

## TESTICULAR TOXICITY OF CYANOBACTERIAL BIOMASS IN JAPANESE QUAILS

Damkova V.<sup>1</sup>, Sedlackova J.<sup>1</sup>, Bandouchova H.<sup>1</sup>, Peckova L.<sup>1</sup>, Vitula F.<sup>1</sup>, Hilscherova K.<sup>2</sup>, Paskova V.<sup>2</sup>, Kohoutek J.<sup>2</sup>, Novotny L.<sup>3</sup>, Pohanka M.<sup>3</sup>, Pikula J.<sup>1</sup>

<sup>1</sup>Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

<sup>2</sup>Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Kamenice 3, 62500 Brno, Czech Republic

<sup>3</sup>Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

Birds may be subject to mortality or adverse biological effects due to the action of cyanobacterial products. Toxic effects of cyanobacterial biomass administered in feed on the reproductive system of male Japanese quails, in particular, were evaluated in this study. The OECD 206 Guideline on Avian Reproduction Toxicity was used for this purpose. The chronic exposure lasted eight weeks with the daily sum of 61.62 µg microcystins (MCs) including 26.54 µg MC-RR, 7.62 µg MC-YR and 27.39 µg MC-LR. A total of 16 control and 16 experimental pairs sacrificed at the end of the experiment were analyzed using histopathology. Eggs laid by these pairs were artificially incubated. While control birds were without pathological findings in the testicular tissue, there was marked atrophy in the testes of cyanobacterial-biomass-exposed males. These results were also supported by findings of biochemical examination aimed at antioxidants and oxidative stress in the testes. Surprisingly, reproductive parameters such as egg viability, hatchability, and the effect of hatching in cyanobacterial-biomass-exposed birds were better than in the control group. This can be explained by the fact that female birds store sperm for days or even weeks in sperm storage tubules. Fertilization of eggs by sperm from experimental males showing testicular atrophy thus had to be due to this sperm storage from early mating. However, further research into this issue is necessary.

The present study was supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno (Grant No. 79/2010/FVHE).

## EFFECT OF GARDOPRIM PLUS GOLD 500 SC ON HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF COMMON CARP (*CYPRINUS CARPIO* L.)

Dobšíková R.<sup>1</sup>, Blahová J.<sup>1</sup>, Modrá H.<sup>1</sup>, Svobodová Z.<sup>1,2</sup>

<sup>1</sup>University of Veterinary and Pharmaceutical Sciences Brno, Department of Veterinary Public Health and Toxicology, Palackého 1/3, 612 42 Brno, Czech Republic

<sup>2</sup>University of South Bohemia, Research Institute of Fish Culture and Hydrobiology Vodňany, Zátíší 728/II, 389 25 Vodňany, Czech Republic

Gardoprim Plus Gold 500 SC is a herbicide preparation used in agriculture to control annual weed in maize. It is a fluid suspense concentrate containing two active substances, namely a chlorotriazine terbuthylazine

(187.5 g/l) and a chloracetanilide S-metolachlor (312.5 g/l). Mechanism of terbuthylazine (N<sup>2</sup>-tert-butyl-6-chloro-N<sup>4</sup>-ethyl-1,3,5-triazine-2,4-diamine) action is a photosynthesis inhibition, S-metolachlor (2-chloro-N-(6-ethyl-o-tolyl)-N-[(1S)-2-methoxy-1-methylethyl]acetamide) inhibits a synthesis of proteins and chlorophyll in plants.

The study was focused on the assessment of Gardoprim Plus Gold 500 SC effects on common carp (*Cyprinus carpio* L.) on the basis of acute toxicity test results and haematological and biochemical examination. One- to two-year-old common carp of 93.66 ± 31.77 g body weight and 18.28 ± 2.15 cm body length were used for the examination. Experimental carp were exposed to Gardoprim Plus Gold 500 SC preparation in the concentration of 13.0 mg/l corresponding to 2.25 mg/l and 3.75 mg/l of terbuthylazine and S-metolachlor, respectively. A 96-hour toxicity test was performed semistatically with water renewal every 24 hours according to OECD 203 Fish, Acute Toxicity Test.

The results of exposed carp were compared with those of carp from control group. Statistical analysis of the data was conducted using the program Statistica 8.0 for Windows (StatSoft, Inc. USA). Values were tested for normal distribution using Kolmogorov–Smirnov test and then log-transformed to improve the homogeneity of variance. A one-way analysis of variance (ANOVA) and Tuckey test were applied.

In haematological profile, the experimental group of carp showed a significant decrease in leukocyte count ( $p < 0.01$ ) and haematocrit ( $p < 0.05$ ). As far as biochemical parameters are concerned, a significant increase in glucose, aspartate aminotransferase, ammonia ( $p < 0.01$ ) and lactate dehydrogenase ( $p < 0.05$ ) levels and a significant decrease in inorganic phosphorus, triglyceride ( $p < 0.01$ ) and chloride ( $p < 0.05$ ) concentrations were found in blood plasma of exposed carp compared to control group.

The exposure of common carp to 13.0 mg/l of Gardoprim Plus Gold 500 SC caused significant shifts in haematological and biochemical profile. The herbicide was classified among substances strongly toxic for fish.

The study was supported by the MSMT grant No. 6215712402.

## ROLE OF THR422 RESIDUE IN PXR TRANSCRIPTIONAL ACTIVITY

Doricakova A., Novotna A., Vrzal R., Pavek P., Dvorak Z.

Department of Cell Biology and Genetics, Faculty of Science, Palacky University in Olomouc, Slechtitelu 11, 783 71, Olomouc Holic, Czech Republic

The steroid and xenobiotic-responsive pregnane X receptor (PXR) plays an integral role in regulation of drug-metabolizing enzymes CYPs, in particular CYP3A4, which catalyzes the metabolism of > 50% of clinically used drugs. However, the regulatory mechanisms of PXR are not entirely understood. There are several indications on the role of phosphorylation in modulation of

PXR transcriptional activity. Although several kinases have been shown to directly phosphorylate PXR and possibly lead to the repression of CYP expression, very little is known about specific site(s) of phosphorylation.

We examined putative phosphorylation site at Thr422, located at the AF-2 surface of the PXR ligand-binding domain (LBD). Side-directed mutagenesis was performed to generate phospho-deficient (PXRT<sup>422A</sup>) and phospho-mimetic (PXRT<sup>422D</sup>) mutants.

We studied changes in human PXR transcriptional activity of PXR mutant as compared to wt-PXR. We measured fold activation of the *p3A4-luc* reporter gene construct transiently transfected together with PXR plasmids to human hepatoma HepG2 cells. We also analyzed the effects of PXRT<sup>422A</sup> PXRT<sup>422D</sup> on basal and inducible levels of CYP3A4 mRNA in HepG2 cells. We observed that both mutations at Thr422 diminished PXR activity and inhibited transactivation of the *CYP3A4* promoter.

Taken together, these data suggest that Thr422 is not primarily responsible to regulation of PXR by phosphorylation. The residuum probably plays a role in a ligand-dependent activation of PXR and in the acquisition of its AF-2 to a conformation that favors interaction with co-regulators or transcription factors at the AF-2 surface. Our future studies will be focused on this interaction.

Our laboratories are supported by the grants from the Czech Scientific Agency GACR 303/07/0128, GACR 503/10/0579 and GACR 304/10/0149.

## ON DIFFERENT ACTIVITIES OF TWO SYNTHETIC COUMARIN DERIVATIVES

Drábiková K. <sup>1</sup>, Jančinová V. <sup>1</sup>, Perečko T. <sup>1</sup>, Nosál' R. <sup>1</sup>, Ambrožová G. <sup>2</sup>, Lojek A. <sup>2</sup>, Šmidrkal J. <sup>3</sup>, Harmatha J. <sup>4</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic

<sup>2</sup>Institute of Biophysics, Department of Free Radical Pathophysiology, AV ČR v.v.i., Královopolská 135, 612 65 Brno, Czech Republic

<sup>3</sup>Institute of Chemical Technology Prague, Department of Dairy and Fat Technology, Technická 5, 166 28 Praha 6-Dejvice, Czech Republic

<sup>4</sup>Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, 161 10 Praha 6, Czech Republic

Coumarins represent an important group of organic compounds with broad pharmacological activities, especially antiinflammatory and antioxidant. Previously, we showed the different inhibitory effect of hydroxyphenyl-hydroxy-coumarin (HHC) and of hydroxyphenyl-hydroxy-dihydrocoumarin (HHDC) on stimulated reactive oxygen species (ROS) generation in human neutrophils [1]. In this work we studied the effect of HHC (0.01, 0.1, 1, 10, 100 µmol/l) and HHDC (0.01, 0.1, 1, 10, 100 µmol/l) on production of reactive nitrogen species (RNS) by lipopolysaccharide (LPS)-stimulated murine macrophages RAW 264.7, measured by the Griess reaction as nitrite (NO) formation in cell supernatants. Changes in inducible nitric oxide synthase (iNOS) expression were determined by Western blot analysis.

Caspase-3 activation is involved in the pathways for apoptosis, which is critical for homeostasis as well as for the control of inflammatory processes. The effect of HHC and HHDC on recombinant caspase-3 activity was evaluated using Caspase-Glo<sup>®</sup> 3/7 Assay (Promega). The potency of HHC and HHDC to affect neutrophil integrity was studied on the basis of ATP liberation by luciferin-luciferase chemiluminescence.

HHC in the concentrations of 10 and 100 µmol/l significantly ( $p < 0.01$ ) reduced NO formation and iNOS expression. The activity of recombinant caspase-3 was significantly ( $p < 0.05$ ) decreased by HHC in the concentrations of 1, 10 and 100 µmol/l, while the integrity of neutrophils was not affected (ATP liberation was not increased). HHDC, on the other hand, failed to influence any function tested.

In light of the inhibitory effect of HHC on iNOS expression, this derivative may be considered as a potential anti-inflammatory agent since NO synthesized by iNOS is related to various pathophysiological processes including inflammation. The obtained results highlighted the different effects of HHC and HHDC on phagocyte functions, which might be due to their diverse free radical scavenging properties and lipophilicity features, as demonstrated [1].

This work was supported by the grants: VEGA No. 2/0003/10, APVV 0315-07, APVV SK-CZ-0034-09 and GAČR 203/07/1227.

REFERENCE: [1] Drábiková K. et al. (2009). Interdisciplinary Toxicology 2: 100.

## RADICAL DEGRADATION OF HIGH-MOLAR-MASS HYALURONAN INDUCED BY ASCORBATE PLUS CU(II) IONS. TESTING OF METHOTREXATE AND CARNOSINE FOR THEIR FUNCTION AS ANTI-OXIDANT

Drafi F. <sup>1</sup>, Valachova K. <sup>1</sup>, Hrabarova E. <sup>2</sup>, Juranek I. <sup>1</sup>, Bauerova K. <sup>1</sup>, Soltes L. <sup>1</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

<sup>2</sup>Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Dúbravská cesta 9, Bratislava, Slovak Republic

Rheumatoid arthritis (RA) is a systemic inflammatory disorder targeting joints. High-molar-mass hyaluronan (HA), a synovial component, is attacked by reactive oxygen species during acute RA. Carnosine (CAR) is an endogenous dipeptide used for its neuroprotective effect. Methotrexate (MTX) is used as a basic anti-rheumatic drug. L-Glutathione (GSH) is an endogenous tripeptide known as an effective •OH radical scavenger. The aim was to test: a) MTX and CAR in comparison to GSH for their potential protective effect on HA degradation induced *in vitro* by the Weissberger's oxidative system – ascorbate plus Cu(II) ions; b) to assess the time- and dose-dependency of dynamic viscosity of the examined HA solutions by applying rotational viscometry. CAR and MTX was studied at the concentrations of 100, 200 and 400 µmol/l. GSH was tested in the concentration of 100 µmol/l, which is equivalent to 100 µmol/l of •OH radicals generated by the above mentioned oxidative

system. When testing CAR at the two lower concentrations, it was shown to have a pro-oxidant effect, followed by a moderate inhibitory action on HA degradation in the later phase of processing. A decreased rate of HA degradation was observed in the initial phase of measuring at the higher concentrations of CAR. MTX, at the two lower concentrations tested, increased the rate of HA degradation. MTX partially acted as a complex scavenger of  $\cdot\text{OH}$ ,  $\text{RO}\cdot$  and  $\text{ROO}\cdot$  radicals in the later experimental phase. The effect of these two substances tested on  $\text{RO}\cdot$  and  $\text{ROO}\cdot$  radical was different: CAR in comparison to MTX was more effective in the prevention of HA dynamic viscosity decrease. In contrast to GSH, neither of the two substances tested had as significant and protective effect against HA degradation as GSH had.

Acknowledgement: VEGA 2/0003/08, 2/0056/10, 2/0090/08, 2/0083/09, COST B35.

### NEUROBEHAVIORAL DYSFUNCTIONS AS A RESULT OF DEVELOPMENTAL IMPAIRMENT OF THE BRAIN

Dubovický M.

*Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic*

Individual characteristics of human nature (e.g. introversion, extroversion, mood, activity, adaptability, aggressiveness, social ability, anxiety) do not need to be primarily innate. They can be determined by the action of various influences and their interactions on functional development of the brain. There is ample epidemiological and experimental evidence that chemical and/or physical factors acting during sensitive time windows of the brain development can cause mental, behavioral, emotional and/or cognitive disorders. Environmental pollutants, addictive substances, drugs, malnutrition, excessive stress and/or hypoxia/ischemia were found to induce functional maldevelopment of the brain with consequent neurobehavioral disorders. Fetal and early childhood exposures to industrial chemicals in the environment can damage the developing brain and can lead to neurodevelopmental disorders, such as autism, attention-deficit hyperactivity disorder (ADHD) and mental retardation. Researchers have found that about 200 industrial chemicals have the capacity to damage the human brain, and they conclude that chemical pollution may have harmed the brains of millions of children worldwide. We can speak of „a silent pandemic“. Nevertheless, the toxic effects of industrial chemicals on children have generally been overlooked. The lecture will provide review on the most significant neurobehavioral manifestations of developmental impairment of the brain during the prenatal, perinatal and early postnatal period in humans and experimental animals. Serious neurodevelopmental disorders, such as cerebral palsy, neurological dysfunctions, epilepsy, and mental retardation, further cognitive disorders and mental disorders, e.g. schizophrenia, anxiety and mood disorders as well as emotional and behavioral disorders,

such as autism spectrum disorder and ADHD and potential risk factors will be presented.

*Supported by the grants VEGA 2/0083/08 and VEGA 2/0086/09.*

### EFFECTS OF HIGH DIETARY LEVELS OF SE – ENRICHED YEAST ON MUSCLE SELENIUM CONTENT AND MEAT QUALITY TRAITS – A MODEL STUDY IN RATS

Fajt Z.<sup>1</sup>, Modrá H.<sup>2</sup>, Baňoch T.<sup>1</sup>, Bláhová J.<sup>2</sup>, Saláková A.<sup>3</sup>, Svoboda M.<sup>1</sup>

<sup>1</sup>Swine Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

<sup>2</sup>Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

<sup>3</sup>Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Selenium is an important trace element. Adequate selenium intake is essential for human health. Its deficiency has been associated with number of health disorders. As pork is consumed in large quantities throughout the world, it would be a convenient Se source for human diet. The selenium content in pork can be increased by feeding pigs with Se-enriched yeast. The standard dose of 0.3 mg/kg of Se has been used so far. It is not clear whether it is possible to increase the dose. The aim of the trial was to evaluate effects of high dietary levels of Se-enriched yeast on selenium concentrations in muscle tissue and on the meat quality traits. It was made in rats because of possible risks of negative effects of high selenium levels in pigs. The experimental feed mixtures were fed to Wistar albino rats for a period of 10 weeks. Sodium selenite and Se-enriched yeast in concentrations 0.3 mg/kg and 2 mg/kg of Se were fed. The use of elevated dietary Se level in the form of Se-enriched yeast resulted in higher Se muscle concentrations accompanied by increased MDA muscle content. The meat quality traits remained unaffected. No significant differences were observed in established biochemical parameters too.

*The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM Project No. MSM 6215712402 and No. 6215712403).*

### THE PROLIFERATIVE PROPERTIES OF SELECTED TIN CONTAINING ORGANIC COMPOUNDS

Ficková M., Mlynářčíková A., Scsuková S., Havránek T., Macejová D., Brtko J.

*Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Organotin compounds are present throughout the environment owing to widespread use in many industrial and agricultural processes. Despite some restrictions on their use, organotins persist in the environment, are absorbed and accumulate in higher organisms. Organotin derivatives are called endocrine disruptors, as they modulate endocrine systems, especially reproduction. Nuclear receptors (NRs) are crucial targets of



organotins. Nuclear retinoid receptors (RAR, RXR) by binding retinoids and other biologically active ligands significantly suppress growth of breast cancer cells. In this study we investigated the effects of tributyltin (TBT) and triphenyltin (TPT) both as RXR ligands on breast cancer cell proliferation. The results are compared with the effects of natural ligand all-*trans* retinoic acid (RAR ligand) and phytanic acid (RXR ligand).

Dose and time dependent effects were studied in human breast carcinoma cells (MCF7 line). During the incubation period (24, 48 and 72 h) the tested compounds were changed for fresh ones every 24 hours. Cell proliferation was measured by mitochondrial metabolic activity (MTT test) and by *de novo* DNA synthesis (BrdU incorporation).

Slightly intensified growth inhibition (MTT test) with time extension was present, while the antiproliferative effect of maximal concentration ( $1 \times 10^{-6}$  M) was similar for both, TBT and TPT, respectively. The effectiveness of tested compounds was considerably different. In all time intervals, the  $EC_{50}$  (nM) for TBT were significantly lower than those for TPT, what proves TBT more efficient than TPT.

*De novo* DNA synthesis moderately declines with time of cell exposition to TBT and TPT; the maximal effect was nearly identical for both compounds. DNA synthesis analyses confirmed TBT more efficient as TPT. The two assays proved TBT more effective than TPT in growth inhibition of MCF7 cells. In both tests no effects of all-*trans* retinoic acid and phytanic acid on cell proliferation were observed.

TBT as the ligand for RXR $\alpha$  could activate the RXR $\alpha$ -PPAR $\gamma$  heterodimer at nanomolar concentrations and induce inhibition of cell growth.

The study was supported by the grants: APVV-0120-07, VEGA 2/0022/08, VEGA 2/0107/10 and the Centre of Excellence (CENDO) grant.

#### EFFECT OF CARVEDILOL AND SMe1EC2 IN THE Fe<sup>2+</sup>/ASCORBIC ACID SYSTEM OR DURING ISCHEMIA IN VITRO: ACTION IN RAT BRAIN TISSUE OF YOUNG AND ADULT ANIMALS

Gáspárová Z.<sup>1</sup>, Ondrejčíková O.<sup>1</sup>, Gajdošíková A.<sup>1</sup>, Gajdošík A.<sup>1</sup>, Štolc S.<sup>1,2</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic

<sup>2</sup>BIONT Inc., Bratislava, Slovak Republic

Carvedilol, a non-selective beta-blocker, is currently used to treat hypertension, heart failure and coronary artery diseases. Besides its cardioprotective and vasculoprotective properties, it has also antioxidant effects. Our aim was to compare the effect of carvedilol and the new pyridoindole derivative SMe1EC2 in two experimental models of oxidative stress *in vitro* in young and adult rats. We found that the basal level of protein carbonylation in the cortex was significantly higher in 10-month-old rats compared to 2-month-old animals. SMe1EC2 inhibited oxidation of protein carbonyl groups induced by the Fe<sup>2+</sup>/ascorbic acid system in the cortex in both age groups of

rats. Compared to SMe1EC2, carvedilol was not effective in inhibition of the Fe<sup>2+</sup>/ascorbic acid induced oxidation of carbonyl groups in any age groups of rats tested. In electrophysiological measurements, carvedilol in the concentration of 10  $\mu$ M significantly increased the resistance of hippocampal slices exposed to transient hypoxia/hypoglycemia followed by reoxygenation in 2-month-old rats, while it had no effect in 10-month-old rats in any concentration tested. SMe1EC2 significantly improved recovery of neuronal response after transient ischemia of the hippocampus *in vitro*, and that even in 18-month-old rats with a similar force of neuroprotective effect as found previously in 2-month-old rats. The results showed a neuroprotective effect of the pyridoindole SMe1EC2 in young and adult rats in both *in vitro* models involving oxidative stress. The mentioned antioxidant properties of carvedilol were confirmed only in the young rat hippocampus exposed to model ischemia *in vitro* where carvedilol was effective only in a 10-times higher concentration than SMe1EC2. An increased basal level of the protein carbonyl formation in the brain cortex of 10-month-old versus 2-month-old rats confirmed age-related changes in neuronal tissue. This could be due to the increased production of reactive oxygen species and a weak level of antioxidant defense mechanism.

The study was supported by the grant VEGA 2/0093/08.

#### PROTECTIVE EFFECT OF MELATONIN ON SPERM PARAMETERS IN MOUSE TREATED WITH BUSULFAN

Ghasemi F.M., Khajeh Jahromi S., Faghani M.

Anatomy & Histology Department, Faculty Of Medicine, Guilan University Of Medical Sciences, Rasht, Guilan, Iran

The aim of this study was to investigate the possible protective role of melatonin on the sperm quality and quantity in chemotherapy-induced spermiotoxicity.

Male adult NMRI mice were divided into four groups. Group 1 (control): mice received vehicle (ethanol 1%) for 5 days; Group2 (busulfan): mice received a single dose of 20 mg/kg busulfan. Group 3 (Melatonin) mice: received melatonin (10 mg/kg) for 5 days. Group 4 (Busulfan+Melatonin): received a 5-day course of melatonin (10 mg/kg) following an initial dose of busulfan (20 mg/kg). Evaluations were made using sperm count, sperm motility, sperm morphology and assay of serum testosterone level.

Busulfan caused a decrease in epididymal sperm concentration and sperm motility, while increasing abnormal sperm rates both in head and tail parts ( $p < 0.05$ ). However, busulfan+melatonin increased sperm count and motility compared to the busulfan-treated animals ( $p < 0.01$ ). In addition, rates of abnormal sperm in the busulfan+melatonin-treated animals decreased in comparison with busulfan alone. A significant decrease in testosterone level was observed in group 2 ( $p < 0.01$ ). Administration of melatonin in group4 significantly increased the levels of testosterone compared with group 2 ( $p < 0.01$ ).

These results indicated that melatonin may have a protective effect against busulfan-induced testicular

damage, partly by effect on hypothalamo-pituitary gonadal axis and reduction of oxidative stresses.

Supported by the GUMS.

#### GENOTOXICITY IN RELATION TO CHROMIUM EXPOSURE AND POLYMORPHISMS OF SELECTED DNA REPAIR GENES

Halašová E. <sup>1</sup>, Mušák L. <sup>1</sup>, Matáková T. <sup>2</sup>, Letková L. <sup>1</sup>, Franecková M. <sup>1</sup>, Vodička P. <sup>3</sup>

<sup>1</sup>Department of Medical Biology, JFMED CU, Martin, Slovak Republic

<sup>2</sup>Department of Medical Biochemistry, JFMED CU, Martin, Slovak Republic

<sup>3</sup>Institute of Experimental Medicine, Academy of Science CR, Prague, Czech Republic

Welders have been chronically exposed to hexavalent chromium with potential consequences on chromosomal integrity. Our study is focused on the level of chromosomal aberrations with respect to chromium level in the blood of welders as well as on the tentative modulating role of polymorphisms in DNA repair genes *XPD* Lys751Gln, *XPG* Asn114His and *XPC* Lys939Gln on chromosomal damage.

The study was performed on 39 welders that have been exposed to chromium for  $10.2 \pm 1.67$  years, and 31 control individuals. Conventional cytogenetic analysis was employed for detection of CAs.

*XPD*, *XPG* and *XPC* polymorphisms were assayed for by Taqman SNP genotyping assay ("Assay-by-Demand") using Real-Time allelic discrimination on AB 7500 equipment.

Chromium analysis in the blood was performed using the atomic absorption spectrophotometer.

Higher frequencies of CAs were detected in exposed individuals than in controls (1.96% versus 1.55%, respectively), but this difference was not significant. In the exposed group, the chromosomal damage consisted predominantly of chromosomal-type of breaks (CSlovak Academy of Sciences; 1.03%), which were approximately two-fold higher as compared to the controls (0.55%). The frequency of chromatid-type breaks was similar in both exposed and control groups (0.92% vs. 1.00%).

Higher frequencies of CTA were found out also in exposed individuals with heterozygous and homozygous variant *XPG* Asn114His genotype then in those with wild-type genotype (1.27% vs. 0.65%), but due to the low frequency of variant allele the results remain inconclusive. We did not find any significant differences between individuals with different genotypes related to *XPD* and *XPG* genes polymorphisms.

The identification of individuals with increased susceptibility to chromium enables to take preventive measures during working process and may contribute to our understanding the effects of chromium on chromosomal integrity.

This work was supported by the Grant of Ministry of Education of Slovak Republic, VEGA No.1/0576/10.

#### EFFECTS OF SUBCHRONIC EXPOSURE TO SPARTAKUS (PROCHLORAZ 450 g/L) ON COMMON CARP (*CYPRINUS CARPIO*)

Haluzová I. <sup>1</sup>, Modrá H. <sup>1</sup>, Blahová J. <sup>1</sup>, Maršálek P. <sup>1</sup>, Šíroká Z. <sup>1</sup>, Groch L. <sup>2</sup>, Svobodová Z. <sup>1</sup>

<sup>1</sup>Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

<sup>2</sup>Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

The aim of our study was to examine toxic effects of sublethal concentrations of fungicide formulation Spartakus (prochloraz 450 g/L) on common carp *Cyprinus carpio*. The fish were exposed to 0.108; 0.36; and 1.08 mg/L Spartakus (prochloraz concentrations of 0.05; 0.15 and 0.38 mg/L) for 28 days. The control groups were subjected to dechlorinated tap water.

Haematological parameters were not found to be influenced by the exposure. A decrease in ALT, total protein, Na<sup>+</sup> and Ca was revealed in fish exposed to formulation of 1.08 mg/L when compared to the control fish ( $p < 0.01$ ), whereas K<sup>+</sup> increased in all prochloraz treated groups ( $p < 0.01$ ).

Spartakus of 0.36 and 1.08 mg/L induced ( $p < 0.05$ ) cytochrome P450 and ethoxyresorufin-O-deethylase activity, with a significant Spearman's correlation ( $r = 0.49$ ) ( $p < 0.01$ ) between the indices. Glutathion was enhanced in fish exposed to 1.08 mg/L ( $p < 0.05$ ), glutathion-S-transferase showed a significant rise ( $p < 0.05$ ) in all concentrations tested. Hepatosomatic index was increased in fish exposed to Spartakus of 0.36 and 1.08 mg/L. The condition factor was not affected by the formulation.

There was venostasis and desquamation of respiratory epithelium in all pesticide treated fish. The fish treated with 1.08 mg/L Spartakus showed a decreased activity of mucous cells in skin. No histological changes were found in samples of hepatopancreas, spleen, head kidney and trunk kidney.

The activity of glutathion-S-transferase and plasma potassium content were found to be the most responsive parameters determined, as they were both statistically significantly influenced by the lowest tested concentration of the formulation. That affected also the gills as the most sensitive tissue examined.

Supported by MSM 6215712402.

#### MONITORING OF RISK METALS CONTENT IN CHUB (*LEUCISCUS CEPHALUS* L.) FROM THE RIVERS IN THE BRNO AREA

Harkabusová V., Čelechovská O., Lavičková A., Malota L.

Department of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1–3, 612 42 Brno, Czech Republic

The aim of this study was to assess the effect of risk metals released to the environment from the town

agglomerations and their accumulation in fish tissues. Metal pollution was studied in fish from different sites of the Svitava and Svatka rivers in and around the city of Brno. Six localities upstream (Bílovice nad Svitavou, Kníničky) and downstream (the Svitava a Svatka rivers before junction, Rajhradice, Židlochovice) were monitored. The samples of chub (*Leuciscus cephalus* L.) were taken from these sites and analyzed. Chemical concentrations of risk metals (Pb, Cd, Cu, Zn, As) were assessed in muscle, liver, kidney and gonad.

The method of high resolution atomic absorption spectrometry (HR-CS-AAS) was used for determination of metals. Analysis was carried out by spectrometer with electrothermic atomization (ContrAA 700, Analytik Jena, AG). Lead, cadmium and copper contents were determined in the graphite furnace, zinc by flame technique and arsenic concentration by hydride generation. Samples were mineralized using wet way before analysis.

The lowest concentrations of risk elements were found in muscle which is related to its low metabolic activity. Zinc, lead and cadmium concentrations declined in order kidney > liver > muscle, for copper the sequence was liver > kidney > muscle. Arsenic content was the lowest of all monitored elements. Concentrations of arsenic were without statistically significant differences ( $P > 0.05$ ) in all tissues. Generally the Svatka river was much more polluted by risk metals than the Svitava river. Contents of all risk elements ranged below their hygienic limits.

This research was supported by the University of Veterinary and Pharmaceutical Sciences IG202111.

#### ASSESSMENT OF SPECIFIC DNA LESIONS BY THE MODIFIED COMET ASSAY WITH HUMAN OGG1 AND ALK D REPAIR ENZYMES

Hašplová K.<sup>1,3</sup>, Hudecová A.<sup>1,3</sup>, Magdolenová Z.<sup>3</sup>, Bjøras M.<sup>2</sup>, Gálová E.<sup>1</sup>, Miadoková E.<sup>1</sup>, Dušínská M.<sup>3,4</sup>

<sup>1</sup>Faculty of Natural Sciences, Department of Genetics, Mlynska dolina, Bratislava, Slovak Republic

<sup>2</sup>Centre for Molecular Biology and Neuroscience (CMBN) and Institute of Medical Microbiology, Rikshospitalet-Radiumhospitalet Medical Centre, Oslo, Norway

<sup>3</sup>Norwegian Institute for Air Research (NILU), PO Box 100, Kjeller, Norway

<sup>4</sup>Slovak Medical University, Limbová 12, Bratislava, Slovak Republic

Environmental pollutants as well as endogenous factors attack DNA which can lead to mutations or may interfere with normal cellular processes such as DNA replication and transcription. It is therefore crucially important to detect this damage and to recognise specific DNA lesions such as oxidised or alkylating bases.

We modified and standardised the comet assay with two lesion specific enzymes – the OGG1 and 3-methyladenine DNA glycosylase (Alk D). The OGG1 gene encodes human DNA glycosylase that is involved in the base excision repair of 8-hydroxy-2'-deoxyguanine (8-OH-dG) resulted from oxidatively-damaged DNA. AlkD belongs to new family of DNA glycosylases; it initiates repair of cytotoxic and promutagenic alkylated bases.

The modification of Comet assay using OGG1 and Alk D enzymes allows to assess specific DNA lesions. The resulting baseless sugars are alkali-labile, and under the conditions of the alkaline comet assay they appear as strand breaks of DNA. In our study, different dilutions of OGG1 and AlkD were tested to find conditions that allow detection of 8-OH-dG or alkylating damage, respectively. To modify the comet assay with OGG1 we treated human cells with Ro 19-8022 (photosensitizer), which induces specific oxidised bases including 8-OH-dG in the presence of visible light. The control of activity OGG1 enzyme in month period showed instability of activity OGG1 enzyme stored in  $-70^{\circ}\text{C}$ . We found out decrease of activity of human OGG1 repair enzyme.

Alkylating agent methyl methan sulfonate (MMS) was used to standardise conditions of the modified comet assay for detection of alkylating damage with AlkD. Standard conditions for the activity of enzyme have been tested. The method is available for genotoxicity testing.

Supported by by VEGA 1/0008/08; 2/0072/09; 1/4467/07; APVT-20-02607; 1253/2009; EC FP7 [Health-2007-1.3-4], Contract no: 201335.

#### CHEMICAL METABOLIZING ACTIVITY OF AN *IN VITRO* HUMAN EPIDERMAL (EPIDERM™) MODEL AND GENOTOXICITY AS DETERMINED BY *IN VITRO* SKIN MICRONUCLEUS ASSAYS

Hayden P.<sup>1</sup>, Kaluzhny Y.<sup>1</sup>, Kandárová H.<sup>1,2</sup>, Letašiová S.<sup>2</sup>, Bolmarcich J.<sup>1</sup>, Jackson G.R.<sup>1</sup>, Cohen H.<sup>1</sup>, Klausner M.<sup>1</sup>

<sup>1</sup>MatTek Corporation, Ashland, MA, USA

<sup>2</sup>MatTek *In Vitro* Life Science Laboratories, Bratislava, Slovak Republic

Human dermal and airway epithelia contain xenobiotic metabolizing enzymes (XMEs) that could cause biotransformation of cosmetic ingredients, hair-dyes and other chemicals into toxic/mutagenic metabolites. The present work evaluated functional expression of XMEs in highly differentiated *in vitro* models of human epidermal (EpiDerm™) and airway (EpiAirway™) epithelia. RT-PCR and quantitative real-time PCR array experiments were conducted to analyze expression of 168 Phase I and Phase II XMEs in the models. To evaluate the functional activity of XMEs, an *in vitro* skin micronucleus assay was also performed with genotoxic chemicals known to require metabolic activation. Phase I enzymes found to be expressed in the models include cytochrome P450 (CYP) isoforms, alcohol dehydrogenases, aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and others. Further expression of some enzymes could be induced by 3-methylcholanthrene (3MC). Phase II enzymes found to be expressed included glutathione S-transferases, glucuronosyl transferases, sulfotransferases, N-acetyl transferases, epoxidases, esterases, and others. *In vitro* skin micronucleus assays conducted on EpiDerm™ tissues topically treated with genotoxins confirmed metabolic activation of four chemicals that are known to require metabolic activation in order to produce genotoxicity. These results show that the EpiDerm™ and



EpiAirway<sup>TM</sup> *in vitro* human skin and airway epithelial models possess functional drug metabolizing activity that can result in biotransformation of chemicals and generation of genotoxicity as determined by an *in vitro* skin micronucleus assay. These models and assays should prove useful for *in vitro* genotoxicity testing of cosmetic formulations as well as *in vitro* testing of chemicals for the REACH program.

## ROSEMARY OIL REDUCES IN RAT HEPATOCYTES DNA-DAMAGING EFFECT OF OXIDATIVE STRESS

Horváthová E. <sup>1</sup>, Slameňová D. <sup>1</sup>, Navarová J. <sup>2</sup>

<sup>1</sup> Cancer Research Institute, Slovak Academy of Sciences, Vlárská 7, 833 91 Bratislava, Slovak Republic

<sup>2</sup> Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovak Republic

Hundreds of plant essential oils have been investigated with the aim of their possible utilization in different fields of human life. Rosemary oil (RO) is one of the most popular essential oils. It has become important to us due to its various health benefits including its ability to stimulate hair growth, boost mental activity, relieve respiratory problems and reduce pain. Research is also being carried out to study its potential in treating various types of cancers.

The aim of our study was to evaluate the ability of RO administration to modulate DNA-damaging effects of oxidative stress induced in primary rat hepatocytes by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), visible light-excited methylene blue (VL+MB) or 2,3-dimethoxy-1,4-naphthoquinone (DMNQ). Primary hepatocytes were isolated from rats supplemented with RO in drinking water for 14 days as well as from control rats. The potential protective effects of RO were tested on the level of DNA using the conventional and modified single cell gel electrophoresis (Comet assay).

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), singlet oxygen (<sup>1</sup>O<sub>2</sub>) and hydroxyl radicals (•OH) are the reactive oxygen species considered most responsible for producing oxidative stress in cells and organisms. H<sub>2</sub>O<sub>2</sub> induces predominantly DNA breaks via the formation of •OH. Photosensitized MB is known to be an efficient producer of singlet oxygen which oxidizes DNA bases. The effects of DMNQ are mediated by the participation in redox cycling. H<sub>2</sub>O<sub>2</sub>, VL+MB and DMNQ demonstrated genotoxic effects in rat hepatocytes resulting in increased level of frank DNA breaks and oxidative DNA lesions which can be identified in modified Comet assay. Further investigations were therefore focussed on possible modulation of these DNA parameters by RO supplementation. We found out that administration to rats of RO, exhibiting free radical-scavenging activity measured by DPPH assay, efficiently and significantly decreased the level of DNA damage induced with H<sub>2</sub>O<sub>2</sub>, VL+MB or DMNQ in hepatocytes.

Based on the DNA-protective effects of rosemary oil against oxidative stress, we conclude that RO represents a plant essential oil contributing to the defence

mechanisms of organisms against civilisation diseases and constituting the effective alternative or complement for the food industry.

Supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic and the Academy of Sciences VEGA grants 2/0072/09, 2/0050/09 and 2/0083/09.

## STUDY OF THE PRO-OXIDATIVE EFFECTS OF PEROXYNITRITE ON BIOLOGICAL SYSTEMS AS A CONSEQUENCE OF ITS *IN VIVO* FORMATION

Hrabárová E. <sup>1</sup>, Juránek I. <sup>2</sup>, Bauerová K. <sup>2</sup>, Šoltés L. <sup>2</sup>

<sup>1</sup>Department of Glycochemistry, Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

<sup>2</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

The study objectives are focused on providing fundamental facts on the contemporary status of knowledge with respect to biosynthesis and reaction pathways of peroxynitrite anion (ONOO<sup>-</sup>). It is the product of nitric oxide and superoxide anion radical reaction, which are both *in vivo* released during SIN-1 (3-*N*-morpholinonyldimethylamine) decomposition. ONOO<sup>-</sup>, as one of the most important endogenous oxidizing and nitrating species, is a mediator of cellular and tissue injury in various pathophysiological situations. Biomolecular pathways of this metabolite, producing free radicals, are co-responsible for the oxidative and nitrosative stress in biological systems. The *in vitro* study of oxidative degradation of high-molar-mass hyaluronan *via* rotational viscometry has been, recently, the subject of an enormously growing concern. The complex biochemical behavior of ONOO<sup>-</sup> is governed by its chemical structure, conformational forms differing in energy content, reactive intermediates released, contribution of carbon dioxide, trace transition metal ions, presence of trace organics, concentration of this species in solution, and, nevertheless, by the reaction kinetics. Toxicological research has brought hypothesis of ONOO<sup>-</sup> formation *in vivo* resulting in a more intense investigation by biochemists, radical, and inorganic chemists. Current elucidating the ONOO<sup>-</sup> role in etio-pathogenetic mechanisms of human diseases, such as cardio-vascular diseases, stroke, cancer, inflammation, arthritic diseases, neurodegenerative disorders, diabetes mellitus, and diabetic complications has still been the subject of intensive investigations.

Supported by the grant VEGA 2/0003/08, 2/0115/09, 2/0083/09, 2/0056/10, and 2/0011/11 of the Slovak Academy of Sciences

## PROTECTIVE EFFECT OF EXTRACT FROM GENTIANA ASCLEPIADEA ON HUMAN CELLS

Hudecová A. <sup>1,2</sup>, Hašplová K. <sup>1,2</sup>, Magdolenová Z. <sup>2</sup>, Gálová E. <sup>1</sup>, Miadoková E. <sup>1</sup>, Dušínská M. <sup>2,3</sup>

<sup>1</sup>Department of Genetics, Faculty of Natural Sciences, Comenius University, Mlynská dolina, Bratislava, Slovak Republic

<sup>2</sup>Norwegian Institute for Air Research (NILU), PO Box 100, Kjeller, Norway

<sup>3</sup>Slovak Medical University, Limbová 12, Bratislava, Slovak Republic

Oxidative stress has been shown to be associated with oxidative damages caused by the free radicals which may be implicated with chronic diseases like cancer, coronary heart diseases, diabetes, neurodegenerative diseases, and even aging. The scientific interest in identifying novel natural antioxidants for use in foods has considerably increased in recent years, in part because the use of synthetic antioxidants as food additives is limited by specific regulations in various countries, established on the basis of their safety. Intensive research has been carried out either to characterize the antioxidant properties of extracts from several plant materials and/or to isolate and identify the compounds responsible for those activities. Many of the herbal extracts are commonly used in herbal medicine as well as in food technology due to their potential for free radical-scavenging activity.

In our study, we have focused on plant extracts from *Gentiana asclepiadea*. This plant belongs to family *Gentianaceae* which is widely used in traditional medicines in many countries. Some of these plants are used as ingredients in Chinese herbal medicines for stimulation of appetite and gastric secretion, gastro-duodenal protection, liver protection, antifungal treatment and in some cases for women's diseases. We have studied antioxidant potential of methanolic and aqueous extracts from haulm and flower, using DPPH and H<sub>2</sub>O<sub>2</sub> challenging assays. DPPH assay determines potential of scavenging DPPH radicals in the cell free extracts solution. H<sub>2</sub>O<sub>2</sub> challenging assay is based on the comet assay. Results obtained from DPPH assay show, that all of the extracts (at the highest concentrations) were able to scavenge more than 80% of DPPH radicals. Methanolic flower extract scavenged almost 90% of the free radicals. Decrease of scavenging activity was shown in lower concentrations. H<sub>2</sub>O<sub>2</sub> challenging assay was carried out on human lymphocytes. Results indicate that extracts themselves (after 30 min treatment) caused DNA damage detected with the comet assay. Combination of pretreatment with extracts and then challenge with H<sub>2</sub>O<sub>2</sub> suggests, that all of the extracts dispose with antioxidant potential depending on the concentration.

Supported by the grants VEGA 1/0008/08; 2/0072/09; 1/4467/07; APVT-20-0260;1253/2009 and by internal NILU grant 106170

### HEME-APOPROTEIN INTERACTIONS AS A POSSIBLE WAY OF CYTOCHROME P450 ACTIVITY MODULATION

Hudeček J.<sup>1</sup>, Hodek P.<sup>1</sup>, Stiborová M.<sup>1</sup>, Anzenbacher P.<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 12840 Praha 2, Czech Republic

<sup>2</sup>Department of Pharmacology, Palacký University, Hněvotínská 3, 77515 Olomouc, Czech Republic

Mammalian liver microsomal cytochrome P450 (CYP) monooxygenases play a major role in metabolism of many drugs and toxic substances. As a part of larger complexes in the membrane of endoplasmic reticulum (in interaction with NADPH:CYP reductase, and

possibly also with the cytochrome b<sub>5</sub>), they catalyze hydroxylation of plethora of nonpolar substrates.

In the past two decades, we witnessed an impressive growth of structural understanding of CYP enzymes, based chiefly on the X-ray crystallographic data for various members of the CYP superfamily, including some partial information about microsomal forms. This might be regarded as a sort of "static" information. At the same time, there is a growing evidence supporting the picture of cytochromes P450 as dynamic, flexible systems, responding to various regulatory signals – resulting e.g. from the interaction with the reductase or cytochrome b<sub>5</sub>, or from binding of effectors (the second molecule of the substrate, inhibitors, etc.).

Such allosteric signals are transferred through the apoprotein skeleton to the enzyme active site, affecting its structure and properties. Quite often, they influence also the electronic distribution on the heme iron, causing changes in its redox potential (as for example the well-known stabilization of the CYP reduced state after binding of substrate).

Possible ways for this information transfer are: (i) direct modulation of the redox potential via changes in the effective dielectric constant in the heme cavity by changes of its hydration or movement of nonpolar side-chains, (ii) electronic the thiolate axial ligand (*i.e.* direct transfers of electronic density to or from the iron), (iii) changes in the geometry of the porphyrin ring or in the position of the iron within the ring (mainly deviations from planarity of this system), and (iv) influence on the heme side chains (in particular, vinyls and propionates).

The possible relative importance of the above-mentioned mechanisms for allosteric regulation of CYP-catalyzed reactions will be discussed in general terms (on the basis of known data for other heme proteins) as well as specifically, based on the experimental evidence and available structural information for various CYP forms.

Supported by GACR grant GA303/09/0472.

### BAL PARAMETERS AND HISTOLOGICAL FINDINGS AFTER EXPOSURE TO INDUSTRIAL MINERAL FIBROUS DUSTS – DOSE DEPENDENCE

Hurbánková M.<sup>1</sup>, Černá S.<sup>1</sup>, Wimmerová S.<sup>1</sup>,  
Kováčiková Z.<sup>1</sup>, Tátrai E.<sup>2</sup>

<sup>1</sup>Institute of Preventive and Clinical Medicine, Limbová 12, Bratislava, Slovak Republic

<sup>2</sup>National Institute of Environmental Health, Budapest, Hungary

Industrial fibrous dusts are applied in many industrial branches and under certain conditions (long-time exposure, higher dose, respirable fibres) they may represent adverse factors in occupational and environmental area. Refractory ceramic fibres (RCF) and glass fibres (GF) are used as asbestos substitutes. Because some of them are respirable – they might represent a potential health hazard by inhalation.

Two types of asbestos substitute mineral fibres – ASMF (refractory ceramic fibres, glass fibers) as well as amosite asbestos were instilled at 4 doses (0,5; 1; 2; 4



mg/animal). Animals (number: 8 per group) were intratracheally instilled with fibrous dust suspension (1 mg in 0,1 ml of saline solution) or only with saline solution (control group). After sacrifice (2 weeks after intratracheal instillation) the animals were anaesthetised with thiopental –150 mg/kg of animal and bronchoalveolar lavage (BAL) was performed. Following BAL parameters were investigated: Inflammatory response biomarkers: count of BAL cells / ml, count of alveolar macrophages (AM) / ml, the differential count of BAL cells (% AM; granulocytes – Gr; lymphocytes – Ly), phagocytic activity of AM, bi- and multinucleated lung cells, phagocytic activity of AM, viability of AM and histology of lungs. Correlation with dose was found for almost all of examined inflammatory parameters. The dose dependence was found also in histological findings: Glass and RCF fibres almost equal – moderately multifocal interstitial inflammation. Between them and amosite are great differences. The morphological changes are dose dependent within 2 weeks. In our two week experiment the number of significant correlations with dose and also the dose dependence lines of BAL parameters were similar after exposure to all examined fibrous dusts. Histology of lung indicated stronger impact of amosite in comparison with the examined substitutes.

*Supported by the grant APVT-21-011164 and MZ SR 2005/29-SZU-07.*

#### **PERILLYL ALCOHOL ATTENUATES 2-ACETOAMINOFLUORINE INDUCED HEPATOTOXICITY: A MECHANISTIC APPROACH**

**Jahangir T., Ahmad S., Sultana S.**

*Jamia Hamdard University, Hamdard Nagar, New Delhi-62, India*

In our everyday life, we are continuously exposed to an array of chemicals that work as a free radical generator. A promising way to control chemical mutagenesis and carcinogenesis is to prevent the formation of reactive metabolites.

There are multiple steps within pathways in which dietary components or natural compounds can alter gene expression and phenotypes of cells and thus are therefore molecular targets for chemoprevention. We proposed and conducted animal study to evaluate the efficacy of Perillyl alcohol (PA) as a protective agent against 2-AAF induced hepatic toxicity.

Eight week old adult male Wistar rats (150–200 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi. All the chemical used were of highest purity grade. Determination of serum toxicity markers, antioxidant levels involvement of intracellular and extracellular apoptosis pathways of PA induced apoptosis.

PA attenuates against 2-Acetoaminofluorine (2-AAF) toxicity in hepatic tissue by modulating serum toxicity markers and simultaneous recovery in antioxidant armory. PA may be used against short -term sub-acute hepatic toxicities as protective agent in cases where there is involvement of oxidative stress and reactive oxygen species.

A 21-day study did not support the PA efficacy as chemopreventive agent but it certainly gives an insight that PA protects the tissue by enhancement of antioxidants and diminution of toxicity markers.

*Acknowledgement: Author is highly thankful to DST govt. of India for providing financial assistance.*

#### **FORMATION OF REACTIVE OXYGEN AND NITROGEN SPECIES IN THE PRESENCE OF PINOSYLVIN – AN ANALOGUE OF RESVERATROL**

**Jančinová V.<sup>1</sup>, Perečko T.<sup>1</sup>, Nosál R.<sup>1</sup>, Lojek A.<sup>2</sup>, Číž M.<sup>2</sup>, Ambrožová G.<sup>2</sup>, Mihalová D.<sup>1</sup>, Bauerová K.<sup>1</sup>, Harmatha J.<sup>3</sup>**

<sup>1</sup>*Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic*

<sup>2</sup>*Institute of Biophysics AS CR, Královopolská 135, Brno, Czech Republic*

<sup>3</sup>*Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo náměstí. 2, Praha, Czech Republic*

The excessive formation of reactive oxygen and nitrogen species participates in many diseases of the cardiovascular system and their complications. It is involved in neurodegenerative diseases, irreversible metabolic failure (diabetes, atherosclerosis), inflammatory diseases (rheumatoid arthritis), neoplastic complications or in complications of organ transplantation. Previously, we found that incubation of human neutrophils with pinosylvlin decreased formation of reactive oxygen species and this effect was related to the diminished activation of protein kinase C. The present study was undertaken to investigate the effect of pinosylvlin *in vivo* – in rats with adjuvant arthritis, as well as to study the this compound on the production of nitric oxide (NO) in macrophages.

The method of chemiluminescence was used for detection of reactive oxygen species produced by resting and stimulated rat neutrophils. Compared to arthritic controls, animals treated with pinosylvlin (28 days, 30 mg/kg) displayed reduction in blood concentration of oxidants, neutrophil count and in hind paw edema. Moreover, the drop in antioxidative plasma activity was less evident in the presence of pinosylvlin. We assume that all these effects may originate in decreased neutrophil count, resulting from interference of pinosylvlin with pro-inflammatory mediators. In light of the fact that increased NO plasma concentrations were found in patients with rheumatoid arthritis, in further experiments we studied the effect of pinosylvlin on the production of this mediator. In murine macrophages (RAW 264.7 cells), pinosylvlin significantly decreased nitrite accumulation as well as expression of inducible NO synthase. No scavenging activity against NO, measured in cell-free system, was observed.

*Supported by grants APVV-0315-07, APVV-SK-CZ-0034-09, VEGA-2/0003/10, GAČR-203/07/1227.*

## THE STUDY OF BIOCOMPATIBILITY AND CYTOTOXICITY OF SILICON NITRIDE ON HUMAN FIBROBLAST CELL LINES

Jantová S.<sup>1</sup>, Matejov P.<sup>1</sup>, Čopan P.<sup>2</sup>, Bakoš D.<sup>3</sup>

<sup>1</sup>Institute of Biochemistry, Nutrition and Health Protection &

<sup>2</sup>Institute of Inorganic Chemistry, Technology and Materials &

<sup>3</sup>Institute of Polymer Materials, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 81237 Bratislava, Slovak Republic

Silicon nitride ( $\text{Si}_3\text{N}_4$ ) has been used in a number of industrial applications, such as engine components, bearings and cutting tools. Now, it has been studied as potential biomaterial for bone tissue reparation. In our previous experiments, three variant discs of  $\text{Si}_3\text{N}_4$  with different porosity (YA3-5, YA3-10 a YA3-20) was prepared.

In this work, the cytotoxicity and genotoxicity of silicon nitride discs with different porosity were evaluated on human fibroblast VH10 and B-HNF-1 cells. The morphology of fibroblast cells growing with three types of silicon nitride – YA3-5, YA3-10 a YA3-20 were assessed by light microscopy. The cytotoxicity was determined by the direct contact test – vital staining, direct counting of adherent and growing cells and the assessment of LDH level. Comet assay was used for genotoxic study. The dishes without  $\text{Si}_3\text{N}_4$  presence were used as negative control.

We found that both used fibroblast cell lines were sensitive to three different types of  $\text{Si}_3\text{N}_4$ . All used methods have indicated slight cytotoxicity. The difference was observed among  $\text{Si}_3\text{N}_4$  with different porosity. The highest inhibition of cell growth was caused by silicon nitride type YA3-5, on the other hand, types YA3-20 and YA3-10 induced the smallest cytotoxicity on both cell fibroblast lines. These changes in viable cell number were also observed when aliquots of the cultures were examined by light microscopy. The microscopic observations shown that control cells grew on the surface of the cultivation flask. The vast majority of them were scattered and exhibited a typical fibroblast morphology, an elongated and polygonal shape. VH10 and B-HNF-1 cells growing around silicone nitride discs were homogeneously distributed on the cultivation surface with good colonization. No significant morphologic changes were found in treated cells, their morphology was completely similar to that of the control cells. The amount of released LDH in cells cultured with three types of  $\text{Si}_3\text{N}_4$  was increased in comparison to the control and was time-dependent. The percent of released LDH was in the range 1.0–32.7%. While type YA3-5 induced the smallest release of LDH from cells, type YA3-10 caused the highest percent of LDH release on both cell lines. Comet assay showed that none type of silicon nitride discs didn't induce DNA damage in comparison to the control.

It can be concluded that silicone nitride with different porosity showed a slight cytotoxicity, none genotoxicity and very good biocompatibility.

Supported by the Slovak State Committee for Scientific Research VEGA, grant 1/0165/10.

## SKIN AND EYE HAZARD TESTED *IN VITRO* IN A GROUP OF CHEMICALS WITH REFERENCE HUMAN SKIN IRRITATION DATA

Jírová D.<sup>1</sup>, Janoušek S.<sup>1</sup>, Kejlová K.<sup>1</sup>, Bendová H.<sup>1</sup>, Kolářová H.<sup>2</sup>, Malý M.<sup>1</sup>

<sup>1</sup>National Institute of Public Health, Srobarova 48, Prague 10, Czech Republic

<sup>2</sup>Faculty of Medicine, Palacký University, Olomouc, Czech Republic

Various *in vitro* protocols, involving tissue and organotypic models, have been assessed in the recent years with the aim to evaluate skin and eye irritation hazard. The key difficulty in determining the validity of alternative *in vitro* methods is that the *in vivo* animal or human data are both scarce and often of limited utility for hazard prediction.

Consequently, recently obtained human 4h patch test data on skin irritation were extended by a number of *in vitro* data related to skin and eye irritation potential. A group of selected chemicals employed in previous EU validation studies and nine cosmetic formulations were subjected to further *in vitro* testing, including Hen's Egg Test – Chorioallantoic Membrane (HET-CAM), Neutral Red Release/Uptake Assays and test using EpiOcular tissue model (MatTek, USA). Human and animal skin/eye irritation hazard data were compared to the results of the *in vitro* methods.

The study revealed, that skin irritants with reference *in vivo* skin irritation data are not necessarily eye irritants. Volatile or solid materials may be misclassified due to their physicochemical characteristics. NRR assay may provide false negative results in case of substances with fixative effect preventing the NR release or in case of chemicals absorbing NR and not removable under standard washing procedure. Microscopical evaluation is recognized as a crucial additional endpoint for correct result assessment. HET-CAM offers valuable results related to conjunctiva. Although overpredictive, it provides the lowest false negative rate. EpiOcular<sup>TM</sup> assay correctly identified the most aggressive cosmetic formulation, in accordance with NRU and NRR results, while HET-CAM correctly identified the mildest formulation.

The *in vitro* models for testing of skin/eye hazard seem to be a useful tool for the prediction of human hazard, particularly for consideration of initial concentrations for confirmatory human patch tests or clinical trials aimed to confirm safety of consumer products. The advantages and limitations of individual *in vitro* methods are discussed with the view of their inclusion in a battery of *in vitro* methods for eye irritation testing.

Supported by grant projects of the Ministry of Health (No. NS9648-4/2008) and Ministry of Education (MSM No. 6198959216).

## THE SULFUR MUSTARD INDUCED DNA CROSSLINKS AND THEIR EFFECT ON CELL VIABILITY

Jošt P., Svobodová H., Štětina R.

Faculty of Military Health Sciences, Hradec Králové, 5000, Czech Republic

The sulfur mustard (SM) is chemical warfare agent and alkylation agent leads to cell death in several hours after

an exposure. Although SM has been intensively investigated, the cellular mechanisms of cell damage remain unclear.

DNA is one of the target molecule of SM in cell. Alkylation actions of SM form DNA cross-links which stop replication and induce repair of DNA. The DNA cross-links were detected using reverse comet assay and cell viability was detected using MTT assay and colony forming ability. For these assays we have used human cells A-549. The cytotoxicity was measured over the broad range of concentrations from 0.12 to 250  $\mu$ M SM.

Results showed that the viability of cells treated with SM depends strongly upon the time interval. Using the colony forming ability assay we have found, that the colony forming capacity is inhibited at much lower concentrations than show the MTT assay after 24 hours. By the comet assay we have found DNA cross-links in SM-treated cells in the lowest concentration. We compare stability of SM in cell culture medium. After 2 hour is SM hydrolyzed on no toxic products. Cells with the damaged DNA do not replicate and do not divide, while they may keep their metabolic activity. We have found the difference between doses inhibiting the basal metabolism (15  $\mu$ M) and doses inhibiting cell division.

*Acknowledgement: this work was supported by the grant of Czech Ministry of Defence: OVUOFVZ200810 and MO0FVZ0000501.*

#### **POSTNATAL CHANGES OF ENERGY METABOLISM IN THE BRAIN OF NEWBORN RATS IN RELATION TO THEIR SUSCEPTIBILITY TO HYPOXIC-ISCHEMIC INSULT**

Juránek I. <sup>1</sup>, Bačiak L. <sup>1</sup>, Kucharská J. <sup>2</sup>, Sumbalová Z. <sup>2</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic

<sup>2</sup>Pharmacobiochemical Laboratory of the 3<sup>rd</sup> Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Hypoxic-ischemic encephalopathy due to perinatal asphyxia is a major cause of mortality and morbidity of newborns. Mammalian brain is considered to be very susceptible to hypoxia, particularly in the neonatal period. However, some data suggest that the neonatal brain may be rather resistant to hypoxic-ischemic insult (HII). In the present study, we tested our original hypothesis that postnatal changes of brain energy metabolism (BEM) may account for the susceptibility of neonates to HII. We used newborn rats aged 1, 4, 7 and 10 days. To evaluate postnatal maturation of BEM, we applied phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS) *in vivo*. Survival rate of rat pups exposed to anoxia was inversely related to the maturity degree of their BEM. To follow acute alterations in BEM, rat pups were challenged with a single 10-min anoxic episode. Decrease of adenosine triphosphate and phosphocreatine and increase of inorganic phosphate reflected an acute BEM failure. Both anoxic BEM failure and reoxygenation BEM deficit inversely depended on BEM maturity. In parallel sets of experiments, activities of respiratory chain

complexes were determined in isolated rat brain mitochondria; they increased with increasing postnatal age and strikingly reached their maximal values at different ages. Mitochondrial levels of  $\alpha$ -tocopherol, coenzyme Q9/Q10 also increased with rat pup age. Efficiency of oxidative phosphorylation was found to increase in the first two weeks of rat life. These findings suggested that oxidative phosphorylation efficiency may contribute to high resistance of newborn rats to HII. In conclusion, our data provide evidence that the susceptibility of the neonatal brain to HII may indeed be a determinant of the maturity of BEM, and these findings are in support of the proposed hypothesis.

*Acknowledgement: The study was partially supported by the Slovak State Program for Research & Development (2003SP200280203) and Grant Agency VEGA (2/0083/09).*

#### **DEVELOPMENT AND EVALUATION OF THE IN VITRO EPIOCULAR EYE IRRITATION TEST (EPIOCULAR-EIT) IN THE FRAMEWORK OF THE COLIPA-ECVAM FUNDED VALIDATION STUDY TO REPLACE DRAIZE EYE IRRITATION TEST**

Kaluzhny Y. <sup>1</sup>, Kandarova H. <sup>1,2</sup>, d'Argembeau-Thornton L. <sup>2</sup>, Klausner M. <sup>2</sup>, Sheasgreen J. <sup>2</sup>

<sup>1</sup>MatTek Corporation, Ashland, MA, USA

<sup>2</sup>MatTek In Vitro Life Science Laboratories, Bratislava, Slovak Republic

*In vitro* tests for assessing ocular irritancy of broad range of chemicals are urgently needed to comply with EU legislation such as REACH and the 7<sup>th</sup> amendment of the Cosmetics Directive. In response MatTek Corporation developed an easy to use and straightforward eye irritation test, EpiOcular-EIT. After the successful pre-validation EpiOcular-EIT entered COLIPA-ECVAM coordinated validation study in 2010.

**Materials and Methods:** The *in vitro* eye irritation test utilizes reconstructed human corneal epithelium tissue (EpiOcular™, MatTek Corp.). EpiOcular-EIT comprised of a single exposure period of 30 minutes with a 2-hour post-exposure incubation (liquids) or 90 minutes exposure followed by 18-hour post-exposure incubation (solids) to various test articles. Tissue viability was determined by MTT viability assay. A single cut-off in relative survival is used for classification. If a tissue viability decreased below 60% a chemical was classified as irritant (R36 and R41), otherwise the chemical was classified as non-irritant. 94 test articles were tested in 2 independent runs to evaluate sensitivity and specificity of the proposed EpiOcular-EIT. Test articles included alcohols, hydrocarbons, esters, ethers, surfactants, and aromatic compounds. In addition to this trial, to demonstrate long-term reproducibility, 5 irritants and 5 non-irritants, were tested using 25 independent lots of EpiOcular model over a 7 months period.

**Results:** Based on the analysis of 94 test articles, EpiOcular-EIT attained sensitivity above 90% and specificity above 60%, thus fulfilling the requirements to enter the formal validation. In addition, independent study performed by seven EU and US laboratories



confirmed excellent inter-laboratory reproducibility, obtaining >99% of agreement in classification.

Based on the analysis of 10 test articles (with positive and negative controls) tested over the 7 months, qualified results (*i.e.* variability < 20%) were obtained in 297 out of 300 independent trials (99.0%) and correct classification was obtained in 98.3% of the trials. This high level of reproducibility mimics data in the EpiOcular quality control database, publicly available since 1996. Therefore, both, the EpiOcular tissue model and the EpiOcular EIT were shown to be functional and valuable for chemical testing for REACH purposes.

#### MEETING REQUIREMENTS OF THE NEW OECD TG FOR *IN VITRO* SKIN IRRITATION TESTING: REPRODUCIBILITY OF THE EPIDERM SKIN IRRITATION TEST (EPIDERM-SIT) FOLLOWING THE ECVAM VALIDATION AND ACCEPTANCE AS A FULL REPLACEMENT METHOD OF THE *IN VIVO* TEST

Kandárová H.<sup>1,2</sup>, Klausner M.<sup>2</sup>, Deng W.<sup>2</sup>, Kubilus J.<sup>2</sup>, Hayden P.<sup>2</sup>, Kearney P.<sup>2</sup>, Letašiová S.<sup>2</sup>, Sheasgreen J.<sup>2</sup>

<sup>1</sup>MatTek In Vitro Life Science Laboratories, Bratislava, Slovak Republic

<sup>2</sup>MatTek Corporation, Ashland, MA, USA

In autumn 2007, an international validation study was performed to evaluate the reproducibility and confirm the predictive ability of the EpiDerm Skin Irritation Test (EpiDerm-SIT) method. In November 2008, ECVAM endorsed the EpiDerm-SIT as a full replacement method for the *in vivo* rabbit skin irritation test. As reflected in a draft of the OECD guideline for skin irritation testing, US and EU regulators have appropriately maintained that a test method must demonstrate reproducibility on an ongoing basis so that regulators and commercial end users are assured that the assay method is stable and continues to give valid test results over time. The purpose of the present study was to investigate the reproducibility of the EpiDerm-SIT post-validation.

Over a 4-month period in 2009, 12 independent lots of EpiDerm tissue were exposed to 3 irritants (alpha terpineol, heptanal, and butyl methacrylate), 3 non-irritants (benzyl benzoate, benzyl salicylate, and isopropanol) and the positive control (sodium dodecyl sulphate) and negative control (Dulbecco's phosphate buffered saline) using the validated SIT protocol. As per the SIT method, tissue viability was determined using the robust and cost effective MTT assay following a single, 60-minute exposure and 42-hour post-exposure incubation.

In all cases, the EpiDerm SIT method correctly identified the irritants and non-irritants. Coefficients of variation (CV) between the tests (n=12) for the tissue viability for all test articles were <8%, except for isopropanol (IPA) which had a CV of 10.3%. Further study of the IPA results, revealed the importance of thoroughly rinsing the tissue following the 60-minute exposure (as outlined in the SIT method). These results together with quality control results for EpiDerm over the past 13 years confirm that the EpiDerm SIT method is REACH ready and compliant with the EU 7th Amendment to the Cosmetic Directive.

#### EFFECTS OF PLANT ALKALOID LOBELINE ON NICOTINIC ACETYLCHOLINE RECEPTORS

Kaniaková M.<sup>1</sup>, Krůšek J.<sup>1</sup>, Vyskočil F.<sup>1,2</sup>

<sup>1</sup>Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague, Czech Republic

<sup>2</sup>Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic

Lobeline (Lob) is one of alkaloids found in plant *Lobelia inflata* and has a long history of therapeutic usage ranging from emetic and respiratory stimulant to tobacco smoking cessation agent. The molecule of Lob possesses some structural features similar to nicotinic ligands. From binding studies at native nicotinic receptors in the CNS it is known, that Lob displaces [3H]nicotine with high affinity ( $K_i=4-30$  nM). However the overall reports about the role of Lob at nicotinic receptors have been inconsistent.

In this study we used the patch-clamp technique in whole-cell configuration to map the effects of Lob at three main subtypes of nAChRs – human muscle  $\alpha\beta\gamma\delta$  receptors (naturally expressed in TE671 cell line), rat neuronal peripheral  $\alpha3\beta4$  and central  $\alpha4\beta2$  receptors transiently transfected in COS1 cells. Lob alone elicits no or hardly detectable responses, up to 1% of maximal response to acetylcholine, at all receptor subtypes tested. However at  $\alpha3\beta4$  receptors Lob, when applied together with acetylcholine, potentiates responses to low agonist concentrations up to 300% of control. Responses to agonist concentrations higher than  $EC_{50}$  are unchanged or slightly inhibited. At  $\alpha4\beta2$  receptors potentiation or inhibition of acetylcholine responses by Lob depends not only on agonist concentration but also on precise application time schedule. When Lob is applied before acetylcholine the resulting effect is always inhibitory, showing apparent noncompetitive mechanism. At muscle receptors no potentiation but only inhibition is observed after Lob treatment. The degree of inhibition depends on agonist / Lob concentrations used but also in this case the application scheme seems to be very important. Models of different modes of Lob action and complex pattern of binding sites occupation by Lob and other agonist are discussed.

Supported by grants No. 202/09/0806, IAA50110905, LC 554 and AV0Z50110509.

#### THE BENEFIT OF CHOSEN COMBINATIONS OF ACETYLCHOLINESTERASE REACTIVATORS FOR THE ANTIDOTAL TREATMENT OF ACUTE POISONINGS WITH NERVE AGENTS

Kassa J.<sup>1</sup>, Zdarova Karasova J.<sup>1</sup>, Caisberger F.<sup>3</sup>, Kuca K.<sup>2</sup>, Pohanka M.<sup>2</sup>, Bajgar J.<sup>1</sup>

<sup>1</sup>Department of Toxicology, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic

<sup>2</sup>Center of Advanced Studies, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic

<sup>3</sup>Department of Anatomy, Faculty of Medicine of Charles University, Hradec Kralove, Czech Republic

Organophosphorus nerve agents are considered to be the most dangerous chemical warfare agents. Their acute toxic effects are based on the phosphorylation

of acetylcholinesterase leading to the irreversible inhibition of its active site and subsequent overstimulation of postsynaptic cholinergic receptors due to the accumulation of the neurotransmitter acetylcholine in synapses of the central and peripheral nervous systems. The current antidotal treatment of nerve agent poisonings usually includes an anticholinergic agent (preferably atropine) to block the overstimulation of muscarinic cholinergic receptors and an oxime to reactivate nerve agent-inhibited acetylcholinesterase. In the last century, several oximes (pralidoxime, obidoxime, trimedoxime, HI-6) were introduced as antidotes against nerve agents but, unfortunately, their potency to counteract the acute toxic effects of some nerve agents (especially tabun, soman and cyclosarin) is rather limited. Therefore, the replacement of commonly used oximes with a more effective oxime with a broader spectrum has been a long-standing goal for the treatment of nerve agent poisonings. During the past several decades, a lot of new oximes were synthesized. Although some of them are considered to be promising oximes against some nerve agents, none of them is sufficiently effective against all nerve agents regardless of their chemical structure.

Another way how to increase the potency of antidotes to counteract acute toxic effects of nerve agents and broaden their spectrum is to combine chosen oximes in the antidotal treatment. In this presentation, the chosen combinations of oximes (HI-6 + obidoxime or trimedoxime and HI-6 + K203) on the reactivating and therapeutic efficacy of antidotal treatment of acute nerve agent poisoning was evaluated. The ability of chosen combinations of oximes to reactivate nerve agent-inhibited acetylcholinesterase and reduce acute toxicity of nerve agents was compared with the reactivating and therapeutic efficacy of antidotal treatment involving single oxime (HI-6, obidoxime, trimedoxime, K203) using *in vivo* methods.

*The study was supported by the grant of Ministry of Defense – MO0FVZ0000501.*

#### **MICRONUCLEUS FREQUENCY IN HUMAN PERIPHERAL LYMPHOCYTES AND OBESITY**

**Kažimírová A.<sup>1</sup>, Barančoková M.<sup>1</sup>, Bielik L.<sup>2</sup>, Wsólová L.<sup>1</sup>, Volkovová K.<sup>1</sup>**

<sup>1</sup>Slovak Medical University, Limbová 14, Bratislava, Slovak Republic

<sup>2</sup>Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Obesity is a condition in which excess body fat has accumulated to an extent that health may be negatively affected. A combination of excessive caloric intake, lack of physical activity, and genetic susceptibility is thought to explain most cases of obesity, with a limited number of cases due solely to genetics, medical reasons, or psychiatric illness. 1/6 of human beings suffer from obesity. According to available information from the Slovak Ministry of Health more than half of adult Slovak people suffer from obesity. According to OECD Slovak Republic is in the seventh position in adult obesity.

The main aim of our work was to compare micronucleus frequency among people with different stage of obesity divided into groups by BMI, waist circumference (normal men <102, women <88, risk men ≥102, women ≥88) and WHR (Waist to Hip Ratio, normal men <0.9, women <0.85, risk men ≥0.9, women ≥0.85). Cytokinesis-block micronucleus assay in human peripheral lymphocytes we used for analysis of micronucleus frequency. SPSS 16.0 we used for statistical analysis.

Total number of volunteers was 283 (185 women and 98 men) in age 40–45 years. According to BMI participants were divided into 4 groups. There were 148 people (74 women, 74 men) in group 1 (BMI 18.5–24.9), 92 people (48 women, 44 men) in group 2 (BMI 25–29.9), 79 people (53 women, 26 men) in group 3 (BMI 30–39.9) and 14 people (10 women, 4 men) in group 4 (BMI 40 and more).

We did not find differences in micronucleus frequency neither between analysed groups according to BMI nor in relationship to waist circumference or WHR. We found positive correlation between micronucleus frequency and WHR index in males (N=98, r=0.256, p=0.011). Women had statistically significantly higher micronucleus frequencies in comparison to men (p<0.001). Data from literatures suggest a complex interaction between BMI and changes in body weight with regard to biomarkers of genotoxicity in both adults and children. The potential impact of such factors on micronuclei and chromosome aberrations formation in peripheral lymphocytes is unclear and thus there is a need for further research.

*This project is supported by MZ SR 2006/07-SZU-0 and Contract no: 201335; the European Commission DG research contract for the study "HEIMTSA", GOCE-CT-2006-036913-2.*

#### **PHOTOTOXICITY OF SUBSTANCES EVALUATED BY METHODS *IN VITRO* AND CONFIRMED IN HUMAN PHOTOPATCH TEST**

**Kejlová K.<sup>1</sup>, Jírová D.<sup>1</sup>, Bendová H.<sup>1</sup>, Gajdoš P.<sup>1</sup>, Kolářová H.<sup>2</sup>**

<sup>1</sup>National Institute of Public Health, Srobarova 48, Prague 10, Czech Republic

<sup>2</sup>Faculty of Medicine, Palacký University, Olomouc, Czech Republic

The phototoxic potential of chemicals, cosmetics and pharmaceuticals is a growing concern in the consumer products industry. The determination of phototoxicity in the 3T3 Neutral Red Uptake Phototoxicity Test (3T3-NRU-PT), accepted in the EU (2000) and OECD Member States (2004), is the first step in the sequential phototoxicity testing strategy. The reconstructed human skin model assays represent an important supplement to the 3T3-NRU-PT which may be used in order to obtain additional information on bioavailability of the chemical in human skin after topical exposure. The human skin models overcome many of the limitations of the 3T3-NRU-PT, as they employ multi-layer tissues that closely parallel human skin morphology. However, further investigations are needed in the extrapolation of *in vitro* results to the human situation.

The aim of this study was the evaluation of phototoxic potential of a group of selected substances (bituminous tars and essential oils used as cosmetic ingredients). The applied tiered testing strategy included chemical analysis of the substances, 3T3-NRU-PT, and the EpiDerm™ Skin Phototoxicity Assay, performed according to the pre-validated phototoxicity test design. In order to clarify the situation in man, the results determined by the EpiDerm skin model were confirmed *in vivo* by means of the human skin photopatch test in a limited group of volunteers.

Both bituminous tars (Ichthyol pale and Ichthammol) were phototoxic in 3T3-NRU-PT, while only Ichthammol elicited phototoxic reactions in the EpiDerm Phototoxicity Assay and subsequently in the human photopatch test. The study on essential oils revealed, that their phototoxicity potential was dependent on the content of photoactive substances, particularly furocoumarins, and the solvent used. Using aqueous dilutions, a phototoxic classification was obtained *in vitro* and in some cases experienced also *in vivo* in the human photopatch test, if the highest non-phototoxic concentrations determined in the EpiDerm Assay were applied. The 3D human skin model test seems to be a useful tool for consideration of initial concentrations for confirmatory human photopatch tests to prove product safety, however, a safety factor of 10 might be considered for extrapolation.

Supported by a grant project of the Ministry of Health of the Czech Republic (No. NS9648-4/2008).

### MERCURY SPECIATION IN FISH TISSUES FROM THE MOST IMPORTANT FISHING LOCATIONS (CZECH REPUBLIC)

Kenšová R., Kružíková K., Pištěková L., Poláčeková L., Svobodová Z.

Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

Mercury is a toxic global pollutant occurring in the environment and it negatively influences living organisms. The most toxic form of mercury is organic methylmercury. It is produced by the sediment microorganisms from inorganic mercury molecules. Methylmercury cumulates in the water organisms and then it is spread through the food chain to final consumers including humans. The occurrence and concentration of mercury in water environment is a hot topic and a close attention is paid to this problem. This attention is primarily focused on contaminated locations, but from the food safety point of view, it is necessary to concentrate also on locations visited and exploited by sport fishers. AWI value (1,6 µg/kg live weight) established by WHO for methylmercury together with the assessed level of methylmercury content in the muscle tissue of analysed fish allows us to determine weekly limit/amount of fish muscle from the particular location which can be consumed.

The aim of the presented study was to determine the amount of muscle tissue from fish from the chosen locations which can be eaten without having any impact on the health of consumers, and to assess the distribution of total mercury (THg) and methylmercury (MeHg) in the tissues of analysed fish. Specimen from six different fish species (chub, roach, common carp, bream, pike, asp) were obtained from seven important fishing locations in the Czech Republic in the spring 2010. Liver, muscle and gonad tissues were taken for further analyses. Locations chosen (river Lužnice–Soběslav; river Lužnice–Suchdol; river Berounka nad soutokem s Vltavou; water reservoirs Jordán and Trnávka, river Otava–Strakonice; river Otava–Sušice) belong to important fishing grounds and are much-frequented by sport fishermen. Total Hg assessment was performed using atomic absorption spectrometry method and AMA 254 analyser (Altec, Prague), speciation of MeHg was performed using gas chromatography with ECD detection (GC2010A chromatograph). Values of THg and MeHg measured were processed statistically. The results were used for the assessment of THg and MeHg distribution in the tissues of different fish species, THg liver/muscle tissue content ratio assessment and further for the health risk assessment.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic MSM 6215712402 and IGA 84/2010/FVHE.

### EFFECTS OF LUTEINIZING HORMONE –RELEASING AGONIST LEUPRORELIN ON SPERMOGENESIS: A TRANSMISSION ELECTRON MICROSCOPIC STUDY

Khajeh Jahromi S. <sup>1</sup>, Ghasemi F.M. <sup>1</sup>, Soleimani Rad J. <sup>2</sup>

<sup>1</sup>Anatomy & Histology Department, Faculty Of Medicine, Guilan University Of Medical Sciences, Rasht, Guilan, Iran

<sup>2</sup>Drug Applied Research Center, Tabriz University Of Medical Sciences, Tabriz, Iran

Gonadotropin releasing hormone analogues (GnRH) suppress the pituitary-gonadal axis and can affect on spermatogenesis or spermiogenesis process. The objective of this study was to investigate the ultrastructural alterations in spermiogenic cells following administration of a single dose of leuprolide acetate.

This study was done on 24 adult males at the age of 8 weeks. The animals were divided into 3 groups. The control group: received no drugs, group 2: received 0.2 ml carboxymethyl cellulose, group 3: received 7.6 mg/kg leuprolide acetate. Study of the spermiogenic cells were made by transmission electron microscopy (TEM) and light microscopy.

The ultrastructural study revealed that alterations were observed in spermiogenic cells. The nuclei and acrosomes in most of round spermatids had been deformed. In some developing spermatids, there were acrosomal vesicles inside the nucleus and ectoplasmic specialization had been partially deleted in some areas. The flagella were deformed in elongated spermatids and their fibrous sheets were discontinuous. In light microscopy the maturity of spermatogenic cells, based



on Johnson's testicular biopsy score, in the control, sham and leuprolide treated groups were  $8.1 \pm 0.53$ ,  $8.04 \pm 0.82$  and  $7.01 \pm 0.57$  respectively and maturation had been significantly reduced in the third group ( $p < 0.01$ ). In addition, all histometric parameters in seminiferous tubules had been decreased compared to the sham and control groups ( $p < 0.01$ ).

Administration of a single dose of leuprorelin during a spermatogenetic cycle in mice is associated with adverse effects on spermatogenesis and it is mostly associated with alterations in spermiogenesis.

### **BOTH ATORVASTATIN AND OMEGA-3 FATTY ACIDS EXHIBIT ACUTE ANTIARRHYTHMIC EFFECTS IN HYPERTRIGLYCERIDEMIC RATS**

**Knezl V., Bacova B.<sup>1</sup>, Radosinska J.<sup>1</sup>, Karas S.<sup>2</sup>, Tribulova N.<sup>1</sup>**  
*Institute of Experimental Pharmacology and Toxicology Slovak Academy of Sciences, Bratislava, Slovak Republic*

<sup>1</sup>*Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic*

<sup>2</sup>*Institute of Measurements, Slovak Academy of Sciences, Bratislava, Slovak Republic*

**Background and purpose:** It has been reported that atorvastatin and omega-3 fatty acids exhibit antiarrhythmic effect in clinic but underlying mechanisms are not elucidated yet. We have previously shown that prolonged treatment of hypertriglyceridemic rats (HTG) with these compounds reduced the incidence of malignant arrhythmias and our findings suggest that myocardial intercellular connexin channels, which ensure electrical coupling and synchronisation, are implicated. To elucidate further how atorvastatin and omega-3 FA may modulate intercellular coupling, this study was aimed to examine whether these compounds exert acute antiarrhythmic effects. **Design and Methods:** Experiments were conducted on male and female five-month-old HTG rats known to be much prone to ventricular fibrillation (VF) than healthy rats. The heart was excised from anesthetized rats and perfused via aorta with oxygenated Krebs-Henseleit solution at constant flow (10–14 ml/min, female-male). Upon equilibration, VF inducibility was tested using electrical stimulation. Repetitive stimulation was performed during 5 min period unless sustained ( $>2$  min) VF occurred earlier. The hearts were perfused with Atorvastatin (Zentiva), eicosapentanoic acid (EPA) or docosahexanoic acid (DHA) (1.5, 7, 15  $\mu$ mol) during 10 min prior el. stimulation.

**Results:** Sustained VF was induced in all HTG rat hearts without treatment. In contrast, the hearts subjected to atorvastatin, EPA and DHA were less susceptible and incidence of sustained VF was reduced to 33%, 71.4% and 80% in male and to 60%, 75% and 60% in female rats. Atorvastatin suppressed VF inducibility in male rats already in concentration 1.5  $\mu$ mol while EPA and DHA were efficient at higher 7 and 15  $\mu$ mol. Strikingly, bolus of either EPA or DHA (150  $\mu$ mol) administered directly to fibrillating heart defibrillated it.

**Conclusions:** Atorvastatin, EPA and DHA exhibit clear antifibrillating and defibrillating efficacy when

acutely applied. This fact suggests that these compounds can likely affect directly connexin channels function in addition to their chronic effects on cell membrane lipid compositions (fluidity) that can affect protein channels conformation and function.

*Supported by the grant APVV 51-059505 and 51-017905, VEGA 2/0086/08.*

### **PHARMACOGENETICS OF THIOPURINES**

**Kolorz M.<sup>1</sup>, Bartosova L.<sup>1</sup>, Suchankova J.<sup>1</sup>, Bartos M.<sup>2</sup>**

<sup>1</sup>*Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, UVPS Brno, Czech Republic*

<sup>2</sup>*Department of Natural Drugs, Faculty of Pharmacy, UVPS Brno, Czech Republic*

Thiopurines (azathioprine, 6-merkaptopurine, 6-thioguanine) are widely used cytotoxic drugs indicated as immunosuppressives (e.g. IBD, RA, and other chronic inflammatory conditions) or cytostatics (e.g. childhood ALL). Because of narrow therapeutic interval therapy of thiopurines is associated with occurrence of side effects, in some cases life threatening. Most of these side effects are attributed to deficiency of Thiopurine S-methyltransferase enzyme activity, which is crucial for metabolism/inactivation of thiopurines and its active metabolites. Enzyme activity is influenced by occurrence of single nucleotide polymorphisms – SNP in gene sequence. Patients with nonstandard gene alleles have higher risk of myelosuppression during therapy with standard doses of thiopurines than patients with standard alleles. There are another enzymes which are responsible for thiopurine metabolism besides TPMT. Enzyme ITPase is probably associated with occurrence of the leucopenia, and other side effects of azathioprine treatment. Lower ITPase activity is associated with SNP C94A.

We studied 3 SNP in TPMT gene (G238C, G460A and A719G) and 1 SNP in ITPase gene (C94A). Our group consists of 174 patients who have been treated with azathioprine (36 individuals with leucopenia during treatment, 138 without leucopenia). Genotypes of TPMT and ITPase were determined by PCR and RealTime PCR.

Chi-squared test confirmed statistical association between the presence of non-standard TPMT alleles and the adverse effect of the azathioprine treatment –leucopenia ( $p = 0.0004$ ), but statistical association was not proven between leucopenia and nonstandard alleles of ITPase gene (94A). However we found statistical association ( $p = 0.043$ ) between the presence of ITPase 94A allele and digestive intolerance to azathioprine, although the number of these patients was very small ( $n = 4$ ).

Our results confirm that presence of non-standard allele of TPMT gene in genome is associated with the risk of leucopenia in patients treated with azathioprine, nonstandard alleles in other genes are associated with less serious adverse effects.

*This project was supported by the IGA VFU grant project No. 2/2010/FaF.*

## CYTOCHROME B<sub>5</sub> – A KEY PROTEIN MODULATING ACTIVITIES OF CYTOCHROMES P450

Kotrbová V.<sup>1</sup>, Mrázová B.<sup>1</sup>, Martínek V.<sup>1</sup>, Frei E.<sup>2</sup>, Stiborová M.<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic

<sup>2</sup>Department of Molecular Toxicology, German Cancer Research Center, 69 120 Heidelberg, Germany

Cytochromes P450 (CYPs) are responsible for the metabolism of a wide variety of xenobiotics. Many CYP-dependent reactions have been shown to be stimulated by another microsomal protein, cytochrome b<sub>5</sub> (b<sub>5</sub>). There are two hypotheses trying to explain its effect. The first one supposes participation of b<sub>5</sub> in processes connected with electron transfer. The second hypothesis proposes the b<sub>5</sub>-mediated induction of conformational changes in CYPs, producing enhanced oxidation. This hypothesis is based on finding showing that not only holoprotein of b<sub>5</sub>, but also its apo-form, which is not capable of electron transfer, can contribute to stimulation effects.

Because apo-b<sub>5</sub> is crucial for studies explaining mechanisms of b<sub>5</sub> action, preparation of apo-b<sub>5</sub> protein was one of the aims of this study. CYPs and b<sub>5</sub> were purified from rabbit liver microsomes. To prepare apo-b<sub>5</sub> several different approaches were used: (i) extraction of heme from b<sub>5</sub> by acid acetone, (ii) heme transfer from b<sub>5</sub> to a protein with higher affinity toward the heme, apomyoglobin and (iii) heterologous expression in *E. coli* in an absence of heme synthesis precursor.

The patterns and amounts of ellipticine metabolites formed by CYP1A1 vary significantly by b<sub>5</sub>. The formation of detoxication products (7-hydroxy- and 9-hydroxy-ellipticine) is decreased, while generation of those responsible for DNA adduct formation (13-hydroxy- and 12-hydroxyellipticine), is increased. The elevated generation of ellipticine-DNA adducts mediated by b<sub>5</sub> was confirmed by the <sup>32</sup>P-postalbeling assay. In the case of Sudan I, production of all metabolites by CYP1A1 is markedly enhanced by b<sub>5</sub>. Analogous experiments were carried out with CYP3A4. Here, formation of all oxidation products of both substrates is higher in the presence of b<sub>5</sub>. The levels of Sudan I metabolites are increased even by apo-b<sub>5</sub>. The b<sub>5</sub> containing protoporphyrines with different metal ions as cofactor or other proteins with or without heme in their molecules do not stimulate oxidation of both substrates. The results indicate that probably both possible stimulation mechanisms mentioned above are involved in the substrate oxidation.

Supported by GACR (203/09/0812, P301/10/0356), Czech Ministry of Education (MSM0021620808 and 1M0505) and GAUK (127208).

## THE EFFECT OF SECONDARY METABOLITES PRODUCED BY INDOOR FUNGI ON LUNG

Kováčiková Z.<sup>1</sup>, Piecková E.<sup>1</sup>, Mesárošová M.<sup>2</sup>, Hurbánková M.<sup>1</sup>, Černá S.<sup>1</sup>, Majorošová M.<sup>1</sup>, Gábelová A.<sup>2</sup>, Tátrai E.<sup>3</sup>

<sup>1</sup>Slovak Medical University, Bratislava, Slovak Republic

<sup>2</sup>Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovak Republic

<sup>3</sup>NIOH, Budapest, Hungary

Exposure to microbes is recognized as a potential cause of inflammation related health problems among occupants of mouldy buildings. As the inhalation route is a possible route of their entry into the organism our attention in experiments were focused on lung. Both spores and secondary metabolites produced by fungi may generate inhalation problems. We studied the effects of secondary metabolites, they represent mixture of compounds, some of them with carcinogenic potency.

*Aspergillus ustus* was used in our experiments. After cultivation under standard laboratory conditions their secondary metabolites were isolated. Their effects were studied *in vitro* on alveolar macrophages (AM) and alveolar epithelial type 2 cells (T2) isolated from rats. Changes in antioxidant status, lectin staining, activity of alkaline phosphatase in type 2 cells and activity of acid phosphatase in AM were found. The results showed toxic effects of tested metabolites on both cell types. The effect was dose dependent, alveolar epithelial type 2 cells were more sensitive than alveolar macrophages.

The same metabolites were used in *in vivo* study. The rats were exposed intratracheally, 4 µg/200 µl were used per dose and the exposure lasted 72 hours. The antioxidant status in lung tissue, cell free fraction of bronchoalveolar lavage fluid and two types of isolated cell (AM, T2) was analysed. In contrast to *in vitro* studies there were found only small differences but the comet assay in isolated cells showed statistically significant enhancement in DNA breaks.

The different results between *in vitro* and *in vivo* experiments can be explained both by some protective mechanisms which were not present in *in vitro* system and by the used concentration. But the *in vivo* experiments revealed significant enhancement in DNA damage especially in T2 cells which are thought to be progenitors of some tumours.

Supported by the grant Science and Technology Assistance Agency under the contract 0322-07 and VEGA contract 2/0051/09.

## INHIBITION OF BENZO[A]PYRENE – INDUCED DNA DAMAGE IN HEPG2 CELLS BY FLAVONIDS

Kozics K., Valovičová V., Slameňová D.

Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute, Slovak Academy of Sciences, Vlárská 7, 833 91 Bratislava, Slovak Republic

Flavonoids are plant derivatives of flavone, which exhibit a variety of biological actions, including anticarcinogenic and antioxidant activities. The chemical structure of these compounds is characterized by various degrees of hydroxylation and glycosidic substitutions. In the present study we investigated the protective effect of flavonoids against-benzo[a]pyrene (BaP)-induced DNA damage in human hepatocellular carcinoma (HepG2) cells, the suitable *in vitro* model of human hepatocytes. BaP-induced genotoxic effects were evaluated using micronucleus test and single cell gel electrophoresis (comet assay). From the all flavonoids tested, fisetin, kaempferol, quercetin, galangin and luteolin

(hydroxylated at the 3',4'-position on the B ring, 3 – position of C ring and on the A ring,  $c=2.5\mu\text{mol/l}$ ) were able to significantly inhibit BaP-induced DNA damage. Chrysin, 7-hydroxyflavone, 7,8-dihydroxyflavone and baicalein (hydroxylated on the A ring) showed inhibition at higher concentrations only (10 and  $25\mu\text{mol/l}$ , respectively). Only rutin (hydroxyl group at the position 3 of C ring is substituted by the sugar rutinose), was not able to inhibit effectively changes induced by BaP in cellular DNA. Our results showed that inhibition of BaP-induced DNA lesions correlates with the structural arrangement and organization of the hydroxyl groups in the molecule structure of the flavonoids tested.

Supported by the Slovak Agency VEGA grant 2/0072/09 and The Agency of the Ministry of Education of the Slovak Republik for the Structural Funds of EU (No. 26240120008).

### DRUG ABUSE AT CHILDREN AND ADOLESCENT IN SLOVAK REPUBLIC

Kresánek K.<sup>1</sup>, Plačková S.<sup>2</sup>, Cagáňová B.<sup>2</sup>, Furková K.<sup>3</sup>

<sup>1</sup>Department of Adolescence Medicine, Slovak Medical University Bratislava, Slovak Republic

<sup>2</sup>National Toxicological Information Centre, Department of Occupational Medicine and toxicology, University Hospital Bratislava, Slovak Republic

<sup>3</sup>Department of Pediatrics, Slovak Medical University Bratislava, Slovak Republic

The drug abusing structure has dramatically changed since 1989. While in 1989 the sniffing of the fluid drugs represented 98% of the global drug abuse, the most abused drugs were: heroin, marijuana, amphetamine and its derivatives. During last 10 years situation with drug abuse has changed. Currently the most abused drugs: amphetamines, cannabinoids, plant drugs (*Datura stramonium*, hallucinogenic mushrooms-*Psilocybe semilanceata*, nutmeg-the seed of *Myristica fragrans*) combined with the alcohol are popular among young abusers. According to an analysis of the phone consultations in our Toxicological Information Centre (TIC) we found out, that the number of intoxications with the plant drugs has increased five times during the last year because of their easy availability, low price and quick spreading of information. The sources of intoxication drugs 2,49%. The situation is currently comparable with other countries within the E.U.

### CHANGES OF MERCURY CONTENT IN FISH FROM THE ŽELIVKA RESERVOIR – SYNDROME OF NEWLY FILLED RESERVOIR

Kružíková K.<sup>1</sup>, Dušek L.<sup>2</sup>, Hejtmánek M.<sup>3</sup>, Vostradovský J.<sup>4</sup>, Svobodová Z.<sup>1</sup>

<sup>1</sup>Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, Brno, Czech Republic

<sup>2</sup>Institute of Biostatistics and Analyses, Masaryk University, Czech Republic

<sup>3</sup>Institute of Chemical Technology Prague, Czech Republic

<sup>4</sup>Rybář s.r.o., Praha, Czech Republic

The accumulation of mercury in the tissues of fish found in recently impounded reservoirs has been known for forty years. Because of the risks to human consumers of these fish, it is important to determine how long after impoundment this will continue to occur.

In the manmade reservoir for drinking water, Želivka (Czech Republic), builded from 1970 to 1974, the systematic investigation of bioaccumulation of mercury in fish from 1974 to 1997 and in 2009 was performed. In the Želivka reservoir, the main fish species were sampled and mercury concentration in the fish muscle and liver were analyzed. The results of the changes of mercury contamination in a time are summarized and discussed in this study. The data for *Rutilus rutilus*, *Perca fluviatilis*, *Sander lucioperca*, *Abramis brama*, *Aspius aspius* were evaluated. The samples were analyzed after acid digestion using method of cold vapor atomic absorption spectrometry.

In the period 1974–1988 have been founded a quite high value (about 1 mg/kg) of mercury content in fish tissues. Although there is not any source of mercury contamination in the Želivka reservoir, about 55% of analyzed sampled (predatory species) exceeded 0.6 mg/kg and all of non-predatory species exceed 0.1 mg/kg. An expressive reduction have been found after twenty years and in 2009 following mercury content in fish tissues was determined (for predatory: most for *Aspius aspius* 0,197 mg/kg in the muscle; for non-predatory: most for *Rutilus rutilus* 0.034 mg/kg). In 2009, any samples did not exceed the hygienic limit 0.5 mg/kg set by Commission regulation 1881/2006.

The hypothesis has been pronounced that in the newly filled reservoir are suitable physico-chemical and biological conditions for change of anorganic mercury to organic mercury and their ingression to the food chain. Mercury levels in fish tissues are establish in time.

Supported by the grant MSM 6215712402.

### IMPACT OF FLAVONOIDS ON CYTOCHROMES P450 AFTER PER ORAL ADMINISTRATION TO MALE RATS

Křížková J., Fousová P., Zawadová D., Stiborová M., Hodek P.

Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 2030, 128 43 Prague 2, Czech Republic

Flavonoids are popular phytochemicals used in various food supplements and drugs. These compounds, however, are foreign compounds modulating the activity of cytochrome P450s (CYPs), xenobiotic-metabolizing enzymes involved in the activation and detoxification of carcinogens.

The objective is to study interactions between flavonoids and CYPs. We focused on the sequential *p.o.* administration, which comprised the administration of a carcinogen preceded by the administration of a flavonoid to male rats.

We decided to use  $\beta$ -naphthoflavone (BNF) as a model flavonoid. The two selected carcinogens were benzo(a)pyrene (BaP) and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). The rats were treated



with BNF and 72h after that BaP (150 mg/ml) or PhIP (50 and 150 mg/ml) were administered. In microsomes prepared from liver and small intestine, the expression and specific activity of CYP1A1/2 were determined using Western blotting and marker activity assays with alkyl-resorufin derivatives.

In liver, BNF caused a significant increase in the specific activity only in combination with BaP, however, it had no effects in combination with PhIP in both of the concentrations. To evaluate the effects of flavonoids on CYPs along small intestine, the tissue was dissected into proximal, middle and distal parts. At the lower concentration of PhIP, the administration of BNF caused an increase in the activity and also in the expression of CYP1A1 in all intestinal parts. Contrary to that, the higher concentration of PhIP caused a decrease of the specific activity.

We have demonstrated that in liver, PhIP at the higher concentration level is an inducer of CYP1A1/2 but to a lesser extent than BaP at the same concentration. In small intestine, we observed the highest CYP1A1 activity in the proximal part with a decrease to the distal part. The effect of BNF will be discussed.

*This work was supported by the Czech Science Foundation (Grant No. 305/09/H008) and the Grant Agency of Charles University (Grant No. 4909).*

#### SEARCHING FOR A UNIVERSAL OXIME FOR TREATMENT OF ORGANOPHOSPHORUS PESTICIDE POISONINGS

Musilek K.<sup>2</sup>, Petroianu G.<sup>3</sup>, Kalasz H.<sup>4</sup>, Zdarova-Karasova J.<sup>2</sup>, Kassa J.<sup>2</sup>, Hrabanova M.<sup>1</sup>, Pohanka M.<sup>1</sup>, Kuca K.<sup>1,2</sup>

<sup>1</sup>Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>2</sup>Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>3</sup>Department of Pharmacology & Therapeutics, FMHS, UAE University, Al Ain, UAE

<sup>4</sup>Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

According to the present knowledge, none of the currently available oximes (pralidoxime, obidoxime, trimedoxime, MMB-4 or HI-6) originally developed for the treatment of the nerve agent poisonings is able to treat organophosphorus pesticide poisoning. Among them, obidoxime seems to be the best candidate, however, its high toxicity disfavors its application in the high quantities.

As byproduct of our searching for the new nerve agent reactivators, we found that oxime K027 seems to be very promising in the case of the treatment of organophosphorus pesticide poisonings. Its reactivation potency is similar or better than that of obidoxime, and moreover, its acute toxicity is lower. Thanks to these results, this oxime seems to be the best candidate for future use as universal reactivator for the treatment of poisonings caused by organophosphorus pesticides.

*This work was supported by the project of Ministry of Defence (Czech Republic) – Project No. MO0FVZ0000501.*

#### DEVELOPMENT OF NOVEL MEANS FOR SKIN DECONTAMINATION

Kuca K.<sup>1,2</sup>, Cabal J.<sup>2</sup>, Marek J.<sup>2,3</sup>, Misik J.<sup>2</sup>, Pavlikova R.<sup>2</sup>, Novotny L.<sup>1</sup>, Pohanka M.<sup>1</sup>

<sup>1</sup>Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>2</sup>Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>3</sup>Vakos XT a.s., Prague, Czech Republic

Decontamination of chemical warfare agents together with several biological pathogens are of high interest nowadays. At our institute, we are focused on preparation of novel detergents having in their structure quaternary nitrogen. Such detergents could be used as part of the decontamination means (thanks to their ability to form micelles which can increase velocity of nerve agent decontamination) and disinfection means (thanks to the cell membrane disruption). Recently, we have prepared several series of novel quaternary detergents derived from benzalkonium and pyridinium salts. These compounds are currently tested for their decontamination and disinfection potency. In this contribution, approaches used at our department for the development of novel decontamination means together with novel decontamination products will be presented.

*This work was supported by the project of Ministry of Defence (Czech Republic) – Project No. OVUOFVZ200803.*

#### PROBIOTIC STRAIN ESCHERICHIA COLI NISSLE 1917 ON ABSORPTION KINETICS OF <sup>13</sup>C-D-XYLOSE IN EXPERIMENTAL PIGS

Kuneš M.<sup>1</sup>, Květina J.<sup>1</sup>, Bureš J.<sup>2</sup>, Voříšek V.<sup>3</sup>, Varayil J.E.<sup>2</sup>, Ali S.M.<sup>2</sup>, Rejchrt S.<sup>2</sup>, Palička V.<sup>3</sup>

<sup>1</sup>Institute of Experimental Biopharmaceutics, Joint Research Centre of Czech Academy of Sciences and PRO.MED.CS Praha a.s., Hradec Králové, Czech Republic

<sup>2</sup>2<sup>nd</sup> Department of Medicine, Charles University Teaching Hospital, Hradec Králové, Czech Republic

<sup>3</sup>Institute of Clinical Biochemistry and Diagnostics, Charles University Teaching Hospital, Hradec Králové, Czech Republic

A number of recent studies have utilized D-xylose test as an investigative tool to study changes of gut absorption capacity. We tried to evaluate the changes in absorption function of pig's gut after E.coli medication and after the induction of small intestinal injury (evoked by indomethacin) using non radioactive <sup>13</sup>C isotope labelled D-xylose.

Female mature pigs (*Sus scrofa* f. domestica, 4 – 5 months old) entered the study: group A=controls (n=6), B=probiotic alone (n=6), C=indomethacin alone (n=5), D=probiotic plus indomethacin (n=5). Probiotic (EcN 1917; 3.5 × 10<sup>10</sup> bacterias / day for 14 days and/or indomethacin (15 mg/kg/day for 10 days) were administered orally prior the xylose test. Xylose- tests were performed after overnight fasting, blood plasma samples were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 and 24 hours after intragastric administration of <sup>13</sup>C-D-xylose (100 mg per animal: Cambridge Isotope Lab. Andover, USA). All

plasma samples were frozen ( $-70^{\circ}\text{C}$ ) and synchronous measurement was accomplished by means of EA/IRMS (elemental analyzer isotope-ratio mass spectrometer Hydra 20-30, SerCon, Crewe UK). Standard  $^{13}\text{C}/^{12}\text{C}$  composition, each bath-sample was bracketed by a set at 200mg/mL of certified sucrose standard solution with final amount of 0.4 mg of carbon in one capsule. Raw data were processed using Callisto software (SerCon, Crewe, UK). Probiotics and indomethacin medication alter (increase) the overall D-xylose absorption comparing to the control. There were found significantly elevated values of parameter  $c_{\text{max}}$  in group C ( $p \leq 0.05$ ) and D ( $p \leq 0.05$ ) and parameter AUC in group B ( $p < 0.05$ ) (compared to control). Although average values of all basic pharmacokinetic parameters were elevated in all medicated groups comparing to controls, there also were found high interindividual variability in absorption of D-xylose.

The study was supported by research grant of the Czech Science Foundation No. 305/08/0535 and by research project MZO 00179906.

#### USE OF ELECTROGASTROGRAPHY FOR TESTING OF DRUGS INDUCED GASTRIC PROKINETIC EFFECT (MODEL DRUG: ERYTHROMYCIN) IN A PRECLINICAL TRIAL (IN EXPERIMENTAL PIGS)

Kvěšina J.<sup>1</sup>, Varayil J.E.<sup>2</sup>, Ali S.M.<sup>2</sup>, Kuneš M.<sup>1</sup>, Tachecí I.<sup>2</sup>, Bureš J.<sup>2</sup>, Rejchrt S.<sup>2</sup>, Kopáčová M.<sup>2</sup>

<sup>1</sup>Institute of Experimental Biopharmaceutics, Joint Research Centre of Czech Academy of Sciences and PRO.MED.CS Praha a.s., Hradec Králové, Czech Republic

<sup>2</sup>Department of Medicine, Charles University Teaching Hospital, Hradec Králové, Czech Republic

Surface cutaneous electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical activity. The aim of this study was working out the methods for EGG in experimental pigs for possibility of pharmacological and toxicological screening of drugs with potential gastric activity.

Six mature female pigs 4–5 months old entered the study. EGG was recorded using a Digtrapper EGG (Synectics Medical AB, Stockholm). All EGGs were carried out under general anaesthesia (i.m. of ketamin and azaperone in introduction, i.v. of thiopental in consequences). Electrodes placement always began with placing the first one roughly within 5 cm of xyphoid process in the centre and then subsequently placing the other two left and right hypochondrium respectively. Seven separate measurements were accomplished in each animal. The first one as a “baseline measurement” (in fasting condition) before erythromycin administration. The second 30-minute measurement was carried out immediately after intragastric administration of prokinetic model drug (1600 mg erythromycin). Another 30 min measurements were made between minutes 30–60, 60–90, 90–120, 240–270, 330–360. Results: Baseline dominant frequency in pigs was  $3.7 \pm 0.5$  cycles per minute. There was a significant prokinetic effect of erythromycin (compared to baseline recording): a statistically significant increase in dominant frequency

at recordings starting 90 min ( $p=0.041$ ) and 330 min ( $p<0.001$ ) after erythromycin administration.

Conclusion: EGG in experimental pigs is feasible. Intragastric administration of therapeutic dose of erythromycin significantly increased the gastric myoelectrical activity. EGG is usable to identify myoelectric changes of the stomach induced by prokinetics drugs.

Supported by the grant GAČR 305/08/0535 and research project MZO 00179906.

#### SELENITE INDUCED *IN VITRO* MODELLING OF NUCLEAR CATARACTOGENESIS IN RATS

Kyselova Z.<sup>1</sup>, Weismann P.<sup>2</sup>, Drimal J.<sup>1</sup>, Stefek M.<sup>1</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

<sup>2</sup>Institute of Anatomy, Faculty of Medicine, Comenius University, Slovak Academy of Sciences, Šinkova 2, 811 08 Bratislava, Slovak Republic

The cataract accounts for approximately 42% of all cases of blindness worldwide. The origination might be related to either of different causes: trauma, chemicals, radiation, genetic predisposition, aging and metabolic diseases (e.g. diabetes mellitus). At present time, the only possible cure for degenerative changes of the eye lens is surgery. However, the risks of subsequent complications are relatively high (e.g.: opacification of posterior capsule, endoftalmitis). Moreover, the uncorrectable optical defects are inevitable. Endeavour to solve cataract pharmacologically is tremendous. The ocular lens appears to be an ideal organ for maintaining culture conditions because of lacking blood vessels and nerves. The lens *in vivo* obtains its nutrients and eliminates waste products *via* diffusion with the surrounding fluids. Lens opacification observed *in vivo* can be imitated *in vitro* by addition of the cataractogenic agent sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) to the culture medium.

The aim of this study was to offer novel alternative approach for the evaluation of eye lens opacification. The main advantages of the proposed procedure are that is available in common biochemical laboratory with an instrumentally undemanding equipment and also is detached from observer's experiences. The tested eye lens of male Wistar rats cultivated *in vitro* were scanned directly on the U-96 plate by spectrophotometer (Infinite M200, Tecan, Switzerland). These measurements were correlated with digital image analysis (morphometric software Analysis, Olympus, Japan) of scanned lens (CanoScan 8800F, Canon, Japan). The scale for quantification cataract stages was established and validated on selenite-induced nuclear cataract in rats. The preliminary data on long-term cultures of epithelial cells isolated from adult rats lens capsule were also acquired.

The eye lens opacity quantification procedure suggested may be helpful in searching for substances potentially efficient in pharmacological preventing or delaying the degenerative cataractous changes of the eye lens.

Supported by the grant VEGA 2/0056/09 and VEGA 2/0001/08.

### ELISA KITS FOR DETECTION OF BISPHENOL A, POLYCYCLIC AROMATIC HYDROCARBONS AND MICROCYSTIN IN ENVIRONMENTAL SAMPLES

Lábaj J. <sup>1</sup>, Černohorská H. <sup>1</sup>, Grígel J. <sup>1</sup>, Šteinhachová M. <sup>1</sup>, Lepša L. <sup>1</sup>, Bláha L. <sup>2</sup>

<sup>1</sup>Vidia Ltd., Research Department, Nad Safinou II/365, 252 42 Vestec, Czech Republic

<sup>2</sup>Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Brno, Czech Republic

One of the biggest problems of our civilization is the decrease of the environmental quality, which is caused not only by the industrial activity but also by the lifestyle of the population. The pollutants become a negative component of water, soil, sediments and sludge and may injure biological functions of the organisms. Polycyclic aromatic hydrocarbons (PAHs) and bisphenol A (BPA) can be included in these kinds of substances.

The primary routes of human exposure to PAHs are inhalation of polluted air and tobacco smoke, as well as ingestion of contaminated food and water. PAHs after metabolic activation cause a broad spectrum of toxic and genotoxic effects in cells and organisms, including DNA mutations, carcinogenesis, teratogenesis, and immune dysfunctions. BPA, endocrine disruptor, is a well-known toxicant whose estrogenic activity has been known for a long time. It was described to be released from polycarbonate flasks. BPA interferes with the normal function of endocrine systems and may have a negative effect on animals and human fertility.

Microcystin-LR is extremely toxic compound produced by cyanobacteria to species of *Microcystis*, *Oscillatoria*, *Nostoc* and *Anabaena*. Microcystin has toxic and genotoxic effects in organisms, including hepatotoxicity, neurotoxicity and possibly potential carcinogenicity. It is also the promoter of local allergic and skin reaction and gastrointestinal problems. This may weak the human immunity and leads to development of so-called summer virus sicknesses. In that, the contamination of water with Microcystin can cause severe health problems to exposed human and animals, it is appropriate to perform monitoring of Microcystin presence in drinking water or water of recreation areas on regular basis. The provisional guideline value by the World Health Organisation is 1 µg/L for drinking water.

The poster presents the quantitative ELISA kits for detection of BPA, PAHs and Microcystin in environmental samples. The principle of all ELISA kits is the competition of pollutants and toxins from calibrator/environmental sample with pollutant/toxin-HRP conjugate for antigen binding site of antibody adsorbed on the wall of the hole of microtitration plate. Intensity of the colour reaction is indirect proportionate to sample concentration. The detection limit of ELISA kits were defined for BPA – 10 µg/L, for PAHs – 10 µg/L, and Microcystin-LR – 0.05 µg/L. The results from detection of pollutants and toxin were compared with other commonly used analytical methods.

Supported by grant EUREKA OE211 of the Ministry of Industry and Trade of the Czech Republic.

### IN VITRO TOPICAL TOXICITY TESTING IN LINE WITH REQUIREMENTS OF EU AND US REGULATORS: RECONSTRUCTED HUMAN TISSUE MODELS

Letašiová S. <sup>1</sup>, Kandárová H. <sup>1,2</sup>, Klausner M. <sup>2</sup>, Hayden P. <sup>2</sup>, Kaluzhny Y. <sup>2</sup>, Kubilus J. <sup>2</sup>, Ayehunie S. <sup>2</sup>, Kearney P. <sup>2</sup>, Sheasgreen J. <sup>2</sup>

<sup>1</sup>MatTek In Vitro Life Science Laboratories, Bratislava, Slovak Republic

<sup>2</sup>MatTek Corporation, Ashland, MA, USA

The potential for substances to cause effects such as corrosion or irritation to skin and eye is a concern of industrial toxicologists in their assessments of possible worker and consumer safety issues. Moreover, national and international regulatory agencies (e.g. ECA, EPA, US DOT), require that substances are labelled as to the toxicity potential to skin or eye. To prevent the unnecessary use of animals for the above-mentioned purposes, EU as well as US regulations recommend the use of alternative tests methods “whenever appropriate and feasible”.

Since reconstructed human tissue (RHT) models closely mimic native tissues, they can be used for reliable estimation of hazard (and in some cases also risk) related to human health. Tests with RHT models for topical toxicity testing are cost-effective and deliver faster and more reproducible results than many of the traditional *in vivo* assays. Another advantage of the commercially available RHT models is that their characteristics can be precisely controlled by established Quality Assurance procedures to insure long-term reproducibility, which is important in the regulatory toxicology [1].

RHT-based assays for skin corrosion and skin irritation testing are validated, moreover, skin corrosion test with RHT models has reached full regulatory acceptance at the OECD level as OECD TG 431: The Human Skin Model Test. A number of *in vitro* RHT-based methods have completed pre-validation testing (photo-toxicity, eye irritation, genotoxicity) or are ready to enter the pre-validation process in the near future. They enable testing without excessive need for laboratory animals, which is of great importance for REACH as well as for EU cosmetic legislation. This presentation will describe currently available RHT-based assays for topical toxicity testing (with discrimination between risk and hazard). Approaches to the development, validation and implementation of these assays into regulatory systems and testing strategies will be discussed.

REFERENCES: [1] Rispin et al., Regul. Toxicol. Pharmacol. 45 (2), 97–103, 2006.

### CYTOCHROMES P450 DETOXICATE CARCINOGENIC ARISTOLOCHIC ACID I IN VITRO AND IN VIVO

Levová K. <sup>1</sup>, Šístková J. <sup>1</sup>, Bárta F. <sup>1</sup>, Frei E. <sup>2</sup>, Arlt V.M. <sup>3</sup>, Phillips D.H. <sup>3</sup>, Schmeiser H.H. <sup>2</sup>, Stiborová M. <sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Prague 2, Czech Republic

<sup>2</sup>German Cancer Research Center, 69 120 Heidelberg, Germany

<sup>3</sup>Institute of Cancer Research, Section of Molecular Carcinogenesis, Sutton, Surrey, SM2 5NG, United Kingdom

Aristolochic acid (AA) causes the development of aristolochic acid nephropathy (AAN) and Balkan endemic



nephropathy (BEN) associated with malignant transformation of the urothelium. These findings highlight the carcinogenic potential of AA to humans. Indeed, AA is among the most potent 2% of known carcinogens.

One of the common features of AAN and BEN is that not all individuals exposed to AA suffer from these diseases. One cause for these different responses may be individual differences in the activities of enzymes catalyzing the detoxication and/or activation of AA.

Using a HRN [Hepatic Cytochrome P450 (CYP) Reductase Null] mouse line, we investigated AAI detoxication *in vivo* and *in vitro*. We found that hepatic microsomes of wild-type (WT) mice oxidize AAI *in vitro* to detoxication metabolite, AAIa, while those of a HRN line were without this effect. Levels of AA-DNA adducts in liver, kidney, lung, spleen, small intestine, colon and urinary bladder of WT mice exposed to AAI were lower than in those of HRN mice. These results suggest that hepatic CYPs decrease the actual concentration of AAI both in these tissues, thereby protecting its activation to AA-DNA adducts. To define the role of CYP enzymes in AAI oxidation, we used besides hepatic microsomes of these mouse models, also those of human and rat. Furthermore, we investigated the modulation of this reaction by specific inducers and selective inhibitors of these enzymes. The efficiency of human recombinant CYPs to oxidize AAI was also tested.

The results demonstrate a major role of hepatic CYPs in AAI detoxication in mouse *in vivo* and that of CYP1A1/2 in this reaction in humans, rats and mice *in vitro*.

Supported by GACR (grants 303/09/0472, 305/09/H008) and Czech Ministry of Education (grants MSM0021620808, 1M0505).

## ALTERNATIVE METHODS IN TOXICOLOGY

Liesch M.

Federal Institute for Risk Assessment (BfR), Unit: 92 - ZEBET,  
Diedersdorfer Weg 1, D-12277 Berlin, Germany

More than fifty years ago the British scientists Bill Russel and Rex Burch propagated the 3R concept ("Refine", "Reduce", "Replace") as a general principle for the ethical conduct of animal experiments in bio-medicinal research. At that time, toxicology was an academic discipline, and no systematic regulatory requirements existed. However, forced by an accident (thalidomide), standardized, internationally harmonized sets of toxicological information requirements were introduced in the 80'ies of the last century for industrial chemicals, pesticides, biocides, medicinal products and medical devices, food additives and non-food consumer products.

Test Guidelines produced and adopted by the OECD since 1981 are used by governments, industry and independent laboratories to determine the hazard or safety of chemicals. The use of toxicological Test Guidelines based on scientifically sound and validated test methods promotes the generation of dependable data for assessment of hazards to *human health* and the *environment*. Besides GLP, dependable data are a crucial prerequisite for the OECD principle of mutual acceptance of data

(MAD). Since their introduction, OECD Test Guidelines are continuously being revised to meet the 3R principle to highest level possible. Today, both, revised (updated) and new OECD Test Guidelines (irrespective if they are animal based or non-animal based) have to be validated according to harmonised principles.

While the definition of the validation process as "...the process by which reliability and relevance of a method are determined for a specific purpose" has been internationally agreed already in 1990, reaching of an international consensus on OECD Guidance Document No.34 on "*Principles of Validation and Acceptance*" has taken until 2005. It was drafted by representatives of OECD Member Countries taking into account advice from OECD stakeholders, such as validation institutions (ECVAM, ICCVAM, and ZEBET), industry (BIAC, TUAC) and the international coalition for animal protection at the OECD (ICAPO).

OECD Guidance Document No. 34 describes general principles, important considerations, illustrative examples, potential challenges and results of the experience gained in the areas of test method validation, independent method peer review and regulatory acceptance. To the extent possible, flexibility is defined to allow adaptation to a given situation. For example, the "Modular Approach" to validation was integrated in GD 34, mainly to help structuring retrospective weight of evidence validation. Definition of "Performance Standards" is today required for any validated and accepted new test method to allow evaluation of protocol modifications (e.g. biological system, endpoint readout). Since, in particular in the area of Alternative Methods, more and more similar tests are being developed for the same toxicological endpoint (e.g. presently more than 10 tests for estrogenic effects), the OECD currently is developing the first "Performance Based Test Guideline (PBTG)" that will describe only the common test principles plus define the required test performance (predictions obtained with a set of reference chemicals).

Finally, the presentation will address an area where other principles of validation are relevant: according to regulations determined by international Pharmacopoeias batch release tests for safety, efficacy and purity of biological medicines can in general only be replaced by an Alternative Method if the new method is validated specifically for each product on the market. This will be shown for Botulinium neurotoxin (BoNT).

## INFLAMMATORY EFFECT OF ROCKWOOL IN OCCUPATIONALLY EXPOSED WORKERS

Liskova A.<sup>1</sup>, Jahnova E.<sup>1</sup>, Kuricova M.<sup>1</sup>,  
Horvathova M.<sup>1</sup>, Ilavska S.<sup>1</sup>, Wsolova L.<sup>1</sup>, Tulinska J.<sup>1</sup>,  
Fuortes L.<sup>2</sup>, Kyrtopoulos S.A.<sup>3</sup>, Dusinska M.<sup>1,4</sup>

<sup>1</sup>Research Base of the Slovak Medical University, Limbova 14,  
833 03 Bratislava, Slovak Republic

<sup>2</sup>University of Iowa, College of Public Health, Iowa City, Iowa, USA

<sup>3</sup>National Hellenic Research Foundation, Institute of Biological  
Research and Biotechnology, Athens, Greece

<sup>4</sup>Norwegian Institute for Air Research, Kjeller, Norway

In the context of cross sectional molecular epidemiology study, inflammatory and immunomodulatory effect of exposure to rockwool man made mineral fibres was examined. Eighty seven workers (mean age 43 years) who produced rockwool fibres used as insulation material in construction have been included. For control group, 36 clerical subjects matched for age and sex were recruited. Current exposure to mineral fibres was assessed using stationary area sampling as well as personal monitoring. All measured levels of rockwool fibres were very low (10 to 1000 times below the Slovak occupational limits).

Markers of the inflammation and cellular immune response were assessed in workers with at least 5 years' exposure to rockwool fibres (average duration of exposure 16 years). Biomarkers examined included lymphocyte subset analysis, expression of adhesion molecules on peripheral blood leukocytes, activation markers on eosinophils, concentrations of interleukins, soluble adhesion molecules and levels of immunoglobulins. Moreover, functional immune assays: lymphoproliferative response to mitogens and antigens, phagocytic activity of leukocytes and cytotoxic activity of natural killer cells were performed. Statistical analysis was done using SPSS software.

In rockwool fibres exposed population, increased serum levels of proinflammatory cytokine interleukin 8 ( $p < 0.001$ ), levels of soluble adhesion molecule ICAM-1 ( $p < 0.001$ ) and elevated levels of immunoglobulin E ( $p < 0.05$ ) were found in comparison with control individuals. On the other hand, serum levels of immunoglobulin M in exposed workers were decreased ( $p < 0.01$ ). Workers in rockwool production plant had significantly enhanced T-cell response of lymphocytes derived from peripheral blood and *in vitro* stimulated with phytohemagglutinin A ( $p < 0.01$ ). Our findings indicate hypersensitivity and an elevated inflammatory status in workers exposed to rockwool mineral fibres.

*We would like to express our gratitude to Viera Vachalkova, Helena Turazova, Olga Liskova and Mikulas Krnac for their excellent technical help. We thank Dr. Katarina Volkovova, Dr. Marta Staruchova, Dr. Alexandra Horska, Dr. Machata, Dr. Sedivcova, Dr. Kopernicka, Dr. Holecka for their help with sampling. We thank also Dr. Petrik and Dr. Kocan for measurement of exposure.*

*This work was supported by the European Commission DG research contract QLK4-1999-01629, GOCE-CT-2006-036913-2, GOCE 018385 and US NIH # 2 D43 TW00621-006.*

## EVALUATION OF A NEW ANTIDOTE TO CHEMICAL WARFARE AGENTS IN MICE

Lucot J.B.<sup>1</sup>, Garrett T.L.<sup>1</sup>, Watson M.D.G.<sup>2</sup>, Helton D.R.<sup>3</sup>

<sup>1</sup>Wright State University, Dayton, OH, USA

<sup>2</sup>WPAFB, AFRL/HEPB, Dayton, OH, USA

<sup>3</sup>Cenomed Research, LLC., Laguna Hills, CA, USA

Treatment of exposure to organophosphate chemical warfare agents (CWAs) relies on a combination of agents that increases the chances of survival and blocks the cholinergic response to CWAs. Protecting the CNS from the long term neurodegeneration produced by CWA exposure is time and dose dependent and current therapy would benefit from the addition of a potent CNS

protective agent. Furthermore, doses of one currently used anti-cholinergic, atropine, produces debilitating side effects, which can be toxic when given in the absence of CWAs. CM-2,550 is a clinically used drug that has both neuroprotective (multiple pharmacology) and anti-cholinergic properties. CM-2,550 is under evaluation in Swiss Webster mice as an add-on to very low doses of atropine. Preliminary data indicate that CM-2,550 is effective as both a pre-treatment and post-treatment when given alone against sarin. CM-2,550 also improves survival when used as a substitute for atropine in the standard treatment of atropine/2-PAM/ diazepam. When added to lower doses of atropine, CM-2,550 produced greater survival than did the standard atropine dose either as a sole treatment or in combination with PAM. CM-2,550 also was as effective as atropine at blocking the tremors produced by the cholinergic agonist oxotremorine. Histological evaluation revealed that CM-2,550 had neuroprotective. These data indicate that CM-2,550 should be further evaluated as a multi-use drug for treatment of CWA exposure and as an agent to reduce or eliminate the side effects of anti-cholinergics such as atropine.

*David Helton is CEO of Cenomed Research LLC which is developing this compound for commercial use. A portion of this research was funded by Cenomed Research LLC through a grant to James B. Lucot.*

## IN VIVO EFFECT OF PINOSYLVIN AND PTEROSTILBEN IN THE ANIMAL MODEL OF ADJUVANT ARTHRITIS

Mačičková T.<sup>1</sup>, Pečivová J.<sup>1</sup>, Drábiková K.<sup>1</sup>, Bauerová K.<sup>1</sup>, Mihálová D.<sup>1</sup>, Harmatha J.<sup>2</sup>, Nosál R.<sup>1</sup>

<sup>1</sup>Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

<sup>2</sup>Institute of Chemical Technology, Technická 5, Praha, Czech Republic

Oxidative stress and inflammation contribute to the pathogenesis of rheumatoid arthritis in an interactive mode. One of the major origins of oxidative stress in inflammatory diseases are neutrophils. Activation of neutrophils induces generation of reactive oxygen species and release of enzymes play an important role in inflammatory and immune processes involved in many diseases, such as allergies, infections and rheumatoid arthritis.

Pinosylvin (PIN) and pterostilbene (PTE) are natural substances from the stilbenoid group. They are chemically related to resveratrol, which is well known by its antioxidative activity. Both substances inhibited significantly the chemiluminescence (CL) of whole blood and CL of isolated neutrophils.

The aim of this study was to evaluate the effects of PIN and PTE on the development of adjuvant arthritis in rats. Adjuvant arthritis was induced by a single intradermal injection of *Mycobacterium butyricum* in incomplete Freund's adjuvant in male Lewis rats. Our experiments included healthy intact animals as reference controls, arthritic animals without any drug administration, and arthritic animals with administration of PIN and PTE in the oral daily dose of 30 mg/kg b.w. The treatment

involved administration of the substances tested from day 0, *i.e.* the day of immunization, to the experimental day 28. The following parameters were monitored: hind paw edema (day 14, 21 and 28), luminol-enhanced CL of the joint and myeloperoxidase (MPO) activity in the hind paw joint homogenates (day 28). Arthritic animals treated with PIN showed decrease in hind paw edema, significantly on day 14 and 28. PIN decreased CL as well as MPO activity of the joint, in comparison with untreated animals. PTE had no effect on hind paw edema and MPO activity in hind paw joint homogenates and exerted only a partial effect on luminol-enhanced CL.

We conclude that the effect of PTE on CL was partial only. However PIN had *in vivo* a beneficial anti-inflammatory and antioxidant effect on oxidative stress induced biochemical changes occurring in adjuvant arthritis, as determined by all three functional parameters.

Supported by the grants VEGA No 2/0003/10, APVV 3015-07, GAČR 203/1227.

#### **LANTHANUM – ACUTE TOXICITY TO FISH DANIO RERIO AND POECILIA RETICULATA**

Máková S.<sup>1</sup>, Babula P.<sup>2</sup>, Plhalová L.<sup>1</sup>, Žáčková K.<sup>2</sup>, Svobodová Z.<sup>1</sup>

<sup>1</sup>Department of Veterinary Public Health and Toxicology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1–3, Brno, Czech Republic

<sup>2</sup>Department of Natural Drugs, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Lanthanides form a group of elements from lanthanum to lutetium, and are widely used in industry. Lanthanide(III) ions have many biological properties, which are based especially on their similarity with calcium ions. These properties include blocking of calcium channels, which results in the inhibition of skeletal, smooth and cardiac muscles contraction, and replacement of calcium(II) ions in the structure of many proteins, which means that these proteins can lose their functions, or, contrariwise, in some cases their function can be activated or increased. Possibility of their interactions with nucleic acids was also demonstrated. Lanthanides are used mainly as contrast agents, but some complexes find the utilization also in the therapy of cancer because of their significant cytostatics properties. Due to these facts, it is evident that lanthanides represent a potential source of environment contamination.

Our work focused on the testing of lanthanum(III) ions toxicity in juvenile and embryonic stages of zebrafish (*Danio rerio*) and juvenile developmental stages of guppy (*Poecilia reticulata*).

Acute toxicity tests were performed on the juvenile aquarium fish *D. rerio* and *P. reticulata* according to method OECD No. 203 (Fish acute toxicity test). For embryo toxicity test was used method OECD No. 212 (Fish short-term toxicity test on embryo and sac-fry stages). The results of toxicity tests (the number of dead individuals at particular test concentrations) were subjected to probit analysis using the EKO-TOX 5.2 programme to determine LC<sub>50</sub> values of lanthanum(III) ions.

The LC<sub>50</sub> values of lanthanum(III) ions ranged between 140–160 mg/l.

Supported by the MSM Project No. 6215712402.

#### **ASSESSMENT OF CYTOTOXIC AND GENOTOXIC POTENTIAL OF NANOPARTICLES USED IN NANOMEDICINE**

Magdolenova Z.<sup>1</sup>, Rinna A.<sup>1</sup>, Fjellsbø L.<sup>1</sup>, Barancokova M.<sup>2</sup>, Kazimirova A.<sup>2</sup>, Hasplova K.<sup>1,3</sup>, Hudecova A.<sup>1,3</sup>, Dusinska M.<sup>1,2</sup>

<sup>1</sup>Norwegian Institute for Air research, Instituttveien 18, Kjeller, Norway

<sup>2</sup>Slovak Medical University, Limbova 14, Bratislava, Slovak Republic

<sup>3</sup>Comenius University, Faculty of Natural Sciences, Mlynska dolina, Bratislava, Slovak Republic

Nanoparticles (NPs) become increasingly used in many areas, but their possible impact on human health is still not known. Until now there is no special regulation for NPs, thus it is necessary to develop testing strategies, to assess the toxicological profile and potential health risks of NPs. We are assessing *in vitro* methods suitable to test NPs used in medical diagnostics with focus on endpoints and markers which can detect genotoxicity. Mechanisms of NPs-induced genotoxicity are not fully understood, both direct interaction of NPs with DNA as well as indirectly through NPs-reactive oxygen species are proposed. The comet assay is one of the most promising methods due to its simplicity, versatility and the ability to detect different DNA lesions. The alkaline comet assay and modified comet assay (using lesion specific enzyme formamidopyrimidine glycosylase able to create breaks at sites of oxidized bases) were used to examine DNA damage induced by NPs together with proliferation activity and plating efficiency (colony forming ability) assays to investigate cytotoxicity. Selected NPs were characterized for their primary and secondary physico-chemical properties. PLGA-PEO NPs, bare iron oxide NPs, and oleic acid coated iron oxide NPs have been tested *in vitro* because currently used in medicine. TiO<sub>2</sub> was used as reference NPs. Short- vs. long-term (2h vs. 24h) consequences of NPs exposure were investigated. First results on Cos-1 monkey kidney and TK6 lymphoblastoid cells (models representing kidney and blood as target organs for NP exposure) will be presented.

Supported by EC FP7 [Health-2007-1.3-4], Contract no: 201335.

#### **PRENATAL ADMINISTRATION OF THE PYRIDOINDOLE SMe1EC2 INFLUENCED ANXIETY AND DEPRESSIVE LIKE BEHAVIOR OF THE OFFSPRING IN THE ADULTHOOD**

Mach M.<sup>1</sup>, Ponechalová V.<sup>1</sup>, Dubovický M.<sup>1</sup>, Navarová J.<sup>1</sup>, Brucknerová I.<sup>2</sup>, Ujházy E.<sup>1</sup>

<sup>1</sup>Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, Slovak Republic

<sup>2</sup>1st Department of Pediatrics, School of Medicine, Comenius University, Limbova 1, Bratislava, Slovak Republic

The pyridoindole derivative 2 ethoxycarbonyl-8-methoxy-2,3,4,4a5,9b-hexahydro-1H-pyrido-[4,3b]



indolinium chloride (SMe1EC2, m.w. 312.79 Da) possesses high antioxidant/antiradical effects and very low toxicity. It is suggestive to use this substance as a protective agent in oxidative stress related diseases, including injuries evoked by chronic intrauterine hypoxia or perinatal asphyxia. However, any chemical substance acting during sensitive periods of the brain development represents a risk for functional impairment followed at later age by neurobehavioral disorders. In the present study, effect of prenatal and neonatal administration of SMe1EC2 on postnatal and neurobehavioral development of rat's offspring was evaluated.

SMe1EC2 increased the time spent in the open arms of the Elevated plus maze ( $p < 0.05$ ) and Fisher LSD post-hoc test revealed most affected group to be the one treated with dose of 50 mg/kg/d. Developmental administration of SMe1EC2 caused increased stress response tested in behavioral despair test (Forced Swim). All treated groups displayed increased depressive-like behavior ( $p < 0.001$ ).

If we take into account the low toxicity and good antioxidant properties, SMe1EC2 may have a good potential in alleviating anxiety. However, our results indicate that this compound administered especially during sensitive periods of the CNS development may influence neurotransmitter systems controlling anxiety and depression.

*The work was supported by the grants VEGA 2/0066/09, 2/0083/08 and Grant UK/275/2010.*

## EMBRYOTOXICITY OF MIRTAZAPINE

Maňáková E. <sup>1</sup>, Hubičková L. <sup>1</sup>, Košťálová J. <sup>1</sup>, Zemanová Z. <sup>2</sup>

<sup>1</sup>CZTIS, Department of Histology and Embryology, 3rd Faculty of Medicine, Charles University in Prague, Czech Republic

<sup>2</sup>Institute of Physiology, Academy of Sciences, Prague, Czech Republic

Many new drugs are introduced into the market every year. Their embryotoxicity is known from animal studies, only. Mirtazapine is an example of the new antidepressant with low acute toxicity but with only little information about its reproductive toxicity. Its wide therapeutic range and only little proved side effects may be an advantage for treatment during pregnancy. Aim of our study was to contribute to the knowledge on possible risks. For embryotoxicity testing we used an alternative method – CHEST that used chicken embryos as experimental model. Fertilized eggs of outbreed Grey Leghorn stock (AVČR farm Koleč) were treated on embryonic day (ED) 4 by Mirtazapine (Sigma), incubated till 9ED, when they were weighed and examined. Summing the proportions of dead and malformed embryos, the beginning of the embryotoxicity dose range was estimated. Doses per embryo were: 0.004 µg, 0.04 µg, 1.2 µg, and 12 µg in 30% DMSO, and 0.2 µg, 0.1 µg, 0.05 µg and 0.03 µg in DMSO only. The first of them corresponds to the therapeutic dose in human (40mg/day). Mirtazapine is not soluble in water or Ringer solution, therefore DMSO was used as a solvent. If Mirtazapine was solved in 15% or 30% DMSO in water, embryotoxicity was lower corresponding data

from preclinical studies. If only DMSO was used, the dose 0.05 µg in 3 µl DMSO resulted in 57% mortality (LD<sub>50</sub>). Typical malformations caused by Mirtazapine were caudal regression syndrome, body wall closure defects, malformation of limbs on left side, which is a place of maximal concentration, and anophthalmia. However anophthalmia and body wall closure defects were more frequent also in control group. We suppose that it is probably done by exchange of hen and lower quality of eggs. Approximation of doses in chick embryos to mammals is complicated by low solubility of Mirtazapine. According to the literature, plasma level is not affected by nourishment however resorption may be limited by solubility and therefore in consequence lower than our doses. Demonstration of plasma concentration and comparison of that is essential for conclusions. If the embryotoxic dose was close to LD<sub>50</sub>, risk at therapeutic doses will be probably low. Mirtazapine according to results of testing and cases published in literature is relatively safe for pregnant women, only higher rate of abortions was demonstrated; however more information is needed to exclude all potential risks.

*Acknowledgements: Work was supported by Grant of Ministry of Education Czech Republic INGO LA08034/2008.*

## DEVELOPMENT OF NOVEL QUATERNARY DETERGENTS AS A PART OF DECONTAMINATION MEANS

Marek J. <sup>1,3,4</sup>, Kuca K. <sup>2</sup>, Cabal J. <sup>1</sup>, Pohanka M. <sup>2</sup>, Dolezal B. <sup>3,4</sup>, Povrazník J. <sup>4,5</sup>

<sup>1</sup>Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>2</sup>Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

<sup>3</sup>Vakos XT a.s., Prague, Czech Republic

<sup>4</sup>ChemProtect a.s., Prague, Czech Republic

<sup>5</sup>Decomkov Praha s.r.o., Prague, Czech Republic

Chemical and biological warfare agents are considered to be threat. Their misuse by terrorists is well discussed and due to this, many institutes throughout the world are interested in development of novel countermeasures against them. At our department, we are interested in development on novel antidotes against nerve agents and development of novel detergents which should be used as a part of the decontamination and disinfection mixtures. Nowadays, we have prepared several series of novel quaternary detergents derived from benzalkonium salts. Instead of benzyl group, we have used pyridinium ring with different substituents. These compounds are currently tested for their decontamination and disinfection potency.

*The work was supported by the grant of SV KTOX.*



## Distributor of Chemicals and Radiochemicals

Full range of products of U.S. producer MP Biomedicals

- Broad portfolio of products from Life Science
- Environmentally Safe Liquid scintillation cocktails (Ecolite, CytoScint, BetaMax)
- IsoBlue™ stabilized nucleotides (32P, 33P, 35S)

Labeled Chemicals 3H, 14C, 32P, 33P, 35S, 36Cl, 45Ca, 51Cr, 57Co, 125I  
MP Biochemicals, Moravek Biochemicals and Radiochemicals, iThemba,  
American Radiolabeled Chemicals

Radiation Safety and Protection Equipment

RIA Kits

Labware and Instruments NanoDrop and FastPrep24 System



If you are interested in the catalog, visit our website and fill out the Request A Free Catalog.



M.G.P. spol s r.o.  
Kvítková 1575  
760 01 Zlín  
Czech Republic

E-mail: [mgp@mgp.cz](mailto:mgp@mgp.cz)  
[www.mgp.cz](http://www.mgp.cz)  
Zelená linka: 800 125 890



## BioVendor

**BioVendor's mission** is to provide researchers and medical experts with top quality immunodiagnostics and related biochemicals – antibodies and recombinant proteins. BioVendor's strength stems from its full control over the whole R&D process conducted by highly qualified researchers in new, fully-equipped labs. Our motivated team is a consistent leader in the development of novel research tools.

### Immunoassays

Our kits are used in research and clinical labs of more than 60 countries including the USA, EU countries and Japan.

**BATS** The *BioVendor Analytical Testing Service* offers comprehensive sample analyses for analytes measured by our assays. BioVendor is a direct assay kit manufacturer, giving our staff a unique understanding of assay performance.

### Proteins

Recombinant proteins produced in human cell line HEK 293 and robust *E. coli* recombinant production systems.

**BIRDS** Our *BioVendor Recombinant Protein Development Service* offer a recombinant protein production platform and skilled staff with a history of over 100 recombinant proteins developed for BioVendor's R&D projects.

### Antibodies

Original and excellent monoclonal and polyclonal antibodies.

**B.ANTS** The *BioVendor Antibody Service* is a service that provides you with highly specific antibodies produced with the latest technologies.

#### » ORGAN DAMAGE MARKERS

Most traditional markers of kidney injury appear only when significant organ damage has already occurred. There is a great need for toxicity biomarkers that can reliably indicate early kidney damage. This need is especially important in the development of new drugs where such biomarkers could predict toxic manifestations and detect damage earlier—improving safety testing and potentially reducing the cost of drug development.

BioVendor has assays measuring of markers indicating damage of:

– kidney    – liver    – cardiovascular system    – brain    – lung

#### » ANIMAL STUDIES

Toxicological studies using experimental animals represent irreplaceable model to assess toxicological effects of various substances. We offer over 125 ELISA kits to investigate samples from experimental animals.