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Chapter

Influences the Aeromath in the Way of Ending Births

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Abstract

Air pollution represents a significant health problem in the Czech Republic (CR). Originally, the most polluted region was Northern Bohemia, later Northern Moravia. These specific conditions were used to study the impact of air pollution to children in those two regions. In Northern Bohemia, the impact of the increased concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) to fetal growth was observed, expressed as intrauterine growth retardation and impact of air pollution to respiratory morbidity and neurodevelopment in children. In Northern Moravia was studied the effect of air pollution to the morbidity of preschool children; to asthma bronchiale—gene expression, children susceptibility to benzo[a]pyrene (B[a]P); to genetic damage in newborns; concentrations of PAHs in the urine of mothers and newborns, content of PAHs in human breast milk and diet.

Keywords: air pollution, polycyclic aromatic hydrocarbons, pregnancy outcome, respiratory morbidity, neurodevelopment, genetic damage, oxidative stress

1. Introduction

Health impact of air pollution to children was studied in heavily polluted parts of the Czech Republic during the last 30 years. The research program analyzed these effects in the region of Northern Bohemia, later in Northern Moravia, using Southern Bohemia as the control region.

It is generally accepted that exposure to air pollution has negative effects on human health, increasing risk of mortality and morbidity from respiratory and cardiovascular diseases [1]. WHO [2] recognized air pollution and particulate matter (PM) in polluted air as a proven human carcinogen. Another human carcinogen in the polluted air is benzo[a]pyrene (B[a]P) from polycyclic aromatic hydrocarbons (PAHs) [3]. According to WHO, air polluted by B[a]P concentrations higher than 1.0 ng/m$^3$ induces DNA damage [4]. Study on city policemen exposed personally to B[a]P over 1.0 ng/m$^3$ proved increased genomic frequency of translocations [5], micronuclei [6], and DNA fragmentation in sperm [7].

2. Northern Bohemia

The Northern Bohemia brown coal basin comprises four mining districts located in the northwestern region of the Czech Republic (CR) (Figure 1). The coal in this region contains usually 1–3% of sulfur and is surface-mined from open-pits. It has
Childbirth

been used especially for coal-fired power plants and local heating. The combustion of this coal together with the heavy industrialization made this region in previous decades one of the most polluted regions in Europe (called together with a similar pollution on the other side of the border with Germany and Poland “Black Triangle”) [8].

Exploratory analysis of the health consequences of environmental pollution in this region prior 1989 suggested shorter life expectancy for males and females (2 years vs. CR), increased frequency of congenital anomalies (5.5–7 vs. 2% in CR), increased frequency of newborns with lower birth weight (<2500 g, 8–9 vs. 4.5% in CR), and increased respiratory morbidity in preschool children (2.90 vs. 0.54 in CR, number of cases/100 children) [9, 10].

After major political changes in the Czechoslovakia in November 1989, a new research program, the Teplice Program, was developed to evaluate the short-term and long-term health impact of air pollution on population. As a model district, the mining district of Teplice was selected, and as a control district, the district of Prachatice in Southern Bohemia was selected with some of the cleanest air in the Czech Republic. The Teplice district had 127,500 inhabitants and an area of 469 km², and a large part of the area has been devastated by the strip mining of coal and associated industrialization. District of Prachatice had 51,500 inhabitants and an area of 1375 km², and 52% of it is covered by forests. For example, in 1993, the average PM10 (<10 μm) concentrations in Teplice were 76 vs. 38 μg/m³, and in Prachatice, PM2.5 concentrations were 64 vs. 32 μg/m³ and B[a]P was 3.7 vs. 2.5 ng/m³, respectively [11].

The Teplice Program was initiated by the Czech Ministry of Environment in 1990, and the research program was prepared in collaboration with US Environmental Protection Agency (US EPA) [12].

2.1 Pregnancy outcome

Initially, a relationship between PM and fetal growth was observed by analyzing data collected during the first 2 years of this study in the highly polluted district of Teplice [13]. When mothers were exposed to PM10 levels >40 μg/m³ or PM2.5 >37 μg/m³ during the first month of gestation, increased risk of intrauterine growth retardation (IUGR) was observed in their children. For each 10 μg/m³ increase
In PM10, the adjusted odds ratio (AOR) of IUGR was 1.25 (95% CI, 1.08–1.56); a similar but weaker association was also observed for PM2.5. This was the first study indicating that exposure to PM10 and PM2.5 affects the fetal growth already in the first months of gestation. But similar association for the risk of IUGR and exposure to sulfur dioxide, nitrous oxides, or ozone was not observed.

Dejmek et al. [14] examined the impact of PM10, PM2.5, and PAHs on IUGR in all single births that occurred in the Teplice and Prachatice districts during the 4-year period from April 1994 through March 1998, which included 3349 pregnancies in the Teplice cohort and 1505 pregnancies in the Prachatice cohort. Compared with exposure to the mean PM10 of <40 \( \mu g/m^3 \) during the first month of gestation, the AOR was 1.44 (95% CI, 1.03–2.02) for the medium-exposure group (PM10 40–<50 \( \mu g/m^3 \)) and 2.14 (95% CI, 1.42–3.23) for PM10 of \( \geq 50 \mu g/m^3 \). Using a continuous exposure, the AOR of IUGR was 1.19 (CI, 1.06–1.33) per 10 \( \mu g/m^3 \) increase of PM10 in the first gestational month. Dejmek et al. [14] further analyzed in both districts the association between carcinogenic PAHs and IUGR. In the district of Teplice, a significant increase of IUGR was observed related to exposures of carcinogenic PAHs (c-PAHs) (benza[a]anthracene, benzo[k]fluoranthen, benzo[g,h,i]perylene, benzo[a]pyrene (B[a]P), chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene) above 15 ng/m³, which corresponds to 2.8 ng/m³ of B[a]P. Similarly as with PM10 and PM2.5, the effect was specific for the first gestational month. Using a continuous measure of exposure, a 10 ng/m³ increase in c-PAH level was associated with an AOR of 1.22 (95% CI, 1.07–1.39). The association between c-PAHs and IUGR was also observed in Prachatice, where such effect was not seen for PM10. Again, the only consistent association between c-PAHs and IUGR was observed in the first gestational month.

The results by Dejmek et al. [13, 14] suggest that the first month was the most sensitive period for the effect of air pollutants. Data by Dejmek et al. [14] imply a critical role of PAHs. It is possible that c-PAHs are responsible for the biologic activity of complex mixtures adsorbed to respirable air particles that can result in IUGR. The results by Dejmek et al. [14] support the hypothesis that the increased IUGR risk is related to c-PAHs. Based on this hypothesis, the association of particles with IUGR risk in Teplice is related to the high correlation of c-PAHs and PM in this area [14]. This finding is consistent with the idea of a primary role for c-PAHs in fetal growth modulation.

Support for the role of PAH in reproductive effects is the findings from many of the biomarker studies [15]. PAH-DNA adduct levels in placentas of nonsmoking mothers were associated with exposure to c-PAHs, and DNA adducts in placenta of IUGR infants were significantly increased [15].

Thus, the first gestational month seems to be the critical period for the association of pollution with fetal growth. The timing of this association is in agreement with the current hypothesis that IUGR pathogenesis is triggered by an abnormal reaction between trophoblast and uterine tissues in the first week of pregnancy [16]. Fine particles and c-PAH levels were associated with IUGR risk. Reduced fetal growth is an important predictor of neonatal morbidity and mortality. Barker [17] showed a relationship between some serious adult risks (viz., non-insulin-dependent diabetes, hypertension, and coronary heart disease) and impaired growth in the prenatal and early postnatal period. He implied that higher exposure to pollutants during the early stages of intrauterine life may be responsible for diseases in the middle age.

2.2 Respiratory morbidity

Stratified random sample of 1492 mother-infant pairs from the “Pregnancy Outcome Study” was recruited to “Immune Biomarker Study,” and samples of
maternal and cord blood were collected at delivery. Later, these children were contacted by pediatric nurses for follow-up at the age of 3 and 4.5 years. Pediatricians and pediatric nurses administered the questionnaires to the family and abstracted the children's medical records. In 2005 the medical records of these children up to the age of 6 years were abstracted; the study continued with 1007 children. The study continued each year until the children born in 1998 reached the age of 10 years. The data covering the period from birth to 10 years were available for 960 children, i.e., 245 boys and 269 girls in the Teplice district and 227 boys and 219 girls in the Prachatice district. Respiratory infections were reported using International Codes of Diseases -10 (ICD-10 codes) [18].

Morbidity differed between children from Teplice town, Teplice district, and Prachatice. Comparing Teplice town vs. Prachatice, at the age 0–2 years, in the Teplice town, incidence of laryngitis, tracheitis, influenza, pneumonia, and otitis media was higher; at the age 2–6 years, laryngitis, tracheitis, and influenza; and at the age 6–10 years, laryngitis, tracheitis, and influenza. Similar differences in the respiratory morbidity were observed between Teplice town and Teplice district for laryngitis, tracheitis, and influenza at the age 0–6 years and bronchitis, laryngitis, tracheitis, and influenza at the age 6–10 years. The prevalence of allergies at the age of 6 years diagnosed as wheezing was higher in the Teplice district (OR 2.0, 95% CI 1.1–3.4), but allergic rhinitis was higher in the Prachatice district (OR 2.7, 95% CI 1.7–4.2) [18].

When this cohort (N = 1105) was followed at the age of 3 years for the impact of air pollution to the children height, indoor coal use was associated with reduced height at age 36 months, adjusted for anthropometric and sociodemographic factors: 1.34 cm for boys and 1.30 for girls [19]. Baker et al. [20] studied in this subcohort (N = 452) incidence of lower respiratory illness (LRI). Maternal prenatal smoking and coal home heating increased significantly the risk for LRI in the first 3 years (RR = 2.77 (95% CI, 1.45–5.37) and RR = 2.52 (95% CI, 1.31–4.85), respectively).

2.3 Neurobehavioral studies

In the mining districts of Northern Bohemia, the first studies on a possible impact of air pollution to children's neurodevelopment started. Symptoms of minimal brain dysfunction (MBD) were analyzed in 5080 children in the second class from the districts of Usti nad Labem, Teplice, and Jablonec nad Nisou. Examination implied increased disturbance of intellect as well as behavioral changes [21]. From polluted districts, 4.8% of children visited special schools, and 10% of children in normal schools were diagnosed with MBD symptoms. Therefore, it may be postulated that the children living in this polluted region are at greater risk for learning disorders than other children in the Czech Republic. Sram [10] hypothesized that in utero exposure to environmental chemicals causes functional changes in the nervous system expressed as developmental disorders or other behavioral dysfunctions. This idea was later confirmed by studies from the USA, Poland, China, and Spain [22]. According to the Czech Statistical Institute [10], in 1988 mental illness was diagnosed in children of age 7–15 years in 4.09% in mining districts vs. 2% in the Czech Republic.

Neurobehavioral functions were assessed using neurobehavioral evaluation system (NES2, computerized assessment battery) [23] in 2nd-, 4th- and 8th-grade students from Teplice and Prachatice (2nd-grade cohort N = 772, 4th-grade cohort N = 322, 7th-grade cohort N = 470 children). Teachers reported that significantly more 2nd-grade children from Teplice (26.6%) than from Prachatice (12.9%) had been referred for clinical assessment of learning and behavioral problems. Similar
results were observed in the 4th-grade children, 27.3% from Teplice and 13.0% from Prachatice, as well as in 7th-grade children, 25.6% from Teplice and 13.1% from Prachatice. Those neurobehavioral studies really indicate poorer performance on neurobehavioral tests and high prevalence of learning disabilities in children from the air polluted mining district [24].

3. Northern Moravia

The Moravian-Silesian Region (MSR) is a heavily populated, industrial area situated in the easternmost part of the Czech Republic, covering 5428 km² with 1.21 million inhabitants [25]. Since the second half of the eighteenth century, the region has been characterized by coal mining, the processing of coal, and metallurgy. Currently, the most important industries are metallurgy, steel and coke production, coal mining, and power generation.

The MSR population is exposed to high concentrations of PM2.5 which exceed the EU standard of 25 μg/m³/year. Similarly, the concentrations of B[a]P in the MSR are several times higher than the EU standard of 1 ng/m³/year. In the district of Ostrava Radvanice-Bartovice (R&B), the concentrations of B[a]P reached the highest in the Czech Republic. Comparing air pollution between 2010 and 2017, it appears that concentrations of PM2.5 decreased in the MSR, but surprisingly, there is no change in the concentration of B[a]P in Ostrava Radvanice-Bartovice [26].

To verify the impact of air pollution on the health of the population in MSR, we analyzed the morbidity of children in three studies: (1) morbidity in children; (2) asthma bronchiale in children; and (3) impact of air pollution on the genome of newborns.

3.1 Child morbidity in the city of Ostrava

The morbidity in the city of Ostrava was studied in 10 pediatric districts in children born from 2001 to 2004 up to 5 years of age (N = 1888) [27]. The pediatricians abstracted medical records in ICD-10 codes. Comparing the detailed age-specific morbidity of 1655 children born and living in the district of Ostrava Radvanice-Bartovice (R&B) vs. children in other parts of Ostrava, a significantly higher incidence of acute illness was observed. Children from R&B experienced a higher incidence of acute respiratory disease in the first year of life (Figure 2) and a higher prevalence of asthma bronchiale (37.1%, N = 170) compared to other parts of Ostrava (10.2–13.2%, N = 1287) [27]. From birth until the age of 5 years, the incidences of pneumonia, tonsillitis, viral infections, and intestinal infectious diseases were also several times higher in children living in the district of Ostrava Radvanice-Bartovice. As Hertz-Picciotto et al. [28] proposed, prenatal exposure to PAHs may alter lymphocyte immunophenotypic distribution in cord blood and change immunoglobulin E levels in the cord serum. We may hypothesize that high concentrations of PAHs affect maturation of the immune system, and children from a more polluted region, therefore, suffer from higher respiratory morbidity, especially in their first year of life.

3.2 Asthma bronchiale in children

This study evaluated the impact of air pollution on gene expression in children and analyzed if there is any specific effect on the origin and development of asthma bronchiale. Specifically, we compared gene expression profiles in the leukocytes of asthmatic children with those in children without asthma,
using Illumina HumanHT-12 BeadChip. The study included a group of 200 children—100 asthmatic and 100 healthy children—aged 6–15 years living in the district of Ostrava Radvanice-Bartovice and a control group of 200 children—100 asthmatic and 100 healthy children—living in the district of Prachatice (Southern Bohemia) [29].

Comparing the first signs of asthma bronchiale (e.g., wheezing), the prevalence in Ostrava was approximately 60% of the cases diagnosed up to the age of 3.5 years, while in Prachatice it was only 25%.

Gene expression was analyzed in 368 samples, and RNA was hybridized on whole genome chips with more than 20,000 coding genes per chip. Samples were evaluated according to locality and disease (i.e., Ostrava-asthma, Ostrava-control, Prachatice-asthma, and Prachatice-control). Differences in gene expression were checked by the statistical tests, t-test and ANOVA. When children were compared, according to locality and the change in the gene expression >1.5, 64 deregulated genes were observed. When Ostrava-asthma children were compared with Ostrava-control, 12 deregulated genes were observed. Comparing Prachatice-asthma children with Prachatice-control, 17 deregulated genes were observed. Using Venn diagrams, genes that were specific to asthma in Ostrava and to Prachatice were found to differ completely, while no gene was observed in both localities. Effects were further observed for the MAPK signaling pathway ($P < 0.01$, 1.5-fold) in Ostrava and for the cytokine-cytokine receptor interaction pathway ($P < 0.01$, 1.5-fold) in Prachatice.

Selected genes were verified using the qPCR method. For asthmatic children from Prachatice, the results showed an increased expression of the genes SIGLEC8, CLC, CCL23, and CACNG6 (relationship of the presence of eosinophils and eosinophilic inflammation is related to the allergic type of asthma) corresponding to the allergic phenotype. For asthmatic children from Ostrava, increased gene expression corresponded to the non-allergic phenotypes DEFA4 (relationship to the presence of neutrophils), AHSP (stabilization of hemoglobin), and HBG2 (part of fetal hemoglobin, with a higher affinity to oxygen). We may check if the increased expression of the genes HBG2 and AHSP is related to hypoxia in Ostrava children or if it is related to changes in hematopoiesis. The significant difference in the gene expression was observed comparing children from Ostrava and Prachatice, which is probably related to the dissimilarity of air pollution between these two regions, especially different concentrations of B[a]P exposure.
This study is unique because it is the first time when whole genome microarrays were used to analyze the relationship between air pollution and asthma bronchiale. The results suggest the distinct phenotype of asthma in children living in the polluted Ostrava region (non-allergic type) compared to children living in Prachatice (allergic type).

Rossnerova et al. [30] studied DNA methylation in the same children. They observed a methylation pattern in 58 CpG sites was significantly different in children from Ostrava compared to children in Prachatice. The methylation of all of these 58 CpG sites was lower in children from Ostrava which indicates a higher gene expression than the control Prachatice region. The patterns of methylation in asthmatic children also differed similarly between both regions.

We may conclude that studying gene expression and DNA methylation in children is a new approach that could allow to better understand the effects of air pollution on human health, as well as to better evaluate the significance of induced changes to the morbidity of children as well as morbidity in adulthood [29].

Choi et al. [31] studied on the same cohort of asthmatic children the effect of 95 candidate genes to contribute to the variability of children susceptibility to ambient B[a]P on doctor-diagnosed asthma. DNA was isolated from sputum. During the period of investigation, B[a]P concentrations in Ostrava were 7.8 vs. 1.1 ng/m³ in Prachatice. Vulnerability to asthma appears to differ according to single nucleotide polymorphisms (SNP) of genotypes CTLA4, STAT4, and CYP2E1. The highest tertile of B[a]P ambient concentration range (>6.3 ng/m³) is associated with a significantly elevated odds of asthma diagnosis. Children with those high-risk genotype are at even elevated risk only when the ambient B[a]P level reaches >6.3 ng/m³. The present study provides first direct evidence of quantified airborne concentration of B[a]P and its risk on asthma and allergies to children.

Choi et al. [32] investigated air pollution-associated risks of childhood asthma among lean, overweight, and obese children within a heavy polluted city (Ostrava) vs. background air quality region (Southern Bohemia). They postulated that (1) airborne PAHs are correlated with biomarkers of oxidative stress (i.e., lipid peroxidation 15-F2 t-IsoP, DNA oxidation 8-oxodG, and protein oxidation carbonyl); (2) PAH exposures pose different risks of asthma among overweight/obese (OV/OB) girls than boys; and (3) oxidant stress biomarkers are associated with sexually dimorphic susceptibility to asthma per unit PAH exposure. The adolescent (>12 years) OV/OB girls were associated with the highest adjusted odds of the asthma (AOR = 15.4; 95% CI, 2.9–29.1; P < 0.001). B[a]P exposure was associated with a large leap in the odds of asthma among the OV/OB adolescents, particularly the girls, after adjusting for 15-F2 t-IsoP and carbonyls. Choi et al. [32] demonstrated for the first time that OV/OB girls might represent the most vulnerable subgroup to airborne B[a]P.

Choi et al. [33] explored molecular signatures and respiratory networks underlying childhood exposure to ambient B[a]P and asthma. Contemporaneous B[a]P concentration, gene expression, and DNA methylation data were analyzed against asthma diagnosis. An elevated B[a]P concentration induced epigenetic suppression of NF-κB (Nuclear factor kappa–B) inflammation, decreased Natural Killer T (NKT) cells and activated anti-inflammatory IL10-secreting CD8+ T effective memory cells. B[a]P was positively correlated with an increased expression of a heme biosynthesis gene, ALAS2, which, in turn, appears to promote concurrent increase of neutrophilic metamyelocyte and mature CD71low erythroid cells. In the urban asthma cases, erythroid-specific master transcription regulator gene (GATA1), glutathione transferase genes (GSTM1 and GSTM3) and eosinophil marker (ILSRA) were simultaneously activated. In children with atopic march onset and diversity, B[a]P exposure enhanced heme biosynthesis, which might
reflect host compensatory mechanism to generate more erythrocytes following B[a]P and other PAH exposure. This comprehensive multiscale network analysis of a large cohort of transcriptomics and epigenetic data in asthma uncovers the global landscape of molecular interactions in asthma as well as the detailed local regulatory circuits in B[a]P-induced asthma.

3.3 Impact of air pollution on the genome of newborns

In the Czech Republic, the Moravian-Silesian Region is the region most polluted by PM2.5 and c-PAHs, as B[a]P is emitted by heavy industry and local heating systems. Accordingly, the impact of air pollution on newborns was studied in two districts: the exposed district of Karvina (MSR, Northern Moravia) and the control district of Ceske Budejovice (Southern Bohemia). This project was very complex, analyzing the concentrations of PAHs in (i) its impact on biomarkers of genetic damage as DNA adducts and gene expression and biomarkers of oxidative stress (8-oxodG adducts and lipid peroxidation); (ii) the urine of mothers and newborns; (iii) the breast milk of mothers; and (iv) ambient air and the diet of mothers. The samples were collected in hospitals in Ceske Budejovice and Karvina. The samples were collected from the normal deliveries (38–41 weeks+) 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) in Ceske Budejovice, a locality with relatively clean air, and 70 mothers (summer) and 73 mothers (winter) in Karvina, a locality with high air pollution. In addition, cord blood and urine samples were taken from 99 newborns (summer) and 100 newborns (winter) in Ceske Budejovice and from 71 newborns (summer) and 74 newborns (winter) in Karvina. c-PAHs bound to PM2.5 were collected by a high volume air sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) for 2 months during the period of biological sample collection [34].

The concentration of PM2.5 was higher in Karvina than in Ceske Budejovice in the summer of 2013 (mean ± SD: 20.41 ± 6.28 vs. 9.45 ± 3.62 μg/m³, P < 0.001) and in the winter of 2014 (mean ± SD: 53.67 ± 19.76 vs. 27.96 ± 12.34 μg/m³, P < 0.001). Similarly, the concentration of B[a]P was higher in Karvina than in Ceske Budejovice in the summer of 2013 (mean ± SD: 1.16 ± 0.91 vs. 0.16 ± 0.26 ng/m³, P < 0.001) and in the winter of 2014 (5.36 ± 3.64 vs. 1.45 ± 1.19 ng/m³, P < 0.001). The concentrations of air pollutants were higher in the winter season than in the summer season for both locations [35].

DNA adducts were determined in the umbilical cord blood by 32P-postlabeling method [36]. DNA adducts were analyzed as total adducts and B[a]P-like adducts. Both categories were significantly higher in Karvina than in Ceske Budejovice (e.g., in winter total DNA adducts in the cord blood were 2.76 ± 1.11 in Karvina vs. 2.32 ± 0.90 adducts/10⁸ in Ceske Budejovice (P < 0.001); B[a]P-like DNA adducts were 0.72 ± 0.28 vs. 0.62 ± 0.28 adducts/10⁸, respectively (P < 0.001)) [37].

Oxidative DNA damage was measured as levels of 8-oxodG (8-oxo-7,8-dihydro-2′-deoxyguanosine) [38]. Levels of 8-oxodG in newborns were more elevated in the Karvina samples than in the Ceske Budejovice samples (mean ± SD: 5.70 ± 2.94 vs. 4.23 ± 1.51 nmol/mmol creatinine, P < 0.001, respectively). This is in agreement with the fact that the concentration of air pollutants was higher in Karvina than in Ceske Budejovice. These results indicate that, in newborns, 8-oxodG levels tend to increase as air pollutant concentrations increase in the winter season [34].

Blood plasma 15-F2t-isoprostane levels (15-F2t-IsoP), a marker for lipid peroxidation, were analyzed using immunoassay kits from the Cayman Chemical Company (Ann Arbor, MI, USA) [39]. Lipid peroxidation in newborn winter
samples in Karvina was significantly higher compared to that in summer samples (15-F2t-IsoP, mean ± SD: 104.26 ± 38.18 vs. 64.24 ± 26.75 pg/ml plasma, P < 0.001, respectively).

When we separately analyzed the impact of air pollution on oxidative stress in newborns in the polluted region of Karvina, the results of multivariate regression analysis showed PM2.5 concentrations to be a significant predictor for 8-oxodG levels. Exposure to PM2.5 and B[a]P was shown to be a significant predictor of the induction of lipid peroxidation [35].

Honkova et al. [40] analyzed whole genome expression in cord blood of leukocytes of 202 newborns from the districts of Karvina and Ceske Budejovice. They aimed to identify differentially expressed genes and pathways in relation to locality and concentration of air pollutants. A pathway analysis revealed a deregulation of processes associated with cell growth, apoptosis or cellular homeostasis, immune response-related processes, or oxidative stress response. They did not find the direct effect of PM2.5 and B[a]P exposure on gene expression of newborns from Karvina; therefore, they assumed that the locality rather than air pollution levels might be a driving force of gene expression modulation. It seems likely that a common environment and complex lifestyle variables mediate long-term effects on gene expression at delivery.

### 3.4 PAH metabolism in urine of mothers and newborns

For the urine of mothers and newborns, monohydroxylated metabolites of PAHs (OH-PAHs) were analyzed [41]. While the content of ΣOH-PAHs in mothers' urine collected in the summer period was comparable in both Karvina and Ceske Budejovice, in the winter period, the samples from the Karvina region showed 1.5 times higher amounts of exposure markers. The amounts of ΣOH-PAHs in newborns' urine samples from highly industrialized Karvina in the winter season were 1.5 times higher than in the summer season collected in the same locality and 3.3 times higher when compared with the less polluted locality of Ceske Budejovice. This was probably related to the air pollution caused by heavy industry and local heating (Figure 3).

In all samples, the highest concentrations of 2-hydroxynaphthalene (2-OH-NAP) was observed. Recently Nie et al. [42] indicated that prenatal exposure to naphthalene decreased birth weight and birth head circumference. Detected concentrations of 2-OH-NAP in urine of mothers from Karvina corresponded to similar level in Taiyuan in China.

### 3.5 Analysis of PAHs in human breast milk and diet

Twenty-four PAHs were analyzed in the human breast milk samples [43]. The results of this unique study focused on a critical assessment of the impact of atmospheric pollution by PAHs in Karvina and Ceske Budejovice within summer and winter on the contamination of breast milk collected from mothers who reside there. As regards c-PAHs, B[a]P was detected only in 19 of analyzed samples and made about 0.4% of the total PAH amount. Comparing the data from winter and summer, in both residential areas, higher concentrations were measured in samples collected in the winter period. Also in the highly industrialized locality with heavily contaminated air, PAH amounts in milk were higher than in the control locality, but the PAH profiles were very similar. The most frequently detected compounds in both areas and seasons were noncarcinogenic phenanthrene, fluorene, fluoranthene, and pyrene. Therefore, the PAH metabolites in breast milk do not represent a significant health risk.
The contribution of ingestion to the total intake is quite variable for individual PAHs, and in summer, between 50 and 95% of the total intake was made, while in winter in the heavily air-contaminated industrialized locality, the inhalation is unambiguously the dominant pathway. Adverse pregnancy outcomes may be affected by lifestyle. The effect of smoking as well as passive smoking is already known [44]. It is understood in the Czech population that pregnant mothers should not smoke. This habit is also affected by education and social standards. Pavlikova et al. [45] studied in those two cohorts of mothers from Karvina and Ceske Budejovice the quality of diet. It showed the low nutritional quality of food consumed: recommended daily intake of vegetables in Karvina was 15.9 vs. 22.8% in Ceske Budejovice and fruits 35.5 vs. 61.8%, respectively. The milk and dairy product intake in both cohorts was sufficient only in 30% of days. (Real samples analyzed for the diet of mothers, 10 in summer 2013 and 10 in winter 2014, 1/4 of daily food intake, were collected for 7 days and 2 weeks before the expected term of delivery in each location). The quality of the diet of mothers and intake of vegetables were negatively correlated with the DNA adduct levels of newborns [46]. These results confirm that sufficient intake of antioxidants may improve the detoxification mechanism of PAHs in pregnant mothers [29].

3.6 Child morbidity in Karvina vs. Ceske Budejovice

Postnatal development and morbidity were compared from birth until the age of 2 years in children born and living in the districts of Karvina and Ceske Budejovice [47]. Maternal consent for the study was obtained during the admission of mothers at the departments of obstetrics in Ceske Budejovice and Karvina. Postnatal follow-up was successfully performed on 178 children (out of 216) registered in 48 pediatric offices in Ceske Budejovice and on 126 children (out of 148) registered in 28 pediatric offices in Karvina. All pediatric offices were visited to provide lists of children, who were selected to the cohort as newborns, as well as the pediatric and maternal questionnaire. The questionnaires were completed for 178 children in Ceske Budejovice and 126 children in Karvina. When postnatal growth and development of children (body weight, length, and head circumference at 3, 6, 12, and 18 months) were compared, there were no differences between children in the two localities. For the analyses of child morbidity, the diagnoses of diseases
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affecting children (presented in the ICD-10) were grouped into 20 classes. The five most frequent illnesses in the first 24 months of life were gastrointestinal infections, dermatitis, tonsillitis, viral infections of the skin and mucous membranes, and viral diseases. The lowest incidence was in the first 6 months. Differences between the incidences of the disease, based on the season that children were born in, were negligible. The highest incidence was observed with the group of diagnoses related to upper respiratory infections (J00, J01, J02, J04, J05, and J06). When expressed as the incidence for 100 children, the incidence of urogenital diseases, dermatitis, viral diseases, and infections of the gastrointestinal system and upper respiratory infections was statistically significantly higher in children living in Karvina than in children living in Ceske Budejovice (Figure 4). Considering other studies on child morbidity, it may be concluded that the above findings are due to Karvina’s more polluted environment.

4. Conclusions

Impact of air pollution to children health was studied in the Czech Republic in two heavily polluted regions: Northern Bohemia and Northern Moravia.

Studies in the Northern Bohemia proved the effect of PAHs to pregnancy outcomes as IUGR, increased respiratory morbidity, and behavioral changes.

Studies in the Northern Moravia observed the impact of air pollution to respiratory morbidity in preschool children, effect of B[a]P to asthma bronchiale in children, increased DNA adducts and oxidative damage in newborns, increased monohydroxylated metabolites of PAHs in urine, increased noncarcinogenic PAHs in breast milk, and low nutritional quality of pregnant mothers.

It should be understood that the present level of air pollution—standard EU 1 ng B[a]P/m³/year was exceeded in the Czech Republic in the year 2017 for 62% of population—will affect the health status of the Czech population already in the next decades. We can assume that functional changes in newborns will be seen as an increased morbidity for cardiovascular diseases in the middle age, i.e., approx. After the next 50 years, the increased load of mutations in genetic material will be transferred to the genetic material of future generations. All these results and new

Figure 4.
Morbidity in children up to 2 years of age in the districts of Karvina and Ceske Budejovice.
Childbirth information should stimulate to decrease the present air pollution and to prevent future morbidity and the economic load for such morbidity.

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