and antipyretics, bronchodilatadors and anti-asthma drugs. Less concentration of antineoplastics, inmunosuprresants and antihistamines were found.

In drinking water were found very low caffeine, nicotine, cotinine, carbamazepine, paroxetine, sulfamethoxazole, ketoporofen, and atenolol concentrations (ng/l).

This project has been funded by the FISCAM (PI 2007/28).

doi:10.1016/j.toxlet.2010.03.238

P103-029
Aristolochic acid suppresses DNA repair and triggers oxidative DNA damage in human kidney proximal tubular cells

S.Y. Chiang, Y.Y. Chen, J.G. Chung, P.Y. Lee, H.F. Huang, C.Y. Hsiang, T.Y. Ho

China Medical University, Taiwan

Aristolochic acid (AA), derived from plants of the Aristolochia genus, has been proven to be associated with aristolochic acid nephropathy (AAN) and urothelial cancer in AAN patients. In this study, we used toxicogenomic analysis to clarify the molecular mechanism of AA-induced cytotoxicity in normal human kidney proximal tubular (HK-2) cells, the target cells of AA. AA induced cytotoxic effects in a dose-dependent manner (10, 30, 90 μM for 24 h) and a time-dependent manner (30 μM for 1, 3, 6, 12, and 24 h). The cells from those experiments were then used for microarray experiments in triplicate. Among the differentially expressed genes analyzed by Limma and Ingenuity Pathway Analysis software, we found that genes in DNA repair processes were the most significantly regulated by all AA treatments. Furthermore, response to DNA damage stimulus, regulation of cell cycle, and apoptosis were also significantly regulated by AA treatment. Among the differentially expressed genes found in the dose–response and time-course studies that were involved in those biological processes, two up-regulated genes (GADD45B, NAIP), and six down-regulated genes (TP53 PARP1, OGG1, ERCC1, ERCC2 and MGMT) were confirmed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Moreover, AA treatment led to increased frequency of DNA strand breaks, 8-hydroxydeoxyguanosine-positive nuclei, and micronuclei in a dose-dependent manner in HK-2 cells, possibly as a result of the inhibition of DNA repair. These data suggest that oxidative stress plays a role in the cytotoxicity and genotoxicity of AA. In addition, our results provide new insight into the involvement of down-regulation of DNA repair gene expression as a possible mechanism for AA-induced genotoxicity.

doi:10.1016/j.toxlet.2010.03.239

P103-030
Safety evaluation of XiXin, a medicinal herb from the Aristolochiaceae family, and its formula in mice

H.C. Wu, S.F. You, W.Y. Tien, P.C. Hsu, Y.Y. Chen, H.N. Cheng, M.Y. Lin, S.Y. Chiang

China Medical University, Taiwan

Aristolochic acid, a known human carcinogen found in plants of the genera Aristolochia and Asarum of the family Aristolochiaceae, is associated with the development of nephropathy and urothelial cancers in humans. XiXin, a medicinal herb of the Asarum genus, contains not only very small amounts of AA but also the hepatotoxic compound, safrole. In the study, an established C3H/He mouse model that exhibits enhanced susceptibility to AA-induced nephrotoxicity was used to assess the safety of XiXin and its formula, Xiao Qing Long Tang. The young mice (about 4–5-week-old) were administered by gavage with different parts of XiXin (roots or whole plant) at the doses of 18 and 180 times of the clinical dose, Xiao Qing Long Tang at the doses of 39 times of the clinical dose, and Aristolochia fangchi (1.5 g crude drug/kg) for 14 days. After 2 weeks treatment in young mice, the Aristolochia fangchi-treated group and the high-dose group of XiXin (whole plant and roots) significantly increased the levels of urine total protein/creatinine and blood urea nitrogen. The levels of GOT and GPT as well as the pathological scores of tubular damage in the Aristolochia fangchi-treated group and the high-dose group of XiXin (whole plant) were significantly higher than those of control, whereas the high-dose group of XiXin (roots) were not. In the group of Xiao Qing Long Tang showed no signs of overt toxicity in all tests examined. The NOAEL of XiXin (whole plant or roots) and Xiao Qing Long Tang in young mice for 14 says were 1.0 g and 17.6 g crude drug/kg/day, respectively. These data may provide a better understanding on the safety of XiXin and its formula and provide some guidelines on how to use AA-containing herbs safely.

doi:10.1016/j.toxlet.2010.03.240

P103-031
Heavy metals and essential elements in bones of three species of penguins from King George Island, Antarctica

S. Jerez[1], M. Motas[1], J. Benzal[2], A. Martínez[2], F. Valera[2], A. Barbosa[3]

[1] *University of Murcia, Spain*, [2] *Estación Experimental de Zonas Áridas, CSIC, Spain*, [3] *Museo Nacional de Ciencias Naturales, CSIC, Spain*

Antarctica is considered one of the few remaining pristine regions of the planet and environmental contamination researches on its ecosystem became of great interest for the international scientific community in the last years. However, a low increase of the contamination levels due to local activities and isolated incidents has been detected. It is important to highlight that, since contamination is a global phenomenon, the transport of pollutants to these remote areas may occur. Moreover, King George Island (62°15′S 58°37′W) is one of the zones of Antarctica with frequent human presence. Unfortunately, information on heavy metal concentrations in Antarctic biota is still scare and fragmented.

Penguins are excellent subjects for examination of metals presence because they are long-lived, have a permanent ecological niche and dominate the avifauna in Antarctica. The different concentrations of heavy metals and essential elements (Cd, Pb, Hg, Cu, Zn, As, Se) has been determined in bones ($n = 44$) of chinstrap penguin (*Pygoscelis antarctica*), gentoo penguin (*Pygoscelis papua*) and adélie penguin (*Pygoscelis adeliae*) from King George Island, obtained between 2008 and 2009 by non-destructive methods. Solutions of bones were analyzed by inductively coupled plasma mass spectrometry.

Bones showed the following order of metal concentrations: Zn > Se > Cu > Pb > As > Cd > Hg (*P. papua*), Zn > Se > Cu > As > Pb > Cd > Hg (*P. adeliae*) and Zn > Se > Cu > As > Cd > Pb > Hg (*P. antarctica*). All the analyzed elements showed the highest average levels in bones of *P. papua* in comparison with the rest of the studied species (Pb in bones of *P. papua*: 0.18 μg/g dry weight; *P. adeliae*: 0.0018 μg/g dry weight; *P. antarctica* 0.0002 μg/g dry weight). This fact probably could be explained by the different feeding habits of these species.

These results became relevant for a better understanding of the current Antarctica's environmental status and the ecotoxicological consequences resulting from the presence of metals in Antarctic ecosystem. This would be useful for the development of future conservation strategies.

doi:10.1016/j.toxlet.2010.03.241

P103-032
Determination of estrogens (estriol, β-estradiol, estrone and 17β-ethynylestradiol) in river water from a rural area in Midwest Brazil

G. Julião Zocolo[1], A. Cicero De Sousa[2], M.N. Teodoro Lopes[3], M.R. Rodrigues De Marchi[1]

[1] *Instituto de Química, UNESP, Brazil*, [2] *Instituto Federal de Educação, Ciência e Tecnologia da Paraíba-IFPB, Brazil*, [3] *Universidade Federal da Grande Dourados – UFGD, Brazil*

Environmental contaminants either of natural (estrogens) or anthropogenic (industrial products) origin are described to affect reproductive biology of vertebrates by mimicking or antagonizing the action of hormones. The environmental presence of these substances in areas without industrial or domestic sewage influence, called Endocrine Disrupting Chemicals (EDCs), has been only rarely related. Nevertheless EDCs are found almost everywhere in the environment and they mainly accumulate in surface waters and sediments. For this reason, permanent or occasional water inhabitants like fish and amphibians face an increased risk of being harmed by EDCs. The objectives this work was to investigate the occurrence of estriol (E3), 17β-estradiol (E2), estrone (E1) and 17α-ethynylestradiol (EE2) in river water from a Brazilian agricultural region with strong influence of livestock in Dourados (MS), Midwest Brazil. The aquatic environment samples were collected in April 2009 from three different sites along Dourados River, that can receive eventually bovine excreta from extensive cattle creation totalizing 32 samples. A procedure was developed for the analysis of estrogens in environmental water. Analytical method included solid phase extraction (Strata X) followed by HPLC-fluorescence analysis. The absolute recovery of estrogens spiked into surface waters using the procedure was 80–106%. RSD of estrogens extraction and analysis in spiked surface waters was 4–10%. Detection limits were 3.3 ng L^{-1} or below for all unless to E1 13 ng L^{-1}, based on a 1 L sample. The most abundant estrogen was estriol, with range concentration between 11 and 130 ng L^{-1}. Presented results are similar to published by other authors for river water with influence of domestic effluents, in order of ng L^{-1}. These findings are an alert to the possible effects of these endocrine disrupters to wildlife in one of the biomass with higher biodiversity in the world (Brazilian Savannah).

doi:10.1016/j.toxlet.2010.03.242

P103-033
Determination of possible genotoxic risks related to toxic substances resulted from coal plant in individuals residing Afsin-Elbistan thermal power plant area via comet assay

E. Durmaz, O.K. Ulutas, İ. Çok.

Gazi University, Faculty of Pharmacy, Turkey

Thermal power plants (TPP) lead to the emission of significant amounts of SO_2 and fine particulate matter and toxic organic micro pollutants, such as polycyclic aromatic hydrocarbons (PAHs) into the environment. These products of combustion represent a risk to both environment and human. Although several experimental studies have indicated the mutagenic and genotoxic effects of some constituents of coal ashes and PAHs in different test systems; only a few studies have been done on the genotoxic risks of individuals residing TTP areas. Afsin-Elbistan A TPP which was located in south-eastern Turkey has been established and on duty without a desulphurization unite since 1984. Also the reduced capacity of particulate filter of the power plant introduce more potential risk for human health as more compounds released to the environment. In our study possible genotoxic risks caused by TPP emissions exposure were evaluated by comet assay. 1-OHP which is thought to be biomarker of exposure to PAH compounds were determined by HPLC method in urine. For this purpose exposed group was consisted of 97 healthy individuals without a history of smoking who were living near Afsin-Elbistan TPP. The results were compared with a control group of 96 healthy individuals without exposure to any known genotoxic agents. Tail intensity levels which were selected as a marker of the genotoxic damage were found significantly higher ($p < 0.001$) in exposed individuals then control group, respectively (8.79 ± 1.88, 7.12 ± 1.88). The mean levels for 1-OHP for exposed group was found significantly higher than ($p < 0.001$) controls, respectively (0.42 ± 0.13, 0.25 ± 0.07).This study was supported by Gazi University Scientific Research Fund (Project No. 02/2007-25)

doi:10.1016/j.toxlet.2010.03.243

P103-034
Activation of biological pathways in the lungs and heart by particulate matter and ozone

E. Thomson, D. Vladisavljevic, R. Vincent, P. Kumarathasan

Health Canada

Air pollution is a complex mixture of gaseous and particulate constituents associated with cardiovascular and respiratory morbidity and mortality. Given the heterogeneous nature of air pollution, constituent pollutants may produce distinct patterns of effects in target tissues. In the present study we used real-time polymerase chain reaction as a sensitive approach to evaluate effects of acute 4 h inhalation exposure to urban particulate matter (0, 5, and 50 mg/m^3 EHC-93), ozone (0, 0.4, and 0.8 ppm), or combinations of particles and ozone in Fisher-344 rats. Pulmonary and cardiac expression of genes involved in a number of biological pathways, including inflammation, oxidative stress, metal-response, xenobiotic metabolism, vasoconstriction, vasodilation, and adhesion, were assessed immediately and 24 h after exposure. Analyses in the lungs revealed immediate effects of particle and ozone exposure on several endpoints, including endothelin-1 (vasoconstriction) and metallothionein-II (metal-response/oxidative stress). Particles increased CYP1A1 expression, consistent with the presence of polycyclic aromatic hydrocarbons, while ozone alone increased expression of the inflammatory mediators interleukin-6 and monocyte chemotactic protein-1. In the heart, particle exposure provoked a transient increase of CYP1A1 and a sustained increase of metallothionein-II. Remarkably, inhalation of ozone, a reactive gas entirely consumed within the lung, resulted in rapid and transient increases of CYP1A1, metallothionein-II, and endothelin-1 expression in the heart. After 24 h recovery, heme oxygenase-1 and inducible and endothelial forms of nitric oxide synthase were modestly increased in the heart, while cyclooxygenase-2 was decreased. Together, the pattern of gene expression suggests increased oxida-