 doi:10.20944/preprints202210.0003.v2

Prenatal Exposure to Polycyclic Aromatic Hydrocarbons and Growth Parameters

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Abstract: *Background and objectives:* The impact of prenatal exposure to polycyclicaromatic hydrocarbons (PAHs) on birth outcomes as weight, length, head circumference, placenta weight, and Apgar. *Materials and Methods:* Two cohorts of children born in the years 2013 and 2014 in Karvina (Northern Moravia, N=144) and Ceske Budejovice (Southern Bohemia, N=198), were studied for the relationship between the prenatal exposure to PAHs and growth parameters up to two years of age. PAHs exposure was evaluated according to the concentration of benzo[a]pyrene (B[a]P) in polluted air and monohydroxylated PAH metabolites (OH-PAHs) in urine of newborns as well as their mothers. Data of growth parameters were obtained from pediatric questionnaires up to 24 months.

Results: Concentrations of B[a]P were significantly higher in Karvina ($p<0.001$). OH-PAH metabolites were significantly higher in the mothers´ as well as in the newborns´ urine in Karvina. The length was shorter in newborns in Karvina at birth ($p<0.001$), but this difference was straightened out during next 3 to 24 months. Birth weight at the delivery did not differ between newborns in Karvina and Ceske Budejovice. Newborns in both locations significantly decreased their weight gain between birth and 3 months after delivery. OH-PAHs metabolites in mother's or newborn's urine did not affect birth weight. Top 25% values of concentrations of 2-OH-FLUO, 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, and the sum of all-OH-PAHs higher than median in the newborns´ urine decreased their length. 2-OH-PHEN top 25% of concentrations in the newborns´ urine decreased their head circumference, 2-OH-FLUO, 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-

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PHEN, 4-OH-PHEN, 9-OH-PHEN, 1-OH-PYR, and all-OH-PAHs decreased placenta weight; 2-OH-FLUO, 1-OH-NAP, 2-OH-NAP, 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, and all-OH-PAHs decreased Apgar 5´. *Conclusions:* We observed that higher concentration of PAHs determined as OH-PAHs metabolites in newborns´ urine decreased their length, head circumference, placenta weight, and Apgar 5´, but did not affect birth weight.

Key words: birth weight, birth length, head circumference, placenta weight, growth parameters, polycyclic aromatic hydrocarbons, monohydroxylated PAH metabolites

1. Introduction

Air pollution poses a serious threat to human health. Respirable particulate matter of aerodynamic diameter ≤ 2.5 µm (PM2.5), 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, PM2.5 particles have the ability to penetrate into the human body via the airways.Compared to larger particles, this is why they represent a significant health risk [1]. PAHs are produced by the incomplete combustion of organic matter. They are widely spread in the environment [2] and some of them have genotoxic [3, 4], mutagenic, carcinogenic [5], and embryotoxic activities [6].

Airborne polycyclic aromatic hydrocarbons (PAHs) can significantly affect birth outcomes. The first paper on this topic was published by Perera et al. 1998 [7] using new methods in molecular epidemiology. The impact of PAHs exposure was determined as PAH-DNA adducts in cord blood leucocytes in Poland (Krakow and Limanowa). Newborns with PAH/DNA adducts above median had significantly decreased birth length, weight and head circumference. Dejmek et al. [8] 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 [9-11] and by the direct interference with placental growth factors [12, 13].

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) OH-PAH concentration in urine.

Studies determining airborne PAHs effect on birth weight only are available in USA [14], and Poland [15]. In addition the effect on fetal growth (intrauterine growth retardation, IUGR) was studied in the Czech Republic by Dejmek et al [8].

Yang et al. [16] published meta-analysis of 11 studies about the association between the prenatal exposure to polycyclic aromatic hydrocarbons and birth weight. They concluded there is no significant relationship between prenatal exposure to PAHs and birth weight. They proposed: further studies are still needed.

Exposure to PAHs in the district of Karvina is similar to data from Krakow, Poland. Therefore, we studied the impact of prenatal exposure to PAHs on birth outcomes, using our previously published data.

2. Materials and Methods

We studied the impact of the prenatal PAHs exposure on subjects from two locations in the Czech Republic: newborns and their nonsmoking mothers living in Karvina, and Ceske Budejovice. The Ceske Budejovice group has been selected as a control group, since the levels of pollution in this locality are significantly lower than in Karvina. Sampling of biological material (urine, plasma) was performed at both locations in two periods with different levels of air pollution: in summer 2013 (low air pollutant levels) and in winter 2014 (high levels of air pollution). PM2.5 and PAHs exposure was assessed. and the oxidative damage to DNA and lipids was analyzed. We observed elevated levels of oxidative damage in the group exposed to higher concentrations of air pollutants in Karvina in the winter season [17].

2. 1. Subjects

We studied birth outcomes in newborns born in the Ceske Budejovice Hospital, Department of Obstetrics and Department of Neonatology, and in the Karvina 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 AS CR in Prague. Each mother signed the written consent. The heating in the homes of mothers was usually by the central heating or gas; open fire places were not used in any house. The samples were collected from normal deliveries (38-41 week+) of nonsmoking mothers and their newborns in the summer and winter season to account for differences in air pollution. The samples included venous blood and urine from 99 mothers (summer) and 100 mothers (winter) at Ceske Budejovice, a locality with relatively clean air, and 70 mothers (summer) and 73 mothers (winter) at Karvina, a locality with high air pollution, and cord blood and urine from 99 newborns (summer) and 100 newborns (winter) at Ceske Budejovice and 71 newborns (summer) and 74 newborns (winter) at Karvina (in both seasons was set of 1 twins). The basic characteristics of the groups studied are shown in Table 1.

Every mother filled a questionnaire about their age, BMI, current residency, eating habits, active smoking during and / or before pregnancy, gestation age, date of child birth, delivery type, child birth weight and gender. Summary information are shown in Tab. I**.**

2. 2. Air sampling and analysis of selected air pollutants

Particulate matter ≤2.5 µm (PM2.5) was collected by a High Volume (HiVol) 3000 Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) in both study localities. The sampling was conducted as previously described {19]. Detailed information on air sampling, extraction of organic complex mixtures (EOM) from the filters, and chemical analysis of B[a]P is given in Topinka et al. [19]. Concentrations of air pollutants were expressed in $\mu g/m^3$ (PM2.5) and ng/m^3 (B[a]P) [17].

2. 3. Information about children

Pediatricians were asked to fulfil out the age of 2 years old children the paediatrician questionnaire including the children medical records, countering the information about weight, length and head circumference at birth, 3 months, 6 months, 18 months, and 24 months.

2. 4. 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.

The total of 575 samples (287 urines from mothers and 288 urines from their newborn children) were obtained [18].

In urine, were analysed polycyclic aromatic hydrocarbons monohydroxymetabolites (OH-PAHs). OH-PAHs metabolites in urine of mothers and newborns were determined by Urbancova et al. [18] 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 obtained from women and their newborn children living in Ceske Budejovice and Karvina.

2. 5. APGAR 5'

APGAR score is determined by pediatrician after 5 minutes after delivery. It evaluates the newborn pulse rate, muscle tonicity, respiratory functions, skin color, and reflex.

2. 6. Statistics methods

Comparison between groups of mothers and children has been performed by using Mann Whitney U Test. Groups were defined by locality (Budejovice and Karvina) and study period (summer 2013 – August – September, and winter 2014 – January - April).

The percentage for categorical parameters of population described in Table 1 are calculated as common mean of 0/1 status values, so it represents percentage of this category in population of the study. Differences between regions has been calculated using logistical regression for categorical instead of Mann Whitney U-test.

Simply Spearman Rank Order Correlation has been used to verify expected relations between childrens´ birth parameters and placenta weight. Presented values are Spearman R with significance marker.

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

Timing frames related for pregnancy months are estimated by known delivery date and gestation age confirmed by medical personal. Air pollution values in these air frames had been individually calculated for every mother-child pair from public data of CHMI [20], related to both localities.

3. RESULTS

We used our previously obtained results about air pollution: During the sampling periods, the mean concentrations of PM2.5 in summer were 21.54±11.78 μg/m^3 in Karvina, and 12.14±7.23 μg/m^3 in Ceske Budejovice, 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 Karvina, and 0.44±0.63 ng/m^3 in Ceske Budejovice, in winter 5.15±5.47 ng/m^3 and 1.43±1.37 ng/m^3, respectively [17]. The concentration of polycyclic aromatic hydrocarbons metabolites (OH-PAHs) in urine of mothers and newborns were determined by Urbancova et al. [18]. Characteristics of study subjects are given in Table 1. Mothers in Karvina were younger than mothers in Ceske Budejovice ($p<0.01$). In Karvina, more mothers had primary and low secondary education ($p<0.01$); 33.1±42.7 mothers were single vs. 20.1±40.2 in Ceske Budejovice. During pregnancy, more mothers were employed in Ceske Budejovice ($p<0.01$). More mothers smoked before pregnancy in Karvina ($p<0.01$), but no differences were observed during pregnancy. Alcohol consumption by mothers between those two regions did not differ before as well as during pregnancy. There were no differences in the type of delivery by vaginal, Cesarean section, or forceps, but in Karvina vacuum extraction was more frequent ($p<0.05$).

Growth parameters as head circumference, length, weight, Apgar 5´ (see Materials and Methods 2.5) and placenta weight are listed in Table 2A, separately for the summer 2013 and winter 2014 in Table 2B. The length was shorter at birth in Karvina vs. Ceske Budejovice in both seasons. In Ceske Budejovice in winter length was shorter at 6, 18, and 24 months compared to summer ($p<0.05$). Apgar score in 5´ was lower in Ceske Budejovice in both seasons. Placenta weight was significantly lower in Karvina in both seasons. In Karvina, placenta weight was significantly lower in winter 2014 compared to summer 2013 ($p<0.001$) which corresponded to higher exposure to PAHs in polluted air; that was indicated by the higher concentrations of OH-PAH metabolites in newborns´ urine (Tab. 4). Placenta weight analysis showed that placenta weight significantly affected birth weight, birth length, and birth head circumference (Tab. 3). These results suggest a very significant role of placenta weight for the fetus development   OH-PAH metabolites for mothers and children in both seasons are listed in Tables 4A and 4B. OH-PAH metabolites were significantly higher in mothers´ as well as in the newborns´ urine in Karvina. 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 Tables 5A and 5B, the impact of mothers´ and childrens´ urine OH-PAH metabolites on growth parameters is analyzed. Top 25 % 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 Ceske Budejovice; 9-OH-

PHEN decreased placenta weight in Karvina. 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 Karvina, 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 6 estimates environmental pollution exposition from 1st month of pregnancy to 24th month after delivery. Concentrations of PM2.5 as well as B[a]P during all this period were significantly higher in Karvina compared to Ceske Budejovice ($p<0.001$).

Table 1. General Overview of Study Subjects

Characteristic			Total		Ceske Budejovice		Karvina	
			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
Count of Pregnancies		count	344	2.0 ± 1.2	199	2.1 ± 1.3	145	1.9 ± 1.1
Maternal Education	Primary	%	344	2.6 ± 16.0	199	1.0 ± 10.0+)	145	4.8 ± 21.5+)
	Lower secondary	%	344	12.8 ± 33.4	199	8.5 ± 28.0++)	145	18.6 ± 39.1++)
	Upper secondary	%	344	45.9 ± 49.9	199	50.3 ± 50.1	145	40.0 ± 49.2
	Studying University	%	344	0.3 ± 5.4	199	0.5 ± 7.1	145	0.0 ± 0.0
	University	%	344	36.1 ± 48.1	199	37.2 ± 48.5	145	34.5 ± 47.7
Maternal State	Single	%	344	25.6 ± 43.7	199	20.1 ± 40.2++)	145	33.1 ± 47.2++)
	Married	%	344	59.0 ± 49.3	199	62.3 ± 48.6	145	54.5 ± 50.0
	Fiancee	%	344	7.6 ± 26.5	199	8.5 ± 28.0	145	6.2 ± 24.2
	Divorced	%	344	5.8 ± 23.4	199	7.0 ± 25.6	145	4.1 ± 20.0
	Widow	%	344	0.0 ± 0.0	199	0.0 ± 0.0	145	0.0 ± 0.0
Employed In Pregnacy		%	344	69.8 ± 46.0	199	75.4 ± 43.2++)	145	62.1 ± 48.7++)
Chronical Disease		%	344	23.0 ± 42.1	199	24.6 ± 43.2	145	20.7 ± 40.6
Risk pregnancy		%	344	16.9 ± 37.5	199	17.6 ± 38.2	145	15.9 ± 36.7
Smoker		%	344	31.4 ± 46.5	199	31.2 ± 46.4	145	31.7 ± 46.7
	Pre Pregnancy	cig/day	337	1.0 ± 3.2	194	0.8 ± 2.9**)	143	1.3 ± 3.5**)
	1st Trimestr	cig/day	337	0.3 ± 1.5	194	0.1 ± 0.7	143	0.5 ± 2.2
	2nd Trimestr	cig/day	337	0.0 ± 0.4	194	0.0 ± 0.4	143	0.1 ± 0.5
	3rd Trimester	cig/day	337	0.0 ± 0.3	194	0.0 ± 0.4	143	0.0 ± 0.3
	ETS	cig/day	306	3.0 ± 8.8	189	2.1 ± 5.7	117	4.6 ± 12.1
Alcohol Consumption	Before Pregnancy	beer	332	0.5 ± 1.0	193	0.6 ± 1.1	139	0.5 ± 0.9
		wine	332	1.3 ± 1.9	193	1.4 ± 2.1	139	1.1 ± 1.6
		dist	333	0.1 ± 0.5	193	0.0 ± 0.3	140	0.1 ± 0.6
	During Pregnancy	beer	334	0.2 ± 0.6	192	0.2 ± 0.5	142	0.2 ± 0.6
		wine	334	0.2 ± 0.5	192	0.2 ± 0.5	142	0.1 ± 0.4
		dist	332	0.0 ± 0.2	191	0.0 ± 0.0	141	0.0 ± 0.3
Delivery Type	Vaginal	%	344	62.5 ± 48.5	199	64.8 ± 47.9	145	59.3 ± 49.3
	S.C.	%	344	33.1 ± 47.1	199	32.7 ± 47.0	145	33.8 ± 47.5
	Forceps	%	344	0.6 ± 7.6	199	1.0 ± 10.0	145	0.0 ± 0.0
	VEX	%	344	2.6 ± 16.0	199	0.5 ± 7.1+)	145	5.5 ± 22.9+)

Results of Mann Whitney U-test compared by region for continuous variable *) p < 0.05, **) p < 0.01, ***) p < 0.001
and Logistic Regression by region for dichotomic +) p < 0.05, ++) p < 0.01, +++) p < 0.001

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Table 2A. Childrens´ Growth Parameters

Growth Parameter					Total		Ceske Budejovice		Karvina
				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
		birth-3m	cm	278	5.8 ± 1.5	171	5.8 ± 1.6	107	5.7 ± 1.2
	gain	3m-6m	cm	282	3.0 ± 1.0	170	3.0 ± 1.1	112	3.0 ± 0.8
		6m-18m	cm	282	4.4 ± 1.1	170	4.5 ± 1.1	112	4.4 ± 1.0
		18m-24m	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-3m	cm	272	12.0 ± 2.3	162	11.8 ± 2.1	110	12.2 ± 2.5
		3m-6m	cm	275	6.6 ± 2.0	167	6.6 ± 2.1	108	6.6 ± 1.8
		6m-18m	cm	280	14.7 ± 3.1	170	14.4 ± 3.2	110	15.0 ± 3.0
		18m-24m	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-3m	g	261	2548 ± 636	163	2478 ± 604**)	98	2665 ± 673**)
		3m-6m	g	259	1666 ± 554	162	1626 ± 545	97	1733 ± 564
		6m-18m	g	256	3622 ± 1032	160	3648 ± 929	96	3580 ± 1189
		18m-24m	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***)

Results of Mann Whitney U-test compared by region *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$
and by period +) $p < 0.05$, ++) $p < 0.01$, +++) $p < 0.001$

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Table 2B. Childrens´ Growth Parameters

Growth Parameter				Ceske Budejovice				Karvina			
				Summer 2013		Winter 2014		Summer 2013		Winter 2014	
				N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Head Circumference	abs	at birth	cm	99	34.6 ± 1.5	98	34.6 ± 1.3	59	34.5 ± 1.4	72	34.4 ± 1.2
		3 months	cm	90	40.5 ± 1.4	81	40.3 ± 1.4	53	40.2 ± 1.1	60	40.2 ± 1.3
		6 months	cm	90	43.4 ± 1.5	80	43.2 ± 1.5	54	43.1 ± 1.3	61	43.1 ± 1.2
		18 months	cm	90	47.9 ± 1.4	81	47.7 ± 1.7	53	47.6 ± 1.5	60	47.4 ± 1.5
		24 months	cm	66	49.1 ± 1.5	72	48.9 ± 1.7	49	48.6 ± 1.4	57	48.7 ± 1.5
		birth-3m	cm	90	5.9 ± 1.6	81	5.7 ± 1.6	47	5.7 ± 1.4	60	5.7 ± 1.2
	gain	3m-6m	cm	90	3.0 ± 0.9	80	3.0 ± 1.2	53	3.0 ± 0.8	59	3.0 ± 0.9
		6m-18m	cm	90	4.5 ± 1.1	80	4.5 ± 1.2	53	4.6 ± 1.0	59	4.3 ± 1.0
		18m-24m	cm	66	1.2 ± 0.7	71	1.2 ± 0.8	48	1.1 ± 0.6	57	1.4 ± 0.8
Length	abs	at birth	cm	96	49.9 ± 1.8*)	96	50.0 ± 1.6**)	71	49.3 ± 2.2*)	73	49.1 ± 2.1**)
		3 months	cm	89	62.1 ± 2.6	78	61.7 ± 2.4	49	61.8 ± 2.6	61	61.2 ± 2.7
		6 months	cm	90	68.9 ± 2.6+)	80	68.0 ± 2.8+)	49	68.3 ± 2.8	62	67.9 ± 2.9
		18 months	cm	90	83.5 ± 3.0+)	81	82.2 ± 3.6+)	53	83.6 ± 3.9	63	82.6 ± 3.6
		24 months	cm	67	89.3 ± 3.5+)	74	88.1 ± 3.6+)	49	89.4 ± 3.9	57	88.7 ± 4.1
	gain	birth-3m	cm	86	12.1 ± 2.1	76	11.6 ± 2.1	49	12.4 ± 2.7	61	12.0 ± 2.4
		3m-6m	cm	89	6.8 ± 1.9	78	6.3 ± 2.3	48	6.6 ± 1.8	60	6.6 ± 1.9
		6m-18m	cm	90	14.6 ± 2.9	80	14.2 ± 3.5	48	15.5 ± 3.2	62	14.7 ± 2.7
		18m-24m	cm	67	5.7 ± 2.3	73	6.0 ± 2.4	48	5.8 ± 3.1	57	6.1 ± 2.2
Weight	abs	at birth	g	99	3477 ± 448	99	3453 ± 438	71	3394 ± 469	73	3363 ± 419
		3 months	g	81	6037 ± 740	82	5860 ± 641	38	6107 ± 799	60	6035 ± 820
		6 months	g	81	7651 ± 860	81	7488 ± 825	38	7873 ± 1024	60	7700 ± 1030
		18 months	g	80	11299 ± 1407	80	11128 ± 1374	37	11519 ± 1850	63	11238 ± 1624
		24 months	g	57	12998 ± 1826	72	12596 ± 1598	33	12909 ± 1689	56	12883 ± 2050
	gain	birth-3m	g	81	2555 ± 601	82	2402 ± 601*)	38	2717 ± 631	60	2632 ± 702*)

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	3m-6m	g	81	1614 ± 434	81	1637 ± 640	38	1766 ± 489	59	1711 ± 610
	6m-18m	g	80	3648 ± 937	80	3648 ± 927	36	3659 ± 1306	60	3532 ± 1121
	18m-24m	g	57	1769 ± 1045	70	1425 ± 683	32	1523 ± 937	56	1601 ± 770
Apgar 5'			90	$9.9 \pm 0.4^{++)}$	92	$9.6 \pm 0.7^{++)***)}$	64	10.0 ± 0.2	65	$10.0 \pm 0.0^{***)}$
Placenta Weight		g	98	566 ± 123	93	$588 \pm 127^{***)}$	69	$556 \pm 130^{++)}$	73	$490 \pm 125^{++)***)}$

Results of Mann Whitney U-test compared by region *) p < 0.05, **) p < 0.01, ***) p < 0.001, by period $^{+}$) p < 0.05, $^{++}$) p < 0.01, $^{+++}$) p < 0.001

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Table 3. Correlations of Birth Parameters with Placenta Weight

	All		Ceske Budejovice		Karvina		All			
							Summer 2013		Winter 2014	
	N	p value	N	p value	N	p value	N	p value	N	p value
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***)

	Ceske Budejovice				Karvina			
	Summer 2013		Winter 2014		Summer 2013		Winter 2014	
	N	p value	N	p value	N	p value	N	p value
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

Spearman Rank Order Correlarion R - *) p < 0.05, **) p < 0.01, ***) p < 0.001

Table 4A. Mothers´ and Children´s Urine PAH OH Metabolites at Birth.

Subject	PAH-OH	Total		Ceske Budejovice		Karvina	
		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***)
Children	3-OH-BaP	284	450,0 ± 0,0	169	450,0 ± 0,0	115	450,0 ± 0,0
	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***)

Results of Mann Whitney U-test compared by region *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$
and by period +) $p < 0.05$, ++) $p < 0.01$, +++) $p < 0.001$

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Table 4B. Mothers´ and Children´s Urine PAH OH Metabolites at Birth.

Subject	PAH-OH	Ceske Budejovice				Karvina			
		Summer 2013		Winter 2014		Summer 2013		Winter 2014	
		N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Mothers	3-OH-BaP	89	450.0 + 0.0	64	450.0 + 0.0	80	450.0 + 0.0	50	450.0 + 0.0
	6-OH-CHRY	89	5.0 + 0.0	64	5.0 + 0.0	80	5.0 + 0.0	50	5.0 + 0.0
	2-OH-FLUO	89	388.3 + 360.3$^{+++)***)}$	64	252.4 + 249.9$^{+++)***)}$	80	558.4 + 591.6$^{+++)***)}$	50	991.6 + 869.7$^{+++)***)}$
	1-OH-NAP	89	699.0 + 1729.8$^{+++)***)}$	64	419.6 + 914.6$^{+++)***)}$	80	917.1 + 1233.3$^{+++)***)}$	50	2368.0 + 4121.0$^{+++)***)}$
	2-OH-NAP	89	6103.0 + 4424.2	64	7861.7 + 7030.7$^{**)}$	80	5806.7 + 4257.3	50	8206.7 + 6066.5$^{**)}$
	1-OH-PHEN	89	405.0 + 348.0$^{***)}$	64	915.1 + 895.5$^{***)}$	80	478.9 + 471.0$^{***)}$	50	813.8 + 624.9$^{***)}$
	2-OH-PHEN	89	184.2 + 152.0$^{+++)}$	64	208.0 + 175.5$^{+++)***)}$	80	308.1 + 359.3$^{+++)}$	49	466.1 + 398.5$^{+++)***)}$
	3-OH-PHEN	89	85.3 + 56.4$^{***)}$	64	64.1 + 87.0$^{***)}$	80	111.5 + 114.3$^{+++)***)}$	50	221.3 + 165.1$^{+++)***)}$
	4-OH-PHEN	89	86.3 + 81.2$^{+++)**)}$	64	115.1 + 119.3$^{+++)**)}$	80	645.7 + 1116.2$^{+++)**)}$	50	1090.1 + 1486.7$^{+++)**)}$
	9-OH-PHEN	89	150.2 + 145.1$^{+++)***)}$	64	39.0 + 73.8$^{+++)}$	80	995.0 + 1106.6$^{+++)***)}$	50	1840.3 + 2502.4$^{+++)}$
	1-OH-PYR	89	203.7 + 142.7	64	213.7 + 125.0$^{***)}$	80	246.0 + 200.0$^{+++)}$	50	359.5 + 178.7$^{+++)***)}$
	All-OH-PAH	89	8301.4 + 6380.1$^{+)}$	64	10077.8 + 7441.3$^{+)***)}$	80	10066.6 + 6318.5$^{+++)}$	50	16346.5 + 12127.6$^{+++)***)}$
Children	3-OH-BaP	89	450.0 + 0.0	65	450.0 + 0.0	80	450.0 + 0.0	50	450.0 + 0.0
	6-OH-CHRY	89	5.0 + 0.0	65	5.0 + 0.0	80	5.0 + 0.0	50	5.0 + 0.0
	2-OH-FLUO	89	127.1 + 106.8$^{+++)***)}$	65	266.2 + 135.7$^{+++)***)}$	80	91.8 + 113.4$^{+)***)}$	50	364.2 + 320.6$^{+)***)}$
	1-OH-NAP	89	104.6 + 247.4$^{+++)***)}$	65	666.2 + 467.8$^{+++)***)}$	80	131.9 + 204.1$^{+)***)}$	50	854.1 + 549.6$^{+)***)}$
	2-OH-NAP	89	2969.6 + 2298.1$^{***)}$	65	5806.4 + 4741.8$^{***)}$	80	3123.1 + 3005.9$^{***)}$	50	4965.9 + 2862.2$^{***)}$
	1-OH-PHEN	89	165.8 + 130.3$^{+++)***)}$	65	872.6 + 654.3$^{+++)***)}$	80	123.8 + 173.4$^{***)}$	50	814.7 + 592.1$^{***)}$
	2-OH-PHEN	89	110.7 + 93.4$^{+++)***)}$	65	490.5 + 290.8$^{+++)***)}$	80	89.3 + 107.3$^{***)}$	50	481.3 + 361.4$^{***)}$
	3-OH-PHEN	89	22.1 + 19.7$^{+++)***)}$	65	69.0 + 50.5$^{+++)***)}$	80	14.4 + 21.0$^{+++)***)}$	50	112.4 + 71.5$^{+++)***)}$
	4-OH-PHEN	89	9.6 + 11.1$^{+++)***)}$	65	36.3 + 42.7$^{+++)**)}$	80	154.4 + 232.9$^{+++)***)}$	50	356.9 + 534.9$^{+++)**)}$
	9-OH-PHEN	89	90.5 + 73.7$^{+++)***)}$	65	297.9 + 160.7$^{+++)***)}$	80	817.8 + 852.9$^{+++)***)}$	50	3769.3 + 3190.1$^{+++)***)}$
	1-OH-PYR	89	37.4 + 54.9$^{***)}$	65	129.9 + 111.5$^{***)}$	80	26.0 + 27.2$^{***)}$	50	173.7 + 166.0$^{***)}$
	All-OH-PAH	89	3612.1 + 2530.9$^{***)}$	65	8627.2 + 5183.9$^{***)}$	80	4548.8 + 3498.9$^{+++)***)}$	50	11885.8 + 5393.9$^{+++)***)}$

Results of 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

 doi:10.20944/preprints202210.0003.v2

Table 5A. Impact of Mothers´ and Children´s Urine PAH OH Metabolites on Growth Parameters

Subject	PAH-OH	Total						Ceske Budejovice						Karvina					
		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

Beta coefficient results of regression between PAH-OH and decrease of Growth Parameters *) p < 0.05, **) p < 0.01, ***) p < 0.001

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Table 5B. Impact of Mothers´ and Children´s Urine PAH OH Metabolites on Growth Parameters

Subject	PAH-OH	Ceske Budejovice												Karvina											
		Summer 2013						Winter 2014						Summer 2013						Winter 2014					
		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'	N	Head Circumference	Length	Weight	Placenta Weight	Apgar 5'
Mothers	2-OH-FLUO	99	0.02	-0.04	0.02	0.18	-0.09	100	-0.03	0.01	0.01	0.00	-0.17	71	-0.19	0.09	0.13	0.09	0.08	74	0.07	0.30*)	0.21	0.05	-
	1-OH-NAP	99	-0.13	-0.17	-0.03	-0.01	-0.04	100	0.04	-0.01	0.02	0.12	-0.13	71	-0.24	-0.03	0.01	-0.11	0.16	74	-0.03	0.27	0.13	0.11	-
	2-OH-NAP	99	-0.01	-0.04	0.03	0.13	0.02	100	-0.11	0.00	-0.12	-0.22	0.00	71	0.06	0.09	-0.05	0.19	-0.23	74	-0.05	-0.06	-0.04	0.03	-
	1-OH-PHEN	99	-0.01	-0.06	0.07	0.16	0.06	100	0.01	0.01	0.07	-0.09	-0.08	71	-0.07	0.10	0.04	0.19	0.02	74	0.24	0.21	0.35*)	0.14	-
	2-OH-PHEN	99	-0.06	-0.04	0.03	0.14	-0.01	100	-0.10	0.08	0.03	-0.09	0.12	71	-0.21	0.02	-0.03	0.32*)	0.08	74	0.20	0.28*)	0.22	-0.01	-
	3-OH-PHEN	99	0.01	-0.03	0.01	0.08	-0.07	100	0.08	0.08	0.17	0.08	-0.10	71	-0.18	0.15	0.11	0.14	0.07	74	0.07	0.12	0.19	0.07	-
	4-OH-PHEN	99	0.04	-0.05	0.04	0.08	0.00	100	0.02	0.03	0.04	0.10	0.14	71	-0.23	-0.09	-0.12	0.13	0.01	74	0.11	0.30*)	0.16	-0.14	-
	9-OH-PHEN	99	-0.13	-0.19	-0.19	-0.21	-0.12	100	-0.10	0.05	-0.10	0.08	0.13	71	0.03	0.22	0.17	0.05	-0.04	74	0.14	0.33*)	0.22	-0.15	-
	1-OH-PYR	99	-0.09	0.08	0.10	0.10	0.01	100	0.14	0.14	0.13	0.22	-0.09	71	0.12	0.15	0.14	0.29*)	-0.02	74	-0.10	0.11	0.03	-0.03	-
	All-OH-PAH	99	-0.01	-0.08	0.03	0.13	0.00	100	-0.09	0.00	-0.11	-0.10	0.01	71	-0.02	0.11	-0.02	0.20	-0.21	74	-0.05	0.13	0.11	-0.03	-
Children	2-OH-FLUO	89	-0.03	-0.17	-0.08	-0.08	0.05	80	-0.08	-0.07	0.03	-0.23	0.03	65	-0.17	-0.16	-0.19	-0.22	-0.10	50	0.11	0.23	0.19	-0.09	-
	1-OH-NAP	89	-0.05	-0.13	-0.11	-0.02	0.09	80	-0.15	-0.14	0.01	-0.24*)	0.00	65	-0.17	-0.05	-0.03	-0.01	-0.05	50	-0.23	0.06	-0.11	-0.09	-
	2-OH-NAP	89	0.12	-0.07	0.08	0.12	0.02	80	-0.11	-0.10	-0.05	-0.38***)	-0.02	65	0.09	-0.06	-0.12	-0.05	-0.34**)	50	0.23	0.09	0.17	-0.19	-
	1-OH-PHEN	89	0.07	0.01	0.03	0.09	0.12	80	-0.03	0.00	0.11	-0.20	0.11	65	-0.24	0.00	0.05	-0.10	-0.13	50	-0.16	-0.11	-0.11	-0.18	-
	2-OH-PHEN	89	-0.04	-0.07	-0.08	-0.08	0.04	80	-0.14	-0.01	0.05	-0.06	0.00	65	-0.20	-0.07	-0.01	0.14	-0.11	50	-0.16	0.08	-0.12	-0.32*)	-
	3-OH-PHEN	89	-0.04	-0.19	-0.08	-0.08	0.11	80	-0.04	-0.02	0.06	-0.27*)	-0.03	65	-0.43**)	-0.19	-0.32**)	-0.11	0.08	50	0.09	0.28*)	0.11	-0.19	-
	4-OH-PHEN	89	-0.09	-0.10	0.01	0.06	0.12	80	-0.20	-0.12	-0.08	-0.28*)	0.02	65	-0.23	-0.18	-0.08	0.00	-0.06	50	0.05	0.11	-0.03	-0.33*)	-
	9-OH-PHEN	89	0.03	-0.16	-0.02	0.09	0.01	80	0.18	0.06	0.20	0.03	0.21	65	-0.13	-0.07	-0.12	-0.14	-0.05	50	0.11	0.20	0.00	-0.30*)	-
	1-OH-PYR	89	-0.06	-0.08	0.01	0.00	0.04	80	-0.05	0.03	0.02	-0.11	-0.02	65	-0.03	0.09	0.08	0.21	-0.03	50	0.09	0.21	0.11	-0.32*)	-
	All-OH-PAH	89	0.12	-0.10	0.06	0.10	0.06	80	-0.13	-0.10	-0.03	-0.37**)	0.02	65	-0.06	-0.09	-0.15	-0.05	-0.31*)	50	0.13	0.17	0.13	-0.35*)	-

Beta coefficient results of regression between PAH-OH and decrease of Growth Parameters *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$

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Table 6. Estimated Environmental Polution Exposure of Mothers

EP estimated values		Month		Total		Ceske Budejovice		Karvina	
				N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
CHMI	PM 2.5µm	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***)
	Benzo(a)pyren	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***)

Results of Mann Whitney U-test compared by region *) p < 0.05, **) p < 0.01, ***) p < 0.001, by period +) p < 0.05, ++) p < 0.01, +++) p < 0.001

4. DISCUSSION

In our study, we did not observe any effect of locations with different level of PAH concentrations in polluted air on birth weight. Higher PAHs exposure expressed as OH-PAH metabolites in newborns decreased length, placenta weight, and Apgar 5´.

Polanska et al. [21] evaluated PAHs exposure as 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, a possible use of 1-OH-PYR in mothers´ urine as the biomarker of PAHs exposure was not confirmed by Al-Saleh et al. [22]. In further study Polanska et al. [23] used several OH-PAHs metabolites in pregnant womens´ urine, but they did not find any statistically significant effect on birth outcomes. Choi et al. [24] suggested the risk of decreased birth weight and birth length during the first trimester, especially first gestational month. This corresponds to Dejmek´s results [8] who observed the increase of IUGR in the first month of gestation. Jedrychowski et al. [25] studied the impact of barbecued meat consumption during pregnancy on birth outcomes; they observed a significant deficit in birthweight. Korean study [26] also proved that higher levels of barbecued, fried, roasted and smoked meats during pregnancy was associated with reduced birth weight. Jedrychowski et al. [15] postulated a more significant effect of PAHs than PM2.5 exposure on birth outcomes, especially birth weight. Yang et al. [27] observed the 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 did not observe any relationship between these metabolites in mothers´ urine and birth length. But 1-OH-PHEN, 2-OH-PHEN, 3-OH-PHEN, 4-OH-PHEN, 1-OH-NAP, 2-OH-NAP, and 2-OH-FLUO in childrens´ urine significantly decreased birth length.

Our study did not confirm results of other studies about the impact of PAHs exposure on decreased birth weight and head circumference. Our results may be innovative to indicate the impact of PAHs exposure on birth length, placenta weight, and Apgar 5´. Probably, the most significant outcome may be seen as the decrease of placenta weight which further affect all birth parameters as birth weight, birth length, and head circumference.

Funding: This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032 (HBM4EU) and by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798).
Acknowledgements: We would like to thank all obstericians, nurses and parents in both districts for the close cooperation, which made our study possible, and Mrs. Kamila Kavka for language editing.

 doi:10.20944/preprints202210.0003.v2

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 Ceske Budejovice, Czech Republic, in June 30, 2017.

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

Disclaimer: This publication reflects only the author's view and the European Commission is not responsible for any use that may be made of the information it contains.
Consent to Publish: All authors approved this text.

Conflict of Interest: The authors declare no conflict of interest.

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

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