Neuroendocrinology Letters Volume 37 Suppl. 2 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

The impact of air pollution in the Southern Bohemia Region on fetuses and newborns

Milos Veleminsky, Jr. [1], **Milan Hanzl** [2], **Radim J. Sram** [3]

1 University of South Bohemia in Ceske Budejovice, Faculty of Health and Social Sciences, Institute of Physiotherapy and Selected Medical Disciplines, Ceske Budejovice, Czech Republic
2 University of South Bohemia in Ceske Budejovice, Faculty of Health and Social Sciences, Institute of Nursing, Midwifery and Emergency Care, Ceske Budejovice, Czech Republic
3 Institute of Experimental Medicine, AS CR, Department of Genetic Ecotoxicology, Prague, Czech Republic

Correspondence to: MUDr. Milos Veleminsky, Jr., PhD.,
University of South Bohemia in Ceske Budejovice, Faculty of Health and Social Sciences, Institute of Physiotherapy and Selected Medical Disciplines
Bozeny Nemcove 585/54, 370 01 Ceske Budejovice, Czech Republic.
e-mail: veleminsky@volny.cz

Submitted: 2016-10-06 *Accepted: 2016-11-05* *Published online: 2016-12-18*

Key words: **air pollution; polycyclic aromatic hydrocarbons; benzo[a]pyrene; pregnancy outcome; DNA adducts; micronuclei; intrauterine growth retardation; lower birth weight**

Neuroendocrinol Lett 2016; **37**(Suppl. 2):52–57 **PMID:** 28233961 NEL371016A08 © 2016 Neuroendocrinology Letters • **www.nel.edu**

Abstract

Air pollution with increased concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs, represented by benzo[a]pyrene, B[a]P) affects fetal development, reduces birth weights (LBW) of newborns, and increases intrauterine growth retardation (IUGR). The Southern Bohemia Region is believed to be one of the least air polluted regions in the Czech Republic. Monitoring air pollution in the city of Ceske Budejovice from 2011–2015, PM2.5 (particulate matter <2.5 µm) decreased from 20.3±14.5 µg/m^3 to 14.3±8.6 µg/m^3, but concentrations of B[a]P did not change between the years 2007–2015: 1.5±0.6 ng/m^3 vs. 1.4±0.6 ng/m^3. Higher B[a]P concentrations the winter induce genetic damage in newborns, increase frequency of micronuclei (chromosomal aberrations), deregulate genes for immunity in umbilical cord blood, and increase incidence of IUGR and LBW in newborns.

INTRODUCTION

The results of research in Europe and the USA have shown that long-term exposure to air pollution affects not only acute symptoms, but also contributes to the development of disease.

The first study on the influence of air pollution on the results of sperm quality, the incidence of bronchitis in children as well as translocations in the genome were performed in the Czech Republic. Program Teplice, from the 1990s, which monitored the effect of air pollution on the health status of the exposed population, was based on air pollution in the Northern Bohemia Region in the 1970s and 1980s (Sram 2001).

MATERIALS AND METHODS

Importance of particulate matter

Particulate matter (PM) is a complex heterogenous group of compounds with specific composition (i.e., size of particle and chemical characterization). The composition of PM varies over time and depends on various sources of emission, chemical processes in the air, and climate conditions. The WHO (2007) recommended using PM2.5 (particulate matter <2.5 μm) as an indicator of health risk. Available evidence shows an association between PM emitted from combustion sources, both mobile and stationary, affects the health status, and increases morbidity and mortality, relative to cardiovascular, respiratory diseases, and atopic reactions to external air. Carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) compounds bind to the surface of PM2.5. c-PAHs in the air are formed from inadequate combustion or pyrolysis of organic material such as diesel, gasoline, natural gas, coal, and wood. The sources of airborne c-PAHs are industry, local heating, and traffic pollution. PM is a mixture of various chemicals, including c-PAHs, which can have negative impact on human health by inducing oxidative damage to DNA, lipids, and proteins (Sørensen *et al.* 2003; Knaapen *et al.* 2006).

Polycyclic aromatic hydrocarbons

Recent research has indicated the influence of prenatal exposure to carcinogenic PAH (c-PAHs) on fetal development (Sram *et al.* 2005) and the incidence of respiratory diseases in children (Herz-Picciotto *et al.* 2007). c-PAHs are one of the main carcinogens in the air. PAH are activated to form reactive derivatives via metabolic enzymes and can lead to chemical reactions that add extraneous chemical groups to DNA (called DNA adducts) and protein adducts. The presence of DNA adducts caused by c-PAHs can lead to mutations. High levels of DNA adducts are associated with an increased risk of carcinogenic diseases (Gammon *et al.* 2004).

c-PAHs also induce the formation of reactive compounds. The mutagenicity of these compounds is linked to the formation of reactive oxygen species (ROS) (Penning *et al.* 1996; Xue and Warshawsky 2005). The level of PAH-induced DNA adducts (the most commonly used marker of c-PAHs exposure) is most often analyzed using the ^{32}P post-labelling method (Binkova *et al.* 1995).

It has been repeatedly shown that exposure to c-PAHs reduces the birth weight of newborns and increases intrauterine growth retardation (IUGR 10th percentile of birth weight for gestational age and gender). It can be assumed that c-PAHs are one of the most important groups of pollutants for human risk assessment.

Rubes *et al.* (2010) found increased DNA fragmentation in mature sperm when exposed to concentrations of B[a]P (benzo[a]pyrene) above 7.5 ng/m^3 for 10 days, which could potentially cause infertility. DNA fragmentation is also linked to the lack of a specific gene, which is involved in detoxification (metabolism) of c-PAHs. Research results from Rubes *et al.* have also shown the impact of air pollution on male fertility.

Molecular epidemiology studies have shown that air concentrations of B[a]P greater than 1 ng/m^3 in air causes DNA damage through increased frequency of translocations in genome (Sram *et al.* 2007), micronuclei in lymphocytes (Rossnerova *et al.* 2009), and DNA fragmentation in sperm (Rubes *et al.* 2010). DNA damage can be repaired, but if not, modified genetic information may lead to diseases that will not manifest for years or decades.

The developing child is increasingly receptive (sensitive) to DNA damage, especially during intrauterine development and later during their school age years.

The risk of c-PAHs exposure was summarized in a WHO document (2011): All the above data shows that exposure to c-PAHs pose a significant risk to human health. Since c-PAHs affect genetic material (via mutations), they can cause long-term damage to the body, which can manifest over a lifetime; additionally, this damage can be transferred to the next generation.

Impact of c-PAHs on pregnancy outcome

Dejmek *et al.* (2000) observed for the first time that c-PAHs in polluted air, at concentrations greater than 2.8 ng B[a]P/m^3, during the first month of pregnancy increases the incidence of children with intrauterine growth retardation (IUGR) and low birth weight (LBW, <2500 g) (Baker 2006). The consequence of this functional immaturity in adulthood is an increased incidence of cardiovascular diseases, diabetes, renal dysfunction, and obesity. A higher incidence of IUGR is also seen by children of the next generation (Painter *et al.* 2008; Boekelheide *et al.* 2012). Today, the effect of the exposure of pregnant mothers to c-PAHs has been explained by the induction of DNA damage and histone modification (Perera *et al.* 2009a). PAH-DNA adducts were observed in the venous cord blood and mothers (Perera *et al.* 2005) as well as in the placentas (Topinka *et al.* 1997) after exposure to c-PAHs in polluted air. Exposure to c-PAHs during pregnancy (through inhalation of c-PAHs) is linked to its toxic effects on the fetus, which is expressed as IUGR, LBW (Dejmek *et al.* 2000; Choi *et al.* 2008b) as well as precocious delivery (Choi *et al.* 2008a). When these newborns are followed through school age, it has been observed that prenatal exposure to c-PAHs affects neurobehavioral development (Perera *et al.* 2009b) and also increases the incidence of bronchial asthma (Miller *et al.* 2004).

Epidemiologic studies observed that fetal growth is programmed from the very early stages of pregnancies; the result is a larger growth deficit as gestation continues (Neufeld *et al.* 1999; Smoth 2004; Milani *et al.* 2005). The consequence of these changes in children with IUGR or LBW is a greater risk of delayed mental development (Van Wassenaer 2005), damage to respiratory functions (Lipsett *et al.* 2006), asthmatic symptoms in childhood (Nepomnyaschy and Reichman 2006), and cardiovascular disease in adulthood (Baker 2006), including hypertension, atherosclerosis, and diabetes (Martin-Gronert and Ozanne 2007).

A study by Choi *et al.* (2012) confirmed the data obtained by Dejmek *et al.* (2000) regarding the impact of increased concentrations of c-PAHs in polluted air being unfavorable to fetal development and the long-term effects of such burdens.

New information indicates that pregnancy outcomes as well as observed DNA damage are affected by the child's genotype and genetic polymorphisms (Sram *et al.* 2006). These results show that when exposed to increased c-PAHs concentrations in the air, some genotypes are more susceptible to DNA damage, which can lead to decreased birth weight (Sram *et al.* 2006). Therefore, it is reasonable to assume that the nature of the child's genome may affect morbidity via differential environmental burdens.

Miller *et al.* (2004) reported on the adverse effect of prenatal exposure to c-PAHs (at concentration 3.53±2.81 ng/m^3) as manifested in respiratory problems of children aged 12–24 months, mainly asthma symptoms. Jedrychowski *et al.* (2005) observed the ability of perinatal c-PAHs exposure to influence an increase in respiratory symptoms such as coughing, wheezing, and ear infections in Krakow. The authors suggested that the immunotoxicity of c-PAHs can damage fetal immunity, which can increase the susceptibility of newborns and children to respiratory infections. These publications showed that the risk of respiratory problems caused by c-PAHs start at a very early age and low birth weight, associated with many respiratory problems, can increase the risk of inflammatory respiratory symptoms or respiratory hyper-reactivity.

<u>Air pollution in Southern Bohemia</u>

The Southern Bohemia Region is believed to be one of the least air polluted regions in the Czech Republic. This was why, at the time of the Teplice Research Project (Sram *et al.* 1996), the district of Prachatice was selected as the control district vs. the highly polluted district of Teplice.

A monitoring station using a VAPS (Versatile Air Pollutant Sampler, URG Corp., Chapel Hill, NC, USA) was installed in the town of Prachatice in 1993 to detect PM2.5 (particulate matter <2.5 μm) and c-PAHs. The concentration of PM2.5 from 1993–2009 was between 10–30 μg/m^3, with lower concentrations from 1999–2005. In the period 2001–2006 concentrations of B[a]P were lower than 1 ng/m^3, and in 2002 they were 0.6 ng/m^3. However, by 2009 the concentration had increased to 1.4 ng/m^3, which was higher than the B[a]P concentration in Prague or Teplice. A probable reason for this change was the increase in natural gas prices that led to a significant number of citizens to return to the use of brown coal for home heating.

The Czech Hydrometeorology Institute started to monitor PM2.5 in the city of Ceske Budejovice in 2004 and B[a]P in 2006. It seems that during the last 5 years the concentration of PM2.5 decreased from 20.3±14.5 μg/m^3 in 2011 to 14.3±8.6 μg/m^3 in 2015. The concentration of B[a]P remained more the less the same from 2007 to 2015 (1.5±0.6 ng/m^3 vs. 1.4±0.6 ng/m^3 (CHMI 2016)).

<u>Impact of air pollution on genetic damage in newborns</u>

We tested the hypothesis that the level of genetic damage, expressed as the level of DNA adducts, the frequency of micronuclei, and changes in the transcriptome varies depending on levels of air pollution. The study was focused on two different locations: Ceske Budejovice (CB) – a regional city in the center of South Bohemia, surrounded by villages and a mostly rural countryside, and Prague (P) – the capital city of the Czech Republic with high, traffic-related, air pollution (Sram *et al.* 2013). South Bohemia is traditionally thought to be a region with moderately clean air (Sram *et. al.* 1996).

The study populations were recruited from the Ceske Budejovice Hospital, a. s. and the Motol University Hospital, in Prague. One-hundred and seventy-eight mothers and their newborns, from both hospitals, all of Caucasian ethnicity, were included. Mothers were aged 30.9±4.1 years [median (range): 31.2 (18.3–49.0) years]. Newborns had an average birth weight of 3435±463 g [median (range): 3430 (2270–5150) g]. Information on the course and outcome of pregnancies were obtained from medical records. The study was approved by the IRB of the Institute of Experimental Medicine AS CR.

Data from Ceske Budejovice were collected between 15 January and 31 March 2009 (92 deliveries). The group was split into mothers living in Ceske Budejovice (40 deliveries) and mothers living in villages and small towns (52 deliveries). All births in both groups were vaginal, from uncomplicated pregnancies.

The levels of B[a]P and benzene, a representative c-PAHs and VOC and PM2.5, for both locations in 2008 and 2009, were obtained from the Czech Hydrometeorological Institute. The mean concentrations of B[a]P, PM2.5, and benzene for the three months before delivery were calculated to estimate the individual exposure of each mother-newborn pair: the B[a]P concentration in Ceske Budejovice was 3.2±0.2 ng/m^3 vs. in Prague 1.9±0.5 ng/m^3 ($p<0.001$), CB PM2.5 24.5±0.7 μg/m^3 vs. P 27.0±2.5 μg/m^3 ($p<0.001$), and CB benzene 2.1±0.1 μg/m^3 vs. P 2.5±0.5 μg/m^3 (Rossnerova *et al.* 2009).

Biomarkers of exposure were determined in the umbilical cord blood (UCB) as DNA adducts using ^{32}P post-labelling (Binkova *et al.* 2007) (Table 1) and biomarkers of effect were determined as micronuclei using automated image analysis (Rossnerova *et al.* 2009) (Table 1). DNA adducts were analyzed as B[a]P-like adducts and total adducts, both categories were significantly higher in Ceske Budejovice vs. Prague ($p<0.001$) (Sram *et al.* 2013). Similarly, the frequency of micronuclei in newborns in Ceske Budejovice vs. Prague ($p<0.001$) was higher. Multivariate logistic regression on the frequency of micronuclei showed a significant impact at the 3-month mean B[a]P before delivery in newborns (OR=3.07 (1.63–5.77), $p<0.001$). The comparison between data coming from cities and villages around Ceske Budejovice and Ceske Budejov-

Tab. 1. DNA adducts and micronuclei in newborns (cord blood) – Prague vs. České Budějovice.

	Adducts/10^8 nucl. (average ± S.D.)			MN frequencies		
	N	B[a]P – like	Total	N	MN/1000 BNC	% AB.C.
Prague	80	0.23±0.18	0.98±0.89	86	2.17±1.32	0.21±0.12
Ceske Budejovice	76	0.41±0.41***	1.40±1.31***	92	3.82±2.43***	0.37±0.23***
City				40	3.45±1.92	0.32±0.17
Villages				52	4.11±2.75	0.40±0.27

*** $p<0.001$; N = number of newborns; MN/1000 BNC = micronuclei/1000 binucleated cells; % AB.C. = % of aberrant cells.

ice itself, showed that there were some differences, but they were not significant (Rossnerova *et al.* 2011).

Can polluted air induce any changes in transcriptome? To answer this question, we used our UCB samples for a pilot study (Sram *et al.* 2013). Non-smoking mothers without exposure to ETS (environmental tobacco smoke) were put into groups, 52 from Ceske Budejovice and 35 from Prague. Total RNA was isolated from leukocytes and gene expression profiles were determined using HumanRef-8 Expression BeadChips (Illumina, San Diego, CA, USA) containing 24,526 transcript probes. Genes with /logFC/ >0.58 (binary logarithm of fold change) and $P<0.01$ were considered as differentially expressed between Ceske Budejovice and Prague. Leukocytes from newborns showed different expression in 104 genes (37 up-regulated and 67 down-regulated genes). Among the down-regulated biological processes were biological processes for the immune and defense response (*KIR2DL3, KIR3DL3, KIR3DL4, KIR2DS5, KLRC3, CLTA4*), negative regulation of proliferation (*CNDKN1A, CTLA4, TGFBR3*), apoptosis (*PRF1, NR4A2, GZMB, TNFAIP3, PP2R2B, DDIT4*), response to oxygen levels, cell migration, organ regeneration, signal transduction (*RGS1, SOCS1, THBS*), cell differentiation (*FLT3, ZBTB16*), and the up-regulated gene encoding *SERPINA1* (which is considered a biomarker of exposure to genotoxic agents). Among biological pathways, natural killer cell mediated cytotoxicity and autoimmune thyroid disease were down-regulated, and the MAPK signalling pathway was up-regulated.

DISCUSSION

These results were surprising, since air pollution in Prague was originally thought to be worse than in Ceske Budejovice. Results of analysis of DNA adducts, micronuclei, and the transcriptome indicate, in the same direction, the significance of exposure to B[a]P in Ceske Budejovice to induce genetic damage in newborns, even when PM2.5 and benzene were higher in Prague. It seems to be a coincidence that the changes observed in Ceske Budejovice were observed when exposure to B[a]P was 3.2 ng/m^3, which exceeds the exposure capable of inducing IUGR (2.8 ng/m^3) (Dejmek *et al.* 2000).

This should lead us to re-evaluate risk of air pollution in Southern Bohemia.

According to the WHO (2011), concentrations of 1 ng/m^3 B[a]P in ambient air can induce genetic damage, which has been demonstrated with several biomarkers. The European Union standard is 1 ng/m^3 B[a]P/year. The concentration of B[a]P in the city of Ceske Budejovice in the year 2015 was, as a yearly average, 1.4±0.6 ng/m^3. Specifically, January, February and December had a B[a]P of 2.7–3.4 ng/m^3, however, from May to September the value fell to 0.0–0.5 ng/m^3. This indicates that B[a]P concentrations in winter reach levels that can induce adverse reproductive outcomes such as IUGR and LBW.

The most likely explanation for this is local heating during the winter, when people burn brown coal and sometimes also waste. Since 2007, this situation seems to be the result of economic policies, which increased the price of natural gas. We can see this effect in the form of higher frequencies of micronuclei in rural villages compared to the city of Ceske Budejovice.

Our results on B[a]P pollution in the winter air of Southern Bohemia showed that B[a]P concentrations during the winter period represent a significant health risk, which could affect pregnancy outcomes and child morbidity.

What are the solutions? The higher concentrations of B[a]P in Ceske Budejovice may partially be caused by outdated central heating systems, which are expected to be continuously modernized, starting this year. Certainly, the most important source of pollution is local heating with brown coal. This situation may improve in the future through actions of the Czech Ministry of the Environment that is financially supporting more effective heating with modern systems. But undoubtedly, the best solution would be the increased use of natural gas for heating.

Adverse pregnancy outcomes can also be affected by lifestyle. The effect of active smoking as well as passive smoking is well known (Dejmek *et al.* 2002). There is no question and it is well understood by the Czech population that pregnant mothers should not smoke. Although, the habit still exists, it can be affected by education and social standards.

Another marker of a healthy lifestyle is diet. A recent study on the quality of diet among pregnant mothers in Ceske Budejovice showed that the nutritional quality of typically consumed food was poor; with a low intake of vegetables – 22.8% of the recommended daily amount (RDA), fruits 61.8% of the RDA, and milk 30.2% of the RDA. These data came from samples of real of diets of mothers, 10 in summer 2013 and 10 in winter 2014; with 25% of daily food intake being collected for the 7 to 14 days before the expected delivery date. The quality of the mothers' diet and intake of vegetables were negatively correlated with DNA adducts of the newborns (Honkova *et al.* 2015). These results confirmed that sufficient intake of antioxidants can improve PAH detoxification in pregnant mothers (Sram *et al.* 2013).

Southern Bohemia is often described a beautiful countryside, with many fish ponds, forests and old castles – an ideal place to relax. We are sorry to say that our data indicates that air pollution in this region during the winter period may represent a risk to fetal development, which may affect the health of newborns and later their morbidity. We are in the early stages in understanding the health effects produced by fetal programming and how these affects manifest later in life. Clearly, it would be of great value to follow newborns from the Ceske Budejovice region for an extended period of times in an effort to study not only the chronic effects of air pollution, but to also see if decreases in pollution and changes in lifestyle can simultaneously reverse or mitigate the genetic damage observed in today's newborns.

CONCLUSION

This overview of air pollution in the Southern Bohemia Region indicates that from 2007–2015 the concentration of the carcinogenic pollutant benzo[a]pyrene was significantly higher than the EU standard, especially during the winter months.

The higher concentrations of B[a]P induce genetic damage in newborns such as DNA adducts or chromosomal aberrations (micronuclei), and deregulated genes needed for immunity, as well as increase the incidence of IUGR and LBW.

ACKNOWLEDGEMENT

The study is supported by the Grant Agency of the Czech Republic 13-13458S. We would like to thank Mr. Kip Bauersfeld for language editing.

Conflict of interest. *The authors report no conflict of interest.*

REFERENCES

1 Baker DJ (2006). Adult consequences of fetal growth restriction. Clin Obstet Gynecol. **49**: 270–283.

2 Binkova B, Lewtas J, Miskova I, Lenicek J, Sram R (1995). DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed populations. Carcinogenesis. **16**: 1037–1046.

3 Binkova B, Chvatalova I, Lnenickova Z, Milcova A, Tulupova E, Farmer PB, Sram RJ (2007). PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair genes polymorphisms. Mutat Res. **620**: 49–61.

4 Boekelheide K, Blumberg B, Chapin RE, Cote I, Graziano JH, Janesick A *et al.* (2012). Predicting later-life outcomes of early-life exposures. Environ Health Perspect. **120**: 1353–1361.

5 CHMI (2016): [online] [cit. 2016-08-11]. Available from: http://portal.chmi.cz/files/portal/docs/uoco/isko/tab_roc/tab_roc_EN.html

6 Choi H, Perera F, Pac A, Wang L, Flak E, Mroz E *et al.* (2008a). Estimating individual level exposure to airborne polycyclic aromatic hydrocarbons throughout the gestational period based on personal, indoor and outdoor monitoring. Environ Health Perspect. **116**: 1509–1518.

7 Choi H, Rauh V, Garfinkel R, Tu Y, Perera F (2008b). Prenatal exposure to airborne polycyclic aromatic hydrocarbons and risk of intrauterine growth retardation. Environ Health Perspect. **116**: 658–665.

8 Choi H, Wang L, Lin X, Spengler JD, Perera FP (2012). Fetal window of vulnerability to airborne polycyclic aromatic hydrocarbons on proportional intrauterine growth restriction. PlosS One. **7**: e35464.

9 Dejmek J, Solanský I, Beneš I, Leníček J, Šrám RJ (2000). The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. Environ Health Perspect. **108**: 1159–1164.

10 Dejmek J, Solansky I, Podrazilova K, Sram RJ (2002). The exposure of nonsmoking and smoking mothers to environmental tobacco smoke during different gestational phases and fetal growth. Environ Health Perspect. **110**: 601–606.

11 Gammon MD, Sagiv SK, Eng SM, Shantakumar S, Gaudet MM, Teitelbaum SL *et al.* (2004). Polycyclic aromatic hydrocarbon-DNA adducts and breast cancer: a pooled analyses. Arch Environ Health. **59**: 640–649.

12 Hertz-Picciotto I, Baker RJ, Yap PS, Dostál M, Joad JP, Lipsett M *et al.* (2007). Early childhood lower respiratory illness and air pollution. Environ Health Perspect. **115**: 1510–1518.

13 Honkova K, Pavlikova J, Rossnerova A, Rossner P, Milcova A, Topinka J *et al.* (2015) Quality of diet of pregnant mothers and its effect on DNA damage in newborns. Proceedings of the 44th Annual Meeting of European Environmental Mutagenesis and Genomics Society, August 23–26, 2015, Prague, Czech Republic, p. 203.

14 Jedrychowski W, Galas A, Pac A, Flak E, Camman D, Raugh V, Perera F (2005). Prenatal ambient air exposure to polycyclic aromatic hydrocarbons and the occurrence of respiratory symptoms over the first year of life. Eur J Epidemiol. **20**: 775–782.

15 Knaapen AM, Gungor N, Schins RP, Borm PJ, van Schooten FJ (2006). Neutrophils and respiratory tract DNA damage and mutagenesis: A review. Mutagenesis. **21**: 225–236.

16 Lipsett J, Tamblyn M, Madigan K, Roberts P, Cool JC, Runciman SI *et al.* (2006). Restricted fetal rowth and lung development: a morphometric analysis of pulmonary structure. Pediatr Pulmonol. **41**: 1138–1145.

17 Martin-Gronert MS, Ozanne SE (2007). Experimental IUGR and later diabetes. J Internal Med. **261**: 437–452.

18 Milani S, Bossi A, Bertino E, Battista ED, Coscia A, Aicardi G *et al.* (2005). Differences in size at birth are determined by differences in growth velocity during early prenatal life. Pediatr Res. **57**: 205–210.

19 Miller RL, Garfinkel R, Horton M, Camman D, Perera FP, Whyatt RM, Kinney PL (2004). Polycyclic aromatic hydrocarbons, environmental tobacco smoke and respiratory symptoms in an inner-city birth cohort. CHEST. **126**: 1071–1078.

20 Nepomnyaschy L, Reichman NE (2006). Low birthweight and asthma among young urban children. Am J Public Health. **96**: 1604–1610.

21 Neufeld L, Pelletier DL, Haas J (1999). The timing hypothesis and body proportionality of the intra-uterine growth retarded infants. Am J Hum Biol. **11**: 638–646.
22 Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DI, Roseboom TJ (2008). Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. BJOG. **115**: 1243–1249.
23 Penning TM, Ohnishi ST, Ohnishi T, Harvey RG (1996). Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. Chem Res Toxicol. **9**: 84–92.
24 Perera F, Tang WY, Whyatt R, Lederman S, Jedrychowski W (2005). DNA damage from polycyclic aromatic hydrocarbons measured by benzo[a]pyrene-DNA adducts in mothers and newborns from Northern Manhattan, the World Trade Center Area, Poland and China. Cancer Epidemiol Biomarkers Prev. **14**: 709–714.
25 Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, Ho SM (2009a). Relation of DNA methylation of 5´-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PloS One. **4**: e4488.
26 Perera FP, Li Z, Whyatt R, Hoepner L, Wang S, Camann D, Rauh V (2009b). Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics. **124**: 195–202.
27 Rossnerova A, Spatova M, Rossner P, Jr., Solansky I, Sram RJ (2009). The impact of air pollution of the levels of micronuclei measured by automated image analysis. Mutat Res. **669**: 42–47.
28 Rossnerova A, Spatova M, Pastorkova A, Tabashidze N, Velemínsky M, Jr., Balascak I, Solansky I, Sram RJ (2011). Micronuclei levels in mothers and their newborns from regions with different types of air pollution. Mutat Res. **715**: 72–78.
29 Rubes J, Rybar P, Prinosilova P, Vezník Z, Chvatalova I, Solansky I, Sram RJ (2010). Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res. 683: 9–15.
30 Smoth GC (2004). First trimester origins of fetal growth impairment. Semin Perinatol. **28**: 41–50.
31 Sørensen M, Autrup H, Møller P, Hertel O, Jensen SS, Vinzents P *et al.* (2003). Linking exposure to environmental pollutants with biological effects. Mutat Res. **544**: 255-271.
32 Sram RJ (ed.) (2001). Teplice Program. Impact of Pollution on Human Health. Prague: Academia.
33 Sram RJ, Benes I, Binkova B, Dejmek J, Horstman D, Kotesovec F *et al.* (1996). Teplice Program – The impact of air pollution on human health. Environ Health Perspect. **104**(Suppl. 4): 699–714.
34 Sram RJ, Binkova B, Dejmek J, Bobak M (2005). Ambient air pollution and pregnancy outcomes: A review of the literature. Environ Health Perspect. **113**: 375–382.
35 Sram RJ, Binkova B, Dejmek J, Chvatalova I, Solansky I, Topinka J (2006). Association of DNA adducts and genetic polymorphisms with birth weight. Mutat Res. **608**: 121–128.
36 Sram RJ, Beskid O, Rossnerova A, Rossner P, Lnenickova, Z, Milcova A *et al.* (2007). Environmental exposure to carcinogenic polycyclic aromatic hydrocarbons – the interpretation of cytogenetic analysis by FISH. Toxicol Lett. **30**: 12–20.
37 Sram RJ, Binkova B, Dostal M, Merkerova-Dostalova M, Libalova H, Milcova A *et al.* (2013). Health impact of air pollution to children. Int J Hyg Environ Health. **216**: 533–540.
38 Topinka J, Binkova B, Mrackova G, Stavkova Z, Peterka V, Benes I *et al.* (1997). Influence of GSTM1 and NAT2 genotypes on placental DNA adducts in an environmentally exposed population. Environ Mol Mutagen. **30**: 184–195.
39 Van Wassenaer A (2005). Neurodevelopmental consequences of being born SGA. Pediatr Endocrinol Reviews. **2**: 373–377.
40 WHO (2007). Health Relevance of Particulate Matter from Various Sources Report on a WHO workshop (Bonn, Germany, March 2007), WHO Regional Office for Europe, Copenhagen.
41 WHO (2011). WHO guidelines for indoor air quality: Selected pollutants. WHO Publ., Bonn 2011, Germany. ISSN 978 92 890 02134, 454 p.
42 Xue W, Warshawsky D (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol. **206**: 73–93.