MMSL
Military Medical
Science Letters

Volume 82, supplementum 1, 2013

MEETING ABSTRACTS

18th INTERDISCIPLINARY CZECH-SLOVAK TOXICOLOGY CONFERENCE - TOXCON 2013

Dear Readers,

this special issue of our journal, Military Medical Science Letters, is aimed at the summary of meeting abstracts presented at the 18th Interdisciplinary Czech-Slovak Toxicology Conference - TOXCON 2013. This meeting was held in Hradec Kralove, Czech Republic, from 19th to 21st June 2013. This particular meeting was organized by Czech Society for Experimental and Clinical Pharmacology and Toxicology and Slovak Toxicology Society with the support of the University hospital Hradec Kralove, Faculty of Medicine, Charles University Hradec Kralove and Faculty of Military Health Sciences, University of Defence Hradec Kralove.

It brought together academic, government and third sector organisations to debate key issues in toxicology. This summary presents all oral and poster contributions which were presented within this meeting. We hope that these abstracts will be useful to those who are working in this area of research.

Kamil Kuca

ČESKÁ SPOLEČNOST
PRO EXPERIMENTÁLNÍ
A KLINICKOU FARMAKOLOGII
A TOXIKOLOGII ČLS JEP

Biochemical and Histopathological Responses of Wistar Rats to Oral Intake of Microcystins and Cyanobacterial Biomass

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Cyanobacteria produce potent and environmentally abundant microcystins, representing an emerging global health issue. We investigated the impact of pure microcystins and cyanobacterial biomass on laboratory rats. All levels of MCs in the liver were close to the detection limit (3–5 ng/g fresh weight) using HPLC MS/MS. Only rats exposed to cyanobacterial biomass had clearly higher hepatic and splenic somatic indexes while oxidative stress markers were significantly increased in the group exposed to the high dose of MCs. Most of the analysed biochemical parameters did not show clear differences among groups. Levels of bilirubin and lipases were significantly increased only after exposure to cyanobacterial biomass and MCs, respectively. Histopathology was dominated by alterations in the hepatic parenchyma and renal cortical tubular system.

Carcinogenic and Nephrotoxic Aristolochic Acid Induces Expression of NAD(P)H:Quinone Oxidoreductase

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Aristolochic acid (AA) causes a specific nephropathy, Aristolochic acid nephropathy, and urothelial malignancies. NAD(P)H:quinone oxidoreductase (NQO1) is the most efficient cytosolic enzyme activating AAI to species forming AAI-DNA adducts. The NQO1 protein and its enzyme activity are induced by AAI in liver and kidney of AAI-pretreated mice and also in rat kidney. Furthermore, the increase in hepatic and renal NQO1 enzyme activity was associated with bio-activation of AAI and elevated AAI-DNA adduct levels in *ex vivo* incubations of cytosols with DNA and AAI. AAI appears to increase its own metabolic activation by inducing NQO1, thereby enhancing its own genotoxic potential.
Supported by 303/09/0472 and UNCE204025/2012.

Toxicity of Selected Pharmaceuticals Occurring in Waters for *Danio Rerio*, with Impact on Selected Biomarkers of Oxidative Stress

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The aim of the study was to determine the toxicity of pharmaceuticals occurring in waters (norfloxacin and ibuprofen) in model organism zebra fish (*Danio rerio*). The tests were performed according to OECD No. 215 (Fish, Juvenile Growth Test) using 30-day old *Danio rerio*. Fish were exposed to a range of sublethal concentrations of pharmaceuticals. Duration of the test was 28 days. For the test was selected determination of glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and malondialdehyde (MDA).
The results show that ibuprofen does not affect the activity of (GR). The increase in ibuprofen concentration correlates with the increase of activity of GP and GST. The MDA contend does not correlate with concentration of ibuprofen. Norfloxacin samples are taken and measurements are under process.
This study was supported by 19/2013/FVHE.

The Effect of Isoflavone Genistein on the Expression of Selected Nuclear Receptors and their Coregulators

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Isoflavones are natural compounds, whose structure is very similar to estradiol, the main female hormone. Soybeans and soy products (tofu, misho, tempeh) represent a main source of isoflavones in human diet. Among the other natural sources of isoflavones also belongs red clover. Isoflavones indicate a weak estrogenic activity, they bind to the estrogen receptor α (ERα) and estrogen receptor β (ERβ), causing inhibition of the estradiol to their cognate

receptors. It was discovered many favourable effects of phytoestrogens on breast cancer cells. There is a strong evidence that isoflavones could have a protective effect on the initiation or progression of breast cancer. In our laboratory, we have investigated the *in vitro* effects of the isoflavone genistein alone/or in combination with other biologically active compounds on the expression of selected nuclear receptors and their coregulators in human breast cancer cell line (MCF-7). The data has shown a marked potency of genistein to affect the expression of selected nuclear receptors.
Supported by the APVV-0160-11, APVV-SK-CZ-0211-11, 7AMB12SK151, VEGA- 2/0008/11, and CEMAN grants.

GFP-RNA Transfection of CF Defective Airway Cells as a Possible Tool for Remedy of Cystic Fibrosis

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The gene therapy is one of promising ways of cystic fibrosis (CF) treatment. To study the delivery and expression of an external gene, mRNA construct containing marker gene coding for green fluorescence protein (GFP) was designed and prepared. CuFi-1 human airway cells with CF genetic disorder were treated with GFP-mRNA combined with a transfection agent, TransIT-mRNA or Lipofectamine RNAiMAX. Both reagents enabled the cell transfection as judged from the developed fluorescence. Comparing data from a fluorescence microscope, TransIT-mRNA was more effective than Lipofectamine in the cell transfection.
The financial support from grants NT12190IGA of the Ministry of Health of the Czech Republic and UNCE204025/2012 of the Charles University in Prague is highly acknowledged.

Alcoholic Liver Cirrhosis Increases the Risk of Left Ventricular Diastolic Dysfunction

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The study is aimed to prove that the incidence of the more unusual and largely under-researched cardiac dysfunction, *i.e.* diastolic, is more frequent in patients with alcoholic cirrhosis. The incidence of left ventricular diastolic dysfunction in medical-ward patients with no prior history of cardiovascular disease to that of the patients with hepatic cirrhosis caused by alcohol abuse was compared. Our findings confirmed that the incidence of left ventricular diastolic dysfunction in patients with alcohol-related liver cirrhosis, classified as Child-Pugh grade A and B, was significantly higher than in the controls without any prior liver disease. Furthermore, we have newly noticed how the severity of diastolic dysfunction affects the morbidity and mortality of patients undergoing such treatments as TIPS, liver transplantation and other surgical interventions resulting from different indications.
Supported by AVOZ 50110509 and 1M0517

Toxicity of Nuclear Retinoid X receptors Cognate Ligands of Organotin Origin in Breast Cancer Cell Lines: Effect on Selected Retinoid/Retinoid X receptor Subtypes Expression

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Ligands for nuclear retinoid X receptors of organotin origin belong to a group of organic pollutants with potent endocrine-disrupting properties. The present study was undertaken to investigate cytotoxicity of TBT-Cl, TPT-Cl, tributyltin bromide (TBT-Br), tributyltin iodide (TBT-I), and non-halides tributyltin hydride (TBT-H), triphenyltin hydride (TPT-H), triphenyltin hydroxide (TPT-HX) in MCF-7 human breast cancer cell line. Moreover, we have investigated the effects of TBT-Cl, TBT-Br, TBT-I, TBT-H on the expression of nuclear all-trans retinoic acid receptor subtypes and their selected coregulators. The IC50 values determined by MTT assay clearly have shown that TBT-Cl is more effective in inhibiting MCF-7 cell proliferation than TPT-Cl. The results suggest that in spite of their toxicity, these compounds may also play an important role in modulation of retinoid receptors expression in MCF-7 cells.
Supported by the APVV-0160-11, APVV-147-10, APVV-SK-CZ-0211-11, 7AMB12SK151, VEGA- 2/0008/11, and CEMAN grants.

Oxidative Stress in Twins

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Oxidative stress (OS) defined as imbalance between prooxidants and antioxidants was re-defined as the state where redox regulation of cellular signaling and redox-sensitive control of cellular functions are disrupted. The aim of the study is to determine the activity of total antioxidant status TAS, values of malondialdehyde (MDA), superoxiddismutase (SOD) and glutationperoxidase (GPX) in a case of 6 identical twins on 1st and 5th day of life (gestational age 33-36 weeks). We confirmed the difference between A to B twins in values of TAS, as well as the difference between the 1st and 5th day of life. Differences in values of MDA, SOD and GPX are discussed.
The form of delivery has a strong influence on differences in specific values of markers of OS. In a case of multiple pregnancy the value of TAS is crucial, and determines the degree of protection of the newborn against the OS due to asphyxia. Values of MDA present the activity of the process of lipoperoxidation.
The work was supported by VEGA 2/008111.

Impact of Paraoxon Followed by Acetylcholinesterase Reactivator HI-6 on the Gastric Myoelectric Activity in Experimental Pigs

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Reactivators of acetylcholinesterase (AChE) are essential compounds in the treatment of organophosphates intoxications. However, their clinical use is limited due to its side effects, including serious gastrointestinal toxicity. The purpose of this study was to evaluate the effect of paraoxon (PAR) and AChE reactivator (Hi-6) on gastric myoelectrical activity (evaluated by surface electrogastrography-EGG) in experimental pigs. Five female experimental pigs (*Sus scrofa f. domestica*, median weight 31.0 kg) entered the study. A baseline 15-minute EGG recording was carried out under general anaesthesia (after i.m. ketamine and azaperone). Intramuscular PAR (1.5 mg) was administrated afterwards and one 10-minute EGG interval was recorded. Hi-6 was administrated 10 min. later (1.5 mg i.m.). Further ten 15-minute-interval EGG recordings were accomplished. PAR induced a non-significant decrease of dominant frequency (DF) (2.8±0.6 vs. 2.6±0.5 cycles per min.; p = 0.092). Subsequent administration of Hi-6 normalised DF to basal values and increased it significantly within subsequent 30 minutes (3.0±0.4; p<0.001). PAR administration did not influence the power (within a 10-minute exposure). However, the amplitudes increased significantly 90 minutes after the Hi-6 administration (819±109 vs. 5054±732 μV^2; p<0.001). AChE reactivator Hi-6 blocked the gastric effect of PAR significantly. Subsequent myoelectric changes in the DF and power were executed by Hi-6. The effect of PAR was non-significant.
The study was supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00179906.

Cyclin-dependent Kinase Inhibitors, Purvalanol A, Olomoucine II and Roscovitine, Enhance Daunorubicin Cytotoxicity in Multidrug Resistant Cell Lines via P-glycoprotein Inhibition

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P glycoprotein (P-gp) efflux pump contributes to multidrug resistance, a major obstacle in successful cancer treatment. We evaluated the cytotoxic effect of three cyclin-dependent kinase inhibitors (CDKi), potential anticancer drugs inhibiting P gp, administered as single agents or in combination with daunorubicin (DNR). We show that purvalanol A, olomoucine II and roscovitine, synergistically potentiate the antiproliferative effect of DNR in MDCKII-ABCB1 and in human carcinoma HCT-8 and HepG2 cell lines. We suggest that this synergism is, at least partly, caused by (i) higher intracellular retention of DNR caused by CDKi-mediated P-gp inhibition and (ii) native cytotoxic activity of the CDKi. Our results indicate that co-administration of the tested CDKi and ABCB1 substrates in the treatment of ABCB1-expressing tumors may allow for significant dose reduction anticancer drugs.
Supported by GAUK 700912/C/2012 and SVV/2013/267-003.

Effect of Antidepressant Venlafaxine on Neurobehavioral Development of Rat Offspring

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Venlafaxine (VENF) is SNRI antidepressant drug. The FDA has classified VENF regarding to pregnancy risk as C category of drug, which means that there are no well-controlled studies examining safety to the developing child. The aim of the study was to evaluate effect of VENF administration during sensitive functional brain development on neurobehavioral development of rat offspring. Pregnant Wistar rats were orally treated with VENF at doses of 7.5, 37.5 and 75 mg.kg^{-1} from day 15 of gestation to day 20 post partum. After weaning, the offspring was tested for motor activity and anxiety- and depression-like behaviors. VENF treatment resulted in a decreased intensity of locomotion in the offspring. In addition, we found reduced anxiety-like behavior in females compared to males exposed to VENF. The results suggest that VENF may gender-dependently interfere with brain development resulting in altered neurobehavioral regulations in a new environment.
Supported by the VEGA 2/0081/11, 2/0084/11 and 2/0107/12

Cytotoxic Effect of Novel Quercetin Complexes on Human Colon Cancer Cells

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Quercetin is the member of the group of natural compounds called flavonoids. It is the subject of intense research on the basis of its antioxidant, anti-inflammatory and anticancer activities. *In vitro* experiments show that quercetin may be effective in treatment of various types of cancer and it may be combined with other anticancer drugs to reduce their doses and subsequently their side effects. However, the degree to which quercetin is absorbed, is not sufficient and is therefore a limiting factor for its action in cells.
We prepared novel quercetin complexes with the aim to overcome this disadvantage. Antioxidant activity was monitored using two spectrophotometric methods. Improved antioxidant properties revealed complexes with caffeic acid and edavarone that showed antioxidant activity by themselves. The cytotoxic activity was determined on human colon cancer cells Caco-2 and HT-29 using mitochondrial cytotoxic test (MTT). Complexes caused cancer cell growth inhibition of both cell lines at comparable level. The most effective were complexes with edavarone and kojic acid. Obtained results indicate, that quercetin complex with edavarone is potent antioxidant and anticancer drug and could be directed to further evaluation.
This study was financially supported by Scientific Grant Agency (VEGA Project 1/0191/12), Slovak Research and Development Agency (APVV Project 0339-10) and The Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the Structural Funds of EU (ITMS 26240220040).

Effect of Glycitein on Phagocyte Activity

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Phytoestrogen glycitein belongs to the isoflavone class of flavonoids and thus it could regulate cellular activities of the inflammation-related cells. Information about its effect on phagocyte activity, particularly on neutrophils, is however rare.
We found that the synthetic isoflavone glycitein reduced reactive oxygen and nitrogen production in stimulated phagocytes, suggesting that it might possess a protective effect against toxic tissue damage. The inhibitory effect of glycitein on stimulated protein kinase C activity in neutrophils and on expression of inducible nitric oxide synthase in stimulated macrophages was indicative of its interference with signalling events included in the regulation of reactive species formation.
Supported by VEGA 2/0010/13, APVV-0052-10 and APVV-0315-07

Low Molecular Weight Precursor Applicable for Alzheimer Disease Drugs Synthesis (AChE and BChE Inhibition, BACE Inhibition, Antioxidant Properties and *In Silico* Modulation)

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Alzheimer disease represents the most common form of dementia, which is manifested by failure of memory and by progressive cognitive decline. The most of the available drugs and newly synthetized drugs are based on inhibition of AChE. For the production of these substances, there are used a lot of inorganic and organic compounds. The main aim of the experiment was to compare the inhibition properties of precursors with the standard drugs.
Properties of these standards drugs for treatment AD were determined and the precursors were tested using four methods: inhibitory ability for AChE and BChE; antioxidant ability was monitored by DPPH assay; fluorescent kit from Cayman Chem was used for assay of beta-secretase inhibition.
In the experiment, standard substances suitable for AD therapy were measured and the IC50 for AChE was found to be in sub-µM concentration. Only huperzine A had great results and it could be said, that it has the same antioxidative ability as the standards. Two compounds, donepezil and pyridine, had comparable value for BACE inhibition like the standard.
Acknowledgment: SV/FVZ201104

Prenatal and Perinatal Manipulation of Monoaminergic Systems Affects Neurobehavioral Development

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Monoamines have been shown to affect a variety of behavioral functions, such as aggression, sexuality, anxiety, mood or learning. In brain development, they play an important organizational role, including cell division, migration, synaptogenesis, maturation of the cortex and development of neuroendocrine systems. In males, for example, serotonin is markedly reduced during the 2nd and 3rd postnatal week what is essential for full masculinization of the brain and behavior. Manipulation of monoaminergic systems by the SSRI and/or SNRI antidepressant drugs represents a risk factor for healthy cognitive, emotional and behavioral development. Our experimental study with venlafaxine showed that it may interfere with brain development by gender-dependant way and affect neurobehavioral adaptations of rat offspring in a new environment.
Supported by the grants VEGA 2/0081/11, VEGA 2/0084/11 and VEGA 2/0107/12

Content of Heavy Metals in Muscle of Some Fish Species from the Danube River, Slovakia

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Anthrophogenic activities lead to environmental contamination with foreign substances. Research of contamination assessment of five fish species (*Barbus barbus, Carassius auratus, Rutilus rutilus, Esox lucius a Acipenser ruthenus*) from Danube river locality Kravany (Slovakia) with selected heavy metals (Cd, Cr, Pb, Zn) was carried in the year 2009. Heavy metals contents in fish did not exceed the valid hygienic limit. The results of this study show that the Danube River is not significantly affected by heavy metals contamination from its main tributaries.
This research was supported by the CENAQUA No. CZ.1.05/2.1.00/01.0024

Alterations of Cell Cycle Related Genes and their Proteins by BPA and BPA-estradiol Mixture in MCF7 Human Breast Carcinoma Cells

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Bisphenol A (BPA) is the representative of synthetic endocrine disruptor (ED) with estrogenic activity that stimulates the proliferation of estrogen receptor (ER)-positive mammary cancer cells. Interfering with the estradiol (E2)-mediated response can promote alterations in cell cycle genes/proteins affecting the G1/S transition. The simultaneous effect of E2 and BPA on cell proliferation have not been studied yet.
We investigated in MCF7 cells the effects of individual E2, BPA ($1x10^{-12}$, $1x10^{-9}$, $1x10^{-6}$M) and the mixture of BPA (as above) with E2 ($1x10^{-12}$M, MIX) on the expression of genes and proteins for cyclin A and cyclin D1. Time dependent changes (24, 48, 72, 96 and 120h) were analyzed by Western blot (proteins) and RT-PCR (mRNA for genes). Increased expression of genes and proteins for cyclin A and D1 by BPA and MIX were time and dose dependent.
Our results demonstrated that BPA alone and in mixture with E2 induces changes at the transcriptional level of the expression of genes involved in cell cycle progression in human breast cancer cells.
The study was supported by the APVV project:-147-10

Impact of Histone Deacetylase Inhibitors on Anticancer Effects of Cytostatics

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Valproic acid (VPA) is known as an anticonvulsant used for treatment of bipolar disorder and epilepsy. Last decades, VPA is also studied as an inhibitor of histone deacetylases (HDACs). Beside VPA, trichostatin A (TSA), is another compound utilized as an HDAC inhibitor. Inhibition of HDAC leads to changes in chromatin architecture that can result in modulation of gene transcription. Inhibitors of HDAC have also an impact on non-histone proteins. They can change their stability and interaction with other proteins or with DNA. We tested the effect of non-toxic concentrations of VPA and TSA on cytotoxicity of different cytostatics to human neuroblastoma cells. VPA increases cytotoxicity of etoposide, cisplatin and staurosporin in co-cultivation experiments. Similar, but lower effects exhibited TSA. VPA seems to influence cell proaptotic signals or regulate reparation machinery of neuroblastoma cells.
Supported by GAUK635712, UNCE204025/2012 and P301/10/0356.

Overcoming of MDR by New Photosensitizers – an *In Vitro* Study

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A major problem with anticancer drug treatment is the development of acquired multidrug resistance (MDR) of the tumor cells. The therapeutic potential of photodynamic therapy (PDT) with new photosensitizers has been be evaluated. Our results showed that propyl-AcrDTU (a derivative of acridine dialkyldithiourea) was more photocytotoxic (1.05 J/cm^2) against a resistant cell line (L1210/VCR) than against the parental cells (L1210).
Supported by APVV-0282-1, VEGA 1/0790/14 and Structural Funds EU (No. 26240220071)

Design, Synthesis and Biological Evaluation of Novel Coumarin-Acridine Hybrids

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Our research group has been involved in the development of cholinesterases inhibitors as potential anti-AD drugs. We have obtained different series of compounds, among which the hybrid structures with a tetrahydroacridine, acridine, and coumarin moiety were the most interesting, as they showed inhibition of both cholinesterases with activities in the micromolar range. To improve the activity, some modifications of heterodimeric molecules, the linker length and incorporation in linker of other groups were introduced.
Financial support for this work from the Slovak Grant Agency VEGA (grants 1/0672/11, 1/0179/11, 1/0001/13) is gratefully acknowledged.

***In Vitro* Interaction of DNA with Estradiol Monitored by Spectrophotometric Methods**

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Group of estrogen pollutants, where the highest estrogen activity is reported at estradiol is characterized by the fact that even at very low concentrations have potential to cause xenoestrogenic effects. Wastewater treatment plants currently do not have a way to break down these substances in the water effectively. Although a certain amount will remain bound in the sludge, the rest is discharged into receiving waters of the wastewater treatment plant. Currently, the research is focused mainly to uncover the relationship between the estrogen receptors binding affinity with an estrogen response element and estradiol. For elucidation of the interaction principles between oligonucleotides and estradiol we have used spectrophotometric methods. Interactions of DNA fragment (10 μg.mL^{-1}) with estradiol (5-500 mm) were carried out in an aqueous environment. Measured were absorption spectra (190-800 nm) at intervals of 10 minutes for 60 min at 25 ° C. Best signals of DNA fragment and estradiol complexes were detected at 260 nm. We observed the different effects of various concentrations of estradiol on selected oligonucleotides sequences.

Acknowledgements: This contribution has been supported by MSMT 6215712402 and IGA VFU 4/2013/FVHE.

The MALDI TOF Study of *In Vitro* Interaction of Oligonucleotides with Estradiol

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With the increasing consumption of contraceptives is increasing also the excretion of estrogen pollutants into the environment. During exposure of excessive amounts of estradiols may be produced the undesirable effects resulting for example in the feminization of males of water organisms. Presence of estradiols in the drinking water implies a risk for the human population in the form of cancer of endocrine systems due to their ability to stimulate mitotic activity in the cancer cells. We have proved interaction study of oligonucleotide sequences, containing promoters for estradiol using MALDI TOF for clarification the principles of interactions between hormone and DNA. 5 μL of the diluted estrogen receptors (ER) solution was incubated for 15 min with 5 μL of estradiol dissolved in deionized water and then was accomplished binding with oligonucleotides (10 μg.mL^{-1}). We have also performed the test of influence of different matrixes on oligonucleotides complexes spectra. Best signals of oligonucleotides and ER complexes were obtained using α-cyano-4-hydroxycinnamic acid (CCA).

Acknowledgements: *This contribution has been supported by MSMT 6215712402 and IGA VFU 4/2013/FVHE.*

Comet Assay Study of the UVA-induced DNA Damage by the Novel Quercetin Derivatives.

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UVA is the main spectral component of the solar UV radiation. Its potential as a damaging agent on the eukaryotic cells is well described in literature. The substantial mediators of the toxic effects of the UVA light are the reactive oxygen species (ROS), formed within the exposed cells. It has been already proved that treatment with various antioxidants might prevent or weakened broad UVA-light toxicity.
Use of the comet assay, a sensitive technique for the detection of the DNA damage at the individual eukaryotic cell level, allowed us to confirm these observations. We have found that murine fibroblast cell line NIH 3T3 cultured in the presence of the plant-derived flavonoid, quercetin, shown certain DNA-protection against the UVA irradiation.
Goal of our study was to find a more potent protectant among our newly synthetized quercetin derivatives, which have been previously tested for their in vitro ROS scavenging activities by the ABTS-test and/or by EPR-spectroscopy.

Supported by: APVV 0339-10 and VEGA 1/0191/12

Individual THC Concentrations Time Profile in Blood after Smoking Marijuana

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Cannabis is one of the oldest and most commonly abused drug worlwide, and its use is associated with pathological and behavioral toxicity. Cannabis consumption may produce dose-related individual impairment in psychomotor and cognitive functions impacting the safety of driving. Delta-9-tetrahydrocannabinol (THC) is responsible for the impairing effects. A question is how long impairment persists after the last drug application. Chronic marijuana smokers store THC in tissues in relation to an individual body constitution and a frequency of doses. Rate limiting step of THC elimination is its slow release from tissues back into the blood and measurable THC concentration may persist in blood even during days of abstinence. Our presentation should demonstrate the individual variability of pharmacokinetics of THC and its metabolites in human volunteers and reveals the discussion whether chronic

cannabis users are eligible to drive safely even after multiple days of abstinence. These findings may impact on implementation of per se legislation with low limits of THC in blood, exceeding of which indicates illegal driving.
This study was supported by the project MVCR VG20122015080

Assessment of Cardiotoxicity Induced by Conventional and High-dose Chemotherapy for Acute Leukemia with Multiple Biomarkers of Cardiac Injury

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Aims: Assessment of chemotherapy-induced cardiotoxicity with multiple biomarkers of cardiac injury – glycogen phosphorylase BB (GPBB), heart-type fatty acid-binding protein (H-FABP), cardiac troponins (cTnT,cTnI), creatine kinase MB (CK-MB mass), myoglobin.
Methods: 47 adult acute leukemia patients were studied – 24 patients treated with chemotherapy (CT) with anthracyclines (ANT) and 23 patients treated with high-dose CT (HD-CT) followed by stem cell transplantation (SCT). Cardiac biomarkers were measured before treatment, after first and last CT with ANT in the first group; after HD-CT and after SCT in the second group.
Results: GPBB increased above the cut-off (7.30 μg/L) in 4 (16.7 %) patients after first CT and in 5 (20.8 %) patients after last CT with ANT. GPBB increased above the cut-off in 5 (21.7 %) patients after HD-CT and after SCT. CTnI became elevated (above 0.40 μg/L) in 2 (8.3 %) patients after first and last CT with ANT. Other biomarkers remained below the cut-offs.
Conclusions: GPBB could become a sensitive biomarker for detection of acute cardiotoxicity associated with conventional and HD-CT for acute leukemia. The predictive value for development of cardiomyopathy is not known and will be evaluated during a prospective follow-up.
Financial support: RO 1011 (FMHS).

The Use of Cardiac Biomarkers in the Detection of Cardiotoxicity Associated with Stem Cell Transplantation for Hematological Malignancies

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Aims: Assessment of cardiotoxicity during stem cell transplantation (SCT) with multiple biomarkers of cardiac injury – myoglobin, creatine kinase MB (CK-MB mass), cardiac troponin I (cTnI), heart-type fatty acid-binding protein (H-FABP), glycogen phosphorylase BB (GPBB).
Methods: 53 patients transplanted for various hematological malignancies were studied. Cardiac biomarkers were measured the day after preparative regimen (PR) and the day after SCT.
Results: We found significant elevations in GPBB (above 7.30 μg/L) in 8 (15.1 %) patients after PR and in 9 (17.0 %) after SCT. H-FABP increased slightly above the cut-off (4.50 μg/L) after SCT in 1 (1.9 %) patient. Other biomarkers remained below the cut-offs. There was a significant correlation between elevation in GPBB and diastolic left ventricular (LV) dysfunction on echocardiography ($r = 0.603$; $p < 0.0001$).
Conclusions: Our results suggest that administration of PR followed by SCT could be associated with myocardial injury manifested by increased release of GPBB from cardiomyocytes which could correlate with diastolic LV dysfunction on echocardiography. Whether these acute changes will have predictive value for development of cardiomyopathy in the future is not clear and will be studied.
Financial support: RO 1011 (FMHS).

Novel Prolyl Oligopeptidase Inhibitors from the Group of Isoquinoline Alkaloids – Possible Candidates for Alzheimer Disease Drug Discovery

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Alzheimer disease (AD) is becoming a priority of public health care, and therefore there is the need of novel effective drugs. Among many thousands of modern drugs, about 40 % are of natural origin. The widest spectrum of action is exhibited by alkaloids. Amaryllidaceae alkaloid galanthamine has been launched onto market in 2001 as a treatment of AD. In the last years, enzyme prolyl oligopeptidase (POP) gained importance as a possible target for the treatment of cognitive disorders in AD patients. In our study, we evaluated biological activity of several isoquinoline alkaloids, isolated from the families Amaryllidaceae (Chlidanthus fragrans, Nerine bowdenii, Zephyranthes robusta), Fumariaceae (Corydalis cava, Fumaria officinalis) and Papaveraceae (Eschscholtzia californica) with the aim to find new type of compounds, modifying POP activity. We found several alkaloids that were POP inhibitors. Tested compounds could be lead structures for the development of new, more efficient AD drugs.
This work was supported by grants SVV UK 267002, Charles University grant Nr.204026/2012/UNCE, the European Social Fund and the state budget of the Czech Republic (CZ): TEAB Nr. CZ.1.07/2.3.00/20.0235, Grant Agency of the CZ Nr. P303/11/1907, and by a long-term organization development plan 1011.

Paroxetin Prenatal Toxicity Assessment Employing the Chick Embryotoxicity Screening Test.

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Antidepressants represent frequent therapy during pregnancy, nearly 3% of pregnant women use this drugs. The teratogenic risk of the antidepressants exposure is not significantly increased in general, however in some exceptions the data are still missing or conflicting. Paroxetin, the selective serotonine reuptake inhibitor, is associated with small increased risk for cardiovascular anomalies, mainly septal defects and right ventricular outflow tract. Some experimental studies document the decerease of proliferation in the cardiomyocytes culture. We used the simple and fast procedure (CHEST – Chick Embryotoxicity Screening Test). Daily dose of paroxetin was approximated and administered intraamniotically to the groups of 3 day old and 4 day old chick embryos. At the end of embryonic period the outcomes were evaluated. Embryotoxic potential was significantly higher in both experimental groups. The group of the embryos treated on 4 day was afflicted by embryonic death (- LD50), teratogenicity (body wall defects prevailed unequivocally) and growth retardation. Exposure of the 3 day old embryos results in multiple organ malformations in comparison to control group, manifesting only moderate body wall defects.
Work was supported by PRVOUK P32.

Study of the Protection Against the UVA-induced Damage in NIH 3T3 Cells by the Novel Quercetin Derivatives.

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Quercetin-type flavonols (primarily as quercetin glycosides), the most abundant of the flavonoid molecules, are widely distributed in the plant kingdom. It can be found elsewhere that *in vitro* studying of their broad and full potential can be highly limited by the deliberation of the aglycon by the metabolic pathways. Therefore, more accurate would be application of the quercetin moiety only. Such approach creates a possibility further synthetically modify the quercetin molecule to modulate and/or potentiate its natural activities.
Quercetin, have been shown to reduce ultraviolet (UV) irradiation-mediated damage in various cell types. UV light in general, as well as UVA light, used in our study, induces the formation of the reactive oxygen species (ROS) and quercetin is declared as a potent ROS-scavenger.
Based on the above, we have studied the protective properties of newly synthetized acyl mono/di-quercetin derivatives against the UVA-induced toxicity. Our goal was to find derivative(s) with higher protective potency compare to quercetin. MTT-assay have been used for primary screening of the viability and proliferation of the UVA-induced murine fibroblast cell line NIH 3T3 cultured in the presence quercetins.
Supported by: APVV 0339-10 and VEGA 1/0191/12

Regulation of Organic Cation Transporter 1 (OCT1, SLC22A1) Expression via Major Nuclear Receptors in Primary Human Hepatocytes

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Organic cation transporter 1 (OCT1) is an important transporter for uptake of cationic drugs into hepatocytes. Its expression in hepatocytes is strongly controlled by hepatocyte nuclear factor-4α (HNF4α). We found that dexamethasone through glucocorticoid receptor (GR) significantly up-regulates OCT1 mRNA and protein in primary human hepatocytes.
We examined direct and indirect transactivation of OCT1 gene in primary human hepatocytes. We also examined which other liver-enriched transcription factors are involved in OCT1 gene expression and if they are regulated by dexamethasone.
Gene reporter construct with 2 kb promoter sequence of OCT1 gene was not responsive to glucocorticoids in cells cotransfected with GR expression construct, but was sensitive for CCAAT/enhancer binding proteins β (C/EBPβ) and HNF4α cotransfection in HepG2 cells. We found that expression of OCT1 mRNA in human livers significantly correlates with C/EBPβ and HNF4α mRNAs expression. We observed that HNF4α is induced by dexamethasone in primary human hepatocytes, but not in hepatocyte-derived cell lines. Neither C/EBPβ nor PGC1α were up-regulated in human hepatocytes by dexamethasone.
We can conclude that OCT1 is induced only through GR nuclear receptor via an up-regulation of HNF4α in primary human hepatocytes.
We acknowledge grant support from the Czech Scientific Agency (Centre Excellence project P303-12-G163).

Proapoptotic Activity of Ecballium Elaterium Juice on CCl4-induced Hepatotoxicity in Rats

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Extract from the fruits of *Ecballium elaterium* is used in the folk medicine in a number of indications as analgetic, antipyretic and hepatoprotective agent. The aim was to investigate the effects of the perorally administrated juice on CCl_4-induced hepatotoxicity model in rats. The content of the active substance cucurbitacin B was assessed, biochemical and histopathological examination with immunohistochemical detection of apoptosis were performed. Reduction of toxic liver tissue damage and proapoptotic effect of the extract were confirmed.
The study was supported by the grant IGA VFU 4/2010/FaF.

The Effect of Atrazine on Early Life Stages of Common Carp (Cyprinus carpio)

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Atrazine and its chloro-s-triazine metabolites are found in surface water, groundwater, even though they are banned in the Czech Republic since 2005. The objective of the study was to determine the subchronic toxic effects of atrazine at concentrations of 0.3; 30; 100 and 300 μg L^{-1} on early life stages of common carp and antioxidant defence enzymes. Atrazine has not been effected the weight and growth rate evolution. Exposure at 0.3 μg L^{-1} was associated with significant increased of activities glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase compared to control. Activity of glutathione reductase (GR) was the slight increases only in the first concentration of atrazine (0.3 μg L^{-1}) and significantly lower ($p < 0.05$) GR were observed in groups exposed to 30, 100 and 300 μg L^{-1} compared to the group exposed to 0.3 μg L^{-1}. The level of oxidized lipids slightly increased in experimental groups at 100 and 300 μg L^{-1} compared to control. Atrazine demonstrated the significant influence on the biotransformation enzyme and oxidative defence enzymes of exposed early life stages of common carp. Based on these results were determine a lowest observed effect concentration (LOEC) = 0.3 μg L^{-1}.
This research was supported by GACR P502/12/P163.

Potential Anticlastogenic Effect of Hyperforin

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Hyperforin is a prenylated phloroglucinol derivative which occurs in the plant *Hypericum perforatum* L. It has several medicinally important properties (antidepressant, anti-inflammatory, proapoptotic, antibacterial and antiangiogenic). To enable its medicinal use, it is necessary to investigate its potential genotoxic effect on human cell lines. We observed also anticlastogenic effect of hyperforin towards the indirect mutagen benzo(a)pyrene. Benzo(a)pyrene is a widespread polycyclic aromatic hydrocarbon. We performed experiments on the human tumor cell line HepG2, using the in vitro mammalian chromosome aberration test. We investigated two cytogenic parameters: the number of aberrant metaphases and the total number of chromosome aberrations. We found out that hyperforin was not genotoxic on human cell line HepG2. The second part of our results implies that hyperforin had anticlastogenic effect against the indirect mutagen benzo(a)pyrene in our experimental conditions. In the future we will continue in our research using another range of hyperforin concentrations, other cell lines and other chemical mutagens.
This study was supported by Grands: VEGA 1/0025/11; VEGA ; APVV-0040-10; SK-BG-0006-10; BG SK/206

Novel Acridine Glycoconjugates Based on Thiosemicarbazides

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New acridine glycoconjugates with glucose, galactose, and mannose connected to the acridine skeleton by thiosemicarbazide, semicarbazide, and isothiosemicarbazide linkers have been prepared and let to react with bifunctial electrophiles, methyl bromoacetate, bromoacetyl bromide and dimethyl acetylenedicarboxylate, to afford novel glycosylated acridinylthiazolidinones. Regioselectivity of studied reactions and structure of products have been extensively studied by NMR. Selected conjugates and cyclic products have proven interesting antitumor activity, inhibition of free radicals and protein aggregation or depolymerization of amyloid fibrils in preliminary tests.

Financial support from the Slovak Grant Agency VEGA, grant 1/0672/11, is gratefully acknowledged.

Oxidation of Carcinogenic Benzo[a]pyrene by Human and Rat CYP1A1 and its Influencing by Cytochrome b5

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Rat liver microsomes, in which CYP1A was induced by Sudan I, generated BaP-9,10-dihydrodiol, BaP-4,5-dihydrodiol, BaP-7,8-dihydrodiol, BaP-1,6-dione, BaP-3,6-dione, BaP-9-ol and BaP-3-ol, which were separated with HPLC. The same metabolites were generated by human hepatic microsomes, but no BaP-9-ol was detected in human microsomes. Rat recombinant CYP1A1 expressed with NADPH:CYP reductase (POR) in Supersomes™ oxidized BaP also to BaP-9,10-dihydrodiol, BaP-4,5-dihydrodiol, BaP-7,8-dihydrodiol, BaP-1,6-dione, BaP-3,6-dione, BaP-9-ol and BaP-3-ol, but additional metabolite (Mx) was eluted by HPLC. Human CYP1A1 expressed with POR in this microsomal system oxidized BaP to BaP-9,10-dihydrodiol, a metabolite Mx, BaP-7,8-dihydrodiol, BaP-1,6-dione, BaP-3,6-dione, BaP-9-ol and BaP-3-ol. BaP-4,5-dihydrodiol has, however, not been detected with human CYP1A1. Addition of cytochrome b5 to the rat and human recombinant CYP1A1 systems led to a more than 2-fold increase in BaP oxidation.
Supported by P301/10/0356, 640712 UNCE204025/2012

Hydroxychloroquine Decreases Toxic Potential of Human Neutrophils

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Activated neutrophils produce and release a great variety of toxic substances (e.g. reactive oxygen species, cytotoxic proteins, proteolytic enzymes), which are operative in the elimination of invading pathogens. However, this "destructive hardware" can cause tissue damage and contribute to persisting inflammation. From this perspective, pharmacological intervention capable to reduce the activity and/or to enhance apoptosis of neutrophils represents a prospective way to support the therapy of chronic inflammation. Hydroxychloroquine, a drug used in patients with rheuma-

toid arthritis, has a potential to reduce the toxic action of neutrophils. This substance reduced the formation of reactive oxygen species, decreased the activity of protein kinase C (the main regulatory enzyme in neutrophils), and enhanced the activity of the executioner caspase-3.
Supported by grants APVV-0052-10, VEGA 2/0010/13 and APVV-0315-07.

1-(3-chlorophenyl)-piperazine (mCPP) - Experiences of our Laboratory

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In the year 2004 member states EU informed European Monitoring Centre for Drugs and Drug Addiction about different new synthetic drugs among which, except other drugs, appeared also derivates of piperazine, concretly m-chlorophenylpiperazine (mCPP), benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP). Effects of the mentioned substances remind effects of methylendioxyamethamphetamine (MDMA) and methamphetamine. In many countries, their use is not controlled, and therefore they have become a legal alternative to MDMA. Since november 2007 mCPP is listed in first class of psychotropic substances of Law 139/1998. The first seizure of piperazine derivate in Slovakia was in August 2005 and until nowdays Institute of Forensic Science registers total amount of 2021 tablets with mCPP content, 239 tablets with mCPP and BZP content and 10994 tablets with mCPP and MDMA content.

Biological Activity of Cholinesterase Modulators and their Interactions with DNA

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A series of cholinesterase modulators was investigated. These compounds showed DNA binding activity ($K=5.0\text{-}8.9\times105\ M^{-1}$). With spectroscopic techniques we indicated that the compounds are act as effective DNA-interacting agents. We have also confirmed their biological activity through experiments focused on their anticancer action. Derivatives 3-5 proved to be effective against HL–60, evoking rapid mitochondrial membrane potential dissipation linked with drop in cell metabolism and loss of viability in concentrations up to 100 μM. The derivative 4 proved to be the most effective as it caused almost absolute eradication of cells at 50 μM concentration. Therefore we presume that some of the derivatives have a potential application as the anti-cancer drugs.

This study was supported by VEGA grant No. 1/0001/13.

Influence of Cranberry on Activity of Cytochromes P450 (CYP) in Mouse Liver Microsomes

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Cranberry (*Vaccinium macrocarpon*) is a fruit which is particularly rich in polyphenolic compounds. The most abundant flavonoids extracted from cranberry fruits are proanthocyanidins (PACs), reported to possess antimicrobial, antiadhesion and antioxidant properties. Because cranberry is known to have a beneficial influence on human health we have studied the effect of long time application of cranberry on enzyme of biotransformation, namely, the levels and activities of CYPs in mouse liver.
Eight months old male NMRI mice were divided to two groups: The controls (A) and to an experimental group (B). Group B was treated with 2% (w/w) cranberry extract mixed with diet for 4 weeks. Control group was fed standard diet. Specific substrates typical for individual human CYP enzymes (1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4) were used to evaluate the influence of cranberry on the respective CYP enzyme activities.
Significant increase was observed in activities of testosterone 6-beta hydroxylase (corresponding to human CYP 3A4) and chlorzoxazone 6-hydroxylase (human CYP 2E1) after administration of cranberry. On the other hand, slight decrease was detected in activity of coumarin 7-hydroxylase (human CYP 2A6).

Financial support from the GACR 303/12/G163 and LF_2013_008 is acknowledged.

Bioscavengers – Efficient Antidotes for Poisoning by Nerve Agents and Organophosphorus Pesticides

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Organophosphorus compounds (OC), such as nerve agents (sarin, tabun, VX) and active oxon forms of some pesticides (chlorpyrifos, parathion, malathion) are strong inhibitors of enzyme acetylcholinesterase (AChE; EC 3.1.1.7). Their acute toxicity is caused by subsequent accumulation of acetylcholine, resulting in cholinergic crisis and possible death of the intoxicated organism. There are two basic ways how to protect the organism against OC: protecting AChE against its inhibition (currently available antidotes) and the decreasing the concentration of inhibitor in blood (enzyme scavengers, commonly called "bioscavengers"). Catalytic (paraoxonase, phosphotriesterase, prolidase and senescence marker protein-30), stoichiometric (AChE, butyrylcholinesterase) and pseudo-catalytic (combination of cholinesterase and its potent reactivator) bioscavengers are intensively investigated for these purposes. These biomolecules can improve the efficacy of available medical protection against highly toxic OC in the near future.

This work was supported by a long-term organization development plan 1011.

Renal Toxicity of Bacterial Lipopolysaccharide is Prevented by IL-1 Antagonist

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Sepsis induced by gram-negative bacteria imposes acute kidney injury (AKI) by lipopolysaccharide (LPS)-mediated activation of severe immune response. In the present study we sought for possibility to prevent such impairment in rats by two potent anti-inflammatory agents, dexamethasone, and anakinra. Untreated LPS rats developed within 10 h typical symptoms of AKI characterized by reduced glomerular filtration, microalbuminuria, increased fractional excretion of sodium, and decreased tubular secretion of azithromycin, the substrate for multidrug transporters Mdr1 and Mrp2. Pretreatment with either drug alleviated these symptoms and restored the azithromycin tubular secretory clearance to control values. This effect was related to up-regulation of basolateral organic anion transporters. Both drugs also reduced elevated levels of plasma cytokines, but only dexamethasone down-regulated iNOS expression. In conclusion, dexamethasone and anakinra were both able to mitigate AKI imposed by LPS and modulated impairment in the expression of major transporters for renal drug elimination. We demonstrated significant role of IL-1β for the development of AKI imposed by LPS.

We acknowledge grant support form the Charles University No. SVV-2013-266901, SVV/2013/267-003, and PrvoUK P37/05.

The Reactivating, Therapeutic and Neuroprotective Efficacy of Two Newly Developed Oximes (K456, K458) in Rats and Mice Poisoned with Tabun – A Comparison with the Oxime K203 and Trimedoxime

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The reactivating, therapeutic and neuroprotective efficacy of two novel bispyridinium compounds (K456, K458) in tabun-poisoned rats and mice was compared with the oxime K203 and trimedoxime. The study showed that the reactivating efficacy of both newly developed oximes is comparable with K203 but lower than the reactivating potency of trimedoxime in diaphragm. In the brain, their potency to reactivate tabun-inhibited acetylcholinesterase is lower compared to trimedoxime and the oxime K203. A comparison of therapeutic efficacy of both novel oximes with the oxime K203 and trimedoxime corresponds to their peripheral reactivating efficacy. Both newly developed oximes (K456, K458) combined with atropine are able to decrease tabun-induced neurotoxicity in the case of sublethal poisonings although they do not eliminate all tabun-induced acute neurotoxic signs and symptoms. Their ability to decrease tabun-induced acute neurotoxicity is slightly higher than that of trimedoxime and the oxime K203 but the difference of neuroprotective efficacy among all oximes studied is not so high to make the decision about

the replacement of commonly used oximes (especially trimedoxime and obidoxime) in the treatment of acute tabun poisonings.
The study was supported by the grant of Ministry of Defence – „Long-term organization development plan 1011“.

The Determination of Lead Ions Encapsulated in Liposomes

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The aim of this study was preparation of liposome complex with encapsulated lead ions. This nanostructure was tested using differential pulse voltammetry (DPV) and atomic absorption spectrometry (AAS). We focused on the effect of the electrolyte pH to open nanostructure. The optimal results were achieved at pH 4. The most efficient encapsulation of lead ions was detected in initial concentration 0.5 – 1 mg/ml of the lead. Lead concentrations detected by DPV were lower (50 %) in comparison to the values determined by AAS. The AAS determined the total lead concentration; however DPV measured only lead released from the liposome structure. Lower concentrations of lead detected electrochemically set are apparently caused only by partial opening of liposome structure. More detail investigation is required and other options of opening of the liposome structure will be examined.

Acknowledgements
This contribution has been supported by MSMT 6215712402.

The Study of Protein Interactions within the MFO System by Photo-Activatable Proteins

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The multienzyme system of mixed function oxidases (MFO) plays a significant role in the metabolism of endogenous compounds and xenobiotics. This system consists of membrane bound cytochrome P450 (CYP), NADPH:cytochrome P450 reductase, cytochrome b_5 (cyt b_5) and NADH:cytochrome b5 reductase. Cyt b_5 can influence the rate of CYP-dependent reactions and even change the pattern of resulting metabolites. To study these effects as well as the membrane organization of MFO components, the approach based on photo-croslinking was employed. For that purpose proteins containing photo-activatable amino acids, e.g. photo-methionine should be prepared. We managed to prepare cyt b_5 with photo-methionine and the expression of photo-activatable CYP2B4 is in progress.

Cytotoxicity of Modified Types of Carboxymethyl cellulose

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Cellulose derivates have been found to be effective, safe, and biocompatible biopolymers. Although they are all based on the same polysaccharide, some differences between modified types of cellulose that could be important in clinical use do occur. The cytotoxicity of six types of carboxymethyl cellulose (CMC) after 24h exposure on human THP-1 cells was examined using erythrosine exclusion test. Results showed that silver-containing CMC exerted significant cytotoxic effect in all tested concentrations. On the contrary, other types of CMC were non-toxic. In conclusion, the use of Ag-containing CMC for anti-hemorrhagic or wound-healing therapy should be considered carefully.
(Supported by TACR grant No. TA01010244)

The Proving of the Taxoid Intoxication of Baboon Using FIA-Q-TOF

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The toxic effect of *Taxus baccata* which is know from Middle Ages is due to presence of cyanogen glycosides and mixture of alkaloids known as taxins. The Taxin B is based as one of the most toxic taxane et all. This taxiod is mostly present in bodily fluids when poisoning by Taxus is occurred. In our work we determined the selected taxols including taxin B as markes of the intoxication of baboon using flow injection analysis with MS detection. Taxin B is detected in the mass m/z 584.2. We determined the urine, gall and blood serum from *Papio Anubis* which was found in the cage lying on the floor and dead. Due to presence of the berries of *Taxus baccata* on the floor of the cage after previous wind storm during all previous night the cause of death by *Taxus* intoxication has been proposed and confirmed by autopsy. Due to selected method we were able to determine a presence of taxinu B/isotaxinu B in all three samples. In the samples of urine and gall the concentration of Taxin was 6 times higher than in serum which is pointing on the advanced state of metabolisation of toxoids and thus it proves the cause of the death.

Acknowledgements: This contribution has been supported by MSMT 6215712402.

Interaction of Anthocyanins with Human Liver Microsomal Cytochromes P450

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Anthocyanins are a largest group of water soluble pigments ubiquitous in the plant kingdom. There have been over 600 anthocyanins identified in nature, featuring six common aglycones - anthocyanidins (cyanidin, delphinidin, malvidin, peonidin, petunidin and pelargonidin) and various types of glycosylations. Anthocyanins possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, all of which are associated with their potent antioxidant property. Interaction of three forms of human hepatic cytochromes P450 - CYP1A2 (7-ethoxyresorufin O-deethylation), CYP2C9 (diclofenac 4′-hydroxylation) and CYP3A4 (testosterone 6β-hydroxylation) with anthocyanins was studied using pooled human microsomes. The most influenced activities were interactions of CYP1A2 with delphinidin, CYP2C9 with pelargonidin, peonidin, malvidin and peonidin and CYP3A4 with all tested anthocyanidins.

Financial support from GACR P303/12/G163 project is gratefully acknowledged.

Modulation of Biochemical Indices in Common Carps (*Cyprinus carpio* L.) Exposed to Toxic Cyanobacterial Biomass

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The aim of this study was to evaluate the influence of toxic cyanobacterial biomass on plasma indices of one-year-old common carps (*Cyprinus carpio* L.). The total dose of microcystins in the experimental group was 27 mg/kg of food, i.e. 0.4 mg/kg of fish weight and day. Eight specimens from experimental and control groups, respectively, were taken on days 1, 7, 14, 21 and 28. Following plasma parameters were evaluated: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine, total protein, glucose, lactate, albumin, bilirubin, urea, cholesterol, triglycerides, Ca, Mg, P, Fe, Na, K, and Cl. Statistical evaluation of the influence of cyanobacterial biomass on biochemical indices of common carps showed only minimal changes between values of control and experimental groups. Only values of aspartate aminotransferase and urea of experimental fish were significantly decreased in comparison with the control group ($p \leq 0.05$).

Oxidative Stress Induced by Arsenic Compounds in Rats - The Influence of Age

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Oxidative stress is suggested as one of the mechanisms of As–induced toxicity. In this study, oxidative stress–related parameters and As retention were examined in the liver and kidney of male Wistar rats (age of 7 and 21 weeks) exposed

to arsenic trioxide, sodium arsenite, and sodium arsenate at a single *ip* dose of 3.8 mg As/kg bw, at 24h post-exposure. In the liver, oxidative injury was manifested by increased lipid peroxidation (LP), decreased catalase (CAT), and altered glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) activities. Significant influence of age was found on the extent of LP (115% vs. 300% of control level in young vs. old rats) and in the effects on GPx and TrxR. Inhibited GPx and TrxR activity was found in young rats, while unchanged GPx and increased TrxR activity was found in old rats. Elevated GSH level and unchanged glutathione reductase activity were estimated in rats of both age. In the kidney, retention of As was higher compared to liver; however, only increased LP and elevated GSH level were found in 21wk-old As(III)–exposed rats.
It is evident, that protective mechanisms against acute As exposure are differently exhibited depending on age of rats. The effects of As exposure on the GPx and TrxR activities may relate to As and Se antagonism.

An *In Vitro* Study of Acetylcholinesterase Inhibitory Effects and Cytotoxicity of New Proflavine Derivatives

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3,6-bis(3-alkylguanidino) acridines (GNDAs) had been synthesized as a new group of proflavines with anticancer activities. However, our experiments showed that these compounds possess only moderate cytotoxicity against neuroblastoma SH-SY5Y cells and in addition, GNDAs decreased activity of acetylcholinesterase (AChE) in SH-SY5Y cells. Their localization in plasma membrane was confirmed. Kinetic studies indicated that GNDAs inhibit AChE by the same mode as tacrine.
Supported by APVV-0282-1.

***In Vitro* Testing of HI-6 Skin Permeation**

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Transant is a transdermal patch used as a protective agent against organophosphate intoxication in the Czech and Slovak armies. It contains HI-6, which is one of the most efficient reactivators for quite a broad spectrum of organophosphates, as its major component. The aim of our presented work was to compare ability of the two HI-6 salts – dimethanesulphonate and dichloride – to permeate the skin. As *in vitro* model, we used static Franz-type diffusion cells and we worked with split-thickness pig abdominal skin. Phosphate buffered saline solution was used as a receptor fluid. Applied HI-6 was dissolved in water, citrate-phosphate buffer or in the phosphate buffered saline, too. Each experiment took the time of 24 hours and samples were collected at one hour intervals. The content of HI-6 in the samples was determined by ion-exchange liquid chromatography.
A long-term organization development plan 1011 is gratefully aknowledged.

Employing Photo-labile Protein Nanoprobe and Mass Spectrometry: Cytochrome P450-cytochrome b5 Membrane Topology Study

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Cytochromes P450 (P450s) - catalyze xenobiotics detoxification, drug metabolism and unfortunately also carcinogen activation – could be modulated by facultative redox partner cytochrome b_5 (cyt b_5) which can mediate the second electron transfer in P450 catalytic cycle.
Photo-labile nanoprobe – cyt b_5 with methionines substituted with photo-labile analog – was expressed in *E. coli* and purified. After reconstitution with P450 2B4 in lipid membrane and photo-activation, the newly formed covalent protein complexes were separated by SDS-PAGE, proteolytically digested and analyzed by liquid chromatography coupled with high resolution mass spectrometry. The existence of mutual interactions of P450 and cyt b5 through their hydrophobic domains was found.
Supported by GAČR P207/12/0627 and UNCE 204025/2012.

The Effect of General Anaesthesia on Gastric Myoelectric Activity in Experimental Pigs

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Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical aktivity (GMA). The aim of this study was to evaluate the impact of different anaesthetic agents on EGG in preclinical experiment. Four 15-minute EGG intervals were recorded and analysed. A baseline EGG recording was started 20 min after intramuscular injection of basic hypnotics, ketamine + azaperone (periods A and B). Four different regimens of general anaesthesia followed immediately after the baseline EGG (5 pigs in each group): thiopental, isoflurane, N_2O and isoflurane + N_2O. EGG recordings followed for the next 30 min under general anaesthesia (periods C and D). The dominant frequencies of slow waves were compared between the baseline intervals A and B and periods C and D. The mean dominant frequency (DF) was within the normal range (2.3 - 3.5 cycles per minute-cpm) in all animals in all regimens. Thiopental did not influence any change of the DF of slow waves. N_2O increased the DF of slow waves in a statistically significant manner (baseline: 2.93±0.53; under general anaesthesia: 3.28±0.34 and 3.28±0.38 cpm; $p < 0,001$, $p < 0,001$). Isofluran slightly increased DF but no significantly (baseline: 3.05±0.28; under general anaesthesia: 3.28±0.33 and 3.28±0.36). N_2O together with isoflurane induced a decrease (statistically not significant) of DF in the last 15-minute interval compared to the baseline recording. All changes of porcine GMA assesed by the DF of slow waves during EGG remained within the normal range although some of them achieved statistical significance. Thus all tested agents used for general anaesthesia can be recommended in preclinical studies with porcine models focused on GMA without any risk of compromising the results. Thiopental seems to be the most suitable as it did not cause any changes at all.

Acknowledgements: The study was supported by research project IGA 14270 from the Ministry of Health (Czech Republic).

Equol; a Possible Way for Modulation of Neutrophils Activity.

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Neutrophils are the most common cells in acute inflammation processes and they provide the first line of defense. Dysregulation in their function does however give rise to damage of host tissue. Thus despite their critical function in protection, activity of neutrophils should not be prolonged to avoid chronic and systemic inflammation responsible for the pathogenesis of a wide variety of diseases. Inhibition of the pro-inflammatory processes of neutrophils seems to be a good way for affecting undesirable mechanisms in cells. Phenolic compounds are currently receiving much attention because of their antioxidant, anti-inflammatory, cardio-protective, cancer chemo-preventive and neuro-protective properties. Equol, as a member of this group, was found to significantly modulate extra- and intracellular redox environments and to affect neutrophil activity during inflammatory processes.
Supported by grants APVV-0052-10, VEGA 2/0010/13 and APVV-0315-07

Effects of Two Isomers of Retinoic Acid and Genistein on the Expression of Selected Nuclear Receptors, their Coregulators and Genes Involved in Apoptosis in ACHN Human Kidney Cancer Cell Line.

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Retinoids/rexinoids, ligands for retinoic acid receptors (RAR) and retinoid X receptors (RXR), have been used in the treatment of various malignant diseases. Retinoid signalling can induce cellular differentiation or apoptosis, which is particularly relevant in the treatment of cancer. Nuclear retinoic acid and retinoid X receptors are transcription factors that play an irreplaceable role in human organism. RXRs which play unique modulatory and integrative roles across multiple regulatory systems, is the only known natural heterodimerizing partner of retinoic acid-inducible transcription factors. Genistein belongs to the isoflavone class of flavonoids and it is also classified as a phytoestrogen with many favourable effects on cancer cells. However, there is a lack of relevant data concerning possible effect of genistein on kidney performance and carcinogenesis. The aim of present study was to investigate the *in vitro* effects of all-*trans* retinoic acid, 9-cis retinoic acid, genistein and their combinations on expression of selected nuclear receptors, their coregulators and genes involved in apop-

tosis in ACHN human kidney cancer cell line.
Supported by the APVV-0160-11, APVV-SK-CZ-0211-11, 7AMB12SK151, VEGA- 2/0008/11, and CEMAN grants.

Determination of New Antiepileptic Drug Lacosamide and Metabolite Desmethyl Lacosamide in Patients with Epilepsy by Liquid Chromatography-tandem Mass Spectrometry

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Introduction: Lacosamide (LCM) is a functionalized amino acid used as unique antiepileptic drug for the adjunctive treatment of partial-onset seizures. New bioanalytical method for determination of lacosamide and its metabolite desmethyl lacosamide (DMLCM) in plasma samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and applied in clinical practice. Method: Sample preparation included the precipitation of 0.05 mL plasma sample using acetonitrile and zinc sulfate. LCM, DMLCM and the internal standard lacosamide-d3 were separated on a Discovery HS F5 column. The mobile phase consists of 75% formic acid 0.1% and 25% acetonitrile. Analytes were detected using a triple quadrupole mass spectrometer with positive electrospray ionization. The quantification was performed using MRM transitions (*m/z*): 251 >116 (LCM), 254>119(lacosamide-d3) and 237>91 (DMLCM). Results: The lower limit of quantification was found to be 1.14 µmol/L (LCM) resp. 1.12 µmol/L (DMLCM). The range of linearity was estimated from 0.0 to 227.7 µmol/L (LCM) and from 0.0 to 224.3 µmol/L (DMLCM). The accuracy and precision reached acceptable values from 90.2 to 105.1 % (recovery) respectively, values were from 1.9 to 10 % (CV). The run time was 8 min. Conclusion: The bioanalytical LC-MS/MS method was employed in epileptics for the drug monitoring of LCM and DMLCM.

Acknowledgements: Development of the method was supported by research project MH CZ-DRO (UHHK, 00179906).

Stereoselective Synthesis of (+)-lactacystin with Using [3,3]-sigmatropic Rearrangements

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The ubiquitin-proteasome pathway is the major proteolytic system in the cytosol and nucleus of all eukaryotic cells. The inhibition of proteasome induced of apoptosis cell. (+)-Lactacystin was the first identified natural 20S proteasome inhibitor where it selectively binds in the β5 subunit through an ester covalent bond. Proteasome targeting recently emerged as a new modality for the potential treatment of diseases ranging from malaria to cancer. The first synthesis of (+)-lactacystin reported in 1992 Corey at al. We present the preparation of precursors for stereoselective synthesis of (+)-lactacystin from D-glucose. The key steps our synthesis are [3,3]-sigmatropic rearrangements performed by conventional or microwave heating.
The financial support from the Slovak Grant Agency VEGA (1/0100/09, 1/0433/11) and UPJS-VVGS 43/12-13, VVGS PF 6/2010/Ch, VVGS 16/2011/Ch VVGS-PF-2013-121 is gratefully acknowledged.

Czech Teratology Information Service (CZTIS) – the Source of Information on the Embryotoxicity.

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CZTIS, Oddělení histologie a embryologie, 3. lékařská fakulta UK v Praze

Czech Teratology Information Service was found in 1996 at the Histology and Embryology Department of 3rd Faculty of Medicine, Charles University in Prague. Service is used by health care providers or women by e-mail or phone inquiries. Majority of questions are focused on exposure during 1st trimester or on exposure during all pregnancy in cases of chronic diseases. In such cases we are contacted in 30% cases before pregnancy. We collect 300 calls in average annually. Inquiries with follow up are used for epidemiological studies. CZTIS conducted studies on psychotropic drugs and appetite suppressant. We also participate on collaborative studies (macrolides, statins, mirtazapine). The evidence-based information concerning the optimal therapy during pregnancy is disseminated among health care providers. The data and our experience in the CZTIS we disseminate among pre-graduate students in the course „Reproductive Toxicology". Students become informed to the CZTIS praxis and the methods of embryotoxicity testing (we use the chick embryo).
Work was supported by PRVOUK P32.

Comparison of *In Vitro* Organ Toxicity in Selected Acetylcholinesterase Reactivators Using Hepatic and Renal Cellular Models.

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The aim of the work was to evaluate organ-specific cytotoxicity of a group of acetylcholinesterase reactivators (RACHE) using two cellular models representing hepatic and renal tissue. Assays based on determination of metabolic and membrane damage indicators were used in the experiments.
In most cases higher IC_{50} values of the tested RACHE were found in the liver cells Hep G2 than in the renal cells LLC-PK1. The substances K027, K203 and HI-6 exerted the lowest toxicity among the tested RACHE. The highest toxicity was found in substances JM-4 and K043. K043 was the most toxic agent for the renal cells, JM-4 for the hepatic cells. IMT showed different toxicities in the particular cellular models.
In summary, the performed in vitro study demonstrated similar results on cytotoxicity of the tested drugs. Even we found some differences in cytotoxicity parameters of the compounds between the hepatic and renal cells, substantial organ-specific differences were not observed.
The study was supported by European social fund and the state budget of the Czech Republic. Project No. CZ.1.07/2.3.00/30.002, and by Charles University in Prague (Project SVV 267003).

Development of Novel Quaternary Detergents as Part of Decontamination Means

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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 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 pyridinum ring with different substituents. These compounds are currently tested for their decontamination and disinfection potency.

The work was supported by the Long Term Development Plan 1011

Synthetic Cannabinoids as an Alternative to Cannabis

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Synthetic cannabinoids were originally synthesized for biomedical research purposes as pharmacological tools for studying the endocannabinoid system. Various synthetic cannabinoids are now used recreationally as an alternative to Cannabis (marijuana). The primary psychoactive compound in Cannabis is the Δ^9–tetrahydrocannabinol (THC), which exibits partial agonistic activity at CB_1 cannabinoid receptors, found mainly in the central nervous system, and CB_2 receptors in the pheriphery. Synthetic cannabinoids may be full agonists and selective for one receptor subtype and thus be possessed of different therapeutic and abuse potentials. Synthetic cannabinoids are marketed as „herbal incense“ or „legal highs,“ often the chemical agonist compounds are being sprayed onto inert plant substrates and smoked. First synthetic cannabinoids were JWH-018, JWH-073, JWH-210…. Nowadays tens of synthetic cannabinoids are synthesized. Identification and quantification of cannabinoids in Cannabis and in blood and urine samples of users, and synthetic cannabinoids in botanical materials, powders, capsules and blood and urine samples are performed by GC-MS, LC-MS and LC-MS-MS techniques.
The study has been supported by the grants MV VG 20122015075, MV VG 20122015080

Effect of Pesticide Exposure on Neopterin Levels in Blood Plasma of Common Carp

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Neopterin belongs to a group of unconjugated pterins, derived from guanosine triphosphate (GTP) by guanosine triphosphate cyclohydrolase. Neopterin is synthesized mainly by activated monocytes/macrophages following stimulation by interferon-gamma cytokine (IFN-γ), which is released by NK cells and T-lymphocytes. The aim of the present study was to investigate the effect of subchronic exposure to prochloraz and propiconazole pesticides on blood plasma concentrations of neopterin in common carp. A total of 60 specimens of juvenile common carp were placed into 4 groups and exposed to prochloraz at concentrations of 0, 50, 150 and 380 µg/L and to propiconazole at concentrations of 0, 70, 180 and 580 µg/L. The total length of exposure was 28 days. Our results identified different trends relating to the neopterin. Plasma neopterin concentrations in common carp were higher in groups exposed to prochloraz compared with the control group. On the other hand, plasma neopterin concentrations in carp from the control group and groups exposed to 70 and 180 µg/L of propiconazole were higher compared with the group exposed to 580 µg/L of propiconazole. The results of our study showed that subchronic exposure to prochloraz and propiconazole pesticides influenced plasma neopterin concentrations.

Effect of Deoxynivalenol on Selected Indicators of the Fish Immune System

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Mycotoxins are secondary metabolites produced by filamentous fungi. Deoxynivalenol (DON), also known as vomitoxin, is a toxic metabolite produced by *Fusarium* species. DON is a common contaminant of animal feed, it is abundant in agriculturally grains, such as wheat, barley and corn. DON affects animal health, in fish it causes decrease in feed intake, reduction in feed conversion and reduction in growth performance. There are differences in sensitivity to DON contaminated feeds in fish. Extremely sensitive is rainbow trout (*Oncorhynchus mykiss*). On the other hand, there is little information about effect of deoxynivalenol on the fish immune system.
This study was supported by IGA 34/2013/FVHE.

Study of Platinum Group Metals Effects on Lettuce Plants (*Lactuca sativa*)

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Ecotoxicological tests are used to obtain information on the level of environmental pollution on organisms at all trophic levels of ecosystems.
The aim of this study was to assess ecotoxicity of PGMs platinum group metals (Pt, Pd, Rh), which are getting into the environment mainly from automobile traffic or hospital and industrial waste. A suitable bioindicator for testing harmful pollutants in soil is lettuce (*Lactuca sativa*).
PGMs were added to the tested substrates in defined concentrations and its toxicity was estimated by measuring the root growth of lettuce seedlings (cv. Safır), which were recorded after incubation at 22°C in darkness for 5 days. The method was modified from ISO 11269.
Based on the results, was observed that inhibitory effect of PGMs depend on the concentration increment. The value of the effective concentration (EC) $PtCl_4$, which causes 5% inhibition of growth compared to the control, after time period of 120 hours, was 1.56 µM and 120hEC50 was 40,93 µM. The value of the (120hEC5) $PdCl_2$ was 1,24 µM, (120hEC50) was not found and the value of the (120hEC5) RhCl3 was 0,53 µM, (120EC50) was not found.
Comparing the obtained results it is possible to say that the most sensitive reaction of lettuce on tested metals was in fallowing order $RhCl_3 \rightarrow PdCl_2 \rightarrow PtCl_4$.
The work has been supported by FRVS966/2013/G4.

Military Incapacitating Agent BZ (3-quinuclidinyl benzilate) in Behavioral Research

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Military incapacitating agent BZ is an anticholinergic compound causing a long-term reversible physiological/mental impairment. Recently, BZ has been applied to behavioural research among other anti-cholinergics. Its effect has been several times used to generate an animal model for investigation of learning and memory deficits in laboratory rodents.
The aim of this study was to evaluate the effect of BZ on cognitive functions of rats performing several behavioral tests. The methodology of behavioural tests was adapted to identify impact of BZ specifically on acquisition, consolidation and retrieval stage of learning and memory. Rats were subjected to the water maze with hidden platform, step-through passive avoidance and the multiple T-maze.

BZ was administered to rats intraperitoneally before training (acquisition test) or immediately post-training (consolidation test) or pre-retention (retrieval test).
BZ significantly impaired acquisition of rats in the water maze and passive avoidance. Surprisingly, consolidation and retrieval were not affected by BZ indicating that BZ affects specifically acquisition. Contrary, the effect on retrieval was found in the T-maze. Nevertheless this effect was rather non-cognitive due to peripheral side effects colliding with the principle of test.

Effects of Endocrine Disruptors Di(2-ethylhexyl) phthalate and Bisphenol A in a Combination with 17β-estradiol on Apoptosis-related Genes Expression in the MCF-7 Breast Cancer Cells

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The role of estrogen signaling in mammary carcinogenesis is well established. Environmental endocrine disruptors (EDs) can exert estrogen-like actions influencing hormone-controlled homeostasis. Di(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA) are chemicals employed in the manufacture of plastics for consumer products (food packaging etc.). Leaching from these sources results in ubiquitous population exposure to these EDs. DEHP and BPA could mimic the effects of 17β-estradiol (E2) through binding to ER, nevertheless, may exert also E2-independent biological actions. The breast tissue is exposed simultaneously to endogenous as well as exogenous agents via blood circulation; therefore, it is important to study effects of their combinations. In the present study, we analyzed the effects of endogenous E2 and environmental estrogens DEHP and BPA on several apoptosis-related genes (Bax, Bcl-2, p53, caspase 3) in the human breast cancer cell line MCF-7. The cells were exposed to E2, DEHP/BPA (all at concentrations 1 pM, 1 nM, 1 μM), or their combinations for 24-120 h. The results indicate that proliferative effects induced by E2 and EDs in MCF-7 cells could partially result from their antiapoptotic action.
This research was supported by the APVV-0147-10 grant.

Effect of Mycotoxin Deoxynivalenol (DON) on Haematological and Biochemical Parameters and Selected Parameters of Oxidative Stress in Piglets

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The Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate region and are commonly found on cereals in the temperate region of Europe. The most frequently detected mycotoxins in the Czech Republic are trichothecenes, especially DON and T-2 toxin. According to Commission Recommendation 2006/576/EC, two concentrations of DON in feedstuff for pigs were chosen: 0,6 ppm (concentration below guidance value) and 2,0 ppm (tree times more than guidance value). Pigs were fed with naturally contaminated feed for 4 weeks.
There were no changes in haematological parameters. Statistically significant increases of ALP activity ($p < 0.01$) and glucose concentration ($p < 0.01$) were found out at the end of the experiment. Statistically significant decreases were found out on day 21 in FRAP ($p < 0.001$) and on day 28 in ceruloplasmin ($p < 0.01$).
The decreases of FRAP and ceruloplasmin denote on decrease of the ability of an organism to scavenge reactive oxygen species.

Heterologous Expression of Cytochrome P450 2S1

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Cytochrome P450 (CYP) 2S1 was evaluated to be prepared by the gene manipulations and heterologous expression in *Eschericha coli*. The conditions suitable for production of plasmid pCMV, which contains the *CYP2S1* gene, were optimized and used for expression of CYP2S1. Further, another expression plasmid containing the modified *CYP2S1* gene, pBAD-A, was also prepared and tested for CYP2S1 expression. Nevertheless, as shown from the results found using a Western blot method, expression of *CYP2S1* gene into the CYP2S1 protein in the used *E. coli* expression system has not been successful. Using mass spectrometry, the unsuccessful expression of CYPS1 was proved.
Supported by P301/10/0356 and UNCE204025/2012

Cholinesterase Reactivators – Current Knowledge and Possible Future Directions

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The reactivators of cholinesterases (AChE, EC 3.1.1.7; BChE EC 3.1.1.8) are crucial components in the treatment of intoxications caused by organophosphates (OP) such as nerve agents and pesticides. These OP inhibitors covalently bind to active site of mentioned enzymes and irreversibly inhibit their activity. The reactivator breaks the inhibitor-enzyme covalent bond and restores its activity. Unfortunately, there is no reactivator applicable for both enzymes and every type of OP inhibitor.
The current standard on the field is represented by 5 reactivators (pralidoxime, methoxime, trimedoxime, obidoxime, asoxime) developed in the 20th century. The disadvantages of available reactivators consist in their limited reactivation ability, poor brain bioavailability or relative toxicity. The future trends are focused on design of non-quaternary reactivators, bioscavengers, catalytic scavengers or pseudo-catalytic scavengers to overlap the known issues. The novel directions will be thoroughly discussed.

The work was supported by the University of Hradec Kralove (a long-term organization development plan) and by the Czech Ministry of Education, Youth and Sports (no. CZ.1.07/2.2.00/28.0118).

Confirmatory Bioanalysis of Amphetamines Using LC-MSn System

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Amphetamines are stimulants of central nervous system and have become one of the most dangerous and widespread drugs in Europe. The golden standard for confirmation of positive results from the immunochemical screening is GC-MS. However, in some cases, new faster and technically easier method for amphetamine confirmation in routine analysis is very desirable, especially in the case of large sequences of samples. Mass spectrometry method based on the principle of atmospheric pressure ionization (ESI and APCI) coupled to linear ion trap combined with liquid chromatography for analytical separation has been developed for identification and quantification of most common amphetamines (amphetamine and methamphetamine) in urine samples. Deuterium labeled analogue methamphetamine-D8 (MAMF-D8) was used as internal standard. Full-scan mass spectra and tenth unit resolution product-ion mass spectra in CID and PQD (trade mark of Thermo Fisher Scientific Corp.) mode were obtained with the linear ion-trap analyzer. We analyzed 30 urine immunochemically positive samples and compared obtained results with conventional GC-MS method.
Work is supported by MH CZ-DRO (UHHK, 00179906) and the programme PRVOUK P37/11

Influence of Arsenic and Cyanobacteria Co-exposure on Haematological Parameters of Rainbow Trouts (Oncorhynchus Mykiss)

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Fish can be exposed to multiple stressors in the environment. The aim of this study was to test the hypothesis that effects of cyanobacterial biomass and arsenic can combine to enhance the toxicity in fish. Development of cyanobacteria is a worldwide problem connected with production of many substances including cyanotoxins. Arsenic is a toxic element that is commonly found in feed mixtures for fish. Rainbow trouts were divided into six groups for this experiment, i.e., control group, cyanobacterial biomass exposed group, arsenic exposed groups (5ppm or 50ppm) and cyanobacterial biomass + arsenic exposed groups (5ppm or 50ppm). Following haematological parameters were evaluated: erythrocyte and leukocyte counts, differential leukocyte counts, haematocrit and haemoglobin values, and phagocytic activity. Samples were collected on days 10, 20 and 30 of exposure. The main statistically significant differences were found in leukocyte counts and in the phagocytic activity in groups exposed to higher concentrations of arsenic and in co-exposed groups.

Photometric Study of Obidoxime in the Presence of $(NH_4)_2PtCl_4$

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Obidoxime is an acetylcholinesterase reactivator which restores enzyme activity upon inhibition by organophosphorous compounds (OPC). The low bioavailability of the ligand could be enhanced by its complexation with metal ions and for that reason we have initiated a broad study on coordination properties of Obidoxime.
Our previous research had shown that oxime converts to oximate anion in the presence of ions of Pd(II). The reaction depends on pH and metal-to-ligand molar ratio. For comparative purposes we evaluated the behaviour of Obidoxime in the presence of Pt(II) ($(NH_4)_2PtCl_4$).
The results revealed that UV absorbance of oxime moiety (285 nm) is batochromically shifted when Pt(II) ions are added (Britton-Robinson buffer, pH 7.4, metal-to-ligand molar ratio from 1-10 to 10-1). As compared to palladium(II), a complete conversion of Obidoxime induced by platinum(II) was observed on 72th hour after mixing reagent solutions.
Acknowledgement: The current study was supported by the National Science Fund (DDVU-02-78/2010).

Reactivation of Inhibited Rat Brain Acetylcholinesterase by Palladium(II)-containing Obidoxime

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The irreversible inhibition of acetylcholinesterase (AChE) by organophosphorous compounds leads to muscular paralysis, convulsions and death by asphyxiation. The enzyme activity can be restored using AChE reactivators.
The main disadvantage of oxime-containing compounds as AChE reactivators is their fast metabolism. The properties of biologically active compounds could be enhanced by complexation with metal ions, and here reported are the results on *in vitro* reactivation of AChE by Obidoxime in the presence of Pd(II) cations.
The rat brain AChE was inhibited by Paraoxon (2.10^{-8}M, c.a. 70% inhibition) and its reactivation was studied by Ellman's method. The experimental data have shown that Obidoxime (4.10^{-5}M) restores enzyme activity (c.a. 65%), while the combination Pd(II)-oxime (at molar ratio 2 : 1) leads to lower reactivation of AChE (c.a. 20%).
Acknowledgement: The study was supported by the National Science Fund (DDVU-02-78/2010).

Impact of Platinum Group Elements on the Soil Invertebrate Folsomia Candida

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Trace concentrations of the platinum group elements (PGE; Pt, Pd and Rh) are nowadays an irreplaceable part of the environmental analysis and assessment. These rare elements are used as effective substances in automotive catalysts to reduce pollution by emissions originating from fuel combustion. Due to their harmful potential, it is necessary to monitor their content and behaviour in different samples. From the assessing of the effects of chemical substances, using ecotoxicological bioassays with different trophic levels can be obtained very valuable information of bioavailability in the ecosystems.
We focused on reproductive effects of platinum ($PtCl_4$), palladium ($PdCl_2$) and rhodium ($RhCl_3$), in particular. Our study is based on a collembolan laboratory breed, test optimalization and validation according to the OECD 232 standards [CSN ISO 11267 – Soil quality - Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants]. The concentrations of PGE tested were as follows: 5, 10, 25, 50 and 100 $\mu M.l^{-1}$. The results were evaluated using the inhibition of reproduction compared with controls. The EC_{50} (effective concentration) was determined after the 28-day test.
Acknowledgement: FRVS 965/2013/G4

Study of Transport of Emtricitabine Across the Rat Term Placenta

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Emtricitabine (FTC), a nucleoside reverse transcriptase inhibitor, is used for the treatment of HIV infection in adults, including pregnant women. To assess potential harmful effects of FTC on developing fetus, it is of importance to have detailed knowledge on transplacental transport of this agent. The drug efflux transporters belong to the most important placental mechanisms actively protecting the fetus

against xenobiotics present in the mother's blood. The aim of this study was to describe transplacental transport of FTC *in vivo* and to assess interactions of this agent with placental transporters employing method of dually perfused rat term placenta. Despite FTC did not interact with placental efflux transporters, we observed low passage of this compound across the placenta using both open- and closed-circuit perfusion system. In conclusions, our data suggest weak permeation of FTC from mother to fetus.

Risk of Combined Exposure by Cyanotoxins, Acetylcholinesterase Inhibitors and Anticoagulants to *Xenopus Laevis* Embryos

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Amphibians can face many risks including natural toxins and anthropogenic contaminants such as cyanotoxins, acetylcholinesterase inhibitors and anticoagulants in the environment.
The aim of this project was to test the hypothesis that co-exposure of embryos *Xenopus laevis* to sublethal doses of cyanotoxins, acetylcholinesterase inhibitors and anticoagulants affect the biochemical and histopathological changes, the malformation increase or mortality more than single exposures to these substances.
The experiment was carried out according to the ASTM (1998) FETAX methodology, with minor modification for our experimental conditions. As the tested substances were selected: anticoagulant bromadiolone (350 µg / l), inhybitor acetylcholinesterase paraoxon (300 µg / l) and cyanotoxins microcystin-LR (500 µg / l) at a dose ¼ LC_{50} for *Xenopus laevis*. In each of 9 experimental groups are used 25 embryos and test was duplicated.
Evaluation of the results was made in these parameters: mortality, comparison the effects of test substances to the growth inhibition of the larvae and the amount of larvae malformations. In addition, histopathological examination was performed.
Acknowledgements: The internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno supported the project 27/2012/FVHE

Ochratoxin A – an Important Mycotoxin in the Light of Recent Knowledge

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Ochratoxin A (OTA) is nephrotoxic, hepatotoxic, teratogenic, immunotoxic and carcinogenic mycotoxin. Now it is discussed in the scientific community the question of the mechanism of action for OTA renal carcinogenicity and whether it is a complete carcinogen (initiator and promoter activity). OTA is widespread in temperate areas and ubiquitous throughout the world and is produced by *Aspergillus* (e.g. *Aspergillus carbonarius*) and *Penicillium* (e.g. *Penicillium verrucosum*) species. OTA is a common contaminant of various foods including cereals, spices, coffee, cacao, beer, wine, raisins, pulses, meat, meat products or edible offal. A new research project „*Ochratoxin A – health risk assessment for selected population groups in the Czech Republic*" is realised right now. The project objective is an assessment of dietary exposure of OTA for ten population groups of age 4-90 years, both sexes.
This study was financially supported by the project (no. NT12051-3/2011) from the Czech Ministry of Health (IGA MZ CR)

Resistance of the Isolative Protective Garment Designated for Specialists´ Protection Against Selected Chlorinated Hydrocarbons

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Chlorinated hydrocarbons are very often used and relatively dangerous substances from healthy risks point of view. Within manipulation with them mainly in big amounts the individual protective equipment (IPE) must be used in a protection position. Users are supposed to know construction material breakthrough time especially in case of long-term usage of personal IPE and in the case when contamination of them is real. Study of connections between a chemical compound structure and the structure of IPE characterised by barrier materials enables to understand of present body protective devices protection quality and gives possibility to choose barrier materials with targeted properties. In an article there are results of breakthrough time of isolating protection folio with butyl rubber barrier layer in relation to chlorinated ethanes. This material is used for protection of specialists of both Fire Rescue Brigades and the Czech Armed Forces Chemical Corps

specialists. The PIEZOTEST device has been used for detection of permeated chemicals. The QCM sensor is a part of PIEZOTEST device.

Microcystin Accumulation by the Fish Tapeworm *Khawia Sinensis*

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Parasites have recently been recognized as accumulation indicators that take up and bio-concentrate substances from environmental pollution. Interestingly, helminths of fish are known to accumulate metals from the ambient environment and to contain several orders of magnitude higher concentrations than hosts. While the majority of reports mention inorganic toxin accumulation in parasites, studies concerning effects of organic pollution are infrequent. Here we show accumulation of microcystin MC RR in the tapeworm *Khawia sinensis*, a parasite of common carps (*Cyprinus carpio*). The tapeworms were dissected from experimental carps orally exposed to cyanobacterial biomass for 20 days. The total dose of microcystins amounted to 27mg / 1 kg of feed, i.e. 0.4 mg / 1kg of fish mass a day. Microcystin RR concentrations in tapeworms and carp liver tissues were analysed using the HPLC MS/MS method and amounted to 2.11±0.74 and 5.78±0.37 ng/g fresh weight ($p < 0.05$), respectively.

Mercury Content in the Parasite-host System: *Ligula Intestinalis* vs. *Abramis Brama* and the Effect of the Parasite on Composition of Fish Muscle

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The bioaccumulation potential of parasites, as a result of competition for chemical elements, including heavy metals, represents a valuable instrument to evaluate functions of the parasite-host system. In the system *Ligula intestinalis* vs. *Abramis brama*, the mean mercury concentration in the biomass of plerocercoids was 0.047±0.023 $mg.kg^{-1}$. It is about 6 times less in comparison with fish muscles. The mean mercury concentration in muscles of infected and non-infected fish was 0.36±0.11 and 0.24±0.1, respectively. Composition of fish muscle and spectrum of fatty acids was not changed. *Ligula intestinalis* takes nutrients from the fish body, but according to our results the withdrawal of nutrients monitored in the present study is uniform without any selection.

Changes in Protein Expression in Human Pancreatic Beta Cells NES2Y after Prolonged Exposure to Non-toxic Concentrations of DDT Using 2D-electrophoresis: Preliminary Data

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Increased incidence of diabetes mellitus represents a serious problem worldwide. The possible connection between levels of pesticide DDT and incidence of diabetes mellitus type II was suggested by number of epidemiologic studies, nevertheless direct impact of DDT on pancreatic beta cells is not well described.
The aim of this study was to explore changes in protein expression in pancreatic beta cells after prolonged exposure to lower levels of DDT.
NES2Y cells were exposed for 1 month to three concentrations of DDT (100nM, 1μM, 10μM). Protein expression was analyzed using 2D-electrophoresis and proteins with changed expression were subsequently analyzed by mass spectrometry.
There were six proteins with changed expression detected, both cytoskeletal and enzymes.

Outcome of the Outbreak of Methanol Poisoning in the Czech Republic in 2012

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Since 1945, methanol poisonings have been sporadic in the Czech Republic. In autumn 2012, an outbreak was caused by adulterated spirits.
From September 3 2012, till January 1 2013, 121 methanol poisonings with total 41 deaths occurred in the Czech Republic. 101 patients (80 men and 21 women) were treated in 30 hospitals; their discharge reports were analyzed using statistical methods. Most frequent symptoms were gastrointestinal, visual, respiratory, coma and chest pain. Treatment included alkalization, haemodialysis, folates, ethanol in 70%, fomepizole in 6%, or a combination of both antidotes in 15% patients. Fomepizole was used in more severely poisoned patients. Total 60 subjects survived without sequels, 21 died and 20 survived with visual damage (9/101), CNS impairment (4/101) or both (7/101). Severe acidosis and coma on admission were strong predictors of death. No significant differences were found between victims and survivors regarding serum methanol and osmolal gap. The groups differed significantly ($p \leq 0.05$) in pCO_2, pH, HCO_3-, lactate and base deficit. In addition, formate and lactate aggregate concentration best reflected the severe metabolic condition of the patients and correlated better with pH and anion gap then formate alone.
Supported with P25/1LF/2.

Oxidative Stress Response in Common Carp after Acute Exposure to Atrazine

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The aim of this study was to investigate the oxidative stress responses of 96h atrazine exposure of common carp. The acute toxicity test was performed on common carp according to OECD No. 203. The experiment was conducted in a semistatic system, and the test solutions were replaced once a day. At the end of the test, blood samples and selected organs (liver, gill and kidney) were taken, immediately frozen and stored at –85 °C until analyses of oxidative stress parameters (ferric reducing potencial of plasma, ceruloplasmin, glutathione reductase, glutathione peroxidase, glutathione S-transferase, catalase, superoxide dismutase and thiobarbituric acid reactive substances). The acute exposure of atrazine to common carp caused significant shifts in oxidative stress markers, especially in liver samples.
This research was supported by GACR P502/12/P163.

An Acetylcholinesterase Inhibitor Tacrine Can Modulate Immunity Response Initiated by Tularemia

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Tacrine is an inhibitor of acetylcholinesterase. In this work, we investigate immune response to a model bacterial disease tularemia for which both innate and specific immunity are necessary to resolve the disease. In the described experiment, we used BALB/c mice divided into eight groups exposed to saline, tacrine in a dose 20.0 - 500 μg/kg, infected with tularemia and challenged by tacrine. The mice were euthanized three days after experiment beginning. We proved significant reduction of interleukin-6 (IL-6) and interferon gamma (IFN-γ) level in a dose response manner in the infected animals in course of tularemia. Moreover, tacrine caused significant increase of bacterial burden in the organs.

Role of Aromatase (CYP19) in Activation of Anticancer Alkaloid Ellipticine

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Aromatase (CYP19) catalyzes the conversion of androgens, namely, testosterone and androstenedione into oestrogens, oestradiol, and oestrone, respectively. Beside healthy tissues, it is localized in breast tumor tissues. CYP19 is also highly expressed in human breast adenocarcinoma MCF-7 cells, which we found to be sensitive to ellipticine. We investigated the efficiency of human CYP19 to activate this drug to species forming covalent DNA adducts that are responsible for its cytotoxicity to MCF-7 cells. CYP19 activates ellipticine to form two major ellipticine-DNA adducts, identical to those derived from 13-hydroxy- or 12-hydroxyellipticine to levels similar to those formed by CYP1A1 and 3A4. The formation of these adducts is dependent on concentration of ellipticine and CYP19. Cytochrome b5 modulates CYP19-mediated activation of ellipticine, increasing its

activation to reactive species forming DNA adducts.
Supported by P301/10/0356, UNCE204025/2012

New Perspectives in the Treatment of Rheumatoid Arthritis Based on Imunomodulators and Antioxidants.

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Rheumatoid arthritis (RA) is a common severe joint disease that affects all age groups, it is thus of great importance to develop new strategies for its treatment. Our research in the last years was focused on evaluation of new substances for the combinations of the classical immunosuppressive treatment with immunomodulators or compounds affecting redox homeostasis. We evaluated different natural (glucans, glycosaminoglycans and coenzyme Q_{10}) and synthetic (stobadine dipalmitate and its derivative SMe1.2HCl) compounds and have achieved beneficial results (reduction of oxidative stress, decreased plasmatic levels of proinflammatory cytokines and reduction of hind paw edema) in experimental arthritis of Lewis rats. Our results have documented pro-oxidative environment in organism during arthritis and showed that therapies, which restore the redox balance could have a beneficial effect on patients suffering RA.
This work was supported by grant: VEGA 2/0045/11

QSAR Approach for Estimation of Estrogen Receptor Binding Affinity

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Impaired reproduction is an important adverse endpoint. Since reproductive test methods are very expensive, the alternatives are developed. Chemical can interacts with protein such as the estrogen receptor (ER) to initiate a cascade of biological effects and perturb endogenous hormone system. Despite the complexity of the endpoints for reproductive impairment, it has been long appreciated that chemical binding to the ER is one important mechanism of interfering with process involved in reproduction.
It is also known that the ER is much less of a lock-and-key interaction than highly specific receptors. The ER is nonspecific enough to permit binding with a diverse array of chemical structures. There are three primary ER binding subpockets, each with different requirements for hydrogen bonding. The steroidal compounds usually interact at two points within the ER using two hydrogen-bonding groups. However, three are also chemicals with one hydrogen-bonding group that bind ER and cause subsequent gene activation.
Defining the boundaries of these chemicals is the challenge for (Q)SAR.

Acknowledgement: The research was financially supported by grants of Internal Grant Agency of Ministry of Health of Czech Republic no.NT 13341-4/2012

Acute Toxicity of Alcohols Determined by Alternative Methods.

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Two alternative methods have been used to estimate the acute toxicity values of selected saturated alcohols.
3T3 NRU Cytotoxicity Test for Identification of Substances not Requiring Classification for Acute Oral Toxicity was performed according to the ICCVAM 2006 Report. Balb/c 3T3 fibroblasts were subcultured for 24 h until reaching confluence ≤ 50%. After application of 8 concentrations of the tested alcohols and subsequent 48 h incubation the Neutral Red Uptake (i.e. cell viability) was determined fluorimetrically The concentration which led to a 50% reduction in cell growth (IC_{50}) was estimated from the concentration-response curve and the LD_{50} value was estimated using formula: log LD_{50} (mg/kg) = 0.372logIC_{50} (μg/ml) + 2.024 .
Tubifex tubifex assay (3 min express test) for acute toxicity determination by using oligochaeta annelid was used. The EC50 for movement inhibition was calculated by concentration-response dependence. Both indexes were correlated with LC50 (Pimephales promelas), IGC50 (Tetrahymena pyriformis) and LD50 (rat, mouse) values available in literature.

Nonclinical Toxicological Studies of Combined Antihypertensive Drug for Registration in the Russian Federation

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Preclinical studies for the purpose of registration in the Russian Federation of a new antihypertensive drug, which is a fixed combination of two active ingredients, amlodipine besylate and metoprolol succinate, are conducted. A combination of two single drug products registered in the Russian Federation and containing the same active ingredients was used as reference drug. General toxic effects of the new combined product as well as its efficiency in comparison with the combination of two drugs for monotherapy were studied. Toxic and tolerable doses were identified. With the use of radio telemetry system it was shown that administration of drugs in the high doses produces a sustained hypotensive effect, which leads to disturbance of the normal circadian rhythm. It was found that the new combined product does not differ on their hypotensive properties and toxic effects from reference drug previously registered and used in clinical practice, and can be recommended for future clinical trials.

Novel Pd(II) Complexes with Anticancer Potency

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Metal complexes targeting DNA have attracted many interests due to their importance in cancer therapy and molecular biology. Numerous anticancer drugs exert their antitumor properties through binding to DNA. Three novel palladium complexes have been prepared and characterized by X-ray crystallography. The deprotonated quinoline-based ligands are coordinated to the Pd atoms *via* the pyridine nitrogen and the phenolato oxygen atoms, other two *cis*–positions are occupied by two chlorido ligands. Spectroscopic instrumental methods were used to investigate the interaction of complexes with DNA. Competitive binding studies showed that the enhanced emission intensity of ethidium bromide in the presence of DNA was quenched by the addition of the investigated complexes. The quenching constants of Pd-complexes suggest that their affinity to DNA was less than the classical intercalators. All three compounds are cytotoxic, and their IC50 values are in the range from 7.4 to 9.7 μM (A2780 cells) and from 22.1 to 24.5 μM (A2780/CP).
This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0280-11, by VEGA 1/0504/12,s Structural Funds EU No. 26240220071 and VVGS PF-2013-114.

The Göttingen Minipigs as a Model for Chronic Dermal Toxicity

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The minipig is acceptable as the non-rodent species in regulatory toxicity studies. It is advisable choice for dermal studies because of his close resemblance of porcine skin to human skin. Test item-loaded patches were administered dermally on the dorsal midline of Göttingen minipigs in our study for nine months. Toxicokinetic data, hematologic, clinical chemistry and immunologic parameters were evaluated. The health condition and behaviour as well as the inspection of surrounding skin at application sites were performed, too. Selected organs and tissues were processed for histopathological examination. Three animals were excluded from the study due to hyperkeratosis affecting the entire dorsal area. Some other animals showed areas of hyperkeratosis of various size surrounding eyes and were localized also on the dorsal neck. Non-specific dermatitis was not related to test substance. Transdermal delivery of test item did not cause any changes of observed parameters. The disadvantage of using Göttingen minipigs in chronic dermal toxicity are the skin changes that occur in young animals. Their primary cause is not exactly known.

Effects of Titanium Dioxide and Silver Nanoparticles on *In Vitro* Steroid Hormone Secretion by Porcine Ovarian Granulosa Cells

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Metal nanoparticles (NPs) become increasingly introduced into consumer products and biomedical applications. The effects of NPs need to be properly understood, especially their reproductive toxicity, because any shortcomings in this regard would be reflected into the next generation. In the present study, we examined the effects of titanium dioxide (TiO_2) and silver (Ag) NPs on *in vitro* steroidogenesis in ovarian granulosa cells (GCs). GCs isolated from porcine ovarian follicles (4-6 mm) were incubated with different concentrations of TiO2 and Ag NPs (0.001-100 μg/ml) in the presence or absence of follicle-stimulating

hormone (FSH, 1 μg/ml) or androstendione (A, 10^{-7} M) for 72 h. At the end of the incubation, progesterone (P4) and estradiol (E2) levels produced by GCs were measured in the culture media by commercial RIA and ELISA kits.
Both NPs exerted significant dose-dependent inhibitory effect on basal and FSH-induced P4 secretion by GCs. Similarly, A-stimulated E2 synthesis by GCs was reduced by the action of tested NPs.
The results indicate that metal NPs might induce changes in steroid hormone secretion by ovarian GCs and thereby could modify reproductive functions.

Supported by grants VEGA 2/0165/11 and APVV-0404-11.

Pre-weaning Handling Influenced Coping with Stress Situations in Adulthood.

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Early stressful experiences significantly affect coping with the stress in adulthood. The key factor is a developmental window, particularly early postnatal period, which is critical. A growing body of data indicates that human susceptibility to mood disorders such as depression and anxiety can be determined early in life.
We examined whether mild stress represented by the daily handling of animals during sensitive period of development (days 0-21 *post partum*) will be reflected in coping with stressful situations in adulthood. The rats after weaning underwent behavioral tests such as open field (OF), elevated plus maze (EPM) and forced swimming test (FST).
Open field activity was influenced by early postnatal handling only in males. Stressed animals were more active and failed to habituate to the new environment represented by open filed arena. In the EPM we observed that handled animals spent more time in the open arms and less time in the closed arms than control animals. In the FS, handled animals spent more time actively trying to climb the walls of the glass cylinder and latency to immobility was significantly longer compared to control.
In conclusion we demonstrated that early postnatal stress influences coping of animals with stress in adulthood.

This study was supported by VEGA 2/0107/12 and VEGA 2/0081/11.

Effect of Thiomersal on Mercury Content in Dog's Hair Before and After Vaccination

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The aim of this study was to determine the content of total mercury in hair of dogs after vaccination with vaccines containing thiomersal preservative. Thiomersal contain 49.55 % of ethylmercury. We were included 48 dogs in the experiment. Hair samples were collected on days 0, 10, 15, 20 and 25. In the monitored individuals, feed samples were also taken. Total mercury content in hair, granules and vaccines was determined by the direct method of cold vapours using an AMA 254 (Altec Ltd., Czech Republic). We used the Wilcoxon test for statistical evaluation. Concentration of total mercury in dog's hair ranged from 0.001 to 0.560 $mg.kg^{-1}$. There is no significant correlation between vaccine with thiomersal preservative and content of mercury in dog's hair. Results show that vaccines do not increase total mercury content in hair of dogs but consumption of feed with fish increases mercury content.

Testing of the Effects of AChE Inhibitors on the Muscarinic M1 Receptors

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The mechanism of Alzheimer's disease (AD) is currently still unclear. The cholinergic hypothesis is related with synaptic dysfunction, especially reducing activity acetylcholintransferase, depletion of acetylcholine in central synapses and reduction of nicotinic (nAChR) and muscarinic (mAChR) receptors. Inhibitors of acetylcholinesterase (AChEI) are used as drugs in the treatment of AD. They increase the concentration of acetylcholine (ACh), the main agonist of nAChR and mAChR.
The aim was to test how the selected AChEI affect M1 mAChR subtype. Their possible agonistic activity could then raise cholinergic activity in AD patients.
The testing was performed using the CHO cell line stably expressing the M1 subtype mAChR. After addition of the fluorescent Ca^{2+} indicator (Fluo-4 AM), agonist carbachol (EC_{80} = $15x10^{-6}$) and a concentration range of AChEI (10^{-7}-10^{-3}) in 96 well plate were measured fluorescence values and relative to control. Agonistic and antagonistic effects of AChEI were analyzed using the statistical program GraphPad Prism6.
Unfortunately, so far, none of the tested AChEI showed ag-

onist activity at M1 receptors. Individual AChEI but also varied in order of inhibition concentrations (IC_{50}).
This work was supported by Ministry of Defence, Czech Republic: A long- term organization development plan 1011.

Effect of Copper Based Pesticides on Oxidative Stress Biomarkers and Histopathological Changes in Liver of Common Carp

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The objective of this study was to evaluate subchronic effect of copper oxychloride and copper sulphate on oxidative stress parameters and histopathological changes in liver of common carp (*Cyprinus carpio*). Fish were exposed to sublethal concentrations of commercial formulation Kuprikol 50 (84 % copper oxychloride) and copper sulphate for 28 days. Selected biomarkers of oxidative stress (catalase, glutathione peroxidase, glutathione reductase, reduced/oxidised glutathion ratio, metallothioneins and lipid peroxidation) and detoxifying enzyme (glutathione-S-transferase) were determined to evaluate oxidative stress in fish liver. Significant differences were found in enzyme activities. Histopathological examination revealed pathological lesions in both tested substances.
This study was supported by IGA 22/2013/FVHE.

Carbamazepine Teratogenic Effects on Cardiomyocytes in *In Vitro* system-Possible Protective Role of Antioxidants

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Carbamazepine (CBZ) use during pregnancy increases cardiovascular anomalies. The chick cardiomyocytes Micromass (MM) system involves culture of fetal heart cells while in other system D3 mouse ES cells were differentiated into cardiomyocytes (ESDC). The CBZ addition in these systems predicts its teratogenic effects on cardiogenesis. In MM the CBZ only inhibits the contractile activity of cardiomyocytes, while in ESDC contractile activity completely ceased around 200µM. The addition of folic acid (FA) and ascorbic acid (AA) to the MM system showed no improvement but in ESDC AA recovered the lost contractile activity. The addition of antioxidant enzyme SOD with CBZ doses showed improved beating in MM with no effects in ESDC system. With CBZ the ROS levels were found to be increased in ESDC system only. The AA and SOD showed protective effects on increased ROS production. But contractile activity was recovered only with AA. In MM the CBZ with AA elevates the ROS production which might involve AA pro-oxidation properties. From results it's observed that the CBZ at the start of cardiogenesis is more toxic compares to late stage cells. While AA along with antioxidant properties also possesses cardiogenic property which has counteracted the CBZ toxic effects in ESDC system.

Influence of Fluoroquinolones on Avian Egg Incubation

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Veterinary antibiotics are used in many countries for treatment of diseases and protection of health of animals. However, excessive application may leave residues of these antibiotics in animal tissues. Huge amounts of antibiotics are used around the world each year, but their influence and the possibility of environmental damage remains unknown. There are also concerns with regards to their potential as a source of environmental pollution including their toxic effects.
The aim of this study was to evaluate the impact of selected fluoroquinolones on avian embryos, their embryonic development and reproduction parameters such as hatchability. The experiment was carried out using fertilized chicken eggs. The groups were exposed to various doses of enrofloxacinum and marbofloxacinum.
The results of this study may help to provide new data on the toxicity of antibiotics for birds and their adverse reproductive effects. Depending on the dose, antibiotics in eggs influenced the development of avian embryos and caused a decrease in hatchability.

Acknowledgements: The internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno supported the project 29/2013/FVHE.

Visual-Man-In-Simulant-Test for the Evaluation of Percutaneous Agent Vapor Exposure Through the Ingress of a Personal Protective Ensemble

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Personal protective ensemble (PPE) when worn is the subject to pressure differentials across the garment due a body movement and ambient wind flow creating ingress effects, which may force hazardous vapor/aerosol through the closures, joints, outlet valves, cracks/holes or the barrier material into the undergarment space and generate percutaneous exposure. The most significant source of variation in susceptibility to percutaneous absorption of toxic agents/simulants is *individual body region variation*, relative to body site, skin thickness and sensitivity. The Czech Visual-Man-In-Simulant-Test (V-MIST) technology enables to detect, localize and quantify penetration-ingress of challenging simulant or CW agent through a PPE, visualized local exposure isodoses on overall body surface and calculate the local and the overall protection factors of a PPE. In this regard the V-MIST can contribute to toxicological assessment of the local and overall *percutaneous vapor exposure* as a part of the Body Region Hazard Analysis and Minimum Required Exposure Dosage. Thus, selection of appropriately PPE´s designs and materials based on differential body region susceptibility to agent vapor exposures is appropriate and requires systematic attention also from the dermal toxicology point of view.

Effects of Terbuthylazine on Oxidative Stress in Common Carp (Cyprinus carpio)

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Terbuthylazine belongs to the triazine herbicides group and it is used as a broad spectrum pre- or postemergence herbicide in agriculture. Effects of terbuthylazine on common carp were observed after 24h exposure (day 1) to high pesticide concentration and after a recovery period of 6 days (day 7). In this study, a commercial herbicide formulation Click 500 SC (terbuthylazine 500 g/l) was used, the test concentration of terbuthylazine was 3.3 mg/l. Biomarkers of oxidative stress such as glutathione (GSH), glutathione-S-transferase (GST), glutathioneperoxidase (GPx), glutathione reductase (GR), catalase (CAT) and the amount of malonyldialdehyde (MDA) were determined in the liver of carp. After 24h-exposure changes of analysed parameters were not found out, on day 7 the activities of GST increased ($p < 0.01$) and values of TBARS decreased ($p < 0.01$). These results showed high regeneration potential of the fish organism after a short-term exposure to terbuthylazine.
This research was supported by IGA VFU 17/2013/FVHE.

Has Crowding Stress a Negative Impact on the Circulatory System of Young Hypertensive Female Rats?

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Genetic hypertension and social stress are supposed to be associated with changes in bioavailability of NO, which can be manifested by alterations in vascular reactivity. The aim of the work was to study the influence of crowding stress on blood pressure (BP) and functional state of the rat aorta of young female spontaneously hypertensive rats (SHR). Crowding was induced in 5-week-old rats by reduction of living space from 200cm^2/100g of body weight (controls) to 70cm^2/100g of body weight. Two weeks later, increased NO synthase (NOS) activity and decreased superoxide production in the aorta was found in the stressed rats but there were no changes in endothelial function and BP. We summarize that changes in NOS activity and superoxide production in the aorta induced by crowding stress were not sufficient for manifestation of endothelial dysfunction and consequently for BP alteration.
This study was supported by the grants VEGA No. 2/0084/10, 2/0086/08 and APVV-0523-10.

Effect of Zeta-cypermethrin on Common Carp

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The aim of this study was to assess the effect of pesticide product Fury 10 EW (containing zeta-cypermethrin

100 g/L) on common carp (*Cyprinus carpio*). The effect was assessed on the basis of the results of acute toxicity tests and on the comparison of results of oxidative stress and antioxidants biomarkers in tissues. The 96hLC50 value of Fury 10 EW was 13.8 μg/L. Oxidative damage was not detected in the experimental carps, however there were significant statistical differences in tissue antioxidant biomarkers after acute exposure of 13.8 μg/L Fury 10 EW compared to control fish. Antioxidants act as a very important protective system against oxidative damage to cells and tissues and keeping them in balance.
Supported by the CENAQUA No. CZ.1.05/2.1.00/01.0024, Project No. 084/2013/Z and Project no. 0804. 0809 UWM.

In Vitro and _In Vivo_ Induced Neurodegeneration by Trimethyltin in Wistar Rats

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Neurodegenerative diseases represent a serious health problem. Patients with Alzheimer-like dementia suffer from memory dysfunctions and in the late state of the disease the quality of their life is highly aggravated. A number of research institutions labor for finding new prospective drugs to slow down brain degeneration. Mechanisms involved in brain impairment are studied on *postmortem* brains or on experimental models of Alzheimer disease (AD), including transgenic rats with plaques and tau pathology, Tg-betaCTF99/B6 or APP/PS1 transgenic mice, etc. Attention has been attracted by a model of trimethyltin (TMT)-induced neurodegeneration which exerts several common features with AD. In the present work we studied *in vitro* and *in vivo* induced neurodegeneration by TMT in male Wistar rats. Concluding, reduced synaptic activity elicited by in vitro TMT action in the hippocampus corresponded with the reduced ability of rats to learn and remember, tested in Morris water maze, after *in vivo* application.
Supported by VEGA 2/0048/11 and 2/0107/12

Pre-weaning Handling Influenced Expression of Oxytocin in Hypothalamic PVN of Adult Rats

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Oxytocin (OT) is important for maternal behavior (labor, lactation, social interaction). Although found in about equal concentrations in both sexes, the physiological importance of OT in males is still unclear. Previous studies have suggested that OT may have important roles during stressful conditions.
In our experiment, we studied effect of early postnatal handling (stress factor) on the expression of OT in adulthood using in situ hybridization OT mRNA as well as behavioral changes (c.f. Sedlackova et al.). Adult offspring underwent stressful condition represented by forced swimming test and were inspected for OT expression changes. The gene expression of OT in the paraventricular nucleus and also anxiolytic effect of OT, which can be realized only by binding to OT receptor, was demonstrably higher in handled rats as compared to controls. These changes were mainly attributed to epigenetic alterations in promoter and regulatory regions methylation.
This work was supported by the Grants VEGA 2/0081/11 and 2/0107/12.

Mechanism of Action of Sulphur Mustard (SM).

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SM –induces massive poly-(ADP-ribosis) synthesis, leading to ATP depletion. These effects of SM are studied in cell cultures using concentrations higher than 100 μM. However, much lower concentrations (0,1-10 μ) induce breaks and cross links (CL) in the DNA. We have followed the concentration and time – dependent effects of SM, measuring the cell viability, colony forming capacity of cells, and the induction and repair of DNA cross links in SM- treated human A549, Chinese hamster ovary (CHO), subline AA8, and its mutant UV-20 defective in DNA repair. We have estimated, that cells are able to form colonies only up to concentration of SM inducing amount of CL that can be repaired (up to 2-3 μM). UV-20 mutant cells are 20 times more sensitive to SM compared to normal AA8 cell, showing the role of DNA repair in the SM effect. However, the UV-20 cells unhook CL with the same efficiency like AA8. Therefore, some other DNA lesions than CL are responsible for the inhibition of cell division.
This work was supported by a long-term organization development plan 1011.

The Effect of Benzo[a]pyrene on Metabolic Activation of Anticancer Drug Ellipticine in Mice

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Ellipticine is an anticancer drug, whose pharmacological efficiency and/or genotoxic side effects are dependent on its cytochrome P450 (CYP)- and/or peroxidase-mediated activation. We evaluated a role of CYP1A and 3A in metabolic activation of ellipticine, using the HRN [Hepatic CYP Reductase (POR) Null] mice, in which POR is deleted in hepatocytes, resulting in the loss of most hepatic CYP function. Moreover, levels of CYP1A, 3A and POR proteins and their enzyme activities were modulated in HRN and wild-type (WT) mice by their treating with benzo[a]pyrene (BaP). Exposure of mice to BaP resulted in an increase in levels of ellipticine-DNA adducts formed by hepatic microsomes of both WT and HRN mice that are generated from 12-hydroxy- or 13-hydroxyellipticine. Treatment of mice with BaP shifts a degree of ellipticine activation due to CYP1A and 3A; a higher contribution of CYP1A to ellipticine-DNA adduct formation than of CYP3A was found in BaP-treated mice.
Supported by P301/10/0356, UNCE204025/2012

Dicoumarol Inhibits Rat NAD(P)H:Quinone Oxidoreductase I*n Vitro* and Induces its Expression *In Vivo*

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Dicoumarol is an inhibitor of NAD(P)H:quinone oxidoreductase (NQO1) and contributes to genotoxicity of nephrotoxic and carcinogenic aristolochic acid I. AAI causes Aristolochic acid nephropathy and also Balkan endemic nephropathy. NQO1 is the most efficient cytosolic enzyme responsible for AAI activation to species forming AAI-DNA adducts. We demonstrate for the first time that dicoumarol can also act as inducer of NQO1 in kidney and lung of rats in vivo. Such an induction increased a potency of these cytosols to activate AAI and elevated AAI-DNA adduct levels in ex vivo incubations of AAI with cytosols and DNA.
Supported by 303/09/0472 and UNCE204025/2012

Carcinogenicity and Nephrotoxicity of Aristolochic Acid I is Dictated by Enzymes Catalyzing its Activation and Detoxication

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Aristolochic acid causes Aristolochic acid nephropathy and associated urothelial malignancies, and is hypothesized to be responsible also for Balkan endemic nephropathy. NAD(P)H:quinone oxidoreductase (NQO1) is the most efficient cytosolic nitroreductase activating AAI. AAI is also activated by cytochrome P450 (CYP) 1A1 and 1A2. Analyzing expression levels of proteins and measuring the marker activities of NQO1, CYP1A1 and 1A2 enzymes in cytosols and microsomes isolated from several organs of genetically modified animal models, we found that these enzymes contribute to both activation of AAI to AAI-DNA adducts and its detoxication to AAIa in these model animals exposed to AAI.
Supported by 303/09/0472, UNCE204025/2012

Cytochrome b_5 is Induced by Aryl Hydrocarbon Receptor Ligands, Benzo[a]pyrene and Ellipticine

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Cytochromes b_5, a protein of the endoplasmic reticulum, is involved in various biosynthesis pathways and in hydrox-

ylation reactions catalyzed by cytochromes P450 (CYP). We found an increase in cytochrome b_5 on the protein and mRNA levels after treating rats and mice with two aryl hydrocarbon receptor ligands, ellipticine and BaP. Cytochrome b_5 modulates CYP1A- and/or CYP3A4-mediated metabolism of BaP and/or ellipticine and increases their activation to reactive species forming DNA adducts. Such an induction resulted in an increase in their pharmacological and/or genotoxic properties.

Supported by P301/10/0356 and UNCE204025/2012

Globin-coupled Heme-based Oxygen Sensor Protein YddV and its Uncommon Heme Oxygenase Activity

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YddV from *E. coli* belongs to the newly described group of heme-based gas sensor proteins. It consists of N-terminal sensor domain detecting O_2 molecule and C-terminal function domain synthetizing important second messenger cyclic di-GMP. During the study of the isolated sensor domain (AA 1–153) of the YddV protein, we found out that substitution of the Leu 65, in the sensing site, for Asn converts this sensor domain into enzyme with heme oxygenase activity. Heme to verdoheme transformation was proved by combination of several methods (UV-VIS spectrofotometry, measurements of the CO gas amount produced during the reaction and mass spectrometry).

Chemical Warfare Agents – Why Today?

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Chemical warfare agents (CWA) (Nerve agents - soman, tabun, sarin; blister agents – sulfur mustards, etc.) are poisonous substances produced absolutely for military purposes – to incapacitate or kill an adversary. Poisoning treatment was therefore the domain of rather military than civilian doctors.
Gradually, this issue has moved towards the civilian sphere: CWA are utilized by various interest groups (terrorist, religious, etc.), commonly used in agriculture where accidental poisoning or targeted suicidal attempts by organophosphate fertilizers may occur and unfortunately chemical or other accidents are no exceptions as well.
The Armed Forces of the Czech Republic, is equipped in terms of CWA poisoning with drugs intended for the first non-professional aid, professional medical first aid and also maintains required supplies of profylactic agents to minimize the threat of intoxications.
The question is – what steps should be followed by medical staff members of prehospital care or Emergency, if they have no military equipment at their disposal?

Free-radical Degradation of High-molar-mass Hyaluronan Induced by Ascortabe plus Cupric Ions: Potential Protective Properties of the Bardejovské Kúpele - Spa Curative Waters – Alžbeta and Klára

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Mineral waters can be classified in many ways according to their distinctive chemical, physical elements, such as temperature, molecular concentration, chemical composition, and mechanisms of therapeutic action. The composition and physical properties of spa waters are varied. Bardejovské Kúpele-spa mineral waters are natural, slightly to moderately mineralized, with healing effects. They are classified as hydrocarbonic - containing chloride and sodium - carbonic, cold, hypotonic, with a relatively high boric acid content. The total mineralization ranges from 1,600 mg/liter to 9,400 mg/liter, and that of carbonic oxide from 2,200 mg/liter to 3,800 mg/liter.

Subchronic Perinatal Asphyxia in Rats: Postnatal Neurobehavioral Study

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Perinatal asphyxia, which is considered a worldwide clinical problem, is one of the most causes of low birth weight, hypoxic ischemic encephalopathy and neuropsychological handicaps. The aim of this study was to assess effects of subchronic perinatal asphyxia (SPA) on behavioral outcomes of offspring in Wistar/DV rats. Pregnant rats were exposed to decreased saturation of oxygen (10.5% O_2) in controlled environment during sensitive stages of brain development (days 19-20 of gestation) for 4 hours a day. We studied effects of SPA on delivery, viability at birth, and parameters of postnatal development (neuromotor and reflex development, sensory function). Behavioral changes were assessed using battery of behavioral tests up to adulthood, i.e. exploratory behavior, learning and memory processes, anxiety and behavioral despair. Results showed that SPA induced intrauterine growth retardation persistent up to day 21 *post partum*. No impairment of neuromotor and reflex development was observed, however hypoxic offspring was less active in one-day open field test and had increased immobility. Working memory tested in water maze was not impaired by SPA. Although the test for anxiety did not reveal statistically significant differences between groups, SPA did cause subtle anxiety-like behavior.
This work was supported by the Grants VEGA 2/0081/11, 2/0084/11 and 2/0107/12.

Trends in CNS Affecting Drugs in the Calls to the Toxicological Information Center in 1997-2012

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Toxicological Information Center (TIC) provides consultations concerning treatment of poisoning due to pharmaceuticals or chemicals. The drugs affecting the central nervous system (CNS) represented the biggest part (39.8%) of calls due to all drug poisonings in 1997-2012.
Whereas the number of calls caused by poisoning with tricyclic antidepressants (TCA) decreased (by 366.7%), the number of calls due to selective serotonin reuptake inhibitors (SSRIs) and other antidepressants overdose increased (by 1347.4%). The toxicity of the dose of SSRIs and other antidepressants was evaluated as lethal in 1.6% calls comparing to 8.6% in TCA.
A significant increase was found in the number of calls due to benzodiazepines (by 359.8%), whereas a decrease was seen in barbiturates (by 340%). The toxicity of the doses of benzodiazepines was considered lethal only in 0.5% calls comparing to 14.6% in barbiturates. The prognosis of poisoning caused by benzodiazepines was rather severe/severe in 0.7% only, which is in contrast to 9.1% in barbiturates.
Current trend of preferential prescription of drugs with improved safety profile to those routinely prescribed in the past brings a beneficial effect in a lower severity and better prognosis of intoxications.
Acknowledgement: Prvouk P25/1LF/2.

Red-wine Flavonoids

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Flavonoids are plant secondary metabolites of varying chemical structure. Here, we deal with flavonoids in red wine (anthocyanins and tanins), which are determining its taste, color and other sensoric properties.
We analyzed (RP-HPLC and CIELab colorimetry) the changes during ageing and oxidation of red wines. Two samples treated with SO_2, obtained from a semi-commercial winery (Svatovavřinecké 2010, 2012), and one sample of a domestically prepared wine (without SO2) were analysed, and followed for 2 months of ageing.
In younger wines, the monomeric and copigmented anthocyanins prevail. With ageing, polymerised pigments increase (70%), and the color shifts to blue-red. It is stabilised against oxidation, and against bleaching with SO_2. The SO_2-untreated wine is darker and contains less malvidine.
We analyzed also a drug preparation (Antistax, Boehringer Ingelheim), used for treatment of leg vein blood circulation, and based on extract of red vine leaves. The principal components were delphinidin and hyperoside.
Acknowledgment: This work was supported by GACR P303/12/G163 project.

Evaluation of the Toxicity of Six Triazine Pesticides to Signal Crayfish (Pacifastacus Leniusculus).

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Toxicity risks of agricultural pesticides to crustaceas are pivotal. Currently, many questions remain unsolved regarding toxicity of triazine pesticides to aquatic freshwater invertebrates like crayfish. The aim of this research was to evaluate the acute toxicity of six triazine pesticides to the signal crayfish (*Pacifastacus leniusculus*), which was chosen as an alternative test organism to fish by using the semi static baths. Signal crayfish of 49.0-81.5 mg weight and 12.8-16.0 mm total length were selected for this bioassay experiments. The trials were tested in triplicates. 96hLC50 values for juvenile signal crayfish were: 12.1 mg/L atrazine, 13.9 mg/L terbutryne, 14.4 mg/L prometryne, 19.5 mg/L hexazinone, 30.6 mg/L metribuzine, and 77.9 mg/L simazine.

This research was supported by the CENAQUA No. CZ.1.05/2.1.00/01.0024 and Project No. 084/2013/Z.

Alternative Method for Assessment of Skin Sensitization: Non-radiolabelled Local Lymph Node Assay LLNA:DA

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Historically, the sensitizing potential of chemicals and consumer products has been evaluated by guinea pig test (GPT) methods. Due to ethical and scientific reasons, a substitute of this test, the Local Lymph Node Assay (LLNA) has been validated in 2002. The basic principle underlying the traditional LLNA is that chemical sensitizers induce a primary proliferation (induction phase) of lymphocytes in the LN draining the site of chemical application which can be quantified by radio-labelled thymidine incorporation into the LN cellular DNA. The newly validated LLNA:DA (2010) is based on the use of measuring ATP content, correlating to the living cell number, by a bioluminiscence method and thus eliminates the potential for occupational and environmental exposure to radioactivity. The LLNA provides a quantitative endpoint, dose-responsive data and allows for prediction of potency. In comparison with GPT, the test procedure uses a reduced number of animals and a shorter test period, stress and injury of the animals is considerably minimized. At the Centre of Toxicology the method was implemented and internally validated using selected chemicals from the ECVAM validation study.

3,6,9-Trisubstituted Acridine Derivatives as New Antitumor Compounds

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Acridine derivatives possess antibacterial, antiviral, and antitumor properties. An important target for anticancer therapy is the enzyme telomerase and telomeres, which form G-quadruplexes as four-stranded DNA structures. Telomere maintenance is crucial for unlimited proliferation of cancer cells, thus design of drugs targeting the telomeric G-quadruplex DNA is a rational and promising approach for the anticancer therapy.

A synthetic approach toward novel 3,6,9-trisubstituted acridine derivatives able to interact with quadruplexes was based on our previous experience with a Jordan-Ullmann reaction. The key step was the preparation of 3,6-diisothiocyanate that was employed in the next reaction leading to bis-thioureas and -ureas and further introduction of amino substituents to the position 9 of acridine. Biochemical and biological properties of synthesized compounds are under study.

The financial support from the Slovak Grant Agency VEGA (1/0672/11, 1/0001/13) and UPJS-VVGS 40/12-13, VVGS-PF-2013-78 is gratefully acknowledged.

Changes of Rat Renal Artery Reactivity Induced by Long-term Intoxication by Carbon Tetrachloride

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Carbon tetrachloride (CCl_4) is a toxic compound known for its noxious effects to the liver and kidney as well as serious damage of endothelial cells. In our experiment we followed possible changes of rat vascular reactivity induced by 10 weeks administration of CCl_4 and pycnogenol (extract from pine *Pinus maritima*). Segments of renal arteries from adult male Wistar rats were subjected to series of contractions induced by successively increasing bolus doses of noradrenaline (0,5; 1; 3; 6; 10 μg). Finally, after precontraction there was induced a relaxation by single bolus dose of acetylcholine (20 μg). Furtherly, recorded contractile responses were assessed by two ways: descriptive method and computational modeling. We found apparent decline in relaxatory responses in the CCl_4 group compared to the control group, not recovered by pycnogenol. Computer-based modeling showed several significant differences in

the characteristic parameters of vessel responses in the CCl_4 group compared to the control group, which determination is unavailable for descriptive evaluations.
Acknowledgment: This work was supported by grants VEGA SR nr. 1/1133/11 and 1/0501/11.

Current State of Drugs of Abuse Bioanalysis in Forensic and Clinical Toxicology – A Review

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Juridical definition of the term drug of abuse is any substance the possession or supply of which is restricted by law. Such drugs are known as schedule substances. Nowadays we are confronted to an increasing occurrence of these substances including many uncontrolled (legal)structural types. Over the last five years forensic and clinical toxicologists have come into contact in their practice with cathinones, piperazine and pyrrolidinophenone derivates, tryptamine designer drugs or some naphthoylindole (JWH) or cyclohexylphenol (CP) type synthetic cannabinoid compounds besides so-called classical amphetamines, methylenedioxymethamphetamine and other designer phenethylamines, opiates, benzodiazepines, natural cannabinoids, gammahydroxybutyrate and related drugs or ketamine. As a result of the increase in abuse of newly synthesized chemicals tandem mass spectrometry have become crucial part of analytical qualitative and quantitative determination in biological matrices. GC-MS is still considered as the gold standard of analytical toxicology, however, polar and semivolatile compounds are difficult to analyze using this technique,so the use of convenient type of LC-MS-MS is recently described as an indispensable analytical tool.
Work is supported by MH CZ-DRO (UHHK, 00179906) and the programme PRVOUK P37/11

Effect of Palmatine on CYP1A Expression in Human Hepatocytes and HepG2 Cells

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The protoberberine alkaloid palmatine is one of the main bioactive constituents of *Rhizoma coptidis*, a widely used traditional Chinese medicine. The aim of our study was to examine whether palmatine affects the expression of cytochrome P450 (CYP) 1A1 and/or 1A2 in primary cultures of human hepatocytes and human hepatoma HepG2 cells. In HepG2 cells, palmatine induced a weak but significant increase in mRNA and activity levels of CYP1A1 after 24 h exposure and at concentrations starting from 10 μM. At 50 μM concentration, palmatine increased the levels of CYP1A1 mRNA and activity to 6.5-fold and 2.6-fold, respectively, compared to control. In contrast, palmatine caused only a slight increase in CYP1A1/2 mRNA levels without affecting the CYP1A activity in human hepatocytes. We conclude that metabolic biotransformation may be responsible for the inactivity of palmatine in human hepatocytes and that the use of palmatine may be considered safe in terms of possible induction of CYP1A enzymes.
The work was supported by grant LF_2013_008.

Methanol Outbreak In The Czech Republic In 2012: 121 Cases and 41 Deaths. Has It Been Different From „Methanol Epidemies“ In Other European Countries?

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Introduction. Mass methanol poisoning with 121 cases and 41 deaths occurred in the CR in 2012.
Methods. In the retrospective study the data were analyzed and compared with data from Estonia and Norway.
Results. The total and hospital mortality (33.9% and 20.8%) in CR was comparable with ones in Estonia (36% and 23%) and Norway (29% and 18%). 75.2% of patients were symptomatic on admission, in Norway and Estonia 77 % and 86 %. Gastrointestinal symptoms were the most common symptoms in the CR (47.5%) and Estonia (49%), followed by visual disturbances, dyspnea, and coma. In Norway the visual disturbances (55%) were on the first place.
Conclusions. The probability of sequelae was lower in intermittent hemodialysis compared to continuous ($p < 0.05$). In the CR and Estonia approximately 60% of patients survived without sequelae (in Norway - 72%), half of the remaining 40% died and half survived with visual or CNS sequelae. The most common were the visual ones (8.9 % CR, 15.3 % Estonia). The significant difference between the CR and Norway was present in the survivors with sequelae (19.8% vs. 9.8%). The Czech patients had the same median pH as Norwegian ones, but compensated worse the acidosis by respiratory mechanisms (median pCO_2 4,1 vs. 2,8 kPa).
Research project No. P25/1LF/2.

Oxime HI-6 Salts (Dichloride and Dimethansulphonate): Hyaluronidase Effect on Bioavaibility after i.m. Application

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Pigs were administered intramuscularly molar equivalents of HI-6 salts (HI-6 dichloride – 10.71 mg/kg and HI-6 DMS 13.59 mg/kg) either with or without hyaluronidase (60 U/kg). Hyaluronidase is supposed to increase tissue permeability and diminishes discomfort caused by the intramuscular injection. Doses of HI-6 salts corresponded with standard HI-6 dichloride dose in one autoinjector (500 mg) and were recalculated for one kilogram of body weight.
Hyaluronidase application had increased tissue absorption and improved pharmacokinetic profile. The C_{max} was significantly higher in case of HI-6 DMS plus hyaluronidase (29.6 ± 2.98 µg/ml) administration increase compared to HI-6 DMS (23.8 ± 3.04 µg/ml) and HI-6 dichloride (19.0 ± 0.93 µg/ml); both without hyaluronidase. Bioavailability calculated as AUC_{total} (HI-6 DMS with hyaluronidase, 4119 ± 647 min.µg/ml) was also significantly higher compared to HI-6 DMS (2259 ± 329 min.µg/ml) and HI-6 dichloride (1969 ± 254 min.µg/ml); both without hyaluronidase.
The results suggest that administration of HI-6 salt with higher solubility is the first step in the improvement of application strategy, but use some substances with spreading effect (hyaluronidase) may also leads to better absorption and better bioavailability.

This work was supported by Internal Grant Agency of Ministry of Health (Czech Republic), grant No. NT 12062.

Influence of Waste Water Treatment Plants on the Activity of Liver Detoxifying Enzymes in Brown Trout from Selected Czech Rivers

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The aim of the study was to assess concentration and activity of liver detoxifying enzymes cytochrome P450 and EROD, respectively. Brown trout was selected as the indicator species. Samples were taken from selected rivers in the Czech Republic, always upstream and downstream from the waste water treatment plants (WWTP) to investigate their influence on the content of pollutants in water and the effect of such chemicals on liver enzymes in fish. Sampling sites were located on small streams (Libotýňský brook, Černý brook, Moravice). There were found statistically significant differences between different locations, but only in Libotýňský brook, the significant difference appeared in EROD also between the upstream and downstream sampling site. Downstream was found significantly higher activity of EROD suggesting the release of pollutants from this waste water treatment plant.

Study was supported by IGA 04/2012/FVHE.

Determination of Oxidative Stress Parameters in Fish After Subchronic Exposure to Acetylsalicylic Acid

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The aim of this study was to investigate the effects of subchronic exposure to acetylsalicylic acid on juvenile development stages in zebrafish (*Danio rerio*) by using selected oxidative stress biomarkers.
We performed a test according to the OECD No. 215 (Fish, Juvenile Growth Test), 30 days old fish were used in the test. The tested concentrations of acetylsalicylic acid were 0.004, 0.4, 40, 120 and 250 mg / l for 28 days. Products of lipid peroxidation and antioxidant enzymes were determined as markers of oxidative stress. Especially changes in the values of biotransformation (Glutathione S-transferase) and antioxidant enzymes (Glutathione reductase, Glutathione peroxidase, Catalase), and products of lipid peroxidation were significantly effected ($p < 0.05$; $p < 0.01$) at a concentrations of 40, 120 and 250 mg / l.

This research was supported by IGA VFU 12/2012/FVHE.