IMPORTANCE OF MOLECULAR CELL BIOLOGY INVESTIGATIONS IN HUMAN MEDICINE IN THE STORY OF THE HUTCHINSON-GILFORD PROGERIA SYNDROME

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Ranged among laminopathies, Hutchinson–Gilford progeria syndrome is a syndrome that involves premature aging, leading usually to death at the age between 10 to 14 years predominatly due to a myocardial infarction or a stroke. In the lecture I shall overview the importance of molecular cell biology investigations that led to the discovery of the basic mechanism standing behind this rare syndrome. The genetic basis in most cases is a mutation at the nucleotide position 1824 of the lamin A gene. At this position, cytosine is substituted for thymine so that a cryptic splice site within the precursor mRNA for lamin A is generated. This results in a production of abnormal lamin A, termed progerin, its presence in cells having a deleterious dominant effect. Depending on the cell type and tissue, progerin induces a pleiotropy of defects that vary in different tissues. The present endeavour how to challenge this terrible disease will be also mentioned.

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THE EFFECT OF UV-A RADIATION ON THE CYTOTOXICITY AND GENOTOXICITY OF CHEMICAL CARCINOGENS

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It is well established that long-wave UV radiation (UVA, 320–400 nm) is able to damage DNA, to cause mutations and induce skin cancer. UVA in contrast to UVB and UVC penetrates deeper into the skin and produces mainly ROS through interaction with endogenous and exogenous photosensitizers. Organic compounds with aromatic ring system such as aromatic hydrocarbons can absorb light in the UVA region. The excited state energy can be then transferred to molecular oxygen or other molecules in the cell to generate ROS, reactive intermediates, free radicals or photo-modified compounds resulting in genotoxicity. UV light-dependent photo-activation of organic compounds might therefore substantially contribute to the adverse health effects of air pollution.

The aim of this work was first, to standardize experimental conditions to study UV light-dependent photoactivation of chemical compounds *in vitro*; second, to investigate the ability of UVA light to activate various chemical carcinogens. Phototoxicity of 2-aminofluorene (2-AF), benzo[a]pyrene (BaP), 7H-dibenzo[c,g]carbazole (DBC), 5,9-dimetyldibenzo[c,g]carbazole (DiMeDBC), and *N*-metyldibenzo[c,g]carbazole (*N*-MeDBC) was studied in human keratinocytes HaCaT. HaCaT cells were exposed to particular carcinogens alone or the carcinogen-treated cells were irradiated with UVA light. Cell viability and DNA strand breakage were determined by MTT assay and by the single cell gel electrophoresis (SCGE), respectively.

Although UVA light (0.6 – 3.4 J/cm^2) did not change substantially survival of HaCaT a slight but significant increase in DNA strand breaks followed with substantial levels of oxidative DNA damage and micronuclei were detected in irradiated HaCaT cells. Irradiation of carcinogen-exposed cells with UVA light (2.4 J/cm^2) resulted in variable but significant reduction of cell viability compared with survival of cells treated with 2-AF, BaP, DBC, DiMeDBC or *N*-MeDBC alone. No DNA breakage was found in cells exposed to particular carconogens alone, however, UVA light increased significantly the DNA strand break levels in 2-AF-, BaP-, DBC- and DiMeDBC- but not in *N*-MeDBC-treated HaCaT cells. The values of combination factor confirmed the photoactivation of chemical carcinogens *via* UVA light.

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NEONATAL EXPOSURE TO POLYMERIC NANOPARTICLE PEG-b-PLA INTERFERES WITH PUBERTAL DEVELOPMENT IN FEMALE WISTAR RATS

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Intentionally engineered polymeric nanoparticles (PNp) present one of the most promising approaches for CNS drug delivery as they have potential to cross blood-brain barrier and they can exert direct actions upon the brain. On the basis of this fact, working hypothesis of our study was: "PNp could have a potential to interact with the processes of sexual brain differentiation and neurotransmission, factors of equal importance with the reproductive aspects".

The aim of the study was to evaluate the *in vivo* effects of PNp PEG-b-PLA [poly(ethylene glycol-*block*-polylactidmethyl ether] on the endpoints related to pubertal development and reproductive function after neonatal exposure in female rats: vaginal and eye opening, and estrous cyclicity and to compare these effects to a known xenoestrogen diethylstilbestrol (DES).

Newborn female Wistar rats were intraperitonealy injected daily with 20 and 40mg/kg b.w. of PEG-b-PLA, vehicle alone (deionized water) or 4 µg/kg b.w. of DES

from postnatal day 4 (PND) to PND 7. In comparison with the vehicle control group, two different doses of PEG-b-PLA significantly accelerated the onset of vaginal opening (VO) in a dose-dependent manner by 3 and 4.92 days. Positive control (DES) significantly accelerated the onset of puberty, VO was observed on PND 10.92 as compared to the control group (PND 32.85). Estrous cycles were monitored in two periods (3 and 5 months of age) during 21 days. Significantly reduced number of regular cycles was detected in the both PEG-b-PLA groups as well as in the positive control (DES) group during first monitoring. The adverse effects of neonatal exposure to low dose of PEG-b-PLA (20 mg/kg b.w.) and DES occurred also long after exposure ceased (second monitoring). Increased pituitary weight was observed in the both PEG-b-PLA groups, in the low dose group (20mg/kg b.w.) in significant extent. Additional *ex vivo* and *in vitro* experiments will support our working hypothesis.

TOXICITY OF TAXOL IN MAGNETIC NANOPARTICLES

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Nanoparticle research is currently an area of intense scientific research. One of the important area is the diagnosis and treatment of cancer. The toxicity and side effects of magnetic nanoparticles are still unknown. In medicine, nanoparticles make it possible to develop novel anticancer drugs. The concentration of drug inside the tumor cells in the targed area increased and therapy may be more effective.

The aim of these experiment was to evaluated toxicity of taxol loaded magnetic PLGA and PLGA-ALB in comparison with free taxol. PLGA and anticancer drug Taxol prepared by the nanoprecipitation method were used. Doses and toxicity of test items was determined after repeated i.v. application in male and female ICR mice by MTD method. Animals were observed individually after dosing periodically the first 6hours and daily thereafter of 14 days. Target organs were weighted. The tissue samples of brain, liver, kidneys, spleen and lung were processed for microscopic examination by standard paraffine technical and stained with hematoxylin and eosin and by Perls Gomori methods for the detection of Fe ions. Taxol loaded magnetic nanoparticles caused symptoms of toxicity at the dose of 10 ml/kg (magnetic PLGA-taxol) and 13.4 ml/kg (magnetic PLGA-ALB-taxol). Clinical changes were observed more frequently in mice treated with magnetic PLGA-ALB-taxol than in animals treated with magnetic PLGA-taxol. We do not observed clinical changes in mice treated with free taxol. A significant increase of the relative weights of spleen and liver was found in mice treated with magnetic PLGA-ALB-taxol and magnetic PLGA-taxol in compare with mice treated with free taxol and control animals. Relative weight of kidneys were increased in mice of all dose groups in comparison with control animals. Spleen was enlarged in mice of the both groups with magnetic nanoparticles. Histopathological evaluation showed diffuse iron accumulation in liver macrophages (Kupffer cells) and massive siderophagia in spleen in all mice treated magnetic PLGA-taxol and magnetic PLGA-ALB-taxol. Results indicated an increase of toxic effects of taxol loaded magnetic nanoparticles in compare with free taxol. Magnetic PLGA-ALB-taxol was more toxic than magnetic PLGA-taxol. Diffuse occurrence of iron was still in liver and spleen in the both "magnetic" groups 14 days after the last application.

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STUDY ON APOPTOTIC EFFECTS OF NEUROTOXIN–ANATOXIN-A ON FISH IMMUNE CELLS

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Anatoxin-a (ANTX-a) is a naturally occurring homotropane alkaloid isolated from toxic stains of freshwater cyanobacteria including *Anabaena flos aquae, Anabaena planktonia*, *Aphanizomenon flos-aquae*, *Cylindrospermum pp.* and *Microcystis* species. It is the cyanotoxin which attracted a lot of attention because of its specificity and potency as an agonist at nicotinic acetylcholine receptors (nAChR). Because ANTX-a is not degraded by cellular enzymes, severe overstimulation of respiratory muscles may result in respiratory arrest and death by respiratory arrest Poisoning episodes caused by this toxin, of wild and domestic animals in North America and in Europe were found in recent years.

The aim of this study was to assess the possible *in vitro* apoptotic effects of ANTX-a on the selected immune cells isolated from the blood of carp. In the experiments pure anatoxin-a was used at concentrations of 0.01, 0.025, 0.05 0.1 and 1 μg/ml RPMI-1640 medium. Apoptosis or necrosis of fish leukocytes (lymphocytes and phagocytes) induced by the toxin was determined by measurement of the activities of caspase-3/7 and the analysis of phosphatidylserine on the outer leaflet of apoptotic cell-membranes using Annexin-V-Fluorescein and Proidium Iodid (PI). Moreover, fluorescent measurement of the release of lactate dehydrogenase (LDH) from cells with a damaged membrane was done.

This study showed that anatoxin-a was cytotoxic and induced apoptotsis and necrosis of immue fish cells depending on used concentrations of this neurotoxin.

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UTILITY OF MOTHERS' HAIR SEGMENTAL ANALYSIS IN EVALUATION OF FETAL EXPOSURE TO BENZODIAZEPINES

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Benzodiazepines as anxiolytics and sedatives are commonly used in pharmacotherapy of major stress disorders. Due to their properties they are also prescribed to the pregnant women in anxiety, insomnia and epilepsy management. American Food and Drug Administration includes these medicines into groups of high risk for the developing fetus, because they permeate the placenta and may have a teratogenic effect. Increased serum concentrations of benzodiazepines can cause oral clefts, muscle weakness, oversedation and sucking difficulties for the newborns. Moreover, their antepartum application can cause breathing difficulties. Exposure to the drug in certain stage of pregnancy is one of the most important factors influencing safety of the treatment. This issue indicated a problem of obtaining plausible data referring to time – drug concentration relationship. The most prevalent biological samples do not provide sufficient information about the history of fetal exposure to xenobiotics. The aim of this study was to develop and evaluate a useful tool for benzodiazepine concentrations assessment in the course of pregnancy. Achieving this goal was possible by means of segmental hair analysis. Pregnant women from the Gynecological-Obstetrical Clinical Hospital in Poznan, who took benzodiazepines during pregnancy were qualified to the experimental group. The women filled in questionnaires which described their medical history. Afterwards hair samples were collected from 50 women with singleton pregnancy and 50 mothers of twins and their respective newborns. In the preliminary study 30 samples collected from the mothers and 30 samples from single newborns were evaluated. Prior to the assay hair samples were decontaminated, segmented (only mothers' hair) and the liquid-liquid extraction was performed. Concentration of diazepam and its metabolite – nordiazepam was determined by means of LC/MS with APCI. The preliminary outcomes show that both substances are incorporated in hair matrix. This fact proves the utility of hair as a biomarker of benzodiazepine exposure. Due to drugs' physiochemical properties they are also present in children's hair – and thus can be used as a unique biomarker of prenatal exposure. It became possible to obtain precise information about the time of exposure by dividing hair shafts into segments, corresponding to three trimesters of pregnancy. The LC/MS – APCI method was very sensitive – concentrations of benzodiazepines at the level of picogram per milligram of hair were determined.

EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF NEUROTOXIN ANATOXIN-A

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Anatoxin-a belongs to cyanotoxins of direct neurotoxic activity. The toxin is a potent pre- and postsynaptic neurotoxin acting as nicotinic agonist binding to the nicotinic acetylocholine receptors (nAChRs). It is an alkaloid of low molecular weight (165 Da) produced mainly by *Anabaena flos-aquae* and *Anabaena planktonia*, but also by *Aphanizomenon, Cylindrospermum, Microcystis* species and benthic *Oscillatoria.* Its final result in acute exposure consists of respiratory arresting and finally the death. In mice the LD50 for anatoxin-a in i.p. application is 200–375 μg/kg bw. While neurotoxic mechanism of its activity is quite well known, there is little or no data on the possible cytotoxic or genotoxic effects of the toxin.

The aim of the study was to evaluate the cytotoxic and genotoxic effects of anatoxin-a (anatoxin-a fumarate, Tocris) at different concentrations after *in vitro* cell exposure. Cytotoxicity towards *Cyprinus carpio* L. erythrocytes was assessed by propidium iodide bounding to DNA after the end of cell exposure, measurement of glutathione (GSH) level and lactate dehydrogenase (LDH) leakage into the culture medium.

Genotoxic effects of anatoxin-a were assessed by umuC test on *Salmonella typhimurium* TA 1535 (pSK1002) and by micronucleus test on carp erythrocytes.

The obtained results at least partially complete the currently existing gap in the data on possible anatoxin-a activity.

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A REVIEW: METALS AS A CAUSE OF OXIDATIVE STRESS IN FISH

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Metals are significant environmental pollutants. They have positive role for organisms as components of transport biomolecules as ceruloplasmin, haemoglobin, transferrin, reserve molecules as ferritin, and enzymes as superoxide dismutase. The negative attribute of metals is ability to act as catalysts in the oxidative damages of biological macromolecules.

Oxidative properties have especially transition metals. The transition metals are the subgroups of elements intervening between groups IIA(2) and IIIA(13) in the periodic table (*e.g.* Fe, Cu, Hg, Cr).

The presence of biomarkers of oxidative stress (antioxidative enzymes, lipid peroxidation´s products etc.) in fish may be helpful in assessing the metal contaminantion in the environment. The analysis fish meat for human nutrition is important for health of people because oxidative stress are often associated with many diseases (*e.g.* Alzheimer´s disease, cancer).

This review summarizes generally knowledges of oxidative actuation of metals and possibilities of fish organism to protect against impacts oxidative pressure.

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CARDIOVASCULAR CHANGES INDUCED BY HIGH-CHOLESTEROL DIET IN HEREDITARY HYPERTRIGLYCERIDEMIC RATS – EFFECT OF SME1EC2

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Elevated plasma lipids belong to the major risk factors for atherosclerosis and coronary heart disease. Experimental model using the Prague hereditary hypertriglyceridemic rats (hHTG) enables to study separately the consequences of metabolic and hemodynamic abnormalities. The aim of this study was to investigate changes in the function of the heart and vessels induced by high cholesterol diet in hHTG rats. Possible beneficial effect of a novel pyridoindole antioxidant – SMe1EC2 was compared to the effect of atorvastatin (ATO), a competitive inhibitor of acyl-CoA cholesterol acyltransferase. Male hHTG rats were fed high cholesterol diet (1% cholesterol + 7.5% lard) for 6 weeks. fSMe1EC2 - 30 mg/kg b.w. and atorvastatin - 50 mg/kg b.w. were administrated *p.o.* Experimental groups: Wistar rats (W), hHTG rats (hHTG), hHTG rats fed high cholesterol diet (CHOL), hHTG treated with SMe1EC2 (S-hHTG), CHOL treated with SMe1EC2 (S-CHOL), CHOL treated with ATO (A-CHOL). After 6 weeks of the treatment, blood pressure (BP) values shown a tendency to increase in the order W< hHTG < CHOL groups. The treated rats had lower BP than CHOL group. The following changes in ECG of CHOL rats were observed: values of PQ and QTc interval were significantly prolonged and SMe1EC2 and ATO improved ECG findings. In the isolated heart according to Langendorff, electrical stimulation treshold decreased significantly in CHOL rats and both treatments returned it to or under the control values. Beneficial effect of SMe1EC2 on evoked ventricular fibrillation was observed. Endothelium-dependent relaxation to acetylcholine of the aortas from HTG rats was decreased compared to W rats. Treatment of hHTG rats reversed relaxation to W rats values.

In conclusions, SMe1EC2 was found to improve functional state of cardiovascular system of hHTG rats treated with high-cholesterol diet.

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THE INTERACTION OF OXIME REACTIVATORS WITH THE NICOTINIC RECEPTORS

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Organophosphate poisoning has been currently treated by the administration of anticholinergic drugs and acetylcholinesterase oxime reactivators. Oximes may interact – except their reactivating effect on cholinesterases – directly with cholinoreceptors. In this study we investigated the effect of various oxime reactivators on the neuromuscular endplate transmission. The results suggest that oxime reativators exert antinicotinic activity, that was demonstrated by the inhibition of muscles twitches during the electrical stimulation of the muscle.

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ECOTOXICOLOGICAL VALUATION OF CHEMICALS ON DUCWEED (*LEMNA MINOR*) USING MICROBIOTESTS

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Due to the enormous number of potentially polluting substances contained in waste waters from municipal and environmental sources, here grows a necessity of providing the information about water quality. Duckweed (*Lemna minor*) is frequently used in series of quality studies to monitor heavy metals and other aquatic contaminants. Ecotoxicology, like many other disciplines follows the trend of miniaturization to satisfy growing demands but also to prevent total environmental burden. The organisms commonly employed in microbiotests are bacteria, protozoa, invertebrates, fish and tissue cultures etc. But standard microbiotest using duckweed as a test organism has not been created yet.

For this purpose we modified EN ISO 20079. We applied polystyrene macroplates with the advantage of requiring lower volumes for the test (10 ml). This macroplates consist of six dimples of maximum volume 15 ml with flattened bottom and with the cover.

The aim of our work was to validate a miniaturized duckweed test using macrotitration plates and compare the values of acute toxicity (168 h EC_{50}) obtained from conventional testing (100 ml) and from microbiotests (10 ml) using reference toxicants: potassium chloride and 3,5-dichlorphenol.

The resulting values 168hEC_{50} for potassium chloride using microbiotest was 8.54 g/l and for 3,5-dichlorphenol it was 4.36 mg/l. The resulting values from conventional tests were 9.78 g/l and 5.71 mg/l, respectively. In our microbiotest we also carried out the validity requirements. Thanks to good correlation between results from conventional tests and microbiotests it is evident that duckweed microbiotests for assessing toxic effect

of chemicals or other hazardous substances are a valid alternative to commonly used ecotoxicological biotests. Using microvolumes can be a good tool to include in a battery of tests for phytotoxicity screening of a wide range of chemicals and environmental samples. The advantages are allowing large numbers of samples to be tested, and generating low volumes of waste.

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EMBRYONIC STEM CELL TEST (EST): DEVELOPMENT, VALIDATION AND IMPLEMENTATION INTO TOXICITY TESTING

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Testing for reproductive and developmental toxicity of drugs and other chemical compounds *in vitro* is an attractive alternative procedure to time-consuming and expensive *in vivo* or *ex vivo* experiments. The mouse embryonic stem cell test (mEST) is the most advanced and promising of *in vitro* embryotoxicity test. Although it has not yet been accepted for regulatory purposes, its use in preclinical drug development is well established. The embryonic stem cell test EST employs the blastocyst-derived embryonic stem cell line D3 from mice which spontaneously differentiate into contracting cardiomyocytes in cell aggregates, termed embryoid bodies (EBs). This morphological feature is used as an endpoint for differentiation together with the measurement of cytotoxicity on D3 cells and 3T3 fibroblasts. In an international validation study funded by the European Center for the Validation of Alternative Methods (ECVAM), this method proved reliable for the prediction of embryotoxicity *in vivo* [1,2]. For a set of 20 reference compounds with different embryotoxic potencies of non-embryotoxic, weakly embryotoxic, and strongly embryotoxic, the EST provided correct classification in 78% of all cases. Remarkably, a predicitvity of 100% was obtained for strong embryotoxicants.

In recent years, the EST has been evaluated by the pharmaceutical industry for testing during research and development, and considerable efforts have been made to improve the EST for this purpose. Moreover, promising molecular endpoints have been established in the mEST, including proteomic and toxico-genomic endpoints. Conversion of the molecular based EST to an automated platform is a near future goal for the field as it does have the greatest potential among *in vitro* embryotoxicity test currently evaluated for high throughput testing.

To date only preliminary results have been obtained with a human EST (hEST). Gabriela G. Cezar and co-workers at the U. of Wisconsin (USA) recently developed a more predictive developmental toxicity model based on an *in vitro* method that utilizes both human embryonic stem (hES) cells and metabolomics to discover biomarkers of developmental toxicity. In this test hES cells were dosed with several drugs of known teratogenicity then LC–MS analysis was performed to measure changes in abundance levels of small molecules in response to drug dosing. Statistical analysis was employed to select for specific mass features that can provide a prediction of the developmental toxicity of a substance. These molecules can serve as biomarkers of developmental toxicity, leading to better prediction of teratogenicity. In particular, our work shows a correlation between teratogenicity and changes of greater than 10% in the ratio of arginine to asymmetric dimethylarginine levels. In addition, this study resulted in the establishment of a predictive model based on the most informative mass features. This model was subsequently tested for its predictive accuracy in two blinded studies using eight drugs of known teratogenicity, where it correctly predicted the teratogenicity for seven of the eight drugs. Thus, the initial data obtained with the new hEST show that this platform is a robust alternative to animal and other *in vitro* models for the prediction of the developmental toxicity of chemicals that may also provide invaluable information about the underlying biochemical pathways.

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CYTOTOXICITY AND GENOTOXICITY OF 7H-DIBENZO[C,G]CARBAZOLE AND ITS TISSUE SPECIFIC DERIVATEVES AFTER BIOTRANSFORMATION WITH HUMAN CYTOCHROME P4503A4 ACTIVATION OF CHEMICAL CARCINOGENS VIA CYP 3A4

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7H-Dibenzo[c,g]carbazole (DBC), a ubiquitous environmental pollutant, is found in a variety of complex mixtures derived from incomplete combustion of organic matter as cigarette smoke, soot, tars, and diesel engine exhaust. DBC, a Group 2B (possible human) carcinogen, is a potent multi-site carcinogen in experimental animals, with both local and systemic effects Recent studies have convincingly demonstrated that DBC is activated by human cytochrome P450 (CYP) 1A family enzymes producing phenols similarly as is the case of mouse and rat metabolism. While CYP1A1 plays an important role in biotransformation of DBC and its sarcomagenic derivatives, CYP1A2-mediated activation is implicated rather in detoxification and exclusion of metabolites. The role of other cytochrome P450 enzymes in DBC metabolism is, however, unknown. Although the extent of human exposure to DBC is unexplored, presence in cigarette smoke and other organic complex mixtures makes DBC an important environmental carcinogen.

Our study is focused on the role of the human CYP3A4, the most abundant P450 enzyme in the human liver, in biotransformation of *7H*-dibenzo[c,g] carbazole (DBC). To better understand the impact of

this enzyme in DBC genotoxicity, two DBC derivatives, 5,9-dimethyldibenzo[c,g]carbazole (DiMeDBC) and *N*-methyldibenzo[c,g]carbazole (N-MeDBC) with specific tropism for the liver and skin, respectively, and the Chinese hamster V79MZh3A4 cell line stably expressing human cytochrome P4503A4 enzyme were used. Lower IC50 values were determined for the liver carcinogens DBC and DiMeDBC (23.3 μM and 7.4 μM, respectively) as compared with *N*-MeDBC, a tissue specific sarcomagen, (>200 μM). Accordingly, a substantial level of micronuclei and slight but significant (p<0.05) number of gene mutations was found in DBC-treated cells. Although DiMeDBC increased significantly the level of micronuclei, no 6-TGr mutations were found in V79MZh3A4 cells. *N*-MeDBC did not produce any micronuclei and only marginal level of gene mutations in these cells. In addition, very low DNA-adduct level was detected in DBC- and *N*-MeDBC- but not in diMeDBC-treated cells. Based on these data we suppose that the human CYP3A4 enzyme is implicated in DBC metabolism. Differences in the cytotoxic and genotoxic effects between diMeDBC and N-MeDBC indicate that either distinct metabolites or at least quantity of intermediates are formed due to CYP3A4 activation.

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ANTIOXIDANT DEFENCE IN RELATION TO OCCUPATIONAL EXPOSURE TO GLASS FIBRES

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Antioxidant vitamins, being effective free radical scavengers, can protect cellular DNA from oxidative damage. Molecular epidemiological study was conducted in a glass fibre factory in Slovak Republic. Altogether 116 subjects were investigated, 36 controls (18 men, 18 women, 22 non-smokers, 14 smokers) and 80 occupationally exposed to glass fibre (39 men, 41 women, 47 non-smokers, 33 smokers). Indicative parameters of lipoperoxidation, DNA damage, and changes in the status of antioxidant defense systems were evaluated in blood samples of monitored subjects.

Activities of antioxidant enzymes in erythrocytes: glutathione peroxidase (GPX), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT); in plasma: levels of vitamin C, vitamin E, carotenoids, ceruloplasmin oxidase activity (CPL), levels of malondialdehyde (MDA); DNA repair rate in the extracts of lymphocytes were measured.

Exposure to glass fibres did not cause any significant changes in the activity of GPX and in levels of vitamins C and E. The activities of CAT was suppressed in the group of exposed men (p=0.028). Exposure caused a decrease in the activity of GST in men (p=0.019) and an increase in women (p=0.038). Levels of MDA in plasma were lower in exposed men compared to the control subjects (p=0.008). DNA repair capacities did not show any differences between exposed/control, men/women or smokers/non-smokers.

There was a significant negative correlation between the activity of GPX and MDA and between levels of vitamin C and MDA in several of groups. CAT activity and SOD correlated positively with MDA. We found significant positive associations between DNA repair and GST, DNA repair and SOD as well as between DNA repair and CAT almost in all subgroups. The detected changes in antioxidant parameters could happen to prevent the increase in lipid peroxidation We observed an interesting parallel between antioxidants and DNA repair capacity. Cells located in highly oxidizing microenvironments appear to have more efficient oxidative defence and repair mechanisms.

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ROLE OF HUMAN ENZYMES IN ACTIVATION AND DETOXICATION OF HUMAN CARCINOGEN AND NEPHROTOXIN ARISTOLOCHIC ACID

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Aristolochic acid (AA) causes the development of aristolochic acid nephropathy and Balkan endemic nephropathy associated with malignant transformation of the urothelium.

Understanding which human enzymes are involved in AA activation and/or detoxication is important in the assessment of an individual's susceptibility to this carcinogen. We identified the major hepatic and renal enzymes responsible for AA-DNA adduct formation in humans and model experimental animals. The phase I enzymes play a crucial role in AA metabolic activation, while a role of the phase II enzymes in this process is questionable. Most of the activation of AA in human hepatic microsomes is mediated by CYP1A2 and/or 1A1, while NADPH:CYP reductase plays a minor role. In human renal microsomes NADPH:CYP reductase is more effective in AA activation. In addition, cyclooxygenase (COX) is another enzyme activating AA in kidney. NAD(P)H:quinone oxidoreductase (NQO1) is the most efficient in the activation of AA in human hepatic and renal cytosols, although a role of cytosolic xanthine oxidase cannot be ruled out. With purified enzymes, the role of all these enzymes in AA activation was confirmed. The orientation of AA in the active sites of human CYP1A1, 1A2 and NQO1 was predicted from molecular modeling and explains the strong reductive potential of these enzymes for AA detected experimentally.

Using several mouse strains, in which both NADPH:CYP reductase (HRN [Hepatic Cytochrome

P450 (CYP) Reductase Null] and CYP1A1 or 1A2 were deleted, we investigated AAI detoxication *in vivo* and *in vitro*. The results demonstrate a major role of CYPs in AAI detoxication in mouse *in vivo* and that of CYP1A1/2 in this reaction in humans and mice *in vitro*. In addition, they also indicate that efficiency of human CYP1A1 and 1A2 are higher than that of their mouse orthologoues both *in vivo* and *in vitro*.

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HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF RAINBOW TROUT BLOOD FOLLOWING REGULATED NITRITE EXPOSURE

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The presence of nitrite in the environment is potential problem due to its toxicity to animals. Increased nitrite concentrations often caused problems in waters with intensive fish husbandry, namely in recirculation systems. Fish are at higher risk of nitrite intoxication than terrestrial animals, since nitrite in the ambient water can be actively taken up across the gill epithelium and can accumulate to very high concentrations in the body fluids where causes several changes.

The aim of this study was to evaluate the favorable effects of chloride during the regenaration period after the nitrite poisoning of rainbow trout by using of haematological and biochemical indices. During exposure period (14 days), two year old fish were exposed to elevated nitrite concentrations (1 mg/l NO_2^-) really in waters with intensive fish husbandry occurring. Exposure period was followed by regeneration period, which was taking next 7 days and was proceed in different water conditions such as: high chloride concentration with current elevated nitrite concentration, high chloride concentration without nitrite addition and nitrite free water without chloride addition.

The prolonged exposure to nitrites in real concentration occurring in recirculation systems (1 mg/l) had effect on biochemical and haematological profile. The recover ability of fish was already observed second day of regeneration period.

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EFFECTS OF STOBADINE ON t-BHP INDUCED OXIDATIVE INJURY OF ISOLATED RAT HEPATOCYTES

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The role of oxidative stress, including uncontrolled reactive oxygen species (ROS) generation, has been widely documented not only under many pathophysiological conditions, such as neurodegenerative diseases, cancer, diabetes, cardiovascular and respiratory diseases, but also 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 within cells and tissues. The purpose of this study was to investigate the effects of the pyridoindole derivative stobadine on t-BHP induced oxidative injury of isolated rat hepatocytes.

The biological system of primary hepatocytes isolated from male Wistar rats was exposed for 1 h to an increasing concentration of t-BHP. Lactate dehydrogenase leakage (LDH) and thiobarbituric acid reactive substances (TBARs) formation were determined as biomarkers of oxidative stress injury of hepatocytes. Double sequential staining with acridine orange and ethidium bromide allowed to discriminate of cells dying either by necrosis or apoptosis and living cells.

Under severe conditions of oxidative stress, there was a large excess of ROS, thus cells died from necrosis (1.0 mM resp. 2.0 mM of t-BHP). Whereas, under mild conditions (0.5 mM of t-BHP) the level of ROS caused cell death mainly by apoptosis. The results showed that pretreatment of hepatocytes with stobadine five min. prior to administration of t-BHP significantly decreased LDH-leakage and TBARs formation. These protective effects of stobadine against t-BHP induced oxidative injury were concentration-dependent.

In conclusion, stobadine seems to be a promising agent for further studies relevant to ROS-induced functional and structural impairments within cells and tissues. The biological model of isolated rat hepatocytes corresponds with possible pathophysiological consequences of different diseases, and might thus serve for prescreening testing of toxic effects of novel substances.

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COMPARISON OF CYTOTOXICITY AND GENOTOXICITY OF SELECTED ANTIDOTES AGAINTS NERVA AGENTS

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The treatment of intoxication with acetylcholinesterase – inhibiting organophosphates consits of reactivators, anticholinergics and anticovulsants. These drugs can be used both as profylactic treatment and as a first aid.

The best reactivator is an oxime HI-6 chloride and is established in the Czech army to the profylaxis and to the first aid. That´s why we choosed it in our study like an initial substance for interactions with other drugs established in the Czech army againts this intoxication. Selected medicaments have been: Pyridostigmine

bromide, Benactyzine chloride, Trihexyphenidyl chloride; Atropine sulphate, Obidoxime chloride; Diazepam; Methoxime chloride.

We have compared the cytotoxicity of HI-6 oxime for different human and rodent cell lines using the colony forming inhibition assay and cell viability assay – the neutral red staining. The genotoxicity of HI-6 oxime was followed by comet assay in combination with endonuclease III, cytosine arabinoside and hydroxyurea.

We have found no significant differences in the cytotoxicity of HI-6 oxime for human A549 or URO cells, subline of CHO cells AA8 and their mutant UV20 (LC50 berween 1.5–2 μg/ml). On the other hand, Hela cells and mouse L929 cells showed slightly higher resistance towards HI-6 oxime (LC50 4.5–5.5 respectively). Similar results were obtained by neutral red assay. In agreement with the cytotoxicity measurements, nearly no DNA breaks were induced in A549 during 24 h of incubation with HI-6 oxime. Quite high number of DNA breaks (up to 60% DNA in tail of comets) was found in L 929 cells, human fibroblasts and URO cells. Nevertheless, the induction of DNA breaks appeared only in cultures incubated in the concentration of HI-6 oxime as high as 1–10 mM.

Because the DNA degradation appeared after 2 hours of incubation already, it may be result of cell necrosis, rather then apoptosis. However, because the neutral red assay did not show any cytotoxicity after 2h of incubation, the possible slight genotoxic effect of HI-6 can not be excluded. Further studies are necessary to prove this possibility.

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METABOLISM OF 2-NITROPHENOL AND *N*-(2-METHOXYPHENYL)-HYDROXYLAMINE, HUMAN METABOLITES OF THE ENVIRONMENTAL POLLUTANTS AND CARCINOGENS *O*-NITROANISOLE AND *O*-ANISIDINE

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2-Nitrophenol (2-NP) is the major detoxification metabolite of an industrial pollutant and a potent carcinogen, *o*-nitroanisole. We characterized the product of 2-NP metabolism catalyzed by human, rat, rabbit and mouse hepatic microsomes containing cytochromes P450 (CYPs) and identified major human CYP enzymes participating in this process. The 2-NP metabolite was characterized by mass spectrometry and co-chromatography on HPLC with a synthetic standard, 2,5-dihydroxynitrobenzene (2,5-DNB) to be 2,5-DNB. No nitroreductive metabolism leading to the formation of *N*-(2-hydroxyphenyl)hydroxylamine or *o*-aminophenol was evident by tested microsomes. Likewise, no DNA binding of 2-NP metabolite(s) measured with the ^{32}P-postlabeling was detectable in hepatic microsomes. Hepatic microsomal CYP enzymes participate in 2-NP metabolism that does not lead to its activation to species binding to DNA. Most of 2-NP oxidation in human liver is attributed to CYP2E1, 3A4, 2A6, 2C and 2D6. Recombinant human CYP2E1, 2A6 and 2B6 were the most effective enzymes oxidizing 2-NP.

N-(2-methoxyphenyl)hydroxylamine is a human metabolite of 2-nitroanisole as well as of another environmental pollutant and carcinogen, *o*-anisidine. We investigated the ability of human, rat and rabbit hepatic microsomes to metabolize this compound. *N*-(2-methoxyphenyl)hydroxylamine is metabolized by microsomes of both species to *o*-aminophenol, *o*-anisidine and two additional minor metabolites, whose exact structures have not been identified as yet. Participation of microsomal enzymes in these reactions was also studied. During incubation of *N*-(2-methoxyphenyl) hydroxylamine with DNA or deoxyguanosine, three adducts measured with the ^{32}P-postlabeling and HPLC, similar to those found to be formed by 2-NA and *o*-anisidine in DNA, were generated. This demonstrates that *N*-(2-methoxyphenyl)hydroxylamine is an activation metabolite responsible for 2-NA and *o*-anisidine carcinogenicity. On the contrary, no DNA adduct were formed by *o*-aminophenol reacted with hepatic microsomes or peroxidases, suggesting its predominant role in *o*-anisidine detoxication.

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HAEMOSTATIC PARAMETERS IN FALLOW DEER (*DAMA DAMA*) AND THEIR IMPORTANCE IN THE DIAGNOSTICS OF DISEASES AND ANTICOAGULANT RODENTICIDES' POISONING

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Frequent reports of wild animals poisoned with anticoagulants emerge every year throughout the world. Especially solid baits in granulated form can result in primary or secondary poisoning of non-target organisms such as small rodents, birds and ruminants. Moreover, there are also infectious diseases which can influence the haemostasis in wild animals.

This study focused on fallow deer (*Dama dama*) which can be affected by anticoagulant rodenticides and in which, *e.g.*, pasteurellosis can lead to haemorrhagic septicaemia. Insufficient data on physiological values of coagulation parameters make then hard to explain the mechanism, role and consequences of changed haemostasis in this species.

In the presented study several parameters of blood clotting were established in clinically normal fallow

deer from a game enclosure in North Moravia (Czech Republic) caught for the planned transportation to a new location. Fibrinogen content measured corresponds with the results obtained by other authors. Thrombin time was assessed as 24.78 ± 3.20 s, which is a completely new information in fallow deer. Activated partial thromboplastin time was 33.76 ± 5.95 s. Prothrombin time with 20.99 ±2.70 s is comparable to data available for mouflon or reindeer, but higher than results obtained by other authors for fallow deer (12.9–16.3 s). The possible explanation for the increased prothrombin time results could be the stress during yarding and handling the animals, which has been reported to be a possible factor causing haemorrhages in deer.

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CACO-2 CELL MONOLAYER INTEGRITY AND EFFECT OF PROBIOTIC ESCHERICHIA COLI NISSLE COMPONENTS

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Different probiotic strains used in clinical trials have shown prophylactic properties in different inflammatory diseSlovak Academy of Scienceses of the gastrointestinal tract. To analyse the interaction of probiotics with transepithelial transport of xenobiotics, this study was aimed to investigate the Escherichia coli strain Nissle 1917 (EcN) components on the integrity of the Caco-2 cell monolayer. The effect of isolated lipopolysaccharide (LPS), a glycolipid of the cell wall of Gram-negative bacteria and supernatant of EcN suspension in a concentration of 0.001, 0.1, 1, 50, 100, 1 000 μg/ml on transport of paracellular marker (^{14}C mannitol) was estimated. Both LPS and supernatant exerted almost the same effect; no effect was shown after high concentrations, decreasing concentrations lowered permeability of ^{14}C mannitol, and the lowest concentration (0.001 μg/ml) significantly decreased monolayer permeability approximately about 20% (LPS) and 30% (supernatant). No changes in P_{app} of ^{14}C mannitol after 2-hour preincubation of the Caco-2 monolayer with LPS in a concentration of 100 μg/ml indicated no disruption of tight junctions of the Caco-2 cells.

To elucidate the observed decreased permeability of the monolayer ("tighter monolayer") induced by lower concentrations of LPS or supernatant, the effect of Ca^{2+}-free transport medium (opening tight junctions) and of medium containing 5, 10, 20, 50, and 100% of Ca^{2+} on the ^{14}C mannitol transport alone and in the presence of low (0.1 μg/ml) and high (100 μg/ml) concentration of LPS was studied. Using Ca^{2+}-free medium, both concentrations of LPS significantly decreased P_{app} of ^{14}C mannitol which indicated that above mentioned changes of ^{14}C mannitol permeability are independent of dimensions of paracellular spaces. These results are in an agreement with hypothesis that probiotics are able to induce restoration of a disrupted epithelial barrier.

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EXTRAPOLATION OF TOXIC INDICES AMONG TEST OBJECTS

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Tubifex tubifex is tubificid segmented worms that inhibits sediments of lakes and rivers, *Tetrahymena pyriformis* is a teardrop-shaped unicellular ciliated protozoan living in aquatic environment, hepatocytes are hepatic cells isolated from rats liver and are responsible for majority of metabolic processes in liver, fathead minow (*Pimephales promelas*) is a species of freshwater fish common in North Amarica and Canada. Absolutly different organisms used for testing toxicity. Despite that the toxic indices can be extrapolated among themselves. For this process a correlation equation for a special effect and, the best, developed for a large heterogeneous series of compounds (QSAR) must be known. Moreover, the disparate effects with rat hepatocytes, viability and ureogenesis in a series of compounds, also correlate. Knowing those correlation equations and their statistic evaluation one can extrapolate among toxic (but generally any biological) effects. The important point is that it is valid just for a series of componds, whithin those series not generally for individual compounds. The reason is a common physicochemical property govering the biological effects – partition coefficient between two unmissible phases, namely between n-octanol and water. Perhaps it means that transport of a compound towards a target is responsible for a magnitude of any effect, and not reactivity which one would presuppose. Various such comparative studies have been published. Moreover, acute toxicity data of 26 aquatic species and 21 compounds were analyzed by pattern recognition analysis to discover factors of toxicity variation among species characteristics. It was confirmed that most variations in toxicological data were due to differences in the compounds, not intrinsic differences between species.

BIOMARKERS OF HEMATOTOXIC AND IMMUNOTOXIC EFFECTS OF GLASS FIBRES EXPOSURE

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Cross sectional molecular epidemiology study was conducted to examine potential hematotoxic and immunotoxic effect of glass fibre exposure in occupationally exposed workers. Seventy five workers (mean age 46 years) who produced glass fibres for at least 5 years (mean duration of exposure 16 years) and 36 clerical subjects have been included to the study. Current exposure to glass fibres was assessed using stationary area sampling as well as personal monitoring. All measured levels of glass fibres were very low (10 to 1000 times below the Slovak occupational limits).

Haematological parameters: white blood cell count including differential, red blood cell count, hemaglobin, hematocrit, mean cell volume and platelet count were measured.

Immune biomarkers examined included lymphocyte subset analysis, expression of adhesion molecules on peripheral blood leukocytes, activation markers on eosinophils, concentrations of interleukins, soluble adhesion molecules and levels of immunoglobulins. Moreover, functional immune assays: lymphoproliferative response to mitogens and antigens, phagocytic activity of leukocytes and cytotoxic activity of natural killer cells were performed. Statistical analysis was done using SPSS software.

Hematotoxic effect of exposure to glass mineral fibres displayed as suppressed total white blood cell count ($p<0.05$) and lower percentage of neutrophils ($p<0.05$) in peripheral blood of exposed individuals. Moreover, enhanced lymphocyte count ($p<0.01$) and higher percentage and number of eosinophils, basophils and monocytes (measured together; $p<0.05$) were observed in exposed population. Red blood cells in exposed workers had increased mean cell volume ($p<0.05$).

Immunomodulatory effect of glass fibres exposure was seen as decreased expression of molecule $CD16^{+}56^{+}$ on lymphocytes. On the other hand, activation of immune response was observed as significantly enhanced T-cell response of lymphocytes *in vitro* stimulated with concanavalin A ($p<0.01$), phytohemmagglutinin ($p<0.01$), and antigen CD3 ($p=0.055$) in workers from glass fibre plant. Increased serum levels of proinflammatory cytokine interleukin 8 ($p<0.001$), levels of soluble adhesion molecule E-selectin ($p<0.001$) and expression of CD66b activation molecule on eosinophils showed inflammatory effect of fibres in exposed population.

Our findings might indicate hematotoxic, immunotoxic and inflammatory status in workers exposed to glass fibres.

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BINDING OF NEW ACETYLCHOLINESTERASE REACTIVATORS K027 AND K203 TO HUMAN MICROSOMAL CYTOCHROMES P450 AND INHIBITION OF THEIR ACTIVITIES

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Spectroscopic methods are often used to detect and evaluate the interactions between biological macromolecules and low molecular weight substances. In the case cytochromes P450 (CYP, the main enzymes of biotransformation of foreign substances as drugs, etc.), the presence of the heme prosthetic group is of great advantage. Here, the heme group exhibits the characteristic absorption band which changes its position and intensity depending on the nature and concentration of the interacting molecule.

Two non-symmetric bispyridine oxime – based reactivators of acetylcholinesterase enzyme, labeled as K027 (1-(4-carbamoylpyridinium)-3-(4-hydroxyiminomethylpyridinium)-propane dibromide) and K203 ((E)-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide) were tested for their potential to inhibit the activities of human liver microsomal CYP. Oximes are known to be potent reactivators of organophosphate-inhibited acetylcholinesterase.

Difference spectroscopy has detected an interaction of both compounds studied with CYP. The compounds were shown to bind to CYP with spectral binding constants of 5.04 ± 1.79 nM (K027) and 5.2 ± 2.6 nM (K203). An enzymological studies were subsequently performed aimed at finding which CYP of the nine most important ones (CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1 and 3A4) is influenced in its activity by this interaction. The results have shown no prominent inhibition of individual CYP activities with both compounds except the CYP2E1 activity and the K203 reactivator. Diagnostic Dixon plot revealed that K203 acted as uncompetitive inhibitor of the CYP2E1 enzyme. However, the inhibition of this activity was less than 50% which makes the possible drug interactions highly unlikely.

Hence, the interaction of K027 and K203 oxime-type acetylcholinesterase reactivators with human liver microsomal CYP enzymes does not seem to be clinically significant and both compounds could be taken in this respect as antidotal drugs with low risk of drug interactions.

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HYGIENIC DUALITY OF FISH FROM THE IMPORTANT FISHING GROUNDS IN CZECH REPUBLIC

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Anthropogenic activities lead to environmental contamination with foreign substances, which subsequently get into the food web. Research of contamination assessment of fish from the 7 important fishing-grounds with selected heavy metals (Hg, Cd, Pb) and persistent organochlorine pollutants (PCB indicator congeners – IUPAC numbers 28, 52, 101, 118, 138, 153 and 180; hexachlorobenzene; α-, β-, γ-isomers of hexachlorocyclohexane, DDT and its degradation products DDE and DDD) was carried out in the Czech Republic in the year 2009. Loading of fish from selected fishing-grounds was different. Mercury can be unequivocaly considered the most serious contaminant of the aquatic environment of the monitored fishing-grounds, from all the extraneous substances studied. Mercury contents exceeded the hygienic limit valid in the Czech Republic in the part of analysed fish muscle samples from Vranov reservoir, Dalešice reservoir and Kořensko reservoir. Values contents of other extraneous substances under study ranged below the respective hygienic limits.

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INTRAUTERINE HYPOXIA INDUCED STRUCTURAL AND BIOCHEMICAL ALTERATIONS IN RAT FETUSES

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Unfavorable conditions in intrauterine development represent a foundation for health problems and morbidity after birth and in later life. Chronic intrauterine hypoxia in rats is an animal model appropriate for studying hypoxia during sensitive stages of development. In the present study, pregnant female Wistar/DV rats were exposed to the lowered oxygen containing environment in a special glass chamber during sensitive stages of organ (days 6–15 of gestation) and brain (days 19–20 of gestation) development. The intrauterine content (weight of fetuses and placentas, number of *corpora lutea*, number of implantations and resorptions, survival rate, skeletal and visceral anomalies) was evaluated on days 20 and 21 of gestation respectively. Moreover, biochemical parameters, *i.e.* N-acetyl-β-D-glucosaminidase (NAGA), content of proteins, malondialdehyd (MDA) and lactate in the maternal serum, brain, liver and lungs as well as in fetal lungs and liver were established. Intrauterine hypoxia caused a significant decrease in fetal and placental weight (p<0.05) after exposure to hypoxia on days 6–15 of gestation compared to control animals. Decreased oxygen supply on days 19 and 20 of gestation resulted in reduction in placental weight (p<0.05). We found an increased incidence of skeletal anomalies (sternebrae, p<0.001) after exposure to hypoxia on days 6-15 of gestation and visceral anomalies (subarachnoidal bleeding, p<0.05) after exposure to hypoxia on days 19–20 of gestation. Biochemical analysis revealed significant changes in specific activity of NAGA in the maternal and fetal liver and also decreased MDA levels in the fetal liver in rats exposed to hypoxia on days 6-15 of gestation (p<0.05) compared to controls. Our results showed that oxygen supply during sensitive developmental stages is crucial for appropriate growth and functional maturation. Proposed animal model provides useful tool for studying mechanisms underlying pathological changes after hypoxia/ischemia insults during pregnancy.

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ASCORBATE AND CU(II) INDUCED OXIDATIVE DEGRADATION OF HIGH-MOLAR-MASS HYALURONAN: PRO- AND ANTIOXIDATIVE EFFECTS OF SOME THIOL XENOBIOTICS AND DRUGS

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Hyaluronan (HA) is a high-molar-mass polysaccharide present in various tissues in vertebrates, especially in skin, umbilical cord, vitreous body, and in joint synovial fluid. High-molar mass HA has antiangiogenic, anti-inflammatory, and immunosuppressive effects. Contrary, HA exposed to oxidative stress is degraded to fragments of lower-molar-mass with angiogenic, inflammatory, and immunostimulatory properties.

In our study HA was degraded by an oxidative system cupric ions *plus* ascorbate and time and dose-dependent changes of HA dynamic viscosity were monitored by the method of rotational viscometry. The aim was to achieve potential inhibition of $^{\bullet}$OH, RO$^{\bullet}$, and ROO$^{\bullet}$ radical induced HA degradation, and for this purpose thiol compounds such as cysteamine and D-penicillamine were tested. The antioxidative effect of the compounds tested alone was also studied by using ABTS and DPPH tests. The results of rotational viscometry showed that cysteamine, in higher concentrations tested, totally scavenged $^{\bullet}$OH, RO$^{\bullet}$, and ROO$^{\bullet}$ radicals. D-Penicillamine was shown to have a biphasic effect. Firstly, it was capable to scavenge all radicals in a dose-dependent manner, and then it acted as a pro-oxidant. ABTS and DPPH tests showed for the substances tested similar properties.

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EFFECTS OF CHRONIC SIMAZINE EXPOSURE ON COMMON CARP (*CYPRINUS CARPIO* L).

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Water pollution is a widespread problem in many aquatic environments. Recently, an environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. As a consequence, residual amounts of herbicides and their metabolites have been found in drinking water and foods, increasing concern for the possible threat to human health posed by exposure to these chemicals.

The aim of the study was to evaluate chronic toxic effects of the simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine) on some biometric, biochemical, haematological parameters and liver biomarkers of the common carp (*Cyprinus carpio* L.). Two year old fish were exposed for 90 days to simazine added to the tank water at four concentrations of 0.06 (real environmental concentration in Czech river), 1, 2 and 4 µg/L. The simazin concentration of 1 µg/L, 2 µg/L, and 4 µg/L corresponding to the $96hLC_{0.00025}$, $96hLC_{0.005}$ and $96hLC_{0.01}$ for carp.

The chronic exposure of simazine in real environmental concentration 0.06 µg/L had not effect on biometric, biochemical, haematological profile and liver biomarkers of common carp. The chronic exposure of common carp of simazine in concentration 1, 2, and 4 µg/L caused significant shifts in biochemical and haematological profile.

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TOXIC STRESS RESPONSE IN THE LIVER TISSUE OF JAPANESE QUAILS EXPOSED TO CYANOBACTERIAL BIOMASS

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Although avian deaths around lakes and rivers associated with cyanobacterial blooms have already been reported, there are only a few experimental studies on the effects of cyanobacterial toxins in birds. Our previous experiments revealed that blood biochemical responses to the cyanobacterial biomass in diet were first observed from day 5 post exposure. The aim of the present study was to evaluate toxic stress response in the liver tissue of adult Japanese quails exposed to cyanotoxins in diet. The OECD 205 Guideline on Avian Dietary Toxicity Test was employed in the experiment. A total of 20 adult male birds were sacrificed on days 0, 5, 10 and 15, respectively, of oral exposure to cyanobacterial biomass. They received the same daily dose of approximately 224.4 ng microcystins per gram of body weight. Ferric reducing antioxidant power (FRAP) and total proteins (TP) were significantly changed on days 10 and 15 of exposure, while glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) remained unchanged in the liver tissue.

The present study was supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno (Grant No. 80/2010/FVHE).

EXAMINATION OF ORLISTAT EFFECTS ON PXR-CYP3A4 SIGNALING IN HEPATIC AND INTESTINAL CELLS

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Due to the increasing obesity world-wide, the drugs for weight loss belong among the most requested ones. One such a drug is Orlistat (*Xenical)*, an anti-obesity drug that inactivates gastric and intestinal lipases, thus, preventing absorption of dietary triglycerides. There are reports indicating that Orlistat reduces bioavailability of Cyclosporin to a clinically relevant degree. Since Cyclosporin is metabolized by cytochrome P450 CYP3A4, we examined whether interaction between Orlistat and Cyclosporin involves induction of CYP3A4. Human Caucasian colon adenocarcinoma cells LS174T and primary cultures of human hepatocytes were used, as *in vitro* models of intestinal and hepatic cells, respectively. Treatment of LS174T cells for 24 h with Orlistat (1 mg/L – 100 mg/L) did not cause induction of CYP3A4 mRNA as compared to control cells while Orlistat (100 mg/L) slightly induced CYP3A4 mRNA in human hepatocytes. Rifampicin, a model CYP3A4 inducer, significantly induced CYP3A4 mRNA in both types of cells. The level of CYP3A4 protein in human hepatocytes was slightly increased by Orlistat after 48 h, while rifampicin strongly induced CYP3A4 protein level. In addition, Orlistat moderately dose-independently activated pregnane X receptor (PXR) in LS174T cells transiently transfected with *p3A4-luc* reporter construct containing the basal promoter (–362/+53) with proximal PXR response element and the distal xenobiotic responsive enhancer module (–7836/–7208) of the *CYP3A4* gene 5´-flanking region. In conclusion, we report here that Orlistat is weak PXR activator and CYP3A4 inducer in human hepatocytes, but it has no effect on CYP3A4 in intestinal cells, implying no role of CYP3A4 induction

in the interaction between Orlistat and Cyclosporin in absorption process.

This work was supported by the grants from the Czech Scientific Agency GACR 303/07/0128, GACR 503/10/0579 and GACR 304/10/0149.

GENOTOXIC AND NONGENOTOXIC EFFECTS OF EXTRACTS AND CHROMATOGRAPHIC FRACTIONS OF DIESEL PARTICULATE MATTER IN LUNG, LIVER AND PROSTATE EPITHELIAL CELLS

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Toxic effects of chemical mixtures have been studied extensively during last decades. However, individual mechanisms of toxicity leading to synergistic and/or antagonistic potencies of chemicals present in these mixtures are still far from being understood. In this study, dichloromethane extract of a diesel particulate matter (standard reference material NIST, SRM 1650b) was chromatographically fractionated and concentrations of an extended set of polycyclic aromatic hydrocarbons (PAHs) and PAH derivatives (including nitro-PAHs and other aromatic compounds) were quantified using GC/MS, HLPC/DAD and LC/MS/MS techniques. An *in vitro* bioassay of the aryl hydrocarbon receptor (AhR)-mediated activity in transgenic hepatoma H4IIE cells, which reflects the "dioxin-like" toxicity, was used to determine relative toxic potencies of mixture constituents, including persistent chlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls, as well as the less persistent PAHs and their derivatives.

Both genotoxic and nongenotoxic effects of crude extract and nonpolar, semipolar and polar fractions of aromatic compounds, benzo[a]pyrene and dibenzo[a,l] pyrene were then evaluated in three cellular models (rat lung epithelial RLE-6TN cells, rat liver epithelial WB-F344 cells and human prostate epithelial cell line LNCaP). We detected significant levels stable PAH-DNA adducts in all three cellular lines, however, only lung and liver cells showed significant induction of DNA damage-related apoptosis. The crude extract and, surprisingly, also polar aromatic fraction induced phosphorylation of p53, Chk2 or Chk1. All of these effects were dose-dependent; as significant induction was observed at the doses 0.1 or 0.5 mg extract equivalent/ml. The studied nongenotoxic parameters, including induction of AhR-dependent gene expression, were induced at much lower concentrations of airborne complex mixtures of aromatic compounds (0.005 mg/ml). Two principal findings are reported: i) prostate epithelial cells are, in spite of significant DNA adduct formation, highly resistant to induction of apoptosis; and ii) nongenotoxic effects related to AhR activation are apparent already at much lower doses that genotoxic effects of model diesel exhaust particulate matter sample.

Supported by the Czech Science Foundation (grant No.310/07/0961); the institutional support was provided by the Academy of Sciences of the Czech Republic (Research Plans AV0Z50040507, AV0Z50040702) and the Czech Ministry of Agriculture (No. MZE0002716202).

VARIOUS ASPECTS OF PISCINE TOXICOLOGY – RESEARCHES IN THE FACULTY OF ENVIRONMETAL SCIENCES AND FISHERIES IN OLSZTYN, POLAND

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In opposition to toxicology of mammals piscine toxicology is closely connected with the conditions of external environment. The aquatic environment is necessary for embryonic development and after hatching during short or long-lasting larval period of most fish species. An aquatic environment is polluted by many industrial and agricultural wastes. Ammonia as a toxic and common compound in water have negative influence for aquaculture especially in intensive fish culture, recirculation system and hatchery facilities. Acute toxicity of ammonia was investigated in carp *Cyprinus carpio* L. and developmental stages of chub *Squalius cephalus* L. Changes in the peripheral blood characteristics and hemopoietic tissues of carp occurred after exposition to ammonia in acute tests and 3, 5 and 10 weeks sublethal concetration. The observed increase of the concentration of most amino acids in fish intoxicated with amonia suggests that the process reflects detoxication of ammonia which takes place both in the brain and muscles after 3 weeks of exposition.

Phenol intoxication tests induced considerable unfavorable changes in the blood and dystrophic and necrobiotic lesions in tissues of fish leading to dysfunction both haemopoietic and reproductive processes.

In study on fish reproduction disruptors the oxygenated polycyclic hydrocarbons (17-β-estradiol, 4,7-dihydroxyisoflavone, 1,6-dihydroxynaphthalene i 1,5-dihydroxynaphthalene) and oxygenated monocyclic hydrocarbons (phenol, 4-n-heptyloxyphenol, 4-n-buthylphenol, 4-sec-buthylphenol; 4-tert-buthylphenol) was assessed using histopathological methods. It was established that examined oxygenated aromatic hydrocarbons both natural (17-β-estradiol and 4,7-dihydroxyisoflavone) and synthetic can disrupt the differentiation of primary and secondary sex traits in pikeperch. The chronic activity of these "biomimetics of estrogen" can lead to the disappearance of natural fish population. *In vivo* and *in vitro* tests were used to exam dibutyl phthalate and butyl benzyl phthalate impact on the development of the reproductive system of pikeperch *Sander lucioperca* L. Additional as multigenerational studies are needed to clarify influence long term exposure of fish to

environmental concentrations of endocrine disrupting chemicals.

Hydrogen peroxide used in fish therapy is known to be toxic for sensitive species. In our work safe concentrations and exposure times was evaluated for ide *Leuciscus idus* L. and pike *Esox lucius* L. fry. The intensity of lesions in gills, skin, pseudobranch and thymus of exposed fish were connected with the time of bath.

Actually anesthetics are routinely required during stressful procedures with fish, but data regarding the safety of individual anesthetics to different fish species are still few and insufficient. The clove oil, MS-222 and 2-phenoxyaethanol anesthesia on fish organism was investigated in our faculty with cooperation with Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic.

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EFFECTS OF HERBICIDES ON TARGET AND NONTARGET VEGETATION

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Pesticides are ubiquitous in the environment and have significant environmental and public health impact. This study examined the target and nontarget effects of herbicide Sekator OD (amido sulfuron and iodosulfuron) to target weed species *Chenopodium album* L. and to two nontarget species – barley *Hordeum vulgare* L. and duckweed *Lemna minor* L. The terrestrial plants were sprayed directly with various dilutions of herbicide sprays (solutions equivalent to 0.1–0.001 of recommended field application rate), the aquatic plants were affected by the same concentrations of herbicide in *Lemna minor* growth medium. The measured endpoints were: survival, height of the shoots, the dry biomass, concentrations of malondialdehydes (MDA) and chlorophyll (a, b), the growth rate of *Lemna minor*. Both (target and nontarget) species had a strong phytotoxic response to herbicide application, though the response of target species was more pronounced. The growth of shoots of *Chenopodium album* and *Hordeum vulgare* was inhibited by 37.1% and by 18.4% respectively. The dry biomass of barley was by 33%, of *Chenopodium album* – by 71,5% lower than in control. *Lemna minor* showed extremely strong response: in the highest concentrations of herbicide the breakdown of all affected colonies was observed. The biomass of *Lemna minor* in lowest concentrations of herbicide was approximately by 31% lower than in control. The regression analysis revealed strong negative relationship between herbicide concentration and the growth of shoots and biomass both for target and nontarget species ($r > -0.7$, $p<0.05$). Application of herbicide provoked an oxidative stress, the concentrations of MDA in *Chenopodium album* and *Hordeum vulgare* were, respectively, by 8.4, and 1.2-fold higher than in control. The study revealed that application of herbicides causes the adverse effects not only in target vegetation, but in nontarget as well.

CYTOTOXICITY EVALUATION OF NATURAL COMPOUNDS ISOLATED FROM *MORUS ALBA*

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Morus alba is a natural source of many compounds with different effects. It has been proposed to possess anti-inflammatory, antioxidant, and hepatoprotective activities. The aim of this study was to evaluate basic and direct toxic effects of three separated flavonoids (MA-12/1, MA-13 and MA-14) on human cells. Recently we have shown strong anti-inflammatory effect of MA-13 (prenylated flavone) on human macrophages caused by the inhibition of key molecules (TNFα, CCL2, NOS2, COX2). Geranylated flavanones (MA-12/1 and MA-14), newly isolated and characterized in our laboratory, showed cytotoxic effect on the cell kinetic and viability, at various time points. The strongest cytotoxicity was found in MA-14 compound at concentrations of 20, 30, and 50 μM ($p<0.001$). This effect increased in time and was expressed also in lower concentrations. MA-12/1 exerted lower cytotoxic effect after 24 h, however, viability was still decreased significantly when compared to control. Interestingly, after 72 h of MA-12/1 treatment the cell number decreased concentration-dependently, but viability was not affected in comparison with control. Our results indicate that flavonoids isolated from *Morus alba* show cytotoxic activity and should be investigated in more detail to reveal potential effects on the cell cycle of tumor cells.

Supported by the grant 3/2010/FaF.

MEMENATINE INHIBITS CYP2D2 METABOLIC ACTIVITY IN RATS, *IN VITRO* AND *IN VIVO* STUDIES

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Many psychotropics are biotransformed by cytochrome P450 (CYP) 2D6 isoenzyme and modification of CYP's activity can be crucial for their efficacy, safety and toxicity. Being not a substrate for CYP, memantine is not supposed to interfere with its activity. In the present study the memantine and combined treatment with fluoxetine used quite often in clinics was determined on the rat CYP2D2, orthologue izoenzyme of human 2D6 activity. The experiments were carried out in adult male Wistar albino rats. The models of perfused rat liver and pharmacokinetic study on the whole animal model were used for *in vitro* and *in vivo* testing. Memantine, combination of memantine and fluoxetine or saline were administered intraperitonealy to animals subdivided

into three groups for 10 days. Memantine as well as fluoxetine were administered at the dose of 5 mg/kg/day. The metabolic activity of CYP2D2 was assessed as a speed of biotransformation of dextromethorphan (DM) into CYP2D2 specific metabolite dextrorphan (DX). Samples of perfusion medium were withdrawn at 30th, 60th and 120th minute in the model of isolated liver. From the living animals samples were collected in the: 10th, 20th, 30th, 40th, 60th, 90th, 125th, 180th, 240th and 300th minute after DM administration. HPLC method was used for measurement of DM and DX levels. Metabolic ratios DM/DX were calculated and compared.

Metabolic ratios were significantly higher in memantine administered group in both *in vitro* and *in vivo* experiments indicating inhibitory memantine on CYP2D2 activity. As it was hypothesised the combined treatment with memantine+fluoxetine showed significantly higher inhibitory effect on the CYP2D2 activity than memantine alone.

To summarize, the results showed inhibition of CYP2D2 metabolic activity caused by memantine which was intensified by fluoxetine. We further conclude that combination of memantine with other drugs from CYP2D6 substrates or inhibitors can lead to clinically relevant interactions with possible toxic consequences.

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MORPHOLOGICAL ALTERATIONS OF *ARTEMIA FRANCISCANA* INDUCED BY IONIZING IRRADIATION

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Our previously published papers have dealt particularly with dependence of the lethality of *Artemia franciscana* on dose of ionizing irradiation. LD_{50} after the exposure to 600–700 Gy was evaluated to 96 hrs, which corresponds to the phylogenic position of this species.

In our experiments we used salt water with the salinity of 47 g/L and pH 7.6 ± 0.1. Freshly hatched nauplii were exposed to gamma irradiation of 100, 250, 500, or 1 000 Gy (^{60}Co, 2.7 kGy/h) and fixed with formaldehyde 72 or 96 hrs after the hatching. Results were documented by the microphotography.

While development of the 2nd larval stage exposed to 100 Gy was not altered at all, the doses of 250, 500 or 1 000 Gy induced the anomalous development (96 hrs after the irradiation).

Another effect of irradiation was the atypical formation of intestinal epithelium. While in the control group the ratio of the thickness of epithel to intestinal lumen was approximately 1 : 1.5–2, in the group irradiated by 100 Gy the intestinal epithelium was thinner with this ratio 1 : 4. In dose of 250 Gy the effect was variable reaching the ratio as low as 1 : 7. And in higher doses the ratio decreased to 1 : 7–10, or even the layer of epithelium was not observed at all (in 500 Gy sporadically, and in 1 000 Gy regularly). The results are in correspondence with similar experiments on vertebrates. This finding may be explained by the high radiosensitivity of epithelial cells in general, caused by their high mitotic activity.

From this point of view the Biotest of 2nd generation may substitute some toxicity tests on vertebrates.

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EFFECT OF PEROXYNITRITE AND PHENOLIC COMPOUNDS ON SERCA ACTIVITY

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Sarco/endoplasmic reticulum Ca-ATPase (SERCA) plays an important role in the relaxation of muscles through transport of cytosolic Ca^{2+} into the sarco/endoplasmic reticulum. Regulation of SERCA activity in skeletal muscle may occur by reactive nitrogen species (RNS) signaling pathways. RNS may be associated with chronic pathological events in skeletal muscle, but in some cases it also may be responsible for SERCA activation.

We studied the effect of peroxynitrite ($ONOO^-$), generated by reaction of nitric oxide with superoxide, on the activity of SERCA from rabbit skeletal muscle *in vitro.* Peroxynitrite concentration-dependently decreased Ca-ATPase activity and induced conformational changes in both the cytosolic nucleotide (ATP-binding) and the transmembrane Ca^{2+} binding sites of SERCA.

Effects of synthetic substances, trolox, pyridoindole derivatives (stobadine, SMe1EC2) and natural standardized extracts of flavonoids (Pycnogenol®, EGb 761) were investigated on SERCA activity both in the absence and presence of peroxynitrite. SERCA activity was protected against peroxynitrite injury by trolox, pyridoindole derivatives and EGb 761. Pycnogenol® showed no protective effect, it even induced SERCA activity inhibition at higher concentrations. In order to understand the mechanism of the effects of phenolic compounds on SERCA, we studied posttranslational modifications (SH-group alterations, tyrosine nitration and protein carbonyl formation) as well as conformational changes of SERCA.

This work was supported by national grants VEGA 2/0001/08 a 2/0083/09 and by the The Agency of the Ministry of Education of the Slovak Republic for the Structural Funds of EU as a part of the Project: Evaluation of natural substances and their selection for prevention and treatment of lifestyle diseases (ITMS 26240220040).

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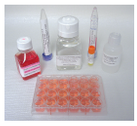

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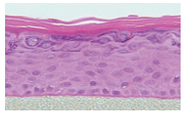

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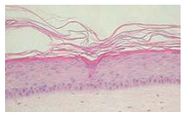

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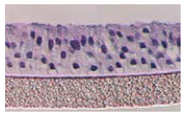

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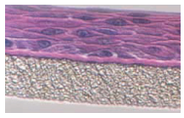

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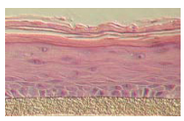

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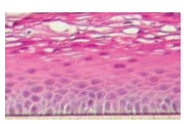

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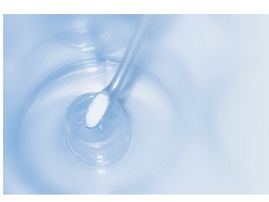

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