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interdisciplinary toxicology

14th Interdisciplinary Toxicology Conference

14th Czech – Slovak Interdisciplinary Toxicology Conference takes place on 1st – 3rd June 2009 in Brno.

The motto of this traditional meeting of toxicologists is on one hand additional contribution to the 90th anniversary of the Veterinary and Pharmaceutical University foundation, on the other hand it is an emphasis on as broadest interdisciplinarity as possible with the subtext that the toxicology has – despite its thematic latitude – many mutual and unifying mechanisms.

The department of poisoning investigation was founded at Veterinary University in Brno already in 1921. Continual increase in number of detected animal poisonings required both personal and material provision of this section. For example in 1953 the number of examined cases exceeded 4 thousands and 1304 of these cases were positive. Together with the development of diagnostics, increasing amount of poisonings and increasing importance of their detection, lectures on toxicology became a part of training at the university in 1950's. In 1953 toxicology became a separate subject taught in 8th semester of studies. The founder of this study field at the Veterinary University was Assoc. Prof. Vojtěch Neumann, DVM., CSc. In 1974, the first textbook of veterinary toxicology for students was published by Prof. Alois Piskač, DVM., DSc. and this at that time excellent work was published also in english version by Elsevier publishing house in Amsterdam (second edition of this text book was released in 1985).

In 2002 a subject called food toxicology extended the curriculum of the training. Currently, both of the subjects – toxicology (2 hours of lectures + 2 hours of practical works a week) and food toxicology (1 hour of lectures + 1 hour of practical works a week) are taught in 5th semester of studies. Both of the subjects are finished with exam.

Postgraduate doctoral study in the branch of veterinary pharmacology and toxicology was commenced in 1990. In 2005 the name and scope of this branch was changed to veterinary toxicology and food toxicology and it was newly accredited. Currently, there is a developed cooperation between the toxicology branch of the Department of public veterinary health and toxicology and the Department of human pharmacology and toxicology at the Veterinary and pharmaceutical university Brno.

The main motto of interdisciplinarity is the statement that there is only one toxicology. One substance (poison) has the same mechanism of action, the same manifestation and we use same antidotes for it in all animal species. Moreover the poisonings in animals and humans very often occur simultaneously. For example during suicidal attempts (mainly by carbon monoxide) the death of man is often followed by the death of his best friend – dog. Similar case from criminalistic field was reported by Prof. Daniela Pelclová, MD., CSc. at 12th Interdisciplinary Toxicology Conference where the man – murderer – tested the effect of thallium sulphate first on his dog and then used it on his wife and daughter. Effects were proven to be the same. Interdisciplinarity of the 14th Interdisciplinary Toxicology Conference will be fulfilled by the fact that it will present contributions from all branches of toxicology - human and veterinary toxicology, industrial and military toxicology, ecotoxicology, basic and applied research, clinical and diagnostic practice.

Interdisciplinary approach is also expressed in the definition of toxicology as it was proposed by Society of Toxicology (SOT) 3 years ago – "Toxicology is the study of the adverse effects of chemical, physical or biological agents on living organisms and the ecosystem, including the prevention and amelioration of such averse effects". In 2008 Ralf Neumann carried out an analysis of publication activity in the field of toxicology during the years 1996 – 2007 (*Lab Times*, issue **6**, 2008; 38 – 40). Results of this analysis show that apoptosis and endocrine disruption emerged as the hottest topics and interdisciplinary approach unambiguously arises out of it too. Ralf Neumann mentions 5 scientific works which were the most cited in the examined period of time. In the first place was mentioned manuscript by Leist, M. *et al.* (1997) Intracellular adenosine triphosphate (ATP) concentration: A switch in the decision between apoptosis and necrosis. *J Exper Med* **185**: 1481–1486, (number of citations: 919). The other 4 articles were dealing with endocrine disruption in water environment. Authors of those articles are Sumpter at al. (number of citations in particular articles varies between 535 and 673). John P. Sumpter from Brunel University, UK, is one of the first pioneers to show that some toxins in the environment cause endocrine disruption in certain animals (fish) leading to, for example, intersexuality.

Czech toxicology was unfortunately found at 19th position among European countries (number of scientific articles: 509, number of citations: 4 152). Nevertheless Czechoslovak toxicology, especially thanks to Prof. Helena Rašková, MD., DSc., belonged to the top and was among founder member of EUROTOX predecessor – European society for the study of the drug toxicity in 1962. We believe that our traditional meetings will contribute to the increased efficiency, production rate and prestige of Czech and Slovak toxicology on both European and worldwide scale.

Prof. Zdeňka Svobodová, DVM., DSc.
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
CZECH REPUBLIC

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interdisciplinary toxicology

Letter to the Editor

Dear Colleagues,

It is an honour to address your conference. I am happy to see this common conference in spite of all the world changes we experienced in the past 100 years. As a pharmacologist I always had to be concerned with the toxicity of drugs and other compounds. The formation of the European Union and the progress in other parts of the world demonstrate how far we have come since the end of the Second World War. The explosion in our fields is due to the formation of international organizations such as the International Union of Pharmacology (IUPHAR) and now also the International Union of Toxicology (IUTOX). The contemporary possibilities of free movement frequently lead us to find suitable places for shorter or longer stays in different countries. Financial realities also play an important role. Nevertheless, there are a number of individuals who seek to broaden their horizons in research and education. I would like to present you with just a few examples of my former pupils or friends. The first example is the Slovak of Hungarian nationality, Prof. Dr. Viktor Bauer, one of my graduate students. Thanks to the visit to Prague by a Japanese scientist who worked for years in Oxford where I had spent some time soon after the war, Bauer was able to work for a considerable time in Japan. After his return, he worked also as the Director of the Institute of Experimental Pharmacology of the Slovak Academy of Sciences. This interval was interrupted for four years when he became the Ambassador of the Slovak Republic to Turkey. Now he once again works at the institute. The second example is Prof. Dr. Rado Nosáľ, again one of my graduate students. He worked for a considerable time in the Department of Pharmacology in Stockholm. His permanent interest for some blood elements brought him not only international acclaim, but he is actually the current Director of the Institute of Pharmacology of the Slovak Academy of Sciences. Next is the Czech, Prof. Dr. Vladislav Eybl, a pupil of Prof. Dr. Zdeněk Köcher and long-term director of the Department of Pharmacology and Toxicology of the Medical Faculty in Plzeň, a part of Charles University. In 1963, he received a one-year stipend offered by the firm Riker to the just formed section of the International Union of Physiology SEPHAR. The Committee had complete freedom to give scholarships to applicants from Europe, USA and other countries. Eybl was one the stipend recipients and worked under Prof. Dr. Otto Krayer at Harvard. This stay and support by Krayer helped him to broaden his horizons and knowledge in toxicology so extensively that he later acted even as auditor for the correspondent agency in USA. Numerous were his activities in developing contacts and actions concerning the steps of activity the later EUROTOX and corresponding activities. The next example is Prof. Dr. Jaroslav Květina who spent a considerable time in the Department of the Istituto Mario Negri under the guidance of the famous Prof. Dr. Silvio Garattini. He also stayed at Nippon University in Tokyo. He not only integrated pharmacology in the Curriculum of the Pharmaceutical Faculty of the Charles University in Hradec Králové as the dean but also gained long-term influence in important International Pharmaceutical Organization. Last but not least, is Prof. Dr. Miloš Kršiak. He spent quite a time at University College in London. He is an important member of the Collegium Neuropsychofarmacologium. His interest concerned the nervous system including pain. I hope all this might inspire some of you to pursue an uneasy, but rewarding scientific career.

I wish you all the best and hope you enjoy the conference.

Prof. Helena Rašková, MD., DSc.
The doyenne of Czech and Slovak toxicology
CZECH REPUBLIC

TOXCON 2009 BRNO

14th Interdisciplinary Toxicology Conference

Brno, Czech Republic

Hotel Myslivna- June 1-3, 2009

Programme & Abstracts

Editors
Ladislava BARTOŠOVÁ
Jana HAVLÍČKOVÁ
Kamila KRUŽÍKOVÁ
Mojmír MACH
Zdeňka SVOBODOVÁ

Organised by

University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
Institute of Experimental Pharmacology & Toxicology, SASc., Bratislava, Slovakia
Section of Toxicology, Czech Society for Experimental and Clinical Pharmacology,
Czech Association J.E. Purkyně, Prague, Czech Republic
Slovak Toxicology Society SETOX, Bratislava, Slovakia

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We would like to express our appreciation and thanks to our sponsors

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Ing. Richard Vachta
Rybářské, hydrobiologické a vodohospodářské služby,
Výstavní 841
389 01 Vodňany

MONDAY – JUNE 1, 2009

13:00 – 13:45
OPENING SESSION

13:45 – 15:45
ALTERNATIVE METHODS IN TOXICOLOGY

DRUG METABOLIZING ENZYME ACTIVITY IN HUMAN IN VITRO DERMAL (EPIDERM™) AND AIRWAY (EPIAIRWAY™) EPITHELIAL MODELS: ALTERNATIVE (NON-ANIMAL) MODELS FOR DETERMINATION OF XENOBIOTIC METABOLISM

Hayden P., Bolmarcich J., Jackson G., Stolper G., Kandarova H., Klausner M.
MatTek Corporation, 200 Homer Avenue, Ashland, MA 01721, USA

IN VITRO MODELS OF ACUTE AND CHRONIC UV INDUCED DAMAGE AND PHOTOAGING OF PRIMARY HUMAN EPIDERMAL KERATINOCYTES AND DERMAL FIBROBLASTS

Hašová M., Dvořáková J., Velebný V., Kubala L.
CPN spol. s.r.o., 561 02 Dolní Dobrouč 401, Czech Republic

SKIN SENSITIZATION: LOCAL LYMPH NODE ASSAY(LLNA) AND ITS NON-RADIONUCLIDE ALTERNATIVES

Plodíková P., Brabníková Z., Koryntová I, Čihák R.
Research Institute for Organic Synthesis, Inc., Centre of Ecology, Toxicology and Analytics, GLP Testing Section, Rybitví 296, 533 54 Rybitví, Czech Republic

MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY IN ANIMAL RESEARCH: NON-INVASIVE DETECTION OF THE EVOLVING CEREBRAL INJURY AFTER HYPOXIC-ISCHEMIC INSULT TO THE BRAIN

Juránek I., Bačiak L.
Department of Biochemical Pharmacology, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

THE DYNAMICS OF GASTROINTESTINAL LESIONS INDUCED BY INDOMETHACIN (NON-SPECIFIC CYCLOOXYGENASE INHIBITOR): THE INTERSPECIES COMPARISON (LABORATORY RAT, EXPERIMENTAL PIG)

Květina J., Kuneš M., Herout V., Novotný L., Bureš J., Kopáčová M., Tachecí I.
Institute of Experimental Biopharmaceutics, Joint Research Centre of PRO.MED.CS Praha a.s. and Czech Academy of Sciences, Heyrovského 1207, 500 03 Hradec Králové, Czech Republic

CURRENT IMPROVEMENT OF CHOLINESTERASE BASED BIOSENSORS

Pohanka M., Drobík O., Křenková Z., Drtinová L., Kuča K., Žďárová-Karasová J.
Faculty of Military Health Sciences, University of Defence, Třebešská 1575, 500 02 Hradec Kralove, Czech Republic

COMPARISON OF EXTRACTION METHODS FOR SCREENING OF DRUGS AND DRUGS OF ABUSE IN BOVINE SERUM SAMPLES USING GC-MS

Marešová V., Chadt J., Nováková E., Holaj R., Švejdová Z., Těšínská H., Dvořáková Y., Formánková J.
Institute of Forensic Medicine and Toxicology, 1st Faculty of Medicine, Charles University and General Teaching Hospital in Prague Studničkova 4, 128 21 Praha, Czech Republic

MODIFICATION OF ALTERNATIVE BIOTEST AT *ARTEMIA FRANCISCANA*

Žďárský M., Dvořák P., Beňová K.
Department of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1- 3, 612 42 Brno, Czech Republic

15:45 – 16:00
COFEE BREAK

16:00 – 17:45
LEGISLATION, QSAR AND REACH

VALIDATION OF QSAR MODELS FOR LEGISLATIVE PURPOSES

Tichý M., Rucki M.
National Institute of Public Health, Centre of Public Health Laboratories, Predictive Toxicology Laboratory, Šrobárova 48, 100 42 Praha, Czech Republic

QSAR APPLICATION TOOLBOX

Rucki M, Tichý M.
National Institute of Public Health, Centre of Public Health Laboratorie, Predictive Toxicology Laboratory, Šrobárova 48, 100 42 Praha, Czech Republic

THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) TEST METHOD FOR IDENTIFYING OCULAR CORROSIVES AND SEVERE IRRITANTS

Brabníková Z, Dynterová A., Valášková R., Rösslerová Z., Čihák R.
Research Institute for Organic Synthesis, Inc., Centre of Ecology, Toxicology and Analytics GLP Testing Section, Rybitví 296, 533 54 Rybitví, Czech Republic

REACH – AN OVERVIEW.

Čihák R.
Research Institute for Organic Synthesis, Inc., Centre of Ecology, Toxicology and Analytics GLP Testing Section, Rybitví 296, 533 54 Rybitví, Czech Republic

SKIN IRRITATION/CORROSIVITY TESTING IN REACH FRAMEWORK

Täublová E.
Research Institute for Organic Synthesis, Inc., Centre of Ecology, Toxicology and Analytics GLP Testing Section, Rybitví 296, 533 54 Rybitví, Czech Republic

RELATION OF REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST TO REACH

Sadloňová I., Flaškárová E., Hózová R., Bačová H., Pätoprstá B., Múčková M.
hameln rds a.s. Modra, Department of Toxicology, Slovakia

PRECLINICAL RESEARCH OF DRUGS, A VIEW FROM PRACTICE

Šlais M.
BioTest s.r.o., Pod Zámkem 279, 28125 Konárovice, Czech Republic

18:00
DINNER

TUESDAY – JUNE 2, 2009

8:00 – 10:00
MOLECULAR, CELL, TISSUE AND ORGANISM MODELS IN TOXICOLOGY PART I.

SELECTED HORMONE INDUCIBLE TRANSCRIPTION FACTORS: *IN VITRO* EFFECTS OF VINCLOZOLIN, GENISTEIN AND BISPHENOL A

Brtko J., Macejová D., Ondková S., Fickova M., Laudet V.
Institute of Experimental Endocrinology, Slovak Academy of Science, Bratislava, Slovakia

ANTIOXIDANT AND GENOTOXIC ACTIVITY OF PLANT ESSENTIAL OILS

Navarová J., Horváthová E., Slameňová D.
Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

PHARMACOKINETICS OF PARA-METHOXYMETHAMPHETAMINE (PMMA) IN RATS AFTER S.C. APPLICATION

Rohanová M., Balíková M., Pálenícek T.
Charles University in Prague, 1st Faculty of Medicine, Kateřínská 32,121 08 Praha, Czech Republic

EFFECTS OF BORNEOL ON PRIMARY RAT HEPATOCYTES *IN VITRO* AND *EX VIVO*

Horváthová E., Slameňová D.
Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute, Slovak Academy of Sciences, Vlárska 7, 833 91 Bratislava, Slovakia

DISRUPTION OF DEVELOPMENTAL SIGNALING PATHWAYS BY TOXIC ORGANIC POLLUTANTS AFFECTING LIVER CELLS

Vondráček J., Procházková J., Bryja V., Richterová M., Kozubík A., Krčmář P., Machala M.
Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

H_1-ANTIHISTAMINES AND OXIDATIVE BURST OF PROFESSIONAL PHAGOCYTES

Nosáľ R., Drábiková K., Jančinová V., Králová J., Lojek A., Číž M., Mačičková T., Pečivová J.
Department of Cellular Pharmacology, Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská 9, 841 04 Bratislava, Slovakia

INTERACTIONS OF OXIDATIVELY MODIFIED CALF SKIN COLLAGEN WITH PLATELETS AND PHAGOCYTES

Číž M., Čížová H., Pejchalová K., Jančinová V., Goshev I., Mihaylova B., Nosáľ R., Lojek A.
Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

CYTOTOXICITY OF MISTLETOE (*VISCUM ALBUM* L.) ON JURKAT CELLS AND ITS INTERACTION WITH DOXORUBICIN

Sabová L., Sabo R., Mojžiš J.
Department of Pharmacy, Pharmacology and Toxicology, University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovakia

10:00 – 10:15
COFFEE BREAK

10:15 – 11:45
MOLECULAR, CELL, TISSUE AND ORGANISM MODELS IN TOXICOLOGY PART II.

CACO-2 CELLS AND BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) FOR PREDICTION OF TRANSEPITHELIAL TRANSPORT OF XENOBIOTICS (MODEL DRUG: CAFFEINE)

Smetanová L., Štětinová V., Kholová D., Květina J., Svoboda Z.
Institute of Experimental Biopharmaceutics, Joint Research Centre of the Academy of Sciences of the Czech Republic and PRO.MED.CS Praha a.s., Heyrovského 1207, 500 03 Hradec Králové, Czech Republic

THE EFFECT OF FLAVONOIDS AND THEIR DERIVATIVES ON OXIDIZED SERCA FROM RABBIT SKELETAL MUSCLE

Viskupičová J., Štrosová M., Horáková L.
Faculty of Chemical and Food Technology, Department of Nutrition and Food Assessment, Slovak University of Technology, Radlinského 9, 812 37, Bratislava, Slovakia

PSYCHOPHARMACOLOGICAL SCREENING OF THE PYRIDOINDOLE DERIVATIVES

Mach M., Ujházy E., Kovačovský P., Brucknerová I., Dubovický M.
Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

TERATOLOGICAL STUDY OF THE PYRIDOINDOLE ANTIOXIDANT SMe1EC2 IN RATS

Ujházy E., Dubovický M., Navarová J., Brucknerová I., Bezek Š., Mach M.
Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

THE STUDY OF EFFECTS OF SMe1EC2 ON ISOLATED RAT HEPATOCYTES OXIDATIVE INJURY

Bezek S., Kyseľová Z., Račková L., Navarová J.
Institute of Experimental Pharmacology & Toxicology Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

PERINATAL AND NEONATAL STRESSFUL STIMULI AFFECT NEUROENDOCRINE AND NEUROBEHAVIORAL DEVELOPMENT OF RAT´S OFFSPRING IN GENDER-DEPENDENT WAY

Dubovický M., Ujházy E., Ježová D.
Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

11:45 – 12:30
POSTER SECTION I.

12:30 – 13:30
LUNCH

13:30 – 14:00
POSTER SECTION II.

14:00 – 15:00
ANTICANCER DRUGS

FURTHER STUDIES ON THE ANTICANCER DRUG ELLIPTICINE ACTION
Stiborová M., Poljaková J., Eckschlager T., Frei E.
Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

A ROLE OF ANTICANCER DRUG ELLIPTICINE IN INDUCTION OF BIOTRANFORMATION ENZYMES
Aimová D., Poljaková J., Frei E., Stiborová M.
Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

SUGGESTED MECHANISMS OF PEROXIDASE RESISTANCE TO A COVALENT MODIFICATION (INACTIVATION) BY FREE-RADICAL METABOLITES
Martínek V., Florian J., Stiborová M.
Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

NOVEL ACRIDINE COMPOUNDS WITH ANTICANCER ACTIVITY ARE DNA LIGANDS AND MODULATORS OF GLUTATHIONE
Paulíková H., Kožurková M., Sabolová D., Imrich J., Vantová Z., Keltošová S., Plšíková J., Janovec L., Hamuláková S.
Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, Bratislava, Slovakia

15:00 – 15:45
ENVIRONMEMTAL TOXICOLOGY - PART I.

CHRONIC HEALTH IMPAIRMENT DUE TO 2,3,7,8-TETRACHLORO-DIBENZO-*P*-DIOXIN EXPOSURE
Pelclová D., Fenclová Z., Ridzoň P., Urban P., Preiss J., Dubská Z., Kupka K., Malík J., Vlček K., Navrátil T.
Department of Occupational Medicine, 1st Medical Faculty, Charles University and General Teaching Hospital, Na Bojišti 1, 120 00 Praha, Czech Republic

NEW MECHANISMS OF TOXICITY OF CYANOBACTERIAL TOXINS
Bláha L., Bártová K., Kohoutek J., Sychrová E., Šídlová T., Hilscherová K., Maršálek B.
Masaryk University, Faculty of Science, RECETOX (Research Centre for Environmental Chemistry and Ecotoxicology), Kamenice 3, 625 00 Brno, Czech Republic

MICROCYSTIN CONTENT IN FISH TISSUES IN SELECTED LOCALITIES OF THE CZECH REPUBLIC
Palíková M., Kopp R., Mareš J., Navrátil S., Bláha L.
Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

15:45 – 16:00
COFFEE BREAK

16:00 – 18:15
ENVIRONMEMTAL TOXICOLOGY - PART II.

SPECIFIC MECHANISMS OF TOXICITY OF COMPLEX CONTAMINANT MIXTURES IN RIVER ECOSYSTEMS

Hilscherová K., Bláha, L., Jálová, V., Jedličková, B.
Masaryk University, Faculty of Science, RECETOX (Research Centre for Environmental Chemistry and Ecotoxicology), Kamenice 3, 625 00 Brno, Czech Republic

HISTOPATHOLOGICAL EFFECTS OF CADMIUM ON *COREGONUS LAVARETUS* L. LARVAE

Wlasow T., Protasowicki M., Krawczak G.
Department of Ichthyology, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10 –719 Olsztyn, Poland

ECOTOXICOLOGICAL EFFECTES OF PHARMACEUTICALS PRESENT IN THE AQUATIC ENVIRONMENT

Li Z., Randak T.
University of South Bohemia České Budějovice, Research Institute of Fish Culture and Hydrobiology Vodňany, Zátiší 728/II, 398 25 Vodňany, Czech Republic

A REVIEW: OXIDATIVE STRESS IN FISH INDUCED BY PESTICIDES

Slaninová A., Smutná M., Svobodová Z.
Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

IS SEA FISH OIL A SAFE AND HEALTHY FOOD SUPPLEMENT?

Smutná M., Kružíková K., Maršálek P., Kopřiva V., Svobodová Z.
Department of Veterinary Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

METALLOTHIONEIS AND THEIR IMPORTANCE IN MONITORING OF LIVING ENVIRONMENT QUALITY

Adam V., Húska D., Hubálek J., Beklová M., Svobodová Z., Trnková L., Kizek R.
Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

ASSESSMENT OF OCCUPATIONAL EXPOSURE TO TOLUENEDIISOCYANATES (TDI)

Dušková Š., Stránský V., Kučera I., Dlouhá B., Mráz J.
National Institute of Public Health, Centre of Public Health Laboratories, Šrobárova 48, 100 42 Praha, Czech Republic

LUNG CANCER AND CHROMIUM EXPOSURE

Halašová E., Matáková T., Kavcová E., Mušák L., Adamkov M., Bukovská E.
Department of Medical Biology, Comenius University, Jessenius Faculty of Medicine, 036 45 Martin, Slovakia

PHYTOREMEDIATION OF HEAVY METALS

Adam V., Shestivska V., Kryštofová O., Diopan V., Zehnálek J., Havel L., Babula P., Kizek R.
Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

18:15 – 19:00
POSTER SECTION III.

19:30
SOCIAL EVENING

WEDNESDAY – JUNE 3, 2009

9:00 – 9:45
CYTOCHROMES

MODULATION OF CYTOCHROME P450 ENZYME SYSTEM BY FLAVONOID COMPOUNDS
Hodek P., Teplá M., Stiborová M.
Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 2030, 128 40 Praha, Czech Republic

COMPARISON OF THE "COCKTAIL APPROACH" WITH SINGLE MARKER ADMINISTRATION IN EVALUATING THE ACTIVITY OF P450 ENZYMES IN RATS
Juřica J., Tomandl J., Kýr M., McCaskey Hadašová E.
Department of Pharmacology, Faculty of Medicine, Masaryk University, Tomešova 12, 602 00 Brno, Czech Republic

STUDY ON MECHANISM OF CYTOCHROME b_5 EFFECTS ON CYTOCHROMES P450
Kotrbová V., Mrázová B., Frei E., Stiborová M.
Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

9:45 – 10:45
DIAGNOSTICS IN TOXICOLOGY - PART I.

INCIDENCE OF ANIMAL POISONING CASES IN THE CZECH REPUBLIC: CURRENT SITUATION
Modrá, H., Svobodová, Z.
Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

CANNABIS AND TRAFFIC RISK. FORENSIC ASSESSMENT OF BLOOD LEVELS OF DELTA-9-TETRAHYDROCANNABINOL IN DRIVERS
Balíková M.
Institute of Forensic Medicine and Toxicology, 1st Medical Faculty and Teaching Hospital, Charles University in Prague, Kateřinská 32, 121 08 Praha, Czech Republic

OBJECTIVE DIAGNOSTIC METHOD OF MUSCARINE INTOXICATION
Merová B., Stříbrný J., Ondra P., Staňková M., Válka I.
Department of Medical Chemistry and Biochemistry, Medical Faculty, Palacký University, Hněvotínská 3, 775 09, Olomouc, Czech Republic

10:45 – 11:00
COFFEE BREAK

11:00 – 11:30
DIAGNOSTICS IN TOXICOLOGY - PART II.

DETERMINATION OF IBOTENIC ACID AND MUSCIMOL IN THE SERUM AND URINE OF A PERSON INTOXICATED WITH *AMANITA PANTHERINA*

Stříbrný J., Sokol M., Merová B., Ondra P.
Military institute of Forensic Medicine, Central Military Hospital Prague, U vojenské nemocnice 1200,169 02 Praha, Czech Republic

LETHAL POISONING CASES FROM YEW TREE NEEDLESS

Staňková M., Bartoš P.
Institute of Forensic Medicine, University Hospital Ostrava, 17. listopadu 1790, 708 52 Ostrava-Poruba, Czech Republic

11:30 – 12:15
OCCUPATIONAL TOXICOLOGY

MONITORING OF 5-FLUOROURACIL IN A HOSPITAL PHARMACY

Opatřilová R., Synek S., Odráška P.
Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

OCCUPATIONAL EXPOSURE OF HEALTH CARE PROFESSIONALS TO ANTINEOPLASTIC AGENTS IN MASARYK MEMORIAL CANCER INSTITUTE, BRNO, CZECH REPUBLIC

Odráška P., Doležalová L., Gorná L., Prudilová M., Bláha L.
Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53 Brno, Czech Republic

OCCUPATIONAL EXPOSURE TO ANAESTHETICS AND CYTOSTATICS, CHROMOSOMAL ABERRATIONS, POLYMORPHISMS OF DNA REPAIR GENES *XPD, XPG, XPC*

Mušák Ľ., Poláková V., Halašová E.[1], Vodičková L., Valachová J.,
Osina O., Hudečková H., Buchancová J., Vodička P.
Department of Medical Biology, Jessenius Faculty of Medicine Comenius University in Martin, Malá hora 4, 037 54 Martin, Slovakia

12:15 – 12:45
GENOTOXICITY

IMPORTANCE OF TPMT GENE POLYMORPHISMS FOR PREDICTION OF AZATHIOPRINE TOXICITY

Kolorz M., Bartošová L., Hošek J., Dvořáčková D., Chýlkova A., Zbořil V., Bartoš M.
*Department of Human Pharmacology and Toxicology, Faculty of Pharmacy,
University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic*

GENOTOXICITY OF THE RADON

Osina, O., Mušák, Ľ., Buchancová, J., Valachová, J., Vičanová, M.
Clinic of Occupational Medicine and Toxicology, Comenius University, Jessenius Faculty of Medicine in Martin, Kollárova 2, 036 59 Martin, Slovak Republic

12:45
CLOSING SESSION & LUNCH

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Adam V., Húska D., Hubálek J., Havel L., Horna A., Trnková L., Kizek R.
Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

2 **MINIATURISED AND AUTOMATED DETECTION OF HEAVY METALS IN LIVING ENVIRONMENT**
Adam V., Kryštofová O., Hubálek J., Babula P., Trnková L., Kizek R.
Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

3 **ALLERGENICITY TESTING USING PLASMACYTOID DENDRITIC CELLS**
Ayehunie S., Snell M., Letašiová S., Klausner M.
MatTek Corporation, 200 Homer Avenue, Ashland, MA 01721, USA

4 **COMPARISON OF SENSITIVITY OF TESTS IN ECOLOGICAL ASSESSMENT OF SEDIMENT EXTRACTS FROM SMALL WATERWAYS IN SUBURBAN LANDSCAPE**
Beklová M., Čelechovská O., Dobšíková R., Králová H., Malá J., Modrá H., Soukupová I., Svobodová Z.
Department o Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

5 **THE COURSE OF ACUTE TOXICITY OF 44BU AND 444 STEREOISOMERS FOR LABORATORY RAT'S MYOCARDIUM**
Beránková K., Pobořilová Z., Bartošová L.
Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, 612 42 Brno, Czech Republic

6 **IMPACT OF SEDIMENT SAMPLES EXTRACTION METHODS ON RESULTS OF ECOTOXICOLOGICAL TESTS**
Beránková P., Kolářová J., Poláková S.
Research Institute of Fish Culture and Hydrobiology Vodňany, University of South Bohemia, Zátiší 728/II, 389 25 Vodňany, Czech Republic

7 **ASSESSMENT OF AQUATIC POLLUTION USING VITELLOGENIN AND 11-KETOTESTOSTERONE IN CHUB**
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Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

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Bořek-Dohalská L., Poljaková J., Rupertová M., Frei E., Stiborová M.
Department of Biochemistry, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

9 **MEVALONIC ACIDURIA AS A CAUSE OF CONJUGATED HYPERBILIRUBINAEMIA IN A CASE OF TERM NEWBORN**
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1st Department of Pediatrics, Medical School, Comenius University, Limbová 1, 833 40 Bratislava, Slovakia

10 **SEASONAL GLUTATHIONE DYNAMICS IN COMMON CARP (*CYPRINUS CARPIO)***

Bušová M., Opatřilová R.
Department of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

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Černá P., Kotyzová D., Eybl V.
Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University in Prague, Karlovarska 48, 301 66 Pilsen, Czech Republic

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Černá S., Hurbánková M., Piecková E., Kováčiková Z., Majorošová M.
Slovak Medical University, Limbová 14, 833 03 Bratislava, Slovakia

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Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia

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Čiernik, M., Hudec, R., Rybár, I, Kriška, M., I., Kyselovič[1], J.
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovakia

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

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

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Dovinová I., Gardlík R., Pálffy R., Kristek F., Čacányiová S., Vantová Z., Pavlíková H.
Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, Bratislava, Slovakia

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Drábiková K., Jančinová V., Perečko T., Jankovičová E., Nosáľ R., Račková L., Šmidrkal J., Harmatha J.
Institute of Experimental Pharmacology & Toxicology Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

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Fajt Z., Svoboda M., Odehnalová S., Drábek J.
Clinic of Pig Diseases, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3 612 42 Brno, Czech Republic

20 **ESTROGEN LIKE EFFECTS OF BISPHENOL A *IN VITRO***

Ficková M., Brezová A., Macho L., Mlynarčíková A.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, Bratislava, Slovakia

21 **EFFECT OF 10-DAY ORAL TREATMENT WITH THE NEW PYRIDOINDOLE ANTIOXIDANT SME1EC2 ON FUNCTIONAL DEFICITS IN RAT HIPPOCAMPUS EXPOSED TO ISCHAEMIA *IN VITRO***

Gáspárová Z., Šnirc V., Mach M., Ujházy E., Štolc S.
Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 01 Bratislava, Slovakia

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

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

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Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University in Prague, Karlovarská 48, 301 66 Pilsen, Czech Republic

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University of Defence, Institute od NBC Defence, Víta Nejedlého, 682 01 Vyškov, Czech Republic

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Hoštacká A., Čižnár I.
Slovak Medical University Bratislava, Limbova 12, 833 03 Bratislava, Slovakia

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Laboratory of Respiratory Toxicology, Slovak Medical University, Limbova 12, 833 03 Bratislava, Slovakia

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Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

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Center of Advanced Studies, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic

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MatTek Corporation, 200 Homer Avenue, Ashland, MA 01721, USA

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Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic

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Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65 Brno, Czech Republic

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Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic

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Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemědělská 1, 613 00 Brno, Czech Republic

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Department of Pharmacology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic

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

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Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

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Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

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Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 128 40 Praha, Czech Republic

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Toxicological Information Centre, Department of Occupational Medicine, General Teaching Hospital, 1st Medical Faculty, Charles University, Na Bojišti 1, 128 00 Praha, Czech Republic

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ABSTRACTS

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METALLOTHIONEIS AND THEIR IMPORTANCE IN MONITORING OF LIVING ENVIRONMENT QUALITY

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Normal function of cell depends on the stability and minimal changes of inner environment. The maintenance of inner environment of all organisms is fundamental for keeping of life. For this purpose, during evolution quite different regulation mechanisms have developed. Probably, one of the most important mechanism is maintenance of metals ions balance and thereby elimination of originating very harmful oxygen radicals. It is well know that especially thiol compounds (compounds containing -SH group(s) in their molecules) play in the area of cell protection essential role. Simple compound – protein glutathione (GSH) belongs to the group of very important thiol compounds. GSH is ubiquitous tripeptide (gamma-glutamylcysteynilglycine). It was proved, that its levels are increased in the presence (after incidence) of stress factors. In addition, very important is reciprocal rate between GSH and GSSG (oxidized glutathione), which determines range of oxidation state. For maintenance of heavy metals ions homeostasis, next important highly specialised molecule of the size 6–8 kDa (in the case of mammals) has importance. For its characterization, this molecule obtained name metallothionein. In animals, their occurrence is observed especially in tissues and organs like liver, kidneys, pancreas and intestine. Significant differences in MT concentrations in dependence on species, tissue type, feeding habits as well as age, ontogenetic state and next, no exactly known mechanisms and factors have been described. In no stressed cells, MT occurs probably in constant quantity (till now works confirming or contradicting this hypothesis do not exist). It is known, that metallothionein is the only naturally occurring compound significantly reducing metals concentrations in living organisms. Due to this fact, molecules of metallothionein are really the crucial, essential molecules in intracellular exchange of various essential as well as nonessential metal ions. Next, inconsiderable role of MT consists in its ability of participation in reactive oxygen species regulation and control. This fact is very intensively investigated especially in brain tissue. Thiol compounds are very important group of proteins occurring across the whole animal kingdom. Till now, their biological role is not wholly clarified. Regulation of essential metals ions level is their very significant role.

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UTILIZATION OF PARAMAGNETIC MICROPARTICLES FOR MONITORING OF STRESS GENES EXPRESSION

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Precise quantitative as well as qualitative mRNA isolation is one of the crucial moments for next molecular-biological study. Unambiguous aim is to identify the expression of genes, searching for specific sequence of nucleic acid as well as determination of point mutation. Nowadays, new technologies in the form of paramagnetic microparticles (MPs) come into laboratories. MPs enable isolation of molecules directly from homogenised cells or from solution, in which target molecules are present, without additional manipulation with sample. The principle of mRNA isolation is hybridization between nucleic acid anchored on MPs. Recent researches predicate, that exposition of organisms to stress (abiotic as well biotic) lead to increasing in expression of certain genes responsible for stress proteins biosynthesis. Quantitative analysis of total mRNA (transcriptome) can be suitable indicator of stress reaction and response.

In our work, we were aimed at mRNA isolation by the help of MPS. Isolated mRNA was quantitative analysed using electrochemical methods in different biological samples.

Primarily, we were focused on preparation of the surface of commercially available MPs before linkage of target molecule; this step consisted in washing. Volume of MPs was 10 μl; this quantity was three times washed by solution of 20 μl 0.1 M NaCl + 50 mM Na_2HPO_4 + NaH_2PO_4 (pH=7). To prepared MPs, samples together with hybridization solution enabling the optimal process of hybridization were added. This hybridization solution consisted of 0.5 M NaCl, 0.6 M guanidium thiocyanate, 0.15 M Tris-HCl (pH=7.5) and 0.05 M phosphate buffer (pH=7.5). Optimal time of hybridization was determined as 30 min. After optimization of the whole procedure,

we applied optimised method to real samples. We determined total mRNA (transcriptome) quantity in hydroponically cultivated plants of maize (*Zea mays* L., *Poaceae*), separately in roots and aerial parts during cultivation after 1 and 3 weeks and after treatment by cadmium(II) ions in concentrations 10, 50 and 100 μM. In addition, we studied effect of Cd(II) ions on total mRNA level in early somatic embryos of Norway spruce (*Picea abies* Karsten).

We determined that level of transcriptome changes during growth (ontogenetic development) as well as Cd(II) ions treatment.

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MINIATURISED AND AUTOMATED DETECTION OF HEAVY METALS IN LIVING ENVIRONMENT

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Heavy metal ions are natural components of Earth's crust. Their content in soil varies from very low (femtograms) to high (milligrams). However, due to anthropogenic activities, their content can be elevated in the site of the action. High concentrations of heavy metal ions can damage human health as well as contaminate living environment. Routine determination of trace metals in complex media is still difficult appeal for many analytical instruments.

The aim of this work was to utilize electrochemical instruments for easy and sensitive determination of heavy metals ions.

Instrument was connected with PC by the help of miniaturised cell with three electrodes. The three-electrode system consisted of carbon electrode as working electrode (carbon tips (Tosch, Japan)), miniaturized Ag/AgCl/3 M KCl reference electrode (CH Instruments, USA) and platinum auxiliary electrode. For smoothing and baseline correction, software PalmSens supplied by PalmSens was utilized. The supporting electrolyte – acetate buffer (0.2 M CH_3COOH + 0.2 M CH_3COONa) – was used. Primarily, we utilized the screen-printed electrodes connected with Biostat instrument for detection of cadmium(II) and lead(II) ions and compared obtained results with the result obtained by the help of standard electrochemical instrument Autolab with hanging mercury drop electrode as working electrode. The resulted calibration curves were strictly linear in cases of both potentiostats (R^2 higher than 0.99). Detection limits were lower than pM. Further, we also used micropotentiostat designed and fabricated by us. Cadmium and lead ions detections were carried out on screen-printed carbon electrodes by the use of cyclic voltammetry. Detection limits were 500 nM and/or 1 μM for cadmium(II) ions and/or lead ions, respectively. Micropotentiostat was tested in experiment with sunflower and maize plants treated by both metals in concentration range 0, 10, 50, 100 and 500 μM; cadmium(II) and lead(II) ions were subsequently quantified in extracts of roots and shoots. Moreover, we utilized PalmSens potentiostat with carbon tip as a working electrode for cadmium(II) and lead(II) ions detection. Detection limits were 50 nM and/or 500 nM for cadmium(II) and/or lead ions, respectively. We tested this potentiostat in analysis of polluted flour samples and waters.

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PHYTOREMEDIATION OF HEAVY METALS

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It is well known that heavy metals represent natural constituents of all ecosystems on the Earth. Above all, thanks to electrotechnic industry, released heavy metals ions came to be serious contaminating factor in living environment. Their physical-chemical properties have biologically important manifestations (cytotoxicity, mutagenesis, and carcinogenity). It is known that heavy metals ions cannot undergo a chemical degradation. They are taken up by living organisms and accumulated in their various parts and organs. In addition, with increasing trophic level of the organism, increasing content of heavy metals in organism is observable. All of these data lead to the searching of technologies focused on removal of these contaminants. Decontamination based on exploitation of contaminated soil is financially extremely expensive and cannot be uneventfully applied to extensive territories. Therefore, phytoremediation technologies come in on the foreground of interest. At phytoremediation, natural ability of plants to degrade or remove toxic compounds from contaminated living environment is utilized, no matter what soil or water. In addition, utilization of plants in this field is by public

accepted very positively and decontaminated area is able to improve aesthetic level of the countryside.

It is interesting that phytoremediation is utilized by human for about 300 years, but for the science is known only since the eighties. Conception of phytoremediation consists in cultivation of plants on afflicted, contaminated areas. Owing to their application, gradual decrease of toxic compounds content in polluted soils based on different mechanisms at decontamination by plants occurs. According to the type of pollution, suitable plant species are chosen. For the present, it is possible to say that phytoremediation is applicable only to moderate or medium serious pollution, because high concentrations of toxic compounds inhibit growth of also naturally resistant plants. For successful phytoremediation, bioavailability of contaminants to plants is very important. Plants can be utilized only for phytoremediation of upper parts of soil (to the 5 metres of depths), where root system of plants can reach. Also next factors, such as bioavailability of contaminant depending on its solubility in water, power of adsorption on soil particles, soil type and next have an effect on process of phytoremediation.

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A ROLE OF ANTICANCER DRUG ELLIPTICINE IN INDUCTION OF BIOTRANFORMATION ENZYMES

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Ellipticines are potent antineoplastic agents used in the therapy of breast cancer and leukemia. Ellipticine mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II and cytochrome P450 (CYP)- and/or peroxidase mediated formation of covalent DNA adducts. The formation of major DNA adducts is mediated by several CYPs, among them CYP3A, 1A and 1B1 are the most efficient to catalyze such reactions. Expression levels of these enzymes are, therefore, crucial for ellipticine pharmacological efficiencies.

The aim of this study was to evaluate the potential of ellipticine to influence the expression of CYP enzymes in two model systems: in rats, animals previously found to be suitable to mimic the fate of ellipticine in humans; and in MCF-7 cell line derived from human breast adenocarcinoma as an example of the target tissue of therapy.

Rats were treated *i.p.* with ellipticine in doses 4–80 mg per kg b.w., which are in the range of dosage in human therapy (80–100 $mg.m^{-2}$). MCF-7 cells were treated four weeks with 0.1 μM ellipticine and these and untreated cells exposed to 0.5 μM ellipticine during 24 or 48 hours. The protein content of CYPs was assessed using Western Blot analysis and the corresponding mRNA content by RT-PCR.

The treatment of rats with ellipticine resulted in strongly induced expression of CYP1A1/2 proteins and elevated CYP1A1 mRNA levels in several tissues. However, levels of CYP1A2 mRNA were unaffected. The CYP1A1/2 protein induction is strongly dependent on the dose of ellipticine administrated. An increase in hepatic CYP1A1/2 protein expression correlates with an increase in EROD activity, a marker for CYP1A1/2 and with the oxidation of Sudan I, a marker for the CYP1A1 activity. Ellipticine was found to be a potent inducer of CYP1A1 and 1B1 expression in human MCF-7 cancer cell line. These results show that CYP1A1 and 1B1 induction by ellipticine can occur in a target tissue of its human therapy, which suggest that a long treatment of humans with this drug might stimulate its own pharmacological efficiency.

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ALLERGENICITY TESTING USING PLASMACYTOID DENDRITIC CELLS

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An *in vitro* predictive test system for assessing the allergenicity potential of substances will have utility throughout industry to monitor products for contact allergenicity. Development of such non-animal alternative assay systems for skin sensitization hazard assessment is within the provisions of the European Union chemicals policy known as REACH (Registration, Evaluation, and Authorization of Chemicals).

We investigated whether phenotypic and functional changes to subset of dendritic cells (DC), plasmacytoid DC (pDC), could be used to identify allergens. To achieve this goal, normal human DC were generated from CD34+ progenitor cells and cryopreserved. Frozen DC were thawed and the pDC fraction (CD123+/CD11c-) was harvested using FACS sorting. The pDC were cultured, expanded, and pulsed with chemical allergens (n=13) or irritants (n=7). Sub-toxic concentrations of each chemical were determined using FACS analysis of propidium iodide stained cells.

Results showed that exposure of pDC (n=2–5 donors) to allergens induced an increased (≥ 1.5 fold) expression of CD86 for 12 of 13 allergens tested. On the other hand, 7 of 7 non-allergens did not result in increased CD86 expression. Based on these results, a preliminary prediction model was developed to identify chemical allergens (sensitivity=91–93% and specificity=93–100%).

In conclusion, CD86 expression in pDC appears to be a sensitive and specific predictor of allergenicity of chemicals. When compared with existing animal models, the assay is advantageous because high throughput screening of chemicals using cells of human origin is possible at low cost.

CANNABIS AND TRAFFIC RISK. FORENSIC ASSESSMENT OF BLOOD LEVELS OF DELTA-9-TETRAHYDROCANNABINOL IN DRIVERS

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Cannabis is one of the most abused drugs throughout the world (UNODC Report 2008). The results of controled experimental and epidemiological studies indicate an increased risk of crashes among drivers consuming cannabis. Delta-9-tetrahydrocannabinol (THC) impairs driving performance in a dose-related manner. The lipophilic properties of THC result in accumulation in fat tissues, where its slow redistribution into the blood stream controls its terminal elimination. It is difficult to correlate driver´s impairment with its THC blood concentration unambiguously. There is a significant time delay of the peak of euphoria related to the peak of THC blood concentration after smoking a marijuana cigarette. The blood THC level peaks within minutes before the end of smoking with subsequent rapid decrease whereas euphoric effects are graduating. Most physiological and behavioral effects return to baseline within 3–5 hours.

In forensic assessment it is important to differentiate impaired drivers from those who are no longer under the influence. Some theoretical models have been developed to estimate time of the last cannabis smoking to differentiate between recent and past consumption, frequent from occassional use. The determination of THC in the blood of occasional cannabis users indicate driving impairment, but in frequent cannabis consumers residual THC in the blood need not document cognitive and psychomotor impairment. Then the behavioral impairment need to be documented by a trained person and toxicological evidence of THC in blood in any amount enable to reveal the accident causation. Cannabis intoxication impairs safe driving and should be prohibited. Controversy remains on what the prohibited analytical THC blood limit should be.

The aim of this review presented should call attention to factors influencing driving performance at cannabis use and inform about international contemporary scientific discussion and conclusions. It may excacerbate necessary consideration and discussion on this important topic also among domestic public. The question how to fairly evaluate driving under influence of cannabis still remains open.

COMPARISON OF SENSITIVITY OF TESTS IN ECOLOGICAL ASSESSMENT OF SEDIMENT EXTRACTS FROM SMALL WATERWAYS IN SUBURBAN LANDSCAPE

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Ecotoxicological assessment of aqueous sediment extracts from selected sites along two small watercourses (Ivanovický potok and Černovický potok) passing through Brno city agglomeration (South Moravian Region, Czech Republic) was made as part of revitalization of small watercourses in suburban areas. Ivanovický potok passes through agricultural countryside. Černovický potok is a right-side tributary of Ivanovický potok.

Sediment samples were collected in spring and in autumn 2008. From sediment samples, standard aqueous extracts were made and used in ecotoxicological tests performed in accordance with methodology laid down in valid standards using organisms from the aquatic environment – the water flea *Daphnia magna,* freshwater green algae *Pseudokirchneriella subcapitata,* common duckweed *Lemna minor,* bacteria *Vibrio fischeri* and embryos of the African clawed frog *Xenopus laevis.*

Freshwater algal growth inhibition test showed an intensive inhibitory effect of aqueous extracts of sediments from the two creeks both in spring and in autumn. Extracts from Ivanovický potok sediments produced a 50% inhibition of algal growth both in spring ($72IC50_A$=45.5 $ml.l^{-1}$) and autumn ($72IC50_A$=98.1 $ml.l^{-1}$). Results of the *Daphnia* acute immobilization tests, on the other hand, were negative. Similarly, no bioluminescence inhibition was found in the *Vibrio fischeri* test. Extracts of sediments collected along the two creeks had stimulatory effects on vegetative growth of common duckweed. FETAX (*X. laevis*) showed a statistically significant embryo growth inhibition in almost all extracts. Increased mortality was found at Černovický potok just upstream its inflow to Ivanovický potok.

Concentrations of selected metals (Cd, Pb, Hg, As) and organic pollutants (non-polar extractive substances PCB_s, PAH_s and organochlorine insecticides) in aqueous sediment extracts were calculated. Results of ecotoxicological test were assessed in their relation to the results of chemical analyses of sediment extracts.

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THE COURSE OF ACUTE TOXICITY OF 44BU AND 444 STEREOISOMERS FOR LABORATORY RAT'S MYOCARDIUM

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The aim of this study was describing of progression of acute cardiotoxicity after intravenous administration of two newly synthesized ultrashort-acting beta-blockers

44Bu and 444 and its isomers. The *in vivo* experiment was performed at Wistar laboratory rats. The general anesthesia for rats was applied by administrating of 1% ketamin (Narkamon® inj. Spofa) and 2% xylazin (Rometar® inj. Spofa). Afterwards the animals were monitored by ECG Seiva Praktik Veterinary along the complete experiment. Tested substances were administered into dissected *vena jugularis* and the intoxication course was observed (arrhytmia occurences, ECG parametres changes and survival time).

For 44Bu compound we founded out significant displays of acute cardiotoxicity

at doses 4.5–5.5 $mg.kg^{-1}$, for 444 compound already at doses 3.5–4.5 $mg.kg^{-1}$. Considerable heart rhythm disorders occured in 30 seconds after *i.v.* administering of tested substance dose.

One of the first remarkable ECG changes was rapid extension of the negative S wave. The occurence of ventricular premature beats were characteristic – especially occurence of bigeminy and trigeminy. Ventricular arrhythmias of the type of Torsade de pointes, which were alternating with AV blocade of the II. and III. grade, were discovered. Sinus rhythm was changed into escape ventricular rhythm in consequence of the blockade of sino-atrial and atrio-ventricular conduction of the 1st degree, and later of the 2nd degree. Ventricular conduction was also affected. We observed widening of QRS complex.

From the point of view of the survival, the 444 compound was stronger. The average survival time in group 444 was 10 minutes, whereas in 44Bu group was 15 minutes. The similar heart rhythm disorders were proven in both stereoisomers.

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IMPACT OF SEDIMENT SAMPLES EXTRACTION METHODS ON RESULTS OF ECOTOXICOLOGICAL TESTS

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Freshwater sediments are contaminated with big amount of substances infiltrating water environment. And thanks to the small versatility of the sediments, they become longstanding reservoirs of these substances and so they are the part of aquatic ecosystems especially suitable for studies of long term pollution.

The aim of this study is to compare the impact of three most used extraction methods of sediment samples upon results of chosen ecotoxicological tests.

Tested samples were prepared by (1) shaking to water medium, (2) soxhlet extraction to DCM, and (3) compensation of DCM. Toxicity was tested with the help of *Daphnia magna*, *Sinapis alba*, and *Scenedesmus subspicata*. SOS-chromtest and induction of micronuclei in *Vicia faba* were used as genotoxicity tests.

The response in genotoxicity tests was significantly higher in extracts in DCM. On the other hand, thanks to low contaminant concentrations, water leach is less suitable for this kind of tests. The similar result is also in toxicity test. And more, extracts in DCM eliminate problem with small diffused particles passing through very fine filters.

It is obvious, that method of sample extraction from freshwater sediments can significantly influence final results of toxicity and genotoxicity tests. Comparing various methods, the most suitable one is the soxhlet extraction in DCM.

This study was supported by the Ministry of Education, Youh and Sports of the Czech Republic (MSM 6007665809) and the Ministry of the Environment of the Czech Republic SP/2e7/229/07.

THE STUDY OF EFFECTS OF SMe1EC2 ON ISOLATED RAT HEPATOCYTES OXIDATIVE INJURY.

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The role of oxidative stress, and accordingly uncontrolled reactive oxygen species generation, have been widely documented in many physiopathologies including neurodegenerative diseases, cancer, diabetes, cardiovascular and respiratory diseases, and in mechanisms of action of environmental toxicants. Tert-butylhydroperoxide (t-BHP) is an organic lipid hydroperoxide analogue, which is commonly used as a pro-oxidant for evaluating mechanisms involving oxidative stress in cells and tissues.

The purpose of this study was to investigate effects of the pyridoindole derivative SMe 1EC2 on isolated hepatocytes oxidative injury induced by t-BHP. The biological model used the isolated hepatocytes from male Wistar rats exposed for 1 h to increasing concentrations of t-BHP. Lactate dehydrogenase leakage (LDH) and Thiobarbituric acid reactive substances (TBARs) formation were determined as biomarkers of hepatocytes oxidative stress injury. Double sequential staining with acridine orange and ethidium bromide allowed cells

dying by necrosis, apoptotic cells, and living cells to be discriminated.

Under severe conditions of oxidative stress where there is a large excess of reactive oxygen species (ROS), cells die from necrosis, whereas under milder conditions, subnecrotic levels of ROS cause cell death mainly by apoptosis. Particular parameters measured showed that pretreatment with SMe1EC2 protected the hepatocytes against t-BHP induced oxidative injury.

In conclusion this novel substance tested seems to be promising agent for further studies relevant to ROS induced functional and structural impairment within cells and tissues corresponding with possible pathophysiological consequences.

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NEW MECHANISMS OF TOXICITY OF CYANOBACTERIAL TOXINS

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Pollution of aquatic ecosystems by nutrients such as phosphorus or nitrogen is a global environmental problem, which results in massive growth of cyanobacteria and development of water blooms. Recent studies document that not only known cyanobacterial toxins (such as peptide microcystins) but also other metabolites may pose significant risks for the environment and human health.

We have studied effects of major cyanobacteria dominating water blooms wordlwide (Microcystis aeruginosa, Aphanizomenon flos-aquae, Planktothrix agardhii and Cylindrospermopsis raciborski etc.) on several cellular mechanisms related to tumor promotion including activation of estrogen receptor (determined with luciferase reporter gene assay with MVLN cells) and two established markers of tumor promoting potencies of chemicals (i.e. inhibition of gap-junctional intercellular communication, GJIC, and activations of Mitogen-Activated Protein Kinases, MAPKs - ERK1 and ERK2).

Cyanobacterial samples upregulated ER-dependent transcription, downregulated GJIC and activated MAPKs. The effects were independent of the presence of known toxins microcystins. With the aim to identify and characterize responsible toxins, we have investigated effects of individual C18 fractions obtained from semipreparatory HPLC. Our study suggests that cyanobacteria are producers of yet unknown hazardous metabolites, which may represent serious health risks in surface drinking water supplies.

Undergoing research (supported by the National Science Foundation of the Czech Republic grant no. 524/08/0496) is focused on further characterization of causative agents and exploration of detailed mechanisms of toxicity.

ASSESSMENT OF AQUATIC POLLUTION USING VITELLOGENIN AND 11-KETOTESTOSTERONE IN CHUB

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The aquatic environment is continually exposed to an expanding range of various pollutants released by urban communities, industry and agriculture. Many of which can interfere with the physiological function of aquatic organisms and also act as endocrine disruptors. The influence on fish exposed to these chemicals can be studied using selected biochemical markers. An important biomarker used to detect fish exposure to estrogenic environmental endocrine disruptors is phospholipoglycoprotein vitellogenin, found in the blood plasma of juvenile and male fish. The natural inductor of hepatic vitellogenin synthesis is 17β–estradiol. However, many environmental estrogens also induce the synthesis of vitellogenin in both males and females. Some studies described that exposure of fish to endocrine disruptors changed the level of sex steroid hormones (e.g. 11–ketotestosterone).

The aim of the present study is to assess aquatic ecosystem contamination using selected biochemical markers – vitellogenin and 11-ketotestosterone in plasma of chub (*Leuciscus cephalus* L.). Seven locations situated upstream (Bílovice nad Svitavou, Kníničky) and downstream (the Svitava and Svratka rivers before junction, Modřice, Rajhradice, Židlochovice) from the Brno agglomeration (Czech Republic) were monitored in 2008. The results of biochemical markers were compared with the levels of the most important inductors of these biomarkers – persistent organic pollutants (HCH, HCB, DDT and its metabolites, PCB and PAH) in bottom sediment, fish muscle and semi-permeable membrane devices.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6215712402, 2B06093).

PRETREATMENT OF HUMAN BREAST ADENOCARCINOMA MCF-7 CELLS BY ELLIPTICINE POTENTIATES ITS OWN CYTOTOXICITY

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Ellipticine is an anticancer agent, which should be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dependent on its cytochrome P450 (CYP)- and/or peroxidase-mediated activation. We demonstrated a new mode of ellipticine action, formation of covalent DNA adducts mediated by its oxidation with these enzymes. Such DNA-adducts are formed *in vitro*, in human breast adenocarcinoma MCF-7, leukemia HL-60 and CCRF-CEM, neuroblastoma cell lines and *in vivo* in animals exposed to ellipticine.

The aim of our study was to investigate the effect of the pretreatment of MCF-7 cells with ellipticine on its cytotoxicity to these cells. We studied the mechanism of ellipticine action explaining its cytotoxicity to MCF-7 cells.

Toxicity of ellipticine to MCF-7 cells cultivated by two procedures [cells treated four weeks with 0.1 μM ellipticine and untreated (wild type) cells] and ellipticine-DNA adducts generated by 2.5 and 5 μM ellipticine in these cells were analyzed. Pretreatment of MCF-7 cells with 0.1 μM ellipticine led to an increase in its toxicity to these cells. The IC_{50} value of ellipticine for the control and the pretreated cells are 1.25 and 0.7 μM, respectively. The ellipticine-DNA adducts were generated in MCF-7 cells cultivated under both two conditions. A pattern of ellipticine-DNA adducts showed formation of two major adducts, identical to adducts derived from 13-hydroxy- or 12-hydroxyellipticine, metabolites formed from ellipticine by CYP enzymes of 3A, 1A and 1B1 or from metabolites generated by peroxidases. Expression of CYP1A1, 1B1 and 3A4 proteins in microsomes isolated from MCF-7 cells was proven with Western blot analysis. Higher levels of ellipticine-derived DNA adducts were found in MCF-7 cells pretreated with 0.1 μM ellipticine. An increase in levels of DNA adducts in MCF-7 cells by their pretreatment with ellipticine correlated with the increase in toxicity of ellipticine to MCF-7 and expression of CYP1B1 in these cancer cells. The results indicate that the pretreatment of MCF-7 cells with ellipticine increases its own pharmacological effect, which results from the increase in CYP-mediated formation of covalent DNA adducts as one of the mechanisms of ellipticine anticancer action.

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THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) TEST METHOD FOR IDENTIFYING OCULAR CORROSIVES AND SEVERE IRRITANTS

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BCOP method is an alternative testing method for ocular irritation/corrosion, development of which was recently seriously impeded by the reason of compliance with REACH requirements. The purpose of the BCOP test method implementation is effort to replace the testing on laboratory animals (rabbits) by *ex-vivo* system using the tissues from slaughtered cattle.

The BCOP test method is organotypic model that provides short-term maintenance of normal physiological and biochemical function of the bovine cornea *in vitro*. In this test method, damage by the test substance is assessed by quantitative measurements of changes in corneal opacity and permeability with an opacitometer and ultraviolet/visible spectrophotometer, respectively. Both measurements are used to calculate an IVIS (*in vitro* irritancy score), which is used to assign an *in vitro* irritancy hazard classification category for prediction of the *in vivo* ocular irritation potential of a test substance. The BCOP test method uses isolated corneas from the eyes of freshly slaughtered cattle. Corneal opacity is measured quantitatively as amount of sodium fluorescein dye that passes across the full thickness of the cornea, as detected in the medium in the posterior chamber. The substances are applied to the epithelial surface of the cornea by addition to the anterior chamber of the corneal holder.

Regional veterinary administration in Pardubice approved the exception to work with animal by-products, category 1 (bovine eyes) according to the EU regulation EC 1774/2002 (article 23) for VUOS a.s. for research purpose. Relevant documents (Project experiments, Technologic process – liquidation of animal by-products, Operating order – manipulation of animal by-products) for work with animal by-products were elaborated. A training period proceeds at our laboratory. During the training period the technicians are learned how to work with eye (e.g. dissect the cornea from bovine eye, identify corneas with defects).

While BCOP is not considered valid as a complete replacement for the *in vivo* rabbit eye test, the BCOP is recommended for use as part of a tiered-testing strategy for regulatory classification and labelling within a specific applicability domain.

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SELECTED HORMONE INDUCIBLE TRANSCRIPTION FACTORS: *IN VITRO* EFFECTS OF VINCLOZOLIN, GENISTEIN AND BISPHENOL A

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The present study was undertaken to investigate the *in vitro* effects of vinclozolin (VIN), genistein (GEN) and bisphenol A (BPA) on expression of nuclear thyroid hormone receptor subtypes (TRalpha, TRbeta), nuclear retinoic acid receptor subtypes (RARalpha, RARbeta, RARgamma), nuclear retinoid X receptor subtypes (RXRalpha, RXRbeta, RXRgamma), and nuclear vitamin D3 receptor (VDR) in MCF-7 cells treated with the above compounds at 1 micromol/l for 24 and 72 h.

The expression of the nuclear receptor subtypes has been analyzed by the RT-PCR technique.

TRalpha expression has been enhanced in MCF-7 cells by VIN after 24 h or 72 h treatment. Expression of TRbeta was increased by VIN when MCF-7 cells were treated for 72 h. VIN also enhanced expression of all RAR subtypes in the cells after 24 h treatment. On the other hand, 72 h treatment with VIN resulted in enhanced expression of RARgamma and RXRbeta and reduced expression of RXRalpha. Treatment of the cells by VIN has shown no effect on VDR expression. GEN decreased expression of both TRalpha and TRbeta when the cells were treated with the compound for 24 h. Also, this compound exerted enhanced TRalpha and TRbeta expression after 72 h treatment when compared to untreated cells. Treatment of the cells with GEN for 24 h did not affect expression of all RAR or RXR subtypes, however, it caused enhanced expression of RARbeta, RARgamma, RXRbeta after treatment of the cells for 72 h. GEN enhanced VDR expression when incubated with the cells for 72 h. We have also shown that TRalpha expression has been enhanced in MCF-7 cells by BPA after 72 h treatment. Similar enhancement of the TRbeta expression has been observed also after 72 h treatment of the cells by BPA. BPA also enhanced expression of RARbeta and RARgamma after 24 h treatment. We have found enhanced expression of all RAR subtypes and RXRbeta and also reduced expression of RXRalpha when treated for 72 h. BPA caused enhanced expression of VDR after 24 h treatment.

The results suggest that these compounds may play a different, but important role in modulation of various nuclear receptors expression in MCF-7 cells.

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MEVALONIC ACIDURIA AS A CAUSE OF CONJUGATED HYPERBILIRUBINAEMIA IN A CASE OF TERM NEWBORN

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Disorders of cholesterol biosynthesis form a heterogeneous group of diseases. Mevalonate kinase deficiency, is severe metabolic disorder which can manifests as severe mevalonic aciduria or as hyper-IgD syndrome with periodic fever.

The aim of this study was to present a clinical and laboratory findings of the term newborn case, with conjugated hyperbilirubinaemia and stress an attention to differential diagnosis.

This term newborn with absent asphyxial signs had a birth weight 2 550 grams, and an increased value of conjugated bilirubin and C-reactive protein in umbilical cord was detected. Diagnosis of mevalonic aciduria was confirmed by urine analysis (mevalonolactone 393 μmol/mmol crea, normal range < 2.0; mevalonic acid 40.5 μmol/mmol crea, normal range < 0.04).

Diferential diagnosis of conjugated hyperbilirubinaemia is often complicated. After examination of infectious or liver disturbances, the metabolic origin of disease has to be evaluated. Mevalonic aciduria can be distinguished clinically, based upon the signs of neurological involvement. It can be also associated with hepatosplenomegaly, lymphadenopathy, anemia, increased erythrocyte sedimentation rates and levels of C-reactive protein, leucocytosis. We showed that urine investigation for organic acids is crucial for patient with conjugated hyperbilirubinaemia of unknown etiology and it should be included in conventional examination strategy.

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SEASONAL GLUTATHIONE DYNAMICS IN COMMON CARP (*CYPRINUS CARPIO*)

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Environmental xenobiotics can invade the organism by various routes. In significantly reactive substances, barriers can be breached and the toxic substances enter

the organism. Detoxification mechanisms are being carried out in the living organisms of animals and plants are capable of preventing organism damage ant their survival. An example of detoxification method that xenobiotics use is a conjugation with glutathione. A change in the concentration of glutathione could signal a change in the water environment or in the quality of feed in the sense of xenobiotic stress on the fish organism.

The goal of our study was establishing the concentration of glutathione GSH in the hepatic/pancreatic system of the common carp. Samples were collected from one location of a fish-farming pond. The objective was to gain an overview of glutathione concentrations in the hepatic and pancreatic system in course of various year periods when fish display varying metabolic activity with regard to the climactic conditions and the season of the year.

Material for analysis was collected shortly after the fish were killed. The extracted hepatic/pancreatic system was then put in ice bath and stored in a freezer box. The content of GSH in the samples was established in a tissue homogenate after deproteinization using Ellman's method on the Cintra 20 UV-Visible Spectrometer device. The GHS concentration was calculated for the total protein count in the samples. The protein concentration was determined using Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich) using bovine serum albumin.

The average glutathione concentration that we detected and calculated for the total protein count were assessed in four sample intervals between October 2005 and September 2006. The average GSH concentrations calculated for the total protein count reached 1.03 μg GSH/g proteins in October, 1.56 μg GSH/g proteins in April, 1.14 μg GSH/g proteins in June and 2.06 μg GSH/g proteins in September. The lowest concentration was found in the hepatic/pancreatic system of an October 2005 common carp, very similar results were also found in samples from April and June. September samples were slightly higher. These values correspond with our expectations of relatively balanced GSH concentrations calculated for total protein count in samples originating from site. We do not consider a slight increase in the September GSH concentrations to be significant. It is a change by one log of microorganisms per one gram of proteins. This slight change corresponds with our expectation of the good condition of the carp population at the end of the productive period with fish in good condition after the period of intensive metabolism and food intake.

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EFFECT OF DEFERIPRONE ON TAMOXIFEN-INDUCED LIVER INJURY IN RATS

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Tamoxifen, nonsteroidal antiestrogenic drug, is widely used in treatment and prevention of hormone-dependent breast carcinoma. Its chronic use may induce a wide spectrum of hepatic lesions (hepatic steatosis, cancer, oxidative damage, iron overload). Deferiprone is orally active iron chelator taken by patients with β-thalassemia. Effective chelation therapy can prevent organ toxicity related to iron overload. The effect of deferiprone (DFP) on tamoxifen (TAM)-induced liver injury was investigated in rats.

Female Wistar rats (150–160g bw., Velaz Prague) were administered by DFP (prepared by Kontoghiorghes G.J.) in the dose of 50 mg/kg once daily for four days. On the 3rd and 4th day, tamoxifen (Tamoxifen Ebewe Pharma, Austria) was given in the dose of 75 mg/kg, always two hours after DFP dosage. Substances were administered intragastrically in 0.5% methylcellulose. At 24h after the last dose of TAM, animals were sacrificed and blood and liver tissue were collected for analyses. In serum, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST) and glutamate-dehydrogenase (GLDH) activities were estimated. The parameters of antioxidant state – lipid peroxidation (LP, measured as malondialdehyde production), reduced glutathione level (GSH), the activities of glutathione peroxidase (GPx) and catalase (CAT) were determined in the liver homogenates. The iron content was measured in the hepatic tissue using AAS.

TAM administration resulted in the increase of ALT activity (by 40%; $p<0.001$), the enhanced level of LP (by 30%; $p<0.05$) and the enhanced hepatic iron content (by 26%; $p<0.05$) compared to control group. Other parameters remained at control level. Treatment with DFP diminished the increase of LP level and completely prevented the increase of Fe content in comparison with TAM-treated group. The DFP treatment enhanced the ALT activity in comparison with TAM-treated animals. The DFP-only administration caused an increase in the CAT activity and a decrease in the activities of GPx and AST compared to controls.

The data suggest deferiprone as a potential hepatoprotective agent in tamoxifen-induced oxidative stress due to its chelating ability.

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EFFECT OF TOXINS OF ASPEGILLI AND PENICILIUM SP. ON CHOSEN CYTOTOXIC PARAMETERS OF RAT BRONCHOALVEOLAR LAVAGE

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Microfungi aspergilli and penicillia are the primary colonizers of mouldy buildings with strong toxic potential to cause ill health of the occupants, while the mechanisms of their action still remains unclear. The aim of this work was to examine the cytotoxic effect of endo – and exometabolites of these moulds on the respiratory tract *in vivo*.

Crude chloroform extracts of biomass (endometabolites – endo) and cultivation medium (exometabolites – exo) of aspergillus versicolor (asp.ver), aspergillus ustus (asp.ustus) and penicilium were dissolved in 0.2% dimethylsulfoxide (DMSO). Male Albino Wistar rats weighing 221.4±23 g were intratracheally instilled by 4 μg of examined metabolites dissolved in 0.2 ml of 0.2% DMSO. The control group was given only DMSO. After three day exposure the animals were exsanguinated in thiopental anaesthesia cutting the vena cava caudalis and the bronchoalveolar lavage (BAL) was performed. Activity of lactate dehydrogenase (LDH) , acid phosphatase (ACP) and cathepsin D (CATD) in cell-free BAL fluid (cfBALF) as well as the activities of ACP and CATD in cells s isolated from BALF (BAL cells) were used for assesment of the lung cytotoxicity of these fungal metabolites.

Activities of examined enzymes measured in cfBALF and BAL cells were higher after exposure to metabolites than after exposure to DMSO. Exposure to metabolites of asp.ver. caused significant increase of ACP and CATD in BAL cells. Changes in activities measured in cfBALF were not significant.. Effect of exposure to metabolites of asper.ustus and penicilium reflected in significant increase of activities measured in cfBALF (except of activity of LDH after exposure to exometabolites). The changes measured in BAL cells were not significant. There were no significant differences beween exposure to endo or exometabolites of asp.ver. and asp.ustus. Exposure to exometabotes of penicilium induced significantly hidher increase of ACP and CATD activities in cfBALF

Increased activities of LDH , ACP and CATD after exposure to examined endo and exometabolites of aspergillus versicolor, aspergillus ustus and penicilium point out on their cytotoxic effect on lung tissue and possibity to cause lung damage.

This research was supported by the grant Nr. 2005/36-SZU-14 by the Slovak Ministry of Health.

HOSPITALIZATION FOR GASTRIC AND DUODENAL ULCER IN RELATION TO CONSUMPTION OF ANTI-INFLAMMATORY DRUGS IN SLOVAK REPUBLIC IN 1996–2007

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A long-time therapy with non-steroid anti-inflammatory drugs (NSAIDs) often results to the gastrointestinal (GI) lesions. These GI lesions can be complicated by GI bleeding or perforation. Mucous abnormalities in GI tract connected by using NSAIDs are characterized with weak correlation among GI clinical symptoms and endoscopic finding. These facts oftentimes lead to the hospitalizations, which are the most serious complication of the NSAIDs therapy.

Our goal was to find out a development of hospitalizations for gastric (K25) and duodenal (K26) ulcer by ICD-10 and studied relation with consumption of anti-inflammatory drugs in Slovak republic in 1996–2007.

Analysis of incidence hospitalizations patients older than 15 years for diagnosis K25 (gastric ulcer), K26 (duodenal ulcer) in relation to consumption of anti-inflammatory drugs: ASA (drugs containing acetylsalicylic acid), NSAIDs (non-steroid anti-inflammatory drugs) and "gastroprotective" NSAIDs from National Health Information Center.

NSAIDs: indometacin, diclofenac, piroxicam, ibuprofen, naproxen, ketoprofen, flurbiprofen

"gastroprotective" NSAIDs: meloxicam, celecoxib, rofecoxib, valdecoxib, etorikoxib, nabumeton, nimesulid.

Results suggest that incidence for complicated gastric and duodenal ulcer in population of Slovak republic older than 15 years in 1996–2007 gradually decrease. This decreasing is prompted in both sex. In men (in K25: from 94 to 53 cases on 100 thousand men, in K26: from 129 to 50 cases on 100 thousand men) and in women (K25: from 57 to 35 cases on 100 thousand women, in K26: from 52 to 26 cases on 100 thousand women). Decreasing incidence of hospitalizations for gastric and duodenal ulcer correlate with decreasing of consumption ASA and also with increasing of consumption "gastroprotective" drugs ($p<0.01$). Simultaneously is necessary that here are also other factors, e.g. continuing eradication of Helicobacter pylori in Slovak population.

Following these results we can say, that incidence on gastric and duodenal ulcer declines. This trend correlate with decreasing of consumption ASA as well with increasing of consumption "gastroprotective" NSAIDs.

ANTI-INFLAMMATORY DRUGS CONSUMPTION DEVELOPMENT IN SLOVAK REPUBLIC IN 1996–2007.

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Non-steroid anti-inflammatory drugs are one of the most using group of all drugs, but gastrointestinal (GI) toxicity is dominating within them averse events. These unfavourable GI effects can befell any part of GI system, most often gastric and duodenal mucous, which are by p.o. application the most exposed areas.

Our goal was to find out development trends in the consumption of anti-inflammatory drugs in the Slovak republic in 1996–2007 from between the subgroups of drugs containing acetylsalicylic acid (ASA), non-steroid anti-inflammatory drugs (NSAIDs) and drugs containing selective non-steroid anti-inflammatory drugs ("gastroprotective" NSAIDs).

NSAIDs: indometacin, diclofenac, piroxicam, ibuprofen, naproxen, ketoprofen, flurbiprofen

"gastroprotective" NSAIDs: meloxicam, celecoxib, rofecoxib, valdecoxib, etorikoxib, nabumeton, nimesulid

The drug consumption data analysis from National Health Information Center and Statistical Office of the Slovak republic.

Upon the available data analysis, the consumption of drugs containing anti-inflammatory substances (calculated upon daily definition dose /1 000 inhabitants/ day) seems to have several phases of its development. The ASA subgroup shows continual decrease from 1996 (decreasing about 2/3, 9.990 vs. 3.353). We assume this is caused mainly by introduction of new anti-inflammatory drugs from NSAIDs subgroups and "gastroprotective" NSAIDs, as well as by the increased use of non-salicylic antiaggregants. The growth in the NSAIDs subgroup till 2002 should also be noticed, however since this year we have registered a progressive decrease of consumption of drugs from this subgroup. It appears to have been caused by the introduction of the COX-2 inhibitors on the market, but also by the increased consumption of so-called COX-2 preferential NSAIDs (the consumption of the drugs containing meloxicam has increased more than 5-times from 2004; 1.540 vs. 7.840; the consumption of the drugs containing nimesulid has increased even almost 6-times, 1.152 vs. 6.750).

NSAIDs belong to the drugs with the highest consumption, but also with a relatively high adverse events incidence, mainly gastrointestinal toxicity. A certainly positive trend of a progressive decrease in consumption of the most expressive, straight gastrointestinal irritative drugs as acetylsalicylic acid can be seen in everyday clinical practice, together with an increased consumption of "gastroprotective" NSAIDs.

INTERACTIONS OF OXIDATIVELY MODIFIED CALF SKIN COLLAGEN WITH PLATELETS AND PHAGOCYTES

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Oxidative modifications of collagen, a main component of extracellular matrix, may be induced by inflammatory processes during which reactive oxygen species are generated by phagocytes. The effects of non-modified and oxidatively modified calf skin collagen type I on platelet aggregation and oxidative burst of phagocytes were examined in the frame of general hypothesis that collagen, platelets and phagocytes cooperate to modulate the oxidative burst of phagocytes and the extent of oxidative stress.

Calf skin collagen type I (1 mg/ml, pH adjusted to 5.0–5.5) was subjected to the oxidative modification by hydrogen peroxide (100 mM, 300 mM) or hydroxyl radical (100mM $FeSO_4$/2mM H_2O_2, 50mM $FeSO_4$/5mM H_2O_2). Thermal denaturation of collagen was performed in a spectrophotometer equipped with temperature gradient device. The heating rate was 0.5°C/min within the range of 22–50°C. Aggregation of isolated human platelets obtained after differential centrifugation was measured using a dual-channel aggregometer. A production of reactive oxygen species by human whole blood phagocytes was evaluated by luminol-enhanced chemiluminescence. Spontaneous and opsonized zymosan particles-, calcium ionophore A23187-, phorbol-12-myristate-13-acetate- or N-formyl-Met-Leu-Phe-activated chemiluminescence of phagocytes was determined.

Oxidative modification of collagen samples was characterised by a decrease in denaturation transition temperature from 37.60°C (non-modified collagen) to 30.80°C and 31.52°C (hydroxyl radical modifications). The modification of collagen with hydrogen peroxide resulted in two denaturation transition temperatures. Oxidatively modified samples showed a modified SDS-PAGE pattern evidencing a significant destruction of the collagen. The non-modified collagen showed an aggregation behavior comparable with that of thrombin. All oxidatively modified collagen samples, independently of the oxidation treatment applied, lost their platelet-aggregating activity. Non-modified collagen itself had a capacity to induce a spontaneous oxidative burst of phagocytes. Oxidatively modified collagen samples lost

their capacity to induce the oxidative burst of phagocytes the changes being independent on the type of oxidative treatment. The effect of collagens on the oxidative burst of activated phagocytes was similar.

The results suggest that reactive oxygen species were able to modify collagen. On the other hand, oxidatively modified collagen lost its activating properties towards platelets and phagocytes.

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EFFECTS OF CYANOTOXINS ON AVIAN REPRODUCTION

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Blue-green algae are becoming an important problem due to the eutrophication of the aquatic environment and production of toxic secondary metabolites – cyanotoxins.

The present study was aimed at evaluating effects of the cyanobacterial biomass administered in feed on reproduction parameters of the Japanese quail (fertilization, hatchability, embryonal mortality, effect of hatching) as well as the toxicology of the chronic 60-day exposure of parent birds.

Effects of cyanobacterial biomass were evaluated using adult Japanese quails (*Coturnix coturnix japonica*) and the OECD method 206. Birds were randomly divided into reproductive pairs and placed into breeding cages at the age of two months. A total of 16 control and 16 experimental pairs (with 32 males and 32 females) were formed. Following a 14-day period of accommodation, the experiment lasting 8 weeks was started. Experimental animals were once a day supplied with a complete feeding mixture supplemented with the cyanobacterial biomass containing microcystins in such a concentration that is environmentally relevant under natural conditions. Control animals were fed the same mixture but without cyanobacteria. Eggs were collected once daily, marked, weighed, and kept under standard conditions until set into the hatching device once a week.

A total of 824 and 821 eggs were laid by control and experimental birds during the eight-week study period. Fertilization rates in control and experimental birds were 77.34±12.15% and 85.51±10.79%, respectively. Hatchability rates in control and cyanobacterial biomass exposed birds amounted to respective values of 83.24±12.61% and 90.07±9.32 while the overall hatching effect values were 65.19±17.74% and 77.73±15.15%. Statistically significant differences on the level of $p<0.05$ were found in fertilization rates and overall hatching effect values.

There was no mortality or any clinical signs of intoxication in cyanobacteria exposed as well as control birds during the experiment. Interestingly, reproduction parameters in experimental birds were better than in the control. We may hypothetize that there were some substances of xenoestrogenic characteristics in the complex cyanobacterial biomass. However, further research into these problems is necessary.

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COMPARISON OF THE SENSITIVITY OF DIFFERENT FISH SPECIES TO THE MEDICAL SUBSTANCES

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In aquaculture there are many substances used for fish treatment. Formaldehyde, sodium chloride and potassium permanganate belong to the most common and that's why we choose these substances for our study. The aim is to define their toxicity in two fish species – *Danio rerio* and *Poecilia reticulata*, and compare this toxicity. These two aquarium fish species are recommended to use as model organisms in toxicity tests. However, their sensitivity could be different.

Potassium permanganate ($KMnO_4$) is a crystalline violet compound. It is used worldwide in aquaculture for treatment and prevention of waterborne external parasitic, bacterial, and fungal diseases. Potassium permanganate is highly reactive under conditions found in the water industry. It will oxidize a wide variety of inorganic and organic substances. Formaldehyde is a colorless, highly flammable gas that is soluble in water. It is used as a fungicide in fish hatcheries to control fungal infections on fish and fish eggs and to control parasitic infestations on fish. Formaldehyde is included in carcinogenic substances. Sodium chloride is common and easy available antiparasitic compound, in fishery can be used to prevent nitrite toxicity in fish, as well. The toxicity of chemicals used to treat fish, as well as their therapeutically effectiveness, are influenced by water parameters such as salinity, pH and water hardness.

To determine acute toxicity of these compounds, the semistatic method according to OECD No. 203 (Fish acute toxicity test) was obeyed. Both zebrafish and guppies were exposed in progressive concentrations series of each substance. In each test series, 4 acute toxicity tests were performed, with 10 fish used for each concentration and for the control group. The results (number of dead fish in individual test concentrations)

were subjected to the probit analysis (EKO-TOX 5.1 software) to determine the 96hLC50 values. Statistical significance of the difference between LC50 values in guppies and zebrafish were evaluated by using the Unistat 5.1 programme.

Toxicity evaluation showed no difference in sensitivity between above mentioned aquarium fish species in any treatment substance.

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ANTIOXIDANT POTENTIAL IN THERAPY OF HYPERTENSION AND CARDIOVASCULAR DISEASES.

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In developed hypertension there are several changes in balance between antioxidant and radical system and in balance between NO- nitric oxide and ROS - reactive oxygen species. In hypertension there are many changes in expression and activity of NADPH oxidase, in main antioxidant enzymes such as superoxide dismutase 1, 2, 3, catalase and glutathione peroxidase as well as in nitric oxid synthase. Experimental studies shows that delivering of external antioxidants, antihypertensives or NO donors can stimulate antioxidative response (Dovinova et al 2009). Such response can be directly regulated throught antioxidant gene transfer or it can be also stimulated throught regulated excercise training. All these approaches lead to different reduction of blood pressure, improvement of endothel function throught eNOS and NO increase and to regression of organic damage of heart, kidney or blood vessels.

The main goals of cardioprotection and blood pressure regulation in hypertension was observed after gene transfer of some antioxidant genes as extraxellular SOD or after endothelial NOS and mitochondrial SOD applied to the region of blood pressure regulation in rostral ventrolateral medulla (*Chu 2003. Kung, 2008).*

Epidemiological studies show that regulated excercice training decreases morbidity and mortality of coronary arthery disease as well as chronic heart failure and/or ischemic heart injury. The regulated excercise affects cardioprotection, significantly reduce ischemic injury, and the effect is linked with changes in mitochondrial SOD activities. Such excercise also induces eNOS expression, SOD1 and SOD3 expression and decrease of NADPH oxidase (*Kojda et al 2005*). The aim of our presentation was to compare approaches in hypertension and cardiovascular treatment with different antioxidant potential.

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COMPARISON OF INHIBITORY ACTIVITY OF TWO SYNTHETIC COUMARINS ON OXIDANT PRODUCTION IN HUMAN NEUTROPHILS

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Neutrophils actively participate in oxidative stress-mediated inflammation and organ toxicity. Suppression of the extensive or inappropriate activation of neutrophils by drugs has been proposed as a way to ameliorate inflammatory diseases. At present, great attention is devoted to the important class of phenolic compounds – coumarins because of their protective effect against oxidative stress.

We compared the ability of two new synthetic coumarins, hydroxyphenyl-hydroxy-coumarin (HHC - 0.01, 0.1, 1, 10, 100 μmol/l) and hydroxyphenyl-hydroxy-dihydrocoumarin (HHDC- 0.01, 0.1, 1, 10, 100 μmol/l) to inhibit reactive oxygen species (ROS) generation in human neutrophils *in vitro*, with respect to some physicochemical characteristics. We tested their effect on phorbol-12-myristate acetate (PMA) stimulated oxidant production in human whole blood and differentiated their effect on extracellular and intracellular oxidant production in isolated neutrophils.

Using luminol-enhanced chemiluminescence (CL), both HHC and HHDC were found to decrease significantly (p<0.01) CL of whole blood stimulated with PMA (0.05 μmol/l) from the concentration of 1 μmol/l, with the effect of HHC being more intensive (58% inhibition of CL) than that of HHDC (24% inhibition of CL). Differences were detected also in isolated neutrophils. HHC and HHDC dose-dependently decreased extracellular ROS production, suggesting their protective effect against toxic tissue damage. On the other hand, intracellular oxidant production (involved in regulation of neutrophil function) was decreased only by HHC.

The different inhibitory effect of HHC and HHDC on human neutrophil ROS generation might be the result of their diverse free radical scavenging properties and lipophilicity features. The higher free radical reducing efficacy of HHC may be attributed to its higher value of parameter ε(HOMO) (–8.67eV, compared to

HHDC,–9.16eV) and more efficient stabilization of the generated phenoxyl radicals as documented by the respective maps of spin densities. The higher values of lipophilicity parameters of HHC (logP=2.755, R_M=0.52) compared to HHDC (logP=2.358, R_M=0.31) suggest a more efficient incorporation of HHC into the membrane than that of the less lipophilic HHDC, thus explaining its insignificant effect on intracellular ROS generation.

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PERINATAL AND NEONATAL STRESSFUL STIMULI AFFECT NEUROENDOCRINE AND NEUROBEHAVIORAL DEVELOPMENT OF RAT´S OFFSPRING IN GENDER-DEPENDENT WAY

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Early exposure to stressful stimuli has been found to affect brain and neurobehavioral and neuroendocrine development of offspring. Neurobehavioral alterations induced by early-stress exposure are manifested mostly by minor changes in behavior which may be gender-dependent. In rodents, exploratory behavior in the new environment was found to be gender-dependent showing higher locomotor activity, lower emotionality, and slower habituation in females compared to their male counterparts. The objective of the present study was to evaluate the effect of neonatal stress on habituation of locomotor activity in the open field both in male and female rats. Sprague-Dawley rat pups of both genders were exposed to combined stressful stimulus (handling, injection of hypertonic saline - 10% solution of NaCl and short-term separation from the mother) on postnatal days 2, 4, 6, 8, and 10. Adult rats were tested in an open field test during 4 consecutive days once daily in 6-min sessions. Between-session and within-session habituation were evaluated. Neonatal stress resulted in decreased habituation rates in males comparable to the levels of female habituation rates. Marked gender differences found in intact controls disappeared and males started to habituate female-characteristic way. On the contrary, females seemed to habituate like males, and their habituation showed certain signs of masculization. Although, the terms feminization and masculization are mostly related to effects of sex hormone administration, it can not be excluded that some behavioral variables could be feminized in males and masculinized in females due to influences operative during neurobehavioral development. Males proved to be more sensitive to neonatal stress and exhibited feminine-like habituation in the new environment. Results also indicate that early stress exposure can alter copying behavior in novelty in gender-dependant way.

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ASSESSMENT OF OCCUPATIONAL EXPOSURE TO TOLUENEDIISOCYANATES (TDI)

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Toluenediisocyanate (TDI, typically a mixture of 2,4- and 2,6-TDI isomers) is a reactive chemical used in the production of polyurethanes, elastomers, glues and varnishes. Nowadays, production of the polyurethane foam components for automotive industry predominantes. Due to severe immunogenic effects even at very low concentration, TDI is the most prevalent cause of occupational asthma in the Western world. Thus, monitoring of exposure to TDI is an important task of the occupational health services.

An array of methods for the assessment of exposure to TDI is available: determination in the workplace air to assess inhalation exposure, determination in the wipes from skin or worksite surfaces to assess dermal exposure (a cause of contact dermatitis), and biological monitoring to assess total exposure regardless of the route of entry to body. Biological monitoring is based on determination of toluenediamines (TDA) in urine and adducts with plasmatic proteins or globin in blood, the latter being used as biomarkers of cumulative exposure to TDI.

In the present study we adopted the above complex monitoring strategy in a plant manufacturing polyurethane foam car seat components using technical TDI (2,4- and 2,6-TDI, 80:20). Sampling of TDI in the workplace air was carried out on filters coated with 1-(2-pyridyl)piperazine, followed by HPLC/UV analysis of the resulting derivatives (OSHA Method 42). Occupational exposure limit (0.05 mg/m^3) was not exceeded in any of the 46 personal and 17 stationary samplings. Wipe sampling and analysis were conducted using coated glass filters and OSHA Method 42 as described above. TDI was detected in all 11 surface wipes and in 9 out of 15 palm or forearm skin wipes. In the biological monitoring, TDA liberated by acidic hydrolysis from urinary acetylderivatives or plasmatic adducts was extracted and converted to heptafluorobutyric derivatives to be determined by GC/MS/NCI. Several fold higher levels of urinary and plasmatic TDA were found in the exposed subjects compared to controls. Biological monitoring enabled to disclose hidden exposures to TDI in some job types.

EVALUATION OF SELENIUM STATUS IN PURE BRED DUROC SOWS AND THEIR PROGE

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Selenium (Se) is an important element in the antioxidant system of the human and animal body. It is involved in the active center of the enzyme glutathione peroxidase. The meat from pigs is the most important source of selenium for human.

The aim of this trial was to determine selenium status in pure bred duroc sows and their progeny and to compare it to Czech Large White×Landrace breed. The pregnant duroc sows (n=12) and pregnant Czech Large White×Landrace sows (n=12) were fed identical diets supplemented with sodium selenite. During lactation significantly higher serum Se concentrations were found in duroc sows and duroc piglets. Also significantly higher serum GSH-Px activities were found in lactating duroc sows and their piglets.

No differences in concentrations of Se in colostrums and milk were found between the two breeds. Our findings support the hypothesis that there may be breed differences in indices of selenium status in pigs.

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ESTROGEN LIKE EFFECTS OF BISPHENOL A *IN VITRO*

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Bisphenol A (BPA) is extensively used as the plasticiser in the manufacture of epoxy resins and polycarbonate plastics for products of daily use. Depolymerisation and subsequent release/leakage of BPA from the final products represent abundant source for human exposure. Due to structural similarity with estradiol (E2), BPA is designated as a xenoestrogene, but the precise mechanism(s) of estrogene like proliferative effects in cancer cells is not known. Simultaneous effects of endogenous E2 together with exogenous BPA on intracellular processes involved in proliferation of malignant cells have not been studied yet.

The aim of the present study was to investigate the effect of BPA alone and in the combination with E2 on gene and protein expression of estrogene receptor (Er) and transcription factor PPARα, regulating genes involved in cell cycle. The specificity of the effects were proved by E2 receptor antagonist, fulvestrant (FUL).

In vitro study consists of preincubation of mammary gland carcinoma cells (MCF7) in the medium without steroid hormones followed by 48 h incubation either with E2 and BPA (1×10^{-12} – 1×10^{-6} M) +/– fulvestrant (1×10^{-6} M). The combined effect was studied with the mixture of BPA (as above) and E2 (1×10^{-9} M). Protein expression was analysed by Western blott and appropriate antibodies, gene expression was determined by RT-PCR.

The effect of BPA on Erα protein expression as well as the inhibitory effect of fulvestrant was similar as for E2. Only the combination of low BPA concentration with E2 significantly reduced Erα protein. No differences in the expression of PPAR protein either by E2, BPA nor their combination were observed.

E2, BPA (1×10^{-9} M) and their mixture significantly reduced gene expression of both, Erα and PPARα. Fulvestrant by increasing the level of receptor mRNA antagonized the effects of E2 and BPA. Simultaneous effect of BPA (1×10^{-6} M) with E2 significantly reduced mRNA for PPARα (vs both E2 and BPA) and increased Erα gene expression (vs E2).

The alterations in protein expression induced by E2 and BPA do not result from the comparable changes of respective genes.

The results indicate additive effect of E2 in combination with low BPA concentration (1×10^{-12} M) on the expression of estrogene receptor protein in MCF7 cells.

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EFFECT OF 10-DAY ORAL TREATMENT WITH THE NEW PYRIDOINDOLE ANTIOXIDANT SMe1EC2 ON FUNCTIONAL DEFICITS IN RAT HIPPOCAMPUS EXPOSED TO ISCHAEMIA *IN VITRO*

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Major interest is currently focused on the development of new effective strategies for the pharmacological therapy of human stroke and cerebral ischaemia. New highly effective neuroprotective agents are being searched. Recently, the new substance 2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido-[4,3b] indolinium chloride with the code SMe1EC2, compared to its maternal pyridoindole antioxidant stobadine, showed enhanced anti-lipoperoxidation activity in rat brain homogenates exposed to Fe^{2+}/ascorbate system, decreased acute toxicity with successful elimination of alpha-adrenolytic activity, improved neuroprotective action in the mouse model of acute head trauma and increased neuroprotective action in rat hippocampal slices exposed to reversible hypoxia/hypoglycaemia. Further, a prenatal developmental toxicity study of SMe1EC2 showed its low toxicity and no embryotoxic and teratogenic effects on developing rats and no signs of maternal toxicity.

The aim of this work was to study the effect of orally applied SMe1EC2 at doses 50 and 250 mg/kg on rat

hippocampal resistance against oxidative stress induced later on.

In this study, 10-day oral treatment of rats with SMe1EC2 resulted in improved resistance of hippocampal neurons to transient 6-min hypoxia/hypoglycaemia *in vitro* compared to neuronal transmission failure in untreated control rats. The responses were determined by extracellular recording from hippocampal slices five days later, expressed by increased recovery of neuronal excitability and synaptic transmission in reoxygenation.

In conclusion, the current study confirmed the neuroprotective effect of the pyridoindole antioxidant SMe1EC2 applied orally, similarly as found previously, when the compound was applied into incubation medium 30 min before and during transient ischaemia/reoxygenation. We suggest that the compound SMe1EC2 exerts not only an acute neuroprotective effect but probably, thanks to its high lipophilicity, it may protect neuronal membranes against oxidative stress for a certain time after its oral application, resulting in improved recovery of neuronal function in the rat hippocampus exposed to oxidative stress.

This research was supported by the Slovak Grant Agency for Science VEGA (2/0083/08, 2/0093/08, 2/0083/09) and APVV Grant (51-017905).

LUNG CANCER AND CHROMIUM EXPOSURE

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The increased occurrence of lung cancer in residents of Dolny Kubin, the North-Slovakia district with ferrochromium industry, compared to the general population of Slovakia, led us to the study assessing influence of the occupational and environmantal exposure to chromium on the lung cancer incidence, respecting also the risk coming from cigarette smoking.

The data of the Department of Oncology of Dolny Kubin Hospital have been analyzed (574 men and 58 women diagnosed for lung cancer). The occurrence of lung cancer in both sexes was compared with the data of National Cancer Registry of Slovakia. The numbers of inhabitants in Dolný Kubín district in the period of analyzed years provided us the Statistical Institute in Martin. These data were nesessarz for rates calculation. Relative values were used to refer to the onset of the illness in analyzed group. The group of men was divided into 3 subgroups according to the level of exposure: Exp 0 included residents of the district Dolný Kubín not particularly exposed to chromium; Exp 1 were workers of ferrochromium Works not directly exposed to chromium; Exp 2 were workers of ferrochromium Works directly exposed to chromium (smelters, tapers, crane operators).

A significant difference was found in the percentage of lung cancer incidence between inhabitants of Dolný Kubín district and Slovak population. The relative risk of lung cancer in men (RR=1.26; CI 95%=1.13–1.32) and women (RR=0.67; CI 95%=0.53–0.78) differ between sexes indicating that lung cancer in our study area affects predominantly male population while RR in females is below the rate in the female Slovak population. The relative risks of all cancers were similar in both sexes (0.76; CI 95%=0.67–0.89 in male and 0.70; CI 95%=0.63–0.87 in female) below the values in the general Slovak population. The rate (75.2 per 100,000) of lung cancer in the subgroup Exp 0 is close to the rate of general Slovak male population (72.6 per 100,000). This rate in the subgroup Exp 1 was 112.5 per 100,000, which is 1.42 times higher than in non-exposed group. In the directly exposed subgroup Exp 2 the rate was 320.1 per 100,000, which is 4.04 times higher than in the non-exposed subgroup. In the group of non-exposed significant difference between the age at the onset of the disease was found between smokers (64.3 years) and non-smokers (67.7 years) (p=0.009). Non-exposed non-smokers had explicitly higher age at the onset of the illness in relation to other groups. In exposed groups (directly and indirectly) significant effect of smoking on the age at the diseases onset was not found (p=0.809 and 0.742, respectively). Small cell carcinoma (SCLC) forms 25.71% of all cases in chromium exposed workers and 16.34% in non exposed individuals.

Occupational exposure to chromium was identified as the main risk factor of lung cancer in Dolný Kubín district even overlaying effect of smoking. Higher percentage of SCLC was found in chromium exposed individuals.

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EFFECTS OF SUBCHRONIC EXPOSURE TO SUCCESSOR® 600 ON COMMON CARP *CYPRINUS CARPIO*

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Substances of anthropogenic origin often affect non-target fauna and flora. SUCCESSOR® 600 (pethoxamid 600 $g.L^{-1}$) is a herbicide formulation with potential to pollute the aquatic environment. The main goal of the present study was to examine its impacts to fish under subchronic conditions.

Exposure of juvenile common carp *Cyprinus carpio* to sublethal concentrations of SUCCESSOR® 600 (0.06; 0.22 and 0.60 mg. L^{-1}) was performed for 28 days. The effects on haematological and biometrical parameters were assessed. Biochemical indices in plasma were measured by biochemical analyzer. Endocrine-disruptive activity of the formulation was evaluated through vitellogenin concentration in male fish plasma, estimated by direct sandwich ELISA. Following indices were determined in liver (hepatopancreas) to reveal mechanism of pethoxamid detoxification in fish: concentration of cytochrome P450 (spectrophotometrically), ethoxyresorufin-O-deethylase activity (spectrofluorimetrically), glutathion content (according to the method of Ellman) and glutathion-S-transferase activity (spectrophotometrically). Histological changes in samples of hepatopancreas, skin, gills, spleen, head kidney and trunk kidney were examined by light microscopy.

Our findings may help to understand the potential risks associated with herbicide exposure in fish organisms.

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CONTENS OF PHTHALIC ACID ESTERS IN FEEDSTUFFS AND THEIR PACKAGES

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Phthalates cause environmental contamination and they migrate from e.g. packaging materials. Phthalates are animal carcinogens and may cause death or tissue deformities. They may compromise liver functions and cause reproduction toxicity. Owing to their lipophilic nature, phthalates are released mainly into foodstuffs containing fat. Feeds became a part of a food chain according to Regulation No.: 178/2003. The aim of this study was monitoring of phthalic acid esters as di-n-butyl phthalate (DBP) and as di-2-ethylhexyl phthalate (DEHP) in the feed chain due to virtually no data on phthalate contamination of products intended for feeding farm animals.

Samples of feed additives and premixtures (n=25) and others feeds (n=13) and their packages (n=38) were taken from the industrial producers in the Czech Republic. Packages consist of only plastic material (n=18); plastic material and paper (n=14); plastic material and aluminium (n=5) and only aluminium (n=1). The concentrations of phthalic acid esters as DBP and DEHP were measured in samples of feeds and their packages. Procedure for the determination of DBP and DEHP consisted, separation of analytes from co-extracts using gel permeation chromatography in Bio-beads S-X3 gel, clean up of extracts or eluates with sulphuric acid and detection and quantification by HPLC determination.

The highest/the lowest concentrations as a sum of DBP and DEHP of feeds were detected in feeding material rape oil 55.76 $mg.kg^{-1}$ / in additive Vitamin A No. 2 (0.06 $mg.kg^{-1}$) respectively. The average contents of DBP and DEHP in feeds was 3.82 $mg.kg^{-1}$. The highest concentration as a sum of DBP and DEHP of packages was detected in additive Vitamin E – No. 1 (526.80 $mg.kg^{-1}$). The lowest concentration as a sum of DBP and DEHP of packaging was detected in additive in additive Cholinchlorid (1.54 $mg.kg^{-1}$). The average content as a sum of DBP and DEHP was 97.13 $mg.kg^{-1}$.

There was no evident relationship between the amount of DBP and DEHP and between feed and packages ($p<0.05$).

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IN VITRO MODELS OF ACUTE AND CHRONIC UV INDUCED DAMAGE AND PHOTOAGING OF PRIMARY HUMAN EPIDERMAL KERATINOCYTES AND DERMAL FIBROBLASTS

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Skin exposure to ultraviolet (UV) component of sunlight induces wide range of detrimental effects including sunburn, premature skin aging (photoaging), immunosuppression, and cancer. The aim of this study was to characterize models of acute UV irradiation of human primary keratinocytes and chronic UV irradiation of human primary fibroblasts.

Cells were irradiated by single or repeated doses of UV and effects on cell proliferation, gene expression of Bcl-2, c-jun, matrix metalloproteinases (MMPs), and release of interleukin 1α (IL-1α), IL-6, IL-8, and transforming growth factor (TGF)-β1 into the cell culture media were evaluated.

Doses of UV light 5 mJ/cm^2 and 10 mJ/cm^2 for acute single exposure of cells and doses of UV light 1.5 mJ/cm^2 and 3 mJ/cm^2 for ten repeated exposures of cells were selected based on cell viability to attain at least 50 percent viable cells up to 48 h after single exposure and 72 h after the last tenth exposure. The acute single exposure to UV irradiation increased the expression of c-jun and MMP-1 and down regulated the expression of Bcl-2 together with an induction of IL-1α, IL-6 and IL-8 release by keratinocytes. The repeated irradiation of fibroblasts by UV induced a release of IL-8 and TGF-β1 which was more significant by repeated exposures of fibroblasts to UV dose 3 mJ/cm^2.

The results indicate that the acute UV irradiation of human primary epidermal keratinocytes and the repeated UV irradiation of human primary dermal fibroblasts may be employed to explore the patophysiology of UV effects on human skin and to evaluate photoprotective properties of various substances.

SELECTED BIOCHEMICAL MARKERS USED IN THE ASSESSMENT OF AQUATIC ENVIRONMENT CONTAMINATION (THE SVRATKA AND SVITAVA RIVERS)

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Biochemical markers can be used for the assessment of aquatic environment quality. Levels of the biomarkers reflect the exposition of organisms to contaminants in the environment. Enzymes of phases I and II of xenobiotics biotransformation [cytochrome P450, ethoxyresorufin-*O*-deethylase (EROD), glutathione S-transferase (GST) and tripeptide glutathione (GSH)] present useful biomarkers widely used for the pollution assessment by organic pollutants in the aquatic environment.

The aim of the study was to assess aquatic ecosystem contamination using the biochemical markers cytochrome P450, EROD, GST and GSH in the liver of chub (*Leuciscus cephalus* L.). Seven locations situated on the Svitava and Svratka rivers (in the Brno conurbation, Czech Republic) were assessed. The results of biochemical monitoring will be compared with the levels of the most important inductors of named biomarkers - organic pollutants: hexachlorocyclohexane, hexachlorobenzene, DDT and its metabolites, polychlorinated biphenyls, polycyclic aromatic hydrocarbons determined in bottom sediment, in fish muscle, in semi-permeable membrane devices (SPMDs) and in polar organic chemical integrative samplers (POCIS).

Cytochrome P450, GST activity and GSH were determined spectrophotometrically while EROD activity was determined spectrofluorometrically. Content of organic pollutants were determined by gass chromatography – mass spectrometry or by high performance liquid chromatography using a fluorescence detector.

The highest levels of cytochrome P450, EROD activity and GST activity were observed at Židlochovice (0.346±0.076 nmol/mg protein, 501.17±90.79 pmol/min/mg protein, 141.26±19.79 nmol/min/mg protein, respectively). The lowest levels of cytochrome P450 and EROD activity were observed at Knínič ky (0.111±0.048 nmol/mg protein, 162.29±72.92 pmol/min/mg protein, respectively). Concerning results of GSH determination, the highest level was revealed at Svratka pod Brnem (4.29±0.91 nmol/mg protein) and the lowest one was observed at Bílovice nad Svitavou (3.17±0.62 nmol/mg protein). Correlations will be added between results of biochemical markers in fish liver and the organic pollutants in fish muscle, bottom sediment, SPMDs and POCIS.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM 6215712402).

DRUG METABOLIZING ENZYME ACTIVITY IN HUMAN *IN VITRO* DERMAL (EPIDERM™) AND AIRWAY (EPIAIRWAY™) EPITHELIAL MODELS: ALTERNATIVE (NON- ANIMAL) MODELS FOR DETERMINATION OF XENOBIOTIC METABOLISM

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Human dermal and airway epithelia contain xenobiotic metabolizing enzymes (XME) that can cause biotransformation of drugs and environmental/occupational chemicals, resulting in altered drug activity or formation of toxic/mutagenic metabolites.

The present work evaluated expression of XMEs in highly differentiated *in vitro* models of human dermal (EpiDerm) and airway (EpiAirway) epithelia. RT-PCR experiments were conducted to evaluate baseline and inducible expression of cytochrome P450 (CYP) isoforms in the epithelial cultures.

EpiAirway cultures constitutively expressed CYP1A1 (weak), CYP1B1, CYP2A6, CYP2B6 (weak), CYP2C8 (weak), CYP2C19, CYP2D6, CYP2E1 and CYP3A5, while CYP3A4 and 3A7 were not detected. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and slightly increased CYP2B6 and CYP2C8 expression in EpiAirway. In EpiDerm, CYP1B1, CYP2C19, CYP2D6, CYP3A4 (weak) and CYP3A5 were constitutively expressed. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and CYP1B1 in EpiDerm. Enhanced metabolism of the CYP1A1 and CYP1B1 substrate ethoxyresorufin confirmed increased activity following treatment with 3MC. Thus CYP expression in EpiAirway and EpiDerm showed a high concordance with CYP expression reported for *in vivo* human airway and dermal epithelia. Total Glutathione S-transferase (GST) activity in the epithelial models was also evaluated by measuring conjugation of glutathione with 1-chloro-2,4-dinitrobenzene and UDP-Glucuronyltransferase activity was determined by 4-methylumbellipherone conjugation. High baseline GST and UDPglucuronyltransferase activity in both models was not further enhanced by 3MC treatment.

The results demonstrate that the EpiDerm and EpiAirway *in vitro* human epithelial models possess *in vivo*-like XME activities and may thus be useful for evaluating epithelial metabolism of drugs and environmental/occupational chemicals.

SPECIFIC MECHANISMS OF TOXICITY OF COMPLEX CONTAMINANT MIXTURES IN RIVER ECOSYSTEMS

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Evaluation of the potential effects of contaminants and their risk assessment in aquatic environment is complicated by their presence in very complex mixtures. Specific problem for risk assessment represents the contamination of river ecosystems, which are by nature rather dynamic, especially when we are concerned with contamination of water and surface sediments from existing primary and secondary sources. Bioassay assessment provides important information since it can integrate the toxic potency of the present pollutants taking into account also their interactions in the complex mixture. The *in vitro* cell bioassays determine contamination by pollutants that act through specific important modes of action. Our studies document significant dioxin-like and endocrine disruptive (anti/estrogenic, anti/androgenic) activity, but also the overall non-specific cytotoxicity in various types of samples from waste water and river ecosystems in the Czech Republic, which are not always clearly linked to the measured contaminant concentrations.

The bioassay approach can serve as an effective screening system to identify samples of interest and provide basic information for further risk evaluation. These bioanalytical approaches provide significant information regarding the biological relevance of mixtures of compounds associated with environmental samples. Complex view (pattern) of possible adverse effects is obtained when applying a series of methods. The results document the advantage and utility of the simultaneous use of bioassays and chemical analysis in risk assessment of complex environmental samples.

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MODULATION OF CYTOCHROME P450 ENZYME SYSTEM BY FLAVONOID COMPOUNDS

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Flavonoids are well known dietary chemicals present in fruits, plants and beverages derived from plants, and in supplements. These compounds belong to popular chemopreventive compounds exerting a great variety of beneficial effects on human health. Their biological activities arise mainly from their antioxidant properties and abilities to modulate several enzymes or cell receptors. Although flavonoids are often considered to be safe because of their "plant origin", ingestion of flavonoids should be taken with caution. Some flavonoids have mutagenic (e.g. quercetin) and/or prooxidant effects, as well as interfere with essential biochemical pathways. Among proteins interacting with flavonoids, cytochromes P450 (CYPs), monooxygenases metabolizing xenobiotics (e.g. drugs, carcinogens) and endogenous substrates (e.g. steroids), play the most prominent role [1]. Flavonoids might inhibit or stimulate activity of several CYPs, and/or induce an expression of certain CYPs. Owing to the structure similarity with estrogen skeleton, certain flavonoids show an estrogenic or anti-estrogenic activity. Like natural estrogens, they bind to estrogen receptor and modulate its activity and/or block CYP19, crucial enzyme of estrogen biosynthesis. Flavonoids effectively inhibit activation of particular carcinogen, however, at the same time they induce CYPs activating the other carcinogen. Hence, detailed study on the flavonoid interactions with CYP multi-enzyme system, namely from the view of the modulation of CYP activity would be helpful in preventing the carcinogen activation and explaining changes in drug metabolism.

α-Naphthoflavone is frequently used as an inhibitor of CYP1A1/2-mediated activation of carcinogens. In our recent experiments this flavonoid stimulated metabolic activation of aristolochic acid I (AAI) into intermediates covalently binding DNA. ^{32}P-Postlabeling technique proved 8 fold increase in DNA adduct formation in the presence of 10 μmol/l α-naphthoflavone [2]. This increase in activation of AAI was attributed to the stimulation of NADPH:CYP reductase, which metabolizes AAI in renal microsomes, tissue expressing low levels of CYPs. These data raised several questions: **i**) whether the stimulatory effect is specific to AAI only, **ii**) how effective are other flavonoids, **iii**) what is the impact on CYP-mediated metabolic activities, and **iv**) what is the mechanism behind the stimulation? In summary, the stimulation of reductase activity via α-naphthoflavone was found also with different electron acceptors (e.g. cytochrome c, potassium ferricyanide) than AAI. Surprisingly, β-naphthoflavone, position isomer, was inefficient in the reductase stimulation, as well as other flavonoids tested. α-Naphthoflavone did not significantly enhanced reaction rate of CYP-mediated monooxygenation of substrates. The nature of the stimulatory effect of NADPH:CYP reductase remains unclear.

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THE EFFECT CF SOME SELECTED CARCINOGENIC METALS ON THE ACTIVITY OF SELENOENZYMES IN THE EXPERIMENT IN RATS

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Selenoenzymes-thioredoxin reductase (TrxR) and glutathione peroxidase (GPx) are important components of the antioxidant defense system of the oganism. In this study the effect of selected carcinogenic metals-Cd, Cr, Ni and FeIII on the activity of those enzymes has been studied in the acute experiment in rats. To the best knowledge of authors there is no comparative study on this topic in the literature.

The experiments were performed in male Wistar rats (130–140g b.w.) divided into 5 groups of 8 animals. The metal compounds were administered *ip* in a single dose equimolar to $CdCl_2.2\ 1/2H_2O$ (4 mg/kg b.w.). Design of the experiment: I–control, II–$CdCl_2.21/2H_2O$, III–$K_2Cr_2O_7$, IV–$NiCl_2$, V–Ferric gluconate. The experiment was finished 24h after the administration of metal compounds. The animals were sacrificed by decapitation and the activity of cytosolic enzymes TrxR I and GPx was determined in the liver, kidneys and brain homogenates. For the determination of TrxR I activity was used the commercial kit (Sigma). The activity of GPx was determined as previously (*V.Eybl et al., Toxicology 2006; 225:150-156*).

The activity of TrxR I in the liver was decreased by Ni (by 8.2%, $p<0.05$) and increased by FeIII (by 12.3%, $p<0.01$). In the kidneys Cd, Cr, and Ni increased the activity of this enzyme by 40.5%, 54.2% and 37.8% respectively ($p<0.01$). Ni and FeIII increased the activity of TrxR I in the brain by 89.2% and 136.5% respectively ($p<0.01$). The activity of GPx in the liver was decreased by Cd (by 9.8%, $p<0.01$) and increased by Ni (by 3.8%, $p<0.05$). In the kidneys the inhibitory effect of Cr and Ni on GPx activity was seen (by 8.3% and 8.5%, $p<0.01$). In the brain inhibitory effect of Cr (by 15.0%, $p<0.05$), Ni (by 25.2%, $p<0.01$) and FeIII (by 15.3%, $p<0.01$) on this enzyme was demonstrated.

The diference in the effect of metals on both selenoemzymes may be caused by different affinity of the metals to those enzymes but also by different kinetics of metals.

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EFFECTS OF BORNEOL ON PRIMARY RAT HEPATOCYTES *IN VITRO* AND *EX VIVO*

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Hundreds of components of essential oils have been investigated with the aim of their possible utilization in different fields of human life. Although biological activity of these natural compounds used in folk and complementary medicine has been known for centuries, their mode of action remains often unclear. For this, the essential oils and their components have been screened for their activities. Borneol, a bicyclic monoterpenoid alcohol, is widely present in the environment as a component of numerous medicinal plants. Interestingly, borneol has been used by many Asian cultures in folk remedies for various purposes, such as the treatment of pains, injuries, burns, ulcerations, skin diseases, haemorrhoids, and in aromatherapy.

The aim of our study was to evaluate genotoxic effects of borneol and its ability to modulate DNA-damaging effects of the model free-radical-generating compound hydrogen peroxide (H_2O_2) in hepatocytes. Primary rat hepatocytes were chosen because the liver is the main organ for the metabolism of foreign compounds and thus represent a suitable system for the evaluation of different influences. Both *in vitro* (treatment of isolated hepatocytes with borneol on Petri dishes) and *ex vivo* (isolation of hepatocytes from borneol-supplemented rats) approaches were used.

Cytotoxicity testing was performed on the basis of trypan blue exclusion. As a measure of genotoxicity, the percentages of DNA in tails of comets by single cell gel electrophoresis were evaluated.

Cytotoxicity of borneol increased in *in vitro* conditions in a concentration-dependent manner and it was associated with DNA-damaging effects at toxic concentrations. While non-toxic concentrations of borneol applied *in vitro* protected cells against H_2O_2-induced DNA damage, cytotoxic concentrations of borneol manifested synergy with H_2O_2, i.e. enhanced DNA-damaging effects of H_2O_2. On the other side, borneol given to rats in drinking water decreased the level of DNA damage induced by H_2O_2 in isolated hepatocytes.

Based on the DNA-protective effects of low concentrations of borneol against DNA-damaging activity of hydrogen peroxide *in vitro* and *ex vivo*, we conclude that at low concentrations borneol represents a component of plant essential oils contributing to the defence mechanisms of organisms against cancer and other civilisation diseases.

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EFFICACY OF STRUCTURAL HOMOLOQUES AND ISOMERS OF PRALIDOXIME IN REACTIVATION OF IMMOBILISED ACETYLCHOLINESTERASE INHIBITED WITH SARIN, CYCLOSARIN AND SOMAN

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The article deals with quantification of efficiency of monopyridinium isomers and homologs derived from clinical used pralidoxim within reactivation of acetylcholinesterase inhibited with organophosphorus nerve agents. This work uses the colorimetric biosensor called Detehit. Biosensor is based on the modificated Ellman method.

The highest reactivation was observed with sarin-inhibited acetylcholinesterase. Substantially lower reactivation was found with the cyclosarin-inhibited enzyme whereas AChE, inhibited by soman could not be effectively reactivated under the given conditions enzyme inhibition for 2 minutes and subsequent treatment with the reactivator for 15 minutes.

Our work enables comparison of efficiency of inhibited acetylcholinesterase reactivators by simple mean. The method allows rapid *in vitro* evaluation of the reactivators without being disturbed by excess of the organophosphate or reactivator.

IMPACT OF CARBON DIOXIDE ON BACTERIAL BIOFILM PRODUCTION

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Biofilm is a microbiological factor that complicates health care particularly by limiting chemotherapeutic treatments of bacterial infections. In microenvironment of biofilm there are several gradients (including gradient of oxygen) that have a significant impact on metabolism and physiology of bacterial cells.

Production of biofilm in clinical isolates of *Pseudomonas aeruginosa, Klebsiella pneumoniae* as well as in environmental strains of *Plesiomonas shigelloides* and *Vibrio cholerae* non-O1 in aerobic atmosphere with CO_2 was evaluated. These bacterial species represent ecological different types with common sign to form biofilm.

Microtiter plate assay with the crystal violet for biofilm formation *in vitro* was applied. The absorbance of eluted dye (A_{550} nm) from bound cells indicated relative concentrations of bacteria in the biofilm.

Our results have shown that biofilm forming capability (the average control-without CO_2) for *P. aeruginosa* was 0.227 (strain 1010), 0.170 (2239), 0.193 (2444), for *K. pneumoniae* 0.210 (61/P), 0.213 (4853), for *P. shigelloides* 0.173 (39/139), 0.179 (47/155), 0.203 (53/176), for *V. cholerae* non-O1 0.335 (10/116), 0.340 (84/233), 0.413 (DO4/1). Biofilm formation in the strains cultivated in aerobic atmosphere with CO_2 was in the majority of cases decreased in direct association with CO_2 concentration (5% <10%<20%). The most significantly suppression of biofilm production was found in 20% CO_2 aerobic atmosphere in *K. pneumoniae* strains. In this case, biofilm production was decreased to 54.5% and 49.8% of the control levels. Only minor decrease in biofilm capability at these conditions was found in *V. cholerae* non-O1 (to 96.2–98.6% of the control levels). Biofilm production in exposed *P. shigelloides* was in the extent of 87.0–94.6% and in *P. aeruginosa* in 81.6–86.2% of the controls.

Carbon dioxide in the majority of cases decreased bacterial biofilm formation. Degree of changes was dependent on its concentration and on bacterial species.

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RESPIRATORY TOXICITY OF THE SELECTED INDUSTRIAL FIBRES – TIME DEPENDENCE

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The time dependence of the effect of amosite (A), glass fibres (GF) and refractory ceramic fibres (RCF) on the respiratory tract was studied. The animals (W-rats) were intratracheally instilled 4 mg of fibres suspended in 0.2 ml of saline solution and sacrificed (under thiopenthal narcosis) after 48 hour, 1 and 3 month exposure to mentioned fibres. Bronchoalveolar lavage (BAL) was performed and folowing BAL and lung tissue parameters were examined: inflammatory, cytotoxic, immunotoxic, clastogenic, genotoxic and parameters of antioxidant status and histology [BAL cell count; alveolar macrophages (AM) count, differential cell count (% of AM, polymorphonuclears and lymphocytes),% of immature AM, binucleated cells, viability and phagocytic activity of AM, frequency of micronuclei (MN), comet assay, FRA (ferric reducing ability) and histology of lung tissue. The results of our work suggest:

The changes of inflammatory parameters in exposed group (to A, GF, RCF) in comparison with control group were evident.

The most important correlation with time were found after exposure tu A.

The activities of examined lysosomal enzymes in group exposed to A,GF, and RCF were generaly higher than in the control group. No significance was found between the group exposed to amosite and the groups exposed to GF and RCF.

The values of FRA positively correlated with time. Course of time dependence in group exposed to particular fibrous dusts was very similar.

We found a significant increase of frequency of micronuclei in animals 48 hours after the instillation by A and GF; after 3 months we detected significant increase of DNA single-strand breaks in animals exposed to GF and RCF compared to control group.

Histological changes of lung tissue were the most expressive after exposure to asbestos-amosite. The most changes occured after 3-month exposure and held over in the same grades also after 6 month exposure.The impact of examined industrial fibrous dust on the lung tissue in our time dependence experiment (evaluated with grades according to Wagner) decreased in following order: A > RCF > GF.

Most of inflammatory parameters showed the significant correlation with the time. The course of time dependence curves of BAL parameters after exposure to all examined industrial fibers was very similar.

This research was supported by the project grants: APVT-21-011164 and MZ SR 2005/29-SZU-07, Slovakia.

NOSYLVIN INHIBITS FORMATION OF REACTIVE OXYGEN SPECIES IN HUMAN NEUTROPHILS

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Pinosylvin (trans-3',5' dihydroxystilbene), one of the naturally occurring resveratrol analogues, is formed constitutively and after UV irradiation or microbial attack in wood and needles of *Pinus* species. The majority of the data available characterizes antifungal, antibacterial and anticancer activities of pinosylvin, yet little is known about its antioxidant and antiinflammatory effects. In this study, the impact of pinosylvin on apoptosis of human neutrophils and formation of reactive oxygen species was investigated and protein kinase C activation was examined as an assumed site of pinosylvin action.

Neutrophil apoptosis was registered by flow cytometry, using double staining with Annexin V-FITC and propidium iodide. Cytotoxic effect of pinosylvin was evaluated on the basis of ATP liberation measured by luciferin-luciferase chemiluminescence. Formation of reactive oxygen species was recorded in whole blood and in isolated neutrophils by luminol- or isoluminol-enhanced chemiluminescence and phosphorylation of protein kinase C was assessed by western blotting, using phosphospecific antibodies.

Under *in vitro* conditions, pinosylvin (1–100 μM) decreased oxidant formation dose-dependently both at extra- and intracellular level and it effectively reduced activation of protein kinase C. The inhibition was not associated with damage of neutrophils, since in the presence of pinosylvin no increase of spontaneous ATP liberation was recorded. Flow cytometric measurements did not reveal any significant effect of pinosylvin on neutrophil apoptosis. Whereas in control samples the percentage of viable, early apoptotic and late apoptotic neutrophils was 90.1%, 9.5% and 0.3%, respectively, in the presence of 100 μM pinosylvin the respective proportions were 85.0%, 13.5% and 0.6%.

The presented results indicate that pinosylvin may reduce formation of oxidants through inhibition of protein kinase C activation, without affecting neutrophil viability and integrity. These effects could contribute to the antiinflammatory activity of pinosylvin. Neutrophils, used as a simple, pure, single primary cell suspension, were found to be a suitable model for pharmacological and toxicological investigation of the interference of drugs or chemicals with cellular functions, activation pathways, phosphorylation events as well as with the viability and integrity of cells.

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DEVELOPMENT OF "PSEUDO CATALYTIC" BIOSCAVENGERS BASED ON OXIME-ASSISTED REACTIVATION OF HUMAN BUTYRYLCHOLINESTERASE AS ANTIDOTES OF ORGANOPHOSPHATE INTOXICATIONS

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Organophosphates (OPs) are used in agriculture as pesticides, in industry in a wide variety of applications including plasticizers, antiwear additives to hydraulic fluids and engine oils and in military as toxic chemical warfare agents (nerve agents). Organophosphorus pesticides are known to cause tens of thousands of deaths every year in the whole world. Oxon forms of pesticides (arising from parent compounds in organism) and nerve agents act as irreversible inhibitors of acetylcholinesterase (AChE; EC 3.1.1.7). For the recovery of inhibited AChE, antidotes from the group of pyridinium or bispyridinium aldoximes (e.g. pralidoxime, obidoxime, HI-6) are used in combination with anticholinergics (mostly atropine) and anticonvulsives (e.g. diazepam). Therapeutic efficacy of aldoxime reactivators (called "oximes") depends on their chemical structure and also type of organophosphorus inhibitor. Relatively new approach in prophylaxis and therapy of OP intoxications

is a use of specific enzymes (e.g. cholinesterases, human paraoxonase, carboxylesterase) called bioscavengers. These enzymes are able to neutralize the molecule of toxic OPs in the bloodstream before they can reach their natural targets – AChE and butyrylcholinesterase (BChE; EC 3.1.1.8).

We tested the ability of currently used oxime reactivators – methoxime, pralidoxime, obidoxime, trimedoxime and HI-6 to reactivate pesticide-inhibited BChE with the aim to find potent oxime, suitable to serve in combination with this enzyme (administered as prophylactic antidote or occurring naturally in blood) as a "pseudo catalytic" bioscavenger. Reactivation potency was tested by *in vitro* screening test using two oxime concentrations (100 μM and 10 μM). Paraoxon, dichlorvos, diisopropylfluorophosphate (DFP), leptophos-oxon and methamidophos were used as appropriate organophosphate inhibitors. Human plasma was used as source of BChE. Our results demonstrated that the best broad-spectrum BChE reactivator after 10 minutes of reactivation is obidoxime, followed by trimedoxime, HI-6 and pralidoxime. The highest reactivation ability among all tested reactivators was measured at higher concentration for obidoxime and it was 12.2% for paraoxon-inhibited BChE. However, such reactivation potency is not sufficient for practical use and therefore, development of new reactivators of BChE is needed.

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MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY IN ANIMAL RESEARCH: NON-INVASIVE DETECTION OF THE EVOLVING CEREBRAL INJURY AFTER HYPOXIC-ISCHEMIC INSULT TO THE BRAIN

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Cerebral hypoxic–ischemic injury is one of the most common causes of mortality and morbidity in industrialized countries. Survivors of the brain hypoxic-ischemic injury usually struggle from severe neurological flaws often resulting in long-life disabilities. The degree of cerebral hypoxic–ischemic injury varies depending on the intensity and duration of the hypoxic–ischemic insult and on the type and size of the affected region. Effective treatment of cerebral hypoxic–ischemic injury requires its timely recognition that lies solely on more or less non-invasive neuroimaging techniques. Yet, the most widely applied neuroimaging approaches using cranial ultrasonography and computed tomography for instrumental examination often fail to reveal the evolving cerebral hypoxia–ischemia pathology at its early stages. Hence, enormous efforts have been put in a search of new non-invasive approaches to assess cerebral hemodynamics, brain tissue oxygenation, etc. Near infrared spectroscopy (NIRS) allows calculations of cerebral blood flow and blood volume within the brain non-invasively. NIRS can also provide information on oxygen consumption by the brain.

Nowadays, other non-invasive, non-destructive and real-time-operating techniques, namely magnetic resonance imaging (MRI) and spectroscopy (MRS) are progressively introduced into clinical practice. They enable to detect efficiently the ongoing cerebral hypoxia-ischemia, to monitor its consequences and also to follow a brain recovery, either spontaneous or therapy-induced, after the hypoxic-ischemic insult. In comparison with the other neuroimaging techniques, MRI and MRS are advantageous in depicting the site and extent of brain hypoxic–ischemic injury (i) more precisely and (ii) at its earlier stages; they also allow (iii) better differentiation of the structurally damaged, functionally altered and non-damaged regions. The present paper intents to provide a general overview on MRI and MRS applications as a useful tool for non-invasive neuroimaging of cerebral hypoxic–ischemic injury to scientists that are working in the field yet are not experts in magnetic resonance. Along with the key pathogenic mechanisms of cerebral hypoxia-ischemia injury, *in vivo* NMR applications, utilized in both the clinical practice as well as in experimental research on laboratory animals, are summarized.

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COMPARISON OF THE "COCKTAIL APPROACH" WITH SINGLE MARKER ADMINISTRATION IN EVALUATING THE ACTIVITY OF P450 ENZYMES IN RATS

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The activity of cytochrome P450 enzymes (CYP) is most often measured using selective substrate of distinct P450 enzyme. Moreover, there are often used more substrates together, so as the activity of multiple P450 enzymes could be measured simultaneously. Despite of the potential risk of interactions and side effects of these markers used *in vivo*, such a "cocktail" approach brings another question – can one marker influence the rate of biotransformation of another one?

The aim of the present work was to compare biotransformation rate of phenacetin and tolbutamide (markers of CYP1A2 and CYP2C6/11 activity) administered either separately or both simultaneously.

The experiment was carried out on male Wistar rats. After 10 days of adaptation to standard laboratory conditions, rats were randomly allocated into 3 groups (A, B, C). The model of isolated perfused rat liver was used. In the Group A, the rate of phenacetin *O*-deethylation was examined to asses CYP1A2 activity. In the Group B, tolbutamide hydroxylation rate was examined to asses CYP2C6/11 activity. In the Group C, both markers (tolbutamide and phenacetin) were given simultaneously, so as to assess activity of both CYP1A2 and CYP2C6/11 under these conditions. The rate of metabolism was assessed as a concentration ratio (metabolic ratio, MR): marker/metabolite, in the 30th, 60th and 120th minute of liver perfusion. Repeated measure ANOVA with Tukey post-hoc test for multiple comparisons was used for the data analysis using Statistica for Windows 8 software.

Our results suggest that phenacetin addition had no significant effect on tolbutamide hydroxylation. On the other hand, tolbutamide addition to the perfusion medium significantly increased the rate of *O*-deethylation of phenacetin. This effect was observed in the 30th ($p \leq 0.01$), 60th ($p \leq 0.05$) and 120th minute of perfusion ($p \leq 0.05$). Detailed mechanism of this phenomenon is unclear and requires further investigation, but may perhaps be explained by effects of positive cooperativity of these substrates, since similar effects have been already observed in CYP1A2.

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DEVELOPMENT, OPTIMIZATION AND VALIDATION OF EPIDERM *IN VITRO* SKIN IRRITATION TESTS FOR CLASSIFICATION AND LABELING OF CHEMICALS ACCORDING TO EU AND GHS RULES

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Recent legislation and a ban on animal testing for cosmetics have heightened the need for validated *in vitro* skin irritation tests (SIT)s. The EpiDerm model has been validated for *in vitro* skin corrosion testing worldwide, and for *in vitro* SIT in the EU in studies sponsored by the European Center for the Validation of Alternative Methods (ECVAM). The EU SIT system distinguishes 2 classifications – skin irritants (R38) and non-irritants (no label). However, a UN treaty endorsed by the US, EU, China, Japan, Australia and others has outlined a GHS of Classification and Labeling of Chemicals. The GHS classifies skin irritancy of chemicals into three categories: non-irritant, slight irritant or irritant. Therefore, additional efforts are underway to validate an EpiDerm SIT for GHS.

15 test chemicals with known *in vivo* Draize skin irritation scores were applied to EpiDerm to identify *in vitro* skin irritation biomarkers and establish a preliminary EpiDerm-GHS-SIT prediction model. Biomarker endpoints evaluated include EpiDerm viability (MTT assay) and inflammatory mediator release by ELISA and/or Multiplex (Bio-Rad BioPlex) assays.

The MTT viability response was the most predictive and least variable biomarker, providing 80% concordance with the *in vivo* Draize classification (i.e. 80% sensitivity and specificity for assigning GHS classifications). Among the mediators investigated, significant levels of IL-1a, IL-1ra, IL-8, IL-18, GROa and PGE2 were produced by EpiDerm tissues. These biomarkers did not improve the classification. This preliminary prediction model will be further tested and refined to form the basis for formal multi-laboratory EpiDerm-GHS-SIT validation studies.

RAMAN SPECTROSCOPY OF DNA INTERACTIONS WITH CERIUM(III), LANTHANUM(III) AND GADOLINIUM(III) IONS

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The biological properties of the lanthanides, based on their similarity to calcium, have stimulated research into their therapeutic application. Up-to-date we have been successfully using at least two pharmaceuticals cerium nitrate as a topical cream with silver sulfadiazene for the treatment of burn wounds and lanthanum carbonate as a phosphate binder for the treatment of hyperphosphatemia. Lanthanides^{3+} compounds have also been investigated for their anti-cancer potential. Cerium(III), lanthanum(III) and neodymium(III) coumarin complexes were synthesized with ligands such as hymecromone, umbellipherone, mendiaxon, warfarin, coumachlor and niffcoumar. Preclinical studies with these compounds have demonstrated cytotoxicity against the HL-60 myeloid cell line. Clinical reports suggested that above mentioned compounds posed cytotoxic effect, however the biochemical mechanism of their action is still unclear.

Therefore, we aim our attention at study of interactions DNA with inorganic salts of cerium(III), lanthanum(III) and gadolinium(III).

Aliquots (4 μl) were sealed in a glass capillary (KIMAX-51, 1.0 mm inside diameter). Spectra were

excited at 514.5 nm using an Argon ion laser (Coherent Innova FreD90C). The radiant power at the sample was 100 mW. Measurements were performed at 25 °C in back scattering setup. Laser line was removed from scattered light by Kaiser Optical notch filter. Remaining signal was analyzed by Jobin Yvon T64000 Raman spectrometer in a single mode using 1 200 lines/mm grating, entrance slit of 0.1 mm wide and Spectrum One CCD3000 LN2 cooled detector. Collection of data on the detector was restricted so that the signals from capillary walls were not collected.

Raman spectroscopy was employed to characterize the perturbations to DNA conformation induced in DNA by three inorganic salts of the above mentioned lanthanides. All studied lanthanides coordinated to N7 of two neighbouring guanine bases, as this nitrogen does not form H bonds with other bases, in the same or in opposite DNA strands. The results of the present work demonstrate that Raman spectroscopy represents a suitable tool to provide insights into structural factors involved in the mechanisms underlying antitumor effects of drugs.

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THE EXPRESION OF NADPH OXIDASES AND PRODUCTION OF REACTIVE OXYGEN SPECIES BY HUMAN LUNG ADENOCARCINOMA EPITHELIAL CELL LINE A549

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Controlled production of reactive oxygen species (ROS) by non-phagocytic cells is recently suggested to significantly participate in regulation of cellular functions. Due to the importance of ROS in a control of wide range of physiological and pathogenic cellular processes it is noteworthy to precisely and accurately detect and quantify formation of ROS. Various methodological approaches are currently used for ROS determinations that vary in sensitivity, specificity and requirements for specialized equipment.

In this study, human lung epithelial cell line A549 was screened to determine expression of ROS-generating NADPH oxidases NOX1, NOX4, DUOX1 and DUOX2 by real-time PCR and fluorimetric, colorimetric, and chemiluminometric methods were applied and optimized to determine ROS production by these cells.

We demonstrated that from all evaluated NADPH oxidases A549 cells expressed significantly only DUOX2. The ROS production in this lung epithelial cell line was possible to detect with fluorometric probes 2',7'-dichlorofluorescein-diacetate, dihydroethidium, and amplex red and colorimetric probe nitrotetrazolium blue. In contrast, any significant reduction of cytochrome c by superoxide was not detected. Similarly, luminol and L-012 amplified chemiluminescence with and without addition of horseradish peroxidase did not reveal sufficient sensitivity to detect ROS produced by A549.

It could be concluded that the most sensitive method for ROS determination in human lung epithelial cell line A549 was fluorometric determination.

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IMPORTANCE OF TPMT GENE POLYMORPHISMS FOR PREDICTION OF AZATHIOPRINE TOXICITY

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Crohn's disease (CD) and ulcerative colitis (UC) are clinical subtypes of the inflammatory bowel disease (IBD). Pharmacological treatment consist of salicylates, cortikoides, immunosuppressives, and biological therapy. Concerning effective and safe farmacotherapy we talk about therapeutical genes coding target structures for drugs or, most commonly participate in drug metabolic pathway. Polymorphisms in this genes lead to changes in a primary structure of a coded protein and thus influences its function. That all result in changes in benefit risk ratio in patients that vary in genome. One of the most studied therapeutical gene is a gene coding Thiopurine S-methyltransferase (TPMT). TPMT is a metabolizing enzyme, that inactivates thiopurine immunosuppressives (e.g. azathioprine, 6-mercaptopurine). Nucleotide substitutions result in lower enzyme activity and leads to lower mercaptopurin degradation. Thus patients with polymorphisms in the genome, but receiving normal doses of azathioprine, are exposed to higher drug levels and side effect following this overdosing. The main symptom of this is serious leucopenia.

The aim of this study was to find a relationship between metabolic enzyme TPMT genotype and clinical output of pharmacotherapy with azathioprine.

Our group consists of 87 patients who have been treated with azathioprine. 21 individuals experienced leucopenia during treatement. We have used PCR, PCR-REA and "real-time" PCR methods to genotyping SNPs in TPMT.

We have fond statistical association between presence of nonstandard TPMT alleles and adverse effect of treatment with azathioprine – leucopenia (p=0.0033).

Our results confirm that TPMT genotyping prior to treatement with azathioprine could predict patients

with predisposition to leucopenia and seems to be a good genetic marker for individualisation of therapy.

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DRUG ADDICTION PROBLEMS AMONG PRISONERS

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The aim of this work was to monitor the drug situation in Kuřim Penitentiary from 2004 to 2008, and to observe and quantify possible differences between convicts motivated to abstain and unmotivated convicts. The work focused on one type of special educative programmes dealing with imprisoned convicts who have problems with drugs. Convicts motivated to solve their drug problems were enlisted into the Voluntary Urine Testing Programme (2004–2006), and later participated in the no-drug zone since 2007. Unmotivated convicts were monitored by means of compulsory urine testing and both groups of motivated as well as unmotivated convicts were checked using general monitoring. Urine samples were biochemically tested for the presence of addictive substances on a monthly basis. Acquired results were put together in the form of graphs and tables showing number of positive and negative samples. Positive samples were divided according to the type of addictive substance. From 2004 to 2008, the total of 1882 samples was examined; 138 were positive, which constitutes 7.4%. The most often tracked substances were benzodiazepines and amphetamines followed by cannabis. Opiates and barbiturates were exceptional. In 2004 the total of 413 samples were examined; 52 samples were positive. In 2005 the total of 370 samples were examined and 42 were positive. In 2006 the total of 427 samples were examined; 14 were positive. In 2007 the total of 251 samples and 16 samples were positive. In 2008 the total of 421 samples were examined; 14 samples were positive. The comparison of acquired data reveals lower occurrence of positive samples among motivated convicts who had drug problems between 2004–2006 (Voluntary Urine Testing Programme) compared to convicts unmotivated to solve their drug problems. In 2007, the No-drug Zone Project was introduced, and it seems to be very beneficial. In the years 2007 and 2008, there was no positive sample revealing addictive substances and intoxicants among the No-drug Zone convicts. The project analysis makes it clear that activities in the area of drug problems among convicts are meaningful and reduce contacts with drugs in the No-drug Zone.

STUDY ON MECHANISM OF CYTOCHROME b_5 EFFECTS ON CYTOCHROMES P450

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Cytochromes P450 (CYPs) are enzymes responsible for the metabolism of a wide variety of xenobiotics. Many CYP-dependent reactions have been shown to be stimulated by another microsomal protein, cytochrome b_5 (b_5). Two hypotheses trying to explain this effect are: (i) an increase in efficiency of processes connected with electron transfers and (ii) an induction of conformational changes increasing the CYP–mediated oxidation. The second hypothesis is based on findings showing that not only holoprotein of b_5, but also its apo-form, which is not capable of electron transfer, can contribute to stimulation effects. It is clear from such investigations that studies utilizing apo-cytochrome b_5 (apo-b_5) are necessary to explain the mechanisms of b_5 effects on CYP-catalyzed reactions.

The aim of the study was to investigate these two proposed mechanisms. Because apo-b_5 is crucial for such studies, preparation of this apo-protein was another aim of this study.

CYPs and b_5 were purified from rabbit liver microsomes and biologically active apo-b_5 prepared by heterologous expression in *E. coli*. CYPs were reconstituted with NADPH:CYP reductase in the presence/absence of b_5, apo-b_5 or other proteins with or without heme in their molecules and used for oxidation of ellipticine or Sudan I. HPLC was used to separate metabolites and ^{32}P-postlabeling to determine ellipticine or Sudan I-DNA adducts.

The patterns and amounts of ellipticine metabolites formed by CYP1A1/2 vary significantly by b_5. The formation of detoxication products (7-hydroxy- and 9-hydroxyellipticine) is decreased, while generation of those responsible for DNA adduct formation (13-hydroxy- and 12-hydroxyellipticine), is increased. The elevated generation of ellipticine-DNA adducts by b_5 was confirmed by the ^{32}P-postalbeling assay. In the case of Sudan I, production of all metabolites by CYP3A4 is markedly enhanced by b_5. The levels of Sudan I metabolites are increased even by apo-b_5. Other proteins with or without heme in their molecules do not stimulate oxidation of both substrates. The results indicate that b_5 affects oxidation of ellipticine and Sudan I not only by the modulation of an electron transport, but also by the alteration of CYP conformations.

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INDOOR FUNGAL CONTAMINATION. THE EFFECT OF ISOLATED FUNGAL SECONDARY METABOLITES ON LUNG CELLS IN EXPERIMENT

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Health complaints in mouldy houses may be associated with microfungal exposure.

The pulmonary health problems can be evoked both by inhalation of spores or their secondary metabolites. The fungal secondary metabolites represent a mixture of different active compounds and the mycotoxins are common part of them.

Our study was focused on the effect of secondary metabolites produces by *Aspergillus versicolor*, a frequent indoor colonizer.

The Wistar rats were intratracheally exposed to isolated metabolites produced by *Aspergillus versicolor* and after 3 day exposure the animals were sacrified and the antioxidant status was estimated in lung tissue and bronchoalveolar lavage fluid (BALF). Except that alveolar macrophages (AM) and alveolar epithelial type II cells – from toxicological point of view the most important cells – were isolated and their antioxidant status and DNA damage were evaluated.

The results did not show statisticaly significant changes of antioxidant status neither in lung nor in the BALF but the DNA damage was enhanced in both types of studied cells.

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SOME FOODS TOXIC FOR PETS (A REVIEW)

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According to world statistics, dogs and cats are the species that owners most frequently seek assistance with potential poisonings, accounting 95–98 % of all reported animal cases. Exposures occur more commonly in the summer and in December that is associated with the holiday season. The majority (> 90 %) of animal poisonings are accidental and acute in nature and occur near or at the animal owner's home. Feeding human foodstuff to pets may also prove dangerous for their health.

The aim of this review was to present common food items that should not be fed (intentionally or unintentionally) to dogs, i.e. chocolate, caffeine, and other methylxanthines, grapes, raisins, onion, garlic, avocado, tomatoes, pottatoes, rhubarb, cat food, alcohol, nuts, xylitol contained in chewing gum and candies, etc. Onion and avocado are toxic for cats, too. The clinical effects of individual toxicants and possible therapy are also mentioned. Knowing what human food has the potential to be involved in serious toxicoses should allow veterinarians to better educate their clients on means of preventing pet poisonings.

It can be concluded that the best advice must surely be to give animal fodder or treats specifically developed for their diets.

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AN ASSESSMENT OF CADMIUM CONTAMINATION OF THE SVITAVA AND SVRATKA RIVERS USING THIOL COMPOUNDS AS BIOCHEMICAL MARKERS

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Heavy metals produced in connection with human activity occur in environment and constitute health risk to living organisms including man. In plants, intervertebrates, fish, birds and mammals there has been detoxification system of stress induced molecules to bind heavy metals and breaks entrance toxic effect of them. A mechanism consists in the interaction of SH-groups of amino-acid cysteine (Cys) in proteins (metallothioneins-MT) and low-molecular weigh nonprotein thiol compounds as glutathione that occur in living body in oxidized (GSSG) and reduced form (GSH). The aim of the presented study was to assess aquatic ecosystem contamination of heavy metals using these selected biochemical markers – MT, GSH, GSSG, Cys measured in liver in fish. Investigation was realized on the seven locations on the Svitava and Svratka rivers (in the Brno conurbation, Czech Republic) in 2007 and 2008. The indicator species selected was the chub (*Leuciscus cephalus* L.). We determined cadmium content by atomic absorption spectrometry (AAS) with electrothermal atomization and content of thiol compounds (MT, GSH, GSSG) and cysteine (Cys) by Brdicka reaction. Cadmium content in fish liver had no significant differences between several localities. The highest values of metallothionein were found at locality situated upstream from the Brno city (Kníničky). The highest values of glutathione in general were found at localities downstream from Brno - GSSG were extremely high at locality Modřice, and GSH highest values were found

at localities Rajhradice, Svitava before junction and Židlochovice, where the values of cysteine were high too. Determination of thiol compounds in fish is suitable for assessment of water contamination of heavy metals, which still represent risk potential for living organisms.

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THE EFFECT OF DIFFERENT MOLECULAR WEIGHT HYALURONAN ON PHAGOCYTES

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Hyaluronan, a high-molar-mass linear glycosaminoglycan, is an abundant component of extracellular matrix. In its native form, the very large hyaluronan polymers have an array of regulatory and structural, mainly anti-inflammatory and anti-angiogenic, functions. Contradictory to high molecular weight hyaluronan, biological effects of fragmented hyaluronan are suggested to be angiogenic, inflammatory and immunostimulatory.

The effects of highly purified hyaluronan of precisely defined molecular weights were tested on mouse peritoneal and lung macrophage cell lines and on human blood phagocytes. Viability of cells, production of nitric oxide and pro-inflammatory cytokines were measured spectrophotometricaly, production of reactive oxygen species was measured by luminol amplified luminescence, and inducible nitric oxide synthase expression was detected by western blot analysis.

The results of this study revealed highly purified hyaluronan of different molecular weight did not have negative effect on the cell viability. Hyaluronan did not exert any stimulatory effect on production of nitric oxide and pro-inflammatory cytokines by mouse macrophages alone or in combination with bacterial endotoxin. Similarly, hyaluronan did not reveal any effect on either spontaneous or activated production of reactive oxygen species by blood phagocytes.

Interestingly, these data are in controversy with most of the recent studies. More experiments should be done to resolve the question of the effect of different molecular weight hyaluronan on phagocytes.

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MERCURY AND METHYLMERCURY CONTENT IN HUMAN HAIR IN COMPARISON WITH FISH CONSUMPTION

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Mercury is extremely toxic and is generally handled with care. Fish show a natural tendency to cumulate mercury and its organic form - methylmercury. Human exposure to mercury and methylmercury occurs mainly via freshwater and sea fish.

The aim of our study was to determinate mercury content in human's hair with different fish consumption. Hair samples were cleaned once in acetone, three times in the water and again in acetone. Total mercury content in hair was determined by the direct method of cold vapor using the AMA-245 analyzer. Total of 299 hair samples from the human (2–66 years old) was analyzed. Total mercury ranged from 0.029 to 3.78 mg/kg and the average was 0.236 mg/kg. Another goal was to develop a method for methylmercury determination in hair samples. The methylmercury was measured after acid digestion with hydrochloride acid and the results show that this method is suitable for measurement of methylmercury. The average content of methylmercury in analyzed hair was 70.2% of total mercury. Every sample was accompanied by answer sheet where the volunteers fill up data about age, gender, region and amalgam filling. The very important part of the answer sheet was information about intake of freshwater and sea fish in the volunteer's diet. The data from answer sheets were evaluated and compare with our result of total mercury and methylmercury. The positive correlation between fish consumption and mercury content in hair was confirmed.

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ACTIVITIES OF SELECTED ENZYMES IN PLANTS TREATED BY HEAVY METALS

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First references about existence of enzymes in literature were published in the half of 19th century in connection with the study of the process of fermentation. Later it was ascertained that enzymes are present in all living organisms, in plants as well as animals. It was determined that enzymes are catalytical proteins, which are able to cleave big molecules onto smaller, or they participate in reactions enabling storage or release of energy. Their involvement into chemical processes in cells and organisms is controlled by a lot of other compounds, which of the total aspect form complicated closed system of metabolical pathways, when malfunction of whichever pathway or enzyme itself can result in nonreversible damage of organism and its resulting death.

Therefore, we in our work focused on the influence of heavy metals on activity of chosen enzymes in different species of plants. In the case of enzymes, activity of transaminases (L alanine(2-oxoglutarate) transaminase (ALT) and L- aspartate(2-oxoglutarate) transaminase (AST)) and urease was monitored.

Transaminases catalyze transfer of amino-groups of amino-acids on 2-oxo-acids and in plants, also very effectively participate in transformations of nitrogen compounds, they are important during biosynthesis of amino-acids from oxo-acids of citrate cycle, photosynthesis and at transport of oxo-acids from roots to the other plant parts. Urease was for the first time isolated from *Cannavalia enzyformis* (*Fabacae*) in 1926 and its name obtained in agreement with its function, consequently enzyme, which is able to degrade urea (carbamide) into ammonia and carbon dioxide. It was determined that this enzyme demonstrates absolute substrate specifity, which means, that hydrolyzes only urea and does not react with other structurally similar compounds. This property is important for correct process of nitrogen metabolism in plant. Thanks to determination of chosen enzymes activities of common sunflower and maize exposed to cadmium and lead ions in concentrations 0, 10, 50, 100, and 500 μM their increased activity was determined in all concentrations in the case of sunflower as well as maize in comparison with control planAs from obtained results unambiguously follows, enzymatic activity plays important role in defence of plants against influence of heavy metals.

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INFLUENCE OF B-HCH ON PLANT MODELS AND THEIR POTENTIAL UTILIZATION FOR PHYTOREMEDIATION

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Industrial boom since the end of 19. century brought into the human understanding and life a large number of discoveries, which have helped to improve our living standard. Compounds, which were in the past considered due to their profitable physical and chemical properties as industrially advantageous (polychlorinated biphenyls, chlorinated aliphatic hydrocarbons, organochlorinated pesticides), nowadays represent important ecological problem. It was determined that these compounds are compounds persistent and toxic, which accumulate in living environment; they can entry food chain and endanger human health. Biological decontamination is one of possible way of their removing.

In our work, we focused on the study of influence of various concentrations of β–hexachlorcyclohexane (β–HCH) on growth and chosen metabolical markers of plants of maize (*Zea mays*) and common sunflower (*Helianthus annuus L.*).

Sunflower and maize plants in our experiments were exposed to the influence of 0, 0.1, 0.3, 0.5, 0.7 and 1 ng/l β–HCH for a period of twelve days under controlled experimental conditions. The influence of various β–HCH concentrations on basic growth characteristics was the first studied parameter. In maize, representing monocotyledonous plants, was all the time of experiment observed significant growth depression in all applied β–HCH concentrations. Except of growth characteristics, we were also interested in the influence of β–HCH on plant metabolism. For this purpose, we monitored activities of chosen enzymes, which are integral components of nitrogen metabolism in plant. These enzymes were urease, L-alanine(2-oxoglutarate) transaminase (ALT) and L-aspartate(2-oxoglutarate) transaminase. In the case of maize, we determined increased activity of enzyme urease, but in the event of ALT and AST their activities were decreased in comparison with control, untreated plants. On the contrary, in the case of sunflower, increased activity of all monitored enzymes was determined. As it was well evident from obtained results, sunflower plants are more resistant to the treatment by β–HCH. Furthermore, sunflower plants prospered in the conditions of β–HCH, they grew without significant symptoms of toxicity, and in comparison with control plants we can say, that this pollutant had stimulative effect on sunflower plants.

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A WAY HOW TO STUDY METABOLOME OF SUNFLOWER PLANTS

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"Metabolomics" is the newest "omics" buzzword in the field of systems biology, joining genomics, transcriptomics and proteomics. The "metabolome," not only the complete set of small (typically less than 1 kDa) molecules (metabolites) but also enzyme activity, concentration of specific aminoacids, peptides or proteins present in cells in a particular physiological or developmental state, is said to be closest to the phenotype. Thus, metabolomics is poised to play a critical role in understanding intricate biochemical and biological systems.

The aim of this study was to suggest and optimize several methods for detection of enzymes closely related with protection of an organism against free radicals.

As an experimental model, we selected maize plants treated with cadmium(II) ions. Moreover, we determined content of low molecular mass thiols such as reduced and oxidized glutathione, phytochelatins, which are able to bind heavy metal ions and transport them to cell spaces, where do not menace yet. Several sunflower cultivars (*Helianthus annuus*) were used in our experiments. High performance liquid chromatography with electrochemical detection consisted of two solvent delivery pumps, chromatographic column and twelve-channel CoulArray electrochemical detector.

Sunflower plants can be used in phytoremediation technologies. However, various cultivars may differ markedly in their ability to withstand adverse effects of heavy metal ions. To select suitable cultivar for following genetic modifications, studying of various protective pathways is needed. We optimized easy-to-use and automatic detection of several enzymes involved in free radicals scavenging and related biochemical pathways. Particularly, we aimed at phytochelatin synthase (EC 2.3.2.15), glutathione transferase (EC 2.5.1.18), glutathione reductase (EC 1.6.4.2), glutathione peroxidase (EC 1.11.1.19), catalase (EC 1.11.1.6), superoxid dismutase (EC 1.15.1.1), ascorbate peroxidase (EC 1.11.1.11), urease (EC 3.5.1.5), aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) and others. Moreover, we compared the altered activity of the above mentioned enzymes with the level of low molecular mass thiols determined by liquid chromatography with electrochemical detection. Based on the results obtained it can be concluded that multi-instrumental approach for evaluation of phytoremediation potential of several sunflower cultivars can bring new interesting point of view on plant metabolome affected by heavy metal ions.

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EFFECTS OF CHEMOPREVENTIVE COMPOUNDS ON HUMAN HEALTH

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Cancer is worldwide a life-threatening disease with one of the highest mortalities. Dietary composition is one of the factors raising the probability of the carcinoma incidence. Since flavonoids and other chemopreventive compounds exert beneficial effects on human health, their consumption rapidly increases. However, they can modulate the activity of xenobiotic-metabolizing enzymes involved in the activation and detoxification of food and environmental carcinogens. Thus, their potential negative effects should be examined.

We investigated the effects of chemopreventive compounds (morin, rutin, quercetin, isoquercitrin, diallylsulphide, biochanin A, curcumin) on cytochromes P450 (CYP1A and CYP2B), namely on their induction and metabolic activities in the small intestine of rat model organism.

The induction effects of selected chemopreventive compounds, administered perorally to rats, on CYP1A and 2B were determined in the small intestine using Western blotting technique. Ethoxyresorufin-*O*-deethylase (EROD), methoxyresorufin-*O*-demethylase (MROD) and pentoxyresorufin-*O*-dependentylase (PROD) activity assays were used as a marker for the activity of CYP1A1, CYP1A2, CYP2B1/2, respectively.

Comparing CYPs expression along small intestine, the highest induction was observed in the proximal part near pylorus with rapid decrease towards the distal part. In response to chemopreventive compounds, the induction of CYP1A1 and CYP2B1 in small intestine was observed after â-naphthoflavone, diallyl sulphide and curcumin treatment. The results of Western blotting detection of CYPs correlate well with their specific enzymatic activities.

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MONITORING OF SILVER NITRATE TOXICITY IN MODEL ORGANISMS FOR ECOTOXICOLOGY

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Silver is a noble metal utilized by man since antiquity. The anthropogenic pollution of the environment comes particularly from the metallurgy and photographical industry. The toxic effect of Ag^+ is known for a long time, especially for microorganisms and water organisms.

The series of published studies reflect about marked toxicity of silver for aquatic ecosystems, it is known that free silver ions belong to one of the most toxic elements, which could cause the acute toxicity of organisms, e.g. fish – after the exposition 10 µg /l Ag^+ die 50% of individuals in 96 h.

Silver nitrate is technically the most important compound of silver. It could be used e.g. for production of other chemical compounds containing silver ions or directly in the photographic industry, in the electronics, jewellery production, chemical industry, health service as dental amalgam. Silver nitrate cauterizes and destroys organic tissues, as it is able to coagulate the proteins. Because of the ability to coagulate the proteins is also used as a disinfection substance in medicine.

The risk with presence of chemical substances and chemical preparations in the environment is determined by using ecotoxicological bioassays.

The main aim of this study was to determine the ecotoxicity of silver nitrate with the battery of bioassays including various trophic levels of aquatic ecosystems – producers (fresh-water alga *Pseudokirchneriella subcapitata*, the vascular water plant *Lemna minor),* consumers *(*the invertebrates- water flea *Daphnia magna*, aquarium fish *Poecilia reticulata),* decomposers (the luminescent bakteria – *Vibrio fischeri*). The possible toxic effects were evaluated testing the inhibition of the growth of white mustard root (*Sinapis alba*).

Results of this study could contribute the explanation of risk, which could be caused by input and storage of these ions in the environment.

While in the case of producers (*Sinapsis alba, Lemna minor* and *Pseudokirchneriella subcapitata*) only weak inhibition effects were observed, in *Daphnia magna* and *Poecillia reticulata* we determined the value of 96 h 50% lethal concentration (96h LC_{50}) of 0.034 $mg.l^{-1}$ and 0.17 $mg.l^{-1}$. For *Vibrio fisheri* a 20% effective concentration after 30 min (30 min EC_{20}) of 1.23 $mg.l^{-1}$ was determined.

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EMPLOYMENT OF DIFFERENT ANALYTICAL APPROACHES FOR DETECTION OF SILVER IN THE ENVIRONMENT

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Silver(I) ions has been shown to be highly toxic to aquatic life. The aim of this work was to detect silver ions by using various electrochemical instruments.

Peptides and proteins such as low molecular proteins called metallothioneins (MT) with heavy metal binding properties could be successfully employed as a biological part of heavy metal biosensors. It was shown previously that metallothionein-hanging mercury drop electrode (MT HMDE) biosensor can be utilized for detection of heavy metals ions. Silver(I) ions demonstrate very strong affinity to MT, which can be characterized by marked changes in differential pulse voltammograms.

The aim of this paper was to compare selectivity and sensitivity of metallothionein biosensors proposed on mercury electrode (HMDE) or with newly suggested miniaturized biosensor on a carbon electrode. Well developed signals of both of MT and MT complex with a heavy metal ion can be observed on HMDE. The detection limits for heavy metal ions are at sub-micromolar levels. HMDE is not suitable for further miniaturization and its using is avoided in many countries around the world. Thus other type of working electrodes had to be employed. Various carbon electrodes were tested. MT gives oxidation signal at potential of 750 mV at carbon electrodes. The detection limit of MT is units of pM. The results obtained lead us to suggesting of biosensor for assessing of heavy metal pollution of aquatic environment.

Biosensor is based on coupling of automated binding of metallothionein by MT-antibody by using paramagnetic beads and screen printed carbon electrodes. Moreover the electrode process is measured by our proposed micro-potentiostat placed nearby electrode system (the size is 25.5 mm×7.2 mm). The proposed biosensor was evaluated on the determination of heavy metal level and MT content at fishes treated with cadmium(II) and silver(I) ions for five day. The results obtained were compared with commonly used techniques for determination of heavy metals (differential pulse voltammetry) and MT (Brdicka reaction) and were in good agreement. In addition, the whole experimental setup was employed to assess the heavy metal contamination of wetlands.

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IMMUNOLOGICAL METHODS FOR STUDY OF METALLOTHIONEIN IN BIOLOGICAL SAMPLE

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Metallothioneins (MT) are low-molecular mass proteins capable to bind heavy metals. They are involved in transporting and/or detoxifying of metal ions. The role of MT in the organism is still understood insufficiently, it is known, that except the essential heavy metals homeostating, toxic heavy metals detoxification and free radicals scavenging MT play a role in many cellular physiological processes, e. g. cell proliferation, enzymes (de)activation, preservation of embryos and reproductive cells, digestive tract and nervous system development, etc. The enhanced MT expression was determined after the exposition of an organism to oxidative stress of various origins (i. g. heavy metals, UV irradiation, radiation, xenobiotics, etc.)

Because their ability to bind heavy metals MTs are often used as biomarkers of heavy metal presence in the environment.

Fish (*Perca fluviatilis*) in approximately weight of 90 g were exposed to 5 μM Cd concentration for four days.

Aim of this work was to determine metallothionein level in samples of fish liver (n=35) and kidney (n=35) by a novel ELISA method employing polyclonal chicken antibodies raised against rabbit MT I and II.

MT levels determined in studied samples were within the range from 2.1 to 357.1 ng/mg of the tissue. The obtained results were verified by a routinely used electrochemical mehod – Differential Pulse Voltammetry-Brdicka reaction, the determined the MT contents in fish tissues were from 12 to 438 μg/g of the tissue.

The correlation coefficient between the signal height of MT standards at ELISA method and Brdicka reaction was higher than 0.99 in concentration range from 7.8 to 62.5 ng/ml. In the case of liver and kidney tissues from carp the correlation coefficient between the results obtained was 0.903 and the relationship between the MT levels in the tissues determined by Brdicka reaction and ELISA can be described by relation: MT(ELISA)=0.751×MT(Brdicka reaction) + 9.968, see Fig. 3. The average difference between the results was 23%.

The results obtained by ELISA were in very good fit with the electrochemical method both in the case of standards and in fish tissue samples.

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MACROSCOPIC AND MICROSCOPIC IMAGE OF GASTROINTESTINAL LESIONS EXPERIMENTALLY INDUCED BY INDOMETHACIN

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It is known that the therapy with non-steroidal anti-inflammatory drugs (e.g. indomethacin) is often accompanied with adverse effects in gastrointestinal tract. Aim of this experimental study was to define the time range of the manifestation of the inflammation in digestion tract (for prospective potential probiotic therapy) and to compare the differences in the manifestation of these adverse reactions in two experimental species. The laboratory rat (Wistar Han II, 200–250 g), and the experimental pig (Sus scrofa f. domestica, 30–35 kg), were used in the study. Indomethacin was administered orally by a single application to the rat (25mg/kg), and repeatedly to the pig for 10 censecutive days (400mg daily). The tissue samples from individual parts of GI tract were collected for histological examination. The pronounced changes in the irritation of the mucous were found in stomach (gastritis, erosions, ulceration) and in caecum (erosions, filiform ulceration) in the pig. In the rat, the gradual development of induced lesions was observed (after 6 hours in stomach; the gradation during 24–72 hours in intestine). Although the relatively comparable dose of indomethacin was administered to the both experimental species, the sensitivity of gastrointestinal tract in the pig seems to be lower in comparison to the rat. The interspecies differences in the manifestation and in the dynamics of the development of gastrointestinal lesions were also observed.

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THE DYNAMICS OF GASTROINTESTINAL LESIONS INDUCED BY INDOMETHACIN (NON-SPECIFIC CYCLOOXYGENASE INHIBITOR): THE INTERSPECIES COMPARISON (LABORATORY RAT, EXPERIMENTAL PIG)

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The experimental simulation of ulcerative gastritis and enteritis was the sence of this methodological research for perspective study of alterations in transport of xenobiotics across the gastrointestinal barrier. For this purpose, the "therapeutically adverse effect" of non-steroidal antiinflammatory drugs was utilized. The laboratory rat (Wistar Han II, 200–250 g), and the experimental pig (Sus scrofa f. domestica, 30–35 kg), were used in this preliminary (toxicological) phase. Following the literature data and our previous experiments, the indomethacin was administered orally by a single application to the rat (25mg/kg), and repeatedly to the pig for 10 censecutive days (400mg daily). The evaluative criteria were macroscopic section and optical light microscopy (after euthanasia) in both experimental species; the stomach was investigated endoscopically (by means of standard video-gastroscopy) and the small intestine by means of wireless capsule endoscopy in pig in addition. The gradual development of induced lesions was observed in the rat (in stomach after 6 hours but no one in intestine; the gradation during 24–72 hours in intestine while in stomach become extinct). In the pig, the most expressive findings were in stomach (petechia, erosions, single ulcers and ulcers chain) and in caecal segment (erosions, filiform ulceration, the perforated ulcer in one instance, enlarged lymph-node). Conclusions: the sensitivity of gastrointestinal tract in the pig seems to be lower in comparison to the rat in the course of relatively comparable dose of orally administered indomethacin. Not only the gradual development of pathophysiological alterations was observed but also the reparative phase, in the rat.

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EPIOCULAR TISSUE MODEL PROTOCOLS FOR *IN VITRO* OCULAR IRRITATION TESTING OF CHEMICALS AND COSMETICS COMPOUNDS

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In vitro tests for assessing ocular irritancy of consumer/household product chemical ingredients are urgently needed to comply with EU legislation such as the 7th amendment of the Cosmetics directive and the REACH directive. Eye care cosmetics (ECC) also need to be non-irritating (i.e. "ultra-mild") in order to be successful in the marketplace. This poster summarizes 2 different protocols that have been developed for use with the *in vitro* EpiOcular tissue model in order to accommodate both purposes.

For REACH irritation testing, a single exposure period is used: 30 minutes with a 2-hour post-exposure incubation (liquids) or 90 minutes with 18-hour post-exposure incubation (solids). A single cut-off in relative survival is used for classification: more than 60%=irritant (I) (R36 and R41); >60%=non-classified (NC). Tissue viability is determined by MTT assay. For ultra-mild testing, exposure times between 8 and 24 hours are used, and tissue viability (ET-50) is also determined by MTT assay.

For irritation screening of chemicals, 99.7% agreement in prediction (NC/I) was obtained from 298 independent trials ac ross seven laboratories (Harbell et al, The Toxicologist 108(1), 2009). For ultra-mild testing of 10 mascaras, a range of ET-50s was obtained from 8.7 hours to > 24 hours. Other formulations with low levels of surfactants known to be irritating at higher concentrations could also be discriminated by ET-50s. Thus, the EpiOcular model appears to function well for both chemical testing for REACH purposes, as well as ultra-mild screening of ECCs and other materials.

ANTIOXIDANT RESPONDS AND PLASMA BIOCHEMICAL CHARACTERISTICS IN THE FRESHWATER RAINBOW TROUT, *ONCORHYNCHUS MYKISS*, AFTER ACUTE EXPOSURE TO THE FUNGICIDE PROPICONAZOLE

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In this study, the toxic effects of propiconazole (PCZ), a triazole fungicide present in aquatic environment, was studied in rainbow trout, *Oncorhynchus mykiss*, by acute test. Compared to the control group, fish exposed to PCZ (96h $LC_{50,}$ 5.04 mg/l) showed significantly higher ammonia and glucose concentration and the activities of plasma enzymes (including creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase), but the total protein content was not significantly different. The oxidative stress indices (levels of lipid peroxidation and carbonyl protein) of muscle in experimental group were not significantly increased compared to the control group, but a significant increase was observed in and brain. A significant decrease was observed in the activities of SOD, CAT, GR and GPx in brain of experimental group, however, opposite tendency in muscle. In short, the PCZ-induced antioxidant responds in different tissues were reflected as changes in the oxidant stress indices and plasma characteristics, but more long term experiments at lower PCZ concentrations will be necessary in the future.

This research was supported by the USB RIFCH Project No. MSM 6007665809 and the Ministry of the Environment of the Czech Republic SP/2e7/229/07.

ACUTE TOXICITY OF PAX-18 FOR JUVENILE AND EMBRYONIC STAGES OF *DANIO RERIO*

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PAX-18 (polyaluminium chloride) is a coagulation agent that is used mainly to precipitates phosphates, prevention of the eutrophication of surface waters and incidence of cyanobacteria. It is applied to the water environment and thus could present potentially danger to fish. The aim of this study was to assess acute toxicity of the preparation PAX for juvenile and embryonic developmental stages of zebrafish (*Danio rerio*). *Danio rerio* was selected as important and often use model species in acute and embryo toxicity tests.

The toxicity tests were performed according to OECD methodology. The acute toxicity tests with juvenile fish aged 2–3 months were realized according to the method OECD No. 203 (Fish acute toxicity test). In embryo toxicity test the method OECD No. 212 (Fish, short-term toxicity test on embryo and sac-fry stages) was used. The semistatic methods were selected. Two series of 5 tests were conducted, one series with embryo and other with juvenile *D. rerio*. The results of toxicity tests (the number of dead individuals at particular test concentrations) were subjected to a probit analysis using the EKO-TOX 5.2 programme to determine LC50 values of PAX. The statistical significance of the difference between LC50 values in juvenile and embryonic stage of *D. rerio* was tested using the Mann-Whitney non-parametric test implemented in the Unistat 5.1 programme.

The LC50 mean value of PAX for juvenile *D. rerio* was 749.7±30.6 $mg.l^{-1}$ and 731.5±94.1 $mg.l^{-1}$ for embryonic stages of *D. rerio*. The study proved statistically comparable sensitivity of juvenile and embryonic developmental stages to polyaluminium chloride.

The work was realized with the support of MSM Project No. 6215712402 Veterinary Aspects of Food Safety and Quality.

EFFECT OF CARVEDILOL ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES IN HUMAN BLOOD CELLS *IN VITRO*

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Activated neutrophils generate superoxide anion including other reactive oxygen species and release enzymes which participate in killing invading microorganisms. On the other hand, they inflict damage on adjacent tissues resulting in pathogenic and toxic significance in a large number of diseases. Carvedilol (CARV) is a combined β_1-, β_2-, and α_1-adrenergic receptor blocking agent, mostly used in the treatment of arterial hypertension, cardiac arrhythmias and angina pectoris. The beneficial effect of carvedilol is assumed to be associated with its antioxidant and anti-peroxidative properties.

CARV dose-dependently inhibited chemiluminiscence in the whole human blood and isolated neutrophils. To extend our knowledge about the antioxidant and cytoprotective effect of CARV we studied its effect on opsonized zymosan (OZ) stimulated superoxide generation and myeloproxidase (MPO) release in isolated human neutrophils, as well as in the mixture of human neutrophils with platelets in the ratio close to physiological conditions (1:50).

Spectrophotometrically, at respective 550 and 450 nm, we measured superoxide generation as superoxide dismutase inhibitable reduction of cytochrome c and MPO release as a oxidation of o-dianisidine in the presence of hydrogen peroxide.

Unstimulated cells showed neither superoxide generation or MPO release after preincubation with the drug. CARV dose-dependently decreased superoxide generation and MPO release from isolated human neutrophils after OZ activation, yet a significant decrease was recorded with CARV concentration of 10 and 100 μmol/l. In our experimental conditions, the coexistence of platelets with neutrophils in OZ stimulated mixture increased superoxide generation in comparison to OZ-stimulated neutrophils and partly decreased the effect of CARV.

Because CARV effectively participates in the decrease of superoxide generation in OZ stimulated human neutrophils and in reduced MPO release, it can be concluded that the toxicity and damage to surrounding tissues would also be diminished.

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PSYCHOPHARMACOLOGICAL SCREENING OF THE PYRIDOINDOLE DERIVATIVES

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Anxiety and depression are the most common mental illnesses affecting more than 35 million Americans each year. More than 27% of adult Europeans are estimated to experience at least one form of mental illness during any one year. According to World Health Organization, by the year 2020, depression is expected to be the highest ranking cause of disease in the developed world.

Pyridoindole derivatives synthesized in our institute have excellent antioxidant properties and the application is primarily to decrease oxidative stress in certain pathological processes. Moreover, their acute and chronic toxicity is very low (LD50 > 2000 mg), making them "safe" substances.

In our study we found that these derivatives possess also anxiolytic potency. We used behavioral battery of tests (open field, elevated plus maze and light/dark exploration) to study anxiolytic activity of the pyridoindole SMe1EC2 derivative at doses of 5, 10 and 25 mg/kg. Diazepam was used as positive control. The results showed that highest dose of SMe1EC2 increased time spent in the open arms as well as increased time spent in light box. These results were comparable with "golden" standard for these tests, diazepam. Moreover, no changes in motor activity were observed indicating no inhibition or activation of central nervous system.

If we take into account low toxicity and good antioxidant properties, this compound could have good potential in alleviating anxiety. However, more research is needed to confirm this finding.

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ACCUMULATION OF ARSENIC IN THE PROCESS OF FISH GROWING

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The toxic effects of arsenic depend on oxidation state, chemical species, exposure and dose, solubility in the biological media, and rate of excretion. Inorganic

compounds of trivalent arsenic As(III) are more mobile, more soluble and some 50times more toxic than pentavalent inorganic arsenate As(V). Organic species of arsenic, as arsenobetaine, arsenocholine and arsenoribosides are effectively non-toxic.

The aim of this study was to monitore the accumulation process of arsenic and its species in the muscles of the Rainbow trout (*Oncorhynchus mykiss*). The Rainbow trout (*Oncorhynchus mykiss*) grew in the drinking water reservoirs. We expected the feeding were the main source of arsenic in the muscles of these fish. The content of arsenic was monitored in the time period of 2–16 months.

The total arsenic concentration was determined by hydride generation atomic absorption spectrometry (HG-AAS), prior to microwave assisted digestion by nitric acid and hydrogen peroxide. The certified reference material DORM-2 (dogfish muscle) was used to check the quality control of the technique. Concentration of arsenic in the certified standard DORM-2 was found to be 17.66±0.789 mg/kg. The repeatability was set from 5 measurements as the RSD=4.47%. The total arsenic content in the fish muscles was from 0.18±0.012 mg/kg to 1.73±0.046 mg/kg. The limit of detection (LOD) and limit of determination (LOQ) of arsenic were 0.108 µg/l and 0.411 µg/l, respectively.

The arsenic species were determined after the extraction by ultrapure deionized water by the method of high-pressure liquid chromatography combined with the hydride generation and fluorescence spectrometry detection (HPLC-HG-AFS). The concentration of arsenobetaine in the samples of fish muscles was in the interval from 0.10±0.003 up to 1.15±0.027 mg/kg. The top of the arsenobetaine concentration was found in the 12 month old fish muscles. The arsenobetaine concentration in CRM DORM-2 was found 17.49±0.008 mg/kg. The limit of detection (LOD) of the HPLC-HG-AFS method and the limit of determination (LOQ) were 9.995 µg/l and 33.318 µg/l, respectively.

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COMPARISON OF EXTRACTION METHODS FOR SCREENING OF DRUGS AND DRUGS OF ABUSE IN BOVINE SERUM SAMPLES USING GC-MS

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Screening for drugs and drugs of abuse in biological samples is very important in clinical and forensic toxicology. Acute toxicity corresponds with the substance concentration in blood and sometimes only blood samples are available.

The object of the study was to compare liquid-liquid extraction (LLE) and solid phase extraction (SPE) techniques for screening for commonly encountered drugs and drugs of abuse in spiked formerly drug-free bovine serum samples using gas chromatography-mass spectrometry (GC-MS).

A mixture of codeine, morphine, ephedrine, 3,4-methylenedioxymethamphetamine, tramadol, dothiepin, cocaine, mirtazapine, clomipramine, alprazolam, zolpidem, clozapine, amitriptyline, citalopram, diazepam, levomepromazine, bromazepam, phenobarbital, and guaifenesin was chosen for the evaluation of the extraction methods. After the addition of internal standards trimipramine-d_3 for basic analytes and hexobarbital for acidic analytes respectively, 2 ml of drug-free bovine serum were spiked with the tested mixture of standards and extracted: **1)** LLE technique: basic and neutral analytes were extracted with 4 mL of ethylacetate:1-chlorbutane:cyclohexane (3:1:1 v/v/v) and acidic and neutral analytes were extracted with 4 mL of ethylacetate:toluene (4:1 v/v/v) **2)** SPE technique: with mixed-mode columns basic and neutral drugs were extracted with dichlormethane:isopropanol:ammonia (8:2:0.2 v/v/v) and acidic and neutral drugs were extracted with acetone:dichormethane (1:1 v/v). The spiked bovine serum samples were liquid-liquid or solid phase extracted, silylated and GC-MS analyzed. Analytes were separated on HP-5ms 30 m×0.25 mm i.d. with 0.25 µm film thickness. The compounds were screened for and identified using a Varian 3400 GC fitted with SPI injector and A200S autosampler connected to a Finnigan MAT MAGNUM ion trap operated in full scan mode.

Studied extraction techniques were evaluated by the extraction recovery assessment. LLE showed extraction efficiency ranging from 64.6 to 96.8 and SPE from 71.2 to 107.1%. The evaluated calibration curves were linear for all drugs and for both extraction techniques covering the concentration range 50–2 000 ng/mL with calibration coefficients above 0.997 for LLE and 0.998 for SPE. The limit of quantification was established as 50 ng/mL and the limit of detection was below this level for both LLE and SPE techniques.

LLE is the most successful method for universality, while SPE is the most useful method for selectivity. The application of the described assay was tested by real samples analysis.

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DETERMINATION OF PTERIN DERIVATIVES IN ANIMAL BLOOD SERUM AFTER NON-INFECTIOUS AGENS ACTION

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The assessment of stress is important for the evaluation of animal welfare. Recent studies have shown that stress situation can influence production of unconjugated pterins (neopterin and biopterin). This study was aimed to determination of neopterin and biopterin as stress markers in piglet during castration. Cortisol was determined as a control stress marker.

Blood samples were withdrawn before castration, 60 min after castration, 4 hours after castration and 24 hours after castration. Blood serum was used for analysis. Neopterin and biopterin were determined by reverse phase HPLC with fluorescence detection (λ_{ex}=354 nm, λ_{em}=438 nm). Cortisol was determined by reverse phase HPLC with with UV/VIS detection.

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SUGGESTED MECHANISMS OF PEROXIDASE RESISTANCE TO A COVALENT MODIFICATION (INACTIVATION) BY FREE-RADICAL METABOLITES

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Peroxidases are enzymes utilizing peroxides to catalyze the single electron oxidation of many substrates.

The aim of our study to investigate two model xenobiotics: carcinogenic azo dye Sudan I and anticancer drug ellipticine.

Both chemicals form free-radical species during their oxidation by peroxidases. Previous studies described that Sudan I is metabolically activated by heme peroxidases to reactive species binding covalently to nucleic acids and proteins. Also the ellipticine forms covalent DNA adducts mediated by peroxidases. Ellipticine is oxidized by peroxidases *via* similar mechanism and this reaction might participate in enhancing its pharmacological efficiencies. The mechanism of peroxidase mediated protein- and DNA-adduct formation consists of 2 parts. First, the enzyme-catalyzed part includes peroxide-dependent activation of the enzyme , organic substrate binding, followed by formation of a primary free-radical of the substrate. These radicals could be immediately released from the active site or undergo a fast conversion in the enzyme binding pocket before the release. The second phase presumably involves radical reactions, radical interconversions, protein- and DNA-adduct formation, oligomerization and radical scavenging or recombination. Since the radical structure and adduct formation process is not directed by the enzyme, the second phase model could consist of xenobiotic derived free-radical species and nucleobase part only.

Reactive free-radicals could covalently modify amino acid residues in the peroxidase active site and consequently irreversibly inhibit its catalytic activity. This is not the case of horseradish peroxidase, which is able to convert thousands of substrate molecules without substantial activity loss. A hypothesis has been proposed that its carbohydrate moieties may play a protective role (spatial shielding) against the binding of the radicals, whereby protect its own enzymatic activity. Nevertheless, even fully glycosylated peroxidase still has a direct contact with its hydrophobic substrates. Possible explanation of why is the peroxidase immune to the inactivation by free-radicals could by in their fast release from the enzyme binding site. Therefore, in order to evaluate whether the free-radical species tend to be readily released from the peroxidase active site, we compare binding free energies of the parental molecules vs. their free-radical products.

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CYTOTOXICITY AND DNA ADDUCT FORMATION BY ELLIPTICINE IN HUMAN U87MG GLIOBLASTOMA CANCER CELLS

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Ellipticine is a potent antineoplastic agent exhibiting multiple mechanisms of action. This anticancer agent should be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dependent on its cytochrome P450 (CYP)- and/or peroxidase-mediated activation. We demonstrated a novel mode of ellipticine action, formation of covalent DNA adducts mediated by its oxidation with these enzymes. Such DNA-adducts are formed *in vitro*, in human breast adenocarcinoma MCF-7, leukemia HL-60 and CCRF-CEM, neuroblastoma cell lines and *in vivo* in rats and mice exposed to ellipticine.

The aim of this study was to investigate the effects of ellipticine on a glioblastoma cell line U87MG and the mechanism of ellipticine action in these cancer cells.

MTS proliferation test was used to determine the IC_{50} values of ellipticine toxic activity to U87MG glioblastoma cells. Cell cycle and apoptotic population were analyzed by FACS after PI staining. Senescence was assessed by detection of senescence-associated β-galactosidase activity. The ^{32}P-postlabeling technique was utilized to determine ellipticine-DNA adducts.

Ellipticine inhibited efficiently proliferation of U87 glioblastoma cell line with IC_{50} of 1.48 μM, due to transient G0/G1 and slight G2/M cell cycle arrest induction. No significant apoptosis was detected neither by Hoechst 33342 dye staining, neither as a subG1 cell cycle population when cells treated by 1 μM ellipticine. In contrast, important senescent population appeared after the ellipticine treatment. As we have shown recently, ellipticine forms covalent DNA adducts after its metabolic activation by CYP enzymes and/or peroxidases. Expression of such enzymatic systems in the U87 cells was found utilizing the Western blot analysis. Moreover, the ellipticine-DNA adducts were generated in these cells exposed to ellipticine in amounts comparable to neuroblastomas or leukemia cells, confirming its metabolic activation in glioblastomas.

Our study confirmed sensitivity of glioblastoma cell line U87 to ellipticine and showed that ellipticine induces rather premature senescence, than apoptosis. Moreover, we proved that ellipticine binds covalently to DNA after its activation in these cells, which is at least one of causes of sensitivity of the U87 cells to the ellipticine treatment.

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PORCINE CYP2A19 AND CYP2E1 FORMS ARE RESPONSIBLE FOR SKATOLE BIOTRANSFORMATION IN THE RECONSTITUTED SYSTEM.

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Skatole (3-methylindole) is naturally occuring microbial metabolite produced from tryptophan in gastrointestinal tract of humans, pigs and ruminats. It is a well known pneumotoxin in cattle and it has important implications in meat production. 5–10% of adult male pigs carry so-called boar-taint (an unpleasant odor liberated when meat is cooked, caused by presence of skatole and adrostenone). On the other hand, uncastrated male pigs are in general more suitable for meat production because of their better fatty acids composition and also better feed conversion compared with castrated pigs. CYP enzymes play crucial role in the metabolism of skatole hence lowering the skatole level, however, there are no studies, which would clearly demonstrated the participation of individual CYP forms on skatole biotransformation in pigs.

According to previously published papers, CYP2A19 and CYP2E1 are probably the most important forms involved in skatole biotransformation. In order to determine which of CYP forms mentioned is responsible for formation of each metabolite, the reconstituted systems with purified porcine CYP2A19 and 2E1 were used. Analyses of formation of major skatole metabolites (indol-3-carbinol, 3-methyloxindole, 2-aminoacetophenon) were performed using an HPLC method with UV detection based on method published by Diaz et al. (Diaz *et al.*, 1999).

According to our findings, the CYP450s responsible for the 3-methyloxindole formation are CYP2E1 and also CYP2A19. For indol-3-carbinol, only CYP2A19 seems to be involved. The 2-aminoacetophenon was not detected in our experiments, suggesting that other CYPs may be involved.

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Diaz GJ et al. Drug Metab Dispos. 1999; **27**(10): 1150–1156.

VIEW OF APC POLYMORPHISMS IN SLOVAK POPULATION

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Mutations in the *APC* gene initiate the early stages of the adenoma-carcinoma sequence in both familial and sporadic colon tumorigenesis. Truncating germ line mutations in the *APC* gene are responsible for most cases of familial adenomatous polyposis, which is characterized by early onset polyposis (the presence of hundreds to thousands of adenomatous polyps in the colon and rectum). Familial adenomatous polyposis is an autosomal dominant inherited disorder that accounts for about 1% of all colorectal cancers. The *APC* gene also acquires somatic mutations in 80% to 90% of sporadic colorectal adenomas. The clinical relevance of the *APC* polymorphisms is also uncertain and previous work suggests that some polymorphisms may either be a low-penetrance allele that increases risk of developing colorectal cancer or a common polymorphism without clinical implication.

The aim of this study was to investigate polymorphism in the *APC* gene and their association with colorectal cancer and colorectal adenoma. Blood samples were collected from 20 FAP suspected families from whole Slovakia, in which *APC* gene mutations

were not identified. For detection of polymorphisms we used single strand conformation polymorphism (SSCP) technique and direct sequencing.

In the cohort of analyzed families were found 12 different types of polymorphisms. The most common *APC* variant was Asp1822Val with frequency of 45%. The functional significance and clinical relevance of the Asp1822Val substitution is unknown, although this amino acid change is located in the center of a β-catenin down-regulation domain. Disregulation of the Wnt/β-catenin signaling pathway leads to nuclear accumulation of β-catenin resulting in aberrant cellular proliferation in the upper crypt. Wnt/β-catenin overactivation, often resulting from mutated *APC*, leads to imbalanced cellular proliferation and differentiation and has been documented in tumors and is implicated in human cancer. The other forms of polymorphism didn't lead into amino acid changes and they were found as a neutral in association to colorectal cancer.

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INFLUENCE OF PROBIOTICS ON RAT LIVER BIOTRANSFORMATION ENZYMES

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Probiotics can be defined as nonpathogenic microorganisms that, when ingested, exert a positive influence on the health or physiology of the host. The effects of probiotics can be direct or indirect through modulation of the endogenous flora or of the immune system. During concomitant medications with other pharmacotherapeutic agents, interactions of probiotics with drug metabolizing systems may take place. Cytochromes P450 (CYP, enzymes metabolizing the majority of drugs) are localized mainly in the liver and their activities may be modulated by the probiotics ingested.

Live bacterial suspension of *E. coli* Nissle 1917 O6:K5:H1 was applied to Wistar rats (10^9 CFU/dose, orally). Three rats were taken as controls (no probiotic applied). Four rats were stressed by oral application of the physiological solution daily for 14 days. The probiotic was applied daily to four animals for 14 days; four rats were given the probiotic only once, the last day of the experiment. Also, the lipopolysaccharide-containing supernatant of *E. coli* was applied to three rats daily for 14 days. Liver microsomal fraction was prepared and activities and relative amounts of selected CYP enzymes were followed by established enzymological techniques and by Western blotting.

The substrates of individual CYP forms such as testosterone, 7-ethoxyresorufin, coumarin, 7-ethoxy-4-(trifluoromethyl)-coumarin, luciferin-H EGE (ethylene glycol ester of luciferin), luciferin-ME (luciferin 6′-methyl ether), luciferin-H (6′-deoxyluciferin), luciferin-ME EGE (ethylene glycol ester of luciferin 6′-methyl ether) and luciferin-BE (luciferin 6′-benzyl ether) were used during the experiment. Changes of activities of CYPs were not statistically significant, however, certain trends were observed (e.g. the 14 day application of supernatant resulted in a decrease of CYP3A1 activity). According to our finding, probiotics do not markedly influence rat hepatic biotransformation enzymes.

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OBJECTIVE DIAGNOSTIC METHOD OF MUSCARINE INTOXICATION

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Muscarine is responsible for the parasympathomimetic effects of some genera of mushrooms (mainly Amanita, Inocybe, and Clitocybe). Typically, symptoms of muscarine poisoning begin within 15 minutes to 1 hour, with headache, nausea, vomiting, and abdominal pain followed by salivation, lacrimation and perspiration (so-called PLS syndrome), miosis and blurred vision. Symptoms generally abate within several hours. Death is uncommon. Nowadays, only microscopic examinations are used to diagnose these mushrooms poisonings.

In this article an objective diagnostic method of muscarine intoxication is presented. In connection with it, the dedicated analytical method for identification (eventually determination) muscarine from human urine, blood or gastric contents was elaborated.

Weak cation-exchange SPE column was used for isolation of muscarine from biological material. For muscarine identification LC-MS-ESI analysis was performed using Gemini C18 column. Retention time of the peak of muscarine was 14.2 min and its base peak of the mass spectra was *m/z* 174. Described method produces distinct muscarine identification and separation from other mushrooms toxins, e.g. ibotenic acid and muscimol.

Three patients urine and gastric contents were analysed for the presence of the muscarine. In the first case, reported method was used for analysis of 55-year-old man`s material, who ingested *Amanita muscaria.* In the

second and the third cases *Clytocybe candycans*, resp. *Amanita pantherina* were eaten by young women (22- and 28-year-old).

Describe sensitive and specific liquid chromatography-mass spectrometry assay proved useful for analysis of muscarine in biological material.

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THE TOXICITY OF MAGNETIC NANOPARTICLES

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Magnetite nanoparticles (MNPs) are used for *in vivo* biomedical application including magnetic resonance, magnetically controlled transport of anticancer drugs as well as hyperthermia generation. In order to prevent aggregation and to increasing the circulation time in the blood stream, the surface of MNPs must be modified with various surfactants to 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 and their capacity to produce oxidative stress in exposed cells. Oxidative stress can lead to oxidative damage of DNA that is supposed to play an important role in degenerative processes such as carcinogenesis and aging. Reactive oxygen species (ROS) can be generated via the Fenton reaction in which iron constitutes the key element. Thereby, magnetite nanoparticles might influence the oxidative status of the cells due to ROS formation.

MNPs were prepared by the coprecipitation method of ferric and ferrous salts in an alkali aqueous medium. X-ray diffraction measurement was performed to identify the crystallographic structure of prepared MNPs and the magnetic properties were characterized by SQUID magnetometer at room temperature. Two surfactans – sodium oleate ($C_{17}H_{33}COONa$) and polyethylene glycol (PEG Mw=1 000) were used for coating of MNPs (10 nm). The biological activity of sodium oleate-coated MNPs (SO-MNPs) and sodium oleate- plus PEG-coated MNPs (SO-PEG-MNPs) was investigated using the human alveolar epithelial carcinoma cell line A549. The cytotoxicity of both MNPs and surfactants were analyzed after short-term (4h) and long-term (24h) exposure. In addition, the oxidative status of exposed and control cells was measured after long-term cell treatment.

Our preliminary results suggested differences in biological activities of nanosphered magnetite particles in dependence on surface coating.

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GLUCOMANNAN FROM *CANDIDA UTILIS* IN METHOTREXATE-BASED COMBINATORY THERAPY OF ADJUVANT ARTHRITIS

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Methotrexate (MTX) is widely used in the treatment of rheumatoid arthritis but its efficacy is often limited by severe side and toxic effects where oxidative stress is involved (1). Glucomannans are polysaccharides with antioxidant and immunomodulatory properties (2–4), which could be protective against the side effects of methotrexate.

We investigated the possible protective effects of glucomannan (GM) from *Candida utilis* in combinatory MTX therapy of adjuvant arthritis (AA).

AA was induced by a single intradermal injection of *Mycobacterium butyricum* in incomplete Freund´s adjuvant in male Lewis rats. MTX in two doses (0.3 mg and 0.5 mg/kg b.w.) was administered twice a week. GM treatment involved daily oral (7.5 mg/kg b.w.) administration. Hind paw volume (HPV), change of body mass, activity of gamma-glutamyltransferase (GGT) in homogenates of spleen and hind paw joint and levels of thiobarbituric acid reacting substances (TBARS) in plasma were monitored.

All parameters diteriorated by arthritis were improved after administration of both MTX doses tested. The results after MTX treatment with GM addition indicate beneficial effects of this combination on arthritis development. Most effective was the dose of 0.5 mg MTX /kg b.w. with GM addition, which in comparison to untreated arthritic rats significantly inhibited the activity of GGT- anti-inflammatory marker – in both tissue homogenates. However the levels of TBARS were not affected. In all groups of animals tested the trends of HPV values, as the main clinical marker of AA, were comparable to those of GGT activity measured in spleen tissue homogenates.

Further experiments should provide insight into the exact mechanisms of MTX and GM interaction in arthritis combinatory therapy.

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METHODS FOR EVALUATION OF ALGICIDE ECOTOXICITY IN AQUATIC ECOSYSTEM – A MINIREVIEW

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Water eutrophication which is closely connected with wide-spread occurrence of cyanobacterial (algal) water-blooms on freshwater bodies represents serious problem. Recently, many chemical methods for the reduction of cyanobacterial mass development have been introduced. There is an effort to find the measures which would be as safe for non-target organisms as possible.

Toxic effects of substances which have been used or seem to be useful for water-bloom management have been studied by many researchers. Single-species laboratory experiments with phytoplankton cultures have been most commonly carried out. They are sensitive and highly reproducible but they do not provide information about effects of tested substances on whole ecosystem, thus they lack environmental realism. On the other hand, microcosm or mesocosm experiments studying the effects of tested substances on community- level have only limited reproducibility.

This minireview provides an overview of methods for evaluation of algicidal substance ecotoxicity in aquatic ecosystem. Advantages and disadvantages of individual testing methods as well as various endpoints used for the measurements are described.

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CYTOCHROMES P450 AND PEROXIDASES OXIDIZE 3-AMINOBENZANTHRONE, THE HUMAN METABOLITE OF CARCINOGENIC 3-NITROBENZANTHRONE

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The nitroaromatic compound 3-nitrobenzanthrone (3-NBA) is the suspected human carcinogen and is one of the most potent mutagens identified in diesel exhaust, ambient air particulate matter, in surface soil and rainwater. The main metabolite of 3-NBA, 3-aminobenzanthrone (3-ABA), was found in the urine of salt mining workers occupationally exposed to diesel emissions, indicating that exposure to 3-NBA can be significant and is detectable. Understanding which enzymes are involved in the metabolic activation and detoxication of 3-ABA is important in the assessment of susceptibility to this compound. Recently, we showed that principal enzymes activating 3-ABA to species binding to DNA in livers are cytochromes P450 (CYP), especially CYP 1A1 and CYP 1A2.

The aim of this study was to investigate the metabolism of 3-ABA *in vitro*, in order to characterize the 3-ABA metabolites as well as cytochromes P450 responsible for their formation

Oxidation of 3-ABA by hepatic microsomes and rat and human Supersomes™ (microsomes containing human and rat recombinant CYPs) and peroxidases was analyzed. The metabolites formed in the reaction were separated by RP-HPLC.

3-ABA is oxidized by rat hepatic cytochromes P450 in microsomes and Supersomes to three metabolites, which were separated by HPLC as distinguish product peaks. Using co-chromatography with synthetic standards, two of them were identified to be oxidative metabolites of 3-ABA, *N*-hydroxy-3-ABA (r.t. of 6.5 min) and 3-NBA (r.t. of 25 min). The structure of another metabolite eluted with r.t. of 18 minute, assigned as M18, remains to be characterized. In this study, we characterized kinetics of their formation and determined the CYP enzymes responsible for 3-ABA metabolite formation. The present study shows the similarity between human and rat hepatic enzymes metabolizing 3-ABA and indicate that rat might serve as a suitable model to mimic the fate of 3-ABA in human. We also found that 3-ABA is oxidized by peroxidases. We compare the efficiencies of three peroxidases, horseradish peroxidase (HRP), lactoperoxidase (LPO) and myeloperoxidase (MPO). The highest efficiency to oxidize 3-ABA was detected with HRP, followed by MPO and LPO.

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IS CYTOCHROME P450 1A1 THE MAJOR ENZYME ACTIVATING BENZO[A] PYRENE *IN VITRO* AND *IN VIVO* ?

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Carcinogenic benzo(a)pyrene (BaP), known to covalently bind to DNA after its activation with cytochromes P450 (CYPs), was investigated for its potential to generate DNA adducts and to induce CYP and NADPH:CYP reductase (POR) enzymes in mouse livers, the main

target organ for DNA adduct formation. CYP1A1 and CYP1B1 are widely accepted to be the most important enzymes in the metabolic activation of BaP. However, current studies show that BaP-induced DNA damage was increased in mice lacking CYP1A1, indicating that *in vivo* the CYP1A1 enzyme plays a detoxification role, and protects mice against BaP toxicity.

The aim of this study was to understand which enzymes are actually involved in the metabolic activation of BaP and whether this compound can influence its own metabolic activation.

HRN ($Por^{lox/lox}$+Cre^{ALB}) mice were constructed in laboratories in Great Britain. Male HRN and WT mice (25–30 g) were treated i.p. with 125 mg/kg body weight of BaP daily for 5 days. BaP metabolites were separated by RP-HPLC. The ^{32}P-postlabelling technique was utilized to determine BaP-DNA adducts.

BaP induces expression of CYP1A 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. The 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 form mono- and di-hydroxylated metabolites of BaP as well as a quinone metabolite of BaP. BaP is also oxidized with purified CYP1A1 reconstituted with NADPH:CYP reductase. A pattern of BaP metabolites formed in this system differs from those formed in the intact microsomes. Taken together, these results demonstrate that by inducing CYP1A1/2 and, perhaps also other enzymes, BaP modulate its own enzymatic metabolic activation and detoxication, thereby modulating its genotoxic potential.

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PREPARATION OF APO-CYTOCHROME b_5 AND STUDY OF ITS FUNCTION

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Cytochrome b_5 (cyt b_5), a component of endoplasmic reticulum membrane, plays a role in modulation of activity of some cytochromes P450 (CYP). To elucidate the mechanism of such modulations it is necessary to evaluate not only the effect of native cyt b_5, but also that of apo-cyt b_5. The effect of apo-cyt b_5 on this enzymatic system has not been investigated in details, because preparation of cyt b_5 as a pure protein failed.

To prepare the native apo-cyt b_5 in a large scale we utilized a protein with higher affinity toward the heme, the apo-myoglobin from the equine skeletal muscle. First, we extracted heme moiety from the native myoglobin by butanone extraction. Than the effect of pH on spontaneous heme release from both proteins was investigated: purified rabbit cyt b_5 as well as equine skeletal muscle myoglobin. The prepared apo-myoglobin was incubated with the cyt b_5 and heme transfer was monitored. The optimal pH range for heme transfer from cyt b_5 into apo-myoglobin was between 4.2 and 5. Native apo-cyt b_5 was separated from myoglobin on a column of DEAE-Sepharose. The apo-cyt b_5 reconstituted with heme reveals the same oxidized and reduced absorbance spectrum as native cyt b_5 and was found to be reduced also with NADPH:CYP reductase.

The experiments investigating the effect of the purified native cyt b_5 and apo-cyt b_5 on oxidation of xenobiotic substrates of CYP3A4 and 1A are under way in our laboratory.

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PREVENTION OF POISONINGS IN CHILDREN

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The Czech Republic belongs to the countries with high child morbidity and mortality due to accidents including household poisonings and intoxications by poisonous plans and mushrooms. For this reason the Ministry of Health of the Czech Republic supports the educational preventive project "Prevention of poisonings in children and young adults".

This prevention program focuses on the increase of the awareness of the risk of poisoning in children; it concentrates on the safe storing and packaging of chemicals at home and the education of teachers and educators. The responsible partner of this project is the Czech Toxicological Information Centre. This educational program is devoted to children of kindergartens and primary schools, aged from 3 to 15 years and to their parents, teachers and educators. The project started in year 2008 and it will continue up to the end the year 2009.

The project includes presentations to the teachers and educators about the danger of children poisonings. It should improve the knowledge of the selected target groups of population (children, teachers, parents) and to increase awareness of the risks in case of ingestion of household product or the plants and the mushrooms by children. The parents and educators are informed about the toxicity of different household products and naturally occurring toxins. In addition they are instructed in providing first aid after ingestion of toxic plants, mushrooms or household products.

Another part of the project is the creation of educational materials to be distributed in school, kindergartens

and mother centers. The project outputs include 8 000 copies of the handbook of K. Mrázová et al.: "Children's poisonings", 1 000 printed cards of the poisonous plans and mushrooms, and 2 000 stickers with phone number to the Czech Toxicological Information Centre. The handbook indicates the most frequent effects of ingestion of dangerous household products, plants and mushrooms and provides advice and recommendations about the first aid in case of potentially toxic ingestions.

The full impact of the project should be seen in several years' horizon. Improvement of the knowledge and behavior after toxic exposures due to the prevention education programs will be tested and evaluated at the end of the year 2009.

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OCCUPATIONAL EXPOSURE TO ANAESTHETICS AND CYTOSTATICS, CHROMOSOMAL ABERRATIONS, POLYMORPHISMS OF DNA REPAIR GENES *XPD, XPG, XPC*

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The workers of anaesthesiological departments and oncological units are periodical, excessively and long-time exposed to low doses of genotoxic agents.

The aim of this study was evaluation of genotoxic risk occupational exposure this agents by means of quantification the total chromosomal aberrations (CAs), and specific types: chromatid-type (CTA) and chromosome-type (CSA). It was attempt to assess the factors of individual susceptibility by polymorphisms of DNA repair genes *XPD*, *XPG* and *XPC*. CAs frequency was detected in peripheral blood lymphocytes by cytogenetic analysis, and polymorphisms of genes by PCR-RFLP based method.

The group exposed to anaesthetics (EXP1) was consisted of 76 persons and the group exposed to antineoplastic agents (EXP2) of 72 persons from Medical Faculty Hospital (MFH) in Martin and Central Military Hospital in Ružomberok. The control included 76 unexposed persons from MFH.

Significantly higher frequency of total CAs was detected in group EXP1 (2.53%±1.46 S.D.) and EXP2 (1.90%±1.34) with comparison to control (1.26%±0.93), respectively, Mann-Whitney U-test, EXP1 p=0.0008, EXP2 p=0.001). In group EXP1 was detected statistically higher frequency of CSA-type aberrations in comparison to CTA-type (1.92%±1.38 vs. 0.61%±0.83, respectively (Mann-Whitney U-test, p=0.0009), however it was´t detected any difference in frequency CTA-type and CSA-type in group EXP2 (0.53%±0.62 vs. 0.73%±0.81). The smoking habitus, gender division and job categorization wasn´t influenced any frequency of total CAs, CTA-type and CSA-type. In polymorphisms of *XPD* gene Lys751Gln was detected slightly increase of CAs, CTA-type and CSA-type in presence of variant allele in group EXP1, but wasn´t detected any difference in group EXP2. In *XPG* gene Asp1104His wasn´t detected any difference in frequency of aberrations in group EXP1, but was detected significantly decrease of total CAs and CTA-type in individuals with variant allele (Mann-Whitney U-test, p<0.05) in group EXP2. In *XPC* gene Lys939Gln wasn´t detected any difference in total CAs, CTA-type and CSA-type in presence of variant allele in both exposed groups.

The detection of individuals with higher susceptibility has the great importance in preventive protection of workers.

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FORMATION AND CHARACTERIZATION OF DEOXYGUANOSINE ADDUCTS GENERATED BY CARCINOGENIC *O*-ANISIDINE AND *O*-NITROANISOLE

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2-Methoxyaniline (*o*-anisidine) and 2-methoxynitrobenzene (*o*-nitroanisole) are industrial and environmental pollutants causing tumors of urinary bladder. The International Agency for Research on Cancer has classified both compounds as carcinogens, which are possibly carcinogenic to humans. Besides their carcinogenicity, they exhibit other toxic effects, including hematologic changes, anemia and nephrotoxicity. We found that both carcinogens are oxidatively activated by cytochromes P450 (CYP) to species binding to DNA *in vitro* and *in vivo*. The same adducts as found in DNA incubated with *o*-anisidine and *o*-nitroanisole and human microsomes containing CYP enzymes *in vitro* were detected in the urinary bladder, the target organ for their carcinogenicity, and to a lesser extent, in liver, kidney and spleen of rats treated with both these carcinogens.

The aim of our study was to prepare, isolate and characterize these adducts. *In vitro* methods for preparation of adducts, their HPLC separation and mass- and/or NMR spectrometry for their characterization were used.

The DNA adducts generated by *o*-anisidine and *o*-nitroanisole were identified as deoxyguanosine

adducts formed from their common metabolite, *N*-(2-methoxyphenyl)hydroxylamine. Three deoxyguanosine adducts prepared by the reaction of this deoxynucleoside and *N*-(2-methoxyphenyl)hydroxylamine were separated by HPLC and their structures were evaluated by mass- and/or NMR-spectrometry. The structure of the major deoxyguanosine adduct was identified to be *N*-(deoxyguanosin-8-yl)-2-methoxyaniline. Using co-chromatography on HPLC, we confirmed that this major adduct is identical with that detected by the ^{32}P-postlabeling assay, found to be formed *in vitro* and *in vivo*. On the contrary, the structures of two minor adducts formed during reactions of *N*-(2-methoxyphenyl)hydroxylamine with deoxyguanosine await further investigation.

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ANTIOXIDANT AND GENOTOXIC ACTIVITY OF PLANT ESSENTIAL OILS

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Plant essential oils, used as flavouring in different preparations for centuries, represent volatile aromatic oils, primarily composed of terpens and their oxygenated derivatives. Many of them were shown to exhibit a range of biological activities. It is however important to evaluate such compounds for their pro-oxidant and toxic properties as their plant origin does not secure their safety for living beings, including humans.

We investigated antioxidant, cytotoxic, genotoxic and potential DNA-protective effects of four components of plant essential oils borneol, carvacrol, eugenol, and thymol. Human hepatoma cells HepG2 were used in our study, incubated *in vitro* with selected components of volatiles for 24 hours.

Antioxidative activities of water and ethanolic solutions of compounds studied were determined by a spectrophotometric method using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Cytotoxicity testing was performed on the basis of trypan blue exclusion. The amount of DNA lesions created in cells treated with selected components of plant essential oils or their combinations with hydrogen peroxide (H_2O_2 – which should reveal DNA protective effects of these compounds) were measured by alkaline single cell gel electrophoresis (comet assay).

Borneol failed to exhibit any antioxidative activity even at the highest concentrations soluble in water or ethanol (<1 000 mM), while eugenol manifested antioxidative activity at many times lower concentrations (5–100 μM). Carvacrol and thymol exhibited lower antioxidative activity in higher concentrations than eugenol (1 000–5 000μM). Trypan blue exclusion technique showed that the cytotoxic effect in HepG2 cells decreased in the order: carvacrol > thymol > eugenol > borneol. The compounds did not induce DNA strand breaks at concentrations ≤IC_{50}. Carvacrol, thymol, and borneol in a whole scale of concentrations significantly protected the cells against DNA strand breaks induced by H_2O_2. Despite the antioxidant activity of eugenol, measured by DPPH assay, it did not manifest any protective effect on HepG2 cells.

To sum up, the cytotoxic effects of the selected components of plant essential oils ($IC_{\leq 50}$) on human hepatoma HepG2 cells were not accompanied with DNA-damaging effect. Carvacrol, thymol, and borneol reduced the level of H_2O_2 induced DNA lesions in the cells.

We conclude that carvacrol, thymol and borneol may be considered potentially active antimutagenic drugs.

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H_1-ANTIHISTAMINES AND OXIDATIVE BURST OF PROFESSIONAL PHAGOCYTES

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Besides their antihistaminic activities, H_1-receptor antagonists possess other pharmacological properties: anti-inflammatory action, inhibition of blood platelet function and antioxidative effects. The mechanism(s) responsible for the nonspecific non-receptor operated activity of H_1- antihistamines is not fully understood and may appear at extra- and intracellular site of neutrophils.

H1-antihistamines (H1-AH) represent a heterogenous pharmacological entity belonging to the group of organic cationic amphiphilic drugs (CAD). Interactions between CAD and blood cells could result in side and adverse drug reactions, which in case of side effects need not be negative. The potential of H_1-antihistamines to inhibit indices of inflammatory cell activation has been studied widely. While individual studies vary in detail, presumably due to differences in experimental conditions and provoking stimuli which may influence the observed results, the effects are likely to be histamine H_1-receptor independent.

Human polymorphonuclear leukocytes (PMNL-neutrophils) have a large homogeneous population of H1 receptors of moderate afinity. Thus PMNL attracted to sites of allergic inflammation have H1-binding sites, which may respond to Hi stimulation. By means of

the amplified chemiluminescence (CL) technique, we demonstrated that activated PMNL responded with a respiratory burst accompanied by both extra- and intracellular generation of reactive oxygen metabolites. Histamine(Hi) and H1-AH tested decreased significantly both the extra- and intracellular component of stimulated CL. We evaluated the ability of acrivastine, antazolin, astemizol, bromadryl, brompheniramine, clemastine, cyclizine, dithiaden, chlorcyclizine, chlorpheniramine, ketotifen, loratadine, oxatomid and pheniramine to suppress production of reactive oxygen species (ROS) and to modulate myeloperoxidase activity by phagocytes and their antioxidative properties in cell free systéme.

Interaction with enzymes (NADPH-oxidase, myeloperoxidase, phospholipase A2, etc.) or interference with PMNL membrane structure may well result in reduction of the CL signal. Depending on the concentration used, some H1-AH were more effective in inhibiting activated CL of whole blood than Hi. In isolated human PMNL, both Hi and H1-AH inhibited stimulated CL dose-dependently, with potentiation observed after specific stimulation (PMA and fMLP). The interaction of H1-AH with PMNL is libely to operate both at extra and intracellular level. The fact that Hi as well as H1-AH decreased the respiratory burst indicates that not only Hi receptors but also non-receptor mechanisms could be involved in the reduction of CL.

The extensive therapeutic use and associated side effects have generated a great deal of interest in understanding the nonreceptor interactions of CADs at cellular and molecular level.

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CHEMILUMINESCENCE RESPONSE INDUCED BY MESENTERIC ISCHAEMIA/REPERFUSION: EFFECT OF PLANT COMPOUNDS *IN VITRO*

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Since reactive oxygen species (ROS) are supposed to contribute significantly to tissue damage, the antiradical activity of any compounds may prove beneficial as it helps to reduce tissue injury. The aim of our work was to study the possible effect of plant origin compounds in the rat model of mesenteric ischaemia/reperfusion (I/R) induced intestinal and vascular injury, along with the role of ROS.

The extent of intestinal damage caused by I/R was recorded and the activity of myeloperoxidase (MPO) was measured in the scraped intestinal mucosa. An increased free radical production was assessed by the luminol enhanced chemiluminescence (CL) response of the ileal samples and of the superior mesenteric artery (SMA). The effect of plant origin compounds was studied *in vitro*: arbutin, extract Mentha villosa, rosmarinic acid and curcumin in a concentration of 5×10^{-4} M were incubated for 30 min with the vascular and intestinal samples taken from the rats exposed to 60 min ischaemia induced by the occlusion of SMA and followed by 30 min reperfusion.

I/R induced pronounced haemorrhagic intestinal injury and an increase of MPO. Comparing to sham operated (control) rats, the CL response increased slightly after I/R, probably in association with neutrophil increase which was indicated by the enhanced MPO activity. All compounds studied significantly reduced the peak values of CL responses of both ileal and vascular samples. Curcumin was found to be the most effective one.

The results obtained showed a similar inhibitory effect of plant origin compounds studied on CL response influenced by mesenteric I/R induced changes. Plant derived phenolics represent good source of natural antioxidants.

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TOXICITY OF ATMOSPERIC POLLUTANTS – SPECIFIC EFFECTS OF EXTRACTS OF AIR PARTICULATE MATTER SIZE FRACTIONS

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Atmospheric pollution poses an important environmental problem nowadays. Although air pollutants were described to cause many toxic effects in exposed organisms, the mostly used approaches for evaluation of level of air pollution are based on chemical analysis that are able to describe neither overall composition nor the toxic effects of the complex mixtures of pollutants. This limitation could be addressed by *in vitro* bioassays that assess overall toxic effect of the complex pollutant mixture and allow relatively extensive screening studies.

In our study, we assessed genotoxicity, dioxin-like activity, anti/estrogenicity and anti/androgenicity of extracts from gas phase of air and six inhalable size fractions of air particulate matter *in vitro*. The air samples have been collected at six localities that differed in type of contamination coming from industrial and combustions sources. We observed significant genotoxic, dioxin-like and antiestrogenic potency of the extracts from particulate air matter. This potency increased with decreasing diameter of the particles. Interestingly, extracts from gas phase of the air samples showed, besides similar effects produced by the particulate matter extracts, also significant antiandrogenicity.

The results suggest that bioassays are suitable for assessment of specific toxic effects of the complex mixtures of atmospheric pollutants. These findings might help to improve the ecological risk assessment that

might become more relevant if it included also other biological endpoints besides genotoxicity.

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OCCUPATIONAL EXPOSURE OF HEALTH CARE PROFESSIONALS TO ANTINEOPLASTIC AGENTS IN MASARYK MEMORIAL CANCER INSTITUTE, BRNO, CZECH REPUBLIC

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Antineoplastic drugs show mutagenicity, carcinogenicity and reproductive toxicity. Due to this fact, occupational exposure of hospital workers handling these drugs should be limited or avoided. This study focused on the surface cytotoxic contamination of the preparation and administration working areas of one of the prominent Czech oncology institutes – Masaryk Memorial Cancer Institute. Main objective was to compare levels of occupational exposure at different workplaces (hospital pharmacy, outpatient and nursing clinic) and to get basic exposure data for the following health risk assessment.

During 7 weeks study, contamination of working tables, floor and objects with frequent hand contact (telephone, door handles etc.) was monitored. Samples were collected by wiping technique utilizing nonwoven swabs moistened with acetate buffer as collection medium. Wipers were extracted in acetate buffer and analysed for cyclophosphamide and 5-fluorouracil by reverse phase HPLC with mass spectrometry detection. Platinum containing drugs (cis-platin, carboplatin etc.) were analysed as a sum of platinum by inductively coupled plasma mass spectrometry (ICP-MS).

Measurable amounts of studied drugs were detected on majority of the sampled surfaces. From the total number of collected samples (n=159), more than 70% were positive for at least one of the drugs. The highest concentrations were found within the pharmacy isolator (safety box used for chemotherapy preparation), where the contamination level often-reached nanograms per cm^2. Contamination of equipment of the preparation room outside the isolators was also very frequent and reached up to tens picograms per cm^2. Interestingly, comparable contamination levels were found also in the outpatient clinic administration areas, where the working tasks are carried out without any special safety measures.

Contamination of the hospital departments by studied drugs was very frequent. The open operations with the drugs during their preparation and administration are the main sources of the contamination. The highest concentrations were measured inside the isolators (in the preparation area) but also on the floor under the infusion stands in administration area, and further efforts should focus on protection of such relatively poorly controlled areas.

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NEUROPROTECTIVE ACTION OF 2,3-DIHYDROMELATONIN (DHM) IN TRANSIENT GLOBAL ISCHEMIA OF GERBIL BRAIN

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Time course of the ischemia/reperfusion (I/R)-induced oxidative impairment in glutathione homeostasis is not uniform in all brain regions. In hippocampus, an early decrease (after 6 h) in the total glutathione (GSx) content is followed by its second delayed decrease after 48–72 hours that is also associated with a significant diminution in the activity of the glutathione reductase (GR) a key enzyme of the redox-metabolism. The decrease in GR activity is attributed to the loss of protection given to this enzyme by GSx. An early I/R-induced depression in GSx content was also shown in the cortex, but the late effect seen in the hippocampus remains absent.

The goal of the present study is to verify whether the early I/R-induced drop in hippocampus GSx leads also in the brain cortex to depression of GR activity and whether similarly to melatonin its dihydro derivative is also capable to stimulate the GR.

Brain ischemia was induced by 12 min bilateral carotid occlusion (BCAO) in male gerbils (60–70g *b wt.*). DHM (10 mg /kg) was administered i.p.20 min before surgery, as well as at the beginning and after 2 and 6 hours of reperfusion. Horizontal locomotor activity was recorded using the open-field test along the experiment.

Content of GSx after I/R decreased in cortex by 28.3% and hippocampus by 33.73% ($p<0.001$). Administration of DHM prevented the decreases in the GSx content ($p<0.05$). Activities of GR in hippocampus and in cortex did not change after I/R irrespectively to DHM administration. In DHM treated animals the horizontal locomotor activity became normalized.

Despite expectations we did not observed any significant changes in the activity of GR in brain cortex and in hippocampus after I/R. We assume that even the decreased level of GSx in both regions investigated remained high enough to protect the GR against an unfavourable shift in the redox state in the applied model of I/R.

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THALLIUM DISTRIBUTION OBSERVED IN PLANTS

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Thallium is a soft, bluish-grey metalloid that was discovered in 1861. It is not an ubiquitous element and it itself is very toxic– its salts are considered to be most toxic compounds that are known. The most important valence state of Tl is Tl(I). In this state, thallium forms many compounds with different solubility that play crucial role in bioavailability. Thallium and its salts are accumulated very often on living environment

The aim of this work was to determine Thallium uptake and accumulation in roots and leaves of maize (*Zea mays* L., *Poaceae – Graminae*) after exposition to Thallium.

The corns as well as plants of maize were exposed to thallium in concentration range from 0 to 50 μmol.l^{-1} in the ontogenetic state of germination. The samples (roots, leaves) were collected after 14-days treatment. For thalium determination in plant tissues, inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used.

Obtained results indicate intense uptake as well as transport of thallium ions in plants. In plants treated by thallium ions in concentration 1 μmol·l^{-1}, thallium concentration 0.1690 mg·g^{-1} in roots and 0.0480 mg·g^{-1} in leaves was determined. Treatment of plants by increasing thallium ions concentration led to increasing thallium concentration in individual plant organs – leaves and roots. It was detemined, that dry matter of roots of plants treated by 10 μmol.l^{-1} thallium ions concentration contained 1.8219 mg·g^{-1} of Tl. Transport of Tl ions into aerial parts of plants is probably limited – in leaves of plants exposed to the identical Tl ions concentration as above mentioned, only thallium concentration of 0.6760 mg·g^{-1} in dry matter was detected. High Tl concentrations were very toxic for plants, concentration 50 μmol.l^{-1} was lethal.

It was found, that thallium already in low concentrations is very toxic and causes growth depression as well as significant decrease of chlorophyll content in leaves. These negative Tl effects can have an impact on agricultural production and can be used as an indicator of environmental pollution.

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MONITORING OF 5-FLUOROURACIL IN A HOSPITAL PHARMACY

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The potential health risks for staff working with anticancer drugs have been known for several decades. Cytostatics have been proven to have mutagenic, carcinogenic and teratogenic effects. Chemical drugs with carcinogenic effect are nevertheless considered a liminal effect factor.

The aim of the paper was to evaluate the level of contamination of areas used for the preparation of cytostatics (entry area, isolator area, exit area), pharmaceutical preparation packaging containing 5-fluororacile (paper box, bottle) and packaging with pre-prepared chemotherapy (bag, syringe), as well as area where cytostatics are not prepared, but which are used by staff that prepare them (WC, office, kitchenette, dressing room).

In the hospital pharmacy at the St. Anne Hospital in Brno potentially contaminated areas were swabbed. Aqua pro injectione was used as a solvent. Samples were analysed using HPLC. 5- fluorouracile content was determined using calibration line. 42 swabs were made, in 19 of them (45.24%) contamination was found. Another 16 swabs were made in areas where protective gloves are not used, 7 of them (43.75%) discovered contamination.

The highest contamination was found on the preparation room desk. Here, however, staf use protective gloves. Relatively high contamination levels were found on the office phone or computer keyboard and kitchen kettle, where gloves and protective suits are not used.

Results produced new decontamination procedures. When tested, no contamination was found, using the same method.

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GENOTOXICITY OF THE RADON

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The aim of this running study is to evaluate the genotoxic risk of the show cave workers employed by the Slovak Cave Administration in Liptovský Mikuláš, who are exposed to radon. Radon (Rn) is a radioactive natural gas, which ensues from decay of radium (^{226}Ra). The isotope ^{226}Ra is a member of uranium decay chain, which occurs naturally as a component of many minerals (uraninite, torbernite, autunite), natural waters and rocks (dark sales, granites, light – colored volcanic rocks, some sedimentary rocks containing phosphate, carbonate and other). Radon is permanently seeping from these materials and inside bounded spaces like are mines or caves where the concentration can be higher. Radon is also the most important source of environmental radiation and he has a potential to generate genotoxic effects, like are chromosomal aberrations. The main adverse health effect of the radon is the possible development of lung cancer after a long-term exposure.

There are several known isotopes of Rn. The half life of most of them is short (^{217}Rn 5.4x10^{-4}s, ^{218}Rn 0.019 s, ^{219}Rn 3.96 s, ^{220}Rn 55.6 s). The most stable is the isotope ^{222}Rn. Its half life is 3.823 days and the energy of its alpha (α) decay is 5.49 MeV. This isotope can be dangerous for the humans and other organisms which are breathing the contaminated air. The energy of alpha particles permits them to travel only for few centimetres through air. The travel is inversely related to the density of tissue. Inside human lungs it is several millimetres. When the alpha particle passes through the cell nucleus it can cause the DNA damage.

The concentrations of ^{222}Rn inside underground spaces (caves) depend on air circulation and have large seasonal variability. Highest concentrations ware measured during the summer. Winter values are only 1/3 to 1/10 of the summertime high values. During the winter is the direction of airflow from underground spaces to free airspace so the underground concentrations of radon decrease.

At the Clinic of Occupational Medicine and Toxicology we examined 15 workers (11 men, 4 women) exposed to radon, with average age 36.87±7.14 years (S.D.). The average exposure time was 10.73±5.99 (EX) years. The control group consisted of healthy employees Of the Martin Faculty Hospital, without exposure to genotoxic agents. We evaluated 1 500 mitoses (100 mitoses per subject). In exposed group we detected statistically higher frequency of total chromosomal aberrations (CAs) in comparison to control (2.27%±0.46 (S.D.) vs. 1.13%±0.53, $p<0.05$). The chromatid (CTA-type) presented 1.67%±0.72 and chromosome (CSA-type) 0.60%±0.83 of aberrations. At four workers (26.67%) we detected higher genotoxic risk. The total number of chromosomal aberrations shows, that in the exposed group was higher exposure to genotoxic agents

MICROCYSTIN CONTENT IN FISH TISSUES IN SELECTED LOCALITIES OF THE CZECH REPUBLIC

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Toxic cyanobacteria represent serious problem for water supply systems, recreation and agriculture also due to production of biologically active compounds including microcystins. Microcystins can accumulate in various aquatic organisms including fish. Majority of microcystins in fish are taken up via the gastrointestinal tract, while the toxin uptake through the gills and skin is less pronounced. Many authors have recently carried out studies concerning the accumulation of toxic cyanobacterial metabolites and microcystins, in particular, in fish tissues. There are substantial differences in toxin concentrations in these studies and some of them addressed the problem of health risks associated with consumption of microcystin-contaminated fish.

The aims of the present study were to analyse microcystin content in the tissues of fish from selected localities in the Czech Republic and to evaluate potential risk of fish consumption.

Studied localities included water dams Vír, Plumlov and Mostiště as well as fishpond Novoveský, a typical hypertrophic pond for intensive breeding of common carp. The toxin content was determined by high-performance liquid chromatography coupled to mass spectrometry.

The highest concentrations were detected in the liver of canivorous fish, mainly in Percidae: in the Mostiště dam in *Perca fluviatilis* 8.6 ng.g^{-1} fresh weight (FW), in the Plumlov dam in *Sander lucioperca* 7.3 ng.g^{-1} FW and in Vír dam in *Perca fluviatilis* 22.7 ng.g^{-1} FW. In the fishpond Novoveský, microcystin was detected in the liver of carnivorous *Sander lucioperca* (15.8 ng.g^{-1} FW) and *Aspius aspius* (4.14 ng.g^{-1} FW) as well as in omnivorous *Cyprinus carpio* (0.6 ng.g^{-1} FW) and herbivorous *Ctenopharingodon idella* (2.0 ng.g^{-1} FW). Concentrations of microcystins in the edible portion of fish tissues (muscles) were generally bellow the limit of detection (2 ng.g^{-1} FW).

In summary, it can be concluded that although the accumulation of microcystins in the fish tissues exist, concentrations of microcystins from monitored localities are low and they do not represent serious health risk to humans.

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THE EFFECT OF URIC ACID ON HOMOCYSTEINE-INDUCED ENDOTHELIAL DYSFUNCTION

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Elevated plasma uric acid is known to indicate an increased risk of cardiovascular diseases associated with endothelial dysfunction. However, the role of uric acid in the pathogenesis of endothelial dysfunction is still a matter of debate. Its potential harmful effect is in contrast with the fact that uric acid is one of the most important antioxidants in body fluids. It is not clear, whether uric acid is a real causative risk factor, an inert marker or even a protective molecule with respect to its antioxidant properties We have studied the effect of uric acid on intact endothelial cells as well as on the cells with homocysteine-induced endothelial dysfunction.

Bovine aortic endothelial cells were treated with uric acid (0.1–0.6 mM) and homocysteine (0.1 mM), or uric acid only for 24 h. Then the cells were stimulated with 0.001 mg/ml of calcium ionophore A23187 and nitric oxide (NO) production was measured electrochemically with the use of NO-sensitive microelectrode. Expression of endothelial nitric oxide synthase (eNOS) and eNOS phosphorylation at Ser1179 was estimated with the use of Western blotting. Interaction between NO and uric acid was measured with NO electrode. Superoxide generation was measured with the use of fluorescence dye MitoSox Red.

24h incubation of the cells with 0.1 mM homocysteine strongly diminished A23187-induced NO release. Concomitant treatment with 0.1 mM uric acid slightly restored NO production. Higher uric acid concentrations were ineffective. Interestingly, a dose-dependent decrease of A23187-induced NO release was observed in the cells treated only with uric acid. Uric acid did not scavenge NO and did not change eNOS expression or phosphorylation at Ser1179, but dose-dependently increased superoxide production in A23187-stimulated cells. In conclusion, uric acid decreased nitric oxide bioavailability and enhanced superoxide generation in A23187-stimulated bovine aortic endothelial cells.

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NOVEL ACRIDINE COMPOUNDS WITH ANTICANCER ACTIVITY ARE DNA LIGANDS AND MODULATORS OF GLUTATHIONE

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Chemotherapy is often the treatment of choice for many types of cancer and the search for new chemotherapeutic agents still plays a major role in the fight against cancer. A worthwhile approach in this area deals with the use of compounds interacting with DNA. In our previous study we found that novel substituted 9-aminoacridine derivatives inhibited cell proliferation of leukemia cell lines by inducing apoptosis [1].

The aim of this work was to compare an anticancer activity of two groups of new acridine substances with DNA affinity and with/without reactivity towards thiols. Conjugates of acridine with thiazolane: methyl-2-[2-(acridine-9-ylimino)-3-R-4-oxo-1,3-thiazolane-5-ylidene]acetate (AcrT; R: *p*-Br-phenyl, *sec*-butyl, *tert*-butyl) and 2′,2′′-[(acridine-3,6-diyl) diimino]- 3′,3′′-R-1,3-diimidazolidine-4-one derivatives (AcrDIM) with alkyl side chains of different length (R: propyl-, butyl-, pentyl-, hexyl-) have been synthesized, their DNA binding properties with purified thymus DNA studied by UV/vis, fluorescence spectroscopy and circular dichroism and the reactivity with thiols monitored. The cytostatic effect of all derivatives against human leukemia HL-60 cells (MTT-assay), the transport of derivatives into cells, the changes of cytomorphology, and, finally, photochemical properties of acridines have all been investigated.

Spectral analysis indicated that AcrDIMs were bound into DNA as intercalators wherein propyl-AcrDIM had the highest DNA-binding constant ($K=1.2\times10^5$ M^{-1}). The screening of cytotoxicity (MTT-assay, human leukemia cells HL-60) showed that the values of IC_{50} (the concentration of the drug inducing 50% inhibition of the cell proliferation) for all AcrDIM were in the range 5.76–20.55 μM. Among them, hexyl-AcrDIM was the most potent antiproliferative derivative ($IC_{50=}5.76\pm0.66$ μM, 48h treatment). A higher affinity to DNA than that of AcrDIMs was observed for AcrTs where the strongest binding was determined for *p*-Br-phenyl-AcrT ($K=5.86\times10^6$ M^{-1}). In addition, AcrTs were able to react with thiols and modulate the level of intracellular glutathione. Also, the cytotoxicity of AcrTs was higher than that of AcrDIMs ($IC_{50=}1.4\pm0.3$ μM for *p*-Br-phenyl-AcrT, 48h treatment). Moreover, we have found that *p*-Br-phenyl-AcrT could be used as a photosensitizer what has been proved by the production of a superoxide radical characterized by EPR.

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HI-6 ANTIDOTE AGAINST NERVE AGENTS AND TULAREMIA DISEASE PROGRESS

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Our experimental work has been engaged with studying modulation effects of HI-6 on infection progress caused by *Francisella tularensis*.

The basic mechanisms of action of the highly toxic nerve agents (sarin, soman, GF, VX) are based mainly on the irreversible inhibition of the enzyme acetylcholinesterase (AChE) thereby causing an accumulation of acetylcholine (ACh) in the synaptic clefts of the cholinergic nervous system. As a result, cholinergic receptors are excessively stimulated, leading to a cascade of postsynaptic events resulting in secondary toxic effects.

Conventional antidotal treatment of nerve agent intoxication consists of anticholinergic drugs (e.g. atropine), to counteract the effects of accumulated ACh at the receptor and oximes (e.g. HI-6) to reactivate nerve agent-inhibited AChE. Here, synergic effect of oxime and infectious disease was studied.

Effect of compound HI-6 on body was estimated using model based on laboratory mice (BALB/c). Mice were divided into five groups. The first four groups were exposed to tularemia by subcutaneous administration of *Francisella tularensis* LVS. The groups 2–5 were challenged by dose of HI-6 (0.4–4 μg/animal). The fifth group was exposed to only HI-6. Significant decrease of mortality due to tularemia was observed when the dose of HI-6 was increased. The achieved data are only preliminary one. It was proven on laboratory animal model. There was also tested effect of HI-6 on growth of *F. tularensis* using cultivation on McLeod agar. HI-6 was able to provide zone of inhibited growth. While bacteriostatic effect was proven, becteriocidic effect was not so clearly visible. In conclusion, HI-6 may be found useful to suppress mortality caused by infectious agent. Modulation of cholinergic antiinflammatory pathway is considered as a possible mechanism of HI-6 action.

LOW-MOLECULAR-WEIGHT ANTIOXIDANTS IN BIRDS EXPOSED TO CYANOBACTERIA

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Low-molecular-weight antioxidants such as ascorbate, uric acid and thiol-bearing substances participate in the protection of the organism against oxidative stress. Exposure to cyanotoxins has been shown to cause oxidative stress in various organisms. Oxidative stress may occur either due to the decrease of cellular antioxidant levels or to the overproduction of reactive oxygen species (ROS). Exposure to microcystins has been linked with increase of ROS production in mammals and fish. It seems, according to some reports, that juvenile birds are more susceptible to the action of cyanotoxins. This fact is probably responsible for their mass mortality as reported in flamingo chicks. It may, therefore, be hypothetized that toxin-induced changes would be more pronounced in juvenile birds than adults.

The aim of the present study was to compare the effects of cyanotoxins in adult and juvenile Japanese quails (*Coturnix coturnix japonica)* regarding biochemical parameters and low-molecular-weight antioxidant levels.

The experiment was started with two-day old quail chicks and 10-week old adults which were fed cyanobacterial biomass containing defined concentrations of microcystins for 15 days. Blood samples were collected every day of exposure to measure biochemical parameters and levels of low-molecular-weight antioxidant.

There were no biochemical changes in experimental juvenile birds as compared with controls, while adults showed an increase in LDH and a drop in glucose. Low-molecular-weight antioxidants were decreased on day 12, 13 and 15 post exposure both in juveniles and adults.

Our hypothesis of juveniles being more susceptible to the action of cyanotoxins than adults was not confirmed. There were only changes of low-molecular-weight antioxidants in juvenile birds.

The present study was supported by the Internal Grant Agency of the Veterinary and Pharmaceutical University Brno, Czech Republic, Grant No. 121/2008/FVHE.

ARBUTIN AND DECREASE OF POTENTIALLY TOXIC SUBSTANCES GENERATED IN HUMAN BLOOD CELLS.

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The compound of bearberry leaf (*Arctostaphyllos uva-ursi*) arbutin, a hydroquinone derivative, is known to have antiseptic properties. Plant based medicines/extracts are usually well tolerated and have less severe side effects compared to highly effective synthetic drugs. Additionally, synergistic or additive pharmacological effects of plant extract might be beneficial by their stronger efectiveness when administered together with a synthetic drug. Since insight into mechanisms of interactions underlying the effectiveness of phytomedicines is rather important, we studied the effect of arbutin on chemiluminescence, superoxide generation and myeloperoxidase release in human blood cells. The effect of arbutin and/or carvedilol (a unique cardiovascular drug with antioxidative properties) on superoxide generation was measured in isolated neutrophils alone and in interactions with blood platelets in the ratio close to physiological conditions.

Whole blood and intra-cellular chemiluminescence enhanced with luminol and extra-cellular chemiluminescence of isolated neutrophils enhanced with isoluminol stimulated with PMA was measured luminometrically. Superoxide generation and myeloperoxidase release was measured spectrofotometrically.

Arbutin alone did not induce chemiluminescence, superoxide generation or myeloperoxidase release from unstimulated cells. It dose-dependently decreased neutrophil oxidative burst in whole human blood and external oxidant generation without affecting oxidative burst arising inside of isolated human neutrophils. The inhibitory effect of arbutin on myeloperoxidase release was lower in comparison to superoxide generation in isolated PMA stimulated neutrophils.

Effect of arbutin on the decrease of PMA stimulated superoxide generation in the mixture of neutrophils and blood platelets [1:50] was lower than in isolated neutrophils alone. The inhibitory effect of carvedilol on superoxide generation in the mixture of neutrophils and blood platelets was only in the higher concentration [100 μmol/l], yet combination with arbutin resulted in inhibition also in lower concentration of carvedilol [10 μmol/l].

Significant decrease of extra-cellular generation of reactive oxygen species and free MPO by arbutin may prove helpfull in controlling inflammation and thus diminishing the damage of surrounding tissues.

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CHRONIC HEALTH IMPAIRMENT DUE TO 2,3,7,8-TETRACHLORO-DIBENZO-*P*-DIOXIN EXPOSURE

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TCDD (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin) is a chemical with an 8 year plasma half-life, chronically affecting especially the cardiovascular system and the central neural system. The aim of this study was to evaluate the consequences of severe intoxication with dioxin in herbicide trichlorophenoxyacetic acid production during the years 1965–1968.

Examination of 11 men (out of about 80 in 1965), mean age 64 years, included: internal and neurological examination, electromyography (EMG), electroencephalography (EEG), visual evoked potentials (VEP), Lanthony test of acquired visual impairment, single-photon emission spectrometry (SPECT) of the brain, neuropsychological examination, ultrasonography of the carotid artery (intima-media thickness), eye fundus examination, and blood lipids. Dioxin level was measured by HRGC/HRMS.

Mean TCDD level in 2008 was still 270±130 pg/g blood lipids (reference level is 2–3 pg/g). All (100%) patients had atherosclerotic changes on the eye fundus, 82% patients had impairment in SPECT of the brain. The same percentage of patients was treated for hyperlipidemia, 73% for hypertension, 55% for diabetes, 45% for ischaemic hearth disease, and 36% for psychic disorders. Mean color confusion index (CCI) in Lanthony test was 1.528, which proofs impairment since 2003, when 1.308 was found. Mean carbohydrate-deficient transferrin (CDT), marker of chronic ethanol intake, was 2.46%, i.e. in the normal range. It did not correlate with CCI. Progression of intima-media thickness from mean 0.84 to 1.06 mm was seen.

The results show that 40 years after intoxication, blood level of TCDD is still 100-fold higher than in the general population. Obviously, the subjects need continuous intense hypolipidemic and neuropsychological treatment.

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THE EFFICIENCY OF GASTRIC LAVAGE AFTER INGESTION OF PHARMACEUTICALS

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Due to the traditional approach, gastric lavage is frequently being performed already before the enquiry to the Toxicological Information Centre (TIC). Nowadays, it is considered controversial and the effect questionable. In overdose of most drugs gastric emptying is recommended usually only up to one hour, in antidepressants with anticholinergic effect up to 4 hours after ingestion.

The objective was to evaluate the results of gastric lavage in terms of noticeable tablets in gastric lavage and to identify the pharmaceuticals, most frequently removed by it.

Data concerning single pharmaceutical overdose were extracted from the documentation of TIC in the past 4 years. During the call to the TIC, question was asked about finding of tablet residues during the gastric lavage previously performed. The time delay after ingestion was also registered.

A total of 39,252 enquiries were answered during the years 2005–2008, 40% from them involved pharmaceuticals. Among the 11,085 drug poisonings with a single pharmaceutical only, gastric lavage had already been completed before the phone call in 1,002 cases, i.e. 16%. Identifiable tablets were seen in 32%; the percentage declined from 66% of residues found up to 1 hour after ingestion, to 26% up to 2 hours, 5% up to 3 hours, and 3% later. However, the percentage of positive findings for toxic and lethal doses increased with latency time; it was 48%, 60%, 62% and 78% up to 1 hour, 2 hour, 3 hours and later, respectively.

The most frequently found drugs in all time intervals were benzodiazepines, neuroleptics, SSRIs and NSA; in the longest delayed time interval (18 cases) benzodiazepines, neuroleptics, SSRIs, NSA, and betablockers.

In the cases of single drug ingestions, where gastric lavage has not yet been initiated, TIC recommended it in 10% of the calls only; in 1% it was contraindicated.

Gastric lavage is more rarely recommended by the TIC. The gastric emptying was optically successful in only 32% of evacuations performed after ingestions of a single pharmaceutical. Toxic and lethal ingestions led more frequently to the positive finding in the longest time interval. Obviously, gastric lavage is futile in many cases of overdose, as the percentage of removed tablets is low; moreover the evidence for efficacy for clinical improvement is lacking. As in other invasive procedures, side-effects have been described. Any effect to prevent repeated suicide attempts has not been proven, either.

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EFFECT OF PTEROSTILBENE ON HUMAN NEUTROPHIL VIABILITY AND ACTIVITY

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The knowledge that consumption of red wine has some health benefits opened the scientific scene for the stilbene type polyphenols. Modification of the basic stilbene molecule with different functional groups gives rise to various natural and synthetic derivatives of stilbene with distinct biological activities.

The aim of this study was to compare the effect of pterostilbene, i.e. 3,5-dimethoxy-4´-hydroxystilbene, on the activity and viability of human neutrophils. We investigated the pro-apoptotic ability of pterostilbene on neutrophils from buffy coat of human plasma. We examined the effect of pterostilbene on phosphorylation of proteinkinase C (PKC) – a regulatory enzyme of reactive oxygen species (ROS) production in neutrophils. Moreover, we explored the effect of pterostilbene on the production of cytotoxic ROS by activated neutrophils.

The effect of pterostilbene on ROS production was examined using the chemiluminiscence method on isolated human neutrophils. The concentrations of pterostilbene was 0.01–100 μM. Due to the different abilities of luminol and isoluminol to pass through the cell membrane, the effect of pterostilbene on intracellular and extracellular ROS was studied. We used phorbol-myristate-acetate (PMA), which activates neutrophils *via* PKC. Western blotting was used to investigate the ability of pterostilbene (100 and 10 μM) to inhibit phosphorylation of PKC of PMA activated neutrophils. For cell viability testing, three different concentrations of pterostilbene (F.C.=1, 10 and 100 μM) and a control sample were incubated with human plasma buffy coat. The cells were then stained and examined immediatly on flow cytometer. From the granulocyte area 5,000 cells were gated and analysed.

Pterostilbene inhibited significantly chemiluminiscence of isolated neutrophils in a dose-dependent manner. At the concentration of 10 μM, pterostilbene inhibited extracellular chemiluminiscence by 68%. In the intracellular space of neutrophils, at the same concentration, the effect changed by 40% inhibition. Pterostilbene did not inhibit phosphorylation of PKC of activated neutrophils. Neither did it significantly affect cell viability compared to control samples.

Pterostilbene significantly inhibited extra- and intra-cellular production of cytotoxic ROS without affecting viability of neutrophils. The inhibition of ROS

production probably does not include inhibition of PKC phosphorylation pathway.

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LEAD TOXICOSIS OF VULTURES IN A ZOO COLLECTION

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This paper is a case report on lead intoxication in a collection of vultures kept at the Zoo of the Capital City of Prague with data regarding the toxicological, biochemical, metallothionein and oxidative stress analyses. Interestingly, effects of therapy on the above parameters were evaluated. Two Egyptian Vultures (*Neophron percnopterus*) and eight Cinereous Vultures (*Aegypius monachus*) were affected. The problem developed after one month of returning the birds to their aviaries following old minium paint removal. The paint dust and chips sanded off the steel aviary construction contaminated the soil in such an extent that the surface layer contained as much as 25 850 µg/g of lead. As vultures feed on carrion from the ground, it was the direct way of their exposure.

A male Egyptian Vulture was found in its aviary showing signs of apathy, polydipsia, polyuria and regurgitation in January 2009. Clinical examination on the next day revealed stupor, foul-smelling fluid flowing freely from the beak and crop distension due to food stagnation and the animal died shortly after being handled. There were many small radiopaque chips in the crop and stomach area visible on the radiograph taken post mortem. Liver, kidney and blood lead concentrations were 12.2, 8.16 and 2.66 µg/g, respectively. No other vultures died or showed clinical signs of lead intoxication. The blood lead levels dropped from the initial value of 1.07 to 0.28 µg/g within two months in the cage-mate female Egyptian Vulture. First, this female was treated with penicillamine that was, because of adverse effects, changed for Ca-EDTA. Toxicological analyses of blood of eight Cinereous Vultures resulted in finding the initial mean value of 1.497 µg/g that decreased to 0.535 µg/g without any therapy; probably due to excretion and deposition in bones.

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COMPARISON OF ACUTE TOXICITY OF TERBUTRYN TO EMBRYONIC AND JUVENILE STAGES OF *DANIO RERIO*

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Terbutryn belongs to triazine selective herbicides, which have been used worldwide to control weeds in agriculture. Although the application of terbutryn has banned in many countries it has still detected in water environment because has a low biological dissolubility. The aim of this study was to determine lethal concentration in the acute toxicity tests and to compare acute toxicity of terbutryn with two different developmental stages of *Danio rerio* – embryonic and juvenile. The embryo toxicity tests were realized according to OECD No. 212 (Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages), in the acute toxicity tests with embryonic stages juvenile fish aged 2–3 months the semistatic method according to OECD No. 203 (Fish acute toxicity test) was used. The results of toxicity tests (the number of dead fish at particular test concentrations) were subjected to a probit analysis using the EKO-TOX 5.2 programme to determine LC50 values of terbutryn. The LC50 terbutryn mean value was 8.32±1.06 mg.l^{-1} for embryonic stages of *D. rerio*, for juvenile fish of *D. rerio* was LC50 terbutryn 8.37 mg.l^{-1}. The LC50 values of these tests were compared. Acute toxicity values of terbutryn for embryonic and juvenile stages seem to be similar for both stages.

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SKIN SENSITIZATION: LOCAL LYMPH NODE ASSAY(LLNA) AND ITS NON-RADIONUCLIDE ALTERNATIVES

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The evaluation of skin sensitisation potential of chemicals was traditionally performed using the Guinea Pig Maximization test or the Occluded Patch Test of Buehler. The murine Local Lymph Node Assay (LLNA) is an alternative to the guinea pig sensitisation test used to identify and characterize dermal sensitizers. This method of testing is required by REACH as a method of first choice.

During the "induction phase" of sensitisation, following exposure to a sensiting test substance, lymphocyte proliferation occurs in the local lymph node. The LLNA measures this phase of sensitization by determining the

increased proliferation of lymphocytes in the auricular lymph nodes that drain the site of exposure (ears). Proliferation is assessed by determining the incorporation of the ^{3}H-methyl thymidine into the DNA of lymph node cells by β-scintilation counting. The ratio of the proliferation in the treated group to that in control group, the stimulation index (SI), is determined. If the SI exceeds the defined limit then a test substance can be further evaluated as a potential skin sensitiser.

Due to the need for radioactive nucleotides to measure lymph node cell proliferation, special licence is needed for the laboratory performing the standard LLNA according to legislation regulating radioactivity issues. To overcame this need additional variants of the LLNA have been developed and validated internally in different laboratories using non-radionuclide techniques for the detection of target cells proliferation: measuring of the ATP-adenosine triphosphate content (LLNA-DA test method), detection of bromodeoxyuridine by flow cytometry (BrdU-FC test method) or by ELISA (BrdU-ELISA test method).

Our laboratory has optimized and internally validated standard (´radioactive´) LLNA and also LLNA with non-radionuclide endpoint – where the target cell proliferation is measured by the cell counting (Celtac alfa). Based on our results both techniques of cell proliferation measuring are able to identify those chemicals that possess a significant potential to cause contact allergy. In addition, the acute inflammatory skin reaction could be detected by evaluation of ear swelling and by weighing of circular biopsies of the ears to identify skin irritating properties of the test chemicals which could interfere with sensitization effect.

This research was supported by Ministry of Environment of the Czech Republic (grant no SP/1b5/87/07)

PRODUCTS OF LIPID PEROXIDATION AND SELECTED BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN PLASMA OF PATIENTS WITH CHRONIC PANCREATITIS

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Chronic pancreatitis is a common digestive disease which is accompanied by chronic inflammation and increased production of cytokines, growth factors, reactive oxygen/nitrogen species and other inflammatory mediators. Chronic inflammation and its products can damage pancreatic tissue. Therefore, the main aim of the study was to determine and compare changes in selected biochemical (pancreatic amylase, amylase, CB protein, cholesterol, triglycerides, HDL, LDL, C-reactive protein) and hematological (leukocytes, erythrocytes, haemoglobin, haematocrit, mean cell volume, blood platelets, differential cell count) parameters, plasma levels of nitrites, total antioxidant capacity and lipid peroxidation products malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE).

One hundred and five patients with chronic pancreatitis and twenty seven healthy controls were included into the study. MDA and 4-HNE adducts with 2,4-dinitrophenylhydrazine were analyzed using HPLC-DAD. The total antioxidant capacity of plasma was evaluated using luminol-enhanced chemiluminiscence that was applied to follow up peroxyl radical scavenging. Nitrites were determined by spectrophotometric Griess reaction. Biochemical parameters were performed by standard methods.

Plasma levels of MDA and 4-HNE were significantly higher in comparison with healthy controls (0.29±0.11 vs. 0.03±0.02 μmol/l for MDA, and 0.30±0.29 vs. 0.16±0.06 μmol/l for 4-HNE, resp.). The total antioxidant capacity did not differ significantly from controls, while the concentration of nitrites in plasma of patients was increased (2.33±2.76 vs. 0.25±0.42 μmol/l). Of the biochemical and hematological parameters tested, mean values of leukocytes, triglycerides and C-reactive protein were found to be elevated to the upper limit of the normal range.

Herein, we proved the formation of lipid peroxidation products and the increase in nitrites and some of biochemical and hematological parameters in patients with chronic pancreatitis. On the basis of these data patients are examined periodically within two-year period to determine time course of changes in these parameters as well as their predictive values.

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CURRENT IMPROVEMENT OF CHOLINESTERASE BASED BIOSENSORS

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Acetylcholinesterase (AChE; EC 3.1.1.7) is an important enzyme in body. It terminates transmission through neurosynapse by hydrolyzing neurotransmitter acetylcholine. Many compounds could inhibit AChE. Irreversible inhibitors of AChE are organophosphates such as nerve agents sarin, soman, tabun, VX, and pesticides such as paraoxon ethyl, paraoxon methyl, diisopropylfluorphosphate, dichlorvos. Other compounds such as aflatoxins, and several drugs such as tacrine,

7-meota or pyrydostigmine are competitive inhibitors with milder effect on AChE than the previous.

Analytical devices based on intercepted AChE, biosensors, are convenient tool for assay of anticholinergic compounds. Here, electrochemical biosensors based on AChE immobilized by sol-gel technology, sorption and precipitation by bifunctional reagents are taken for perspective tools. The biosensors were performed for assay of pesticides, nerve agents and some drugs.

The principle of assay, immobilization procedure and the achieved analytical parameters are presented. The current effort to construct biosensor is compared with novel trends. Perspectives of biosensors based on cholinesterase are stated and the future trends of biosensors performance in assay of anticholinergic toxins and drugs evaluation are estimated in the contribution.

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IRON IN THE ECOSYSTEM OF THE GREAT LAGOON (SZCZECIN LAGOON, NW POLAND) – A HEALTHY OR ADVERSE ELEMENT TO ICHTHYOFAUNA?

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Compounds containing iron, which occur in aquatic ecosystems in the aqueous phase both in the dissolved and suspended forms as well as appearing in sediments as dissolved salts in interstitial waters and bound to solid phase particles, fulfil a very important catalytic role in self purification processes. They play the role of the agent transferring oxygen between the oxidized water phase and bottom sediments in processes of organic matter mineralization. On the hand, an excess of iron in the environment may lead to excessive concentrations of iron in interstitial waters and subsequently in the overlying water column, this being a threat to ichthyofauna of respiratory dysfunction called "żelazica", resulting from Fe accumulation on gills. The threat of gill dysfunction occurs even in basins devoid of oxygen deficiency and especially for fish feeding close to the muddy sediments, e.g. big carp bream (*Abramis brama* L.). The pool of iron in sediments of the Szczecin Lagoon has been studied profoundly (iron compounds contribute up to 6%) but the speciation of iron, dissolved and suspended, in water of the Szczecin Lagoon has been poorly recognized because the analyses were usually limited to total iron concentration. No information was available either on distribution coefficients K_D, defined as the ration between iron in the suspended form to dissolved form.

In the years 2004–2006, water samples were collected from surface layer at a number of locations in the Szczecin Lagoon: in Roztoka Odrzańska (buoy 17) and in the northern part of the Lagoon (buoy MOS), the sampling was done during the vegetation season (April–October). The concentrations of total iron (Fe_{tot}) and dissolved iron (Fe_{diss}) were determined together with suspended matter (m_{part}). The data obtained from these analysed allowed to calculate the contribution of iron in the suspended matter (Fe_{part}) and distribution coefficients K_D. Besides the described parameters, water temperature, oxygen concentration, dissolved organic matter concentration (COD-Cr) and Cl^- concentration were determined as well. Water saturation with oxygen (%) and coefficients of seawater and riverine water mixing (kg seawater/kg riverine water) were calculated additionally.

Linear regression analyses were carried out to find out relationships between response functions i.e. Fe_{tot}, Fe_{diss}, m_{part} and Fe_{part} and the determined environmental factors as well as selected climatic elements, particularly wind direction and speed (wind speed determined the appearance of Langmuir circulation in the Szczecin Lagoon). Statistically significant relationships were found between response functions and dissolved organic matter concentration, water mixing coefficient, Langmuir circulation and wind directions facilitating gravitation water flow out of the estuary. The values of distribution coefficient K_D were similar to those obtained in other European rivers in near mouth areas.

The concentrations of total iron in all analysed water samples were below the threat level to ichthyofauna. Water quality classification in the Szczecin Lagoon, regarding iron content was usually in the I classification class according to the Polish legal classification standards.

Research was supported by University of Szczecin.

ANTICANCER AGENT ELLIPTICINE COMBINED WITH HISTONE DEACETYLASE INHIBITORS, VALPROIC ACID AND TRICHOSTATIN A, IS AN EFFECTIVE DNA DAMAGE STRATEGY IN NEUROBLASTOMA CELL LINES

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Valproic acid (VPA) and trichostatin A (TSA) exert antitumour activity as histone deacetylase inhibitors, whereas ellipticine action is based mainly on DNA

intercalation, inhibition of topoisomerase II and formation of covalent DNA adducts mediated by cytochromes P450 (CYPs) and peroxidases. Both two types of antitumour compounds are cytotoxic to human neuroblastoma cells.

The aim of this study was to investigate the effects of VPA and TSA on cytotoxicity of ellipticine to neuroblastoma cells.

The effects of these compounds in combination therapy on growth of human neuroblastoma UKF-NB-3 and UKF-NB-4 cancer cell lines and the molecular mechanisms of such treatment were investigated. The results demonstrate that the anticancer activity of ellipticine to neuroblastomas was synergically increased by 24 hour pretreatment with VPA and TSA. Combination therapy more effectively inhibited the growth of both neuroblastoma cells than single-agent (ellipticine) treatment, decreasing values of IC_{50} for ellipticine in a dose-dependent manner. Whereas VPA at concentrations of 2 mM is able to increase the cytotoxicity of ellipticine up to 10-fold, the only concentration of 100 nM TSA produced up to 2-fold higher cytotoxicity in neuroblastoma cells. A higher sensitivity of neuroblastoma cells to ellipticine correlated with an increase in formation of covalent ellipticine-derived DNA adducts that was found to be one of the most important DNA-damaging mechanisms of ellipticine action in neuroblastomas. VPA and TSA incubated with ellipticine, DNA and human microsomal CYPs or peroxidases inhibited and/or had no effect on ellipticine-derived DNA adduct formation, which results from the effect of these compounds on ellipticine oxidation to individual metabolites. Protein expression of CYP1A1, the enzyme participating mainly in ellipticine detoxication was not affected by VPA, but it was increased by TSA. The effects of VPA and TSA on other CYPs metabolizing ellipticine were also investigated.

The results show that stimulation effects of VPA and TSA on toxicity of ellipticine to neuroblastoma cells follow from their ability to make cell DNA more accessible to the ellipticine–mediated damage and not from their effects on expression and activities of enzymes activating this drug.

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TESTING OF NEWLY ISOLATED NATURAL SUBSTANCES HAVING POTENTIAL ANTIDIABETIC EFFECT *IN VIVO*

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The tested natural substance – geranylated flavanone mimulone, was extracted from *Paulownia tomentosa* fruits. Based on specialised literature, the substance is characterised by antimicrobial and anti-hyperglycemic activity as well as proved cytotoxic effect.

The aim of the work was to assess the mimulone impact on histological picture of liver, kidney and pancreas tissue following the artificial inducing of diabetes 1 type..

The set included 60 laboratory inbred male mice divided into 6 groups (n=10).

Control/Intact group (N) was divided into two subgroups. The first groups remained unmedicated, the second subgroup had intraperitoneally applied (IP) 10% dimethylsulfoxid (DMSO), the tested substance solvent.

Control/diabetic group (A) had type 1 diabetes induced by means of intravenous application of alloxan.

Diabetes was in the same way induced in the groups A+M-IP and A+M-PO.

The group A+M-IP had IP applied mimulone and 6 hours later alloxan.

The group A+M-PO had perorally (PO) applied the substance and 12 hours later alloxan.

The group M-PO had PO applied only mimulone, and the group M-IP had the substance applied IP. Application of the tested substance and DMSO was repeated every approximately 24 hours for 5 days. Animals were destroyed after 5 days and samples of tissue and blood were taken.

Alloxan application (toxic to B-cells of Langerhans islets (LO) pancreas) resulted in the type 1 diabetes, which was characterised by hyperglycaemia confirmed by checking the glucose levels in blood. The reference diabetic group showed characteristic attributes of LO extinction. Degraded LO in various extinction stage were observed.

The groups, which had the tested substance applied prior to diabetes inducing, showed hyperglycaemia as well, but the measured values were lower compared to the reference group. Histological examination proved that LO destruction was not so radical, quite a few LO without morphological changes were observed.

The tested substance has probably favourable impact on endogenous component of pancreas; however, it is not suitable for more intensive intraperitoneal application because it causes massive peritonitis.

PHARMACOKINETICS OF PARA-METHOXYMETHAMPHETAMINE (PMMA) IN RATS AFTER S.C. APPLICATION

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Para-methoxymethamphetamine (PMMA) is an amphetamine abused psychedelic compound with reports on several intoxications and deaths caused by its ingestion. However, only partial information on its pharmacokinetics and biotransformation based on a controlled study in animal models is available.

The experimental study was designed for the temporal disposition profile of PMMA and its metabolite (4-methoxyamphetamine) in blood and brain in rats.

Male Wistar rats were administered single 5 and 20 mg/kg doses of PMMA hydrochloride dissolved in physiological solution subcutaneously. The animals (10 animals at each time interval) were sacrificed at 0.5, 1, 2, 4 and 8 hours after dosing and plasma and brain samples were collected and stored at –20°C until analyses. To extend this study, higher dose of 40 mg/kg of PMMA hydrochloride was given to rats. Animals were sacrificed (3 animals at each time interval) and samples were collected as mention above. PMMA and its metabolites were confirmed in samples and quantitatively evaluated.

The absorption of PMMA into blood stream was rapid with maximum plasma concentration reached 30 minutes after all dosing, whereas the appearance of the metabolite were rather delayed. In brain, PMMA and its metabolite 4-methoxyamphetamine penetrated the blood/brain barrier sufficiently and for both drugs after each dose the maximum brain/serum ratio value exceeded 8. The drug's ability to persist in brain may correspond to reported psychotropic effects. As the dose of PMMA administered was increased, rats showed rises in PMMA concentration that did not follow the same proporcionality which could be indicative of kinetic nonlinearity. The disposition of PMMA was characterized by its estimated half-life ranging from 0.4 to1.0 h, volume of distribution ranging from 5.7 to 6.4 L/kg and clearance ranging from 4.4 to 9.8 L/kg.h. To our knowledge, our findings provide estimation of kinetic data of PMMA and its metabolites based on controlled animal experiments.

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QSAR APPLICATION TOOLBOX

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As part of the OECD activities to increase the regulatory acceptance of (Q)SAR methods when data are lacking, the OECD has started the development of a (Q) SAR Application Toolbox as a means of making QSAR technology readily accessible, transparent, and less demanding in terms of infrastructure costs.

The OECD QSAR Application Toolbox is a software application intended to be used by governments, the chemical industry and other stakeholders to fill gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical workflow. Grouping chemicals into chemical categories is crucial to this workflow.

The Toolbox contains:

- databases with results from experimental studies,
- a library of QSAR models,
- tools to estimate missing experimental values by read-across, i.e. extrapolating results from tested chemicals to untested chemicals within a category, and
- tools to estimate missing experimental values by trend analysis, i.e. interpolating or extrapolating from a trend (increasing, decreasing, or constant) in results for tested chemicals to untested chemicals within a category.

The Toolbox allows a user to systematically group chemicals into categories according to the presence or potency of a particular effect for all members of the category. The Toolbox is able to quickly evaluate chemicals for common mechanisms or modes of action as well as for common toxicological behavior or consistent trends among results related to regulatory endpoints.

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EFFECTS OF ANATOXIN-A ON THE SELECTED IMMUNE FUNCTIONS OF THE FISH CELLS

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One of the most important consequences of water eutrophication is the occurrence of toxic cyanobacteria (blue-green algae) water blooms causes potential stress and hazard risk to health of aquatic animals. The alkaloid neurotoxin, anatoxin-a, is one of the main cyanotoxin produced mainly by *Anabaena flos aquae* or *Anabaena planktonia*, but also by *Aphanizomenon*, *Cylindrospermum*, *Microcystis* species and benthic *Oscillatoria*. This toxin is the potent pre – and post-synaptic neurotoxin acting as nicotinic agonist binding to the nicotinic acetylocholine receptors (nAChRs). Relatively little is known about the interactions between the exposure to anatoxin-a and health of fish.

The aim of this study was to assess the possible *in vitro* effects of anatoxin-a on the selected immune function of the cells isolated from the blood of carp. In the experiments pure anatoxin-a was used at concentrations of 0.01, 1 and 5 μg ml^{-1} RPMI – 1640 medium. Cytotoxicity of toxin on leukocytes (lymphocyte and phagocytes) was analyzed by quantification of intracellular ATP. Lymphocyte proliferation was determined by chemiluminescent immunoassay, based on the measurement of BrdU incorporation during DNA synthesis. The phagocytes were assayed for intracellular production of reactive oxygen species.

This study showed that anatoxin-a has a suppressive effect on lymphocyte and phagocyte. After exposure to the toxin we observed the decrease of phagocytic cells activity and significant changes on the proliferative

ability of lymphocytes. Moreover, the viability of the cells exposed only to the highest concentration of AnaX was significantly decreased.

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THE USE OF PARASITES AS BIOINDICATORS OF PESTICIDE EXPOSURE

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The most sensitive species are used in risk assessment of pesticides. Scientists are trying to find out the most sensitive system in nature to detect environmental pollution. Parasites are under normal conditions present in surrounding environment, so their stages are exposed to environmental pollution.

The aim of this study was to observe the effect of two commercial pesticides products (containing glyphosate, resp. tolylfluanid) to larval stages of four small ruminant parasites species (*Cooperia curticei*, *Ostertagia circumcincta*, *Haemonchus contortus* and *Trichostrongylus axei*). Product, containing glyphosate, is used as herbicide and second commercial product, containing tolylfluanid, was used as fungicides (nowadays not included on ANNEX I list of approved active substances in EU).

Larvae (L3) were obtained from Veterinary Laboratories Agency, Weybridge, UK. There were two concentrations tested for each product (registered maximal concentration of active substance per ha and 10 times higher concentration) vs. control group (distillated water) in this study. Total amount of 500 individuals were dosed with automatic micropipette per each tested concentration to Petri dishes (diameter of 40 mm). The number of larvae was microscopically calculated and if needed number of individuals per Petri dish was not reached, larvae were dosed again to reach correct number. After application of pesticides concentrations, Petri dishes were incubated by average temperature of 27°C in laboratory incubator (TER 80). Effects were regularly observed by using microscope (Nikon) every day for 42 days. If the effect of pesticides was present, larvae were found dead, without movement after microscope lighten. Results were statistically evaluated by using Contingency table.

Larvae react to pesticides concentrations in different ways. From four tested ruminant parasites, *T. axei* larvae seemed to be the most resistant ones to tolylfluanid exposure. There was no statistical significance in both tested concentrations of tolylfluanid after 42 days of exposure (p=1.000). 100% of dead larvae were found on 37. day of experiment at higher concentration, resp. on 33. day at lower tested concentration of glyphosate.

C. curticei, *O. circumcincta* and *H. contortus* showed same statistical significance in both pesticides tested. There was high statistical significance (p<0.0001) in both concentrations of glyphosate and only at higher tested concentration of tolylfluanid. Larvae of *C. curticei* and *H. contortus* were found dead, spiral shaped and without movement at all concentrations tested, spiral shape was not observed in other two tested larvae. Larvae of *O. circumcincta* reacted to pesticides exposure very fast, rapid loss of live larvae was recorded on second day of experiment at both concentrations of glyphosate and at higher tested concentration of tolylfluanid. From all tested ruminant parasites (L3), larvae of *O. circumcincta* seem to be the most sensitive ones and need to be continued in experiments.

This research was supported by National Reference Laboratory for Pesticides, Košice, Slovakia.

CYTOTOXICITY OF MISTLETOE (*VISCUM ALBUM* L.) ON JURKAT CELLS AND ITS INTERACTION WITH DOXORUBICIN

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Mistletoe preparations are frequently used by cancer patients in German–speaking countries because of their ability to stimulate the immunity and to improve the quality of life. Moreover mistletoe and its active substances (especially lectins) possess cytotoxic effect on various cancer cell lines. However, only little is known about its interaction with anticancer drugs. Therefore the aim of this study was to investigate *in vitro* cytotoxicity of aqueous mistletoe extract (VA) and its interaction with doxorubicin (DOXO) in Jurkat cells.

We used VA extract and anticancer drug doxorubicin (Adriablastina® PFS 10 mg/5 ml, Pharmacia Italia S.p.A, Italy). VA extract was prepared according to the modified method of Park and co-workers (1999) from dried mistletoe tops (over-the-counter drug) produced under the trade name: "Imelo biele vňať" (Hanus, Nitra, Slovakia). Jurkat cells (human acute T-lymphoblastic leukemia cells) were kindly provided by Dr. M. Hajdúch (Olomouc, Czech republic). Cells were cultured in supplemented RPMI medium. Cytotoxicity was assessed by modified MTT assay (introduced by Mosmann in 1983), which is based on reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to dark-blue, insoluble formazan in mitochondria of the living cells. Apoptosis-inducing effect was determined by DNA fragmentation assay.

Our results show that VA extract as well as DOXO exert cytotoxic effect on Jurkat cells in dose-dependent manner. Cytotoxicity of DOXO was much stronger (LC_{50}=11.68 ng/ml) then that of VA extract (LC_{50}=35.67 μg/ml). Their combination led to synergism only at those concentrations that were highly cytotoxic alone. Both substances (alone and in combination) induced DNA fragmentation in Jurkat cells.

In conclusion, aqueous extract prepared from mistletoe tops exerted cytotoxic and apoptosis-inducing effect on Jurkat cells alone as well as in combination with DOXO. Therefore additional *in vitro* and *in vivo* studies on mistletoe extract and its interactions are needed to clarify the role of mistletoe in adjuvant cancer treatment.

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RELATION OF REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST TO REACH

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On basis of new European Community regulation on chemicals and their safe use (EC 1907/2006) all chemicals produced or imported per year in volume 10–100 tonnes have to be tested for health and safety and registered with a new central Europen authority by selected methods. One of these methods is Reproduction/Developmental Toxicity Screening Test (OECD 421). This screening test is used to provide initial information about possible effects of chemicals on male and female reproductive performance such as gonadal function, mating behaviour, conception, growth and development of the F1 offsprings from conception to day 4 post-partum. We tested two chemicals used as stabilizers and antidegradants in rubber industry. The test substances were administrated to rats Wistar of both sexes in 3 graduated doses per os daily for seven days a week. Dosing of both sexes began 2 weeks prior to mating and continued during the mating period. After 2 weeks of mating period all males were struck dead. Daily dosing of the females continued throughout pregnancy to 3 day post-partum. Throughout the test period general clinical observation was recorded. Males and females were weighted once a week. Live pups were counted and sexed and litters weighted within 24 hours of parturition and on day 4 post-partum. All were killed at day 4 post-partum and they were examined externally for gross abnormalities. At the time of sacrifice the adult rats were examined macroscopically for any abnormalities or pathological changes. The number of corpora lutea in the ovaries and of implantation sites in uterus were recorded. The testes and epididimides of males were weighted. All organs of the reproductive system were examined using histological techniques. Administration of two chemicals did not cause mortality and did not have adverse effects on reproductive performance.

MODULATION OF TULAREMIA DISEASE PROGRESS BY THE CHOLINESTERASE REACTIVATOR HI-6

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Cholinesterase reactivator HI-6 is a drug commonly used to treat individuals exposed to nerve agents.

This experimental work has been engaged with studying modulation effects of HI-6 on infection progress. We used BALB/c mice and the causative agent of tularemia, i.e., *Francisella tularensis*, to induce a model bacterial infection.

Cultivation tests confirmed bacteriostatic effects of HI-6. *In vivo* experiments revealed intriguing effect differences resulting from HI-6 administration to mice. While the HI-6 dose of 7 mg *pro toto* induced no statistically significant effects on infection progress, the much lower dose of 8 μg of HI-6 *pro toto* clearly reduced mortality caused by tularemia infection in experimental mice as compared to only *F. tularensis*-infected controls (comparison of survival curves using the logrank test, chi square=4.335, df=1, p=0.0373).

The chemical structure of HI-6 reactivator and expected roles of its organic groups are also discussed in the present study.

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CYANOTOXINS INFLUENCE ON THE NEUROENDOCRINE AND IMMUNE SYSTEMS

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Cyanotoxins are the metabolites of cyanobacteria belonging to different chemical groups and of diverse mechanisms of toxicity. Generally they are divided into hepatotoxins, neurotoxins and dermatotoxins/irritant toxins. There is a growing evidence, that except the above mentioned organs and effects of their toxicity, prolonged exposure to very low levels of different cyanotoxins may also include molecular and cellular damage promoting tumours and cancer, may lead to reproductive dysfunctions and teratogenic effects or induce the disruption of endocrine and immune systems. The latter

ones are often difficult to detect as the observed effects are usually indirect. Immune effects observed after cyanotoxin intoxication may be a result of direct influence on immune cell functions or disorders induced by nervous and/or endocrine interactions.

The purpose of that paper is to sum up the current information about the influence of cyanotoxins on neuroendocrine and immune systems obtained from the literature and from our own studies.

FORAGE AS A PRIMARY SOURCE OF MYCOTOXINS IN THE FOOD CHAIN

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The work objective was to assess the content of mycotoxins entering the food chain from the fodder. The content of mycotoxins was assessed in fresh herbage and then in subsequently produced silages. The silages were made of the wilted herbage of festucoid hybrid (*Festuca arundinacea×Lolium multiflorum*), loloid hybrid (*Lolium multiflorum×Festuca pratensis*) and perennial ryegrass (*Lolium perenne)*. Preservatives used in the production of silages were the chemical conservative kemisile (formic acid, propionic acid, amonnium formate) and the biological conservative silall. The content of mycotoxins was established by Elisa method. Mycotoxins assessed were Ochratoxin A, Fumonisin, Aflatoxin, Zearalenon and T2 toxin. These mycotoxins were not detected in fresh herbage samples from swards harvested at the beginning of June that were taken immediately after the harvest. Samples of subsequently produced silages showed the presence of zearalenon and T2 toxin after seventy days of conservation. The contents of ochratoxin A and aflatoxins were below the limit for detection. Fumonisil was not detected. The highest content of zearalenon was found in silages made of perennial ryegrass (55.7 ppb) and the lowest content of zearalenon was observed in the festucoid hybrid silages (48.0 ppb). By contrast, the content of T2 toxin in the ryegrass silages amunted to only 9.2 ppb while in the festucoid hybrid silages amounted to 17.6 ppb. The highest contents of zearalenon and T2 toxin were established in silages untreated with preservatives (66.9 ppb and 37.8 ppb, resp.) while the lowest contents were found in silages treated with the chemical preservative (34.4 ppb of zearalenon and <LOQ of T2 toxin). Silages treated with the biological preparation showed the content of zearalenon at 60.6 ppb and the content of T2 toxin below the limit for detection. The use of preservatives in the production of silages had a statistically significant influence (p<0.05) on the contents of zearalenon and T2 toxin. Gradual sampling of the fresh herbage in July, October and November revealed namely the presence of zearalenon, which was highest in October (364 ppb and 310.7 ppb in the loloid hybrid and perennial ryegrass, respectively). The festucoid hybrid exhibited the zearalenon content below the limit for detection. The results showed a beneficial influence of silage additives on the reduction of mycotoxins in silages produced from the wilted herbage of grasses. At the same time, they point to a lower contamination of the fresh herbage from grass stands in summer and to an increased risk of the occurrence of mycotoxins towards the end of the growing season, especially in the genus of *Lolium*.

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USE OF THE BLOOD COAGULATION PARAMETERS' DETERMINATION IN ANIMAL DISEASE AND POISONING DIAGNOSTICS

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Blood coagulation is a process essential for life. It maintains inner environment of the body and protects it from bigger losses of blood. Although there are many variations of this system among different animal species, the principle of blood clotting in all of them remains the same.

There may occur situations and diseases when amount or function of coagulation factors is impaired and both hypo- and hypercoagulation conditions are causing health problems and may be dangerous. Detection of such cases and establishment of physiological and pathological values of clotting parameters is vital for further research, diagnostics and successful treatment of diseases and poisonings connected with changes in coagulation profile.

Hypercoagulation conditions can be sometimes found in animals, it was confirmed that they suffer from similar mutation of factor V – Leiden mutation – as humans.

Hypocoagulation is more common status and one of the most typical reasons of it might be intoxications by anticoagulant rodenticides, mainly warfarin and its derivates. They occur frequently in many animal species including pets, livestock and wild animals.

There are also inherited and inborn malfunctions of coagulation cascade. Most common are haemophilias which also occur in animals, similarly to humans mostly concerning lack of factor VIII or IX in blood. Certain species were reported to have deficiency of factor VII and XI too.Nutrition (presence of aflatoxins in feed) or

several virus diseases are also described to alter haemostatic parameters.

We often lack information on how often poisonings by anticoagulants happen in wild animals and physiological values of coagulation parameters are still not assessed in them, so the determination of those parameters will be a task for our future research. The aim of our study will be determination of coagulation times (Quick test, aPTT) and fibrinogen, factor VIII and IX concentrations in selected wild animal species – vole, bank vole, field mouse, fallow deer and mouflon – and use of these values in the diagnostics and treatment of diseases and poisonings where change in blood coagulation is involved.

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OXIDATIVE STRESS IN FISH INDUCED BY PESTICIDES

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Oxidative stress is the phenomena in the last years. In human medicine is it associated with process aging and many diseases (e.g. atherosclerosis, arthritis, and cancer and Alzheimer´s disease). Knowledge of oxidative stress has a great importance for environmental and aquatic toxicology. Prooxidant endogenous and exogenous factors action and antioxidant defenses in fish organism can be used to assess specific area pollution or world sea pollution.

Oxidative stress comprises an important mechanism of chemical toxicity and cellular defense. It is induced of many diverse chemicals and results in a diverse health outcomes including damages or death of cells. In oxidative stress play important roles reactive oxygen species (ROS) as superoxid radical, hydroxyl radical, alkooxyl and peroxyl radicals, and reactive nitrogen species (RNS) such as nitric oxide, antioxidant defense systems, and the deleterious impacts of ROS.

Oxidative stress is evoked by many chemicals involving also pesticides. Therefore are biomarkers of this stress an important focus for assessment environmental pollution of pesticides.

This review summarizes current knowledge and new facts in understanding of oxidative processes in fish organism after exposure of pesticides. The relieving ROS in fish organism has been shown especially after exposure of organochlorine pesticides, organophosphates, bipirydil herbicides and triazine herbicides.

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CACO-2 CELLS AND BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS) FOR PREDICTION OF TRANSEPITHELIAL TRANSPORT OF XENOBIOTICS (MODEL DRUG: CAFFEINE)

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The human epithelial Caco-2 cell monolayer model is widely used as a standard screening tool for studying the mechanism of cellular drug transport. Caffeine was chosen as a model drug; caffeine is rapidly and nearly completely absorbed from the gastrointestinal tract and is supposed to be class I of the Biopharmaceutics Classification System (BCS). Our study was conducted 1) to characterize the transepithelial transport of caffeine across the intestinal barrier, 2) to precise classification of caffeine according to BCS, 3) to predict caffeine absorption in humans.

Caco-2 cells were cultured in DMEM at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. For transport studies, Caco-2 (passages 75–78) were seeded onto the Transwell inserts (2.5×10^5 cells/cm^2) and grown to late confluence (22–24 days). The monolayer integrity was checked by 500 μM phenol red permeability measured by spectrophotometry (558 nm) before the experiment and by ^{14}C mannitol (0.5 μCi/ml) permeability simultaneously during the transport studies. Caffeine transport (100, 300, 1 000 and 10 000 μM) was studied in apical to basolateral (AP-BL) and basolateral to apical (BL-AP) direction, under iso-pH 7.4 and pH-gradient (6 vs. 7.4) conditions. The relative contribution of the paracellular route was estimated using Ca^{2+}- free transport medium (opening tight junctions).

The results showed that caffeine transport is independent of transport direction, of concentration and pH, with high permeability coefficient (P_{app}): in AP-BL direction P_{app}=46.3–53.5×10^{-6} cm/s; in BL-AP direction P_{app}=45.6–49.4×10^{-6} cm/s. Thus, the transport seems to be transcellular mediated by passive diffusion. Using Ca^{2+}- free transport medium, the tight junctions were opened (confirmed by increased P_{app} of mannitol) but the caffeine P_{app} did not change. Thus, the paracelular route is only a minor way of caffeine transport. Estimated high solubility and high permeability of caffeine rank it among well absorbed compounds and class I of BCS which is in a good agreement with the literature data.

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IS SEA FISH OIL A SAFE AND HEALTHY FOOD SUPPLEMENT?

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Omega-3 polyunsaturated fatty acids are highly valued because human desaturases are not able to supply their need in sufficient range. These are particularly eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). Satisfactory intake of EPA and DHA in fish oil is vital for health protection and improvement. On the other hand, there is concern for heavy metals (mercury and methylmercury) contamination and persistent compounds (e.g. DDT, HCH, PCB) occurrence in food.

The aim of our study was to determine and discuss content of mercury, methylmercury and persistent compounds in sea fish oil and in tinned cod liver including their own oil and sauce from food safety and quality point of view. Analyzed samples were capsules filled with fish oil, most frequently from salomon. Tinned cod liver from Poland and Denmark were bought in the Czech market. Total mercury content was analyzed by AMA 254 analyzer, methylmercury, DDT (including its metabolites), four isomers of HCH and seven indicator congeners of PCB were analyzed using gas chromatography. The average values of total mercury and methylmercury in cod liver ranged from 0.011 $mg.kg^{-1}$ to 0.22 $mg.kg^{-1}$ and from 0.0024 $mg.kg^{-1}$ to 0.014 $mg.kg^{-1}$, respectively. Total mercury was found in capsules of sea fish oil in trace values (0.92 $ng.g^{-1}$ max.). Persistent chlorinated compounds both in capsules and cod liver were under the limit of detection.

Following our results sea fish oil and cod liver can be recommended as safe and healthy food supplements.

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IN VIVO EFFECTS OF SESAME OIL IN THE MODEL OF ADJUVANT ARTHRITIS

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In the latest nutraceutical studies the traditionally well-known health value of sesame was described, including the application of sesame oil. As oxidative stress is known to contribute to the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis, utilization of antioxidant activity of sesame oil (1) might be of importance in anti-arthritic therapy.

The goal of this study was to evaluate the effect of sesame oil on functional damage induced by adjuvant arthritis and on changes of selected biochemical parameters reflecting oxidative tissue injury.

To induce adjuvant arthritis, *Mycobacterium butyricum* in incomplete Freund's adjuvans was intradermally administered to Lewis male rats. Functional parameters as hind paw edema and endothelium-dependent relaxation of the aorta, identified as the response to acetylcholine *in vitro*, were determined on experimental days 14 and 28. Further we assessed biochemical markers of oxidative stress: plasmatic levels of TBARS, gamma-glutamyltransferase (GGT) activity in the joint and spleen tissue homogenates, level of protein carbonyls and total antioxidant activity in plasma, as well as activity of the lysosomal enzyme N-acetyl-glucosaminidase (NAGA) in serum and the kidney. The effect of sesame oil (1ml/kg, daily oral administration) was evaluated on experimental day 28.

The beneficial effect of sesame oil on markers of oxidation stress accompanying adjuvant arthritis was demonstrated by significant decrease of plasma TBARS and decrease of GGT activity in the joint and spleen tissue homogenates. Level of protein carbonyls, total antioxidant activity in plasma and activity of NAGA in serum and in the kidney were improved, yet not significantly. In the hind paw edema the maximal increase was found on day 28 of adjuvant arthritis, accompanied by significant decrease of aortic endothelium-dependent relaxation. Administration of sesame oil resulted in mild, non-significant decrease of hind paw swelling and in significantly increased acetylcholine-evoked relaxation.

We conclude that sesame oil has beneficial effects on oxidative stress induced biochemical changes occurring in adjuvant arthritis and importantly on two functional parameters – hind paw edema and endothelium-dependent relaxation of the aorta.

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EFFECT OF CISPLATIN ON METALLOTHIONEIN LEVEL IN FISHES

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The study of platinum metals is demanding much more of our attention, especially in evaluating their harmful effects to the environment. One source of these metals is automobile catalytic converters. Within the context of the application of platinum-based cytostatics, which have been widely used for the treatment of malignant diseases, we can surmise that these metals, migrate into sewage systems and thus pollute aquatic ecosystems.

In our work we studied sublethal effects of cisplatin on zebrafish (*Danio rerio*) using ecotoxicological biotests. Specifically the metal's transition from aquatic ecosystems to its distribution in fish bodies. The impact of cisplatin was found by observing the most significant protein – metallothionein (MT). MT plays a key role in transport of essential heavy metals, detoxification of toxic metals and protection of cells against oxidation stress. It is a cystein-rich protein and its sulhydryl groups have the ability to create stable complexes with different metals and decrease metal concentration in intracellular and extracellular spaces.

In our experiment fishes were exposed for seventy days to the influences of different rates of cisplatin concentrations (10, 20, 40 μM) and one fish was captured per week. We prepared three samples: from the head part, the entrails and the muscles. For this purpose we employed differential pulse voltammetry (Brdickova reaction).

The highest content of MT was measured in samples from the entrails. The content was lower in samples from the head, and the lowest was from the muscle samples. The average value of MT from the entrails in our control samples was 1 642.9 μg/g, in a 20 μM concentration the value increased to 1 766.9 μg/g. In a 10 μM concentration the content decreased to 1 409.3 μg/g and also decreased in a 40 μM to 1 508.1 μg/g.

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LETHAL POISONING CASES FROM YEW TREE NEEDLESS

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The yew tree (Taxus baccata L.) is widely spread all over central and southerm Europe. The toxicity of yew has been well known.

The authors describe two cases of lethal poisoning from tree needles and method of toxicological determination poisoning with yew tree.

Cases history: A 18-year-old young woman drank up a decoction of taxus needles from small yew tree in flowerpot which she bought in a garden-tillage. About 2 hours after her father found her in a comatose state. About 6 hours after drinking of the decoction she died in hospital. The case was closed as suicide.

A 20-year-old man was found dead in the mental home area, where he was treatmented on a schizofrenic disorder.

For toxicological investigation we used gastric contents, livers, urines and bloods. Biological materials were extracted using SPE and analyzed with LC-MS method. As comparative standards was prepared crude taxine extract and standard of taxine B and isotaxine B was obtained from Institute für Rechtsmedizin, Münster. Analysis was focused on determination of taxine B and isotaxine B with Mr=583 and other pseudo-alkaloids of taxine fraction.

In the stomach contentas all of taxine pseudo-alkaloids of "taxine fraction" were determined. In liver, urine and bloods there were identification taxine B and isotaxine B.

FURTHER STUDIES ON THE ANTICANCER DRUG ELLIPTICINE ACTION

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Ellipticine is an antineoplastic agent, which should be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dictated by its cytochrome P450 (CYP) and/or peroxidase-mediated activation in target tissues. Namely, among the multiple modes of ellipticine antitumor action, metabolic activation of ellipticine mediated by its oxidation with CYPs and peroxidases was found to be the predominant mechanism of cytotoxicity to human breast adenocarcinoma MCF-7 cells, leukemia cells (HL-60 and CCRF-CEM lines) and several neuroblastoma cells.

The aim of this study is to increase our knowledge on the molecular mechanism of ellipticine action in cancer cells.

The DNA-targeting mechanism of ellipticine is based on its oxidation by CYPs and peroxidases to metabolites responsible for formation of ellipticine-derived DNA adducts. In addition, besides the formation of such covalent ellipticine-derived DNA adducts, participation of the mechanisms in ellipticine toxicity, such as intercalation into DNA and inhibition of DNA topoisomerase II, which were found to be additional DNA-mediated mechanisms of ellipticine antitumor, mutagenic and cytotoxic activities, cannot be excluded. Whereas 9-hydroxy- and 7-hydroxyellipticine formed by CYPs and the major product of ellipticine oxidation by peroxidases, the ellipticine dimer, are the detoxication metabolites, two carbenium ions, ellipticine-13-ylium and ellipticine-12-ylium, derived from other two

metabolites, 13-hydroxy- and 12-hydroxyellipticine, generate two major deoxyguanosine adducts in DNA seen *in vivo* in rats and mice treated with ellipticine. Such DNA-adducts are also formed in cancer cell lines *in vitro*, namely, in human breast adenocarcinoma MCF-7, leukemia HL-60 and CCRF-CEM and neuroblastoma cell lines and *in vivo* in breast carcinoma of rats bearing this type of tumor and exposed to ellipticine. Ellipticine is also a strong inducer of CYP1A and CYP1B1 enzymes, modulating levels of detoxication and activation metabolites and thus its own genotoxic and pharmacological efficiencies. Furthermore, cytochrome b_5 dictates the pattern of ellipticine metabolites generated by CYP3A4 and mainly by CYP1A1 and 1A2 enzymes, increasing formation of ellipticine metabolites forming DNA adducts. The study forms the basis to further predict the susceptibility of human cancers to ellipticine.

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DETERMINATION OF IBOTENIC ACID AND MUSCIMOL IN THE SERUM AND URINE OF A PERSON INTOXICATED WITH *AMANITA PANTHERINA*

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Ibotenic acid and muscimol are isoxazole alkaloids which mostly participate in the psychotropic properties of *Amanita pantherina* and *A. muscaria*. *Amanita pantherina* poisoning is in the majority of cases accidental because it can be easily mistaken for the edible species (*Amanita rubescens*, *A. spissa* and *Macrolepiota procera*). *Amanita muscaria* poisoning is mostly intentional for recreational purposes. The prognosis of poisoning is generally good; lethal cases are rare.

Mushroom poisoning is often proved by microscopic examinations of spores in the stomach or gut content. Authors of this presentation introduce the instrumental method of proving *Amanita pantherina* or *A. muscaria* poisoning. Toxins responsible for the effects of these *Amanitas*, ibotenic acid and muscimol, are rapidly absorbed from the gastrointestinal tract and readily excreted in urine. This study describes the isolation of ibotenic acid and muscimol from the serum and urine, and the determination of these compounds by gas chromatography/mass spectrometry. Isolation of these alkaloids from the biological material was performed on a strong cation exchanger Dowex®, and the elution and derivatization of the alkaloids were made in one step with ethyl chloroformate in aqueous solution of sodium hydroxide with the addition of ethanol and pyridine.

By this method, ibotenic acid and muscimol were determined in the serum and urine of the person intoxicated by *Amanita pantherina*. The mass spectra of derivatized ibotenic acid and muscimol (main peaks m/z 113, 185, 257 and 98, 113, 130 respectively), the extraction efficiency and the limit of detection are described.

THE EFFECT OF PERACETIC ACID ON HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF RAINBOW TROUT

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Since the use of malachite green was banned in many European countries, new alternative treatments have been tested to lower the parasite burden and mortality level of infected fish. We tested the effect of Persteril (with peracetic acid as active component) to haematological and biochemical indices of rainbow trout (*Oncorhynchus mykiss*). The total of 30 fish (mean weight 66±8g) were divided into 3 groups and placed into 200 l aquaria. Persteril was added once (group 2) and two-times (group 3) daily to reach the concentration of 1ppm in both tanks. The control fish (group 1) were placed in the water without adding of Persteril. Fish were examined after 4 days in all groups. The main haematological response of fish to peracetic acid was observed at higher application frequence (group 3). There was significant decrease ($p<0.05$) in haematocrit (0.34±0.02 LL^{-1}), erythrocyte count (0.91±0.11 TL^{-1}) and significant increase in mean corpuscular haemoglobin concentration (0.159±0.006 LL^{-1}, $p<0.01$). Less marked changes were observed in group 2, where significantly increased ($p<0.05$) the values of mean corpuscular haemoglobin (62.11±7.25 pg) and mean corpuscular volume (460.0±73.2 fL). The biochemical values differed most in group 2, where significantly higher ($p<0.01$) values of enzymatic activities (μkat/l) of alanine aminotransferase, aspartate aminotransferase, creatin kinase and lactate dehydrogenase were measured. Compared with the control group 1, group 2 showed more considerable changes in biochemical values and group 3 in haematological values. This could be caused by various responses of fish to different interval of Persteril application.

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SIGNIFICANCE OF PORK AS A SOURCE OF SELENIUM FOR HUMAN CONSUMPTION – EVALUATION OF SITUATION IN THE CZECH REPUBLIC

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Selenium is an esential nutriet important for the maintenance of antioxidant and metabolit functions. As pork is consumed in large quantities throughout the world, it would be a convenient selenium source in human diet.

The aim of this paper was to review current knowledge of significance of pork meat as a source of Se for human consumption and to evaluate the selenium content in pork meat produced in the Czech Republic. The selenium has an important role in human health. Pork could be an important source of Se for human diet. The Se content in meat can be increased by higher dietary intake of selenium. The magnitude of increase is higher when organic Se from Se-enriched yeast is fed. Selenium intoxication in pigs occurs only when very high Se concentrations are used in the diet (more than 5 mg of Se / kg). The organic Se source produces fewer clinical signs of intoxication than inorganic sodium selenite. The organic Se has positive or at least no negative effect on meat quality.

Altogether 135 pork samples from 9 different herds in the Czech Republic were collected and analyzed for Se content.

The average selenium content in pork found in our study was 84.35±12.3.μg.kg^{-1} Because of the high average annual consumption of pork in the Czech Republic, the annual selenium intake from pork by humans represents a significant part of requirement of men per year.

The results of our study show that pork contributes significantly to selenium intake in human population of the Czech Republic.

The study was supported by the project MSM 6215712403.

HUMAN AND RAT CYTOCHROMES INVOLVED IN OXIDATION OF 2-NITROPHENOL, A METABOLITE OF CARCINOGENIC 2-NITROANISOLE

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2-Nitroanisole (2-methoxynitrobenzene, 2-NA) is an important industrial pollutant. It is used as a precursor in the synthesis of *o*-anisidine, an intermediate in the manufacture of many azo dyes. 2-NA exhibits carcinogenic activity, causing neoplastic transformation in the urinary bladder and, to a lesser extent, in spleen, liver and kidneys in rodents. 2-NA is oxidized by rat, rabbit and human hepatic microsomes to 2-nitrophenol (2-NP), 2,5-dihydroxynitrobenzene (2,5-DNB) and 2,6-dihydroxynitrobenzene (2,6-DNB), which are suggested as detoxication 2-NA metabolites. 2-Nitrophenol is the main detoxication metabolite of 2-NA.

The aim of this study was to investigate the efficiency of rat hepatic CYPs to metabolize 2-NP, to determine the metabolites formed during such a metabolism and to compare the efficiencies of rat CYPs with those of human and the effect of the selective CYPs inducers and inhibitors on 2-NP oxidation.

2-NP is oxidized by rat liver microsomes to one metabolite, 2,5-dihydroxynitrobenzene. To define the role of CYPs in 2-NP oxidation we investigated the modulation of this reaction by specific inducers and selective inhibitors of these enzymes. Treating rats with an inducer of CYPs of a 2B subfamily (PB) leads to an increase in 2-NP oxidation, while that with β-NF (inducer of a 1A subfamily) and ethanol (inducer of CYP2E1) had lower effects. Most of inhibitors of individual CYPs tested in this study, α-naphtoflavone (for CYP1A), diamantane (for CYP2B), sulfaphenazole (for CYP2C), quinidine (for CYP2D), diethyldithiocarbamate (for CYP2E1) and ketoconazole (for CYP3A) influenced the 2-NP metabolism. Diethyldithiocarbamate, quinidine, ketoconazole and diamantane inhibited generation of 2,5-DNB, whereas α-naphtoflavone and sulfaphenazole were almost without this effect. Using microsomes from Baculovirus transfected insect cells expressing recombinant rat and human CYP enzymes, we found that rat recombinant CYP2E1, 2C11, 2B1, 1A2, 1A1 and 3A4 were the most effective to oxidize 2-NP. Similarly, human CYP2E1, followed by CYP2A6, 2C6, 3A4 and 2D6 were the most efficient to oxidize 2-NP.

The present study shows the similarity between human and rat enzymes metabolizing 2-NP to 2,5-DNB and indicate that rat might serve as a suitable model to mimic the fate of 2-NP in human.

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IN VITRO TESTING INHIBITION POTENCY OF ACETYLCHOLINESTERASE REACTIVATORS

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Organophosphate inhibitors of acetylcholinesterase (AChE; 3.1.1.7), are typical pesticides moreover, it could be abused as nerve agent in wars. The main mechanism of action is known, it is irreversible inhibition of enzyme AChE. These compounds covalently bind on the AChE resulting in break its physiological function with subsequent increase of level of neurotransmitter acetylcholine. This situation may cause cholinergic

crisis and even death of intoxicated patient. Standard treatment of organophosphate poisoning consists of an anticholinergic drug (mainly atropine) in combination with AChE reactivator (oxime). Reactivators are nucleophilic reagent, which are able to dephosforylate inhibited AChE so it return its original activity. Oximes are also highly hydrophilic compounds with quaternary nitrogen in their structure. These physico-chemical disadvantages depress the ability of these compounds to penetrate through the blood-brain barrier. Due to the oximes ability penetrate into active site of AChE, reversible inhibition is expected. The present study is aimed at estimation of oximes inhibiting potency.

The inhibition potency was tested by standard spectrophotometric Ellman method. IC_{50} of reactivators was measured by Spectrophotometer Helios Alpha, Electroncorporation (GB). Eel AChE was used throughout experiments.

The aim of our study was to compare the ability of tested reactivators (change in linker; C1–C12) to study their potential to inhibit AChE and find some structural dependence. The higher ability to inhibit AChE was found in molecule with linker C11 ($IC50=1.57\times10^{-4}$). The reactivators with short C1–C4 or longer linker than C11 had low potency to be inhibitor of AChE. This knowledge will be used for preparation of newly synthesized inhibitors of AChE. Inhibitors of AChE with low ability to penetrate through the blood-brain barrier may be suitable as a potential therapeutics of Myasthenia gravis.

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CYTOCHROMES P450 METABOLIZE CARCINOGENIC ARISTOLOCHIC ACID I FORMING ITS DETOXICATION METABOLITE AND DECREASING LEVELS OF AA-DNA ADDUCTS

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Aristolochic acid (AA) causes the development of two kidney diseases, aristolochic acid nephropathy (AAN) and the Balkan endemic nephropathy (BEN), which are associated with urothelial cancer. One of the common features of AAN and BEN is that not all individuals exposed to AA suffer from these diseases. We suggested that one cause for these different responses may be individual differences in the activities of enzymes catalyzing the detoxication and/or activation of AA.

The aim of this study was the understanding which enzymes are involved in AAI activation and/or detoxication.

HRN [Hepatic Cytochrome P450 (CYP) Reductase Null] ($Por^{lox/lox}+Cre^{ALB}$) mice were constructed in laboratories in Great Britain. Male HRN and WT mice (25–30 g) were treated i.p. with 10 or 50 mg/kg body weight of AAI in one dose and with 125 mg/kg body weight of benzo[a]pyrene (BaP) daily for 5 days. AAI metabolite AAIa was separated from AAI by RP-HPLC. The ^{32}P-postlabelling technique was utilized to determine AAI-DNA adducts.

Using a HRN mouse line, we investigated AAI detoxication *in vivo* and *in vitro*. We found that hepatic microsomes of wild-type (WT) mice demethylate AAI *in vitro* to detoxication metabolite, AAIa, while those of a HRN line were without this effect. Levels of AA-DNA adducts in livers and kidneys of WT mice exposed to AAI were up to ten-fold lower than in those of HRN mice. These results suggest that hepatic CYPs decrease the actual concentration of AAI both in liver and kidney, thereby protecting its activation to AA-DNA adducts. To define the role of CYP enzymes in AAI oxidation, we used besides hepatic microsomes of these mouse models, also those isolated from these mice pre-treated with B(a)P, and those of human and rat. Furthermore, we investigated the modulation of this reaction by specific inducers and selective inhibitors of these enzymes. The efficiency of human recombinant CYPs to oxidize AAI was also tested. The results demonstrate a major role of hepatic CYPs in AAI detoxication *in vivo* and that of CYP1A1/2 in this reaction *in vitro*.

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PRECLINICAL RESEARCH OF DRUGS, A VIEW FROM PRACTICE

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Development of new frugs is complicated time and money consuming process. From 10 000 promising substances or molecules at the beginning, only 1 successfuly pass through testing phases and reach the market. This process takes 10–15 years and costs $500–$1 000 milions. The process starts with basic research, followed with preclinical phase, clinical phases and is completed with authorities (EMEA, FDA) approval of new drug.

Preclinical phase of new drug development is complex of studies based on legislation, which evaluate the success of drug during first steps of development. Aim of preclinical phase of testing is to assess adverse effects of substance, to describe mechanisms of toxicity in target organs, to define extent and dose-response relationship and to determine reversibility of effects. Obtained data are used for setting of tests in following clinical phases. Preclinical phase takes 2–4 years and costs about 10% of total costs.

There are many *in vitro* and *in vivo* biological models for preclinical testing of toxicity comprising cell and

tissue cultures, invertebrates, rodents, rabbits, ferrets, dogs and primates. Cell and tissue cultures are mostly used for genotoxicity screening. Vertebrates are used for testing of acute, subchronic or chronic toxicity. Based on results of this studies additional studies of reproduction and embryonal toxicity or carcinogenicity are performed. Toxicokinetics as a part included in some of this studies provide information about pharmacokinetics, pharmacodynamics and ADME properties of tested substance.

Testing on biological systems has it's own limitations. Extrapolation animal toxicity to human is not easy applicable, due to interspecies differencies in anatomy, physiology, genetics and metabolism. Ethical reason becames more and more relevant. As a reaction several alternative tests reducing using of laboratory animals are approved or just validated.

EFFECT OF PHENOLIC SUBSTANCES AND THEIR DERIVATIVES ON SERCA ACTIVITY FROM RABBIT SKELETAL MUSCLE

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Sarcoplasmic reticulum Ca-ATPase (SERCA) from skeletal muscle is calcium regulating single-chain transmembrane protein with easily measurable function, present in high concentration in sarcoplasmic reticulum (SR) vesicles. SR is potentially a regulator of apoptosis, at least for two reasons: it is the main intracellular store of Ca^{2+}, a second messenger, and is physically and functionally interconnected with mitochondria. Modulation of SERCA activity may be a contributing factor in the development of some cardiovascular, neurodegenerative or skeletal muscle diseases.

Effects of phenolic antioxidants, synthetic (trolox, pyridoindole stobadine and SMe1) and flavonoids (quercetin, rutin, naringin, phloridzin, esculin) and their lipophilic derivatives (rutinpalmitate (R-16:0), rutinstearate (R-18:0), naringinstearate (N-18:0), phloridzinstearate (P-18:0), esculinstearate (E-18:0), rutinoleate (R-18:1), rutinlinolenate (R-18:3)) were investigated on the activity of Ca-ATPase from SR of rabbit skeletal muscle to examine their potency to modulate its enzyme activity.

SR vesicles were isolated from New Zealand rabbit fast-twitch skeletal muscle. Enzyme activity of SERCA was measured spectrophotometrically at 37°C by NADH-coupled enzyme assay. Lipophilic flavonoid derivatives were prepared by lipase-catalyzed esterification of flavonoids with fatty acids.

Rutin and naringin, flavonoids with similar structural features, elevated the SERCA activity in comparison to control. Trolox, stobadine, SMe1, quercetin, phloridzin and esculin did not significantly influence the SERCA activity. Lipophilic derivatives of flavonoids with incorporated fatty acid chain, such as rutinpalmitate, rutinstearate, naringinstearate, phloridzinstearate, esculinstearate, rutinoleate and rutinlinolenate significantly decreased the activity of Ca-ATPase incorporated into SR vesicles. The inhibitory effect was independent on the incorporated fatty acid chain length and the degree of saturation. Free fatty acid like stearic acid (C18:0) had no effect on Ca-ATPase activity. This fact may suggest that studied derivatives can bind into the lipidic membrane through fatty acid chain and thus indirectly influence the function of SERCA. The flavonoids unmodified with fatty acids probably directly influence the Ca-ATPase activity.

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COMPARISON OF THE GENOTOXIC EFFECTS OF TWO FUNGICIDES

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Fungicides represent the most problematic chemicals in terms of potential health hazard since about 90% of all fungicides used either currently or in the recent past have been determined to be carcinogenic in experimental animals. The cytokinesis-block micronucleus (CBMN) assay is the preferred method for measuring micronucleus frequency (MNi) in cultured human and/or animal cells because scoring is specifically restricted to once-divided cells.

In this study the fungicides dichlofluanid and tolylfluanid were investigated for their ability to induce MNi *in vitro* using sheep peripheral lymphocytes.

For the micronucleus assay, heparinised blood (100 IU/mL) from 7 clinically healthy donors (18-month-old female Merino sheep), obtained by *jugular vein* puncture, was used. Whole blood 0.4 mL was added to 7.0 mL chromosome medium supplemented with fetal calf serum, phytohemagglutinin-L, L-glutamine, penicillin G (100 IU/mL), streptomycin (100 μg/mL) and 7.5% $NaHCO_3$. Lymphocyte cultures from each donor were divided into treated cultures, solvent and positive controls and incubated at 37 °C for 72 h. Treated cultures were exposed to dichlofluanide and tolylfluanid for 48 h. The tested concentrations of fungicides were 1×10^{-4}, 1×10^{-5} and 1×10^{-6} M/L. Cytokinesis was blocked by Cyt-B which was added 44 h after culture initiation to get a final concentration of 6 μμg/mL. The cells were treated with hypotonic solution (0.035 M/L KCl) for 20 min at 37 °C and fixed with cold methanol:glacial acetic acid (3:1, v/v). The slides were air-dried and stained with 10% Giemsa Romanowski in Sörensen phosphate buffer, pH 6.8, for 3–6 min. Using a Nicon microscope (CCD-100 camera system, Mitsubishi) the coded slides were scored

blinded at magnifications of 400× and 1 000×. MNi were counted in 1 000 binucleated cells per donor and per concentration. Effects on the cellular proliferation rate were estimated by calculating nuclear division index (NDI). 500 lymphocytes per donor were scored to evaluate the percentage of cells with 1–4 nuclei and NDI was calculated according to Eastmond and Tucker (1987). The Sigma Stat program (Statistical software™, Jandel Scientific) was employed for statistical evaluation of the results. The mean frequency of micronuceated cells and the NDIs were calculated based on the results of the independent experiments, and the statistically significant differences between control and treatment values were determined using analysis of variance (ANOVA) followed by Tukey´s test at the 95% confidence level.

After exposure to dichlofluanid the micronucleus frequency was not significantly increased at the concentrations tested ($p<0.05$). At the concentration of 1×10^{-4} M/L the number of MNi was 17.5±13.66/1 000 binucleated cells, at 1×10^{-5} M/L was 12.25±6.55/1 000 binucleated cells, at 1×10^{-6} M/L presented 16.00±7.35 MNi/1 000 binucleated cells versus 11.75±6.65 micronuclei/1 000 binucleated cells in DMSO control. Many of the treated cells also possessed multiple MNi.

In lymphocyte cultures exposed to fungicide tolylfluanid it was observed that at the lowest concentration (1×10^{-6} M/L) the frequency of micronuclei was not significantly different (32.33±3.51/1 000 at this concentration versus 30.33±2.82/1 000 in DMSO control, $p=0.44$). Higher concentrations of fungicide (1×10^{-4} and 1×10^{-5} M/L) resulted in a significant dose dependent increase in the number of MNi in comparison with control (74.0±13.0/1 000 and 52.67±10.12/1 000 versus 30.33±2.82/1 000 in DMSO control, $p=0.005$ and 0.020, respectively, ANOVA test). Many of the treated cells also possessed multiple MNi. Tolylfluanid did not affect nuclear division index (NDI) at all treatment concentrations.

Our results thus demonstrate a weak genotoxicity of tolylfluanid, whilst dichlofluanid has not got any significant effect on the frequency of MNi in sheep peripheral lymphocytes *in vitro*.

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SKIN IRRITATION/CORROSIVITY TESTING IN REACH FRAMEWORK

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Skin irritation/corrosivity testing in REACH framework is managed by Regulation (EC) No. 1907/2006 of the European Parliament and of the Council (Dec 2006). Tendency of the regulation in the testing of this endpoint is to come on by a tier strategy with the aim to save animals.

For quantity 1–10 tonnes/year the following information is demanded: an assessment of the available human and animal data, an assessment of the acid or alkaline reserve, *in vitro* study for skin corrosion, *in vitro* study for skin irritation. From 10 tonnes/year testing *in vivo* is necessary with except of listed special cases.

In our laboratory, we employ ourselves with implementation of alternative tests. According to the tier strategy the evaluation alkaline/acidic reserve is the first step of testing of a chemical substance. If pH is higher 11.5 or lower than 2 the substance is considered as corrosive. At pH in intervals 10–11.5 or 2–4 the measuring of alkaline/acidic reserve is advised. Parameters for calculation are determined, along which corrosivity/irritation of the test substance can be specified. In case of positive result, the testing is finished and the substance is labelled as corrosive/irritant.

In case of negative result, the next step is corrosivity determination *in vitro* on human epidermal model.

Artificially grown human epidermis originated from human keratinocytes is used for testing. Principle of the test is formazan formation (blue) from MTT (yellow). Test materials are placed directly to stratum corneum of epidermal model (one sample for three tissue discs) in two exposure times (3 min and 1 hour). After rinse, tissues are incubated with MTT, where it is reduced by mitochondrial dehydrogenase to blue formazan precipitate. In damaged cells this reduction is decreased proportionally to harm. Consequently, extraction is performed overnight by isopropanol and evaluated by spectrophotometr (λ=570 nm) Viability of tissues treated with a test chemical is compared with that of untreated controls and the percent of viability is calculated. This value is used for the classification according to prediction model. The test is able to differentiate between R35 a R34 EU classification groups.

In the case of positive result, of the *in vitro* corrosivity test the test substance is labelled as corrosive and testing is finished. In the case of negative result, skin irritation *in vitro* is going to be determined in the next step.

Principle of the skin irritation method on artificial human epidermis is the same as in case of corrosivity testing. Design differs by using one exposition time only (1 hour) which is followed 42-hours post incubation period for regeneration and inflammatory mediators release, what serve as supportive indicators. Turnover of colour is evaluated by the same way as in skin corrosivity test.

In the case of negative result the testing continues in one animal *in vivo*. In the case of positive result the substance is labelled as skin irritant; in the case of negative result, *in vivo* testing is completed by using of two other animals. Testing is then over.

In our laboratory, skin corrosivity method is established; verification of the skin irritation test is recently in progress.

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VALIDATION OF QSAR MODELS FOR LEGISLATIVE PURPOSES

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Validation of *in silico* methods related to chemical safety programs for legislative usage is described. A group of experts preparing criteria for results of QSAR (Quantitative Structure – Activity Relationships) was recommended during the 34. joint meting of Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in 2002 in Paris. During 2003–2004 meetings organized by European Centre for Validation of Alternative Methods (ECVAM) at Joint Research Centre of Europen Committee prepared a base for validation of QSAR models for legislative usage. Five principles were recommended and agreed, which must QSAR models fulfilled: a defined end point, an unambiguous algorithm, a defined domain of applicability, appropriate measures of goodness-of-fit, robustness and predictivity, a mechanistic interpretation if possible. A cross validation is reccommended for the validation.

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INTERACTION OF TWO ACETYLCHOLINESTERASE REACTIVATORS WITH HUMAN LIVER MICROSOMAL CYTOCHROMES P450

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Two non-symetric bispyridine oxime – based reactivators of acetylcholinesterase enzyme (EC 3.1.1.7), labeled as K-48 (1-[4-hydroxyiminomethylpyridinium]-4-[4-carbamoylpyridinium]-butane dibromide) and HI-6 (1-[2-hydroxyiminomethylpyridinium]-3-[4-carbamoylpyridinium]-2-oxa-propane dichloride) were subjected to an interaction with human liver microsomal cytochromes P450 (CYP).

Difference spectroscopy has detected an interaction of both compounds studied with cytochromes P450. The compounds were shown to bind to microsomal cytochromes P450 with spectral binding constants of 0.25±0.05 (K-48) and 0.54±0.15 (HI-6) μM. To find which cytochrome P450 from the human liver microsomal fraction interacts with these compounds, an inhibition of enzyme activities specific for nine individual CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) was studied. The results have shown no inhibition of the CYP1A2, 2A6, 2B6, 2C8, 2C9, 3A4, activities; in the case of K-48, relatively small inhibion of the CYP2C19 and CYP2D6 was found (20% decrease of the respective activities). For the HI-6 reactivator, the only the CYP2E1 activity was inhibited, in this case down to 60% of the control.

This fact is, on the other hand, advantageous for the compounds studied – they apparently will not interact with metabolism of other compounds at the level of the nine most important CYP enzymes.

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TERATOLOGICAL STUDY OF THE PYRIDOINDOLE ANTIOXIDANT SMe1EC2 IN RATS

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Administration of antioxidants *via* maternal organism may be rational approach to protect embryo and fetus during period of increased risk of oxidative stress (pre-eclampsia, iron imbalances, infections, gestational diabetes) as well in prevention and treatment of diseases associated with oxidative stress (bronchopulmonary dysplasia, idiopathic respiratory distress syndrome, asphyxia, hypoxia, etc.).

The 2-ethoxycarbonyl-8-methoxy-2,3,4,4a5,9b-hexahydro-1H-pyrido-[4,3b] indolinium chloride (m.w. 312.79 Da, chemical purity more than 99%, SMe1EC2) is a prospective antioxidant and neuroprotectant drug. The aim of the study was to evaluate the effects of SMe1EC2 on embryo-fetal development.

The substance tested was administered orally to Wistar/DV rats from day 6 to 15 of gestation (organogenesis) at the doses 5, 50 and 25 mg/kg/day. The females were sacrificed on day 20 of gestation and uterine content was inspected (corpora lutea, implantations, early and late resorptions, live and dead fetuses, pre- and post-implantation loss). Live fetuses were examined for gross, skeletal and visceral anomalies.

Administration of SMe1EC2 did not induce any signs of maternal toxicity. No adverse effect of the substance tested was found on reproductive variables. Morphological examination of fetuses revealed no evidence of teratogenicity. The prenatal toxicity study showed that the substance SMe1EC2 did not have embryotoxic and teratogenic effects.

The substance tested is suggestive to use in prevention and treatment of diseases and injuries associated with hypoxia/ischemia including developmental disorders due to oxidative stress as well.

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INCREASED LEAD EXPOSURE IN GLASS FACTORY EMPLOYEES

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The occupational lead (Pb) exposure of workers to lead as well as exposure of the general population is well researched. The occupational lead exposure may occur at this time still quite often. The examination of lead in blood has considered as a useful biological exposure test.

Aim of this work was to examine lead (Pb) concentrations in blood of the glass factory employees. The employees have been producing glass from liquid lead glass at different working positions more than one year. It is very likely that the concentrations of mentioned metal will be higher than in general population because a lot of working positions were classified in risk category 3 and 4.

During one year the authors examined content of Pb in blood of 282 glass factory employees. Average age of the exposed workers was 37.52±7.86 years (x±SD). The used analytical method was the atomic absorption spectroscopy in graphite furnace (GFAAS). The collection of 5 ml heparinized blood was realized in 8 terms. The workers with values more than 1.930 μmol.l^{-1} were invited on The Clinic of Occupational Medicine in Martin for mobilization treatment.

The average blood concentration of our group was 1.609±0.819.86 μmol.l^{-1}. The increased concentrations over 1.930 μmol.l^{-1} were found in 101 cases that was more than 35.82%. The highest values (more than 3.400 μmol.l^{-1} = 700 μg.l^{-1}) were found in five glass blowers. This was higher than a binding biological value which must not be exceeded (Slovak government decree No.355/200).

Blood lead levels in the general population of Slovakia have been decreasing over the past 2 decades as regulations regarding Pb paint, fuels, water piping and Pb–containing plumbing materials. The marked increased values of Pb in blood in exposed group against the general population indicated increased professional exposure of this metal. In spite the glass production in this factory might be terminated we find important to monitor the exposed group and we recommend to repeat the analyses after 1 year.

EFFECTS OF SUBCHRONIC SIMAZINE EXPOSURE ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF THE COMMON CARP (*CYPRINUS CARPIO* L)

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The worldwide use of simazine as a pre-emergent herbicide on a broad variety of crops and for weed control in industrial areas and effluents from manufacturing sites have resulted in its release into the environment in various waste streams. Recently, the environmental pollution, especially water ecosystem, by pesticides has become a serious problem. Due to their heavy use in agriculture and to their persistence, many of these compounds present in surface and ground waters, and have to be considered a potential risk for water life as well as for drinking water quality.

The aim of the study was to evaluate subchronic toxic effects of the simazine (6-chloro-*N*^{2},*N*^{4}-diethyl-1,3,5-triazine-2,4-diamine) on some biochemical and haematological parameters of the common carp (*Cyprinus carpio* L.). Two year old fish were exposed for 28 days to simazine added to the tank water at four concentrations of 0.06 (real environmental concentration in Czech river), 4, 20 and 50 μg.l^{-1}.

The subchronic exposure of simazine in real environmental concentration 0.06 μg.l^{-1} had not effect on biochemical and haematological profile of common carp. The subchronic exposure of common carp of simazine in concentration 4, 20 50 μg.l^{-1} caused significant shifts in biochemical and haematological profile.

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ISOLATION AND PARTIAL CHARACTERIZATION OF CATECHOL-1,2-DIOXYGENASE OF *CANDIDA TROPICALIS* YEAST PARTICIPATING ON PHENOL BIODEGRADATION

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Biodegradation is highly effective method of decontamination of a fouled environment. The process is based on efficiencies of organisms (both prokaryotic and eukaryotic) to utilize xenobiotics as a source of

carbon and energy without formation of any secondary waste products. *Candida tropicalis* yeast has been considered to be able to metabolize phenol and utilize it as the only source of carbon and energy. Degradation of phenol by *C. tropicalis* yeast comprises of several steps and the final products (succinate and acetyl-CoA) are implemented into intermediate metabolism of the yeast. The enzymes responsible for the first step of degradation (oxidation of phenol to catechol) are: (i) cytochrome P450 (EC 1.14.15.1), the enzyme of the mixed function monooxygenase system localized in the membrane of endoplasmic reticulum and cytoplasmic NADPH-dependent phenol hydroxylase (EC 1.14.13.7). During the second step of phenol degradation, intra-diol cleavage of catechol to cis,cis- muconic acid occurs, being catalyzed by cytosolic catechol-1,2-dioxygenase (EC.1.13.11.1).

The aim of our study was to isolate and partially characterize the enzyme responsible for the second step of phenol biodegradation, catechol-1,2-dioxygenase.

C. tropicalis yeast Ct2 used in our laboratory was isolated from soil contaminated with aromatic hydrocarbons. The method of purification of catechol-1,2-dioxygenase consisted of preparation of cytosolic fraction from *C. tropicalis* yeast by centrifugation, chromatography and re-chromatography on a DEAE-Sepharose column (linear gradient of 0–0.3 M NaCl was used for separation of proteins), dialysis to remove the low-molecular particles followed by gel permeation chromatography. The enzyme activity was determined by HPLC or spectrophotometrically. The purity of enzymes was controlled with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The molecular mass of the enzyme was determined by SDS-PAGE and gel chromatography on Sephadex G-100 column.

pH optimum for the formation of cis,cis-muconic acid catalyzed by catechol-1,2-dioxygenase was determined to be 7.7 and the optimal temperature for this reaction was 30°C. Molecular mass determination suggests that catechol-1,2-dioxygenase is a dimer, whereas a molecular mass of a subunit is 30±5 kDa. The kinetic characteristics of the enzyme were also investigated.

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THE EFFECT OF FLAVONOIDS AND THEIR DERIVATIVES ON OXIDIZED SERCA FROM RABBIT SKELETAL MUSCLE

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Ca^{2+}-ATPase from sarco/endoplasmic reticulum (SERCA) belongs to important calcium regulating proteins because it maintains calcium homeostasis within cells. Intracellular calcium level plays a role in signalling function. Modulation of SERCA activity is of a great significance due to the ability of regulation of many cellular processes, such as modification of free radical formation or induction of apoptosis. Flavonoids owing to a wide range of various biological activities may be suitable candidates for SERCA activity modulation.

The main aim of the present work was to evaluate the effect of different flavonoids and their derivatives on SERCA activity after oxidation by biological inflammatory oxidants, hypochlorous acid (HOCl) and peroxinitrite ($ONOO^-$).

Ca^{2+}-ATPase activity was measured spectrophotometrically at 37°C using NADH-coupled enzyme pyruvate kinase/ lactate dehydrogenase assay. Peroxynitrite was synthesized by mixing $NaNO_2$, acidified H_2O_2 and NaOH at 4°C. The second oxidant, NaOCl, was used as a precursor of HOCl. Flavonoid derivatives were prepared by lipase-catalyzed esterification of flavonoids with fatty acids. SERCA was oxidized by HOCl (3 min) or $ONOO^-$ (30 s) after previous treatment with flavonoids (2 min) at 37°C.

The IC_{50} values of Ca^{2+}-ATPase activity decrease were found to be 50 and 150 μmol/l for HOCl and $ONOO^-$, respectively. Out of the tested phenolic compounds (rutin, quercetin, trolox) and its lipophilic derivatives (rutinpalmitate, rutinstearate, rutinoleate, and rutinlinolenate), only rutin and trolox had protective effect on SERCA in both oxidation models at concentration range (15–250 μmol/l). Quercetin protected SERCA from HOCl-induced oxidation (15–250 μmol/l), while no protection was observed in peroxynitrite system. The lipophilic rutin derivatives were found to have inhibitory effect on SERCA to different extent (from 15 to 75 μmol/l) when HOCl as oxidant was used. Rutinpalmitate protected SERCA from $ONOO^-$-induced inhibition at low concentrations (15–50 μmol/l), while enhanced inhibition at high concentrations (150–250 μmol/l). The other rutin derivatives had additional inhibiting effect on ATPase activity in $ONOO^-$-injured SERCA from concentration 100 μmol/l. The results suggest that flavonoids may be used for different therapeutic purposes depending on their structure. While unmodified flavonoids with protective effect on SERCA could be useful in anti-inflammatory protection, lipophilic derivatives may represent therapeutic agents for apoptosis induction in antitumor treatment due to its strong inhibitory properties on Ca^{2+}-ATPase activity.

THE COMPARISON OF NEW IMPEDANCE-BASED METHOD OF CELL PROLIFERATION WITH COMMONLY USED TECHNIQUES

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Precise determination of cell numbers is a crucial step in studies focused on regulation of cytokinetics and cell toxicity. The most utilized methods for determination of viable cell numbers are methods based on cell metabolism. Currently a new impedance-based system is available which was compared with the above mentioned methodological approaches.

Colorimetric assay based on reduction of tetrazolium salts (MTT) to dark blue formazan and chemiluminiscent assay based on whole cell lysate ATP determination were compared with a new impedance-based system allowing label free, dynamic monitoring of viable adherent cells in real time. Normal human epidermal keratinocytes and normal human dermal fibroblasts together with NIH/3T3 mouse fibroblasts were employed.

Impedance-based method was found to be applicable for determination of cell proliferation in all tested skin cells. Impedance-based method provided high-quality data about cell proliferation, the growth curves obtained in time periods of several days were significantly diverse between experimental groups utilizing different cell seeding densities. Comparison of this method with ATP and MTT determinations showed significant correlations.

The study shows, the new impedance-based determination of viable adherent cells is a valuable approach for cytokinetics and pharmacological studies.

DISRUPTION OF DEVELOPMENTAL SIGNALING PATHWAYS BY TOXIC ORGANIC POLLUTANTS AFFECTING LIVER CELLS

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Liver progenitor cells have been shown to play an important role in the liver development, regeneration and disease. However, relatively little is known about the impact of organic environmental toxicants on this specific liver cell population. Ligands of the aryl hydrocarbon receptor (AhR), such as dioxins or dioxin-like polychlorinated biphenyls (PCBs), have been shown to disrupt cell proliferation control in a model of liver progenitors – rat liver epithelial WB-F344 cell line. In the present study, we provide evidence that activation of AhR leads to alteration of expression of a number of genes participating in signaling pathways involved in regulation of liver development and function. PCB 126, a dioxin-like PCB congener, induced expression of follistatin (extracellular activin inhibitor), while simultaneously decreasing mRNA levels of activin A receptors type 1 and 1B. Activin A signaling is known to contribute to numerous pathologic liver conditions. Exposure to PCB 126 has further led to a significant increase of Necl-5 (PVR/TagE4) mRNA, a member of the nectin family of immunoglobulin (Ig)-like cell adhesion molecules. This protein is known to be cell density regulated and it might be involved in the observed disruption of contact inhibition. Necl-5 upregulation has been also shown to play a role in liver regeneration and acute liver injury. PCB 126 was also a potent inducer of expression of hairy and enhancer of split 1, a target of Notch signaling. We next observed a significant down-regulation of Met tyrosine kinase, which, as a hepatocyte growth factor receptor, plays a crucial role in the control of liver regeneration. Importantly, analysis of microarray data indicated that PCB 126 induced downregulation of a number of target genes of canonical Wnt/beta-catenin signaling. This pathway has been shown to play a crucial role in activation and expansion of liver progenitor cells and this indicated that AhR ligands may affect this process. Taken together, numerous signaling pathways involved in liver development and regeneration seem to be affected by AhR ligands and future studies should explore the functional significance of the observed changes in gene expression.

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NEUTROPHILIC DIFFERENTIATION MODULATES CYTOTOXIC EFFECT OF BUTYRATE IN HL-60 CELLS

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Retinoic acid (RA) is known to induce neutrophilic differentiation of promyelocytic leukemia cells. The molecular target of RA in promyelocytic leukemia is histone deacetylase (HDAC) and HDAC inhibitors show synergism when combined with RA to overcome the block in cell differentiation. Further, neutrophilic differentiation is accompanied by changes in the function of mitochondria. Because these organelles are involved in the regulation of cell death, we examined the cytotoxicity of butyrate, an HDAC inhibitor, in human promyelocytic leukemia HL-60 cells undergoing neutrophilic differentiation. Flow cytometric analysis of cells stained with a mitochondria-specific dye tetramethylrhodamine ethyl ester showed that in undifferentiated HL-60 cells butyrate induced a loss of the inner mitochondrial membrane potential. At the concentration of 2 and 5 mM and 24 h exposure, butyrate increased the proportion of cells with low mitochondrial potential from 6% (control) to 42% and 71%, respectively. At the same experimental conditions, 1 μM RA augmented the toxic effect of butyrate while 76% and 77% of cells with reduced mitochondrial potential was detected after treatment with 2 and 5 mM butyrate, respectively. On the other hand, in HL-60 cells differentiated for 3 days with 1 μM RA

and incubated for additional 24 h with RA and 2 or 5 mM butyrate, a dissipation of mitochondrial potential was found respectively only in 30% and 27% of cells. Moreover, as shown by annexin V/propidium iodide dual cell staining flow cytometry, treatment of undifferentiated HL-60 cells with butyrate and RA resulted in an appearance of both early apoptotic cells and late apoptotic/necrotic cells whereas in the differentiated cells the treatment elevated only the proportion of early apoptotic cells. Similar changes in the response of differentiating HL-60 cells were also found after treatment with camptothecin, a topoisomerase I inhibitor, and valinomycin, a potassium ionophore. We conclude that neutrophilic differentiation attenuates but not abrogates the effects of cytotoxic compounds in HL-60 cells.

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HISTOPATHOLOGICAL EFFECTS OF CADMIUM ON *COREGONUS LAVARETUS* L. LARVAE

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Cadmium is an important xenobiotic of the aquatic ecosystems. As other heavy metals it causes an abundance of effects on aquatic organisms. There is much information on lethality to fish, uptake, organ distribution and excretion of cadmium. In embryogenesis and larval period of fish toxic effects of metals can be observed using histological methods which allow for examining specific target organs.

The aim of this study was to evaluate the cadmium toxicity in larval period of endangered Coregonid species by utilizing histological biomarkers.

Larvae of lake whitefish *Coregonus lavaretus* L. aged 12 and 26 days after hatching were exposed to 100 µg Cd dm^{-3} for both 48h and 72h. Fishes were obtained from experimental rearing of Coregonid fishes of Faculty of Environmental Sciences and Fisheries in Olsztyn. Before tests larvae were fed on zooplankton. Static bioassay tests were carried out in small and aerated laboratory tanks supplied with de-chlorinated tap water at room temperature after 48h acclimation of experimental and control fish. Cadmium nitrite ($Cd(NO_3)_2 . 4 H_2O$) was chosen as a toxicant. The larvae were fixed in Bouin's solution. Fish were embedded in paraffin – wax sectioned at 5µm and stained with haematoxylin and eosin. The following organs were examined for histological lesions: skin, gills, thymus, pseudobranch, liver, kidney and intestine.

The skin lesions were observed in 12 days (younger) and 26 days (older) larvae especially in longer exposition to xenobiotic. In the gills and pseudobranch – respiratory related organ stronger lesions occurred in younger fish and after exposition for 72h. The thymus gland due to its exposition to branchial cavity suffered both in younger and older larvae. Mobilization of secretory cells in organs of branchial cavity took place after longer exposition to cadmium. Focal areas of necrosis, hyperemia and structural disruption in nuclei and cytoplasm of hepatocytes occurred in liver of exposed to cadmium larvae. Necrotic changes were observed in glomeruli and renal tubule too. Intestinal lesions were more evident in fish after longer exposition to intoxication.

However cadmium is mainly distributed to the kidney and to the liver of fishes all analyzed tissues of larval lake whitefish were vulnerable to this metal.

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INHIBITION OF DEXTROMETHORPHAN'S METABOLISM BY ARIPIPRAZOLE IN RATS

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Alterations in the activity of cytochrome P450 (CYP450) are often mentioned within the context of drug-drug interactions. Thus knowledge, whether drugs affect CYP450 mediated metabolism can be of high clinical importance. Antipsychotic aripiprazole is metabolized via CYP450. There is a strong probability that aripiprazole or other drugs co-administered and metabolized by CYP450 enzymes can on repeated administration elicit a changed efficacy with possible toxic effect occurrence.

The aim of our recent work was to determine the influence of aripiprazole on the activity of CYP450 2D2 and 3A4 measuring biotransformation of the specific marker dextromethorphan in rats.

Saline as a control or aripiprazole at the dose of 3 mg/kg/day were administered orally for 4 weeks. The model of isolated perfused rat liver was used for determination of CYP450 activity. Dextromethorphan (DEM) was added into the perfusion medium as a substrate of CYP450 and recirculating perfusion apparatus was used. Perfusion lasted for 2 hours and samples were collected after marker addition in the 30^{th}, 60^{th} and 120^{th} minutes. Levels of dextrorphan (DEX) and hydroxymorphinan (CYP2D2 specific metabolites) and methoxymorphinan (CYP3A4 specific metabolite) were analyzed in withdrawn samples by HPLC methods.

Aripiprazole pretreatment significantly decreased DEM levels only in the 30^{th} minute of perfusion. In samples withdrawn in the 60^{th} and 120^{th} min concentrations of DEM were similar to the controls. Metabolic conversion of DEM into DEX was significantly inhibited by aripiprazole. DEX measured levels were decreased by 37% to 48% during the whole perfusion in aripiprazole

treated group. This result to some extent correlated with a trend of lowered hydroxymorphinan levels after aripiprazole. Amounts of methoxymorphinan were not detectable in samples of aripiprazolee treated rats perhpas due to blockage of its formation via CYP3A4.

Our results confirmed, that aripiprazole inhibits the metabolism of dextromethorphan by both CYP2D2 and CYP3A4. This should be considered in prescription of aripriprazole and combined treatments with other drugs metabolized by these enzymes.

This project was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM 0021622404).

CYTOTOXICITY OF NEWLY SEPARATED NATURAL COMPOUNDS EVALUATED ON HUMAN CANCER CELL LINE

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The basis of each new therapeutically active molecule testing *in vivo* is the cytotoxic effect assessment on animal tissue. The cytotoxicity level determination allows evaluation of new compounds effects in biologically safe concentrations. The tissue culture methods represent the most convenient tool enabling assessment of wide range of tested compounds in various concentrations.

The aim of our work was to clarify the cytotoxicity of 15 newly separated natural compounds in increasing concentrations evaluated on human monocytic leukaemia cell line THP-1. This specification is useful for further characterization of tested substances *in vivo* and for non-toxic levels used in biological systems.

The tested compounds belong to the group of prenyl phenol type and non-prenylated phenolics: prenylated and geranylated compounds from Moraceae and Scrophulariace families (*Morus* spp. *Maclura pomifera, Paulownia tomentosa*), pterocarpans from *Pterocarpus rohrii* and *Schizandra chinensis* dibenzocyclooctadiene lignans. Extracts from these plants have been prepared previously in the laboratory Department of Natural Drugs. Cells were maintained in RPMI 1640 medium enriched with 10% of fetal bovine serum and 1% antibiotics mixture, and treated with tested compounds in the range of concentrations 1 μM–50 μM. Subsequently, the effect of newly separated substances on cell viability and kinetics parameters was evaluated.

Based on our results tested compounds were divided into the three categories, potentially having: 1. cytostatic effect, 2. cytotoxic effect, and 3. unspecified effect. First group substances were able to suppress cells growth without affecting cells viability in contrast with control group. Compounds from the second category showed a toxic effect on cells growth kinetic and viability in increasing concentration manner. In the third category natural substances exerted effects other than from two previous groups, e.g. improved cells growth and viability.

THE INFLUENCE OF *TRANS*-RESVERATROL ON THE INTERSEXUAL DIFFERENCES IN ACTIVITY OF CYTOCHROME P450 IN RATS.

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Differences in metabolism between males and females are known for a long time. The principle of such variation has not been clarified yet, but hormonal regulation of cytochrome P450 activity is supposed to be involved. Estrogenically active is t*rans*-resveratrol, a natural polyphenol with many protective biological effects including for instance protection of the brain from free radical attack and from beta-amyloid neurotoxicity, implicated in the present study.

The aims were to evaluate the role of sex on metabolic activity of cytochrome P450 2D2 in rats and focuse on *trans*-resveratrol influence on this isoenzyme separately in male and female rats.

Wistar albino rats of both sexes were treated with *trans*-resveratrol at the intraperitoenal dose of 5 mg/kg/day/10 days prior to liver isolation. Marker substance dextromethorphan (DEM) and its 2D2 specific metabolite dextrorphan (DEX) were measured during perfusion.

In our results the activity of 2D2 in male rats was higher than in females: levels of DEX in perfusion medium of control males were significantly increased in the 30th and 120th minute of perfusion. This fact corresponds with elevated DEM concentrations in females in the 120th minute. *Trans*-resveratrol significantly inhibits the activity of CYP2D2 in males: DEM levels were significantly higher than in controls during the whole perfusion and in addition DEX levels were decreased in the control males in the 120th minute. In females, resveratrol acted similarly on DEM levels, but without influencing DEX level. There were no changes in DEX levels in *trans*-resveratrol treated animals between males and females, while DEM levels were significantly increased during the whole perfusion in females.

In conclusion, the results confirmed gender differences in the metabolic activity of CYP450 2D2 with higher rate in rat males. *Trans*-resveratrol acted as an inhibitor, however again with greater impact in males than in females. This metabolic divergence found could be a cause for different sensitivity or even toxicity of drugs metabolized by the CYP450 2D2.

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TESTING OF TERATOGENIC EFFECT OF NEW SYNTHETIC DRUGS

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The aim of this work was to test a teratogenic effect of new synthetic drug 444 and 44Bu, which belong to ultra short acting beta blocker and distinct only by substitution in aliphatic molecular chain (n-butyl v. terc-butyl).

To test those substances was used FETAX test, that is carry out on embryos of the South African clawed frog *Xenopus laevis*. It is concerned in 96 hours test, which is used to evaluate the developmental toxicity of a test material. The embryos grow in a solution of testing substance in various strength. As a control are the embryos developed in standard FETAX solution. Every 24h are solutions changed and dead embryos despatched, to protect the multiplication of micro-organisms, which can negatively induce the live ones. During hole test should be sustained constant temperature and a 12-h day/12-h night cycle. At the end of the test live embryos are killed by CO_2 and fixated in formaline solution. In every embryo is evaluated his length and occasional malformation. The conclusions are statistically interpreted, there is calculated lethal concentration of testing material LC50 and concentration of a material, which cause growth inhibition.

Substances 444 and 44Bu were tested in strength 1, 10, 100, 300 and 500 mg/l. For 44Bu substance were ascertained lethal concentration LC50 12 mg/l. In the lowest testing material concentration 1 mg/l was not ascertained any statistically significant distinction in embryos length neighter against control nor was observed enhanced mortality of testing subjects. There was detected 100% appearance of malformation- in most cases it was about axial flexure of the tail and medium gut abnormalities. In concentration 10 mg/l was found out statistically very significant (p<0.01) growth inhibition against control and concentration 1 mg/l. There were uncovered malformations in every tested subjects, except axial flexure of the tail and gut abnormalities there was also observed head, thoracic and abdominal edema.

Material 444 recorded lower toxicity than 44Bu. LC50 was determined on 14 mg/l. Increased malformation incidence was not found in concentration 1 mg/l nor in concentration 10 mg/l. In concentration 10 mg/l was ascertained statistically very significant (p<0.01) growth inhibition in comparison with control and concentration 1 mg/l.

It seems to be, that even a minimal change in a chemical structure could have sense for biological effect of material, like its teratogenity.

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MODIFICATION OF ALTERNATIVE BIOTEST AT *ARTEMIA FRANCISCANA*

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To monitor ecotoxicologic and pharmaco-toxicologic effects it is necessary to use the highest number of individuals tested. Under the standard conditions you must extend the test period to the maximum time. Furthermore, the number of tested vertebrae must be reduced to the minimum.

The alternative generation II biotests at *Artemia franciscana* (previously called *A. salina*) comply with such demands. Five-day and ten-day biotests including the validation criteria are described in this paper. The capabilities of such tests are very wide. Additionally to the standard application in ecotoxicology, there is also a possibility of modelling pharmacologic effects.

In this test, the nauplii stages are placed into the disposable Petri dishes. Live nauplii are counted every 24 hours. The test validity is determined by the percentage of killed individuals in the control group. Both fresh and long-term stored biologically treated salt water was applied in this test. Water consists of the following chemicals with p.a. purity [$g.L^{-1}$] 23.9 NaCl; 10.83 $MgCl_2 \cdot 6H_2O$; 2.25 $CaCl_2 \cdot 6H_20$; 0.68 KCl; 9.06 $Na_2SO_4 \cdot 10\ H_2O$; 0.20 $NaHCO_3$; 0.04 $SrCl_2 \cdot 6H_2O$; 0.099 KBr; 0.027 H_3BO_3. This gives salinity of 49 $g.L^{-1}$ with a high purification capability at pH-value of 7.6±0.1. It is also possible to use much lower salinity of 9 $g.L^{-1}$ for applications in pharmaco-toxicology. A solution of glucose with a concentration of 3% in dish can be used as the alternative nutrition of the organisms tested. The volume of solution in the dish with all added and tested materials was always 5 ml or 10 ml.

The statistic evaluation of the significant evidence differences was performed according to the Wayland and Hayes test.

The individual test modifications have the different possibilities of applications. For substances with impaired dissolubility in salt water, salinity of 9 $g.L^{-1.}$ should be used. Low-toxic dissolvents are the other possibility of application. However, the prolongation of tests with the addition of glucose at low salinity is not possible.

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EXPOSURE TO 17B-ESTRADIOL AND TESTOSTERONE CAUSED INDUCTION OF VITELLOGENIN AND GONADAL IMPAIRMENT IN CHUB (*LEUCISCUS CEPHALUS* L.)

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A controlled laboratory study was carried out to quantify vitellogenin (VTG) concentrations in a common cyprinid freshwater fish, the chub (*Leuciscus cephalus* L.), exposed to steroid hormones. The effect of 17b-estradiol and testosterone was investigated on vitellogenin induction. Gonad status was also determined. Per oral exposure to estradiol and a testosterone-estradiol mixture increased ($p<0.01$) blood plasma concentrations of VTG, indicating that vitellogenic response in the chub is sensitive to steroid hormone exposure. The testosterone-estradiol mixture had a negative effect on the investigated chub gonads. The effects were signified by histological negative changes when compared to control fish.

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DETERMINATION OF PLANT EXTRACTS BIOLOGICAL ACTIVITIES

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Many natural compounds occurring in plant extracts offer a great potential in the fight against cancer through a range of mechanisms including antioxidant and antimutagenic activities, enzyme, cell cycle, gene expression and apoptosis modulation, as well as P-glycoprotein activation. Further understanding of the natural phytochemicals role and mechanism of action could lead to new prevention strategies for cancer and other degenerative diseases.

Research on the curative effects of herbal medicine has grown faster than any other form of alternative medicine. Crude extracts from plants belonging to the families Lamiaceae have been used in traditional medicine for alleviating many ailments. These extracts have been the subject of several studies due to their remarkable biological properties: antiinflammatory, antioxidative, antifungal, mitogenic and tumor inhibitory ones. The aim of this research was evaluation of antioxidant, antimutagenic, cytotoxic and apoptotic activities of extracts from *Salvia officinalis* L. and *Origanum vulgare* L. using tumor cell lines.

The potential antioxidant activity of extracts was proved by the 1,1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging assay. The antimutagenic effect of extracts against ethylmethane sulphonate was determined by the cellular and acellular comet assay. The cytotoxicity of extracts was measured by the cell growth inhibition assay using murine leukemia L1210 cell line and human promyelocytic HL-60 leukemia cells. Apoptosis-inducing effect was determined by fluorescence microscopy (chromatin condensation).

On the basis of results obtained we concluded that the extracts from *Salvia officinalis* L. and *Origanum vulgare* L. had a strong antioxidant and antimutagenic potential and exerted the antiproliferative and proapoptotic activities on leukemia cells.

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ANTIOXIDANT EFFECT OF SOME PLANT ETRACTS

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The use of plants in traditional medicine is still widespread because many of them represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. It is commonly accepted that, in a situation of oxidative stress, reactive oxygen species (ROS) such as superoxid, hydroxyl and peroxyl radicals are generated, and due to antioxidant acivities of medicinal plants can be eliminated.

The aim of this study was to compare biological activity of methanolic extracts of some plants belonging to the *Papaveraceae* and *Gentianaceae* families. These plants have been widely used in traditional medicine in many countries. Among them, the roots of *Gentiana scabra*, *Gentiana triflora* and *Gentiana rigescens* have been used as ingredients in Chinese herbal medicine for stimulation of appetite and gastric secretion, gastro-duodenal protection, liver protection, antifungal treatment, and in some cases for women´s diseases. It is known that *Gentianaceae* contain secoiridoid glucosides, flavone C-glucosides, and xanthones as well as their glucosides. It has been reported that gentiopicroside, a compound present in many Gentiana species, has free radical scavenging activity. *Papaveraceae* are herbal plants traditionally used as a cure for coughs and for the symptomatic treatment of various neurotic states in adults, particularly for minor sleep disorders. Their alkaloids are dopaminergic antagonists and

neuroleptids. For the *Papaveraceae* family is characteristic the presence of flavonoids and other phenolic components about which is known that they have the same activity as gentiopicroside.

We have determined antioxidant activity of methanolic extracts from parts of *Papaver rhoeas* (flower and stem) and *Gentiana asclepiadea* (flower and stem) by the DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay using ascorbic acid as a control. Besides, IC50 was defined, the dose-response effects of extracts tested were proved.

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