110.7	169	32.0 ± 44.2***	115	148.9 ± 138.9***
	All-OH-PAH	284	6480.4 ± 5136.8	169	4055.5 ± 3054.8***	115	10044.0 ± 5497.7***

Note: Mann–Whitney *U*-test compared by region * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and by period ⁺ $p < 0.05$, ⁺⁺ $p < 0.01$, ⁺⁺⁺ $p < 0.001$.

Table 4. Impact of mothers' and children's urine PAH OH metabolites on growth parameters

		Total						České Budějovice						Karviná					
Subject	PAH-OH	*N*	Head circumference	Length	Weight	Placenta weight	Apgar 5'	*N*	Head circumference	Length	Weight	Placenta weight	Apgar 5'	*N*	Head circumference	Length	Weight	Placenta weight	Apgar 5'
Mothers	2-OH-FLUO	283	–0.06	0.05	0.04	0.00	–0.09	169	–0.01	–0.02	0.01	0.12	–0.18*	114	–0.12	0.08	0.07	–0.09	0.14
	1-OH-NAP	283	–0.13*	–0.02	–0.01	–0.06	–0.08	169	–0.09	–0.11	–0.02	0.05	–0.13	114	–0.19	–0.01	–0.02	–0.18	0.19
	2-OH-NAP	283	–0.03	–0.04	–0.06	–0.01	0.04	169	–0.05	–0.02	–0.04	–0.03	0.02	114	0.01	0.02	–0.05	0.10	–0.17
	1-OH-PHEN	283	0.02	–0.04	0.06	0.01	0.07	169	0.00	–0.02	0.06	0.04	–0.05	114	0.08	0.15	0.18	0.18	0.01
	2-OH-PHEN	282	–0.08	0.00	0.01	0.01	0.06	169	–0.08	0.01	0.02	0.06	0.01	113	–0.08	0.07	0.02	0.03	0.12
	3-OH-PHEN	283	–0.04	0.04	0.07	–0.01	–0.04	169	0.04	0.02	0.09	0.09	–0.10	114	–0.13	0.05	0.04	–0.07	0.13
	4-OH-PHEN	283	–0.03	0.00	–0.01	–0.05	0.04	169	0.01	0.00	0.02	0.11	–0.01	114	–0.08	0.05	–0.03	–0.17	0.09
	9-OH-PHEN	283	–0.05	0.12	0.00	–0.08	–0.07	169	–0.11	–0.05	–0.14	–0.02	–0.04	114	0.00	0.15	0.08	–0.21*	0.08
	1-OH-PYR	283	–0.01	0.04	0.06	0.06	0.01	169	–0.01	0.10	0.10	0.16*	–0.06	114	–0.02	0.07	0.05	0.02	0.05
	All-OH-PAH	283	–0.06	–0.03	–0.04	–0.02	0.03	169	–0.05	–0.05	–0.04	0.05	–0.03	114	–0.07	0.08	0.00	0.00	–0.09
Children	2-OH-FLUO	284	–0.04	–0.14*	–0.06	–0.22***	0.18**	169	–0.04	–0.12	–0.02	–0.16*)	0.09	115	–0.01	0.07	0.01	–0.14	–0.05
	1-OH-NAP	284	–0.12	–0.22***	–0.12	–0.20***	0.16*	169	–0.10	–0.14	–0.06	–0.10	–0.01	115	–0.20*	–0.02	¬–0.06	–0.08	–0.01
	2-OH-NAP	284	0.04	–0.13*	–0.04	–0.19**	0.09	169	0.02	–0.08	0.02	–0.14	0.00	115	0.15	0.00	–0.02	–0.09	–0.29**
	1-OH-PHEN	284	–0.05	–0.17**	–0.04	–0.19**	0.25***	169	0.04	0.01	0.08	–0.06	0.14	115	–0.20*	–0.04	–0.01	–0.13	–0.10
	2-OH-PHEN	284	–0.12*	–0.15*	–0.09	–0.18**	0.16*	169	–0.07	–0.05	–0.02	–0.08	0.05	115	–0.19	–0.01	–0.06	–0.09	–0.06
	3-OH-PHEN	284	–0.06	–0.13*	–0.07	–0.24***	0.20***	169	–0.03	–0.09	0.01	–0.19*	0.07	115	–0.08	0.13	–0.04	–0.11	0.03
	4-OH-PHEN	284	–0.11	–0.19**	–0.09	–0.24***	0.21***	169	–0.11	–0.10	–0.02	–0.10	0.12	115	–0.11	–0.05	–0.06	–0.19*	–0.01
	9-OH-PHEN	284	0.01	–0.04	–0.03	–0.14*	0.01	169	0.04	–0.02	0.04	0.09	–0.06	115	–0.02	0.03	–0.07	–0.30**	0.05
	1-OH-PYR	284	–0.06	–0.07	–0.03	–0.14*	0.00	169	–0.06	–0.02	–0.01	0.02	–0.14	115	–0.02	0.06	0.03	–0.17	0.07
	All-OH-PAH	284	–0.02	–0.18**	–0.07	–0.25***	0.14*	169	0.00	–0.10	0.01	–0.12	0.01	115	0.01	0.00	–0.05	–0.24*	–0.19

Note: Beta coefficient results of regression between OH-PAH and decrease of growth parameters * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5. Estimated environmental pollution exposure of mothers

EP estimated values		Month		Total		České Budějovice		Karviná	
				N	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
CHMI	PM$_{2.5)}$	1st of pregnancy	μg/m^3	289	26.2 ± 18.8	172	17.6 + 4.6***	117	38.9 + 24.0***
		2nd of pregnancy		289	27.7 ± 18.6	172	20.0 ± 7.4***	117	39.2 ± 23.6***
		3rd of pregnancy		289	29.6 ± 17.3	172	22.3 ± 8.8***	117	40.2 ± 20.8***
		4th of pregnancy		289	28.3 ± 14.6	172	21.2 ± 8.3***	117	38.8 ± 15.5***
		5th of pregnancy		289	25.6 ± 13.9	172	18.2 ± 6.3***	117	36.6 ± 14.8***
		6th of pregnancy		289	23.2 ± 13.8	172	15.9 ± 4.9***	117	34.0 ± 15.3***
		7th of pregnancy		289	23.2 ± 14.2	172	16.0 ± 5.0***	117	33.8 ± 16.6***
		8th of pregnancy		289	24.5 ± 13.2	172	18.7 ± 5.7***	117	32.9 ± 16.3***
		9th of pregnancy		289	25.7 ± 14.5	172	18.0 ± 6.0***	117	37.0 ± 15.9***
		0–3 after delivery		289	24.1 ± 13.0	172	15.1 ± 3.5***	117	37.4 ± 10.2***
		4–6 after delivery		289	23.5 ± 12.8	172	17.9 ± 5.1***	117	31.9 ± 15.9***
		7–18 after delivery		289	21.0 ± 8.0	172	14.5 ± 0.7***	117	30.6 ± 0.7***
		19–24 after delivery		289	22.1 ± 9.1	172	17.0 ± 4.7***	117	29.6 ± 8.9***
CHMI	Benzo(a)pyrene	1st of pregnancy	ng/m^3	273	2.9 ± 3.5	172	1.9 ± 1.7***	101	4.5 ± 4.8***
		2nd of pregnancy		251	2.5 ± 2.8	172	2.1 ± 1.8**	79	3.4 ± 4.2**
		3rd of pregnancy		235	2.2 ± 2.0	172	2.0 ± 1.7**	63	2.8 ± 2.7**
		4th of pregnancy		235	2.8 ± 3.1	172	1.7 ± 1.2***	63	6.0 ± 4.3***
		5th of pregnancy		259	3.2 ± 3.9	172	1.3 ± 0.9***	87	6.9 ± 4.8***
		6th of pregnancy		282	3.4 ± 4.7	172	1.3 ± 1.1***	110	6.8 ± 6.0***
		7th of pregnancy		289	3.5 ± 5.0	172	1.4 ± 1.3***	117	6.5 ± 6.6***
		8th of pregnancy		289	2.9 ± 3.9	172	1.4 ± 1.4***	117	5.2 ± 5.2***
		9th of pregnancy		289	3.4 ± 4.1	172	1.2 ± 1.3***	117	6.6 ± 4.6***
		0–3 after delivery		289	3.3 ± 3.6	172	1.2 ± 0.6***	117	6.5 ± 3.7***
		4–6 after delivery		289	3.0 ± 3.4	172	1.6 ± 1.4***	117	5.1 ± 4.4***
		7–18 after delivery		289	2.4 ± 1.5	172	1.2 ± 0.0***	117	4.1 ± 0.4***
		19–24 after delivery		289	2.0 ± 1.2	172	1.3 ± 0.7***	117	2.9 ± 1.1***

Note: Results of Mann–Whitney *U*-test compared by region * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a comparison by period was not statistically significant.

Table 6. Correlations between birth parameters and placenta weigh

	All						All			
	All		České Budějovice		Karviná		Summer 2013		Winter 2014	
	N	Spearman R	*N*	Spearman R	*N*	Spearman R	*N*	Spearman R	*N*	Spearman R
Birth weight – placenta weight	333	0.45***	191	0.51***	142	0.34***	167	0.53***	166	0.36***
Birth length – placenta weight	327	0.37***	185	0.39***	142	0.24**	164	0.40***	163	0.33***
Birth head circumference – placenta weight	320	0.37***	190	0.39***	130	0.30***	156	0.48***	164	0.26***

	České Budějovice				Karviná			
	Summer 2013		Winter 2014		Summer 2013		Winter 2014	
	N	Spearman R	*N*	Spearman R	*N*	Spearman R	*N*	Spearman R
Birth weight – placenta weight	98	0.57***	93	0.45***	69	0.43***	73	0.28*
Birth length – placenta weight	95	0.41***	90	0.37***	69	0.37**	73	0.17
Birth head circumference – placenta weight	98	0.46***	92	0.33**	58	0.52***	72	0.18

Note: Spearman rank order correlation R; *p*-value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

In this study, we hypothesized that prenatal PAH exposure, expressed by eleven OH-PAH metabolites in the urine of mothers and their newborns born in localities with a significantly different level of air pollution, may be related to placental weight, Apgar 5', and growth parameters from birth to 24-month-old children.

Polanska et al. (2010) evaluated PAH exposure at the level of 1-OH-PYR in the urine of pregnant women. Such prenatal exposure adversely influenced birth weight, birth length, and head circumference. However, the possible use of 1-OH-PYR in mothers' urine as the biomarker of PAHs exposure was not confirmed by Al-Saleh et al. (2013). Polanska et al. (2014) studied several OH-PAHs metabolites in pregnant women's urine, but did not find any statistically significant effects on birth outcomes. Meanwhile, Choi et al. (2012) suggested a risk of decreased birth weight and birth length during the first trimester, especially the first gestational month in connection with OH-PAH metabolites, which corresponds to Dejmek's et al. (2000) results, who observed an increase of IUGR in the first month of gestation. Jedrychowski et al. (2017) postulated a more significant effect of PAHs than $PM_{2.5}$ exposure on birth outcomes, especially birth weight. Yang et al. (2018) observed decreased birth length due to prenatal exposure to 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, 9-OH-PHEN, 2-OH-FLUO, 9-OH-FLU, 1-OH-NAP, 2-OH-NAP and 1-OH-PYR in mothers' urine.

We confirmed that not only in the time frame of sampling in 2013–2014, but also in the new evaluation of time frames related to individual months of pregnancy up to the 24th month of the child's age, the differences in the level of air pollution between Karviná and České Budějovice were significant. They also differed significantly between seasons. $PM_{2.5}$ and B[a]P concentrations in Karviná in summer were comparable to those in České Budějovice in winter.

Significantly lower values of some birth outcomes (length, placental weight) were observed in Karviná and in the winter period, which can correspond to higher exposure to PAHs in polluted air; this was also indicated by the significantly higher concentrations of OH-PAH metabolites in newborns' and mothers' urine in Karviná. Similarly, our previous study of newborns and mothers also assessed the impact of prenatal exposure to air pollution on oxidative damage to DNA and lipids. Increased levels of oxidative damage were observed in the group exposed to higher concentrations of air pollutants in Karviná during the winter season (Ambroz et al., 2016).

Comparing summer to winter, the concentrations of OH-PAH metabolites were higher in both localities in winter. To clarify, the levels of OH-PAH metabolites in mothers were higher than in newborns; 9 out of 11 measured metabolites were significantly elevated in Karviná newborns compared to their mothers, in whom only 5 out of 11 metabolites were elevated. This indicates that the effect of OH-PAH metabolites was more pronounced in children.

The highest number of measured OH-PAH metabolites in newborns' urine was associated with a reduction in length (7 metabolites) and placental weight (9 metabolites). The Apgar 5' value was significantly lower in relation to the 6 metabolites.

In contrast to several of the studies mentioned above, we did not observe any effect of OH-PAH concentrations on birth weight. On the other hand, this negative result is consistent with the meta-analysis by Yang et al. (2018), who also observed no effect of exposure to PAHs on birth weight.

The most significant association was observed between several OH-PAH metabolites in newborns' urine and a reduction of placental weight, but without any relation to the study localities. Since placental weight also significantly influenced other birth parameters, the role of placental weight in fetal development is clearly crucial, and our results show that it is also highly influenced by PAH exposure.

Although our study did not confirm the findings of other studies on the effect of PAH exposure on the reduction of birth weight and head circumference, the results are innovative regarding the effect of PAH exposure on birth length, placental weight, and Apgar 5'. The most significant outcome is the reduction in placental weight, which can potentially affect all growth parameters, such as birth weight, length, and head circumference.

Conclusion

Our study is unique among similar epidemiological studies investigating the effects of air pollution, by the use the sensitive estimation of 11 metabolites of OH-PAHs in the urine of mothers and their newborns. Furthermore, newborns were born in two localities mainly differing by significant levels of air pollution. We linked the assessments of PAH exposure to growth parameters obtained from birth questionnaires, as well as pediatric questionnaires at the age of two years.

The obtained results indicate that newborns' growth parameters are negatively affected in those who were born in winter and in a high air polluted locality. Concentrations of OH-PAH metabolites in the urine of mothers and newborns reliably reflect the level of exposure to polluted air. The OH-PAH metabolites are strongly associated with birth length, placental weight, and Apgar 5', regardless of the studied localities.

Our study is limited and can be considered a pilot study that should be replicated in another, similar population. To conclude, the effect of PAHs on growth parameters is important and requires further investigation.

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Ethical approval
The study was conducted according to the 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, on June 30, 2017.

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

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This publication reflects only the view of the authors. The European Commission is not responsible for any use that may be made of the information the publication contains.

Data availability statement
All data in our paper and from the corresponding author are available 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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