

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 –  $4 \times 10^{-4}$  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 –  $106.6 \pm 14.23$ ; T1 –  $85.16 \pm 11.72$ ; T2 –  $71.30 \pm 14.53$ ; T3 –  $75.36 \pm 6.02$ ) as well as in the tail region (Co –  $248.11 \pm 25.75$ ; T1 –  $209.08 \pm 30.21$ ; T2 –  $143.41 \pm 46.71$ ; T3 –  $136.73 \pm 39.75$ ). In addition, the values of sperm transit in

the head/body region showed a significant increase in T3 group ( $5.84 \pm 0.22$  days) when compared with controls ( $4.03 \pm 0.91$  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 (DHP) in human urine has not been successfully proved yet. A possible explanation is that DHP 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 DHP.

**Methods:** We incubated 20 and 50  $\mu$ M 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  $\mu$ M 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. DHP and 4-NP formation was measured by liquid chromatography-tandem mass spectrometry.

**Results:** A distinct positive linear correlation was observed for DHP formation with increasing time or serum concentration. Chemical hydrolysis was measured and subtracted from enzymatic hydrolysis. Maximum DHP formation was 21 and 9.2 pmol/min/ $\mu$ l serum from EHDPP and TPHP, respectively, compared to 88 pmol 4-NP/min/ $\mu$ l 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. DHP was more quickly produced from EHDPP than from TPHP, which should be taken into consideration for interpreting DHP levels in human urine. The possibility of similar PFRs contributing to DHP formation needs to be investigated for a complete assessment of DHP as a biomarker of exposure.

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