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ABSTRACT BOOK

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L-01

DEVELOPMENT AND VALIDATION OF THE EPIDERM *IN VITRO* SKIN IRRITATION PROTOCOL FOR THE ASSESSMENT OF MEDICAL DEVICES EXTRACTS

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Assessment of dermal irritation is an essential component of the safety evaluation of medical devices. Reconstructed human epidermis (RhE) models have replaced rabbit skin irritation testing for neat chemicals (OECD TG 439). However, medical device (MD) extracts are dilute solutions with low irritation potential, therefore the validated RhE-methods needed to be modified to reflect needs of ISO 10993.

A protocol employing RhE EpiDerm was optimized in 2013 using known irritants and spiked polymers (Casas et al., TIV, 2013). In 2014 a second laboratory assessed the transferability of the assay. After the successful transfer and standardization of the protocol, 17 laboratories worldwide were trained in the use of the protocol in the preparation for the validation. All laboratories produced data with almost 100% agreement of predictions for the selected references.

In 2016, an international round robin validation study was conducted to confirm the ability of the RhE models to correctly predict the intra-cutaneous irritation of extracts from MDs. Four irritant polymers and three non-irritant controls were tested. Blinded polymer samples were extracted with sesame oil and saline per ISO 10993-12. Positive and negative solvent controls were included.

The EpiDerm tissues were able to correctly identify virtually all of the irritant polymer samples either in the saline or in the sesame oil or in both solvent extracts. Our results indicate that RhE tissue models can detect the presence of skin irritants at low concentrations in dilute medical device polymer extracts. The use of the reconstructed tissue models, as replacements for the rabbit intra-cutaneus test if being implemented into the ISO 10993 standards used to evaluate medical device biocompatibility. The work will be published in a special issue of Toxicology *in Vitro* in 2018.

L-02

RECONSTRUCTED 3D HUMAN SMALL INTESTINE MODEL FOR PREDICTION OF GASTROINTESTINAL TOXICITY AND DRUG ABSORPTION

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Small intestine is an important gateway through which many nutrients, drugs and other substances enter blood flow. In fact, about 90% of orally administered drug absorption occurs in the small intestine. Thereby there is a need of good and reliable *in vitro* model capable to predict drug toxicity and absorption/metabolism patterns. However, currently available *in vitro* intestine models are neither organ nor species-specific, relying predominantly on the use of cell lines generated from the colon or kidney. In addition they lack a proper 3D architecture and functionality, which in turn affects their ability to properly predict drug absorption and toxic effects.

Here we present the reconstructed 3D human small intestine model – EpiIntestinal, which mimics morphology and cell-type composition of normal human small intestine. As opposed to organoid models, EpiIntestinal is polarized and allows studying bidirectional drug penetration through intestinal wall. It expresses proteins involved in active drug transport and metabolism at physiological levels, which makes it ideal for modelling of complex drug absorption profiles, including the permeation, metabolism, drug-drug interaction and adverse effects of drugs on epithelium.

Comparative studies revealed that the absorption of drug in EpiIntestinal mimics the in vivo profile much closer than the currently used Caco-2 model. In another study aimed at adverse effects of drugs, EpiIntestinal was able to predict toxicity with much higher specificity and sensitivity than animal model. All in all, this model represents a promising tool to model complex processes occurring in small intestine.

L-03

SUCESFULL EVALUATION OF TWO EPIOCULAR EYE IRRITATION TEST PROTOCOLS IN THE INTERNATIONAL PROJECT CON4EI - CONSORTIUM FOR *IN VITRO* EYE IRRITATION TESTING STRATEGY

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Assessment of the acute eye irritation potential is part of the international regulatory requirements for testing of chemicals. The objective of the CON4EI (CONsortium for *in vitro* Eye Irritation testing strategy) project is to develop tiered testing strategies for eye irritation assessment for all drivers of classification. For this, a set of 80 reference chemicals (38 liquids and 42 solids) was tested with eight different alternative methods. Here, the results obtained with reconstructed human cornea-like epithelium (RHCE) EpiOcular and the EpiOcular Eye Irritation Test (EIT) -adopted as OECD TG 492 - are shown.

The primary aim of this study was an evaluation of the performance of the test method to discriminate chemicals not requiring classification for serious eye damage/eye irritancy (No Category) from chemicals requiring classification and labelling (Category 1 and 2). In addition, the predictive capacity in terms of *in vivo* driver of classification was investigated. In a second step, it was investigated whether the EpiOcular EIT can be used as part of a tiered-testing strategy for eye

irritation assessment. The chemicals were tested in two independent runs by MatTek *In Vitro* Life Science Laboratories.

For the EpiOcular EIT (OECD TG 492), a sensitivity of 96.9% and specificity of 86.7% with an accuracy of 95% was obtained overall and for both runs separately (100% concordance). For the EpiOcular ET-50 method, the overall accuracy of 74.5%, an FNR of 3.1% (Classified versus Not classified) and FPR of 3.4% (Classified versus Not classified) were achieved. Furthermore, about 79% of the Cat 1 liquids and 69% of the Cat 1 solids and 68% of the Cat 2 liquids and about 61% of the Cat 2 solids were identified correctly. The results of these studies seem promising with regard to the evaluation of inclusion of these test methods in an integrated testing strategy (ITS) for eye irritation assessment.

This research was funded by CEFIC-LRI. We acknowledge Cosmetics Europe for their contribution in chemical selection. The research has been recently published in the special issue of Toxicology *in Vitro*, CON4EI: Consortium for *in vitro* Eye Irritation testing strategy, Edited by: An Van Rompay, Sandra Verstraelen. Volume 49, Pages 1-116 (June 2018).

L-04

SELECTED BISPHENOLS AND PHTHALATES SCREENED FOR ESTROGEN AND ANDROGEN DISRUPTION BY *IN SILICO* AND *IN VITRO* METHODS

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Endocrine disruptors are substances capable to bind to specific receptors similarly to endogenous hormones, thus potentially contributing to endocrine system disturbances and consequent system disorders. Sources of exposure may come from industry or agriculture, including consumer products, e.g. food packaging materials, thermopaper, plastics, household products or cosmetics. Interaction of ligands with receptors is a molecular initiation event that leads to complex effects. The physiological receptor mechanism may be affected either by direct receptor binding of the exogenous ligand to the receptor, resulting in activation (agonistic activity) or inhibition (antagonistic activity), or consequent modulation of associated signaling pathways regulation. Human receptors may share ligands with variable affinity and efficacy. Certain substances may be persistent, resulting in bioaccumulation in the food chain as well as in the organism. Others may be quickly metabolized and act for a limited time. Therefore, exposure to endocrine disruptors cannot be simply investigated in humans since it is influenced also by individual environmental, medical and social factors. These facts complicate the detection of negative effects *in vivo*. Development and use of *in silico* screening tools and *in vitro* methods is therefore effective for first-level screening and should be used more intensively. In our pilot study, selected bisphenols and phthalates were tested using OECD QSAR Toolbox, Stably Transfected Transactivation *In Vitro* Assay to Detect Estrogen Receptor Agonists (OECD TG 455) and yeast-based microplate assay (in compliance with Draft ISO/DIS 19040) in order to determine the interactions of the tested chemicals with human estrogen and androgen receptors. *In vitro* results correlated well with *in silico* prediction for phthalates predicted as non binders, while predictions for bisphenols differed slightly. Both *in vitro* biological methods exhibited good concordance of results regarding the estrogenic activity. Minor discrepancies were detected for certain bisphenols due to cytotoxicity elicited in higher concentrations. Substances showing strong estrogenic activity exhibited parallel activity on the androgen receptor. The research article also summarizes recent developments in legislation with reference to *in vitro* methods suitable for screening of endocrine disruption.

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L-05

FORMULATION OF APOFERRITIN NANOCARRIER WITH ENCAPSULATED ELLIPTICINE AND STUDY OF ITS PROPERTIES

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One of the approaches to decrease the adverse effects of drugs is their encapsulation inside a suitable nanocarrier, allowing for a targeted delivery to tumour tissue whereas avoiding healthy cells. Apoferritin is the iron-free form of ferritin, a naturally occurring iron-storage protein. Using apoferritin as a nanocarrier has the potential to move undetected through the body without inducing any resistance from the immune system of the patient. Furthermore apoferritin can be modified with recognition ligands to achieve tumor-specific targeting. The simple-to-use encapsulation protocol (creating ApoElli) was developed and the prepared nanocarrier was characterized. The nanocarrier exhibits narrow size distribution, which suggests being suitable for entrapping of the hydrophobic molecule of ellipticine. The release of ellipticine at acidic (6.5) and neutral (7.4) pH was studied. Ellipticine is gradually released from its ApoElli form into the water environment under acidic pH; more than 80% ellipticine was released after 48 hrs incubation at pH 6.5. In contrast at pH 7.4 less than 20% was released. ApoElli is also stable after its storage at physiological pH (7.4) up to 1 month at 4 °C. The presence of membrane particles accelerates release

of ellipticine from Apo-Elli and makes it possible to be transferred into microsomes even at pH 7.4. Microsomal cytochromes P450 are capable of oxidizing free ellipticine and/or its ApoElli form to its metabolites and generating covalent ellipticine-derived DNA adducts, both under pH 7.4 and 6.5. The form of ellipticine plays essentially no role in these processes. The ApoElli is toxic to UKF-NB-4 neuroblastoma cancer cells but exhibits significantly lower toxicity for non-malignant cells (non-malignant fibroblasts, HDFn cells).

The work was supported by GACR (17-12816S) and Charles University (204025/2012).

L-06

APOFERRITIN NANOCAGE FOR ELLIPTICINE DELIVERY TO NEUROBLASTOMA CELLS

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Recently, nanoparticles have been widely investigated for delivery of anticancer drugs. Apoferritin is a natural nontoxic iron carrier and has a natural hollow structure that can be used to store small molecules such as cytostatics. The aim of our study was to compare the cytotoxic effects of anticancer drug ellipticine loaded in apoferritin (APOELLI) and free ellipticine (ELLI) on neuroblastoma cells UKF-NB-4, chemoresistant sublines derived from this cell line, and normal human fibroblasts (HDFn), as a model of non-malignant cells. We show here that the cytotoxicity of APOELLI is lower than cytotoxicity of free cytostatic, but APOELLI induces more double strand breaks than free ELLI in neuroblastoma cells. Moreover, cytotoxicity of drug loaded apoferritin is significantly lower for HDFns. Further, using fluorescence microscopy, we have shown that apoferritin can deliver drugs inside cells and the drug exerts their effect thereof. Fluorescence intensity of ELLI/APOELLI in nuclei of neuroblastoma cells is significantly higher than in those of HDFn, because ELLI/APOELLI is more sequestrated in lysosomes in fibroblasts. The extent of APOELLI enter into the UKF-NB-4 cells correlated with formation of covalent ELLI-derived DNA adducts in these cells; the levels of ELLI-DNA adducts generated by APOELLI were 67% of those formed by free ELLI. The results found in this study seem to be promising, because encapsulation does not affect toxicity of cytostatic and improves drug stability. We suppose that apoferritin with encapsulated ELLI is targeted to the several cancer cells including neuroblastoma through receptors TfR 1 and/or SCARA 5 which are expressed in many cancers.

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L-07

DNA ADDUCTS FORMATION BY PLANT ARISTOLOCHIC ACID IS UNIQUE BIOMARKER OF EXPOSURE AND EXPLAIN THE INITIATION PHASE OF UPPER UROTHELIAL CANCER

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Aristolochic acid (AA) is an alkaloid causing aristolochic acid nephropathy (AAN) and Balkan endemic nephropathy (BEN) that are renal diseases often associated with upper urothelial cancer (UUC). The formation of covalent DNA adducts by carcinogens is considered to be one of the earliest steps in the initiation phase of cancer development. Moreover, the covalent binding of carcinogens to DNA, which is causally related to tumorigenesis, is now considered as a central dogma of chemical carcinogenesis. This belief is supported by various observations, such as the facts that: (i) the carcinogenic properties of many carcinogens is dependent upon their activation to reactive electrophilic derivatives, which react with nucleophilic sites within DNA; (ii) the extent of DNA adduct formation can frequently be correlated with the magnitude of carcinogenic responses; and (iii) mutations in certain tumour suppressor genes and the activation of several proto-oncogenes can be mediated by the interaction of carcinogens with DNA. However, since humans are exposed not only to one but to a complex mixture of carcinogens, direct proofs of an association of exposure to the development of a specific cancer type are rare. The plant carcinogen AA is one of the rare examples where a distinct environmental exposure is linked to tumour development in humans. This study demonstrates the significance of AA-derived DNA adducts in the aetiology of UUC leading to specific A:T to T:A transversion mutations (mutational signature) in AAN/BEN-associated tumours, which are otherwise rare in individuals with UCC not exposed to AA. Therefore, such DNA damage produced by AA-DNA adducts is one rare example of the direct association of exposure and cancer development (UUC) in humans, confirming that the covalent binding of carcinogens to DNA is causally related to tumourigenesis. Even though aristolochic acid I (AAI), the major component of the natural plant extract AA, might directly cause interstitial nephropathy, enzymatic activation of AAI to reactive intermediates capable of binding to DNA is a necessary step leading to the formation of AA-DNA adducts and subsequently AA-induced malignant transformation. Therefore, AA-DNA adducts can not only be utilized as biomarkers for the assessment of AA exposure and markers of AA-induced UUC, but also be used for the mechanistic evaluation of its enzymatic activation and detoxification. Differences in AA metabolism might be

one of the reasons for an individual's susceptibility in the multi-step process of AA-mediated carcinogenesis and studying associations between activities and/or polymorphisms of the enzymes metabolising AA is an important determinant to identify individuals having a high risk of developing AA-mediated UUC.

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L-08

CHANGES IN EXOSOME PRODUCTION AFTER CHEMOTHERAPY TREATMENT

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Intercellular communication is one of the most important processes commonly found among all organisms. It is secured by extracellular vesicles among which we include exosomes. Exosomes are nanosized vesicles of intracellular origin with size in range of 30-150 nm. They mediate communication between cells by transferring their cargo.

The aim of our work was to test isolated vesicles from ovarian and breast cancer cell lines for exosome markers and to measure the size and concentration of isolated vesicles. As additional objectives, we chose to test the effect of drugs on the production of exosomes form various cell lines and their effect on viability of cells. We verified the expression of CD63, CD9 or TSG101 as markers enriched on exosomes, while we registered significant changes in their expression as well as change in the amount of produced exosomes while being affected by several specific drugs - cisplatin (CP) and inhibitor of survivin (YM155). The treatment of cells with specific drugs also changed the amounts of exosomes produced into the extracellular milieu, which size and concentration were tested on NanoSight NS500 device. The potential of exosomes to mediate intercellular communication was tested as the ability of a tumor or non-tumor cell line to internalize heterologous. This effect was observed by confocal microscopy. Alterations in exosome secretion reflect different cell and tissue states in the whole organism and adapt to the individual's lifestyle. In the future, tailored validation of patients' exosome profiles will be necessary.

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L-09

ALTERED EXPRESSION OF BIOTRANSFORMATION ENZYMES IN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is killing 250,000 to 1 million patients per year and originates in the key cell population for biotransformation, the hepatocytes. Despite that, studies on biotransformation enzymes in HCC are scarce. It is known that malign transformation leads to a significant deregulation of gene expression and alteration of metabolic functions in hepatocytes. Advanced stages of the disease are frequently associated with liver failure and threaten the patients with a severe impairment of (drug) metabolism. The impact on efficacy and toxicity of drug in such patients is unknown. This study aimed at analysis of the most important enzymes of both phases of drug metabolism, in hepatocellular carcinoma samples as compared to their para-tumour non-cancerous tissue. Whole transcriptome profiles were obtained for protein coding genes and long non-coding RNAs using gene expression microarrays Agilent SurePrint G3 8x60k and for a panel of 754 miRNAs using qPCR TaqMan Low Density Arrays v3.0. Selected genes were validated at mRNA level using qPCR and at protein level by western blotting. Results show significant alteration of several drug metabolism-associated pathways as well as a significant drop of gene expression across the whole P450 superfamily (up to several orders of magnitude) in the HCC tissue compared to surrounding non-cancerous tissue in approximately half of the patients, correlating with expression of expression levels of regulative nuclear factors and the histological grade of the tumours.

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L-10

INSIGHTS ON THE RELATIONSHIP BETWEEN STRUCTURE OF PHENYLETHANOID GLYCOPYRANOSIDES AND THEIR ACTIVITIES USING CELL-FREE ASSAYS AND HUMAN CELLS CULTURED *IN VITRO*

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Prevention of cancer remains the most promising strategy for reducing both its incidence and the mortality. The study of protective potential of selected natural compounds and their synthesized analogues, which could be used in the prevention and health protection, might be therefore of great importance. Plants are a rich source of phytochemicals possessing such properties. Salidroside as the main natural phenylethanoid glycopyranoside

(PEGP) present in plants of the genus *Rhodiola* is characterized by many beneficial pharmacological effects. The structure of the compound, a wide range of its biological activities and limited availability of the most productive species has inspired organic chemists to synthesize salidroside (SALI - tyrosol β-D-glucopyranoside) and PEGPs: tyrosol β-D-galactopyranoside (TYBGAL), tyrosol α-D-galactopyranoside (TYAGAL), tyrosol α-D-mannopyranoside (TYAMAN), hydroxytyrosol α-D-mannopyranoside (HOTAMA), homosyringyl β-D-glucopyranoside (HSYGLU), hydroxytyrosol β-D-xylopyranoside (HOTXYL) and hydroxysalidroside (HOSALI).

The objectives of our study were (i) to prepare PEGPs by chemical or less conventional enzymatic procedures; (ii) to determine their reducing power, radical scavenging and chelating capacities using cell-free approaches and (iii) in experimental system utilizing human hepatoma HepG2 cells to evaluate their cytotoxicity (MTT test) and protective potential against lesions induced by hydrogen peroxide (H_2O_2; comet assay).

Glycosylated hydroxytyrosols (HOSALI, HOTAMA, HOTXYL) and HSYGLU manifested the highest reducing power and DPPH radical scavenging capacity and they were most active in protection of HepG2 cells against free-radicals generating agent H_2O_2, particularly at the lower concentrations used. On the other hand, pre-treatment of HepG2 cells with SALI had protective effects even though SALI displayed neither reducing power nor DPPH radicals scavenging activity. We suppose that protection induced by SALI is achieved by the effects on other cellular processes distinct from antioxidant action.

Differences in the effectiveness of the phenylethanoid glycopyranosides found in this study revealed that structures of their molecules in terms of aglycone combined with sugar moiety can affect and contribute to their activities.

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L-11

METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS PLAYS A MAJOR ROLE BOTH IN THEIR GENOTOXICITY AND IN THEIR NON-GENOTOXIC EFFECTS

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Polycyclic aromatic hydrocarbons (PAHs) are an important group of abundant environmental pollutants formed by a variety of combustion processes. Both individual PAHs, such as benzo[a]pyrene (BaP), and their complex mixtures, have been associated with adverse human health effects, including cancer. Their carcinogenicity is primarily attributed to their genotoxic effects, linked with formation of covalent DNA adducts, for which PAHs require activation to their electrophilic metabolites, dihydrodiol epoxides, in order to exert mutagenic or carcinogenic effects. However, other metabolites of PAHs have received considerably less attention, although they may induce a number of toxic effects linked with generation of oxidative stress, alter the activity of important intracellular signaling pathways (including e.g. activation of receptor tyrosine kinases or mitogen-activated protein kinases) or activate specific intracellular receptors. This presentation aims to provide an overview of known non-genotoxic effects of PAHs, which could be linked with their metabolism. In the second part, we will illustrate the important role of metabolism in estrogen-like effects of PAHs and/or their mixtures. We studied both metabolism and the estrogen receptor (ER)-mediated effects of model PAHs, such as BaP (and benz[a]anthracene; BaA, in human breast cancer cell models in the presence or in the absence of enzymatic activity required for their metabolization. In cells without active PAH metabolism, BaP formed significantly lower amounts of most of its metabolites, including hydroxylated-BaPs; this was linked with suppression of estrogen-like effects, such as ER-dependent modulation of cell cycle progression or induction of the ER-dependent luciferase reporter gene. These results suggest that more attention should be paid to the role of metabolism of PAHs in their toxic modes of action, as it may alter also their non-genotoxic effects.

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L-12

ANTIGENOTOXIC ACTIVITY OF *POLYSCIAS FILICIFOLIA* EXTRACTS

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Searching for antigenotoxic compounds, which are able to decrease or even remove the mutagenic effects, represents a rapidly expanding field of the cancer research. Traditional medicinal plants are an important source of active compounds with a potential antigenotoxic activity. Biotechnological methods in plant cell and organ cultures offer the possibilities to improve productivity of useful compounds, one of them is the elicitation. We evaluated the antigenotoxic potential of extracts of south Asian traditional herb, *Polyscias filicifolia* Bailey, growing *in vitro* with an addition of elicitors. The extracts contain wide range of biological active compounds like phenolic acids: chlorogenic acid (CGA), caffeic acid (CA) and ferulic acid (FA) derivatives

or triterpenoid saponins, which aglycone is oleanolic acid (OA). Additionally, we evaluated the antigenotoxic activity of phenolic acids and OA. The evaluation was made using short-term bacterial *umu*-test, toward three different mutagens: 4-nitroquinoline-N-oxide, mitomycin C and 2-aminoanthracene (2AA). The tested extracts exhibited high antigenotoxic potential if the assay was performed with 2AA and metabolic activation. However, the extracts after a hydrolysis of saponins were slightly active. Phenolic acids: FA and CA slightly decreased 2AA-genotoxicity, while CGA increased the effect. Therefore, phenolic acids probably are not responsible for the extracts activity and more attention should be put on triterpenoid saponins. We evaluated antigenotoxic activity of free aglycone (OA) and no effect was observed. Based on the obtained results it can be conclude that *Polyscias filicifolia* extracts are a potential source of antimutagenic compounds, but further studies are necessary to demonstrate the activity of triterpenoid saponin fractions.

L-13

CYANOBACTERIA – BASED DIETARY SUPPLEMENTS QUALITY MONITORING

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The dietary supplement Spirulina, used worldwide as a natural product for overall health improvement, represents a dried biomass of cyanobacteria. It has no therapeutic value, but does have value as a source of nutrients, e.g. vitamins, minerals and other substances with a nutritional or physiological effect. *Cyanobacteria* are prokaryotic gram-negative bacteria whose rapid and excessive growth associated with water eutrophication can lead to the formation of aggregations, referred to as a "water blooms". Some of these bloom-forming cyanobacteria genera produce cyanotoxins which may cause various health problems. Spirulina is the common name for cyanobacteria–based dietary supplements that contain, in particular, dried biomass from two species, *Arthrospira platensis* and *Arthrospira maxima.* These non-toxic cyanobacteria grow under controlled conditions, so are generally considered to be safe. They must meet the requirements of European and Slovak food legislation, however, which sets limits for levels of contaminants. Based on the confirmed contamination of other cyanobacteria-based products, concerns have also been raised about the safety of Spirulina dietary supplements. To ensure the protection of human health, the Public Health Authority of the Slovak Republic in Bratislava assessed the levels of selected contaminants (heavy metals and polycyclic aromatic hydrocarbons). In addition to the legislation, the declared cyanobacteria *Arthrospira* was microscopically examined in eight randomly selected samples, where its presence was confirmed, but with observed differences in level within individual samples. The samples were also analysed for the presence/absence of the specific cyanotoxins, microcystins (LR, YR, RR). Ecotoxicity monitoring optimised extract preparation conditions and ecotoxicity testing, and evaluations were made. The results of ecotoxicological tests performed on the test organisms *Vibrio fischeri, Thamnocephalus platyurus* and *Sinapis alba* revealed the possible presence of contaminants in some of the cyanobacteria-based dietary supplement samples.

L-14

PROLONGED EXPOSURE TO NON-LETHAL DOSES OF DDT AND DDE SHOWED DIFFERENT EFFECTS ON INSULIN PRODUCTION AND PROTEIN EXPRESSION IN PANCREATIC BETA CELLS

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Diabetes is one of the most prevalent diseases of civilization nowadays. An influence of pollutants on development of diabetes worldwide is suspected but an exact level and mechanism of their effect is yet to be determined. Among compounds with suspected pro-diabetic effects belong also pesticide DDT and its metabolite DDE. The aim of the present work was to investigate effects of prolonged exposure to non-lethal doses of DDT and DDE on insulin production and protein expression in pancreatic beta cells. To achieve that, we exposed rat pancreatic beta cells INS1E to non-lethal doses (10 μM) of DDT or DDE for 1 month. Changes in glucose-induced insulin secretion were determined by ELISA kit and changes in protein expression were examined using 2D-electrophoresis and western blot. Cells exposed to DDT for 1 month completely failed to increase insulin secretion after exposure to higher glucose concentrations. WB showed significant decrease in expression of both proinsulin and hexameric insulin in cells exposed to DDT. Cells exposed to DDE for 1 month secreted insulin at the level of control cells. WB showed significant decrease in expression of proinsulin but not of hexameric insulin in cells exposed to DDE. 2D-electrophoresis showed one protein with upregulated expression in both cells exposed to DDT and cells exposed to DDE: vitamin D-binding protein (VDBP). To conclude, DDT and DDE showed different effect on insulin secretion. A possible modulator of DDT and DDE effect on expression of proinsulin in pancreatic beta cells can be vitamin D-binding protein. Further work needs to be done to elucidate mechanisms of described phenomena.

L-15

TOXICITY OF HOSPITAL WASTEWATER ASSESSED BY DIFFERENT BIOASSAYS

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The purpose of this study was to determine toxicity of wastewater from hospitals in the Czech Republic using traditional and alternative toxicological methods.

The pilot study comprised weekly dynamics of sewage ecotoxicity of treated wastewater from one large hospital in two different seasons (May vs. November). A detailed investigation of wastewater ecotoxicity, genotoxicity and reprotoxicity followed in five different hospitals. The toxicity classification system based on the calculation of TU (toxic unit) was applied to evaluate ecotoxicity.

The seven following bioassays were used in this study: algal growth inhibition test with *Desmodesmus subspicatus* (EN ISO 8692), *Vibrio fischeri* luminescent test (EN ISO 11348-2), immobilization test with *Daphnia magna* (EN ISO 6341), *Allium cepa* assay, Bacterial Reverse Mutation Test – Ames Agar Plate Test (OECD TG 471), Comet assay (single-cell gel electrophoresis) and YES/YAS – Yeast Based Reporter Gene assay.

In the pilot study, the wastewater ecotoxicity during one week showed no significant differences in separate working days, however, higher toxicity values were recorded in May compared to November. In the following study, samples from two of the five hospitals were classified as toxic (III.toxicity class), the others as non toxic (I.toxicity class). Genotoxicity has not been confirmed neither by Ames test, nor Comet assay in any sample. In several cases, wastewater samples exhibited agonist activity to the estrogen and androgen receptors.

The study demonstrated different levels of toxicity of treated hospital wastewater. Variable sensitivity of individual bioassays for tested wastewater samples was recognized. It can be assumed that the results of Ames test, Comet and YES/YAS assays may be influenced by sample sterilization (by filtration) which might have caused a loss of genotoxic and reprotoxic activity as certain chemicals may be captured on the filters. The study will continue with optimization of sample preparation. A more extensive study including proposal for improvement of hospital wastewater treatment within the Czech Republic can be recommended with the aim to decrease the discharge of toxic chemicals into the local sewage system and environment.

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L-16

FORMATION AND ELIMINATION OF *N,N*-DIMETHYLFORMAMIDE ADDUCTS WITH BLOOD PROTEINS IN HUMANS

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N,N-Dimethylformamide (DMF), an industrial solvent with hepatotoxic properties, undergoes metabolism to a reactive intermediate, *N*-methylisocyanate, that binds covalently to blood protein globin to produce stable adducts, *N*-methylcarbamoylvaline (MVU) at the globin *N*-terminus and N_{ε}-methylcarbamoyllysine (MLU). Earlier we have shown in rats that physiological removal of the erythrocytes is followed by a hydrolytic cleavage of globin resulting ultimately in the excretion of free MVU and N_{α}-acetyl-MLU (MLU-Ac) in the urine. In the current study with human volunteers, DMF was used as a testing adduct-forming compound to verify a theoretical model based on our previous experiment on rats, which describes the fate of the protein adducts in blood including their degradation and excretion in the urine. Following ingestion of 500 mg DMF in aqueous solution, the subjects (n=7) provided multiple blood and urine samples during next five months. In addition to MVU and MLU in globin, protein-bound MLU was also measured in total plasma. Both globin and plasma were worked-up by acidic hydrolysis followed by HPLC-ESI-MS^2 or GC/MS analysis. Maximum levels of MVU and MLU in globin were attained 7 days post-exposure (17.5±3.7 nmol/g and 15.7±3.5 nmol/g, respectively) and then followed by an almost linear decline to the pre-exposure levels ca. 130 days later, reflecting the life span of human erythrocytes. MLU levels in plasma peaked on day 3 (3.8±0.7 nmol/ml) and then declined with a half-life of ca. 25 days, close to turnover rate of human serum albumin. Analyses of urine samples for MLU-Ac and MVU, based on SPE clean-up and HPLC-ESI-MS^2 or GC/MS, are currently in progress. In addition to their role in deriving the above toxicokinetic model in humans, urinary MVU and MLU-Ac are considered as promising non-invasively accessible biomarkers of cumulative exposure to DMF in occupational biomonitoring.

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L-17

NANOPARTICLES – POTENTIAL ECOTOXICOLOGICAL THREAT FOR THE ENVIRONMENT

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Nanotechnologies and different kinds of nanomaterials belong nowadays to intensively investigated materials due to their unique properties and several potential applications as electronics, medicine or imaging methods. However, with the research of their possitive properties there is a growing demand for investigation and evaluation of their impact on the environment and possible harmful properties for organisms. This report summarizes possible toxic effects of developed nanomaterials on environment and living organisms. It deals with their possibility of bioaccumulation in plants or animals, ecotoxicity and mechanisms of toxicity. Main goal of this report is to highliht the potential environmental complications in case of nanomaterials use and the necessity of research about their toxic effects.

Supported by the grant scheme for supporting excellent teams of young researchers under the conditions of STU in Bratislava: „Mikropolutanty a rezistentné kmene baktérií ich monitoring a možnosti použitia inovatívnych postupov na ich odstránenie – nanomateriály a železany."

L-18

MICROPLASTICS AS CONTAMINANTS - SCOPE, FATE, AND ENVIRONMENTAL IMPACTS

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Microplastics represent emerging pollutants of global importance. They are small enough to be ingested by a wide range of organisms and at nano-scale, they may cross some biological barriers. As well as microplastics, chemical additives added to plastics during manufacture which may leach out upon ingestion, can enter food chains and potentially cause humans serious health problems. In this article, we discuss the sources of plastic microparticles, summarize known informations about their behaviour at wastewater treatment plants and potential impacts of microplastics in the environment.

Supported by the grant scheme for supporting excellent teams of young researchers under the conditions of STU in Bratislava: „Mikroplasty a ich účinné odstránenie pomocou progresívnych postupov."

L-19

A COMPARISON OF THE REACTIVATING, THERAPEUTIC AND NEUROPROTECTIVE EFFICACY OF TWO NOVEL BISPYRIDINIUM OXIMES (K305, K307) WITH TRIMEDOXIME AND THE OXIME K203 IN RATS AND MICE POISONED WITH TABUN

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The ability of three original bispyridinium oximes (K305, K307, K203) and one currently available oxime (trimedoxime) to reactivate tabun-inhibited acetylcholinesterase and reduce acute toxicity of tabun including neurotoxic signs and symptoms was evaluated in tabun-poisoned rats and mice. The oxime-induced reactivation of tabun-inhibited acetylcholinesterase was measured in diaphragm and brain of tabun-poisoned rats. The results showed that the reactivating efficacy of two recently developed oximes (K305, K307) does not achieve the level of the reactivation of tabun-inhibited acetylcholinesterase induced by the oxime K203 and trimedoxime. Generally, the reactivating efficacy of all oximes studied is higher in diaphragm compared to the brain. The therapeutic efficacy of all oximes studied roughly corresponds to their reactivating efficacy. While both recently developed oximes were able to reduce acute toxicity of tabun less than 1.55 fold, another original oxime K203 and commonly used trimedoxime reduced the acute toxicity of tabun more than 1.6 fold. Thus, the differences between therapeutic efficacy of all oximes studied are not so high as in the case of reactivating efficacy. Only one newly developed oxime (K307) combined with atropine was able to markedly decrease tabun-induced neurotoxicity in the case of sublethal poisoning although it did not eliminate all tabun-induced acute neurotoxic signs and symptoms. Its ability to decrease tabun-induced acute neurotoxicity was only slightly lower compared to the oxime K203 and trimedoxime. On the other hand, the neuroprotective efficacy of the oxime K305 was negligible. In conclusion, the reactivating, therapeutic and neuroprotective efficacy of both newly developed oximes does not prevail the effectiveness of the oxime K203 and trimedoxime and, therefore, they are not suitable for their replacement of commonly used oximes for the antidotal treatment of acute tabun poisoning.

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L-20

THE MONOQUARTERNARY REACTIVATORS FOR THE TREATMENT OF ORGANOPHOSHOROUS INTOXICATION

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Mono- and bis-pyridinium aldoximes are the only causal antidotes that are designated for the treatment of organophosphate (OP) poisoning. Intoxication by OPs is caused either by pesticides or by the nerve agents, the latter belong to group of chemical warfare agents. These compounds irreversibly inhibit enzyme acetylcholinesterase (AChE) that is no more able to fulfill its physiological function. Mono- and bis-pyridinium aldoximes are able to restore catalytic function of AChE. The

reactivating ability of aldoximes is limited by several drawbacks like low blood-brain barrier permeation, low reactivation potency against specific nerve agents etc. In order to obtain efficient treatment of OP, the introduction of novel AChE reactivators raised as an important issue. For over 60 years of intensive research, none of the reactivators reached sufficient activity. Herein, we present novel mono quaternary reactivators that possess excellent *in vitro* activity to restore AChE activity after intoxication with different nerve agents as well as pesticides. The molecular docking simulations, total synthesis and biological evaluation will be discussed.

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L-21

OXIME ADMINISTRATION AT 100% MTD OR 5% LD_{50} DOSES- THEIR THERAPEUTIC EFFICACY IN SARIN INTOXICATION TREATMENT

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The poisoning with nerve agent (e.g. sarin, tabun, VX) represents a life threating danger. Compounds with oxime moiety attached to a quaternary nitrogen pyridinium ring are able to reactivate acetylcholinesterase (AChE; 3.1.1.7) inhibited by organophosphorus agents. However, there are some evidence, other mechanisms not related with reactivation may lead to survival.

The main aim of this work was a comparison of efficacy two different doses (100% maximal tolerated dose-MTD and 5% lethal dose-LD_{50}) of obidoxime and HI-6 after sarin intoxication. 100% MTD is higher than standardly used 5% LD_{50}. Thus, the higher concentration of oxime in the central nervous system and their direct effect on cholinergic receptors were assumed. Accordingly, behavior mice Balb/C and their symptoms of sarin intoxication were observed. *In vivo* determination of reactivation in peripheral (blood) and central (brain) compartment showed that administration of 100% MTD does not provide significant benefit.

This work was supported by the Long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence.

L-22

DEVELOPMENT OF NOVEL DISINFECTANTS AGAINST PATHOGENS BASED ON QUATERNARY AMMONIUM SALTS

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In this project, we tried to develop new compounds or mixture of compounds based on quaternary ammonium salts (QAS) with a strong disinfectant potential against bacterial, fungal and viral pathogens which ma occur in the hospital environment. We have prepared, approximately 60 analogues of quaternary ammonium salts (QAS) containing quaternary nitrogen and long carbon chain. It is assumed that their effect is based on ability to interfere with the stability and functionality of microbial cell membranes of a wide range of infectious agents, which is ideal for topical use as disinfectants. According to the literature and our preliminary data, QAS were found to be effective against both bacteria and fungi. We observed that the most promising anti-G+ and G-bacteria agents were the series 31, 32, 27, 20 and 18. Against anaerobic bacteria the highest efficacy showed series 33 and 32. Although efficacy on viruses in quaternary ammonium salts is relatively rare, one of 32 series reduces the virus titer by 5 orders of magnitude after 5-minute virus exposure. A little less effective was series 28 and 33. Highest efficacy against yeasts was observed for series 32, 18, 16 and 32, against fibrous fungi then for series 18 and 16. Supplemental efficacy against green algae was very high for series 32, 27, 18 16 and 27. On the basis of the identified efficacy, combinations of substances were proposed to cover the entire spectrum of pathogens. These mixtures have been formulated and their efficacy against bacteria, viruses and fungi was confirmed. Furthermore, less skin irritability has been observed for novel mixtures in comparison to standard Ajatin at 0.1%.

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L-23

DECONTAMINATION OF WARFARE AGENTS

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Decontamination of chemical, biological and radiological agents is one of the basic measures to prevent poisoning or infection of affected persons. CBRN agents can be misused during war conflict (e. g. Gulf War, Syrian Civil War) or for terrorist reasons. The need for new combined decontamination and disinfecting agent results both from the obsolescence of existing solutions and from the requirements for improved protection of soldiers and civilians. The armed forces of advanced countries are usually equipped with primary sorption agents such as IPB-80. The sorbent is commonly inorganic material or modified activated carbon (charcoal). Relatively rare are the sorbents combined with reactive agents. In addition, various types of decontamination solutions are used - from purely aqueous solutions through water/organic solvent mixtures to completely non-aqueous solutions. Active decontamination agents are most often based on nucleophilic reagents such as alcoholates, phenolates, organic or inorganic peroxides, oximes and complex metal compounds. Liquid agents introduced into military equipment in the last decades

are characterized by the use of highly viscous polyethylene glycol-type solvents (Canadian RSDL or Russian IPP-11). At present, the Czech Army is equipped for personal decontamination by the IPB-80 anti-chemical package, which contains activated montmorillonite and is based on the sorption principle. Although this package device has a high exploitation value and low production cost, its weaknesses are known. It has limited use in the interior of combat vehicles, sporadic efficiency in decontamination of worn equipment and open wounds. Decontamination system, based on solutions of chemically reactive (active) agents can circumvent these handicaps. Furthermore, they combine antimicrobial efficacy with hydrolytic activity and by that can be used as polyvalent agents against both biological and chemical warfare agents.

We are focused on the development of novel quaternary ammonium compounds as a part of decontamination means. Quaternary cationic surfactants are compounds that are widely used in many industries. This large group of structurally different chemicals provides a number of interesting features. The most commonly used group are benzalkonium salts (*N*-alkyl, *N*-benzyl and *N*,*N*-dimethylamines, where the alkyl is most often in between C_{10}-C_{18}). We have prepared many of novel compounds that will be tested soon.

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L-24

HYDROCORTISONE REDUCES VESICATION BY MECHLORETHAMINE IN VIVO

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Extravasation reactions (EVRs) occur in approximately 6% of cancer patients receiving intravenous chemotherapy, with dermatotoxic effects ranging from tissue swelling and localized inflammation, to blistering (vesication) and ulcer formation. Current treatments aimed at reducing undesired EVRs remain grossly inadequate. Our long-term goal is to decipher the molecular mechanisms that regulate vesication and cutaneous inflammatory responses to cytotoxic chemotherapy drugs such as mechlorethamine (HN2), and to identify novel medical interventions to reduce EVRs. The purpose of the present study was to determine the vesicant countermeasure potential of hydrocortisone (HC). To this end, the mouse ear vesicant model (MEVM) was used, with male Swiss Webster mice serving as the test strain. Compared to control ears, mouse ears exposed to a single dose of HN2 (0.500 µmol/ear) showed an increase in wet weights, ear thickness, edema, hyperplasia, vesication and inflammatory cell infiltration after 24 h. Tissue expression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP-9) were upregulated in response to HN2. Fluorescence microscopy of TUNEL stained sections showed that the occurrence of apoptosis extended from the epidermis of the HN2 treated side all the way to the contralateral epidermis. In contrast, HN2 exposed ears treated topically with HC at a test dose of 0.031 mg/ear showed a significant decrease in wet weight (12.6% less than HN2 alone), morphometric thickness (16.5% less than HN2 alone) and vesication (60.0% for HN2 reduced to 33.3% after HC). Taken together, our studies suggest that lose-dose HC may serve as an effective countermeasure to chemotherapy EVRs.

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L-25

SEARCHING FOR NEW OPPORTUNITIES IN THE TREATMENT OF DEPRESSION AND ANXIETY: PRECLINICAL EVALUATION OF COMPOUNDS WITH PYRIDOINDOLE STRUCTURE

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DAnxiety and depression represent a serious mental health problem with globally increasing prevalence. Although the etiology of depression and anxiety is not yet completely understood, it is well established that serotonin (5-HT), norepinephrine (NE), and dopamine (DA) systems play a key role in their treatment.
Using *in silico* analysis of molecular structures, the pyridoindole derivatives (PDs) with code number 144, 143 and 104 were designed to test their potential antidepressant (PD144) and anxiolytic (PD143, PD104) properties. All PDs properties were tested in Wistar rats, subjected to behavioral assessment after acute administration (open field – OF, elevated plus maze – EPM, light/dark test – L/D, stress-induced hyperthermia – SIH and forced swim test – FST). In addition, in selected brain regions HPLC-ED analysis of monoamines levels, Fos-immunostaining and *in vivo* electrophysiology were evaluated.

PD144 caused significant changes of SIH and distance travelled in OF as well as decreased immobility time in FST. 5-HT and NA levels were increased in the hippocampus, whereas HVA/DA turnover was increased in the prefrontal cortex and the striatum.
Anxiolytic properties of PD143 were observed in all tests designed to identify the anxiety-like behavior, whereas PD104 showed significant changes only in the OF. Both, PD143 and PD104, decreased DOPAC/DA as

well as 5HIAA/5HT turnover in the hypothalamus and the amygdala. In addition, PD104 induced an increase in 5-HT level in the hippocampus and the hypothalamus. All tested substances following an acute administration increased the number of immunoreactive cells displaying c-Fos expression in the central nucleus of the amygdala and the hypothalamic paraventricular nucleus. Both of these structures are involved in the pathogenesis of depression and anxiety.

Electrophysiological investigations showed that PD144 dose-dependently suppressed the excitability of 5-HT neurons of the dorsal raphe nucleus, NE neurons of the locus coeruleus, and DA neurons of the ventral tegmental area. These results associate with the mechanism of action of the triple reuptake inhibitors.
In conclusion, our results represent a unique approach for preclinical evaluation of the tested compounds. We were able to identify anxiolytic and antidepressant properties, whereas the best results were achieved by PD144.

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L-26

MODULATING THE EFFECT OF PRENYLATED PHENOLIC COMPOUNDS ON PANCREATIC INS-1E CELLS

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Phenolic compounds are of considerable biomedical interest due to their antioxidant properties and potential in prevention and possibly treatment of many chronic diseases. The fruits, leaves and root bark of Morus nigra (Moraceae), the black mulberry tree, have a long history of use for various therapeutic purposes in traditional medicine worldwide. The roots of the plant are known to be a rich source of phenolic compounds with a particularly high chemical diversity [1]. Seven prenylated phenolic agents isolated from Morus nigra were tested for SERCA activity in non-cellular system, and in pancreatic ß cells for viability and apoptosis. Albanol A and B, kuwanon E and U and morusin induced reduction of SERCA activity in non-cellular system which correlated with decrease of cell viability and initiation of apoptosis in pancreatic β cells. These properties may be useful in cancer treatment. On the other hand, moracin P and R only slightly decreased SERCA activity, increased cell viability and did not induce apoptosis, suggesting that they might have a potential use against cardiovascular diseases and/or diabetes.

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[1] Zoofishan, Z., Hohmann, J. & Hunyadi, A. Phytochem Rev (2018). https://doi.org/10.1007/s11101-018-9565-1

L-27

IMPACT OF PRE-GESTATIONAL STRESS ON OFFSPRING NEUROBEHAVIORAL DEVELOPMENT AND HIPPOCAMPAL FUNCTIONING

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Depression during pregnancy or even prior to gestation can negatively affect offspring´s neurobehavioral development. Several studies have shown, that offspring who had experienced excessive stress during gestation had higher rates of cognitive, social and mood disorders later during adolescence or even in adulthood. Hippocampal neurons play a crucial role in the regulation of behavior, mainly anxiety-related behaviors and spatial learning and memory. Excessive stress could interfere with sensitive developmental processes in the brain and may affect hippocampal functioning with severe neurobehavioral consequences in later life. The aim of this work was to investigate the effects of pre-gestational stress on emotional and cognitive behaviors of the offspring. We also investigated primary neuronal cultures prepared from hippocampi of the newborn offsprings of the stressed dams to investigate hippocampal excitability. Moreover, we studied neurogenesis in the dentate gyrus of hippocampus in adolescent and adult offspring. Our results have shown, that pregestational chronic unpredictable stress affected selected reproductive and neurobehavioral variables of the rat offspring. Offspring exposed to maternal stress prior to conception showed altered behavior in a new and anxiogenic environment, spatial memory deficit and reduced neurogenesis in the dentate gyrus of hippocampus. Pre-gestational stress also affected both spontaneous and depolarization-activated action potential firing of hippocampal neurons. This work suggests, that maternal stress even prior to gestation can interfere with functional brain development of the offspring and can cause long-term behavioral changes at the level of neurobehavioral adaptations.

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L-28

ANALYSIS OF ECG RECORDED FROM RATS PRENATALLY EXPOSED TO STRESS AND VENLAFAXINE

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The prevalence of gestational depression currently oscillates between 10–18% among pregnant women all over the world. The most frequently prescribed antidepressants are selective serotonin reuptake inhibitors (SSRI) and selective serotonin and noradrenaline reuptake inhibitors (SNRI), including venlafaxine. Venlafaxine is not considered as a human teratogen. However, it has been documented, that prenatal exposure to venlafaxine, could possibly lead to congenital malformations, especially heart defects. On the other hand, untreated depression also represents a great risk for both mother and her child. The aim of this study was to focus on the effect of the maternal stress as a model of depression and treatment with/without venlafaxine (10 mg/kg/day, p. o.) on the electrical activity of the heart of both male and female rat offspring. The ECG was recorded from anesthetized rats. In males, prenatal exposure to stress led to significant decrease in P wave amplitude, but on the other hand, P wave and PQ interval prolongation, compared to control. Prenatal exposure to venlafaxine caused significant increase in duration of P wave and QRS complex. Venlafaxine showed tendency to potentiate the effect of stress, which resulted in significant prolongation of P wave, PQ interval. Compared to the control group, we observed no significant changes in QT or QTc interval duration in any of male groups, however QRS complex duration was prolonged. Female offspring prenatally exposed either to stress or venlafaxine did not display significant changes of selected ECG parameters. Prenatal administration of venlafaxine combined with prenatal exposure to stress led to significant increase in P wave amplitude, but decrease in duration of P wave and PQ interval. Moreover, QRS complex shortening and QT interval prolongation was present in this group, compared to control. In conclusion, we observed differences between males and females in reactions to prenatal exposure to stress or venlafaxine, but it is important to remark that both factors, when present during pregnancy, may affect the electrical activity of the heart of offspring.

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L-29

PROPOSED *IN SILICO* APPROACHES FOR MIXTURES

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Various safety assessment approaches are used for botanicals varying from reliance on post market surveillance of adverse events to tiered strategies. As the first tier in a newly proposed decision tree approach for the risk analysis for botanicals, the literature is searched for the available safety data on the botanical and its primary constituents. Data gaps exist for most botanicals, in particular, individual chemical constituents and repeat exposure information and vulnerable subpopulations (children, pregnant women, elderly). This lack of data drives one to a second tier in which the plant's compounds are assessed individually, including extraction methods. These chemicals need to be identified and evaluated for toxicity. Tools are constantly being developed that identify what often are called 'compounds of concern' or compounds with known biological activity. Additionally, harvesting methods and seasons, plant parts, and extraction methods all lead to different extracts which in turn may lead to different chemicals of concern. This approach together with better chemical characterization allows for more precise identification of these compounds. In the third step, these identified compounds undergo individual and specific risk assessment. Key elements for evaluating the safety of botanicals include better information on history of use, systematic assessment of weight of evidence, use of *in silico* approaches, inclusion of threshold of toxicological concern considerations, and adoption of *in vitro* and *in vivo* physiological based pharmacokinetic modeling.

L-30

ZEBRAFISH AS A MODEL FOR IDENTIFICATION OF TOXIC MECHANISMS WITH SINGLE CHEMICALS AND MIXTURES

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The zebrafish, *Danio rerio*, is an increasingly used model organism in biomedical research, high-throughput pharmacological screenings and toxicology due to several reasons: genetic tools are available, its transparent larval stages are amenable for imaging techniques, high fecundity and fast development allow investigating a large number of animals without long waiting periods and their small body size allows for the continuous measurement of behavior with full control of the environment. Moreover, major brain structures and physiological processes are conserved among phyla, allowing basic findings to be translated to other vertebrates including humans. Toxicity tests with zebrafish embryos and early larval stages have been gaining more attention through the standardization of testing methods in an OECD guideline (zFET OECD 236). The rapid extrauterine development of this vertebrate model enables the convenient assessment of numerous sub-lethal and lethal endpoints. Moreover, since larval stages up to 120 hours post fertilization, i.e. upon feeding independently, are not protected by law, they are considered alternatives to animal testing. Next to dissecting detailed toxic mechanisms with single chemicals and mixtures using the toolbox available, larval stages are amenable to high-throughput automatic devices which allow exposing the animals to a wide range of defined chemical mixtures. While zebrafish is an excellent model organism for the mechanistic dissection of complex processes, there are certain limitations with respect to exposure routes and application of chemicals through the medium. However, because of its numerous advantages the zebrafish can be used as a model to study both human and environmental health.

L-31

ADVANCED *IN VITRO* APPROACHES FOR COMPARATIVE ASSESSMENT OF HEATED TOBACCO PRODUCTS

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This presentation highlights the successful integration of omics approaches with cellular-based assays for the comparative assessment of a candidate modified-tobacco product, the Tobacco Heating System 2.2 (THS2.2) *in vitro*. Advanced human air-liquid interface cellular models of the aerodigestive tract (buccal, bronchial, and nasal organotypic cultures) were exposed to THS2.2 aerosol or cigarette smoke (CS) at similar nicotine concentrations. Less cytotoxicity and lower concentrations of secreted inflammatory mediators were observed in all cultures following exposure to THS2.2 aerosol compared with those following exposure to CS at comparable doses of nicotine. Global gene expression profiling and computational network enrichment analysis demonstrated mechanistic changes following exposure to CS including the impact on xenobiotic metabolism response, oxidative stress, and inflammatory response; at comparable nicotine concentrations, THS2.2 aerosol exposure elicited reduced and more transient effects on these processes. The identification of mechanisms by which potential modified risk tobacco products can reduce the impact on biological systems compared to cigarette smoke exposure is of great importance in understanding the molecular basis of the harm reduction paradigm. The presented systems toxicology approach is a robust methodology enabling detection of not only subtle exposure effects, but also identification of relevant biological mechanisms across tissues and exposure conditions.

L-32

EXTRAPOLATING NEW APPROACHES INTO A TIERED APPROACH FOR MIXTURE RISK ASSESSMENT

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Current risk assessments of chemicals do not generally consider exposure to multiple substances but rely instead on the assessment of individual substances in individual foods and other commodities. This is consistent with approaches to the assessment of chemical mixtures in most environmental media, such as soil, water and air. In reality, humans are routinely exposed simultaneously to numerous chemicals during normal daily activities. These mixtures can be variable and constantly changing and defining them presents a challenge. Several approaches to this problem have been proposed, including the use of high through-put assays or Tox21 approaches. In addition, structure activity relationships, read-across procedures and the Threshold for Toxicological Concern (TTC) concept can often shed light on the toxicity of otherwise obscure contaminants. *In vivo* models such as the nematode, *C. elegans,* or the zebrafish, *Danio rerio,* assays are rapid and inexpensive, and might be used to facilitate mixture assessment, either independently or in an integrated manner to assess health impacts. Exposure to chemical mixtures is the rule, not the exception. Although guidelines exist to sort our way though this sticky wicket, improvements are always welcome and, indeed, needed. Fortunately, new methods offer a significant improvement in the hazard assessment of chemical mixtures, and with the appropriate exposure determination, should continue to promote the credible protection of public health and the environment.

EFFECT OF HOP-DERIVED PRENYLFLAVONOIDS ON EFFICIENCY OF SELECTED CYTOSTATICS *IN VITRO*

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Prenylflavonoids, a unique class of naturally occurring flavonoids, are secondary metabolites of plants. They are formed by attaching of prenyl group to the flavonoid backbone. Various biological activities of prenylflavonoids have been described, including anti-cancer, antibacterial, anti-inflammatory, osteogenic, estrogen-like and antioxidant activity. Prenylflavonoids possess also inhibitory effect on a number of enzymes. Their biological activity and presence in food make interaction, both positive and negative, with concurrently used therapy possible. In the present study, we explored whether prenylflavonoids xanthohumol, isoxanthohumol, 6-prenylnaringenin and 8-prenylnaringenin could enhance the antitumor effect of classical cytostatics 5-fluorouracil, irinotecan and oxaliplatin in two colorectal carcinoma cell lines *in vitro*. Effect of the prenylflavonoids was compared to that of naringenin, their structural analogue lacking prenyl group. Two isogenic human cell lines, one established from a colorectal adenocarcinoma (SW480) and the other from its lymph node metastasis (SW620), were used. Effect of studied compounds on the cancer cell viability, activity of caspases and reactive oxygen species (ROS) formation was evaluated. Cell viability was monitored by neutral red uptake test, activity of caspases was determined by Caspase glo assay kit, and ROS formation was assessed using dichlorofluorescein assay. Combinations of prenylflavonoids and naringenin with individual cytostatics were set according to the recommendation of Chou and the type of interaction was evaluated by CalcuSyn software. IC_{50} of prenylflavonoids were determined below 40 μM. Most of the prenylflavonoids exerted antagonistic effect with classical cytostatics, except for the 8-prenylnaringenin, which showed synergistic effects with 5-fluorouracil and irinotecan. The 8-prenylnaringenin was also the only prenylflavonoid, which was able to significantly increase activity of caspases 3, 7, 8, and 9. Described *in vitro* interactions indicate possibility of side effects during chemotherapy. This should be verified by *in vivo* experiments.

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LEVELS OF SELECTED CONTAMINANTS IN FISH MUSCLE FROM UPPER NITRA RIVER

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The purpose of this study was to determine the levels of Zn, Cu, Ni, Cr, Pb, Cd and Hg in the muscle of three common fish species – brown trout (Salmo trutta m. fario), Alpine bullhead (Cottus poecilopus) and grayling (Thymallus thymallus), which inhabiting the upper course of the Nitra River. The concentrations of analysed metals ($mg.kg^{-1}$ wet weight) ranged as follows: brown trout – Zn 5.86–12.97, Cu 0.51–0.76, Ni 0.00–0.37, Cr 0.18–0.41, Pb 0.00–0.34, Cd 0.03–0.13, Hg 0.04–0.07; Alpine bullhead – Zn 7.02–13.68, Cu 0.34–0.62, Ni 0.00–1.13, Cr 0.19–0.24, Pb 0.00–0.37, Cd 0.03–0.09, Hg 0.06–0.18; grayling – Zn 3.38–6.36, Cu – 0.46–0.62, Ni 0.04–0.22, Cr 0.13–0.22, Pb 0.00–0.25, Cd 0.02–0.09, Hg 0.05–0.12, respectively. Statistically significant differences among individual fish species have been recorded. For individual fish species the statistically significant correlations between analysed metals and standard length, weight and age were detected. Permissible limits for safe consumption in the case of Pb, Cd and Hg were exceeded in 10%, 63% and 0%, respectively.

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THE EFFECT OF ANTHOCYANIN RICH WHEAT ON RAT CYTOCHROMES P450

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Wheat is one of the most important agricultural crops worldwide. The nutritional quality of wheat grain may be improved by the use of colored varieties with the presence of polyphenols, particularly anthocyanins. Anthocyanins, a subclass of flavonoids, are beneficial for human and animal health being e.g. mild antioxidants. Anthocyanins exhibit anti-inflammatory and anti-carcinogenic effect, prevent cardiovascular disease, control obesity and mitigate diabetes properties. Because of the possibility of metabolic interaction of anthocyanins with other foreign compounds, we decided to determine whether the consumption of wheat enriched with anthocyanins does not affect the activity of phase I biotransformation enzymes, namely cytochromes P450 (CYP) in the liver.

Rats were fed by Novosibirskaya wheat (control), ANK-28A, ANK-28B (red grain colour) and Aoi-Yu (blue grain colour) and had an access to tap water *ad libitum*. After 75 days, rats were anesthetized and exsanguinated. Livers were taken for further analyses. Microsomal fractions were prepared (pooled from each cage) and used for the study of CYP activity. Activities of rat CYPs (CYP1A, CYP2E1, CYP3A, CYP2D, CYP2C, CYP2B) were measured using their specific substrates [1].

Experiments have shown that eating foods enriched with anthocyanins does not result in clinically significant changes of CYP activity. On the contrary, a slight inhibition of some enzymes, e.g. CYP2E1 by anthocyanins, may have protective effect on the organism, as inhibition of CYP2E1 may be reflected in decrease of ROS formation.

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SEX AND AGE SPECIFIC EFFECTS OF MATERNAL DEPRESSION AND VENLAFAXINE TREATMENT ON ANXIETY-LIKE BEHAVIOR AND HIPPOCAMPAL NEUROGENESIS OF RAT OFFSPRING

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The prevalence of mental stress-related disorders, such as depressive and anxiety disorders, is rising, particularly in developed countries. Women are twice as vulnerable as men to experience a major depressive disorder during their lifetime. The character of early development in the uterus, and perhaps earlier, creates conditions for effects of risk factors for the physical and mental health of offspring. Risk factors include maternal depression as well as antidepressants that pass through the placenta, haematoencephalic barrier and also into the breast milk. Thus, gynecologists and obstetricians have a dilemma whether to treat or not to treat depression during pregnancy. At present, more findings suggest that risk of antidepressant medication during pregnancy and lactation is lower compared to risks of untreated depression. However, the effects of prenatal and early postnatal treatment with antidepressants on late postnatal development are poorly understood. Venlafaxine, the serotonin and noradrenaline reuptake inhibitor, is a new line of antidepressant therapy in pregnancy. However, the effects of venlafaxine treatment in gestation and lactation on the fetal and neonatal development are not fully known.

The aim of the present work was to study effects of maternal depression (chronic unpredictable stress) and venlafaxine (10 mg/kg/day from day 15 of gestation to day 21 *post partum*) during gestation and lactation on behavioral variables with focus on anxiety-like behavior and hippocampal neurogenesis of rat offspring in various postnatal developmental stages.

The results of our studies showed that maternal depression had anxiolytic effect on behavior in adolescent but anxiogenic effect in adulthood, exclusively in males. Maternal depression reduced neurogenesis in the dentate gyrus of hippocampus in males. Venlafaxine treatment affected only anxiety-like behavior in adolescent females.

The results of our studies suggest that the risks of untreated maternal depression may outweigh the risks of antidepressant treatment. The negative effects of untreated maternal depression can not occur immediately after birth, but under challenging conditions in adulthood.

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NITRIC OXIDE-DEPENDENT AND INDEPENDENT MECHANISMS OF EPICATECHIN-INDUCED VASORELAXATION

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(–)-Epicatechin (Epi) is a major bioactive cocoa flavanol, which is absorbed well in the gastrointestinal tracts of both humans and rodents. This study elucidated the mechanisms underlying vasodilatory effects induced by chronic and acute Epi treatment in the femoral arteries (FA) of adult spontaneously hypertensive rats (SHR).

We determined mechanisms associated with depressor effect of Epi in SHR after 10-day Epi treatment (250 mg/kg/day) and underlying mechanism after the acute Epi administration (up to 10 mmol/l) in the isolated FA using the wire myograph. Chronic Epi-treatment led to significant blood pressure reduction, elevated nitric oxide (NO) production and increase of NO-dependent component of acetylcholine-induced vasorelaxation without the alterations in the vascular smooth muscle cell function. Acutely administered Epi fully relaxed the FA pre-contracted with high-potassium salt solution (KPSS) or serotonin. However, NO synthase and soluble guanylate cyclase inhibitors (L-NMMA, ODQ) or estrogen receptor antagonists (fulvestrant, G15) failed to attenuate the relaxant effect of Epi. In contrast, Epi fully relaxed KPSS pre-contracted FA similarly as did nifedipine (specific L-VDCC inhibitor). Moreover, pre-treatment with Epi or nifedipine substantially inhibited the KPSS-induced contractions.

In conclusion, the results showed different vascular mechanisms of Epi when administered chronically *per os* or acutely on isolated arteries. Chronic Epi-treatment was associated with improved vascular NO bioavailability and NO-dependent mechanisms of vasorelaxation. In contrast, *in vitro* studies failed to confirm direct NO-dependent mechanism of Epi action, which was associated with a blockade of calcium entry through L-VDCC located on the vascular smooth muscle cells.

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INFLUENCE OF HUMULENE, B-CARYOPHYLLENE AND CARYOPHYLLENE OXIDE ON THE MRNA AND PROTEIN EXPRESSION OF PHASE I XENOBIOTIC-METABOLIZING ENZYMES IN PRECISION-CUT HUMAN LIVER SLICES

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Sesquiterpenes α-humulene (HUM), β-caryophyllene (CAR) and caryophyllene oxide (CAO) are important constituents of *Humulus lupulus* (hops) and *Syzygium aromaticum* (clove) essential oils. They are often present in various folk medicines and dietary supplements. Numerous biological activities, including anticancer, anti-inflammatory and pro-apoptotic, of these sesquiterpenes have been reported. Moreover, their inhibitory effect on the activity of several phase I xenobiotic-metabolizing enzymes in human and rat subcellular fractions have been described. Therefore, the aim of this study was to evaluate their influence of selected phase I drug-metabolizing enzymes in precision-cut human liver slices. In our experiments, five human liver samples received from surgery were used to obtain precision-cut liver slices, which were cultivated for 24 hours in presence of HUM, CAO and CAR in the 10 μM concentration. The mRNA expression of selected isoforms of cytochrome P450 (CYP 3A4, 2B6, 2C), carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C (AKR1C) were detected using real-time quantitative PCR and protein levels of these enzymes was detected using immunoblotting. Studied compounds slightly altered the level of mRNA as well as protein of CYP3A4, CYP2B6 and CYP2C. HUM caused significant inhibition in CBR1 and AKR1C mRNAs in one liver sample. In addition, protein expression of CBR1 was remarkably inhibited by HUM in all liver samples, while that of AKR1C3 was markedly increased in three samples. The observed effects of HUM, CAR and CAO were inconsistent, and they reflected rather inter-individual variability among liver donors.

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BIRT TRAUMA – STILL A CURRENT PROBLEM IN NEONATOLOGY

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As the birth trauma is refer foetal and neonatal injury during labour caused by mechanical forces. We may notice it during the first investigation of the newborn, or it may also be a hidden injury that will occurs during hours or days. The worst complication of the birth injury is asphyxia. Risk factors are divided into maternal, foetal and joined with delivery.

Retrospective analysis of the causes of birth trauma in newborns admitted to Neonatal Department of Intensive Medicine (NDIM). 123 newborns were analysed retrospectively during January 1st, 2015 – December 31st, 2016. The analysis was focused on the presence of risk factors (gestational age, birth length and weight; maternal risk factors – primipara, hypertension, infection, preeclampsia, diabetes mellitus, cefalopelvic disproportion, drug abuse, not cooperated mother during delivery; foetal risk factors – macrosomia; form of delivery – instrumental delivery, the necessity of using external expression, prolonged delivery, pelvic position of the newborn) and form of the birth trauma.
From 123 newborns (36.58% premature newborns; 63.42% term newborns) 68.30% were born spontaneously and 31.70% by caesarean section (in two cases in pelvic position and in 6 cases delivery was complicated by dystocia of arms). Primipara was the most frequent risk factor (63.41%). Macrosomia was confirmed in 12.20% (n=15) of cases of prenatally hypertrophic newborns. In less then 5% – dislocation of the nasal cartilaginous portion of the septum, bleeding into the adrenal glands, suffusion, subarachnoid bleeding, subdural bleeding, incision, fracture of femur, fracture of humerus, torticollis, torsion of testes, lesion of brachial plexus, fracture of clavicle; ≥5–10% – haemorrhage into retina, oedema, haemorrhage; ≥10–20% – caput succedaneum, cefalhaematoma, haematoma of soft tissue and ≥20% – haematoma and incisions different forms of birth trauma were presented.
Despite the increasing level of perinatal care, we are still experiencing traumatic neonatal injury. Birth injuries of the newborn may affect the newborn's quality of life both in the short and the long follow up. Some forms of postpartum trauma leave permanent consequences. Through monitoring of the foetus during pregnancy and delivery and early recognition of possible complications contribute to a reduction in the incidence of postnatal trauma as well as of severe asphyxia.

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EFFECT OF BISPHENOLS ON SELECTED NUCLEAR RECEPTOR EXPRESSION IN HUMAN GRANULOSA CELL LINE COV434

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Bisphenols are chemical plasticizers and monomeric constituents of polycarbonate plastics and epoxy resins. Bisphenol A (BPA) is a well-known endocrine disrupting compound, capable of affecting the normal function and development of the reproductive system, breast, adipose tissue, etc. Recently, other bisphenols have been introduced as replacements for BPA usage in many applications but the data show that they might also exert various adverse biological effects. In spite of the diverse identified bisphenol effects, little is known about the

molecular mechanisms of their action but experimental data show that BPA binds with a significant number of different receptors. Members of the nuclear receptor superfamily play roles in numerous physiological and patophysiological processes, including reproduction, metabolism, and in the genesis and progression of cancer.

In the present study, we used the human ovarian granulosa cell line COV434 to investigate whether bisphenols BPA and BPS are able to influence the viability of the cells and the expression of the selected nuclear receptors related to reproductive processes (*LRH-1, NURR1, RORA, TRA*). The agents were tested in several concentrations (1 nM, 100 nM, 10 μM) and the cells were treated for 48 h. The results indicate that bisphenols might afflict cellular processes via their influence on nuclear receptor ecosystem, and further detailed studies are needed for elucidating the consequent effects.

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THE EFFECT OF SILVER NANOPARTICLES AND IONS ON ZEBRAFISH EMBRYOS (*DANIO RERIO*)

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In present days, significant advancement in the field of nanotechnologies is noted. Silver nanoparticles are used in many different ways, for example in medicine, agriculture, construction and production of variety of consumer goods, such as electronics and textiles. It is due to their characteristics, such as antibacterial, antivirotic and antimycotic activity. With growing production and usage, a rise of concentrations of silver nanoparticles in the environment is expected. There is a concern, that silver nanoparticles in the ecosystem might have a negative impact on non-target species, such as fish. Therefore, we conducted fish embryo toxicity tests according to the modified OECD 236 guideline, using two different species of silver nanoparticles and silver nitrate, as a source of silver ions. 96hLC_{50} values were recorded, showing great differences in toxicological effects of tested substances. 96hLC_{50} for silver nitrate was 58.44 μg/l. 96hLC50 calculated for silver nanoparticles stabilized with maltose and gelatine was nearly 100 times higher – 4.31 mg/l. 96hLC50 for silver nanoparticles stabilized with polyvinylpyrrolidone exceeded 100 mg/l. Other sublethal effects, such as presence of oedemas and deformations, bradycardia, lags in development and delayed hatching, were observed as well.

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UNPREDICTABLE CHRONIC MILD STRESS AS AN ANIMAL MODEL OF DEPRESSION

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Depression is characterized by affective, cognitive and behavioral impairments and it affects approximately 20% of pregnant and lactating women. Untreated depression during gestation represents a higher risk for maternal morbidity, including arterial hypertension leading to preeclampsia or eclampsia, suicide attempts and *post-partum* depression. Most of the experimental models resemble the dysfunctions observed in human depression, such as significant weight loss, increased anxiety, as well as anhedonia, although, hallmarks of the disorder such as depressed mood, low self-esteem or suicidality are hardly accessible in non-humans. Therefore, the need for well-controlled animal studies dealing with consequences of pre- and perinatal effects of stress is crucial. For experimental purposes there is a need for reliable and reproducible tests to measure behavioral phenotypes in animals, such as depression and anxiety–like behavior, anhedonia and behavioral despair. The objective of the study was to induce depression status in pregnant and lactating female rats.

Female Wistar rats were subjected to two-week chronic unpredictable stress induced by random stressors. Moreover, they were treated with SNRI antidepressant venlafaxine orally at a dose of 5 mg/kg twice a day from day 15 of gestation to day 21 *post-partum*. Mothers were tested in the open field eight weeks after chronic unpredictable stress procedure in a single 15-min session as well as in sucrose preference test to evaluate anxiety status. Subsequently, hippocampal neurogenesis was investigated by immunohistochemistry essay using DCX staining. Results of the present study showed that two-week chronic unpredictable stress altered behavior of the dams demonstrating elevated anxiety-like behavior and lead to a lower litter numbers. Stressed dams had lower hippocampal neurogenesis, while venlafaxine treatment reversed this lowering.

Our results suggest that stress and antidepressant therapy can have significant impact on behavior and hippocampal neurogenesis in rat dams and that unpredicted mild chronic stress represents reliable animal model of depression.

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ASSESSING THE TIME DEPENDENT DERMATOXICITY OF MECHLORETHAMINE USING THE MOUSE EAR VESICANT MODEL

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Mechlorethamine (HN2) is an alkylating agent and sulfur mustard mimetic which is also used in anticancer therapy. Dermal exposure of HN2 is associated with extravasation and tissue blistering reactions that can lead to secondary infections in treated patients, thereby limiting the clinical use of this chemotherapeutic agent. The purpose of the present study was to investigate the time dependent dermatotoxicity of HN2 using the mouse ear vesicant model (MEVM). To this end, our operational definition of dermatotoxicity included tissue responses to HN2 consistent with an increase in the wet weights of mouse ear punch biopsies, an increase in the morphometric thickness of H&E stained ear sections and an elevation in histopathological scoring values for tissue edema, hyperplasia, inflammatory cell infiltration and vesication. The ears of male Swiss Webster mice were exposed to a single dose of HN2 (0.500 µmol/ear) or DMSO and the mice were then euthanized at 15 min or 1, 2, 4, 8, 12 and 24 hr following HN2 exposure. Mouse ears exposed to HN2 at all time points showed an increase in wet weight, morphometric thickness, edema, inflammatory cell infiltration and signs of vesication. The incidence in tissue vesication sharply increased between 4 and 8 hr after exposure, revealing that tissue vesication is well established by 8 hr and remains elevated at 12 and 24 hr after exposure. It is worthy to note that ears treated with DMSO vehicle also exhibited an increase in wet weight and morphometric thickness at 15 min, 1, 2 and 4 hr following treatment; however, these vehicle effects eventually subsided by 8 hr. The majority of published studies using the MEVM have examined tissue responses at 24 or 48 hr after vesicant exposure. The results of the present study provide a more holistic understanding of the kinetics of vesication and indicate that time points earlier than 24 hr may be useful to assess the effects of medical countermeasures to mustards, as well as investigate the toxic mechanisms involved in the process of vesication.

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CHARACTERIZATION OF CADMIUM INDUCED APOPTOSIS

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Cadmium (Cd) is a heavy metal which is highly toxic. Cadmium has nephrotoxic and hepatotoxic effects linked with induction of oxidative stress. Acute cadmium exposure causes apoptotic cell death. Cd in cells activates pro-apoptotic factors and leads to the activation of caspases and DNA damage, hi our study, we focused on comparison of methods for detection of apoptosis in cells treated with Cd. We used Human Kidney-2 cells (HK-2) treated with Cd at concentrations 25–100 pM $CdCl_2$ for time periods (6–24 h). In our work, we used various methods for detection of apoptosis such as: detection of mitochondrial membrane potential (JC-1 probe). DNA condensation (Hoechst 33258), detection of DNA fragmentation (TUNEL assay) and detection of caspase 3/7 activity (Caspase 3/7 kit). We observed the decrease of mitochondrial membrane potential in cells treated with 100 pM Cd after 24 h. We observed condensation of nucleus in both concentrations of Cd (25 and 100 pM) at both time duration (6 and 24 h). DNA fragmentation was observed in cells treated with 100 pM Cd after both time periods. Finally, caspase activity was increased at both tested Cd concentrations after 6 h. We conclude, all four methods detected apoptosis in Cd treated cells. After comparison of the methods, we found a strong correlation – the most sensitive apoptosis assay in Cd toxicity characterization was caspase 3/7 activity assay.

THE EFFECT OF THE EXPOSURE OF RATS TO THE ANTICANCER AGENTS VANDETANIB, LENVATINIB AND ELLIPTICINE ON THE EXPRESSION OF CYTOCHROMES P450

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Inhibitors of tyrosine kinases (TK) have become the novel tool in cancer therapy because of their ability to disrupt cell signaling pathways that regulate the processes of tumor growth and metastasis in cancer tissues. The TK inhibitors vandetanib and lenvatinib are oral anticancer drugs used for the treatment of certain tumors as so-called targeted therapy. Very little is known about the impact of these two compounds on the expression of biotransformation enzymes crucial for metabolism of drugs. Because combination of drugs is often used in the anticancer treatment and drug-drug interactions may play an important role in therapeutic effect of the administrated drugs, the Wistar rats were exposed to vandetanib or lenvatinib either alone or in combination with DNA-damaging cytostatic chemotherapeutic drug ellipticine to investigate the effect of these drugs on gene and protein expression of major biotransformation enzymes *in vivo*.

First, we focused on the expression of cytochromes P450 (CYP) 1A and 3A, the enzymes dominantly responsible for metabolizing drugs, including all three studied compounds, and other xenobiotics, in rat livers. Our results confirmed that ellipticine is a potent inducer of CYP1A1 and CYP1A2 that was reflected in the significantly increased gene and protein expression as well as activity of these enzymes. Co-treatment of vandetanib or lenvatinib with ellipticine had no effect on ellipticine induction potential. Interestingly, vandetanib and lenvatinib alone enhanced the mRNA and protein levels of CYP1A1 but at least 10-times less efficiently than ellipticine. All treatments led to slightly increased gene expression of CYP3A1 and marker activity of CYP3A, 6β-hydroxylation of testosterone. Furthermore, we studied the effect of the drugs on the gene expression

of CYP family 2 that also plays an important role in metabolism of drugs. Among rat isoforms CYP2A2, 2B1, 2C11, CYP2D1 and CYP2E1, only the gene expression of *CYP2C11* was 2-times decreased by ellipticine administrated to rats either alone or in combination with TK inhibitors.

It should be taken into account that by altering the expression of the enzymes involved in the metabolism of the administrated drugs, their pharmacological efficacy might be modulated.

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ACCUMULATION OF SELECTED METALS POLLUTION IN AQUATIC ECOSYSTEMS IN THE BASIN SMĚDÁ, CZECH REPUBLIC

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In 2016 was carried monitoring the environmental pollution of the river Smědá. The study present the concentrations of Hg, Pd, Co and Cd in the water, sediment and muscle tissue of brown trout– *Salmo trutta* morpha *fario* at 10 localities. The potential ecological risk of heavy metal concentrations in the sediments indicated that five sites in the middle and lower reaches posed small bis moderate ecological risk, but do not exceed the legal limits valid in the Czech Republic. Only two sites (2,3) represents a potential higher ecological risk (Hg content in sediment 0.057 mg/kg and Pb in sediment mg/kg) caused by the historical occurrence of the glassworks factory.

The contents of the analysed metals in fish muscles were low for all sites and did not exceed the values of limits admissible in the Europien comision regulation 1881/2006/ES and 629/2008/ES.

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IMPACT OF ANTHELMINTICS ON ACTIVITY OF ANTIOXIDANT ENZYMES IN SOYA (*GLYCINE MAX*)

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Veterinary anthelmintics, widely used drugs against parasitic worms, represent risk to environment as they may affect non-target organisms including plants. Plants are exposed to veterinary pharmaceuticals in pastures with treated animals or in fields fertilized with dung from treated animals. Anthelmintics enter plant body and can induce stress and consequent response. The production of reactive oxygen species (ROS), alterations in plant cell redox state and antioxidant mechanisms are usually the first responses to environmental stress. The antioxidant enzymes, including superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione peroxidase , glutathione reductase, and glutathione S-transferase, which are responsible for ROS elimination, may be affected as well. The aim of this project was to find out the impact of anthelmintics unwantedly occurring in environment (fenbendazole, FBZ; ivermectin, IVM) on soya (*Glycine max*) antioxidant enzymes. The changes in activities of antioxidant enzymes were measured in different parts of soya plant, in roots, leaves, seeds, and pods. The soya plants were harvested after the cultivation with 10 μM FBZ or 10 μM IVM for 3 months in greenhouse, and 20,000 g supernatant was prepared. The results showed the significant decrease of peroxidase and ascorbate peroxidase activity after FBZ and IVM treatment of *Glycine max* in roots, leaves, seeds, and pods. The activity of glutathione S-transferases was increased in soya treated with both anthelmintics. In addition, FBZ and IVM also markedly increased glutathione reductase activity in soya seeds. Some changes in the activity of other antioxidant enzymes were observed as well. In conclusion, veterinary anthelmintics can induce stress in plants and might affect their antioxidant systems.

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BIOSYNTHESIS OF MAGNETIC NANOPARTICLES USING PLANT EXTRACTS AND THEIR POTENTIAL ANTIBACTERIAL ACTIVITY

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Plant contains abundant natural compounds such as alkaloids, flavonoids, saponins, steroids, tannins and other nutritional compounds. These naturally occurring biomolecules have been identified as a potential reducing and capping agent for wet synthesis of nanomaterials in non-hazardous ways. In addition, green fabrication of magnetic nanoparticles is single step, rapid and cost-effective as compared to other physico-chemical technologies, which make it a promising technology. In this study, eco-friendly method was introduced to synthesize magnetite iron oxide nanoparticles using the aqueous extracts of geen tea leaves and *Cannabis sativa* seeds in alkaline conditions. The synthesized nanoparticles were characterized by UV–VIS spectroscopy and XRD analysis. These biogenic nanoparticles exhibited potential antibacterial activity against pathogenic bacteria presented in real waste water, therefore could be beneficial for potential applications in various fields

such as drug delivery, antibacterial drug, biomedical fields or wastewater treatment.

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HOMO- AND HETERODIMERS OF ACRIDINE DERIVATIVES AS INHIBITORS OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN ALZHEIMER´S DISEASE

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In the last decade much attention has been paid to the development of homo- and heterodimers containing two identical or different structural units linked by chain for simultaneous interaction with the active and the peripheral binding sites of acetylcholinesterase (AChE). A series of novel monotacrine, tacrine-tacrine, tacrine-acridine, tacrine-coumarin, acridine-coumarin and tacrine-quinoline ligands were designed, synthesized, and biologically evaluated as inhibitors of both AChE and butyrylcholinesterase (BChE) [1–3]. Among of monotacrine ligands, compound in which tacrine is connected to an (benzylpiperazinyl)etyl unit exhibited excellent inhibitory activity against hBChE (IC_{50}=0.4 nM) [2]. From tacrine-tacrine homodimers, inhibitors with buthylene-thiourea and hexylene-thiourea linker showed a strong acetylcholinesterase activity, with an IC_{50} value of 2 and 8 nM, resp [2]. The most effective inhibitors of hAChE within tacrine-acridine dimers were the derivatives joined with alkylenepiperazine linker with an IC_{50} value of 3 and 6 nM [2]. The structure-activity relationship studies showed clear correlation between the structure of homo/heterodimers and their inhibition potential against hAChE/BChE.

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ESTIMATION OF REDOX STATUS IN KIDNEY CELLS TREATED WITH CADMIUM

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Cadmium is a highly toxic heavy metal. The toxic effect of cadmium is most commonly detected in kidney. Acute exposure of the cells to cadmium can lead to apoptosis. Cadmium-induced cell death is linked with oxidative stress. Glutathione is the main intracellular nonprotein thiol with antioxidant properties in cells playing an essential role in protection against oxidative stress. Therefore, glutathione levels may be reduced in presence of cadmium-induced oxidative stress. Our aim was to characterize the redox changes in cells after cadmium treatment. A human proximal tubular cell line was used for studying nephrotoxicity *in vitro.* Proximal tubular cells were treated with $CdCl_2$ at concentrations 0–200 μM for 2, 6 and 24 h. To evaluate the induction of oxidative stress after $CdCl_2$ treatment, we measured intracellular reactive oxygen species (ROS) production and glutathione levels using intracellular probes and bimanes, respectively. ROS production increase was detected in cells treated with 100 and 200 μM $CdCl_2$ after 2 and 6 h. After 24 h, no increase in ROS production was detected in 100 μM and 200 μM $CdCl_2$ treated cells. After 24 h of treatment with 25 μM $CdCl_2$, we found substantial enhancement of ROS production. Significant depletion of intracellular glutathione appeared in all incubation periods following the increase in ROS production. However, the finding of ROS production increase was not linked with glutathione depletion after 24 h of treatment with 25 μM $CdCl_2$. We conclude that $CdCl_2$ is a potent inducer of oxidative stress in tubular cells associated with ROS production and reduction of glutathione levels. The level of oxidative stress is depends on the dose and time of $CdCl_2$ treatment.

THE HIGH-FRUCTOSE DIET DIFFERENTLY AFFECTS MICRORNAS EXPRESSION IN LEAN AND OBESE MICE

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Fructose is a highly lipogenic sugar naturally occurring mainly in fruits, vegetables or honey. The major problem is steady increase in fructose intake in soft drinks, sweets or prepackaged food. Fructose intake, compared to glucose, does not increase the levels of insulin and leptin, hormones involved in long-term regulation of energy homeostasis and body adiposity. In addition, dietary fructose is transported to the liver, where it bypasses the major control point of glycolysis, enzyme phosphofructokinase, and this way is becoming an unregulated source of glycerol-3-phosphate and acetyl-CoA. Fructose accelerates de novo lipogenesis and due to the molecular instability of furanose ring, it promotes the formation of reactive oxygen species (ROS). Taking together, the excessive consumption of fructose might contribute to the development of many serious metabolic disorders and diseases. The molecular mechanisms of these effects have not been fully elucidated yet. In our study, we focused on changes in miRNAs expression caused by high-fructose intake. MiRNAs have emerged as a key regulators of metabolic homeostasis over the past decade and the abberant

expression may be associated with many disorders and diseases, including obesity and related pathologies. These small, endogenous, single stranded, non-protein coding RNAsgene products, present in genomes of all eukaryotic organisms regulate RNA silencing and post-transcriptional gene expression through binding mainly to 3´UTR region of mRNA. For our experiments, we used lean mice and mice with obesity induced by high-fat diet, both with or without fructose administration in drinks. The panel of tested miRNAs was selected based on their involvement in obesity, metabolic syndrome, NAFLD and other related pathologies. The changes in miRNAs expression we observed in plasma, liver tissue, and white, brown and subcutaneous adipose tissues.

IMPACT OF PLATINUM NANOPARTICLES ON THE AQUATIC ORGANISMS

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In recent years several nanoparticles types have been studied intensively, but only very few studies have focused on the toxic effect of platinum nanoparticles (PtNPs) to aquatic organisms. PtNPs have many industrial and biomedical applications. Due to this extensive use, emission of PtNPs into aquatic environment must be expected. This may lead to undesirable environmental effects. Therefore, investigating the potential aquatic toxicity of nanomaterials has become an important issue. In this study we investigated the toxicity of three PtNPs of nominal size (3.1–24.4 nm) on organisms representing all trophic levels of the aquatic ecosystem: Duckweed *Lemna minor*, water crustacean *Daphnia magna* and marine bacteria *Vibrio fischeri*. The experiments were carried out on the basis of OECD 221; OECD 202 guideline and ISO 11348–2. Concentrations for each organism were chosen on the basis of the range finding test. The marine bacteria responded most sensitively to the presence of platinum nanoparticles. The effective concentration of Pt1 (3.1–10 nm) that caused 50% inhibition in bioluminescence of *Vibrio fischeri* was 135.47 $\mu g.L^{-1}$ and the effective concentration of Pt3 (8.7–24.4 nm) was 254.64 $\mu g.L^{-1}$. The concentration of Pt1 that caused 50% growth rate inhibition after 168 hours ($168hEC_{50}$) to *Lemna minor* was 10.67 $\mu g.L^{-1}$ and the concentration of Pt3 that caused 50% growth rate inhibition was <130 $\mu g.L^{-1}$. The acute toxicity ($48hEC_{50}$) of Pt1 for *Daphnia magna* caused concentration <410 $\mu g.L^{-1}$, Pt2 (4.2–21 nm) <415 $\mu g.L^{-1}$ and for Pt3 775.32 $\mu g.L^{-1}$. The ecotoxicity of platinum nanoparticles varies considerably according to the test organisms and particle size. The lowest toxicity of all tested samples was observed in *Daphnia* while the highest toxicity was in bacteria.

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HUMAN GUT MICROBIOME – XENOBIOTICS METABOLISING SYSTEM

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There is accumulating evidence indicating functional interactions between the human gut microbiome and ingested xenobiotics. These interactions may result in changes of effects of therapeutics and increase of toxicity and carcinogenicity of environmental compounds. Thus, it is getting accepted that human gut microbiome acts as an „additional organ" involved in metabolism of xenobiotics. Myricetin (MYR) is one of the key flavonoids present in various human foods and beverages including vegetables, teas and fruits. Its reductive analogue, dihydromyricetin (DHM), is highly effective in counteracting acute EtOH intoxication and in reducing excessive EtOH consumption. As some 2,3-unsaturated flavonoids undergo reductive metabolismus by gut microbiota, e.g. reduction of quercetin to taxifolin, we tested whether MYR is converted to reductive metabolite DHM, too. Our experiments with human fecal microbiota indicate that MYR was metabolized very fast, even when applied into the reaction mixture as a solid. Only negligible amount of the parent compound was left after 6 hrs. It seems that under anaerobic conditions MYR may serve as a convenient source of energy for microbiota. Alas, no DHM was detectable in the reaction mixture either. Thus MYR does not undergo reductive metabolism and cannot be used as a DHM precursor. In addition, the microbiota composition has changed in response to the MYR presence. It is not clear whether the shift in bacteria composition is originating from MYR antimicrobial effect on some bacterial genera and/or whether MYR is promoting the growth of other genera.

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THE IMPACT OF ANTIDEPRESSANTS ON FISH

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Even so the usage of antidepressants in world is on the rise, the information about impact on water organisms is limited. Psychopharmaceuticals are commonly found in surface waters in amount between ng/l to µg/l, thus they were chosen for this study for assessment of the potential impact on non-target organism *Danio rerio*. Embryos in different stages of development were

used and two different concentrations of venlafaxine were chosen; the 300 ng/l representing the environmental concentration (low) and the 30 µg/l as 100x higher (high) concentration for evaluation of dose depending effect. For the gene expression evaluation, genes ABCB4, CYP1A, CYP3A65, GST, ABCC1 and PXR were chosen, each of them representing one of the phase zero to III of xenobiotic biotransformation. The results of assay showed that the impact of venlafaxine on the zebrafish embryos is the most evident in the time of hatching (96 hours post fertilization); this period is one of the most threating period for the embryo development due to the first contact with the exogenous environment. In this time, the results of gene expression showed increase in mRNA amount of ABCB4 and GST in both concentrations of venlafaxine. The CYP1A, CYP3A65, ABCC1 and PXR gene revealed increase in mRNA amount in high concentration of venlafaxine; however, in contrast the low concentration for these genes revealed the decrease in mRNA amount. The second increase in gene expression was observed in 144 hours post fertilization for both concentrations of venlafaxine and all genes; this period is the time of transition to exogenous nutrition of embryo and thus the first oral exposure to xenobiotics in environment. To sum it, the study showed that venlafaxine can affect the gene expression of biotransformation enzymes of *Danio rerio* embryos already in environmentally relevant concentration.

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EXPRESSION AND PURIFICATION OF HUMAN ACETYLCHOLINESTERASE

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Human acetylcholinesterase (*h*AChE, EC 3.1.1.7) is the target of numerous natural and synthetic inhibitors, including therapeutics and a wide range of toxic esters as organophosphorus pesticides and nerve agents.

Current study is focused on new Expi293 expression system which is used for production of recombinant protein. This expression model promised to achieve expression levels near to 1 g/L of protein. The Expi293F™ cells were transfected with a pcDNA™3.4 TOPO® vector encoding the *h*AChE and incubated at 37°C8% CO2, 80% humidity and 120 rpm stirring speed. The cells produced and secreted complete *h*AChE within seven days. Progressive level of protein was supervised by Ellman's method. Protein was purified by Sepharose/Procainamide affinity chromatography. Subsequently, protein was analyzed by western blot using an anti-His tag antibody. The purity enzyme was determined via SDS-PAGE. The kinetic parameters of a recombinant enzyme were validated by value of IC_{50} of standard inhibitor e.g. donepezil, which was compared with published results.

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OPTIMIZATION OF PREPARATION OF VANDETANIB ENCAPSULATED IN AN APOFERRITIN

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Vandetanib is a once-daily oral agent using for the treatment of tumours of the thyroid gland. It acts as a tyrosine kinase inhibitor affecting signalling of epidermal growth factor receptor, vascular endothelial growth factor receptor or rearranged during transfection. Adverse effects connected with this drug are vomiting, nausea, insomnia, etc. The most dangerous adverse effect is connected with long QT interval. One way how to minimize side effects is targeting of drugs to tumour tissues. An apoferritin, the apo-form of naturally occurring protein ferritin, represents a suitable transporter for such targeting. It is a protein composed of 24 polypeptide subunits, structurally arranged to create an internal cavity with size of 8 mm in diameter. The structure is remarkably stable and is able to withstand biologically extreme temperatures (up to 70°C) and a wide pH range (pH 2–10). Furthermore, apoferritin can move undetected through the body without any immune response. It is also possible to modify its surface by ligands specific for targeting tissues. Unfortunately, such modification can stimulate immune responses.

Herein, the ability of the apoferritin to encapsulate vandetanib (creating ApoVan) was studied. At a constant concentration of drug and an increasing concentration of apoferritin more vandetanib is incorporated into apoferritin internal cavity. With increasing concentration of drug, the concentration of ApoVan reaches the maximum and starts to decline. These results indicate that the efficiency of encapsulation is dependent on ratio of vandetanib to apoferritin. The prepared ApoVan samples were characterized by transmission electron microscopy and quasielastic dynamic light scattering. The nanocarrier exhibits narrow size distribution and spherical shape. The surface zeta potential (ζ-potential) was also determined. All the characteristic indicate that apoferritin is a suitable nanotransporter for vandetanib.

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METABOLISM OF A TYROSINE KINASE INHIBITOR VANDETANIB BY HUMAN CYTOCHROMES P450 AND FLAVIN MONOOXYGENASES *IN VITRO* AND ITS EFFECT ON FORMATION OF DNA ADDUCTS GENERATED BY AN ANTICANCER DRUG ELLIPTICINE

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Vandetanib is a tyrosine kinase inhibitor (TKI) indicated for the treatment of symptomatic or progressive medullary thyroid cancer in patients with unresectable locally advanced or metastatic disease. It inhibits signalling of epidermal growth, vascular endothelial growth factor or rearranged during transfection. In this study, oxidation of vandetanib by human hepatic microsomes and recombinant cytochromes P450 (CYPs) and flavin-containing monooxygenases (FMOs) expressed in Supersomes™ was studied. The vandetanib oxidation products were separated by HPLC and identified by mass spectroscopy. Human hepatic microsomes oxidize vandetanib to *N*-desmethylvandetanib, but not to vandetanib-*N*-oxide. Of all tested human CYP enzymes, the CYP1A1, 2C8, 2D6, 3A4 and 3A5 enzymes, mainly in the presence of cytochrome b_5, oxidize vandetanib to *N*-desmethylvandetanib. No vandetanib-*N*-oxide was generated by tested human CYPs. However, FMO enzymes were able to generate this metabolite. Of three human FMOs tested (FMO1, FMO3 and FMO5), FMO1 and FMO3 oxidize vandetanib to vandetanib-*N*-oxide. FMO1 was more effective than FMO3 in this reaction. The results found in this study approved the knowledge showed by the preliminary studies, suggesting that vandetanib is oxidized to *N*-desmethylvandetanib and vandetanib-*N*-oxide, and specified the efficiencies of individual CYPs and FMOs in the reactions. Moreover, they indicated an essential role of cytochrome b_5 in oxidation of vandetanib to *N*-desmethylvandetanib by CYP3A4. Because this CYP is the most important enzyme activating also another anticancer agent that is effective against certain tumours of the thyroid gland, DNA-damaging drug ellipticine, the effect of vandetanib on metabolic activation of this drug was investigated. An inhibition effect of vandetanib on the most efficient anticancer effects of ellipticine, formation of covalent ellipticine-derived DNA adducts, was found. Cytochrome b_5 plays an important role also of this CYP3A4-mediated activity.

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TYROSINE KINASE INHIBITORS VANDETANIB, LENVATINIB AND CABOZANTINIB MODULATE METABOLISM OF AN ANTICANCER AGENT ELLIPTICINE BY CYTOCHROMES P450

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Vandetanib, lenvatinib and cabozantinib are tyrosine kinase inhibitors (TKIs) targeting VEGFR subtypes 1 and 2, EGFR and the RET-tyrosine kinase, thus considered as multiple TKIs. These TKIs have already been approved for treating patients suffering from thyroid cancer and renal cell carcinoma, and further clinical trials are ongoing for prostate cancer and glioblastoma multiforme. Ellipticine and its derivatives are other anticancer agents that are effective against certain tumors of the thyroid gland (anaplastic thyroid carcinoma), ovarian carcinoma, breast cancer and osteolytic breast cancer metastasis. Ellipticine anticancer efficiencies are dependent on its metabolism leading both to the activation metabolites causing DNA damage (covalent DNA adducts) and their detoxification to products that are excreted. Ellipticine is oxidized by microsomal cytochrome P450 (CYP) enzymes and peroxidases. Oxidative activation by CYP3A4 leads to formation of 12-hydroxy- and 13-hydroxyellipticine, reactive metabolites that dissociate to ellipticine-12-ylium and ellipticine-13-ylium, binding to DNA, while formation 9-hydroxyellipticine by CYP1A1 and the ellipticine dimer by peroxidases are considered to be detoxification products. A number of studies testing the effectiveness of individual anticancer drugs alone or in a combination with other cytostatics demonstrated that such combination can have additive and/or even synergistic effects on treatment regimen. The aim of this study was to study the effect of TKIs vandetanib, lenvatinib and cabozantinib on oxidative metabolism of ellipticine. All tested TKIs inhibit oxidation of ellipticine catalyzed by hepatic microsomes and individual CYPs, but not by peroxidases (horseradish peroxidase, lactoperoxidase and myeloperoxidase). The mechanism of these effects is studied in details. The study might provide a rationale for the clinical evaluation of the combination of TKIs and DNA-damaging anticancer drugs.

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CYTOTOXICITY OF ENDOCRINE DISRUPTORS IN TM3 LEYDIG CELL LINE

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Environmental contaminants altering the function of the endocrine system and exhibiting adverse health effects on the organism are defined as endocrine disruptors (EDs). Alkylphenol ethoxylates, a class of non-ionic surfactants, are microbially degraded into alkylphenol diethoxylates and alkylphenol monoethoxylates. These are subsequently degraded into alkylphenols (4-octylphenol; 4-nonylphenol) and along with other sub-products, are known to persist in the environment for a long time. Exposure to other EDs such as bisphenols has been also shown to cause adverse effects on the male reproductive system in humans and numerous animal species. A decrease in semen quality was the first reported alteration and from this moment on an informative expansion was launched on the potential consequences of bisphenol exposure. Both may disrupt not only spermatogenesis, by interfering with germ cells and sperm-supporting cells, but may also affect steroidogenesis occurring in Leydig cells. The aim of the present study was to investigate the potential impact of selected EDs on the male reproductive system in mice. Our study was initiated to evaluate the role of alkylphenols and bisphenols on Leydig cell function *in vitro* at lower experimental concentrations (0.04–5.0 μg/mL). Subsequently, we determined the metabolic activity, membrane integrity and lysosomal activity in TM3 cell line after 24 h cultivation. The cell viability was assessed using the metabolic activity (AlamarBlue^{TM}) assay, while the membrane integrity of exposed cells was evaluated by CFDA-AM assay. Determination of lysosomal activity was monitored by neutral red assay. A slight decrease in cell viability and membrane integrity of TM3 Leydig´s line was recorded after alkylphenols (1.0–2.5 μg/mL) treatment. Significantly decreased lysosomal activity was observed following exposure to the whole applied range of alkylphenols *(P<0.05; P<0.001).* Bisphenol´s exposure to TM3 Leydig cell line has shown a similar tendency. All the monitored parameters representing cellular health were negatively affected by experimental doses (0.04–5.0 μg/mL) of bisphenol A and bisphenol B.

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SURFACE WATER QUALITY AND THE EFFECT OF MUNICIPAL WASTEWATER TREATMENT IN THE SVRATKA RIVER (CZECH REPUBLIC)

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The aim of this work was to determine the selected physical-chemical indicators of surface water quality. Sampling was carried out during four seasons of the year at three different localities of the Svratka River – in the town of Tišnov, in the town of Veverská Bítýška, and close to the selected wastewater treatment plant (WWTP) in order to assess the impact of the selected town or location on the water quality in the river. At the same time, selected indicators were determined directly in the waste water from the WWTP near the town of Tišnov.

Among the parameters of interest were chosen basic elements set in the legislation such as chemical oxygen demand (COD), total nitrogen and total phosphorus, and some other substances such as nitrates, sulphates, chlorine, etc. These indicators were in surface water and wastewater determined by the method of spectrophotometry, using certain reagent and cuvette sets from Merck.

The results were compared with the permissible limit values specified in the present legislation of the Czech Republic, especially the Decree no. 401/2015 Coll., and with the values according to CSN 75 7221 – Classification of surface water quality. According to these regulations, we have classified the Svratka River to the class III of surface water quality (impure water). Based on the obtained results, it was also possible to assess the effectiveness and efficiency of selected WWTP to remove contaminants from waste water, which could negatively affect water quality in the river and thus the whole water ecosystem.

CYTOTOXIC EFFECTS OF NOVEL NITRO-HYDROXYNAPHTHANILIDES ON CANCER CELL LINES IN THE CONTEXT OF THE DIFFERENT SUBSTITUTION PATTERN

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The structure of three new series of nitro-substituted hydroxynaphthanilides was designed based on ring analogy with salicylanilides, derivatives possesing many pharmacological effects including promising anticancer activity, although their mechanism of action is still not fully understood. Our study focused on the evaluation of their potential effects on viability and proliferation of human cancer cell lines in the context of differences in the substitution pattern on naphthalene or anilide part of the structure.

Our results revealed that the position of the β-ring of naphthalene towards the carboxanilide or phenolic scaffolds and the position of nitro substituent both affected the intensity of cytotoxic activity in cancer cell lines. In all three series of compounds, we found their increased potency towards impaired cell viability and antiproliferative effect with the positioning of the substituent

as follows: *ortho* < *meta* < *para*. Their antiproliferative activity was also quantitatively comparable to that activity of another nitro-substituted salicylanilide, niclosamide. In addition, compounds with such effects induced accumulation of cells in G1 cell cycle phase in a dose-dependent manner in both THP-1 and MCF-7 cell lines. The higher concentration of 10 μmol/l of the most potent compound did not affect metabolic activity of nontumor cell line 3T3-L1 but at the same time it induced the phosphatidylserine externalization and activation of caspase 3 in cancer cell line THP-1. On the other hand, activation of caspase 8 upon that treatment was not observed. Further analysis showed the ability of that compound to induce cytochrome C release and cleavage of pro-caspase 9. That effects were also accompanied by a loss of mitochondrial membrane potential.

Thus our results indicated the pro-apoptotic effects of tested compounds in cancer cell lines, their significant advantage also lies in the fact that such an activity seems to be effectively modulated by appropriate structure modification.

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IN VITRO PAMPA AND MDCK MODELS FOR THE PREDICTION OF BLOOD BRAIN BARRIER PENETRATION

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The study of drugs acting on the targets in the brain can be precisely determined only by the *in vivo* study. Unfortunately, this way is very expensive and ethical aspect must be considered because of the high consumption of laboratory animals. The development of new potential drugs targeting the brain requires screening to assess permeability through the blood brain barrier (BBB). Two screening methods used for this purpose, PAMPA (Parallel Artificial Membrane Permeability Assay) and MDCK (Madin-Darby Canine Kidney) cells permeability assay, have been used in this experiment for evaluation of standard drugs set. Both PAMPA and MDCK permeability assays are used to evaluate the ability of compounds to diffuse from a donor compartment through the appropriate membrane into an acceptor compartment. PAMPA´s membrane is a brain polar lipid layer while MDCK assay uses MDCK cell monolayer. Then, the concentration in acceptor compartment has been determined by UV-VIS spectrophotometry. The transport through PAMPA is based exclusively on physico-chemical properties mostly represented by the logP but completely ignores the active mechanisms present in the BBB of living cells. These mechanisms involve active transport, efflux transporters and enzymes. This fact limits PAMPA´s use because a lot of compounds interact with them. MDCK BBB model improves evaluation using living cells which form the tight junction similarly to the BBB endothelial cells. Here we compare the two models for the BBB penetration assessment.

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EFFECT OF NEURAMINIDASE TREATMENT OF LUNG EPITHELIUM ON BINDING OF PSEUDOMONAS AERUGINOSA

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Cystic fibrosis (CF) is one of the most common human autosomal recessive diseases. This genetic disorder is associated with increased susceptibility of lungs to bacterial infections, which frequently develop to life-threatening conditions for CF patients. In this regard Pseudomonas aeruginosa (PA) is the most dangerous bacterial pathogen. Among PA virulence factors lectins seem to play crucial role in the PA adherence on CF airway epithelium. PA lectins, PAIL and PAIIL, showing high affinity for D-galactose and/or L-fucose, are assumed to be namely involved in the bacteria binding. Likely, the low-sialylation of glycoconjugates on CF epithelial cells provides exposed saccharide residues suitable for the lectins interaction. To study the PAIL and PAIIL involvement in the adherence of PA on airway epithelium the mouse model mimicking CF lung conditions is being developed. The status of low-sialylation was induced by the intratracheal instillation of neuraminidase, the enzyme cleaving terminal sialic acid. For our experiments lectins PAIL and PAIIL were recombinantly expressed and coupled with high-performance fluorescent label, DyLight 488. The saccharide binding ability of the DyLight 488 labeled lectins was checked by using a red blood cell agglutination assay. The PAIL and PAIIL labeling did not alter the lectin saccharide binding affinity. Next, tissue slices of lungs from neuraminidase-treated and untreated mice were mounted on microscope slides and incubated with DyLight 488 labeled lectins. Finally, tissue specimens were examined on an inverted fluorescence microscope (Nikon Eclipse TE2000-U). In the neuraminidase-treated group the cuboidal epithelium lining the surface of respiratory bronchioles showed an apparent fluorescence DyLight 488 labeled PAIIL when compared with untreated mice. On the other hand, when DyLight 488 labeled PAIL lectin was used (for neuraminidase treated mouse), fluorescence was observed both on the epithelium of bronchioles surface and on the pulmonary alveoli surface.These results support the assumption that PAIL and PAIIL mediate the PA binding on CF airway epithelium. Thus, lectins PAIL and PAIIL are a candidate target in preventing PA lung infections of CF patients.

IMPLEMENTATION OF *IN VITRO* METHODS FOR REGULATORY TOXICOLOGY TESTING AND RESEARCH AT HAMELN RDS A.S.

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Hameln has set up a research programme to implement toxicology methods that follow the 3Rs principles (Refine, Reduce and Replace animal testing) as requested by the EU regulation Directive 2010/63/EU. In this programme, hameln (as member of EU-NETVAL) implemented particular *in vitro* methods described by the regulatory standards.

Amongst the tests, the OECD TG 431 (*in vitro* skin corrosion test), TG 439 (*in vitro* skin irritation test), TG 492 (*in vitro* eye irritation test), TG 471 (bacterial reverse mutation test – Ames test), TG 473 (*in vitro* mammalian chromosomal aberration test), TG 476 (*in vitro* mammalian cell gene mutation test), TG 487 (*in vitro* mammalian cell micronucleus test) are those most frequently used *in vitro* assays at hameln. Implementation of these methods requires evaluation of benchmark materials and controls as defined by the OECD Test Guidelines. Hameln also actively collaborates with external parties to obtain trainings in the *in vitro* methods in order to accelerate their implementation.

This poster will present data and experiences obtained during this implementation process and will discuss the benefits and pitfalls of the implementation of the 3R methods into the GLP certified laboratory for both regulatory and scientific purposes.

EFFECT OF CEMTIRESTAT ON Ca-ATPASE (SERCA1) ISOLATED FROM ZUCKER DIABETIC FATTY RATS

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Cemtirestat, 3-mercapto-5H-1,2,4-triazino[5,6-b] indole-5-acetic acid, has been identified as one of the efficient inhibitors of aldose reductase, the enzyme involved in the polyol pathway, representing a promising therapeutic target in prevention of diabetic complications linked with glucose toxicity [1]. Impairment of calcium ATPase by high glucose or its derivatives was observed in human diabetes as well as in experimental animal models. Moreover, acute increases in glucose level strongly correlate with oxidative stress and may thus influence conformational changes in proteins [2]. We investigated sarcoplasmic reticulum (SR) calcium ATPase (SERCA1) which transports calcium ions from the cytoplasm into the SR, and thus plays a key role in calcium homeostasis and cell signaling. *In vivo* effects of cemtirestat on calcium pump SERCA1 isolated from skeletal muscles of Zucker diabetic fatty rats, a type 2 diabetes model, were examined. Results indicate a significant decrease of SERCA1 activity and attenuation of the enzyme expression accompanied by its post-translational modifications in diabetic rats compared to control animals. Yet treatment with cemtirestat failed to affect either activity decrease or declined expression of SERCA1 in diabetic rats.

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EFFECT OF BILBERRY EXTRACT (*VACCINIUM MYRTILLUS L.*) ON CONJUGATING ENZYMES IN RATS

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Vaccinium myrtillus L. (bilberry) fruit is a blue-colored berry with high content of anthocyanins, bioactive secondary metabolites composed of a sugar moiety and anthocyanidins (flavonoid structure). Bilberries are widely studied due to their health-promoting properties, especially for their positive effect on night vision, vascular permeability and capillary fragility. However, very little is known about bilberry effect on biotransformation enzymes. The aim of this study was to evaluate the effect of bilberry extract on the mRNA expression and activity of main phase II biotransformation enzymes: glutathione S-transferase (GST), sulfotransferase (SULT), UDP-glucuronosyl transferase (UGT) and catechol-O-methyltransferase (COMT) and predict possible impact on the action of together administered drugs. The *in vivo* study, where rats were exposed to bilberry extract (two concentrations, 0.15 or 1.5 mg/mL) in drinking water for 29 and 58 days, was performed to fulfil the objective. Mild decrease of COMT mRNA level was observed in samples with lower concentration of bilberry extract after 58 days. All other assayed mRNA levels remain unchanged. GST, COMT and UGT activities did not show response to bilberry extract. Slightly modified activity was observed in SULT, where activity was increased after 58 days by both concentrations (118±0.6% of control – in samples with lower concentration; 115±0.7% of control – in samples with higher concentration). The results suggest that bilberries as food supplement possess very low, if any, potential for food–drug interactions with respect to conjugating enzymes. This conclusion is positive particularly for bilberries regular consumers.

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ANTIEPILEPTIC DRUGS DURING PREGNANCY. CZTIS EXPERIENCE

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Antiepileptic drugs are suspect teratogens with various risk of malformations. They are used for epilepsy treatment but also for treatment of psychiatric diseases and migraine. Aim of our work was to evaluate spectra and changes over the time in indications for individual drugs. Majority were calls before planned pregnancy or during first trimester. Proportion of calls for valproate in epileptic women is stabile reaching approximately 20%. It is probably result of disease severity or maternal fears from malformation. Spectra of drugs has been changed to safer and newer drugs, i.e. lamotrigine and levetiracetam. Spectra of antiepileptic drugs for psychiatric indications are very similar with relatively high occurrence of valproic acid and lamotrigine. Topiramate and clonazepam were more frequently used in psychiatric indication then for epilepsy. Antiepileptic drugs in both indications are combined, more often in psychiatric indication. Psychiatric drugs are less studied, information on reproductive toxicity are not available, evaluation of the risk therefore cannot be exact. We add three cases of psychiatric patients (depression, bipolar disorder and obsessive-compulsive disorder) treated by antiepileptic drugs with follow up. Case 1: 33 years old woman suffering from depression was treated by alprazolam, venlafaxine, clonazepam, bupropion, zolpidem and trazodone. She gave birth girl smaller for gestational age with small hemangioma. Case 2: Woman 34 years old suffering from bipolar disease and sclerosis multiplex was treated by valproic acid, sertraline, and methylprednisone. She used sporadically paracetamol with caffeine. She gave birth healthy girl in term. Case 3: 28 years old woman suffering from obsessive- compulsive disorder was treated by sertraline, lamotrigine, flupentixole and sulpiride. She gave birth premature girl (36th week) without malformation.

Conclusions: We found that polytherapy, drugs without known effect on fetus, and anticonvulsant with risk of teratogenicity are used for treatment psychiatric indications. Improvement of knowledge about optimal pharmacotherapy during pregnancy in psychiatrists is therefore essential.

The work was supported by Progres Q16.

THE CHANGES IN MICRO-RNA EXPRESSION IN PLASMA AND HEART OF MICE TREATED WITH CARDIOTOXIC ANTICANCER DRUGS

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Cardiovascular toxicity remains a major cause of drug failure during preclinical and clinical development and it also contributes to the post-approval withdrawal of medicines. Moreover, many drugs widely used in clinical practice have potentially toxic effects on the heart in some patients. MicroRNAs (miRNAs), small endogenous non-coding RNA, seem to be promising tool regarding drug-induced cardiotoxicity. Cardio-specific miRNAs can serve as markers in the identification of potentially toxic compounds through *in vitro* preclinical screening and the miRNAs circulating in plasma can be useful in the identification of patients with subclinical cardiotoxicity. In our project, we focused on two anticancer drugs, doxorubicin (DOX) with proven cardiotoxicity and imatinib (IMT) with potential cardiotoxicity. Mice were treated with DOX (3mg/kg, every other day, i.v.) or IMT (100mg/kg, every day, p.o.) for 9 days. The control groups were treated only with the solvents. The last day of experiment, mice were sacrificed, blood was sucked out and hearts were removed. Plasma samples and heart homogenates were used for miRNAs isolation and quantification using miRNA-microarray and qPCR. In plasma, concentration of troponin T was also assayed. The results showed significant increase of troponin T in mice treated with DOX. DOX treatment led to up-regulation of miR-208b and miR-367 in heart. In plasma of DOX-treated mice, levels of several miRNAs (namely miR-34a, miR-133a,b, miR-1a, miR-7058) were increased, while others (e.g. miR-6240, miR-339, miR-6236) were decreased in comparison to controls. IMT treatment caused also similar increase in troponin-T plasmatic level and miRNAs expression, but the changes were not statistically significant due to marked inter-individual differences.

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THE EFFECT OF PRENATAL AND PERINATAL EXPOSURE TO PHTHALATE MIXTURE ON SOCIABILITY, ANOGENITAL DISTANCE AND TESTOSTERONE PRODUCTION IN ADULT RATS

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Phthalates (Pht) are chemicals belonging to the group of endocrine disruptors that are capable of interfering with the endocrine system of animals. Testosterone (T) is the hormone important for brain development and its levels are negatively affected by exposure to Pht. For this reason, there is a growing concern of Pht having detrimental impact on brain development, and behaviour of individuals. Our goal was to investigate the effect of prenatal and perinatal exposure to Pht mixture on sociability, anogenital distance (AGD), and plasma T levels in adult rats. Pregnant Wistar rats were divided into two groups: control (Ctrl) and exposed to the mixture of phthalates (Ft) – Di(2-ethylhexyl) phthalate

(DEHP), Diisononyl phthalate (DiNP), and Di-n-butyl phthalate (DBP) in dose of 4.5 mg/kg/day each. Mixture was diluted in peanut oil as vehicle, and delivered to an animal orally from gestational day 15 to postnatal day 4. Social interaction test was performed in adult rats (Ctrl: ♂ n=12, ♀ n=10; Ft ♂ n=10, ♀ n=12) to assess sociability. AGD was measured between the groups (Ctrl: ♂ n=19, ♀ n=25; Ft ♂ n=21, ♀ n=22) to assess changes in prenatal T levels. Postnatal plasma T levels were measured in adulthood (Ctrl: ♂ n=15, ♀ n=26; Ft ♂ n=18, ♀ n=22) using radioimmunoassay. Our results show that offspring from Ft group spent a less time in social interaction (p<0.001), had a decreased frequency of social contacts (p<0.001), shorter AGD (p<0.05), and decreased levels of plasma T when compared to Ctrl (p<0.05). These results suggest a possible role of phthalates in decreasing T levels, which could lead to abnormalities in brain development, leading to changes in sociability, aspect of behaviour which was shown to be impaired in several neurodevelopmental diseases.

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IN VITRO CHARACTERIZATION OF ACETYLCHOLINESTERASE REACTIVATORS

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Acetylcholinesterase (AChE; 3.1.1.7) reactivators play a key role in the treatment of organophosphate poisoning. The reactivators disrupt the covalent bond between organophosphorus compounds and AChE and restore the physiological function of this enzyme. On the other hand, the reactivators possess their own toxicities, whose mechanisms are not fully understood.

The objective of our study was to compare the cytotoxicity of asoxime, methoxime, obidoxime, pralidoxime, trimedoxime, K027, K074, K075, and K203 using hepatocellular carcinoma cell line HepG2. MTT assay and real-time cell viability assay were used to measure cytotoxicity of selected compounds, which was expressed as toxicological index IC_{50}. Fluorogenic 2,7-dichlorofluorescein diacetate dye and dihydroethidium were utilized to measure generation of reactive oxygen species (ROS). The microcapillary flow cytometry was used for determination of change in apoptotic activity.

The tested reactivators showed different cytotoxicity with HI-6 being the most and K027 being the least toxic. Changes in intracellular ROS were also detected; however, they did not correlate with substances´cytotoxicity.

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THE TOXICITY OF SELECTED ANTIDEPRESSANTS ON THE *DANIO RERIO* LARVAE

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In recent years observed increased interest in the presence of active substances in surface waters. The presence of drugs in surface waters is associated with a significant and ever-increasing use of medicines worldwide, which in turn is mainly due to the increasing prevalence of civilization diseases and an aging population. The main source of pharmacologically active substances in surface waters are treated municipal wastewater, which are introduced into watercourses from surface waters treatment plants. Pharmacologically active compounds, when they enter surface waters, may affect aquatic organisms. It is worth noting that these organisms are often vulnerable not to one, but to several pharmacologically active compounds occurring in concentrations of μg/L and ng/L throughout their lives. A group of drugs that is particularly interesting are antidepressants. According to the global health estimates by WHO 2015 the proportion of the global population with depression in 2015 is estimated to be 4.4%. Antidepressants can even affect aquatic organisms even at low concentrations of ng/L.

The aim of this study was to determine the influence of selected antidepressants and their mixtures on larvae Zebrafish as well as to determine the effect of low concentrations of sertraline, paroxetine, fluoxetine and mianserine and their mixtures on physiological or histological responses of Danio rerio larvae.

The fish embryo toxicity test (FET) was carried out in 6-well plates according to OECD 236 guideline. The plates were incubated at 27–0.5°C.for 96 h. The proliferative activity of hepatocytes was analyzed in fish embryo tissues by immunohistochemical staining of the nuclear proliferation cell antigen (PCNA).

Exposition to the analyzed pharmaceuticals did not influence the survival of embryos and larvae during 96 h of the test at the concentrations tested (5–25 μg/L). In some cases it has been reported pathologies such as scoliosis or pericardial edema. PCNA test revealed that significantly lower proliferation (3-times) of hepatocytes occurred in larvae exposed to paroxetine, mianserin, sertraline and the mixture of the pharmaceuticals at the highest concentrations (25 μg/L of each compound).

The results suggests that selected antidepressants sertraline, paroxetine and mianserin may adversely affect the organogenesis of fish.

INFLUENCE OF NEWLY DEVELOPED HEMOSTATIC AGENTS ON THE INFLAMMATION RESPONSE IN TRAUMATICALLY INJURED KIDNEY

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Various surgical procedures require application of an effective hemostatic material. Appropriate agent should prove high hemostatic activity and besides also biocompatibility and potential bioresorbability. The aim of this study was evaluation of the local tissue reaction in rat's kidney after surgical intervention and application of hemostatic textiles. We used a common model of partial nephrectomy. We assessed new cellulose nonwoven textiles based on oxidized cellulose and sodium salt of carboxymethylcellulose in comparison with a fibrilar form of regenerated oxidized cellulose. Materials were attached to the wound to effectively stop the bleeding and left in the same location while the peritoneum was sutured. Main purpose was to investigate the influence on the renal parenchyma after 3 and 30 days from the surgery. We leaned on the results from renal histology and immunohistochemistry considering histological changes and kidney cytokines TNF-α, TGF-β as inflammation markers. Obtained results revealed better hemostatic activity of the new carboxymethylcellulose material together with beneficial effect on the healing tissue.

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INTERFERENCE OF EXOGENOUS BROMIDE WITH THE METABOLISM OF IODINE AND THYROID HORMONES IN THE RAT

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With the aid of several radioanalytical methods, using ^{82}Br and ^{131}I or ^{125}I radiotracers, we studied the effects of an enhanced bromide intake on various aspects of iodine metabolism and, consequently, on the metabolism of thyroid hormones in the rat. In particular, we followed the influence of both an extremely high bromide intake (> 160 mg bromide per animal per day) and also of lower intakes, under conditions of sufficient iodine supply (standard diet B) and of iodine deficiency (Remington type diet R). In addition to adult male rats, we used also lactating rat dams and their pups.

Here, we summarize the effects of excessive bromide intake on the thyroid function and on the whole-body metabolism of iodine. Discussed is especially the influence of a high bromide intake in lactating rat dams on the production rate of mother's milk and on iodine and bromide transfer through mother's milk to the suckling young. Moreover, the impact of high levels of bromide in the organism of lactating dams on their performance in the course of the lactation period and in particular on the prosperity of their pups is also described. We have proved that bromide, similarly to iodide, readily penetrated into rat milk and via mother's milk was transferred in a large extent into the body of suckling young. We have also confirmed the earlier observation that bromide toxicity is dependent on the state of iodine supply into the organism.

In the adult rats we followed the effects of exogenous bromide on the uptake of ^{131}I-iodide by the thyroid glands and by various others organs and tissues (for more comprehensive results, *see* [1]). In addition, we have also found a complex, biphasic relationship between the extent of bromide intake in the rats and the specific peroxidase activity in their thyroid glands (*see* the accompanying poster).

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[1] Pavelka S. in: COMPREHENSIVE HANDBOOK OF IODINE: NUTRITIONAL, BIOCHEMICAL, PATHOLOGICAL AND THERAPEUTIC ASPECTS (Preedy VR, Burrow GN and Watson RR, eds.). Oxford: Academic Press, 2009, pp. 199–206 (Chapter No. 20) and 587–595 (Chapter No. 61)

BIPHASIC INFLUENCE OF EXOGENOUS BROMIDE ON THE THYROID PEROXIDASE ACTIVITY

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Thyroid peroxidase (TPO) is the key enzyme in the biosynthesis of thyroid hormones in thyrocytes. Recently, we have proved potent goitrogenic effects of exogenous bromide and perchlorate ions in the rat [1] (*see* also the accompanying poster). Here, we followed in detail, with the aid of an improved radiometric enzyme assay for TPO, the influence of different bromide and/or perchlorate intake in the animals, maintained on diets with diverse iodine content, on their TPO activity.

Unexpectedly, we found that the influence of exogenous bromide on the TPO activity in the rat thyroids was complex, biphasic with regard to the extent of bromide intake. An increase (up to 3-fold) in TPO activity was measured in rats with a low or moderate bromide intake (below ca. 60 mg per animal per day), while in animals with very high bromide intake (over ca. 160 mg) its thyrotoxic effects prevailed and TPO activity was reduced. The inhibitory effect of bromide was markedly increased in animals maintained on iodine-deficient diet. Interestingly, elevated TPO activity was found in all rats administered with perchlorate alone, regardless of the type of diet (the content of iodine in the diet, respectively). At the whole-body level, in rats administered with bromide and perchlorate we measured a consistent increase in relative weight of their thyroids with increasing time and concentration

of applied bromide, and a sharp reduction of the 24-h uptake of [^{131}I]-iodide by their thyroids. In these animals, we also determined a steady decline in serum total thyroxine concentration.

Supported by the Academy of Sciences of the Czech Rep. (Research project No. AV0Z50110509)

[1] Pavelka S. in: COMPREHENSIVE HANDBOOK OF IODINE: NUTRITIONAL, BIOCHEMICAL, PATHOLOGICAL AND THERAPEUTIC ASPECTS (Preedy VR, Burrow GN and Watson RR, eds.). Oxford: Academic Press, 2009, pp. 199–206 (Chapter No. 20) and 587–595 (Chapter No. 61)

INTERACTION OF ANTIDEPRESSANT DRUG FLUOXETINE WITH THE METABOLISM OF TRI-IODOTHYRONINE

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Thyroid hormones (TH) are supposed to control the activity of some neurotransmitters (e.g., serotonin), which are hypothetically involved in the pathogenesis of depressive illness. Namely, one of the pathogenic factors of depression might be inadequate activities of brain enzymes iodothyronine deiodinases (IDs) that could lead to local insufficient concentration of 3,3',5-triiodo-L-thyronine (T_3). This hypothesis led to the development of a new group of non-tricyclic antidepressant drugs known as selective serotonine re-uptake inhibitors. The most frequently used representative of this group of pharmaceuticals is fluoxetine (Fluox). We studied the interaction of Fluox with the metabolism of TH in the rat with the aid of our newly developed radiometric assays for deiodinating enzymes, IDs of types 1, 2 and 3 (D1, D2 and D3), as well as of adapted radiometric enzyme assays for conjugating enzymes iodothyronine sulfotransferases (ST) and uridine 5'-diphospho-glucuronyltransferase (UDPGT). Effects of subchronic administration of Fluox by itself, T_3 alone or in combination with Fluox, on T_3 production and degradation in the CNS and in different peripheral rat tissues were followed both, at the level of whole organism and at the molecular level.

In samples of liver microsomes of rats treated with Fluox, we found about two-fold higher UDPGT activities in comparison with control rats. Even more profound changes in enzyme activities were found in case of IDs, especially in the pituitary and cerebellum. The treatment of rats with Fluox alone caused a moderate increase in D2 and, in turn, a slight decrease in D3 activities in cerebellum. On the other hand, the administration of T_3 by itself caused, in accordance with our expectation, a substantial decrease in pituitary D2 activity and a simultaneous increase in D1 and D3 activities practically in all tissues studied. In conclusion, the elaborated radiometric assays for IDs, UDPGT and ST were found very useful for the assessment of enzymatic changes at the molecular level, caused by the administration to the rats of Fluox, T_3 or the combination of these pharmaceuticals.

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GESTATIONAL HYPOXIA ALTERED POSTNATAL DEVELOPMENT AND INDUCED LONG-LASTING BEHAVIORAL CHANGES IN RATS

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Lowered blood oxygen level during pregnancy may have long-lasting or even permanent effect on health of the offspring. The aim of our study was to assess effect of gestational hypoxia on postantal development and behavioral changes (activity, anhedony, anxiety) of the rat offspring. Hypoxia (10.5% O_2) was induced on GD19 and 20 for 8 h per day. The weight of hypoxic group was significantly elevated compared to control group and this increase in weight persisted up to adulthood. Sensorimotor development of hypoxic pups was delayed, seen as significantly lower percentage of appropriate reaction at air-righting and startle reflex test. The activity of hypoxic pups in the open field at weaning was significantly lower than control, especially in female rats. However, this activity did not differ from control group on postnatal day 85. Although hypoactivity was normalized at adulthood, other hypoxia-induced behavioral changes were apparent. Hypoxic group showed anxiety-like behavior with significantly more entries to dark zone in light-dark test and anhedony in sucrose preference. In conclusion our model showed crucial role of oxygen supply within last days of gestation for proper behavioral development and coping strategies.

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CYTOTOXICITY AND ANTI-INFLAMMATORY EFFECT OF CHLOROQUINE AND HYDROXYCHLOROQUINE IN BV-2 MICROGLIAL CELLS: ROLE OF REDOX HOMEOSTASIS CHANGES

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Chloroquine (CQ), a commonly used anti-malaria drug, was also shown to have immunomodulatory properties. In addition, oxidative stress-promoting effects were reported for CQ cytotoxicity along with protective effects of antioxidant co-treatment. In this study, CQ and its derivative hydroxychloroquine (HCQ) were evaluated for their cytotoxic and anti-inflammatory properties in murine BV-2 microglial cells, an accepted model of the brain-resident macrophages playing a key role in neuroinflammation.

The 24 h incubation with CQ caused some more profound viability decline of BV-2 cells than did HCQ, as confirmed by MTT assay. However, this was not associated with upregulation of intracellular reactive oxygen species (ROS) levels, as confirmed by dichlorodihydro-fluorescein diacetate (DCFH-DA) assay and flow cytometry. The co-treatment with antioxidant SMe1EC2 suppressed the basal ROS levels and enhanced MTT conversion in CQ-treated BV-2 cells that might be ascribed to proliferation-promoting effect of SMe1EC2.

The non-toxic concentrations of CQ and HCQ showed a mild suppression of lipopolysaccharide (LPS)-stimulated NO release by BV-2 microglia, as documented by measurement of nitrite levels in media by Griess method. This effect was associated with promotion of intracellular ROS production with a more profound effect confirmed for CQ. The preliminary data from Western blot analysis point to negligible influence of either CQ or HCQ on expression of proteins iNOS, phospho-Erk1/2/Erk1/2 and HO-1. However, LC3-II/LC3-I ratio was increased in CQ- and HCQ-treated activated cells in comparison to LPS-stimulated control, pointing to inhibition of autophagy flux.

In conclusion, the results of our study point to a role of intracellular oxidative stress in modulation of inflammatory profile of microglial cells by CQ and HCQ with some higher relevancy for CQ.

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BIOTRANSFORMATION OF IVERMECTINE IN SOYA *(GLYCINE MAX)*

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Veterinary pharmaceuticals are used in large amounts in modern husbandry for treatment and prevention of diseases in animals. These drugs represent important source of environmental pollution as they can reach environment through the treatment processes, inappropriate disposal of used containers, unused medicine or livestock feed, and manufacturing processes. Pharmaceutical as well as other xenobiotics enter plant body and can induce stress and consequent response. The production of reactive oxygen species (ROS), which are associated with the direct damage of various biomolecules, and subsequent alterations in plant cell redox state and antioxidant mechanisms are usually the first responses to environmental. The ivermectine was chosen according to their importance in anthelmintic therapy, occurrence in waste water and documented negative effects on non-target organisms.

Soybean (Glycine) intake has received interest due to its health benefits, such as lowering the risk of chronic diseases – heart diseases and cancers, especially breast and prostate cancers, osteoporosis, and diabetes. Soy antioxidant activities and the role of soya antioxidants such as isoflavones have received increasing interest since it has been recognized that soybeans may have therapeutic activities in addition to health promotion.

The objectives of this study were to identify the IVM metabolites and IVM biotransformation pathways in soya (*Glycine max*). Plants were incubated with IVM (10 μM) for 2 and 4 weeks. Before analysis, homogenized samples were subjected to liquid–liquid extraction. The samples were analysed using UHPLC/MS (QqQ) in positive-ion mode. The results showed that IVM entered plant and enzymatic systems of plant were able to transform IVM via several reactions.

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ESTROGEN AND ANDROGEN RECEPTOR BINDING AFFINITY OF BISPHENOLS ESTIMATED BY QSAR

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Chemicals might interact with proteins such as the estrogen receptor (ER) or androgen receptor (AR) resulting in initiation of a cascade of biological effects and perturbation of the endogenous hormone system. Despite the complexity of the endpoints for reproductive impairment, it has been long appreciated that chemical binding to the ER or AR is one of the important mechanisms of interference with the reproductive process. Since reprotoxicity test methods are very expensive and time-consuming, alternative procedures are being developed.

It is also known that the ER or AR are much less of a lock-and-key interaction than highly specific receptors. The ER and AR are 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, there are also chemicals with one hydrogen-bonding group that bind ER and cause subsequent gene activation. For AR the most important parameter seems to be distance between nucleophilic sites in the molecules, the oxygen atom connected to a cyclic carbon atom (associated with the 3-position in the steroidal skeleton) and the oxygen atom in the hydroxyl group (associated with the 17-position in the steroidal skeleton). Also partition coefficient log Kow (n-octanol-water) of binding chemical plays significant role.

Defining the boundaries of these chemicals is the challenge for (Q)SAR and computational chemistry. The aim of the presented work was to characterize the groups of bisphenols with potential to bind to ER or AR and cause adverse effects in the human organism. All the evaluated bisphenols showed high and even very high affinity to the ER binding subpockets.

These compounds may act as a gene activators and cause adverse effect in the human, especially during the pregnancy, breast-feeding and developing.

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NEW (PYRIDINE-2-SULFONATE) SILVER COMPLEX: DESIGN, DNA BINDING AND CYTOTOXIC ACTIVITY

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In recent years, more attentions focused on the coordination compounds as metallo-pharmaceuticals with antitumor activity. It culminated with the discovering of cisplatin and its derivatives as the antitumor drug and carried on with the finding of novel other non-platinum based chemotherapeutic agents. These pharmaceuticals emerging from the interface of pharmacology, toxicology, biochemistry and inorganic chemistry, have achieved access over traditional organic drugs.

Toward this goal, we have prepared new silver pyridine-2-sulfonate complex, which was characterized by X-ray and variety of analytical techniques. We have investigated the antiproliferative effect of new complex, free ligand and silver sulfadiazine on mouse leukaemia L1210 by MTT assay. The highest cytotoxic effect of Ag complex was observed after 48 h cultivation against S cells (IC_{50} = 1.4 μM) and R cells (IC_{50} = 1.2 μM). On the other side, no cytotoxic effect of free ligand was observed against all tested cells (IC_{50} >5 μM after 24, 48 and 72 h cultivation). Moreover, the DNA-binding properties of this new metal complex were investigated by electronic absorption, fluorescence, and CD spectra. To establish the mechanism of anticancer action of complex, also topoisomerase (Topo I) inhibition assay were conducted. Topo I inhibition study have shown that Ag complex inhibits its activity at concentration of 30 μM. Our results provide useful information about complex-DNA interactions, which are valuable for the rational drug designing having enhanced activity and greater clinical efficacy.

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DOSE-RESPONSE RELATIONSHIP OF SELECTED ANTIDEPRESSANTS ON PARAMETERS OF ISOLATED PERFUSED RAT HEARTS

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Depression is a very common and disabling disease with important social and economic implications affecting 16–17% of population – prognosis for 2020 is 40% (1). From economical point of view depression belongs among five most expensive diseases over the world (2). Mortality of mentally diseased people is higher in comparison to corresponding aged-matched individuals (3) and is probably related to high incidence of cardiovascular disorders (4). Antidepressants (AD) are drugs used for the treatment of major depressive disorder. The earliest and probably most widely accepted scientific theory of antidepressant action is the monoamine hypothesis which states that depression is due to an imbalance (most often a deficiency) of the monoamine neurotransmitters (namely serotonin, norepinephrine and dopamine). In our study we focused on selected AD citalopram (serotonin reuptake inhibitor), venlafaxine (serotonin–norepinephrine reuptake inhibitor), melatonin (affecting dual melatonergic-serotonergic pathway) and amitriptyline (tricyclic antidepressant) used as a standard and their dose-response relationship on functional parameters of isolated spontaneously beating perfused rat heart. Doses of applied substances were in concentration range between 10^{-9} – 10^{-4} mol.l^{-1} and each concentration was applied for 10 minutes. Increasing concentration of tested AD slowed heart rate, decreased the incidence of all forms of ventricular premature beats, except citalopram, in which opposite slope of dependence and even episodes of tachycardia were observed. There was a tendency to decrease the left ventricular developed pressure and contractility index, as well as heart product, with increasing concentration of AD. The coronary flow was mildly suppressed by tested drugs. According to the obtained data we determined 10^{-7} mol.l^{-1} as the effective concentration (EC50). The character of changes in selected parameters depended on the type of representative AD.

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EFFECTS OF SELECTED METAL NANOPARTICLES ON MOUSE TESTIS LEYDIG CELLS *IN VITRO*

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Despite a great potential benefit of silver (Ag) and gold (Au) nanoparticles (NPs) in the areas of biomedical, pharmaceutical and industrial applications, there has been an increased interest in studying their possible deleterious effects in biological systems. Current data have suggested that NPs may pose adverse effects on male reproductive functions, mainly due to modification of the testicular structure, impairment of spermatogenesis and alteration in the biosynthetic and catabolic pathways of testosterone.

The present study aimed to investigate a potential toxicity of two different sizes (20 nm and 100 nm) of Ag and Au NPs on mouse somatic Leydig TM3 cells, the testosterone-producing cells of the testis. TM3 cells were cultured with the different concentrations of Ag NPs (0.1–10 μg/ml) or Au NPs (0.16–16x10^{10} or 0.19–19x10^{8} particles/ml) in the absence or presence of luteinizing hormone (LH, 100 ng/ml) for 24, 48, and 72 h. Cell viability was assessed by MTT and CytoTox-ONE Homogenous Membrane Integrity (LDH) assays. The Apo-ONE Homogeneous Caspase-3/7 Assay was used to measure the activities of caspase-3 and -7 for assessment of cell apoptosis. Testosterone levels in culture media were measured by radioimmunoassay commercial kits. Treatment of TM3 cells with Ag and Au NPs induced a significant concentration- and time-dependent inhibition of cell viability and increase in cell apoptosis. The smaller NPs showed more deleterious effects. Alterations in testosterone secretion by TM3 cells were observed by the action of Ag and Au NPs. The mechanisms involved in Ag and Au NPs-induced toxicity should be further investigated.

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MICRORNAS IN DIAGNOSIS AND THE PREVENTION OF DRUG-INDUCED CARDIOTOXICITY

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Drug-induced cardiotoxicity is a serious problem associated with the administration of many drugs. Prediction of the onset of heart damage is still mainly based on the detection of a circulating cardiac troponin. MicroRNAs (miRNAs) have been reported to be affected by drugs and other xenobiotics, and the potential of miRNAs as biomarkers and diagnostic tools has been considered. In recent years, an association of certain miRNAs with the cardiotoxicity of some drugs, namely anthracyclines, bevacizumab, cyclosporine A and isoprenaline, has already been found.

We have reviewed available information about the changes in miRNAs levels induced by cardiotoxic drugs. We have focused on three aspects: the altered expression of miRNAs in the heart upon treatment with cardiotoxic drugs, circulating miRNAs as promising early biomarkers of cardiotoxicity, and the potential of miRNAs in the prevention and/or attenuation of drug-induced cardiotoxicity. The targeted changes in the level of certain miRNAs by antagomiRs and miRNA-mimics are also described and evaluated. In addition, the cardioprotective mechanism of various natural compounds via their effect on miRNA levels are examined.

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OXIDATION OF AN ANTICANCER DRUG ELLIPTICINE BY HUMAN AND RAT CYTOCHROMES P450 LEADING TO ITS DETOXIFICATION AND ACTIVATION: A COMPARATIVE STUDY

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Ellipticine is a drug exhibiting significant antitumor and anti-HIV activities. The prevalent mechanisms of antitumor, mutagenic and cytotoxic activities of ellipticine were suggested to be intercalation into DNA, inhibition of DNA topoisomerase II activity and formation of covalent adducts with DNA after being activated with CYPs. Here, we compare the efficiency of human and rat recombinant CYP enzymes expressed in Supersomes^{TM} (microsomes from Baculovirus transfected insect cells containing recombinantly expressed human CYPs and NADPH:CYP reductase with or without cytochrome b_5) to oxidize ellipticine to individual metabolites and to form DNA adducts. Moreover, we evaluated whether oxidation of ellipticine to metabolites generating DNA adducts (13-hydroxy-, 12-hydroxyellipticine and ellipticine N^2-oxide) correlated with formation of these adducts. All tested recombinant CYPs oxidized ellipticine to up to five metabolites: 9-hydroxy-, 12-hydroxy-, 13-hydroxy-, 7-hydroxyellipticine and ellipticine N^2-oxide. 13-Hydroxyellipticine, the metabolite forming the major ellipticine-deoxyguanosine adduct in DNA, was generated predominantly by a CYP3A subfamily of both humans and rats, followed by CYP1A1, 1A2, rat 2D1 and human CYP2D6*1. Differences were found in efficiencies of CYPs of a 2A subfamily in both species. While rat CYP2A1 and 2A2 oxidize ellipticine to 13-hydroxyellipticine with efficiency similar to CYP2D1, human CYP2A6 was much less active. 12-Hydroxyellipticine was produced by rat CYP2A1 with the highest efficiency, followed by human CYP2C19 and rat CYP1A1. Among the CYP enzymes tested, human recombinant CYP2D6*1 was the most efficient enzyme generating ellipticine N^2-oxide followed by human CYP3A4 forming about four times lower amount of this ellipticine metabolite. CYP3A2 generated the highest amounts of this metabolite among the rat CYP forms. Here, we also showed that all CYPs activate ellipticine to form DNA adducts. Two major DNA adducts found to be formed on deoxyguanosine from 13-hydroxy- and 12-hydroxyellipticine, were generated. Formation of 13-hydroxyellipticine correlated with generation of the DNA adduct 1. In the case of adduct 2, situation is more complicated as there are two ellipticine metabolites responsible for its production. Therefore, the correlation coefficients for the formation of adduct 2 and 12-hydroellipticine (or ellipticine N^2-oxide), were less significant.

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ANTHELMINTICS IN PLANTS – THE EFFECT ON TRANSCRIPTOME

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Anthelmintics, the drugs against parasitic worms, are widely used in human and veterinary medicine, nowadays. The usefulness of anthelmintic drugs is indisputable, but at the same time they pose a risk to ecosystems. With excrements of treated animals, anthelmintics can get into the environment and there affect non-target organisms – free-living invertebrates and wild plants. In our project, the most frequently used anthelmintics (albendazole, fenbendazole, flubendazole, ivermectin, monepantel) were used, and different plant species were tested, also the model plant *Arabidopsis thaliana (*wild type, *Brassicaceae).*

The presented work is the part of this project. The aim of the study is to get information about the effects of anthelmintics on hydroponics cultures of *Arabidopsis thaliana* and changes in plant transcriptome. The broad-spectrum benzimidazole anthelmintic fenbendazole was first used. Hydroponics cultures was stressed by 5 μM fenbendazole. The effect was studied after 24 and 72 hours of stress. The microarray analysis was performed. For general expression at the transcription level were used Agilent-based microarrays.

Exposure to fenbendazole in 5 μM concentration resulted in up-regulation of 104 and down-regulation of 64 transcripts in roots after 24 hours, up-regulation of 10 and down-regulation of 20 transcripts in roots after 72 hours. Significantly stronger response was recorded n rosettes where transcription of 193 genes was increased and 272 genes was decreased after 24 hours, 393 genes was increased and 403 genes was decreased after 72 hours.

Now we are working on the same experiment with the anthelmintic drug ivermectin. It is a macrocyclic lactone. It is used in human and veterinary medicine against parasitic nematodes and some ectoparasites. The results will be presented at the poster section. It is almost the first study of the effect of ivermectin on plants.

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PHARMACEUTICALS AND PLANTS: TOXIC EFFECT OF IVERMECTIN ON PLANT PROTEOME

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In environment, ivermectin (IVM), a broad spectrum anthelmintic used especially in veterinary medicine, may impact non-target organisms. The plants uptake, metabolise and accumulate IVM as another organic xenobiotics. Nevertheless, the informations about IVM effects in plants are limited. Therefore, we investigated the effect of IVM and its metabolites on the protein expression in model plant *Arabidopsis thaliana.*

Hydroponics cultures were stressed by 5 μM fenbendazole. The effect was studied after 24 and 72 hours of stress. Comparative proteomic analysis (nano LC-MS) was provided to identify the proteins expressed under this stress. More than 5600 proteins were identified there. The proteins with more than 2-fold change in expression in comparison to control, and proteins, which were expressed only in treated samples (at least 3 from 4) or only in control were selected. Exposure to IVM in 5 μM concentration resulted in up-regulation of 51 and 100 and down-regulation of 42 and 51 proteins in roots after 24 and 72 hours, respectively. In rosettes expression of 42 and 34 proteins was increased and expression of 79 and 80 proteins was decreased after 24 and 72 hours, respectively. In the roots were predominantly up-regulated proteins from plastids (loucoplats) involved in various biological processes as electron transport and energy generating pathways. On the other hand down-regulated were the proteins with structural molecule activity. In the leaves, enzymes localized in the cell wall were up-regulated, especially after 24 hours and down-regulated the proteins of endoplasmatic reticulum. Increased expression was found in only few biotransformation enzymes (e.g. superoxide dismutase). There was little difference between 24 and 72 hours.

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EFFECT OF *CIS*-NEROLIDOL, *TRANS*-NEROLIDOL AND FARNESOL ON THE MRNA AND PROTEIN EXPRESSION OF PHASE I XENOBIOTIC-METABOLIZING ENZYMES IN PRECISION-CUT HUMAN LIVER SLICES

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Sesquiterpenes *cis*-nerolidol (cNER), *trans*-nerolidol (tNER) and farnesol (FAR) comprise a group of plant secondary metabolites with numerous biological and pharmacological activities, among which an inhibitory effect on several phase I drug-metabolizing enzymes in humans have been observed. Being a part of plant essential oils, they are regularly consumed in food or dietary supplements. Moreover, they are officially approved food flavors and are widely used in cosmetic industry. They may, therefore, enter the human body in concentrations that may eventually lead to serious

drug interactions in treated patients. Accordingly, we investigated the effect of cNER, tNER and FAR on mRNA and protein expression of several important phase I drug-metabolizing enzymes in human liver. For the investigation, precision-cut human liver slices were cultivated in a medium supplemented by cNER, tNER or FAR in 10 μM concentration for 24 hours. Known inducers of cytochrome P450 enzymes (CYP) rifampicine and β-naphthoflavone were used as positive controls. The mRNA expression of three CYP isoforms, namely CYP 3A4, CYP 2B6 and CYP 2C, as well as the expression of carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C (AKR1C) was detected using real-time quantitative PCR. Protein levels of these enzymes were detected using western blot technique. A statistically significant inhibitory effect of tNER and FAR on the mRNA expression in one human liver sample was observed. In both cases, the studied compounds inhibited the mRNA expression of CYP 3A4, CYP 2C, CBR1 and AKR1C. However, their effect on CYP 2B6 was rather contradictory. While tNER acted as an inhibitor and caused a significant decrease in the CYP 2B6 mRNA expression, FAR, on the other hand, caused a considerable increase in the mRNA expression. Moreover, the mRNA expression inhibition of CYP 2C and AKR1C by FAR in two different human liver samples was detected. The results, however, differ among individual human liver samples presumably due to possible inter-individual variability.

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INTERACTION OF NEW POTENTIAL ANTICANCER DRUGS WITH HUMAN LIVER MICROSOMAL CYTOCHROMES P450

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Cytokinins are group of phytohormones that are involved in many processes in plants. These processes including e.g. growthing, differentiation and leaf senescence. However, they also have various activities in animals and humans. Three cytokinin derivates (BPA-302, BP-21 and BP-117) were tested for their potential to inhibit activities of human liver microsomal cytochromes P450 (CYP) *in vitro*. All activities (CYP1A2, CYP2A6 and CYP2C9) were determined according to established protocols. The results have shown no prominent inhibition of individual CYP activities with either compounds except in the case of CYP2C9 and BP-117. CYP2C9 plays a large part in drug biotranformation and its inhibition by another drug could lead to drug-drug interactions. However, this should be verified by further experiments and *in vivo*.

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WATER FLOWERS EXTRACTS OF *CORNUS MAS* AND *CORNUS KOUSA* INHIBIT ALDOSE REDUCTASE, WITHOUT ANY EFFECTS ON LIPOTOXICITY

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The effective inhibition of aldose reductase of leaves extracts from *Cornus mas* and *Cornus kousa* as well as some biological activities including antidiabetic activity of fruits from *Cornus* species were evaluated in the recent studies. In contrast, the biological effects of flowers from *Cornus* sp. have not been studied yet. The inhibitors of aldose reductase, the first enzyme in polyol pathway, are considered to be potential therapeutical agents in the development of chronic diabetic complications. Diabetes mellitus could be accompanied by elevated blood level of free fatty acids, which could cause lipotoxicity. Our study is focused on evaluation of potential inhibitory efficacy of water flower extracts from *Cornus mas* and *Cornus kousa* on isolated rat aldose reductase *in vitro*. The extracts were studied in the cell model of lipotoxicity as well, which presents a risk during diabetes. The cytotoxicity of the extracts on mouse fibroblasts cell line was evaluated. Both extracts showed effective inhibition of rat lens aldose reductase in non-toxic low concentrations. In contrary, the non-toxic concentrations of both extracts caused almost no effects in the lipotoxicity cell model induced by palmitic acid.

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COPPER-CHELATORS ARE ALSO RELATIVELY POTENT ZINC CHELATORS

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Zinc (Zn) is an essential metal that is involved in numerous physiological processes. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in protein and DNA synthesis, cell division, wound healing and immunity. Zinc deficiency is characterized by growth retardation, loss of appetite, suppressed immune function, diabetes, etc. One of the causes of zinc deficiency can be the long-life use of metal chelators which are mostly non-selective. Such example are copper-chelators used in rare inherited disorder resulting in body copper excess – Wilson's disease.

The aim of this work was to study possible ability of D-penicillamine, trientine and ammonium tetrathiomolybdate (ATM) to bind zinc by a spectrophotometric method based on the competition between the tested compound and dithizone as an indicator. Various physiologically relevant pH levels ranging from 4.5 to 7.5 were tested. compound and dithizone as an indicator. Various

physiologically relevant pH levels ranging from 4.5 to 7.5 were tested.

All the compounds shown the non-selectivity for copper and the capacity to bind zinc. Experiments showed that the most potent Zn chelator was trientine. It can bind approximately 65% of Zn when is mixed with zinc ions in the molar ratio of 1:1 at pH 7.5. D-penicillamine and ATM showed lower chelating capacity. Surprisingly all of the tested compound showed higher capacity to form a complex with Zn ion in comparison with ability of D-penicillamine to chelate Cu ions.

Clinically used copper-chelators as well ATM are also relatively potent zinc chelators and hence they long term use can possibly result in toxicity associated with zinc deficiency.

INTERMITENT HYPOXIA *IN UTERO* AND FREQUENCY OF SKELETAL AND VISCERAL ANOMALIES IN THE RAT FOETUSES

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Chronic or intermitent hypoxia is a common pregnancy complication associated with intrauterine foetal growth restriction that may influence respiratory outcome at birth. An experimental model of intermitent intrauterine hypoxia is proposed. The objective of this study was to determine the effect of early (eGIH) and late gestational intermitent hypoxia (lGIH) on foetal growth, and incidence of skeletal and visceral anomalies. Pregnant Wistar/DV rats were exposed to the lower oxygen containing (10%O_2 for 8 hr/day) in a special hypoxic chamber during late gestational period (days 15–16 or 19–20 of gestation) or to the lowered oxygen containing environment for 12 hr on day 16 or 20 of gestation. On day 21 of gestation were assessed : the weight of live foetuses and frequency of skeletal and visceral anomalies. No gross malformations observed after repeated 8 –hour GIH or single 12- hour GIH. Pups exposed to intermitent hypoxia *in utero* weiged significantly less than the control pups only in the late GIH. Skeletal anomalies consists of anomalies of sternebrae (split, unossified, aditional, reduced ossification), phalanges (reduce ossification of metacarpals and metatarsals) and ribs (wavy, 13th or 14th pair of accessory thoracolumbar rudimentary ribs). Inspection of visceral anomalies revealed nasal anomalies (unseparate nasal conchae) brain and skull anomalies (internal hydrocephalus of moderate degree and not complet dilatation or undilatation of lateral brain ventricles). The frequency of skeletal and visceral anomalies is similar to the control group in early and late intermitent hypoxia without statistical significance. These data demonstrate that brief, intermitent periods of intrauterine hypoxia have no significant effect on incidence of skeletal and visceral anomalies in rat fetus.

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ANTIDEPRESSANT SERTRALINE AS A POTENTIAL POLLUTANT IN WATER ENVIRONMENT

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The aim of this article is to describe potential risk of sertraline as a pollutant in water environment. Sertraline is type of antidepressant from a group of selective serotonin reuptake inhibitors, which primarily affect the nervous system, mainly brain. Its usually taken by people as pharmaceuticum for the treatment of depression, panic disorders, anxiety disorders, anorexia, social phobia and panic stress. In recent years, due to to people‘s hurried lifestyle the rate of use sertraline and other antidepressants in the human population has been steadily increasing. Sertraline presents a certain ecology danger for aquatic environment, where it can continually get throw wastewater treatment plants and persists in range between ng/l to μg/l. So far many side effects of sertraline to non-target water organisms were described. Studies have shown that sertraline causes changes in swimming activity, behavioral modification, enzyme activity (SOD, CAT, GST, AchE), feeding rate and food consumption in fish. The main affected organs of fish were brain, kidneys and liver. For this reason it is very important to constantly monitor the environmental concentrations of sertraline as well as to study its other negative risks.

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STUDY OF PROTECTIVE EFFECTS OF ANTIOXIDANTS TARGETING AND NOT TARGETING MITOCHONDRIA

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3T3 cells and tumor cells MCF-7 were exposed to the action of various concentrations of ascorbic acid (0–6 mM) and cupric ions (0–6 μM) individually to show their effect. Viabilities of the cells were determined by using the MTT test. The results showed dose-dependent decrease in cell viability when examining ascorbic acid. Both cell strains were almost equally sensible to ascorbic acid. Similar results were observed when examining cupric ions alone. The percentages of the viability of 3T3 and MCF-7 cells damaged by ascorbic acid in the presence of cupric ions were ca. 50% and 30%, respectively.

The addition of *N*-acetylcysteine did not result either in higher viability of 3T3 cells or in higher damage of MCF-7 cells.

Further, 3T3 and VH10 cell lines were subjected to the oxidative damage by Cu(II) ions and ascorbate, which form hydroxyl radicals. MitoQ as a mitochondrially targeted antioxidant was examined to prevent cell death and was compared with trolox as a reference standard serving as an antioxidant. MitoQ at lower concentrations (up to 500 nM) increased viability of cells. In contrast, MitoQ at higher concentrations (over 500 nM) decreased the viability of both cell strains. The former observation indicates that the application of MitoQ should be evaluated also from the point of its cell-destructive potential.

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COMPARISON OF INHIBITORY EFFECT OF RESVERATROL AND ITS DERIVATIVES ON ENZYME ACTIVITIES IN HUMAN LIVER MICROSOMAL CYTOCHROME P450 3A4 USING TWO INDEPENDENT SUBSTRATES

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Resveratrol and its derivatives belong to stilbenoids which are polyphenols naturally occurring in grapes, wine, berries or peanuts. Stilbenoids are common parts of human diet and are known to be health benefit agents for human. Due to their anti-inflammatory, anti-asthmatic, anti-diabetic, hypolipidemic and antioxidant properties are these polyphenols in forefront of interest.

We investigated the comparison of mechanism of inhibitory effect of eight stilbenoids (dihydroresveratrol, oxyresveratrol, cis-resveratrol, trans-resveratrol, pinostilbene, pterostilbene, cis-piceatannol and trans-piceatannol) on human hepatic cytochrome P450 CYP3A4/5 present in human microsomes using two independent substrates (midazolam and testosterone). Final concentration of compounds examined was chosen in the range 0 – 100 micromolar in the reaction mixture.

All compounds studied influenced CYP3A4/5 activity with substrate testosterone. Oxyresveratrol has been shown to be a mixed inhibitor and pterostilben a competitive inhibitor of CYP3A4/5, other compounds tested have been found to be noncompetitive inhibitors of this activity of CYP3A4/5 with substrate testosterone. With substrates midazolam, only oxyresveratrol, trans-resveratrol and pinostilben influenced activity of CYP3A4/5 and these compounds have been shown to be the noncompetitive inhibitors of this activity of CYP3A4/5.

The differences in mechanism of inhibitory effect are related to the promiscuity of CYP3A4/5 active site. CYP3A4/5 has a relatively large and flexible active site cavity that can accommodate multiple substrate molecules to achieve optimal activity. Hydroxylation site of testosterone molecule is oriented differently to this site in midazolam molecule. This could be the reason why the mechanisms of inhibitory effects with substrates testosterone and midazolam are not the same.

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THE EFFECTS OF NATURAL PSEUROTINS ON SELECTED IMMUNE CELL FUNCTIONS

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Pseurotin A is a secondary metabolite produced by many species of fungi, mainly by Aspergillus sp. and Penicillium sp. During the pseurotin A biosynthesis, a large number of closely related bioactive compounds, such as pseurotin D or synerazol is also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly, a few studies suggested effects of pseurotins in eukaryotes, such as antiangiogenic activity. In this study, we focused on effects of natural pseurotins on physiological functions of immune cells. Our results employing endotoxin-activated myeloid RAW264.7 cells (murine peritoneal macrophages) show that pseurotins (Pseurotin A, Pseurotin D and some structure analogs) were able to significantly reduce NO production in a concentration-dependent manner, both at the level of nitric oxide (NO) production and at the level of inducible NO synthase expression. These pseurotins also inhibited expression of early response cytokine interleukin (IL)-6 but not tumor necrosis factor α. Moreover, pseurotins were able to inhibit proliferation of RAW264.7. Other tested immune cells were mouse B-lymphocytes. They were isolated by sorter Aria II based on CD19 positivity. Interestingly, we show that pseurotins inhibited immunoglobulin E production of B-lymphocytes activated by a combination of E. coli endotoxin and IL-4. These effects were also related to changes in proliferation of B-lymphocytes via inhibition of JAK/STAT signaling pathway. We did not see any cytotoxic effects of pseurotins on these cells. It can be concluded that natural pseurotins are able to reduce oxidative stress, inhibit production of cytokines, NO and are able to modulate B-lymphocyte immune response.

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METABOLISM OF A TYROSINE KINASE INHIBITOR LENVATINIB BY HUMAN CYTOCHROMES P450 *IN VITRO*

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Lenvatinib is an oral, multitargeted tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptors (VEGFR1-VEGFR3), fibroblast growth factor receptors (FGFR1-FGFR4), platelet-derived growth factor receptor (PDGFR)α, rearranged during transfection (RET), and v-kit (KIT) signaling networks implicated in tumor angiogenesis. It is used for treatment of certain types of tumors of the thyroid gland and metastatic renal cell carcinoma. Based on preliminary studies using human hepatic microsomes, lenvatinib was suggested to be oxidized by cytochromes P450 (CYPs), mainly by CYP3A4, to its *O*-demethylated metabolite, a desmethylated form of lenvatinib. However, no direct prove of this suggestion was demonstrated. Therefore, the aim of this study was to investigate the metabolism of lenvatinib by human microsomal enzymes *in vitro* in detail. The metabolism of lenvatinib by human hepatic microsomes and recombinant CYPs expressed in Supersomes^{TM} was investigated. The lenvatinib metabolites were separated by HPLC and identified by mass spectroscopy. Utilizing human hepatic microsomes *O*-desmethyllenvatinib, *N*-depropylated lenvatinib and one additional metabolite were produced. Of all tested human CYP enzymes, the CYP1A1, 1A2, 2C19 and 3A4 enzymes oxidize lenvatinib to its metabolites. *O*-desmethylated lenvatinib was generated by CYP1A1, 1A2 and 3A4, while CYP2C19 forms another metabolite; its structure has not yet been identified. CYP1A1 and 3A4 are also responsible for oxidation of lenvatinib to *N*-depropylated metabolite. Cytochrome b_5 plays an essential role in the CYP2C19 and 3A4 activities to oxidize lenvatinib. Besides CYPs, aldehyde oxidase (AO) oxidizes lenvatinib forming one metabolite; its structure has not yet been identified. Further characterization of structures of all lenvatinib metabolites formed by the tested enzymatic systems is under way in our laboratory.

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EFFECT OF HEXAHELICENE ON THE EXPRESSION OF CYP1A1

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The helicenes are polycyclic aromatic hydrocarbons (PAHs) with non-planar screw-shaped structures composed of ortho-fused benzene rings. Since many planar PAHs activate the aryl hydrocarbon receptor, this study examined whether [6]helicene and its derivative, 1-butyl-3-(2-methyl[6]helicenyl)-imidazolium bromide (compound **1**), affect the expression of cytochrome P450 1A1 (CYP1A1) in human hepatoma HepG2 cells. The MTT reduction assay showed that both [6]helicene and compound **1** significantly decreased the viability of HepG2 cells after 24 h of exposure. Compound **1** was less cytotoxic than [6]helicene with the IC50 values reaching 8.4 and 0.9 μM, respectively. After 24 h of HepG2 cell treatment with 5 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a prototypical CYP1A1 inducer, we found a 386-fold increase in the level of CYP1A1 mRNA and a 40-fold increase in CYP1A1 activity. In contrast, 24 h of exposure to [6]helicene (0.001–1 μM) and its derivative **1** (1–5 μM) resulted in mild or negligible changes in the mRNA levels and activity of CYP1A1. At the highest concentrations tested, CYP1A1 mRNA levels induced by [6]helicene and compound **1** increased 2.9-fold and 2.7-fold, respectively, and the activity of CYP1A1 reached 1.0-fold and 1.4-fold of the control values. We conclude that [6]helicene and its derivative **1** have a weak effect on the CYP1A1 pathway in HepG2 cells.

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CLIMATE CHANGE IMPACT ON THE TOXICITY OF PHENOXY HERBICIDES

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Climate change is a major concern for sustainable agriculture and food security. Crop productivity strongly depends on crop protection measures such as use of herbicides. Climate change could influence the fate and ecotoxicity of herbicides by altering their environmental partitioning and degradation, distribution and abundance of weeds and growth and development of weeds and crops. Differential responses of crops and weeds to elevated temperature and CO_2 may also cause shifts in their response to herbicidal application and their competitive interactions. The aim of the study was to examine the influence of elevated temperature and CO_2 on the effects of phenoxy herbicide (Chloro-2-methylphenoxyacetic acid (MCPA)) to spring barley (*Hordeum vulgare* L.) and common lambsquarter (*Chenopodium album* L.). Two climate scenarios were investigated: current climate (21 °C, 400 ppm CO_2) and future climate (25 °C, 800 ppm CO_2). *Ch. album* and *H. vulgare*, growing together in the microcosms, were sprayed with herbicide sprays solutions equivalent to 0.5–2 of field application rate. The growth and response of antioxidative defence system were evaluated after 2 weeks. Antioxidant enzymes superoxide dismutase

(SOD), catalase (CAT) and glutathione reductase (GR) were measured. Oxidative stress parameters, such as the concentrations of malondialdehyde were determined. Phenoxy herbicide severely inhibited the growth of *Ch. album*, altered activity of antioxidative enzymes and induced oxidative stress. Less pronounced effects of herbicides were also found in non-target *H. vulgare*, though the majority of effects were statistically insignificant. Moreover, reduced interspecific competition due to dramatic decrease in *Ch. album* growth at high herbicide dose led to an increase in *H. vulgare* biomass of roots and shoots. The results of our study show that the ongoing increases in temperature and atmospheric CO_2 concentration may have important consequences for crop-weed competition.

SELECTION OF SUITABLE REFERENCE GENES FOR GENE EXPRESSION STUDIES IN HUMAN LIVER SLICES

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Precision-cut liver slices are an interesting model due to its multicellular composition, preserved tissue architecture and intercellular communication. Its applicability to human tissues allows us to avoid interspecies differences and directly apply human tissues into multiple experimental designs. Despite a short time when liver slices are able to keep its viability, functionality and physiological processes (up to 24 hours), it is still a very good model for gene expression studies. However, there exists no study validating this model for selection of a suitable reference gene (or their combination). Therefore, we decided to perform a validation study, since selection of inappropriate reference gene can influence the trend and deviation of results. In our experiments, three human liver samples received from surgery were used to obtain precision-cut liver slices (8 mm diameter, 150 μm thickness), which were cultivated for 24 hours in 85% O_2/ 5% CO_2 atmosphere and samples were collected after 0, 4, 8, 12, 18 and 24 hours. Known cytochrome P450 (CYP) inducers β-naphthoflavone and rifampicine were used as positive controls at all time points. To verify the viability of liver slices, ATP content and lactate dehydrogenase leakage were measured. For reference gene validation, GAPDH, SDHA, ACTB, B2N, HPRT and YHWAZ were chosen. These genes were compared using RefFinder, a free web tool that uses several other softwares, such as geNorm, Normfinder, BestKeeper and the comparative Ct method, and gives those genes a comprehensive gene ranking. The gene stability was compared for the whole 24-hour interval and also for each time point separately. The gene expression of CYP3A4, 2B6, 1A2 and 2C was also determined.

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