Original research

THE OSTRAVA REGION, CZECH REPUBLIC: IMPACT OF AIR POLLUTION ON CHILDREN'S HEALTH

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ABSTRACT

Introduction: The Moravian-Silesian Region (MSR) is heavily polluted, especially due to heavy industry and local heating, and concentrations of benzo[a]pyrene (B[a]P) are among the highest in the European Union.

Methods: To verify the impact of air pollution on the health of the population in the MSR, the morbidity of children was analysed in three studies which are reviewed: 1) morbidity in children; 2) asthma bronchiale in children; and 3) impact of air pollution on the genome of newborns. Morbidity in children was studied in 10 pediatric districts in Ostrava City. Asthma bronchiale was studied in children from Ostrava-Radvanice and the control district of Prachatice, southern Bohemia, by gene expression profiles. The impact of air pollution on the genome of newborns was studied in the district of Karvina and the control district of Ceske Budejovice.

Results: In asthmatic children from Ostrava, increased gene expression corresponded to the non-allergic type of asthma. Concentrations of benzo[a]pyrene in the winter of 2014 were 5.36 ± 3.64 vs. 1.45 ± 1.19 ng/m^3 in CB, P < 0.001 in Karvina. In newborns in the polluted district of Karvina, increased oxidative damage affecting immunodeficiency pathways and the neurotrophin signalling pathway, higher amounts of OH-metabolites of PAHs in urine, as well as increased respiratory morbidity in children up to two years of age were observed.

Conclusion: Studies in the Moravian-Silesian Region indicate a significant impact of air pollution on children's health

Keywords: AIR POLLUTION, GENE EXPRESSION, OXIDATIVE DAMAGE, PAHS IN URINE, RESPIRATORY MORBIDITY IN CHILDREN
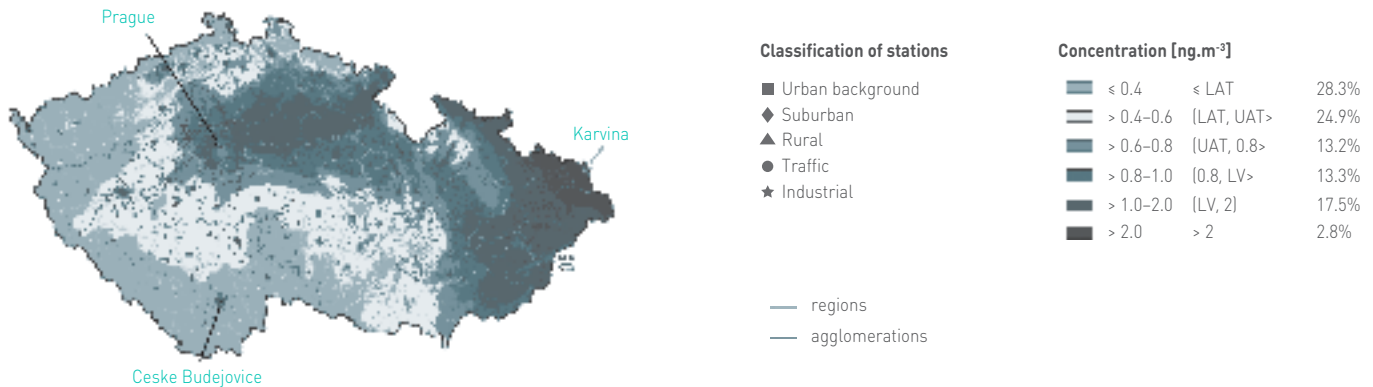
INTRODUCTION

MORAVIAN-SILESIAN REGION

The Moravian-Silesian Region (MSR) is a heavily populated, industrial area situated in the easternmost part of the Czech Republic (CR), covering 5428 km² with 1.21 million inhabitants (1). The MSR is situated in a basin bordered by mountains to the west, east, and partially to the south, with frequent temperature inversions occurring in the winter. The MSR administrative structure consists of six districts including, from west to east, Bruntal, Opava, Novy Jicin, Ostrava City, Karvina and Frydek-Mistek. The Karvina district is one of the most densely populated districts in the Czech Republic, with 712 inhabitants/km². Since the second half of the 18th 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 population density in the MSR is also associated with a high intensity of local vehicular transport and local heating. Approximately 50% of the inhabitants use central heating, 34% natural gas, 10% coal, 3% electricity and 3% wood (2).

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

FIG. 1. BENZO[A]PYRENE CONCENTRATIONS IN THE CZECH REPUBLIC, YEAR 2015 (3)

Classification of stations
- Urban background
- Suburban
- Rural
- Traffic
- Industrial

Concentration [ng.m⁻³]

≤ 0.4	≤ LAT	28.3%
> 0.4–0.6	(LAT, UAT>	24.9%
> 0.6–0.8	(UAT, 0.8>	13.2%
> 0.8–1.0	(0.8, LV>	13.3%
> 1.0–2.0	(LV, 2)	17.5%
> 2.0	> 2	2.8%

— regions
— agglomerations

pollution on the genome of newborns. The methods used in these studies are detailed in the original references. This paper is the review of the results obtained from these studies.

The MSR population is exposed to high concentrations of $PM_{2.5}$ which exceed the EU standard of 25 mg/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 (Fig. 1). In the district of Ostrava Radvanice-Bartovice, the concentrations of B[a]P are the highest in the Czech Republic. Comparing air pollution between 2010 and 2015, it appears that concentrations of $PM_{2.5}$ decreased in the MSR, but surprisingly, there is no change in the concentration of B[a]P in Ostrava Radvanice-Bartovice (3).

Monthly concentrations of B[a]P in Ostrava Radvanice-Bartovice and Karvina during the winter period are higher than 20 ng/m³, with the potential to significantly affect the pregnancy outcome (4) as well as the DNA fragmentation in sperm (5).
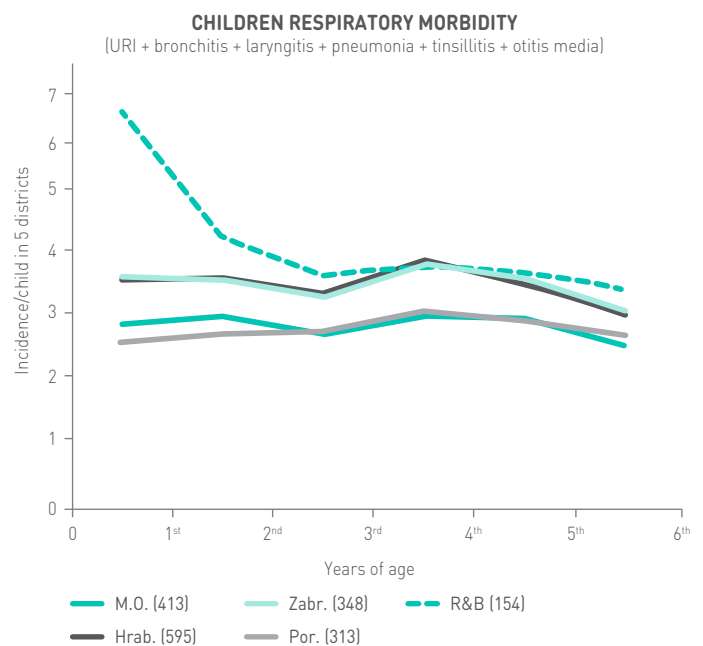
RESULTS/APPROACH

MORBIDITY IN CHILDREN

In 10 pediatric districts in the city of Ostrava, morbidity was monitored in children born from 2001 to 2004 up to five years of age (n = 1888) (6). The pediatricians abstracted medical records in ICD-10 codes (International Codes of Diseases). Comparisons of the detailed age-specific morbidity of 1655 children born and living in the district of Ostrava Radvanice-Bartovice showed a significantly higher incidence

of acute illness compared to children in other parts of Ostrava. These children suffered from a higher incidence of acute respiratory disease in the first year of life (Fig. 2) and a higher prevalence of asthma bronchiale (37.1%, n = 170) compared to other parts of Ostrava (10.2–13.2%, n = 1287) (6). From birth until the age of five 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. Prenatal exposure to PAHs may be associated with altered lymphocyte immuno-phenotypic distribution

FIG. 2. CHILDREN RESPIRATORY MORBIDITY IN THE DIFFERENT DISTRICTS OF OSTRAVA CITY

CHILDREN RESPIRATORY MORBIDITY
(URI + bronchitis + laryngitis + pneumonia + tinsillitis + otitis media)

- M.O. (413)
- Zabr. (348)
- R&B (154)
- Hrab. (595)
- Por. (313)

(Districts: M.O.: Moravska Ostrava, Hrab.: Hrabuvka, Zabr.: Zabreh, Por.: Poruba, R & B: Radvanice/Bartovice)

in cord blood and possible changes in cord serum immunoglobulin E levels, as proposed by Hertz-Picciotto et al. *(7)*. We can hypothesize that high concentrations of PAHs affect maturation of the immune system. Therefore, children from a more polluted region suffer from higher respiratory morbidity, especially in their first year of life.

ASTHMA BRONCHIALE IN CHILDREN

The task of this study was to evaluate the impact of air pollution on gene expression in children, and to analyse 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. This included a group of 200 children – 100 asthmatic and 100 healthy children – aged 6 to 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) *(8)*.

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 analysed 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, we observed 64 deregulated genes. When we compared Ostrava-asthma with Ostrava-control, we observed 12 deregulated genes. Comparing Prachatice-asthma with Prachatice-control, we observed 17 deregulated genes. Using Venn diagrams, genes that were specific to asthma in Ostrava and to Prachatice were found to differ completely, while no one gene was observed in both localities. Effects were further observed for the MAPK signalling 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 to the presence of eosinophils–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 haemoglobin), and *HBG2* (part of fetal haemoglobin, with a higher affinity to oxygen). Accordingly, we may ask 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 in the level of B[a]P exposure.

This study is unique because it is the first time when whole genome microarrays were used to analyse the relationship between air pollution and asthma bronchiale. The results suggest the distinct phenotype of asthma in children living in the polluted Ostrava region compared to children living in Prachatice.

Rossnerova et al. *(9)* studied DNA methylation in the same children. They observed a significantly different methylation pattern in 58 CpG sites 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 in comparison with the control Prachatice region. The patterns of methylation in asthmatic children also differed similarly between both regions.

Studying gene expression and DNA methylation in children is a new approach that allows us to better understand the effects of air pollution on human health, and to evaluate the significance of induced changes to the morbidity of children as well as morbidity in adulthood *(10)*.

IMPACT OF AIR POLLUTION ON THE GENOME OF NEWBORNS

In the Czech Republic, the Moravia-Silesian Region is the region most polluted by $PM_{2.5}$ 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). Biological material from newborns and their mothers was collected in the summer and winter seasons. This project was very complex, analysing the concentrations of PAHs in: (i) ambient air and the diet of mothers; (ii) the breast milk of mothers; (iii) the urine of mothers and newborns;and (iv) its impact on biomarkers of genetic damage as DNA adducts and gene expression, biomarkers of oxidative stress (8-oxodG adducts and lipid peroxidation).

The samples were collected 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 Committee of both hospitals and the Institute of Experimental Medicine CAS in Prague. The samples were collected from the normal deliveries (38-41 week+) of non-smoking 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.

AIR POLLUTANTS EXPOSURE
c-PAHs bound to $PM_{2.5}$ were collected by a High Volume Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) for two months during the period of biological sample collection (11).

In Karvina, the concentration of $PM_{2.5}$ was higher 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 (12).

DETERMINATION OF 8-OXODG
Oxidative DNA damage was measured as levels of 8-oxodG (8-oxo-7,8-dihydro-2'-deoxyguanosine) (13). Levels of 8-oxodG were determined through the use of a Highly Sensitive 8-OhdG Check ELISA kit (JaICA, Shizuoka, Japan).

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 (12).

15-F2T-ISOPROSTANE IMMUNOASSAY
Blood plasma 15-F2t-isoprostane levels (15-F2t-IsoP), a marker for lipid peroxidation, were analysed using immunoassay kits from the Cayman Chemical Company (Ann Arbor, MI, USA) (14).

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 analysed the impact of air pollution on oxidative stress in newborns in the polluted region of Karvina, the results of multivariate regression analysis showed $PM_{2.5}$ concentrations to be a significant predictor for 8-oxodG levels. Exposure to $PM_{2.5}$ and B[a]P was shown to be a significant predictor of the induction of lipid peroxidation (12).

GENE EXPRESSION PROFILES OF AIR POLLUTION EXPOSURE IN NEWBORNS
The modulation of gene expression profile was analysed and several specific genes were detected in groups of 231 newborns in Karvina and Ceske Budejovice during the winter and summer seasons. RNA was isolated from frozen umbilical cord blood and hybridized on Illumina HumanHT-BeadChip. This unique study compares whole genome changes caused by air pollution in newborns at the time of delivery. Differences in gene expression were checked by t-test

and ANOVA. Deregulated genes were identified using a linear model and study groups were compared. We observed differences in gene expression > 1.5 (upregulated) and < 0.67 (downregulated) for 75 specific genes in Karvina newborns compared to those in Ceske Budejovice, and 127 specific deregulated genes in Karvina newborns compared between the winter and the summer period. These groups of genes were analysed for function annotation of biochemical pathways and we found locality and season-specific pathways (15).

Significant affected pathways were primary immunodeficiency pathways (five genes in pathway, P < 0.05 in the Karvina winter compared to the Ceske Budejovice winter) or the neurotrophin signalling pathway (nine genes, p < 0.07 in the Karvina winter compared to the Karvina summer). The affecting of the neurotrophin signalling pathway can play an important role in the development of neurons in the brain, because the crucial gene of pathway *BDNF* (Brain-derived neurotrophic factor) coding proteins influences differentiation, growth and survival neurons. A recent study describes the lower placental expression of *BDNF* with an increase *in utero* exposure of $PM_{2.5}$ (16). The affecting of neurodevelopment processes may result in incidences of epilepsy, neurodegenerative diseases or impacts on memory (17). The downregulation of *BDNF* was observed using the qPCR method for Karvina (2.1 times decreasing in winter and 1.9 times decreasing in summer) compared to Ceske Budejovice. Through other deregulated genes, we observed higher expression *IL10* (interleukin 10) in the Karvina winter compared to the Karvina summer (FC 1.73, P < 0.01) which can symbolize a higher activity of inflammatory immune processes.

Using multivariate regression analysis, 3865 genes were found which correlate with exposure data ($PM_{2.5}$ and B[a]P). One significant (0.51, P < 0.001) gene *CHD8* (Homo sapiens chromodomain helicase DNA binding protein 8) was found in relation to B[a]P. *CHD8* is a repressor of transcription by remodelling of the chromatin structure, especially in fetus development. A current study (18) finds that mutation of the *CHD8* gene is associated with an increased risk of autism spectrum disorders (ASD) and this supports the hypothesis that *CHD8* may play a central role in neuronal cell development and ASD risk.

ANALYSIS OF OH-PAHS IN URINE

For the urine of mothers and newborns, monohydroxylated-metabolites of PAHs (OH-PAHs) were analysed (19). While the content of ∑OH-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 ∑OH-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 (Fig. 3).

FIG. 3. CONCENTRATIONS (NG/G CREATININE) OF DETECTED OH-PAHS IN THE URINE SAMPLES OF MOTHERS AND NEWBORNS
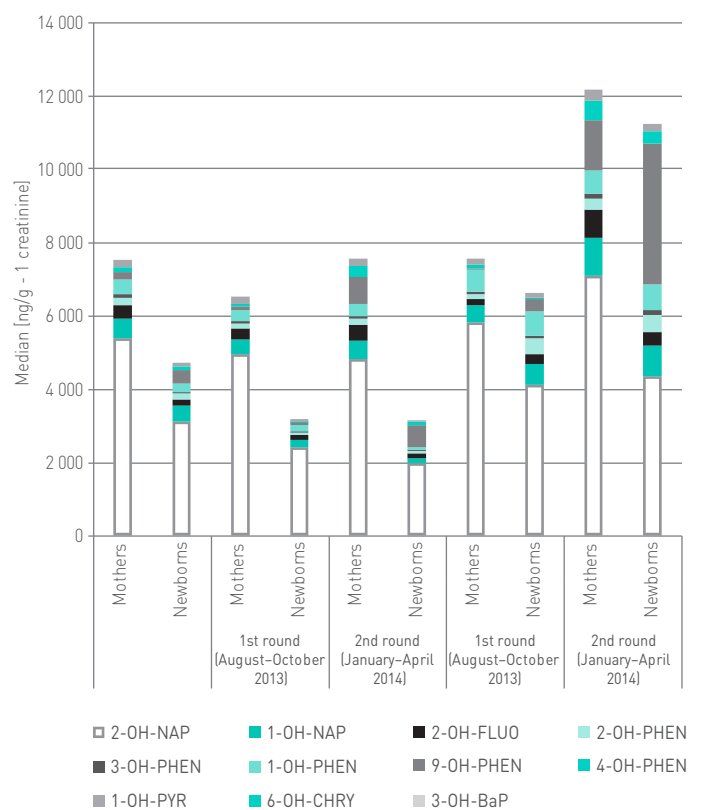
ANALYSIS OF PLASMA LIPIDOME

In addition to 'classic' parameters monitored in previous studies, a novel approach, based on non-target metabolomic fingerprinting, was involved in this case. The objective was to get more comprehensive information on the effect of polluted

air on exposed individuals. It is estimated that there are approximately 3000 endogenous or common metabolites in the human metabolome (by definition, this involves all low molecular weight compounds – up to approximately 1500 Da – occurring in tissues or biofluids). In this study, lipidome, consisting of various lipid classes, was investigated, since they are dominating in human plasma. It is worth noting that, in general terms, metabolome is inherently very dynamic: small molecules are continuously absorbed, synthetized, and degraded, and interact with other molecules, both within and between biological systems, and with the external environment.

Plasma from mothers and newborns was investigated (after deproteination) employing ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS). The obtained data were subjected to multivariate statistics in search of specific patterns of compounds for sample groups, which were defined by the sampling locality and season. Apart from the obvious differences between the lipidome of mothers and newborns, several other patterns could be observed. The most pronounced difference in the mothers and newborns groups related to the season, while the effect of the locality was rather negligible. As an example, the grouping of newborns in the winter season by locality is shown in Fig. 4. One of the key compounds, but not the only one, contributing to the separation of the two groups was hydroxy eicosatetraenoic acid. The concentration of this metabolite was below the detection limit in the plasma of newborns from Ceske Budejovice, while it was fairly higher in the plasma/serum of newborns from Karvina. Higher oxidation stress in Karvina during the winter season might be the conceivable cause. In any case, the research on this challenging fact is ongoing (20).

ANALYSIS OF PAHS IN HUMAN BREAST MILK AND DIET

Parent compounds (24 PAHs) were analysed in human breast milk samples (21). The results of this unique study focused on a critical assessment of the impact of atmospheric pollution, by PAHs, in Ceske Budejovice and Karvina, in the summer and winter, on the contamination of breast milk collected from resident mothers. As regards c-PAHs, B[a]P was detected in only 19 of 324 of the analysed samples, constituting about 0.4% of the total PAHs amount. Comparing the

FIG. 4. STATISTICAL SEPARATION OF A SAMPLE SET OBTAINED ON NEWBORNS IN THE WINTER SEASON: SAMPLES COLLECTED IN CESKE BUDEJOVICE ARE IN GREY; SAMPLES COLLECTED IN KARVINA ARE IN GREEN.
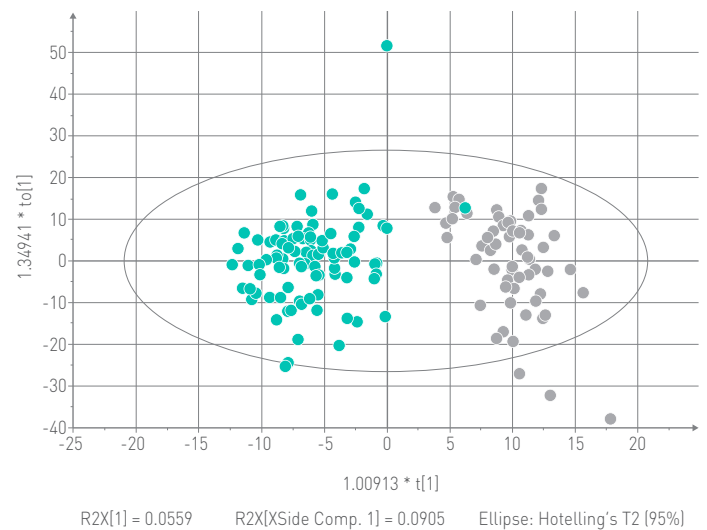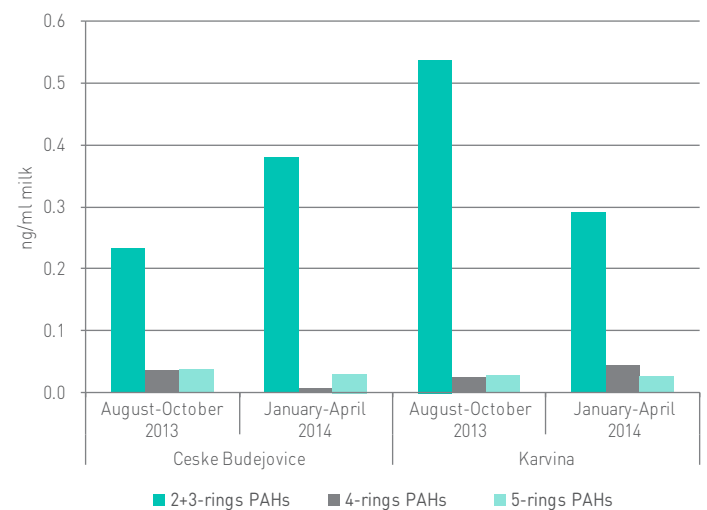

R2X[1] = 0.0559   R2X[XSide Comp. 1] = 0.0905   Ellipse: Hotelling's T2 (95%)

FIG. 5. COMPARISON OF PAH LEVELS IN HUMAN BREAST MILK IN DIFFERENT LOCALITIES AND SAMPLING PERIODS

(2 + 3-rings PAHs: NA, AC, ACL, FL, PHE, AN; 4-rings PAHs: FA, PY, BaA, CHR, BcF, 5MC; 5 + 6-rings PAHs: BbFA, BkFA, BjFA, BaP, DBahA, IP, BghiP, CPP, DBalP, DBaeP, DBaiP, DBahP)

winter and summer data, in both localities, higher concentrations were measured in winter samples (Fig. 5). The significant difference between the sampling localities is also documented in this figure: higher concentrations of PAHs were measured in milk samples from the Karvina district, which corresponded to PAH amounts in air, but the PAHs profiles were very similar in both localities.

The obtained data were used further for the estimation of the contribution of ingestion to total intake. This is quite variable for individual compounds, and in the summer, constituted between 50% and 95% of the total intake, while in the winter, in the heavily air-contaminated industrialized Karvina locality, inhalation was unambiguously the dominant pathway. Adverse pregnancy outcomes may be affected by lifestyle. For example, the effects of smoking and passive smoking are known *(22)* and it is understood among Czechs that pregnant mothers should not smoke. This habit is also affected by education and social standards.

Another marker of a healthy lifestyle is diet. A recent study on the quality of diet for pregnant mothers in Ceske Budejovice revealed the low nutritional quality of food consumed: regarding recommended daily doses, vegetables constituted only 22.8%, fruits 61.8%, and milk 30.2%. Here, real samples of the diet of mothers – 10 in the summer of 2013 and 10 in the winter of 2014, representing 25% of daily food intake – were collected for seven days and two weeks before the expected term of delivery. The quality of the diet of mothers and intake of vegetables were negatively correlated with the DNA adduct levels of newborns *(23)*. These results confirm that a sufficient intake of antioxidants may improve the detoxification mechanism of PAHs in pregnant mothers *(10)*.

CHILD MORBIDITY

Postnatal development and morbidity were compared from birth until the age of two years in children born and living in the districts of Karvina and Ceske Budejovice. 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 179 children (out of 216) registered with 48 pediatric offices in Ceske Budejovice, and on 121 children (out of 148) registered with 28 pediatric offices in Karvina. Also taking part in the study were 28 pediatricians and their nurses in Karvina, and 48 pediatricians and their nurses in Ceske Budejovice. 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 affecting children (presented in the International classification codes: 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 skin and mucous membranes, and viral diseases. The lowest incidence was in the first six 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. Considering other studies on child morbidity, including the previous study in the frame of the UFIREG program of the European Union *(24)*, it may be concluded that the above findings are due to Karvina's more polluted environment. However, this hypothesis should be supported by further analysis including the influence of indoor and family factors *(25)*.

FIG. 6. MORBIDITY IN CHILDREN UP TO TWO YEARS OF AGE IN THE DISTRICTS OF KARVINA AND CESKE BUDEJOVICE
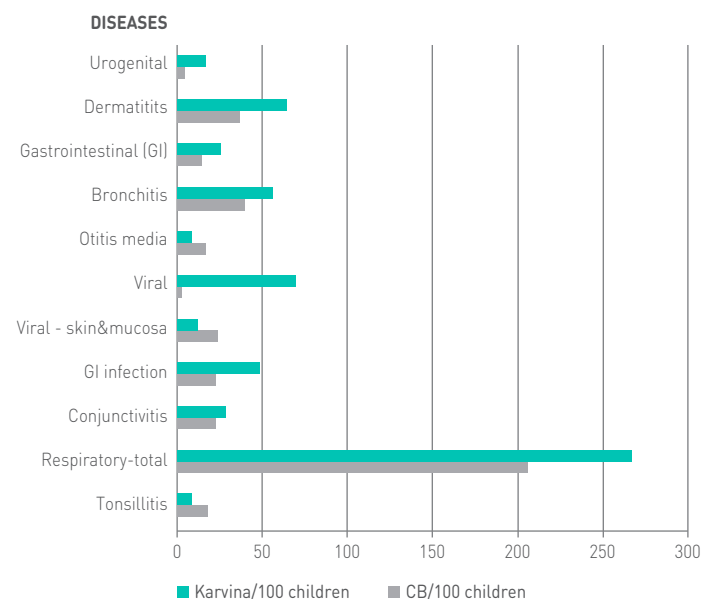
CONCLUSION

Studies in the Moravian-Silesian Region indicate a significant impact of air pollution on children's health. During the past years, new and original results were obtained related to:

a) Changes in gene expression in asthmatic children due to increased concentrations of B[a]P.

b) Increased oxidative damage in newborns.

c) The impact of air pollution in deregulating gene expression in immunodeficiency pathways and the neurotrophin signalling pathway.

d) The impact of PAHs in polluted air on the OH-metabolites of PAHs in urine.

e) The impact of air pollution on metabolomics fingerprinting.

f) The impact of air pollution on increased respiratory morbidity in children up to two years of age.

Disclaimer: The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or policies of the World Health Organization.

Abbreviations: ASD, autism spectrum disorders; B[a]P, benzo[a]pyrene; *BDNF*, brain-derived neurotrophic factor; 15-F2t-IsoP, 15-F_{2t}-isoprostane; MSR, Moravian–Silesian Region; 8-oxodG, 8-oxo-7,8-dihydro-2-deoxyguanosine; OH-PAHs, monohydroxylated-metabolites of PAHs; $PM_{2.5}$, particulate matter of aerodynamic diameter <2.5 µm; PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; SPE, Solid Phase Extraction; WHO, World Health Organization.

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