analyzed by ANOVA followed by Tukey's *post hoc* test, adopting $p < 0.05$ as a limit of significance.

Results: We found a significant decrease in AOPP levels in 192RR subjects (0.64 nmol/ml, $p < 0.01$) compared to WT (1.76 nmol/ml) and 192QR (1.60 nmol/ml) workers; serum AGE were lower in 192RR workers (147,221 AU/ml, $p < 0.05$) than in WT (161,400 AU/ml) and 192QR (152312 AU/ml) subjects. The analysis of the allele frequencies distribution demonstrated that 192Q (WT) wild-type allele was significantly more frequent than 192R mutated allele (0.74 vs 0.26) in the sample. WT allele was mainly represented by homozygote genotype 192QQ (51%), the mutated one by heterozygote 192QR (45.4%).

Conclusions: These results are comparable with those reported in an our previous study conducted in a smaller sample of farmers employed in a farm located in Sicily; they also confirm that chronic OP pesticide exposure may result in long-lasting oxidative stress.

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P03-098
The impact of polycyclic aromatic hydrocarbons in polluted air and diet on newborns

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Northern Moravia is a region of the Czech Republic with the highest ambient air concentrations of particulate matter (PM2.5) and carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), including benzo[a]pyrene (B[a]P). The sources of pollution include heavy industry and local heating. In the city of Karvina (K) located this region we studied the impact of air pollution on newborns. Subjects from a clean city of Ceske Budejovice (CB) were used as controls. Milk, blood and urine samples from 100 mothers and cord blood and urine from 100 newborns were collected in winter and summer in each district. Concentrations of c-PAHs were assessed in PM2.5 collected by HiVol samplers. The following parameters were studied: bulky DNA adducts, oxidative DNA damage (8-oxodG), lipid peroxidation, cotinine, gene expression profiles. PAH concentrations were determined in breast milk, urine and diet. Concentrations of PM2.5 were as follows: summer: 21.54 ± 11.78 mg/m^3 and 12.14 ± 7.23 mg/m^3, winter: 55.35 ± 38.74 mg/m^3 and 26.39 ± 16.85 mg/m^3, in K and CB, respectively ($p < 0.001$). Concentrations of B[a]P were as follows: summer: 1.31 ± 1.26 ng/m^3 and 0.44 ± 0.63 ng/m^3, winter: 5.15 ± 5.47 ng/m^3 and 1.43 ± 1.37 ng/m^3, in K and CB, respectively ($p < 0.001$). DNA adduct levels in the cord blood in winter samples were: 2.76 ± 1.11 and 2.32 ± 0.90 adducts/10^8 nucleotides, in summer samples: 2.34 ± 0.64 and 1.87 ± 0.75 adducts/10^8 nucleotides in K and CB, respectively ($p < 0.01$). 8-oxodG adducts (nmol/mmol creatinine) in the urine of newborns were: winter: 5.66 ± 2.90 and 4.23 ± 1.51 ($p < 0.001$), summer: 4.69 ± 2.40 and 4.66 ± 1.43 ($p = 0.203$) in K and CB, respectively. In both localities and seasons lower than recommended fruits and vegetables intake was observed in mothers diet. DNA adduct levels were negatively associated with the intake of vegetables. Our observations indicate genetic damage in newborns, which may significantly affect their morbidity. Supported by the Grant Agency of the Czech Republic 301/13/458S.

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P03-099
Evaluation of biomarkers of oxidative stress in gasoline station workers: A case-control study

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Question: Benzene is an important industrial chemical and a well-known environmental pollutant which can induce leukemia, aplastic anemia and cancer. It is widely accepted that benzene metabolism plays a crucial role in its toxicity, with involvement of one or more of reactive metabolites. The enzymatic activation of benzene and its reactive metabolites lead to continuous production of reactive oxygen species (ROS), which damage nucleic acids, proteins and other biological macromolecules, decreases antioxidant activity and increases oxidative stress. Modification of biological molecules creates new compounds which can serve as biomarkers of oxidative stress, as advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and 8-hydroxy-deoxyguanosine (8-OHdG) as a marker of oxidative DNA damage. This study aims to evaluate oxidative stress level in a group of workers exposed to benzene.

Methods: Study population consisted of 50 workers employed in gas stations and 45 age-matched controls, not exposed to benzene either in occupational or living environment. Urinary concentration of t,t-muconic acid, a metabolite of benzene, was determined by HPLC to assess actual exposure level in exposed and control subjects. Serum levels of AGEs and AOPPs were determined by a spectrofluorimetric and spectrophotometric automatized assay respectively, whereas 8-OHdG concentrations were measured by a colorimetric ELISA kit.

Results: Urinary t,t,-muconic levels in exposed workers were higher than controls, confirming low-dose actual benzene absorption in the exposed group. All biomarkers of oxidative stress were significantly increased in the benzene-exposed group.

Conclusions: In conclusion, our results showed that chronic benzene exposure may result in long-lasting oxidative stress, as demonstrated by the presence of statistically significant correlations between benzene exposure and levels of biomarkers (AGEs, AOPPs, t,t-muconic acid, 8-OHdG). Therefore, the early identification of these biomarkers can be very useful in order to promote programs on health protection and prevention for those populations more susceptible to the adverse effects of benzene exposure.

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P03-100
Action of a polyamine putrescine in genetic material of *Allium cepa*

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Among the consequences of high population growth is the need for increasing areas for the burial of human bodies. The areas destined for the implementation of cemeteries usually have inadequate hydrogeological characteristics, which might cause serious health and environmental problems. The main causes of environmental contamination is the liquid called necrochurume which is

released by bodies decomposition process, which can percolate into the soil and contaminate the groundwater supplies. This liquid is formed by different organic substances among which the putrescine ($C_4H_{12}N_2$), a polyamine which acts as an intracellular messenger and is related to cell growth and differentiation processes. The aim of this study was to evaluate the cytotoxicity and genotoxicity of the polyamine putrescine through the chromosomal aberrations and micronuclei induction test in meristematic regions of *Allium cepa* roots. The seeds were germinated in Petri plates, exposed to different concentrations of putrescine (23 mg/L, 18 mg/L, 13 mg/L, 8 mg/L, 3 mg/L, 1.5 mg/L and 0.75 mg/L). The negative control was performed with ultrapure water and the positive control was methylmethanesulfonate (MMS – 4×10^{-4} M). After the exposure period the roots were fixed and processed according to cytogenetic techniques. A total number of 5000 cells/treatment were scored and the statistical analyses for the frequency of cytotoxicity and genotoxicity was Performed by ANOVA/Dunn ($p < 0.05$). None cytotoxic potential was verified (mitotic index and cell death frequency). The genotoxicity (chromosomal aberrations and micronuclei frequency) was significant only for 23 mg/L concentration. The data showed genotoxic potential of polyamine putrescine in these experimental conditions. The results will be of great importance for understanding the mechanisms of action of this substance and relevant to define its supposed potential as an environmental pollutant.

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P03-101
Evaluation of diamine putrescine effects on epididymal sperm count and transit in Wistar rats

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Cemeteries are a major source of groundwater contamination due to the percolation of necrochorume resulting from corpses putrefaction. This compound is rich in several chemical substances including the diamine putrescine ($C_4H_{12}N_2$), responsible for performing important physiological role in cell growth, differentiation and apoptosis, process that, when compromised, can lead to health damage. Exposure to exogenous chemicals through the water consumption can affect reproductive function in different ways of acting which may lead to infertility. The aim of this study was to assess the effect of diamine on sperm counting and transit in the epididymis of Wistar rats. For this, the rats were exposed to putrescine for 56 consecutive days, organized in three experimental groups with different concentrations (T1 – 46.3 mg/kg, T2 – 138.9 mg/kg and T3 – 231.5 mg/kg), and a control group (Co-drinking water). The organs were homogenized for sperm count using Neubauer chamber, and the results were submitted to statistical analysis (ANOVA/Tukey post-test, $p < 0.05$). The results showed a dose–response curve with a significant decrease in the number of sperm in the head/body (Co – 106.6 ± 14.23; T1 – 85.16 ± 11.72; T2 – 71.30 ± 14.53; T3 – 75.36 ± 6.02) as well as in the tail region (Co – 248.11 ± 25.75; T1 – 209.08 ± 30.21; T2 – 143.41 ± 46.71; T3 – 136.73 ± 39.75). In addition, the values of sperm transit in the head/body region showed a significant increase in T3 group (5.84 ± 0.22 days) when compared with controls (4.03 ± 0.91 days) while in the tail region there was no significant difference. The results show negative effect of this amine in the reproductive function of animals and emphasize the importance of toxicological studies with this compound.

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P03-102
Hydrolysis of triphenyl phosphate and 2-ethylhexyl diphenyl phosphate by human serum enzymes

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Question: Phosphate flame retardants (PFRs) are abundant chemicals in house dust but little is known about their biological levels and their relationship with house dust concentrations. These relationships provide insight into major exposure pathways and potential health risks. The correlation between triphenyl phosphate (TPHP) in dust and its metabolite diphenyl phosphate (DPHP) in human urine has not been successfully proved yet. A possible explanation is that DPHP is not a specific biomarker of TPHP, but also of other PFRs, such as 2-ethylhexyl diphenyl phosphate (EHDPP). Since serum enzymes are capable of hydrolyzing organophosphate structures, such as paraoxon (PXN), we compared their activity towards TPHP and EHDPP in production of DPHP.

Methods: We incubated 20 and 50 μM for TPHP and EHDPP each separately with diluted human serum (1:50, v/v) in TRIS buffer (2 mM $CaCl_2$). The assay was based on optimal conditions for paraoxonases (Furlong et al., 1983). 20 μM PXN was used as positive control (metabolite 4-nitrophenol, 4-NP), while negative controls were prepared without serum. Reactions were started by addition of parent compound and quenched by mixing with acetonitrile (1:1, v/v) and internal standard solution. DPHP and 4-NP formation was measured by liquid chromatography-tandem mass spectrometry.

Results: A distinct positive linear correlation was observed for DPHP formation with increasing time or serum concentration. Chemical hydrolysis was measured and subtracted from enzymatic hydrolysis. Maximum DPHP formation was 21 and 9.2 pmol/min/μl serum from EHDPP and TPHP, respectively, compared to 88 pmol 4-NP/min/μl serum.

Conclusion: TPHP and EHDPP were hydrolyzed in serum and this should be taken into account for the determination of biotransformation rates in humans for PBPK modeling. DPHP was more quickly produced from EHDPP than from TPHP, which should be taken into consideration for interpreting DPHP levels in human urine. The possibility of similar PFRs contributing to DPHP formation needs to be investigated for a complete assessment of DPHP as a biomarker of exposure.

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