Neuroendocrinology Letters Volume 43 No. 1 2022
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

A possible link between cognitive development in 5 years old children and prenatal oxidative stress

Barbora Blazkova[1], Antonin Ambroz[2], Alena Milcova[2], Anna Pastorkova[1,2], Pavel Rossner, Jr.[2], Ivo Solansky[1], Milos Veleminsky, Jr.[1,3], Milos Veleminsky[1], Radim J. Sram[1,2]

1 Faculty of Health and Social Sciences, University of South Bohemia, 370 05 Ceske Budejovice, Czech Republic
2 Institute of Experimental Medicine CAS, 142 20 Prague 4, Czech Republic
3 Hospital Ceske Budejovice, a.s., 370 01 Ceske Budejovice, Czech Republic

Correspondence to: Radim J. Sram
Faculty of Health and Social Sciences, University of South Bohemia,
370 05 Ceske Budejovice, Czech Republic
TEL: +420 724 185 002, E-MAIL: radim.sram@iem.cas.cz

Submitted: 2022-03-07 Accepted: 2022-05-02 Published online: 2022-03-10

Key words: **Neuroendocrine neoplasms; inflammation; complete blood count; neutrophils; platelets; lymphocytes; inflammatory index**

Neuroendocrinol Lett 2022; **43**(1):27–38 **PMID:** 35490423 NEL430122A05

Abstract

BACKGROUND: To study the impact of oxidative damage associated with particulate matter< 2.5 μm (PM2.5) during prenatal period on the cognitive development in five years old children.

METHODS: Two cohorts of children aged five years, born in the years 2013 and 2014, were studied for their cognitive development in the polluted district Karvina and the control district Ceske Budejovice. Exposure to PM2.5 in the ambient air was measured for each mother during the 3rd trimester of pregnancy. Oxidative damage was determined from the level of biomarkers at delivery in mothers´ and newborns´ urine as 8-oxo-7,8-dihydro-2´-deoxyguanosine (8-oxodG) and in plasma as 15-F2t-isoprostane levels (15-F2t-IsoP). The Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test) were used as psychological cognitive tests.

RESULTS: Average concentrations of PM2.5 ± SD in the 3rd trimester of mothers´ pregnancies were 37.7 ± 14.7 μg/m^3 and 17.1 ± 4.8 μg/m^3 in Karvina and Ceske Budejovice, respectively ($p < 0.001$). The maternal level of 15-F2t-IsoP in plasma at the time of delivery was significantly associated with the results of the RCPM test ($p < 0.05$) and the BG test ($p < 0.05$) in five years old children.

CONCLUSIONS: Lipid peroxidation in maternal plasma at the time of delivery has an adverse effect on the results of psychological cognitive tests in five years old children.

INTRODUCTION

Oxidative stress results from the imbalance between the formation of reactive oxygen species (ROS) and the ability of the organism to readily detoxify the reactive intermediates as well as to repair the resulting damage (Mazzoli-Rocha *et al.* 2010). The source of ROS can be endogenous (cellular metabolism, inflammation) and exogenous (air pollution and other environmental factors). To maintain the physiological level of ROS in the organism, cells use a wide range of antioxidant mechanisms. However, resulting high levels of ROS, when the antioxidant mechanisms are insufficient, can cause an oxidation of cellular biomolecules: DNA, lipids, proteins. The biomarkers of oxidative damage to biomolecules can predict human health problems (Dizdaroglu, 2015).

ROS (e.g. hydroxyl or superoxide radicals), can interact with all structures of DNA resulting in a variety of DNA modifications such as a single base and sugar-phosphate damage, as well as strand breaks and DNA cross-links. Among DNA bases, guanine is the most susceptible to oxidation. One of the predominant modified bases, 8-oxoguanine (8-oxoGua), is highly mutagenic due to mispairing with adenine. Guanine is oxidized by ROS in the presence of other components of the redox cyclic system (NADPH and copper ions). If 8-oxoGua is not identified and repaired by base excision repair (BER), it would induce GC-TA and AT-GC transversions. This modification is fixed in the subsequent replication cycle, this modification may be the onset of a process of carcinogenesis (Weiss *et al.* 2005). 8-hydroxy-2' -deoxyguanosine (8-oxodG), excreted in urine, is generally perceived as an indicator of the total oxidative DNA damage (Loft & Poulsen 1999).

Cellular or organelle membranes and their polyunsaturated fatty acids (PUFA) are among the first targets of ROS attack. Due to the formation of lipid peroxides which can later decompose into a wide range of products (short-chain alkanes, aldehydes, alkenyls, isoprostanes, etc.), the properties of biological membranes are changed (Ayala *et al.* 2014). 15-F2t-isoprostane (15-F2t-IsoP) is considered to be a reliable biomarker of membrane lipid damage in vivo due to its stability (Roberts & Morrow 2000). It is formed by non-enzymatic oxidation of arachidonic acid abundant in membranes (Morrow *et al.* 1990). 15-F2t-IsoP is subsequently removed from the site of action by phospholipase A2 (PLA2), can be quantified in body fluids: urine or plasma (Morrow *et al.* 1992). Although urine collection is an easily available and non-invasive method, the determination of 15-F2t-IsoP in plasma provides a more accurate view of the overall oxidative damage of the organism (Lee *et al.* 2016).

In the ambient air, the particulate matter (PM) represents a major factor that upon inhalation may induce oxidative stress in the organism. The small size of fine PM (PM of aerodynamic diameter of < 2.5 μm; PM2.5) facilitates its transport through the respiratory tract. The organism defense in the lower respiratory tract against infiltrating PM is mediated by a non-specific immune response involving phagocytic alveolar macrophages. During the elimination of PM by macrophages, proinflammatory and other mediators are released and ROS are formed, thus contributing to oxidative stress in the lungs (Valavanidis *et al.* 2013). It is assumed that proinflammatory mediators released during PM phagocytosis in the pulmonary alveoli may eventually enter the bloodstream and cause systemic inflammation and oxidative stress (Mills *et al.* 2009). The effects of these proinflammatory mediators are also manifested in the central nervous system (CNS) under specific conditions and are associated with neurodegenerative diseases (Block *et al.* 2009). Oxidative damage might be also induced by polycyclic aromatic hydrocarbons (PAHs) metabolized to reactive intermediates which exert their mutagenic and/or carcinogenic effects. PAHs are adsorbed onto PM and significantly contribute to toxicity of the polluted air. The o-quinone pathway of PAHs metabolism leads to redox cycling and the formation of ROS and therefore oxidative damage of cellular macromolecules (Burczynski *et al.* 1998).

The link between ambient air pollution and oxidative stress and their impact on mothers and their newborns has been repeatedly investigated. Ambroz *et al.* (2016) studied the impact of air pollutants on oxidatively damaged DNA and lipid peroxidation in two groups in two localities: the district of Karvina with high air pollution levels and the control district of Ceske Budejovice. Recently, the Karvina region was listed the 3rd most polluted location by PM2.5 for premature mortality in Europe (Khomenko *et al.* 2021). 8-oxodG in urine and 15-F2t-IsoP in plasma were determined as biomarkers of oxidative damage. The results of the multivariate regression analysis showed PM2.5 concentrations to be a significant predictor of 8-oxodG excretion as well as 15-F2t-IsoP levels in newborns from Karvina. The effect of 8-oxodG excretion was observed only in mothers at delivery. These results suggest that concentrations of PM2.5 affect the oxidative damage in newborns in the polluted region.

A recently published review indicates the impact of fine particulate matter on neurological disorders as dementia and cognitive function, stroke, depression, multiple sclerosis, schizophrenia, ADHD and neurodevelopment, implying the effect of air pollution during pregnancy on brain development (Kim *et al.* 2020). Rivas *et al.* (2019) observed an association between early life exposure to air pollution and working memory and attention by studying the effect of PM2.5 exposure during prenatal period to – seven to ten years old children in Barcelona (N = 2 221). PM2.5 concentrations during pregnancy were 16.5 ± 3.0 μg/m^3. In another study in Barcelona, Mortamais *et al.* (2019) analyzed the effect of PM2.5 prenatal exposure on the corpus callosum volume (CC) in eight to twelve years

Tab. 1. Overview of tested confounders

		All		CB		Karvina		Boys		Girls	
		N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
Maternal Characteristics											
Maternal Age	years	168	31.9±4.5	99	32.7±4.4	69	30.8±4.4 *)	78	31.6±4.0	90	32.2±4.9
ETS - pregnancy	cig/day	167	0.06±0.24	99	0.04±0.20	68	0.09±0.29	78	0.06±0.25	89	0.06±0.23
ETS - 1st child year	cig/day	167	0.08±0.27	99	0.03±0.17	68	0.15±0.36 **)	78	0.09±0.29	89	0.07±0.25
ETS - 2nd child year	cig/day	167	0.11±0.32	99	0.06±0.24	68	0.19±0.40 **)	78	0.12±0.32	89	0.11±0.32
Maternal University Education	%	167	37±48	99	34±48	68	41±50	78	42±50	89	33±47
Birth Characteristics											
Vaginal Delivery	%	168	69±46	99	70±46	69	68±47	78	69±46	90	69±47
Gestation Age	weeks	168	39.8±1.8	99	39.5±1.5	69	40.1±2.0 ***)	78	39.7±1.3	90	39.9±2.1
Birth Weight	g	162	3434±439	97	3464±452	65	3389±417	76	3502±439	86	3374±432 ++)
Birth Length	cm	159	49.7±2.1	94	50.0±1.9	65	49.2±2.3 **)	76	50.1±2.1	83	49.3±2.0 ++)
Birth Head Perimeter	cm	158	34.4±1.4	97	34.4±1.5	61	34.4±1.3	74	34.7±1.5	84	34.2±1.4
Apgar 5′		146	9.9±0.5	88	9.8±0.6	58	10.0±0.1 ***)	66	9.8±0.6	80	9.9±0.3
Hyperbilirubinia	%	168	9±29	99	6±24	69	13±34	78	8±27	90	10±30
TBC Primovaccination	%	168	8±27	99	4±20	69	13±34 *)	78	5±22	90	10±30
Children's Diseases											
GIS	%	168	23±42	97	16±37	71	31±47 *)	75	23±42	93	23±42
Viral Diseases	%	168	24±43	97	4±20	71	51±50 **)	75	19±39	93	28±45
Otitis	%	168	10±29	97	13±34	71	4±20	75	12±33	93	8±27
URD	%	168	76±43	97	71±46	71	82±39	75	76±43	93	75±43
Bronchitis	%	168	26±44	97	24±43	71	30±46	75	27±45	93	26±44

Results of Mann Whitney U-test compared by region *) $p < 0.05$, **) $p < 0.01$, ***) $p < 0.001$ and by gender +) $p < 0.05$, ++) $p < 0.01$, +++) $p < 0.001$. ETS - environmental tobacco smoke, GIS - gastrointestinal diseases, URD - upper respiratory diseases

old children. Brain MRI showed that increase of PM2.5 level decreased CC volume which could increase behavioral problems. Johnson *et al.* (2021) reviewed the impact of air pollution on children health and highlight associations between prenatal fine particles exposure and adverse neurodevelopment. Gangwar *et al.* (2020) reviewed the impact of particulate matter pollution on oxidative stress in human studies over the last five years. All these studies show the significance of oxidative stress for PM2.5-associated neurodevelopmental changes.

In several studies, PM2.5-induced oxidative stress was identified as a developmental neurotoxicant (Block & Calderón-Garcidueñas, 2009; Genc *et al.* 2012; Liu & Lewis 2014; Chiu *et al.* 2016; Zhang *et al.* 2018). A potential link between prenatal oxidative stress and behavioral outcomes was assessed by Rommel *et al.* (2020) in four years old children as followed: the level of urinary biomarkers was determined in mothers´ urine in the third trimester of pregnancy. Prostaglandin F2α (PG F2α), 8-iso-prostaglandin F2α (8-iso-PG F2α) and metabolite 2,3-dinor-5,6-dihydro-15-F2t-isoprostane were measured as biomarkers of oxidative stress. Parents completed the Behavior Assessment System for Children (BASC) Parent Rating Scale in order to evaluate behavior of their children (Reynolds 2010). The third trimester oxidative stress measured as 8-iso-PG F2α was associated with social impairments at the age of four years, specifically ASD (autism spectrum disorders). Inflammation-related response, measured as PG F2α was associated with ADHD (attention deficit hyperactivity disorder) symptoms. These associations were pronounced in children of more educated mothers. Rommel's *et al.* (2020) paper is the first one indicating the association between oxidative stress during prenatal period and neurobehavioral development in children. Until now, the impact of air pollution on cognitive development has been considered to be related to exposure to PAHs (Jedrychowski *et al.* 2003; Perera *et al.* 2003; Perera *et al.* 2006; Perera *et al.* 2009; Edwards *et al.* 2010; Perera *et al.* 2012; Jedrychowski *et al.* 2015; Peterson *et al.* 2015).

Recently, we have analyzed the impact of PAHs in ambient air at the time of delivery on the cognitive development in five years old children. Exposure to PAHs was evaluated according to the concentration of benzo[a]pyrene (B[a]P) in ambient air during the last trimester and OH-PAH (hydroxy-PAH) metabolites in the urine of newborns at the time of delivery. Similarly to Ambroz *et al.* (2016), we studied two cohorts of children from Karvina and Ceske Budejovice. We did not observe any significant effect of prenatal PAH exposure on results of psychological cognitive tests in five years old children. However, lower scores in cognitive tests results were associated with the higher exposure to PM2.5 during the third trimester in girls in Karvina (Blazkova *et al.* 2020 a).

Results of the Rommel *et al.* (2020) have inspired us to analyze possible relationship between oxidative damage at the time of delivery and cognitive functions in children later in their development.

We assessed in two cohorts from polluted and control districts oxidative stress in mothers and newborns in urine and plasma at the time of delivery (Ambroz *et al.* 2016). The average concentration of PM2.5 in Karvina was 38.1±27.3 μg/m^3, in Ceske Budejovice 18.3±14.7 μg/m^3. For carcinogenic benzo[a]pyrene (B[a]P), the values were 4.5±1.9 ng/m^3 and 1.5±0.6 ng/m^3, respectively (CHMI 2013). The levels of 8-oxodG (oxidatively damaged DNA) and 15-F2t-IsoP (lipid peroxidation) were determined as biomarkers of oxidative stress and correlated with mothers' PM2.5 exposure levels in the third trimester. We used these results to study a possible association between oxidative stress during prenatal development and cognitive development of five years old children.

MATERIALS AND METHODS

Subjects

The cohorts were formed in summer 2013 and winter 2014 from mothers and their newborns born in the Ceske Budejovice Hospital, Department of Obstetrics and Gynecology and Department of Neonatology and in the Karvina Hospital, Department of Obstetrics and Gynecology and Department of Neonatology. The samples were collected from normal deliveries (week 38-41) of nonsmoking mothers and their newborns in the summer and winter season to account for monitored seasonal differences in air pollution. When the mothers were invited to take part in the study, they signed the informed consent. The samples included venous blood and urine from 99 mothers (summer) and 100 mothers (winter) obtained in Ceske Budejovice, a locality with relatively clean air, and 70 mothers (summer) and 73 mothers (winter) from Karvina, a locality with high air pollution; and cord blood and urine from 99 newborns (summer) and 100 newborns (winter) collected in Ceske Budejovice and 71 newborns (summer) and 74 newborns (winter) from Karvina. Venous blood and urine from mothers were collected during the time of delivery. For newborns, cord blood was obtained and urine was collected during the first or second day after delivery. Blood was drawn to EDTA tubes to isolate DNA and plasma. Urine samples were collected into 50 mL tubes (Greiner Bio-one) and stored at -20°C until transported to the Institute of Experimental Medicine. Aliquots (1-2 mL) of urine were frozen at - 80°C until analysis. The study was approved by the Ethics Committees of both hospitals and the Institute of Experimental Medicine CAS in Prague (Ambroz *et al.* 2016).

Between November 2018 and November 2019, 199 mothers from Ceske Budejovice district and 143 from Karvina district who provided samples from their children in 2013 and 2014, were approached to take part in the psychological cognitive testing of their children. Undertaking the psychological test was optional. Out of the total amount of 342 potential subjects, 140 refused to participate, and 31 were impossible to contact. In the present study, data from 99 children from Ceske Budejovice and 70 children from Karvina were collected. The final sample therefore included 169 children (1 set of twins). The confounders of oxidative stress markers in the groups studied are shown in Table 1. This study was approved by the Ethics Committee of the Faculty of Health and Social Sciences, University of South Bohemia, Ceske Budejovice.

Air sampling of PM2.5

PM2.5 was collected by a High Volume (HiVol) 3000 Air Sampler (model ECO-HVS3000, Ecotech, Australia) on Pallflex membrane filters (EMFAB, TX40HI20-WW) in both study locations (Topinka *et al.* 2011). The detailed information regarding the air sampling is described in Topinka *et al.* (2011). In order to estimate PM2.5 exposure for each mother during the last trimester, filters were collected every 3rd day during the urine sampling, i.e. from August 2018 to November 2019. Concentrations of PM2.5 were expressed in μg/m^3.

Analysis of oxidative stress

8-oxodG ELISA

Solid phase extraction (SPE) of urine samples was performed as described by Rossner *et al.* (2013). Levels of 8-oxodG were analyzed by Highly Sensitive 8-OhdG Check ELISA kit (JaICA, Shizuoka, Japan). ELISA was conducted according to the manufacturer's instructions with some modifications: samples were incubated with the primary antibody at 4°C overnight, each sample was analyzed in duplicate using a 50 μL sample/well. 8-oxodG concentration was expressed in nmol 8-oxodG/mmol creatinine. Creatinine levels were measured in urine samples by the reaction with picric acid - the Jaffe method (Jaffe, 1886; Delanghe & Speeckaert, 2011). The samples were analyzed in triplicate, 50 μL sample/well and creatinine concentrations

were expressed in mmol/L (Ambroz *et al.* 2016). A repeated sample was analyzed in every experiment to assure quality control and reproducibility. An inter-assay variability did not exceed 10%.

15-F2t-isoprostane immunoassay

Blood plasma 15-F2t-IsoP were analyzed using immunoassay kits from Cayman Chemical Company (Ann Arbor, MI, USA) according to the manufacturer's protocol. Each sample was analyzed in duplicate (Rossner *et al.* 2008). The 15-F2t-IsoP concentrations were expressed as pg 15-F2t-IsoP/ml plasma (Ambroz *et al.* 2016). A repeated sample was analyzed in every experiment to assure quality control and reproducibility. An inter-assay variability did not exceed 10%.

Cotinine level

Plasma cotinine levels as a marker of active and passive smoking were analyzed by radioimmunoassay (Langone & Van Vunakis, 1982) to check the tobacco smoke exposure reported in the lifestyle of mothers´ questionnaires (Ambroz *et al.* 2016). Concentration of cotinine was expressed in ng/ml. Cotinine level > 14 ng/ml indicated active smoking, while cotinine levels between 1 and 14 ng/ml described passive smoking (environmental tobacco smoke, ETS) (Bearer *et al.* 1997; Pichini *et al.* 2000). A repeated sample was analyzed in every experiment to assure quality control and reproducibility. An inter-assay variability did not exceed 5%.

Measures of child visual-motor functioning and intellect

To examine the potential effect of oxidative damage on cognitive development in five years old children, two psychological assessment instruments were used, namely the Bender Visual Motor Gestalt test (BG test) and Raven Colored Progressive Matrices test (RCPM test). These standardized methods have been broadly used by child psychologists for decades and have been found to be well perceived by five years old children. Moreover, compared to other potentially suitable assessment tools, the BG test and the RCPM test were manageable in one session. These non-verbal, culture-free tests can be used for both healthy five years old children and those with some kind of physical or mental impairment.

Each child from the cohort was tested individually by a child psychologist. The psychologist did not know the results of the analysis of the oxidative damage in children urine at the time of testing. The BG test was used to measure the extent of visual-motor functioning of each child from the cohort. This method has been widely applied to assess the level of motor functioning and visual perception (Bender & Strnadova, 1974). The children were presented with various geometric shapes drawn on nine cards with increasing level of difficulty. The task was to draw the best copy possible on a blank sheet of paper. The reproductions were scored based on the accuracy and organization according to the BG test manual (Bender & Strnadova, 1974). 169 children from our cohort completed the test.

Right after the completion of the BG test (which on average took about 5-10 minutes), the related RCPM test followed. The test has been widely used worldwide to assess reasoning and problem-solving ability in children, elderly, and mentally or physically impaired people. This intelligence test consists of 36 tasks with increasing level of difficulty. Each task is a matrix with eight patterns and one missing of the total of nine. The missing piece should be determined from multiple answer options. Children were asked to choose the missing element from six options in a drawing. One point was given for each correct answer. The total

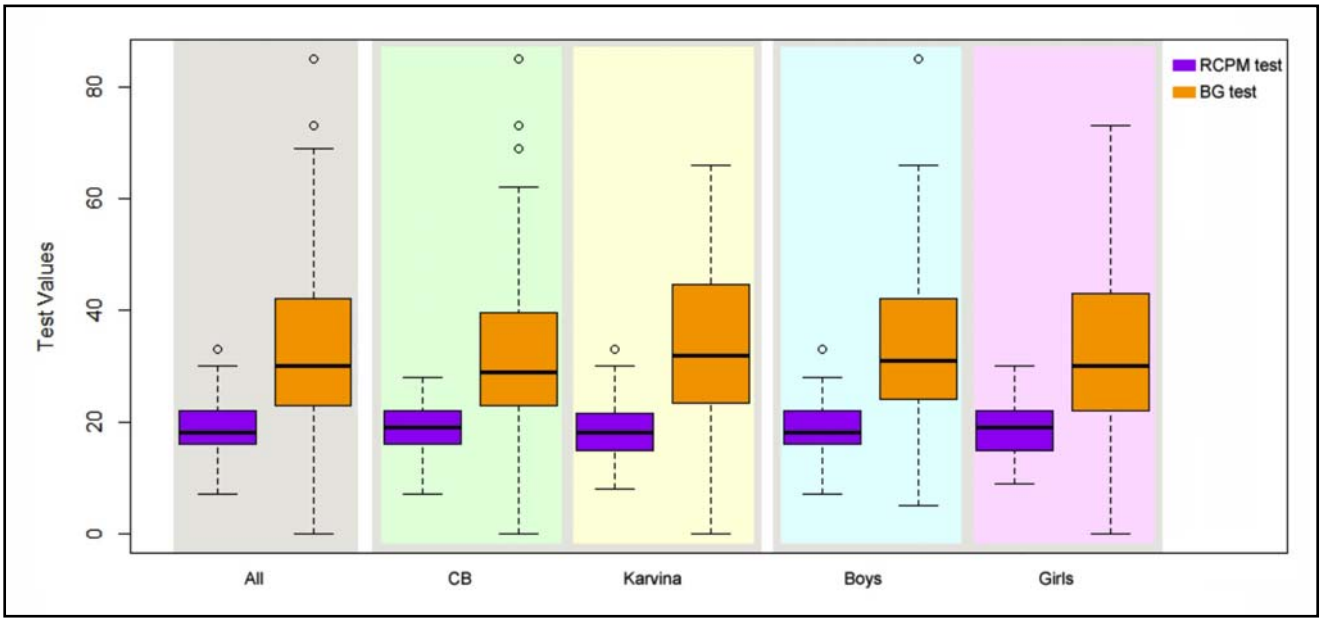

Fig. 1. Results of Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test)

score represents the total number of matrices that were completed correctly, the range of possible scores is between 0 to 36 (Raven *et al.* 1990). Since one of the children tested was unable to understand the RCPM test logic based on standardized instructions presented and one was unable to finish the whole test, out of 169 tested children 167 children at the age of five years completed the test.

Both the RCPM test and the BG test provide single total score. In general population the results are distributed normally (Smirni, 2020). In both psychological assessment tools used the higher score means better result.

Questionnaire for mothers

Mothers involved in the study provided information about the social environment of the family, breast-feeding and eating habits, and child´s medical history. Similarly, the data regarding gestational age, birth weight, birth length, head circumference, and Apgar score were collected in order to be taken into account when analyzing the psychological tests results.

Statistical analysis

Two statistical methods for the evaluation of differences between the cohorts were used.

(1) Mann-Whitney U-test (Wilcoxon rank-sum test) was applied to a direct comparison of the RCPM test and the BG test results, PM2.5 values, and oxidative damage values between cohorts.
(2) Logistic regression was used to estimate the bivariate and multivariate impacts of oxidative damage and other confounders on the scores of the RCPM test and the BG test as dependent variables. In order to convert scores of the test values, of PM2.5 concentrations and oxidative damage values into binary scale, the values were divided by medians of appropriate group distribution.

8-oxodG concentration in urine and 15-F2t-IsoP in plasma showed the probability which children would achieve the RCPM test and the BG test scores above the median in their cohorts in association with the oxidative damage from environmental pollution as calculated odds ratios (OddRs), where OddR > 1 expresses positive and OddR < 1 negative associations (Szumilas, 2010). Multiple parameters from health and social status of mothers as mostly related to maternal questionnaires such as: maternal age, maternal ETS (passive smoking), various maternal health status parameters, children birth parameters and birth procedures, and child illnesses in period from birth to two years of age were checked as potential confounders. Additional test has been calculated from parameters related to moth-er's pregnancies such as following: medically confirmed risk pregnancy, long term sick leave and illness during pregnancy, mother's permanent medications, maternal work and employment risk factor during pregnancy, medical risk factor and birth complication. Maternal alcohol consumption during pregnancy according to the information obtained by the questionnaire has been also considered, but no strongly significant relations were found. The impact of the type of delivery and the mothers´ education were studied separately (Blazkova *et al.* 2020b).

RESULTS

The tested confounders are shown in Table 1. The district of Karvina differed from the district Ceske Budejovice as follows: mothers from Karvina were younger ($p < 0.05$), ETS (environmental tobacco smoke) exposure was higher during the first and second child year ($p < 0.01$), gestation age was longer ($p < 0.001$), children were shorter ($p < 0.01$), Apgar 5´ was higher ($p < 0.001$), and gastrointestinal and viral diseases in children were more frequent ($p < 0.01$). Birth weight and birth length was lower in girls vs. boys ($p < 0.01$) in both cohorts.

The results of psychological cognitive tests are presented in Figure 1. Neither results of the RCPM test nor the BG test differed in children in Karvina vs. Ceske Budejovice, or boys vs. girls.

Concentration of PM2.5 was significantly higher in Karvina when compared with Ceske Budejovice ($p < 0.001$) (Figure 2). When estimating the impact of PM2.5 concentrations during the third trimester on psychological test scores in children using logistic regression, no significant effect was observed in the RCPM test, while the BG test scores showed decreased cognitive functions in girls in Karvina (OddR = 0.25, $p < 0.05$) (Figure 3) and also for Karviná and all indicates not significant decreasing of BG cognitive test results for upper levels of PM 2.5.

Mean and median levels of oxidative stress markers and cotinine in mothers´ and children´s urine or plasma after delivery are presented in Table 2. There were no differences in levels of 8-oxodG in urine and 15-F2t-IsoP in the plasma of mothers in either district. Cotinine levels were higher in the plasma of mothers from Karvina ($p < 0.001$). The concentration of 8-oxodG in urine of newborns was higher in girls ($p < 0.01$) in both districts, while 15-F2t-IsoP concentration in plasma was higher in newborns from Ceske Budejovice ($p < 0.001$). Comparing the level of oxidative stress markers between mothers and children, they were significantly higher in children.

The associations between oxidative stress markers and psychological test scores are reported in Table 3. The RCPM test results above-median values were significantly lower in children born to mothers with above-median levels of 15-F2t-IsoP in plasma for all (OddR = 0.45, $p < 0.01$) and for boys (OddR = 0.30, $p < 0.01$). Surprisingly, the results of the RCPM test were positively associated with 8-oxodG levels in urine of girls

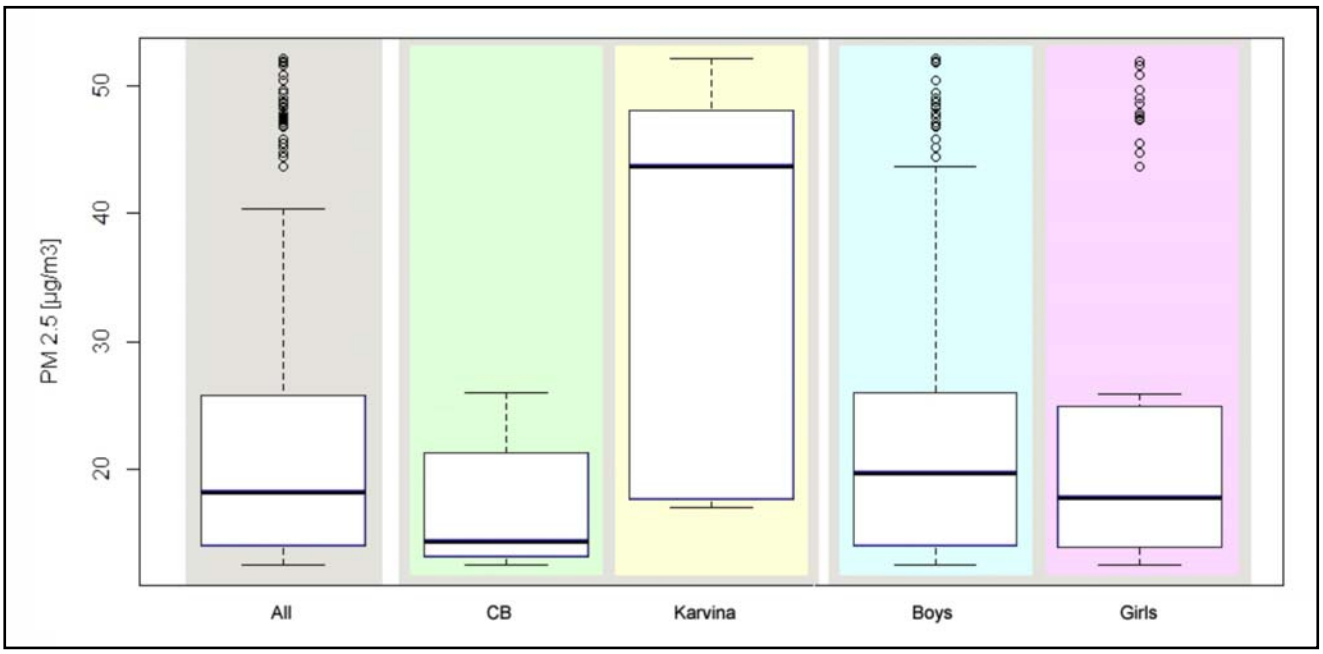

Fig. 2. Concentration of PM2.5 in 3rd trimester of mother's pregnancies, delivery in years 2013-2014

(OddR = 2.69, $p < 0.05$). The results of the BG test were negatively impacted by the maternal above-median levels of 15-F2t-IsoP in plasma (OddR = 0.54, $p < 0.05$). Models have been adjusted for maternal education, delivery type, and cotinine level. Impact of delivery type and education was described in (Daniel, 1990). Oxidative damage is correlated with values of PM 2.5 during 3rd trimester.

Results of BG test were significantly affected by children delivery season, better results were observed in children born in summer against those born in winter in both cohorts (OddR = 3.11, $p < 0.01$), in Karvina (OddR = 4.81, $p < 0.05$) and in girls (OddR = 4.38, 0.01). A June-August period has been considered summer season, December-February has been considered winter season (Figure 4).

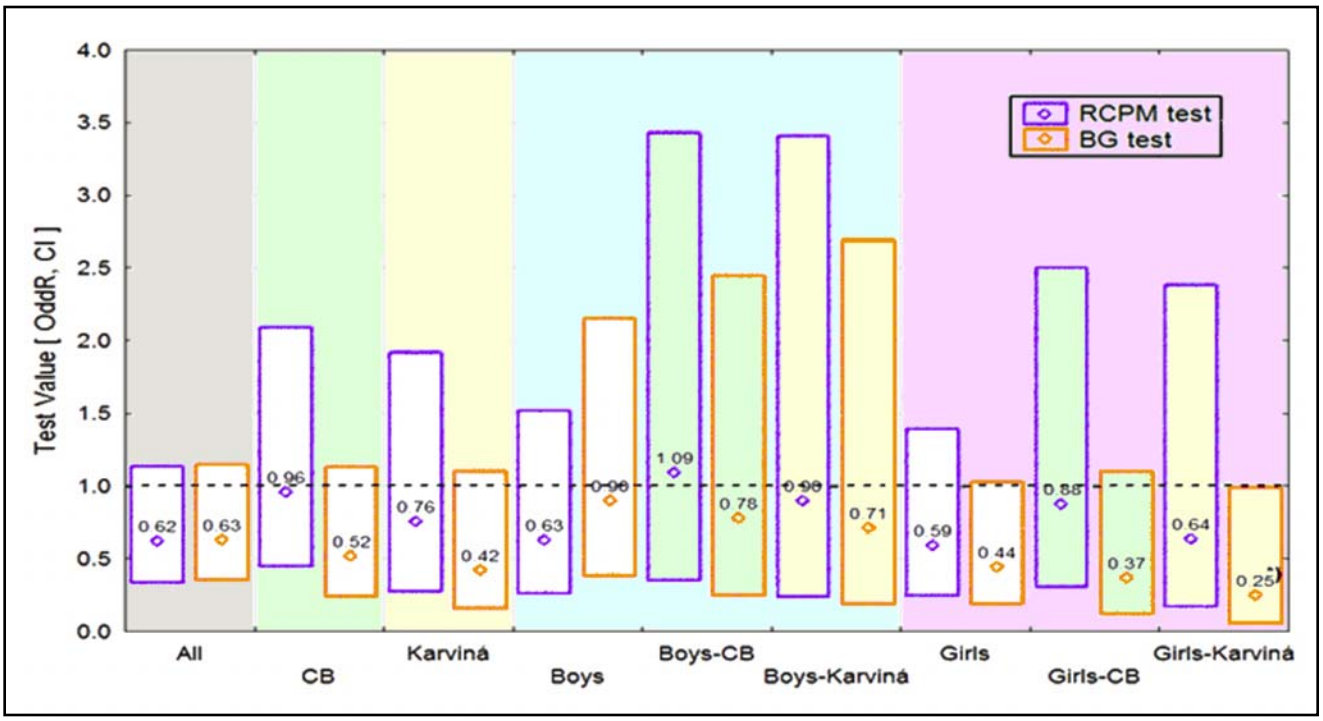

Fig. 3. Estimated impact of PM2.5 in 3rd trimester of mother's pregnancies to The Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test) testing values.

Tab. 2. Concentration of oxidative stress markers and cotinine in samples collected from mothers´ and children´s urine and plasma at delivery in 2013-2014

			All		CB		Karvina		Boys		Girls	
			N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
Maternal	8-oxodG	[nmol/ mmol creatinine]	168	1.75±0.84	98	1.75±0.91	70	1.74±0.73	77	1.90±0.99 +)	91	1.62±0.66 +)
	15-F2t-IsoP	[pg/ml plasma]	169	61.00±25.96	99	61.69±28.05	70	60.02±22.83	77	63.70±27.80	92	58.73±24.23
	Cotinine	[ng/ml]	169	2.29±14.78	99	0.14±0.28	70	5.33±22.71	77	1.19±7.84	92	3.22±18.70
Child	8-oxodG	[nmol/ mmol creatinine]	162	4.82±1.99	98	4.67±1.51	64	5.05±2.56	75	4.41±1.61 ++)	87	5.18 ±2.22 ++)
	15-F2t-IsoP	[pg/ml plasma]	168	93.26 ±35.57	98	99.69 ±32.74***)	70	84.26 ±37.60***)	76	91.34 ±35.74	92	94.85 ±35.54
			N	Median (5-95%)	N	Median (5-95%)	N	Median (5-95%)	N	Median (5-95%)	N	Median (5-95%)
Maternal	8-oxodG	[nmol/ mmol creatinine]	168	1.49 (0.96-3.15)	98	1.48 (0.91-3.25)	70	1.58 (1.00-3.06)	77	1.56 (0.96-3.47)	91	1.48 (0.89-2.72)
	15-F2t-IsoP	[pg/ml plasma]	169	57.4 (26.6-112.8)	99	56.7 (25.1-115.8)	70	57.7 (30.9-106-8)	77	60.9 (26.6-116.6)	92	55.9 (23.6-105.0)
	Cotinine	[ng/ml]	169	0.09 (0.05-2.78)	99	0.10 (0.05-0.37)	70	0.10 (0.04-28.0)	77	0.11 (0.05-0.44)	92	0.09 (0.05-28.0)
Child	8-oxodG	[nmol/ mmol creatinine]	162	4.35 (2.56-8.02)	98	4.35 (2.62-7.33)	64	4.32 (2.51-11.09)	75	4.02 (2.47-7.47)	87	4.74 (2.76-8.95)
	15-F2t-IsoP	[pg/ml plasma]	168	90.4 (42.0-150.3)	98	96.3 (54.5-152.0)	70	84.6 (38.0-148.6)	76	87.3 (41.6-150.0)	92	91.0 (42.0-157.1)

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

DISCUSSION

We aimed to study the impact of PM2.5 exposure on oxidative stress induction and its relation to cognitive development in five years old children. We did not observe any significant effect of PM2.5 concentrations in the third trimester on the RCPM test results in any group, but we found worse cognitive performance of girls from Karvina as measured by the BG test. Increased levels of 8-oxodG in girls were associated with the results of the RCPM test. Air pollution during the third trimester increased lipid peroxidation in children (Ambroz *et al.* 2016). We thus may assume that oxidative damage-induced changes were more pronounced in girls. Our results correspond to previously mentioned reviews, indicating the impact of oxidative damage by PM2.5 during prenatal period on the child neurodevelopment.

Our results indicate that cognitive functions in children measured by the psychological cognitive tests were associated with the levels of 15-F2t-IsoP, the marker of lipid peroxidation in maternal plasma. In contrast, no association of cognitive functions with oxidatively damaged DNA, determined as 8-oxodG in mothers´ urine, was observed. Our data are comparable with the results gathered by Rommel *et al.* (2020) who also measured 8-iso-prostaglandin F2α. As recently shown, the detected increase in oxidative damage, probably due to air pollution (PM2.5), might have a negative impact on cognitive development in children. Corona (2020) reviewed the role of oxidative stress in ADHD (attention-deficit/hyperactivity disorder). Bjorklund *et al.* (2020) postulated the significant role of oxidative stress in autism spectrum disorder.

The results of the RCPM test were inversely related to the cotinine level in mothers´ plasma. As we selected non-smoking mothers, it was probably the effect of passive smoking. That would correspond with a previous idea that maternal smoking during pregnancy was associated with neurotoxic effects on children (Weitzman *et al.* 2002). Increased level of cotinine corresponds to the increased level of nanoparticles (<0.1 μm) in tobacco smoke which also increases the oxidative stress.

The outcome of the RCPM test was significantly associated with maternal education. Mothers´ university degree substantially improved the results of the psychological tests used, probably due to hereditary

Tab. 3. Estimated impact of oxidative damage in the mother´s and children´s urine and plasma on psychological testing scores

			All	CB	Karvina	Boys	Girls
			OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)	OddR (95% CI)
RCPM test	Maternal	8-oxodG	0.72 (0.38-1.35)	0.85 (0.39-1.87)	0.63 (0.21-1.87)	0.89 (0.34-2.31)	0.64 (0.27-1.54)
		15-F2t-IsoP	0.45 (0.24-0.86) **)	0.63 (0.28-1.41)	0.59 (0.20-1.69)	0.30 (0.10-0.84) **)	0.59 (0.25-1.37)
		cotinine	0.55 (0.29-1.04)	0.79 (0.35-1.80)	0.49 (0.17-1.42)	0.79 (0.29-2.10)	0.43 (0.18-1.04)
	Child	8-oxodG	1.52 (0.80-2.86)	1.78 (0.79-3.98)	1.85 (0.63-5.44)	0.74 (0.28-1.98)	2.69 (1.13-6.40) **)
		15-F2t-IsoP	0.61 (0.32-1.16)	0.72 (0.32-1.63)	0.64 (0.22-1.87)	0.63 (0.24-1.66)	0.57 (0.24-1.35)
BG test	Maternal	8-oxodG	1.39 (0.76-2.54)	1.46 (0.66-3.22)	0.63 (0.23-1.70)	1.31 (0.53-3.26)	1.56 (0.68-3.61)
		15-F2t-IsoP	0.54 (0.30-1.00) **)	0.59 (0.27-1.32)	0.50 (0.19-1.34)	0.54 (0.21-1.37)	0.50 (0.22-1.15)
		cotinine	1.17 (0.63-2.15)	1.72 (0.74-3.98)	0.65 (0.24-1.72)	1.07 (0.42-2.72)	1.31 (0.56-3.05)
	Child	8-oxodG	1.44 (0.79-2.64)	1.61 (0.72-3.61)	1.93 (0.71-5.19)	1.52 (0.59-3.96)	1.56 (0.69-3.55)
		15-F2t-IsoP	0.72 (0.39-1.31)	0.77 (0.34-1.74)	0.85 (0.32-2.24)	0.55 (0.22-1.39)	0.85 (0.38-1.91)

Logistic Regression results *) $p < 0.05$, **) $p < 0.01$, adjusted for maternal education and delivery type

factors and possibly higher willingness of mothers to devote resources to enhance the cognitive development of their child. However, this effect was not observed in the BG test results. Due to a limited number of children in our cohorts, the results can only be generalized with caution.

The results of the RCPM test were also significantly affected by the type of delivery: lower level of oxidative damage was observed in children born vaginally. The impact of Cesarean and vaginal delivery on results of psychological tests was already described by Blazkova *et al.* (2020 b).

Our study has some limitations that need to be mentioned. As this investigation is only the second one indicating the impact of oxidative stress induced by air polluted by PM2.5 on cognitive functions in children, the results should be interpreted with caution. It is necessary to confirm the observed effects also in other regions and population groups, preferably on a larger research sample. In addition, as oxidative stress is affected by the level of antioxidants in diet, the diet of mothers as well as their blood levels of vitamins A, C, D, and E should be studied and the results should be adjusted to these parameters.

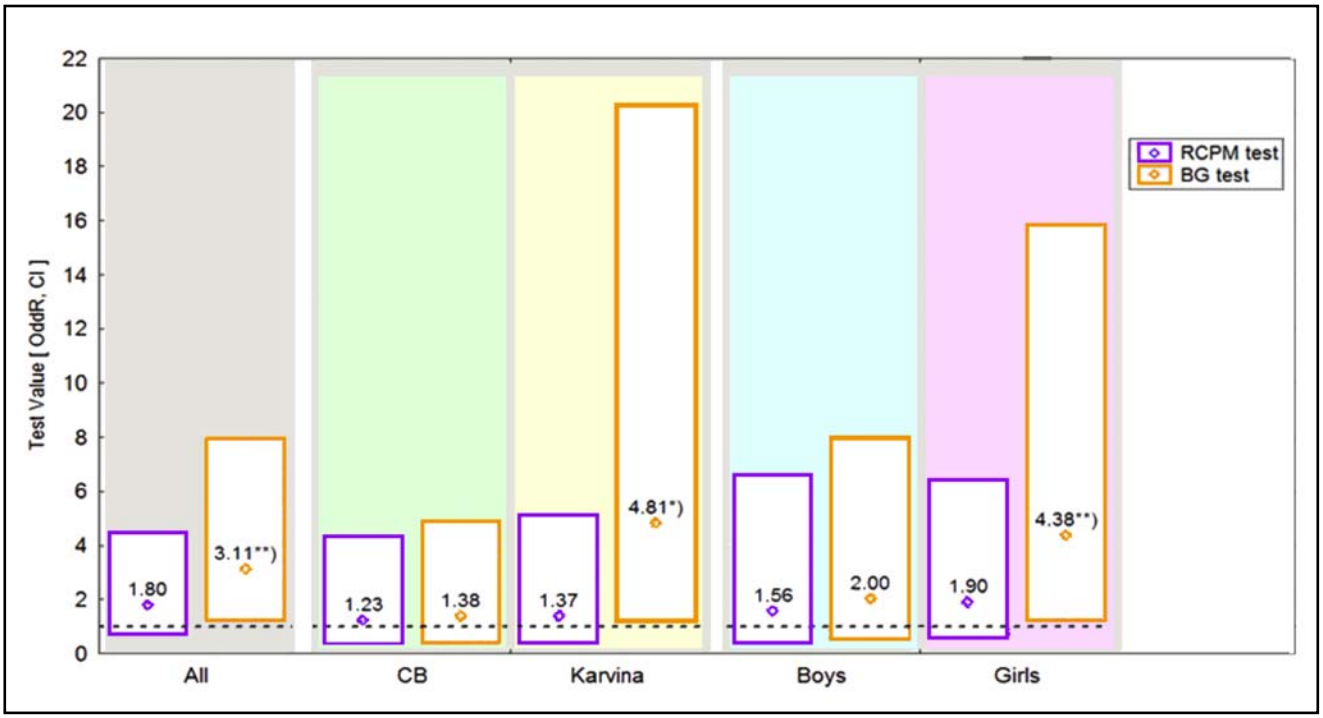

Fig. 4. Estimated impact of seasonality of children´s delivery on The Bender Visual Motor Gestalt Test (BG test) and the Raven Colored Progressive Matrices (RCPM test) testing scores.

Since the study took five years starting with the first contact with mothers of newborns ending with contacting them to take part in psychological assessment of their five years old children, we were only able to analyze 169 cases. 140 mothers refused to take part in the psychological examination and 31 were impossible to contact. The reasons for not being able to contact were either moving to another district or changing the phone number. To check whether there could be any possible systematic bias between the group of those who refused to continue participating in the study and those who agreed to get their children assessed, we analyzed potential differences in multiple confounders between mothers and children included into this study and those who refused or had been elusive. Except for a small but significant difference in maternal age we did not find any substantial difference between groups. It is also possible that there might had been mothers afraid that their children would be unable to perform well at psychological assessment and refused to take part in the assessment.

In any case, a larger group of participants shall be tested in the future to get more representative numbers.

CONCLUSIONS

In this study, we observed for the first time that oxidative damage, seen as increased lipid peroxidation in maternal plasma, can correlate negatively with psychological cognitive test results of five years old children. We may hypothesize that oxidative damage induced by air pollution by PM2.5 during the prenatal development observed by Ambroz *et al.* (2016), may be deleterious for the child brain development. This effect on children´s cognitive functions is more noticeable as their development continues. These innovative findings show the importance of reducing air pollution in order to protect the children´s neurodevelopment. However, further research conducted on a larger cohort will be needed to support the hypothesis mentioned above. The impact of oxidative stress on neurobehavioral development in children seems to be a new significant topic which should be studied in a complex way in the future: new methods related to epigenetics as DNA methylation and other biomarkers like telomere length, mitochondrial DNA content (mtDNA), and inflammation markers might be used (Desai *et al.* 2017).

AUTHOR CONTRIBUTIONS

RJS and BB contributed to the concept and design of the study, BB carried out the psychological tests, AP organized contacts with pediatricians and mothers, AA and PR analyzed oxidative damage in urine and plasma, AM determined the concentration of cotinine, IS performed the statistical analysis, RJS and MV, Jr., organized sampling of biological materials and databases processing related to newborns and their mothers, RJS, BB, MV, Jr., and MV wrote sections of the manuscript. All authors wrote the first draft of the manuscript and approved the submitted version.

FUNDING

This work was supported by Ministry of Health of the Czech Republic, grant nr. NV18-09-00151. and by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02 .1.01/0.0/0.0/16_019/0000798).

ACKNOWLEDGEMENTS

We would like to thank all pediatricians, nurses and parents in both districts for the close cooperation, which made our study possible, and Mrs. Kamila Kavka for language editing.

ETHICAL APPROVAL

The study was conducted according to guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Faculty of Health and Social Sciences, University of South Bohemia in Ceske Budejovice, Czech Republic, in June 30, 2017.

CONSENT TO PARTICIPATE

Informed consent was obtained from the parents of all subjects involved in the study.

CONSENT TO PUBLISH

All authors approved this text.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1 Ambroz A, Vlkova V, Rossner P, Rossnerova A, Svecova V, Milcova A et al. (2016) Impact of air pollution on oxidative DNA damage and lipid peroxidation in mothers and their newborns. Int J Hyg Environ Health. **219**: 545–556; doi: 10.1016/j.ijheh.2016.05.010.

2 Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 360438.

3 Bearer C, Emerson RK, O'Riordan MA, Raitman E, Shackleton C (1997) Maternal tobacco exposure and persistent pulmonary hypertension of the newborn. Environ Health Perspect. **105**: 202–206.

4 Bender L, Strnadova M (1974.) Bender-Gestalt Test: příručka pro administraci, interpretaci a vyhodnocení testu. Psychodiagnostické a didaktické testy: Bratislava, Czechoslovakia (In Czech).
5 Bjorklund G, Meguid NA, El-Bana MA, Tinkov AA, Saad K, Dadar M et al. (2020) Oxidative stress in autism spectrum disorder. Mol Neurobiol. **57**: 2314–2332; doi: 10.1007/s12035-019-01742-2.
6 Blazkova B, Pastorkova A, Solansky I, Veleminsky J, Jr., Veleminsky M, Urbancova K et al. (2020 a) Effect of polycyclic aromatic hydrocarbons exposure on cognitive development in 5 year old children. Brain Sci. **10**: 619; doi: 10.3390/brainsci10090619.
7 Blazkova B, Pastorkova A, Solansky I, Veleminsky M, Jr., Veleminsky M, Rossnerova A et al. (2020 b) The impact of cesarean delivery and vaginal delivery on results of psychological cognitive test in 5 years old children. Medicina. **56**: 554; doi: 10.3390/medicina56100554.
8 Block ML, Calderón-Garcidueñas L (2009). Air pollution: mechanisms of neuroinflammation and CNS disease. Trends Neurosci. **32**(9): 506–516.
9 Burczynski ME, Harvey RG, Penning TM (1998) Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: oxidation of trans-7, 8-dihydroxy-7,8-dihydrobenzo[a]pyrene to the activated o-quinone metabolite benzo[a]pyrene-7,8-dione, Biochemistry. **37**: 6781–6790; doi: 10.1021/bi972725u.
10 Chiu Y-HM, Hsu H-HL, Coull BA, Bellinger DC, Klog I, Schwartz J et al. (2016) Prenatal particulate air pollution and neurodevelopment in urban children: examining sensitive windows and sex-specific associations. Environ Int. **87**: 56–65; doi: 10.1016/j. en vint.2015.11.010.
11 Corona JC (2020) Role of oxidative stress and neuroinflammation in attention-deficit/hyperactivity disorder. Antioxidants. **9**: 1039; doi: 10.3390/antiox9111039.
12 CHMI (Czech Hydrometeorological Institute) (2013) Concentrations of PM2.5 and B[a]P in Karvina and Ceske Budejovice. https://www.chmi.cz/files/portal/docs/uoco/isko/tab_roc/2013_enh/pollution_my/CZCB.html.
13 Daniel WW (1990) Spearman rank correlation coefficient. Applied Nonparametric Statistics. Boston: PWS-Kent. pp. 358–365. ISBN 978-0-534-91976-4.
14 Delanghe JR, Speeckaert MM (2011) Creatinine determination according to Jaffe—what does it stand for? NDT Plus. **4**: 83–86; doi: 10.1093/ndtplus/sfq211.
15 Desai G, Chu L, Guo Y, Myneni AA, Mu L (2017) Biomarkers used in studying air pollution during pregnancy and perinatal outcomes: a review. Biomarkers. **22**(6): 489–501; doi: 10.1080/1354750X.2017.1339294.
16 Dizdaroglu M (2015) Oxidatively induced DNA damage and its repair in cancer. Mutation Res Rev. **763**: 212–245.
17 Edwards SC, Jedrychowski W, Butcher M, Camann D, Kieltyka A, Mroz E et al. (2010). Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children intelligence at 5 years of age in a prospective cohort study in Poland. Environ Health Perspect. **118**: 1326–1331; doi:10.1289/ehp.0901070.
18 Gangwar RS, Bevan GH, Palanivel R, Das L, Rajagolopan S (2020) Oxidative stress pathways of air pollution mediated Toxicity: Recent insights. Redox Biol. **34**: 101545; doi: 10.1016/j. redox.2020.101545.
19 Genc S, Zadeoglulari Z, Fuss SS, Genc K (2012) The adverse effects of air pollution on the nervous system. J Toxicol. **2012**: 782462; doi: 10.1155/2012/782462.
20 Jaffe M (1886) Ueber den Niederschlag, welchen Pikrinsäure im normalen Harn erzeugt, und über eine neue Reaction des Kreatinins. Zeitschrift für Physiologische Chemie (In German). **10**: 391–400.
21 Jedrychowski W, Whyatt RM, Camman DE, Bawle UV, Peki K, Spengler JD et al. (2003) Effect of prenatal PAH exposure on birth outcomes and neurocognitive development in a cohort of newborns in Poland. Study design and preliminary ambient data. Int J Occup Med Environ Health. **16**: 21–29; PMID: 12705714.
22 Jedrychowski W, Perera FP, Camann D, Spengler J, Butscher M, Mroz et al. (2015) Prenatal exposure to polycyclic aromatic hydrocarbons and cognitive dysfunction in children. Environ Sci Pollut Res. **22**: 3631–3639; doi: 10.1007/s11356-014-3627-8.
23 Johnson NM, Rodrigues Hoffmann A, Behlen JC, Lau C, Pendleton D, Harvey N et al. (2021). Air pollution and children Health – a review of adverse effects associated with prenatal exposure from fine to ultrafine particulate matter. Environ Health Prev Med. **26**: 72; doi: 10.1186/s12199-021-00995-5.
24 Khomenko S, Cirach M, Pereira-Bardoza E, Mueller N, Barrera-Gomez J, Rojas-Rueda D et al. (2021) Premature mortality due to air pollution in European cities: a health impact assessment. Lancet Planet Health. **19**: S2542–S196(20)30272-2. doi: 10.1016/S2542-5196(20)30272-2.
25 Kim H, Kim W-H, Kim Y-Y, Park H-Y (2020) Air pollution and central nervous system disease: A review of the impact of fine particulate matter on neurological disorders. Front Public Health. **8**: 575330; doi: 10.3389/pubh.2020.575330.
26 Langone JJ, Van Vunakis H (1982) Radioimmunoassay of Nicotine, Cotinine, and Gamma-(3-Pyridyl)- Gamma-oxo-N-Methylbutyramide. Methods Enzymol. **84**: 628–640. http://dx.doi.org/10.1016/0076-6879(82)84050-0.
27 Lee YY, Galano JM, Oger C, Vigor C, Guillaume R, Roy J et al. (2016) Assessment of isoprostanes in human plasma: technical considerations and the use of mass spectrometry. Lipids. **51**(11): 1217–1229.
28 Liu J, Lewis G (2014) Environmental toxicity and poor cognitive outcomes in children and adults. J Environ Health. **76**: 130–138; doi: 10.11648/j.cmr.20140305.13.
29 Loft S, Poulsen HE (1999). Markers of oxidative damage to DNA: antioxidants and molecular damage. Methods Enzymol. **300**: 166–184.
30 Mazzoli-Rocha F, Fernandes S, Einicker-Lamas M, Zin WA (2010) Roles of oxidative stress in signaling and inflammation induced by particulate matter. Cell Biol Toxicol. **26**: 481–498; doi: 10.1007/s10565-010-9158-2.
31 Mills N, Donaldson K, Hadoke PW, Boon NA, MacNee W, Cassee FR, et al. (2009) Adverse cardiovascular effects of air pollution. Nat Clin Pract Cardiovasc Med. **6**(1): 36–44.
32 Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd (1990) A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA. **87**(23): 9383–9387.
33 Morrow JD, Awas JA, Boss HJ, Blair IA, Roberts LJ (1992) Noncyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. Proc Natl Acad Sci USA. **89**(22): 10721–10725.
34 Mortamais M, Pujol J, Martinez-Vilavella G, Fenoll R, Reynes C, Sabatier R et al. (2019) Effects of prenatal exposure to particulate matter air pollution on corpus callosum and behavioral problems in children. Environ Res. **178**: 108734; doi: 10.1016/j. envres.2019.108734.
35 Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D et al. (2003) Effects of transplacental exposure to environmental pollutans on birth outcomes in a multiethnic population. Environ Health Perspect. **111**: 201–205; doi: 10.1289/ehp.5742.
36 Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D et al. (2006) Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner/city children. Environ Health Perspect. **114**: 1287–1292; doi: 10.1289/ehp.9084.
37 Perera FP, Li Z, Whyatt R, Hoepner L, Wang S, Camann D et al. (2009). Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics. **124**: e195–e202; doi: 10.1542/peds.2008-3506.
38 Perera FP, Tang D, Wang S, Vishnevetsky J, Zhang B, Diaz D et al. (2012) Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6-7 years. Environ Health Perspect. **120**: 921–926; doi: 10.1289/ehp.1104315.
39 Peterson BS, Rauh VA, Bansai R, Hao X, Toth Z, Nati G et al. (2015) Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on development of brain white matter, cognition, and behavior in later childhood. JAMA Psychiatry. **72**: 531–540; https://doi.org/10.3390/brainsci10090619
40 Pichini S, Basagana X, Pacifici R, Garcia O, Puig C, Vall O et al. (2000) Cord serum cotinine as a biomarker of fetal exposure to cigarette smoke at the end of pregnancy. Environ Health Perspect. **108**: 1079–1083.

41 Raven JC, Court JH, Raven J (1990) Manual for Raven's Progressive Matrices and Vocabulary Scales - Section 2: ColouredProgressive Matrices. Oxford Psychologists Press: Oxford, UK.
42 Reynolds CR (2010) Behavior assessment system in children. In The Corsini Encyclopedia of Psychology (pp. 1 – 2) Hoboken, NJ, USA: John Wiley & Sons, Inc.; doi: 10.1002/9780470479216.corpsy0114.
43 Rivas I, Basagana X, Cirach M, Lopes-Vicente M, Suades-Gonzales E, Garcia-Esteban R et al. (2019) Associatio between Early life exposure to air pollution and working memory and attention. Environ Health Perspect. **127**(5): O5702; doi: 10.1289/EHP3169.
44 Roberts LJ, Morrow JD (2000) Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med. **28**(4): 505–513.
45 Rommel AS, Milne GL, Barrett ES, Bushi NR, Nguyen R, Sathyanarayana S et al. (2020) Associations between urinary biomarkers of oxidative stress in the third trimester of pregnancy and behavioral outcomes in the child at 4 years of age. Brain Behav Immun. **90**: 272–278; doi: 10.1016/j.bbi.2020.08.029.
46 Rossner P, Jr, Svecova V, Milcova A, Lnenickova Z, Solansky I, Sram RJ (2008) Seasonal variability of oxidative stress markers in city bus drivers - Part II: Oxidative damage to lipids and proteins. Mutat Res. **642**(1–2): 21–27; doi: 10.1016/j.mrfmmm.2008.03.004.
47 Rossner P, Jr, Mistry V, Singh R, Sram RJ, Cooke MS (2013) Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine values determined by a modified ELISA improves agreement with HPLC-MS/MS. Biochem Biophys Res Commun. **440**(4): 725–730; doi: 10.1016/j.bbrc.2013.09.133.
48 Smirni D (2020) The Raven's Coloured Progressive Matrices in Healthy Children: A Qualitative Approach. Brain Sci. **10**(11): 877. Doi: 10.3390/brainsci10110877.
49 Szumilas M (2010) Explaining Odds Ratios. J Can Acad Child Adolesc. **19**: 227–229.
50 Topinka J, Rossner P, Jr, Milcova A, Schmuczerova J, Svecova V, Sram RJ (2011) DNA adducts and oxidative DNA damage induced by organic extracts from PM2.5 in an acellular assay. Toxicol Lett. **202**: 186–192; doi: 10.1016/j.toxlet.2011.02.005.
51 Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S (2013) Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. **10**(9): 3886–3907.
52 Weiss JM, Goode EL, Ladiges WC, Ulrich CM (2005) Polymorphic variation in hOGG1 and risk of cancer: a review of the functional and epidemiologic literature. Mol Carcinog. **42**(3): 127–141.
53 Weitzman M, Byrd RS, Aligne A, Mosswe M (2002) The effect of tobacco exposure on children behavioral and cognitive functionining: Implications for clinical and public health policy and future research. Neurotoxicol Teratol. **24**: 397–406; doi: 10.1016/s0892-0362(02)00201-5.
54 Zhang Q, Li Q, Ma J, Zhao Y (2018) PM2.5 impairs neurobehavior by oxidative stress and myelin sheaths injury of brain in the rat. Environ Pollut. **242**: 994–1001; 10.1016/j.envpol.2018.07.031.