## DETERMINATION OF PERFLUOROOCTANE SULPHONATE AND PERFLUOROOCTANOATE IN BLOOD BY GAS CHROMATOGRAPHY WITH ION TRAP MASS SPECTROMETRY

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Perfluorooctane sulphonate (PFOS) and perfluorooctanoate (PFOA) belong to the group of perfluoroalkyl substances (PFAS) which represent a group of man-made chemicals constituting persistent and accumulative compounds of considerable negative effects to living organisms and human health. The aim of this work was to develop a method for determination of PFOS and PFOA in blood using gas chromatography with ion trap mass spectrometry.

Sample preparation was based on solid phase extraction (SPE) with mixed-mode weak cation-exchange SPE cartridges. Due to the high polarity and low volatility of perfluoroalkyl substances, perfluorooctane sulphonate and perfluorooctanoate were converted into more volatile and less polar derivatives using method of silylation. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used as a derivatization reagent. Separation, identification and quantification of PFOS and PFOA were carried out by means of gas chromatography with ion trap mass spectrometry. VF-5ms (30 m × 0.25 mm) column was used for separation. The following parameters of the method were established: detection limits for PFOA and PFOS are 4.1 ng/ml and 11.4 ng/ml, respectively; l*inearity for PFOA and PFOS* is 13.7–1×10$^3$ ng/ml and 38.0–1×10$^3$ ng/ml, respectively; recovery *for PFOA and PFOS is 89% and 87%, respectively.*

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## EFFECTS OF PROBIOTIC *ESCHERICHIA COLI* NISSLE 1917 ON EXPRESSION OF CYTOCHROMES P450 IN THE GASTROINTESTINAL TRACT OF MALE RAT

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*Escherichia coli* strain Nissle 1917 (serotype O6:K5:H1) is a probiotic agent with beneficial effects on the gastrointestinal tract. Cytochromes P450 (CYP) are the most important enzymes of drug metabolism and are localized mainly in the liver, however also in the intestine. There is a possibility of interactions with concomitantly taken pharmacotherapeutic agents. This is why the aim of the study was to find whether the *E. coli* strain Nissle 1917 influences the expression of CYP in the rat intestine. Live bacterial suspension of *E. coli* strain Nissle 1917 was applied to healthy Wistar rats daily for 7 days. Other rats were stressed by oral application of the physiological solution daily for 7 days as well. Sections of the duodenum, jejunum, ileum, caecum and of the colon have been taken from each experimental animal. With all individual samples, microsomal fraction has been prepared and expression of selected CYP was followed by Western blotting. It was found that the expression of CYP enzymes change in a different way along the intestine. CYP1A1, 2B1/2 and 2E1 are present mainly in the duodenum and at the beginning of the jejunum; on the other hand, CYP2C6 is expressed mainly in the caecum and colon. CYP3A1 was found all over the intestine. The results show that there are no significant differences between control samples and samples from *E. coli* treated rats, only the expression CYP3A1 protein in the jejunum and colon appears to exhibit a small, but significant tendency to decrease. This conclusion is important for evaluation of possible changes in the gastrointestinal tract after intake of the probiotics in human food showing that there is most likely either little or no effects of probiotics to drug metabolism by CYP enzymes in the gastrointestinal tract.

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## POSSIBILITIES OF THE SEPARATION AND THE CAPTURE OF *AMANITA PHALLOIDES* TOXINS IN BIOLOGICAL MATERIALS BY LC-MS METHOD

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*Amanita phalloides* commonly known as the death cap, is one of the most poisonous of all known toadstools. It is widely distributed across Europe. These species is now known to contain two main groups of toxins, both multicyclic (ring-shaped) peptides, spread throughout the mushroom tissue: the amatoxins and the phallotoxins. Of the amatoxins, α-amanitin is the chief component and along with β-amanitin is likely responsible for the toxic effects. Their major toxic mechanism is the inhibition of RNA polymerase II, a vital enzyme in the synthesis of messenger RNA (mRNA) and small nuclear RNA. Without mRNA essential protein synthesis and hence cell metabolism grind to a halt and the cell dies. The liver and the kidneys are the principal organs affected. Of the phallotoxins, phalloidin is the most important compound. Phallotoxins are higly toxic to liver cells, they have since been found to have little input into the death cap´s toxicity as they are not absorbed through gut.

In clinical and forensic toxicology it is very important to quickly distinguish the intoxication caused by *Amanita phalloides* (which is lethal in majority cases of intoxication) from the one caused by *Amanita muscaria*

and *Amanita pantherina* (psychoactive fungi, the poisoning is rarely lethal). Therefore our study solves the problem of the isolation and the detection of α-amanitin, β-amanitin, phalloidin and phallacidin from biological materials (such as urine or blood) in one step with muscarine (*Amanita muscaria* and *pantherina* toxin). The experiments were performed using an LC-MS and an LC-MS-IT-TOF systems equipped with ESI in positive ion mode.

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## BIOLOGICAL ACTIVITY OF MAGNETITE NANOPARTICLES *IN VITRO*

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Magnetite nanoparticles (MNPs) have been widely used for various biological application. In nanomedicine, a great effort has been focused on development of magnetic resonance imaging contrast enhancement, hyperthermia cancer therapy, nanovectors for targeted drug delivery, increased cytostatic drug uptake and accumulation in malignant tissue. The surface of MNPs must be modified with various surfactants to prevent aggregation and make them more stable, biodegradable and non-toxic. Multiply functionalized MNPs, prepared by loading of specific ligands, antibodies, peptides and drugs may offer an exciting tool to make MNPs target-specific and increase their therapeutic benefit.

The objective of this study was to investigate the biological activity of nanospheric superparamagnetic magnetite particles ($Fe_3O_4$, 10 nm in diameter) in dependence on surface modifications in the human alveolar epithelial carcinoma cell line A549 and human embryonic lung fibroblasts HEL after short-term (4h) and long-term (24h) exposure. This study is focused on MNP cytotoxicity, genotoxicity, uptake and distribution in these two cell lines.

MNPs prepared by the coprecipitation of ferric and ferrous salts in an alkali aqueous medium were coated with different surfactans – sodium oleate (SO, $C_{17}H_{33}COONa$), polyethylene glycol (PEG, Mw=1000) and copolymer poly(lactic-co-glycolic acid) (PLGA). The uptake and distribution of SO-MNPs, SO-PEG-MNPs and SO-PEG-PLGA-MNPs have been investigated by transmission electron microscopy (TEM) and atomic absorption spectroscopy (AAS). Cytotoxicity of individual MNPs and surfactants was evaluated by MTT and LDH assays, genotoxicity by the comet assay and micronucleus test. Differences in the biological activity of MNPs were found in dependence on the surface modification and time of exposure.

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## COMPARATIVE STUDY OF TWO STILBENE DERIVATIVES IN THE EXPERIMENTAL RAT MODEL OF ARTHRITIS BASED ON EVALUATION OF CLINICAL PARAMETERS

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Stilbene derivates (1,2 diphenyl-etene), as resveratrol, are known for their anti-oxidative and anti-inflammatory properties. Two other stilbene derivates – pterostilbene (PTE) [3,5-dimethoxy-4′-hydroxystilbene] and pinosylvin (PIN) [3′, 5′-dihydroxystilbene], structural analogues of resveratrol, were compared as to their antirheumatic effect in this study.

Adjuvant arthritis (AA) – an experimental rat model of arthritis – was induced by single intradermal application of *Mycobacterium butyricum* in Lewis rats. Immediately after AA induction, pterostilbene (AA-PTE) and pinosylvin (AA-PIN) were administered daily in the oral dose of 30 mg/kg b.w. during 28 experimental days. The group of healthy rats (CO) was compared with untreated arthritic rats (AA) and AA was compared with treated groups.

During the experiment, clinical parameters were monitored– change of body weight (CBW), hind paw volume (HPV) and arthrogram (ART), along with the immunological marker MCP-1 level on day 14 in plasma. After completing the experiment on day 28, we measured activities of gamma-glutamyltransferase (GGT) in spleen and joint tissues as well as levels of MCP-1 and thiobarbituric acid reacting substances (TBARS) in plasma. CBW was not modified by PTE and by PIN only to a small extent. HPV was significantly decreased in AA-PIN *versus* AA on day 14 and also on day 28. This result corresponds with the decreasing activity of GGT in joint tissue on day 28. ART was modified by PIN administration on all days monitored (14, 21, 28), but PTE was without effect. Both compounds decreased MCP-1 plasmatic levels as found on day 14, yet the effect was significant only for PIN. The results of this comparative study showed that PIN in comparison to PTE was more effective in all parameters tested and should be further studied for its application in rheumatoid arthritis therapy.

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## FLOW-CYTOMETRIC ASSESSMENT OF HYDROGEN PEROXIDE TOXICITY IN CYANOBACTERIUM *MICROCYSTIS AERUGINOSA*

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Some of previously published papers suggested great algicidal potency of several photodynamic agents for the reduction of cyanobacterial water-blooms in the aquatic environment. Hydrogen peroxide which has been shown to be selectively toxic to cyanobacteria (approximately ten times higher toxicity to cyanobacteria than to green algae) represents probably the most prospective substance from this group of chemicals. It has been confirmed recently that the exposure of cyanobacteria to hydrogen peroxide inhibits cyanobacterial photosynthesis and that its toxicity is strongly dependent on the illumination regime used. Aim of our study was to evaluate hydrogen peroxide toxicity based on flow-cytometric investigation of cyanobacterial metabolic (esterase) activity and cell membrane integrity. For the measurement of the further parameter, fluorescein diacetate (FDA) was used as a substrate, the later parameter was measured using cell membrane impermeable fluorescent dye SYTOX Green (SYTOX). Our results suggested that the exposure of cyanobacteria to hydrogen peroxide can affect both investigated parameters. While the esterase activity decreased immediately from the beginning of the exposure, percentage of SYTOX positive cells (*i.e.* cells with damaged membrane) started to increase after cca five hours of the exposure. Our results confirmed the dependency of hydrogen peroxide toxicity on the rate of hydrogen peroxide decomposition kinetics in the culture medium. It was also detected that if cyanobacteria were exposed to low (sublethal) initial concentration of hydrogen peroxide, their esterase activity can recover after the period of the time which is necessary to decomposition of hydrogen peroxide in the medium.

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## XENOBIOTIC METABOLITE DETECTION USING HIGH RESOLUTION TANDEM MASS SPECTROMETRY

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Efficient metabolite identification requires combination modern mass spectrometric techniques and advanced software tools. A novel software approach for single run metabolite detection from high resolution liquid chromatography/mass spectrometry data will be presented.

In contrast to traditional post-acquisition processing techniques this approach neither requires prediction of biotransformation reactions from parent drug structure nor uses any molecular mass shifts resulting from oxidation, reduction, hydrolysis, glucoronization or other common metabolism reactions.

With accurate mass data, metabolite peaks can be selectively resolved and identified from background matrix ions by searching ions related with fragmentation products of parent structure. It has been shown [1] that parent drug and its metabolites exhibit number of identical fragments ions even if their precursor ions are different. In the first step fragment ions are automatically predicted from parent structure using specialized fragmentation tool, in our case Mass Frontier software. In the next step a table consisting of accurate m/z values of predicted fragments is generated and subsequently applied as an extraction filter to the data dependent high resolution/accuracy chromatographic run. This approach results in selective removal of vast majority of matrix-related background ions.

Even though parent drug and its metabolites share common fragment ions, depending of sample preparation and experimental parameters $MS^n$ spectra of parent drug may not provide sufficient number of indicative ions since they can be buried under the threshold level. To eliminate this effect we used not only ions extracted from parent drug spectra but also fragment ions automatically generated from parent structure. Predicted fragment ions dramatically enhanced the number of selective m/z values of potential metabolites and naturally significantly increased detection efficiency. Even if the chromatographic peaks in the experimental data are poorly resolved, FISh detects metabolites with a comparable reliability and success rate as the emerging industry standard MDF. Presented approach can be fully automated while the only input data are chromatographic run and parent drug structure.

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## CYTOCHROMES P450 1A1/2 AND NAD(P)H:QUINONE OXIDOREDUCTASE ARE INDUCED BY CARCINOGENIC AIR POLLUTANT 3-NITROBENZANTHRONE AFTER INTRATRACHEAL INSTILLATION IN RATS

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3-Nitrobenzanthrone (3-NBA) is a carcinogen occurring in diesel exhaust and air pollution. Using the $^{32}$P-postlabelling method, we found that 3-NBA and its human metabolite, 3-aminobenzanthrone (3-ABA), are activated to species forming DNA adducts by cytosols and/or microsomes isolated from rat lung, the target organ for 3-NBA carcinogenicity, and kidney and liver. Each compound generated identical five DNA adducts.

The importance of hepatic, pulmonary and renal NAD(P)H:quinone oxidoreductase (NQO1) to reduce 3-NBA to species that are further activated by *N*,*O*-acetyltransferases and sulfotransferases was demonstrated. Cytochromes P450 (CYP) 1A1 and 1A2 are the essential enzymes for oxidative activation of 3-ABA in microsomes of all these organs. NQO1, CYP1A1 and 1A2 were found to be induced in rats after their i.p. treating with 3-NBA. Here, 3-NBA was also investigated for its ability to induce these enzymes in livers, lungs and kidneys after intratracheal instillation of 0.2 and 2 mg of 3-NBA per kg of body weight, and for the such induction on DNA adduct formation by 3-NBA and 3-ABA. Intratracheal instillation of rats with 3-NBA simulates well the exposition of human population to this carcinogenic air pollutant (inhalation of air with this compound). A concentration-dependent increase in protein expression levels and enzymatic activities of NQO1 and CYP1A1/2 was found. These results demonstrate that 3-NBA is capable to induce NQO1 and CYP1A1 in liver, lungs and kidney of rats thereby enhancing its own genotoxic and carcinogenic potential.

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## ANTICOAGULANT RODENTICIDES POISONING IN GULL (*LARUS RIDIBUNDUS)* FROM CHOMOUTOV LAKE, CZECH REPUBLIC: A CASE STUDY

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Surface application of pesticides poses a high risk for wildlife animals and the environment. Preparations for the plant protection enter the foodchain easily and thus can contribute to the chemical load of consumers, including humans.

The application of rodenticides on the fields is an important and frequent source of poisoning in wildlife birds. In the Czech Republic, three active rodenticide substances for the application on field crops are registered currently: zinc phosphide, bromadiolone, aluminium phosphide.

In April 2010, massive deaths of gulls were observed in the nature preserve Chomoutovské jezero in the territory of Protected landscape area Litovelské Pomoraví. The deaths occurred due to the poisoning after the agricultural use of rodenticide Lanirat Micro containing 0,005% bromadiolone. Bromadiolone belongs to the group of anticoagulant rodenticides and causes blood coagulation imbalances after the repetitive intake.

Lanirat Micro was applied on the fields in the immediate vicinity of the sanctuary. Gulls, searching for the food in the treated area, ate bait granules and also poisoned rodents. Repetitive intake of bromadiolone caused chronic poisoning and death of 1500 birds from the colony normally counting 6–10 thousands of individuals. Massive bleeding to the gastrointestinal tract was detected during the autopsy; bromadiolone was measured in the tissue samples using HPLC method. Samples were positive and bromadiolone poisoning was proven.

As a prevention of the repetition of such poisoning, it is necessary to ban surface applications of dangerous substances in the area surrounding nesting colonies of gulls.

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## METABOLIC ACTIVATION OF CARCINOGENIC BENZO[A]PYRENE BY CYTOCHROME P450 1A1 IS DICTATED BY COMPOSITION OF THE MIXED-FUNCTION-MONOOXYGENASE SYSTEM

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Carcinogenic benzo(a)pyrene (BaP) was investigated for its potential to generate DNA adducts and to induce cytochrome P450 (CYP)and NADPH:CYP reductase enzymes in livers.

BaP induces expression of CYP1A1/2 enzymes in livers of experimental models, which leads to increase in their enzymatic activity. In addition, this compound is capable of generating DNA adducts, predominantly in livers of studied organisms. As determined by $^{32}$P-postlabeling analysis, 6.4-fold higher DNA binding of BaP was observed in the livers of HRN™ mice than in WT mice. This finding suggests a detoxication role of CYP1A in BaP metabolism *in vivo*. In *in vitro* experiments, DNA adduct formation in calf thymus DNA was up to 25-fold higher in incubations of BaP with microsomes from pretreated animals than with controls. This stimulation effect was attributed to induction of CYP1A1/2 and/or enzymes, which are responsible for oxidative activation of BaP to the metabolites generating major DNA adducts *in vitro*. BaP is oxidized by hepatic microsomes from pretreated and control animals to six metabolites. BaP is also oxidized with purified CYP1A1 reconstituted with NADPH:CYP reductase generating only five metabolites, but forming two DNA adducts. Activation of BaP by CYP1A1 is supposed to be dictated by enzymatic composition of the mixed-function-monooxygenase system of the endoplasmic reticulum. Therefore, the effects of individual components of this system (CYP, POR, cytochrome $b_5$ and epoxide hydrolase) on BaP metabolic activation is one of the aims of this study. Another target of our study is to evaluate a role of CYP2S1 in reactions activating BaP in the mouse microsomal system.

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## ROLE OF CYTOCHROMES P450 AND PEROXIDASES IN METABOLIC ACTIVATION AND DETOXICATION OF THE ANTICANCER DRUG ELLIPTICINE IN LIVER, LUNG AND KIDNEY

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Ellipticine exhibits significant antitumor and anti-HIV activities. The prevalent DNA-mediated mechanisms of its antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA, (ii) inhibition of DNA topoisomerase II activity and formation of covalent DNA adducts after being enzymatically activated with cytochromes P450 (CYP) or peroxidases.

Human and rat CYP1A and 3A were found to be the predominant enzymes catalyzing oxidation of ellipticine *in vitro* either to metabolites that are excreted (7-hydroxy- and 9-hydroxyellipticine) or that form DNA adducts (12-hydroxy- and 13-hydroxyellipticine). Of the mammalian peroxidases, cyclooxygenase (COX)-1, COX-2, lactoperoxidase and myeloperoxidase efficiently generated ellipticine-derived DNA adducts. However, the actual impacts of these enzymes *in-vivo* depend on several additional factors. One of them might be the presence of various patterns of individual CYP and peroxidase enzymes and/or even the presence of other proteins influencing their activities in target and non-target tissues. The CYP and peroxidase enzyme patterns depend also on a known phenomenon that ellipticine is a strong inducer of CYP1A enzymes in several tissues including cancer cells, which catalyze its own metabolism

Here, we have used the Wistar rats, a suitable model mimicking the fate of ellipticine in humans, to examine the pulmonary and renal CYP- and peroxidase-dependent metabolism of ellipticine and compare it with that found in the liver. We examined ellipticine metabolism and DNA adduct formation *in vitro* using microsomes isolated from hepatic and extra-hepatic tissues of this animal model either control (untreated) or treated with 40 mg/kg b.w. of ellipticine. We also expand former study investigating the induction of CYPs by ellipticine and examining the actual contributions of CYPs and peroxidases to DNA adduct formation by this drug in lung and kidney.

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## PROTECTIVE EFFECT OF ARBUTIN AND ROSMARINIC ACID AGAINST TOXICITY INDUCED BY THE ANTICANCER DRUG CYCLOPHOSPHAMIDE

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Cyclophosphamide (CP), an alkylating drug, has been in clinical use for the treatment of malignant and non-malignant disorders for over 40 years. However, toxic metabolites of CP can cause toxicity also for normal cells. Reactive oxygen species play an important role in CP-induced toxicity. CP disrupts the redox balance of tissues, resulting in oxidative stress.

The purpose of the present study was to investigate *in vitro* the antioxidative and *in vivo* potential protective effects of natural antioxidants arbutin and rosmarinic acid against CP-induced oxidative stress in ICR mice.

The *in vitro* antioxidative activity of arbutin and rosmarinic acid was evaluated spectrophotometrically by the use of the DPPH radical. Rosmarinic acid exhibited higher DPPH radical-scavenging activity than arbutin. After 60 min, the scavenging activity of rosmarinic acid was 95% at $5\times10^{-4}$ and 51% at $5\times10^{-5}$ mol/l, whereas the scavenging activity of arbutin was 50% at $5\times10^{-4}$ and 23% at $5\times10^{-5}$ mol/l.

Female ICR mice (23–25 g, 8–10 weeks old, Breeding station Dobrá Voda, Slovak Republic) were used in an *in vivo* study. The mice were orally pretreated with arbutin and rosmarinic acid in the dose of 3 mg/kg for 7 consecutive days. Oral treatment was continued while cyclophosphamide in the dose of 80 mg/kg was administered twice intraperitoneally (24-hour interval). The animals were sacrificed 24 h after the second CP injection.

Malondialdehyde (MDA) and the activity of the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAGA) were determined in various organs of ICR mice to detect the toxicity induced by CP.

Treatment with CP induced a rise of NAGA activity in serum and in the spleen of mice and increased the level of MDA in the liver, kidney and heart. In agreement with the known lower CP hepatotoxicity, the activity of NAGA in the liver remained unchanged. Administration of arbutin and rosmarinic acid ameliorated the biochemical changes.

We conclude that the favourable effect of the natural compounds studied is based on their scavenging activity of free radicals resulting from CP metabolism.

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## OXIDATIVE BURST OF HUMAN NEUTROPHILS IS SUPPRESSED BY N-FERULOYLSEROTONIN ISOLATED FROM SEEDS OF *LEUZEA CARTHAMOIDES* (WILD) DC

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Neutrophils are the first immune cells to arrive at the site of infection and inflammation. When approprietaly stimulated, phagocytic neutrophils display respiratory burst in which they produce reactive oxygen species (ROS). Pharmacological intervention with oxidative

burst is of utmost importance during inflammatory processes to protect the tissue against ROS damage. The aim of this study was to investigate the effect of N-feruloylserotonin(N-f-5HT) on stimulated human neutrophils *in vitro*.

N-f-5HT was isolated from seeds of *Leuzea carthamoides* (Wild) DC by solvent extraction followed by column chromatography and by further HPLC separations. N-f-5HT inhibited dose-dependently oxidative burst of human whole blood and isolated neutrophils *in vitro*, as measured by luminol and/or isoluminol enhanced chemiluminescence, in the rank order of stimuli phorbol-myristate-acetate(PMA)>opsonized zymosan>$Ca^{2+}$ ionophore A23187. In isolated neutrophils stimulated with PMA, N-f-5HT was effective against extracellular as well as intracellular reactive oxygen species. Liberation of ATP, analysis of apoptosis and recombinant caspase-3 activity revealed that N-f-5HT in the concentrations used (up to 100 μM) did not alter the viability and integrity of isolated neutrophils. Western blot analysis documented that N-f-5HT in concentrations of 10 and 100 μM significantly decreased PMA-induced phosphorylation of protein kinase C (PKC) alpha/beta II.

In light of the presented results, N-f-5HT isomers seem to represent interesting naturally occurring compounds with the pharmacological effect of inhibiting respiratory burst of human neutrophils in extra- and intracellular compartments. The results suggest that N-f-5HT represents an effective substance and could be further investigated for its pharmacological activity against oxidative stress in ischaemia-reperfusion, inflammation and other pathological conditions.

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## CONSTRUCTION OF STABLY-TRANSFECTED REPORTER CELL LINES

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Primary human hepatocytes express majority of cytochrome P450 (CYP) enzymes involved in metabolism of wide range of endogenous substrates as well as detoxification/metabolic activation of exogenous compounds. Restricted accessibility of primary human hepatocytes, *in vitro* phenotypic instability and limited life-span seriously complicate their use in routine testing. Human liver-derived cells are used for drug metabolism study given their availability, unlimited life-span, stable phenotype and easy handling. Although human hepatoma cell lines show many liver specific functions their biotransformation capacity is low. Therefore, we have employed strategy to enhancement of expression level of CYP genes with help stable transfection of transcription factors into hepatoma cells. We focused on CYP3A4 gene that plays major role in drug biotransformation in humans and is responsible for oxidative metabolism of more than 50% of clinically available drugs. Stable transfected MZ-Hep-1 cells with p(3A4-CLEM4/XREM/prox)-luc reporter gene construct containing basal promoter with the proximal PXR response element (ER6), the distal xenobiotic responsive enhancer module XREM and CLEM4 region of the CYP3A4 gene were co-transfected with human pregnane X receptor (PXR). After selection under neomycin resistance, we studied human PXR transcription activity. We measured the luciferase activity in response to rifampicin in sexteen clones and chose three clones showing the highest CYP3A4 induction. The expression level of PXR protein was confirmed by Western immunoblotting.

We have developed stable cellular model that can provides specific and reproducible *in vitro* system for monitoring CYP3A4 inducibility by compounds in the drug development process.

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## PROLONGED OXYTOCIN TREATMENT ON HEART TISSUE

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Our previous studies have shown that acute treatment with hormone oxytocin diminishes myocardial injury induced by ischemia/reperfusion in isolated rat heart [1]. In addition, exposure to intensive stressors such as immobilization results in elevated concentrations of oxytocin in plasma [2]. It is therefore important to elucidate the effects of oxytocin on the heart tissue. The aim of the present studies was to evaluate the changes in selected signaling pathways in the heart following prolonged treatment with oxytocin *via* osmotic minipumps.

Osmotic minipumps were implanted subcutaneously and released oxytocin (3.6 ug/100 g body weight/day) into the bloodstream for 14 days. Oxytocin concentrations in plasma were analysed by a specific radioimmunoassay. The levels and activation of proteins and atrial natriuretic peptide (ANP) were determined by immunoblot assay, the MMP-2 activities in left ventricle heart tissue were measured by zymography.

Oxytocin treatment led to an increase in circulating oxytocin levels approaching those observed during immobilization stress. Detection with a phosphospecific antibody showed increased activation of Akt kinase in oxytocin-treated rats. Treatment with oxytocin resulted in a significant increase in specific phosphorylation of p38-MAPK. The activation of p38-MAPK pathway was confirmed also by the determination of phosphorylated Hsp27, a physiological substrate for MAP kinase-activated protein kinase 2, which is activated by p38-MAPK. The results show significant increase in

phosphorylated Hsp27 in oxytocin-treated animals. In addition, increased levels of ANP were observed in the left heart ventricle of oxytocin-treated rats. Oxytocin treatment failed to influence activities of ERKs and matrix metalloproteinases.

In conclusion, prolonged treatment with oxytocin stimulates intracellular Akt kinase and p38MAPK signaling pathways, as well as the levels of the ANP in the left heart ventricle. These changes may participate on protective effects of oxytocin and may be important in possible involvement of oxytocin in reducing the negative consequences of stress on the heart.

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## IMMUNOMODULATORY EFFECTS OF CYANOTOXINS IN THE LABORATORY RAT

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Aims of the present study were to evaluate impacts of feeding experimental rats with diet containing microcystins in variable concentrations in the form of complex biomass, isolated microcystins and also the fish meat presence/absence on their state of health. Six different exposure variants were investigated: A – negative control; B – commercial diet with 25% of fish with cyanobacterial bloom biomass which contained final total nominal MC concentration 25000 ug/kg of food; C – clean fish (commercial diet with 25% of fish from the locality with no occurrence of cyanobacteria and microcystins); D, E – clean fish with MC – externally added microcystin in two nominal doses corresponding to 700 ug/kg and 5000 ug/kg; F – contaminated fish (commercial diet with 25% of fish from the locality with heavy cyanobacterial bloom). The exposure lasted for 28 days. At the end, animals were weighed, sacrificed and tissues and blood collected for further toxicological, biochemical and haematological as well as histopathological analyses. Hereinafter, only haematological results are presented. There was a decrease in the total leukocyte count in group E; significantly different, however, only from group D. There were also differences in lymphocyte subpopulations of peripheral blood and lymphoid organs. Peripheral blood NK cells and γ/δ lymphocytes counts were significantly increased in group E compared to C. Group F showed a change in the ratio of CD 4+ and CD 8+ lymphocytes. Significant changes such as the rise of B lymphocytes and all T lymphocytes subpopulations were noted in the spleen of group E animals. NK cells of the spleen were lower in group D. Changes of thymic lymphocyte subpopulations were documented in groups E and F.

Our results demonstrate some immunomodulatory effects of higher cyanotoxin doses, in particular.

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## VITAMIN LEVELS IN A SELECTED POPULATION IN THE CZECH REPUBLIC

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The work objective was to monitor nutritional habits in the observed group of professional soldiers with the focus on eating food with the content of antioxidant carriers. Then to show present state of health and nutrition in the group on the basis of anthropometric measurements and biochemical examinations and finally to observe the level of antioxidant vitamins in the observed group of professional soldiers.

The group included 171 healthy individuals, 152 men and 19 women. Their average age was 34.2 ± 7.9 years. The venous blood was taken for biochemical examinations in all individuals on a fast. Anthropometric measurements (weight, height, caliperation, waist circumferences), blood pressure and puls were taken continually in all individuals. Simple questionaires were administered to all participants for the complete evaluation of present health and for the registration of eating habits of the observed persons.

The study results show that retinol and α-tocoferol levels in the observed group were within a normal range. The average conentration of vitamin C in this group was 54 mmol/l and reached nearly the values given in other European countries. But concentrations of β-caroten and lycopen in serum were up to 50% lower in comparison with concentrations in population in the countries of West Europe. Higher vitamin C and β-caroten serum levels were found in individuals who respond in a questionnare they eat fruit and vegetables or supplements of vitamin preparations every day. Statistically lower levels of vitamin C, β-karoten and lycopen in the group of obese people (compared with the group of normal weight people) show decreased level of antioxidant protection of the organism and the risk of cardiovascular diseases.

The results show that it is necessary to ensure optimal food not only with an energetic diet value but also with a proper input of antioxidant carriers in the form of fresh vegetables and fruit every day.

## *IN VITRO* EFFECT OF PINOSYLVIN AND PTEROSTILBEN ON HUMAN NEUTROPHILS

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Neutrophils play a central role in innate immunity but they may be also deleterious in acute inflammation. Myeloperoxidase (MPO), by auto- and paracrine mechanisms, perpetuates the inflammatory response. Moreover, it is implicated to prolong the life span of neutrophils, thereby delaying the resolution of inflammation.

Since insight into mechanisms underlying the effectiveness of a number of natural compounds-phytomedicines is rather important, we studied the effect of pinosylvin, a natural stilbenoid (trans-3',5' dihydroxystilbene, a component of pine leaf with antibacterial and antifungal activity) and pterostilbene (3,5-dimethoxy-4'-hydroxystilbene, a naturally occurring phenolic derivative with distinct health-enhancing activities) on human neutrophil functions (degranulation and respiratory burst). Neutrophils were isolated from fresh blood (obtained at a blood bank from healthy male donors without any medication for at least 7 days) preincubated with the drugs studied and their effect on superoxide generation and myeloperoxidase release was recorded after stimulation with PMA [1 µmol/l] or FMLP [0.1 µmol/l]. Both compounds dose dependently decreased superoxide generation and MPO release. The use of inhibitors, staurosporine and wortmannin, revealed involvement of proteinkinase C rather than phospholipase D signaling pathway. Our results suggest that the effects of pinosylvin and pterostilbene may prove helpful in controlling inflammation.

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## CHEMICAL COMPOSITION AND THE ARYL HYDROCARBON RECEPTOR-DEPENDENT ACTIVITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR DERIVATIVES IN COMPLEX AIRBORNE MIXTURES

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Polycyclic aromatic hydrocarbons (PAHs) are a highly important group of contaminants in various environmental samples. They are relatively easily metabolized, as compared to persistent organic pollutants (POPs), such as polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans. Many PAHs are known or suspected mutagens and carcinogens. Both PAHs and POPs have been reported to be potent inducers of aryl hydrocarbon receptor (AhR). Because the AhR-dependent induction of gene expression is widely used as a biomarker of dioxin-like toxicity, we used the chemical-activated luciferase reporter gene assay to determine AhR-mediated ("dioxin-like") activity.

Previously, we have determined the AhR-inducing potencies of a large number of principal individual PAHs and PAH derivatives, which allow calculating theoretical induction equivalents related to the reference toxicant 2,3,7,8-TCDD (IEQ values). In this study, we performed a combined chemical and *in vitro* bioassay analysis of extracts and chromatographic fractions of airborne particulate matter samples from five cities in the Czech Republic (Třeboň, Praha, Ostrava-Poruba, Ostrava-Bartovice, Karviná). The samples were extracted and fractionated into five fractions with increased polarity. The highest AhR-dependent activity was found in the neutral aromatic fraction containing both PAHs and POPs, with PAHs being the principal inducers of dioxin-like activity. The sampling site Ostrava-Bartovice was the most contaminated area, with IEQ value 21-times higher than in the extracts of the sample collected in Třeboň. These results were in a good agreement with HPLC or GC/MS analysis and with IEQs calculated based on combination of concentration data with the relative AhR-inducing potencies of individual PAHs and derivatives found in the airborne samples.

Within this study, we also developed novel induction equivalency factors for nitro-PAHs and several additional PAHs found at significant levels in the airborne samples, in order to facilitate a more complete analysis of IEQ values. The most prevalent and AhR-active PAHs included both US EPA PAHs (benz[a]anthracene, indeno[1,2,3-cd]pyrene, chrysene, and benzo[a]pyrene) and emerging PAH toxicants (methylbenzanthracenes, dibenzoanthracenes); the latter group of PAHs contributed by approximately 50% to the total AhR activity of measured PAHs.

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## INHIBITION OF OXIDANT PRODUCTION IN RAT ADJUVANT ARTHRITIS WITH PTEROSTILBENE

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Within the stilbene type polyphenols, resveratrol is well known because of its antiinflammatory and

anticancerous activity, yet its bioavailability is low. Pterostilbene is a dimethoxy derivative of resveratrol with higher lipophilicity. Pterostilbene is known due to its anticancerous, antidiabetic, antifungal and anti-inflammatory properties, but little is known about the effects of pterostilbene on activated neutrophils. These cells are considered to be the first line defense of innate immunity.

The aim of this study was to investigate the effects of pterostilbene on oxidative burst of neutrophils in the adjuvant arthritis model in Lewis rats. Blood was sampled in weekly intervals during 28 days. Production of reactive oxygen species was measured using luminol-enhanced chemiluminescence. Moreover, the effects of pterostilbene on neutrophil number and activity were determined. Ex vivo stimulation with phorbol-myristate-acetate (PMA) was used to investigate the response of inflammatory primed neutrophils on the stimulus.

The increase in spontaneous chemiluminescence in the arthritic group was significantly ($p<0.001$) higher compared to the control group. Pterostilbene in the daily dose of 30 mg/kg p.o., administered over 28 days, nonsignificantly decreased the production of oxidants in blood, except day 28. Stimulation with PMA (0.005–0.5 µmol/l) increased the production of oxidants. Likewise, pterostilbene nonsignificantly decreased the production of reactive oxygen species in PMA stimulated neutrophils. However, pterostilbene decreased the number of neutrophils in whole rat blood, with significant effect on day 14. There was no difference between the arthritic group and the pterostilbene treated arthritic group in neutrophil activity, which was determined as chemiluminescence of one neutrophil in the sample.

One may hypothesise that the lower chemiluminescence in the pterostilbene-treated arthritic group may be due to the lower number of neutrophils in this group.

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## ACUTE PARACETAMOL POISONINGS REPORTED TO THE TOXICOLOGICAL INFORMATION CENTRE IN BRATISLAVA

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The National Toxicological Information Centre (NTIC) in Bratislava has frequently been consulted for advice on paracetamol exposures. To obtain more information about paracetamol poisonings in Slovak Republic, we performed a retrospective analysis of all the telephone calls to our Centre.

All the telephone inquiries involving paracetamol exposures were extracted from our database for the year period 2003–2008. The following data were analysed: age, sex, intent of exposure (accidental or suicidal), substances ingested, the clinical severity, a type of first aid provided before professional medical treatment, the intoxication treatment chosen. All intoxications were classified in accordance with the Poison Severity Score.

The population under review comprised 423 intoxication cases recorded from the medical consultations provided by the NTIC over telephone and from the hospital discharge reports. Paracetamol exposures in females (64%) were more prevalent than those involving males. Intoxications in adults made up 51% of cases, with the majority of cases being suicidal intoxications. Intoxications in children up to the age of 6 accounted for 11% of cases from the population under review. These were accidental intoxications, often caused by exceeding the recommended daily therapeutic amount. 34% of cases were made up of intoxications of patients of the age 6 to 18 years. Suicidal cases (64%) mostly involved the combination of paracetamol with other drugs or with alcohol. Accidental intoxications (22%) were caused by paracetamol alone. First aid before professional medical treatment was provided only in 15% of cases. Therapeutic medical treatment was carried out in these forms: ingestion of activated charcoal (55% of cases), ingestion of a laxative (25%), gastric lavage (19%), physiological saline infusion (14%), haemoperfusion (4%), forced diuresis (4%), haemodialysis (2%), ingestion of hepatoprotective drugs (3%). N-acetylcysteine as a paracetamol antidote was given in 37% of cases. 51% of intoxications were accompanied by mild, transient and resolving symptoms (PSS 1). There were no fatal cases (PSS 4).

Obligatory reporting of every poisoning to the NTIC including cases of poisonings not resulting in a consultation with the NTIC came into force in October 2006. Previous to this measure we received only 30% of feedback information on poisonings about which we were consulted, which did not enable us to carry out the full analysis of the efficacy of the treatment.

## SIMAZINE TOXICITY TO *DANIO RERIO*: EFFECTS OF SUBCHRONIC EXPOSURE ON FISH

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The aim of this study was to investigate effects of subchronic exposure to sublethal levels of simazine on growth of *Danio rerio*. Simazine belongs to symmetrical triazine herbicides used extensively in agriculture and non-agricultural sites, primarily to control broadleaf and some grassy weeds, that have become ubiquitous contaminants of the environment.

The juvenile growth tests were performed on *Danio rerio* according to the OECD guideline No. 215. *D. rerio* is one of the model organisms most commonly used in toxicity test. Fish at the age of 20 days were exposed

for 28 days to the simazine environmental concentration commonly detected in the Czech rivers (0.06 µg/l) and the range of sublethal concentrations of simazine (0.6, 6.0 and 60 µg/l). Fish were fed with dried *Artemia salina* without nutshells in amount of 8% of their body weight per day, the food ration was based on initial fish weights and was recalculated after 14 days. At the end of the tests fish were weighed again and their length was determined. Tank-average specific growth rates were calculated using formula according to the OECD No. 215. Results were analysed using the statistical programme Unistat 5.1. Data was subjected to one-way ANOVA and subsequently to Dunnett`s test in order to assess the statistical significance of differences in tank-average fish specific growth between test groups with different concentrations and that of the control group. Experimental procedures were in compliance with national legislation (Act No. 246/1992 Coll., on the protection of animals against cruelty, as amended and decree No. 207/2004 Coll., on the protection, breeding and use of experimental animals, as amended).

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## ANTIDOTES AGAINST ORGANOPHOSPHATES MODULATE OXIDATIVE STRESS ACCOMPANYING INTOXICATION AND CAUSE SHIFT OF SELECTED BIOCHEMICAL MARKERS, ANTIOXIDANTS AND STRESS MARKERS IN TREATMENT

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Organophosphates are chemical toxins used as drugs, pesticides and chemical warfare agents able to cause hyperstimulation of cholinergic nervous system by inhibition of acetylcholinesterases (AChE) in body. The current therapy can be causative or symptomatic. Atropine is an antidotum reducing symptomatic manifestation of nerve agent intoxication by reduction of overstimulation of muscarinic acetylcholine receptors. Causative treatment is available by oxime reactivators returning activity of AChE. Though the organophosphate intoxication and treatment process were considered as impact on cholinergic nervous system only, the recent investigations appoint at multiple impact on organism.

In the first experiment, investigation was aimed at overall impact of drug HI-6 on laboratory beagle dog organisms. Markers of oxidative stress such as malondialdehyde (MDA), reduced and oxidized glutathione and ferric reducing antioxidant (FRAP) power in hematocrite and plasma were assayed in time intervals 0–60–120–240 minutes. Multiple biochemical markers such as alanineaminotransferase, aspartate aminotransferase, glutathione peroxidase, glutathione S-transferase, alkaline phosphatase, amylase, lactate dehydrogenase as and haematological parameters were investigated in serum. In the second part of experiments, wistar rats intoxicated with soman or cyclosarin were treated with atropine and/or oxime reactivators. The mentioned stress and biochemical markers were assayed again. In a conclusion, metabolic disorders and oxidative stress could play an important role in organophosphate intoxication as well as the consequent treatment process. The used drugs as well as nerve agents were affecting animal organism and triggering oxidative stress. On the other hand, oxime reactivators were found to be able cause the serious disorders and oxidative stress arising after organophosphate intoxication.

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## ELLIPTICINE CYTOTOXICITY TO HUMAN THYROID CANCER CELL LINES

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Ellipticine, an alkaloid isolated from *Apocyanaceae* plants, exhibits significant antitumor and anti-HIV activities. The prevalent DNA-mediated mechanisms of its antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA, and (ii) inhibition of DNA topoisomerase II activity. We have demonstrated that ellipticine also covalently binds to DNA after being enzymatically activated with cytochromes P450 (CYP) or peroxidases. Human and rat CYP1A and 3A were found to be the predominant enzymes catalyzing oxidation of ellipticine *in vitro* either to metabolites that are excreted (7-hydroxy- and 9-hydroxyellipticine) or that form DNA adducts (12-hydroxy- and 13-hydroxyellipticine). Of the mammalian peroxidases, human cyclooxygenase (COX)-2, ovine COX-1, bovine lactoperoxidase and human myeloperoxidase efficiently generated ellipticine-derived DNA adducts.

The ellipticine-derived DNA adducts were found in human breast adenocarcinoma MCF-7 cells, leukemia HL-60 and CCRF-CEM cell lines, neuroblastoma IMR-32, UKF-NB-3 and UKF-NB-4 cell lines and glioblastoma U87MG cells *in vitro* and *in vivo* in rats and mice exposed to ellipticine. Here, the cytotoxicity of ellipticine to human thyroid cancer cell lines BHT-101, B-CPAP, 8505-C and this compound to cell cycle distribution were investigated. Furthermore, the effect of hypoxic conditions on the ellipticine toxicity to these cells was also examined. The toxicity of ellipticine to thyroid cancer cells cultivated under the standard conditions was higher than that to the cell lines grown

under hypoxia (1% oxygen). Another target of this work was to evaluate the effect of ellipticine on expression of enzymes and proteins participating in activating ellipticine, namely on CYP1A1, 1B1, 3A4, cytochrome $b_5$ and peroxidases COX-1 and thyroid peroxidase (TPO). Using the $^{32}$P-postlabeling assay, the covalent modification of DNA isolated from the studied cells exposed to ellipticine was investigated.

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## ASSESSMENT OF THE EFFECTS OF PHARMACEUTICALS DETECTED IN THE CZECH RIVERS ON AQUATIC ORGANISMS

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In recent years, several studies have reported the occurrence of pharmaceuticals in surface waters in Europe. The data obtained from the Czech Hydrometeorological Institute show that the concentrations of these compounds in the Czech river water samples ranged from 50 to 400 ng/l and the most frequent occurence and highest concentrations were detected for ibuprofen, carbamazepine, sulfamethoxazole and diclofenac.

The aim of this study is to evaluate the effects of selected pharmaceuticals on aquatic organisms, to assess their acute toxicity, *i.e.* to determine 96-h LC50 (concentrations lethal to 50% tested fish and 50% tested embryos). The aim of frog embryo teratogenesis assay – *Xenopus* (FETAX) is to assess 96-h EC50 (concentrations causing malformation in 50% tested embryos), MCIG (minimum concentration to inhibit growth) and TI (teratogenic index).

Acute toxicity tests on *Danio rerio* and *Poecilia reticulata* fish (according to OECD No. 203), and FETAX tests on *Xenopus laevis* embryos (according to ASTM E1439-98) are performed to assess acute toxicity, embryotoxic and teratogenic effects of pharmaceuticals. Parameters determined by FETAX tests will be supplemented by the analysis of biochemical markers which may inform about the mechanism of action of tested substances, their metabolism and potential for causing cell damage due to oxidative stress. The overproduction of reactive oxygen species is a mechanism of toxicity of many xenobiotics. Therefore, glutathione content and glutathione-S-transferase activity will be determined in the *Xenopus laevis* larvae body homogenates.

Results of our study will contribute to the extension of current knowledge about the effects of pharmaceuticals on aquatic organisms.

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## TESTING OF THE BIOLOGICAL ACTIVITY OF SUBSTANCES WITH POTENTIAL CYTOSTATIC EFFECT ON MOUSE MODEL OF LEUKEMIA (L-1210)

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The aim of the study was to introduce a model of mouse lymphocytic leukemia for the testing of newly synthesized substances with potential cytostatic effect.

The experiment was performed on CBA/J SPF laboratory mice (leukemia-sensitive). Animals were housed with free access to standard diet and water. After one week acclimatization, suspension of mouse leukemic cells (L-1210) was administered at the dose of $1 \times 10^5$ intraperitoneally. For a period of 29 days health state and weight were observed and peripheral blood samples were taken for leucocyte screening examination once a week. After 29 days animals were killed by cervical dislocation, necropsy was performed and organs for histopatological examination were taken.

Despite the viability of cell culture was estimated only to 30%, the disease was succesfully induced, only in milder form than literature describes. Normal and tumor lymphocytes, damaged neutrophil granulocytes, thrombocytes and a large amount of monocytes were detected in peripheral blood. Progenitor leukemic cells were observed after 14 days.

Histological examination of the liver and kidneys didn't show tumor infiltration, only inflammatory reaction with vasodilatation, venostasis and diffuse interstitial hemorrhages. Splenic marginal zone reaction was detected. The most distinctive changes were found in gut-associated lymphoid tissue (GALT). The definite B-cell reaction was revealed within the enlarged lymph nodes. The numerous extranodal tumor infiltrations were observed in the gastrointestinal tract.

Conclusion: Acute lymphocytic leukemia was induced in spite of the application of lower concentration of viable tumor cells. Although mice didn't exhibit external symptoms of the disease, this model can be considered as suitable for *in vivo* testing of newly synthesized substances with potential cytostatic effect.

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## ORGANIC POLLUTANTS ON EARTHWORMS *EISENIA FETIDA*

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Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants contaminating all components of the

environment. Earthworms, common soil organisms, were used to evaluate the load of this environmental component by these pollutants. It is known that adverse effects of pollutants in soils may result in direct (lower reproduction, higher mortality) as well as indirect (bioaccumulation) effects.

The aim of this study was to examine the relation between soil contamination by PAHs and soil organisms represented by the eartworm *Eisenia fetida*.

The PAHs on *Eisenia fetida* was evaluated according to the OECD methods No. 207 („Earthworm Acute Toxicity Tests", 4 April 1984). Eartworms were exposed to these pollutants dissolved in hexane for 14 days. A standard mixture containing 45 PAHs was used in the test. PAHs were administered into defined substrates in concentrations of 20, 40, 60 µg/kg of the dry substrate. A total of 10 earworms ranging from 380 to 425 mg in weight were inserted into each concentration and controls. The relation between soil contamination and soil organisms, *i.e.* the bioavailability of PAHs, was evaluated on the basis of determining their content in tissues of earthworms and in the substrate.

Samples of the substrate and tissues were homogenised, dehydrated using anhydrous sodium sulphate and extracted by dichloromethane. PAHs were then determined following cleaning the extract by colon chromatography using gas chromatography with mass detection.

There was no acute toxicity during the 14-day test with PAHs. The content of PAHs in tissues and soils was evaluated as the sum of PAHs for each concentration separately. Fresh tissues of earthworms contained 321.9 µg/kg, 419.1 µg/kg and 629.9 µg/kg sum of PAHs, while the dry substrate contained 41.4 µg/kg, 220.8 µg/kg and 384.5 µg/kg sum of PAHs, respectively. Our analyses document the fact that PAHs show a higher affinity to animal tissues than soils.

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## OXIDATIVE STRESS AND VULNERABLE B CELL: PERSPECTIVES OF UTILIZATION OF *ZINGIBER OFFICINALE* ROSC.

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Oxidative stress plays a critical role in diabetes type 1 and 2. With regard to their reduced antioxidant reserves, pancreatic insulin producing β cells represent one of its primary targets.

In traditional medicine, several medicinal plants or their extracts have been used to treat diabetes. *Zingiber officinale* Roscoe (family, Zingiberaceae), known commonly as ginger, is consumed worldwide in cookeries as spice and flavoring agent. It has been used as spice and medicine for thousands of years.

The present study was undertaken to investigate the potential protective effect of *Zingiber officinale* in a simple model of oxidative damage of INS-1E β cell line. Unlike to isolated fraction of essential oil, both low cytoxicity and the remarkable protective effects on β cell viability followed by lowering oxidative stress markers (as documented by several colorimetric and fluorescent methods) were found for the ethanolic extract *Zingiber officinale* Rosc.

The present study is the first pilot study to assess the potential of *Zingiber officinale* in a model of cytotoxic conditions imposed by diabetes in β cells.

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## ARE COMMON STPS IMPORTANT SOURCES OF POLLUTION OF AQUATIC ENVIRONMENT?

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Sewage water treatment plants (STPs) are frequently associated with releasing of xenobiotics to aquatic environment and consequently with effects on exposed aquatic organisms.The impact of 8 STPs on water quality and on exposed brown trout (*Salmo trutta v. fario* L.) was assessed during 2007–2009. Fish were caught upstream (US) and downstream (DS) of STPs situated on small streams. The effect of pollution on fish was examined by measuring hepatic ethoxyresorufin-O-deethylase, vitellogenin, calculating gonadosomatic and hepatosomatic indexes. Additionally, passive samplers - POCIS (pharmaceuticals, perfluorinated compounds, pesticides) and SPMD (PCBs, PAHs, OCPs, Triclosan and MeTriclosan) were deployed to describe pollution by above mentioned compounds. DS sampling sites showed significantly higher contamination than US sites in most of measured parameters. The effects of chemical compounds on the brown trout examined were observable and higher in the sites with lower dilution of STP effluent. Data documented that common STPs are an important source of pollution of the aquatic environment, especially pharmaceuticals.

*Supported by the grant MSM 6007665809 and MZP SP/2e7/229/07.*

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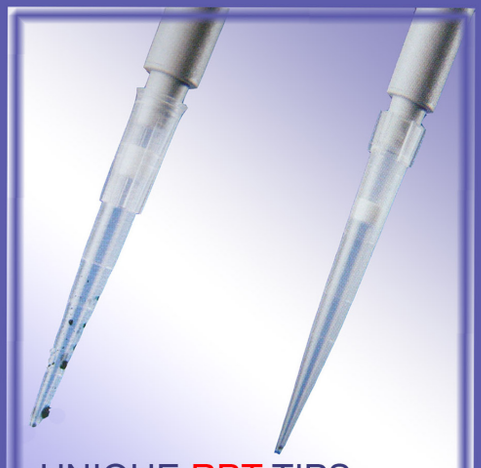

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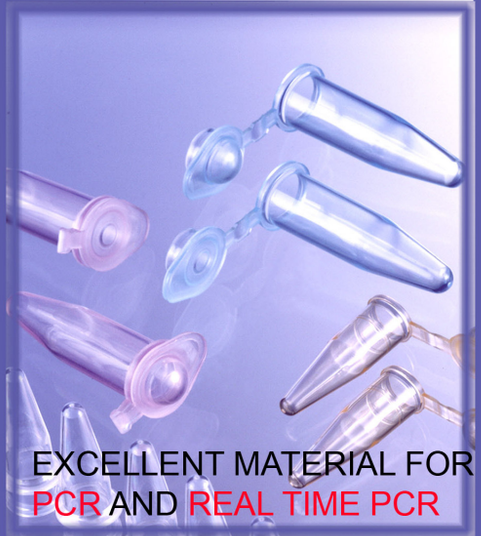

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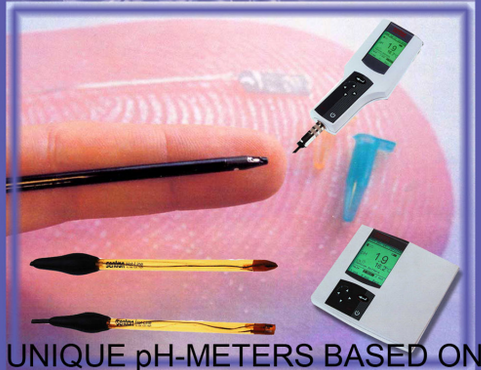

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