

Research Article

The European Hot Spot of B[a]P and PM_{2.5} Exposure—The Ostrava Region, Czech Republic: Health Research Results

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The Ostrava Region in the Czech Republic is a heavily polluted industrial area. Concentrations of PM₁₀, PM_{2.5}, and benzo[a]pyrene (B[a]P) significantly exceed limit values. To investigate the impact of these levels on human health, epidemiological, molecular epidemiology, and in vitro studies were done in 2008–2011. Morbidity of children was followed in 10 pediatric districts. In the most polluted district, children suffered higher incidence of acute respiratory diseases in the first year of life, and higher prevalence of asthma bronchiale. Gene expression was studied in children from Ostrava and from a control rural area. Genes specific to asthma bronchiale differed, suggesting a different molecular phenotype in children in the polluted region compared to children in the control area. A molecular epidemiology study showed adverse effect of the Ostrava exposures, but also an increased expression of XRCC5, which probably protects these exposed subjects against the degree of genetic damage that would otherwise be expected. In vitro studies clearly related concentration of B[a]P from PM_{2.5} extracts to induced PAH-DNA adducts. These studies clearly demonstrate that under the present local environmental conditions, the health of the population is severely impaired and will likely remain so for a significant period of time.

1. Introduction

The Moravian-Silesian Region (MSR) is a heavily populated industrial area situated in the easternmost part of the Czech Republic (CR), covering 5 427 km² with 1,25 million inhabitants [1]. The MSR is situated in a basin bordered by mountains from west, east, and partially from south, with frequent temperature inversions in winter. Since the 2nd half of the 18th century, the region is characterized by coal mining, processing of coal, and metallurgy. The MSR administrative structure consists of six districts (from the west: Bruntál, Opava, Nový Jičín, Ostrava city, Karviná, and Frýdek-Místek). The Karviná district is one of the most densely populated districts of the Czech Republic (789 inhabitants/km²). The most important current industries are metallurgy, steel, coke ovens, coal mining, and power generation. The population density in the MSR is also associated with high-intensity local vehicular transport and local heating. Almost fifty percent of the inhabitants use

central heating, 34% natural gas, 10% coal, 3% electricity, and 3% wood [2].

This paper provides an overview of air pollution levels in the Ostrava Region (OSTR, city of Ostrava and the district of Karviná) for the period (2002–2011), and a summary of findings from health effects studies and in vitro investigations done in the region in years 2008–2011. In addition, we review relevant results from earlier investigations, and studies done in the bordering similarly polluted region in Poland.

2. Air Pollution Situation in OSTR

Figure 1 shows the relative burden of PM₁₀ (particulate matter with aerometric diameter < 10 μm) in OSTR compared to the whole Czech Republic (CR) in 2011. For PM₁₀, the concentrations were continuously above 40 μg/m³ annual average in 2002–2011 (Figure 2), and considerably higher than urban background in the largest city of CR; Prague. Concentrations higher than 50 μg/m³ PM₁₀ were recorded

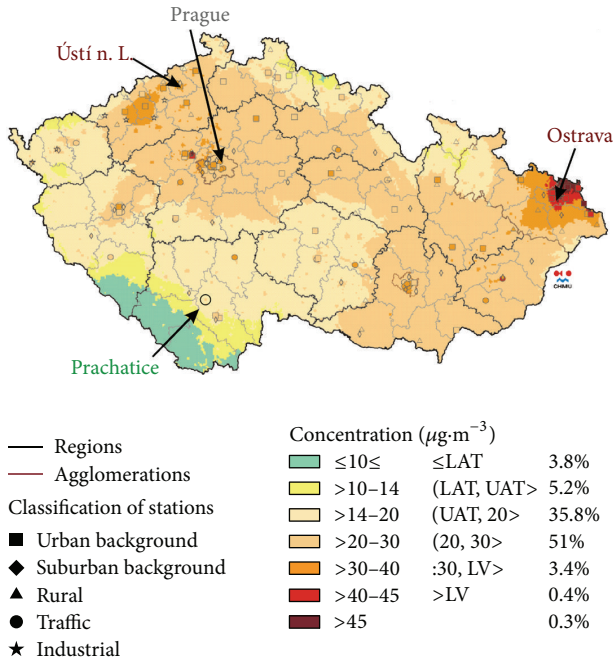


FIGURE 1: Field of annual average concentrations of PM_{10} in ambient air in 2011 (source: CHMI [3]).

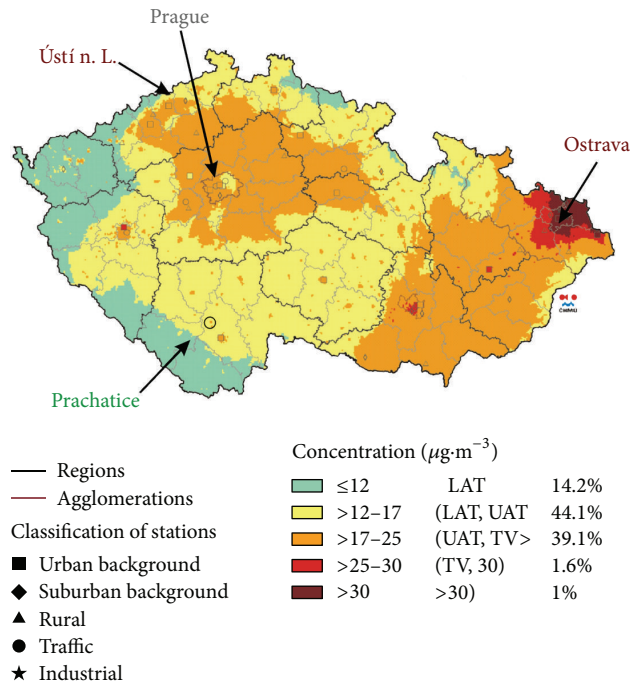


FIGURE 3: Field of annual average concentrations of $\text{PM}_{2.5}$ in ambient air in 2011 (source: CHMI [3]).

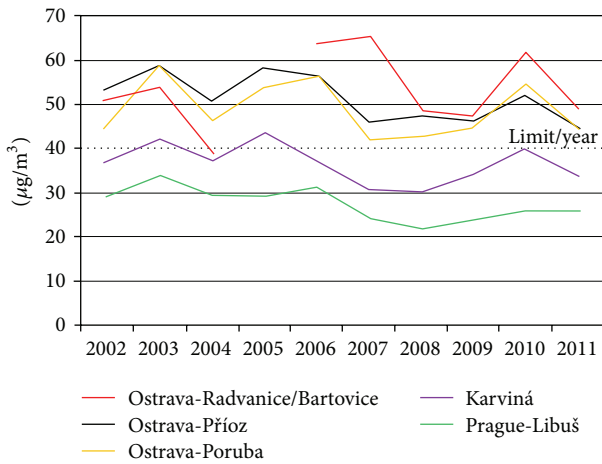


FIGURE 2: Annual average concentrations of PM_{10} in the ambient air on selected monitoring stations in OSTR and Prague, years 2002–2011 ([3]).

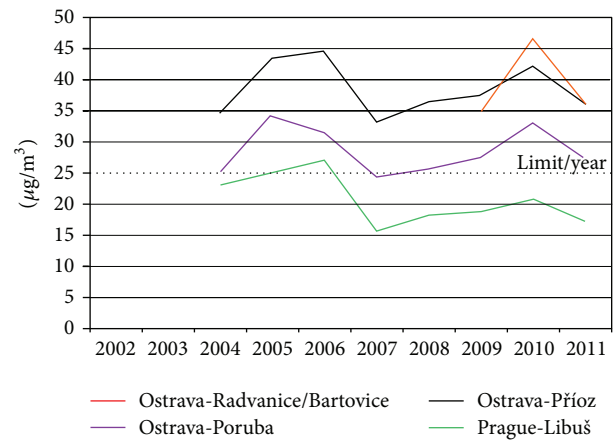


FIGURE 4: Annual average concentrations of $\text{PM}_{2.5}$ in ambient air on selected monitoring stations in OSTR and Prague, years 2002–2011 [3].

during the year 2011 for 100 days at two stationary monitoring stations in MSR, Karviná, and Ostrava-Radvanice.

Similarly to PM_{10} , population in this region is exposed to high concentrations of $\text{PM}_{2.5}$ (particulate matter with aerometric diameter $< 2.5 \mu\text{m}$), that are higher than those observed on a background station in Prague (Figure 3). In the period 2004–2011 the limit of $25 \mu\text{g}/\text{m}^3/\text{year}$ was exceeded on all three $\text{PM}_{2.5}$ stationary monitoring stations in OSTR (Figure 4).

Concentrations of benzo[a]pyrene (B[a]P) in OSTR are the highest in all of the Czech Republic (Figure 5). The highest concentrations were detected in Ostrava-Radvanice

(Figure 6). The limit of $1 \text{ng}/\text{m}^3/\text{year}$ B[a]P was exceeded on all OSTR monitoring stations in all years 2004–2011. In the city of Karviná, comparing period 2002–2005 with 2009–2009 shows that B[a]P concentrations increased. It is surprising that comparing years 2010 and 2011, in Ostrava-Radvanice and Karviná concentrations of both PM_{10} and $\text{PM}_{2.5}$ decreased while B[a]P increased—in Ostrava-Radvanice by 40%, in Karviná by 20%.

Other pollutants: benzene—the limit of $5 \mu\text{g}/\text{m}^3/\text{year}$ is continually exceeded in Ostrava-Privoz; nitrogen dioxide (NO_2) concentrations were lower on all measuring points in the period 2006–2011 than the limit of $40 \mu\text{g}/\text{m}^3/\text{year}$;

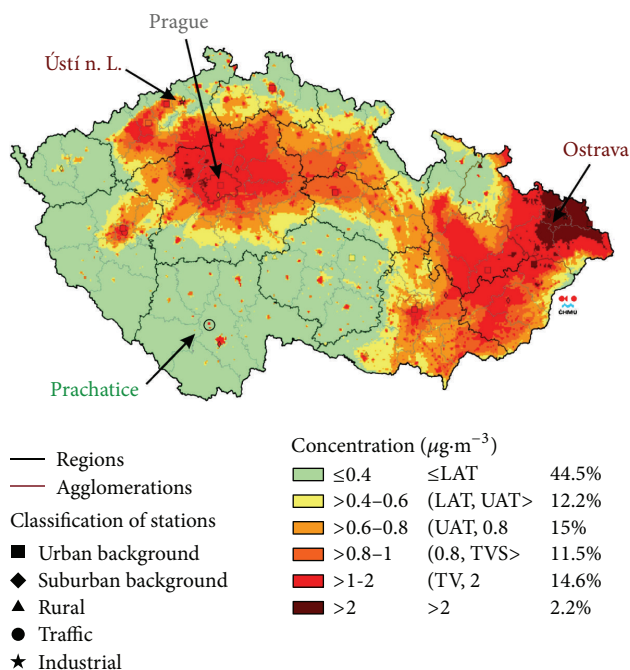


FIGURE 5: Field of annual average concentrations of B[a]P in ambient air in 2011 (source: CHMI [3]).

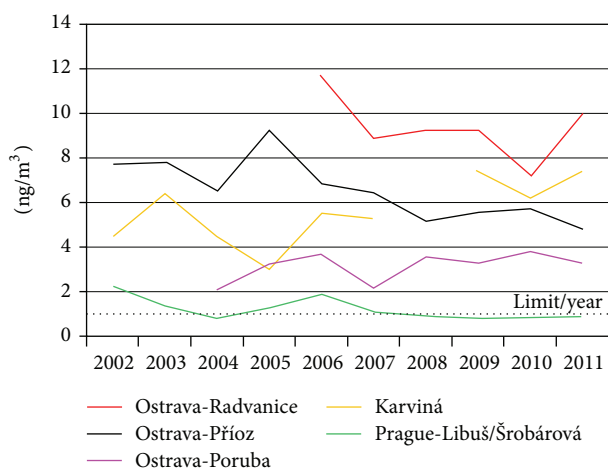


FIGURE 6: Annual average concentrations of B[a]P in ambient air on selected monitoring stations in OSTR and Prague, years 2002–2011 [3].

arsenic (As) determined in PM_{10} was lower than the limit of $6 \text{ ng}/\text{m}^3/\text{year}$ on all monitoring stations in the period 2006–2011.

Air pollution concentrations are available from the Czech Hydrometeorological Institute website starting 1997 [3]. A comparison can be made between the highly polluted Ostrava-Radvanice and Ostrava-Poruba, considered as a clean part of Ostrava. In 1997–2007, only PM_{10} was monitored in Ostrava-Radvanice. Average concentrations of PM_{10} were in Ostrava-Radvanice $36 \mu\text{g}/\text{m}^3$, in Ostrava-Poruba $32 \mu\text{g}/\text{m}^3$ in 1997–2000, in years 2001–2004 $47 \mu\text{g}/\text{m}^3$ versus $40 \mu\text{g}/\text{m}^3$,

and in years 2006–2007 $65 \mu\text{g}/\text{m}^3$ versus $34 \mu\text{g}/\text{m}^3$, respectively. PM_{10} concentrations increased by 80% in the period 2006–2007 compared to 1997–2000 in Ostrava-Radvanice, but not in Ostrava-Poruba. It is likely that these differences in concentrations of PM_{10} also corresponded to significantly different exposures to $\text{PM}_{2.5}$ and PAHs (polycyclic aromatic hydrocarbons). Another specificity of Ostrava-Radvanice has been high concentrations of B[a]P. Considering general information about sources of PAH [4], this specific situation could be associated with the steelwork complex operating coke ovens, located approximately 2 km from Ostrava-Radvanice, with prevailing winds going mostly just to this part of the city [5].

3. Prior Evidence of Health Risks Related to the Observed Pollution Levels

The pollution levels observed in OSTR give rise to severe concerns regarding health risks. Two main sources of information allow us to identify possible health risks prior to carrying out own studies: the WHO assessments and air quality guidelines that provide general guidance, and research that documents possible human biological effects of air pollution in areas with similar source profiles.

WHO [6] recommends to use concentrations of $\text{PM}_{2.5}$ as an indicator of health risk. The association between $\text{PM}_{2.5}$ (resulting from, e.g., incomplete combustion in mobile as well as stationary sources) and health effects such as increased cardiovascular morbidity and mortality has already been established.

$\text{PM}_{2.5}$ acts as a carrier of complex mixtures containing carcinogenic PAHs (c-PAHs). These compounds, formed by incomplete combustion of organic material as oil, petrol, gas, coal, and wood, are adsorbed on the fine particulate fraction. It has been shown that health impact of $\text{PM}_{2.5}$ is related directly to content of reactive oxidative species (ROS) [7] and c-PAHs, inducing oxidative damage and inflammation [8, 9].

Using ^{32}P -postlabeling and HPLC analysis of DNA adducts in in vitro acellular assay, Binková et al. [10] observed that in the extract from PM_{10} 50% of total radioactivity from all DNA adducts corresponded to PAH-DNA adducts derived from c-PAHs. Regarding health effects of air polluted with high concentrations of c-PAHs, other effects than cancer were observed only during the last twenty years: molecular epidemiology studies indicate that air pollution with concentrations higher than $1 \text{ ng}/\text{m}^3$ B[a]P affects genetic material (DNA) by increasing genomic frequency of translocations [11, 12], micronuclei in peripheral lymphocytes [13], and DNA fragmentation in sperm [14]. Elevated exposure to B[a]P has been associated with higher level of PAH-DNA adducts, urinary 1-hydroxypyrene, DNA strand breaks, and DNA repair capacity [6]. Increased concentrations of c-PAHs were associated with elevated risk of intrauterine growth retardation (IUGR) and low birth weight (LBW) during the first gestational month of pregnancy ($\text{B[a]P} > 2.8 \text{ ng}/\text{m}^3$) [15]. Figure 7 illustrates the impact of B[a]P levels in the year 2011 in districts Ostrava-Radvanice and Ostrava-Poruba [3]. Ambient PAHs and fine particles were associated with early-life susceptibility to bronchitis. Associations were stronger for

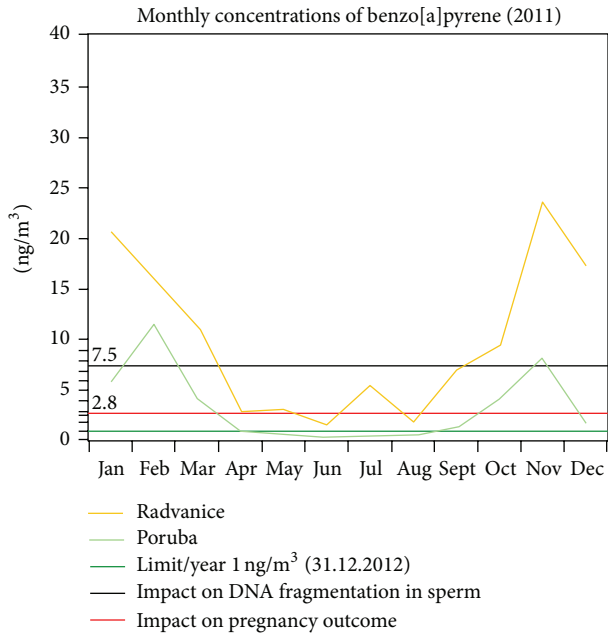


FIGURE 7: Monthly concentrations of B[a]P in 2011 in Ostrava-Radvanice and Ostrava-Poruba: impact to pregnancy outcome and DNA fragmentation in sperm ([3, 14, 15]).

longer pollutant-averaging periods and, among children >2 years of age, for PAHs compared to fine particles [16]. All these data indicate that air pollution by B[a]P poses a very significant health risk in the Ostrava Region.

It is surprising that there is no published information about the impacts of air pollution in OSTR except for Leonardi et al. [17]. They carried out a cross-sectional study in four areas in Ostrava in school children aged 7–11 years in 1996. In 1996, prevalence of pediatrician-diagnosed asthma in Ostrava-Radvanice was 3.0%, asthma or asthmatic, spastic or obstructive bronchitis 10.8%, compared do Ostrava-Poruba with frequency 2.5% and 8.8%, respectively. Later data are available from the National Institute of Public Health nationwide monitoring of allergic diseases in children aged 5–17 years, for 2006. Kratenova and Puklova [18] observed a prevalence of asthma 10.0% versus 7.9% in the Ostrava Region ($N = 1189$) versus other cities in the Czech Republic, with the highest prevalence of asthmatic children in Ostrava-Radvanice (30.8%).

Signs of a possible impact of air pollution were observed by a pediatrician in Ostrava-Radvanice (pediatric district with approx. 1200 children). During the period 2001–2007, the incidence of diagnosed asthma increased from 10% to 30% in children aged up to 17 years, from which 60% of children were under the age of 3.5 years (Figure 8). We did not find any authors that report similar high incidence of asthma. It may be a coincidence, but PM_{10} increased from $39.2 \mu\text{g}/\text{m}^3$ to $65.4 \mu\text{g}/\text{m}^3$ in the period 2003–2007. These data were obtained using ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th revision), prior to the new recommendations in 2008 for both diagnostics and treatment of asthma in children under

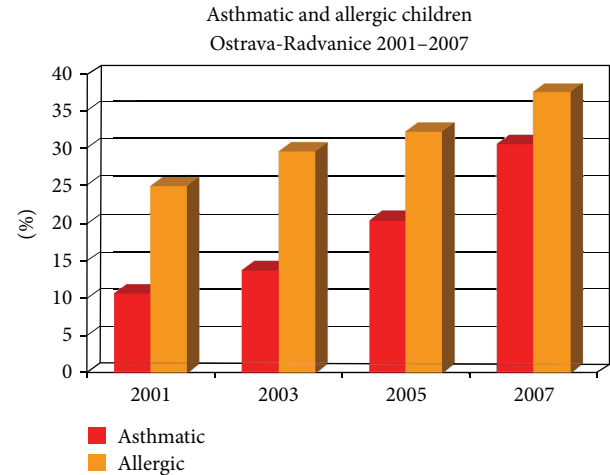


FIGURE 8: Newly diagnosed asthma bronchiale and allergy cases in children in Ostrava-Radvanice/Bartovice in years 2001–2007.

five, proposing to use the term wheezing instead of asthma bronchiale for such young children [19].

4. Program Ostrava

To elucidate situation in OSTR, Program Ostrava was designed to investigate the impact of air pollution on human health in this region. It was funded by the Ministry of the Environment and the Ministry of Education of the Czech Republic [25]. The aim was to evaluate if air pollutants adsorbed on fine particles (c-PAHs, carcinogenic polycyclic aromatic hydrocarbons) as well as VOC (volatile organic compounds) affect human health, and if new information can be obtained using genomics methods.

Program Ostrava consisted of 4 projects: (1) morbidity in children, (2) asthma bronchiale in children, (3) molecular epidemiology study: impact of air pollution on genetic damage, and (4) in vitro study: mechanisms of toxic activities of chemicals adsorbed on respirable particulate matter.

5. Morbidity in Children

In 10 pediatric districts in OSTR, morbidity was followed in children born 2001–2004 up to 5 years of age ($N = 1888$). The pediatricians abstracted medical records in ICD-10 codes. Comparisons of detailed age-specific morbidity of 1655 children born and living in the district of Ostrava-Radvanice (R and B) showed significantly higher incidence of acute illnesses than in children in other parts of Ostrava. They suffered higher incidence of acute respiratory diseases in the first year of life (Figure 9) and higher prevalence of asthma bronchiale (37.1%, $N = 170$) compared to other parts of Ostrava (10.2–13.2%, $N = 1287$) [26]. Prenatal exposure to PAHs may be associated with altered lymphocyte immunophenotypic distribution in cord blood and possible changes in cord serum immunoglobulin E levels as proposed by Hertz-Picciotto et al. [27]. We can hypothesize that high concentrations of PAHs affect maturation of the immune system.

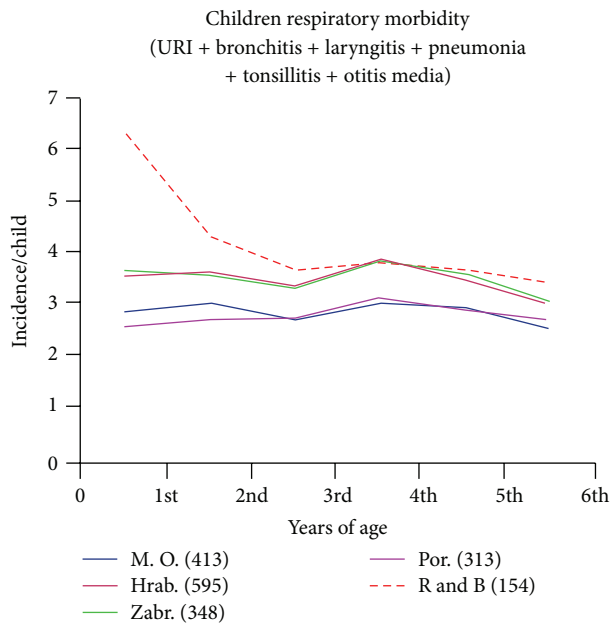


FIGURE 9: Morbidity of children in Ostrava 2001–2009 [26] (Ostrava districts: M.O.—Moravian Ostrava, Hrab.—Hrabůvka, Zabr.—Zábřeh, Por.—Poruba, R and B—Radvanice and Bartovice).

Therefore, children from a more polluted region suffer higher respiratory morbidity especially during the first year of their life.

6. Asthma Bronchiale in Children

In order to investigate specific effects on the origin and development of asthma bronchiale, we have analyzed the impact of air pollution in OSTR on gene expression, micronuclei (MN), and oxidative damage in children. Specifically, we used gene expression profiling technique to study changes in transcript levels in leukocytes of asthmatic children compared with those in children without asthma, in a group of 200 children living in Ostrava-Radvanice (100 asthmatic and 100 healthy children, age 6–15 years) and in a control group of 200 children living in Prachatice (rural district of Southern Bohemia) (100 asthmatic and 100 healthy children) [28].

Gene expression changes were analyzed in 368 blood samples using HumanHT-12 v3 BeadChips (Illumina) containing probes for more than 48 K transcripts. Samples were statistically evaluated according to disease and locality (Ostrava-asthma, Ostrava-control, Prachatice-asthma, and Prachatice-control). A comparison of both Ostrava groups versus both Prachatice groups revealed 64 differentially expressed genes (P value < 0.01 , fold change > 1.5) corresponding to the effect of locality.

Comparison of asthma groups with their corresponding controls within each locality (Ostrava-asthma versus Ostrava-control and Prachatice-asthma versus Prachatice-control) showed 12 differentially expressed genes for Ostrava Region and 17 differentially expressed genes for Prachatice region. Surprisingly, deregulated genes specific to asthma in Ostrava and asthma in Prachatice completely differ; no

transcript was observed simultaneously in both localities. In Ostrava, MAPK (mitogen-activated protein kinases) signaling pathway ($P < 0.01$; 1.5-fold) and in Prachatice cytokine-cytokine receptor interaction pathway ($P < 0.01$; 1.5-fold) were affected (Figure 10).

In asthmatic children living in Ostrava, the results show an increased gene expression related to the nonallergic immune response (*DEFA4*—relationship to the presence of neutrophils; neutrophilic inflammation is associated with the non-allergic type of asthma) and a response to hypoxia (*AHSP*—stabilization of haemoglobin α , *HBG2*—part of fetal haemoglobin (subunits 2α and 2γ , higher affinity for oxygen)). On the other hand, in asthmatic children from Prachatice we observed increased expression of *SIGLEC8*, transmembrane protein involved in the apoptosis of eosinophils. Recently, the association between sequence variants of *SIGLEC8* and total levels of serum IgE has been suggested, indicating the role of this gene in the susceptibility to asthma [29]. Enhanced expression of other genes (*CLC*, *CCL23*, and *CACNG6*) having a relationship with the presence of eosinophils has also been observed. Eosinophilic inflammation is related to allergic immune response and thus corresponds to the allergic phenotype of asthma. These results suggest a different phenotype of asthma in children living in the industrial Ostrava Region as compared to children living in rural Prachatice.

This study is unique because for the first time, whole genome chips were used to analyze the relationship between air pollution and asthma bronchiale. Comparing expression on profiles of children from both regions, we observed deregulation of 64 genes, which affect biological processes and metabolic pathways. Rossnerova et al. [30] studied DNA methylation in the same children. They observed significantly different methylation pattern in 58 CpG sites in children from Ostrava compared to children in Prachatice. The methylation of all 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 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 for morbidity of children as well as morbidity in adulthood [31].

To investigate, if asthma bronchiale is related to biomarkers of genetic damage, a subset of the same Ostrava cohort ($N = 175$) was followed in November 2008, when the mean daily concentration of B[a]P measured by stationary monitoring was $11.4 \pm 9.8 \text{ ng/m}^3$ (samples taken every 6 days). The frequency of micronuclei (MN) in binucleated cells, measured by automated image analysis, as well as markers of oxidative damage to DNA, lipids, and proteins was not associated with asthma. Higher levels of MN were associated with increased levels of protein carbonyl groups. The frequency of MN does not differ between asthmatic and control children. A hypothesis, that asthmatic children may be more affected by exposure to B[a]P was not confirmed [32].

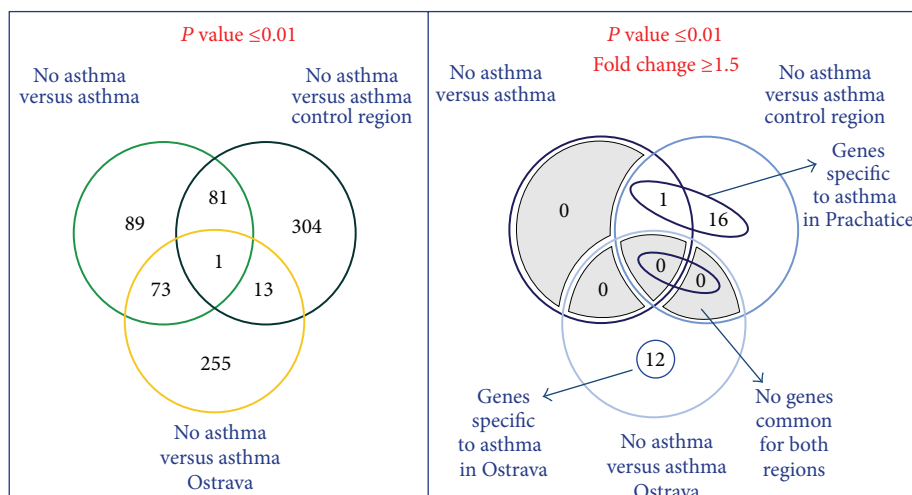


FIGURE 10: Comparison of “no asthma” versus “asthma” *t*-test results. (In the Venn diagrams shown, the *t*-test results obtained using all experiments either with a *P* value cutoff of 0.01 or a *P* value cutoff of 0.01 and at least a 1.5-fold change are compared.)

TABLE 1: DNA adducts in subjects from Prague and Ostrava Region in winters 2009 and 2010 [20] (data for controls are unexposed subjects from [21]).

	N	DNA adducts					
		B[a]P (ng/m ³)		DNA adducts/10 ⁸ nucleotides		Total	
		2009	2010	2009	2010	2009	2010
Prague	64	0.80 ± 0.55	2.86 ± 1.87	0.21 ± 0.06	0.25 ± 0.12	1.30 ± 0.41	1.37 ± 0.47
Ostrava	98	2.73 ± 2.60*	14.8 ± 13.3*	0.28 ± 0.08*	0.16 ± 0.06*	1.37 ± 0.37	1.03 ± 0.33*
Controls	42	0.80 ± 0.62		0.10 ± 0.03		0.76 ± 0.20	

**P* < 0.05.

7. Molecular Epidemiology Studies

We investigated the impact of high level of environmental air pollution on selected biomarkers. Exposure was measured as follows: PM_{2.5} by stationary monitoring, c-PAHs (B[a]P) and VOC (benzene) by personal and stationary monitoring. Personal exposure to c-PAHs was defined using outdoor concentration, ETS exposure, indicator of home heating by coal, wood or gas, frequency of exhaust fan use, cooking habits, and commuting by a car [23].

Cotinine in plasma, triglycerides, total, HDL and LDL cholesterol, and vitamins A, C, E were used as lifestyle indicators. The following parameters were analyzed: DNA adducts by ³²P-postlabeling as biomarkers of effect, chromosomal aberrations by FISH (fluorescent in situ hybridization) MN as biomarkers of effect, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) as a marker of oxidative DNA damage, 15-F2t-isoprostane (15-F2t-IsoP) as a marker of lipid peroxidation, protein carbonyls as a marker of protein oxidation, and genetic polymorphisms as biomarkers of susceptibility. Sampling was done in winter 2009, summer 2009, and winter 2010. Volunteers were recruited from office workers in Ostrava city, city policemen from Havírov and Karviná (*N* = 98), and in 2010 also from general population of Ostrava-Radvanice (*N* = 28). City policemen from Prague (*N* = 65) served as a control group.

During all sampling periods, the study subjects from OSTR were exposed to significantly higher concentrations of B[a]P and benzene than subjects in Prague as measured by personal monitoring. Taken separately, B[a]P levels were lowest in Prague in 2009. Prague winter 2010 concentrations were about equal to the lower Ostrava 2009 levels, and levels in Ostrava in winter 2010 were 5-fold higher. Despite higher B[a]P air pollution in OSTR during all sampling periods, the levels of B[a]P-like DNA adducts per 10⁸ nucleotides were significantly higher in the Ostrava subjects only in winter 2009 (mean ± SD: 0.21 ± 0.06 versus 0.28 ± 0.08 adducts/10⁸ nucleotides, *P* < 0.001 for Prague and Ostrava subjects, respectively) (Table 1, data for controls are unexposed subjects from [21]). During the other two sampling periods, the levels of B[a]P-like DNA adducts were significantly higher in the Prague subjects (*P* < 0.001). Multivariate analyses done separately for subjects from Ostrava and from Prague, combining all sampling periods in each location, revealed that exposure to B[a]P and PM_{2.5} significantly increased levels of B[a]P-like DNA adducts only in the Ostrava subjects [20].

Despite severalfold higher concentrations of air pollutants in the Ostrava Region, the levels of stable aberrations (genomic frequency of translocations per 100 cells (*F_G*/100), percentage of aberrant cells (% AB.C.) were comparable

TABLE 2: Chromosomal aberrations in peripheral lymphocytes by FISH in subjects from Prague and OSTR in winters 2009 and 2010 [22] (data for controls are unexposed subjects from [12], % AB.C.: percentage of aberrant cells, $F_G/100$: genomic frequency of translocations/100 cells).

	N	Genomic frequency of translocations (FISH)					
		B[a]P (ng/m ³)		% AB.C.		$F_G/100$	
		2009	2010	2009	2010	2009	2010
Prague	60	0.80 ± 0.55	2.86 ± 1.87	0.27 ± 0.18	0.25 ± 0.15	1.43 ± 1.15	1.39 ± 1.03
Ostrava	98	2.73 ± 2.60	14.8 ± 13.3*	0.26 ± 0.19	0.22 ± 0.18	1.44 ± 1.23	1.25 ± 1.18
Controls	42	0.80 ± 0.62		0.21 ± 0.16		1.13 ± 1.01	

* $P < 0.05$.

TABLE 3: Lipid peroxidation in subjects from Prague and Ostrava Region in winters 2009 and 2010 [22].

N	Oxidative stress 15-F2t-isoprostane (pg/mL)			
	B[a]P (ng/m ³)		15-2Ft-IsoP	
	2009	2010	2009	2010
Prague 60	0.80 ± 0.55	2.86 ± 1.87	165.9 ± 41.7	256.5 ± 104.7*
Ostrava 98	2.73 ± 2.60*	14.8 ± 13.6*	279.3 ± 303.6*	279.5 ± 124.5

* $P < 0.05$.

(Table 2, controls are unexposed subjects from [12]). The frequency of unstable aberrations measured as number of micronuclei was unexpectedly significantly lower in the Ostrava Region subjects in both seasons of 2009. Urinary excretion of 8-oxodG did not differ between locations in either season. Lipid peroxidation measured as levels of 15-F2t-IsoP in blood plasma was elevated in the Ostrava subjects sampled in 2009, similarly increased in Prague samples in 2010 (Table 3). Multivariate analyses conducted separately for subjects from Prague and Ostrava showed a negative association between the frequency of micronuclei and concentrations of B[a]P and PM_{2.5} in both regions. A positive relationship was observed between lipid peroxidation and air pollution [22].

In contrast to the above results, changes were observed in a group of 4 subjects from Prague who spent 3 weeks in Ostrava just in the period of inversion in winter 2010, when the average daily concentration of B[a]P reported by stationary monitoring was 14.7 ± 13.3 ng/m³. The frequency of micronuclei in peripheral lymphocytes in those individuals increased approximately 50% (Table 4) [24], and similar increase was observed for genomic frequency of translocations.

The relationship between exposure to B[a]P and the level of DNA adducts and chromosomal aberrations in winter 2010 in Ostrava inhabitants was surprising, as the results did not correspond with the expected dose-effect relationship. Therefore we put forward a hypothesis about a possible adaptive response, indicating that this outcome may be affected by DNA repair.

This hypothesis was tested by Rossner et. al. [33] who further investigated in 64 subjects from Prague and 75 subjects from Ostrava the levels of oxidative stress markers (8-oxodG, 15-F2t-IsoP, protein carbonyls) and cytogenetic parameters [$F_G/100$, % AB.C. and acentric fragments (ace)], and their

TABLE 4: The frequency of micronuclei in peripheral lymphocytes in subjects from Prague after 3-week stay in Ostrava in January 2010 [24].

Groups	Sampling	Frequency of micronuclei		
		N (6000 c./person)	% AB.C. with MN	MN/1000
Controls	(i)	4	0.81 ± 0.15	8.32 ± 1.63
	(ii)	4	0.80 ± 0.14	8.47 ± 1.55
Exposed	(i)	4	0.74 ± 0.43	7.96 ± 4.92
	(ii)	4	1.14 ± 0.55	12.91 ± 6.49*

* $P < 0.05$.

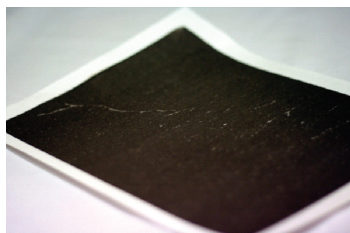
relationship with the expression of genes participating in base excision repair (BER) and nonhomologous end joining (NHEJ) by quantitative PCR. Multivariate analyses revealed that subjects living in Ostrava had increased odds of having above-median levels of *XRCC5* expression (OR; 95% CI: 3.33; 1.03–10.8; $q = 0.046$). Above-median levels of 8-oxodG were associated with decreased levels of vitamins C (OR; 95% CI: 0.37; 0.16–0.83; $P = 0.016$) and E (OR; 95% CI: 0.25; 0.08–0.75; $P = 0.013$), which were elevated in subjects from Ostrava. They suggest that air pollution by c-PAHs affects *XRCC5* expression, which probably protect subjects from Ostrava against the induction of a higher frequency of translocations; elevated vitamin C and E levels in the Ostrava subjects decrease the levels of 8-oxodG. Such changes in gene expression were not observed in the 4 subjects from Prague after 3-week-stay in Ostrava; their reaction differed from subjects with long residence time in OSTR.

For the first time, this study measures the levels of biomarkers in subjects exposed to air pollutants. Simultaneous assessment of oxidative stress markers, chromosomal aberrations, and measurement of DNA repair gene expression is a new approach that can bring more clarity to the mechanisms of pollution effects.

8. In Vitro Studies

A wide variety of in vitro systems was developed in order to study the genotoxicity of chemicals and their mixtures, including complex mixtures of environmental pollutants adsorbed onto respirable air particles (PM_{2.5}). Complex mixtures of organic compounds to which humans are exposed through air pollution are only partially characterized with

Locality	Month and year of collection	Volume of air (m ³)	PM _{2.5} (μg/m ³)	B[a]P (μg/m ³)	c-PAH (ng/m ³)
Ostrava-Radvanice	03/2009	29900	36.7	13.6	81.6
Ostrava-Poruba	03/2009	35200	25.8	4.28	27.2
Karviná	04/2009	47400	n.a.	1.88	12.1
Třeboň	11-12/2008	44700	11.4	1.11	7.92



Filter from Ostrava-Bartovice

FIGURE 11: Characteristics of PM_{2.5} particles collected in winter 2008-2009 in Ostrava-Radvanice/Bartovice, Ostrava-Poruba, Karviná, and Třeboň [38].

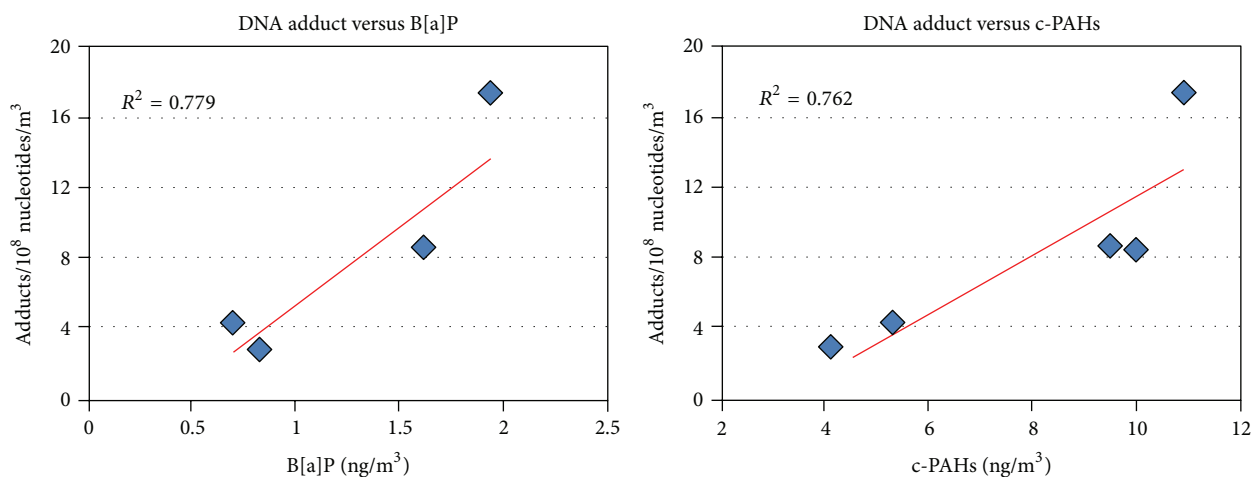


FIGURE 12: The relationship between the PAH-DNA adducts versus concentrations of B[a]P and c-PAHs in extracts from PM_{2.5} from samples from Ostrava-Radvanice/Bartovice, Ostrava-Poruba, Karviná, and Třeboň [38].

respect to their chemical composition due to difficulties with chemical analysis of the individual components. Therefore, alternative assays based on biological effects of complex mixture components may be a suitable alternative to a circumstantial chemical analysis. Using rat liver microsomal fraction (S9), it has been repeatedly shown that PAHs formed DNA adducts after metabolic activation by P450 enzymes to diol epoxides. This activation system may be used in acellular assay coupled with ³²P-postlabeling to assess genotoxic potential of complex environmental mixtures via the analysis of DNA forming activity of the mixtures in native DNA [34–37].

PM_{2.5} particles were collected by high-volume samplers in MSR (localities Ostrava-Radvanice/Bartovice, Ostrava-Poruba, and Karviná) and in the locality exhibiting a low level of air pollution—Třeboň—a small town in the nonindustrial region of Southern Bohemia (Figure 11). PM_{2.5} was extracted (extractable organic matter—EOM) and c-PAHs contents in the EOMs were determined. DNA adduct levels and

oxidative DNA damage levels (8-oxodG) induced by EOMs in an acellular assay of calf thymus DNA coupled with ³²P-postlabeling (DNA adducts) and ELISA (8-oxodG) in the presence and absence of microsomal S9 fraction were used as markers of genotoxic potential. Twofold higher DNA adduct levels (17.2 adducts/10⁸ nucleotides/m³ versus 8.5 adducts/10⁸ nucleotides/m³) were induced by EOM from Ostrava-Bartovice (immediate proximity to heavy industry) compared with that from Ostrava-Poruba (mostly traffic emissions). PAH-DNA adducts are highly correlated with the content of B[a]P and c-PAHs in EOM (Figure 12). Oxidative DNA damage induced by EOM from Ostrava-Bartovice was more than fourfold higher than damage induced by EOM from Třeboň (8-oxodG/10⁸ dG/m³: 0.131 versus 0.030 for Ostrava-Bartovice versus Třeboň, respectively). c-PAH contents in EOMs were the most important factors relating to their genotoxic potential [38].

These results clearly demonstrated that EOM extracted from PM_{2.5} induces bulky DNA adducts as well as oxidative

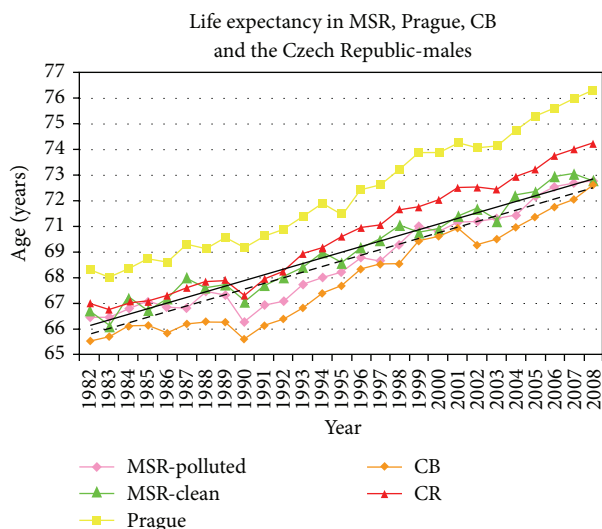


FIGURE 13: Life expectancy in two sub-regions of MSR (“polluted”, “clean”), Prague, Coal Basin compared to average life expectancy for the Czech Republic (males).

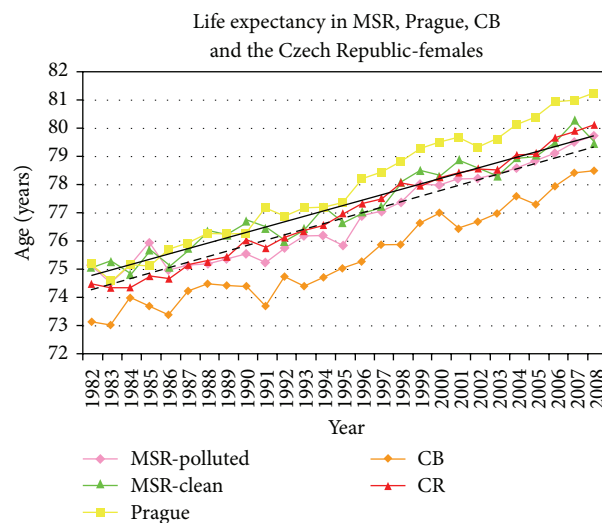


FIGURE 14: Life expectancy in two sub-regions of MSR (“polluted”, “clean”), Prague, Coal Basin compared to average life expectancy for the Czech Republic (females).

DNA damage as measured by the levels of 8-oxodG. Both effects are enhanced by metabolic activation by microsomal cytochrome P-450 enzymes. Since $PM_{2.5}$ particles collected in various localities differ in their c-PAHs content and c-PAHs significantly contribute to genotoxicity and DNA oxidative damage, it may be suggested that monitoring of $PM_{2.5}$ levels is not a sufficient basis to assess genotoxicity of respirable aerosols. This further indicates that the industrial emissions prevailing in Ostrava-Bartovice represent a substantially higher genotoxic risk than traffic-related emissions in Ostrava-Poruba. B[a]P and c-PAH contents in EOMs are the most important factors for their genotoxic and DNA oxidative potential.

9. Regional Studies Outside Program Ostrava: Air Pollution and Mortality

Impact of air pollution on life expectancy was repeatedly established in USA [39, 40], as well as in the mining district of Usti Region in the Czech Republic (coal basin, CB) [41]. A long-term study done in the period 1982–2008 shows a significant increase of life expectancy in the Czech Republic starting around 1990 when major measures were taken to reduce emissions from the district’s most prominent sources (brown coal fired power plants). This increase was approx. 7 years for males and 6 years for females. These trends are similar also in the CB, but 2 years lower for each gender when we assess the whole period 1990–2008. In 1990, MSR life expectancy of males living in MSR-polluted districts was 1 year shorter to the national average, in 2008 life expectancy of males was 2 years shorter, for females it corresponded with the trends in the Czech Republic (Figures 13 and 14) [42].

The association between daily, cardiovascular and respiratory mortality in men and women and increase of $100 \mu\text{g}/\text{m}^3$ PM_{10} was studied in MSR, Usti region (coal basin,

CB) and Prague for the period 1997–2009 [43]. The step of $100 \mu\text{g}/\text{m}^3$ reflects the observed variability of daily concentrations. In men, the daily total mortality increases (4 days mean) significantly with association with $100 \mu\text{g}/\text{m}^3$ PM_{10} by 8.3% in MSR, 5.9% in CB, and 7.9% in Prague. This increase was higher in men older than 65 years (13.2% in MSR, 8.4% in CB, and 8.5% in Prague). The relationship between daily mortality and PM_{10} was stronger for cardiovascular mortality (12.9% in MSR, 9.0% in CB, 9.7% in Prague), and even more pronounced in men older than 65 years (18.4% MSR, 12.8% CB, and 10.5% Prague). In women, the results are quite different. No increase of daily total and cardiovascular mortality associated with $100 \mu\text{g}/\text{m}^3$ increase of PM_{10} was observed. It may be proposed that other factors are more important, for example, differences in exposure to other pollutants in occupational, ambient and home environment, smoking habits, diet, education and economical status.

10. Regional Studies Outside Program Ostrava: Long-Term Effect of Air Pollution

Dejmek et al. [15] observed for the first time the effect of increasing concentrations of c-PAHs in polluted air on intrauterine growth retardation (IUGR) and low birth weight (LBW, <2500 g). Today, this is interpreted as the effect of pregnant mothers being exposed to c-PAHs that induces DNA damage and histone modification [44]. PAH-DNA adducts were detected in cord and maternal blood [45] and placentas [46] due to exposure to c-PAHs from polluted air. Exposure to c-PAHs during pregnancy is associated with the toxic effect to fetus, inducing IUGR, LBW [15, 47], and premature birth [47]. When those children were followed until the school age, it was observed that prenatal exposure to c-PAHs impaired neuropsychic development [48] and increased the incidence of asthma bronchiale [44].

Choi et al. [49] published results of a study on pregnant mothers from Cracow, Poland, which is a region bordering MSR and similar in its industrial status. The air is polluted by local heating and power plants using coal. Personal exposure to B[a]P in the first trimester was during March-May 2.11 ng/m^3 , during December-February 7.21 ng/m^3 [50], (this concentration corresponds with environmental burden in Ostrava-Poruba in 2011 [25]). This study showed a significant negative impact of c-PAHs on growth of fetus during the first trimester.

Epidemiologic studies indicate that the growth of fetus is programmed already in the very early stages of pregnancy and that impairment in the first trimester results causes a larger deficit of growth during further gestation [51–53]. The consequence of these changes in children with IUGR or LBW is a higher risk to delay neurodevelopment [54], affect lung functions [55], increase asthmatic symptoms in childhood [56], and increase of cardiovascular diseases [57] and diabetes [58] in adulthood.

Study by Choi et al. [49] confirms the data by Dejmek et al. [15] about the impact of increased c-PAHs concentrations in polluted air on fetus' unfavorable development and the long-term effect of such burden.

It is already recognized that pregnancy outcome and DNA damage are affected by the child genotype, genetic polymorphisms [59]. This study indicates that increased ambient concentration of c-PAHs may induce more significant DNA damage in children with certain genotypes (alleles), which is expressed as the decrease of birth weight. Therefore, we may expect that the quality of genome, under a different environmental stress, may affect also the child morbidity.

Miller et al. [60] observed adverse effect of prenatal exposure to c-PAHs on respiratory symptoms for children aged 12–24 months, especially asthmatic symptoms, already at concentrations $3.53 \pm 2.81 \text{ ng c-PAHs/m}^3$. Jedrychowski et al. [61] observed the effect of perinatal exposure to c-PAHs in Cracow to increase respiratory symptoms as cough, wheezing, and ear infections. They explain this observation by immunotoxic activity of PAHs, which impairs fetal immune functions and is later responsible for increased susceptibility of newborns and preschool children to respiratory infections. These data suggest the risk of exposure to c-PAHs in very early age. Low birth weight associated with impaired lung functions may increase the risk of inflammatory respiratory symptoms or hyperreactivity of respiratory airways.

11. Conclusions

The specific pollution situation observed in the Moravian-Silesian Region (especially in OSTR) is a result of high population density and activities of heavy industries. Combined evidence indicates that health impact of air pollution is associated specifically with high concentrations of c-PAHs. Concentrations of B[a]P have been exceeding the annual limit value of 1 ng/m^3 in the whole study period, in some localities severalfold. These levels of air pollution, and especially of B[a]P, significantly increase respiratory morbidity in children of preschool age, asthma bronchiale in children, and cardiovascular mortality. These levels have shown associations

with long-term biological effects manifesting themselves in different forms and ages, from effects observable in foetus to decrease in life expectancy in adults.

The health and biological effect studies clearly demonstrate that under the present environmental conditions in the MSR, the health of the population is severely impaired and will likely remain so for a significant period of time. Recent studies imply that B[a]P [62] and air pollution [63–65] induce gametic mutations. It means that induced DNA damage in human gametes is transferred to next generations [66]. According to Barker [57], changes induced during the fetal growth increase in adults the risk of cardiovascular diseases and diabetes. It may be therefore postulated that the effect of present air pollution in MSR will affect the health of population for the next several decades.

The results presented here provide evidence of an association between industrial pollution and deteriorated health and point strongly at an urgent need to mitigate the pollution in the region. Considering that, in 2011, levels of B[a]P exceeding European limit values affected approximately 60% of all Czech population [67]; the results presented here should give rise to a national concern.

Acknowledgments

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