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Abstracts

The EUROTOX 2006/6 CTDC Congress

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Satellite Symposium	Dermal Exposure to Chemicals and Health Risk Assessment

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Keynote Lecture

1
DNA repair systems and genetic toxicology

Miroslav Radman [1,2]

[1] *Université de Paris-Descartes, Faculté de Médecine, Paris, France;* [2] *Mediterranean Institute for Life Sciences, Split, Croatia*

The discovery of DNA repair mechanisms has changed the notion of genetic risk of environmental agents, or of particular life styles, because the genotoxic impact is dependent on the efficacy of many DNA repair systems. Not only do the error-free constitutive and inducible DNA repair activities decrease the impact of the initial DNA modifications, but also the activities of inducible error-prone DNA replication (tolerance) systems determine the final genetic consequences of such damage. Whereas error-free DNA repair systems increase the effective dose of genotoxic agents, the error-prone systems decrease the effective dose. Error-free systems act to increase cell survival and decrease the genotoxic outcome, error-prone lead also to an increase in survival but at the expense of increased induced mutation or recombination rates. Hence, the mutant organisms deficient in error-free repair (e.g., NER and BER) are sensitive to genotoxic agents and are hypermutable, whereas mutants in error-prone repair are sensitive but non-mutable. We shall compare bacterial and mammalian mutants of those kinds.

Furthermore, some repair systems cause the cell killing by specific lesions, e.g., the mismatch repair system activity kills bacterial and mammalian cells treated with simple alkylating agents (methylation or *cis*-platin). Mismatch repair, and specifically the level of the MutL, controls the frequency of chromosomal deletions mediated by homologous recombination between directly repeated sequences (M. Elez, I. Matic and M. Radman, unpublished). Mismatch repair can also become saturated by a critical level of certain kind of DNA damage (e.g., base analogs, alkylations, etc.) having a generalised indirect genomic genotoxic effect resulting from the reversible mismatch repair deficient phenotype. Some extremophile organisms, e.g., *Deinococcus radiodurans* have an exceedingly efficient error-free recombination repair system, but lack error-prone, mutagenic, DNA polymerases resulting in an extraordinary resistance to genotoxic agents with little or no mutagenic consequence.

These are examples of known DNA repair systems which profoundly modify the genotoxic consequences of DNA damaging agents. Thus, the risk assessment must include the diagnostic of the DNA repair systems active in the relevant organism (e.g., humans). In other words, risk assessment will be one day individualised just like the general medicine.

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Thursday, September 21, 2006

PS1 Plenary Session

2
New approaches to toxicologic mechanisms by the application of genomics, proteomics and metabolomics

Christopher Gerner, Olga Teufelhofer, Wolfram Parzefall, Rolf Schulte-Hermann

Institute of Cancer Research, Internal Medicine Clinics I, Medical University of Vienna, Vienna, Austria

Application of omics technologies in toxicological research holds great promise as to provide novel information about biological mechanisms from a holistic point of view.

Direct comparison of genomics data and proteomics data collected from the same experimental setup clearly highlights how different genes and proteins are regulated. This may be explained by the fact that proteins may be regulated in very different ways, such as by induction of synthesis, modifications, translocation and proteosomal degradation. Here, it will be demonstrated, how proteome data may accurately indicate the biological functionality of a cell. We designed a combined proteome analysis approach including metabolic labelling of cells and tissues, subcellular fractionation, 2D gel-based separation of proteins, fluorographic and autoradiographic protein detection and quantification in addition to shotgun proteomics. Protein identification was accomplished by nano-liquid chromatographic separation of peptides and fragmentation analysis with an ion-trap mass spectrometer. In addition to several *in vitro* human and rat model systems, this methodology was applied to investigate the proteome of *in vivo* rat model and human tissue specimen. We have designed strategies to most sensitively detect alterations induced by various toxicants and directly compared *in vivo*-effects and *in vitro*-effects of such toxicants on different cell types. Observed characteristic responses of the cells may allow to design diagnostic strategies focussing on critical pathophysiological events. The strategy includes the identification of specifically secreted proteins and induced enzymes, which may serve as biomarkers. Secreted proteins may

directly serve as biomarkers in body fluids, while the induction of catalytic enzyme activity may allow the directed search for the resulting, potentially accumulating, metabolite.

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W1 Reactive Oxygen Species in Toxicity

3
Oxidant stress and liver injury

Hartmut Jaeschke

University of Arizona Tucson, Arizona, USA

Oxidant stress is often discussed as the cause of liver cell injury during drug- or chemical-induced hepatotoxicity. However, many details of the mechanism remain poorly defined. Acetaminophen (APAP) hepatotoxicity, which is the most frequent cause of acute liver failure in the US and UK, is used as a model to study oxidant stress and its consequences in the liver. After APAP overdose, a reactive metabolite is formed, which first depletes cellular glutathione (GSH) and subsequently covalently binds to cellular proteins, especially mitochondrial proteins. This leads to mitochondrial dysfunction with formation of superoxide and peroxynitrite in mitochondria. The selective mitochondrial oxidant stress and peroxynitrite formation have three major consequences: (1) loss of mitochondrial DNA; (2) Mitochondrial membrane permeability transition (MPT) pore opening and collapse of the mitochondria membrane potential; (3) release of endonuclease G and apoptosis-inducing factor from the mitochondria and translocation to the nucleus where these endonucleases cause DNA fragmentation and karyolysis. If enough mitochondria are affected within a hepatocyte, these events lead to cell death by oncotic necrosis. If the recovery of intracellular, in particular mitochondrial GSH levels is accelerated by administration of GSH or *N*-acetyl cysteine after the metabolic activation of APAP, peroxynitrite is effectively scavenged and the loss of mitochondrial DNA, the nuclear DNA fragmentation, the MPT pore opening and cell death is prevented. On the other hand, loading of mitochondria with Vitamin E had no protective effect. These findings indicate that antioxidants are only effective when they are able to scavenge specific reactive oxygen species in a particular cellular location relevant for the individual toxicant.

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4
Reactive oxygen from macrophages and chemical carcinogenesis

Rolf Schulte-Hermann, Olga Teufelhofer, Wolfram Parzefall, Constanze Freiler, Bettina Grasl-Kraupp, Christopher Gerner

MUW, IKF Vienna, Vienna, Austria

Liver injury and inflammation are key factors in the development of human liver cancer. Hepatocytes are widely regarded as the primary target of toxic and carcinogenic chemicals. However, also Kupffer cells, the macrophages of the liver, are important mediators of injury and a major source of reactive oxygen in the liver. Published reports indicate that Kupffer cells are directly and/or indirectly activated by various chemicals including CCl_4, ethanol, paracetamol, peroxisome proliferators, cadmium and arsenic. We have recently identified nitrosamines and other carcinogens as direct activators of Kupffer cells. Activation results in formation of superoxide by a specific NADPH oxidase (PHOX). In addition, activated Kupffer cells release NO and various proinflammatory cytokines which contribute to chemically induced liver injury and may affect the development of liver cancer. We have studied the role of superoxide for liver toxicity, genotoxicity, and tumor formation by using P47 PHOX knockout mice which cannot produce superoxide by Kupffer cells and granulocytes. The results show that these mice are largely protected from DNA damage, cytotoxicity and protein nitration, in response to the genotoxic carcinogen diethylnitrosamine. Effects on liver cancer formation will be presented. Overall, the results confirm the important role of reactive oxygen from the hepatic microenvironment for liver injury and tumor formation by toxic compounds.

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5
Oxidative damage of DNA: Repair and implications

Bernd Epe

Institute of Pharmacy, University of Mainz, D-55099 Mainz, Germany

Basal levels of oxidative DNA base modifications such as 7,8-dihydro-8-oxoguanine (8-oxoG) are observed in all types of cells, most probably due to a continuous generation of reactive oxygen species (ROS) in the cellular oxygen metabolism, and it has long been suspected that they might play an important role in the initiation of carcinogenesis. Their repair proceeds mostly via base excision

repair (BER), which in the case of 8-oxoG is initiated in mammalian cells by the repair glycosylase OGG1. Here, we show that the removal of these lesions from the chromosomal DNA can also be accomplished by a mechanism involving poly(ADP-ribose)polymerase-1 (PARP1) and Cockayne syndrome B protein (CSB), but independent of OGG1, CSA and XPC. In the livers of *Ogg1*$^{-/-}$*/Csb*$^{-/-}$ and *Ogg1*$^{-/-}$*/Parp1*$^{-/-}$ double-knockout mice, the accumulation of endogenously generated oxidative purine modifications (8-oxoG) is two-fold higher than in *Ogg1*$^{-/-}$ mice. This effect can be exploited to analyse the consequences of the endogenous oxidative base damage for spontaneous mutagenesis and the initiation of carcinogenesis. In the transgenic non-transcribed *lacI* gene of BigBlue *Ogg1*$^{-/-}$*/Csb*$^{-/-}$ mice, the spontaneous mutation frequencies were found to be nearly two-fold higher than in the same locus in *Ogg1*$^{-/-}$ mice. Most of the spontaneous mutations were GC → TA transversions, which are characteristic for 8-oxoG. Treatment of the mice with the peroxysome proliferator WY-14,643 induced preneoplastic foci, the number of which was much higher in *Ogg1*$^{-/-}$*/Csb*$^{-/-}$ mice than in wild-type mice.

The data indicate that (i) relatively few additional oxidative base modifications already double the spontaneous mutation rates and that (ii) these spontaneous mutations translate into increased tumour incidence if cell proliferation is stimulated by a non-genotoxic promoter.

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6

H_2O_2 signaling by redox modifications of cysteine residues

Michel B. Toledano

Laboratoire Stress Oxydant et Cancer, Service de Biologie Moléculaire Systémique, DBJC, Commissariat a l'Energie Atomique, CEA-Saclay, 91191 Gif-sur-Yvette, France

Microbial H_2O_2 sensors are regulators of cellular H_2O_2 homeostasis, being activated by oxidation when H_2O_2 levels increase and setting the expression of oxidant-scavengers in response to this increase. Such regulation, meant to prevent oxidative cellular damage has the hallmarks of a homeostatic control. In mammals, regulators activated by mechanisms similar to those of microbial H_2O_2 sensors are being described. However, their function is to regulate cellular pathways unrelated to H_2O_2 metabolism. The yeast *S. cerevisiae* Orp1-Yap1 and *S. pombe* Tpx1-Pap1 are two-component H_2O_2 sensors sharing an overall common mechanism of activation. Their regulatory component, the Yap1 and Pap1 transcription factors, are activated by reversible disulfide bond formation. H_2O_2-induced oxidation of Yap1 and Pap1 is not direct involving, respectively, the thiol-based peroxidases Orp1, a GPx-like enzyme, and Tpx1, a peroxiredoxin, which relay the peroxide signal by means of a thiol-oxidation cascade. Orp1 and Tpx1 are thus peroxide sensors and redox transducers. Pap1 but not Yap1 activation is restricted within a narrow range of H_2O_2 concentration. This is due to Tpx1 oxidation to an inactive cysteine-sulfinic acid form, eventually reversed by ATP-dependent reduction by sulfiredoxin. These mechanisms illustrate the built-in high specificity of cysteine-based redox regulation and suggest the existence of specific pathways of cysteine oxidation.

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7

A toxicogenomics approach for risk assessment of glutathione depletion and oxidative stress-induced nongenotoxic hepatocarcinogenesis in the rat liver

Takeki Uehara[1], Naoki Kiyosawa[1], Atsushi Ono[1], Toshikazu Miyagishima[1], Tetsuro Urushidani[1], Taku Nagao[1,2]

[1] *Toxicogenomics Project, National Institute of Biomedical Innovation, Japan;* [2] *National Institute of Health Sciences, Japan*

In Japan, National Toxicogenomics Project has been on going, where National Institute of Health Sciences and 15 pharmaceutical companies had a 5-year collaborative research from 2002. Now, we are constructing a large-scale toxicogenomics database, which consists of gene expression data on the rat liver, kidney, or rat and human hepatocytes using Affymetrix GeneChip and conventional toxicology data about 150 chemicals, in order to predict the toxicity of new chemicals in the early stage of drug development. Oxidative stress arisen from glutathione depletion in the hepatocyte has been proposed as a major mechanism of action for hepatotoxicity caused by drug overdosing, possibly including participation in nongenotoxic carcinogenesis. This report presents one of the toxicogenomics approaches for risk evaluation of glutathione depletion and oxidative stress-induced nongenotoxic hepatocarcinogenesis in the rat liver by using toxicogenomic biomarker sets. At first, we selected a total number of 130 glutathione depletion-responsive genes whose expression profile showed inverse correlation with the hepatic glutathione content in the phorone-treated rat liver. The expression of these genes was

markedly altered by treatment with well-known oxidative stressors, such as acetaminophen, bromobenzene, thioacetamide, methapyrilene and coumarin. This result indicated that these genes would be useful for evaluation of drug-induced hepatic glutathione depletion from microarray data. In the next step, we identified a classifier of 112 genes by a supervised analysis, namely Prediction Analysis for Microarrays, that yielded the greatest predictive accuracy of over 95% in classifying the presence or absence of hepatocarcinogenicity. DNA damage and cell cycle regulation as the sign of regeneration by sustained oxidative stress were common characteristics of the oxidative stress-mediated nongenotoxic hepatocarcinogenesis. Our large-scale toxicogenomics database would be invaluable for toxicologists as a resource of candidate toxicogenomic biomarker sets to be identified.

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8

Identification of genes and proteins associated with trans, trans-2,4-decadienal induced oxidative stress in human bronchial epithelial cells

Pinpin Lin [1], Louis W. Chang [1], Pao-Chi Liao [2], Hsin-Yi Wu [2], Yu-Chang Tyan [2], Hui-Ti Tsai [1]

[1] *National Health Research Institutes Zhunan, Taiwan;* [2] *National Cheng Kung University, Taiwan*

Trans, trans-2,4-decadienal (*tt*-DDE), a specific type of dienaldehydes, is abundant in heated oils or cooking oil fumes. Ingestion of heated oils and exposure to cooking oil fumes has been suggested to have great health impact in variety of organs, including lung. We demonstrated that long-term treatment with *tt*-DDE increased cell proliferation and cytokines secretion via oxidative stress in human bronchial epithelial cells BEAS-2B. Furthermore, these effects were prevented by co-treatment with anti-oxidants *N*-acetylcysteine (NAC) and Vitamin C. Utilizing the proteomic techniques, the objective of this study was to identify protein biomarkers associated with *tt*-DDE-induced oxidative stress and cytotoxicity in human bronchial epithelial cells BEAS-2B. Treatment with *tt*-DDE for 48 h dose-dependently reduced cell viability in human BEAS-2B cells, but not in lung cancer cell lines. Co-treatment with antioxidant *N*-acetylcysteine tremendously reduced *tt*-DDE-induced in BEAS-2B cells. Protein extracts from DMSO or *tt*-DDE treated cells were separated on 2D gel and differentially expressed proteins were identified by mass spectrometry. A total of 30 proteins with known functions were identified.

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S1 Alcohol and Diet Interactions

9

An overview of Italian studies of Mediterranean diet and cancer

Carlo La Vecchia [1,2]

[1] *Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy;* [2] *Istituto di Statistica Medica e Biometria, Università di Milano, Milano, Italy*

Various aspects of the Mediterranean diet are considered to have a favourable effect on several common epithelial cancers. These aspects were analyzed using data from a series of case–control studies conducted in Northern Italy on over 12,000 cases of several major cancer sites and 10,000 controls. For most digestive tract cancers, the risk decreased with increasing vegetable and fruit consumption, with relative risks (RR) between 0.3 and 0.7 for the highest versus the lowest tertile, and the population attributable risks for low intake of vegetables and fruit ranged between 15% and 40%. A number of antioxidants and other micronutrients showed an inverse relation with cancer risk, but the main components responsible for the favourable effect of a diet rich in vegetables and fruit remain undefined. Fish tended to be another favourable diet indicator. In contrast, subjects reporting frequent red meat intake showed RRs above unity for several neoplasms. Whole grain food intake was related to reduced risk of several types of cancer, particularly of the upper digestive tract. This may be due to a favourable role of fibre, but the issue is still open to discussion. In contrast, refined grain intake and, consequently, glycaemic load and index were associated with increased risk of different types of cancer, including digestive tract and hormone-related ones. Further, olive oil, which is a typical aspect of the Mediterranean diet, has been inversely related to cancers of the colorectum and breast, and mainly of the upper digestive and respiratory tract neoplasms.

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10

Molecular aspects of hepatic ethanol metabolism and toxicity as modulated by dietary factors

Magnus Ingelman-Sundberg

Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Ethanol metabolism is to the main part catalysed by ADH and to a minor part by the ethanol-inducible CYP2E1. All together there is no significant induction of hepatic metabolism by the alcohol. Continuous infusion of ethanol into rats causes fluctuating ethanol concentrations with peaks at about every 6 days. Although some ethanol radicals are formed in the liver, the main toxic consequence of ethanol metabolism is oxidative stress to a great part exerted through the induction of CYP2E1 which can form a high extent of free oxy radicals in the perivenous area of the liver. This causes activation of Kupffer cells leading to a cascade of proinflammatory cytokines which in turn can activate Stellate cells for production of collagen and also cause a subsequent differentiation into myofibroblasts. Protection from ethanol induced liver injury is obtained by agents that inhibit the action of Kupffer cells and proinflammatory cytokines. In addition the toxicity is to a great extent reduced at least in animal models, by agents exerting antioxidant properties in the liver. The lecture will provide with an updated review in the field with emphasis on the action of nutritional factors and mechanisms behind ethanol induced hepatotoxicity.

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11

Genetic and nutritional aspects of alcohol initiated organ damage

Felix Stickel, Helmut K. Seitz

Department of Clinical Pharmacology, University of Berne, Switzerland

Chronic alcohol consumption causes organ dysfunction through various mechanisms. Among these, malnutrition caused by interactions with micro- and macronutrients is highly important. Interactions with lipid metabolism result in dyslipidemia, truncal obesity, and fatty liver. Enhanced oxidative stress through increased lipid peroxidation may lead to liver fibrosis. Thiamine deficiency represents a nutritional emergency situation among heavy drinkers potentially precipitating Wernicke–Korsakoff encephalopathy. Folate metabolism is markedly impaired by chronic alcohol ingestion via poor dietary intake, decreased intestinal uptake, aberrant hepatic storage, altered methylation, and increased urinary excretion. This may ellicit macrocytosis, defective one-carbon metabolism, decreased methylation capacity, reduced antioxidant defense, and changes in epigenetic control of genes involved in alcohol-mediated cocarcinogenesis. Heavy drinkers frequently present severe Vitamin A deficiency. Clinically important are resulting night blindness, reproductive insufficiency, and retinoid effects on cell proliferation and differentiation. Recently, polar retinoid metabolites generated through oxidative metabolism of retinoids via alcohol-induced cytochrome P450 2 E1 were found to precipitate hepatocyte apoptosis by causing mitochondrial damage.

Severe liver damage develops in only a minority of heavy drinkers and evidence from twin studies indicate that genetic factors account for at least 50% of individual susceptibility. The contribution of genetic factors to the development of diseases may be investigated either by means of animal experiments, through linkage studies in families of affected patients, or population based case–control studies. With regard to the latter, single nucleotide polymorphisms of genes involved in the degradation of alcohol, antioxidant defense, necroinflammation, and formation and degradation of extracellular matrix are attractive candidates for studying genotype–phenotype associations.

However, many associations in early studies were found to be spurious and could not be confirmed in stringently designed investigations. Therefore, future genotype–phenotype studies in alcoholic liver disease should meet certain requirements in order to avoid pure chance observations due to a lack of power, false functional interpretation and insufficient statistical evaluation.

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12

Alcohol promoted mammary cancer–mechanistical studies and potential preventive effects of dietary phytochemicals

Jose A. Castro

Centro de Investigaciones Toxicológicas (CEITOX), CITEFA-CONICET. Juan B. de La Salle 4397, B1603ALO Villa Martelli, Buenos Aires, Argentina

There is abundant evidence in literature about chronic alcohol (ethanol) consumption associated with an increased risk of breast cancer even if consumed in

moderate doses. However, the biological and molecular mechanisms mediating this association remain unknown and speculative. Several studies support the hypothesis that ethanol use may increase breast cancer risk at least partially through an increasing effect in sex steroid levels and increases in cell division. Under this assumption, the nature of the mutational events responsible for the initiation step of the process would remain unclear. Recent results from our and other laboratories showed that effects of ethanol in the process may not necessarily be only indirect. For example, our laboratory recently demonstrated that rat mammary cytosolic xanthine oxidoreductase (XOR) is able to bioactivate ethanol to acetaldehyde and free radicals and that the microsomal fraction exhibited a lipoxygenase (LO) – peroxidase like mediated enzymatic pathway for the metabolism of ethanol to acetaldehyde. We also showed that repetitive ethanol drinking enhanced the activity of both, the XOR and the LO mediated metabolic pathways and promoted oxidative stress in mammary tissue. In addition, other laboratories reported the presence in mammary tissue of some type I alcohol dehydrogenase and of CYP2E1. In the studies of our laboratory we also showed that each one of those activation processes can be inhibited by small concentrations of phytochemicals present in food.

In summary, the available results suggest that different pathways of ethanol metabolism to the mutagen acetaldehyde present in mammary tissue; the promotion effect of oxidative stress and the proliferative action of estrogens might be involved in ethanol induced mammary cancer. Known inhibitory actions of some phytochemicals on each of these processes might have a preventive potential which deserves to be explored.

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13
The protective effects of moderate red wine consumption

Alan Crozier

Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Red wine is a rich source of a wide range of phenolic compounds derived from the seeds, skin and flesh of black grapes. These include anthocyanins, flavan-3-ol monomers and polymers, flavonols, hydroxycinnamates, phenolic acids and stilbenes as well as some anthocyanin-flavan-3-ol complexes which form during maturation of the wine. The nature of these compounds will be discussed along with information on which contribute to the high antioxidant capacity of red wines. The fate of some of these compounds in the body following ingestion will be covered along with a summary of the evidence from a variety of sources for the protective effects of moderate red wine consumption.

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S2 Molecular Mechanisms of Organophosphates

14
Introductory overview on mechanisms of organophosphate toxicity and detoxication with emphasis on studies in Croatia

Elsa Reiner [1], Zoran Radić [2], Vera Simeon-Rudolf [1]

[1] *Institute for Medical Research and Occupational Health, Zagreb, Croatia;* [2] *UCSD, La Jolla, CA 92093, USA*

Organophosphates (OP) are highly toxic compounds that are used as pesticides, drugs, and also as warfare nerve agents. Cholinesterases (acetylcholinesterase, AChE, and butyrylcholinesterase, BChE) and phosphoric triester hydrolases (paraoxonase, PON, and DFPase) are involved in the toxicity and detoxication of these agents. AChE and BChE are inhibited by OPs, while PON and DFPase hydrolyse OPs. The molecular structure of these enzymes and their mechanisms of interaction with OPs have by-and-large been resolved and generally understood. Present studies are mainly directed towards details concerning enzyme structure, role of individual residues in the enzyme interaction with OPs, search for effective antidotes and decontaminating agents against OPs, and developing methods to identify OPs in humans and in the environment.

Studies in Croatian laboratories have comprised several approaches relevant for the toxicity of OPs. Due to individual differences in the susceptibility to OPs, and to some other pharmacologically active compounds, the catalytic properties and distribution profiles of BChE and PON variants were analyzed in healthy population groups in Croatia. Activities and distribution profiles of variants were also analyzed in relation to certain diseases. Over several decades, about 150 compounds were synthesised and tested (on rodents) as antidotes, particularly against the warfare agents Sarin, Soman, Tabun, and VX. These compounds contained pyridinium, imidazolium or quinuclidinium moieties with one or two oxime groups, and some achieved a satisfactory therapeutic effect as compared to the conventional antidotes. The kinetics of in vitro reactivation of phosphy-

lated cholinesterases and the protection of the enzyme against inhibition by OPs was studied. Reaction models concerning binding of reversible ligands (including oximes) to the catalytic and/or allosteric enzyme sites were proposed and tested. Several methods for measuring cholinesterase activities in human blood were validated and recommended as field methods for assessing OP absorption. Quality control studies for measurement of BChE activities and for identifying BChE variants were conducted, and introduced in several clinical laboratories in Croatia.

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15

Neurodegeneration involving neuropathy target esterase (NTE)

Paul Glynn

MRC Toxicology Unit, University of Leicester, UK

In various animals, including man, certain organophosphates (OP) induce delayed neuropathy with paralysis and long nerve degeneration. Neuropathic OPs react covalently with NTE. However, in mice, these same OPs cause no clinical signs of neuropathy and only minimal histopathological changes. By contrast, ethyloctylphosphonofluoridate – a highly potent NTE inhibitor – causes severe subacute neurotoxicity and extensive brain oedema in mice. Finally, genetic deletion of NTE from mouse neural cells causes a progressive neurodegeneration. Data will be presented which address the question: are all these diverse syndromes caused by inactivation of NTE?

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3D structure of mammalian paraoxonase at 2.2 Å resolution

Joel Sussman, Amir Aharoni, Michal Harel, Leonid Gaidukov, Boris Brumshtein, Olga Khersonsky, Shai Yagur, Lilly Toker, Israel Silman, Dan Tawfik

Weizmann Institute of Science, Department of Structural Biology, Rehovat, Israel

Members of the serum paraoxonase (PON) family display a wide range of physiologically important hydrolytic activities, including drug metabolism and detoxification of nerve agents. PON1 and PON3 reside on high-density lipoprotein (HDL, 'good cholesterol') and are involved in the prevention of atherosclerosis. We describe the first crystal structure of a PON family member, a directly evolved variant of PON1, at a resolution of 2.2 Å. PON1 is a six-bladed β-propeller with a unique active-site lid that is also involved in HDL binding. The three-dimensional structure and directed evolution studies permit a detailed description of PON1's active site and catalytic mechanism, which are reminiscent of secreted phospholipase A2 and squid DFPase, and of the routes by which PON family members diverged toward different substrate and reaction selectivities.

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Acetylcholinesterase: emergence from a vulnerable target to a template for antidote and detection development

Palmer Taylor [1], Zrinka Kovarik [2], Elsa Reiner [2], Zoran Radić [1]

[1] *University of California, San Diego, USA;* [2] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Applications of recombinant DNA technology, chemical synthesis on biological templates and high throughput detection provide unexplored avenues for development of antidotes and arenas for remote detection for exposure to organophosphate nerve agents and pesticides. Our research strategy is based on modifying acetylcholinesterase (AChE), the very target of toxicity, so that it serves in antidotal therapy and as a remote detection sensor. We discuss here how acetylcholinesterase, through appropriate mutations, becomes more susceptible to oxime reactivation. Since the reaction between organophosphate and the mutated enzyme remains rapid, regeneration of active enzyme by oxime becomes the rate-limiting step in the process to complete a catalytic cycle for generation of active enzyme. Accordingly, "Oxime-assisted Catalysis" by AChE provides a potential means for catalyzing the hydrolysis of organophosphates in the plasma prior to their reaching their cellular target site. In turn, AChE, when conjugated with organophosphate, can be employed as a template for 'click chemistry' synthesis of new nucleophilic reactivating agents that could potentially prove useful in reactivation at the target site as well as in catalytic scavenging. Finally, substituted acetylcholinesterase molecules can be conjugated to fluorophores giving rise to shifts in emission spectra for detection of exposure to organophosphates. Since reagents do not have to be added to detect the fluorescence change, the modified enzyme would serve as a remote sensor.

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Neuromuscular function and acetylcholinesterase activity in OP poisoning

Horst Thiermann[1], Ladislaus Szinicz[1], Peter Eyer[2], Thomas Zilker[3], Franz Worek[1]

[1] *Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany;* [2] *Walther-Straub-Institute of Pharmacology and Toxicology, Munich, Germany;* [3] *Toxicological Department of 2nd Medical Clinic, Technical University, Munich, Germany*

The most important toxic mechanism in poisoning by organophosphorus compounds (OP) is inhibition of acetycholinesterase (AChE), leading finally to death due to respiratory arrest. The disturbance of physiological functions at the level of muscarinic receptors may be antagonized competitively by antimuscarinics as atropine. However, at the respiratory muscle, namely the diaphragm, nicotinic transmission dominates, calling for other strategies, e.g. reactivation of inhibited AChE, to cope with paralysis.

For this purpose, oximes were introduced in therapy about 50 years ago. Although good effectiveness was demonstrated in vitro and in animal studies, their use in human therapy is a matter of debate until now. Such a dispute calls for unravelling the reasons for this ambiguous judgement.

In numerous experimental assays it was shown that several oximes are able to reactivate AChE inhibited by several OPs. However, it has to be taken into account that oxime effectiveness is dependent on the oxime itself, the respective OP and its post-inhibitory reactions (ageing, spontaneous reactivation), as well as on the presence of OP. By using the phrenicus-diaphragm preparation of the mouse, it could be shown that obidoxime was able to reactivate muscle AChE inhibited by paraoxon and to restore muscle force when the poison load was not too high. An increase in muscle AChE-activity was associated with an increase in muscle force and almost complete recovery of neuromuscular transmission at AChE levels higher than 40% of control. To assess whether similar effects can be observed in OP-poisoned patients, valuable information have to be extracted from individual clinical cases. To this end, the clinical course of poisoning as well as effectiveness of antidotal therapy were investigated in patients with need of artificial ventilation being treated with atropine and obidoxime. The analysis revealed that sufficient reactivation of OP-inhibited non-aged AChE was possible, if the poison load was not too high and the effective oxime concentrations were administered early and long enough. When RBC-AChE-activity was higher than some 30%, neuromuscular transmission was undisturbed indicating absence of nicotinic signs and symptoms.

Accordingly, oximes at appropriate doses, should be given as early as possible and as long as reactivation may be expected. Furthermore, investigation of neuromuscular transmission during OP-poisoning should be included in clinical routine programmes to monitor the course of OP-poisoning. Finally, it was concluded that low atropine dosing (several milligrams) should be sufficient to cope with muscarinic symptoms during oxime therapy.

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W2 Mercury—Exposure and Health Effects

19

Exposure to methylmercury due to consumption of fish in the Mediterranean

Milena Horvat, Darija Gibičar

Jozef Stefan Institute, Ljubljana, Slovenia

Daily intakes and retention of mercury from food is generally difficult to estimate accurately. In most food stuffs Hg concentration is below 20 ng/g. Mercury is known to bioconcentrate in aquatic organisms and it is biomagnified in aquatic food webs. For example, mercury in small fish is normally bellow 100 μg/kg, while in sward fish, shark and tuna values frequently exceed 1200 μg/kg. For that reason, it is generally assumed that population groups dependent on fish protein intake are exposed to higher Hg intake.

There are a number of studies carried out at the national level to estimate daily intakes of toxic substances. In case of mercury the main problem is that these reports provide data on total mercury concentrations and the percentage of Hg as monomethylmercury (MeHg) is not known. In some surveys the percentage of Hg originating from fish is provided and it is assumed that the percentage of Hg as MeHg is from 60% to 90%. Therefore fish and fish products represent the major source of MeHg to general population. However, in Hg contaminated sites other food produced in contaminated soil may also contribute considerably to intake of inorganic Hg and MeHg. The Mediterranean region is well known for the elevated presence of Hg due to natural sources. A number of Hg mines were operational for centuries, fos-

sil fuel exploitation (such as oil and natural gas, where Hg is present as a by-product) is also one of the important reason for mercury emissions into the environment. In such regions co-exposure of inorganic Hg and MeHg may occur.

The paper will address the outcomes of studies on mercury in fish carried out in the Mediterranean region and compare them with other oceans of the world. Apart from the literature data, the main source of data presented will obtained through Regional Seas Programme of UNEP and the International Atomic Energy Agency. In particular, the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL) carried out by the Mediterranean Action Plan (MAP) of UNEP through its various phases since 1975 up to now will be presented. This also includes the data management through UNEP-MAP and various studies carried out by the World Health Organization in the period from 1982 to 1988 as well as recent national studies (2000–2005) carried out in Northern Italy and Greece where mercury intakes and consequently elevated Hg concentrations were reported.

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Health effects of inorganic mercury

Lars Barregard

Salgrenska University Hospital and Academy, Gotenberg, Sweden

The most common exposure to inorganic Hg is by inhalation of mercury vapour (Hg^0) in occupational settings or from dental amalgam fillings. Low-level exposure occurs via Hg^0 in ambient air, and inorganic Hg in the diet. Exposure to methyl mercury (MeHg) results in some inorganic Hg, since MeHg is demethylated in vivo. Exposure is usually assessed using urinary Hg excretion. Typical mean U–Hg levels in the general population of Europe and the US are 0.5–2 μg/g creatinine (μg/gC).

The major target organs for inorganic Hg are the central nervous system (CNS) and the kidneys. In the CNS, high exposure causes unspecific symptom, emotional changes, memory loss, and tremor. After short-term exposure these effects are generally reversible, but persisting CNS damage may occur after long-term exposure. As for the kidney, immunologically mediated nehpritis is rare. Much more common is the effect on renal tubules with an increase in urinary enzymes or low molecular weight proteins. This early effect is generally reversible and not necessarily adverse.

Recent advances in knowledge are the reports of the growing impact of gold mining on exposure to Hg^0 with adverse health effects at heating of gold–mercury amalgam in South America and Asia. For the general population in Europe and the US, high exposure to Hg^0 from dental amalgam has been shown in long-term chewing gum users. In contrast, a large EU-funded project (EMECAP) showed that the contribution of Hg^0 from air around chloralkali plants is small.

In low-level exposed Norwegian and Swedish chloralkali workers reversible effects on renal tubules were recently found at exposure levels of only 10–15 μg/gC. There are also effects on selenium metabolism and on the thyroid (inhibition of Se-containing deiodinase), although probably not adverse. Low Se populations may be more vulnerable regarding renal effects of mercury. Some US studies indicate that dentists with very low U–Hg may suffer subtle CNS effects, but this was not confirmed by others. It has been proposed that certain polymorphisms (e.g. $CPOX_4$) could affect the sensitivity of CNS to effects of mercury.

Two high-quality epidemiological studies on low-level exposure to Hg^0 were presented this year. More that 1000 children in the US and Portugal were followed for 5–7 years after placement of amalgam or composites, in controlled randomized clinical trials. No effects of amalgam could be shown on the CNS (neurobehavioral tests) or kidneys (microalbuminuria).

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Mercury exposure in the Amazon, Brazil: Contributions from health studies

Volney de M. Camara [1], Elizabeth Santos [2]

[1] *Federal University of Rio de Janeiro,Rio de Janeiro, Brazil;* [2] *Evandro Chagas Institute, Brazil*

This is presenting some studies developed by UFRJ and IEC. A study on Hg exposure levels in 3020 newborns (NB) and mothers from Itaituba, Pará showed: mercury mean in mothers' blood 11.53 μg/L and the 16.68 μg/L for newborns. The correlation between the Hg concentration in newborn and mothers was strongly positive ($r = 0.8019$; $p = 0.000$). A comparative research on indoor mercury exposure ($n = 365$) pointed 13 individuals presenting mercury urine levels up to 86.0 μg/L. A research project for preventing indoor burning of mercury–gold amalgams decreased urinary Hg in residents from 2.90 to 1.49 μg/L. Researchers in four riverine communities not impacted by goldmining suggested

a higher background Hg concentration in the Amazon. Mean Hg hair reached 45.59 μg/g for 203 inhabitants from Caxiuanã and Hg concentration in carnivorous fishes varied from 0.006 to 2.529 μg/g.

There are a still a limited number of health effect studies. A thesis pointed higher Hg concentrations in riverine exposed community (São Luiz dos Tapajós) than not exposed riverine community (Aldeia) and association between symptoms versus mercury in hair. A comparative cross-sectional study was performed for investigating a battery of neurological development tests in two groups of 209 riverine children (exposed and less exposed) from 3 to 7 years old. Both groups showed a high proportion with "non-normal" performance, suggesting that this type of test (possibly other) presented limitations for use with Amazon communities due to cultural and educational reasons. Also important to emphasize that Hg exposure imposes multiple ecological, entomological and social impacts in the Amazon ecosystem, increasing some diseases, such as malaria.

Efforts should make for poverty reduction, malaria control, the empowerment of isolated river based populations, decrease mining exposure, etc. Health studies should discuss how the presence of selenium, consumption of fruits, Omega 3 PUFA and the genetic Amazon pattern, among others, could act as a protective agent against the toxicity of Hg in the Brazilian Amazon. Cohort (retrospective/prospective) study must be developed to observe possible changes on the pattern of mercury exposure and health effect.

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Human health effects from exposure to methylmercury from seafood

Philippe Grandjean

Institute of Public Health, IPH Research Unit of Environmental Medicine, University of Southern Denmark, Odense, Denmark

Environmental exposure to methylmercury originates from seafood and freshwater fish. Several prospective studies have assessed neurobehavioral deficits associated with prenatal exposures from the maternal diet during pregnancy, while studies in adults have focused on cardiovascular functions and mortality. All of these studies have strengths and weaknesses. One major issue is exposure assessment, which should ideally reflect the dose accumulated in the target organ, at the time of greatest susceptibility. Effect outcomes are generally non-specific and therefore susceptible to confounding, and their sensitivity to methylmercury toxicity must be considered in the light of long-term health consequences. These issues will be illuminated from data obtained in prospective studies of birth cohorts from the Faroe Islands. Exposure information was gathered from dietary questionnaires, and from mercury analysis of umbilical cord blood, cord tissue, maternal hair, and postnatal samples from the child. Clinical examinations have included neuropsychological tests, neurophysiological parameters, and cardiovascular functions. Standard statistical analysis of such data assumes that the exposure has been measured without any imprecision. Although imprecision can be included in sensitivity analyses, knowledge has been missing about the actual level of imprecision. Random or non-directional imprecision will tend to bias a dose–effect relationship toward the null hypothesis. We used structural equation model analysis to assess the imprecision of the major exposure biomarkers. Although the analytical imprecision is about 5% (coefficient of variation), we found that total imprecision of the cord–blood mercury concentration was 25–30%, and that of hair–mercury was about twice as high. In agreement with these findings, regression analyses showed that the cord blood concentration was the best predictor of methylmercury-associated neuropsychological deficits, and that correlations with hair mercury concentrations were weaker. Adjustment for confounders needs to take into account the beneficial effects of seafood, which may mask the adverse effects of methylmercury, and the possible effect of other seafood contaminants, such as polychlorinated biphenyls. The overall findings suggest that subtle adverse effects exist at low levels of exposure, and they support the conclusion of the European Food Safety Authority, that exposures to methylmercury should be minimized.

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The effects of As(III), Cu(II) and Hg(II) on the expression of MRP-transporters in long-term cultures of normal human bronchial epithelial cells

Felix Glahn [1], Michaela Jambor [1], Abdel W Torky [1], Ekkehard Stehfest [1], Stefan Hofmann [2], Heidi Foth [1]

[1] *Institute of Environmental Toxicology Halle/Saale, Germany;* [2] *Cardio-Thoracic Surgery Halle/Saale, Germany*

Human lung has to cope with exposure to low doses of heavy metals from various sources and the chronic uptake might lead to adaptation. As multidrug resistance associated proteins (MRP) transport a wide range of

substrates including some glutathione–metal complexes they might protect cells by extrusion of metal complexes. The ubiquitously expressed mammalian ABC-halftransporter (UMAT) has high sequence similarity to the fisson yeast heavy metal tolerance protein hmt1, thus it is assumed to be involved in metal ion homeostasis.

We had the rare opportunity to conduct long-term experiments with primary cultures of NHBEC from three patients (B182/1, B201/1, B222/1) which we incubated with As(III), Cu(II) and Hg(II) in concentrations proven to be non-toxic in the MTT-assay before. Cells were cultivated for 6 weeks in the presence of the investigated metals and RNA was isolated after each splitting. The expression of different MRP-isoforms and UMAT was determined by real-time RT-PCR.

The first week of treatment with As(III) down-regulated MRP1 (0.5-fold), MRP3 (0.6-fold; $p < 0.01$) and MRP4 (0.6-fold; $p < 0.05$) in B201/1. In cultures derived from B182/1 treatment with As(III) led to an up-regulation of MRP3 (1.3-fold), MRP4 (1.8–2-fold) and MRP5 (2.3-fold). After the second week of treatment with As(III) the expression of MRP1 (0.4-fold; $p < 0.01$), MRP3 (0.5-fold; $p < 0.01$; 2.5 μM), MRP4 (0.4-fold) and MRP5 (0.5-fold) was decreased in B201/1. In the cultures derived from B182/1 MRP1 was down-regulated significantly (0.75-fold, $p < 0.05$). The first week of incubation with Cu(II) significantly down-regulated the expression of MRP1 (0.6-fold; $p < 0.05$) in B201/1. In B182/1 incubation with Cu(II) slightly increased expression of MRP3. Incubation (5 days) of NHBEC B201/1 with Hg(II) significantly down-regulated MRP1-expression (0.5-fold; $p < 0.01$; 2.5 μM), whereas in B182/1 Hg(II) increased expression of MRP3 significantly (2.1-fold, $p < 0.05$). Comparing the results of cultures derived from different patients clear inter-individual differences in the reactions to As(III), Cu(II) and Hg(II) become obvious.

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Evaluation of biomarkers of exposure and effects of mercury using machine-learning methods

Darija Gibičar[1], Dragi Kocev[2], Bernard Zenko[2], Milena Horvat[1], Saso Dzeroski[2], Vesna Fajon[1], Barbara Mazzola[3]

[1] *Institute "Jozef Stefan", Department of Environmental Sciences, Jamova 39, 1000 Ljubljana, Slovenia;* [2] *Institute "Jozef Stefan", Department of Knowledge Technologies, Ljubljana, Slovenia;* [3] *Scuola Superiore Sant'Anna, CRIM Laboratory, Pisa, Italy*

During the EU funded project European Mercury Emissions from Chlor Alkali Plants (EMECAP) we evaluated the exposure to mercury in subjects living close to the mercury cell chlor-alkali (MCCA) plant and in occupationally exposed MCCA workers, compared to controls from the reference areas. Beside urinary mercury as a biomarker of exposure also biomarkers of effect were assessed: albumin, alpha-1-microglobuline (A1M) and *N*-acetyl-beta-D-glucosaminidase (NAG) in urine as indicators of kidney function damage and 8-hydroxydeoxyguanine (8-OH-dG) in urine as an indicator of DNA damage. In addition selenium in urine was analysed because of its known antioxidant protective role.

The dataset consisted of total 269 subjects: 57 chlor-alkali plant workers, 94 subjects living in 1.5 km diameter from the chlor-alkali plant, and 118 controls living 20 km south of the plant. All subjects completed a questionnaire about the location of their residence and workplace, occupational history including possible exposure to mercury, the number of teeth with amalgam fillings, as well as consumption of various types of fish, smoking habits, consumption of vegetables from their backyard gardens and questions connected to their medical history. Subjects with kidney diseases, diabetes, hypertension or extreme levels of creatinine were excluded from the data analysis.

We used one-way ANOVA to evaluate the differences between the groups in all observed parameters. In order to find associations between the attributes, machine-learning methods were used. We used WEKA's model trees and regression trees, which were validated using 10-fold cross-validation.

Results have shown significantly higher concentrations of mercury and NAG in the urine of chlor-alkali workers and lower concentration of their urinary selenium, compared to the subjects living close to the MCCA and the controls.

Urinary mercury was positively associated with number of teeth with amalgam fillings and negatively associated with age of the subjects and working years in the plant. Associations between A1M and NAG concentrations, teeth with amalgam fillings and urinary selenium were found.

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W3 Current Issues in Air Pollution and Health

25
Toxicity of automotive fine and ultrafine particles

F.R. Cassee

National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Ambient particulate matter (PM) has been linked with augmentation of respiratory and cardiovascular diseases and increased mortality rates. In particular diseased people (e.g. asthmatics) and elderly are at increased risk. Traffic exhaust, but also wood smoke has been suggested to contribute in particular to these health effects. The wealth of recent toxicological studies provides sufficient plausibility to explain the modes of action and underlying mechanism of these health effects. However, there has been little or at least conflicting evidence that the toxic effects also occur at lower, closer to ambient levels of PM. In addition, toxicology has shown that at an equal mass basis PM from different places or sampled at different seasons have different toxic properties. It has also been shown that size matters: particles <0.1 um seem to be more toxic than larger, yet still inhalable particles. There is evidence for source specific particle toxicity. The currently dominated discussion relates to combustion type particles resulting from various sources such as traffic (gasoline and diesel engine exhaust, lubrication oil, tire and brake ware dust). Besides that there is evidence that mineral dusts re-dispersed from, e.g. road surfaces express different toxic potentials. Even though there still are admitted gaps of understanding, the general principle of induction of the viscous circle of oxidative stress and inflammation provides a reasonable causality of almost all respiratory and cardiovascular diseases associated with PM exposure. This paper will review recent toxicological information on traffic related ambient PM.

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School children's exposure to ambient air pollution in Bangkok, Thailand

Mathuros Ruchirawat

Chulabhorn Research Institute, Bangkok 10210, Thailand

Urban air pollution resulting from traffic is a major problem in many cities in Asia including Bangkok, Thailand. These rapidly expanding cities tend to have significantly more traffic congestion than most others. Such pollution originates mainly from incomplete fossil fuel combustion, the composition of which is very complex and some elements of which are carcinogenic in experimental animals and in man. Polycyclic aromatic hydrocarbons (PAHs) and benzene are among the major carcinogenic compounds found in urban air pollution from motor vehicle emissions.

In major cities in Asia, the levels of PAHs and benzene are relatively high compared with those in Europe or in the United States. People living in such cities, therefore, are exposed to higher levels of these carcinogenic pollutants.

The potential health effects of exposure to PAHs and benzene in air pollution have been studied in school children attending schools in inner-city Bangkok compared with those attending schools in the rural areas.

Bangkok school children are exposed to total PAHs at levels more than six-fold higher than those in the rural area. Urinary 1-hydroxypyrene, a metabolite of PAH, was also significantly higher, while PAH-DNA adducts in lymphocytes were four-fold higher in Bangkok children than rural school children.

Benzene exposure in Bangkok school children was approximately two-fold higher than in rural school children. This is in agreement with the levels of biomarkers of internal benzene dose, i.e. blood benzene and urinary *t,t*-muconic acid.

The potential health risks from exposure to genotoxic substances were assessed through DNA-damage levels and DNA repair capacity. DNA strand breaks and 8-OHdG levels were significantly higher, whereas DNA repair capacity was significantly reduced in Bangkok children. This indicates that children living in major cities may have an increased health risk of the development of certain diseases due to exposure to genotoxic substances in air pollution.

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Benzene metabolites block gap junction intercellular communication

Edgar Rivedal [1], Gisela Witz [2]

[1] *Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway;* [2] *Department of Environmental and Community Medicine, Robert Wood Johnson Med School/UMD, NJ, USA*

Benzene exposure has been related to bone marrow depression and development of leukemia. Metabolism of benzene has been shown to be required for the toxicological effects. We have investigated the effect of benzene metabolites, *trans,trans*-muconaldehyde (MUC), four MUC metabolites and hydroquinone (HQ), on gap junction intercellular communication (GJIC) in rat liver epithelial IAR cells, expressing connexin 43 (Cx43). Inhibition of GJIC is considered a possible predictor of tumor promoters and non-genotoxic carcinogens, and has also been associated with perturbation of hematopoiesis.

The benzene metabolite MUC was found to be a strong inhibitor of GJIC ($EC_{50} = 12\ \mu M$) with potency similar to that of chlordane ($EC_{50} = 7\ \mu M$). HQ inhibited GJIC with EC_{50} of 25 μM, and the metabolite OH/CHO with EC_{50} of 58 μM. The other MUC metabolites tested, CHO/COOH and OH/COOH were weak inhibitors of GJIC, while COOH/COOH had no effect. Benzene itself showed no effect on GJIC when tested in concentrations up to 20 mM.

Relative potency of the metabolites on inhibition of GJIC appears similar to their hematotoxic effects. MUC was also observed to activate ERK-1/2 and induce a dramatic loss of Cx43 from the cells. Substances with such ability have previously been observed to interfere with normal hematopoietic development. Thus, the ability of benzene metabolites to interfere with gap junction functionality, and especially the rapid loss of Cx43, should be considered in relation to benzene's hematotoxicity and development of leukemia.

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Exposure modeling—Using operational air pollution models

Ole Hertel [1], Steen Solvang Jensen [1], Martin Hvidberg [1], Matthias Ketzel [1], Ruwim Berkowicz [1], Mette Sørensen [2], Steffen Loft [3], Ole Raaschou Nielsen [2]

[1] *National Environmental Research Institute, P.O. Box 358, Frederiksborgvej 399, DK-4000 Roskilde, Denmark;* [2] *Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen East, Denmark;* [3] *Institute of Public Health, Copenhagen University, Øster Farimagsgade 5A, DK-1014 Copenhagen K, Denmark*

For the modelling of air pollution exposures in the Danish cohort studies, a number of locally developed models have been applied. These models are including the regional scale model DEHM, the Urban Background Model UBM, and the Operational Street Pollution Model OSPM. In the first Danish exposure studies input data for the calculations were obtained manually or using information from questionnaires to the local authorities. Recently a GIS based tool, AIRGIS, has been developed. AIRGIS takes advantage of information from the unique Danish registry databases, as well as information from Danish digital maps for building images and road network. The basic requirements for calculating air quality levels at a particular address are the availability of digital maps of streets with traffic data, buildings with building heights and addresses. Data on building heights may in AIRGIS be obtained in different ways: (a) object heights above sea level of buildings minus terrain heights; (b) heights obtained from the Building and Dwelling Registry using property limits to geo-code buildings; (c) heights obtained from Digital Elevation Models. For population exposure assessment, population data can be linked to an individual address using the Central Population Register. Currently, calculations are being performed for about 20,000 addresses and in later projects more than 200,000 Danish addresses in various epidemiological cohorts. The AIRGIS system may, however, also be used to trace an individual's air pollution exposure along a route in the urban area. This has been used to test the system towards individual exposure measurements in Danish experimental studies.

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Pulmonary toxicity of *Stachybotrys chartarum* in experiment

Zuzana Kovacikova [1], Elena Pieckova [1], Erzsebet Tatrai [2], Marta Hurbankova [1], Zuzana Pivovarova [1], Silvia Cerna [1], Mirjana Matausic-Pisl [3], Jana Tulinska [1]

[1] *Slovak Medical University, Bratislava, Slovakia;* [2] *National Institute of Environmental Health, Slovakia;* [3] *Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Microbial growth affects indoor air quality and concerned occupants and workers often report respiratory symptoms. Our experiments were focused on *Stachybotrys chartarum* what has been linked to chronic intoxications, allergies, especially in babies. *Stachybotrys chartarum* produce several different metabolites with different effects, some of them can act synergistically. They may represent a potential but still poorly documented hazard. During study of indoor fungal colonization in Slovakia also several cases of *S. chartarum* were found. Isolates were cultivated and tested for their ability to produce toxic metabolites (endometabolites – concentrated in the fungus and exometabolites – released to the cultivation media). The extracts of both were tested *in vivo* in rats and *in vitro* in lung cells isolated from rats and organ cultures from chick tracheas. *In vivo* experiment: Wistar rats were intratrachealy exposed for 3 days to 4 μg of metabolites in 0.2% DMSO. After finishing the exposure the bronchoalveolar lavage was performed. It revealed lowered viability of alveolar macrophages and enhanced activity of lysosomal enzymes in exposed groups in comparison to the control one. *In vitro* experiments: alveolar macrophages and lung epithelial type II cells were isolated from rat lung and cultivated in the presence of various concentrations of metabolites. After 24 h cultivation the activity of acid phosphatase in alveolar macrophages was decreased, the production of MCP-1 and TNF-α by both cell types was changed, the number of alkaline positive type II cells was decreased and the histochemical staining with Maclura pomifera showed changes of type II cell membranes. All differences were dose-dependent. The ciliostatic activity of metabolites was followed for 3 days in organ culture from chick trachea. The effect was time- and dose-dependent and the toxicity of exometabolites was higher. The toxic effects of metabolites isolated from *Stachybotrys chartarum* were proved in all experimental models used in our experiments.

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In vitro airlifted interface exposure of human derived lung cells to indoor priority pollutants

Grazia M. Sacco, Fabrizio Pariselli, Diana Rembges

European Commission, Joint Research Centre, IHCP-Physical and Chemical Exposure Unit, Ispra, Italy

Volatile organic compounds (VOCs) are important indoor air contaminants which evaporate into the atmosphere at room temperature, and are detected in the indoor environment at concentrations often much higher than outdoors. These substances commonly found in occupational or non-occupational environments are potential causes of acute symptoms such as allergies, asthma, mucous irritation, headaches and tiredness and may substantially contribute to the increase of cancer incidence in the population. Their potential toxicity is increased by their strong lipophilicity with a capacity to concentrate in fat deposits throughout long-term exposures and to accumulate in the lipid bilayer of the cellular membranes. Hence, there is a pressing need to find "stress indicators" to rigorously evaluate the impact of these xenobiotics on biological processes.

The aim of this work was to develop a reproducible *in vitro* dynamic exposure model (using the CULTEX® device) where human tumor lung epithelial cell (A549) lines, representative of VOCs target tissue, were exposed in an airlifted interface to air pollutants in order to investigate the effects of single VOCs and their mixtures, as a simulation of *in vivo* inhalation exposure.

The study started with the application of two priority air pollutants, toluene and benzene, selected in the frame of the INDEX project ("Critical appraisal of the setting and implementation of EU INDoor EXposure limits"). Exposure to toluene and benzene concentrations ranging from 0.1 to 0.6 ppm showed reproducible and dose-related direct toxic effects (determined with LDH assay) on A549 cells. Moreover, benzene and toluene induced an inflammatory response (IL-8 stimulation), in accordance to data reported in literature.

The results obtained so far, show the sensitivity and specificity of the overall *in vitro* CULTEX® exposure set-up and suggest that this experimental model will allow us to extend the work to air pollutants mixture effects.

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S3 Perturbation of Epigenetic Status by Toxicants

31
Introduction—Relevance of epigenetic mechanisms for toxicology

Jonathan G. Moggs

Syngenta, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK

Epigenetics describes heritable changes in gene function that occur in the absence of a change in DNA sequence. The principle way in which epigenetic information is stored and propagated is via methylation of DNA at cytosine residues, to form the modified base 5-methylcytosine, and through post-translational modification of histone proteins that package genomic DNA into chromatin. Specific patterns of these epigenetic marks form the molecular basis for developmental-stage and cell-type specific patterns of gene expression that are hallmarks of distinct cellular phenotypes. Thus, whilst the DNA sequence of our genomes (genetic code) represents "stored" information, epigenetic marks (epigenetic code) represent the way in which the genetic code is organised and read. Recent research has begun to uncover the molecular basis for how our cells read and write epigenetic codes and has also revealed a close association between epigenetic changes and the predisposition to, and development of, a range of human diseases, including cancer. The importance of epigenetic mechanisms in human disease has prompted the initiation of world-wide efforts to map the human epigenome.

Perturbation of epigenetic status alters the spectrum of genes and proteins expressed in a cell, which in turn leads to alterations in cellular phenotype. It is becoming apparent that a wide range of environmental factors including diet, stress, behaviour and exposure to both natural and synthetic chemicals can influence the epigenome. An emerging body of data suggest that epigenetic perturbations may also be involved in the adverse effects associated with some toxicants, including certain classes of non-genotoxic carcinogens. Importantly, the epigenetic status of the genome can be stably propagated through mitosis and cell division, and therefore toxicants that perturb epigenetic status can potentially have long lasting effects on the phenotype of a somatic cell population. The possibility that certain environmental factors are associated with epigenetic changes that can be transmitted via the germline has also been suggested. Thus, the potential for perturbation of epigenetic status by xenobiotics represents an important area of future investigation for toxicologists.

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Cancer epigenetics: From DNA methylation to histone modifications

Manel Esteller

Cancer Epigenetics Laboratory, Molecular Pathology Program, Spanish National Cancer Centre (CNIO), Mardid, Spain

We are in an era where the potential exists for deriving comprehensive profiles of DNA alterations characterizing each form of human cancer. DNA methylation is the main epigenetic modification in humans. Tumor cells show aberrant methylation of several CpG islands, but global demethylation versus the counterpart normal cells. We have combined a candidate gene and biochemical approach to determine the overall aberrant DNA methylation in transformed cells. Our results show that CpG island promoter hypermethylation has a tumor-type specific pattern, where each gene tends to be methylated in the cancer cells driven from a particular tissue but not from others. Epigenetic silencing affects all cellular pathways: DNA repair (hMLH1, MGMT, BRCA1), cell cycle ($p16^{INK4a}$, $p14^{ARF}$, $p15^{INK4b}$, p73), apoptosis (DAPK, TMS1), hormone receptors (ER, PR, AR, RARB2, CRBP1), cell adherence (CDH1, TIMP3), detoxifiers (GSTP1) and many more (APC, LKB1, SOCS-1, ...). Promoter hypermethylation of particular genes has important consequences for the biology of that particular tumor. This is for example the case of the DNA repair gene MGMT which methylation-mediated silencing leads to transition mutations, but, at the same time, "marks" those neoplasms that are going to be more sensitive to the chemotherapy with alkylating drugs. Hypermethylation can be observed in hereditary tumors, where it may account for the "second hit" of the tumor suppressor gene. We have also developed massive genomic screenings to find new hypermethylated genes in cancer cell. From these assays we have identified new candidate tumor suppressor genes with important potential roles in the pathogenesis of human cancer.

Second, we have studied the global methylcytosine content of a large collection of normal tissues and sporadic and hereditary primary tumors. The picture that emerges shows that 85% of human cancer cells are hypomethylated when compared to the original normal cells. We have also found that the 5-methylcytosine DNA content and the number of CpG islands hypermethylated

in a given tumor are not random, but it involves environmental factors and genetic predisposition.

Overall, our data demonstrate that human tumors suffer a profound, but specific disturbance in their DNA methylation and chromatin patterns.

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Dietary modulation of epigenetic status

Simonetta Friso [1], Roberto Corrocher [1], Sang-Woon Choi [2]

[1] *Department of Clinical and Experimental Medicine, University of Verona School of Medicine, Verona, Italy;* [2] *Vitamins and Carcinogenesis Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA*

Epigenetics refers to noncoding changes within the genome transmitted through mitosis and affecting gene expression without altering genome structure. DNA methylation, histone modification and chromatin compartments remodeling are the main epigenetic features of DNA. Methylation of cytosine is the major epigenetic mechanism in mammalian DNA and it usually occurs within C:G sequences rich regions, the so called CpG islands. Differently from the rest of the genome, CpG islands are predominantly present in promoter regions or first exons, are usually unmethylated and associated with euchromatin and gene expression. Vitamins such as methoinine, choline, betaine, Vitamin B6, Vitamin B12, folate, and oligoelements including zinc and selenium are cofactors in metabolic pathways related to nucleic acid metabolism as well as DNA methylation. Folate, a water-soluble B vitamin involved in one-carbon metabolism, has gained increasing interest for its crucial role in synthesis, repair and methylation of DNA, all of which are essential mechanisms for sustaining the adequate regulation of genome function. The significance of folate metabolism is related to its function in providing one-carbon units for the synthesis of nucleic acids and *S*-adenosymethionine (*S*-AdoMet), the universal methyl donor for biological methylation reactions including that of DNA. Folate depletion and supplementation have been documented to affect DNA methylation. A more composite mechanism of genes and nutrients interrelationship, however, appears to be involved. A paradigm for the complexity of such relationships is the model of a gene–nutrient interaction between folate status and a polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) which has been reported to modulate genomic DNA methylation. The MTHFR enzyme is essential in one-carbon metabolism for its position at the intersection between DNA synthesis and DNA methylation pathways, since its substrate 5,10-methylenetetrahydrofolate serves for the assembly of thymidylate and purines and its product, 5-methyltetrahydrofolate for the synthesis of methionine and *S*-AdoMet. The common *677C>T* mutation in *MTHFR* leads to a thermolabile and less functioning enzyme that limits the methyl supply and affects DNA methylation, although only if associated with impaired folate status. This observation suggests that the interaction between a nutritional status and a mutant genotype may modulate DNA methylation and potentially affect gene expression in a gene–nutrient interaction manner. Understanding the patterns of DNA methylation through the interaction with nutrients is a critical issue, not only to provide pathophysiological explanations for the study of cell growth regulation, tissue specific differentiation and disease development, but also to identify at-risk individuals to conduct targeted nutritional-based interventions.

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Epigenetics—The missing link between genetics, disease and the environment

Stephan Beck

Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK

Following the sequencing of the human genome, one of the key tasks ahead for biomedical research is to identify and understand functional variants that define susceptibility to or protection from disease. From monozygotic twin studies, we know that the genetic contribution to many common diseases only amounts to 40–70%, leaving 30–60% to non-genetic contributions such as epigenetic and environmental contributions. While it remains challenging to measure and quantify environmental variants, novel approaches have recently been developed to identify and quantify epigenetic variants with great accuracy.

DNA methylation is the most stable type of epigenetic modification and, therefore, is a very suitable target for such analyses. Occurring naturally on cytosine bases at CpG dinucleotides, DNA methylation is intimately involved in diverse biological processes and the aetiology of many diseases, particularly cancer. It can affect genome function under exogenous influence and hence may constitute the main (and so far missing) link between genetics, disease and the environment. Differentially methylated cytosines give rise to distinct patterns and

profiles thought to be specific for gene activity, cell type and disease state. Like single nucleotide polymorphisms (SNPs), such methylation variable positions (MVPs) are informative epigenetic markers that promise to significantly advance our ability to understand and diagnose human disease.

I will present relevant public resources providing such genetic and epigenetic data and discuss our current thinking on how such data can be combined into an integrated (epi)genetic approach to common disease.

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Altered DNA methylation: An epigenetic mechanism underlying carcinogenesis

Jay Goodman

Michigan State University East Lansing, Michigan, USA

The involvement of mutagenesis in carcinogenesis needs to be reconciled with the fact that not all carcinogens are mutagens and the view that nonmutagenic events also play key roles in the transformation of a normal cell into a cancer cell. This apparent paradox can, in part, be resolved by considering the roles that altered DNA methylation, an epigenetic mechanism, play in carcinogenesis.

Gene expression is not determined only by DNA base sequence; it also depends on epigenetic mechanisms, i.e., heritable gene-regulating mechanisms not involving a change in DNA base sequence. Inheritance occurs on two levels. The transmission of genes either in the somatic sense or from generation to generation is distinct from mechanisms involved in transmission of alternative states of gene activity. Epigenetics describes the latter and involves regulation of temporal and spatial control of gene activity, e.g., changes in gene expression during development, imprinting, segregation of gene activities such that daughters of a cell exhibit different patterns of gene expression, and mechanisms that permit the somatic inheritance of a specific set of active and quiescent genes.

DNA methylation (the presence of 5-methylcytosine (5MeC) as compared to cytosine) is an epigenetic mechanism controlling gene activity. Changes in DNA methylation are not mutations because 5MeC and cytosine base pair with guanine. In general, increased methylation of a gene is associated with deceased transcription (e.g., may silence tumor suppressor genes, functionally equivalent to inactivation due to point mutation or allelic loss) and decreased methylation may up-regulate gene expression (e.g., may increase expression of oncogenes). Thus, altered DNA methylation can facilitate the aberrant gene expression underlying carcinogenesis.

The key role of DNA methylation in the regulation of gene expression will be presented in conjunction with a discussion of how altered methylation is a fundamental, epigenetic mechanism involved in carcinogenesis. Data from my laboratory will be used to illustrate how we are testing the hypothesis that susceptibility to carcinogenesis is related inversely to the capacity to maintain normal patterns of DNA methylation, and implications for safety assessment will be emphasized.

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Round Table Discussion Image of Toxicology

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The importance of the NAME in the toxicology subject area

Torbjörn Malmfors

Malmfors Consulting AB

Words do not only convey information but also affect our thinking. However, both functions are dependent on the meaning associated with the words. But words do not have any meaning by themselves but get their meaning from how they are used. By using different synonyms, with similar meaning, we convey the same information but could induce different thoughts, which among different things results in various perceptions of the meaning of the words. For instance “child”, “offspring” or “kid” is synonyms but give rise to different thoughts, both negative and positive ones depending on which associations we have to the different words. Thus, it is not unimportant which words we chose when communicating a message cf “risk” vs “safe”. Furthermore, if the use of words changes over time there might be further inconsistencies in the use of words, which seriously distort communication. “Toxicology” is a word, which besides not being well known also has come into disrepute, both because it arises negative associations and has got a changed usage and meaning. This problem has now been brought up on the agenda to improve the image of the discipline we call toxicology. There are different proposals among, which stipulating a meaning of “toxicology” will have little success mainly because “toxicology” probably always will induce negative associations. A more radical proposal is to find a more appropriate term, which easily can be connected to what we do and what aim for, has a positive image among the public and decision maker and is short enough to fit the mass media and easy to pronounce and remember. It is difficult to judge the outcome of such an

attempt but it is a fair guess that doing nothing will never get toxicology up from the trenches. An attractive term could be CHEMOBIOLOGY as biology has a positive connotation, and the term could be associated to interactions between chemicals and living organism and to how chemicals can be used compatibly with life rather than causing adverse effects.

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W4 Biological Monitoring of Carcinogens

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Methodological improvements in the biological monitoring of carcinogens

Jaroslav Mráz

National Institute of Public Health, Šrobárova 48, 100 42 Prague, Czech Republic

The biomarkers most relevant for evaluation of health risk due to exposure to carcinogens are the biological end-points. Low specificity of these biomarkers may be an advantage if the carcinogenic agent has not been identified or upon exposures to complex mixtures. However, assessment of the no-effect exposures to individual compounds is only possible by tracing the xenobiotic molecule in the body. Specific carcinogen-DNA adducts can be detected through analysis of the tissues or blood, or the adducted bases or nucleosides removed from DNA due to repair processes can be measured in the urine. For various reasons, none of these methods became a practical tool for routine human biomonitoring. On the other hand, determination of the adducts of carcinogens with proteins offers several principal advantages over the DNA adducts or urinary metabolites, the major advantage being their long-term persistence in the body. Important prerequisites for the use of the protein adducts in occupational/environmental health are applicability in health risk assessment and availability of adequate analytical procedures and instrumentation.

In the present paper we will focus on the state-of-the-art developments in monitoring of the carcinogen adducts with the blood protein globin. Depending on the analytical approach, the species to be determined include (a) the carcinogen molecule itself, following detachment from the protein, (b) the carcinogen adduct with a single amino acid or peptide and (c) the native carcinogen-globin adduct. Currently, the most widely used procedure is determination of the adducts of various carcinogenic alkylating agents (ethylene oxide, dimethyl sulfate, 1,3-butadiene, acrylonitrile, acrylamide) at the N-terminal valine of globin using the modified Edman degradation procedure. In this area, the typical methodological improvements include further chemical modification of the Edman products, clean-up procedures, and application of more sensitive and/or selective mass spectrometric techniques. Although immunoanalytical methods are a simple, rapid and cost-effective alternative to chromatography/mass spectrometry, very few immunoassays for the carcinogen-globin adducts have so far been developed.

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Quantitation of environmental chemical exposure using biological monitoring dater

Jürgen Angerer

Insitut für Arbeits-, Sozial- und Umweltmedizin, Erlangen, Germany

Biological monitoring is one of several tools for ensuring health protection in the event of exposure to chemical substances. With the aid of biological monitoring internal exposure and biochemical and biological effects can be measured. Unlike ambient monitoring biological monitoring is a measure of the amount of substance actually taken up by human tissues. Further assumptions and worst case scenarios are not necessary as long as some knowledge of sources of exposure, metabolism and kinetics is available. Biomonitoring can help to accurately access and communicate health risk, take reasonable measures for reducing exposures and omitted excessive decontamination measures.

Biological monitoring is especially important in case of carcinogenic substances which may cause fatal outcome. In the case of carcinogenic substances not only internal exposure can be accessed, e.g. by measuring metabolites in urine. Today in many cases the reaction products of carcinogenic substances with haemoglobin, the so called Hb-adducts, can be determined. Hb-adducts are surrogates of DNA-adducts which are thought to be the initial step of carcinogenesis. Hb-adducts are markers of biochemical effects which principally should more reliably than internal exposure reflect carcinogenic risk.

The possibilities of biological monitoring shall be shown on hand of such carcinogenic substances that are of great relevance for public health. Internal exposure and biochemical effects of groups of the general population shall be shown on hand of polyaromatic hydrocarbons (PAH), aromatic amines, acrylamide, tobacco smoke, etc.

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Assessment of occupational exposures and biological variability by using biological monitoring

Paola Manini

Laboratory of Industrial Toxicology, Istituto Superiore per la Prevenzione e la Sicurezza sul Lavoro (ISPESL) Research Center, University of Parma, Parma, Italy

Biological monitoring (BM) and biomarkers are used in occupational toxicology for a more accurate risk assessment of groups of workers. Although acceptable exposure limits have been fixed for the working environment, it has become clear that various factors can affect exposure, including additional skin absorption, differences in individual uptake, the degree of working practice, different workload, and the use of personal protection devices. BM is mainly aimed at (i) defining the existence of an occupational exposure; (ii) quantifying the level of internal dose; (iii) verifying that exposure limits (BEI®, BAT, BLV) are respected. As compared to ambient monitoring, BM is more expensive and complex. Several biomarkers are available for the same chemical and the meaning of the marker may depend on the sampling time. Therefore, practical issues, including cost and selection of an adequate sampling strategy, should be dealt with when planning a BM program for specific purposes. In addition, several biological and analytical sources of variability may influence biomarker levels, thus making the interpretation of BM data a difficult task. If analytical variance could be kept under control by quality assurance programs, inter-individual differences in uptake, biotransformation, susceptibility to damage, and repair capacity can result in different dose–response relationships for different groups of individuals. However, we should recognize that the main aim of BM is not to reduce, but to explain biological variance. Finally, the decreasing trend in occupational exposure levels highlighted the specificity problems of traditional biomarkers of exposure and prompted the research to the development of new biomarkers, e.g. unchanged volatile compounds in urine, minor metabolites, DNA and protein adducts. Depending on the scope and context (research or routine) different requirements of biomarkers can be envisaged in terms of validation and acceptable variability.

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Monitoring of genetic effects of occupational toxicants and modulating factors

Maria Dusinska[1], Alena Kazimirova[2], Magdalena Barancokova[2], Zuzana Dzupinkova[2], Alexandra Horska[2], Katarina Volkovova[2], Andrew Collins[3]

[1] *Research Base of Slovak Medical University, Institute of Preventive and Clinical Medicine Bratislava, Slovakia;* [2] *Slovak Medical University;* [3] *Medical University Oslo, Norway*

Sequence variations in a number of genes for DNA repair and phase I/phase II metabolising enzymes have recently been studied in many biomonitoring studies as putative biomarkers of individual susceptibility to cancer (and possibly other diseases), measured alongside markers of exposure and effect. This facilitates the quantification of potential risk of exposure at the level of individuals. We are monitoring single nucleotide polymorphisms (SNPs) to investigate how environmental exposure, nutrition and genetic factors together can influence genomic stability. This molecular epidemiological approach will allow us to assess the potential risk of environmental exposure and other factors at the level of individuals.

We conducted a biomonitoring study in three factories in Slovakia producing asbestos, glass fibres and rockwool. Altogether 239 exposed and 148 controls were investigated. Polymorphism in glutathione *S*-transferase *GSTP1 a* and *b* were determined in the A → G transition at nucleotide +313 by PCR. *GSTM1 and GSTT1* deletions were characterised by multiplex PCR. SNPs in five DNA repair genes were also determined: *XRCC1* (exon 10, G/A, Arg399Gln); *XPD* (exon 10, G/A, Asp312Asn and exon 23, A/C, Lys751Gly); *XPA* (5′ non-coding region, 23A/G); and O^6-methylguanine-DNA methyltransferase (*MGMT*, promotor-enhancer, 1099C/T). We also measured DNA damage (strand breaks, base oxidation and alkylation, using modified comet assay); individual DNA repair capacity in lymphocyte extracts; micronuclei and chromosome aberrations; cellular defences (intrinsic antioxidants, antioxidant enzymes); humoral and cellular immune markers, growth factors and proinflammatory mediators.

We analysed the association between SNPs in repair genes and the various biomarkers of DNA stability,

and found several interesting associations that appeared simultaneously in different subgroups. Presence of the *XPA A* allele was associated with higher levels of DNA damage as well as with higher activity of OGG1 repair enzyme. OGG1 repair activity also increased with age, but when analysed according to *XPA* genotype, the increase was observed only in those individuals with an A allele. While *XPA* is known as a protein involved in nucleotide excision repair of UV-induced damage and bulky DNA adducts, it may also have a role in the repair of oxidised bases. Our results also show that *GST* polymorphisms may be involved in oxidative DNA damage. The individuals carrying the *GSTP1 b/b* and *GSTT1* null showed a higher level of oxidised bases in smokers' DNA. The association found in many subgroups suggests that the investigated genotypes may affect genomic stability and thus may contribute to individual susceptibility and variation in response to endogenous and exogenous factors.

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Gene expression alterations in human lung tissue associated with chronic tobacco smoking: Possible mechanisms involved in smoking related diseases

Kent Hart, David Ryberg

National Institute of Occupational Health, P.O. Box 8149, Dep., Oslo, Norway

Tobacco use is a major cause of death from cancer, cardiovascular and pulmonary diseases. Although these associations have been known for decades and numerous studies have been conducted focusing on tobacco smoking and disease mechanisms, these mechanisms are not fully elucidated. One complicating factor may be the extreme complex mixture of chemicals in tobacco smoke. Available relevant model system is also a problem: the adverse effects are probably the results of complex interactions between many factors, both chemical and biological. In this study we have used Affymetrix chips to measure the expression of nearly 9000 genes. Samples from non-tumorous lung tissue were obtained from surgically treated lung cancer patients. The study groups comprised 32 current smokers and 25 ex-smokers with at least 10 years of abstinence. The two groups were otherwise matched with respect to age, sex and occupational history. The expression data from all the chips were normalized and summarized using the GCRMA module developed in the statistical package R. Two different approaches were used for the biological interpretation of genes differently expressed among smokers and ex-smokers, both based on analysis of gene sets. In the first method we selected only significantly different expressed genes (FDR < 0.1) and used this list for the analysis of enriched GO terms (gene ontology) and overrepresentation of genes in known metabolic and signaling pathways. In the second approach we used all genes and expression values in a gene set enrichment analysis (GSEA) using all known metabolic and signaling pathways. A variant of this method was also used for analysis of gene regulatory networks based on documented transcription factors. Together these methods gave biological interpretations of the expression data which were very consistent.

Our results indicate the lung tissue from chronic tobacco smokers have significantly alterations in fatty acid and cholesterol metabolism, which may affect both the composition and properties of its membranous systems. This also seems to affect cell adhesion and the recruitment and function of immunological cells. Our data indicate involvement of specific cell signaling systems and transcription factors behind these alterations.

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Exposure to high level-benzene and ultrafine particulate matter present in ambient air in Cotonou, Benin: Assessment of biological markers

Patrice Avogbe [1], Lucie Ayi-Fanou [1], Benjamin Fayomi [2], Peter Moller [3], S. Loft [3], P. Vinzents [3], Herman Autrup [4], Ambaliou Sanni [1]

[1] *Laboratoire de Biochimie et de Biologie Moléculaire, Université d'Abomey-Calavi, Benin;* [2] *Unité d'enseignement et de Recherche en Science de la Santé, Université d'Abomey, Calavi, Benin;* [3] *University of Copenhagen, Institute of Public Health, Copenhagen N, Denmark;* [4] *University of Åarhus, Department of Environmental and Occupational Medicine, Åarhus, Denmark*

Air pollution has worsened over the past two decades in Cotonou, the economic capital of Benin, where two stroke motorbikes represent the major form of transportation. Very few studies have been conducted to understand the effect of air pollutants on human's health

in developing countries. This is the first time such a study has taken place in the Republic of Benin.

Exposure was characterized by measuring ultrafine particulates matter (UFP), benzene and polycyclic aromatic hydrocarbons (PAHs), as well as urinary excretion of *S*-phenylmercapturic acid (*S*-PMA), a biomarker of benzene exposure. Oxidative DNA damage was investigated in mononuclear blood cells as DNA strand break (SB) and formamidopyrimidine DNA glycosylase (FPG) sensitive sites in taxi-moto drivers ($n = 28$), subjects living near highly trafficed roads ($n = 37$), and village residents ($n = 28$). Genetic polymorphisms in glutathione *S*-transferase (GST) and NADPH quinine oxidoreductase 1 (NQO1) on biomarker levels were investigated.

Air measurements showed high levels of benzene (in excess of 70 $\mu g\, m^{-3}$), PAHs and UFP in Cotonou compared to the village. High levels of *S*-PMA were obtained in urine from subjects living in Cotonou compared to those living in the rural areas. Taxi-moto drivers had higher level of FPG sensitive sites than the other groups.

Genotyping analysis revealed that subjects with GSTT1 null genotype had lower urinary *S*-PMA excretion than subjects carrying the plus genotype.

The correlation between *S*-PMA and SB was strongest in subjects with the NQO1*1/*2 and *2/*2 genotypes ($R = 0.37$), and between *S*-PMA and FPG sensitive sites in subjects with the GSTP1*B/*B genotype ($R = 0.39$).

In conclusion, the air in Cotonou is poor, containing high levels of benzene and UFP. Subjects living in Cotonou had an elevated level of SB and FPG sites, and that NQO1 and GST genes may modulate the effect.

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S4 Developmental Neurotoxicity and Food Contaminants

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Food contaminants and the developing nervous system: Human evidence

Gerhard Winneke [1], Michael Wilhelm [2], Jürgen Wittsiepe [2], Ulrich Ranft [1], Ursula Krämer [1]

[1] *Heinrich-Heine-Universität, Düsseldorf, Germany;* [2] *Ruhr-Universität Bochum, Germany*

The most comprehensive set of human research data dealing with dietary exposure to neurotoxic contaminants in the environment is available for organic mercury, namely methylmercury (MMC), and for polychlorinated biphenyls (PCBs), both of which are poorly and slowly degradable and therefore have a potential to accumulate in the general environment and in the food chain. In both cases long-term cohort studies in infants and young children have been performed worldwide. Environmental exposure to MMC is primarily via marine food items, whereas more general PCB-exposure occurs through diets high in animal fat including marine food and dairy products. The focus of this lecture will be on PCB-associated neurodevelopmental adversity.

In addition to more historic cohort studies at least six more recent studies are available; despite some discrepancies in detail neurodevelopmental delay/deficit was observed to be associated with PCB-exposure in most of them (see review by Schantz et al., 2003). Our own experience is based on two cohorts of between 170 and 230 healthy mother/infant-pairs established between the years 1993 and 1995 on the one hand (Walkowiak et al., 2001; Winneke et al., 2005), and between 2000 and 2003 on the other (ongoing). In the earlier cohort pre-/perinatal exposure was in terms of indicator PCBs (CB138, 152, 180) in cordblood and maternal milk, respectively, whereas in the later cohort a larger spectrum of PCBs and PCDD/Fs was measured in maternal blood and milk (Wittsiepe et al., 2006). The Fagan Test of Infant Intelligence (FTII), the Bayley Scales of Infant Development (BSID) and the Kaufman Achievement Battery for Children (K-ABC) were administered as early measures of cognitive development. The quality of the home environment (HOME) and maternal intelligence were taken as important environmental and genetic confounders, respectively.

At median PCB-levels of 404 ng/g milk fat in the earlier study significant PCB-related BSID- and KABC-decrements were observed at 30 and 42 months of age, but no longer at school age. At median PCB-levels of 177 ng/g milk fat in the 2nd study no exposure-related BSID-decrement was seen at 12 and 24 months, any more. It is concluded that PCBs or PCB-associated PAHs as food contaminants have a negative impact for early cognitive development. The clarification of the mechanistic basis, the possible role of PCDD/Fs, and the persistence of such adversity still remains a challenge for future research, however.

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In vitro systems to study developmental neurotoxicity of food contaminants

S. Ceccatelli, C. Tamm, R. Tofighi, C. Johansson

Division of Toxicology and Neurotoxicology, Institute of Environmental Medicine, Karolinska Institutet, SE-17177 Stockholm, Sweden

The developing brain is particularly vulnerable to toxic agents, even at exposure levels that have no lasting effects in the adult nervous system. Because of a growing recognition of an apparent increase in the incidence of developmental disabilities, considerable attention has being focused on the effects of exposures to environmental pollutants, including food contaminants such as methylmercury and PCBs. The use of cell cultures has proven to be a powerful approach to study and elucidate the mechanisms of toxicity. However, the complexity of the nervous system requires the use of various cellular models representing specific *in vivo* targets. We have used cultures of neural stem cells (NSCs) as *in vitro* models to investigate the developmental neurotoxicity of food contaminants. The neural progenitor cell line C17.2 cells and primary cultures of embryonic cortical-NSCs (cNSCs) were exposed to methylmercury (MeHg) to evaluate the effects on survival and differentiation. The results show that NSCs are highly sensitive to MeHg, especially cNSCs. MeHg induces apoptosis in both models via Bax-activation, cytochrome *c* translocation, and caspase and calpain activation. Remarkably, exposure to MeHg at concentrations comparable to the current developmental exposure (via cord blood) of the general population in many countries, inhibits spontaneous neuronal differentiation of NSCs. The effects of low concentrations of MeHg, relevant to human exposure, on spontaneous neuronal differentiation of NSCs point to the need for further investigations of NSCs exposed to sub-toxic doses of neurotoxic contaminants. By confirming the *in vivo* data on the increased sensitivity of the developing nervous system to MeHg, our study shows that cultures of NSCs are good *in vitro* models to identify the neurodevelopmental effects of toxic substances. The difference in sensitivity to MeHg that we have observed in NSCs compared to other neural cells further strengthens the need for multiple cellular models when *in vitro* studies are used for identifying the toxic effects of potential neurotoxicants.

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Mechanisms of developmental neurotoxicity: Molecular and behavioral correlates

Blanca Piedrafita, Pilar Monfort, Omar Cauli, Marta Llansola, Regina Rodrigo, Carmina Montoliu, Nisrin El Mlili, Vicente Felipo

Laboratory of Neurobiology, Centro de Investigacion Principe Felipe, Valencia, Spain

Prenatal and neonatal exposure to neurotoxicants may affect brain development and function. Neurotoxic agents present in the environment and especially in the food chain may reach the brain of the fetus or the newborn during critical periods of brain development. These agents may affect cerebral function and development resulting in long-lasting or permanent deficits in cerebral function that can be reflected at different ages (young ness, adulthood or elderly) as alterations in motor function or coordination and/or altered intellectual function with alterations in learning ability and/or memory. These neurological alterations would be consequence of alterations in the function of one or more neurotransmitter systems which, in turn, are due in many cases to alterations in signal transduction associated to receptors of different neurotransmitter systems. Our group has been studying in the last years the molecular mechanisms by which exposure of rats to neurotoxic agents leads to alterations in learning ability. We have found that several neurotoxic agents affect the function of the glutamate–nitric oxide–cGMP pathway in brain in vivo as well as in primary cultures of neurons. Activation of the NMDA type of glutamate receptors increases intracellular calcium which binds to calmodulin and activates nitric oxide synthase, increasing production of nitric oxide (NO), which activates soluble guanylate cyclase (sGC), increasing cGMP formation, part of which is released to the extracellular fluid. This glutamate–NO–cGMP pathway modulates cerebral processes such as long-term potentiation and some forms of learning and memory. Different neurotoxicants alter the function of this pathway at different steps and by different mechanisms. The alterations in the function of this pathway in brain in vivo (analysed by brain microdialysis in freely moving rats) strongly correlate with the alterations in the ability of the rats to learn a conditional discrimination task in a Y maze. Pharmacological manipulation of this pathway to normalize its function allows to restore learning ability in rats. The results obtained show that the glutamate–NO–cGMP pathway is a main target for different neurotoxic agents and that alterations in this pathway are responsible for some of

the cognitive deficits induced by exposure to some neurotoxicants.

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Contaminants in fish: Risk–benefit considerations

L.G. Costa [1,2], L. Manzo [3]

[1] *University of Parma, Italy;* [2] *University of Washington, Seattle, WA, USA;* [3] *University of Pavia and Fondazione Salvatore Maugeri, Pavia, Italy*

Fish provide a healthful source of dietary protein and are high in nutrients such as omega-3 fatty acids. There is evidence of long-term cardioprotective and reproductive benefits associated with fish consumption. Yet, benefits may be offset by the presence of contaminants, such as methylmercury (MeHg), polychlorinated biphenyls (PCBs) and several other halogenated persistent organic pollutants (dioxins, PBDEs, PFOS). MeHg is a known developmental neurotoxicants, as evidenced by several animal studies and episodes of human intoxication in Japan and Iraq. Fish represents the main source of exposure to MeHg for the general population. Large predatory fish (swordfish, tuna) have the highest levels of MeHg contamination. Studies in populations where fish is a major food source are suggestive of adverse health effects in children. Based on these findings, provisional tolerable weekly intakes of 0.7–1.6 μg/kg have been set by regulatory agencies. These limits may be exceeded if a diet rich in contaminated fish is consumed. Farmed and wild-caught fish appear to have similar levels of contaminants. The possibility that other contaminants present in fish (e.g. PCBs) may have additive or synergistic effects with MeHg on development has also been suggested. Advisories are in place that recommend limited consumption of certain fish in children, pregnant women and women of childbearing age. Yet, fish consumption during pregnancy and in childhood has been shown to have beneficial effects on behavioral development. Most often, fish that have high levels of deleterious contaminants also have high levels of beneficial omega-3 fatty acid, and vice versa. Careful risk–benefit considerations should foster fish consumption while minimizing exposure to neurotoxic contaminants (supported in part by PRIN 2004 and DEVNERTOX-CONTRACT no. FOOD-CT-2003-506143).

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Chronic administration of benzo(*a*)pyrene (BaP) modulates specific behaviours and expression of *N*-methyl-D-aspartate (NMDA) receptor genes in mice

Henri Schroeder [1], Nathalie Grova [2], Emmanuel Prodhomme [2], Sophie Farinelle [2], Anne Valley [1], Claude P. Muller [2]

[1] *Neurosciences Comportementales, URAFPA, INRA UFC340, Nancy, France;* [2] *Institute of Immunology, LNS, Luxembourg*

The behavioural performances of adult female mice chronically exposed to BaP (0.02–200 mg/kg/day, 10 days, i.p.) were monitored in tests related to spatial learning and memory (Y maze, Morris water maze), anxiety (elevated-plus maze, hole–board test), locomotor activity (open-field), and motor coordination (Locotronic apparatus). At low doses (0.02 and 0.2 mg/kg), BaP impaired short-term learning and memory capacities in both mazes. Surprisingly, in the Y maze, performances of mice exposed to the highest dose of BaP (200 mg/kg) were similar to those of control animals. A desinhibition state, possibly related to an anxiolytic-like effect of BaP was observed in these two situations and may have blurred the learning deficit in these mice when they were faced with a new situation. In the elevated-plus maze and the hole–board apparatus, BaP-treated mice (200 mg/kg) appeared to be less anxious than controls, reflected by significant increases of the time spent in the open arms (+19.5%), and the total number of head-dips (+21%). None of these effects are related to motor impairments because of the lack of effects of BaP on locomotor activity and motor coordination. Blood and brain levels of BaP and its metabolites were investigated using an HPLC method. The results showed a statistical correlation between the dose and the blood and cerebral levels of BaP and its metabolites. The chronic administration of BaP was also demonstrated to modulate the gene expression of glutamate NMDA receptor NR1, NR2a and NR2b subunits in various brain areas. In hippocampus, NR1 subunit mRNA expression increased up to 17-fold in a dose-dependent manner whereas NR2a and NR2b expression remained unchanged. Moreover, an overexpression of NR2a in temporal cortex was observed at the two lowest doses (0.02 and 0.2 mg/kg) followed by a decrease at 2–200 mg/kg of BaP. In conclusion, these results suggest a relationship between the behavioural impairments induced by a chronic exposure to BaP, its blood and brain concentrations, and the regional expres-

sion of NMDA-NR1, NR2a and NR2b mRNA in the brain.

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Effects of developmental exposure to methylmercury and PCB153 on cholinergic receptors at weaning and puberty in the rat

A.F. Castoldi [1], E. Roda [2], L. Manzo [1,2], T. Coccini [1]

[1] *Toxicology Division, IRCCS Maugeri Foundation, Italy;* [2] *University of Pavia, Pavia, Italy*

The susceptibility of the developing nervous system to methylmercury (MeHg) and *ortho*-substituted PCBs is well established according to epidemiological and experimental evidence.

The effects of the oral administration to rat dams of MeHg (0.5 mg/kg/day) and/or PCB153 (5 mg/kg/day) from GD7 to PND21 were investigated on the density (B_{max}) of cholinergic muscarinic receptors (MRs) in the cerebral cortex, cerebellum, hippocampus and striatum of male and female offspring on PND21 (weaning) and on PND36 (puberty) by saturation ^{3}H-QNB binding studies.

In cerebral cortex, MeHg and PCB153, administered either alone or combined, produced delayed (PND36) receptor changes, in that all treatment types decreased the density of MRs to a similar extent (10–20%) in both genders and no additive/synergistic effects were observed. At the earlier time point (PND21), only PCB153 alone affected MRs, causing a 10% decrease and a 20% increase in B_{max} values in male and female rats, respectively (control values: 714 ± 45 and 605 ± 34 fmol/mg protein, respectively).

In cerebellum, a common finding to males and females (control $B_{max} = 71 \pm 14$ and 76 ± 42 fmol/mg protein) was the MeHg-induced 15% decrease in MR density, in the absence of any effect elicited by PCB153, either alone or combined, on PND21. Notably, MeHg decreasing effect persisted in the male cerebellum up to postnatal day 36. Besides in the same gender there was also a delayed decrease in MR number caused by PCB153 alone and combined with MeHg. At variance, at the same developmental age, the female cerebellum did not display any alteration in MR number. Binding experiments in striatum and hippocampus are still ongoing.

Low doses of MeHg and PCB153, given alone throughout the prenatal and lactational periods, affect the MRs density of weanling and pubertal rats in a brain area- and gender-dependent fashion: notably, some changes with early onset persist up to puberty, while other modifications become manifest only at the delayed time point. Concerning mixture, no additive or sinergistic effects of the individual compounds are observed on this endpoint.

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S5 Pharmacovigilance in Developing Countries

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Pharmacovigilance: The French Experience

Jacques Descotes

Poison Center and Pharmacovigilance Unit

Pharmacovigilance was started in France in 1974 and became fully operational in 1984. Today, there is a total of 31 regional centres located in major university hospitals. These centres are mainly financed by each hospital administration and an annual grant of the Ministry of Health. They serve several functions, namely the identification, evaluation, prevention and reduction of adverse drug reactions (ADRs). Data on ADRs is collected either from spontaneous reporting (reporting of severe or new ADRs is a legal obligation), or periodical visit to selected hospital wards. After medical validation including causal relationship assessment, this data is entered in a national computerized databank. This confidential data can be accessed online by each centre. All centre directors meet every other month as a Technical Pharmacovigilance Committee in the Ministry of Health to share new information and identify urgent issues that require further in-depth inquiries. When judged necessary, the conclusion of inquiries is presented to a multidisciplinary panel of experts, the National Pharmacovigilance Committee, to suggest measures to be taken by health authorities. Another important function of regional centres is information toward health professionals. Indeed, most ADRs are reported via inquiries from medical doctors or pharmacists who need assistance for the diagnosis of a pathological condition in a given treated patient. Therefore, Pharmacovigilance centres often serve as drug information centres and this includes, for instance, advice on drug therapy in pregnant or lactating women, the risk of drug associations and drug treatment in at-risk, e.g. elderly patients. Information is also accomplished by participating in postgraduate and continuing education programmes, and publishing Pharmacovigilance bulletins distributed locally or regionally. More recently, a new role of Pharmacovigilance centres emerged: the evitability of ADRs in relation to inappropriate use,

inaccurate prescription, or inadequate labelling. Most regional centres function within clinical pharmacology departments, and a few in poison centres. The staff comprises of medical doctors, usually clinical pharmacologists, and pharmacists. Because of their expertise in the management and validation of ADRs, they often serve as experts to local health authorities, e.g. drug hospital committees, and the French Agency on Health Products (AFSSAPS).

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WHO perspective on pharmacovigilance in developing countries

Mary R. Couper

World Health Organization, Geneva, Switzerland

Development of pharmacovigilance in resource poor settings of Africa faces several challenges. The biggest challenge is the absence of qualified personnel to carry out pharmacovigilance work and the acute lack of resources and political commitment. Other problems include poor communication facilities, unbridled and uncontrolled supply of medicines and absence of functional national drug regulatory authorities.

The WHO Programme for International Drug Monitoring, which was established in 1968 has, to date, 79 countries as full members to the programme and 19 associate members. Of these only six full member countries are from Sub-Saharan Africa where pharmacovigilance is still often considered a luxury. However, in many of these countries, treatment programmes are being expanded rapidly following the release of significant funding for procuring medicines for treating malaria, tuberculosis and HIV/AIDS. Not infrequently, several disease control initiatives, involving the administration of several medicines, are carried out among the same population, with little or no understanding of how these various medicines could interact. Moreover, the conditions and populations in which many of these medicines were tested often differ radically from the conditions and populations of large-scale treatment programmes. Recognizing this and conscious of the resource constraints in introducing a fully-fledged 'generic' pharmacovigilance programme, WHO is now seeking to roll-out a programme of 'disease-driven' pharmacovigilance; disease programme managers are being trained to monitor and manage adverse reactions to medicines in specific public health programmes as a first step towards introducing pharmacovigilance into countries. It is hoped that with time, individuals thus trained would teach others the basics of ADR surveillance and help set-up nationwide monitoring for all types of medicines. WHO is now organizing a series of training workshops, for introducing pharmacovigilance into public health programmes.

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Issues with pharmacovigilance in Eastern Europe countries: The industry perspective

Ivana Hanzl Dujmovic, Ivana Starešinić Šernhorst

Belupo, Pharamaceuticals and Cosmetics, Koprivnica, Croatia

The new EU pharmaceutical legislation emphasises the importance of ensuring the continued safety of medicinal products in use with the main purpose to protect the public health. To fulfill this task the Member States are obliged to establish pharmacovigilance systems, which could be continually adapted to take account of scientific and technical progress. The Eastern European countries are oriented towards the same goal on their way to the community. They are building the legal framework, which could enable the transposition of the European standards into the national legislation on medicines. At the same time, they are establishing or adapting their national pharmacovigilance system with the aim to make it as close as possible to the European model.

Structure and functioning of the pharmacovigilance system in Croatia will be presented. Croatia has a long tradition in the follow-up and reporting of adverse drug reactions. It has been the WHO collaborative center for ADRs reporting since 1974. As the EU accession country, Croatia is in a phase of harmonization of the national legislation on medicines with the new European requirements. It has also established the new pharmacovigilance system in which the Croatian Agency for Medicines and Medical Devices is acting very actively involving all stakeholders—pharmaceutical industry, healthcare professionals and patient organizations.

As the Croatian pharmaceutical company, Belupo is faced with a lot of changes in the field of pharmacovigilance in Croatia as well as in the Eastern Europe countries outside the EU, which are traditionally Belupo's target markets. Belupo's accumulated experience from the mentioned countries will be compared with its experience of the EU system (EudraVigilance, data sources, PSURs, etc.).

Pharmacovigilance is an important task for pharmaceutical industry as well as for all other stakeholders. The current system in the all mentioned countries could be

improved to rationalize and strengthen the system, making more efficient use of the resources available within pharmaceutical industry and the Regulatory Authorities, especially related to the medicinal products which have been on the market for a relatively long period of time ("established" products).

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EUROTOX-SOT Debate

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OMICS research adds substantially to the safety assessment of chemicals: The case against

Alan R. Boobis

Imperial College London, UK

Omics technologies, which include genomics, transcriptomics, proteomics and metabonomics, offer tremendous potential in toxicological research. However, the logistical, statistical, bioinformatic and biological challenges in their application and interpretation of the data are formidable. Their very power, and the amount of information that can be generated, demands that fitness for purpose be established before they be used to help make key decisions on the safety of chemicals. The potential contributions of omics approaches in hazard identification, hazard characterisation, exposure assessment and risk characterisation will be addressed and the reasons why omics have not yet added substantially to the safety assessment of chemicals will be discussed.

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Motion: "Omics are of Value in Safety Evaluation"

Dan Nebert

University of Cincinnati, Cincinnati, OH, United States

Pro the Motion: The two most important factors affecting interindividual risk of an environmental disease (toxicity or cancer) are [a] the amount and type of environmental exposure and [b] one's genes. Highly accurate (**'omics**) tests for genetic susceptibility to toxicity and cancer have been sought, in order to identify individuals at increased risk**;** this type of research represents the leading edge of genotype-phenotype association studies, and is a major goal of public health and preventive medicine programs. The latest promising advances, as well as shortcomings—in the fields of genomics, transcriptomics, proteomics, and metabonomics—will be examined. How soon will we be able to determine with certainty an unequivocal phenotype or genotype?

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S6 Factors Governing Susceptibility to Chemical Allergy

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Impact of the route and characteristics of exposure on the acquisition of sensitisation to chemical allergens

Rebecca J. Dearman

Syngenta CTL Macclesfield, Cheshire, UK

Many chemicals cause skin sensitisation resulting in allergic contact dermatitis, a delayed type hypersensitivity reaction. Chemical respiratory allergy is also an important occupational health problem, but there are currently available no validated methods for hazard identification. This is partly due to the fact that the relevant cellular and molecular mechanisms of sensitisation of the respiratory tract remain uncertain, with particular controversy surrounding an obligatory role for IgE. There is now increasing evidence that respiratory sensitisation is associated with the preferential activation of type 2 T lymphocytes and the expression of type 2 cytokines. Type 2 cell products favour immediate type hypersensitivity reactions, serving as growth and differentiation factors for mast cells and eosinophils, the cellular effectors of the clinical manifestations of the allergic reactions, and promoting IgE antibody production. In contrast, chemical contact allergens induce type 1 cytokine expression profiles. For chemical respiratory allergens in particular, there is debate also regarding the routes through which exposure may result in the acquisition of respiratory sensitisation. Although inhalation is probably the most important and the most common route of exposure to chemical respiratory allergens, there is experimental evidence, and some limited clinical data to suggest that skin contact may result in sensitisation of the respiratory tract. Trimellitic anhydride (TMA), a known respiratory allergen, and 2,4-dinitrochlorobenzene (DNCB), a potent contact allergen that is considered not to cause sensitisation of the respiratory tract, have been used as reference allergens and induced immune responses characterised in BALB/c strain mice. It has been demonstrated that DNCB and TMA provoke type 1 and type 2 responses, respectively, with respect to antibody isotype and cytokine profiles, regardless of whether sensitisation is via inhalation or the skin. These data demonstrate that the ability of chemi-

cal allergens to provoke divergent qualities of immune response in mice is not a function of the route through which primary sensitisation is acquired, but is related to the inherent properties of the different classes of chemical sensitiser.

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Heritable and acquired factors influencing susceptibility to skin sensitization

Axel Schnuch

Information Network of Departments of Dermatology (IVDK), University of Göttingen, Germany

Only a part of the general population exposed to ubiquitous allergens and only a part of workers exposed to occupational allergens are sensitized to contact allergens. Under the condition that allergen exposure (in terms of dose and potency) is adequately considered, this subgroup of individuals can be qualified as being more susceptible. Although it is established, that acquired and inherent "factors" are involved, the nature of these factors governing susceptibility is not fully understood. The most important *acquired factor* increasing susceptibility is pre-existing dermal inflammation very probably enhancing (a) permeation of an allergen and (b) the specific immune response ("danger signal"). Certain inflammatory dermatosis like irritant contact dermatitis (e.g. through wet work), or stasis dermatitis and perianal dermatitis (probably caused by alterations of the venous blood flow) are associated with sensitization to even weak allergens. In addition to acquired factors, *inherent* (*genetic*) *factors* must play a role in increased susceptibility for allergic contact dermatitis (ACD), as was shown by numerous experimental and epidemiological studies, in particular experimental sensitization in twin studies. Yet the "genetic factors" themselves, neither in terms of genotype nor phenotype, were not identified. The lack of conclusive results in the study of the genetics of ACD is probably due to an insufficient definition of "susceptibility", as, in principle, the immune system of every human is capable to mount a cellular immune response, which indicates a graded susceptibility rather than an "all or none" disposition, as in certain genetic diseases. We propose the phenotype of "multiple sensitization" (MS) to be studied further. Patients with MS are sensitized more easily to dinitrochlorobenzene (DNCB) (Moss et al.), and, according to our studies (IVDK), are at a greater risk: (a) to be sensitized to a further allergen, (b) to exhibit stronger allergic reactions, and (c) to be sensitized to even weak allergens. First findings from the IVDK of functionally relevant genetic polymorphisms (TNF-alpha, IL-16) in MS are promising steps in the future study of the genetics of ACD.

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Heritable and acquired factors influencing susceptibility to chemical respiratory allergy

Piero Maestrelli

University of Padova, Padova, Italy

Chemical respiratory allergy can affect lungs and nose causing occupational asthma (OA) and rhinitis. These occupational diseases are probably the result of multiple environmental, behavioral and genetic influences.

Occupational exposure is the most important determinant of whether respiratory allergy develops. In general, the higher the level of exposure the more likely is the occurence of sensitization. However, there is still a lack of information regarding the risk of sensitization at low concentrations and the existence of a "no-effect level". Even if the level of exposure is a critical factor for the development of OA, given the same level of exposure, only a small proportion of workers will develop respiratory allergy, suggesting that host susceptibility may be involved.

Various host risk factors for OA have been established. Cigarette smoking has been reported to be associated with the development of OA in workers exposed to platinum salts and anhydride compounds, which are chemicals that cause asthma through an IgE mechanism. In contrast, cigarette smoking does not increase the risk of asthma caused by other low-molecular-weight agents, such as diisocyanates and red cedar, for which a specific IgE is not usually considered the main mechanism of the development of the disease.

Whereas skin reactivity to common inhalants is a predisposing factor in workers exposed to high-molecular-weight agents, atopy is not a risk factor for asthma induced by low-molecular-weight agents such as western red cedar or diisocyanates.

Major histocompatibility complex class II proteins may be important factors in the individual response to occupational agents such as acid anhydrides, diisocyanates, western red cedar, complex platinum salts, natural rubber latex, and animal proteins. A second pool of genes that could be involved in respiratory allergy to diisocyanates is the superfamily of glutathione *S*-transferase, a family that is critical for protecting cells from oxidative stress products. However, the associ-

ation between glutathione *S*-transferase genotype and susceptibility to occupational asthma induced by diisocyanates remains controversial. A genetic variation in *N*-acetyltransferase, another antioxidant enzyme system, was shown to be associated with the risk of diisocyanate asthma, with slow acetylators being more susceptible.

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Incorporation of susceptibility factors in the development of differentiated risk assessments

G. Frank Gerberick, Petra Kern, Cindy A. Ryan, Pauline M. McNamee

The Procter & Gamble Company, OH, USA

A number of chemicals used in consumer products today have the potential to cause allergic contact dermatitis (ACD). However, the fact that a chemical is a skin sensitizer does not mean it cannot be formulated safely into consumer products. In recent years, the application of the principles of exposure-based quantitative risk assessment (QRA) has been introduced in the contact dermatitis literature. This work has progressed well due to our understanding of the chemical, cellular and molecular mechanisms underlying ACD. Thus, it has been possible to develop a QRA approach to determine safe levels of ingredients identified as potential skin sensitizers (e.g., some fragrances ingredients, some preservatives) in different consumer product types. Key steps of the QRA process are determination of known safe benchmarks, application of sensitization assessment factors and calculation of consumer exposure through normal product use. Using these parameters, an acceptable exposure level (AEL) can be calculated and compared with the consumer exposure level (CEL). The ratio of the AEL to CEL must be favorable to support the safe use of an ingredient identified as a potential skin sensitizer. This ratio must be calculated for the ingredient in each product type. This presentation describes the key principles and steps of an exposure-based QRA for induction of skin sensitization: (1) determination of known safe benchmarks (no expected sensitization induction level or NESIL); (2) application of sensitization assessment factors (SAFs); (3) calculation of an acceptable exposure level though application of the SAFs to the NESIL; (4) calculation of consumer exposure through product use; (5) comparison of the AEL to the CEL and (6) the impact of the AEL to CEL ratio are described. In addition, the presentation will focus on the various susceptibility or assessment factors that must be carefully considered for conducting a sound QRA for skin sensitization (e.g., inter-individual variability).

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S7 Aryl Hydrocarbon Receptor (AhR) Novel Molecular and Toxicological Aspect

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AhR-dependent gene expression

Allan Okey

University of Toronto, Department of Pharmacology, Toronto, Ontario, Canada

The aryl hydrocarbon receptor (AHR) is a ligand-responsive transcription factor that regulates induction of multiple xenobiotic-metabolizing enzymes and also mediates all major toxic effects of dioxin-like compounds. Dioxin toxicity is triggered by alteration of gene expression but the specific genes whose dysregulation by dioxins leads to toxicity are not yet known. Our laboratory is using gene expression arrays to identify the full spectrum of dioxin-responsive genes in tissues from rodents treated *in vivo* with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Hundreds of genes exhibit altered expression (both "upregulation" and "downregulation") in livers of TCDD-treated rats or mice. In order to determine which of these dioxin-responsive genes are mechanistically important in dioxin toxicity we are contrasting gene expression in dioxin-sensitive rat strains with gene expression in the Han/Wistar (*Kuopio*) rat strain which is very highly resistant to TCDD toxicity. The resistant H/W rat strain has a polymorphism that deletes 38 or 43 amino acids from the AHR transactivation domain. Genes in the conventional AH gene battery (e.g. *CYP1A1*, *CYP1A2*, *CYP1B1*, *ALDH3A1*, *NQO1* & *UGT1A1*) remain responsive to TCDD in H/W rats despite the large deletion in the transactivation domain. However, the deletion may selectively alter the receptor's ability to dysregulate particular genes that are key to dioxin toxicity. In order to identify the full suite of genes whose response to dioxins is truly dependent on the AHR we used expression arrays to compare gene expression in livers of *Ahr*-null mice (*Ahr*−/−) with that in mice with wildtype AHR (*Ahr*+/+). Expression of over 400 genes was significantly altered by TCDD in an AHR-dependent manner, including high induction of the flavin-containing monooxygeneases, Fmo2 and Fmo3, previously considered to be uninducible. Very few genes were responsive to TCDD in *Ahr*−/− mice, confirming that the AHR is required for virtually all transcriptional responses to TCDD. In the absence of TCDD,

AHR status *per se* affected constitutive expression of about 400 genes including the metallothioneins Mt1 and Mt2 and a serine proteinase inhibitor, Serpina12. These array experiments are narrowing the list candidate genes to be further tested for their roles in dioxin toxicity and in normal development.

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A possible role for the AhR in regulating stress hormones and pigmentation

Agneta Rannug

Karolinska Institutet, Stockholm, Sweden

The overall aim of our current research is to advance the knowledge of the endogenous function of the aryl hydrocarbon receptor (AhR). Ligands for the AhR up regulate cytochrome P450 enzymes, in particular CYP1A1 that metabolically convert many exogenous substances to hydrophilic metabolites. However, the ubiquitous nature and evolutionary conservation of the AhR and its involvement in multiple forms of cross-talk that regulate important physiological functions suggest a role of the AhR other than a mere defense against environmental contaminants. Our hypothesis defines a function for the AhR in adapting biological functions to changes in environmental light. The hypothesis is based on the finding of tryptophan UV photoproducts with very high AhR binding affinity, which are substrates for the AhR-battery of biotransformation enzymes and extremely active transient activators of AhR-regulated responses. These photoproducts are formed also with visible light in the presence of the photosensitizing vitamin riboflavin. Based on the observations of photoproducts as potent AhR ligands and the known toxic effects of metabolically inert AhR ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the skin of exposed humans, we focus the studies on the role of the AhR and its ligands in light-regulated functions in skin cells in vitro. TCDD causes severe toxicity due to activation of peripheral as well as systemic stress responses and interaction with cell cycle progression. One such stress response, which we have detected in primary human melanocytes is activation of the melanin producing enzyme tyrosinase at 1 nM concentration of TCDD. The tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole, FICZ, also seems to activate melanogenesis. These results speak in favour of a role for the AhR in adaptation to light.

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Physiological roles of AhR in murine reproductive and immunological processes

Yoshiaki Fujii-Kuriyama [1], Takashi Baba [1], Junsei Mimura [2], Masayuki Yamamoto [3], Ken-ichiro Morohashi [4]

[1] *TARA Center, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan;* [2] *National Institute for Basic Biology, Higashiyama 5-1, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan;* [3] *SORST, JST, Honcho 4-1-8, Kawaguchi, Saitama 332-0012, Japan;* [4] *ERATO, JST, Honcho 4-1-8, Kawaguchi, Saitama 332-0012, Japan*

AhR was originally found as a binding factor for 2,3,7,8-tetrachloro-*p*-dioxin (TCDD) with great avidity and later identified as a ligand-activated transcription factor which activates the expression of xenobiotic-metabolizing *CYP1A1* gene by binding to an inducible enhancer, XRE in the promoter. Gene disruption experiments revealed that in addition to induction of the xenobiotic-metabolizing enzymes, AhR mediates pleiotropic toxicological and pharmacological effects by polycyclic aromatic hydrocarbons (PAHs) such as teratogenesis, tumor promotion, immunosuppression, and endocrine disruption. Despite its mediator functions in these adverse biological effects by PAHs, AhR is conserved well in the animal kingdom from nematode to mammalians, suggesting that AhR plays an important role in animal physiological processes. These adverse effects may be thought to be the reverse side of the multiple physiological functions of AhR. Recently, many scientific efforts have been devoted to these lines of investigations such as liver angiogenesis, immunology and reproduction.

Here, focusing on the reduced fertility of female AhR-null mice, we carried out the experiments to show that AhR plays a crucial role in female reproduction by regulating the expression of ovarian P450 aromatase (CYP19), a key enzyme for estrogen synthesis. Interestingly, AhR activated *CYP19* gene transcription cooperatively with a nuclear receptor family protein, Ad4BP/SF-1 as revealed by the *in vitro* transient DNA transfection assay and *in vivo* chromatin immunoprecipitation (Chip) assay using ovarian granulosa cells.

We have recently found that AhR functions downstream of the TLR signal transduction pathway and will also discuss the functional role of AhR in the immunological processes.

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A role for the aryl hydrocarbon receptor (AhR) in the homeostatic control of circadian behavior

Lynn T. Frame [1], Ying Liu [2], Min Lian [2], Wang Li [3]

[1] *Texas Tech Health Sciences Center Lubbock, Texas, USA;* [2] *Texas Tech University, Department of Environmental Toxicology, Texas, USA;* [3] *Texas Tech University Health Sciences Center, Department of Pharmacology and Neurosciences, Texas, USA*

Polyhalogenated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are persistent environmental contaminants, which bioaccumulate in the food chain and concentrate in human adipose tissue. The toxic effects of "dioxin-like compounds" are mediated by the aryl hydrocarbon receptor (AhR), an evolutionarily conserved transcription factor related to circadian clock proteins. Much of the available experimental data point to endogenous functions for the AhR. However, no overarching "definition" exists to explain its co-evolution with circadian clock proteins, its importance for normal development, and its strategic position as mediator of environmental disease risks. These gaps in our understanding of AhR biology limit our ability to assess risks associated with dioxin exposure and, in turn, limit our ability to formulate policies that protect environmental and human health. Several years ago, we proposed a role for the AhR in the homeostatic control of circadian behavior. In this research update, we summarize data from recent experimental and epidemiological studies, which lend further support to this hypothesis. We focus on data from studies in laboratory rodents, which indicate a role for AhR signalling in the consolidation of behavioral states and adaptation (or "entrainment") to environmental cycles of varying duration. In our "working model", therefore, we propose that the AhR co-evolved with other PAS-domain containing proteins for behavioral adaptation to environmental stress. We close with an overview of research options for the future.

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Mechanisms of AhR-dependent carcinogenicity in rats

Dieter Schrenk

Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC, 1997). TCDD acts as a liver carcinogen in female but not in male Sprague–Dawley rats (Kociba et al., 1976) and as a potent tumour promoter in two-stage rat initiation-promotion assays for hepatocarcinogenicity. Most if not all of TCDD's effects are mediated by activation of the cytosolic arylhydrocarbon receptor (AhR) a nuclear transcription factor which enhances the transcription of a wide spectrum of genes, including cytochrome P450 (CYP) 1A1, 1A2 and 1B1. The tumour-promoting action of TCDD is probably related to its ability to suppress apoptosis in preneoplastic liver foci (Stinchcombe et al., 1995). In UV-treated rat hepatocytes in primary culture addition of 1 nM TCDD significantly suppressed apoptosis (Woerner and Schrenk, 1996). Furthermore, it led to a pronounced attenuation of the p53 response otherwise observable after UV-treatment. Concomitant hyperphosphorylation of p53 indicates that an AhR-dependent kinase activity may be involved in these effects.

Both permanent ligand-independent AhR activation (in transgenic mice; Andersson et al., 2002) as well as TCDD treatment without preceding application of an initiator can lead to tumour formation. In TCDD-treated female but not male Sprague–Dawley rats, estradiol-dependent increases in oxidative DNA damage (8-oxo-deoxyguanine; 8-oxo-dG) were observed (Wyde et al., 2001). In rodent but not in human hepatoma cells TCDD treatment also led to enhanced formation of reactive oxygen species (ROS) and 8-oxo-dG. In female-derived rat hepatocytes this effect was enhanced by co-treatment with estradiol for 48 h. Furthermore, estradiol can also be metabolised by AhR-regulated CYPs to estradiol-3,4-quinone, which can bind covalently to DNA.

In summary, TCDD acts as a rat liver carcinogen probably via several mechanisms: (1) it suppresses apoptosis of preneoplastic or otherwise damaged hepatocytes via not completely elucidated, probably AhR-dependent, signalling pathways, (2) it leads to enhanced formation of ROS and oxidative DNA damage which is facilitated

by estradiol, and (3) it may lead to increased formation of estradiol metabolites covalently binding to liver DNA.

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W5 Effects of Ultrafine Particles Epidemiology and Toxicology

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Exposure to ultrafine particles and respiratory health effects

Juha Pekkanen [1,2]

[1] *Environmental Epidemiology Unit, National Public Health Institute, Kuopio, Finland;* [2] *Department of Public Health and Clinical Nutrition, University of Kuopio, Finland*

There is convincing evidence from numerous time series studies that daily variations in ambient air fine particulate pollution ($PM_{2.5}$, PM_{10}) are associated with respiratory health. However, it is not known which characteristics of particulate matter are responsible for this association, is it the chemical composition of particulate matter and/or the large number concentration of ultrafine particles (<0.1 μm in diameter) in urban air. Vehicular traffic is the main source of ultrafine particles in urban air.

There have been relatively few epidemiological studies on ultrafine particles. Most have been panel studies looking at daily variation in respiratory symptoms and lung function. Several, but not all, of these studies have suggested that daily variation in ultrafine particles number concentrations are more strongly or as strongly associated with daily variation in respiratory health than PM_{10} or $PM_{2.5}$. There are also few studies suggesting associations with respiratory mortality.

A major factor that may produce bias in these studies is the measurement of human exposure. Time series studies usually use ambient air measurements at one central site of a city as proxy for the time variation in personal exposure. However, there are no studies on the correlation between the ambient air and personal exposure concentrations of ultrafine particles. Theoretical consideration and studies looking at indoor ultrafine particles suggest that this correlation is worse that for $PM_{2.5}$, for which the correlation has been shown to be fairly good. This suggests the need to further develop methods to assess exposure to ultrafine particles.

This presentation reviews and presents new data on epidemiological studies on the association between ultrafine particles and respiratory health and on exposure assessment of ultrafine particles.

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Association between ultrafine particles and cardiovascular diseases

Francesco Forastiere

Department of Epidemiology, Rome E Health Authority, Rome, Italy

The short-term effects of air pollution on daily mortality and hospital admissions have been well established on the basis of the large multi-city studies conducted in the US and in Europe. Consistent associations have been found when gravimetric measurement of particulate matter has with particles of diameter ≤ 10 μm or in some investigations with particles ≤2.5 in diameter. Ultrafine particles (diameter < 0.1 μm) in urban air represent a potentially important health risk, and are not well characterised by mass measurements like PM_{10} or $PM_{2.5}$. However, there are only scanty data on the association between ultrafine particles and mortality or morbidity end-points. Wichmann et al. (2000) reported that over a 3-year period the concentrations of both ultrafine (<0.1 μm in diameter) and fine particles (0.1–2.5 μm) in the ambient air of Erfurt, Germany, were similarly associated with mortality in a distributed lag model where the contribution of the previous 4–5 days was evaluated. In two-pollutant models, associations of ultrafine and fine particles were largely independent of each other. In a recent investigation in Rome, the association between daily ambient air pollution levels (particle number concentration (PNC)—a proxy for ultrafine particles (diameter < 0.1 μm), PM_{10}, CO, NO_2, and O_3) and the occurrence of fatal non-hospitalized coronary events was evaluated. A 7.6% increase (95% CI 2.0–13.6) for an interquartile range of PNC (27,790 particles/cm^3) was found but a strong effect of PM10 was also detected (Forastiere et al., 2005). Finally, in a cohort of post-myocardial infarction survivors recruited in five European cities, the risk of subsequent cardiovascular rehospitalization was associated with both PM_{10} and ultrafine particles (von Klot et al., 2005). We have evaluated the association of PNC and PM_{10} with both daily mortality and hospital admissions for cardiovascular and respiratory causes in the Rome population during the period 1998–2004. Results are presented in order to examine whether the two pollutants

exert an independent effect also considering the lag structure.

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Translocation and accumulation of nanoparticles in secondary target organs after uptake by various routes of intake

Wolfgang G. Kreyling

GSF-National Research Center for Environment and Health, Institute of Inhalation Biology, and Focus Network Aerosols and Health, D-85746 Neuherberg/Munich, Germany

Nanoparticles are increasingly used in a wide range of applications in science, technology and medicine. Since they are produced for specific purposes which cannot be met by larger particles and bulk material they are likely to be highly reactive, in particular, with biological systems. On the other hand a large body of know-how in environmental sciences is available from toxicological effects of ultrafine particles after inhalation. Since nanoparticles feature similar reactivity as ultrafine particles a sustainable development of new emerging nanoparticles is required.

Cardio-vascular effects observed in epidemiological studies triggered the discussion on enhanced translocation of ultrafine particles from the respiratory epithelium towards circulation and subsequent target organs, like heart, liver, spleen and brain, eventually causing adverse effects on cardiac function and blood coagulation, as well as on functions of the central nervous system. There is clear evidence that nanoparticles can cross body membranes and reach and accumulate in the above mentioned secondary target organs.

To quantitatively determine accumulated fractions in such organs the ultimate aim is to quantitatively balance the fractions of nanoparticles in all interesting organs and tissues of the body including the remainder body and total excretion collected between application and autopsy. Substantial uncertainty remains if only selected organs are analyzed. Furthermore, in case of analysis based on a label (radioactive, fluorescent, magnetic, etc.) firm fixation of the label to the nanoparticles need to be demonstrated. Since these gross determinations of nanoparticle contents in organs and tissues do not provide microscopic information on the anatomical and cellular location of nanoparticles such studies are recommended to be complemented by electron microscopy analysis.

In addition, the role of particle parameters determining this translocation dynamics remains to be not fully understood. Nanoparticle parameters such as size, hydro-/lipophilicity, surface charge, surface ligands and their possible exchange in various body fluids need to be considered. The current knowledge on systemic translocation of ultrafine particles in man and animal models and an estimate of accumulating particle number, surface area and mass in secondary target organs during short-term and chronic exposure will be discussed in order to demonstrate the relevance of translocated fractions of nanoparticles.

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Inflammatory effects and oxidative stress caused by ultrafine particles in experimental systems

Ken Donaldson

Queens Medical Research Institute, University of Edinburgh, Edinburgh, UK

A substantial literature demonstrates that the main ultrafine particles found in ambient urban air are combustion-derived nanoparticles (CDNP) which originate from a number of sources and pose a hazard to the lungs through their potential to cause oxidative stress, inflammation and cancer. They also have the potential to redistribute to other organs following pulmonary deposition and also affect the cv system via inflammatory effects. CDNP show considerable heterogeneity in composition and solubility, meaning that oxidative stress may originate from different components depending on the particle under consideration. Key CDNP-associated properties of large surface area and the presence of metals and organics all have the potential to produce oxidative stress. CDNP may also exert genotoxic effects, depending on their composition. CDNP may translocate to the brain and also the blood, and thereby reach other targets such as the cardiovascular system, spleen and liver. Nanotechnology promises to revolutionise our lives and one of its main products is nanoparticles. These new types of manufactured nanoparticles, e.g. nanotubes need to be evaluated for their hazard. Research is being directed towards understanding whether the manufactured NP have ability cause oxidative stress and inflammation and translocate from their site of deposition, analogously to the CDNP.

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DNA damage and cytotoxic effects induced by respirable quartz in human lung epithelial cells

Delia Cavallo [1], Carla Fanizza [2], Cinzia Lucia Ursini [1], Emilia Paba [2], Aureliano Ciervo [1], Arianna Di Francesco [1], Raffaele Maiello [1], Paolo De Simone [2]

[1] *Department of Occupational Medicine, Italy;* [2] *Department of Occupational Hygiene-ISPESL, National Institute of Occupational Safety and Prevention Monteporzio Catone, Rome, Italy*

Respirable quartz is known to cause silicosis. Some epidemiological studies seem to indicate an increase of respiratory cancer risk in long-term exposed workers. IARC classification of quartz as human carcinogen highlights the need to perform further investigations in the field and to clarify the mechanisms of action of respirable quartz at cellular level. Several in vitro studies demonstrated a cellular internalization of quartz particles after 4 h of exposure. The aim of our study was to assess cytotoxic effects and direct-oxidative DNA damage induced on a human lung epithelial cell line (A549) by exposure to quartz. The cells were exposed to SRM1878a (respirable standard α-quartz from NIST) at 25, 50 and 100 μg/ml for 4 h and analysed by scanning electron microscope (SEM) and LDH release assay for cytotoxic effects evaluation. Cells were also exposed to 10, 25, 50 and 100 μg/ml of the same respirable quartz for 2 and 4 h and analysed by Fpg enzyme-modified comet test to evaluate direct and oxidative DNA damage. Observations by SEM showed a detectable dose–effect response. Cells exposed to 25 and 50 μg/ml of quartz showed small blebs, while microvilli got shorter and embedded in the cell surface at the highest dose (100 μg/ml). A slight LDH release was found only at 100 μg/ml. Fpg modified Comet test showed a dose-related oxidative DNA damage in cells exposed for 2 h to quartz. Cells exposed for 4 h at the same concentrations showed a dose-related direct DNA damage and the presence of oxidative DNA damage at lower doses particularly at 25 and 50 μg/ml. The induction of oxidative DNA damage by short-term exposure at lower doses of quartz suggests the oxidative stress as early effect of respirable quartz followed by direct DNA damage due to DNA strand-breaks at longer exposure times and at the highest dose. The bleb induction on cell surface evidenced by SEM at 25 and 50 μg/ml correlates with the presence of oxidative DNA damage at 4 h. The cell surface modifications found by SEM at 100 μg/ml indicate that high doses of quartz induce cytotoxic effects confirmed by LDH analysis and correlate with the genotoxicity showed by comet assay.

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Possible genotoxic risk in coal workers' pneumoconiosis

Ozge Cemiloglu Ulker [1], Aylin Ustundag [1], Yalcin Duydu [1], Berran Yucesoy [2], Asuman Karakaya [1]

[1] *Ankara University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey;* [2] *Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA*

Occupational exposure to coal dust causes coal workers' pneumoconiosis (CWP) which is a chronic inflammatory and fibrotic lung disease. In recent years chronic inflammation is accepted a common important factor in the pathogenesis of neoplasia. The chronic inflammation provides dynamic setting for oxidative stress and formation of free radicals. Interaction of reactive oxygen species (ROS) with DNA augments the likelihood of DNA structural and transcriptional errors. To investigate the genotoxic risk of occupational exposure to coal dust in CWP patients, sister chromatid exchange (SCE) and micronucleus (MN) tests were performed on CWP patient group ($n = 23$), coal workers group ($n = 29$) and unexposed control group ($n = 29$). Both SCE and MN frequencies in CWP patients were found significantly higher than in coal workers and unexposed group. There were no differences between coal workers and unexposed groups' SCE and MN frequencies. While positive correlations between SCE frequency and the exposure duration time or age were found in coal workers, no correlations were found in other two groups. There was no effect of smoking on the frequencies of SCE and MN in all three groups. Based on these results, it can be suggested that development of CWP related to occupational exposure to coal mine dust leads to a significant induction of cytogenetic damage in peripheral lymphocytes of CWP patients.

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Round Table Discussion Dose Dependent Transition in Mechanisms of Toxicity

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Dose-Dependent Transitions in Mechanisms of Toxicity Round Table Discussion – Introduction

Nancy G. Doerrer

ILSI Health and Environmental Sciences Institute

Analysis of dose-response curves for many agents demonstrates that multiple mechanisms exist. For example, critical, limiting steps in any given mechanistic pathway may become overwhelmed with increasing exposures, signaling the emergence of new modalities of toxic tissue injury at higher doses. Kinetic- and/or dynamic-mediated responses may be altered in a non-linear manner with increasing dose. Chemical-specific case studies show that, as the dose of an agent increases, dose-dependent transitions such as receptor interactions, altered homeostasis, and saturation of pharmacokinetic and repair mechanisms can and do occur. Such dose-dependent transitions in the principal mechanism of toxicity could have significant impact on the interpretation of reference data sets for risk assessment, as well as effects seen at high doses in traditional toxicity testing, particularly if such doses do not reflect relevant human exposure levels.

The ILSI Health and Environmental Sciences Institute (HESI) undertook a project in 2001 to explore dose-dependent transitions in mechanisms of toxicity. In partnership with the US Environmental Protection Agency, the US Agency for Toxic Substances and Disease Registry, Health Canada, the US National Institute of Environmental Health Sciences, the American Chemistry Council, and the Society of Toxicology, HESI convened a scientific workshop in 2003. The purpose of the workshop was to demonstrate the existence of dose-dependent transitions in mechanisms of toxicity through examination of case studies, and provide a forum for multi-sector discussion of data needs, experimental design, and principles for incorporating dose-dependent transitions into risk assessment decisions. Two scientific papers were published in *Toxicology and Applied Pharmacology* in late 2004 as a result of the multi-year effort.

Speakers will present principles for identifying, interpreting, and applying data on dose-dependent transitions in mechanisms of toxicity, and will use case studies to demonstrate how these data are used in a risk assessment context.

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Dose-Dependent Transitions in Mechanisms of Toxicity: General Principles

Kendall B. Wallace

Biochemistry & Molecular Biology, University of Minnesota Medical School, Duluth, MN, USA

Current regulations mandate that evaluations of chemical safety are based on sound and comprehensive knowledge of mechanism of action cast in the context of a well-characterized dose-response relationship. This makes for particular challenges when extrapolating from experimental toxicity tests performed at relatively high doses to more subtle and perhaps prolonged exposures of human populations. Although isolated steps along the pathway from exposure to biological response can be linearized and extrapolated beyond the defined dose-response curve, extrapolating the composite of multiple steps is oftentimes frustrated by the non-linear nature of the complex dose-response relationship. The source for this concern is that the path from exposure to biological response is most usually a multivariate course involving a host of sequential and branching processes, most of which have finite, self-limiting capacities. For example, absorption and disposition of an exposed dose oftentimes requires active membrane translocation across a finite number of transporter proteins each with distinct affinities for the ligand. Metabolic transformation, whether it be activation or inactivation, is another example of active processes with finite capacities and affinities, as is the association of the ultimate pharmacophore with the appropriate receptor. Finally, tissue defense and repair processes, including regenerative repair, also have finite capacities, which once exceeded constitute a departure from linear behavior. The fundamental constant underlying a linear dose-response relationship is the mechanism of action. However, saturation of any one of the multiple steps in the pathway constitutes a departure from one mechanism of action to another possibly unrelated mechanism of action exhibiting a completely different relationship to exposed dose. Hence, exploration of the full dose-response relationship for competing and saturable steps in the typical mechanism of action constitutes a complex relationship between exposure and biological response, one that is seldom a simple linear function of dose. There are numerous well-documented examples where the mechanism of action is a function of the dose, a few of which will be presented. The primary objective that challenges the projection of risk is to devise a method for incorporating considerations of dose-dependent tran-

sitions in mechanisms of action into regulatory practice.

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Dose-Dependent Transitions in Mechanisms of Toxicity: Zinc Case Example

John M. Rogers[1], George P. Daston[2], Janet Uriu-Adams[3], Lynn A. Hanna[3], Carl L. Keen[3]

[1] *Reproductive Toxicology Division, NHEERL, ORD, United States Environmental Protection Agency, Research Triangle Park, North Carolina, United States;* [2] *Procter and Gamble Company, Cincinnati, OH, United States;* [3] *Department of Nutrition, University of California, Davis, CA, United States*

Zinc (Zn) is an essential trace element. Maternal Zn deficiency can result in complications of pregnancy and inadequate supply of Zn to the conceptus can interfere with the development of numerous organ systems. Maternal dietary Zn deficiency has been shown to be teratogenic in all species tested. *In utero* Zn deficiency may result from inadequate maternal dietary Zn intake (primary Zn deficiency) or from disturbances in maternal Zn metabolism that decrease availability of Zn to the embryo/fetus (secondary Zn deficiency). Derangement of maternal Zn metabolism induced by chemical exposure is the topic of this presentation. Metallothionein (MT) is a metalbinding protein present in a number of tissues, particularly the liver. Hepatic MT can be induced by exposure to metals or a variety of organic molecules, and MT induction is part of the acute phase response to tissue damage, infection or severe stress. We have demonstrated that a variety of non-metal chemicals can induce a maternal acute phase response, including hepatic MT induction. Induction of the acute phase response leads to sequestration of maternal circulating Zn to hepatic MT, reducing maternal serum Zn and therefore Zn availability to the conceptus. Thus, induced (secondary) *in utero* Zn deficiency can be a significant mode of action for chemicals in standard developmental toxicity bioassays, particularly agents given at or near maternally toxic levels, and may also be important for women with multiple chemical exposures, infections, and/or stress during pregnancy, particularly if their dietary Zn intake is marginal or inadequate.

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Pesticide Case Example

Angelo Moretto

University of Padova

Xenobiotics may exert different toxic effects depending on dosing and route of exposure. Consequently a dose-effect relationship may be identified for each compound. In addition, for each effect a dose-response relationship exists which may overlap or intersect the dose-response relationship of other effects. This may cause transition in mechanisms of toxicity that are relevant for the understanding of the toxicity of the compound and its interpretation for risk assessment. It is also possible that certain pathway may be saturated with increasing doses, giving way to the expression of other toxic effects not to be seen without such saturation. Examples of pesticides that will be discussed showing such dose-dependent transitions include the fungicides thiocarbamates and the organophosphorus insecticide dichlorvos. These will be discussed and the importance of understanding the mode of action in relation with species-specific (patho)physiology and condition of expected human exposure will be underlined. In particular, the effects on thyroid hormones homeostasis and its consequences by thiocarbamates will be evaluated in the context of the different characteristics of rat and human thyroid function, showing that dose-dependent transition to cancerogenesis only occur at higher doses in rats. The effects of dichlorvos on esterases and DNA occur at different rates and with different concentrations. The possibility that dose-dependent transition from inhibition of nervous system acetylcholinesterase to formation of DNA adducts at higher doses (concentrations) will be evaluated in terms of plausibility in actual exposure conditions.

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Dose-dependent transitions in mechanisms of toxicity: Concluding remarks

Alan R. Boobis

Imperial College London, UK

There is ample evidence that for many compounds there are transitions in response over the experimental dose range. The challenge is to take account of this adequately in risk assessment. The session highlights the importance of deeper mechanistic understanding of toxicological processes and the need for better study design with suit-

able choice of doses, particular in the region of the dose transition. The central role played by metabolism, and toxicokinetics more generally, is emphasised as is the value of physiologically-based toxicokinetic models in exploring inter-relationships over a wide range of scenarios. There is still a need to develop clearer strategies for incorporating such information into risk assessment, and to move away from default assumptions of linearity of response above any point of departure. An area of major difficulty is estimating where on the dose-response curve human exposure is likely to be. There will also be a need to improve understanding of some of the approaches used, so that risk assessors can evaluate the information appropriately. The impact of new information on dose-dependent transitions for DNA-reactive carcinogens varies with the risk assessment policy of the region. This is an issue that would benefit from greater harmonisation.

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Saturday, September 23, 2006

PS2 Plenary Session—Bo Holmstedt Memorial Lecture

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Future challenges for toxicologists

Lewis L. Smith

Global Head of Regulatory Sciences, Syngenta plc, Switzerland

Although the past is not necessarily a useful indicator of the future, it may be of interest that by 1500 B.C. the Egyptians had an extensive knowledge of the toxic and curative properties of natural products. By 400 B.C. there is evidence that the Hippocrates understood the importance of reducing absorption of a substance for the alleviation of poisoning. In Roman times, poisoning became a common political weapon and these practices survived to the Middle Ages, where in Italy poisoning for political purposes and financial gain was well documented in the city of Venice.

Modern toxicology is probably best symbolised by the work of Paracellsus in the 16th Century and by the mid 18th Century, the Spanish chemist and physician Bonaventura Orfila, was the first recorded scientist to make systematic use of animals as test subjects and develop methods for the chemical analysis of specific poisons in tissues and body fluids. In the 20th Century the systematic testing of chemicals became increasingly regularised and more precise in terms of specific protocols which had to be followed. This allowed consistency in the regulatory testing of chemicals and drugs. However, regulatory toxicology is not necessarily defined by the science of toxicology. For example, the evaluation of mutagenic compounds uses tests that were devised in the 1970s and has been used successfully for the past 30 years. This is both convenient and comfortable, since the tests are well understood, the hurdles recognised and from the results both industry and the regulator can predict what the fate of a molecule should be. However, the science of toxicology has moved on in the last 30 years. Our understanding of the genomic and molecular control of pathways has increased almost exponentially. The endpoints used in many tests have not taken account of this new knowledge nor how pathways can predict the nature of cellular damage. This means that for many mutagenic tests the science is essentially observational and devoid of an understanding of the mode of action or mechanism of toxicity which the chemical exhibits.

Testing for carcinogens has evolved over the last 100 years. Only two or perhaps three different species of experimental animal are now used to identify carcinogens. This is, in part, driven by economic pressure and restriction on the use of experimental animals. The relevance of positive findings for non-genotoxic carcinogens has been problematic for several decades. The endpoints used have barely changed in half a century. The use of pharmacokinetic data has been largely confined to the evaluation of pharmaceuticals and only recently extended to pesticides and chemicals. The frightening lack of concordance between the organs targeted by carcinogens in rodent bioassays versus the site of cancers in humans is still barely addressed. Strain differences, within rodent species, to the response to a single chemical mean the choice of strain are almost as important to the result as the choice of chemical. The reality remains that when tumours are observed in animals the default assumption is that it will cause the same effect in humans unless evidence is produced to the contrary. However, if the chemical is an important industrial molecule, or drug, this often leads to an investigation of the mode of action or mechanism of toxicity of the chemical to establish if the results are relevant to humans. If the result of the original bioassay is negative, no additional studies are undertaken.

The future of toxicology should be driven by the identification and prioritisation of the issues which we expect to impact on human health and a more mechanistic approach to hazard characterisation. Only by understanding the mechanisms through which injury occurs and the relevance of this to humans, will

toxicology improve the predictability of injury to humans.

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W6 Biological Pesticides

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Fungi in biocontrol of plant diseases

Leif Sundheim

Norwegian Institute for Agricultural and Environmental Research, Plant Health and Protection Division, Aas, Norway

Fungi are the most important causal agents of plant diseases. Antagonistic fungal species are also becoming an increasingly important alternative to chemical pesticides in disease control. Antibiosis, mycoparasitism and competition are natural mechanisms that control the interaction between fungi in soil and on plant surfaces. The biocontrol fungi currently available employ one or more of these antagonistic mechanisms. The number of fungi commercialized as biocontrol agents increases from year to year.

Antagonistic fungi have been identified in cropping systems and in natural ecosystems. Screening of fungal isolates for biocontrol efficacy is followed by identification of promising strains. Morphology, enzymatic activity and molecular methods are employed in classification of the antagonistic fungi into existing or new taxa. Once the identity of the antagonist is established, it is desirable to screen different isolates using methods as close to practical disease control as possible. Fungal isolates should be tested under a number of different environmental conditions. Also, screening a wide genetic basis of potential biocontrol strains increases the likelihood of finding highly antagonistic strains for disease control *in vivo*.

Mass production of biocontrol strains without changes in the genetic integrity and biocontrol potential is another challenge. It is essential to develop a quality control system that monitors genetic stability over time. Regulations for registration of biocontrol agents vary from country to country. Strain authentication is necessary to protect intellectual property rights and commercial values. Molecular methods facilitate registration and commercialization of biocontrol fungi.

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Regulatory issues for biological pesticides

Ian C. Dewhurst

Pesticides Safety Directorate, York, UK

The majority of pesticides used for plant protection are based on synthetic chemicals. Regulatory assessments for potential human health effects are performed by comparing the findings in an extensive range of routine toxicity studies, designed for testing chemicals, with estimates of exposures. Recently there have been significant moves towards developing natural/biological alternatives. Biological pesticides (those based on viable organisms or simple plant extracts) present the regulator with a different set of challenges to those raised by most chemical pesticides. In particular, there are no internationally recognised test guidelines for pathogenicity and infectivity, and testing strategies are generally adapted from those for relatively pure chemicals rather than being specific to biological products.

The concerns associated with biological pesticides vary greatly between plant extracts and those based on viable micro-organism, and from one organism to another, requiring an almost case-by-case approach. However, it is possible that certain classes of biopesticides, such as viruses pathogenic to insects, might present a very low risk to human health and could be assessed accordingly. The known toxicity of certain bio-molecules (e.g. nicotine, bacterial toxins) and the pathogenicity of certain organisms (e.g. *Plasmodium* spp., *E.coli* 0157) underlines the need for a risk assessment for biological pesticides even though they are 'natural'. The main aspects of a health risk assessment for a product based on viable organisms are characterisation of the organism, levels of contamination (chemical and viable), infectivity, pathogenicity, sensitisation and production of toxic secondary metabolites. For a plant extract the main aspect is frequently related to the variations in composition associated with different growing/extraction conditions and the associated difficulty in relating commercial material to that used in toxicity tests. Reliable data on (lack of) effects associated with naturally occurring (background) exposures can sometimes provide considerable reassurance for human health risk assessments of both viable organisms and plant extracts.

This paper describes the current EC scheme together with work at the OECD level and highlights important aspects by presenting examples of biological pesticides that have been assessed under these procedures.

The views expressed are those of the author and should not be taken as being the agreed policy of the

Pesticides Safety Directorate, the UK Government or the European Commission.

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Status of biopesticides—Indian scene

P.K. Gupta

Toxicology Consulting Services Inc., C-44 Rajendranagar, Bareilly-243 122, UP, India

It is well known that most of the chemical pesticides are responsible for a variety of known and unknown toxic problems in animal and human health. These chemicals also have adverse effects on our environment. Despite large consumption of chemical pesticides, it is estimated that crop losses vary between 20% and 30% due to pests alone. Keeping this in view, it was considered that biopesticides offered as environmentally benign alternatives to chemicals. In India, till date only 12 biopesticides (such as *Bacillus*, *Trichoderma*, *Pseudomonas*, *Beauveri* species, *NPV* of *Helicoverpa armigera*, *NPV* of *Spodoptera litura*, Neem based pesticides, and *Cymbopogan*) have been registered against 194 chemicals which are used as chemical pesticides. Most of these biopesticides find use in public health for the control of malaria, except a few that are used in agriculture. The other technologies include: (i) use of transgenic plants for pest management and (ii) a number of beneficial insects and worms that fight the nastier ones is being encouraged. In addition, the Centre for Indian Knowledge Systems (CIKS) has attempted to identify the technologies that farmers were using for pest control before chemical pesticides came into the picture. The preparation of these products is extremely simple, as is their application. In terms of efficiency. CIKS' work has been to see that the extracts developed by farmers in their own backyards are as efficient as or more efficient than what is available in the market. Its motive has been one of self-reliance. Therefore, the thrust of the discussion will be on the future strategies of India to increased agriculture production and restore public health and the environment through the use of alternative methods such as biopesticides.

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Stepwise shifting to application of biopesticides in Egypt

Sameeh A. Mansour

National Research Centre Dokki, Pesticide Chemistry Department, Cairo, Egypt

Egypt is facing the challenge of maximizing its own limited resources in a cost-effective way. Specifically, the responsibility of stakeholders is to seek options and alternatives for securing food and fiber per unit area without destroying the environment and sustainability. This may interpret introducing biotechnology to the Egyptian agricultural sector since 1990. Benefits of genetically modified (GM) crops versus certain risks, lead Egypt to deal with this technology as slow as the country has not produced any commercial biotechnology crops up till now. Political, economical and social considerations have shared the slow passage of GM crops from experimental, to trials, to commercialization. The most pronounced accomplishments of the Agricultural Genetic Engineering Research Institute (AGERI) are represented by producing tuber moth-resistant potatoes; virus-resistant squash and tomatoes; corn borer-resistant maize; drought-tolerant wheat; and insect-resistant long-staple GM cotton strain. Also, AGERI has successfully managed to manufacture its first biopesticide "Agerin", which has the potential for sales on a worldwide scale. In the field of botanical pesticides, the hundreds of published articles did not due to a marketed product up till now. A way to promote shifting to application of biopesticides in the Egyptian agriculture is to support establishing of "centers of excellence" with complimentary facilities to conduct food and environmental safety. Oriented research with end users is required to make benefits from the bench-scale research in the area of new pest controlling agents. The private/public partnership is crucial for achieving nationally commercialized biopesticides.

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Main problems of modern pesticides and agrochemicals toxicology in Ukraine

Mykola G. Prodanchuk, Nadiya Nedopylanska, Peter G. Zminho

Medved's Institute of Ecohygiene and Toxicology, Kiev, Ukraine

In the recent fore decades the tasks and methods of pesticides toxicology have been established finely. The cumulative experience according to quantitative criteria of pesticides dangerous, its absorption, distribution, excretion and opinions about mechanism of action, cumulative, combine action and devious influence to human health have generalized. Therapy of the poison by the main groups of pesticides and prevention of negative influence to health was determined.

The problem of pesticides toxicology in Ukraine now has as fundamental so engineering development. Integration into world science in field of methodology of toxicological investigation and in legislation has being piecemeal.

The priority directions of pesticides toxicological investigations are the study of mechanisms of toxic, selective and combine action; ability to endocrine disruption; immunological action; the study of aging sensibility to pesticides; evolving toxicogenomic of pesticides; the development of the new approaches to test the pesticides of new generation, which used in super-low doses; the methodology of integration of risk assessment for human health; the safe methods of testing and neutralization the unusable and forbidden pesticides; the improvement of diagnostics and treatment of acute and chronic poisons, professional diseases with chemical etiology, antidote therapy.

According to Ukrainian requirements for any pesticide (as a new one, so a generic) it should be submitted full extend information about active ingredient (EI) and formulation. A special part of information documents refer to toxicological properties of EI and formulations of pesticides (acute toxicity, skin and eye irritation, sensitization – for EI and formulations and also chronic toxicity/carcinogenicity, reproductive toxicity, teratogenicity, neurotoxicity, mutagenicity and metabolism – for EI). The main institution in the problem of health safety pesticides in Ukraine is the Institute of Ecohygiene and Toxicology named by L.I. Medved is able to execute all toxicological, hygienic and chemical investigation.

Current legislation procedures and criteria for assessment of pesticides are based on the "Law of Ukraine about pesticides and agrochemicals". The main principle of state politic in pesticide sphere is the priority of health and environmental security above economic effect.

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Risk analysis of combustion products of plant protection chemicals

Cristina Andreoli, Massimiliano Picciolo, Antonella Bassi, Valentina Gobbi, Giovanni Lionetti, Alfredo Nunziata

Research Department, BAT-Italia S.p.A., Italy

The plant protection products are a class of chemical substances used in agriculture, from the sowing to the harvesting until the conservation, to the aim to ensure the production of a sufficient amount and adequate quality of agricultural products. On the other hand, the use of these chemicals implies a certain degree of risk both for farmers and consumers, and for the environment, in terms of potential chronic effects in humans and in animals and persistence in the environment.

Because of these agrochemicals can be found as contaminants in food, they have been subjected to a rigorous evaluation of risk assessment by the main regulatory agencies in the world. This process allowed the availability of a lot of reliable data on their toxicity. The totality of these information concerns the plant protection products and pesticides themselves or their biological metabolites.

The plant protection products can be introduced in the environment and constitute a source of risk for the human health also in the burnt form, through processes of agricultural product incineration, or as contaminants in the food products during the cooking, or for other uses, such as during the tobacco's combustion in the smoke products.

Our work presents the results of a preliminary analysis of risk assessment for chemical class of compounds derived by the combustion of Aldicarb, pyrethroids, pesticides containing one aromatic ring and benzimidazolyl carbamates, identified through thermogravimetric analysis and contemporaneous execution of mass spectrum. During the combustion, the molecules are subjected to processes of degradation (pyrolysis and oxypyrolysis) and formation (pyrosynthesis and addition) which lead to the fragmentation in low molecular weight species and/or to the production of new molecules through aggregations. The results showed that the thermal decomposition of the above mentioned plant protection compounds, determines the formation of small molecules, such as CO, HCN, CH_3OH, CH_3SH and of other molecules, such as *n*-nitrous derivatives, sul-

phured compounds, halogenated compounds and benzyl groups. A preliminary risk assessment of the compounds formed by termodegration of Aldicarb was performed. The pyrolysis of the Aldicarb leads to the formation of *N*-methylcarbamate and Nitrile Aldicarb, that further split up into four compounds, among them methylmercaptane. Both *N*-methylcarbamate and Methylmercaptane are neurotoxic after inhalation in humans and rats.

The study will continue with the risk analysis of the combustion products of other class of commonly used pesticides.

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W7 Novel techniques in environmental biomonitoring

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A high density flounder (*Platichthys flesus*) cDNA microarray as a tool in the identification of expression changes in gene sets predictive of exposure to pollutants

James K. Chipman [1], Steven G. George [2], Tim D. Williams [1], Amer M. Diab [2], Victoria Sabine [2], Fernando Ortega [1], Francesco Falciani [1]

[1] *School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK;* [2] *Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK*

Traditionally a number of useful biomarkers of pollutant responses in the environment have centred on the induction of stress-responsive genes. Genomic technologies offer opportunities to gain a more global assessment of the health status of an organism through an understanding of the functional pathways that are responding to pollutant exposure. This may provide additional predictive biomarkers for environmental monitoring as well as identifying toxic mechanisms. Toxicogenomics in organisms of environmental relevance is a worthy challenge. We have developed a 13,000 cDNA microarray for the European flounder (EU-GENIPOL Project). Flounder taken from different sites in Northern Europe (and of different pollution status) can be distinguished according to their hepatic gene expression profile using bioinformatic approaches. To determine which gene expression differences may relate to pollutant impact, we are completing complementary laboratory exposures of flounder to selected toxicants and determining the associated gene expression profiles. This will be demonstrated in relation to response to sublethal acute exposures to cadmium and to estrogens. In addition to the detection of changes in known biomarkers, gene ontology analyses have indicated alteration of a range of pathways. In the case of cadmium, many of the changes seen can be associated with an oxidative stress including modulation of genes involving chaperones, protein synthesis and degradation, cytoskeleton, apoptosis and cell cycle. Importantly, interactive effects have been discovered, e.g. between polycyclic aromatic hydrocarbons, estrogens and metals. A major challenge is the integration of such studies into risk assessment for example in the derivation of novel batteries of biomarkers. However, to achieve this, it is necessary to distinguish between compensatory and toxic responses and these issues will be illustrated with reference to CYP and HSP induction. This work was funded by the NERC, CEFAS and EU and has involved GENIPOL collaborations.

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Molecular ecotoxicology: From the understanding of toxicity mechanism to the application in biomonitoring programmes

Aldo Viarengo, Francesco Dondero

Università del Piemonte orientale "Amedeo Avogadro", Alessandria, Italy

A combination of genomic/proteomic/metabolomic and cellular techniques was used to assess the effects of heavy metals exposure in amoebic cells of the slime mould *Dictyostelium discoideum*. Our data demonstrated that sublethal concentrations of heavy metals affect important physiological parameters such as cytosolic free calcium concentration, lysosomal membrane stability and endocytosis, without altering the cell replication rate. A 10 K DNA microarray – covering about 90% of the *Dictyostelium* genome – was used to unveil molecular adaptations and mechanisms of toxicity evoked in Hg challenged amoebae. At fully sublethal concentrations (low-medium dose: 0.5–2 μM) Hg exposure determined the prompt activation of genes coding for glutathione neo-synthesis and glutathione transferase (gst) enzymes, and transmembrane extrusion proteins (ABC). These genes are likely to represent the first defence line against heavy metals toxicity. Other up-regulated genes were involved in heavy metal transport, toxicity resistance, detoxification, energy metabolism (mitochondrial electron transport), lipid neo-synthesis and several redox processes. At higher and lethal concentration of Hg (10 μM: LC10) we could observe the downregualtion of particular gene classes, such as the ribosomal component proteins.

In addition, extracts of control and treated cells exposed to a sublethal Hg concentration able to affect the cellular biomarkers were analysed by two-dimensional electrophoresis. A reference map containing some 900 soluble proteins was obtained. Identification of excised spots was performed by MALDI-TOF peptide mass fingerprinting, and the identity of some 150 proteins was revealed. Fifteen proteins involved mainly in detoxification and signal transduction pathways as well as a hypothetical protein possibly related to energy metabolism showed a highly significant increase in Hg-treated cells. The same extract were analyzed by high throughput metabolomic analysis showing no differences in metabolic profiles of control cells and treated cells, and therefore suggesting that changes at proteomic levels were able to keep unaltered steady states of cellular metabolites.

In conclusion, the systems biology approach can represent a potent tool to characterize adaptive responses to hazardous pollutants present in the environment, to unveil clues on their mechanism of toxicity and to develop new more sensitive tools for ecotoxicological surveys.

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Proteomics: In search of new pollution biomarkers

Juan López Barea

Department of Biochemistry and Molecular Biology, University of Cordoba, Spain

Biomarkers routinely used in pollution monitoring need a full and prior knowledge of toxicity mechanisms, being somehow biased. Proteomics may identify proteins altered by pollutants that, once validated, can be used as new unbiased biomarkers. This approach is also limited, since sequences of most studied organisms are poorly represented in sequence databases, thus rendering difficult protein identification.

Initial studies of this type were made in animals exposed to model pollutants, and lead to the concept of protein expression signatures. For instance, in *C. gallina* clams, pollutants alter 15 proteins, 4 of them identified as cytosqueletal. In spite of the clear responses to model chemicals, studies in animals from ecosystems with different pollution loads are scant. Thus, in *M. edulis*, PAHs and PCBs from polluted sites increase the HSP levels in mussels, and SELDI shows the increase of 26 peptides in animals from polluted areas.

Proteomics is being used to assess possible effects on Doñana National Park of metals from a previous mining spill and of pesticides routinely used at nearby crops, using *M. spretus* mice, *P. clarkii* crayfish and *S. plana* clams as bioindicators. Over 2000 soluble proteins are fully resolved in 24 cm wide 2-DE gels (pH 4–7). About 1% of them show differences in animals sampled at areas with different pollution loads. In crayfish and mice, maximum effects are found, respectively, in animals from watercourses and marhses and land areas exposed to the mining accident or with high pesticide use. In clams from the Guadalquivir estuary, the number of altered proteins directly correlates to the metal load derived from river deposits.

Many proteins have been shown to be altered by pollutants. They are related to heat shock, cytosqueleton, oxidative stress or biotransformation, ribosomal structure or function, proteolysis, synthesis of S-containing amino acids or GSH, and general metabolism. Different sources of protein alteration can be envisaged. Higher protein expression has been shown in several cases, but there are increasing indications that several modifications may shift protein position in 2D electrophoresis. They include limited proteolysis, oxidative damage (carbonyl or glutathionyl formation), or other modifications, including phosphorylation, nitration, or alteration of gene expression via binding to Ah receptor and its cross talk to other signal transduction pathways.

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Inhibitors of the ABC transport proteins as emerging pollutants—Determination and ecotoxicological relevance

Tvrtko Smital [1], David Epel [2], Smiljana Britivic [1], Sanja Krca [1], Branka Pivcevic [1], Roberta Sauerborn Klobucar [1], Roko Zaja [1]

[1] *Laboratory for Molecular Ecotoxicology, Division for Marine and Environmental Research, Rudjer Boskovic Institute, Zagreb, Croatia;* [2] *Hopkins Marine Station of Stanford University, Pacific Grove, CA, USA*

One of the most intriguing defense strategies evolutionary developed in aquatic organisms is the activity of the Multixenobiotic resistance (MXR) mechanism, first described in early 1990-ties by Kurelec and coworkers. As in the case of the multidrug resistance (MDR) phenomenon expressed in various mammalian tissues, it has been shown that specific transmembrane ABC (ATP Binding Cassette) transport proteins are key mediators of the MXR activity. Among them, the P-glycoprotein (P-gp, ABCB1) and the multidrug resistance-associated proteins (MRPs, ABCCs) are the best known and most

studied efflux transporters. With remarkable substrate (non)specificity these proteins actively export a diverse group of natural products, chemotherapeutic drugs and hydrophobic peptides across the plasma membrane, contributing to the selective pharmacological and toxicological barrier and providing resistance to aquatic organisms living in polluted environments. However, the main disadvantage of the MXR defense is its sensitivity to the presence of specific compounds, so-called chemosensitizers or MXR inhibitors. By blocking the P-gp and/or MRPs transport activity they can cause an increase in intracellular accumulation and toxic effects of other xenobiotics normally effluxed by the ABC transport proteins. Enhancement of MXR inhibition with concomitant detrimental effects has been shown in several studies with aquatic organisms exposed to both model MXR inhibitors and environmental pollutants. Furthermore, the presence of MXR inhibitors has been demonstrated in environmental samples from polluted locations at concentrations that could completely abolish P-gp and/or MRPs transport activity. Ecotoxicological significance of MXR inhibition is being additionally highlighted by their presence among both conventional and emerging man-made pollutants—in addition to pharmaceuticals some pesticides and synthetic musk fragrances show extremely high MXR inhibitory potential at environmentally relevant concentrations. Consequently, the MXR-inhibitors might have important implications on key environmental parameters (exposure, uptake, internal dose, bioaccumulation, response, synergism, toxicity) and within this presentation I will try to illustrate why the property to inhibit MXR defense classifies these substances among top-hazardous environmental pollutants.

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Chronic noise stress-induced alternations in the expression of Hsp70, c-fos, DNA damage and Fas/Fasl expressions in discrete brain regions of Albino rats

Sundaramahalingam Manikandan, Narayanaperumal Jeya Parthasarathy, Ramasamy Srikumar, Rathinasamy Sheeladevi

Dr. ALM PG Institute of Basic Medical Sciences, University of Madras Chennai, Tamil Nadu, India

Brain is more susceptible to stressors than any other organ. Exposure to continuous loud noise is a serious environmental health problem due to excess production of oxygen free radicals. The aim of the present work was to evaluate the effects of oxidative stress in discrete (cerebral cortex, cerebellum, midbrain, pons-medulla, hippocampus and hypothalamus) brain regions and neuronal dendritic changes after the rats were exposed to chronic noise (100 dBA/4 h/day for 30 days). Expression of *Hsp70*, *c-fos* mRNA (RT-PCR) and Fas/FasL protein expression (immunoblotting and immunohistochemistry) in discrete brain regions were studied. Results showed that neuronal dendritic count in the hippocampus and medial prefrontal cortex were reduced significantly ($P < 0.01$) in the second and third order dendrites after 30 days of noise exposure when compared to control animals. Excessive free radical generation produced by noise stress led to increases in lipid peroxidation level, superoxide dismutase activity, *Hsp70*, *c-fos* mRNA expression, Fas/FasL protein expression and concomitant decreases in the activity of catalase, glutathione peroxidase and depletion of reduced glutathione in all the brain regions. This study suggests that 30 days of noise exposure causes oxidative stress in all the brain regions and alteration in neuronal communication in HIP and mPFC and this finding may be applicable to human, are working/living in noisy environment.

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Effect of waterborne strontium on calcium-dependent proteases in fish

Liudmila A. Bondareva [1], Nina N. Nemova [1], Elena I. Kaivarainen [1], Nikolay A. Kashulin [2]

[1] *The Institute of Biology, Karelian Research Centre of Russian Academy of Sciences, Pushkinskaya, 11, Petrozavodsk 185910, Russia;* [2] *The Institute of Industry Ecology Problems of North, Kolski Research Centre of RAS, Russia*

The release of strontium (Sr) to the environment is of concern due to the strong accumulation of this calcium resembling element in the bone and other tissues. The uptake of Sr^{2+} in the whitefish, *Coregonus lavaretus* and *Coregonus albula*, was studied in chemically defined freshwater lakes in North-Western Russia including highly contaminated by Sr^{2+} Covdor Lake. The accumulation rate of Sr^{2+} in fish tissues is strongly correlated with the activity of Ca^{2+}-dependent proteases, calpains. This effect is attributed to the Sr^{2+} resembling capacity in the relation to Ca^{2+} in calcium-dependent processes and can be suggested as a part of a complex mechanism of Sr^{2+} toxicity. Calpains, having both dagradative and regulatory roles, are believed to participate in signal transduction, skeletal muscle growth, and degenerative disease. The regulatory mechanisms governing calpain activity are complex and it is known that

Ca^{2+} can be involved in cellular components binding, proteolytic activity, autolysis, dissociation, and calpastatin (specific calpain inhibitor) binding. It was shown that all indicated Ca^{2+}-dependent steps in calpain activity are affected by Sr^{2+} uptake in the *in vivo* experiment. Sr^{2+} was found to activate fish calpains *in vitro* to 60% of Ca^{2+} activity. Our results show that Sr^{2+} can affect calpain activity in fish following diverse pathological features in cellular metabolism. Some population effects of extremely high Sr^{2+} accumulation in fish due to metallurgy industry sewage are discussed.

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S8 Arsenic Toxicity

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Effect of arsenic exposure on reproductive outcome and infant mortality: Findings from cohort studies in Bangladesh

Anisur Rahman [1,2], Marie Vahter [1], Eva-Charlotte Ekström [2,3], Shams El Arifeen [1], Mahfuzar Rahman [1], Mohammed Yunus [1], Lars Åke Persson [3]

[1] *ICDDR, B: Centre for Health and Population Research, Dhaka, Bangladesh;* [2] *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden;* [3] *International Maternal and Child Health, Uppsala University, Sweden*

Millions of peoples world-wide are exposed to arsenic through drinking water. Despite the large number of studies showing severe health effects of arsenic exposure, little attention has been given to possible adverse effect of arsenic exposure on early human development. Arsenic is known to pass the placenta, both in experimental animals and in humans. A few human studies reported association between arsenic in drinking water and adverse reproductive outcome, but those studies are generally ecological in design and, based on recall outcome data and small sample size. Our ongoing research in Bangladesh aims at evaluating the effect of arsenic exposure via drinking water on pregnancy outcome, fetal growth, birth anthropometry and child development including morbidity and mortality. The studies are conducted in rural Matlab, Bangladesh, where a health and demographic surveillance system (HDSS) has been operating for decades. The first study was based on a cohort of 29,134 pregnancies, identified longitudinally by HDSS in 1991–2000. Data on individual arsenic exposure was assessed by drinking water history, obtained at personal interviews in a separate survey of the prevalence of arsenic exposure (2002–2003), and analysis of the arsenic concentration in the tube-wells used by all the women during pregnancy. Data on reproductive events including fetal loss, and infant mortality were ascertained from HDSS. The second prospective cohort study was nested into a supplementation study on pregnant women identified in 2001–2004. Arsenic exposure was assessed by arsenic metabolites in urine collected at early and late gestation. Out of 5483 urine samples collected at early pregnancy, 3570 have been analyzed for arsenic to date. Data on fetal loss, birth anthropometry and child health are being collected prospectively. The results indicate that drinking tube-well water with more than 50 μg/L of arsenic during pregnancy significantly increased the risk of fetal loss (relative risk (RR): 1.14; 95% confidence limits (CL): 1.04, 1.25) and infant death (RR: 1.17; 95% CL: 1.03, 1.32). Analysis is in progress to evaluate the association between arsenic metabolites in urine and fetal loss, birth anthropometry and child health. The increasing evidence that arsenic exposure via drinking water may have public health implications for reproductive and infant health emphasize the need for urgent mitigation activities.

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Arsenic, a global public health problem

Marie Vahter [1], Li Li [1], Barbro Nermell [1], Anisur Rahman [2], Shams El Arifeen [2], Mahfuzar Rahman [2], Lars Åke Persson [3], Eva-Charlotte Ekström [3]

[1] *Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden;* [2] *Centre for Health and Population Research, Dhaka, India;* [3] *Uppsala University, International Maternal and Child Health, Uppsala, Sweden*

Millions of people world-wide are exposed to arsenic through drinking water or industrial emissions. While the environmental pollution of arsenic because of industrial emissions and occupational exposure has decreased considerably in most western countries, there is a continuous reporting of the occurrence of arsenic in ground water used for drinking purposes. The situation is particularly serious in many low-income countries, where people are prone to use ground water for drinking purposes because of water constrains or pollution of available surface water

sources. Often, mitigation possibilities are limited. The use of arsenic-containing ground water for irrigation leads to wide-spread contamination of land and additional exposure via food. The health consequences of chronic arsenic exposure include various forms of cancer, e.g. skin, lungs, urinary bladder and kidney. The estimated cancer risk at the current WHO drinking water guideline, 10 μg/L, is in the range 1/1000 to 1/100. Non-cancer effects associated with arsenic exposure include diabetes, skin diseases, chronic cough, toxic effects in liver, kidney, cardiovascular system, peripheral- and central nervous systems. Although effects on reproduction have been indicated in several studies, little is known about effects on fetal and child development. Reported mechanisms or mode of action of arsenic include enzyme inhibition, including DNA repair enzymes and enzymes involved in cell cycling, oxidative stress, modification of DNA methylation, interactions with nuclear receptors, and un-coupling of cellular respiration. Apparently the mode of action is highly dose-dependent. There seems to be a vide variation in susceptibility to arsenic toxicity. Known risk modifying factors include gender, age, genetic predisposition, nutrition and metabolism of arsenic. Arsenic is metabolized by a series of reduction and oxidative methylation reactions, obviously modulating the toxicity.

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Gender-specific gene expression in arsenic exposed mice

Erminio Marafante [1,2], Graziella Cimino-Reale [1,2], Casati Barbara [1,2], Marie Vahter [1,2]

[1] *Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy;* [2] *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*

Arsenic is a potent carcinogen, which frequently occurs in ground water used for drinking purposes. The mechanism of arsenic toxicological action *in vivo* is poorly understood, in particular in relation to dose, type of tissue and gender. We used the Applied Biosystems Expression Mice Genome Survey Microarrays of 28 K genes to elucidate tissue- and gender-dependent responses of gene expression in the liver, kidneys, lungs, adrenals, bone marrow and urinary bladder of mice exposed to environmentally relevant doses of arsenic in drinking water. The mice were exposed for 4 months (starting at weaning) to arsenate in drinking water at a concentration of 1000 μg As/L, which is commonly occurring in many areas of the world. Arsenic exposure caused clear modulation of gene expression in all the tissues tested and there were remarkable sex differences. The observed gender-dependent profiles of gene expression may indicate that arsenic interferes with the basal sex-dependent regulation of cell physiology, e.g. steroid hormones. If so, it is likely that the toxic response to arsenic exposure may differ between males and females.

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Gene-environment interactions for arsenic

Karin Broberg

Department of Occupational and Environmental Medicine, Lund University, Lund, Sweden

There is a large variation in susceptibility among individuals to chronic inorganic arsenic exposure. This is probably related to individual differences in arsenic biotransformation, which causes heterogeneity in retention and tissue spread of noxious metabolites. Hereditary factors seem important for this inter-individual variation in arsenic metabolism. For example, there is evidence that variants in the gene encoding arsenic (3+ oxidation state) methyltransferase (AS3MT) affect arsenic metabolite pattern in urine. Moreover, it is generally thought that many of the toxic effects associated with arsenic exposure are mediated through arsenic-induced oxidative stress. Genetic differences in the defense against oxidative stress, or DNA repair capacity likely modify the toxic effects of arsenic as well. The knowledge of genetic effect-modifications for arsenic is at present limited, but such information is of importance, not only for understanding the mechanisms for arsenic toxicity, but also for proper risk evaluation and identification of vulnerable individuals and population groups. This review examines the present evidence for gene-environment interactions for arsenic.

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S9 Risk Benefit Assessment Methodologies

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Dealing with allergens in food: A risk analysis based approach

Geert F. Houben

TNO Quality of Life, Zeist, The Netherlands

Food allergy currently evokes strong interest among regulators as well as consumers and industry. An issue of

concern is the possible risk associated with unintended presence of allergens, e.g. through cross-contact.

Knowledge of minimum eliciting doses (MEDs, thresholds) is recognised as a key element in assessing and managing allergen risks. However, application of knowledge about MEDs, and in particular, extrapolation of data from the study dose ranges to low doses is subject to several important limitations and uncertainties. Estimation of MEDs on a population basis and their use in deterministic risk assessments will in many cases lead to a conclusion that an allergic reaction due to a certain (even very low) level of allergen in a food product cannot be excluded. An approach aiming at zero-risk, leads in practice to zero-tolerance of residual allergen. This not only poses serious technical, practical, and financial problems for all stakeholders, but will likely fail to achieve its stated aim.

To address this dilemma, the *ILSI Europe Food Allergy Task Force* established the *Expert Group on Determination of Eliciting Doses*. This Expert Group examined the applicability of a probabilistic risk assessment model to evaluate the consequences of defined amounts of allergen in food. A modelling framework developed by the Dutch TNO Quality of Life, and population distributions of MEDs using different statistical fits were used. Further development and exploration of this risk assessment approach may open options for an approach for managing allergens in food based on the concept of tolerable risk.

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Risk assessment of substances that are both genotoxic and carcinogenic

A.G. Renwick [1], S. Barlow [2], J. Kleiner [3]

[1] *University of Southampton, School of Medicine, Bassett Crescent East, Southampton SO16 7PX, UK;* [2] *8 Harrington Road, Brighton, East Sussex BN1 6RE, UK;* [3] *European Food Safety Authority, Palazzo Ducale, 43100 Parma, Italy*

The European Food Safety Authority (EFSA) and the World Health Organization (WHO), with the support of the European Branch of the International Life Sciences Institute (ILSI Europe), organised an international conference on 16–18 November 2005 to discuss how regulatory and advisory bodies evaluate the potential risks of the presence in food of substances that are both genotoxic and carcinogenic.

Currently, there are three main approaches used to assess the risks from substances that are genotoxic and carcinogenic. The advice offered depends on the technical approach adopted by the regulatory or advisory body providing the advice, and also on the legal background in the region or country concerned. The conference discussed the options for risk assessment related to low intakes of substances that are both genotoxic and carcinogenic, how the results should be interpreted in relation to human health, whether the available approaches meet the needs of risk managers and how to provide practical advice in situations where exposure cannot be completely eliminated and the magnitude of risk cannot be readily determined.

There was consensus at the meeting that:

(i) ALARA (as low as reasonably achievable) provided advice based solely on hazard identification and did not take into account either potency or human exposure;
(ii) quantitative low-dose extrapolation of dose–response data from an animal bioassay raises numerous uncertainties related to selection of the most appropriate mathematical models to fit the data and to extrapolate down to levels of human exposure and the possibility of non-linearity in the intake-response relationship over the range of extrapolation;
(iii) the margin of exposure (MOE) was the preferred approach at present as it is based on the available animal dose–response data, without extrapolation, and on human exposures; the MOE can be used for prioritisation of risk management actions but is difficult to interpret in terms of health risk.

Whichever approach is adopted, decisions on risk management actions are likely to take into account other aspects, such as cost and feasibility.

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Future Assessment: What are the options and how can they be applied?

Gerhard Rechkemmer

Technische Universitaet Muenchen, Freising, Germany

The relationship between food and human health so far was primarily focussed on the identification and characterization of hazards originating from substances naturally occurring or present as contaminants in food, and the assessment of the associated risk, taking into account exposure. An EC-funded concerted action dealing with the risk assessment of chemicals in food and

diet was recently conducted (FOSIE). The risk assessment methodology and principles have been described in great detail and a risk assessment paradigm has been proposed.

The increasing development of functional foods has now put the focus also on the characterisation of health benefits from foods or food components. However, in contrast to the commonly accepted principles and methods for risk assessment, a generally applicable benefit assessment is lacking so far. In a further EU-funded concerted action a process for the assessment of scientific support for claims on foods (PASSCLAIM) has recently been developed. The PASSCLAIM project focussed on the benefits of food and food components for human health, well-being and the reduction of disease risk. PASSCLAIM addressed diet-related cardiovascular disease, bone health and osteoporosis, physical performance and fitness, body weight regulation, insulin sensitivity and diabetes, diet-related cancer, mental state and performance, and gut health and immunity. It has resulted in common criteria to evaluate the benefits of foods and food components were developed.

It will be important to develop new methodologies to enable a common and concerted risk-benefit assessment by integrating the principles now already in place for separate assessment of risks and benefits. The identification and validation of specific biomarkers for health, well-being and disease risk reduction is of great importance. Furthermore new methods should be introduced, based for example on parameters like quality-adjusted life years (QALY) or disability-adjusted life years (DALY) as tools for risk-benefit assessment.

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The threshold of toxicological concern concept in risk assessment

Robert Kroes

IRAS, Utrecht University, Utrecht, The Netherlands

The concept that "safe levels of exposure" for humans can be identified for individual chemicals is central to the risk assessment of compounds with known toxicological profiles. The traditional approach to risk assessment is separated into hazard identification, hazard characterisation, exposure assessment and risk characterisation, and data from toxicity studies on the specific chemical under evaluation are necessary for hazard identification and characterisation.

The Threshold of Toxicological Concern (TTC) is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical-specific toxicity data, below which there would be no appreciable risk to human health. The concept proposes that a low level of exposure with a negligible risk can be identified for many chemicals, including those of unknown toxicity based on knowledge of their chemical structures.

The TTC principle was examined for general toxicity end points as well as for specific end points including carcinogenicity, teratogenicity, reproductive toxicity and immunotoxicity. In addition, consideration was given to structural alerts for high potency carcinogens, endocrine disrupting chemicals, food allergens and to the potential for metabolism and accumulation. A decision tree approach, which incorporates a tiered approach for applying the TTC principle, has been proposed as a preliminary step in food safety evaluation (Kroes et al., 2000 and 2004).

The TTC principle, its use to date, its potential future applications and the incorporation of the TTC principle in the Risk Assessment paradigm are described.

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Intake assessment of a food additive on food consumption data provided by retailer's fidelity card

Herve Nordman, Yves Haldemann, Massimo Sardi, Hans-Albrecht Bartels, Peggy Schumann, Bernard Bottex

Ajinomoto Switzerland AG, Zug, Switzerland

The colorant Sunset yellow (E110) has been selected as a test additive to develop a new approach to estimate the intake of chemicals in food. First all E110 containing products sold by the Swiss food retailer Migros have been identified. Migros as a retailer is unique as it produces practically all the manufactured foods it sells, thus allowing for an easy quantification of the ingredient in question and on top of it has an efficient fidelity card data bank. Of the 3.1 million households in Switzerland, 2.3 million participate in Migros' fidelity card programme known as Cumulus. Analysis of the Cumulus database showed that 27,440 households had purchased at least one food product containing E110 during the 15 days of the survey. 6860 Of these households purchase most of their foods from Migros. Among those with the highest E110 intakes, 1204 cardholders have been interviewed by telephone. Formal consent of the cardholders was obtained, and the household composition was described.

Eventually the survey covered 2390 household members. The analysis revealed that 65% consumed some but

less than 0.07 mg kg-body-weight^{-1} day^{-1} of E110; 8% between 0.07 mg and 0.13 mg; 3% between 0.13 mg and 0.20 mg; 3% between 0.20 mg and 0.67 mg, and 0.4% more than 0.67 mg. E110 intake has been estimated for the total sample population, for the German, French and Italian speaking groups, for age groups, per food category, per gender and per status of household. The food group that contributed the most to E110 intake was the marzipan category: 1.33 mg kg-body-weight^{-1} day^{-1}.

An important quality of the above intake assessment method, as opposed to existing estimates obtained with the Danish budget method or the Monte Carlo modelling, is that it is closer to actual intakes. The method is also much less time and resource consuming. However, further validation of this promising intake assessment method is needed, by comparison with other methods and other ingredients.

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Utility of post-market monitoring of novel foods

Paul Hepburn, Heiner Boeing, Andrew Cockburn, Anne Constable, Agnès Davi, John Howlett, Nynke de Jong, Bevan Moseley, Regina Oberdörfer, Claire Robertson, Hans Verhagen, Jean-Michel Wal

Unilever Bedford, Bedfordshire, UK

Prior to the marketing of novel foods a thorough evaluation of nutritional and toxicological safety is conducted. However, the conventional safety assessment approach that has been classically used for chemicals poses different challenges when applied to whole foods, including GM and novel foods, and accordingly this has resulted in modified testing protocols. Post Market Monitoring (PMM) is increasingly being requested by regulatory authorities, as part of the approval to accompany the introduction of certain novel foods (e.g. phytosterol-esters) into the market.

PMM is a tool that can be used as part of the risk management process to confirm the assumptions made in the risk assessment and obtain consumer use information following the launch of a product. Two essential elements can be addressed by PMM methodology, (i) tracking or estimating food consumption under actual conditions of food use, and (ii) tracking or identifying any potentially food-related health effects. Information on food consumption is generally derived from three sources: food supply data, data from household food expenditure/ consumption surveys, and results of surveys of individual consumers. Tracking or identifying potentially food-related health effects requires access to health surveillance systems (e.g. disease registers, population health surveys), epidemiological studies or through contact centres established by the food companies. Successful PMM provides reassurance that the product does not cause adverse health effects.

From the analysis of a number of case studies, which have been reported, some conclusions on the applicability of PMM have been drawn. In particular, PMM can be considered as a potential tool that can be used to complement the pre-market risk assessment. This is achieved by providing information which can be used to confirm the assumptions which were made around intake and absence of health effects. However, PMM cannot be used as a tool to replace any steps in the pre-market risk assessment process. Further, the need for PMM must be judged on a case-by-case basis in which the objectives, the circumstances under which it is undertaken and the methodology are considered.

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S10 Gene–Teratogen Interactions in Chemically-Induced Congentital Malformations

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Teratogenic effects of ethanol: Interaction with retinoid metabolism

Françoise Gofflot [1], Jabier Gallego-Llamas [2], Pascal Dollé [2]

[1] *Institut Clinique de la Souris (ICS), Strasbourg, France;* [2] *Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Strasbourg, France*

The pathophysiological mechanisms involved in the embryonic and fetal alcohol syndrome are not fully understood. Maternal alcohol (ethanol) administration during early embryogenesis can induce a spectrum of developmental defects in animal models. Clearance of ethanol involves the activity of alcohol dehydrogenases (ADHs), a class of developmentally expressed enzymes that have also been implicated in the first oxidative step generating retinoic acid (RA) from circulating retinol (Vitamin A). RA is the main active vitamin A metabolite, and many lines of evidence have shown that this signaling molecule has to be synthesized in specific tissues and at appropriate levels to ensure normal embryonic development. Vitamin A deficiency – or genetic conditions that affect RA signaling – can lead to pleiotropic embryonic and fetal defects, some of which being sim-

ilar to the teratogenic effects of ethanol. It has therefore been suggested that some of the developmental effects of excess ethanol may involve an interference with endogenous RA synthesis by competiting with ADH enzymes. Our work aims at demonstrating such an interference in vivo. We are using a mouse model with a targeted disruption of the gene coding for retinaldehyde deshydrogenase 2 (RALDH2), the main enzyme acting downstream of ADHs for embryonic RA synthesis. Homozygous disruption of this enzyme is early embryonic lethal. Heterozygous mutant mice are viable, however, despite an impaired ability to synthesize RA during embryogenesis due to *Raldh2* haploinsufficiency. We have investigated whether administration of ethanol at subteratogenic doses may lead to selective defects in *Raldh2* haploinsufficient embryos. We found that such embryos exhibit developmental abnormalities similar (albeit sometimes less severe) to those occurring spontaneously in *Raldh2* homozygous mutants. These results indicate that ethanol exposure is particularly deleterious in individuals partly deficient in their ability to synthesize RA during development. These analyses will be refined through the use of a RA-sensitive reporter transgene that will help define the critical stages and embryonic tissues sensitive to ethanol exposure in a *Raldh2*-deficient background.

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Valproic acid-induced skeletal malformations: Associated gene expression cascades

Valentina Massa[1], Robert M. Cabrera[2], Francesca Di renzo[1], Richard H. Finnell[2], Erminio Giavini[1]
[1] *Department of Biology, University of Milan, Milan, Italy* [2] *Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston, TX, USA*

Valproic acid (VPA) is an anticonvulsant drug widely used therapeutically for a variety of neurological conditions, including epilepsy. VPA is also well known for its teratogenic potential in both humans and experimental animal models. The typical malformations observed following VPA exposure include neural tube defects (NTDs), craniofacial and skeletal malformations. Nevertheless, the mechanisms underlying VPA's anticonvulsant efficacy or its teratogenicity remain to be elucidated. In order to study valproic acid-induced teratogenic effects, two approaches were utilized. In the first project, gene-expression profiles were analyzed, whereas the second study was focused on histone acetylation status. Using anti-sense RNA amplification and cDNA microarrays, we examined the expression of approximately 5700 genes in the first six postotic somites of control and treated embryos at 6, 12, 18 and 24 h after the 8.5 days post coitum (d.p.c.) VPA treatment (1.36 and 2.72 mmol/kg). Analysis indicated that several ontological groups (histone deacetylase complex, GTPases, cell proliferation, apoptosis and cytoskeletal) have significantly enriched gene expression changes in response to the teratogenic insult.

The histone deacetylase (HDAC) enzymes participate in the nucleosome structure control. Several studies showed that VPA is a strong inhibitor of HDAC activity in cell and animal models, producing histone hyperacetylation. In order to better define the correlation between HDAC inhibition and teratogenicity, pregnant mice were treated i.p. on 8.5 d.p.c. with 400 mg/kg VPA. One hour after treatment, embryos were processed for Western blotting and immunohistochemical analysis, using antibodies anti hyperacetilated histone H4. VPA exposure in utero induced hyperacetylation of embryonic proteins, specifically those localized in the caudal neural tube and in the somites, the main target organs of VPA teratogenic effects.

The results of these works suggest that valproic acid induces congenital malformations through gene-expression alterations by chromatin structure misregulation.

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The genetic basis of mammalian neurulation

Andrew Copp

Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK

Neurulation is a key event in central nervous system development, in which bending and subsequent fusion of the edges of the neural plate culminates in the formation of the neural tube, the precursor of the brain and spinal cord. Failure of neural tube closure results in neural tube defects (NTDs) in which the lumen of the neural tube remains open to the amniotic fluid environment. Degeneration of the neuroepithelium occurs later in gestation leading to neurological deficit by the time of birth, and severe handicap from the newborn period onwards. While a strong genetic predisposition to human NTDs is generally accepted, the genes that mediate this predisposition have been difficult to identify. In contrast, over 100 genes are known to be required for mouse neural tube

closure, by virtue of the NTD phenotypes when they are inactivated in knockout or mutant strains.

In this talk, three types of genetic mouse NTDs will be described. (1) Failure of the initial step of neurulation, leads to the most severe NTD, craniorachischisis, in which the neural tube remains open from midbrain to low spine. Recent studies show that initiation of neurulation requires signalling via the non-canonical Wnt/dishevelled (planar cell polarity) pathway. (2) Disruption of dorso-lateral neural plate bending in the low spinal region prevents the neural fold tips from coming into apposition, leading to lumbo-sacral spina bifida. Our studies demonstrate an essential role for signalling via the BMP and Sonic hedgehog pathways in regulating neural plate bending. (3) Re-opening of the closed neural tube can yield a variety of NTDs, including isolated thoracic spina bifida, not previously seen in mouse genetic models. Studies in a new genetic knockout strain demonstrate that NTDs can result from re-opening as well as failure of neural tube closure.

Primary prevention of NTDs is now practised clinically using peri-conceptional folic acid therapy. However, a proportion of NTDs in humans and mice do not respond to folic acid, and we have described the use of inositol as an alternative, adjunct therapy where folic acid is ineffective. Studies of the action of inositol in preventing NTDs in mice will be described, as well as a new clinical trial to evaluate inositol as a preventive agent for NTDs in human pregnancy.

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TGF beta signaling in neural crest cells in development and diseases

Lukas Sommer

ETH Zurich, Switzerland

Stem cells are undifferentiated cells with the potential to generate multiple specialized cell types, and with the capacity to self-renew and thus to produce undifferentiated progeny that are stem cells again. Due to these properties stem cells can yield appropriate numbers of differentiated cells in developing organs and can regulate tissue maintenance, regeneration, and repair. These functions are not only of interest to basic science, but also have implications for clinically oriented research. However, the mechanisms that govern stem cell self-renewal and differentiation are still poorly understood. Recently, we have shown that specific inactivation of TGFβeta signaling in neural crest stem cells (NCSCs) results in cardiovascular defects, thymic, parathyroid, and craniofacial anomalies, as well as developmental eye disorders. All these malformations represent features of DiGeorge syndrome, the most common microdeletion syndrome in humans, and Axenfeld Rieger's anomaly, characterized by compound malformations in the anterior eye segment. Consistent with a role of TGFβeta in promoting non-neural lineages in NCSCs, mutant neural crest cells migrate into the pharyngeal apparatus and into the developing anterior eye, but they are unable to acquire non-neural cell fates. Thus, TGFβeta signal modulation in neural crest cells might play a crucial role in the etiology of the congenital disorders DiGeorge syndrome and Axenfeld Rieger's anomaly. The combined data are consistent with a role of TGFβeta in suppressing NCSC maintenance, thereby promoting stem cell differentiation in a context-dependent manner.

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Structure-activity relationship for the reactivators of acetylcholinesterase inhibited by nerve agent VX

Kamil Kuca [1], Daniel Jun [1], Kamil Musilek [2], Jiri Bajgar [1]

[1] *Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic;* [2] *Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic*

Nerve agents such as sarin, VX and tabun are organophosphorus substances able to inhibit an enzyme acetylcholinesterase (AChE; EC 3.1.1.7). AChE reactivators (pralidoxime, obidoxime and HI-6) and anticholinergics (atropine mainly) are generally used as antidotes in the case of intoxication with these agents. Among the nerve agents, agent VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) belongs to the most toxic organophosphorus nerve agents ever prepared. This very high toxicity is caused by the disability of blood hydrolases to cleave this nerve agent. There are many studies dealing with the relationship between structure of nerve agents antidotes and their biological activities. However, there is lack of studies aimed especially at the structure-activity relationship of antidotes, especially AChE reactivators, against VX agent intoxication.

Due to this, in this work, reactivation potency of 25 structurally different AChE reactivators was tested in vitro and subsequently, relationship between their chemical structure and biological activity was outlined. As

resulted, the most important structural factor in reactivator's molecule seems to be position of the functional oxime group (position four), followed by presence of quaternary nitrogens influencing the affinity to intact and inhibited AChE.

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Comprehensive evaluation of pediatric susceptibility to 18 industrial chemicals

Ryuichi Hasegawa [1], Mutsuko Hirata-Koizumi [1], Micheal Dourson [2], Akihiko Hirose [1], Satoshi Nakai [3], Eiichi Kamata [1], Makoto Ema [1]

[1] *National Institute of Health Sciences Tokyo Japan;* [2] *Toxicology Excellence for Risk Assessment, Cincinnati, USA;* [3] *Yokohama National University, Yokohama, Japan*

To elucidate relative pediatric susceptibility to chemicals, we first established a protocol for an 18 day repeated dose study in newborn rats; doses for eighteen industrial chemicals, mostly phenolic substances, were administered by gavage from postnatal Days 4 to 21. The toxicity results from these newborn studies were then compared to those of young rat studies for 28 days starting at 5–6 weeks of age. Both studies were conducted under the same experimental conditions as far as possible, such as the same strain from the same breeding farm, the same Lot Number of chemicals, and the same administration solutions preparation. For evaluation, in addition to main study, the results of dose-finding study were very carefully taken into account, considering the lack of information from dose-finding studies such as the lack of histopathological examination in both newborn and young studies. Two new toxicity endpoints specific to this comparative analysis were identified; the first, presumed no observed adverse effect levels (pNOAELs) were estimated based on the results of both main and dose-finding studies, and the second, presumed unequivocally toxic levels (pUETLs) were defined as clear toxic doses giving similar severity in both newborn and young rats. With analyses of both pNOAEL and pUETL ratios, six or five chemicals demonstrated lower or nearly equal sensitivity (less than two-fold variation) in the newborn as compared with the young rat. However, 11 chemicals were clearly more sensitive (two- to eight-fold) in newborn rats when compared with young rats, and newborn rats were exceptionally sensitive for 1 chemical (more than 25-fold). In addition, Benchmark Dose Lower Bound (BMDL) was calculated as an alternative endpoint for pNOAEL. Most BMDLs were comparable to the corresponding pNOAELs and the correlation coefficient was 0.904. In conclusion, newborn rats demonstrated greater susceptibility (at most eight-fold) to nearly two thirds of chemicals, and less or nearly equal sensitivity to the others, for repeated oral exposure. It should be noted that there was one exceptional case in which toxicity appeared only in newborn rats.

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S11 Pneumoconiosis in Developing Countries

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Pneumoconiosis: Some historical highlights and a perspective into particle toxicology of the future

Paul J.A. Borm

Institut fuer Umweltmedizinische Forschung an der Universitaet Dusseldorf und Centre of Expertise in Life Sciences (CEL), Zuyd University, Heerlen (NL), the Netherlands

Particle research has been historically closely connected to industrial activities or materials, such as coal, asbestos, man-made mineral fibers causing pneumoconiosis. More recently ambient particulate matter (PM) is ruling the waves in scientific journals, since it causes acute mortality in susceptible groups. In supporting research on particle-induced respiratory effects and mechanisms, research programs of the European Community on Steel and Coal (ECSC) have played a tremendous role. Now current particle research in Europe and the USA is dominated by the PM issue, and within a few years the testing and effects of manufactured nanomaterials will drive particle toxicology. It is the purpose of this presentation to put current development in particle toxicology within the historical context of its development in Europe.

Differences between historical and current research in particle toxicology include the exposure concentrations, particle size, target populations, endpoints, and length of exposure. Inhaled particle effects are no longer confined to the lung, since particles are suggested to translocate to the blood while lung inflammation invokes systemic responses. Particle size and concentrations have both been reduced about 100-fold from 2–5 mg/m^3 to 20–50 $\mu g/m^3$ and from 1–2 μm to 20–100 nm (ultrafine) as domestic fuel burning has decreased and vehi-

cle sources have increased and attention has moved from coal mining industry to general environment. Secondly there is the major issue of poorly soluble particles (including coal mine dust, carbon black, TiO_2) that cause lung cancer in rats. So far, risk assessment has tremendous problems in extrapolating these findings to humans and it historical data on dust-overloaded coal miners' lungs are used to resolve this issue.

Finally, Nanotechnology continuously produces new materials in the ultrafine range. Although inhalation exposure is considered to be minimal in this technology, some particles are being used for carrier purpose in medical applications. While coal mining was the black gold of the early 20th century, Nanotechnology is up to even higher expectations in the 21st century. However, learning from the past and current insights into the adverse effects of particles, we have to invest in new methods both in testing and risk assessment to allow a sustainable development of nanomaterials. Before jumping into this pool of opportunities a lesson of the past and especially in coal mine induced respiratory diseases is obligatory.

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Genetic susceptibility in pneumoconiosis

Berran Yucesoy, Michael I. Luster

Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA

Silicosis and Progressive Massive Fibrosis (PMF) are chronic interstitial lung diseases with a complex etiology that can occur after cumulative dust exposure. A large number of mediators such as cytokines, antioxidants and growth factors have been implicated in the pathogenesis of experimental and human pulmonary fibrosis. Common functional polymorphisms in these genes have been shown to influence individual susceptibility against various lung pathologies including, cancer, asthma, and chronic obstructive pulmonary disease. In light of this, we investigated associations between cytokine, antioxidant and fibrogenic gene variations and the development and severity of dust-induced pulmonary fibrosis in ex-coal miners with silicosis and PMF. A significant association was found between silicosis and the TNFα −238, TNFα −308 and IL-1RA +2018 variants. Also, an association between accelerated decline in lung function and genetic variations in cytokine genes were investigated in firefighters. There is a broad range of rates of longitudinal decline in lung function among firefighters, and this cannot be explained with differential occupational smoke exposure. Genetic differences in pulmonary response to respiratory toxicants likely play a role in determining the rate of longitudinal decline. Our results showed that the presence of IL-1β +3953, IL-1RA +2018 and TNFα −308 variants are associated with the decline rate of lung function as measured by FEV1. These findings suggest that specific variants of cytokine genes may influence individual susceptibility to occupational pulmonary diseases.

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Asbestos and asbestosis in Croatia: Past, present and future

Ivančica Trošić

Institute for medical Research and Occupational Health, Zagreb, Croatia

This study gives a historical review of the national use of asbestos, the first and later appearance of asbestos-related diseases in the country, health risk derived from para-occupational exposure to asbestos, and the national and European Union (EU) regulations on the asbestos issue. Between 1945 and 1990 Croatia had a strong shipbuilding and asbestos cement industry. The first cases of asbestosis were reported in 1960, and 317 cases were recorded from 1990 to 2000. The Croatian Cancer Registry recorded a total of 248 cases of pleural mesothelioma between 1991 and 1997, two-third of which were attributable to occupational exposure to asbestos. Regulations on maximal allowable concentrations for harmful agents in the work environment and biological limit value, including all forms of asbestos were issued in 1993. By including asbestos on the Health Ministry's list of toxic materials, Croatian government banned the manufacture, trade and use of asbestos, effective January 1, 2006. This ended the manufacture, trading and use of asbestos and asbestos products, suspended all asbestos production in Croatia and brought Croatia into line with the member states of the EU. On February 14, 2006, the Ministry of Health released a revised version of the List, whereby the sale and commercial use of asbestos remain off limits, but the production of asbestos products is allowed. Besides, para-occupational exposure seems to be at least as dangerous as occupational because the majority of people are not aware of it. Major asbestos exposure continues today in building, renovation, demo-

lition, maintenance, brake repair, household repairs, and do-it-yourself construction. The Institute for Medical Research and Occupational Health in Zagreb identifies asbestos by presence and type in bulk materials using a certified standard method (Health and Safety Executive Book, UK, Method for the Determination of Hazardous Substances-Series 77—Asbestos in bulk materials; HRN EN ISO 9001:2002; HRN EN ISO/IEC 17025:2004). The article includes recommendations for proper procedure in response to positive asbestos findings. The future approach to the asbestos issue in Croatia will by all means depend on revised regulations, which are expected to conform to the recommendations of the European Union.

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Mechanistically identified suitable biomarkers of exposure, effect and susceptibility for silicosis: Validation of biomarkers of early effect

Mary Gulumian[1,2], Kerry Downs[1], Berry Girdler-Brown[3], Jill Murray[4,5], Paul Borm[6], Val Vallyathan[7], Vince Castranova[7], Ken Donaldson[8]

[1] *Department of Toxicology, NIOH, P.O. Box 4788, Johannesburg 2000, South Africa;* [2] *Department of Haematology and Molecular Medicine, Wits University, South Africa;* [3] *School of Health Systems and Public Health, University of Pretoria, South Africa;* [4] *Department of Pathology, NIOH, South Africa;* [5] *School of Public Health, Wits University, South Africa;* [6] *Particle Research Core, medizinische Forschung, gGmbH, Dusseldorf, Germany;* [7] *NIOSH and West Virginia, University Medical Center, Morgantown, WV, USA;* [8] *ELEGI Colt Laboratory, Wilkie Building, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH9 8AG, United Kingdom*

Clinical detection of silicosis is currently dependent on radiological abnormalities, a late manifestation of disease. A need for markers of prediction and early detection of pneumoconiosis is imperative for the early evaluation of dust allaying strategies. Understanding of the underlying mechanisms of the etiology of silicosis was instrumental in proposing numerous biomarkers that have been evaluated to assess effects following exposure to crystalline silica dust. Human validation studies have substantiated some of these proposed biomarkers and have argued in favour of their use as biomarkers for crystalline silica-induced pneumoconiosis. A number of 'ideal' biological markers of effect were identified through literature review, viz. TNF-α, IL-8 and ROS measurement by chemiluminescence (monocyte release), CC16, 8-isoprostanes, total antioxidant levels measured by TEAC, glutathione, glutathione peroxidase, glutathione *S*-transferase, and PDGF (serum). These identified biomarkers were then evaluated in South African gold miners exposed to silica with and without clinical evidence of silicosis and in control group of men with no silica dust exposure.

Results showed that three out of the 10 biomarkers investigated were significantly affected by exposure to silica dust, independently of any effects due to HIV infection, ARV treatment, smoking or age, to an extent that would enable them to be used as biomarkers of early effects due to exposure to crystalline silica.

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Risk groups and pneumoconiosis in Turkey

Arif Çımrın

Dokuz Eylul University Medical School, Department of Pulmonary Medicine, Izmir, Turkey

In Turkey as a developing country, 4,220,000 (20.7%) of a total number of 51,202,000 economically active population work in the industry (Home Population Workforce Statistics, 2005; www.die.gov.tr). Beside this, 98.1% of 850.928 workplaces are small-scale enterprises which have less than 50 employees (www.ssk.gov.tr). As a result of this, Turkey is a late industrized country at where agricultural employment is still important, an artificial condition occurred in the service sector and small-scale enterprises are dominating.

According to official data, the diagnosed occupational diseases is decreasing during the past few years, the total number of occupational disease is 384 at 2004 and the incidence is less than 0.001%. Regarding to the European Union data of 2002 which reveals the incidence of occupational diseases is 0.3–0.5%; the number of diagnosed occupational diseases every year in Turkey should be at least 20,000.

The pneumoconiosis related studies in Turkey are limited. Most of them are work place based and cross-sectional studies. At the last evaluation, the CWP preva-

lence changed from 1.23% to 6.23% and the incidence is changed from 0.17% to 2.18% at the bituminous coal region, in the last 20 years (Öztürk et al., 2005). Two studies at the lignite mine workers had been done at the enterprises run by the state. The prevalence was found as 13.5%. At one of the studies, a correlation between the dust exposure time and CWP had been shown (Çımrın et al., 2005, 2004). One of the studies at the work places where there is quartz exposure was consisted of the enterprises around Cine. Silicosis prevalence was found as 15% at quartz mill workers (Şener et al., 1993). Silicosis frequency at two different ceramic factory workers were found as 6.0% and 6.5% (Çımrın et al., 1999; Sakar et al., 2005); and the workers who make glass sandblasting and metal sandblasting at small enterprises were found as 36.4% (Sevinc et al., 2003). At 12 denim processing enterprise in İzmir, silicosis related changes were found at the 23.8% of the cases (Çımrın et al., 2006). At a cross-sectional study with 500 workers at Cine region in 2004, the pneumoconiosis frequency at quartz and feldspar grinding work was found as 23% (Öztürk et al., 2006). At the cross-sectional studies done at the workplaces at where there is coal and quartz exposure, pneumoconiosis cases which are severe and complicated as it can cause morbidity were not determined. The dominant profusion was 1 and less than this, there was profusion at the level of 2.

The pneumoconiosis case reports from the clinics are very limited. One of them is two cases who have the history of working as dental technicians reported by Kartaloğlu et al. There is morbidity (Kartaloğlu et al., 2003). Other reports come from textile and dental laboratories (Akgün et al., 2005; Gur et al., 2005; Karaman et al., 2006).

As a conclusion, in order to realize the real situation of occupational diseases and to create a healthy working environment in Turkey; employers', related sites' cooperation is necessary.

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PS3 Plenary Session

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The 2005 WHO re-evaluation of toxic equivalency factors for dioxin like compounds—Implications for risk assessment and limitations of the concept

Martin Van den Berg

World Health Organization Collaborating Centre for Research on Environmental Health Risk Assessment and Institute for Risk Assessment Sciences, Faculties of Veterinary Medicine, Science and University Medical Center, Universiteit Utrecht, The Netherlands

An WHO-IPCS expert meeting was held in June 2005 (Geneva, Switzerland) during which a re-evaluation took place of the toxic equivalency factors (TEFs) for dioxin like compounds, including some polychlorinated biphenyls (PCBs). Evaluation of existing WHO-TEF values were based on a combination of unweighted relative effect potency (REP) distributions, expert judgement and point estimates. Changes in TEF values were decided by the expert panel for a number of environmentally significant congeners such as 2,3,4,7,8-pentachlorodibenzofuran (PnCDF), octachlorodibenzo-*p*-dioxin (OCDD) and octachlorodibenzofuran (OCDF), and mono-*ortho* substituted PCBs. The new 2005 WHO TEF values have some impact on the total toxic equivalency (TEQ) with an overall decrease between 10% and 25% for most human food matrices. An important prerequisite of the TEF concept is additivity and the WHO expert panel recognized that this was further confirmed by recent in vivo mixture studies. It was also recognized that new studies provide evidence that non dioxin-like AhR agonists/antagonists that might impact the overall toxic potency of dioxin like compounds. Several (groups of) compounds were further identified that might possibly be included in the TEF/TEQ concept in the future. Concern by the WHO panel was also expressed about the application of the TEF/TEQ approach to abiotic environmental matrices such as soil, sediment, etc., as these values and associated methodology are primarily meant for estimating exposure via dietary intake. A number of future approaches to determine alternative or additional TEFs were also identified. In this presentation the outcome of this WHO re-evaluation will be presented in more detail, while in addition points of discussion

as identified at the meeting will be brought forward also.

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S12 Chemical Influence on Cell Cycle Control

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p53 is a key molecular node in the inflammatory stress response network

Curtis C. Harris

Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD 20892-4258, USA

Free radicals are ubiquitous in our body and are generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms. Free radicals can also inflict cellular damage. Several defenses have evolved both to protect our cells from radicals – such as the p53 and the Rb tumor suppressor pathways, and antioxidant scavengers and enzymes – to repair DNA damage. Nevertheless, many chronic inflammatory diseases, e.g., ulcerative colitis, are associated with increased cancer risk. Understanding the relationship between chronic inflammation and cancer provides insights into the molecular mechanisms involved. In particular, we highlight the interaction between nitric oxide and p53 as a crucial pathway in inflammatory-mediated carcinogenesis.

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Apoptosis and proliferation in chemical toxicity

Alex Bell, Mark Graham, Ruth Roberts, Tim Hammond

SAUK Astrazeneca, Astrazeneca, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Most cells in the body exist in the "resting" or Go state of the cell cycle. It is a combination of internal and external factors that control the fate of the cell with respect to prolonging its survival, stimulating its proliferation or pushing it towards a controlled programmed cell death. Exposure of an organism to a xenobiotic can dramatically alter the normal fate of the cell with the resultant cellular phenotype manifesting itself as a toxicological lesion or regenerative response to toxic damage. The action of the xenobiotic on cell fate may result from the primary pharmacology of the drug target and in this scenario an understanding of disease context in which the drug is to be used relative to its pharmacological action are important. Conversely, the action of the chemical drug may result from "off-target" interactions with other proteins. In this scenario an understanding of the mechanism of the off-target interactions are necessary to either determine relevance to man and/or to provide opportunities to screen out the toxicological liability.

Three themes of chemical induced changes of cell fate will be highlighted and the molecular mechanisms currently understood to be involved in these cell fates will be considered. The proliferative response of agonism of the aryl hydrocarbon receptor (AhR), a transcription factor important in the mechanism of toxicity of some environmentally and pharmaceutically important chemicals that shows species differences in pleitropic response will be explored. Peroxisome Proliferator Activated Receptor (PPAR) involvement in cell proliferation and suppression of apoptosis in relation to receptor mediated carcinogenesis will be presented, and lastly antagonism of IκB kinase (IKK2) pathway will be used as an illustrative example of "on-target" effect where toxicities in the rat are driven by pharmacology.

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DNA repair in defence against genotoxin-induced apoptosis

Bernd Kaina, Manuela Briegert, Steffen Naumann, Markus Christmann, Gerhard Fritz, Wynand Roos

Department of Toxicology, University of Mainz, Mainz, Germany

DNA damaging agents are powerful inducers of apoptosis. To identify critical lesions responsible for the apoptotic response, we studied apoptosis in various DNA repair deficient cell systems. Cells defective in nucleotide excision repair are hypersensitive to UV-C-induced apoptosis, showing that DNA lesions and not death receptor activation on its own trigger apoptosis. Cells defective in MGMT are hypersensitive to methylating agents showing that O^6-methylguanine (O^6MeG) triggers apoptosis. In this process, mismatch repair (MMR) and DNA replication are essentially involved. O^6MeG is hypothesized to be converted via MMR and DNA replication into DNA double-strand breaks (DSBs) that act as a critical downstream trigger of apoptosis. In rodent fibroblasts, DSBs provoke Bcl-2 decline, caspase-9/-3 activation, PARP cleavage and finally DNA degradation. This occurs independent of p53. ATM is not required since ATM deficient cells are hypersensitive to

methylating genotoxins when MGMT is depleted. p53 and c-Fos knockout mouse fibroblasts are more sensitive to UV light than the corresponding wild-type cells, indicating that in fibroblasts p53 and c-Fos play a protective rather than a pro-apoptotic role, which is due to regulation of DNA repair genes. Human lymphoblastoid cells and malignant glioma cell lines are more sensitive to methylating agents if they are wild-type for p53 compared to p53 mt. Methylation-induced apoptosis in lymphocytes and glioblastoma cells was preceded by DSB formation, p53 stabilization, and death receptor (Fas/CD95/Apo1) upregulation. O^6MeG lesions do not induce immediate early-signaling. This is in contrast to cisplatin which is highly effective in inducing the MAP kinase pathway provoking sustained JNK/p38 kinase activation and death receptor ligand (Fas-L) upregulation. For methyl methanesulfonate, JNK/P38 kinase activation requires DNA-PK and CSB protein that appear to be involved in sensing critical DNA damage. Overall, the data show that non-repaired DNA damage triggers apoptotic cell death in a cell-type and lesion-specific way by activating the death receptor or mitochondrial damage pathway. Although many downstream players may have impact on cellular sensitivity, DNA repair seems to be most important in determining the genotoxin-induced apoptotic response.

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Effects of dietary flavonoids on detoxyfing cell system

Ksenija Durgo [1], Lidija Vuković [3], Gordana Rusak [2], Maja Osmak [3], Jasna Franekić Čolić [1]

[1] *Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia;* [2] *Faculty of Natural Science, University of Zagreb, Zagreb, Croatia;* [3] *Ruđer Bošković Institute, Zagreb, Croatia*

Flavonoids are phytochemicals widely distributed in plants. In this work we have investigated five structurally similar flavonoids for their ability to: (i) to cause antioxidant/prooxidant effect in cells; (ii) to change basal level of total cellular glutathione and glutathione-*S*-transferases; (iii) to cause cytochrome 1A1 expression and (iv) their influence on antiapoptotic proteins expression (survivin and Bcl-2) and their ability to cause PARP degradation.

For this purpose, laryngeal carcinoma cell lines HEp2 and drug resistant cell line CK2 were used.

Previously, we have concluded that cell line CK2 was more resistant to cytotoxic effect of investigated flavonoids than parental, HEp2 cells. Quercetin, luteolin and fisetin have acted as prooxidants in cell lines where cytochrome 1A1 was highly expressed (cell line HEp2). In such conditions, fisetin and luteolin have caused enhanced prooxidative response when cells were treated with free radicals of other origin. In cells where cytochrome 1A1 was slightly expressed, quercetin has caused protein PARP degradation and survivin expression. Luteolin has caused PARP degradation and antiapoptotic proteins expression in cells with low cytochrome 1A1 and glutathione expression. In cells where expression of cytochrome 1A1 is low and glutathione-*S*-transferases activity and glutathione level high, fisetin has caused PARP degradation and antiapoptotic proteins repression. These results show clear evidence of proapoptotic nature of fisetin. Naringin has shown antioxidant property, it has caused cell membrane stabilisation and higher selectivity. In cells where cytochrome 1A1 was highly expressed, together with total cellular glutathione and glutathione-*S*-transferases, naringin has caused Bcl-2 repression, and has shown proapoptotic action. Myricetin did not cause prooxidative damage; it has caused expression of phase II enzymes and Bcl-2 expression, pointing its antiapoptotic nature.

In conclusion, small differences in chemical structure of flavonoids lead to drastic change of their biological effects. These effects are strongly dependent of cell type as well as test systems used. Extensive studies on structure–function relationship of flavonoids in different test systems could provide rational approach to drug and chemopreventive agent design.

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FISH for micronuclei analysis in barley cells

Jolanta Juchimiuk, Barbara Hering, Jolanta Maluszynska

University of Silesia, Poland

Chromosomal aberrations (CA) can be detected with simple, classical chromosome staining methods, nevertheless fluorescent *in situ* hybridization method (FISH) provides new tools for their analysis. The identification of individual chromosomes or chromosome arms using FISH with specific probes is very helpful in the detection and detailed characterization of chromosome rearrangements. Additionally, the possibility of the detection of chromosome or chromosome fragments in interphase

nuclei in order to analysis of chromosomal aberrations, is unquestionable advantage of FISH technique.

Until now, FISH is not widely applied in plant mutagenesis for detection and precise localization of chromosome aberrations, as DNA probes required for chromosomes of particular plant species are very limited. The application of FISH in chromosomal aberrations analysis is more common in human cytogenetics and carcinogenicity studies in mammals. However, there are plant species, which most of the chromosomes can be distinguished by presence/specific location of available DNA probes and thus make possible detailed CA analysis with FISH.

In present study fluorescent *in situ* hybridization (FISH) using telomere- and centromere-specific DNA and rDNA (5S and 25S rDNA) as probes was used to compare the cytogenetic effects of two chemical mutagens: *N*-nitroso-*N*-methylurea (MNU) and maleic acid hydrazide (MH) on root tip meristem cells of *Hordeum vulgare* ($2n = 14$). The micronucleus test (MN) combined with FISH allowed quantitative analysis of the involvement of chromosome fragments in micronuclei formation and thus enabled explanation the origin of mutagens-induced micronuclei. The analysis of the frequency of micronuclei with signals of investigated DNA probes did not show significant differences between the MH- and MNU-induced micronuclei. Additionally, especially the application of rDNA as probes to FISH enabled more detailed study of the contribution of particular chromosomes/chromosome fragments in micronuclei formation.

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W8 Food Safety in Relation to Chemical Contaminants in Developing Countries

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Common adulterants and food safety status in India

Mukul Das

Food Toxicology Laboratory, Industrial Toxicology Research Centre, Lucknow 226001, India

Food is required for the growth and maintenance of living organisms. The chemical constituents of food that are important to safety include a wide spectrum of substances. These chemical substances may exist as naturally occurring components, or as contamination of the environment in which the food is produced and stored, or as additives introduced by man in the course of food manufacture and preparation, or as deliberate adulterants added for the purpose of earning undue profits by unscrupulous traders. Broadly, the common pollutants causing concern to food industry and consumers could be classified as (a) intentional additives/adulterants and (b) non-intentional contaminants. The commonly encountered food adulterants which pose a serious health hazard are: (i) Non-permitted colours in sweets, savoury, crushed ice, hard boiled sugar confectioneries, spices, etc. (ii) Adulterants in edible oils such as Argemone seed oil; butter yellow for colouration of cheaper colourless oil; tricresyl phosphate, an odourless and colourless industrial chemical. (iii) *Lathyrus sativus* pulse and its flour in *Cajanus indicus* pulse and dehusked Bengal gram flour. Among the contaminants of concern are: (i) polycyclic aromatic hydrocarbons, a group of ubiquitous environmental pollutant in several foodstuffs including several edible oils. (ii) Toxic metals in various raw and processed foodstuffs. (iii) Pesticide residues especially organochlorine compounds in food grains, vegetables, fruits, milk and edible oils. (iv) Mycotoxins which represent a major group of chemicals that can occur in a variety of plant food especially in tropical countries. Among these compounds, aflatoxin B1 appears to be the most hazardous. Thus, there is need to restrict the presence of various contaminants and adulterants in food within the maximum permissible levels. It is only after ensuring good quality food, by keeping the contaminants as low as achievable, that we shall be able to fulfill the basic requirement of safe food for humans.

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Food safety nets in Latin America

Elizabeth de Souza Nascimento

Faculty of Pharmaceutical Sciences/University of São Paulo, São Paulo, Brazil

Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. The four pillars of food security are availability, stability of supply, access and utilization. There are many nets involved with attacking hunger, and they are necessary as FAO estimates that 852 million people worldwide are undernourished: 815 million in developing countries, 28 million in the countries in transition and 9 million in the industrialized countries. It is a fact that the percentage of population undernourished in the Latin America and Caribbean decreased from 19 millions in 1967 to 10 million at the turn of the new millennium. So, supply food to hunger people still an issue in this part of the world. But also food safety is of

concern, for it defines the degree of confidence that food will not cause sickness or harm to the consumer when it is prepared, served and eaten according to its intended use. In order to assure that, there are nets of food safety involved to assess hazardous chemicals in food, including naturally occurring toxicants, agro-industrial contaminants and food additives. Examples of these nets in Latin America are CYTED—American Iberian Program of Science and Technology. The signatary members are Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Spain, Guatemala, Honduras, Mexico, Nicaragua, Panamá, Paraguay, Peru, Portugal, Dominican Republic, Uruguay and Venezuela, and in the area of food and agriculture houses investigations such as risk evaluation of arsenic and other toxic metals in food, among others. Some of this countries developed programs to assure food safety, and Brazil is among them, carrying on programs, such as the Analysis of Pesticide Residues in Foods, coordinated by the Health Ministry, in which from 2001 to 2004 analyzed 4001 food samples. The results showed that 2032 samples presented pesticide residues, 71% in accordance to Brazilian regulations and 28.5 were not. This presentation will focus on food safety nets and their accomplishments in Latin America.

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Evaluation of the daily nitrate intake through infant formulas in Brazil

Elede Martins Elias, Susanne Rath, Felix G.R. Reyes

State University of Campinas, Campinas, Brasil

Whey from milk with added nitrate in the cheese production may contain higher levels of nitrates, and since it is used as ingredient in infant formulas, it could be a source of nitrate in this food. Nitrate is considered a compound of relatively low toxicity and its risk to the human health depend on its reduction to nitrite, which can lead to the formation of methemoglobinemia or *N*-nitroso compounds. Breast milk is the fundamental food for the healthy development of infants, but in some cases the use of infant formula is necessary. The baby bottles prepared with infant formula thus becomes the main source of nitrate exposure for babies less than 3 months old. A method to quantify nitrate using flow injection analysis (FIA), with spectrophotometric detection, based on the formation of $FeSCNNO^+$, was developed and validated. The nitrate was extracted from the infant formula with water at 50 °C and treated with Carrez reagent. The clean-up of the extract was performed using solid phase extraction on octadecil cartridge. For the method validation the following parameters were evaluated: linear range (0.50–3.00 mg L^{-1}), linearity 0.9917; intra-assay precision (R.S.D. 1.60%; $n = 10$), detection limit (0.3 mg kg^{-1}), quantification limit (1.00 mg kg^{-1}) and recovery (94–108%). The method was used for the determination of nitrate in infant formulas available in the retail market of the state of São Paulo, Brazil. The nitrate level in the samples ($n = 30$) was lower than 21.8 mg kg^{-1}. Nine samples presented a nitrate level lower than the quantitation limit of the method and 21 showed an average value of 7.0 ± 0.35 mg kg^{-1}. The daily nitrate intake was estimated considering the average consumption of baby bottles prepared with the infant formula, for children aging 0–12 months old. For this purpose, the nitrate content in the water used in the preparation of the baby bottles was considered and ranged from 0.37 to 2.69 mg/kg b.w./day. It should be emphasized that the higher values of ingestion were found for infants less than 3 months old. The results indicate the need to establish specific legislation in Brazil for the presence of nitrate in infant formulas, in particular for those destined to infants up to 12 weeks old, in order to be able to conduct actions to control the presence of nitrate in this food, since infants younger than 3 months old are particularly susceptible to the formation of methemoglobin due to nitrate exposure.

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Cytotoxic and apoptotic effects of fumonisin B_1, beauvericin and ochratoxin A on porcine kidney PK-15 cells

Maja Šegvić Klarić [1], Stjepan Pepeljnjak [1], Lada Rumora [2], Danica Ljubanović [3]

[1] *Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia;* [2] *Department of Medical Biochemistry and Haematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia;* [3] *Department of Pathology, University Hospital Dubrava, Zagreb, Croatia*

Fumonisin B_1 (FB_1), beauvericin (BEA) and ochratoxin A (OTA) are widespread mycotoxins, which contaminate food and feed, particularly maize. Their co-occurrence was established in 6% (two toxins) and 2% (three toxins) of maize samples in Croatia with average concentrations 0.62 mg/kg of FB_1, 0.39 mg/kg of BEA and 0.02 mg/kg of OTA. Fumonisin B_1 and OTA could be implicated in development of various diseases in animals and humans, including nephrotoxicosis and carcinogenesis. Cytotoxic

and apoptotic activity of BEA was observed in few cell lines including human B-lymphocytes, rodent colangiocytes and human cell lines of myeloid origin. However, possible nephrotoxic effects of BEA have not been investigated yet. In this study, we explored cytotoxic (cell viability) and apoptotic (index of apoptosis and activation of caspase-3) effects of FB_1, BEA and OTA in PK-15 cells (porcine kidney epithelial cells). Cells were treated with lower concentrations of mycotoxins (0.05, 0.5 and 5 μg/mL) for 24 and 48 h. Decrease of cell viability shows that FB_1, BEA and OTA are cytotoxic to PK-15 cells in dose dependent manner. Cell viability was significantly decreased after 24 h of exposure to 5 μg/mL of FB_1 (25%), BEA (30%) and OTA (40%), as compared to untreated control cells ($P < 0.05$). On the other hand, index of apoptosis was increased by 110–140% after 48 h of exposure to individual mycotoxins (5 μg/mL), as compared to control samples ($P < 0.05$). OTA activated caspase-3 after 24 h of treatment with 0.5 μg/mL (84%), while BEA (319%) and FB_1 (419%) significantly affected this enzyme after prolonged exposure ($P < 0.05$). Combined treatment with two or three mycotoxins (0.05 and 0.5 μg/mL) showed additive effects on cell viability. Beauvericin and OTA (5 μg/mL) synergistically increased apoptotic index after 24 h of treatment. Synergistic effects on caspase-3 activities were observed after 24 h of exposure to BEA + OTA and FB_1 + BEA + OTA (0.05 and 0.5 μg/mL), as well as to FB_1 + BEA and BEA + OTA (5 μg/mL). From the mycotoxicological risk point of view, frequent maize contamination by fungal species that produce FB_1 and BEA (*Fusarum* spp.), as well as OTA (*Aspergillus* spp. and *Penicillium* spp.), is one of the greatest concern. Co-occurrence and accumulation of these mycotoxins in grains and their synergistic action might be an important trigger for development of chronic renal diseases, especially after long-term exposure of consumers.

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S13 Toxic Contaminants Generated from Heat-Treatment of Food

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Chemical formation pathways of toxic contaminants from heat-treatment of food

Bronislaw Wedzicha

University of Leeds, Procter Department of Food Science, Leeds, UK

Acrylamide is a potentially toxic chemical which is formed when plant foods are subjected to heat. There is evidence to confirm that the population generally is exposed to small but significant levels of acrylamide; the way that it is metabolised in the body suggests that it is probably a carcinogen in man. For this reason there has been considerable interest in acrylamide since its discovery in food. Whilst the highest levels are associated with potato products, significant levels are observed in cereal products and some has been detected in plant foods in general.

The principal pathway for the formation of acrylamide in foods is by the reaction of asparagine with intermediates in the Maillard reaction. Asparagine is the predominant free amino acid in many plant-derived foods, and the Maillard reaction is critical to colour and flavour development in heat-treated foods. The chemical mechanisms of these processes will be explained, and it will be shown how reducing sugars (e.g., glucose, fructose, maltose) and ascorbic acid, and amino acids, react to form precursors of acrylamide. The possibility that other ingredients may contribute to acrylamide yield will also be explained critically.

The amount of acrylamide formed in food is under kinetic control, i.e., the amount formed at the end of a heating process depends on the rate of formation and the length of the heating process. The kinetic approach identifies the rate limiting steps in any process, and examines how these steps depend on the reaction conditions: pH, temperature, concentrations of reactants, etc. The relationship between the kinetics of browning and acrylamide formation will be explained, and methods whereby the kinetic approach can be used to suggest ways of minimising acrylamide formation will be discussed. The model itself consists of a number of pathways which compete with acrylamide formation and a process by which acrylamide is 'lost' either physically or by reactions with food components.

Acrylamide is by no means the only substance which has been implicated in this way. Heterocyclic amines and polycyclic hydrocarbons are both well known heat-

induced toxicants. Their mechanisms of formation will also be explained. More recently, attention was drawn to furan. The possibility that other (and as yet unknown) thermally generated toxicants exist in food will be considered briefly. The discovery of acrylamide in food required a specific chemical derivatisation procedure for it to be detected. This discovery depended on a specific association of ideas. Whilst the identification of acrylamide leads one to suggest a number of related chemicals as candidates for investigation, the real interest lies in other classes of compounds formed by as yet unknown mechanisms. The increasing use of LC–MS and other hyphenated techniques will increase our chances that such new substances might be revealed in the future.

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Formation and biochemistry of genotoxic heterocyclic aromatic amines in cooked meats

Robert J. Turesky

Division of Environmental Health Disease Prevention, Wadsworth Center, P.O. Box 509, Albany, NY 12201-0509, USA

Heterocyclic aromatic amines (HAAs) are potent genotoxicants that occur in grilled meats and tobacco smoke condensate. The concentrations of HAAs in cooked meats are dependent upon the type of meat, the temperature, the duration of cooking and can range from one up to 500 parts per billion. HAAs that contain the *N*-methyl-2-aminoimidazole moiety form through the reaction of pyridine or pyrazines, heat-catalyzed degradation products of amino acids, with sugars and creatine, a compound found in muscle-meats, to produce the "IQ-type" compounds. Other HAAs, such as 2-amino-9*H*-pyridole[2,3-*b*]indole, are derived directly from the pyrolysis of proteins or amino acids heated at high temperature.

Some HAAs are carcinogenic in rodents and nonhuman primates and induce tumors at multiple sites that include: the liver, stomach, lung, colorectum, prostate and mammary glands. The bioactivation of HAAs occurs by cytochrome P450-mediated *N*-oxidation of the exocyclic amino group, followed by phase II enzyme catalysis to produce reactive esters of the *N*-hydroxy-HAAs, which can covalently adduct to DNA and can lead to mutations. The human P450s preferentially catalyze *N*-oxidation (bioactivation) of HAAs, while rodent P450s are more efficient in detoxication of HAAs through oxidation of the heterocyclic ring systems.

Several epidemiological studies have linked frequent consumption of red meats with increased risk of developing colorectal and breast cancer, and the highest risk has been observed for individuals who frequently eat meat grilled well-done. Moreover, a higher incidence for colon cancer has been reported in individuals who possess both rapid *N*-acetyltransferase and P450 1A2 *N*-oxidizer phenotypes; these phenotypes are associated with enzymes that bioactivate HAAs. The large-interindividual variation in phase I and II enzymes involved in bioactivation and detoxication of HAAs suggest that health risks associated with dietary exposure to HAAs may greatly vary amongst individuals.

Recent advances in mass spectrometry (MS) techniques have allowed us to discover six novel HAAs in grilled meats and urine of meat-eaters and demonstrate that the exposure to HAAs is higher than currently accepted estimates. The employment of sensitive MS techniques for the measurement of biomarkers of HAAs, such as urinary metabolites, DNA and protein adducts, may aid to clarify the role of HAAs as a critical dietary factor in the initiation of several common forms of human cancer.

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Mechanisms of action of carcinogenic heterocyclic amines

Nigel J. Gooderham, Sandra N. Lauber, Stuart K. Creton, Huijun Zhu

Molecular Toxicology Group, Division of Biomedical Sciences, Imperial College London, London SW7 2AZ, UK

It has been known for quite some time that there is a strong association between diet and the aetiology of human cancer, particularly cancer of the colon, breast and prostate. The contribution that diet makes to cancer has been estimated to be as high as 45%, thus attempts to identify causal diet-borne agents has been an area of high activity. In the early eighties a family of chemicals, the heterocyclic amines, were found to be generated when meat was cooked, and their extreme mutagenicity in short-term assays prompted speculation of their role as human carcinogens. Since then numerous experimental and epidemiology studies have examined the relationship between diet, meat, heterocyclic amines and the incidence of cancer. The studies confirm that consumption of meat correlates with tumour formation, particularly cancers of the colon, breast and prostate, and a number of these studies directly implicate the

heterocyclic amines. Furthermore, every cooked-meat heterocyclic amine examined thus far has been shown to induce tumours in laboratory animals. One such heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), is a complete carcinogen that induces tumours of the colon, breast and prostate in the rat. Heterocyclic amines like PhIP are readily absorbed after consumption of cooked meat, and extensively metabolized. Humans efficiently activate the heterocyclic amines to their genotoxic *N*-hydroxy derivatives, which are then metabolically detoxified and excreted. Each of these steps, from exposure to excretion varies from individual to individual and thus may contribute to susceptibility to cancer if the heterocyclic amines are causal agents. With a good understanding of these events, clinical trials and case control studies have attempted to evaluate the potential contribution of heterocyclic amines to human tumour incidence. For human colorectal cancer, studies that have examined exposure support a role for the heterocyclic amines yet studies that have used a pharmacogenetic approach do not. These confounding results imply that the case for the heterocyclic amines being causally linked to human colonic cancer is equivocal. However, recent studies that have examined the molecular and cellular toxicology of the most abundant cooked-food heterocyclic amine, PhIP, have demonstrated powerful biological activities at very low exposure levels (sub-nM). These latter properties are hormone-like and offer a mechanistic explanation for the unique tissue-specific toxicity of this heterocyclic amine.

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Effect of acrylamide and glycidamide on intestinal tumourigenesis in Min mice

Hege B. Ølstørn, Jan Erik Paulsen, Jan Alexander

Department of Food Toxicology, Norwegian Institute of Public Health, Oslo, Norway

Acrylamide (AA), which is metabolised to the genotoxic metabolite glycidamide (GA) was some years ago also found in small amounts in heat treated carbohydrate rich foods. In this study, we used the *Min* mouse, which has shown to be particularly sensitive to intestinal carcinogenesis early in life. The *Min* mouse is a murine model of familial adenomatosis coli and has a germ line truncation mutation in one *Apc* allele. Tumourigenesis is believed to start upon inactivation of the remaining wt *Apc* allele, either by loss of heterozygosity or mutation. Because about 80% of sporadic colorectal cancers show mutations in *APC*, this model is also relevant for these cancers. The Min mice were exposed by s.c. injection to 50 mg/kg bw of AA or GA either via the dam once during the last week of pregnancy, or twice during the first two neonatal weeks or both. The number, size and location of the lesions were scored 8 or 12 weeks after birth. GA given postnatally increased the number of small intestinal tumours 1.3-fold and flat dysplastic aberrant crypt foci (ACF) in the colon were increased 1.5-fold and classical ACF 4.8-fold. Combined pre- and postnatal exposure to GA in particular increased the number of flat dysplastic ACF and tumours of the large intestine. AA did not increase the tumour number in any of the exposures, but significantly increased the number of flat dysplastic ACF of the colon, indicating a tumour initiating potential.

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Heterologous and transgenic models for studying genotoxic effects of contaminants produced by heat-treatment of food

Hansruedi Glatt, Yasmin Sommer, Gisela Dobbernack, Walter Meinl

German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany

Genotoxicants formed by heat-treatment of foods usually require metabolic activation to exert their effects. In collaboration with various partners we have expressed nearly 100 human and rodent xenobiotic-metabolizing enzymes and transmembrane transporters in bacterial and/or mammalian target cells of in vitro test systems. In this lecture, we shall present findings on heterocyclic aromatic amines (HA) and furfuryl alcohols (FA). The activation of HA usually involves two steps. The first step, the hydroxylation of the exocyclic amino group, is uniform; it is often followed by a conjugation reaction—this second activation step can strongly differ between different HA and, to a lesser extent, between various species. The most common activating phase-II enzymes are sulphotransferases (SULT1A1 and 1A2 in humans, Sult1c1 in rats and Sult1d1 in mice) and acetyltransferases (NAT2 in humans and Nat1 in rats). Specificities are very high at low concentrations of HA and N-OH-HA (fM to nM—relevant for human exposures), whereas enzymes are more promiscuous at high concentrations (μM—as used in many animal experiments and biochemical studies conducted in vitro). FA are present in foods at 10,000 higher levels than HA. They are activated by SULT to mutagens. Sulpho conjugates of different FA enormously differ in their half-life times and their ability

to penetrate biological membranes. Likewise, enzymes involved in their formation show pronounced species-dependent differences. Recently we have constructed mouse models expressing various human SULT with human-like tissue distribution to obtain more insight into the bioactivation of some HA and FA. Initial results will be presented.

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Poster Sessions

Thursday, September 21, 2006

P1 Biotransformation

P1-01
Ethanol single dose provoked acetaldehyde accumulation and oxidative stress potential in rat mammary tissue
A consequence of a limited breast ability for detoxication of alcohol

Gerardo Daniel Castro, Aurora M. Delgado de Layño, Silvia L. Fanelli, María E. Maciel, María I. Díaz Gómez, José A. Castro

Centro de Investigaciones Toxicológicas (CEITOX), CITEFA-CONICET, Buenos Aires, Argentina

In previous studies from our laboratory the presence of several pathways of metabolic activation of ethanol to acetaldehyde and hydroxyl free radicals as well as the promotion of oxidative stress were reported. Other laboratories also reported the presence of type I alcohol dehydrogenase and of CYP2E1 and a limited presence of aldehyde dehydrogenase. In the present studies, we tested the possibility that after a single dose of alcohol, acetaldehyde accumulated in mammary tissue to reach concentrations higher than in blood. Three different doses of alcohol were tested and acetaldehyde concentrations in breast, liver and blood were measured at times ranging from 1 to 24 h. We also determined alcohol dehydrogenase; aldehyde dehydrogenase and *p*-nitrophenol hydroxylase activities. Oxidative stress induced hydroperoxyde formation studies, as determined the xylenol orange procedure are in course for each dose at different times of exposure and at present led to positive results at some times for the highest dose tested. The obtained results showed that acetaldehyde concentrations at the three alcohol doses tested (low, medium and high) were always higher than in blood. Peak concentrations of acetaldehyde in liver, while higher than those in breast, appeared to decrease to blood levels following a similar time sequence. Limited activities of alcohol dehydrogenase and aldehyde dehydrogenase in mammary tissue were observed. The microsomal CYP2E1-mediated *p*-nitrophenol hydroxylase in mammary tissue was several times smaller than in liver.

In summary, results suggest that the mutagen acetaldehyde, either formed in situ or, even in small amounts, continuously arriving via blood, tends to accumulate in mammary tissue as a consequence of a limited capacity of it for detoxication. During the period of alcohol exposure, the GSH levels in mammary tissue were not decreased.

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P1-02
Potentiation acetaminophen hepatotoxicity in rats treated with ethanol and under alcoholism

Alla K. Voronina, Valentina Kovalenko, Ganna Shayakhmetova, Irina Blazchuk

Institute of Pharmacology and Toxicology, Kyiv, Ukraine

Acetaminophen is widely used as an analgesic and antipyretic agent, and it is assumed to be safe when taken in recommended doses. However, in isolated cases in which alcohol has presumably played a synergistic role with acetaminophen in production liver toxicity after ingested drug for therapeutic reasons. It is known that P-4502E1 metabolizes acetaminophen, and that it can be induced after by inducers such as ethanol. The purpose of this study was to clarity the effects of ethanol ingestion and chronic ethanol consumption on the hepatotoxicity of acetaminophen. Preliminary studies in mice support the concept that acetaminophen hepatotoxicity may be enhanced by ethanol ingestion. A significant reduction in the LD_{50} was seen in the alcohol-treated mice. The influence of alcohol on the hepatotoxicity of acetaminophen was studied in male albino rats. Rats were treated acetaminophen with ethanol and under alcoholism by intragastric administration. In treated ethanol rats (3.2 mg/kg b.w.), the activities of aniline-*N*-hydroxylase (a marker of cytochrome P-450 2E1 activity) in liver microsomes significant increased after acetaminophen (dose—400 mg/kg b.w.) administration compared with control animals. Among

these glutathione and protein SH-groups contents as well as liver glutathione reductase and catalase activity were decreased. The rate of induced malonodialdehyde (MDA) formation, hydroperoxides and super oxide anion contents were increased in comparison to the control. Alanine aminotransferase (AlAT) activity elevated in blood serum of 1.7-fold the upper limit of normal. After chronic ethanol consumption feeding for 6 months, aniline-*N*-hydroxylase, glutathione reductase, AlAT activities and MDA formation was increased significantly, whereas content of glutathione were decreased compared with control. After parallel administration of acetaminophen (dose—500 mg/kg b.w.) the activities of aniline-*N*-hydroxylase followed up 65% and the serum ALAT activities increased of 5.6-fold. In acetaminophen-treated animals, ethanol-fed rats showed a significant decreased hepatic glutathione levels. Our results suggest that ethanol may be an additional risk factor for developing acetaminophen hepatotoxicity. The observation that acetaminophen and alcohol intensify the induction of CYP2E1 in a synergistic manner may help to understand and to prevent an additional risk factor for developing acetaminophen hepatotoxicity.

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P1-03

Rat liver microsomal monooxygenase system after ethanol and ethylene glycol administration

Andrzej Plewka[1], Joanna Kowalowka-Zawieja[2], Jedrzej Przystanowicz[2], Danuta Plewka[1], Grazyna Nowaczyk-Dura[1], Barbara Zielinska-Psuja[2], Jerzy Orlowski[2]

[1] *Medical University of Silesia, Katowice, Poland;* [2] *Poznan University of Medical Sciences, Poznan, Poland*

Ethylene glycol (EG) is a popular component of antifreeze coolants, windscreen cleaners and de-icers used in automotive industry. Ingestion of EG solutions results in conversion of relatively non-toxic glycol into highly toxic metabolites. Ethanol (EtOH) administered i.v. restricts first step of EG biotransformation by competitive inhibition of alcohol dehydrogenase. This is a key enzyme in metabolic route of both xenobiotics, a smaller amount of them is transformed by microsomal monooxygenase system.

We evaluated the effect of separate and simultaneous per os administration of ethylene glycol (3830 mg/kg b.w.) and ethanol (1000 mg/kg b.w.) on CYP450 and cytochrome b_5 concentrations and activities of their reductases in rat liver. Tests were performed 8, 12, 18, 24, 36 and 48 h after exposure.

In rats exposed to EtOH amount of microsomal protein was elevated to 115% of control after 8 h and 150% at the end of experiment. EG administration, single or simultaneous with EtOH, resulted in substantial decrease of protein amount in period 24–48 h to 85% and 80%, respectively.

A continuing decrease to 66% of control in CYP450 concentrations in EG group was measured in 24–48 h period. EtOH elevated CYP450 concentration to 110% and 145% of control after 8 and 48 h, respectively. Exposure to both xenobiotics resulted in decrease of concentration to 70–80% in whole time of experiment.

Activity of CYP450 reductase increased to 125% and 150% after 48 h in EG and EG with EtOH group, respectively. After EtOH administration the activity decreased to 70% after 18 h, to reach 90% of control after 48 h.

Cytochrome b_5 concentrations after EG administration were 90–65% of control in 18–48 h without effect on cytochrome b_5 reductase. Similar results were obtained after EtOH administration (80–70% of concentration in control group). Simultaneous exposure to both alcohols resulted in decrease of cytochrome b_5 concentration and its reductase activity (80% after 8 h and 60% after 36 h).

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P1-04

The effect of ethanol and ethylene glycol administration on CYP450 and cytochrome b_5 concentrations and their reductases activities in rat kidney

Andrzej Plewka[1], Joanna Kowalowka-Zawieja[2], Jedrzej Przystanowicz[2], Danuta Plewka[1], Grazyna Nowaczyk-Dura[1], Barbara Zielinska-Psuja[2], Jerzy Orlowski[2]

[1] *Medical University of Silesia, Katowice, Poland;* [2] *Poznan University of Medical Sciences, Poznan, Poland*

Ethylene glycol (EG) is extensively metabolised into acid and aldehyde compounds what results in direct renal failure. Ethanol is commonly used in acute EG poisonings to prevent toxic metabolite formation.

The aim of the study was to assess the influence of ethylene glycol and ethanol on microsomal monooxygenase system in kidney.

Male Wistar rats were exposed separately or simultaneously *per os* to EG (3830 mg/kg b.w.) and ethanol (1000 mg/kg b.w.). Cytochrome P450 and

cytochrome b_5 concentrations and activities of NADPH-cytochrome P450 and NADH-cytochrome b_5 reductases were measured in microsomal fraction of kidney homogenates 8, 12, 18, 24, 36 and 48 h after xenobiotics administration.

The amount of microsomal protein in kidneys remained unchanged after ethanol administration and decreased to 80% of control in EG and EG with ethanol groups. Exposure to EG resulted in decrease in CYP450 concentration to 88% and 62% after 8 and 48 h, respectively. A transitory elevation of concentration after 24 h in ethanol group was observed. Decrease to 80–70% of control in 12–48 h was measured after exposure to both xenobiotics.

Ethanol administration increased activity of CYP450 reductase to 130% of control in the 24–48 h period. In groups exposed to ethylene glycol CYP450 reductase activity decreased to 60% and 70% of control after single and simultaneous with ethanol administration, respectively.

Cytochrome b_5 concentration and activity of its reductase were decreased to 75–90% in all examined groups.

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P1-05

Role of serum albumins in the detoxication of the carbamate carbaryl

Miguel A. Sogorb, Carlos Álvarez-Escalante, Victoria Carrera, Eugenio Vilanova

Universidad Miguel Hernández, Elche, Alicante, Spain

The carbaryl hydrolyzing activity (carbarylase) associated to bovine serum albumin (BSA) was studied at toxicologically relevant concentrations (15–300 μM). The 1-naphthol released during the hydrolysis of carbaryl was monitored using gas chromatography coupled to mass spectrometry. BSA (1–7.5 mg/mL) hydrolyzed carbaryl in a time-progressive way. Carbarylase was also dependent of enzyme and substrate concentration. The estimated turnover number and Michaelis–Menten constant were $1.6 \times 10^{-4}\,s^{-1}$ and 430 μM, respectively. Therefore, the second order rate constant was $0.37\,M^{-1}\,s^{-1}$. At BSA concentrations of 7.5 mg/mL and substrate concentrations ranging between 50 and 300 μM about 80% of substrate was hydrolyzed in 3 h of incubation at 37 °C. At lower substrate concentrations (15 and 30 μM carbaryl) also significant carbarylase is detected at the highest BSA concentration (7.5 mg/mL), even when these substrate concentrations were 30 and 15 times lower than Michaelis–Menten constant. Although the efficacy of the carbarylase associated to BSA is low, the extrapolation of our results to physiological albumin concentrations (around 40 mg/mL) suggests that the hydrolysis of carbaryl by serum albumins plays a critical role in the detoxication of this carbamate at in vivo toxicologically relevant concentrations.

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P1-06

Interactions of TNF-alpha and AhR ligands it rat liver epithelial 'stem-like' cells

Lenka Umannova [1], Miroslav Machala [2], Pavel Krcmar [2], Jan Vondracek [1], Alois Kozubik [1]

[1] *Institute of Biophysics, 612 65 Brno, Czech Republic* [2] *Veterinary Research Institute, 62132 Brno, Czech Republic* [3] *Faculty of Science, Masaryk University, Brno, Czech Republic*

TNF-alpha is a pleiotropic pro-inflammatory cytokine produced in liver mainly by activated macrophages, such as Kupffer cells. This cytokine is involved in regulation of the immune system as well as in maintenance of homeostasis. Among other effects, pro-inflammatory cytokines, such as TNF-alpha can influence expression of cytochrome P-450 (CYP) monooxygenases, which play an important role in xenobiotic metabolisms. The gene expression of CYP1A and CYP1B1, major enzymes of metabolic activation of many promutagens, including polycyclic aromatic hydrocarbons, is controlled by aryl hydrocarbon receptor (AhR).

We studied the effects of interaction of TNF-alpha and AhR ligands, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), on cell proliferation, cell cycle regulation and expression of CYP1A1 and CYP1B1 enzymes in model of rat liver progenitor cells—WB-F344 cell line. Our results indicate that TNF-alpha may cause a temporary down-regulation of the expression of CYP1A1, which could be due to mutual inhibitory interactions between the AhR and NF-κB, which is activated by TNF-alpha. In contrast, the expression of CYP1B1 was enhanced by TNF-alpha. We also found that TNF-alpha significantly potentiated disruption of contact inhibition induced by TCDD in WB-F344 cells.

Taken together, our results indicate that inflammatory cytokine may significantly modulate effects of a potent liver tumor promoter TCDD, suggesting that AhR ligands may be even more effective under inflammatory conditions, which are encountered during various liver disease states.

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P1-07

Three-dimensional quantitative structure–activity relationship (3D-QSAR) analysis of CYP2B6 enzyme

Hannu Raunio [1], Laura Korhonen [1], Miia Turpeinen [2], Minna Rahnasto [1], Carsten Wittekindt [1], Antti Poso [1], Risto Juvonen [1]

[1] *University of Kuopio Kuopio;* [2] *University of Oulu, Finland*

The human cytochrome P450 2B6 (CYP2B6) enzyme metabolises numerous compounds, including some widely used pharmaceuticals (e.g. bupropion, cyclophosphamide, propofol) and several toxic agents. The aims of this study were to generate a 3D-QSAR model of the CYP2B6 enzyme and to find novel potent and selective inhibitors of CYP2B6 for in vitro studies.

Twenty-five compounds were first analyzed for inhibition potency. Based on this series of compounds, an initial Comparative Molecular Field Analysis (CoMFA) model was created. Based on this CoMFA model and structures of known potent CYP2B6 inhibitors we further selected and tested 16 new pyridine and phenylalanine derivatives. The human CYP selectivity of the three most potent inhibitors was tested. To obtain a more precise picture of the key molecular characteristics of CYP2B6 inhibitors, we carried out a second CoMFA analysis with all 41 compounds. The prediction power of this CoMFA model was evaluated by estimating pIC_{50} values for an external test set of compounds.

Lower than 1 μM IC_{50} values were obtained with 4-(4-chlorobenzyl)pyridine, 4-benzylpyridine and 4-(4-nitrobenzyl)pyridine (<1 μM). They were found to be also very selective inhibitors towards CYP2B6, especially 4-(4-chlorobenzyl)pyridine as the IC_{50} values against other human CYP enzymes were higher than 100-fold. The created CoMFA model was of high quality with the following statistical values with two components: $q^2 = 0.71$, $S_{PRESS} = 0.64$, $r^2 = 0.85$. The sterically favored area was located around substitution at carbon 4 of the benzyl ring of benzylpyridine in the CoMFA model. A broad partial negative charge near to a nitrogen atom and around benzyl tends to increase the inhibition potency. The model predicted accurately the IC_{50} values of a series of test compounds.

The CoMFA model yielded novel structural information about the CYP2B6 enzyme. The developed model accurately predicted the inhibition potencies of several structurally unrelated compounds. In addition, novel potent and selective inhibitors of CYP2B6 were found.

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P1-08

Expression of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human CD34$^+$ bone marrow stem cells

Ulrike Bernauer [1], Henk Garritsen [2], Ursula Gundert-Remy [1]

[1] *Federal Institute for Risk Assessment Berlin;* [2] *Staedtisches Klinikum Braunschweig, Dept of Transfusion Medicine, Braunschweig, Germany*

Background: The bone marrow represents an important target tissue for the toxic and haematopoietic effects of chemicals and pharmaceuticals (e.g. benzene, dapsone). CYP enzymes are involved in the metabolism of these compounds. Local metabolism within the target tissue may play a role in the haematotoxic effects of xenobiotics, especially in cases where it seems to be unlikely that metabolites generated in the liver will survive carriage into the bone marrow. Therefore, it was our aim to investigate the possibility of CYP-dependent xenobiotic metabolism in the human bone marrow. CD34$^+$ bone marrow stem cells are thought to be the target cells for the toxic effects of some chemicals and therapeutic agents. We investigated the expression pattern of xenobiotic-metabolizing CYP enzymes in a panel of CD34$^+$ cells samples from different individual donors.

Methods: Human CD34$^+$ bone marrow stem cells from 42 donors were obtained after immunomagnetic separation from leukapheresed blood samples after informed consent of the donors. Total cell protein was separated by SDS-polyacrylamide gel electrophoresis and probed with commercially available antibodies specific for the following CYP enzymes: CYP1A1/2, CYP1B1, CYP2A6, CYP2B6, CYP2C10, CYP2D6, CYP2E1 and CYP3A4/5/7.

Results: Bands indicative for CYP3A5, CYP2E1, CYP1A2 and CYP2C9 CYP1A2 were present in all samples investigated pointing to their constitutive expression in human CD34$^+$ stem cells. Expressions of CYP 1A1, CYP2C8, CYP2C19 and CYP1B1 proteins were below detectable levels. There was also evidence for low expression levels of CYP2A6, CYP2B6 and CYP2D6. It

can be deduced, that human $CD34^+$ bone marrow stem cells possess the ability to locally metabolize foreign compounds.

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P1-09

Changes in brain and liver cytochrome P450 after multiple cocaine administration, alone and in combination with calcium channel blocker

V. Vitcheva, M. Mitcheva

Laboratory of Drug Metabolism and Drug Toxicity, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

The objective of the following study is to trace the possible changes, caused by multiple cocaine administration, in cytochrome P450 quantity, both on brain and hepatic level. Cocaine is extensively metabolized, primarily in the liver, and one of the metabolic pathways is *N*-demethylation to norcocaine, a process catalyzed by CYP3A4. Since there are literature data about use of calcium channel blockers in maintaining different types of drug dependence and withdrawal, it was interesting to trace the possible metabolic interactions of cocaine and 1,4-dihydropiridine calcium channel blocker Nifedipine for which is known to be a substrate of CYP 3A4.

For the experiment, male Wistar rats were used. The animals were divided in four groups: control; treated with nifedipine (10 mg/kg i.p., once daily, for 5 days); treated with cocaine (15 mg/kg once daily, for 5 days); treated with nifedipine (10 mg/kg i.p.) and half an hour later, with cocaine (15 mg/kg i.p). Total quantity of cytochrome P450 was measured spectrometrically in brain and liver microsomes. Multiple administration of cocaine, alone and in combination with nifedipine, did not change the brain cyt P450 significantly. On the hepatic level, nifedipine, compared to the control group, increased P450 quantity, statistically significant by 28%. Cocaine decreased P450 level by 17%, statistically significant, versus control. In the combination group, cyt P450 quantity was increased statistically significant by 13%, compared to the control and by 35%, compared to the pure cocaine group.

According to the results of this study we suggest interactions of the studied compounds on the metabolic level.

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P1-10

Alterations of hepatic xenobiotic metabolizing enzyme activities in combined selenium plus iodine-deficient rats by fenvalerate exposure

P. Erkekoglu, A. Caglayan, B. Giray, F. Hıncal

Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

This study was undertaken to investigate the effects of fenvalerate (FV), a pyrethroid insecticide, on hepatic xenobiotic metabolizing enzyme activities in combined selenium and iodine-deficient rats. Three-week-old rats were used in all experiments. The animals were divided into four groups and feeding period was 7 weeks. (i) Control group was fed with regular diet and drinking water. (ii) FV group (CF) was fed with regular diet and drinking water, and during the last week of feeding period the rats received 100 mg/kg/day, i.p., FV. (iii) Selenium plus iodine-deficient group (ISeD) received both Se-deficient diet and 1% sodium perchlorate containing drinking water. (iv) Selenium plus iodine-deficient group exposed to FV (ISeD-F) was fed selenium-deficient diet and iodine deficiency was introduced by 1% sodium perchlorate-containing drinking water and received 100 mg/kg/day, i.p., FV during the last week of feeding period. Microsomal aniline hydroxylase (CYP2E1), EROD (CYP1A1/1A2), PROD (CYP2B1/2B2), P450R and cytosolic GST activities were determined in hepatic tissue. In combined deficiency, all enzyme activities examined were found to be decreased compared to control group, but the decrease in CYP 2E1 activity was not statistically significant. FV exposure of normal rats caused significant elevations of EROD (180%), PROD (100%) and GST (12%) activities. In ISeD-F rats, activities of P450R (173%), EROD (226%), PROD (140%) and GST (457%) increased significantly compared to ISeD group, however CYP2E1 did not change. These results showed that hepatic xenobiotic metabolizing enzyme activities of rats are significantly affected by combined iodine plus selenium deficiency, and the inductive effect of fenvalerate exposure might change the metabolism of concominantly exposed xenobiotics—including drugs, as well as endogenous substrates in both normal and deficiency states.

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P1-11

Nifurtimox induced ultrastructural and biochemical alterations in rat heart
Their potential relevance in the treatment of Chagas' disease

Laura C. Bartel, María Montalto de Mecca, Carmen Rodríguez de Castro, Silvia L. Fanelli, Edith G. Díaz, José Alberto Castro

CITEFA-CONICET, Centro de Investigaciones Toxicológicas (CEITOX), Argentina

Chagas' disease is an endemic parasitic disease in some areas of Latin America. About 16–18 million people suffer this sickness and more than 100 million are living at risk of getting the infection. Life threatening myocarditis can occur during the acute phase of the disease. About 20–25% of the surviving patients of the acute phase develop the chronic phase, which is characterized by potential lethal cardiopathy. There are available two drugs for the ethiological treatment of the disease, Nifurtimox (Nfx) and Benznidazol (Bz). They are being used in the acute phase and more recently they were used in the asymptomatic intermediate phase. This is developed after the acute and before the chronic phase and when the deleterious effects of the disease in heart could be already in course. Both nitroheterocyclic drugs have serious toxic side effects which compromised their use. The mechanism of toxicity is associated with their nitroreduction and the generation of reactive metabolites. However, their potential effects on cardiac function are yet not known. In this study, we describe initial experiments to test the acute effects on rat heart. Male Sprague–Dawley rats (18 weeks, 280–320 g bw) were treated intragastrically with Nfx at a dose of 100 mg/kg bw suspended in 1% carboxymethylcellulose. Control rats received the same amount of the vehicle. We observed that the administered drug reached the heart tissue at 1, 3 and 6 h after treatment. Studies on Nfx nitroreductase activity showed that the microsomal fraction had the ability to nitroreduce Nfx. With respect to the biochemical effects due to protein oxidation processes, we observed an increase in protein carbonyl content of treated rats at 1 and 3 h and a protein sulfhydryl decreasing content was observable only at 3 h after treatment, being undetectable at 1 and 6 h. No increases in the *t*-buthyl hydroperoxide induced chemiluminiscence were observed at 1, 3, 6 and 24 h after Nfx administration. However, at 24 h after treatment ultrastructural alterations were observable in the heart. They consisted in a marked vacuolization of the cytoplasm, the separation and loss of myofibrils and mitochondrial swelling too. Furthermore, most cells showed clumping of the chromatin adjacent to the nuclear membrane. Results suggest that Nfx administration might aggravate pre-existing adverse cardiac conditions.

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P2 Clinical Toxicology

P2-01

Acute effects of hexanal, acetic acid and dioxane in humans, examples of volatile chemicals studied in our exposure chamber

L. Ernstgård [1], Anders Iregren [2], Birher Lind [1], Bengt Sjögren [1], Gunnar Johanson [1]

[1] *Work Environment Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden;* [2] *National Institute for Working Life, Stockholm, Sweden*

Chemosensory irritation of the mucous membranes is an important endpoint in the risk assessment of volatile chemicals. Information on health hazards associated with the handling of hexanal, acetic acid and 1,4-dioxane is sparse. We have performed controlled short-term chamber exposures with the intention to evaluate acute health effects of these chemicals, with emphasis on irritation.

In pilot studies, the subjects where exposed to stepwise (10 min per level) increasing levels of each chemical vapour. A questionnaire with visual analogue scales (VAS) was used for graded ratings of irritation and central nervous system symptoms. Based on the ratings, the subjects where exposed for 2 h at rest to vapours of hexanal (0, 2 and 10 ppm), acetic acid (0, 5 and 10 ppm) and 1,4-dioxane (0 and 20 ppm), respectively. In addition to the VAS ratings, effects in the airways were assessed by spirometry, nasal swelling was studied by both acoustic rhinometry and nasal blocking index, blink frequency was measured by electromyography and inflammatory markers in plasma (C-reactive protein and interleukin-6) were analysed.

Exposure to 20 ppm 1,4-dioxane did not significantly affect any of the measured parameters. For hexanal, ratings of discomfort in the eyes and nose, solvent smell and headache increased significantly with exposure level (2 and 10 ppm). Blinking frequency was significantly increased at 10 ppm. Acetic acid caused significantly increased ratings of nasal irritation and smell at 5 and 10 ppm. No effects on pulmonary function,

nasal swelling or on plasma inflammatory markers were detected in any study.

In conclusion, minor effects were detected after exposure to hexanal (10 ppm) and acetic acid (10 ppm). No effects were detected for 1,4-dioxane at 20 ppm, the highest level tested.

The major advantages of this kind of experimental design are that the volunteers are exposed under well-controlled conditions, with respect to exposure level and duration as well as temperature and humidity, and that they serve as their own controls. This leads to less variability and, hence, increased statistical power to detect adverse effects.

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P2-02

Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide workers

Shahin Shadnia, Mohammad Abdollahi, Ebrahim Azizi

Loghman-Hakim Hospital, Tehran, Iran

The widespread use of pesticides in public health and agricultural programmes has caused severe environmental pollution and potential health hazards including severe acute and chronic cases of human poisonings. Many dangerous effects of OPs in acute poisoning cases result from inhibition of blood cholinesterase activity. It has been reported that OPs may induce oxidative stress in humans and animals. Good evidence supports the hypothesis that oxidative stress may be involved in pesticide-induced cell injury. On the other hand, some epidemiological studies have shown the association between chronic exposure to pesticides and recorded cases of human malignancy. Exposure to known genotoxic compounds could induce DNA damage not only directly but also through other mechanisms, such as oxidative stress or inflammatory processes.

The aim of this study was to evaluate genotoxicity and oxidative stress in workers who formulate organophosphorus (OP) pesticides. In this survey, blood leukocytes and erythrocytes of a group of 21 pesticide formulating workers and an equal number of control subjects were examined for genotoxicity and oxidative stress parameters. The mean comet tail length and mean comet length were used to measure DNA damage. Lipid peroxidation level, catalase, superoxide dismutase (SOD) and glutathione peroxidase activities in erythrocytes were analysed as biomarkers of oxidative stress. In addition, the acetylcholinesterase activity was measured as a biomarker of toxicity. The average duration of employment of workers in the factory was 97 months. Results indicated that chronic exposure (multiple-dose, greater than or equal to 6 months duration) to OP pesticides was associated with significant increase in activities of catalase (32.93 ± 2.35 versus 15.96 ± 1.81), SOD (84.14 ± 6.80 versus 40.13 ± 3.82) and glutathione peroxidase (29.30 ± 1.70 versus 24.94 ± 0.80) in erythrocytes of workers in comparison to controls. The level of lipid peroxidation ($83.85 \pm /9.10$ versus 79.92 ± 3.73) and acetylcholinesterase activity (7.969 ± 0.26 versus 8.39 ± 0.33) did not show any significant differences between the two groups. The results also indicated that chronic exposure to OP pesticides was associated with increased DNA damage. Comet tail length (13.87 ± 0.44 versus 5.57 ± 0.28) and comet length (33.62 ± 0.57 versus 24.83 ± 0.37) was increased significantly in workers to controls. It is concluded that human chronic exposure to OP pesticides may result in stimulated antioxidant enzymes and increased DNA damage in the absence of depressed acetylcholinesterase levels. Routine genotoxicity monitoring concomitant to acetylcholinesterase activity in workers occupationally exposed to OP insecticides is suggested.

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P2-03

The latent effects of leaves plant extracts on the adults of *Galleria mellonella* L. (Lepidoptera: Galleriidae)

M.S. Khalil[1], M.A. Taha[2], E.E. Seliem[2], A.A. Abd_Elwhab[2]

[1] *Faculty of Science, Menofeya University, Egypt;* [2] *Faculty of Science (for girls), Al-Azhar University, Egypt*

The treatment of larvae of *Galleria mellonella* L. (Lepidoptera: Galleriidae) by fresh and dry ethanolic plant leaves extracts of *Melia azedarach* (*Melliaceae*), *Venca rosea* (*Apocynaceae*), *Allium sativum* (*Liliaceae*) and *Calcasia antiqurum* (*Araceae*) cause significant hazard effects by reduction of adult longevity and prolongation of its life spans through retardation of development of immature stages. It was comparable for all tested plant, except that of *A. sativum* which was more effective than them. The reductions in female's longevity lead to reductions in fecundities by decreasing the periods of ovipositors. Whereas the reductions in males longevity lead to reductions in chance of mating by reductions in mating periods, and consequently reductions in fertile eggs of females. These both reductions cause hazard effects on this pest and considered as a good manner for control this insect.

The life span of this pest increased by increasing of concentrations of both fresh and dry leaves extracts. These increasing were independent on sexes. And comparable for all tested plants, except extracts of *M. azedarach* which cause more prolongation in its life span than other plants, through its more potent for retardation and delaying the developments of immature stages than other plants. These results indicated that the ability of using these extracts for control of this series pest in a safety way for both honeybees and environment.

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P2-04
Verification of tramadol abuse in patients on methadone substitution therapy

Vesna Horvat, Sanja Mandic, Dario Mandic, Ksenija Bojanic, Neda Majetic-Cetina

Clinical Hospital Osijek, Osijek, Croatia

Methadone is a synthetic opioid, often used in the treatment of narcotic addiction. The oral use of methadone can suppress withdrawal symptoms, but in contrast to heroin, does not give any euphoric effect. However, this sensation can be induced by combination with alcohol, benzodiazepines, tramadol, etc. Therefore, regular urine testing should be carried out. We analyzed urines of 25 patients on methadone therapy with 3 methods: enzyme multiplied immunoassay (EMIT), thin layer chromatography (TLC) and gas chromatography mass spectrometry (GC–MS). We assessed the efficacy of these methods in verification of tramadol abuse in patients on methadone substitution therapy.

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P2-05
Body-packers: Report of a case and mini review of the literature

Mohammad-Reza Faiaz-Noori, Shahin Shadnia, Abdolkarim Pajoumand

Loghman-Hakim Hospital Poison Center, Faculty of Medicine, and Medical Toxicology Research Center, Shaheed-Beheshti University of Medical Sciences (SBUMS), Kamali Avenue, South Karegar Street, Tehran 13334, Shebeen El-Koom, Iran

Body-packers are people who illegally carry drugs, mostly cocaine, and opium and/or heroin, concealed within their bodies. The packets can be made of various materials, but most often are condoms, which are easily available on the market. The packets are inserted in the mouth, rectum or vagina in order to get across borders without being detected.

In this presentation, we report a case of opium body-packer and review the available scientific literature by focusing in treatment approach.

The patient was a 35-year-old Afghanian man who was found unconscious by emergency personnel. The patient had lethargy, respiratory rate: 8 min^{-1}, pulse rate: 120 beat/min, blood pressure: 80/50 mmHg, axillary's temperature: 38.5 °C and pinpoint pupils. The remainder of the examination was unremarkable. Because the individual was alone and unresponsive, no past medical history was obtained and the only positive history was his travel from Afghanistan 2 days earlier that he gave to emergency personnel before arriving at our hospital.

The hemoglobin, platelets, leukocyte count, blood sugar, serum electrolytes, and serum creatinine, were within normal range. Liver function test and coagulation profile were normal. In the emergency department the patient was treated with oxygen, naloxone and hypertonic glucose. One dose of activated charcoal (1 g/kg) was administered orally. After intravenous injection of naloxone (4 mg) the lethargy, respiratory depression and miosis resolved. The patient was admitted to the intensive care unit and 90 min after admission the patient redeveloped respiratory distress and lost consciousness, for which he was intubated and mechanically ventilated, due to our suspicious of body packing.

Plain abdominal X-ray showed multiple packets throughout the gastro-intestinal (GI) tract. Eighty-one packets were removed by surgery and three of them were leaking.

After removing the packets, the patient was treated conservatively. He suffered a pulmonary infection (aspiration pneumonia) and he regained his consciousness after 4 days. Upon recovery the patient was seen by a psychiatrist and later on he was taken to the prison.

Body-packers who present with opioid poisoning can usually be treated conservatively with infusion of an opioid antagonist. Prompt surgical intervention is only indicated for bowel obstruction, perforation, esophageal obstruction, and perforation, but our experience has shown that there is a high mortality rate after packet leakage or rupture and we now recommend surgery for patients who have significant signs or symptoms of toxicity. If the patient admitted in intensive care unit, where the patient can be intubated and ventilated, we prefer not to administer naloxone.

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P2-06

Comparing experimental designs for benchmark dose calculations for continuous endpoints

Kristi Kuljus[1], Dietrich von Rosen[2], Salomon Sand[3], Katarina Victorin[3]

[1] *Uppsala University Uppsala, Sweden;* [2] *Swedish University of Agricultural Sciences, Uppsala, Sweden;* [3] *Karolinska Institutet, Stockholm, Sweden*

There are basically two statistical methods that are used in risk assessment of non-genotoxic chemical compounds: the no observed adverse effect level (NOAEL) approach and the benchmark dose (BMD) method. The BMD method is based on dose–response modeling. To take uncertainty in the data and model fitting into account, the lower confidence bound of the BMD estimate can be used as a point of departure in health risk assessments. Mathematical modeling of the dose–response data may be difficult when toxicological studies are performed with a limited number of dose levels. The main aim of the present study was to investigate whether an increased number of dose groups and at the same time a decreased number of animals in each dose group improves conditions for estimating the benchmark dose.

We have used Bayesian design approach for comparing experimental designs for benchmark dose calculations in case of continuous endpoints. We exemplify the method by considering the class of Hill models, which has been acknowledged in applications relating to risk assessment of chemicals and BMD analysis. In the study, the BMD was defined as corresponding to 5% change in response relative to the total effect size. Since Hill models are nonlinear, the optimum design for estimating the benchmark dose depends on the values of the unknown parameters. For this reason we have considered Bayesian designs and assume that the parameter vector has a prior distribution. A natural design criterion is to minimize the expected variance of the BMD estimator. We present an example where we have calculated the value of the design criterion for several designs and try to find out how the number of dose groups, the number of animals in the dose groups and the choice of doses affects the criterion value for different Hill curves.

It follows from our calculations that to avoid the risk of unfavorable dose placements, it is good to use designs with more than four dose groups (including the control group). We can also conclude that any additional information about the expected dose–response curve should be taken into account when planning a study, because it can improve the design.

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P2-07

Chronic inhalation of ethylene glycol monoethyl ether affected the reproduction of male rats

Rui-Sheng Wang, Megumi Suda, Katsumi Ohtani

Japan National Institute of Occupational Safety and Health, Kawasaki 214-8585, Japan

Ethylene glycol monoethyl ether (EGEE) is one of a family of glycol ethers widely used as an organic solvent for resins, paints and dyes, and as a thinner in industry. It is known that EGEE can cause damage in the testes of several animals, as demonstrated by the testicular atrophy and decreased sperm count. In the present study, we examined the dose-dependent effects of chronic inhalation of the solvent on reproduction and sperm motion in male rats, and the recovery after termination of the exposure. Male Sprague–Dawley rats at 8 weeks old were exposed to EGEE at 0, 80, 250 and 700 ppm by inhalation, 8 h/day, 6 days/week, for 9 weeks. The body weight gain was not affected in any EGEE groups. However, the weight of testes and epididymides was significantly lower in the high EGEE group than in controls. The percentages of motile sperm and progressive sperm in cauda epididymis were dose-dependently decreased in the three EGEE groups, with significant difference between control and the medium and high EGEE groups. Besides the two motion parameters of sperm, the velocity parameters (curvilinear velocity, average path velocity, and straight line velocity), parameter showing the swimming pattern of spermatozoa as head movement (amplitude of lateral head displacement and beat cross-frequency), and parameters such as straightness and linearity all indicated damage of sperm to some extents. The percentages of sperm with rapid motion were lower in medium and high EGEE exposure, while the static sperm was significantly increased in the two groups. With the sperm from spermaducts, similar results were obtained as in the cauda epididymides. On the other hand, the blood concentration of androgens (testosterone and progesterone) showed little change in the EGEE groups, suggesting that Leydig cells were not damaged. Part of the animals was mated with female rats after the termination of exposure, and the pregnancy and fetus were checked. Also, the recovery of the male rats 10 weeks after the termination of EGEE exposure was evaluated.

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P2-08

Dose depending pyrazinamide effects on peroxidation processes and lipid contents in rats

G.M. Shayakhmetova, L.B. Bondarenko, N.A. Saprykina, V.M. Kovalenko

Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

Metabolism of xenobiotics and its accessibility for the male's reproductive system are one of the least investigated problems of toxicology. Numerous xenobiotics and its metabolites can permeate through blood–testicle barrier. These substances may negative affected on biochemical and proliferative processes in reproductive organs' tissues. We have recently reported that the antituberculosis drug, pyrazinamide (PZA), caused morphofunctional damages of testis in male rats and a dose dependent CYP P450 2E1 induction in their liver (Toxicol. Lett. 158 (Suppl. 1) (2005) 123).

In the present work, we investigated the influence of PZA on testis, liver and serum biochemical parameters of male Wistar rats. PZA was administered intragastrically in doses 500 and 1000 mg/kg during all period of spermatogenesis. It was shown that PZA caused dose dependent increasing of ascorbate (to 1.3 times) and NADPH-dependent lipid peroxidation (to 1.5 times) in liver microsomal fraction with simultaneous decrease of serum free cholesterol (to 2.2 times) and increase of total lipids contents (to 1.2 times) as compared with intact animals. At the same times PZA administration (1000 mg/kg) decreased the content of glutathione in liver (by 45%), increased the level of ceruloplasmin in serum by 20% and decreased the relative intensity of EPR-signals of transferrin ($g \approx 4.2$) by 20% and iron–sulfur proteins ($g \approx 1.94$), mainly mitochondrial enzymes, by 10% in testis.

Results of the study indicate that in addition to CYP P450 2E1 induction many kinds of biochemical abnormalities may be underlie the negative influence of PZA on male's reproductive system. These effects may be connecting with the state of pro- and antioxidant systems and the transport of iron, as well as disturbances of bioenergetics processes in germinal cells. Further experiments will suppose for explanation of correlation CYP P450 2E1 induction or inhibition effects on male's reproductive system.

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P2-09

Epidemiological profile of acute poisoning in a University Hospital in Zaragoza (Spain)
Prospective study of 10 years

A. Ferrer-Dufol, M. De Azúa-Jimenez, N. Fernandez-Letamendi, R. Royo-Hernandez, M. Rivas, E. Civeira-Murillo

Clinical Toxicology Unit, University Clinic Hospital, Zaragoza, Spain

We present the profile of the toxic incidents studied in a prospective way by means of a specific toxicology file in the Emergency Department of a General Hospital covering a population of 360,000 inhabitants. Toxic cases represent near 2% of all the emergency cases. During the period 1995–2004, 9321 cases have been attended. Mean age is 33.39 (±16): males 65.66% and females 34.33%. Children under 16 years account for 865 cases (9.28%). The cause of poisoning has been an overdose of drugs of abuse in 57.77%, suicidal in 21.96%, a domestic accident in 10.46%, occupational 1.38%, other toxic accidents in 1.79% and unknown 6.63%. The main chemical compounds are medicaments (29.04%) and drugs of abuse (63.58%). Among the rest of substances (12.99%) the most common are caustics cleaners producing 337 cases. Other toxicants such as carbon monoxide, solvents, pesticides or detergents are much less frequent. The main individual agent is ethanol, which has produced 5100 cases (56.60%) followed by benzodiazepines with 1300 cases (14.91%). The route of exposure is oral in 6921 cases, respiratory 820 cases, cutaneous 123 cases and intravenous 338 cases. A total of 6647 cases (71.31%) have had symptoms, mainly neurologic, digestive and cardiovascular. Analytical confirmation has been carried out in 6253 cases (67.08%). Specific treatment was used in 2363 cases: gastric decontamination has been used in 1781, antidotes in 1491, enhanced elimination in 33. Some symptomatic treatment has been employed in 4033 cases. Most cases have had a good outcome, with only 24 deaths (0.26%), most of them suicidal cases (12), some overdoses by drugs of abuse (4) and 2 accidents. In conclusion, acute poisoning is a not too high but usual pathology in the Emergency department in a general Hospital in Spain. This pathology affects to a relatively young population, it is mainly intentional, with low figures of accidents, and the outcome is generally good.

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P2-10

Comparison of clinical adverse drug reactions of three hypotensive drugs (indapamide, isosorbid-5-mononitrate, molsidomine) after their single dose oral administration to healthy volunteers

Dagmar Kholova [1], Karel Macek [2], Ladislav Vyslouzil [1], Jiri Kopecky [1], Jaroslav Kvetina [1]

[1] *Institute of Experimental Biopharmaceutics, Hradec Kralove, Czech Republic;* [2] *Teaching Hospital, Hradec Kralove, Czech Republic*

The aim was to compare adverse drug reactions (ADRs) of tablet formulations containing drugs with hypotensive effect: indapamide (5 mg), isosorbid-5-mononitrate (IS-5-MN, 40 mg) and molsidomine (4 mg). The data were obtained retrospectively as a secondary product from three clinical pharmacokinetic bioequivalence studies. All drugs were administered in a single oral dose to 24–25 healthy volunteers. Volunteers were asked about ADRs occurrence, their duration and severity during three days after the drug administration. Statistically significant differences of the ADRs frequency (χ^2 test, $p = 0.05$) were found between indapamide (50%) and IS-5-MN (79%) and between indapamide and molsidomine (80%). ADRs frequency did not differ between IS-5-MN and molsidomine. Only four types of ADRs occurred after indapamide, six types of ADRs were registered after IS-5-MN and eight types after molsidomine administration. The most common ADRs were sleepiness (8×) and headache (4×) in the case of indapamide, headache (17×) and nausea (2×) in the case of IS-5-MN, headache (21×) and sleepiness (2×) in the case of molsidomine.

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P2-11

Analytical and demographic profile of the toxic incidents related to alcohol in a University Hospital in Zaragoza (Spain)
Prospective study of 10 years

Ana Ferrer-Dufol, Sebastian Menao-Guillén

Clinical Toxicology Unit, University Clinic Hospital, Zaragoza, Spain

We present the analytical and demographic profile of the toxic incidents related to alcohol studied in a prospective way by means of a specific toxicology file in the Emergency Department of a General Hospital covering a population of 360,000 inhabitants. During the period 1995–2004, 9321 toxic cases have been attended which represent near 2% of all the emergency cases. Ethanol related cases are 56.60% of total cases. Among the 5100 cases with a clinical diagnosis of ethanol intoxication mean age is 33.75 ± 13.18 years: males 78% and females 22%. A total of 3452 cases have been confirmed analytically showing a mean ethanol concentration in blood of 1.54 ± 0.79 g/L. Among them, 1148 are cases related to traffic events and present a mean ethanol blood concentration of 1.36 ± 0.61 g/L (the legal limit for driving in Spain is 0.50 g/L). There is a clear rise in the number of cases per year. The mean age has slightly risen and the mean ethanol concentration in blood is constant all along the 10 years. The sex profile has changed with a significant rise of females from 14.8% to 22.7%.

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P2-12

Intoxication with the antipsychotic drug clozapine: Plasma concentrations before and after a deliberate overdose

Agnes G. van Velzen, Jan-Willem Fijen, Irma de Vries, Jan Meulenbelt

National Institute for Public Health and the Environment, The Netherlands

The Dutch National Poisons Information Centre receives around 2000 enquiries a year about intoxications with antipsychotic drugs. Many of these intoxications concern deliberate overdoses in adults. The atypical antipsychotics (e.g. quetiapine, olanzapine, clozapine) are the group of antipsychotic drugs most often involved in these intoxications. Clozapine was the first atypical antipsychotic to be introduced onto the Dutch market. In 2005, the Dutch poisons centre received 73 enquiries about clozapine overdose (of which 62 in adults). Here we present the case of an 18-year-old woman who was admitted to our intensive care unit (ICU) after taking a clozapine overdose in an attempted suicide. The woman was being treated with clozapine in the psychiatric ward. She had a history of schizofrenia and cocaine abuse, but no epilepsy. At 11.45 a.m. she was discovered having seizures in the psychiatric ward. The seizures were treated with diazepam. When it appeared that she had ingested 3 g of clozapine in a suicide attempt, she was transferred to the emergency department. Upon examination she showed signs of sinus tachycardia (140 bpm) with QT prolongation. She had a metabolic acidosis with partial respiratory compensation. Other lab values were within normal range. Here urine was negative for cocaine. Activated charcoal and sodium sulphate were administered via a gastric tube to hamper further absorption of clozapine from the gastro-intestinal tract. Subse-

quently, she was submitted to the ICU for observation. From less than one hour following the seizures until several days later, blood samples were taken to determine the clozapine plasma concentration. The clozapine plasma concentration 35 min after the seizures was 3.86 mg/L, while one day before the overdose it had been only 0.19 mg/L. The next day the QT interval had normalized and her heart rate had decreased to 100 bpm. The clozapine plasma concentration had decreased to 0.40 mg/L at 10 a.m. and 0.26 mg/L at 8.40 p.m. In the evening, the patient was discharged to the psychiatric ward.

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P2-13

Quantitative and statistical analysis of differences in sensitivity between Long-Evans and Han/Wistar rats following long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Salomon Sand [1], Nicholas Fletcher [1], Dietrich von Rosen [2], Katarina Victorin [1], Matti Viluksela [3], Jouni T Tuomisto [3], Jouko Tuomisto [3], Agneta Falk Filipsson [4], Helen Håkansson [1]

[1] *Karolinska Institutet, Stockholm, Sweden;* [2] *Swedish University of Agricultural Sciences, Uppsala, Sweden;* [3] *National Public Health Institute, Department of Environmental Health, Kuopio, Finland;* [4] *Swedish Chemicals Inspectorate, Sundbyberg, Sweden*

In this study, differences in sensitivity between dioxin sensitive Long-Evans (L-E) and dioxin resistant Han/Wistar (H/W) rats following long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were statistically and quantitatively investigated. Sensitivity differences were analyzed by comparing benchmark doses (BMDs) for the two strains considering a number of toxicological endpoints including data on body and organ weights, volume fraction of hepatic foci, hepatic CYP1A1 induction, as well as retinoid parameters. The dose–response relationships for L-E and H/W rats, described by the Hill function, were assumed to be fundamentally similar (i.e. parallel), differing only in terms of their location on dose scale. This assumption was generally supported according to statistical analysis. It was concluded that L-E and H/W rats differed statistically in their response to TCDD treatment. Differences between the strains were most pronounced for volume fraction of hepatic foci; L-E rats were approximately 80 times more sensitive than H/W rats. For body and organ weight parameters, L-E rats were approximately 10–20 times more sensitive than H/W rats. For retinoid parameters and hepatic CYP1A1 induction estimated differences between the strains were generally about five-fold, and associated with a low uncertainty. In conclusion, the present study employs a dose–response modeling approach suitable for statistical evaluation of strain and species differences in sensitivity to chemical exposure. The study demonstrates, statistically and quantitatively, differences in sensitivity between the L-E and H/W rat strains following long-term TCDD exposure.

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P2-14

Inflammatory and epithelial changes in lungs from apolipoprotein E deficient mice after chronic cigarette mainstream smoke exposure

Stefan Lebrun [1], Walter Stinn [1], Horst Weiler [1], Baerbel Friedrichs [1], Ansgar Buettner [1], Klaus von Holt [1], Thomas Wallerath [1], Raymond R. Schleef [2], Hans-Juergen Haussmann [3]

[1] *PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany;* [2] *Philip Morris USA, Richmond, VA, USA;* [3] *Roesrath, Germany Roesrath Germany*

Inflammation plays a key role in the atherosclerotic process. As part of a study on the influence of cigarette mainstream smoke (MS) in combination with high-fat diet on the development of atherosclerosis, we investigated the inflammatory response and histopathological alterations in lungs from apolipoprotein E-deficient ($ApoE^{-/-}$) mice, a classic model for atherosclerosis. Male $ApoE^{-/-}$ mice were whole-body exposed for 12 months (6 h/day, 5 days/week) to diluted MS at total particulate matter (TPM) concentrations of 100 and 200 μg/l or to filtered fresh air. Each exposure group was fed either a chow diet or a milk-fat-enriched diet. Bronchoalveolar lavage (BAL) was performed and inflammatory cells were quantified in BAL fluid (BALF); lungs were evaluated histopathologically. Mice exposed to 100 μg TPM/l MS showed no statistically significant inflammatory and epithelial changes in the lung. Mice exposed to 200 μg TPM/l MS showed statistically significant inflammatory changes, i.e., a constant elevation of neutrophils and a continuous increase in lymphocytes in BALF with both diets. In the high-fat diet groups, there was a tendency to a greater increase in neutrophils and lymphocytes. Pathomorphological findings observed in the lungs of mice exposed to 200 μg TPM/l MS included multifocal alveolar histiocytosis present as single macrophages and pigmented macrophage nests (PMN). Hypertrophy/hyperplasia of the alveolar epithelium was seen only in single cells

directly associated with the PMN. The incidence of these pathomorphological findings increased from 20% after 3 months of exposure to 80% after 12 months of exposure in both diets. Interstitial lymphocytic infiltrates were present in 80% of these mice only after 12 months of exposure. The pathomorphological alterations were not influenced by high-fat diet.

In summary, our data indicate that chronic exposure of $ApoE^{-/-}$ mice to 200 μg TPM/l MS results in non-neoplastic pathomorphological changes in the lung and an inflammatory response, which is a mixture of innate and adaptive immune responses, as indicated by an increase in BALF neutrophils and lymphocytes.

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P2-15

A new and vital antidotal pathway for paraquat poisonings more than 60 years later: Induction of lung P-glycoprotein

Ricardo Jorge Dinis-Oliveira[1,3], Fernando Remião[1,3], José Alberto Duarte[2], Rita Ferreira[2], Amparo Sánchez Navarro[3], Maria de Lourdes Bastos[1,3], Félix Carvalho[1,3]

[1] *REQUIMTE, Department of Toxicology, Faculty of Pharmacy, University of Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal;* [2] *Department of Sport Biology, Faculty of Sport Sciences, University of Porto, Rua Dr. Plácido Costa, 91, 4200-450 Porto, Portugal;* [3] *Faculty of Pharmacy, Avenida Campo Charro s/n, 37007 Salamanca, Spain*

The widespread use of the non-selective contact herbicide paraquat (PQ) has been the cause of thousands of deaths from both accidental and voluntary ingestion. The main target organ for PQ toxicity of is the lung. This is mainly due to a characteristic polyamine uptake system in this organ for which PQ is a preferential substrate. Due to the lack of antidotes for PQ poisoning, the prognosis has been primarily based on the plasma and urine concentration of PQ within the first 24 h of intoxication. No antidote or effective treatment was developed until now to decrease the PQ accumulation in the lung or to disrupt its toxicity. In the present study, a procedure that conducts to a remarkable decrease of PQ accumulation in the lung, together with an increase of faecal excretion and a subsequent decrease of several biochemical and histopathological biomarkers of toxicity, is described. The administration of dexamethasone (100 mg/kg i.p.) to Wistar rats, two hours after PQ intoxication (25 mg/kg i.p.), decreased the PQ lung accumulation to about 40% of the only PQ-exposed group, and led to an improvement of tissue healing in just 24 h as a result of the induction of *de novo* synthesis of P-glycoprotein (P-gp). The involvement of P-gp in these effects was confirmed by Western blot analysis and by the use of verapamil (10 mg/kg i.p.), a competitive inhibitor of this transporter, which given one hour before dexamethasone blocked its protective effects, causing instead an increase of PQ lung concentration and an aggravation of toxicity. In conclusion, the induction of P-gp, leading to a decrease in lung levels of PQ and the consequent prevention of toxicity, seems to be a new and promising treatment of PQ poisonings.

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P2-16

Drug pharmacokinetic in hepatodysfunction: A possible way to toxicity

Berta São Braz, Ana L. Rodrigues, Adriana Belas, A. Jorge Silva

Faculty of Veterinary Medicine/CIISA, Lisboa, Portugal

When a medicine marketing authorization is asked to Competent Authorities, a therapeutic scheme must be established and proposed. Pharmacokinetic studies needed to achieve that goal are performed in healthy animal not taking in account potential interferences of disease states. Hepatic diseases and subsequent hepatic dysfunction must be responsible by a modified hepatic metabolizing activity, which can modulate pharmacokinetics with influence in drug therapeutic activity and toxicity.

This study was delineated in order to determine influence of hepatic dysfunction on pharmacokinetic of antipyrine (AP), a low-clearance compound, and hexobarbital (HB) an intermediate high-clearance compound.

Hepatic dysfunction was induced by intraperitoneal administration (IP) of galactosamine (hepatotoxin used as acute hepatitis model) to Wistar male rats. AP (50 mg/kg, IP) and HB (100 mg/kg, gavage) were administered to animals 24, 48 and 72 h after hepatotoxicity induction, after what blood samples were collected. Antipyrine analytical determination in plasma samples was performed by enzymatic hydrolysis (with Limpet Acetone Powder), extraction (chloroform/ethanol mixture (9/1, v/v)), evaporation to dryness and dilution (phosphate buffer and methanol) before injection onto HPLC column (Nova Pack C18 column Waters), with

determination performed at 245 nm. Analytical determination of HB blood concentration, after *n*-butyl acetate extraction, was performed by gas chromatography in split mode, using promazine as internal standard, a capillary column Hp-5 (5% phenyl methyl siloxane) and FID as detector.

Our results show that hepatic dysfunction, namely 48 h after galactosamine administration (time at which hepatotoxicity clinical signals were more evident) induce a more slow metabolization and more elevated plasmatic concentration with increase of elimination half-lives for both drugs.

So it can be concluded that careful administration of hepatic metabolized drugs must be done in case of hepatic dysfunction, particularly in acute disease, in order to avoid toxicity induction by drug accumulation.

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P2-17

Changes in daily activity, learning and motivational behavior in male mice exposed to methylmercury during development

N. Onishchenko[1], C. Bergkvist[1], M. Vahter[2], T. Hökfelt[3], S. Ceccatelli[1]

[1] *Division of Toxicology and Neurotoxicology, Institute of Environmental Medicine, Sweden;* [2] *Unit of Metals and Health, Institute of Environmental Medicine, Sweden;* [3] *Department of Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden*

Methylmercury (MeHg) is an environmental contaminant that is known to be neurotoxic in humans and laboratory animals, particularly during brain development.

In the present study, we investigated the effect of prenatal and early postnatal exposure to MeHg on mouse behavior. For this purpose, we analyzed young and adult MeHg-exposed mice in acute tests for spontaneous locomotion and motor functions, as well as with monitoring of basic activities and learning abilities using an automated system (IntelliCage) developed for continuous long-term recording of behaviour in the home cage.

Pregnant female mice received MeHg (0.5 mg/kg/day) via drinking water from gestational day 7 till day 7 after delivery, and offspring's behavior was studied as mentioned above. We found no difference between 5-week-old MeHg-exposed and control offspring, neither in locomotor activity, estimated as a distance walked during 1 h, nor in performance in rotarod test. However, the analysis of behavior performed in the IntelliCage revealed that MeHg-exposed male, but not female, mice were less active in exploration of a new environment and that their daily locomotor/exploratory activity, monitored for 45 days, was lower than in controls. Also, we found an impaired learning ability in the MeHg-exposed male mice using a complex test involving visual discrimination, conditioned learning and working memory.

MeHg-exposed males had lower sucrose preference than matching controls, a putative indicator of anhedonia, and longer immobility time in the forced swimming test, which can be considered as a predisposition to depressive behavior. Similar analysis of female offspring did not reveal any of the changes in behavior described above.

Behavioral alterations in laboratory animals exposed to neurotoxicants have always been important indicators of toxicity, but slight changes in behavior can be evaluated only by complex tests involving different brain functions. The results of the present study show that prenatal and early postnatal exposure of mice to MeHg can affect learning ability and motivation-dependent behavior in a gender-dependent manner.

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P2-18

Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after peginterferon α-2b plus ribavirin therapy

Levent Gorenek, Ali Acar, Ahmet Aydin, Can Polat Eyigun, Aytac Cetinkaya, Ayse Eken, Ahmet Sayal, Onur Erdem, Cemal Akay

Gülhane Military Medical Academy, Ankara, Turkey

Oxidative stress could play a role in pathogenesis of hepatitis C virus infection. The aim of our study is to determine oxidant/antioxidant status of patients with chronic hepatitis C (CHC), and the effect of peginterferon α-2b plus ribavirin combination therapy on oxidative stress. Nineteen patients with chronic hepatitis C virus (HCV) infection and 28 healthy controls were included in the study. In control and patient groups, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, erythrocyte malondialdehyde (MDA) levels, erythrocyte CuZn-superoxide dismutase (SOD), erythrocyte glutathione peroxidase (GSH-Px) activities were measured. After peginterferon α-2b and ribavirin combination therapy for 48 weeks, these parameters were measured again in the patient group. Serum

MDA levels is increased significantly in chronic hepatitis C (CHC) patients (*n*: 19), before treatment when compared with healthy subjects (*n*: 28) (9.28 ± 1.61, 4.20 ± 1.47 nmol/ml, $p < 0.001$, respectively. MDA concentration decreased significantly ($p < 0.001$) after treatment as well as ALT, AST activity, in erythrocytes of these patients. Average antioxidant enzymes (superoxide dismutase and glutathione peroxidase) were statistically significantly lower in erythrocytes of patients with CHC before treatment compared with control group (both, $p < 0.001$). Chronic hepatitis C patients after peginterferon α-2b and ribavirin therapy showed values of SOD, GSH-Px were significantly higher than pretreatment levels (both, $p < 0.001$). Our results show that patients with chronic HCV infection are under the influence of oxidative stress associated with lower levels of antioxidant enzymes. These impairment returns to level of healthy controls after peginterferon α-2b plus ribavirin combination therapy of CHC patients. Although interferon and ribavirin are not antioxidants, their antiviral capacity might reduce viral load, and inflammation, and perhaps through this mechanism might reduce virus-induced oxidative stress.

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P2-19

Craving in alcoholism

Role of naltrexone and platelet monoamine oxidase B activity (pMAO-B activity)

P. Bo [1,2], T. Coccini [3], A. Costa [1,2], P. Chimento [1], L. Manzo [2,3], A. Moglia [1,2]

[1] *Institute of Neurology IRCCS C. Mondino, Pavia, Italy;* [2] *University of Pavia, Pavia, Italy;* [3] *Toxicology Division, IRCCS Maugeri Foundation, Pavia, Italy*

Aims: Naltrexone has been proposed as useful treatment of alcohol abstinence, and previous evidence has suggested that low levels of pMAO-B activity can be used for diagnostic assessment of alcoholism, regardless of gender and smoking status. The first aim of this study was the evaluation of influence of chronic naltrexone treatment versus placebo in maintaining the abstinence over a 3-month period. The second aim was to correlate abstinence/relapse with pMAO-B activity.

Subjects and methods: Twenty alcohol-dependent inpatients, diagnosed according DSM-IV criteria and evaluated using an ad hoc questionnaire—VAQUA, aged 30–60 years, heavy smokers (>20 cigarettes/day), were enrolled. Patients with liver cirrhosis, psychosis, cognitive impairment (MMSE), CNS diseases not related to alcoholism, pregnancy and breast feeding were excluded. The temporal pattern of pMAO-B activity, radiochemically measured, was evaluated on the first and 7th day of abstinence, and after 3 months. All patients underwent a brain CT scan, and were randomly assigned to treatment with either naltrexone 50 mg/day or placebo.

Results: Abstinence/relapse was independent of treatment. Twelve hours after last alcohol intake, all patients showed low pMAO-B activity levels (5.94 ± 2.80 nmol/mg protein/h). On the 7th day of abstinence this value increased to 9.25 ± 4.95 nmol/mg protein/h. In the 3rd month, patients with alcohol abstinence (measured by VAQUA and serum GGT), showed an average level of pMAO-B activity of 7.9 ± 2.9 nmol/mg protein/h. Notably, relapsing patients showed pMAO-B activity levels similar to those obtained on the first determination.

Widespread cortical and subcortical atrophy, significantly more marked in frontal lobes, were detected by CT scans.

Conclusions: Data obtained in this small but selected sample population, suggest that naltrexone treatment does not affect abstinence/relapse in alcoholics, and that the higher levels of pMAO-B 7 days after cessation of drinking remain relatively stable 3 months later in the group of recovering alcoholics.

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P2-20

Effects of in utero and lactational exposure to methylmercury and PCB153 on cerebral dopaminergic receptors in rats at weaning and puberty

E. Roda [1], T. Coccini [2], M.V. Vettori [3], D. Poli [3], A. Caglieri [3], L. Manzo [1,2], A.F. Castoldi [2]

[1] *University of Pavia, Italy;* [2] *Toxicology Division, IRCCS Maugeri Foundation, Pavia, Italy;* [3] *University of Parma, Italy*

The central dopaminergic system is a likely target for the neurodevelopmental toxicants methylmercury (MeHg) and ortho-substituted PCBs. The effects of MeHg (0.5 mg/kg/day) and/or PCB153 (5 mg/kg/day), orally administered to rat dams from GD7 to PND21, were investigated on the density (Bmax) of cortical and striatal dopamine D1-like (D1-R) and D2-like receptors (D2-R) in the rat offspring at PND21 and at PND36, by saturation binding studies. Concomitantly, the levels of Hg and PCB153 in brain areas were determined by cold vapor analysis and by gas chromatography with an electroncapture detector, respectively.

In PND21 males the number of both cortical and striatal D1-Rs was decreased to a similar extent (15–30%) by MeHg and PCB153, either alone or combined, without additivity. All alterations completely disappeared by PND36. Concerning D1-Rs in females, the most evident effects at both time points were a 15% decrease in Bmax caused by the PCB + MeHg mixture in cortex and a 15–20% reduction in Bmax elicited by MeHg alone in striatum.

In PND21 cerebral cortex Hg levels (μg/g tissue) were 0.25–0.89 in MeHg alone group and 0.94–1.40 in MeHg + PCB153 group. Such values were reduced by 5–6-fold 15 days later. Similar values and a similar declining trend with time were detected in the striatum. In rat cortex PCB153 levels in rats treated with PCB153 ± MeHg were 15–17 μg/g tissue on PND21 and 4.7–8.1 on PND36.

In both males and females the density of cortical D2-Rs (control Bmax = 48–57 fmol/mg protein) was highly enhanced (+50%) by PCB153, either alone or combined with MeHg, in a delayed fashion (PND36), while MeHg was uneffective. In striatum a common finding to males and females (control Bmax = 200 ± 54 and 189 ± 42 fmol/mg protein) was the MeHg-induced delayed decrease in D2-R density (10%), which was masked in the co-presence of PCB153.

At both ages the number of D1 and D2-Rs is affected by MeHg and/or PCB153 in cortex and striatum. The susceptibility of such receptors to MeHg and/or PCB153 is gender- and time-dependent and the effects of the two contaminants are not additive.

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P2-21

Preventive effect of aminoguanidine on cisplatin-induced nephrotoxicity in rats—Comparison to Vitamins E and C

Suna Atasayar, Hande Gürer Orhan, Hilmi Orhan, Bora Gürel, Gözde Girgin, Hilal Özgüneş

Hacettepe University, Faculty of Pharmacy, Ankara, Turkey

Aminoguanidine is a potent antioxidant that has many biological effects. Cisplatin is widely used chemotherapeutic agent and its nephrotoxicity is recognized as the most important side effect. In this study, the antioxidant effect of aminoguanidine on nephrotoxicity of a single dose of cisplatin is investigated and compared with the effects of well-known antioxidants Vitamins E and C combination. When kidney and liver tissues were investigated histopathologically, it has been observed that there are tubular damage and perivascular inflammation in kidney samples of cisplatin-administered groups. All antioxidants, aminoguanidine, Vitamins E and C are capable to prevent these effects of cisplatin. Liver tissues of all groups were intact. In order to investigate both toxicity and prevention mechanisms, malondialdehyde (MDA) levels in erythrocyte, plasma, kidney and liver, and glutathione (GSH) levels in erythrocyte, kidney and liver samples were measured. Cisplatin-induced nephrotoxicity was evidenced by significant decrease of total sulfhydryl content measured as GSH and significant increase in lipid peroxidation measured as MDA levels. Consecutive repeating administration of both aminoguanidine or vitamin combination with cisplatin decreased MDA levels. Administration of antioxidants with cisplatin prevented the decrease in liver GSH levels. The nephrotoxicity was confirmed biochemically by significant elevation of serum urea and creatinin levels. Although application of Vitamins E and C combination prevented the increase in serum urea levels, aminoguanidine did not. It has been concluded that, at the used concentrations, vitamin combination is more effective in terms of preventing nephrotoxicity compared to aminoguanidine. Secondly, the data obtained are convinced enough that oxidative damage takes place in this side effect of cisplatin.

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P2-22

Towards developing efficient testing strategies—Analyzing the decision relevance of different toxicity tests

Anna Nordberg, Christina Rudén

Royal Institute of Technology, Stockholm, Sweden

In REACH, the forthcoming chemical legislation in the European Union, all new and existing chemicals will be subject to the same legislation. As a result testing will be required for many previously untested chemicals. Since the potential scope of REACH is over 100,000 industrial chemicals, this has put scientific and regulatory focus on how testing should effectively be performed in the regulatory context. How should chemicals be selected for testing? How extensive testing should be required? What tests should be prioritized? There is a pressing need to improve current test strategies in order to be more resource efficient.

One way to address this is to analyze which tests are most influential for risk management, i.e. in the European classification and labelling system. The classification and labelling directive is the major system for risk management in chemicals control. The system applies to both new and existing industrial chemicals, it is harmonized within the European Union and is currently undergoing global harmonization. Furthermore, it is an important tool for conveying information about health hazards to the users of chemicals.

This is a report from an ongoing study in which we compare classifications of chemicals having different data-sets in order to investigate the relative influence of different toxicity tests on the classification of chemicals. For example: if data from both an acute toxicity study and from a 28-day study are available; how often will this result in a classification on the basis of (a) the acute toxicity study only, (b) the 28-day study only, or (c) both these studies?

To do this we utilize information on data availability for more than 2500 individual chemicals notified as new substances according to the current European Union chemical legislation. The purpose of this exercise is to identify which tests that contains most information relevant for risk management decision making, assuming that such tests should be prioritized in the regulatory context, given the limited resources available for testing.

The overall objective is to provide knowledge that may help develop more efficient test strategies for industrial chemicals.

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P2-23

The effects of date palm gemmule on sperm quality and sex hormone levels on partial sterile male rats as experimental model

Sogra Bahmanpour, Fereidon Kavoosi, Mohammad Reza Panjehshahin, Tahera Talaei Khozani

Anatomy Department, Medical School, Shiraz University of Medical Sciences, Tehran, Iran

Date palm gemmule has been used in traditional medicine to improve the semen quality and treat infertility. Their components remedies those are rich of antioxidant and vitamins can influence spermatogenesis. The aim of this project was to evaluate the effects of date palm gemmule on sperm quality. To do these, seventy male rats were divided into seven groups. The groups were injected with 5 mg/kg of busulfan. The experimental groups were treated with 50, 100, 150 and 200 mg/kg of hydroalcoholic extract of date palm gemmule for 48 days. The control was injected with busulfan without any other treatment. After this period the blood samples were taken for hormonal assay. The semen was collected from distal part of the ductus deferens. The sperm count and motility were measured and the smears were prepared. The sperm smears were stained with acridin orange, aniline blue, eosin and chromycin A3.

The results indicated that estradiol decreased significantly after treatment with 50 mg/kg of the extract. The percent of the sperm with good morphology and normal chromatin histone were increased significantly.

It is probable that the extract with its content like phytoestrol effect the estradiol level and by this way influence the spermatogenesis and as a result the sperm quality. The extract may improve the sperm quality because of its content like Vitamins A and C, glucose and calcium.

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P2-24

Graft function in long-term cyclosporine or tacrolimus treatment
A comparative study with nephrotoxicity markers

Z Marchewka[1], Jakub Kuźniar[2], K Falkiewicz[2], B Szymańska[1], M Zynek-Litwin[2], Anna Długosz[1], Marian Klinger[2]

[1] *Department of Toxicology, Wroclaw Medical University, Wroclaw, Poland;* [2] *Department of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland*

The object of this study was to compare the effect of cyclosporine (CsA) and tacrolimus (TAC) treatment on the transplanted kidney. This was done by examining nephrotoxicity markers in urine: lysosomal enzymes of proximal tubular cells (NAG, NAG-B, GAL, β-Gr) and brush border enzymes (AAP, GGT). The study involved 120 CsA-treated patients and 99 TAC-treated patients. Four groups of patients were identified depending on the time since transplantation (I: up to 1 year after transplantation; II: 1–2 years; III: 2–3 years; IV: more than 3 years). The control group consisted of 31 people with normal kidney function.

Nephrotoxicity markers density in urine was examined by length of treatment in both CsA- and TAC-treated patients. The same method was applied to assess nephrotoxicity markers' density in relation to graft function (as measured by creatinine serum level). The authors also sought to correlate CsA and TAC level in serum and the enzymes activity for all groups as well as the entire pop-

ulation. The TAC group showed a significant negative association between the time from transplantation and the creatinine level. No such association was found for the CsA-treated group.

Significantly higher activity of NAG and NAG-B in urine was detected for the CsA- and TAC-treated groups as oppose to the control group. Peak enzyme activity for both CsA- and TAC-treated patients occurred in group I. As for groups III and IV, NAG-B excretion was found higher for the CsA group. A marked correlation ($p < 0.05$) between NAG activity in urine and serum creatinine level was found in the CsA group. Likewise, an association between NAG excretion and graft function was demonstrated (higher NAG occurs in worse graft function) in the TAC group, but this correlation was not significant statistically. An important negative association between NAG activity in urine and the time after transplantation ($p < 0.001$) was found in the TAC group. The results of the study testify in favour of tacrolimus-based treatment. However, doubts remain regarding assessment of cyclosporine and tacrolimus toxicity, as the results of research are neither unequivocal nor simple.

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P2-25

Plant and mushroom exposures reported to the Slovak toxicological information centre in the years 1993–2005

Silvia Plackova, Blažena Cagáňová, Jaroslav Kresanek

National Toxicological Information Centre, Bratislava, Slovak Republic

Objective: Every year the National Toxicological Information Centre (NTIC) in Bratislava responds to about 2500 inquires from all over Slovakia. Mushroom poisoning represents 6%, plant exposures represent 4% of all cases collected by NTIC. Mushroom intoxications can be the serious often resulting in death. To obtain more information we performed a retrospective analysis of all telephone calls to our centre.

Methods: Review of cases reported to the NTIC in the years 1993–2005.

Results: During the 13-year period 1313 mushroom intoxications were reported to the Slovak TIC. The majority of cases (69%) were adults. A gastrointestinal syndrome was noted in 72% of the cases. The second most frequent kind was the cyclopeptide syndrome (12%), 37 cases resulted in death (25 adults and 12 children), which was caused by Amanita phalloides or other amatoxin-containing mushrooms. In 781 cases a mycological screening was performed in our NTIC. During this period 691 plant and herbal exposures were reported. Adults corresponded to 19% and children to 81% (58% of them were less than 5 years old). Unintentional exposures were 79%, abuse 20.5%, suicidal attempt (0.34%). Ingestion was the route more usually involved (96% of cases). The plants most frequently implicated in decreasing order were: *Datura stramonium* (185 cases), *Dieffenbachia* (56 cases), *Atropa bella-donna* (46 cases), *Viscum album*, *Lonicera xylosteum*, *Mahonia aquifolium*, *Convallaria majalis*, *Taxus baccata*, *Laburnum anagyroides*. 80.2% of patients were asymptomatic, 16.5% of patients developed minor symptoms, 3% of patients developed moderate symptoms. One patient developed severe symptoms.

Conclusion: Most plant ingestions were not associated with the development of symptoms. Amanita poisoning was mostly confused with agaricus species. This analysis showed the severity of mushroom poisoning. It also showed the problem of people tending to underestimate the problem of mushroom ingestion because of the latent period before onset of gastrointestinal effects. This resulted in late presentation to hospital and late treatment.

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P2-26

Short- and long-term effects of a neonatal exposure to benzo(*a*)pyrene (BaP) or 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) on behaviour of rat pups

Jérôme Boussel[1], Nathalie Grova[2], Cyril Feidt[1], Didier Desor[1], Guido Rychen[1], Henri Schroeder[1]

[1] *Neurosciences Comportementales, URAFPA, INRA UFC340, Nancy, France;* [2] *Institute of Immunology, LNS, Grand Duchy of Luxembourg, Luxembourg*

While PCBs are described as potential neurotoxic compounds, little is known about the effects of the polycyclic aromatic hydrocarbons like BaP on the developing brain. Therefore, we examined both the short- and long-term effects of an early chronic treatment with PCB126 or BaP at similar doses to those observed in the human feeding, on behaviour in developing rats. Rat pups received a subcutaneous injection of PCB126 (2–20 μg/kg) or BaP (2–20 mg/kg) every third day from postnatal day 3 (P3) to P21. Control animals received an equivalent volume of vehicle. Several tests of reflex and motor coordination development were performed in pups at P4, P9, P10 and P20. At P50, rats were tested for spatial memory in the eight-arm maze, anxiety in the elevated plus-maze,

and exploration in the open-field. PCB126 (20 μg/kg) and Bap (20 mg/kg) induced significant decreases of body (19–20%) and brain weight (6–8%) at P50. During development, significant differences in locomotor and coordination capacities were observed between PCB126 (20 μg/kg), BaP (20 mg/kg) and controls, reflected by increases of the time needed by the pup to right itself at P4, and the time used to face the top of the board of an inclined plane at P9. At the adult stage, each compound did not modify the exploratory activity and the spatial learning and memory abilities of the animals whatever the dose used. In the elevated-plus maze, BaP-treated rats (20 mg/kg) were less anxious, as reflected by increases in the number of entries (+20%) and time spent (+24%) in the open arms. The opposite was observed in PCB126-injected rats (20 μg/kg), suggesting an increase of their anxiety level. This anxiogenic-like activity of PCB126 was also observed in the eight-arm maze in which the time need by these animals to achieve the test was increased compared to controls when they are faced for the first time to this novel environment (+14%, $p < 0.05$). In conclusion, an early chronic exposure to PCB126 or BaP induces short- and long-term specific behavioural alterations, asking the question about their potential toxicity on the developing brain.

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P2-27

Expression of renal organic anion transporters OAT1 and OAT3 in ochratoxin A-treated rats

Vilim Žlender, Marija Ljubojević, Daniela Balen, Davorka Breljak, Naohiko Anzai, Radovan Fuchs, Ivan Sabolić

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Introduction: Ochratoxin A (OTA), a mycotoxin produced by several *Aspergillus* and *Penicillium* species, is nephrotoxic, hepatotoxic, teratogenic and immunotoxic. In the mammalian kidney, OTA primarily targets proximal tubules (PT). Studies have shown that in PT OTA inhibits the transport of *p*-aminohyppurate (PAH) and that the secretion of both OTA and PAH is mediated by organic anion transporters OAT1 and OAT3 (subfamily of SLC22 drug transporters) that reside in the PT basolateral membrane (BLM). These findings indicate that OAT1 and OAT3 may be involved in OTA nephrotoxicity, but this possibility has not been clarified.

Methods: To study cell morphology and expression of OAT1 and OAT3 along the nephron in an *in vivo* experimental model of OTA nephrotoxicity, adult male rats were treated with different OTA doses (0, 50, 125, 250 or 500 μg/kg, every 2nd day, p.o.) for 10 days. Using specific antibodies, OATs were studied by Western blotting (WB) in total cell membranes (TCM) isolated from the pooled cortex (C) and outer stripe (OS) tissues, and by immunocytochemistry (IC) in tissue cryosections. The specific protein bands were evaluated densitometrically.

Results: OTA caused a dose-dependent damage (cell desquamation and degradation) of the PT S3 segments in medullary rays. In WB of TCM, at lower or higher OTA doses the respective OAT1 content significantly increased (∼50% at 125 μg/kg) or strongly decreased (∼70% at 500 μg/kg). The content of OAT3 exhibited a strong, dose-dependent increase, reaching maximum (∼300% above the controls) at 125–250 μg/kg. The content of actin in TCM remained unchanged. IC showed heterogeneous OAT1 staining in the PT BLM which strongly diminished in rats treated with 500 μg OTA/kg, whereas OAT3 staining in the C and OS tubules matched the respective WB data. At 500 μg OTA/kg, a dramatic loss of OAT3 was observed in the heavily damaged S3 segments in medullary rays.

Conclusions: OTA treatment in rats causes: (a) dose-dependent cell damage in S3 in medullary rays, and (b) dual effect on renal OAT1 an OAT3: low doses upregulate and high doses downregulate the expression of these transporters in the cortical tubules.

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P2-28

Does titanium oxide as nanomaterial penetrate the cellular membrane of cell culture and Vitrolife-skin?

Hiroshi Tokunaga, Tadashi Uchino, Yoshiaki Ikarashi

National Institute of Health Sciences, Tokyo, Japan

The use of the nanomaterial such as titanium oxide is rapidly advanced in cosmetic without checking their safety in recent years. In order to estimate the penetration of titanium oxide to the cellular membrane or three-dimensional culture skin (Vitrolife-skin), two kinds of titanium oxide at the particle size of 20 nm (LU175) and of 250 nm (LU205) were exposed to the CHO cells, the A431 cells and Vitrolife-skin.

CHO cells at 5×10^4 cells/mL or A431 cells at 4×10^4 cells/mL were suspended into 0.1 mL medium in the 96-well microplate and cultured for 24 h under 5% CO_2 incubator at 37 °C. The suspensions of titanium oxide which ranged from 0% to 1% were added onto the 96-well microplate. After cultivating each cell into the 96-well microplate, the cell viability was estimated. IC50s of CHO cell were 0.4% at LU175 and exceeded

1% at LU205. Also IC50s of A431 cell were 0.4% at LU175 and 0.55% at LU205 but did not show the cell toxicity. The cell toxicity about LC175 depends on CHO or A431 cells.

CHO cells at 5×10^4 cells/mL or A431 cells at 4×10^4 cells/mL were suspended into 0.1 mL medium in the 60-mm dish and cultured for 2 days for CHO cells or 5 days for A431 cells under 5% CO_2 incubator at 37 °C. When 5 mL of 0.2% LU175 suspension was added onto CHO cells' dish, 1.9% LU175 was present on the cellular mixture. The located amounts of cellular membrane, microsome and cytosol were 133, 0.27 and 57 μg/dish. On the same manner for A431 cells, 10.6% LU175 was on the cellular membrane, microsome and cytosol. Their located amounts were 622, 13 and 436 μg/dish.

0.1 mL of 0.2% LU175 suspension was put onto the donor side of Vitrolife-skin and the cumulative amount of LU175 was examined in the receiver side after 24 h. 2.4 μg of LU175 was detected in the receiver side. Neither LU175 nor LU205 showed the cell toxicity against Vitrolife-skin. Vitrolife-skin could not observe due to the resistance of the stratum corneum on Vitrolife-skin.

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P2-29

Cardiovascular toxicity in acute tricyclic antidepressant (Amitriptyline) overdose

Branimir Pavlovski, Niko Becarovski, Letka Melovska, Nestor Popovski, F Licoska, Aleksandra Babulovska

Clinic of Toxicology, Urgent Internal Medicine, Clinical Center, Skopje, The Former Yugoslav Republic of Macedonia

Background: Amitriptyline (TCA) continues to be a leading cause of significant morbidity and mortality in acute suicidal poisonings involving all antidepressant drugs.

Objective: The aim of this study was to evaluate electrocardiographic changes, urine and serum concentrations in corelation to clinical pictures in patients acutely intoxicated by Amitriptyline.

Material and methods: Over a 18 months period, 36 consecutive patients (27 (75%) males and 9 (25%) females, with a Amitriptilyine overdose were admitted to the Toxicology Clinic. The mean age of this group was (37.2) from 18 to 60 years. Severity of coma scale (Glazgow Coma Scale) on admission, cardiac, neurologic, respiratory complications, urine, plasmatic concentration of the drug, length of coma, other possible complications were all evaluated for each patient. ECG, blood pressure and respiratory rate were continually monitored. Urine concentrations of the drug were determined using (TLC) thin layer chromatography as a screening test and serum concentrations were determined with fluorescence polarization immunoassay (FPIA) technique.

Results: ECG changes—sinus tachycardia was the most common adverse outcome for all group. RaVR was greater in those patients who had arrhythmias or seizeres than in those who did not (4.1 mm versus 1.6 mm, $P < 0.01$). Seizeres occurred in four (11%) patients and ventricular arrhythmias in two (5%) patients. Artificial ventilations for four patients were made. GCS from 3 to 12 for all groups were ranged.

Serum concentrations using FPIA technique were range from 222 to 850 ng/ml.

Conclusion: There is no correlation between severity of clinical picture and urine and serum concentrations of the drug. RaVR of 3 mm or more was the only ECG change that significantly predicted these adverse outcomes.

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P2-30

Cadmium toxicity on glutamate and possible melatonin protective role

A. Lafuente, A. Romero, M. Velasquez, T. Cabaleiro

Laboratorio de Toxicología, Facultad de Ciencias, Universidad de Vigo, Campus de Ourense, Las Lagunas s/n, 32004 Ourense, Spain

Cadmium toxicity and free radicals are related, and melatonin seems to be a scavenger of these radicals. The aim of this study was to determine possible protective melatonin role in the alterations of glutamate concentration in hypothalamus and median eminence induced by cadmium exposure. For this purpose, four groups of adult male rats were used: (a) rats treated with i.p. injections of melatonin (30 μg/rat/day), using like vehicle saline solution (0.9%) and absolute ethanol (90/10, v/v); (b) animal treated with 25 mg/L of cadmium chloride ($CdCl_2$) in the drinking water; (c) rats treated with both melatonin and cadmium; and (4) double control group (received drinking water without adding $CdCl_2$ and the vehicle). Duration of theses treatments was 30 days. At the end of the treatments, rats were sacrificed and the hypothalamus (anterior, mediobasal and posterior) and median eminence were removed. Glutamate concentration was measured by high performance liquid chromatography (HPLC) using fluorescence detection after pre-column derivatization with OPA. After cadmium exposure, glutamate concentration increased in both anterior and median eminence, but decreased in mediobasal hypothalamus. In the animals treated with melatonin glutamate

content was increased in both anterior and mediobasal hypothalamus, although decreased in median eminence. However, in animals which received cadmium and melatonin, glutamato content in mediobasal hypothalamus and median eminence are similar to the observed levels in the control group. These data suggest that melatonin could have a protective role in cadmium effects on glutamate.

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P2-31

Subchronic effect of refractory ceramic fibres (man-made vitreous fibres) on the selected inflammatory and cytotoxic parameters of bronchoalveolar lavage in rats

Marta Hurbankova, Silvia Cerna, Sona Wimmerova, Margareta Sulcova

Slovak Medical University, Bratislava, Slovakia

Industrial fibrous dusts are applied in many industrial branches and represent adverse factors in occupational and environmental area. Refractory ceramic fibers (RCFs) – amorphous alumina silicates – are used as one kind of asbestos substitutes. Because RCFs are relatively durable and some RCFs are respirable, they may present a potential occupational and environmental health hazard by inhalation. The aim of present work was to find out the subchronic effect of RCFs on selected parameters of bronchoalveolar lavage (BAL) in W-rats, confirm the biopersistence of RCFs after 6 month instillation and contribute to the understanding of the pathomechanism of lung injury after fibrous dust exposure. Wistar rats were intratracheally instilled with 4 mg/animal of RCFs – exposed group and with 0.4 ml saline solution/animal – control group. Animals were sacrificed after 6-month exposure. Bronchoalveolar lavage (BAL) was performed and selected BAL parameters (mainly inflammatory and cytotoxic) were examined. Following treatment with RCFs was observed: statistically significant increase of proportion of lymphocytes and polymorphonuclears as well as percent of immature alveolar macrophages (AM) and phagocytic activity of AM; statistically significant decrease of viability of AM and proportion of AM (from the differential cell count) in comparison with the control group. The results of this study indicated that RCFs even 6 months after intratracheal instillation very significantly changed the majority of examined BAL parameters. The presence of inflammatory and cytotoxic response in lung may signalize beginning or developing process of injury.

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P2-32

Health impact of ^{90}strontium on tobacco industry workers

Vera Garaj-Vrhovac [1], Martina Đurinec [1], Vlatka Brumen [2], Vesna Kancelir-Miletić [3]

[1] *Institute for Medical Research and Occupational Health, 2 Ksaverska St., 10000 Zagreb, Croatia;* [2] *Occupational Medicine Specialist, Medical College University of Zagreb, SPH "Andrija Štampar", 4 Rockefellerova St., 10000 Zagreb, Croatia;* [3] *Outpatient Clinics Rovinj, Istarska bb 52210 Rovinj, Croatia*

Within the process of cigarettes' manufacturing, tobacco paper thickness is controlled using closed radioactive source, i.e. ^{90}Sr. On that ground, the question of possible health impact of such an occupational exposure was risen. According to the codes of practice exercised within the radiation protection frame, all workers dealing with ^{90}Sr have been provided with personal film-dosemeters, collected and read out in regular 3 month intervals by an authorised institution. The workers in question are also placed under regular medical surveillance carried out by the local Occupational Medicine Services. Since radioactive sources are also assumed to have a certain genotoxic impact, such an assumption was verified using single cell gel electrophoresis (SCGE). The study embraced a total of 20 workers dealing with ^{90}Sr, out of which 12 males and 8 females, aged 39.6 years on the average (range 20–61 years), with an average duration of employment of 20.3 years (range 3–38 years). Over the entire follow-up period, physical dosimetry unexceptionally revealed doses falling bellow the detectibility range. Medical reports revealed no clinically significant changes in overall health status. SCGE revealed no signs of ^{90}Sr genotoxicity (mean tail length ranging from 10.37 to 15.85 μm). Results obtained by this survey provided no reason whatsoever to believe that ^{90}Sr, present in such an occupational setting, poses as a health risk. However, constant ambient and biomonitoring of the exposed personnel, and close observation of all the established codes of conduct regarding radioactive sources, should not be neglected at any time.

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P2-33

Toxicity assay in repeated doses of *Dermatophagoides siboney* and *Blomia tropicalis* allergens extracts in Cenp:NMRI mice

Yana González [1], Bárbara González [1], Ana M. Bada [1], Dasha Fuentes [1], Avelina León [1], Edilis Santana [1], María E. Arteaga [1], Alexis Labrada [2]

[1] *Centro de Toxicología y Experimentación Animal (CETEX), Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB), Cuba;* [2] *Departamento de Alergénos, Centro Nacional de Biopreparados (BIOCEN), Cuba*

Allergen extracts are used for hyposensitiveness or immunotherapy treatments, reducing significantly the clinical symptoms of the disease. Because of its wide use in immunoallergen therapy, and because of the importance of its use, the objective of this work is to evaluate the *Dermatophagoides siboney* and *Blomia tropicalis* allergens extracts for establishing the possible harmful, functional and morphological effects that its repeated subcutaneous administration to Cenp:NMRI mice could bring about. In both assays there were established 2 experimental groups, a control and a treated group (20 animals each). Animals were daily observed to detect toxicity signals. At the end of the assay, there were carried out hematological and blood chemistry exams on all animals, and anatomo-pathological examination. There were not detected any significant variations neither in corporal weight nor in water and food consumption, as well as, neither it was shown any variation in the hematology parameters. Statistical variations in the uric acid, urea and glucose were observed, not being of biological relevance. Anatomo-pathological results showed hemorrhagic and inflammatory lesions, which were observed in both experimental groups. It could be concluded that the used dose of 166.6 UB did not cause lethality or toxic effects in the employed biomodel.

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P2-34

Quantitative control of UV filters in cosmetic products in regards to their adverse health effects

Karolina Lyubomirova [1], Stoyanka Uzunova [1], Anton Tachev [1], Lidia Metchkueva [1], Nora Ianakieva [1], Vessela Vitcheva [2]

[1] *National Center of Public Health Protection, Sofia, Bulgaria;* [2] *Department of Toxicology and Pharmacology, Pharmaceutical Faculty, Medical University, Sofia, Bulgaria*

Overexposure to sun radiation is widely accepted as the underlying cause for adverse health effects on skin, eyes and immune system. This is the reason of an overuse of UV filters in different cosmetic products, particularly in sun protection products. However, many of the common used UV filters are known for their endocrine disruption activity and sensitizing potential. Regulation measures have been taken in regards to the possible health risk of UV filters. The concentration of UV filters is strictly determined by the European Directive (76/768/EEC) and the harmonized Bulgarian legislation (Ord. No. 26/2001, No. 36/2005). However, in Bulgaria, as in other countries, there are not validated methods for determination of the concentration of UV filters in cosmetic products.

Aim: The aim of the study was to develop a method for precise determination and control of the concentration of UV filters used in different types of cosmetic products.

Materials: Three types of cosmetic products—day cream, night cream and emulsion were assessed. The products contained one of the UV filters: octylmetoxycinnamate (UV-1) and 1-(4-tert-butylphenyl)-3-(-metoxyphenyl)propane-1,3-dion (UV-2).

Methods: The new developed spectrophotometric method is based on the ability of ethanol solutions of the investigated UV filters to absorb ultraviolet radiation with a definite wavelength. The value of the light absorption at wavelength 308 nm is proportional to the concentration of UV-1, and 357 nm to UV-2, respectively.

Results: The validated method covers the concentration range of 2.5–30 $\mu g/cm^3$ (1.25–15%) for both filters. The limit of detection of the method is from 0.042% to 0.53% for UV-1 and 0.038% for the products containing UV-2. The limit of quantification is from 0.082% to 0.60% for the three tested products containing UV-1 and 0.072% for UV-2. The recovery of products containing 2% UV-1 is 90–95% and of those, containing 4–90–100%. The recovery of products containing 2% UV-2 is 80–93% and of those, containing 4% is 80–96%.

Conclusion: The developed method allows precise determination of the concentration of UV filters used in different cosmetic products in market control. The method will contribute to decrease the possible risk of adverse effects due to the overuse of UV filters.

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P2-35
Epidemiology and management of acute intoxications in emergency department

Marijana Grgic Medic, Marijan Merkler, Vladimir Gasparovic

Department of Internal Medicine, Division of Emergency Medicine and Intensive Care, Clinical Hospital Center Zagreb, Zagreb, Croatia

Acute intoxication is clinical condition characterized by the distress of one or more organs due to the toxin or its metabolites. The aim of this study was to analyze retrospectively the frequency, causes and outcome of acute intoxications in the Department of emergency medicine and intensive care of the Clinical hospital center Zagreb in the period of 2 years (2004 and 2005).

The study included patients referring to the Emergency department (ED) for suspected voluntary or accidental acute poisoning. The incidence of intoxications, toxic substances, and clinical outcome were analyzed.

Out of 35513 patients admitted to the ED, 435 (1.2%) patients, 213 (45%) were observed due to acute poisoning. The median age was 34 years (range 4–88 years).

Drugs were the most frequent toxic agent, detected in 209 (48%) patients, whereas ethanol was the second leading agent, found in 136 (31%) patients. Ten (2.2%) patients ingested a combination of alcohol and psychoactive drugs. In 36 (8.2%) of patients a history of drug addiction was recognized. Most of them referred for opiate intoxication (7.8%), one patient was admitted for cocaine and one for amphetamine intoxication. Mushrooms, carbon monoxide, corrosives and pesticides were the less common toxic agents, detected in 6 (1.4%), 10 (2.2%), 9 (2.07%) and 1 (0.22%) patient, respectively. In 18 patients (4.1%) the toxic agent could not be determined.

Suicidal attempt was the cause of intoxication in 102 (23.4%) patients, but the data about the reason for intoxication were missing in about 40% of patients. The incidence of suicidal attempts among acutely intoxicated patients admitted to the intensive care unit (ICU) in the year 2005 was as high as 70%, indicating that overall incidence could also be higher.

Most of the patients (310, 71%) were treated in ED and dismissed within 24 h. Seventy-four (17.01%) patients required the admission to ICU; 43 (9.88%) patients were admitted to the psychiatric ward and 5 (1.1%) to the internal medicine ward.

Seven patients (1.6%) died within 72 hours following the admission to the ICU. The causes of death were pneumonia and ARDS, following the ingestion of psychoactive drugs (1 patient) and corrosives (3 patients), and coma following opiate intoxication (3).

Conclusion: Almost 50% of all intoxications were drug intoxications, which indicates that prescription of medication should be controlled more cautiously.

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P2-36
Hemorrhagic complications in warfarin use—Intoxication or hypersaturation?

Marijana Grgic Medic, Marijan Merkler, Vladimir Gasparovic

Department of Internal Medicine, Division of Emergency Medicine and Intensive Care, Clinical Hospital Center Zagreb, Zagreb, Croatia

Many common cardiovascular disorders have a relationship to thromboembolic diseases, including ischemic heart disease, atrial fibrillation, valvular disease, and atherosclerotic vascular disease, and usually require antithrombotic therapy. Warfarin has been the standard anticoagulant used in numerous clinical settings. The major complication associated with the use of warfarin is bleeding due to excess anticoagulation. Several factors, such as age over 75 years, active cancer, heart failure, liver disease or alcoholism as well as concomitant use of drugs such as NSAID or antibiotics have been recognized as risk factors for warfarin hyperanticoagulation.

The aim of the present study was to analyze the patients admitted to emergency department (ED) for hemorrhagic diathesis (bleeding and/or PV below the therapeutic range) during warfarin therapy, in a period of 1 year and to identify the possible risk factors for hemorrhagic diathesis.

Totally, 19 patients with warfarin related hemorrhagic diathesis were identified. Their mean age was 70.4 ± 11.2 years. The indication for warfarin treatment was chronic atrial fibrillation ($n = 6$), pulmonary embolism ($n = 6$), deep venous thrombosis ($n = 2$), cardiac valve replacement ($n = 3$), cerebrovascular insufficiency ($n = 2$). The duration of warfarin therapy prior to the bleeding episode was in 13 patients (68.4%) over 1

year, without data of previous bleeding episodes or the regularity of PV control.

The median PV at admittance was 0.05 (range 0.01–0.2). The most frequent symptoms were gastrointestinal bleeding ($n = 5$, 26.3%), hemathuria ($n = 5$, 21%), nose bleeding ($n = 3$) hemathoma ($n = 1$), and hemoptoa ($n = 1$). Four patients were asymptomatic and low PV values were detected on regular blood controls.

In nine (47%) cases, active malignancy was registered. Four patients had chronic renal insufficiency and three patients the acute urinary infection was present prior to the onset of bleeding (all patients presented with hemathuria).

The conditions significantly associated with warfarin related hemorrhagic diathesis were age above 70 years and active malignancy, which is in accordance with the literature. The occurrence of anticoagulation during infection, observed in three (15%) patients could be explained by the concomitant use of antipiretics (NSAID) or antibiotics.

Conclusion: Hemorrhagic complications in warfarin use are not intoxication; therefore, the terms hypersaturation or hyperanticoagulation should be used instead.

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P2-37

Biochemical evaluation of the placental transfer of a single oral dose of chlorpyrifos-methyl in pregnant rats

EL-Hussein Naguib El-Khatib [1], Alaa El-Din Bayoumi Abdel-Khalek [2], Zidan Hindy Abdel-Hamid [2], Reem Ziada [1]

[1] *Mammalian Toxicology Department, Central Agricultural Pesticides Laboratory, Giza, Egypt;* [2] *Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

Chlorpyrifos-methyl is a commonly used anticholinesterase insecticide in Egypt, and therefore the potential for human exposure is high. The placental transfer of chlorpyrifos-methyl and its effects on certain biochemical markers was studied in pregnant (at the 18th day of gestation) females of laboratory strain white rats. Chlorpyrifos-methyl (1/10 or 1/30 of LD_{50}) were given as a single oral dose. Pregnant females and their corresponding fetuses were sacrificed after 10, 30 min, 1, 3, 6, 12, 24 and 48 h. The obtained results indicate that (1) chlorpyrifos-methyl was transferred through the placental route and was able to produce a significant decrease in the activity of brain and/or serum ChE of pregnant dams and their foetuses. The effect was dose and duration dependent. (2) Total glutathione (GSH) content showed significant increase in dams and foetuses liver, while significant decrease was observed in the dams placental GSH. (3) Significant alterations were observed in pregnant dams placenta and serum, and in fetuses liver glutathione *S*-transferases (GST) activity, such alterations were dose and time-dependent. (4) Administration of chlorpyrifos-methyl to pregnant dams produced a significant decrease in brain ATP-ases activity, while their fetuses brain ATP-ases activities were increased as an effect of the same treatment. The present study suggests that pregnant females and their corresponding fetuses may be at risk of significant biochemical alterations following a single oral dose of chlorpyrifos-methyl.

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P2-38

Inflammatory effects of two quartz samples after intratracheal instillation in a 90-day study with rats

O. Creutzenberg [1], G. Oberdörster [2], L. Coogan [3], W.F. Moll [3], R. Hamilton [4], C. Ziemann [1], H. Muhle [1]

[1] *Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany;* [2] *University of Rochester, Rochester, NY, USA;* [3] *Sorptive Minerals Institute, Washington, DC, USA;* [4] *Johns Manville Corp., Technical Center, Littleton, CO, USA*

Respirable crystalline silica was classified by IARC (1997) as human carcinogen. However, as different polymorphs react differently in lungs, assays are needed for differentiation. The objective of this 90-day study was to characterize differences in biological activity between two quartz species, ground quartz particles (well-characterized Positive Control DQ12) versus a quartz-containing material (geologically ancient Quartz Isolate from bentonite). Total doses of 15.2 mg/kg body weight of the Positive Control and Quartz Isolate were administered to rats by intratracheal instillation. Controls received saline only. Bronchoalveolar lavagate analysis showed that, relative to the controls, the total leukocyte counts at 3 days were significantly elevated in both the Quartz Isolate and Positive Control groups. At 28 and 90 days, the Quartz Isolate values were no longer statistically different from the control values whereas the corresponding Positive Control values were about 12 and 65 times greater than control values. At 3, 28 and 90 days, the percentages of polymorphonuclear neutrophils (PMNs) were 18%, 25% and 32% for the Quartz Isolate group and 39%, 45% and 46% for the Positive Control

group, respectively. Histopathologically, the Quartz Isolate group showed moderate effects compared to controls after 28 days with no progression of severity at 90 days. In contrast, the Positive Control group exhibited more severe effects at 28 days and a progression in intensity at 90 days. In conclusion, in the Positive Control group, a persistent inflammation (interstitial fibrosis, alveolar lipoproteinosis) was observed.

Conclusions: (i) Controls did not induce lung inflammation. (ii) The Positive Control produced significant and progressive lung inflammation. (iii) The Quartz Isolate induced a significantly weaker inflammatory response than the Positive Control (not progressive). From a regulatory point of view, these substance-specific toxicological effects of Quartz Isolate may need to be considered for its classification.

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P2-39

A new method for chronic measurement of respiratory function in the telemetered monkey

S. Milano, E. Chalencon, O. Boucheix, P. Lege, K. Broudic-Labay

MDS Pharma Services, Lyon, France

In safety pharmacology studies, the assessment of the respiratory function in the conscious, non-restraint large animal species is extremely difficult to approach. An attempt to measure the respiratory function in the telemetered monkey by measuring pleural pressure was recently developed by Murphy and co-workers.

In our hands, this method was not accurate enough to quantify ventilatory changes, mainly because the amplitude of the recorded pleural pressure was greatly influenced by the posture of the animal.

Using a conventional electrophysiology approach, it has been shown that the electromyographic activity of the diaphragm is a very good index of tidal volume and bronchoconstriction status, both in humans and animals.

In the monkey, we developed a method of measurement of the respiratory function by the recording of the diaphragm electromyogram using telemetry. Two bio-potentials of a model TL11-M3-D70-CCTP radio transmitter (Data Sciences International; USA) were implanted into the costal part of the left diaphragm. The electromyographic activity of the respiratory muscle (EMG) was simultaneously recorded with ECG, blood pressure and body temperature.

Intravenous administration of the respiratory depressant morphine, respiratory excitant buspirone, exposure to the respiratory stimulant hypercapnia and administration of the bronchoconstrictive agent methacholine verified that the diaphragmatic EMG is a suitable tool for measuring the respiratory function in the telemetered monkey.

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P2-40

The effect of 90-day repeatedly intravenous injection ginsenoside Rh_2 on serum total cholesterol, creatine kinase and globin in beagle dogs

Zhifeng Liu, Chun Mei Li, Guishen Li, Dalei Li, Min Li, Ke Liu

Yantai University, Yantai, Shandong province China

Ginsenoside Rh_2, a purified dammarane-type tetracyclic triterpenoid soponin, was prepared from total saponins of the leaf and stem of *Panax ginseng* and *P. notginsen* by alkaline degradation. In our laboratory, we found it exhibited anticancer effects both in vitro and in vivo, which implicated its potential anti-tumour clinical potential. This study first clarified that the Rh_2 could increase the levels of blood serum total cholesterol, creatine kinase and globin, which were observed in the test of its subchronic toxicity by 90 days intravenous injection of beagle dogs. Results have shown that after 90 days intravenous injection of Rh_2, the blood serum creatine kinase increased significantly in high dose group (HDG, 125 mg/kg) and middle dose group (MDG, 42 mg/kg), and slight increase was also found after administered the drug for 45 days, implicating that the increases are time-dependent. On the 45th day and 90th day of the drug administration, the blood serum total cholesterol increased markedly in HDG and MDG; and the blood serum total protein and globin increased in MDG and low dose group (LDG, 14 mg/kg). After withdrawing the drug for 28 days, the increased blood serum creatine kinase and total cholesterol and globin recovery back to the control level. No changes on levels of GLU, ALP, TBIL, BUN, ALT, AST, γ-GT, TG, Create on any dose of drug treatment were observed on either the last day of drug administration or 28 days after drug withdrawal.

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P2-41

Investigation of the cause of corneal opacities encountered in continuous IV infusion studies in rats

Sterenn Goubin, Lucie Reynaud, Fanny Bellebeau

MDS Drug Safety Assessment, Les Oncins, 69210 St. Germain sur L'Arbresle, France

Corneal opacities, sometimes associated with neovessels, have frequently been encountered in rats under continuous IV infusion in our laboratories. The rats in these studies are surgically implanted with indwelling catheter in a femoral vein. The catheter is tunnelled subcutaneously to exit in the intrascapular area and runs through a tether system attached to a jacket worn by the rat. The eye opacities usually appear a few days after surgery and then worsen throughout the study. Several investigations were undertaken to establish the cause of these lesions. An initial study was performed to investigate the potential benefits of the use of an artificial tear solution applied to the eyes during general anaesthesia. Six groups of rats were included in this study: group 1 was an absolute control, group 2 was equipped with an infusion jacket only, group 3 was anaesthetised with isoflurane but not operated, group 4 was anaesthetised with isoflurane and received the artificial tear solution, group 5 was anaesthetised and equipped with a complete infusion system and group 6 was equipped with a complete infusion system and received the artificial tear solution during anaesthesia. Corneal opacities, associated or not with neovessels, were noted only in the animals equipped with a jacket (groups 2, 5 and 6). The artificial tear solution did not reduce the incidence and severity of the ocular lesions. In fact, the incidence was higher in the group receiving the solution. A second experiment was performed to compare the effects of a Spandex infusion jacket (Lomir) with a silicone rubber harness (Covance). The harness induced less severe ocular lesions than the jacket, but unfortunately resulted in superficial sores in the axilla region of some rats. A third study investigated the benefits of an alternative system of catheter routing using a tail cuff in comparison with the Spandex jacket. The tail cuff did not induce corneal opacities, but did result in slight swelling and sores on the tail. Some refinement is still necessary, but the tail cuff method of tether attachment appears to present several advantages over current methods for deep IV infusion in the rat.

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P2-42

Altered penetration of polyethylene glycols into compromised skin

Ivone Jakasa, Maarten Verberk, Sanja Kezic

Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DD Amsterdam, The Netherlands

The human skin acts as an important, but only partial barrier to exogenous compounds. Most of the available skin permeability data are from normal intact human and animal skin. Data on permeability of chemicals in the compromised skin, in particular in humans in vivo, are scarce.

The objective of this study was to investigate the differences in percutaneous penetration of polyethylene glycols (PEG) in subjects with normal and compromised skin in relation to molecular weight (MW). Two models of compromised skin were investigated: skin damaged with detergent sodium lauryl sulphate (SLS) and skin of patients with atopic dermatitis (AD).

Twenty healthy subjects and 20 AD patients were exposed on the volar forearm to PEGs (MW of 150–590 Da) for 6 h. After the end of exposure the stratum corneum (SC) was totally removed by means of tape stripping and the concentration of PEGs were determined in each strip. Using the solution to the Fick's second law of diffusion the penetration parameters were deduced.

The diffusion of PEGs decreased with the increasing MW in normal skin, skin of AD patients and SLS compromised skin. Both models of compromised skin showed increased diffusion of PEGs compared to controls. The partition coefficient showed no MW dependence in normal and AD skin; however, in the skin compromised by SLS the partitioning showed unexplained increase with increasing MW.

The studies on percutaneous penetration of PEGs demonstrated altered skin barrier in SLS compromised skin and in the skin of AD patients which was visibly not affected by disease. Compromised skin showed not only increases absorption, but facilitated entrance of larger molecules which normally would not be able to pass through the skin. Since a compromised skin barrier due to environmental damage or skin disease is not uncommon, when evaluating the health risk associated with skin exposure, penetration of higher molecular weight compounds should be considered.

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P2-43

Epidemiology of acute pesticide poisoning admitted in ICU II Toxicology—Emergency Clinical Hospital Bucharest between 1997 and 2005

Radu Alexandru Macovei, Ilenuta Danescu, Mihail S. Tudosie, Victor A. Voicu

ICU II Toxicology, Emergency Clinical Hospital Bucharest, Romania

Objective: Acute pesticide poisoning, a medical emergency requiring prompt treatment, is a common problem in our country due to widespread use of these compounds. We present an epidemiological profile of acute pesticide poisoning admitted in our department between 1997 and 2005.

Methods: We retrospectively analyzed the records of pesticide poisoning admitted in ICU Toxicology.

Results: During 9 years, 818 cases of acute pesticide poisoning were recorded. The frequency in total poisoning was: 1997—3.13% (106 cases), 1998—4.05% (126), 1999—3.31% (89), 2000—4.80% (118), 2001—5.03% (107), 2002—3.98% (84), 2003—4.93% (74), 2004—4.38% (58), 2005—4.98% (56). The most common occupations were associated with agriculture. The seasonal distribution peaked in spring and autumn months. In most cases oral ingestions was reported. The majority (86%) was suicide attempts and the remaining (14%) was accidental exposures. The most frequent implicated was 21–30 year group for both males and females. Of the patients studied, 49.27% was females and 50.73% males. The products involved were: organophosphates 42%, carbamates 30%, other insecticides and herbicides 18%, rodenticides 8%. In 76% of admitted patients were severe symptoms (coma 46%, respiratory failure 42%, cardiac troubles 18%). 46% underwent mechanical ventilation. 62% received atropine, 40% received fresh frozen plasma, 8% received cholinesterase reactivators. Blood pseudocholinesterase measurements were regularly performed. The mortality was 4% in the total of poisonings, through cardiac arrhythmias and respiratory disturbances, higher in males group and in the patients with associate morbidities. All patients with suicide attempts were referred to a psychiatry specialist.

Conclusions: The acute pesticide poisoning counts for 4.12% in the total number of poisoning admitted in our department. The number of patients decreased in the last years because the legal measures of availability of these products and the use of more non-toxic substances. Most of the cases admitted were suicidal with oral route of exposure and in severe condition. Organophosphates show a higher proportion of severe and fatal cases. To decrease the morbidity and mortality through pesticide poisoning, it is needed a more severe control of the sale and use of these products.

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P2-44

Acute myocardial infarction in a patient using bupropion to aid smoking cessation

Yuksel Gokel, Akkan Avci, Mehmet Kanadası

Cukurova University School of Medicine, Balcali, Dana, Turkey

Bupropion is a new monocyclic antidepressant that has seen increased usage in smoking cessation. Bupropion has cardiovascular, neurologic, and gastrointestinal toxicity. The case is reported of a patient who had been using bupropion to aid smoking cessation. The patient suffered from chest pain, pruritus, and rashes. Electrocardiographic examination revealed ST segment elevation in DI, V5, and V6 leads. Cardiac enzymes, CK-MB and Troponin T levels are elevated. The patient was considered as acute myocardial infacrtion. This case is the second case of acute myocardial infarction in a patient using bupropion to aid smoking cessation.

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P2-45

Therapeutic effect of bis-pyridinium oximes against tabun poisoning

Bozica Radic [1], Ana Lucic Vrdoljak [1], Radovan Fuchs [1], Kamil Kuča [2]

[1] *Institute for Medical Research and Occupational Health, Zagreb, Croatia;* [2] *Faculty of Military Health Sciences, Zagreb, Croatia*

Organophosphorus compounds are widely used as pesticides and unfortunately as nerve agents in chemical warfare. They are known inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) an enzyme that hydrolysis the neurotransmitter acetylcholine in the nervous system. The clinical signs of AChE inhibition manifest as hypersalivation, lacrimation, diarrhoea, tremor, respiratory distress, convulsion and seizures. Signs are dose-dependent, leading to severe incapacitation and rapid death. Together with atropine, pyridinium oximes are known to be successfully used to treat poisoning with many organophosphorus compounds.

In this paper three new bis-pyridinium compounds: K033 [1,4-bis (2-hydroxyiminomethylpyri-

dinim) butane dibromide], K027 [1,4-hydroksyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide], K048 [1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide] were tested as potential antidotes in tabun poisoned mice. Their antidotal effect was compared with TMB-4 [1,3-bis (4-hydrxyiminomethylpyridinium) propane dibromide], which is the best-known antidote in tabun poisoning.

In all experiments, oxime K033 in doses of 1/4 or 5% of its LD_{50} was used for the pre-treatment 15 min before tabun-intoxication. Also, one or 5 min after tabun application experimental animals received oxime K027, K033 or K048 (5% or 1/4 of its LD_{50}) plus atropine sulphate as therapy. The antidotal efficacy of tested compounds was expressed as therapeutic factor (TF) and therapeutic dose (TD). Under same experimental conditions, our experiment selected compound K048 as the most protector/reactivator of tabun inhibited AChE. Namely, this study has shown that the therapeutic regimen consisting of K033 as preatretment and 1/4 of LD_{50} of K048 plus atropine as treatment had the highest TF and TD. The TF was 13.3 LD_{50} of tabun; TD was 10 LD_{50} of tabun and insurance survival of all tested animals.

In conclusion, treatment with these new bispyridinium oximes seems to be a very good alternative for current treatment in tabun poisoning. For this reason, these and other similar compounds require further investigation.

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P2-46

Interventional environmental study and health survey in metal pickling process

Amal Kamal El Safty, Nabil Abdel Maksoud, Neveen Abdel Maksoud, Sawsan Helal

Department Occupational Medicine, Faculty of Medicine, Kasr El Eini Hospital, Cairo University, Egypt

Pickling is a process for the removal of a scale, oxides, or other impurities from a metal surface by immersion in an inorganic acid, usually sulfuric acid, hydrochloric acid, nitric, hydrofluoric, or phosphoric acid. This research aims at environmental assessment, health survey and biological monitoring of metals for workers engaged in the process of metal pickling in steel industry. Our study design is an interventional study that includes environmental assessment of the work place for hydrochloric acid and chlorine gas, clinical evaluation, ECG, ventilatory functions and biological measurements of metals (Ca, Pb, Cd, Cu and Mn). We suggested ventilation means to ameliorate workplace conditions. Reassessment of air quality is tested. Very high environmental measures of HCL and Cl_2 were found in metal pickling ward that responded dramatically to enhanced exhaust ventilation means ($P < 0.01$). Metal screening revealed low mean value of calcium both total and ionized levels, 15 cases of high Pb, 3 cases of high Cd and 3 cases with high Cu. We concluded that environmental and engineering control measures besides the use of personal protective equipment are important in minimizing exposure hazards. Exposure to metals leached from steel surfaces during pickling is a great hazard affecting the level of body trace elements. We recommend enhancing the ventilation and the use of personal protective equipment (PPE). Raising the awareness of all workers about the importance of use of PPE is mandatory.

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P2-47

Investigation of blood toxicity in association with aescin (the horse chestnut seed extract)

Li Chun-mei [1,2], Liu Zhi-feng [1,2], Gao Yong-lin [2], Liu Ke [1,2]

[1] *School of Pharmacy, Yantai University, Yantai Shandong Province 264003, China;* [2] *Shandong Engineering Research Center for Natural Drugs, Yantai Shandong Province 264005, China*

Object: To investigate the blood toxicity of aescin and evaluate its safety in SD rat.

Methods: SD rats were treated with different doses of aescin (15, 10 and 5 mg/kg, i.p.) once time per day for 7 days. Hematology indices (white blood cell, red blood cell, platelet and hemoglobin) and blood coagulation indices (Prothrombin time, Thrombin time, activated part thromboplastin and coagulation time) were selected as observational indices.

Results: Comparing with the control, rats treated with aescin, the number of white blood cell was obviously decreased ($p < 0.05$, <0.01), the number of red blood cell and platelet, and the content of hemoglobin enhanced markedly ($p < 0.05$, <0.01). At the same time, all the blood coagulation indices in rats treated with aescin 10 and 15 mg/kg shortened significantly ($p < 0.05$, <0.01), and rats treated with 5 mg/kg, Prothrombin time and Thrombin time were evidently reduced ($p < 0.05$, <0.01).

Conclusion: There was significant blood toxicity to SD rats treated with high dose of aescin.

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P2-48

Influence of the timely quantitative analysis in ethylene glycol or methanol suspected poisonings

Andrea Giampreti, Davide Lonati, Valeri Petrolini, Raffaela Butera, Carlo Locatelli, Luigi Manzo

Poison Centre and National Toxicology Information Centre, Toxicology Unit, IRCCS Maugeri Foundation and University of Pavia, Italy

Background: Guidelines for ethylene glycol (EG) and methanol (M) poisoning suggest that antidotic treatment should be based on history, serum EG/M levels, osmolar gap and metabolic acidosis. In Italy, EG/M analysis is performed on 24 h/day only in two laboratories, and measured osmolality (freezing point depression) is not at all available in emergency services.

Objective: To evaluate retrospectively the influence on confirmation/exclusion of poisoning of the quantitative EG/M serum analysis in two groups of patients (group 1, mild to moderate risk and no antidotic treatment; group 2, severe risk and antidotic treatment) with suspected EG/M poisoning.

Methods: All potentially poisoned cases of EG/M exposure referred to Pavia Poison Center during 2 years were reviewed. Patients were evaluated for: history (the main criterion used to start antidotic treatment), clinical presentation, metabolic acidosis, antidotic treatment, serum EG/M levels, time to obtain analytical result.

Results: Between 63 cases of EG/M suspected exposure, 38 patients had a history and/or signs/symptoms consistent with a potentially toxic exposure. All patients underwent gastrointestinal decontamination; osmolal gap was never available. In group 1 (23/38; 61%) none showed metabolic abnormalities (amount ingested 0.5 ± 0.4 ml/kg); only for six patients serum EG/M levels were available (within 4.4 ± 1.1 h) and returned <20 mg/dl. In group 2 (15/38; 39%) clinical findings were highly suggestive for severe EG/M poisoning requiring antidotic treatment (amount ingested 1.2 ± 1.0 ml/kg): EG/M levels obtained 14.2 ± 18.5 h after sampling allowed discontinuation of treatment in 10 cases while confirmed it in 5 patients.

Conclusion: The unavailability of timely EG/M testing may lead to unnecessary treatment in a considerable proportion of cases. However, significant delays in antidotic treatment can occur if therapy is withheld until toxicological analysis is obtained. The availability of timely EG/M testing may permit to differentiate patients with or without toxic exposure and to identify cases in which hospitalization, specific monitoring (metabolic acidosis) and antidotic therapy is needed.

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P2-49

Amatoxin poisoning: Evaluation of *N*-acetylcysteine, forced diuresis and multiple doses activated charcoal combined regimen in 54 patients

Carlo Locatelli, A Giampreti, Davide Lonati, Valeria Petrolini, Raffaela Butera, Luigi Manzo

Poison Centre and National Toxicology Information Centre, Toxicology Unit, IRCCS Maugeri Foundation and University of Pavia, Italy

Background: The interest of *N*-acetylcysteine (NAC) in amatoxin poisoning has been documented in previous clinical studies although results from experimental models are conflicting.

Objective: To describe the outcome of a prospective cohort of amatoxin poisoned patients treated with NAC as monochemotherapy, associated to forced diuresis and activated charcoal intestinal dialysis.

Methods: Consecutive cases of confirmed amatoxin poisoning observed from 2002 to 2003 were eligible for the study. Patients were included if they were treated according to a therapeutic protocol consisting, apart from general supportive care, of (i) gastrointestinal decontamination, (ii) multiple dose activated charcoal, (iii) forced diuresis until negative urinary amanitin levels, and (iv) acetylcysteine 150 mg/kg followed by 300 mg/kg/day at least until the third day after mushroom ingestion in patients without hepatitis and as long as AST values are <200 UI/l in patients with hepatic damage.

Results: Among 56 eligible cases, 2 were excluded. Fifty-four patients (53.0 ± 18 years) were studied. NAC was started 36.2 ± 17.0 h after poisoning (range 14–80 h). Twenty-five patients (25/54, 46%) did not develop liver damage; in 13 patients (13/54, 24%), serum transaminases peak values were <2000 UI; in the remaining 16 patients (16/54, 30%), severe liver damage occurred. The mortality rate was 2% (1/54) in the entire case series, and 6% (1/16) in the group of patients with severe liver damage.

Conclusion: The observed mortality rate was lower than in published case series (10–30%) in which NAC was not used; this is confirmed even when subgroups of severe patients are compared. However, the improvement over time in general management should be considered too. In amatoxin poisoning NAC may favorably act as GSH precursor: preclinical in vitro and in vivo studies documented a reduction in cellular GSH content

after amatoxin exposure. Moreover, indirect evidences including interactions among alpha-amanitin, TNF and NAC, and NAC effectiveness in fulminant hepatic failure, support the use of NAC in this poisoning.

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P2-50

Clinical gradation and overall management of 80 viper bitten patients in Italy

Andrea Giampreti, Davide Lonati, Carlo Locatelli, Raffaela Butera, Valeria Petrolini, Luigi Manzo

Poison Centre and National Toxicology Information Centre, Toxicology Unit, IRCCS Maugeri Foundation and University of Pavia, Italy

Objective: To describe clinical course of *Vipera* envenomated patients in order to identify a correlation between clinical gradation and evolution after viper bite.

Methods: A retrospective analysis of all cases of viper bite referred to Pavia Poison Center over a period of one year was performed. Patients were evaluated for: time of presentation after bite, clinical gradation according with severity of local and systemic manifestations, clinical evolution and overall management. Severity of envenoming was assessed on admission using a validated Grading Severity Score (GSS).

Results: Eighty patients aging from 3 to 80 years old were included in the study, 18 patients were lost during follow-up. Forty-five (45/80; 57%) patients presented at a first evaluation with only fang marks (GSS 0); 29 (29/80; 36%) patients had also local edema (GSS 1); 5 (5/80; 7%) patients presented regional edema and (2/5) systemic (vomiting, diarrhea, diplopia, hypotension) manifestations (GSS 2); one fatal case was reported (1/80; 1.3%). Between patients with GSS 0, 5/45 (11%) evolved in local signs, and one presented also a systemic effect requiring antidotic treatment; dry bite was assessed in 30/45 patients (67%). Twenty patients with GSS 1 (20/29; 69%) developed worsening of local edema, 5/29 cases (17%) presented also systemic symptoms, 4/29 (14%) needed antidotic treatment. Four/five patients with GSS 2 (80%) evolved in severity of local and systemic symptoms; 3/5 were treated with antivenom. Time of presentation after bite in patients with GSS 0, 1 and 2 ranged from 30 min to 7 h, from 30 min to 6 h and from 3 to 12 h, respectively. Worsening of clinical picture was observed in 11% of GSS 0 patients and in 69% of GSS 1 patients during the first 12 h; the severity of envenomation increased in 80% of GSS 2 patients during the following 24 h.

Conclusions: Viper envenomation is a potentially serious event that requires immediate hospital care. Patients also with low GSS at presentation have a not negligible chance of clinical worsening in following hours after viper bite. An observation of at least 12 h is advisable for a correct management in all cases of viper bitten patient.

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P2-51

Anti-tubercular drug-induced hepatotoxicity in HIV-positive and negative patients

G. Yimer[1,3], A. Aseffa[1], E. Aklillu[5], E. Makonnen[3], W. Amogne[3], K. Bobosha[1], K. Golka[2], A. Rane[5], L. Lindquist[4], G. Aderaye[3]

[1] *Armauer Hansen Research Institute, Addis Ababa, Ethiopia;* [2] *Institute of Occupational Physiology, University of Dortmund, Germany;* [3] *Addis Ababa University, Medical Faculty, Addis Ababa, Ethiopia;* [4] *Department of Internal medicine, Infectious Disease Unit, Karolinska Institute, Sweden;* [5] *Department of Clinical Pharmacology, Karolinska Institute, Sweden*

Objective: To assess and compare the prevalence, severity and prognosis of anti-TB drug induced hepatotoxicity (DIH) in HIV-positive and negative tuberculosis (TB) patients in Ethiopia.

Design: In this study, 103 HIV-positive and 94 HIV-negative TB patients were enrolled. All patients were evaluated for different risk factors and monitored biochemically and clinically for development of DIH.

Results: Biochemical hepatotoxicity was observed in 17.3% of the patients and 8 out of the 197 (4.1%) developed clinical hepatotoxicity. Seven of the eight were HIV-positive and two for HBsAg. Biochemical hepatotoxicity was significantly associated with HIV co-infection ($p = 0.002$), concomitant drug intake ($p = 0.008$), decrease in CD4 count ($p = 0.001$), and being rapid acetylator ($p = 0.026$). Clinical hepatotoxicity is also significantly associated with being female ($p = 0.027$), HIV co-infection ($p = 0.043$), concomitant drug intake ($p = 0.003$), HBsAg ($p = 0.046$), and decrease in CD4 count ($p = 0.025$). However, hepatotoxicity was observed to have no significant association with alcohol intake, age, body mass index, and type of TB.

Conclusion: We conclude that anti-TB DIH is a major problem in the management of tuberculosis and there is a need for a regular biochemical and clinical follow up for those patients who are at risk.

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P2-52
Emergency hemodialysis in the management of intoxication

Salim Satar, Nezihat Rana Alpay, Ahmet Sebe, Yuksel Gokel

Cukurova University School of Medicine, Balcali, Dana, Turkey

Management of intoxicated patients has many aspects in a wide spectrum; beginning with decontamination processes and basic supportive care. The most logical therapeutic approach is probably the specific antidotes, when available and/or applicable. On the other hand, many chemicals and drugs, can be removed from the body by means of hemodialysis or hemoperfusion while treating vital sign abnormalities and electrolyte and acid-base disturbances of the patient.

In this paper, we described 11 cases of intoxication; 6 with methyl alcohol, 3 with lithium and 2 with salicylate, admitted to emergency department and treated with hemodialysis between January 1, 2002, and December 31, 2004. We reviewed their medical charts and detailed demographic data, medical history, type of exposure, clinical and laboratory presentations, duration of hemodialysis performed and the outcome.

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P2-53
Ingestion of caustic substances by adults

Salim Satar, Metin Topal, Nalan Kozaci

Cukurova University School of Medicine, Balcali, Dana, Turkey

Caustic products are responsible for the most serious cases of poisoning, which are always emergency cases. In this paper, we review demographic features and endoscopic results of the patients admitted to a university emergency department with a history of caustic substance ingestion between January 2000 and June 2003. Thirty-seven patients were included in this study. Twenty-one of the patients were female and 16 were male. The mean age of the patients was 30.9 ± 14.7 years. The agents included sodium hypochlorite in 24 patients and hydrochloric acid in 13 patients. All the patients ingested these agents orally. The mean interval time of admission to emergency department after ingestion of caustic agent was 5.4 ± 5.6 h. Endoscopy was attempted in 37 patients. Endoscopic results were as follows: grade 0 in 8 (21.6%) patients, grade 1 in 17 (45.9%) patients, grade 2a in 5 (13.5%) patients, and grade 2b in 7 (18.9%) patients. We believe that early signs and symptoms after caustic substance ingestion are not consistent with the extent of damage, and endoscopy is the only reliable method to assess injury. It is important that efforts should be made to educate the public about the dangers of caustic substances so that their threat may be diminished.

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P2-54
Elimination of heavy metals intoxication sequential micronutrition

M. Lugand Bourgeois, A. Cornely

Bioresearch and Partners, Route de l'Ile au Bois, 1870 Monthey (CH), Switzerland

By restoring homeostasis through the use of a nutritional new way, Bioresearch and Partners is to make a major contribution for the management of metal-induced toxicities, should they be punctual or chronic. The studied population focused on patients suffering from heavy metal-induced pathology. At the beginning and during the nutrition intervention period, patients were monitored for several other patho-physiological and psychosomatic symptoms as well as for blood hypersensitivity to metals.

Method study: Six-month open trial.

Inclusion: Twenty-six patients (18–75) with significant metal-induced symptoms: MELISA positive (Hg, Al, Ni, Au, Cd, Pd, Sn) blood test measuring hypersensitivity to metals that works by placing a range of metals into contact with T lymphocytes and monitoring the reaction as well as for several patho-physiological and psychosomatic symptoms fatigue, pain and inflammatory symptoms. Six capsules per day.

Monitoring 3–6 months: Patient patho-physiological and psychosomatic symptoms, MELISA test.

Result: Between 3 and 6 months, patients recorded a significant improvement in their pathological symptoms.

At 6 months:

Fatigue (75% of the patients before treatment): 90% reported significant reduction in asthenia.
Gastro-intestinal symptoms (50% of the patients before treatment) symptoms disappeared in 70% of the patient population.
Rhumatoid pain (60% of the patients before treatment) symptoms disappeared in 50% of the patient population

- Stimulation of T lymphocytes by Hg, Pd, Al, Sn, and Au, in the MELISA assay, returned to normal values in all patients after 6 months (Ni and Cd: 12 months).

Conclusion: Elimination of symptoms and normalisation of the MELISA test (lymphocytes reaction) offer a satisfactory response to the required therapeutic aims in a population presenting intoxication to heavy metals that can be used in monotherapy or in combination with other metal elimination approaches.

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P2-55
Skin irritation by kerosine

Chantal J.G.M. Smulders, Peter J. Boogaard

Shell Health Services, Shell International B.V., The Hague, The Netherlands

Kerosine is a middle distillate with carbon numbers ranging from C9 to C16 and boiling from 145 to 300 °C. Kerosines are complex mixtures of many thousands of individual hydrocarbons. The analytical data that do exist show that the major components of all kerosines are branched and straight chain paraffins and naphthenes (cycloparaffins). Aromatic hydrocarbons, mainly alkylbenzenes and alkylnaphthalenes only comprise a minor part of kerosine streams. The predominant use of kerosine in Europe is as aviation jet fuel for civilian (Jet A-1) and military (JP-8) aircraft.

Human studies and case reports as well as animal studies show that kerosine may act as a skin irritant. The degree of irritancy is preparation-, dose- and exposure-time dependent and symptoms range from very faint erythema to severe irritation in humans. Since primary irritation appears to be one of the key effects in the toxicity of kerosines and jet fuels, the mechanisms of the irritation and the following inflammatory reaction have been studied extensively. The aim of this study is to gain insight in the factors determining the degree of irritation.

It has been hypothesised that the degree of irritation depends on the irritant properties and absorption kinetics of the individual compounds in kerosine.

Dermal application of kerosine or jet fuel generally shows that the aromatics and aliphatics are well absorbed into the skin. Subsequently, the aromatics penetrate the skin at a higher rate than the alkanes. The flux of the kerosine components through the skin depends on the lipophilicity and the molecular weight of the kerosine component. It is shown that the steady state flux of the components was proportional to their concentration. However, it is evident that, although many kerosine components will be absorbed into the skin, not all kerosine components will actually permeate the skin.

One study investigated the kinetics, irritant properties and the effect on biomarkers of inflammation of paraffinic kerosine constituents. The results show that the degree of irritation and the ability to activate biomarkers of inflammation depends on the number of carbon atoms. However, the constituent with the higher dermal permeation and retention produced less irritation. The combined results from different studies suggest that the irritant properties of kerosine not only depend on the concentration and kinetics of the constituents, but also on the specific structural configuration of the constituents.

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P2-56
Conjunctival dichlorvos poisoning

Ahmet Sebe[1], Salim Satar[1], Zikret Koseoglu[2], Hasan Yesilagac[1]

[1] *Cukurova University School of Medicine, Department of Emergency Medicine Balcali, Adana, Turkey;* [2] *Adana Numune Research and Education Hospital, Emergency Department Adana, Turkey*

Organophosphates (OP) are toxic substances that frequently cause poisoning in humans. Worldwide, 3 million cases of acute, severe pesticide poisoning are reported each year, resulting in 220,000 deaths. In Turkey, more than 200 chemicals are registered as pesticides. Among these, OPs are the more important and most widely used group in our country.

OP compounds are cholinesterase inhibiting compounds that produce serious human toxicity. Clinical manifestations depend on type of the agent, its concentration and the degree of exposure. The route of exposure in organophosphate poisoning varies. These compounds caused poisoning by oral, dermal, parenteral, conjunctival, gastrointestinal, and respiratory routes. However, there are few reports in the literature with conjunctival route. In this paper, we reported a case of severe intoxication from dichlorvos by conjunctival route, accidentally who is treated successfully.

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P2-57

Rat liver and kidney pools of free amino acids in norm and with different doses of pyrazinamide

Larysa B. Bondarenko, Natalia A. Saprykina, Valentina M. Kovalenko

Institute of Pharmacology and Toxicology Academy of Medical Science of Ukraine, Ukraine

At present, in view of tuberculosis caused morbidity growth in the whole world, problem of its chemotherapy schemes optimization with simultaneous minimization of antibacterial drugs prolonged introduction negative functional and biochemical consequences is very acute. One of the sensing parameters of early complex estimation of metabolic processes disturbances in tissues and organs is the pool of free amino acids.

The aim of this work was investigation of rat liver and kidney pools of free amino acids in norm and with introduction different doses of pyrazinamide.

Experiments were carried out on Wistar male rat (160–200 g of body weight). Pyrazinamide (1000 and 2000 mg/kg of body weight) was introduced per os during 60 days. The contents of free amino acids were determined on the amino acid analyzer AAA-881 (Czechia).

Rat liver and kidney pools of free amino acids changes investigation allowed getting complex evaluation of pyrazinamide effects on amino acids, proteins, nucleotides, nucleic acids and energy metabolisms in these organs. In liver dose 1000 mg/kg of body weight of pyrazinamide caused maximal quantity of these parameters changes. Part of them could be considered as a result of organism's compensatory answer to xenobiotic introduction. Pyrazinamide dose increasing to 2000 mg/kg of body weight resulted to exhaustion of organism's adaptive abilities. In kidney under the effect of pyrazinamide there were dose dependent changes in pools, which could be considered as evidence of this compound specific effect first of all on processes of amino acids reabsorption, nitrogen-containing compounds transformations and energy metabolism.

The detected changes in pools of free amino acids at pyrazinamide different doses introduction can serve complementary criterion of estimation of both organism's internal reserves, degree of metabolism disintegration under the influence of xenobiotic and adaptive abilities of target organs and organism as a whole.

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P2-58

Use of prazosin in the treatment of scorpion envenomation: A case report☆

Zikret Koseoglu

Adana Numune Education and Research Hospital Emergency Department, Turkey

Although scorpion bite is usually lethal in children under 6, sometimes it might cause death for those over 6. Acute lethal systemic seizures (cardiovascular and central nervous systems) have been frequently reported from the countryside of developing countries. Numerous researchers have suggested quite different approaches to the treatment of scorpion bites. In this report, we describe a case brought to the emergency service after the patient had been bitten by a scorpion and had significant sympathetic nervous system symptoms. The patient was treated with prazosin successfully.

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P2-59

Validation of GC/MS method for analysis of methadone in human urine

Sanja Mandic, Vesna Horvat, Dario Mandic, Zeljko Debeljak, Neda Majetic-Cetina

Institute of Public Health, Osijek, Croatia

A fast method for qualitative analysis of methadone in human urine using gas chromatography–mass spectrometry (GC–MS) is described. Urine samples were extracted by liquid–liquid extraction using Toxi-tubes A, and were not derivatized. Limit of detection was 25 ng/ml. Method performance in terms of recovery, repeatability and intermediate precision was studied and found to be acceptable for clinical analysis of methadone.

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P2-60

Effect of halothane anesthesia on canine BUN and creatinin

Siavash Sharifi [1], Ahmadreza Mohamadnia [1], Mehrdad Pourjafar [1], Amin Derakhshanfar [2]

[1] *Shahrekord University, Saharekord, Iran;* [2] *Shahid bahonar University of Kerman*

Inhalant anesthetics are widely used for producing general anaesthesia. Inorganic fluoride is a common metabo-

lite of halogenated inhalation anesthetics produce renal toxocity in mice, rats, guinea pigs, dogs, and human.

Fifteen healthy dogs (20.1 ± 1.2 live body weight) were anesthetised. Dogs were randomly assigned to three experimental groups (groups A, B and C) for 1, 3 and 5 h of anaesthesia with halothane (5 dog/group), respectively. All anesthesia were repeated in the same group every 48 h as totally three anesthesia (D1, D2 and D3) were performed in each group. Prior to anaesthesia, cephalic vein was catheterized for subsequent fluid administration and venous blood sampling. For induction of anaesthesia, halothane in 100% oxygen (4 L/min) was delivered via a fitted facemask. The concentration of halothane was gradually increased (0.5% every 30 s) until a vaporizer setting of 4% was reached. Intubated animals connected to a rebreathing system and a medium plane of anaesthesia, as determined by palpebral and pedal reflexes, was maintained with halothane (1–1.2%) in oxygen (1.5 L/min).

Venous blood samples were obtained before (time 0) and 1, 3, 5 and 24 h after induction of anaesthesia. Serum BUN and creatinin concentration was measured by biochemical spectrophotometric method.

No significant differences were observed in serum BUN and creatinin concentration in group A, in different sampling times compared with time 0 during study.

In group B, serum BUN and creatinin were increased 3–24 h compared to time 0 in third anesthesia (D3) ($P < 0.05$).

In group C, serum BUN and creatinin were increased five hours after anesthesia in D2 and 3–24 h after anesthesia in D3 ($P < 0.05$).

In our study, serum BUN and creatinin increased following 3 and 5 h of halothane anaesthesia, compared with baseline values in secound and third anesthesia. This suggests that repeated halothane anaesthesia within a long period has significant effects on the extent of halothane metabolism in dog and delivery inorganic flourid produce renal toxicity.

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P2-61
Formalin effects on the nose and throat of personnel of anatomical sciences departments in Iran medical schools

Mohammad Reza Namavar, Farzaneh Dehghani, Soghra Bahmanpour, Aghdas Poostpasand

Shiraz University of Medical Sciences, Shiraz, Fars, Iran

Formaldehyde (and its compositions) is a chemical substance that is widely used in chemical industries (detergents, cosmetics and so on), medicine (for sterilization of operation room and its instruments and fixation of histological specimens and cadavers) and even in textiles and papers.

In recent years many studies were done in formalin effects on people health. But anybody did not study formalin effects on nose and throat in persons that have direct contact with this substance in Iran. For this reason; personnel of anatomical sciences departments in medical schools were selected as research group. Questionnaires were prepared that had following indexes: Nasal signs (rhinorrhea and smell sense changes), throat signs (throat sore), job, sex, age, formalin contact (duration and direct or indirect contact) and diseases history. These questionnaires were sent to anatomical sciences departments of medical schools in Iran. Data were analyzed by Chi-square test.

Results showed that contact with formalin causes decrease (or loss) of smell sense and increase of rhinorrhea and throat sore. These changes have direct relation with contact duration and direct contact. Relation between contact with formalin and these changes is significant ($P < 0.05$). It is suggested that people do not have any direct contact with formalin. Also, departments of anatomical sciences use low formalin (or formalin free) agents for fixation of cadavers.

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P2-62
Fatal intoxication by alcohol and heroin

Davorka Sutlovic, Marija Definis Gojanović

Split University Hospital, Split, Croatia

At the recent time, in Split–Dalmatia County, drug abuse with alcohol consumption is in increase.

We reported two cases of three deaths due to fatal combination of heroin and alcohol. The first case was a couple, 30-year-old man and 28-year-old woman, with a history of drug use which families claimed that they were in treatment program. They were found in a car

surrounded by cans from different alcohol drinks. A numerous needles were beside the bodies. The second case was a 29-year-old man, found dead in an old house, who attempted suicide recently. Three fresh injection marks were found on his right arm, and two needles were near his body.

Multiple samples were collected and stored at 4 °C until analysis in order to establish drug distribution. Solid-phase extraction was performed using Amberlite XAD-2, polyaromatic adsorbent resin (Supelco; SIGMA ALDRICH, Taufkirchen, Germany). The analysis was performed using a Shimadzu GC-2010, with ion trap mass spectrometer. Blood–alcohol concentration (BAC) was measured by Shimadzu GC-2010 with headspace and flame ionization detector (FID). Absorbancies at 541, 560 and 576 nm were measured by spectrophotometer (Ultrospec 2000. Pharmacia Biotech (Biochrom) Ltd. Cambridge, England) to evaluate the amount of carboxyhemoglobin (COHb).

CASE 1

Blood alcohol and urine concentration in man was 1.60 and 2.93 g/kg respectively. Concentration of blood alcohol in woman was 1.81 while urine alcohol concentration was 2.48 g/kg. Heroin, meconin, papaverine and caffeine were found in both syringes. Meconin was detected in all samples. Monoacetylmorphine was found only in woman's urine, bile and hair, while in the man's hair noscapine was found. In the samples of man and woman blood the amount of COHb was 25% that could contribute to their death.

CASE 2

Blood alcohol concentration was 1.67 and urine alcohol concentration was 2.03 g/kg. Heroin, theophyline, meconin, acetaminophen, 3-acetyl-morphine, 6-acetylmorphine, codeine, noscapine, papaverine and caffeine were detected in syringes. Meconin was found in blood, urine and bile samples. There was presence of 3-acetylmorphine, 6-acetyl-morphine and codeine in the blood. Concentrations of morphine were much higher in tissue samples from three injection marks than in any other samples.

Ethanol in the blood together with morphine drastically augments the risk of rapid death due to respiration failure. It can also lead to a relatively high risk of overdosage in experienced drug abusers.

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P2-63
Suicide by Fentanyl

Davorka Sutlovic, Marija Definis Gojanović

Split University Hospital, Split, Croatia

Fentanyl is potent, short acting narcotic analgesic widely used as surgical anesthetic. To our knowledge, death due to the intravenous injection of fentanyl has not been reported previously.

A case in which fentanyl was injected is presented. A 41-year old nurse, an employee at the hospital emergency department, was found dead at home. She had no known history of drug and alcohol abuse. Two syringes, one empty and second filled with a clear liquid, were found near the body, while a needle was stuck into her hand.

Multiple samples were collected and stored at 4 °C until analysis in order to establish drug distribution. Samples were screened for ethanol, common drugs of abuse and other basic drugs. Quantification and confirmation analyses were performed by first isolating fentanyl by extraction of 1–5 ml/g specimen. Solid-phase extraction was performed using Amberlite XAD-2, polyaromatic adsorbent resin (Supelco; SIGMA ALDRICH, Taufkirchen, Germany). Underivatized specimens were analysed on an Shimadzu GC-2010, with ion trap mass spectrometer (mass selective detector, MSD).

Quantification was performed by Selective ion mode (SIM) with external standard curve preparing with 0.0785 mg/ml Fentanyl citrate (eq. to 0.05 mg/ml fentanyl). For fentanyl three ions were monitored m/z: 245, 146 and 189. Blood–alcohol concentration (BAC) was measured by Shimadzu GC-2010 with headspace and flame ionization detector (FID).

Toxicological analysis was positive of fentanyl poisoning. In blood concentration was 540 μg/L and in stomach tissue mixed with blood was 40 μg/g. Blood alcohol concentration was 0.0 g/kg. No other organic bases were detected. Testing of the syringes and needle found at the scene was positive for fentanyl. Fentanyl overdose was listed as the cause of death and the manner of death was classified as suicide.

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P2-64

Self-reported versus documented side effects of antiretroviral drugs in a sample of HIV-infected patients in Pretoria, South Africa

Ntambwe Malangu

University of Limpopo, Tshwane, South Africa

Objective: To determine the level of concordance between self-reported and documented side effects in HIV-infected patients on ART.

Method: a cross-sectional survey of patients who started ART between July 2004 and August 2005, who consented to be interviewed for the collection of information on sociodemographic characteristics, side effects and other data.

Results: The 180 patients who consented to be interviewed had the following characteristics: mean age of 36.7 (±8.1) years old, 68.8% female, 86.7% unemployed, 73.9% with high school level of education, and 77.8% single. Overall, The average number of side effects documented was 2.2 (±1.5) versus 2.6 (±1.4) for self-report. Twenty-five side effects were documented in 89% of patients' files versus 19 side effects reported by 94% of respondents. Some 14 (73.7%) of these 19 side effects were also documented; while 11 (44%) of the symptoms documented were not reported, and five (26.3%) of reported symptoms were not documented.

Conclusion: The majority of side effects self-reported by patients were also documented suggesting that that patients' self-report could be used as a credible source of information for pharmacovigilance purposes.

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P2-65

Distribution and concordance of phenotypically and genotypically determined acetylation status on patients taking anti-tuberculosis drugs in Ethiopia

Getnet Yimer[2,3], Klaus Golka[3], A. Aseffa[2], E. Aklillu[3], E. Makonnen[4], W. Amogne[3], K. Bobosha[2], A. Rane[4], L. Lindquist[4], G. Aderaye[3]

[1] *Institut für Arbeitsphysiologie, Universität Dortmund, Germany;* [2] *Armauer Hansen Research Institute, Adis Abeba, Ethiopia;* [3] *Adis Abeba University, medical faculty, Adis Abbeba, Ethiopia;* [4] *Karolinska Institute, Stockholm Sweden*

Knowing polymorphisms of drug metabolizing enzymes is important in selection of drugs and in predicting treatment outcome. *N*-Acetyltransferase (NAT) is one of the polymorphic drug metabolizing enzymes which metabolizes different drugs and carcinogens. NAT-2 gene shows a significant ethnic variation in different ethnic groups and there is no report so far on the genotypic and phenotypic distribution of NAT-2 gene in Ethiopians. In our study we tried to show the distribution and concordance of NAT-2 gene in newly diagnosed tuberculosis patients taking anti-tuberculosis drugs from Ethiopia. We used PCR RFLP and allele specific PCR for genotyping to see mutations at 191, 341, 434, 590, and 857 positions and caffeine based assay for phenotyping. Out of 128 individuals on whom genotyping was done 68 (53.1%) individuals were rapid acetylators and 60 (46.9%) were slow acetylators. From these 128 individuals on whom genotyping was done 55 individuals undergo phenotyping and we found that 30 (54.5%) were slow acetylators and 25 (45.5%) were fast acetylators. Unlike other studies which show a very high concordance rate, in our study the concordance rate of phenotypically and genotypically determined acetylation status was only 50.9%. This may be because of the concomitantly taken anti-tuberculosis drug which may either induce or inhibit caffeine metabolism. This has an implication that caffeine is probably not a good probe for phenotypic determination of acetylation status in patients taking drugs that may inhibit or induce its metabolism. Another interesting finding from our study is that none of our Ethiopian study participants showed the Black specific SNP (NAT2*14).

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P2-66

Improved elimination of formate in methanol poisoning by intravenous infusion of formate dehydrogenase conjugated with linear mono methoxy poly ethylene glycol

Arumugam Muthuvel, Rathinam Rajamani, Rathinasamy Sheeladevi

Department of Physiology, Dr. A.L.M. Postgraduate Institute of Basic Medical Sciences, University of Madras, Chennai-600113, Tamil Nadu, India

Elimination of formate, a highly toxic metabolite in methanol poisoning, is one of the principles of clinical management in methanol poisoning. Formate dehydrogenase (EC 1.2.1.2) acts over formate and converts formate into CO_2 in the presence of NAD. Rapid elimination of formate by single bolus intravenous infusion of native Formate dehydrogenase (FD), isolated from Candida boidinii has been reported in our earlier study. Carbicarb buffer was used to correct metabolic acidosis. In order to prevent immunological reactions which might

be produced by multiple dosing of Formate dehydrogenase and to prolong the serum half life of the enzyme, the *N*-hydroxysuccinimidyl ester of methoxy polyethylene glycol propionic acid (mPEG-SPA 5000) was conjugated to native Formate dehydrogenase. PEGylation reactions were run at 20 °C for 30 min in a reaction buffer (0.2 M sodium phosphate buffer, pH 8.3). The PEGylated molecules were purified from unreacted PEG with Amicon Ultra—4 (10 kDa) and by Sephacryl S-300 HR gel-filtration chromatography. Unreacted Formate dehydrogenase molecules were removed by DEAE Sepharose FF anion-exchange chromatography. PEG-FD enzyme molecules obtained from reacting ratio of FD/PEG of 1/40 had an enzyme activity of 68% of unmodified enzyme. Immunogenicity of PEGylated and native enzyme was evaluated by ELISA. Plasma half life based on biological activity was also evaluated. In vivo efficacy of PEG-FD or native FD was comparatively evaluated by single intravenous administration of PEG-FD or native FD in folate deficient methanol intoxicated albino rats along with carbicarb buffer infusion. Methanol and formate were estimated at specific time points, respectively, with HPLC and Fluorescence spectrophotometer. PEG-FD had comparatively longer plasma half life, lower immunogenicity and lesser allergenicity than native FD. PEG-FD had better in vivo efficacy than native FD in eliminating the formate in methanol poisoning.

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P2-67

Monitoring of the heroine addict patients during methadone substitution therapy

Daniela Luiza Baconi, Anne Marie Ciobanu, Maria Barca, Dan Balalau, Cristina Hudita, Gina Manda[2], Mihaela Ilie[1], Mihai Ionica[3]

[1] *"Carol Davila" University of Medicine and Pharmacy, faculty of Pharmacy, Bukharest, Romania;* [2] *"Victor Babes" National Institute, Bucharest, Romania;* [3] *Clinical Emergency Hospital, Bucharest, Romania*

The drug addiction treatment is a long-term process that implies multiple interventions and has as final aim the abstinence. Addiction treatment, including diagnosis, medical assistance, and social reintegration of addicts, has the purpose of improving the health state and the quality of life, by diminishing the drug usage, the morbidity and mortality due to the addiction, by facilitating the access to public services and full social reintegration.

The study aims at a complex evaluation (clinic, paraclinic, and psychologic) of the heroin addict patients during methadone substitution treatment conducted at Sf. Stelian Center for Evaluation and Treatment of Addictions, Bucharest. This study presents evaluation of 32 cases (29 men and 3 women) during methadone detoxication treatment; these patients fulfilled the inclusion criteria and were eligible for the study. Socio-demographic, toxicological, and clinical characteristics of the patients included in the study are presented.

Complete hematological and biochemical analyses are performed for each patient; the psychologic profile of the patients is evaluated and the prevalence and the type of psychiatric morbidity at the heroin addicts are identified.

The analytic diagnostic of drug abuse is provided by using GC-MS chromatographic methods and the methadone plasma levels are determined by HPLC and GC-MS methods.

The presented methodology is continued during methadone maintenance therapy.

In the study group the mean age is 24.53 years (range 16–32) and the mean length of i.v. heroin use is 4.78 years (range 1–9); the mean levels of heroine metabolites in urine was 9940.1 ng/mL (in 6 cases the levels was higher than 30,000 ng/mL) and the mean methadone dose was 17.41 mg (range 7.5–40 mg). HVC infection was present in 75% of patients. Cocaine use has been reported in 25% of patients, marijuana or hashish use in 50% of patients and ecstasy use in 28% of patients.

The results of the study identified some of the factors associated with enrolment into methadone treatment and give an insight into profiles of the patients who enter in the treatment.

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P2-68

Alanine transaminase and prothrombin time abnormalities following mushroom poisoning

Daniela Chaparroska, Niko Becaroski, Aleksandra Babulovska

Department of Urgent Internal Medicine and Toxicology, University Clinical Centre, Skopje, The Former Yugolav Republic of Macedonia

Aims: Toxic mushroom poisoning leads to a variety of clinical outcomes ranging from self-limited gastrointestinal symptoms to fulminant hepatic failure requiring orthotopic liver transplantation. We reviewed the outcomes of patients with severe acute hepatitis secondary

to amanita phalloides poisoning, treated with contemporary modalities.

Methods: We retrospectively reviewed patients admitted to our institution over a 5-year period (2000–2005) with elevated transaminase levels (>1000 IU/L) attributed to recent mushroom ingestion. The patient's clinical course, laboratory data, and treatment regimen were recorded and analyzed.

Results: The mean peak serum levels were: aspirate transaminase 6520 IU/L, alanine transaminase 8860 IU/L, total bilirubin 11.5 mg/dL, creatinine 535 Mmol/L, and prothrombin time- international normalized ratio (PT-INR) >10. Three patients developed acute renal failure requiring hemodialysis and hemoperfusion. The other 17 patients survived without significant morbidity.

Conclusions: Patients with severe hepatitis from amanita phalloides poisoning are thought to have a poor prognosis and frequently need liver transplantation for survival. We suggest that with early and aggressive multidisciplinary care, such patients recovered from severe hepatitis caused by amanita phalloides poisoning, without liver transplantation.

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P2-69

Acute intoxications in the ICU—What has changed in a 5 years period?

Marijan Merkler, Marijana Grgić Medić, Radovan Radonić, Vladimir Gašparović

Clinical hospital center Zagreb, Zagreb, Croatia

Acute intoxication is a condition characterized by the distress of one or more organ systems due to the toxin or its metabolites. Patients with severe and life threatening intoxications, requiring intensive treatment and surveillance, are admitted to the Intensive care Unit at of the Clinical hospital center Zagreb.

The aim of this study was to analyze the acute intoxicated patients admitted to the ICU in the year 2005 and to assess the changes in the structure of acute intoxication in the 5 years period, by comparing these data with the data from the year 2000.

During the year 2005 (1st January–December 31st 2005), 44 patients (23 male and 21 female) with severe intoxications were admitted to the ICU. The median age was 40.6 years (range 17–86 years). Thirty-nine (88.6%) patients survived and 5 (11.4%) patients died. The intoxication occurred most frequently in the suicidal attempt, in 32 (72.7%) patients, overdose was registered in 10 (22.7%) patients, and accidental poisoning in 2 (4.6%) patients. Toxic agents were in most cases (18 patients, 40.9%) drugs, narcotics were detected in 10 (22.7%) patients, alcohol in 5 (11.4%), corrosive agents in 5 (11.4%) and other agents in 6 (13.6%) cases (pesticides, carbon monoxide, ethylene-glycol, etc.).

The most frequent indications for admissions into the ICU were acute confusional states (21 patients, 45.4%), and 17 (38.6%) patients were comatose at admission.

The median duration of treatment in the CU was 2.95 days (range 1–9 days). The most frequent complications were respiratory insufficiency, and pneumonia was registered in eight (18.2%) patients. Twenty patients (45.4%) continued the treatment in the psychiatric ward following the dismissal from the ICU.

These data were compared with our data from the year 2000. The proportion of patients with acute drug intoxications remained unchanged (40.2% in 2000 versus 40.9% in 2005) and was the highest in both years. The increase in the proportion of patients with acute narcotic intoxications increased from 15.1% to 22.7% whereas the proportion of acute alcohol intoxication decreased from 22.6% to 11.4%. The median duration of treatment did not change significantly (3.1 days versus 2.9 days).

The significant increase of patients admitted due to narcotic abuse was detected. Nearly half of all intoxications are caused by drugs. The most common complications of acute intoxications in human are observed on respiratory tract.

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P2-70

National antidotes stockpile for chemical emergencies in Italy

Valeria Petrolini [1], Carlo Locatelli [1], C. Lonati [1], L. Mela [1], Andrea Giampreti [1], Raffaela Butera [1], A. Volpini [2], Luigi Manzo [1]

[1] *Pavia Poison Centre and National Toxicology Information Centre, Toxicology Unit, IRCCS Maugeri Foundation Hospital and University of Pavia, Italy;* [2] *Department of Civil Protection, Rome, Italy*

In the last 5 years concern about health risks of hazardous accidents has increased: the existing plans to face an industrial chemical disaster has been reviewed and extended worldwide to counteract potential terrorist attacks involving chemical agents. In Italy, within the context of an agreement between the Italian Department of Civil Protection and the Pavia Poison Center, a system for antidote stoking, supply, and use in chemical (conventional and/or non conventional) emergencies has been set up. Sixteen different antidotes, unavailable in appropriate quantity in the Italian emergency depart-

ments, and required to treat possibly poisoned patients by chemicals of greatest concern were identified and acquired. A defined amount of every antidote has been stocked in a three level stockpile: level (A) 152 sites all over Italy where a quote of high priority antidotes are stocked in an amount adequate to treat 100 seriously poisoned patients for almost 24 h, level (B) 21 sites (at regional level) where a larger amount of some high priority antidotes are stocked together with some second priority antidotes, and level (C) 2 sites where a large amount of almost all antidotes and second priority antidotes are stocked. A national database links all the antidote stockpiles and is managed by the Civil Protection Department and by the Pavia Poison Centre toxicologists to ensure, together with a 24 h/day clinical service consultation, a proper and immediate drug delivery to the accident site throughout the Italian territory.

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P2-71

Predictive parameters of severity for carbamazepine cardiotoxicity

Z. Pereska, L. Petkovska, C. Bozinovska, J. Naumovski, D. Petrovski, A. Babulovska

Clinic of Toxicology and Urgent Internal medicine, Skopje, Macedonia

Acute carbamazepine intoxications are presented with marked cardiovascular and neurological disturbances. The aim of the study is to observe possible influence of demographic factors on clinical features, as QTc being a parameter of carbamazepine cardiotoxicity in young patient's poisoning.

Materials and methods: Thirty-two patients (pts) admitted at the Clinic of Toxicology because of carbamazepine poisoning were included in the study. Eighteen pts (56.25%) were female and 14 (43.75%) of the patients were male, aged 26.13 ± 10, 763 years. Twelve-lead ECG-gram performed at admission was analyzed and QTc was calculated by Bazzet's formula (431.63 ± 26.314 ms). Carbamazepine serum concentrations in blood samples taken on admission were considered in the analysis (80–215 μmol/l). Conscious disturbances were graduated as somnolent (14 pts—43.75%), soporous (10 pts—31.25%) and comatose (8 pts—25%). The usefulness of the regression model was testing by ANOVA analysis (d.f. = 4, F 19.012, $p = 0.000$), the influence quantification of serum concentration, age, gender and consciousness on the QTc interval were analyzed by regression analysis. Results showed that 37.5% of patients have QTc > 450 ms; increase of the serum concentration for every μmol/l induced increase of QTc for 0.781 ms; patients who are soporous had longer QTc for 16.434 ms compared to patients that are somnolent; and in females this showed to induced longer QTc for 12.859 ms in comparison with males in acute carbamazepine poisoning in especially pointed in young population. Carbamazepine serum concentrations have positive correlation with the level of coma ($r = 0.487$, $p = 0.007$). Its concentrations in comatose patients (>140 μmol/l) were almost linear combination of the other independent variables so its influence could not be quantified with this statistical method.

Conclusion: Carbamazepine cardiotoxicity in acute poisonings is a multifactorial induced conducting disturbance. Higher carbamazepine serum concentration, female gender and conscious disturbance are related to higher level of QTc prolongation. Although carbamazepine serum levels should be always performed in this kind of intoxications as a golden standard for standard of care and legislatively, at bed site estimating QTc can be used as a practical parameter in making first conclusions about the severity of carbamazepine overdose. This would be a very practical point in smaller hospitals and units that could not provide immediate levels or have these toxicology results as send outs.

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P2-72

Cyclosporine induced lipid disorders

L. Petkovska, N. Ivanovski, Z. Pereska, Dz. Naumovski, C. Bozinovska, A. Babulovska

Clinic of Toxicology and Urgent internal medicine, Medical fakulty, Skopje, Macedonia

Background: The maintence immunosuppressive therapy in transplant recipients is associated with various lipid disorders. The causes and mechanisms of post-transplant hyperlipidemia are complex and not fully understood. Objective of this study was to investigate weather the higher concentrations of the cyclosporine A (CsA) is associated with more prominent lipid disorders.

Methods: We measured lipids and lipoprotein lipids 3 months after renal transplantation in two groups of pts with statistically significant different CsA concentrations. All pts were on equal doses of other immunosuppressive agents, and had stable graft function.

Results: In the first group (15 pts), CsA trough level was 289.01 ± 71.21. In the second group (17 pts), CsA

trough level was 159.12 ± 12. The most prominent disorder was elevated total cholesterol with average value 7.29 ± 1.9 mmol/l in the first and 6.07 ± 1.2 in the second group. Also the LDL was more elevated in the first (4.83 ± 0.81) then in the second group (3.81 ± 1.19). The mean value of TG and HDL were higher in the first than in the second group, but these differences were not ss.

Conclusion: Higher CsA trough levels are associated with remarkable lipid disorders.

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P2-73

Combustive toxicology: The new concept of people death and the probable remote consequences at fires

L.M. Shafran, D.P. Timoshina, I.A. Harchenko, D.I. Leonova, J.V. Nekhoroshkova, S.V. Novak, I.S. Presnjak, N.G. Selivanenko, E.V. Tretjakova

Ukrainian Scientific and Research Institute of Medicine on Transport, Odessa, Ukraine

Traditionally, in combustive toxicology four major factors of a fire are examined: high temperature, smoke, toxic burning products of and lack of oxygen. Studying combined and complex actions of these factors is carried out usually from positions of the fatal poisonings risk assessment. This direction is actual for Ukraine where daily perishes at fires six to eight persons. The analysis of the death causes of people death has shown that in 70% of cases the death is caused by toxic burning products. It is connected first of all to wide application in construction, on transport, in municipal sphere polymeric and synthetic materials. Our researches have shown that at burning not only carbon oxide (II), but also not less than in 50% of polymeric materials subjected to tests, the death of animals came from the combined action of several chemical substances. So, for polyurea polymers the contribution to fatal effect CO makes 44–68%, and the other part falls at a share of cyanic hydrogen, oxides of nitrogen, chloride hydrogen both others vapors and gases. At rubbers among minor components sulphurous anhydride, styrene, cyanic and chloride hydrogen prevail. It is important to emphasize, that a number of toxic organic components which contribute to fatal effect, are not determined owing to biotransformation, sorption and other reasons. The problem is not limited to cases of fatal poisonings at fires. On each victim by results of the carried out researches it is necessary till 20–30 victims with attributes not a fatal poisoning. The contribution of psycho-emotional stress to clinical semiology and consequences of defeat of people at fires is not taken into account. Authors develop the new concept of toxicology of burning at fires, which is based on a combination of experimental researches on animals with clinical and psychophysiological supervision among victims and firemen. Results of researches and experience of carrying out of medical and psychological rehabilitation have confirmed efficiency of spent actions, which allow to lower number of sanitary losses at fires.

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P2-74

Poisonings with mercury—A lasting problem

D.V. Bolshoy, E.G. Pykhteeva, L.M. Shafran, G.F. Burlak

Ukrainian Scientific and Research Institute of Medicine on Transport, Odessa, Ukraine

The relation to the danger estimation and risk assessment of mercury (Hg) poisonings is ambiguously. Hygienists and toxicologists, working on the problem, the International organizations determining a policy in the field of occupational health and safety of the environment insists on the maximal reduction of Hg use and it replacement, where it is technologically possible, with less toxic metals or other connections. On the other hand, application of Hg is economic, for example, the mercury medical thermometer is approximately in six times cheaper then electronic one. Use of Hg at observance of security measures can give the big economic benefit, therefore requirements of ecologists and toxicologists encounter resistance of businessmen and industry. Due to the measures undertaken the WHO, sharp poisonings with Hg became the big rarity. However, for last 5 years we had diagnosed 28 poisonings with Hg. These poisonings had occupation related character in 23 cases and five persons have poisoned in a municipal conditions. During 2004, we have surveyed 114 workers, who worked with heavy metals. In 29 of cases content of Hg in urine was above 10 μg/l (WHO recommended standard), including three cases of excess of the content of Hg in urine higher than 25 μg/l (WHO recommended minimal toxic level). Probably, these people were exposed with Hg in the municipal or environmental conditions. In January, 2006 in our laboratory were examined 14 crew members of ship "Carmen" for the determination of Hg content in biosubstrata. According to the received information on the vessel faulty Hg devices were used and floods of this metal in ship working premises took place. In seven surveyed seamen, the levels of mercury in blood were found above 25 μg/l and in three seamen, higher then 50 μg/l. The maximal found Hg concentration in blood was 124.1 μg/l. The contents of Hg in urine in six sea-

men exceeded 25 μg/l. At repeated research in 14 days the contents of Hg in urine at all surveyed seamen was within the limits of the hygienic permitted level. But in the urine of four surveyed seamen, the content of Hg continued to remain high enough. The carried out analysis of radical hair area in 2 months after the first reference has shown excess of the content of Hg in seven persons. In blood and in the urine content of Hg was within the hygienic permitted limits. In nine surveyed seamen proteinuria it was observed. Also the increased content of lipid peroxidation products was found in urine, that is, a marker of oxidative stress and the worsening of kidneys functions.

Conclusion: The analysis of blood is informative only at the first 48 h after receipt of mercury. Much longer (till 2 weeks) keeps the analysis informatively the urine. Later on it is possible to diagnose a poisoning by means of hair analyses, selecting corresponding area in view of speed of their growth. It is necessary also to pay attention to such biomarkers of kidneys function as proteinuria, enzymuria, level of the lipid peroxidation products increasing in urine, etc.

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P3 Immunotoxicology

P3-01
Evaluation of the allergenic potential of iodinated radio contrast media using animal models

Hiroyuki Hattori, Megumi Tamanaka, Michiyuki Kato, Kazuhisa Furuhama

Daiichi pharmaceutical Co., Ltd., Japan

The incidence of late adverse reactions, such as maculopapular rash, urticaria, angioedema and fever, to iodinated radio contrast media (RCM) has been reported to be 2–5%. There has been recently increasing attention to the mechanism of the adverse reactions. For a few case reports, a hypersensitivity mechanism is postulated, but the allergenic potential of RCMs has never been shown in animal models. Therefore, the present study was conducted to detect the potential of RCMs using guinea pigs and mice.

The following six RCMs were used: two non-ionic monomers, iohexol (IH) and iopamidol (IP); two non-ionic dimmer, iotrolan (IT); two ionic monomers, amidotrizoate (AT) and iothalamate (IM); one ionic dimmer, ioxaglate (IX). The allergenic potential of RCMs was evaluated by maximization test and delayed type skin test in female Hartley guinea pigs as models for detecting Type IV allergic reaction. Furthermore, popliteal lymph node assay (PLNA), which has been reported to be a useful model, was carried out in mice. In the maximization test, all RCMs induced positive reaction with high incidences of 50%, 80%, 40%, 70% and 80% for IM, IX, IH, IP and IT, respectively. Positive delayed type skin reactions with infiltration of mononuclear cells in the superficial dermis were observed in animals sensitized and challenged with IP (43%), IT (20%), IM (7%) and AT (10%). Negative reactions were noted in animals sensitized with vehicle and challenged with RCM in both the maximization and delayed type skin tests. As for PLNA, increases in lymph node weights and cell numbers were observed in mice injected with IH and IT.

These results clearly showed that RCMs possess the potential to induce allergic reactions, especially of Type IV, and raise the possibility that these three animal models may be useful for screening of new RCMs having weaker allergenic potential than the aforementioned RCMs.

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P3-02
Methanol intoxication on splenic lymphocyte subsets, serum cytokines, Hsp70, c-fos, iNOS and Fas/FasL expressions in HPA axis organs of experimental rats

Narayanaperumal Jeya Parthasarathy, Ramasundaram Srikumar, Sundaramahalingam Manikandan, Govindarajulu Sathya Narayanan, Rathinasamy Sheela Devi

Immunology laboratory, Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600113, India

It is well known that the nervous system has increased susceptibility to methanol intoxication. Our previous studies reveal that the methanol intoxication affects the non-specific and specific immune systems. The present study reveals the effect of methanol intoxication on splenic lymphocyte subsets (B and T-Lymphocytes, CD4, CD8), MHC class II expressed cells and macrophages and serum cytokines levels (TNF-α, IFN-γ, IL-2 and IL-4). In addition, mRNA expression of Hsp70, c-fos, iNOS (RT-PCR) and Fas/FasL protein expression (immunoblotting and immunohistochemistry) in HPA axis organs (hypothalamus, adrenal) and spleen. Male Wistar albino rats were intoxicated with methanol (2.37 g/kg b.wt., i.p.) for 30 days and immunized with SRBC (5×10^9 cells). Administration of methanol showed significant decreases in the Pan T cells, CD4, macrophage counts, serum TNF-α, IFN-γ and IL-

2 on the other hand, the significant increase in the MHC class II molecule expressed cells, B cells and serum IL-4 of the methanol intoxicated group animals was compared with the corresponding control animals; there was no significant change in CD8 cells compared with respective controls. Furthermore, the increased mRNA expression of Hsp70, c-fos iNOS and Fas/FasL in hypothalamus, adrenal and spleen upon 30 days methanol intoxicated groups compared with control animals. From this study, it can be concluded that repeated exposure to methanol could alter the immune system through HPA axis.

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P3-03
Immunotoxicity screening of AMPA antagonists

Gyula Szűcs, Mihály Albert, Éva Rónai, Dezső Dányi

EGIS PLC, Budapest, Hungary

Drug therapy might have suppressant effects on immune system in laboratory animals and also in patients. Evaluation of potential adverse effects of human pharmaceuticals on the immune system should be incorporated into standard drug development. For lead optimization, a rapid and easy in vivo screening method was developed in female rats for the detection of drug-caused lymphoid atrophy at our laboratory. Female rats were used in these studies because females proved to be more sensitive than males in pilot studies. Seven-day repeated dose, oral comparative studies were performed with 2,3-benzodiazepine type AMPA antagonist drug candidates to test their possible toxic effect on the immune system. Dexamethasone, a well-known agent causing lymphoid atrophy served as positive control to prove model sensitivity. An oral dose of 30 mg/kg/day was applied both in case of test substances and the positive control. The following parameters were evaluated in the 1-week toxicity studies for signs of immunotoxicity: haematology (total leukocyte counts and absolute differential leukocyte counts), serum chemistry (globulin levels and A/G ratios), gross pathology (lymphoid organs/tissues), organ weights (spleen, thymus, popliteal lymph nodes and adrenal glands) and histology (spleen, thymus, popliteal lymph nodes, adrenal glands and mesenteric lymph nodes).

Three 2,3-benzodiapine type examples, namely EGIS-11372, -11639 and -11788 were selected to demonstrate the characteristics of the responses.

Based on our results, it can be stated that thymus and spleen weights and histology proved to be the most sensitive indicators of lymphoid organ toxicity for this type of compounds.

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P3-04
Modulatory effect of *Trigonella foenum-graecum* L. extract on deltamethrin-induced low dose immunosuppression in mice

Hasibur Rehman, Rizwan A. Ansari, Sheikh Raisuddin

Jamia Hamdard (Hamdard University), New Delhi, India

Trigonella foenum-graecum L. (fenugreek) has been shown to possess several medicinal properties. It is widely reported as a demulcent, tonic, laxative, expectorant, anti-inflammatory, nutritive, blood purifier and aphrodisiac agent. In clinical studies, it has shown hypoglycemic and anti-diabetic properties. Immunomodulatory effect of fenugreek extract has also been demonstrated in mice. We investigated modulatory effect of *T. foenum-graecum* seed extract on the immunotoxic effects of deltamethrin in mice. Swiss albino male mice were treated per os with the aqueous extract (100 mg/kg, b.wt. daily for 15 days). Deltamethrin was administered orally in a single dose of 18 mg/kg b.wt. in corn oil. Body weight, relative organ weight, lymphoid organ cellularity, hemagglutination titre (HT), plaque forming cell (PFC) assay and quantitative hemolysis of SRBC (QHS) assay were studied in the treated animals. Deltamethrin showed significant suppressive effect on lymphoid organ weight and cellularity and humoral immune functions. Plant extract itself produced no immunotoxicity at the above dose whereas it resulted in restoration of humoral responses in deltamethrin-treated animals as shown by a significant ($p < 0.01$) increase in PFC response as well as QHS in deltamethrin-treated animals. The results suggest that exposure to deltamethrin causes immunosuppression in mice and fenugreek extract has modulatory effects on these parameters. The antioxidant property of fenugreek seeds might be contributing to modulatory action resulting in its protective effect in immunosuppresed mice.

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P3-05

Cytokine genes in susceptibility to coal workers' pneumoconiosis

Asuman Karakaya[1], Ilker Ates[1], Berran Yucesoy[2], Sinan H. Suzen[1]

[1] *Ankara University, Faculty of Pharmacy, Tandogan, Ankara Turkey;* [2] *Toxicology and Molecular Biology Branch, National Institute for Occupational Safety and Health, WV, USA*

Coal workers' pneumoconiosis (CWP) and silicosis are the most widespread fibrotic lung diseases whose etiopathogenesis are not well defined. Pro-inflammatory cytokines play a pivotal role in the onset, progression and termination of the reactions such as inflammation, fibroblast proliferation and extracellular matrix (ECM) synthesis in CWP and silicosis. Recent studies suggest that these diseases may share genetic risk factors. In this study, the frequency of single nucleotide polymorphisms (SNPs) in the genes coding for TNF-α, IL-1α, IL-1β and IL-6 and their genotype associations with CWP and disease severity were determined in a 56 Turkish coal workers with CWP (34 pneumoconiotic, 22 fibrotic) and 99 healthy controls without any apparent inflammation or other pulmonary disease. According to the genotyping results, regardless of disease severity, TNF-α (−238) and IL-1β (+3953) polymorphisms affected CWP occurrence (OR's 1.86 and 1.52, respectively). On the other hand, both TNF-α (−238) and (−308) variants were associated with an increased risk of developing PMF (OR's 3.44 and 1.47, respectively). IL-1β (+3953) variant was also associated with the development of simple CWP (OR: 1.90). No significant association was found between variations in IL-6 and IL-1α genes and the occurrence and progression of CWP and PMF.

In conclusion, this study suggests that TNF-α and IL-1β polymorphisms may be considered as susceptibility factors for CWP.

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P3-06

Bone marrow differentials—Comparison of flow cytometry and manual counting in rats treated with compounds inducing hematological changes

Andre Forichon, Fabienne Condevaux, Marie-Francoise Perron-Lepage, Jacques Descotes

Poison Center and Pharmacovigilance Unit, France

Bone marrow analysis is recommended to screen for hematotoxic as well as immunotoxic pharmaceuticals. Because manual counting is time-consuming and lack sensitivity, a flow cytometry method has been developed to investigate bone marrow cell-specific populations in rats. A series of monoclonal antibodies (Pharmingen, Becton-Dickinson) was selected for immunostaining, which allowed accurate identification of the lymphocyte, erythroid and myeloid populations based on the differential expression of the cell surface antigens CD71, CD3, CD45, CD45RA and CD45R. Bone marrow samples were taken from groups of eight 8-week-old male Sprague–Dawley rats treated with the reference compounds, erythropoietin (EPO), dexamethasone, doxorubicin or LPS, and examined for lineage-specific changes in cell populations. Samples from each rat were evaluated by flow cytometry (FACScan, Becton Dickinson) as well as manual counting from bone marrow smear examination. Expected changes, i.e. lymphopenia (dexamethasone), myelosuppression (doxorubicin), leucocytosis (LPS) or increased immature erythroid population (EPO) were clearly identified by flow cytometry. Bone marrow differentials obtained by flow cytometry were similar to those obtained by manual counting. Cell separation was confirmed by cell sorting (FACS Vantage, Becton Dickinson). Thus, flow cytometry appears to be a sensitive and cost-effective alternative to manual counting for the initial screening of toxic effects on the bone marrow in preclinical safety studies. In addition, this method provides an evaluation of bone marrow cellularity and absolute values for each sub-population.

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P3-07

Effect of phenol and hydroquinone associated exposure on leukocyte migration into allergic inflamed lung

Alexandre Ferreira, Sandra Manoela Dias Macedo, Suellen Cristine Moreira Vaz, Wothan Tavares Lima, Sandra Helena Poliselli Farsky

University of São Paulo, Sao Paulo, Brazil

Benzene is metabolized by the liver generating phenol (PHE) and hydroquinone (HQ), which is substrate to myeloperoxidase in the bone marrow resulting in 1,4 *p*-benzoquinone. These metabolites are linked to immunotoxic effects, however, their effects on leukocyte mobilizations during inflammatory processes are not fully understood. We previously showed that in vivo HQ exposure for an extended period of time (50 mg/kg, i.p., 16 daily doses, with 2-day intervals every 5 doses) impairs the leukocyte migration into lung during an allergic response in rats. Now, we investigated the effect of lower doses of HQ, PHE or association of both on leukocyte mobilization into inflamed lungs. Adult, male Wistar rats were sensitized with OA (10 mg; aluminum hydroxide solution, i.p. route) at the 10th day after beginning phenolic compounds (5 mg/kg, i.p., 16 daily doses, with 2-day intervals every 5 doses) or vehicle (ethanol:saline solution 1:20) exposures. Twenty-four hours after last doses, animals were challenged (1% PBS solution; 15 min inhalation). Broncheoalveolar lavage (BAL) was collected 24 h after challenge. HQ or PHE exposure induced impairment on number of polymorphonuclear (PMN, 50%) cells into BAL. The reduced cell migration was not modified by HQ and PHE associated exposure. These data suggest that in vivo lower dose of both phenolic compounds impairs the PMN recruitment to inflamed lung and PHE/HQ association does not promote synergic effect.

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P3-08

The skin allergenic properties of chemicals may depend on contaminants
Evidence from studies on coumarin

Francois E. Floch

ITEConsult, Lyon, France

Background/aims: Positive patch tests are considered as representative of a contact allergy to the tested chemical. However, contaminants and derivatives rather than the suspected chemical itself could be responsible for the skin allergic reactions. Here, we tested the importance of contaminants in the sensitizing and allergenic properties of coumarin in mice and humans. Coumarin, an ingredient of cosmetics and fragrances, was chosen as the reference chemical since conflicting results have been obtained regarding its ability to induce contact allergy. This could be explained by the presence, in some chemical preparations, of coumarin derivatives endowed with allergenic properties.

Methods: In mice, three different coumarin preparations were tested in the local lymph node assay. In humans, we assessed the irritant and allergenic properties of highly pure coumarin in non allergic and fragrance allergic patients.

Results: Pure coumarin did not exhibit irritant or sensitizing properties in the LLNA. In contrast, two other commercially available coumarins and three contaminants that were detected in these coumarin preparations were identified as weak and moderate sensitizers, respectively. In humans, pure coumarin was extremely well tolerated since only 1 out of 512 patients exhibited positive patch test to the chemical.

Conclusions: These results indicate that coumarin cannot be considered as a common contact allergen and further emphasize that purity of chemicals is mandatory for assessment of their allergenicity.

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P3-09

The KLH-assay as alternative to the PFC-assay: A comparative study with cyclophosphamide

André Hendrikus Penninks, Jolanda H.M. van Bilsen, Mary-lene M.H. Brouwer, Karin - Junker, Frieke C. Kuper

TNO Quality of Life, Zeist, Utrecht, The Netherlands

To date the T cell-dependent antibody response (TDAR) is regarded to be the most predictive assay for immunotoxicity testing. The IgM-plaque forming cell (PFC) assay using sheep red blood cells (SRBC) as antigen is a widely accepted and validated standard test. However, this assay has a number of drawbacks. Therefore, alternative assays like the KLH-assay using Keyhole Limpet Hemocyanin (KLH) as antigen are being developed and validated. In an Immunotoxicity Inter-Laboratory Project (IILP), a common design for a KLH-assay was established using well known immunosuppressive compounds. In this study we assessed for cyclophosphamide (CY) the robustness and sensitivity of the KLH-assay

and compared it with the PFC-assay. For both the KLH- and PFC study, Wistar rats were exposed to 1, 3 and 10 mg cyclophosphamide/kg bw by gavage for, respectively, 33 and 28 days. Primary immunization with KLH (300 μg i.v.) was performed on day 14 and the secondary immunization was performed on day 28. The IgM and IgG antibody responses were determined in blood collected at days 19, 28 and 33. Next to these endpoints, the effects on body weight, hematology, lymphoid organ weight and pathology will be presented. To assess if KLH immunization will affect routine histopathology of the lymphoid organs an additional top dose group was included that was not immunized with KLH. On day 19, the anti KLH-IgM titers were dose-dependently decreased, which were significant at the two top doses of CY. The primary anti KLH-IgG titers at day 28 were still low and only significantly reduced at the top dose of CY. At day 33, the secondary anti KLH-IgM titers was again dose-relatedly reduced and was significant at all dose levels. The secondary anti KLH-IgG titers was significantly decreased at the two top doses of CY. In the PFC-assay, the rats were immunized with SRBC on day 24 and the number of IgM-PFC in the spleen were assessed on day 28. In the PFC-assay, the number of IgM-PFC in the spleen was dose-relatedly decreased which was significant at the two top doses of CY. Based on the obtained results it can be concluded that upon exposure to CY the KLH- and PFC-assay were equally sensitive, and that KLH immunization did not affect the histopathology of the lymphoid organs.

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P3-10

Effects of hydroquinone exposure on allergic lung inflammation in rats

Sandra Manoela Dias Macedo, Suellen C.M. Vaz, Emerson Luis bothelho Lourenço, Ana Paula Ligeiro de Oliveira, Gabriela Cavriani, Wothan Tavares de Lima, Sandra Helena Poliselli Farsky

University of São Paulo, Sao Paulo, Brazil

We investigated the effects of hydroquinone (HQ) exposure on leukocyte migration during a lung allergic inflammation. Male Wistar rats were intraperitoneally exposed to vehicle or HQ (50 mg/kg; once a day, 22 days with a 2-day interval every 5 days). OA-sensitization (10 μg suspended in 10 mg $Al(OH)_3$; i.p. injection) was carried out on day 10 and OA-challenge (1% OA-PBS solution) on day 23 after beginning of exposures. Numbers of polymorphonuclear (PMN) and mononuclear (MN) leukocytes in the bronchoalveolar fluid and number of PMN in the pulmonary tissue 24 h after challenge were reduced in HQ-exposed rats. The inability of leukocyte migration into inflamed tissue was not influenced by changes in the number of circulating leukocytes or in the expression of adhesion molecules on circulating leukocytes or pulmonary endothelium. Nevertheless, the effect may be dependent on impaired OA-IgE mediated mast cell degranulation. This hypothesis was based on: (1) reduced circulating OA-IgE levels were detected in HQ-exposed rats, as shown by higher concentration of serum from HQ-exposed rats to mount passive anaphylaxis cutaneous reaction; (2) diminished mast cell degranulation, since tracheal contraction ability evoked by in vitro administration of OA was lower in the tissue from HQ-exposed rats. Together, our data show that in vivo HQ prolonged exposure impairs the development of allergic inflammation in a mechanism depending, at least in part, of impaired OA-IgE mediated mast cell degranulation.

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P3-11

Modulation of the bioavailability and adverse effect of benzo[*a*]pyrene (BaP) in mice actively immunized with BaP-carrier conjugates

Nathalie Grova, Sophie Farinelle, Emmanuel Prodhomme, Claude P. Muller

Institute of Immunology, Laboratoire National de Santé, Luxembourg, Grand Duchy of Luxembourg

The influence of carcinogen-specific antibodies on the bioavailability and biological activity of BaP was investigated using polyclonal antibodies. Mice were actively immunized with BaP-derivatives conjugated to protein carriers (such as diphtheria toxoid). The effectiveness of this strategy was initially evaluated by ^{3}H-BaP measures after an intra-peritoneal administration (0.1 μg/kg) in pre-immunized female Balb/c mice. After 24 h, the level of radioactivity associated to BaP and its metabolites in the blood and liver increased by 20.5 and 2.5 times, respectively, compared to the control group ($p < 0.01$). This preliminary study showed that polyclonal antibodies modified the bioavailability of BaP within the organism and may interrupt the metabolic activation pathways at both levels of the pro-carcinogen BaP and its activated metabolites. These results were further confirmed by evaluating the pharmacokinetics of polyclonal antibodies after chronic administration of BaP in mice. Quan-

tification of specific anti-BaP antibodies (direct Elisa) revealed that an excess administration of BaP (150 times, 20 mg/kg) lead to a decrease of 26% of the level of polyclonal antibodies in the blood at day 3 to 42% at day 29 ($p < 0.001$). Similarly, equimolar administration of BaP (0.2 mg/kg) showed a decrease of 20% ($p < 0.05$) during the first week followed by a similar level of specific polyclonal antibodies in treated and untreated groups up to day 29, suggesting that the major part of BaP (under native or the metabolised forms) should be bound to specific antibodies. The complex probably stays in the blood and seems to be degraded in the same way than unbound antibodies. Analysis of BaP and its metabolites in sera by HPLC are currently underway to confirm these hypotheses. The biological relevance of BaP redistribution by specific antibodies was demonstrated by the reversal of BaP-induced inhibition of proliferation peripheral blood lymphocytes of these mice. All these results confirm the ability of this immunoprophylactic strategy to decrease the part of BaP available in the blood, mediating its redistribution within organism.

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P3-12
The research of antidiabetic drugs immunotoxic potential

Valentina Kozar, Maria Kudrya, Irina Anatoliyevna Palagina

Institute of Endocrine Pathology Problems, Kharkov, Ukraine

The immune system effectiveness is determined by its components balance, which are specific and non-specific defence factors, which is crucial to take into consideration while evaluating possible immunotoxic drugs effect.

The antidiabetic drugs (ADD) of different pharmacological groups: glyklaside (sulpha urea derivation), original phensuccinal compound (siccine acid derivation) and diacamph (camphor acid derivation) were studied.

The character and the changes orientation of immunologic resistance indexes with the peroral ADD administration to rats in therapeutic and toxic doses were studied. The duration of the experiment is 30 days.

The effectiveness of natural killer cells (NKC), the effectiveness of phagocytic and metabolic neutrophils, the cytokine level of TNF-α and IL-4 were studied. They found out that phensuccinal and diacamph decreased the effectiveness of NKC in two to three times as in therapeutic as in toxic doses. All ADD in toxic doses and phensuccinal and diacamph even in therapeutic doses increased the effectiveness of phagocytic and metabolic neutrophils ($p < 0.05$). In the studied doses, phensuccinal decreased the TNF-α content ($p < 0.05$). Glyklaside increased the level of TNF-α only in therapeutic doses ($p < 0.05$). All ADD in toxic doses and glyklaside in therapeutic doses increased IL-4 content ($p < 0.05$). Phensuccinal decreased IL-4 content ($p < 0.05$) in therapeutic doses.

Thus, glyklaside produces the unilateral effectiveness on IL-4 content, on phagocytic and metabolic neutrophils, phensuccinal produces the unilateral effectiveness on the effectiveness of NKC, the level of TNF-α, diacamph produces the unilateral effectiveness on the effectiveness of NKC and on phagocytic and metabolic neutrophils effectiveness independently of doses.

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P3-13
Chemical respiratory allergen-induced gene expression profiles following topical exposure of mice

Rebecca Jane Dearman, Catherine J. Betts, Fei Ling Lim, Richard Currie, Ian Kimber

Syngenta CTL Macclesfield, UK

Prolonged (13 day) topical exposure of BALB/c strain mice to the chemical respiratory allergen trimellitic anhydride (TMA) results in expression of a polarized T helper 2 cytokine phenotype consistent with the development of IgE-mediated immune responses. The kinetics of early (24–120 h) changes in gene expression induced by TMA have been assessed using oligonucleotide microarrays. Mice received a single topical exposure of 10% TMA, or of vehicle alone, on the dorsum of each ear and auricular lymph node tissue was isolated 24 to 120 h later. Further control mice were left untreated (naïve). Marked increases in lymph node activation (cellularity and proliferation) were recorded at 48 h, reaching maximal levels at 72–96 h. Lymph nodes were pooled per treatment group, total RNA was prepared and cRNA was synthesized, biotinylated and fragmented. Fragmented cRNA was hybridized to Mouse Genome 430 2.0 GeneChips® (Affymetrix; 45,101 probe sets) and analyzed using GeneChip Scanner 3000 and GeneChip Operating Software. Data were normalized for intensity and genes showing two-fold or greater changes compared with the median expression value for that gene across all samples identified. Although 15,513 probe sets were detected in lymph node tissue, only 86 probe sets (representing 83 unique genes)

displayed statistically significant ($p < 0.05$) allergen-specific changes in gene expression. Of these genes, 50% were up-regulated 24–48 h after treatment, 36% were up-regulated at 72 h and 13% were down-regulated 72–120 h post-exposure. In general, early (24–48 h) up-regulated genes were associated with immune or inflammatory responses; including immunoglobulin heavy chain gene IGH-4, certain chemokine ligands and Granzyme B (which exhibited the greatest fold increase of 8.4-fold). These changes were followed at 72 h by up-regulation of cell cycle genes, such as cyclins B1 and B2. Up-regulated genes returned to control levels within 120 h, or in some cases gene expression in allergen-activated tissue was markedly lower than control levels at these later time points, suggestive of the existence of feedback control mechanisms. The utility of selected genes as markers for the identification of chemical respiratory allergens is being explored further.

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P3-14
Stereological study of the morphine on the lymph node

Farzaneh Dehghtani, Zahra Vojdani, Ali Noorafshan, Fatemeh Bahaedini, Fatemeh sSeredi

Anatomy Department, Shiraz Medical School, Shiraz, Iran

Morphine is a narcotic analgesic drug that can suppress immune system. Quantitative study of the lymph node implies this matter. Therefore, the objectives of this study is the stereological study of the morphine on the lymph node. Nineteen male mice (25–30 g) were divided into two groups. Two bottles of water for the control group and two bottles of water and 0.5% morphine solution were put for the experimental groups. On day 71, after physical dependent test, the animals were dissected and lymph node were removed. After histological processing, the sections in 0.1 length distance with thickness of 5 μm were removed and stained with haematoxylin–eosin. Total volume of the lymph node and volume density was estimated by cavalieri and point—counting methods. Then the data were analyzed by Mann–Whitney test.

The results showed that chronic morphine administration could decrease the total lymph node volume and its cortex as compared to the controls. Moreover, the absolute number of the circulating lymphocytes decreased in the experimental group.

It seems that chronic morphine treatment can decrease lymph node volume due to decreasing of volume of its cortex.

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P3-15
Toxic effect of cyclosporine A on the volume of thymus in mice (a stereological study)

M.R. Panjehshahin, S. Bahmanpoor, F. Dehghani

Pharmacology and Anatomy Department, Shiraz Medical School, Shiraz, Iran

Cyclosporine A is one of the immunosuppressant drugs, which can decrease the number of T-lymphocytes. Thymus is a part of lymphatic system, so it seems that cyclosporine may reduce the volume of this organ. Therefore, the aim of this study is to measure volume of thymus by stereological method. For this purpose, 32 balb/C mice were randomly divided into three experimental and one control groups. Cyclosporine A was orally administered to experimental groups in doses of 10, 30 and 50 mg/kg for 7 days. Control group was given 1 ml of saline. Then animals were sacrificed under deep anesthesia and their thymus were removed. Five micrometer serial sections were prepared and stained with H and E. Volume of thymus was determined by using Cavalieri Principle and point counting method. Results showed that the number of T-cells decreased but there was no difference in the volume of thymus between experimental and control groups. Further studies should be performed to clarify the issue.

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P3-16
The respiratory local lymph node assay (LLNA) as a tool to study respiratory sensitizers

Josje Arts, Wim de Jong, Marcel Schijf, Arja de Klerk, Henk van Loveren, Ruud A. Woutersen, Frieke Kuper

TNO Quality of Life Zeist, Utrecht, The Netherlands

The LLNA is used to test the potential of low molecular weight (LMW) compounds to induce sensitization via the skin. Stimulation rates of three-fold the control values in relation to the dose (EC3 values) have been suggested to establish and compare potency. All LMW respiratory allergens known to date have also tested positive in this dermal assay, but it is unknown if potential and potency via the dermal route are comparable to those via the inhalation route. In the present study, male BALB/c mice were exposed nose-only to 30 mg/m^3 aerosols of

the respiratory allergen trimellitic anhydride (TMA) or the skin allergen dinitrochlorobenzene (DNCB) during 3 days for 45, 90, 180 or 300 min/day. The dermal route (ear application) was used as a positive control. Negative controls were exposed by ear application of the vehicle (acetone–olive oil 4:1) and by inhalation to the vehicle (acetone) for 300 min/day for 3 days. The animals were necropsied 3 days after the last exposure, the local lymph nodes were excised and harvested cells were processed with ^{3}H-thymidine to determine proliferation. In the inhalation groups, lymph nodes draining various parts of the respiratory tract, including nasal passages and nasopharynx, larynx/trachea and trachea/bronchi/bronchioli, were examined because the impact of compounds in the respiratory tract and (thus) their exact draining pattern are often not fully known. The auricular lymph nodes were sampled in the positive control group. Dosage (concentration × time)-related increases in thymidine incorporation were observed in the lymph nodes draining the nasopharyngeal region for both TMA and DNCB resulting in lymph node stimulation rates up to more than 10-fold above vehicle-treated controls. Testing of other compounds is underway. The experimental results with DNCB and TMA indicate that the potent contact allergen DNCB is at least of comparable potency as TMA in the respiratory LLNA. In analogy to the dermal LLNA, this result suggests that strong contact allergens such as DNCB can *also* act as potent sensitizers by inhalation, provided that such compounds are inhaled.

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P3-17

Stereological study of the morphine on the spleen

Zahra Vojdani, Farzaneh Dehghani, Ali Noorafshan, Fatemeh Seyedi, Faegheh Bahaedini

Shiraz University of Medical Sciences, Iran

Introduction: Morphine as a narcotic analgesic drug in long-term administration can suppress immune system including spleen. Quantitative studies can be an appropriate indicator of assessment of organ disturbances. Therefore, in this research, chronic morphine treatment on the volume of spleen were studied by the stereological methods.

Materials and methods: Nineteen male mice (25–30 g) were divided into two groups. Two bottles of water for the control group and two bottles of water and 0.5% morphine solution were put for the experimental group. On day 71, after physical dependent test, the animals were deeply anesthetized, dissected and Spleen were removed. After histological preparation, the sections in T distance with thickness of 5 μm were removed and stained with haematoxylin–eosin. Total volume of the spleen and volume density was estimated by Cavalieri and point-counting methods. Then the data were analyzed by Mann–Whitney test.

Results: The results showed that chronic morphine administration led to a decrease in the total spleen volume and mean white and red pulp volume. In addition, white blood cell count were decreased in the experimental group.

Conclusions: It seems that morphine can reduce spleen size by decreasing white pulp volume.

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P3-18

In vitro effects of methadone on lymphocyte proliferation following a short-term treatment with methotrexate-loaded liposomes in a murine model of arthritis

Maria Barca [1], Anne Marie Ciobanu [1], Dan Balalau [1], Daniela Luiza Baconi [1], Mihaela Ilie [1], Monica Neagu [2], Gina Manda [2]

[1] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Bucharest, Romania;* [2] *"Victor Babes" National Institute, Bucharest, Romania*

Due to the complex pathogenesis, the treatment of the rheumatoid arthritis is a continuous challenge. Recently, opioid use in the treatment of the severe non-malignant pain, especially in rheumatoid arthritis, has been approved. The opioid agents not only control the pain, but they can also modulate the immune response; thus, the opioids exert their effects indirect (via hypothalamus–hypophysis–adrenal axis) and direct, interacting with opioid receptor on the immune cells.

We investigated in vitro the effects exerted by methadone 20 ng/mL on the proliferative capacity of the splenic mononuclear cells, isolated from the rats treated with methotrexate in a murine model of arthritis. In a rat model of arthritis, three different doses of methotrexate injection solution or liposomes loaded with hydro-soluble methotrexate (sodium salt) and hydrophobic methotrexate (methotrexate itself) have been administered weekly for 3 weeks.

For in vitro experiments investigating the effects of methadone, the splenic lymphocytes have been activated

with Con A and the proliferation has been evaluated by the uridine incorporation method.

The results show that the immunosuppressive effect of methadone on the splenic lymphocyte activation/proliferation is obvious in the animals treated with low doses of hydro-soluble methotrexate (injectable solution or loaded in liposomes). This effect is reversible, probable related to the treatment or to the evolution of the immune state between two methotrexate doses. Methadone exerts persistent inhibitory effect at high doses of methotrexate injectable solution. At the same time, methadone can induce the activation of the splenic lymphocytes, isolated from the animals treated with hydrophobic methotrexate loaded in lyposomes.

The results of the study suggest that high doses of hydro-soluble and hydrophobic methotrexate can modulate in both ways the cells reactivity to methadone.

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P4 Necrosis and cytotoxicity

P4-01
Effects of CO_2 *Viscum album* L. leaves extract on *in vivo* oxidative stress of Ehrlich tumour cells

T. Cebovic [1], M. Popovic [2], S. Spasic [3], G. Leposavic [3]

[1] *School of Medicine, Biochemistry Department, Hajduk Veljkova 1-3, 21000 Novi Sad, Serbia and Montenegro;* [2] *Faculty of Sciences, Chemistry Department, Trg D, Obradovica 5, 21000 Novi Sad, Serbia and Montenegro;* [3] *School of Pharmacy, Bulevar Vojvode Stepe 345, 11000 Beograd, Serbia and Montenegro*

European mistletoe or *Viscum album* L. extracts have been known as secondary medicaments, widely used in therapy of hypertension. Also, they have been reported to exert cytotoxic and immunomodulatory effects *in vitro* and *in vivo*. The mechanism of anti-tumoral activity is, however, largely unknown. As most of the studies on mistletoe concern its polar extracts, rich in lectins and viscotoxins, it was decided to focus on the nonpolar constituents of family Viscaceae. In this study, we tested the hypothesis that CO_2 extract from the European mistletoe, exhibit mentioned properties due to induction of oxidative stress in Ehrlich tumour cells *in vivo*. CO_2 extract of *V. album* L. was given to experimental animals in three different ways—as a pre-treatment, treatment and post-treatment. We have observed significant reduction of cancer incidence in all groups that received mistletoe extract in comparison to Ehrlich control. Number of tumour cells was decreased up to almost 50% in male animals that received mistletoe extract before implantation, and up to 35% in female animals. Reduced number of EAC cells was also obtained in animals with developed carcinoma. Levels of antioxidative enzymes in EAC cells did not confirm the presence of oxidative stress, as well as intensity of lipid peroxidation. In contrast, oxidative stress of EAC was increased in a rather high degree after administration of *Viscum* extract. This was in accordance with the increase of damaged cells percentage. These results indicate that oxidative stress, defined by the levels of antioxidative enzymes, might play an important role in *in vivo* cytotoxic properties of misltletoe grown on plums extracts. In addition, applied mistletoe extract might also possess beneficial effects on restoration of impaired oxidative balance in normal tissues. Our data suggest that examined mistletoe extract may be potentially useful in the prevention of the tumour development, but emphasise the need of further elucidation of mechanisms of its action.

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P4-02
The effect of schisandrin B on mercuric chloride-induced nephrotoxicity in rat

Alessandra Stacchiotti, Elisa Borsani, Francesca Ricci, Eleonora Foglio, Rita Rezzani, Luigi F. Rodella, Rossella Bianchi

University of Brescia, Brescia, Italy

Schisandrin B (SCH.B) is a dibenzocyclooctadiene derivative isolated from the fruit of *Schisandra chinensis*, a traditional Chinese herb (kindly gift by Prof. Ko, Hong Kong University, China) successfully used to treat viral and toxic hepatitis and ischemic injury. Its ability to protect against free radical-mediated damage has been related to the enhancement of mitochondrial glutathione system and to the induction of stress proteins. Mercuric chloride ($HgCl_2$) is a nephrotoxic agent that affects the straight portion of proximal tubules and induces stress proteins.

The purpose of this study was to find out the beneficial effect of SCH.B pretreatment in rat treated by a single acute nephrotoxic dose of $HgCl_2$. In particular, we focused here on the renal distribution of two different stress proteins, HSP72 and HSP25, after $HgCl_2$ alone respect to mercury plus SCH.B treatments.

Four groups of four male Wistar rats (200–250 g) were used according to the following procedure: Group 1: saline i.p. injected rat as controls; Group 2: $HgCl_2$ (1 mg/kg) i.p. once at day 9; Group 3: SCH.B p.o. 5mg/kg daily for 9 days; Group 4: SCH.B + $HgCl_2$ as above, all rats were sacrificed at day 10. Saline and SCH.B

groups showed normal renal histopathology and almost similar faint stress proteins pattern. In contrast, after mercury HSP25, the major actin-chaperone and HSP72, an inducible cytoprotective stress protein, were moderate in cortical affected tubules. Moreover, by Perls, a histochemical staining, specific for iron, we detected scattered blue deposits inside epithelial tubular cells. Remarkably, after SCH.B pretreatment, HSP25 and HSP72 expressions persisted and were intense in cortical proximal tubules together with a partial morphological recovery. Perls signal was strong in scattered proximal straight tubules together with lysosomal accumulation.

Taken together these data suggest that SCH.B pretreatment attenuated $HgCl_2$-induced nephrotoxicity in rat by the persistence of the tubular expression of specific cytoprotective stress proteins.

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P4-03
Expression of Ras in cadmium-induced nephrotoxicity
Effects of the natural antioxidant quercetin

Sánchez-González P., Vicente-Sánchez C., Pérez-Barriocanal F., López-Novoa J.M., Morales A.I.

Department of Physiology and Pharmacology (Toxicology), University of Salamanca, Spain

Cadmium (Cd) is an ubiquitous environmental toxicant that affects biological systems in various ways. The molecular mechanisms of its toxicity are not yet well defined. We have recently reported in an experimental model of chronic cadmium administration in rats, that quercetin, a potent oxygen free radical scavenger, has a protective effect on cadmium-induced nephrotoxicity. Quercetin's strong antioxidant activity could be responsible for the protective effect. However, Cd activates multiple signal transduction pathways related to cellular responses to stress. Ras is a member of a family of small GTPases with a great variety of functions including regulation of gene expression and cell proliferation. Our aim in this work was to study the effect of Cd and quercetin on the proliferation related to Ras-mediated signal transduction pathways. Experiments were carried out in male Wistar rats during nine weeks. Rats were distributed in four experimental groups: (1) control rats; (2) Cd (1.2 mg Cd/kg/day s.c.); (3) quercetin (50 mg/kg/day, i.p.); (4) Cd + quercetin (Cd and quercetin at the same doses as in the Groups 2 and 3, respectively). Renal toxicity was evaluated by measuring urinary excretion of proteins, albumin, glucose and enzyme markers of tubular necrosis, as well as plasma concentrations of creatinine and urea. Renal expression of Ras and Ras activation was assessed by Western blot and immunohistochemistry. Assessment of cell proliferation was evaluated by detection of the Ki67 antigen. Animals that received both Cd and quercetin showed a better renal function than those receiving Cd alone. Cd-induced tubular endothelium lesions were markedly reduced in rats that also received quercetin. On the other hand, Cd increased Ras activation and cell proliferation. Quercetin treatment reduced Ras activation and the number of cells in proliferation. Our results show that suppression of Ras signal transduction pathway activated and cell proliferation by quercetin is associated with the protective effect on cadmium-induced nephrotoxicity.

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P4-04
Cytotoxicity of PPAR ligands on renal proximal tubular cell lines

Hector Giral[2], Ricardo Villa-Bellosta[2], Julia Catalan[2], Ana Ferrer-Dufol[1], Victor Sorribas[2]

[1] *Clinical University Hospital, Toxicology Unit, Zaragoza, Spain;* [2] *Laboratory of Molecular Toxicology, University of Zaragoza, Spain*

Peroxisome-proliferator activated receptors (PPAR) are nuclear transcription factors involved in the metabolism of fatty acids and glucose. Several ligands have been synthesized and used pharmacologically to control dislipidemias and insulin resistance, such as fibrates and glitazones, which are common activators of PPAR-alfa and PPAR-gamma, respectively.

Several reports have shown the development of apoptosis by different PPAR activators. Because the kidney contains most of the known PPAR isoforms and it is a critical organ in the course of dislipidemias and diabetes, we have tested whether several PPAR activators affected viability of renal cell lines. Three models of proximal tubular cells were chosen: opossum kidney (OK) cells, pig LLC-PK1 cells and murine MCT cells. Each cell line was assayed in Hank's balanced salt solution (HBSS) with two different PPARα (WY14643 and clofibrate) and two PPARγ (pioglitazone and ciglitazone) activators. Total cell death was quantified by the activity of lactate dehydrogenase (LDH) in HBSS: Twelve hours of incubation with 50–150 μM WY14643 increased LDH activity, and the effect was maximal at 250 μM. Clofibrate, however, did not affect cell viability, even up to 500 μM. Ciglitazone increased LDH activity at 10 μM and it was maximal at 50 μM in all three lines, while pioglitazone did not induce cell death at any of the con-

centrations assayed. These cytotoxic effects were maintained even in the presence of epithelial growth factor, cholera toxin and BSA in the HBSS. Direct analysis of the cells by phase contrast and Nomarski optics pointed to cell death by necrosis, and several assays were carried out to a better characterization of the cell death.

Phalloidin staining showed that actin filaments were disrupted by WY14643 and ciglitazone, and no stress fibers were observed at either 6 or 16 h. Caspase-3 activity (an apoptotic early event) was also not modified by WY14643 or ciglitazone at either 6 or 16 h. Annexin V-EGFP fusion protein assay coupled to propidium iodide staining, double staining of the cells with acridine orange and ethidium bromide, and DNA laddering in agarose electrophoresis did not show evidence of apoptosis.

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P4-05

Does the storage time of human blood have any impact on the outcome of the red blood cell lysis cytotoxicity test?

Jens Lichtenberg, Inge Birgit Jørgensen, Ninna Willestofte Berg

Novozymes A/S, Safety & Toxicology, Bagsvaerd, Denmark

The red blood cell (RBC) lysis test is a well established in vitro model for the evaluation of general cytotoxicity. The RBC lysis test is based on the INVITTOX protocol IP-99 and we have used the model to screen new industrial enzyme and peptide preparations for haemolytic capacity. In brief, a 2% RBC suspension in PBS is prepared from human blood. The test article is diluted and mixed with the RBC suspension for testing at various concentrations. Following incubation for 1 h at 37 °C, the samples are centrifuged and aliquots of 200 μL supernatant are measured at 540 nm. The results are expressed relative to 100% lysis. Sodium dodecyl sulphate (SDS) is included as positive control.

Following the above procedure, it is speculated if the susceptibility of RBC to the positive control SDS may change over time indicating that the robustness of RBC decreases as the storage time of the RBC increases. In the present investigation, RBC were obtained from different male ($n = 2$) and female ($n = 2$) donors and tested every third day in the RBC lysis test over a period of 12 days. The susceptibility to SDS at the concentrations of 25, 50 and 100 μg/mL SDS was measured at each test day.

At a concentration of 50 μg/mL SDS the hemolysis of the RBC on days 5 and 12 is increased by 20% or 40% versus the hemolysis measured on fresh blood (day 1). The present data obtained from our laboratory suggests that fresh RBC should always be used when conducting the RBC test as the robustness of the RBC is decreasing over time, which may disturb standard curve of the positive control.

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P4-06

The toxicological study on PC-3M cells attacking nude mice

Zu-Yue Sun, Jian-Hong Chu, Zhi-Ling Li, Xiang-Yun Liu, Xiao-Fang Zhang, Jian-Hui Wu, Yan Zhu, Lin Cao

Shanghai Institute of Planned Parenthood Research, National Evaluation Center for the Toxicology of Fertility and Regulating Drugs, Shanghai, PR China

The purpose of these studies was to explore the toxicity of human prostatic carcinoma line PC-3M, while attacking the nude mice. Some methods below were handled: (1) PC-3M cells (human prostatic carcinomia cell line) was implanted into the prostates (orthotopic implantation) of male nude mice. (2) After inoculated, the tumor cells were isolated and cultured from one primary prostate tumor and lymph node metastasis confirmed by histological examination in the same nude mouse with orthotopic implantation of PC-3M cells, respectively. Cell invasion and adhesion ability in vitro were first compared between two cell lines. (3) Then human metastasis-related genes differentially expressed between them were analyzed by utilizing cDNA microarray technique. It could be observed that: (1) at sacrifice, tumors formed in prostates, as well as regional and distant lymph nodes were extracted for histological analysis. It has been demonstrated that the tumors in the prostate grew well with 10/10 frequency and 7/10 of them happened lymph node metastasis after 40 days from attacked. (2) The in vitro cell invasion and adhesion potential of tumor cells from lymph node metastasis was significantly higher than those from primary tumor, metastasis-related genes differentially expressed between those two cell lines were identified, all of them were up-regulated in the tumor cells from lymph node metastasis and could be categorized as: (1) genes encoding cellular matrix-degrading proteolytic enzyme including cathepsin and MMP. (2) Genes encoding transcription factors. (3) Genes related to heterotypic adhesion of tumor cells. (4) Genes encoding cell surface receptors. Moreover, four genes were chosen for semi-quantitative RT-PCR analysis, they showed a consistent expression pattern with that of cDNA microarray analysis. It might be concluded that prostatic carcinoma line PC-3M has

an toxicological effect to nude mice, include the tumors formed in with 10/10 frequency in the prostate and 7/10 of lymph node metastasis. It also be found furtherly that the lymph node metastasis in nude mice given an injection of PC-3M cells in the prostate is a selective process favoring the survival and growth of a special subpopulation derived from primary tumor with specific genetic alterations. Identification and further characterization of the toxicology of the PC-3M may allow a better understanding of the tumor cells in toxicology.

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P4-07

Expression of vascular endothelial growth factor isoforms and receptors throughout acetaminophen-induced liver toxicity and regeneration

Vasilios P. Papastefanou [1], Evangelos Bozas [4], Zoi Papoutsi [3], Agni Grypioti [1], Stavros Garyfallidis [5], Polyxeni Nikolopoulou-Stamati [2], Michael G. Mykoniatis [1]

[1] *Department of Experimental Pharmacology, Medical School, University of Athens, Greece;* [2] *Department of Pathology, Medical School. University of Athens, Greece;* [3] *Department of Biochemistry, Medical School, University of Athens, Greece;* [4] *Pediatric Research Laboratory, Faculty of Nursing. Univesity of Athens, Greece;* [5] *Patision General Hospital, Athens, Greece*

Acetaminophen is a widely used analgesic and a known hepatotoxic agent. Vascular endothelial growth factor is a growth factor with multiple functional roles. The aim of this study was to determine the expression of VEGF and its receptors in liver after the administration of a toxic dose of acetaminophen in rats.

Eleven groups of adult male rats received a dose of 3.5 g/kg b.w. of acetaminophen per os. The rats were killed post administration at 0–288 h. Blood and liver tissue were extracted. Determination of the serum enzyme levels of ALT, AST and ALP was performed. Liver injury and regeneration were assessed with haematoxylin–eosin specimens, thymidine kinase assay and Ki-67 expression. RT-PCR, Western blotting and immunohistochemical methods were used for assessment of VEGF isoforms and VEGFR1 and VEGFR2 expression.

Maximal expression of AST and ALT was observed at 24–48 and 24–36 h, respectively, with another peak of expression at 192 h post administration. ALP was increased post 72 h peaking at 192 h. Centrilobular necrosis was observed at 48–72 h and thorough restoration of the liver microarchitecture was observed at 288 h. Liver regeneration lasted from 36 to 144 h according to the results from thymidine kinase and Ki-67. VEGF and VEGFR2 m-RNA and protein levels presented with a three-peak pattern of expression at 12–24, 72–96 and 192–240 h post administration. Significant difference was noted between periportal and perivenular immunohistochemical expression.

VEGF proves to be a critical molecule during acetaminophen-induced liver regeneration. It presents with three peaks of expression. The first two peaks are associated with the initial inflammatory reaction to the noxious stimulus and the hepatocyte regenerative process where as the third one could be important for remodeling of the tissue architecture.

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P5 Neurotoxicity

P5-01

Evaluation of in vitro neurotoxicity of methyl mercury, PCB153 and PCB126

A. Vitalone [1], G. Giordano [2], L.G. Costa [1,3]

[1] *University of Bari, Italy;* [2] *University of Washington, Seattle, WA, United States;* [3] *University of Parma, Italy*

Methyl mercury (MeHg) and polychlorinated biphenyls (PCBs) 126 and 153 are persistent environmental pollutants, and seafood represents the main source of human exposure. These compounds are known developmental neurotoxicants, as evidenced by animal studies and observations in humans. Aim of this study was to evaluate the effect of MeHg (0–5 μM) and PCBs (0–100 μM) in a battery of in vitro cell systems, with the goal of developing a potentially useful in vitro screening system. Cell viability was assessed by measuring MTT reduction and Trypan blue exclusion. The studies were carried out in six different rat or human cell lines: rat PC12 pheochromocytoma cells and human SH-SY5Y neuroblastoma cells were used as models of neuronal cells; rat C6 glioma cells and human 1321N1 astrocytoma cells were used as model of astrocytic cells; rat 3T3 fibroblasts and human prostate PZ-HPV-7 cells were used as non-nervous-system cell lines. Additionally, primary rat astrocytes, as well as hippocampal, cortical and cerebellar neurons were also utilized. The results obtained with MTT indicate that: (1) MeHg is more toxic than PCB126 and PCB153; (2) nervous system-derived cell lines are more sensitive than non-nervous cell lines to all contaminants; (3) neuronal cells are more sensitive than astrocytic cells in case of MeHg, but not PCBs; (4) human neuronal cells

are more susceptible to the toxicity of MeHg and PCBs than rat-derived neurons; (5) primary neurons, but not primary astrocytes, are more sensitive to MeHg and PCBs toxicity. Results obtained with the Trypan blue exclusion assay reflect similar effects, though these are seen at higher concentrations. These in vitro results are in agreement with in vivo findings for MeHg, but further studies are needed to elucidate the differences between PCB126 (dioxin-like PCB) and PCB153 (NDL-PCB).

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P5-02

Chronic noise stress-induced alterations in the expression of *Hsp70*, *c-fos*, DNA damage and Fas/FasL expressions in discrete brain regions of albino rats

Sundaramahalingam Manikandan, Narayanaperumal Jeyaparthasarathy, Ramasamy Srikumar, Rathinasamy Sheeladevi

Neuroscience laboratory, Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Chennai 600113, India

Brain is more susceptible to stressors than any other organ. Exposure to continuous loud noise is a serious environmental health problem due to excess production of oxygen free radicals. The aim of the present work was to evaluate the effects of oxidative stress in discrete (cerebral cortex, cerebellum, midbrain, pons-medulla, hippocampus and hypothalamus) brain regions and neuronal dendritic changes after the rats were exposed to chronic noise (100 dBA/4 h/day for 30 days). Expression of *Hsp70*, *c-fos* mRNA (RT-PCR), Fas/FasL protein expression (immunoblotting and immunohistochemistry) and DNA damage in discrete brain regions were studied. Results showed that neuronal dendritic count in the hippocampus and medial prefrontal cortex were reduced significantly ($P < 0.01$) in the second and third order dendrites after 30 days of noise exposure when compared to control animals. Excessive free radical generation produced by noise stress led to increases in lipid peroxidation level, superoxide dismutase activity, *Hsp70*, *c-fos* mRNA expression, DNA damage, Fas/FasL protein expression and concomitant decreases in the activity of catalase, glutathione peroxidase and depletion of reduced glutathione in all the brain regions. This study suggests that 30 days of noise exposure causes oxidative stress in all the brain regions and alteration in neuronal communication in HIP and mPFC and this finding may be applicable to human, are working/living in noisy environment.

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P5-03

Cytokine response in repeated skin irritation measured by stratum corneum tape stripping

Cindy Maria de Jongh [1], Maarten M. Verberk [1], Carien E. Withagen [1], John J. Jacobs [2], Thomas Rustemeyer [3], Sanja Kezic [1]

[1] *Academic Medical Center, Coronel Institute of Occupational Health, The Netherlands;* [2] *University of Utrecht, Utrecht, The Netherlands;* [3] *VU University Medical Centre, The Netherlands*

Cytokines play an important role in immune and inflammatory reactions. Little is known about cytokines involved in chronic irritant contact dermatitis. Our aim was to investigate the cytokine response in chronic skin irritation measured by a non-invasive stratum corneum (SC) tape stripping method. A repeated sodium lauryl sulfate (SLS) exposure test was used as a model for chronic irritation.

Methods: Eighteen healthy volunteers were exposed to SLS on the volar forearm. A patch with 0.1% SLS was applied for 6 h, 4 days a week, during 3 weeks. Four days after the last exposure the SC at the treated and an untreated control site was removed by means of 20–30 times tape stripping. Presence of interleukin-1α (IL-1α), IL-1 receptor antagonist (IL-1RA) and IL-8 was analyzed using ELISA. The cytokine concentrations for each strip were normalized for soluble protein content.

Results: IL-1α decreased by 30% after repeated exposure compared to untreated skin ($p = 0.04$), while IL-1RA increased 10-fold and IL-8 increased 4-fold (both $p < 0.001$). The IL-1RA/IL-1α ratio for the SLS-treated skin increased 15 times ($p < 0.001$).

Discussion: The balance between IL-1RA and IL-1α is important in the down-regulation of the inflammatory response. An increase in this ratio has also been described in other chronic disorders like atopic dermatitis and psoriasis. We found that the response in IL-1RA and IL-1α after a repeated irritation is opposite to that after a single irritation (Perkins et al., 2001). The increase in IL-8 was found in both single and repeated irritation.

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P5-04

Ecstasy-induced cell death in cortical neuronal cultures is 5-HT_{2A}-receptor-dependent and potentiated under hyperthermia

João Paulo Capela [1], Karsten Ruscher [2], Marion Lautenschlager [3], Dorette Freyer [2], Ulrich Dirnagl [2], Ana Rita Gaio [4], Maria Lurdes Bastos [1], Andreas Meisel [2], Felix Carvalho [1]

[1] *REQUIMTE, Toxicology Department, Faculty of Pharmacy, University of Porto, Porto, Portugal;* [2] *Neurology Department, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [3] *Psychiatry Department, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [4] *Department Pure Mathematics, Faculty of Science, University of Porto, Porto, Portugal*

Studies on 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") induced neurotoxicity mainly focus on damage of serotonergic terminals. Less attention has been given to neuronal cell death produced by MDMA and other amphetamines in areas including the cortex, striatum and thalamus. Previous studies showed, using cortical neuronal cultures, that MDMA-induced neuronal death was accompanied by activation of neuronal apoptotic pathways, namely endonucleosomal DNA cleavage and differential expression of antiapoptotic and proapoptotic bcl-xL/S splice variants. Therefore we investigated MDMA-induced neurotoxicity in neuronal serum free cultures from rat cortex. Since ecstasy intake induces hyperthermia in both animals and humans, the experiments were performed under normal (36.5 °C) and hyperthermic conditions (40 °C). Cell viability was accessed using morphology, the LDH release assay and the MTT assay. The cell death mechanism was also evaluated by means of ethidium bromide/acridine orange staining. Our findings showed a concentration-, time- and temperature-dependent apoptotic cell death induced by MDMA in cortical neurons. MDMA-induced damage was potentiated under hyperthermia. The neurotoxicity was reduced by the 5-HT_{2A}-receptor antagonists, ketanserin and R-96544, in both normothermic and hyperthermic conditions. DOI, a model agonist for the 5-HT_{2A}-receptor, also induced a concentration- and time-dependent apoptotic cell death. Again, protection was provided by ketanserin and R-96544 against DOI-induced neurotoxicity, thereby indicating that the MDMA stimulation of the 5-HT_{2A}-receptor leads to neurotoxicity. As the misuse of MDMA as a recreational drug (particularly in crowded and hot environments) can induce hyperthermia, the results presented here corroborate the serious concern previously reported in the literature. Since MDMA 5-HT_{2A}-receptor agonistic properties lead to neuronal death, clinically available atypical antipsychotic drugs with 5-HT_{2A}-antagonistic properties could be a valuable therapeutic tool against MDMA-induced neurodegeneration.

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P5-05

Effect of increasing doses of fenofibrate on BCKDH kinase

M. Knapik-Czajka, A. Gozdzialska, P. Knapik, J. Jaskiewicz

Department of Analytical Biochemistry, Collegium Medicum Jagiellonian University, Krakow, Poland

Introduction: BCKDH complex catalyzes the oxidative decarboxylation of branched chain α-ketoacids originating from valine, leucine and isoleucine. Two regulatory enzymes are associated with BCKDH, a specific kinase (BK) catalyzing phosphorylation and inactivation of the complex and a specific phosphatase catalyzing dephosphorylation and reactivation of BCKDH. BK activity is regulated by physiological factors and by different xenobiotics including fibrates. Fibrates decrease BK activity and protein expression leading to increase in both BCKDH activity and branched chain amino acid catabolism. So far all studies have been done with only one dose of the chosen fibrate. The goal of our study was to investigate the effect of increasing doses of fenofibrate on BK activity in rat's liver.

Methods: For 14 days, fenofibrate was administrated to groups of Wistar male rats (fed chow containing 8% protein) at one of the daily doses: 5, 10, 20 and 50 mg/kg b.w. Control group was given only vehicle (0.3% methylcellulose). It is established that low-protein diet stimulates BK activity and increases BK amount associated with BCKDH leading to complex inactivation. BK activity was determined spectrophotometrically in extracts of rats' livers. BK protein amount was determined by Western blot analyses.

Results: Comparing to control group BK activity was lower 1.5-, 4.8-, 5.9- and 11.9-fold in groups receiving doses of 5, 10, 20 and 50 mg/kg b.w. fenofibrate, respectively. There was no significant difference in the amount

of BK protein between fenofibrate-treated and control groups.

Conclusions: It can be concluded that fenofibrate in condition of protein malnutrition inhibits BK activity directly or/and change the amount of BK protein amount associated with BCKDH complex. This toxic, opposite to physiological effect of fenofibrate on BK is dose-related. That results in higher BCKDH activity leading to increase of branched-chain amino acid catabolism and decrease in their levels. Since leucine plays an important role in up-regulation of protein synthesis in muscle the reduced level of this amino acid may be one of the factors involved in pathomechanism of miopathy observed during treatment with fenofibrate.

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P5-06
Occurrence and levels of environmental chemicals in human milk in the general population

Tina Jannice Branting [1], Christina Rudén [1], Mattias Öberg [2], Christina Björkdahl [1]

[1] *Institution of Philosophy and the History of Technology, Stockholm, Sweden;* [2] *Karolinska Instituet, Stockholm, Sweden*

This is a report from an ongoing project aiming at increasing the understanding of the occurrence and levels of environmental chemicals in human milk in the general population.

The World Wildlife Foundation concluded in the report "Chemical trespass: a toxic legacy" published in 1999 that "over 350 contaminants have, at some time, been found in human breast milk". A closer look at these data reveals that for a large number of these contaminants, information about actual levels are lacking and/or have only been found in a small number of breast milk samples. This raised the question to what extent this number of 350 contaminants found in human milk can be considered relevant for the general population.

To clarify this, we have created a database in which we have gathered available and relevant information about the number and levels of environmental chemicals found in human milk in the general population, globally. Detailed information about the studies and the participants has been stored in the database. The inclusion criteria were based on a protocol developed by the WHO for investigating POPs in human milk. A minimum of 50 breast milk donors in each study was used as one criterion to ensure that levels of contaminants are relevant for the general population. So far, the database includes quantitative data for 140 individual chemicals identified in milk fat samples from 68 areas in 26 countries worldwide. To mention a few, the chemicals include several organochlorine pesticides, PCBs, PBDEs, dioxins, furans and hexachlorocyclohexanes.

Our conclusion is that approximately 150 chemicals, rather than 350, can be considered a relevant number of chemicals found in human milk in the general population today. The general focus on chemicals in human milk is clearly limited to a small number of POPs. Levels in human milk in the general population for hundreds of other chemicals are still unknown and should therefore be further investigated.

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P5-07
Nitric oxide in diquat neurotoxicity

Marijana Curcic, Mirjana Djukic

Institute of Toxicological Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia and Montenegro

Leading by the similarity in chemical structure of diquat (DQ) and paraquat (PQ), bipyridylium herbicides, widely used for weed growth control, we assumed that its neurotoxicity might be mediated by the similar mechanisms. PQ is well known redox-cycling compound, which interferes NO metabolism. Therefore, the goal of the study was to determine nitrate, as a longlasting end-products of nitric oxide (NO) metabolism, in vulnerable brain regions of Wistar rats poisoned with DQ.

According to regional distribution of enzyme nitric oxide synthase (NOS) in the brain, nitrate were determined in hippocampus, cortex and striatum of Wistar rats, after intrastriatally (i.s.) administration of single dose of DQ (50 mg/kg), in both ipsilateral and contralateral side, 30 min, 24 h and 7 days after the poisoning.

After 30 min, nitrate values were about three times higher than physiological, in all three brain regions, on both sides. In cortex and striatum, nitrate decreased until 24th hour and remained the same until the 7th day. Unlike, in hippocampus, nitrate started to decrease later, form 24th hour to 7th day. All measured nitrate values are statistically significant higher than physiological. One-way ANOVA and post hoc Tukey test were used for statistical data processing.

Obtained elevated and similar nitrate concentrations, in ipsilateral and contralateral sides, indicate that: (a) oxidative/nitrosative stress mediated by DQ toxicity was

propagated evenly and promptly; (b) DQ impairment of NO metabolism. The reason for different shape of nitrate decreasing in hippocampus v.s. cortex and striatum was probably due to enlarged regional NOS distribution in hippocampus.

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P5-08

Neurotoxicity of ecstasy metabolites in rat cortical neurons, and influence of hyperthermia

João Paulo Capela[1], Andreas Meisel[2], Artur Reis Abreu[3], Paula Sério Branco[3], Luísa Maria Ferreira[3], Ana Maria Lobo[3], Fernando Remião[1], Maria Lurdes Bastos[1], Félix Carvalho[1]

[1] *REQUIMTE Toxicology Department, Faculty of Pharmacy, University of Porto, Porto, Portugal;* [2] *Neurology Department, Charité-Universitätsmedizin Berlin, Berlin, Germany;* [3] *REQUIMTE/CQFB, Chemistry Department, Faculty of Science and Technology, University of Nova de Lisboa, Monte de Caparica, Portugal*

3,4-Methylenedioxymethamphetamine (MDMA or "Ecstasy"), is a widely abused, psychoactive recreational drug. Metabolism of MDMA involves *N*-demethylation to 3,4-methylenedioxyamphetamine (MDA). MDMA and MDA are *O*-demethylenated to *N*-methyl-α-methyldopamine (*N*-Me-α-MeDA) and α-methyldopamine (α-MeDA), respectively, both of which are catechols that can undergo oxidation to the corresponding *ortho*-quinones. In the presence of glutathione (GSH), *ortho*-quinones may be conjugated with GSH to form a glutathionyl adduct. In this study, we evaluated the neurotoxicity of MDMA and of three of its metabolites, obtained by synthesis, *N*-Me-α-MeDA, α-MeDA and 5-(GSH)-α-MeDA (5-(Glutathion-*S*yl)-α-methyldopamine) in rat cortical neuronal serum free cultures under normal (36.5 °C) and hyperthermic (40 °C) conditions. Our study shows that these metabolites are more neurotoxic than the parent compound MDMA. They induced programmed cell death in cortical neurons and their neurotoxic effect was potentiated under hyperthermic conditions (40 °C). *N*-Acetylcystein, an antioxidant and precursor of GSH, protected against MDMA metabolites-induced neurotoxicity, indicating that GSH depletion may render the cells more exposed to the effects of these reactive metabolites. These data suggest that MDMA metabolism and MDMA-induced hyperthermia, leading to the formation of ROS/RNS and/or toxic oxidation products may contribute for the neurotoxicity exerted by "Ecstasy".

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P5-09

Exploration of the monoaminergic neurotransmission: Localisation of several receptors and transporters in the Marmoset brain

Sandrine Chalon[1], Denis Guilloteau[2], Sylvie Bodard[2], Sandrine Ferry[1]

[1] *Pfizer, Amboise, France;* [2] *INSERM U619*

Marmoset is a small nonhuman primate, which has many interests as non-rodent species in toxicology, such as its brain morphologically and functionally close to that of human, and its size leading to easy manipulation. Nevertheless, to date few data are available on the localisation of brain receptors and transporters in this animal species.

The rapid development of device aimed to in vivo imaging methods in small animals is of major value in the study of brain affections. Scintigraphy allows non-invasive and repetitive imaging of the spatio-temporal cerebral distribution of specific molecular neurotransmitter targets in a single living animal.

To explore the monoaminergic systems in the Marmoset brain, we validated the use of specific radioactive iodinated probes useful in SPECT studies, i.e. (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy -3β-(4′-methylphenyl) nortropane (PE2I) as dopamine transporter (DAT) ligand, iodobenzamide (IBZM) as dopamine D_2 receptor (D_2R) ligand and 2((2((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine (ADAM) as serotonin transporter (SERT) ligand. Tracers were labeled with ^{125}I, purified and obtained with high specific activity (74 TBq/mmol). For ex vivo quantitative autoradiographic studies, Marmosets (2/ligand) were i.v. injected with 3–5 MBq of [^{125}I]-labeled tracer. The animals were euthanized and their brains were removed at 1 h (PE2I, ADAM) or 2 h (IBZM) post-injection, according to the known in vivo kinetics of products. Twenty micro-thickness brain sections were cut using a cryocut and exposed on films for 2–3 weeks. After revelation and fixation, sections were colored with cresyl violet in order to use anatomical references for the selection of regions

of interest before autoradiographic quantification. The DAT density was distributed in the following order: caudate = putamen > nucleus accumbens > substantia nigra > cortex, with ratios region/cortex of 16, 16 and 9, respectively. The D_2R density was distributed in the following order: caudate = putamen > nucleus accumbens > cortex with ratios region/cortex of 13, 14 and 11 for the caudate, putamen and nucleus accumbens, respectively. The SERT density was rather homogeneous and distributed as follows: caudate = amygdale = nucleus accumbens = hippocampus = thalamus > cortex. Therefore, all three iodinated tracers PE2I, IBZM and ADAM were able to cross the blood–brain-barrier and bind to the expected molecular targets. Their distribution in the Marmoset's brain corresponds to the known repartition in Cynomolgus. These results will contribute to the cartography of the DAT, D_2R and SERT in the Marmoset brain in order to establish an anatomic atlas and references for future in vivo PET exploration of these cerebral targets.

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P5-10
Effects of thimerosal on rat brain development

Seung-Jun Kwack, Yo Woo Choi, Chi Won Song, Chae-Hyung Lim, Hyung-Sub Kim, Jae Ho Oh, Yong Na Yeom, Kui Lea Park, Seung-Hee Kim, Dong Deuk Jang

Toxicological Research Department, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, Republic of Korea

The purposes of this study were to evaluate the toxicity of the thimerosal on neuro-development. Thimerosal (or mercurothionate) is a mercury-containing compound used to prevent bacteria from contaminating vaccines, especially in multi-dose vaccine vials. The toxicity of mercury is well known and those most at risk are occurred in the nervous system and brain. Exposure to mercury from vaccines containing thimerosal in the first 6 months of life ranges up to 187 μg based on which vaccines are administered. These exposure levels exceed the limit of EPA guidelines, and possibly other guidelines, for the infants.

However, the toxic mechanism of thimerosal has not been cleared. The present studies were performed to explain its cytotoxicity and toxic mechanisms. Thimerosal induced cytotoxicity and inhibited the proliferation and differentiation of the midbrain cells. The ROS generation was increased by thimerosal treatments. However, antioxicants, such as Vitamine E and propyl gallate, protected from oxidative damage on midbrain cells and was recovered midbrain cell proliferation and differentiation.

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P5-11
The relationship between ecstasy use and pre-existing psychiatric factors

Ionescu M. Daniela

University of Medicine and Pharmacy Victor Babes Timisoara, Romania

Objective: Recreational ecstasy (3,4-methylenedioxymethamphetamine, MDMA) use has been associated with a number of psychiatric and psychological problems. This paper aimed to assess whether psychiatric history and patterns of ecstasy use were related.

Material and methods: In a territorial centre of addiction during 2004–2005, we identified 351 subjects, of 15–36 years old: group A, 179 (100 males and 79 females) problematic ecstasy users who had reported problems attributable to their ecstasy use and group B 172 (100 males and 72 females) subjects with non-problematic ecstasy consummation. We applied a specific questionnaire, which ascertained personal and family psychiatric disorders and the Brief Symptom Inventory score. Data analysis was conducting using SPSS 10. 1.3.

Results: Problematic ecstasy users exhibited a higher score on anxiety, depression, obsessive-compulsive disorders, phobia, panic attacks, eating disorders, schizophrenia and alcohol and/or others drug dependence. The data indicated that a greater number (89, 55% group A) of them have a personal and a family history of psychiatric illness compared to non-problematic group users (35, 61% group B).

Conclusions: The relationship between drug use, vulnerability to psychopathology and pre-existing mental problems is a multifactorial clinical situation. The current data suggests this is a potentially vital concern in the exploration of problematic ecstasy use.

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P5-12
Peripheral neurotoxicity in human viper bite envenomations

D. Lonati, A. Giampreti, C. Locatelli, R. Butera, V. Petrolini, L. Manzo

Poison Centre and National Toxicology Information Centre, Toxicology Unit, IRCCS Maugeri Foundation and University of Pavia, Italy

In the last years, cases involving peripheral neurological symptoms (PNS) after European *Vipera* species envenomation have been reported both in Italy and South-Eastern France. PNS can be related to the presynaptic toxicity of phospholipases A2 (PLA2), viper venom neurotoxins that cause neuromuscular paralysis. PNS involve mostly cranial nerves and have always been observed in patients with moderate to severe non-neurological systemic symptoms.

Objective: To identify and describe the characteristics of PNS observed in patients referred to Pavia Poison Center after viper bite.

Methods: Patients observed over a 2 years period were reviewed. Cases were assessed for presence, severity and time course of local, neurological and non-neurological associated signs, overall management and outcome.

Results: Six adult patients with PNS were observed. PNS included bilateral (3/6 patients) or monolateral (1/6) ptosis, diplopia (3/6), dysphagia (1/6) and bilateral deficit of masseter muscles (1/6). All patients showed local signs from mild (2/6), to moderate (3/6), to massive limb edema (1/6). Systemic non-neurotoxic effects (vomiting, diarrhea) occurred in 4/6 patients; 2 patients showed local signs and PNS in absence of any other systemic symptoms. PNS were observed 4–30 h after the bite, while systemic non-neurotoxic effects occurred earlier (40 min–17 h). Antivenom was administered in 3/6 patients as soon as PNS were observed. In treated patients, PNS started to improve between 6 and 11 h, and resolved between 24 and 26 h after the onset. In untreated patients PNS improved and resolved later (between 7 and 44, and between 55 and 64 h after the onset, respectively).

Conclusion: PNS observed seems to be strictly connected with the mechanism of action of PLA2; PNS can occur with a delayed onset and even in patients presenting only with local effects so a thorough clinical evaluation also for these patients is advisable. Antivenom administration seems to be beneficial in shortening the persistence of PNS, but its efficacy needs to be further investigated in a larger series.

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P5-13
In vitro comparison of eight acetylcholinesterase reactivators to reactivate VX inhibited ACHE

Daniel Jun, Jiri Bajgar, Kamil Kuca

Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

A comparison of one mono-(pralidoxime) and seven bis-(methoxime, obidoxime, K027, K074, K075, HI-6 and HS-6) quaternary acetylcholinesterase reactivators inhibited by VX agent was performed. As a source of the acetylcholinesterase, a rat brain homogenate was taken. There were significant differences in reactivation potency of all tested oximes. The oxime K075 seems to be the most efficacious followed by K074, HI-6, HS-6, K027, obidoxime, MMC and 2-PAM. In addition, the results of this study showed, that the reactivation potency of the tested reactivators depends on their structural factors—such as the number of pyridinium rings (two are better than one), the position of functional oxime groups (position four is preferred), as well as the length of the linker bridge between two pyridinium rings (three and four membered chain is preferred).

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P5-14
Effects of low dose methylmercury administration during postnatal brain spurt in rats

Pietro Borracci [1], Addolorata Coluccia [1], Michele Persichella [1], Arcangela Giustino [1], Mineshi Sakamoto [2], Maria Rosaria Carratù [1]

[1] *Department of Pharmacol. and Human Physiology-Medical School-University of Bari, Italy;* [2] *National Institute for Minamata Disease, Minamata, Japan*

Rapid brain growth occurs primarily during the third trimester of pregnancy in humans, whereas in rats it occurs mainly after parturition. In particular, the rat cerebellum is vulnerable to methylmercury during late brain spurt. In the present experiments the effects of low dose methylmercury treatment were studied during the postnatal developing phase in rats. Male Sprague–Dawley rats were orally administered 0.75 mg/kg/day methylmercury chloride (MMC) on postnatal day 14 for 10 consecutive days. This dose level, resulting in MMC brain concentration of 0.82 ± 0.05 μg/g tissue, did not cause the typical symp-

toms of cerebellar dysfunction, i.e. hind-limb crossing and ataxia. Moreover, no change in body weight gain was observed between MMC-treated and control rats. Locomotor behaviour, which involves many different brain systems, also including the cerebellum, was studied in the Opto-Varimex apparatus. MMC-exposed animals were tested in the Opto-Varimex cage for 10 min on the day after the final MMC administration and every week thereafter until postnatal day (PD) 45. Comparison with the age-matched control rats showed a significant reduction in the number of rearings in MMC-treated rats starting from PD31 until PD45 ($p < 0.05$ for PD31 and 38; $p < 0.01$ for PD45, Bonferroni's Multiple Comparison test). MMC exposure did not alter the number of crossings, resting time and the time spent in the center of the arena. The present results demonstrate that MMC treatment during late brain spurt, at a dose level approximating the safe intake limits established by the European Food Safety Authority, induces a persistent deficit in the exploratory behaviour, further underlining the serious potential hazard for the exposed children.

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P6 Occupational Toxicology

P6-01
Serum essential metal changes among aluminum workers

F.M. Metwally [1], M.S. Mazhar [2]

[1] *Industrial Medicine and Occupational Health Department, National Research Center, Egypt;* [2] *Community and Occupational Health Department, Al-Azhar University, Egypt*

This study was done to investigate the possible changes in essential metals among a group of workers occupationally exposed to aluminum "Al" fumes. It included 60 subjects working in the pot-room working in one of the aluminum companies and 60 employees not occupationally exposed to Al as a control group. The results showed that the mean levels of serum copper, calcium, zinc and iron were significantly lower among the exposed group compared to that of the control group. Also, the mean levels of plasma and urinary Al were significantly higher in the exposed employees than in the control. A statistically significant negative correlation was found between plasma and urinary Al on one hand and the studied essential metals on the other. It is evident from this work that Al exposure has an adverse effect on human essential metals with its subsequent impact on the cellular enzymatic and metabolic processes.

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P6-02
Respiratory effects of occupational exposure to man made vitreous fibres among employees of a fibreglass industry in Shiraz, Iran

Masoud Neghab

School of Health, Shiraz, Fars, Iran

Evidence for associations between exposure to mineral fibers (fiberglass) and either respiratory symptoms or functional impairment has not been conclusive. Additionally, the potential adverse pulmonary effects of this compound have not been extensively studied. This study was, therefore, undertaken to evaluate the possible effects of inhalation exposure to this chemical on the respiratory system of a group of fiberglass-exposed workers. The study population consisted of a group of 49, randomly selected, male workers with current occupational exposure to fiberglass fibers and 45 healthy male office workers without history of past or present exposure to this material that served as the control group. The average (mean $\pm$ S.D.) age (years) and the duration of exposure to dust for the exposed group were 39.6 ± 7.3 and 11.4 ± 5.6, respectively. The corresponding values for the control group were 42.8 ± 7.6 and 0 ± 0, respectively, and there was no statistically significant difference between the mean values of age for both groups. Subjects were interviewed and respiratory symptom questionnaires, as suggested by the American Thoracic Society (ATS, 1978), were completed for all of them. They were classified to smokers and non-smokers and underwent chest X-ray and lung function tests according to the guidelines given by the ATS, 1979. Furthermore, using standard methods, personal dust monitoring for airborne inhalable and respirable fractions was carried out at different worksites. Atmospheric concentrations of fiberglass fibers in the breathing zone of exposed workers were estimated to be 44.5 and 6.27 mg/m^3 in line and tissue units, respectively. These values exceeded the current standards. Analysis of the data revealed that there was no significant difference between the prevalence of respiratory symptoms among exposed and unexposed workers. Similarly, the prevalence of abnormal radiographic findings such as chronic inflammatory process, calcification and fibrosis in both groups was not significantly different. Furthermore, the results of spirometry demonstrated that lung function parameters, i.e. vital capacity (VC), forced vital capacity (FVC), forced expiratory volume

in the first second (FEV1) and the percentage ratio of FEV1 to FVC in exposed workers were comparable with those of control subjects. In conclusions, our data provide further evidence in favor of the notion that exposure to fiberglass fibers is unlikely to be associated with respiratory symptoms and functional impairments.

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P6-03

Comparative study of two dosage regimens of pralidoxime in human organophosphate poisonings

Hafed Thabet[2], Pascal Houzé[1], Baud Frédéric[1], M. Amamou[2]

[1] *INSERM U705, Paris, France;* [2] *Reanimation Medicale e Toxicologique, Tunis, Brazil*

Standard therapeutic protocols of organophosphate (OP) poisonings include decontamination, symptomatic treatment, and antidote as atropine and pralidoxime (PRX). As previously reported, the efficiency of PRX depends on its plasma concentrations. In the present study, we compared two dosage regimens of PRX aiming at: (1) establishing the different kinetics of PRX; (2) comparing the efficacy of these two treatments on the reactivation of serum cholinesterase; (3) proposing a dosage regimen with clinical efficacy. Thirty-eight patients (>15 years old, sex ratio F/H = 0.9) were included in a randomized prospective study between April and November 2005 in Intensive Unit Care. On admission, OP poisoning was assessed by the decrease of cholinesterase activity and clinical findings. The severity was graduated from 1 to 4. All patients received atropine and a loading dose of PRX (5 mg kg^{-1}) followed by continuous intravenous infusion using two doses: 25 mg kg^{-1} j^{-1} (G1, $n = 19$) or 50 mg kg^{-1} j^{-1} (G2, $n = 19$). Blood collection was performed from H0 (after loading dose), H6, H12 up to H24 to determine PRX concentrations and serum cholinesterase activity (SCA). All results are expressed as mean ± S.E.M. Statistical analysis was performed using Student's t and Chi^2 tests with $p < 0.05$ as significant value. In all cases, ingestion was observed in a suicidal attempt. On admission, muscarinic syndrome was observed in 100% cases associated with nicotinic (36.8%) and central (39.5%) syndromes. The mean SCA value was 702 ± 602 IU L^{-1} (normal range: 5000–12,000 IU L^{-1}). Atropine was infused in 89.4% of patients and mechanical ventilation was required in 23.6% of cases (mean duration: 27 ± 23 h). The kinetics of PRX showed that in the G1 group, the mean values for all times were constant between 2.5 and 2.8 mg L^{-1}. In G2 group, PRX values were upper than 3 mg L^{-1}. Between two groups no statistical differences were observed except for the duration of mechanical ventilation duration ($p < 0.05$). SCA was returned in normal range 24 h (G2) and more than 36 h (G1) after start of PRX infusion. Our study confirms that cholinesterase activity was not correlated with clinical outcome and PRX efficiency. However, PRX efficiency seems dose-related as proved by fasted cholinesterase reactivation, decrease of duration of mechanical ventilation when the 50 mg kg^{-1} j^{-1} dose was used. But, co-administration of atropine precludes clearly comparing the efficiency of the two PRX dosage regimens.

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P6-04

Ventilatory disorder induced by formaldehyde exposure

Abdolreza Rajaeifard, Masoud Neghab

School of Health, Shiraz, Fars, Iran

The potential of formaldehyde to produce chronic respiratory tract disease is a controversial issue and evidence for associations between exposure to formaldehyde and either respiratory symptoms or functional impairment has not been conclusive.

The main purpose of this study was, therefore, to evaluate the possible respiratory effects of formaldehyde exposure. The study population consisted of a group of 80, randomly selected, male workers with current occupational exposure to formaldehyde vapours and 80 healthy male office workers without present or past exposure to formaldehyde. Subjects were interviewed and respiratory symptom questionnaires as suggested by the American Thoracic Society (ATS), were administered to them. Furthermore, pulmonary function tests were carried out with a calibrated Vitalograph spirometer.

Respiratory symptom questionnaires revealed that exposed workers compared to control subjects had higher prevalence of wheezing and shortness of breath. Similarly, analysis of the data demonstrated statistically significant reduction in lung function parameters (i.e., vital capacity, $p < 0.0001$, forced vital capacity, $p < 0.0001$, FEV1, $p = 0.0013$ and FEV1/FVC ratio, $p < 0.0001$) of exposed subjects compared to the corresponding values for their control counterparts.

These findings, which are in full agreement with our previous observations, provide further evidence in favour of the notion that chronic exposure to formaldehyde induces respiratory symptoms and ventilatory disorders.

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P6-05
Combined effect of electromagnetic radiation of extremely high frequencies and chemical compounds on biological objects

Svetlana M. Rogacheva, Pavel E. Kuznetsov, Ulia A. Malinina, Era B. Popyhova, Svetlana A. Denisova, Alexander U. Somov

Saratov State Technical University, Saratov, Russian Federation

Weak electromagnetic radiation of extremely high frequencies (EMR EHF) causes significant modification in the functioning of biological objects of different organization levels. The most effect was discovered at "resonant" frequencies. Water structure disorganization was shown to play an important role in this effect. The aim of the work was to study the combined effect of EMR EHF and chemical compounds with different physiological activity on erythrocytes and infusoria *Paramecium caudatum*. There were chosen alkaloid nicotine and antimicrobial drug metronidazole (1-(2′hydroxiethil)-2-methil-5-nitroimidazole). These compounds were shown to possess contrary effect on the subsuface water of cell and model membranes.

Hemolytic stability of erythrocytes and hemotaxis activity of *P. caudatum* under the effect of different concentrations of the substances (10^{-15} to 10^{-3} M) and different frequencies of EMR (52–75 GHz) were studied. The density of the radiation current was 120 μW/cm^2. There was used pyramidal horn-type antenna with 12 cm length and aperture 42 cm × 50 cm. The erythrocyte suspensions in phosphate buffer, pH 7.2, with initial $D_{670} = 0.8$ were affected by EMR or/and the compound solution for 150 min at a room temperature. The optical density was measured every 30 min by spectrophotometer Specol 221, its changing indicated the hemolytic stability of erythrocytes. The hemotaxis activity of *P. caudatum* was measured using the equipment for bioassay "Biotester-2", Russia.

It was shown that metronidazole protected erythrocytes and microorganisms from the negative effect of EMR at resonant frequencies 55 and 65 GHz. There was no protective effect of metronidazole from electromagnetic fields at non-resonant frequencies. The effect of nicotine was on the contrary to the same of metronidazole. The preliminary experiments carried out with laboratory rats proved the results obtained using the described test systems.

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P6-06
Excretion of unchanged toluene, ethylbenzene, xylene and mesitylene in urine after experimental exposure of human volunteers

Beata Monika Janasik, Marek Jakubowski

Institute of Occupational Medicine, Lodz, Poland

The results reported recently in the literature show that the measurement of urinary excretion of unchanged solvents in urine provides highly sensitive and specific index of occupational exposure to volatile organic compounds (VOC's). However, there is insufficient information at present on the kinetics of excretion of VOC's in urine.

The aim of this study was to evaluate the kinetics of urinary excretion of unchanged toluene, ethylbenzene, xylene and mesitylene after termination of inhalation exposure and the precision of evaluating of exposure on the basis of determination of unchanged toluene ethylbenzene, xylene and mesitylene in urine and in blood as well as the metabolites in urine.

The subjects of this study were male volunteers. Volunteers were exposed to toluene, ethylbenzene, xylene and mesitylene in concentration of 200 mg/m^3 for 4 h at rest. Urine samples were collected before the onset of exposure, every 2 h during exposure and within 10 h after its termination and after 24 h from the onset of exposure. Capillary blood samples obtained from the finger tips were collected before the onset of exposure and at 0, 5, 30 90 min and 16 h after termination of exposure.

The obtained results describe for the first time the kinetics of excretion of unchanged VOC's after experimental inhalation exposure. The excretion curves of unchanged toluene, ethylbenzene, xylene and mesitylene and within the period of 4 h are characterized by single-phase process of half-lives of 1.45 h (toluene), 1.18 h (ethylbenzene), 1.47 h (xylene) and 2.07 h (mesitylene), respectively.

The obtained result suggest that determination of unchanged VOC's in urine can be used as an exposure test even in the range of concentrations of VOC's in the air of occupational settings are much lower than the present recommendations. The arguments for the use of this method are the noninvasive specimen collection, the possibly minor kinetic influence in comparison with VOC levels in blood, and the simultaneous quantification of mixture compounds in a single urine sample.

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P6-07
Effects of maternal exposure to PCB 153 during gestation and lactation on growth and functional development of the offspring

Krystyna Sitarek, Sławomir Gralewicz

Nofer Institute of Occupational Medicine, Łódź, Poland

Polychlorinated biphenyls (PCB) are environmental pollutants. They are toxic for the central nervous system and the endocrine system, especially during development. PCB 153 is the congener present in breast milk. The purpose of the present work was to study the effect of PCB 153 exposure during gestation and lactation on the postnatal offspring's development. Female rats were exposed orally to PCB 153 at doses 1.0 mg/kg/day (low exposure) or 5.0 mg/kg/day (high exposure) starting from day 7 of pregnancy to day 21 post partum. A battery of routine tests was used for assessment of the morphological and functional development of the offspring from the day of delivery to weaning. The reproductive performance of dams was not overtly affected by the exposure. However, the litters delivered by dams of the PCB exposed groups were noticeably heavier than the litters delivered by control dams. Results of the tests and measurements indicate: (i) a faster rate of the body weight increase in the offspring of the high exposure group, (ii) a faster incisor eruption, pinna detachment and eye opening, in the male and female progeny of the low exposure group, (iii) a faster progress in the negative geotaxis in males of both exposure groups and (iv) a poorer performance in the forepaw suspension test in males and females of both exposure groups. In summary, neither high (5.0 mg/kg/day) nor low (1.0 mg/kg/day) exposure to PCB 153 from GD7 to PND21 affects adversely health status and reproductive performance of the exposed dams. PCB 153 exposure during gestation and lactation results in accelerated physical development of the offspring. It results, however, in some transient functional deficits, i.e. lower muscular strength and/or endurance.

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P6-08
Exposure to lead and indicators of biological effect

Ligia Fat [1], Ludovic Gyorffy [2], Mariana Ghiurca [2]

[1] *Institute of Public Health, Cluj Napoca, Cluj, Romania;* [2] *District Hospital, Baia-Mare, Romania*

Objectives: The medical literature shows that both occupational and environmental exposure to lead affect the human health. Once in organism, lead initial produce the physiological absorption state, than the high absorption state that can be diagnosticated using toxicological investigations. Clinical manifestations appear when the absorbed lead is higher than the eliminated lead, generating the acute or chronic intoxication. The transition from one state to another depends on the exposure time and noxes concentration. The aim of this study is to analyze comparative, using some specific and precocious affected biological indicators, two groups of people occupationally and environmentally exposed to lead.

Methods: We investigated 195 workers from the "melting" sector of a non-ferrous metallurgical plant with the mean age = 31.81 ± 8.19 years and the mean time of exposure = 9.03 ± 6.67 years, and 255 people who work in the administrative sector of the same factory and live in the proximity, with the mean age = 35.66 ± 8.49 years and the mean time of exposure = 7.73 ± 6.16 years. We made clinical exam, biotoxicological investigations, urinary delta amino levulinic acid (ALA-u), zinc protoporphyrin (ZPP), standard questionnaires regarding the effects of lead on the human organism, neurological and psychological exams. The air lead exceeded 71.3 times the maximum accepted values.

Results: We found high values of ZPP, over 10 μg/l, in 78.6% exposed workers and 28.2% people from the environmental group. In the environmental group (aged 20–35 years and length of service under 5 years), 19.1% had ZPP over 10 μg/l. High values of ALA-u, over 10 mg/l, were found in 68.1% of the exposed workers and 27.4% from the environmental group. In the group aged 20–35 years and length of service under 5 years, 62.9% workers and 16.6% controls had values over 10 mg/l. There were concordance between ZPP and ALA-u values ($r = 0.567$, $p < 0.001$).

Conclusions: There were significant differences between the exposed and control group regarding the values of the intoxication marker ZPP ($p < 0.001$) and also for the values of ALA-u, which is specific for the lead intoxication ($p < 0.001$). There were also significant differences regarding ZPP and ALA-u values between the two groups (aged 20–35 years and working length under 5 years) ($p < 0.001$). The most affected and sensible were

the younger people. The general and professional morbidity correspond with the clinical and toxicological data.

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P6-09

Symptoms and immunological markers in vulcanization workers in the southern Sweden rubber industries

Lena S. Jönsson, Karin Broberg, Anna Axmon, Bo A.G. Jönsson, Margareta Littorin

Occupational and Environmental Medicine, Lund University, Sweden

Workers in the rubber industries have generally an increased risk of developing several diseases, such as cardiovascular and respiratory diseases, as well as cancer. However, the prevalence of diseases in the Swedish rubber industry today is largely unknown. In addition, the exposure situation is complex and exposure assessment is limited and it is therefore in many cases unclear which agents induce the different diseases. One way to handle this is to use index substances, i.e. a specific substance as a marker of a larger group of compounds. One such substance could be 2-thiothiazolidine-4-carboxylic acid (TTCA), which traditionally has been used as a marker of carbon disulphide (CS_2), present in vulcanization fumes.

We examined 166 exposed workers and 117 unexposed controls. Medical and occupational histories were obtained by structured interviews. Symptoms, which had occurred during the past 12 months, were recorded and immunological markers in blood analyzed. Urinary levels of TTCA were analyzed by liquid chromatography tandem mass spectrometry.

Compared to controls, the exposed workers had increased risks of symptoms from the eyes (itching, running and/or burning; odds ratio (OR) 3.0), as well as nose bleeding (OR 4.2), throat burning and dryness (OR 3.1), hoarseness (OR 2.2), severe dry cough (OR 3.6), nausea (OR 4.5) and headache (OR 2.5). No significantly (p-value <0.05) increased risks of other nasal symptoms or of dyspnea, wheezing or chest tightness were observed. When dividing the exposed workers into three equally sized groups according to TTCA levels and comparing each group with the controls, we observed the highest risk among exposed workers with intermediate TTCA levels. Furthermore, exposed workers in all three TTCA subgroups had significantly increased concentrations of total plasma IgG compared to controls. Increased risks of elevated concentrations of leukocytes, neutrophils or eosinophils were observed in the group with high levels of TTCA.

This study suggests that workers in the Swedish rubber industries have an increased risk of several symptoms and have elevated levels of some immunological markers. Additionally, it shows that urinary levels of TTCA are useful for biomonitoring of exposure–symptoms relationships in the rubber industry.

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P6-10

Interactions between chemicals and drugs in the workplace
Is there a cause for concern?

Claude Emond, Caroline Lapointe, Adolf Vyskocil

Université de Montréal, Montreal, Canada

Workers are frequently exposed to mixtures of chemicals. Additionally, they intentionally use different prescription and over-the-counter drugs to treat various health conditions. Acetylsalicylic acid (AAS), acetaminophen and cimetidine are the most non-prescription drugs used. In respect of worker's life privacy and because of ignorance, the potential interactions between drugs and workplace chemicals are not usually considered. It appears that a better understanding of interactions between drugs and workplace chemicals may contribute to diminish the risk of toxic effects. The objective of this study was to identify binary interactions between drugs and regulated toxic substances found in workplaces (principally solvents and pesticides). Information was drawn from primary references available on TOXLINE™ and PubMed™ databases. Human as well as animal studies were evaluated. Since at massive doses most chemicals would cause polysystemic effects, the interactions were evaluated only for realistic exposure concentrations of workplace chemicals up to the short-term exposure limit or ceiling value or five times the 8-h time-weighted-average (TWA) permissible exposure limit (PEL) for human data and up to 100 times the 8-h TWA PEL or ceiling value for animal studies. Only few reasonable human and animal studies were identified involving almost exclusively non-prescription drugs. Thus, 26 pairs of binary co-exposures of drugs and workplace chemicals from 20 studies were identified. From them 7 pairs presented potentiation effects, 7 presented kinetic inhibitions and 12 reported no interactions. For example, AAS potentiates ototoxic and developmental effects of toluene and phenobarbital potentiates hepatotoxic effects of trichloroethylene and carbon tetrachloride. This review highlights a lack

of toxicological studies evaluating co-exposure of drugs and workplace chemicals for realistic exposure concentrations. An effort in this field might help scientists to understand changes in the metabolism and toxic effects of workplace chemicals due to co-exposure to drugs.

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P6-11

Operator exposure risk assessment of benzimidazole fungicides on Korean agricultural condition

Je Bong Lee [1], Jin Sup Shin [1], Mi-Hye Jeong [1], Yeon-Ki Park [1], Geon-Jae Im [1], Kyu Young Kang [2]

[1] *National Institute of Agricultural Science and Technology, Suwon, South Korea;* [2] *Department of Environ. Biotechnology, Gyeong Sang National University, Jinju, South Korea*

Pesticide risk assessment for pesticide operators as well as for consumers has become one of the pesticide regulatory tools to reduce any unreasonable adverse health effects from pesticide use. The risk for pesticide operators can be quantified by comparing the acceptable operator exposure level (AOEL) with exposure level during pesticide application.

This study is to evaluate the risk of benzimidazole fungicides application worker. The exposure level of pesticide applicators were calculated using Japanese operator exposure study tested with EPN 45% EC. The AOELs for pesticides were obtained dividing relevant lowest no observed abuse effect levels (NOAELs) for the exposure scenario into uncertainty factor, 100. For the non-cancer and cancer occupational risk assessment, Q_1^* produced by US/EPA and life time average daily dose (LADD) calculated from average daily dose (ADD), treatment days per year, worked years for life time were used. Operator exposure for benzimidazole fungicides application were benomyl 0.2, carbendazim 0.36 and thiophanate-methyl 0.42 mg/kg/day. Short-term AOELs for benomyl, carbendazim and thiophanate-methyl were 0.3, 0.1 and 0.2 mg/kg/day, and long-term AOEL were 0.025, 0.025, 0.08 mg/kg/day, respectively. LADDs were benomyl 0.0038, carbendazim 0.0067, thiophanate-methyl 0.0081 mg/kg/day. The ratios of exposure to AOEL were 0.28–1.5 for short-term and 3.73–9.88 for long-term. Cancer risk for operator were 9.12×10^{-6} for benomyl, 1.61×10^{-5} for carbendazim and 1.13×10^{-4} for thiophanate-methyl by the standard application scenario. The result showed three fungicides exceed the risk criteria, 1.0×10^{-6}. The above risk assessments were based upon conservative assumptions and therefore are believed to be protective of the applicator. To refine the risk at the more actual conditions, further risk assessment with more realistic data would be needed.

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P6-12

Circulating levels of tumor necrosis factor-α in lead occupationally exposed workers

Concettina Fenga [1], Sebastiano Gangemi [2], Giovanna Spatari [1], Anna Familiari [1], Matteo Valentino [3], Venerando Rapisarda [1], Irene Polito [1], Domenico Germanò [1]

[1] *Department of Social and Environmental Medicine-Section of Occupational Medicine, University of Messina, Italy;* [2] *School of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Italy;* [3] *Department of Molecular Pathology and Innovative Therapies, Clinic of Occupational Medicine, Polytechnic University of Marche, Ancona, Italy*

Lead is a common environmental contaminant that is found both in occupationally exposed workers and in populations not directly exposed to lead. It may affect humoral and cellular immunity, although not at the concentrations normally found in industrialized countries. Cytokines have a role in the immune balance. We measured the plasma levels of tumor necrosis factor-α (TNF-α) a pro-inflammatory cytokine. TNF-α serum concentrations were measured in 30 healthy workers with low exposure to lead (mean PbB: 24.7 ± 15.18 μg/dl) operating in a exhaust lead battery storage factory and in a group of 30 healthy donors.

The serum levels of TNF-α was significantly higher in exposed than controls (3.15 ± 1.87 pg/ml versus 2.13 ± 1.31 pg/ml; $P = 0.005$).

The increase of TNF-α showed in this study may be correlated to a direct action of lead on membrane TNF-mononuclear cell receptors. Lead is known to have a high affinity for cysteine membrane proteins and may thus interact with membrane receptors, modifying the membrane fluidity of erythrocytes in lead-exposed workers in the same way as it modifies the membrane fluidity of polymorphonuclear cells incubated with lead.

In order to better understand the effects of lead to the immune balance further studies are needed.

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P6-13

Evaluation of genetic damage in workers employed in a rubber tyres production utilizing the comet assay

Joao Teixeira, Blanca Laffon, Susana Silva, Joana Roma-Torres, Carla Costa, Patricia Coelho, Josefina Mendez, Olga Mayan

The production of rubber and rubber products is a large and diverse industry. A broad spectrum of chemical compounds belonging to many structural and use categories is employed during rubber processing (vulcanization agents, accelerators, activators, colorants, solvents, etc.) usually at high temperatures. Convincing evidence of an excess of certain forms of cancer among rubber workers has been provided. The objective of this study was to determine the genotoxic effects in a group of individuals engaged in the production of rubber tyres from a Portuguese factory. Peripheral blood samples were collected from 32 exposed workers and 32 controls, and micronucleus (MN) test, sister chromatid exchanges (SCE) and comet assay were performed. Urinary thioethers were measured as a general biomarker of exposure to electrophilic compounds, and genetic polymorphisms in metabolizing enzymes (CYP2E1 *Dra*I, EPHX1 codons 113 and 139, GSTP1 codon 105, and GSTM1 and GSTT1 deletion polymorphisms) were analyzed as susceptibility biomarkers. Thioethers excretion was found significantly higher in rubber workers. No significant increase was observed in MN or SCE frequencies in the exposed population as compared with control individuals, although MN frequencies were higher in the exposed group. Comet assay data showed decreased TL values in the exposed population with regard to the control group that might indicate the induction of crosslinks by the substances present in the workplace environment. Significant increase in MN frequency was obtained for GSTT1 null exposed individuals with regard to positive ones, and interaction with GSTP1 polymorphism was found. Higher levels of cytogenetic tests frequencies were observed in epoxide hydrolase expected low activity donors with regard to medium and high activity individuals. No effect of CYP2E1 or GSTM1 variants was obtained in the biomarkers analyzed.

The DNA samples from exposed workers and control population will be used for further quantification of DNA adducts using gold nanoparticle probes.

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P6-14

Surveillance of respirable dust in limestone and dolomite surface mines

Potharaju Venkata Sai Prabhakara Murty, Kalaga V. Lakshman Rao

Visakhapatnam Steel Plant, Andhrapradesh, India

Occupational exposure to respirable crystalline silica over a period of time may lead to silicosis and other respiratory ailments. Occupational Exposure Limits (OEL) for respirable crystalline silica is established by many organizations throughout the world. However, the values are not harmonized to assess the occupational risk and to provide similar levels of worker health protection in different regions of the world. Sampling locations within the mines, differences in the number of samples, duration and frequency of the sampling, are the key factors in assessing OELs. Workplace respirable dust monitoring is conducted in captive mines of Visakhapatnam steel plant as a part of occupational health and hygiene surveys. The gravimetric dust sampler (MRE NCB), type 113A, Serial Number 021872, Casella, London, was used for the collection of airborne respirable dust. Flow rate of the sampler is 2.5 l/min. Results presented here represent the years 2001–2005 at 12 different locations of two mines. Results are presented in 8 h time weighted average (TWA) concentration ($mg\,m^{-3}$). In Indian context as per mines act, permissible limit of respirable dust should not exceed (15/% of respirable silica) to treat workplace as harmless. Free silica content in the respirable dust samples was analyzed by X-ray diffraction analysis. All the workers are subjected to periodical medical examination, which include spirometric and chest X-ray evaluations. The chest X-rays are classified as per standard International Labor Organization (ILO) classification. In the limestone mine the average values range from 2.1 to 11.2 $mg\,m^{-3}$ and in dolomite mine average values range from 2.7 to 10.1 $mg\,m^{-3}$. It revealed that there is a significant improvement in the work practices as the levels are low in the year 2005 at all locations compared to other locations in the preceding years. X-ray diffraction analysis indicated that the presence of free SiO_2 in the samples is negligible. In the year 2005 the concentrations are below the permissible levels 7.5 $mg\,m^{-3}$ at all locations in the two mines. Periodical medical examination results have not shown any evidence of abnormalities related to exposure to free silica. There is a need to conduct questionnaire survey on respiratory symptoms, occupational history and to integrate with the medical screening results to identify the possible occu-

pational health and safety interventions at the workplace.

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P6-15

Gynaecological disturbances among females engaged in the manufacture of sex hormones

Mona Sobhy Siha, Mona Mohamed AbdelBadii

Faculty of Medicine, Cairo, Egypt

Introduction and objective: Numerous studies have established as association between exposure to sex hormones and many gynaecological troubles. The aim of this work is to investigate the different gynaecological disturbances which may affect female workers occupationally engaged in the manufacture of contraceptive pills and other hormonal preparations.

Materials and methods: The total number of female workers was 214, a control group of 220 subjects was taken of comparable age and socioeconomic status and not exposed to any external source of hormones. All workers were subjected to a prepared questionnaire including present, past, family and occupational history. Gynaecological examinations were carried out for married female workers who agreed to cooperate with the team (137 exposed, 180 control). Virgins were excluded.

Results and discussion: This study showed the presence of masculinizing signs among the exposed group. There was also positive statistically significant difference between exposed and control on comparing gynaecological disorders. Hysterectomy was done to 11.2% of exposed workers versus 3.6% of non-exposed workers. Our study showed a significant positive relationship between duration of exposure and the prevalence of hysterectomy cases. About 51% of married exposed workers had reproductive disorders. Gynaecological examination showed that exposed workers suffered from vulvovaginitis (46.7%), cervical erosion (3.9%) and leucorrhea (62.8%) ($P < 0.05$). About 12% of the exposed workers complained of some family health disturbances in the form of precocious puberty in female children, gynaecomastia in male children and husbands.

Recommendations: We recommend health education for exposed workers for the importance of the use of protective equipments and enclosure of machines. Periodic medical examination should be carried out regularly for early detection of affected personnel.

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P6-16

Occupational hazards and illnesses among working women in semiconductor industries at Cavite Export Processing Zone: Baseline Data Gathering and Design of a Health Program

Jinky Del Prado-Lu

Occupational Health Program of the National Institute of Health, University of the Philippines, Manila, Philippines
College of Arts and Sciences, UP, Manila, Philippines

This is a cross-sectional study on occupational hazards and illnesses among women workers in 10 semiconductor industries in a Cavite Export Processing Zone. Stratified sampling techniques were used based on industry size. Workplace ambient monitoring, biological monitoring (blood lead monitoring), survey questionnaires, interviews, focused group discussions and a review of medical records were done. Results showed that noise was the most prevalent physical agent across all the industries (100%, 80% and 67% in small, medium and large-scale industries, respectively). Meanwhile, particulate matter was the most common chemical agent while repetitive motion was the most widespread ergonomic hazard (100% in all industries). Common illnesses and injuries identified were allergies (94.8%), tension headache (86.8%), cough and colds (60.4%), conjunctivitis (71.4%), rhinitis (71.8%), hypertension (79.1%), gastroenteritis (50.4%), UTI (98.2%), dysmenorrhea (90.6%), muscle pain (50.1%), anemia (98.1%) and cuts (65.5%).

Based on the interviews, common problems of women workers are: lack of ventilation and protective equipment, low wages, long working hours, need for overtime, injuries during night shifts, prolonged standing, joint, muscle and low back pain, colds, headache and eye strain. Questionnaires revealed that the top five most frequent occupational hazards among female workers are: backpain, excessive work, heat, poor ventilation and use of chemicals, while the most prevalent illness were cough and colds (59%).

Blood lead levels of 285 subjects revealed that 42.2% of samples were in the 21–40 μg/dL range which is considered by the Department of Health as detrimental to health. The most vulnerable age group was the 21–30 category, which represented 87.5% of the total respondents. The mean blood lead level was 20.7 μg/dL.

When hazards and illness were correlated with alpha set at 0.05, high noise levels in the workplace was associated with migraine and hearing loss ($p = .000$). Heat in the workplace was also shown as statistically significant

in the occurrence of skin allergies. Arthritis was associated with extreme cold temperature ($p = .003$). Other significant associations included tiredness and fatigue with vibration of machines, radiation exposure with bone pain, extreme fatigue and skin allergies, dust exposure with eye strain, viral exposure with cough and colds, PTB and fatigue. These results show that hazards are prevalent in the workplace and may be associated with certain occupational illnesses among female workers.

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P6-17
Occupational exposure to lead in a pigments manufactory

Luciana Daniela Tamaian

Prophylactic Medicine Center, Cluj-Napoca, Romania

Introduction: In countries in a social and economic transitional period, a large number of workers are exposed to lead. Lead is one of the most toxic metals. In industrial environments, particularly in pigment manufactories, workers are professionally exposed to lead.

Objective: The aim of this study is to analyze the effects of the occupational exposure to lead, in a pigment factory.

Materials and methods: The study was conducted in 200 men, working in a pigments factory (the exposed group), and 145 randomly selected men, without professional exposure to lead (control group). None is expose to noise. The lead concentration in the manufactory exceeded by 20–40 times the maximal admissible values (0.1 mg/m^3). We analyzed medical history, biomonitoring data (urine and blood lead levels), urinary DAL, the hemoglobin concentration (Hb), the count of red blood cells (BBC) and color index (CI). The two groups were tested by air and bone conducted audiometer in a sound isolated chamber.

Results: The biochemical tests performed showed high levels of blood lead to 75 workers exposed (mean 36 ± 8 μg%) and normal levels of blood lead in the control group (10 ± 2 μg/dl). There were high levels of PbU and DAL to workers of the exposed group (PbU: mean 123 ± 2 μg/l, DAL: mean 29 ± 4%). The mean of Hg in workers exposed was 11.5 g/dl, and from control group 13.9 g/dl. The count of RBC was 4×10^6 ml^{-1} for the exposed group and 4.89×10^6 for the control group. CI was 1.00 for the exposed group and 1.09 for the controls. One hundred and seventy subjects from the exposed group reported vertigo, weakness and lack of appetite. The audiometer showed an important hearing loss (40–50 dB for high frequencies) to 75 workers exposed to lead and a mild hearing loss (25–30 dB for high frequencies) to others 72 workers exposed. Only two non-exposed workers had a mild hearing loss (10–15 dB for 4 kHz).

Conclusions: Workers in pigments factory are professional exposed to lead. Workers with high exposure to lead have neurasthenia, anemia and hearing loss.

Discussion: Early diagnosis and treatment, rearrangement of working condition and personal hygiene are very important in order to prevent the occupational diseases in pigment factories.

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P7 Oxidative Stress

P7-01
Dimethyl sulphoxide reduces hydroxyurea induced abnormal development in mouse embryos

R. Pérez-Pasten [1], E. Martínez-Galero [2], J.A. Morales-González [1], G. Chamorro [2]

[1] *Pharmacy Academic Area, Health Sciences Institute, Autonomous University of the State of Hidalgo, Mexico;* [2] *Preclinical Toxicology Laboratory, National School of Biological Sciences, National Polytechnic Institute, Mexico*

Dimethyl sulphoxide (DMSO) has been well recognized for its antioxidant activity. This research was conducted to assess the protective effect of DMSO on abnormal mouse embryo development resulting from hydroxyurea (HU) *in vitro* exposure.

Early somite embryos were cultured in immediately centrifuged rat serum (culture medium), having added HU 2 μM 1 h later, with and without 0.1% DMSO (v/v), and were then incubated for an additional hour. Embryos were then replaced in fresh culture medium for 48 h, followed by growth and differentiation evaluations to determine developmental and embryonic defects. DMSO did not alter embryonic growth and development, while embryos exposed to HU only, showed significant viability reduction, developmental retardation and a high incidence of neural closure tube defects, among other abnormalities. Comparatively, co-treatment with DMSO–HU increased embryonic viability, DNA content and development score. Furthermore, it reduced the incidence of major abnormalities such as neural tube defects and rotation failure, but it was not able to decrease the incidence of other specific abnormalities, as well as overall embryonic growth, which did not reach normal values. However, values were significantly higher then those achieved with HU alone.

The partially protective effect of DMSO against HU induced developmental damage was shown. Therefore, the effect observed could be related to the antioxidant activity of DMSO. HU induced teratogenic insults have been shown to be probably mediated by DNA synthesis inhibition, in addition to oxidative damage. More research is needed to clarify the mechanism involved in DMSO antiteratogenic effects.

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P7-02

Morphologic effects of Vitamin E on hepatic regeneration induced by partial hepatectomy in ethanol exposed rats

R. Perez-Pasten [1], J.A. Morales González [1], P.R. Saldaña [2], J.B. Romero [3], S.R. Mendiola [2], R.I.S. Domínguez [2], A.K. Bernardino González [2]

[1] *Pharmacy Academic Area, Health Sciences Institute, Autonomous, University of the State of Hidalgo, Mexico;* [2] *UNAM;* [3] *Vicente Guerrero, Hospital-IMSS*

The morphological effect of Vitamin E during ethanol-induced hepatic regeneration (HR) inhibition was investigated.

Partial hepatectomy (HP) was performed on adult Male Wistar rats. After HP, animals were treated with ethanol alone or ethanol plus Vitamin E at different doses. HP and sham HP rats were used as controls. Ethanol 36% (v/v) was dispensed for 7 days, while Vitamin E was administered daily at the following doses: 100, 200, or 400 international units (IU). At day 7, rat livers were obtained for weight gain determination, in addition to light microscopy analysis.

Light microscopy demonstrated that HP induces cellular disorganization and an increase of mitosis. Ethanol provoked an increase in fat droplets, inflammatory infiltrate and congested cells, as well as absence of mitosis. On the contrary, Vitamin E administration reverted ethanol-induced changes, this effect was evident at the 200 and 400 IU.

These data demonstrate that Vitamin E possesses a protector effect on ethanol-induced morphologic changes during hepatic regeneration. To ascertain the mechanism of action of Vitamin E additional studies at cellular as well as at molecular levels are needed.

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P7-03

Effect of adrenaline and oxygen free radicals on calcium tolerant cardiomyocytes: Formation of glutathione adducts

Vera Costa [1], Márcia Carvalho [2], Félix Carvalho [1], Maria Lourdes Bastos [1], Rui Carvalho [3], Fernando Remião [1]

[1] *Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;* [2] *Faculdade de, Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal;* [3] *Centro de Neurociências de Coimbra, Coimbra, Portugal*

Cardiovascular diseases are one of the most common causes of death in the Western World. During the ischemic/reperfusion process in the heart, large amounts of catecholamines are released and reactive oxygen species are generated. Freshly isolated cardiomyocytes have been used as a model for cardiotoxicity studies and previous studies reported that the catecholamine oxidation process was deleterious to these cells. One of the aims of the present work was to establish the effects of catecholamines in the presence or absence of a system capable of generating oxygen free radicals. Superoxide radical was generated using the xanthine (0.1 mM) and xanthine oxidase (0.01 U/mL) system (X/XO). The oxidative stress insult was assessed by evaluating intracellular glutathione levels. Incubation of cardiomyocytes with adrenaline (0.5 mM) for 3 h induced a significative depletion of total and reduced glutathione. While the X/XO system had no observable effect, the concomitant incubation of X/XO and adrenaline resulted in a significant potentiation of adrenaline's mediated effect on lowering the levels of glutathione. Furthermore, glutathionyl-adrenaline adduct was found by HPLC with electrochemical detection both in the cells and in the extracellular medium in the suspensions incubated with adrenaline in the presence or absence of superoxide radicals. In spite of this deleterious effect, there was no change in cardiomyocytes viability during the end-points tested.

In conclusion, the reactivity of adrenaline, in presence or absence of superoxide, in freshly isolated cardiomyocytes leads to oxidative stress, reflected by the loss of intracellular glutathione homeostasis. The formation of adrenaline adducts with nucleophilic groups, as glutathione, decreases cell's defence levels, which may ultimately result in cardiotoxicity.

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P7-04

In vivo investigation of efficiency and preventive role of selenium and zinc on aspirin induced impairment on antioxidant system, hepatic and renal toxicity

Onur Erdem, Ahmet Aydin, Ahmet Sayal, Akay Cemal, Vural Kesik, Ahmet Emin Kurekci

Gulhane Military Medical Academy, Ankara, Turkey

Aspirin (acethylsalicylic acid) is an antiinflammatory drug that is widely used in arthritis because of its cheapness and efficiency since 1899. Aspirin impairs the antioxidant system, shifts the balance between oxidants and antioxidants towards oxidants, causes hepathic and renal injury. Our study was undertaken to determine whether aspirin induced antioxidant damage, renal and hepathic injury can be prevented or decreased by selenium and zinc supplementation. Wistar albino male rats were used in the experiments. Rats were randomly divided into 5 groups that each group consists of 15 rats. The rats in group I were treated orally with 75 mg/kg/day aspirin, group II were treated orally with 75 mg/kg/day aspirin, 0.1 mg/kg/day selenium and 1 mg/kg/day zinc, group III were treated orally with 50 mg/kg/day aspirin, group IV were treated orally with 50 mg/kg/day aspirin, 0.1 mg/kg/day selenium and 1 mg/kg/day zinc, group V were untreated and selected as control group. All groups were treated at the same dose for 2 weeks and in the remaining 3 weeks the aspirin dose was reduced %25 for each week while selenium and zinc doses were same. The treatment lasted at 5 weeks time and the rats were sacrified at the end of the treatment. Blood, liver and kidney tissues were taken for antioxidant enzyme and pathologic investigation. The results of this study have confirmed that erithrocyte superoxide dismutase and erithrocyte glutathione peroxidase enzyme activities were decreased, erithrocyte malondialdehyde levels increased both high and low dose aspirin treatment. Erithrocyte malondialdehyde levels increased both high and low doses but the protective effect of selenium and zinc was seen. The levels of ALT, uric acid, direct bilirubine which were reflecting indirectly the renal and hepathic injury were increased and no protective effect of selenium and zinc were seen. There were minor changes in pathologic specimens which were not statistically significant reflecting that aspirin has no toxic effects on liver and kidney at theuropathic doses. The results revealed that aspirin made changes in antioxidant system even at theuropathic doses, made no changes in hepathic and renal pathology and selenium and zinc has a protective effect on keeping the antioxidant status.

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P7-05

Parameters of oxidative stress after ethylene glycol and ethanol oral administration in rats

Joanna Kowalowka-Zawieja, Jedrzej Przystanowicz, Barbara Zielinska-Psuja, Jerzy Orlowski, Maksymilian Kulza

Department of Toxicology, Poznan University of Medical Sciences, Poland

Ethylene glycol (EG) is widely used in industry, it is also a basic component of many automotive fluids: antifreeze coolants, windscreen washers and de-icers. Accidental or intentional (as ethanol replacement) ingestion of these liquids leads to intoxication, which is often fatal.

In Poland, i.v. ethanol administration is a basic method of treatment of EG poisoned patients. Ethanol acts as a competitive inhibitor of alcohol dehydrogenase, an enzyme converting ethylene glycol into very toxic metabolites. Ethanol therapy is effective but causes many adverse effects, such as central nervous system depression, apnea or hypoglycaemia.

In the present study we investigated changes in oxidative stress parameters in male Wistar rats. We measured activity of superoxide dismutase (SOD), glutathione reductase (GSSG-Rd), glutathione *S*-transferase (GST), glutathione peroxidase (GSH-Px) and lipid peroxidation (as TBARS) in animals exposed separately or simultaneously to ethylene glycol and ethanol. Both xenobiotics were administered by gavage once, ethylene glycol in dose 3830 mg/kg b.w. (1/2 DL_{50} in Wistar rats) and ethanol in dose 1000 mg/kg b.w.

Pro- and antioxidative balance tests were performed in cytosolic fraction of homogenates of liver and kidneys taken from rats at 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 h after administration.

Disturbances in pro- and antioxidative balance were revealed in all examined groups. Activities of GSSG-Rd, GSH-Px and GST in liver and kidneys measured with reference to control group were significantly higher in most time points, mainly in 1–6 h period. In samples both from liver and kidneys significantly higher level of TBARS was observed. SOD activity was significantly lower in kidneys of animals exposed to ethanol

only, in other groups SOD activity was significantly higher.

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P7-06

Validation of a HPLC-ECD method for the detection of adrenaline-GSH adducts in biological samples

Renata Silva [1], Vera M. Costa [1], Sílvia Boldt [1], Helena Carmo [1], Félix Carvalho [1], Márcia Carvalho [2], Maria de Lourdes Bastos [1], Fernando Remião [1]

[1] *Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;* [2] *Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal*

Introduction: Sustained high levels of circulating catecholamines may lead to neurotoxicity and cardiotoxicity. Neurotoxic effects induced by dopamine oxidation are involved in Parkinson's disease. Cardiotoxicity can occur indirectly via adrenoceptor overactivation although, increasing evidence also points toward direct oxidative mechanisms. We have shown that the catecholamines oxidation products can conjugate with GSH in isolated rat cardiomyocytes and hepatocytes leading to the formation of more toxic species. A HPLC method to analyse catecholamines GSH adducts in biological samples was developed and this methodology validation is now presented for the adrenaline-GSH adducts.

Methods: Human serum samples were incubated with a reaction mixture of adrenaline, tyrosinase and GSH at room temperature. After 12 min of reaction, the adducts were extracted by alumina adsorption. The extracts were injected into an HPLC equipped with a coulometric detector. A Spherisorb S5 ODS2 column and an isocratic mobile phase (10% methanol, 50 mM citric acid and 0.46 mM octanesulfonic acid, pH 3.0) at 1 mL/min were used. The validation parameters were evaluated, in both extracted and unextracted samples including, instrumental reproducibility, overall procedure reproducibility, linearity, limit of detection and extraction recoveries.

Results and discussion:

(1) The method showed good instrumental reproducibility (<3% CV obtained after 20 injections of the same sample) and good overall procedure reproducibility (<5% CV obtained after injection of 20 different samples at the same adduct concentration).
(2) A linear response was obtained (triplicate analysis of five different concentrations (0–0.1 mM)).
(3) The alumina extraction removes interferences and improves chromatogram analysis in serum samples.
(4) The limit of detection was greatly improved by the extraction (2 pmol and 0.0625 pmol for the 2′ and 5′ mono GSH conjugates, respectively).
(5) The extraction recoveries were different for both adrenaline-GSH monoconjugated adducts (between 47 and 75%, depending on the tested concentration).

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P7-07

The mixture of *p*-xylene and toluene gives antagonistic effect on lipids peroxidation *in vitro*

Sawicka Ewa [1], Długosz Anna [1], Marchewka Zofia [1], Kuźniar Jakub [2]

[1] *Department of Toxicology, Wroclaw Medical University, Wrocław, Poland;* [2] *Department of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wrocław, Poland*

Introduction: Xenobiotics can induce the formation of reactive oxygen species, either directly or by causing cell damage and, thus, activating intracellular sources. Some organic solvents may express their toxicity by inducing free radical process. The interaction may take place in the metabolism of chemical, especially when the similar chemical structure is observed.

It is examined whether free radicals sweepers could be good antidotes against chemicals that generate free radicals. Especially in occupational exposure, this protection against peroxidation could be useful.

Our previous report showed increased lipid's peroxidation process in workers employed at paint and lacquer industry, exposed to mixture of solvents, measured as lipid peroxidation product: malonyldialdehyde with 4-hydroxynonenal (MDA + 4HNE) concentration in the blood. Moreover study on coenzyme Q_{10} (CoQ_{10}) supplementation in the same group of workers employed at paint and lacquer factory showed the significant reduction of increased lipids peroxidation after 4-weeks CoQ_{10} treatment.

The aim of presented study was to examine, whether the exposition to toluene and *p*-xylene could be responsible for increase in MDA. The influence of toluene and *p*-xylene on MDA and hydroxyl radical ($^{\bullet}OH$) generation, both a single and combined exposure, was investigated. The effect of CoQ_{10} on MDA in exposure to toluene and *p*-xylene or their mixture on human placental model was examined.

Material and methods: The research was performed on *in vitro* human placenta mitochondria. MDA was measured spectrofotometrically with thiobarbituric reactive substances (TBARS) method. The level of $^{\bullet}$OH was evaluated by deoxyribose degradation method.

Conclusions: p-Xylene cause increased MDA and $^{\bullet}$OH concentration in mitochondria with the statistically significant correlation dose-answer. Moreover the correlation between MDA and $^{\bullet}$OH after exposure to *p*-xylene is noted. The mixture of toluene and *p*-xylene gives antagonistic effect on lipids peroxidation, decreasing the MDA level.

Coenzyme Q_{10} at a concentration: 3.0 and 12.0 μg/ml successfully inhibits MDA level increased by *p*-xylene.

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P7-08

Oxidative and immune processes in mechanisms of anti-diabetic drugs toxicity

I. Palagina, V. Kozar, M. Kudrya, N. Ustenko

Institute of Endocrine Pathology Problems, Kharkov, Ukraine

Phensuccinalum (*PhS*) and Diacamph (*DC*) are original anti-diabetic drugs (ADDs) with antioxidant activeness representing the dicarboxilic acids derivatives. With an aim of their industrial introduction we have established criteria of their inhalation toxicity considering shiftings in oxidant status and non-specific immune response of Wistar rats. The studied air concentrations of *PhS* (2 months) and *DC* (3 months), respectively, 1.51 ± 0.38 and 3.5 ± 0.08 mg/m^3, were close to forecasted $Limc_{ch}$ levels. State of free radical peroxidation (FRPO), lipoperoxidation (LPO), systems of anti-radical (ARP) and antioxidant protection (AOP) were assessed by chemiluminescence parameters, levels of intermediate and final LPO products, levels of reduced glutathione and Vitamin E, activeness of superoxide dismutase (SOD) and catalase in liver microsomes and homogenate, lung homogenate, serum and whole blood. Non-specific resistance was evaluated by phagocytic and metabolic activeness of neutrophiles and level of heterophilic agglutinins of the blood. We found that under inhalation of *DC* (1 month) the FRPO processes in liver microsomes are activated, and in lung homogenate are depressed accompanied with SOD activeness increase. Two months' inhalation of *PhS* and 3 months' of *DC* proved to change chemiluminescence and biochemical indices of lung homogenate and of liver microsomes and homogenate which fact witnesses a decrease in FRPO and LPO processes intensity. One month after termination of ADDs inhalation in lungs we registered a decrease in SOD activeness (*PhS*) and in Vitamin E content (*DC*) accompanied with activating FRPO and speeding up LPO products oxidation processes. Within inhalation and aftertreatment periods, *DC* weakened phagocytic activeness and oxygen-dependent mechanisms of neutrophiles bactericidal effect, and *PhS* proved to cause opposite changes. We concluded that under *PhS* and *DC* inhalation the oxidant–antioxidant balance state in rats is characterized by a decrease of FRPO and LPO processes intensity accompanied in liver and lungs with activation of ARP and AOP systems; in aftertreatment period LPO processes prevail against insufficiency of AOP components. We proved that activation of LPO processes and depression of AOP system activeness may be used as adequate criteria of ADDs inhalation toxicity.

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P7-09

Effect of nicotine on metabolic processes and pro-antioxidant status in male rat
Age-related differences

Roman Kireev[1], Ekaterina V. Stepanova[2], Olga V. Slyzova[2], Elena I. Solodovnikova[2], Lyubov V. Doroshenko[2], Yulia V. Soldatova[2]

[1] *Research Institute General Pathology and Pathophysiology, Moscow, Russia;* [2] *Saratov State University, Department of Biochemistry and Biophysics, Saratov, Russia*

The present studies were aimed at the identification of possible age-related differences in the effect of nicotine. Adolescent and adult male rats were injected subcutaneously with nicotine at the dose 0.5 mg/kg during a 10 day-period. Control rats were administered subcutaneously 1 ml normal saline. The animals were sacrificed after 24 h following the last injection.

The measured parameters were tissue, serum and erythrocytes TBARS and reduced glutathione (GSH); in erythrocytes—glutathione-*S*-transferase (GST); in serum—γ-glutamyltransferase, ALT, AST activity, level ceruloplasmin and urea.

The results indicated that nicotine induced hypothermia in both adolescent and adult rats. Nicotine significantly increased urea level and AST activity in serum in both adolescent and adult male rats. After of nicotine injection the ALT activity in serum was increased only in adult male, but not in adolescent ones. Cholinesterase activity was decreased more in adolescent male than in the adult.

Erythrocytes GST activity and γ-glutamyltransferase activity in serum and liver were significantly increased in both adolescent and adult male after nicotine treatment. The level of thiobarbituric acid reactive substances in erythrocytes, serum, heart and liver was increased in all experimental groups of male. However, these changes were strongly marked in adult male group. In addition, nicotine treatment resulted in significantly higher erythrocytes and heart glutathione concentrations than in controls. Serum ceruloplasmin level was significantly increased by nicotine treatment only in the adolescent male, but not in adult male group.

The obtained results testify to the fact that nicotine treatment leads to the reliable changes in the metabolic processes and antioxidant system in the group of adolescent males, as well as in the group of adult animals. However, proceeding from the obtained results, we can come to the conclusion, that young male rats are more resistant to nicotine effects.

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P7-10

Oxidative stress, DNA damage and DNA repair capacity in children with Type 1 diabetes mellitus

Rudolf Stetina[1], Jana Varvarovska[2], Zdenek Rusavy[3], Renata Pomahacova[2], Jaroslav Racek[4], Silvie Lacigová[3], Ladislav Trefil[4], Konrad Siala[2], Frantisek Stozicky[2]

[1] *Faculty of Military Health Sciences Hradec Kralove;* [2] *Pediatric Department, Charles University Hospital, Plzen, Czech Republic;* [3] *Ist Clinic of Internal Diseases, Charles University Hospital – Diabetology Center, Plzen, Czech Republic;* [4] *Institute of Clinical Biochemistry and Haematology, Charles University Hospital, Plzen, Czech Republic*

Oxidative stress (OS) connected with diabetes mellitus (DM) results in damage to lipids, proteins as well as to DNA. We have studied the levels of oxidative stress, the DNA damage as well the capacity to repair the oxidative damage to DNA in group of children (8 girls, 12 boys, age 13.26 ± S.D. 2.98) with diabetes mellitus Type 1 (T1DM). The average HbA_{1c} was 9.50 ± S.D. 2.52% (DCCT). Markers of oxidative stress investigated were followed: superoxide dismutase (SOD), glutathione peroxidase (GPx), plasma antioxidant capacity (AOC), reduced glutathione (GSH) and malondialdehyde (MDA). DNA breaks in peripheral lymphocytes were measured using the comet assay. The capacity to remove the oxidative DNA damage was measured in extracts prepared from these lymphocytes. Paediatric patients' results were further compared with those of a group of 23 adult diabetic individuals without microvascular complications, and with 30 adult T1DM patients with any of the following complications: retinopathy, nephropathy and/or neuropathy.

We have found significantly lower levels of SOD, MDA and GSH in diabetic children, compared with healthy controls. However, increased DNA breaks in peripheral lymphocytes of diabetic children were observed compared to healthy controls. The capacity to repair the oxidative damage of the DNA (DNArc) was significantly increased in diabetic children ($p < 0.05$). In comparison with adults, children had lower numbers of DNA breaks. DNArc of peripheral lymphocytes in children was significantly greater than in both adult groups ($p < 0.01$ and $p < 0.001$).

We have confirmed that children and adult patients with T1DM have increased parameters of OS and of DNA breaks compared with the age-related healthy population. In all diabetic groups, DNA repair capacity was significantly higher than in healthy controls. The DNA repair capacity was inversely related to the age of the patient, being significantly higher in children compared to adults.

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P7-11

Effect of quercetin in cadmium-induced hepatotoxicity

A.I. Morales, J. Egido, C. Vicente-Sánchez, F. Pérez-Barriocanal, J.M. López-Novoa

Department of Physiology and Pharmacology (Toxicology), University of Salamanca, Spain

Cadmium (Cd) is a common environmental pollutant and its wide distribution has led to an increased interest in its toxicity and biological effects. The liver is a target organ where Cd is mainly accumulated and the principal site of synthesis of metallothioneins (MTs). Oxidative stress can play a key role in Cd-induced dysfunctions. Quercetin, one of the most abundant flavonoid present in vegetables, is known as a potent oxygen free radical scavenger and a metal chelator. Our aim was to study the effect of quercetin on Cd-induced alteration in the function of the liver, on oxidative stress and on the synthesis of MTs, in an experimental model of rats chronically treated with Cd. This study was performed in Wistar rats that were administered during 9 weeks with either cadmium (1.2 mg Cd/kg/day s.c), quercetin (50 mg/kg/day, i.p.) or cadmium + quercetin. Hepatic toxicity was evaluated by enzymatic markers

of hepatic damage. Malondialdehyde concentration in plasma was employed as an index of lipid peroxidation. Plasma total antioxidants were also measured. Liver samples were used to analyse changes in the activity of superoxide dismutase and glutathione-reductase. Metallothionein hepatic expression was assessed by Western blot. Hepatic expression of metallothionein 1 and 2 (MT-1, MT-2) mRNA was assessed by Northern blot. Our results show that the administration of cadmium for 9 weeks induced an increase of activity enzymatic markers of hepatic damage in both groups, cadmium and Cd + quercetin. However, the administration of quercetin with cadmium prevented hepatic lipid peroxidation and alteranions in enzymatic markers of oxidative stress which were increased in rats receiving only cadmium. The concentration of antioxidants in plasma was higher in the group that received cadmium and quercetin. MT proteins and MT-1 and MT-2 mRNA levels in liver were substantially increased during treatment with Cd, being even higher when the animals received Cd and quercetin. Our results show that although quercetin treatment prevents oxidative stress and increases MT expression, it is not able to protect from Cd-induced hepatotoxicity, suggesting that other mechanisms could be involved in hepatic damage.

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P7-12

Hyperthermia-induced toxicity in freshly isolated mouse hepatocytes: Involvement of oxidative stress and cellular signalling

M.J. Santos-Marques [1], C. Sousa [1], R. Vitorino [2], R. Ferreira [3], F. Remião [1], F. Amado [2], J.A. Duarte [3], F. Carvalho [1], M.L. Bastos [1]

[1] *REQUIMTE, Toxicology Department, Faculty of Pharmacy, University of Porto, Porto, Portugal;* [2] *Chemistry Department, University of Aveiro, Porto, Portugal;* [3] *Biochemistry Department, Faculty of Sport Sciences and Physical Education, University of Porto, Porto, Portugal*

The hepatotoxic effects of hyperthermia have been associated with the over-generation of reactive oxygen species (ROS) and depletion of cellular antioxidants leading to oxidative stress. There are multiple mechanisms or processes for cell and tissue protection against ROS. In addition to direct antioxidant defence systems, the genes for heat shock proteins (HSPs) can also be upregulated in response to cellular trauma, resulting in cellular protection and enhanced survival. This effect is mediated by the activation and DNA binding of heat shock factors (HSF), notably HSF1.

The aim of this study was to determine the usefulness of the freshly isolated mouse hepatocytes as a model for the study of the time course of hyperthermia-induced oxidative stress and cellular signalling. Freshly isolated mouse hepatocytes obtained from Charles River CD1 male adult mice were incubated both at normothermic (37 °C) and hyperthermic (41 °C) conditions for 4 h. The toxic effects were evaluated by measuring cellular viability (Trypan Blue exclusion and LDH leakage) and oxidative stress, through the measurement of glutathione redox status and lipid peroxidation. Hyperthermia-induced cell signalling was evaluated through the measurement of HSF1 activation and HSP70 expression. The results accomplished demonstrated that mild continuous hyperthermia (41°) leads to oxidative stress and loss of cellular viability in a time-dependent manner, with significant effects already observed 1 h after treatment. Additionally, it was also found that hyperthermia was capable to induce a heat shock response reflected by the activation of HSF1, which emerged before the formation of HSP70 levels.

In conclusion, in the present study, it was demonstrated that this cellular model is suitable for the evaluation of hyperthermia induced oxidative stress and cell signalling through the transcriptional activation of HSP70 via HSF1.

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P7-13

Effect of Vitamin E supplementation in phenytoin induced developmental toxicity in rats

Eduard Ujházy [1], Mojmír Mach [1], Michal Dubovický [1], Jana Navarová [1], Štefan Bezek [1], Marián Zemánek [2]

[1] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia;* [2] *Zentiva J.S.C., Hlohovec, Slovakia*

Phenytoin (PHT) is known as a human and animal teratogen affecting the embryos by intrauterine hypoxia. The aim of the present study was to test the hypothesis that a high dose (500 mg/kg) of the natural antioxidant Vitamin E (VitE) could reduce developmental toxicity of PHT in rat offspring. PHT (150 mg/kg) was administered

by oral gavage daily from day 7 to 18 of gestation and VitE prior to PHT orally on the same days. PHT administration resulted in decreased survival rate and lower body weight of pups on day 21 *post partum* (PP). Moreover, PHT slightly changed somatic growth and pups failed to present dynamic air righting on days 15–20 PP. VitE supplementation did not alleviate these changes and itself induced persisting body weight reduction on the days 21 PP and 100 PP. The vertical exploratory activity was decreased in the PHT group with significance in females. We observed also decreased brain wet weight in the PHT and VitE + PHT groups compared to controls and increased dopamine levels in males of the PHT group on day 100 PP. We concluded that prenatal supplementation with 500 mg/kg of VitE did not ameliorate the developmental toxicity of PHT and failed to protect postnatal development of rat foetuses. Further, occurrence of persistent body weight gain depression up to adulthood in the group supplemented with VitE indicates its possible interference with somatic growth regulation.

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P7-14

Influence of smoking and amount of cigarette consumption on oxidative stress, protein carbonyl content and biochemical parameters

Vügar Aliyev [1], Banuçiçek Yücesan [2], Şebnem Ş. Çeçen [1], Ayşe Karakuş [1], Serap Yalçın [1], Tülin Söylemezoğlu [1]

[1] *Ankara University, Institute of Forensic Medicine, Ankara, Turkey;* [2] *Refik Saydam Hygiene Center, Blood & Blood Prod. Quality Cont. Lab., Ankara, Turkey*

This study was objected to evaluate the effect of cigarette smoking and degree of consumption on oxidative damage, protein carbonyl content, kidney damage, liver injury, lipid profile by clinical and biochemical procedures. Groups were arranged according to smoking status which was determined with smoking indices and calculated as Σ number of cigarettes consumed per day × average duration of smoking in years. The groups were as follows: Group A (nonsmoker); Group B (SI = 1–400, light smokers); Group C (SI = 400–800, moderate); Group D (SI = >800, heavy). No significant difference in MDA levels was found between nonsmokers/light smokers ($p = 0.725$) and moderate/heavy smokers. However a significant increase was observed in MDA levels if nonsmokers/moderate smokers ($p = 0.007$) were compared. Also degree of cigarette consumption increased MDA levels significantly when light smokers were compared with moderate ($p = 0.018$) and heavy smokers ($p = 0.014$). Accordingly, a significant decrease was observed in TSH levels when nonsmokers were compared with moderate ($p = 0.016$) and heavy smokers ($p = 0.004$). Furthermore, degree of cigarette consumption like in MDA case, affected TSH levels in volunteers by decreasing the levels significantly if light smokers were compared with moderate ($p = 0.028$) and heavy smokers ($p = 0.018$). A significant increase was observed in protein carbonyl content if evaluated overall ($p = 0.018$). Kidney (urea, creatinine, uric acid) and liver (AST, ALT, GGT) function tests were studied, however no significant difference was found in any one of these clinical parameters. No significant difference was found in terms of cholesterol, LDL and VLDL. However HDL levels of smokers were significantly lower than that of nonsmokers ($p = 0.008$) whereas triglyceride levels of smokers were higher ($p = 0.032$). Our results indicate that cigarette smoking and degree of consumption induces oxidative stress by increasing MDA levels and protein carbonyl content and decreasing TSH levels. Smokers in our population were found to be dyslipidaemic therefore we may say that they are under the risk of coronary artery disease.

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P7-15

Formation of reactive oxygen species (ROS) in TCDD- and estradiol-treated hepatoma cells and RAt primary hepatocytes

M. Göttel, D. Schrenk

Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was classified as a human carcinogen by the International Agency for Research on Cancer (IARC). TCDD acts as a liver carcinogen in female but not in male Sprague–Dawley rats. Its effects are mediated by activation of the cytosolic aryl hydrocarbon receptor (AhR). The activated AhR enhances the transcription of a wide spectrum of genes including cytochromes P450 (CYP) 1A1, 1A2, and 1B1. There is good evidence for a role of estrogens in the mechanism of TCDD-induced hepatocarcinogenesis in the rat. Previous studies have shown that TCDD treatment leads to an estrogen-dependent formation of 8-oxo-2′-deoxyguanosine (8-oxo-dG) in female rat liver. It was postulated that estrogens can act as genotoxic procarcinogens. In particular, the catechol estradiol metabolite 4-hydroxyestradiol, formed by CYPs, can generate

DNA-damaging ROS via redox cycling and the corresponding estradiol-3,4-quinone can form covalent DNA adducts. In our study, treatment of the hepatoma cell lines H4IIE (rat) and Hepa1 (mouse) with 1 nM TCDD for 48 h resulted in significantly enhanced ROS formation measured as oxidation of 2′,7′-dihydrodichlorofluorescein (added as acetate; H_2DCFDA) in permeabilized cells. Co-incubation of 100 nM estradiol with TCDD did not lead to a further increase beyond that of TCDD alone. In contrast, in human HepG2 hepatoma cells only slightly, not significantly increased levels of ROS formation were detected after TCDD incubation. In rat primary hepatocytes from male and female rats TCDD treatment significantly enhanced ROS formation, which was further increased by addition of estradiol to male-derived, and to a higher extent, to female-derived cells. Previously determined 8-oxo-dG levels in nuclear DNA of HepG2 cells and rat hepatocytes after TCDD are in good agreement with our DCF data. Induction of 7-ethoxyresorufin-*O*-deethylase (EROD), the catalytic CYP1A activity, was observed in all tested cells after TCDD incubation indicating that CYP1A induction is not the only relevant factor. In summary, our findings suggest that TCDD can lead to enhanced ROS formation in rodent but not in human hepatoma cells. Estradiol enhanced this effect mainly in female-derived hepatocytes in culture.

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P7-16

Hepatoprotective effect of thiol reductants against toxicity of azathioprine

Hossein Niknahad, Maliheh Parsa, Akram Jamshidzadeh

Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran

Azathioprine (AZT) is used for controlling autoimmune diseases and prevention of rejection of transplanted organs. Many cases of hepatotoxicity have been reported during the administration of AZT, but the mechanism of its hepatotoxicity is not fully understood. In the present study, we have used freshly isolated rat hepatocyte to investigate the mechanism of AZT-induced cytotoxicity. AZT was toxic towards hepatocyte in a dose and time-dependent manner and caused about 50% cell death at 2 h with a concentration of about 500 μM. AZT depleted the hepatocyte content of GSH to less than 30% and 15% of the initial level at 2 and 3 h, respectively. It also depleted the protein thiol content of the cells to less than 25% and 10% of the control at 2 and 3 h, respectively. Incubation of hepatocytes with external GSH, *N*-acetylcysteine, or dithioteritol, a thiol reductant, strongly prevented AZT-induced cytotoxicity. Addition of dithioteritol, but not external GSH, or *N*-acetylcysteine, even 30–60 min after AZT prevented cytotoxicity and returned the blebby damaged hepatocytes to the normal shape. Dithioteritol restored protein thiol and GSH content of the cells. The results suggest that the main mechanism of AZT-induced hepatotoxicity is by depleting protein thiol and intracellular GSH content of hepatocytes. Restoring protein thiol content by thiol reductants such as dithioteritol could prevent AZT-induced hepatotoxicity, or even cure the initial damages induced to hepatocytes.

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P7-17

HeLa cells respond to sublethal cadmium and arsenic concentration preferentially by up-regulation of metallothionein and heme oxygenase transcripts
Gene delivery of g-glutamylcystein ligase improves the metabolic activity of cells exposed to each metal

José Macias-Barragán, Selene Huerta-Olvera, Martha E. Ramos-Márquez, Juan Armendáriz-Borunda, Fernando Siller-López

Molecular Biology and Gene Therapy Institute, Health Sciences Center, University of Guadalajara, Guadalajara, Mexico

Cadmium and arsenic generate oxidative stress as a mechanism of toxic action. Cells have diverse elements to cope with this toxicity mainly by the synthesis of stress proteins and antioxidants. In this work we analyzed the response to the *in vitro* exposure to each metal and the effect of delivering the gene of γ-glutamyl cystein ligase (γ-GCL), key enzyme for the synthesis of glutathione, as a protective element against metal toxicity.

Methods: HeLa cells were exposed 24 h after seeding to 5 μM Cd^{2+} and 20 μM As^{3+}, and 24 h later were collected for analysis of gene expression of several stress and antioxidant-related enzymes by RT-qPCR. Also, the catalytic and regulator subunits of the γ-GCL gene were cloned in a plasmid and deliver to cells and protection conferred to metabolic activity to increasing concentrations of each metal was analyzed by the MTT assay 72 h post-transfection (24 h post-metal exposure).

Results: Cd^{2+} significantly increased the expression of metallothionein (MTA1), heme oxygenase (HOX1) and glutathione *S*-transferase (GSTA1) (150-, 100- and 43-fold, respectively). As^{3+} increased HOX1 (44 times) and MTA1 (22-fold). No significant changes were observed on the expression of the catalytic and modulator subunits of γ-GCL, glutathione synthetase, superox-

ide dismutase (SOD) and glutathione peroxidase (GPx). When γ-GCL was transfected, a significant protection to Cd^{2+} and As^{3+} toxicity was observed through the recovery of the metabolic activity of the cells.

Conclusion: At the time point analyzed, the enzymes mainly involved in the response to reactive oxygen species like SOD, GPx and those related to GSH synthesis were not up-regulated by both metals as did those involved in the acute stress response like MTA1, HOX1 and GSTA1, which could indicate that toxicity is partially solved by this type of primary response. Noteworthy, cadmium showed a higher induction response than arsenic at the same IC50 for metabolic activity. On the other hand, transfection with γ-GCL significantly improved the metabolical activity of cells, indicating that the increased synthesis of γ-GCL and therefore of GSH might be a potential gene therapy alternative against chemical and free radical related diseases.

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P7-18

Effect of various propolis on erythrocyte superoxide dismutase and catalase activities: A preliminary study

Gozde Girgin [1], Terken Baydar [1], Asli Ozkok Tuylu [2], Kadriye Sorkun [2], Bekir Salih [3], Gonul Sahin [1]

[1] *Hacettepe University, Faculty of Pharmacy, Toxicology Department, Ankara, Turkey;* [2] *Hacettepe University, Faculty of Science, Biology Department, Ankara, Turkey;* [3] *Hacettepe University, Faculty of Science, Chemistry Department, Ankara, Turkey*

Propolis (bee glue) is a natural product derived from plant resins collected by honey bees that has a strong characteristic smell and taste. Propolis may contain more than 160 constituents such as flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, ketones, sesquiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. Its content shows different chemical composition due to geographical and botanical origin. So far, many effects of propolis including antiseptic, bacteriostatic, antimycotic, antiprotozoan, antiviral, spasmolytic, astringent, anti-inflammatory, immunostimulatory, anticancer and antimicrobial activities have been elucidated. Recently, it is also reported that propolis has antioxidant effects, especially its scavenging activity was determined. The major antioxidant enzymes, superoxide dismutase and catalase, directly catalyze the transformation of peroxides and superoxide to non-toxic species and they are known as protective enzymes. In the present study first of all, content of alcohol extract of each propolis sample was determined by gas chromatograph coupled with mass spectrometry (GC–MS). Additionally the major aim of this study was to evaluate if there was any effect of propolis content on the activities of superoxide dismutase and catalase in human erythrocytes, in vitro. For this purpose, 22 propolis samples belonged to *Apis mellifera* colonies were collected from different regions of Turkey. Catalase and superoxide dismutase assays were carried out by spectrophotometry according to Marklund & Marklund and Aebi methods, respectively. According to our preliminary results, superoxide dismutase activity changed from 93.9% to 409.2% while catalase activity ranged between 80.8% and 112.1% of the control.

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P7-19

Interactions between the *OGG1* Ser326Cys polymorphism and intake of fruit and vegetables in relation to lung cancer

Mette Sørensen, Ole Raaschou-Nielsen, Rikke Dalgaard Hansen, Anne Tjønneland, Kim Overvad, Ulla Vogel

Danish Cancer Society, Institute of Cancer Epidemiology, Copenhagen, Denmark

Oxidative stress induced by tobacco smoke is thought to cause lung cancer. The enzyme 8-oxoguanine glycosylase 1 (OGG1) repairs oxidatively damaged DNA, and a single nucleotide polymorphism in the *OGG1* gene ($Ser^{326}Cys$) has previously been associated with the risk for lung cancer. We examined the role of the *OGG1* polymorphism and interactions with intake of fruits and vegetables and smoking in the development of lung cancer, by genotyping blood samples from 431 lung cancer cases and 796 persons in a comparison group, which were identified within a prospective Danish cohort on 57,000 cohort members. We found no overall association between the *OGG1* polymorphism and lung cancer. There was a statistically significant interaction between the *OGG1* polymorphism and dietary intake of vegetables, with a 54% decrease in lung cancer risk per 50% increase in vegetable intake among homozygous $Cys^{326}Cys$ carriers and no decrease in risk among carriers of $Ser^{326}Ser$ or $Ser^{326}Cys$. Though statistically insignificant, the same tendency was seen in relation to intake of fruit. There were no statistically significant interactions between the *OGG1* polymorphism and smoking duration or smoking intensity. This sug-

gests that the beneficial effect of vegetables and fruits is highest among susceptible individuals.

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P8 Risk Assessment

P8-01
Assessment of the health risks from non-compliance with arsenic drinking water parametric value

Joyeux Michel[2], Baert Alain[3], Cabillic Pierre-Jean[4], Casellas Claude[5], Creppy Edmond[6], Montiel Antoine[2], Robin Alban[7], Sauvant-Rochat Marie-Pierre[8], Seux René[9], Grimault Laurent[10], Arnich Nathalie[1]

[1] *AFSSA Agence française de sécurité sanitaire des aliments, France;* [2] *Eaux de Paris (SAGEP), Paris France;* [3] *Centra Anti-poison, CHU Pontchaillou-Rennes, France;* [4] *Service sante-environment, DDASS du Morbihan-Vannes, France;* [5] *Faculte de Pharmacie, Montpellier, France;* [6] *UFR des Sciences Pharmaceutiques, Bordeaux, France;* [7] *Bureau des Eaux, Direction Generale de la sante, Paris, France;* [8] *Faculte de Pharmacie, Clermont-Ferrand, France;* [9] *laboratorie d'Etude de Recherche en environment et sante, ENSP, Rennes, France* [10] *Agence francaise de Securite des aliments, Maisons Alfort, France*

In the context of the Directive 98/83/EC on the quality of water intended for human consumption, Member States can provide exemptions to cover non-compliances with the parametric values if there are no other reasonable means of maintaining the water supply and if ingestion of the water does not constitute a potential hazard to human health.

Within this context, the French Food Safety Agency (AFSSA) was requested to assess the health risks from non-compliance with several parametric values in drinking water, included arsenic.

The drinking water quality database SISE-EAUX (French Ministry of Health) shows for a 4-year period (01/99 to 12/02) that at least one non-compliant result was registered for 3.4% of the distribution units (serving less than 722,000 persons). Near 2300 analyses were higher than the parametric value (10 μg/L). Half of these results are below 18 μg/L.

Exposure to arsenic is mainly from food (seafood, meat). Comparison of food and drinking water arsenic intake is complex because of differences on chemical form and bioavailability in these matrices. The inorganic forms of arsenic, more toxic, are predominantly present in drinking water compared to the organic forms mainly present in food. Unfortunately, results are generally expressed in total arsenic.

Based on the most sensitive endpoint of arsenic for human health (i.e. skin cancer), an Excess Unit Risk of 1.5×10^{-3} $(\mu g/kg\ b.w./d)^{-1}$ is proposed by the US-EPA. The risk level associated with consuming drinking water at the parametric value of 10 μg/L would be around 6×10^{-4} for lifetime exposure (2 L, 70 kg, 70 years). This guideline value of 10 μg/L is provisionally retained by WHO taking into account uncertainty over the actual risks at low concentration and conventional treatment feasibility. Based on this EUR and the US-EPA approach to take into account the susceptibility of children and infants, the working group estimated the sanitary risk related to exceeding 10 μg/L for limited periods (from 3 to 9 years).

Finally, AFSSA considers that the excess cancer risk associated with the parametric value is significant. Consequently, ingestion of water with a concentration higher than 10 μg/L does not appear to be acceptable. No derogation will be allowed.

This risk assessment highlights the need for more knowledge on these non-compliances (speciation of arsenic in drinking waters according to geographical areas) and the need to assess the benefit/cost aspects of the treatments proposed.

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P8-02
Risk assessment of white phosphorus in military training areas, a probabilistic approach

Øyvind Albert Voie, Kjetil S. Longva, Arnljot E. Strømseng, Arnt Johnsen

Norwegian Defence Research Establishment, Kjeller, Norway

White phosphorus is a highly toxic compound used in various pyrotechnic products. Ammunitions containing white phosphorus are widely used in military training areas where its unburned products contaminate soil and local ponds. Traditional risk assessment methods presuppose a homogenous spatial distribution of pollutants. The distribution of white phosphorus in military training areas is spotty and heterogeneous, which makes it less probable to be exposed by, e.g., soil ingestion. Another problem posed by spotty distribution of pollutants is the difficulty of taking statistical representative environmental samples. The current approach suggests a Bayesian network (BN) as a risk assessment tool. The probabilistic reasoning supported by BN allows us to take into account the spotty distribution of white phosphorus. Further, by

BN one can combine empirical data and expert knowledge, which makes it possible to include all kind of data relevant to the problem. As an example data from environmental sampling and analysis can be supplemented by firing records and knowledge on the persistence of white phosphorus in the environment, which together can be used to estimate the density of hot spots and white phosphorus concentration. The current work includes an example of use where the risk for white phosphorus poisoning in grazers at a military shooting range in Northern Norway is calculated.

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P8-03

Vehicle effects of DMSO in two strains of CBA mice in murine local lymph node assay

W. Wang-Fan, L.G. Ullmann, E. Deparade, J. Rapaport

RCC Ltd., Toxicology, Zelgliweg 1, CH-4452 Itingen, Switzerland

This poster is one part of our series investigations on vehicle effects in murine local lymph node assay (LLNA). Based on our vehicle- and positive-control validation studies, (some of the results have been presented separately at previous annual meetings of the SOT, the J-SOT and the 10th ICT Meeting), DMSO shows a significantly high DPM/LN level in female CBA/CaOlaHsd mice in LLNA tests.

In this presentation, the vehicle effect of DMSO were assayed and compared using two strains of female CBA mice: CBA/CaOlaHsd and CBA/CaHsdRcc (SPF). The results are summarized in the Table 1.

The vehicle control validation studies demonstrated that DMSO shows a significantly high DPM/LN level compared with that in the naive control group. Consistent results have been obtained in two different strains of CBA mice. The test results showed there exist differences of the proliferative capacity of the draining lymph node cells between CBA/CaOlaHsd and CBA/CaHsdRcc (SPF) mice, when the animals are topically treated by DMSO, which is one of the recommended vehicles used in LLNA tests.

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P8-04

Identification of a critical dose level for risk assessment: Developments in benchmark dose analysis of continuous endpoints

Salomon Sand [1], Dietrich von Rosen [2], Katarina Victorin [1], Agneta Falk Filipsson [1] [1] *Institute of Environmental Medicine, Stockholm Sweden* [2] *Swedish University of Agricultural Sciences, Uppsala, Sweden*

In this study developments in benchmark dose (BMD) analysis from continuous experimental data are proposed. The suggested approach defines the BMD as the dose at which the slope of the S-shaped dose–response relationship changes the most in the low-dose region, i.e. the dose corresponding to the "maximum slope-change point". This dose resides in a region where the sensitivity to chemical exposure may start to change noticeably. It is shown that the response (defined as a % change relative to the total size of response) at the "maximum slope-change point" depends on the geometrical shape of the dose–response curve; the response becomes lower as the curve becomes more asymmetrical and threshold-like in the low-dose region. Given a symmetrical case, described by the Hill function, the response associated with the dose of interest becomes 21%. According to a limiting case of asymmetry and threshold-like characteristics, reflected by a Gompertz curve, the response corresponding to the dose of interest becomes as low as 7.3%. Use of a response in the range of 5–10%

Table 1
Comparison of DPM/LN levels between naïve animals and animals treated by DMSO in two strains of CBA mice

Strain of mice	No.	Group	DPM/LN		*t*-Test ($N = 10$, $G = 2$, $t = 2.31$)	Significance [at $p \leq 0.05$ (two sides)]
			Mean	S.D.		
CBA/CaOlaHsd	5	Untreated	182	78	$t = 3.25$	Yes
CBA/CaOlaHsd	5	DMSO	406	133		
CBA/CaHsdRcc (SPF)	5	Untreated	189	54	$t = 6.87$	Yes
CBA/CaHsdRcc (SPF)	5	DMSO	682	151		
CBA/CaOlaHsd	5	Untreated	182	78	$t = 0.16$	No
CBA/CaHsdRcc (SPF)	5	Untreated	189	54		
CBA/CaOlaHsd	5	DMSO	406	133	$t = 3.07$	Yes
CBA/CaHsdRcc (SPF)	5	DMSO	682	151		

when estimating the BMD conservatively accounts for uncertainties associated with the proposed strategy, i.e. such a definition results in a dose that is close to, or below, the "maximum slope-change point" given typical models considered, and may be appropriate in a risk assessment point of view. The present investigation also indicated that a BMD defined according to the suggested procedure may be estimated more precisely relative to BMDs defined under other approaches for continuous data.

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P8-05

Concentration of polycyclic aromatic hydrocarbons as factor of risk assessment for therapeutically applied peat

Boguslaw Wilkomirski, Malgorzata Malawska

Warsaw University, Warsaw, Poland

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds that are present as pollutants not only in air or food, but also in different types of soils and deposits, including peat. Natural peat are formed by complex processes and deposited in many areas of the world, also in Poland. Due to different positive medical effects of peat many patients have got beneficial results by peat therapy. Hence it is very important to use the peat maximally free from any pollutants which are potentially dangerous for human. The purpose of this work is to define the concentration of PAHs in different peats from Polish peatlands and consider the PAHs content as a potential risk factor in a peat therapy. Peat samples were collected along the whole stratigraphic profiles. During 3 years of investigations the peat cores from 23 peatlands situated in north-eastern and southern Poland were collected. The investigated samples differed by anthropogenic PAHs concentration, which varied from very low (39 ng/g) to very high (3746 ng/g). In the latter deposit the concentration of benzo[*a*]pyrene reached 316 ng/g. In all investigated peatlands the highest concentration of PAHs (except perylene, which can be effectively formed by biotransformation of isoprenoids precursors and was taking into account separately) was found in the highest layers of deposits suggesting the anthropogenic origin of these pollutants. Different and sometimes high concentration of carcinogenic PAHs in peat suggest the necessity of PAHs determination before the use of peat for medical purposes and/or use the peat from deeper layers as a rule.

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P8-06

Risk estimation of metals released from medical implants

Michaela Zeiner[1], Peter Zenz[2], Felix Lintner[2], Wolfgang Schwägerl[2], Ilse Steffan[1]

[1] *University of Vienna, Vienna, Austria;* [2] *Otto Wagner Spital, Vienna, Austria*

It has been documented that wear debris as well as corrosion products of surgical implants may have profound consequences which can cause an implant failure. Alloys used for biomaterials have to be well studied in terms of mechanical and corrosion properties, and cytotoxicity prior to medical applicability, nevertheless adverse effects may occur in an organism after implantation. The determination of the concentrations of the alloys' constituents in various organs and body fluids showed a systemic distribution of wear particles throughout the whole body. A previous study performed in Switzerland by Burian et al. (2004) pointed out that wear debris is capable to elicit acute inflammation which may result in endothelial damage and subsequent failure of microperfusion.

The presented study is focused on the fate of niobium (Nb) and molybdenum (Mo) in human organisms with a total hip replacement (cobalt–chromium–molybdenum alloy with 1‰ Nb). Independently from the fact whether the alloys are articulating against themselves or against ultra-high-molecular weight polyethylene, considerable amounts of wear debris are released from the prostheses and have to be regarded as a cause of long-time problems. As found in a preceding investigation (Zeiner et al., in press) Mo and Nb represent the elements with the highest tendency to be accumulated among the constituents of the alloy under study.

Mo is an essential trace element for humans being a component of certain metalloflavoproteins. Metabolism and toxicological studies in humans are usually limited to oral administration. The absorption of Mo via the digestive tract depends on many factors resulting in a wide range (30–80%). After absorption Mo rapidly appears in blood and many organs. Regarding persons with metallic implants elevated Mo levels could be found in brain, lung and lymphatic nodes. High Mo concentrations may cause mineral imbalances. Haematological effects as well as disorders in growth and development have been reported for animals.

Nb has been considered as biological inactive for a long period. Its oral absorption is very low (up to 2%) and the half life time four months. Nowadays, low toxicity is assumed for Nb since it elicits cardiovascular effects

as well as liver changes (Scansetti, 1992). Due to the distribution of wear debris in the body elevated Nb levels were determined in brain, kidney, lung, lymphatic nodes, and spleen.

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P8-07

Mechanistic study on aniline-induced erythrocyte toxicity

Harpal Singh, Elissa T. Purnell

Savannah State University, Savannah, GA, USA

Bioindicators are useful endpoints of toxicity for assessing health risks. Strategies for the use of indicators in the prediction of environmental damage should include mechanistic research to determine the earliest steps leading to toxicity. This study presents mechanistic data on the relationship between the chemical structure and hemotoxic markers as side-effects of industrial chemicals such as aniline and its halogenated analogs. Chemical-induced methemoglobinemia, loss of circulating blood cells, blood stability, glutathione depletion and the membrane cytoskeletal changes were assessed following exposures to halogenated aniline analogs. Parent compounds are usually converted to their toxic metabolites (*N*-hydroxylamine) and react with oxyhemoglobin with consequent reduction of molecular oxygen to active oxygen species leading to hemotoxic damage. Whole blood was collected from male Sprague–Dawley rats and cells were washed (×3) with 50 ml of phosphate buffered saline supplemented with glucose (PBSG, pH 7.4). The percentage of methemoglobin was determined spectrophotometrically at 635 nm. For the electrophoretic study, red blood cell ghosts were prepared from 2 ml aliquots that were incubated with various concentrations of aniline analogs for 2 h at 37 °C. Cells were washed with buffer solution and lysed in 20 ml of phosphate buffer (5 mM, pH 8.0) and centrifuged for 10 min. Analysis of membrane proteins was performed by SDS-PAGE. Results showed dose- and time-dependent changes in the induction of methemoglobin and loss of circulating red cells by aniline-derivatives. The minimum dose required to induce these effects vary with the test agent based on their electronegativity potential. Analysis of red cell skeletal membrane proteins showed changes in protein bands 2.1, 2.2, 3, 4.1, 4.2, 5, and 6. Specifically, Band 2.1 became broader where as band 2.2 diminished completely in some treatment. Band 3 was also affected in aniline analogs treated cells. Changes in the skeletal membrane proteins may target the red cells for premature removal. Erythrocytes were more stable in buffer solution than in treated blood cells. Depletion of reduced glutathione in treated red cells was also noticed. Dose- and time-dependent changes suggest the use of these hemotoxic end points as potential biomarkers for assessing chemical and drug safety.

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P8-08

Daily intake of ethanol increases lesions in pancreas and liver after repeated administration of a mild acute toxic dose of dibutyltin dichloride (DBTC) at intervals of 3 weeks in rats

J. Merkord [1], H. Weber [2], G. Hennighausen [1]

[1] *Institute of Toxicology, University of Rostock, Germany;* [2] *Institute of Clinical Chemistry, University of Rostock, Germany*

A single i.v. administration of 4 mg/kg DBTC induces a mild interstitial pancreatitis after 2–4 days followed by a restitutio ad integrum after 21–28 days. The repeated administration of 4 mg/kg DBTC i.v. at intervals of 3 weeks induced an acute interstitial pancreatitis and biliopancreatic duct lesions after 6 weeks and pancreatic fibrosis and liver lesions (intrahepatic bile duct hyperplasia, inflammation of portal tract and necrosis) after 12–18 weeks.

The present study was done to determine the additional influence of daily ethanol intake (15% in drinking water ad libitum) on the lesions of biliopancreatic duct, pancreas and liver of rats after repeated administration of 4 mg/kg DBTC i.v. at intervals of 3 weeks. The histopathological changes of pancreas and liver were examinated by lightmicroscopy 1, 4, 7 days and 2, 3, 4, 6, 9, 12, 15 and 18 weeks after administration of DBTC. Furthermore pathobiochemical parameters of pancreatitis (amylase and lipase activity in serum), liver lesions (alkaline phosphatase activity and bilirubin in serum) and of fibrosis (hyaluronic acid in serum) were studied.

Ethanol increased the toxic effects of repeated low DBTC doses on pancreas and liver of rats after 9–12 weeks. The fibrotic process in pancreas around the main duct started earlier (after 9 weeks) and was significant stronger than in the repeated DBTC-group after 18 weeks. The liver lesions (intrahepatic bile duct hyperplasia, parenchymal necrosis and beginning fibrosis) in the DBTC/ethanol-goup were stronger than in the DBTC-group after 18 weeks. Elevated serum levels of alkaline

phosphatase, bilirubin and hyaluronic acid were found after 9 weeks in the DBTC/ethanol group.

This study demonstrates that the daily ethanol intake increases the DBTC toxicity on pancreas and liver of rats by repeated administration of lower doses at long intervals.

In man the repeated exposure of ethanol drinking people to organotin compounds may increase the risk of toxic effects on pancreas and liver.

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P8-09

Gangliosides-based cancer vaccines: Toxicological assays

A. Bada [1], A. Casacó [2], A. Mancebo [1], B. González [1], D. Fuentes [1], O. Hernández [1], M.E. Arteaga [1], J. Hernández [1], C. González [1], N. Subirós [1], Y. González [1], C. Mesa [2], L.E. Fernández [2]

[1] *Centro Nacional para la Producción de Animales de Laboratorio, La Habana, Cuba;* [2] *Centro de Inmunología Molecular, La Habana, Cuba*

We have evaluated two ganglioside-based cancer vaccines: first GM3, an ubiquitous antigen which overexpressed in several tumor types; and *N*-GlycolylGM3, a more tumour specific molecule, not being expressed in normal tissues and present in several neoplasic cells. We carried out seven studies in order to evaluate the toxicity of both cancer vaccines: acute intramuscular toxicity test, repeated dose intramuscular and subcutaneous toxicity test (in Sprague–Dawley rats), and a 12-month study in *Macaca fascicularis* monkeys. All animals were inspected daily for clinical signs. Body weight and rectal temperatures were measured during the test. In monkeys, systolic blood pressure, respiratory and cardiac rates were measured. Anti-GM3, anti-DNA and anti-nuclear antibodies were determined at 0, 2, 6 and 12 months of the study in monkeys. Blood samples were collected for hematological and serum biochemical determinations. Gross necropsy was made in rats. Monkeys were not sacrificed. Organ weights were measured for the thymus, adrenals, testis, ovary, heart, lung, kidneys, spleen, liver and brain. These organs, administration site and abnormal tissues were processed for histopathological examinations. There were neither death nor observable differences regarding body weight, rectal temperature, systolic blood pressure, respiratory and cardiac rates. Local damage at injection sites was observed in intramuscular route studies. A slight decrease in hemoglobin and hematocrit was observed in female rats treated intramuscularly with GM3 vaccine. One monkey developed a slight and temporary anemia. Rats treated with *N*-GlycolylGM3 and monkeys had increased white blood cell and neutrophils. All treated rats showed an inflammatory reaction in the administration sites. All treated monkeys consistently developed IgM and IgG anti-GM3 antibodies; and no monkey had evidence of anti-DNA or anti-nuclear antibodies. No other tissues in any group showed signs of toxicological lesions. In conclusion, GM3 an *N*-GlycolylGM3 vaccines were confirmed to have a low toxicity.

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P8-10

Development of *in silico* genotoxicity predicting system on chromosomal aberration for existing industrial chemicals

A. Hirose [1], E. Kamata [1], H. Akiyama [2], M. Takahashi [1], M. Ema [1], M. Hayashi [1]

[1] *National Institute of Health Sciences, Tokyo, Japan;* [2] *CTC Laboratory Systems Co. Ltd., Tokyo, Japan*

A huge kinds of chemicals exist in our environment, and the risk of the almost chemicals have not been evaluated. We needed development of an instantaneous decision system for the risk of each chemical. The (quantitative) structure activity relationships (QSAR) approach would resolve this issue, but individual QSAR system has not so powerful to judge administratively. We have recently developed the mutagenesis predicting schema by using a combination of three *in silico* systems (DEREK, MultiCASE and ADMEWorks). Next, we tried the similar approach for developing the chromosomal aberration predicting system. Before evaluating the each *in silico* system, the each system was optimized for chromosomal aberration prediction by using learning set of chemicals. As a results, each *in silico* system indicated around 65–77% of concordance for chromosomal aberration. In combination approach, the concordance of prediction reached to the ratio of 91%, when prediction results are all same in three *in silico* systems. However, the applicability decreased to 36%, which meant that only a third of the chemicals were evaluated. If the criterion of predicting decision was set the case of obtaining the same results from two or more *in silico* systems, the applicability increased to 86%. In this case, the concordance decreased to 77%, which indicated similar concordance level to the results of individual evaluation using single *in silico* system. Further improvement of accuracy in each *in silico* system is required. As another possible predicting system, ClassPharmer (Bioreason) was investigated. The system can classify the chemicals

based on common substructure, and has predicting tool based on analyzing the correlation between common substructures and biological activities. We evaluated two optimized prediction models in the ClassPharmer, and the concordance lead to around 70–75%. Adding this new prediction system to the above combination approach may be useful for increasing applicability. Additionally, in the course of optimizing the prediction models by using ClassPharmer, we found some possible toxicophores, which may be correlated to potency of chromosomal aberration. The information would be helpful for improvement of accuracy on the above three *in silico* systems.

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P8-11

A proposed framework for the interpretation of biomonitoring data

P.J. Boogaard [1], P.B. Farmer [2], M. Holt [3], L.E. Knudsen [4], C.D. Money [5], L. Onyon [6], D.E. Owen [7], S.H. Robison [8], G. Schoeters [9], G.D. Stropp [10], M.F. Wilks [11], W. Will [12]

[1] *Shell International, Netherlands;* [2] *University of Leicester, UK;* [3] *ECETOC, Belgium;* [4] *University of Copenhagen, Denmark;* [5] *ExxonMobil, Belgium;* [6] *World Health Organization, CH, Switzerland;* [7] *Shell Chemicals, UK;* [8] *Procter and Gamble, USA;* [9] *VITO, Belgium;* *10*Bayer HealthCare, Germany; *11*Syngenta Crop Protection, CH, Switzerland; *12*BASF, Germany

Biomonitoring (BM) becomes more frequently used and has the potential to increase our knowledge on the extent of human exposure to chemicals. This creates a number of opportunities for improving human health risk assessment (HRA). Not only by giving further insights into exposure to chemicals, but also by triggering research investigating links between low-level exposures, adverse health effects and vulnerable subpopulations. However, it also creates a number of challenges, not least because the integrity of BM data is heterogeneous. Therefore, it should be ensured that BM data are interpreted within the boundary in which they can be reliably applied. Given the emerging trend for increased availability of BM data, ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals, a scientific non-profit organisation) formed a Task Force to develop a framework in which BM data can be consistently evaluated and which can be used to foster a consistent basis for the application of BM data in HRA.

A document ("Guidance for the Interpretation of Biomonitoring Data", ECETOC, Brussels, 2005) was developed that sets out the considerations which enable the relevance of any BM result to be reliably interpreted. Specifically, four elements are identified that allow any set of BM data to be evaluated with respect to the extent to which it can be applied in different stages of the HRA process. The elements are: (1) analytical integrity, (2) ability to describe exposure (toxicokinetics), (3) ability to relate data to effects, and (4) overall evaluation and weight of evidence.

The framework builds off the general guidance contained in an earlier publication ("Biomarkers and risk assessment: concepts and principles", Environmental Health Criteria #155, WHO-IPCS, Geneva, 1993) and describes the required knowledge for each element to apply in HRA, notably to: (1) establish exposure trends, (2) characterise the nature of exposures, (3) investigate linkages between exposure and adverse health effects, and (4) facilitate risk management and standard setting. It was validated with a series of illustrative case studies identifying (1) where different types of BM data can be reliably used, (2) their relative importance, (3) where and how they can consistently interpreted whilst accounting for the current level of understanding of the supporting science.

This approach intended both to offer a considered view of the available science and to serve as a catalyst for stimulating discussion on some of the broader issues presented by the application of BM technologies today.

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P8-12

Risk assessment of flavouring substances used in foods

Karin Norby, Vibe Beltoft, Krestine Greve, Trine K. Reffstrup, Jørn Gry

Danish Institute for Food and Veterinary Research, Soborg, Denmark

The aim of the present project, the FLAVIS project, is to perform risk assessment of chemically defined flavouring substances. The evaluations are then presented to the European Food Safety Authority (EFSA) for final adoption in its Scientific Panel on food additives, flavourings, processing aids and materials in contact with food. The regulatory background for the work is found in the European Parliament and Council Regulation No. 2232/96 laying down a procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all others in the EU.

In application of this Regulation, a Register of about 2800 flavouring substances used in or on foodstuffs in the EU Member States was adopted and are currently being evaluated according to the evaluation programme laid down by Commission Regulation. The EU Safety Evaluation Procedure is derived from the approach developed by the "Joint FAO/WHO Expert Committee on Food Additives" (JECFA) and referred to in Commission Regulation EC No. 1565/2000. First, the 2800 flavouring substances are divided into groups of structurally related substances. The Procedure is then a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds), that are considered not to present a safety concern, have been specified. In the project a very comprehensive database (the FLAVIS database) has been developed for the evaluation. It compiles information on the about 2800 flavouring substances used in Europe: specifications, structural class, food categories used in, intake data, natural occurrence as well as metabolism and toxicological data. The EFSA Panel has now adopted most of the safety evaluations of the Register compounds, but still another 435 flavouring substances need to be evaluated in the FLAVIS project and adopted by the Panel in due time before July 2007, where the EU Commission (DG SANCO) plans to issue the first European Positive list on flavouring substances.

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P8-13
Causality assessment of drug-related liver toxicity

J. Schulze [1], C.-P. Siegers [2]

[1] *Office of the Dean, Medical Faculty, Theodor Stern-Kai 7, 60590 Frankfurt, Main, Germany;* [2] *Institute of Experimental and Clinical Pharmacology and Toxicology, Ratzeburger Allee 160, 23538 Lübeck, Germany*

On two recent occasions in Germany, drug regulatory decisions for herbal drugs (kava kava, chelidonium extracts) have been based on hepatotoxicity as suspected adverse effects. Causality was mainly based on reported time coincidence between drug intake and symptom development, as well as a "positive dechallenge" indicating improvement after hospitalization. However, incidentally elevated enzymes or decreased hepatic protein synthesis are common, with GOT or GPT increases observed in up to 1/3 of all outpatients. For drug hepatotoxicity, two principally different modes of actions can be separated with relevance to incidence and population risk. For liver damage exerted by toxic mechanisms (i.e. direct reaction of a drug or its metabolites) only an increase in liver damage (e.g. GPT, GOT increase, coagulation decrease) should be taken as proof for a causal correlation requiring stringent data collection criteria; detection of enzyme elevation at the time of hospitalization alone cannot be sufficient. Additionally, in the cohort of affected patients a dose response correlation should be observed. Idiosyncratic liver damage on the other hand is dependent upon antibodies or leukocytes specifically reacting with drugs or drugs bound to liver membranes. In both cases the presence of specific antibodies or immune cells can be proven by rather simple laboratory methods (antibody binding, lymphocyte proliferation), as has been shown in one of the kava cases under discussion. The presence of specific reaction in either of these tests strongly suggests immunologically mediated mechanisms whereas the absence does not necessary rule out immunologically mediated hepatotoxicity. Whereas toxic liver damage may be limited by intake limitation, restricting daily intake is no preventive strategy for idiosyncratic reactions. Whether or not regulatory actions should be taken ought to be based on proven (or likely) causal association indicated by the parameters given above; also the availability of safer therapeutic alternatives should be considered in a risk–benefit analysis.

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P8-14
Assessment of environmental health risks of chemicals associated with industry: The experience of EDF and Gaz de France

Sylvaine Ronga-Pezeret, Gaëlle Guillossou, Magali Boize, Pierre-André Cabanes

Industry EDF-Gaz de France, Paris, France

At the beginning of the 1990s, Electricité de France (EDF) and Gaz de France began conducting health risk assessments of the effects of chemicals released into the environment in the course of energy production. From 2000 onward, French regulations have required studies to detail the environmental impact and management of sites and polluted soils.

Because of its tasks of surveillance, research, and management support, the Medical Studies Department of EDF-Gaz de France has a privileged viewpoint of this

practice and has conducted an initial assessment of these studies.

We approach environmental health risk assessments here by examining concrete cases involving atmospheric chemical emissions and chemical effluent from electricity plants (nuclear and fossil-fuel energy), ash piles, and old manufactured gas plants. From these examples, we see that health risk assessments encourage both thorough assessments of chemical discharges and interdisciplinary relations; they also nourish public debate, enable health criteria to be integrated into industrial choices, and stimulate new research. These studies are also likely to feed the future database foreseen by the European REACH regulations, which can recommend assessment of the risks associated with the use of chemical substances.

Nonetheless, several critical points became clear. Interdisciplinary work, because it requires the sharing of concepts, necessitates special training and organization. The particular position of companies that inform the public of the health effects they may cause requires specific analysis. Finally this experience shows the importance of toxicology research that takes into account the needs of environmental health risk assessors.

An initial conclusion is the need to obtain aid from toxicology experts to promote a structured, accessible interpretation of these study results. That is, the numeric indicators obtained from health risk assessments may give a false impression of precision that promotes their abusive use. Moreover, the use of health risk assessments requires recognizing their limitations and knowing how to integrate appropriately all of the available tools to preserve health public.

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P8-15

Consumer risk assessment of contaminants and residues in animal feed using transfer factors

Winfried Leeman, Monique Rennen, Geert Houben

TNO Quality of Life, Department of Food and Chemical Risk Analysis, Zeist, The Netherlands

In recent years, increasing attention has been paid to the risk posed to animal feed by chemical contamination. Cases concerning the contamination of milk, eggs and other animal products by potentially hazardous chemicals have sparked massive interest. Assessment of the risk to consumers posed by chemical contaminants or residues in animal feed has often been hampered by a lack of knowledge about how contaminants and residues behave when consumed by livestock. In assessing consumer risk, the transfer of contaminants or chemical residues from animal feed to animal products is predominantly an unknown factor. To gain a better understanding of this transfer we performed a meta-analysis of public literature. The relevant data on the transfer of various groups of chemicals from animal feed to products of animal origin were gathered and recorded in a database.

The database can be used to derive chemical specific transfer factors (defined as the ratio of the concentration of a chemical in an animal product to the concentration of the chemical in animal feed), to assess the relative vulnerability of animal matrices, or to derive transfer factors based on the physico-chemical properties of defined classes of chemicals using statistical analysis. In the absence of compound-specific information, this provides the basis for a probabilistic assessment of the carryover of substances.

Compared to the commonly used worst case scenarios, the use of database derived transfer factors offer a more accurate risk assessment. It is noted that even if little information is available, scientifically founded transfer factors can be derived using the data of comparable chemicals (in terms of either contaminant class or physico-chemical properties). Furthermore, subselection is possible for specific feeding periods, feeding concentrations, or animals, making the applicability of transfer factors highly attractive for rapid risk management decision-making and/or intervention.

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P8-16

Within-animal variation as a minimal critical effect size for continuous toxicological parameters applicable in the Benchmark dose approach

Susan Dekkers, Jan Telman, Monique Rennen, Marko Appel, Harrie Buist, Cees de Heer

TNO Quality of Life, Department Food and Chemical Risk Analysis, Zeist, The Netherlands

One of the major issues in the Benchmark dose (BMD) approach for continuous data is the determination of the maximum change in a toxicological parameter considered as non-adverse or acceptable, at the level of the individual organism. This breaking point is defined as the critical effect size (CES). With the use of the CES, the critical effect dose (CED) can be estimated as the dose at which the average animal shows the CES defined for a particular toxicological parameter. We propose to use the within animal variation to derive the CES, as it reflects the normal physiological variations in individual animals of the toxicity parameter concerned.

The within-animal variation in routinely studied continuous toxicological parameters was estimated from temporal fluctuations in individual healthy non-exposed animals. Assuming that these fluctuations are non-adverse, this within-animal variation may be indicative of the minimal magnitude of the critical effect size. The total variation in the data from individual non-exposed animals was divided in variation parts due to known factors (differences in sex, animal and day) and a residual variation, by means of analysis of variance (ANOVA). Using the residual variation and the estimated analytical measurement error of a toxicological parameter, the within-animal variation can be estimated. The data showed within-animal variations ranging between 0.6% and 34% for different clinical chemistry and haematological parameters in 90-day rat studies. This indicates that different (minimal) CES values may be applicable for different parameters. Six of the 26 parameters studied showed a within animal variation bigger than 10%, implying that a default 10% CES would overestimate hazard. For most parameters studied, the within animal variation was greater than the between animal variation.

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P8-17
Towards improved chemicals regulations—Designing efficient test systems

Christina Rudén, Sven Ove Hansson

Royal Institute of Technology, Stockholm, Sweden

The combined force of three overall objectives, namely: (1) to fill data gaps for a large number of general industrial chemicals within the REACH system, (2) to reduce the use of animals for regulatory required toxicity testing and (3) to develop predictive testing for new endpoints of concern, has put recent focus on the need for improve regulatory test requirements. How should chemicals be selected for testing? How extensive testing should be required? What tests should be prioritized?

Producing (eco)toxicological test data to enable a full risk assessment (i.e. including long-term and multi-generation testing) involves high costs. Still, the analysis of how to combine different tests into efficient and scientifically well-founded test systems for regulatory use is largely an unexplored field, lacking a comprehensive approach.

Individual toxicological and ecotoxicological tests can be described in terms of their *validity*, *reliability* and *sensitivity*. Every test is a trade-off between at least some of these factors, and all tests give rise to a certain frequency of false positive or false negative results. Furthermore, the *cost* of a test needs to be taken into account.

The traditional way of designing test systems is to prioritize low cost at low tier(s), to enable testing of many compounds, whereas the validity of the data is given higher priority at higher tier(s), to enable well-founded risk management decisions. It is also part of the standard strategy to choose lower tier test methods so that false negatives are minimized, while allowing for some false positives.

The frequencies of false positive and false negative results that a certain test produces is usually not known in any detail. Therefore, systematic investigations of the relations between different tests, both in terms of what toxicological mechanisms that they cover, and in terms of statistical correlations between their outcomes in different substance groups need to be performed. Only with major efforts along these lines will it be possible to construct cost-efficient and reliable test systems that can deal efficiently with the needs of risk managers and the public.

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P8-18
Evaluation of health hazards by inhalation of mineral wools

Elsa Nielsen, Ole Ladefoged, Pia Nørhede

Danish Institute for Food and Veterinary Research, Soborg, Denmark

"Mineral wool" is a subgroup of man made vitreous (or mineral) fibres (MMVF) composed of tangled, discontinuous, inorganic, non-crystalline fibrous materials manufactured from glass, stone (rock), slag or other processed minerals. MMVF have been on the market for over 60 years and a large number of human and animal studies are available. Despite the apparently large toxicological database, the evaluation of health hazards by inhalation exposure to MMVF presents a number of difficulties. The available animal studies are generally not suitable for a hazard assessment as (1) the fibres (types, dimensions) are not always adequately characterised, (2) exposure levels are generally not expressed as fibre concentration in the air, and (3) difficulties in extrapolation of results from animal studies to the human situation because of differences in anatomy and physiology of the respiratory tract and possibly also in sensitivity to the fibres. The available epidemiological studies have generally focused on respiratory diseases including lung cancer, predominantly in two large cohorts of MMVF production workers in the US and Europe. A

cross-sectional study has also been conducted in the US cohort. Studies have also been conducted in end-users. Evaluation of fibre exposure levels and dose-response relationships are complicated by factors such as, e.g. (1) exposure measurements were not performed as a part of a specific study but reference was made to general estimates for the facility, (2) co-exposure to other fibres (e.g. asbestos) or chemicals has been documented in several studies, and (3) smoking as a possible confounder. Because of their similarity with asbestos fibres, MMVF have been considered carcinogenic. However, MMVF are of lower bio-persistence and cleared more rapidly from the lung than asbestos, and IARC has recently concluded that the most recent epidemiological studies in the cohorts have not provided consistent evidence between exposure to MMVF and risk of lung cancer. The critical effect following inhalation exposure to MMVF is thus non-malignant respiratory tract effects (irritation and obstructive lung disease). A NOAEL cannot be established for irritative effects due to limitations in the available data. No evidence of decreased pulmonary function (as well as of pneumoconiosis and pleural abnormalities) was observed in the cross-sectional study in which the mean concentration of airborne respirable fibres was estimated to be 0.03×10^6 fibres/m^3. In conclusion, a NOAEL of 0.03×10^6 fibres/m^3 is considered for respiratory tract effects of mineral wools in humans.

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P8-19

Bridging the gap between science and politics—The role of precaution in chemicals control

Christina Rudén [1], Per Rosander [2], Sven Ove Hansson [1]

[1] *Royal Institute of Technology, Stockholm, Sweden;* [2] *International Chemicals Secretariat, Stockholm, Sweden*

The *precautionary principle* is declared to be underpinning the REACH proposal. This principle urges us to take actions to reduce potential unwanted effects from chemicals even if the existence of that effect is not scientifically proven. To define this principle, and apply it in chemicals control, it is necessary both to determine the level of proof needed for triggering precautionary actions and to decide what actions should be taken when that level has been reached.

It is a common misunderstanding that since the precautionary principle is a risk management principle, it does not influence risk assessment. As we see it, it is the task for risk assessors and scientists to evaluate the weight of the available scientific evidence in the risk assessment phase. Whereas in the risk management phase, when the appropriate actions aiming risk reduction (if any) are determined, criteria outside of the purview of science, such as technical and economical feasibility must also be taken into account. In order to help risk managers to implement the precautionary principle in a systematic fashion risk assessors should:

- *Be explicit about uncertainties*. Information about uncertainties and knowledge gaps constitutes important information to decision-makers.
- *Heed scientifically sound early warnings*. We do not need to await full scientific proof before considering actions to reduce risk.
- *Recognize patterns*. If a chemical is known to be hazardous, its chemical relatives should be considered in this context.

Generally speaking, it is the task for risk assessors to provide risk managers with the information they need to make their decisions according to the criteria they have chosen.

The NewS research programme (A New Strategy for Risk Assessment and Management of Chemicals) and the International Chemicals Secretariat have developed a series of information material (brochures and web-based information) with the aim to clarify the role of precaution in chemicals control. For further information please contact cr@infra.kth.se or info@chemsec.org.

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P8-20

Use of intake fraction to improve dioxin risk assessment

Jouko Tuomisto, Olli Leino, Hannu Kiviranta, Jouni T. Tuomisto

KTL (National Public Health Institute), Department of Environmental Health, P.O. Box 95, FI-70701 Kuopio, Finland

Toxicity equivalency factors (TEF) help to assess the toxicity of PCDD/F mixtures. TEFs are based primarily on potency, but because long-term data are preferred in establishing TEFs ("intake-TEF"), kinetic factors (absorption, distribution, elimination) are also involved. The use of TEqs (TCDD equivalents) for various matrices such as emissions, sediments and contaminated soil ignores the fact that all congeners are not conveyed similarly to human beings. A congener specific intake fraction (iF) might help to involve different transport of different congeners from sources to humans. iF denotes

the fraction of emission that is inhaled or ingested by human population. It is used especially in air pollution management as a robust and easily understandable tool.

Changes in congener spectra from one matrix level to another were tested in high consumers of Baltic herring. Congener concentrations were assessed in air emissions, in fish and in fishers. Accumulation of each congener into a later matrix was compared with that of TCDD (=1). Concentrations of most congeners were lower in fishers than in emissions, relative to TCDD, down to a factor of 1/60. This was sorted out stepwise from emission to fish and from fish to fisher. Transport from emission to herring was lower for congeners (especially higher chlorinated PCDD/Fs) than for TCDD. On the other hand, levels of higher chlorinated dioxins were higher in fishers relative to TCDD than in fish, and furans were lower than dioxins. This may be due to long half lives of higher dioxins and shorter half lives of furans.

The likelihood of different congeners to reach people thus varies by orders of magnitude, and kinetic differences modify accumulation. Therefore relevance would be improved by dividing intake fraction, kinetic factors and TEF to separate entities. An internal TEF would be here more appropriate than intake TEF. This gives the formula: MS-TEq = iF × B × iTEF × A, where MS-TEq is matrix-specific TEq, iF intake fraction specific to the matrix and congener, B toxicokinetic factor, iTEF internal TEF, and A is the amount of the congener in the matrix. This segregates intake, kinetics and effect to separate entities and TEF describes only the last step directly relevant to relative human toxicity.

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P8-21

Serum nicotine and cotinine levels as passive smoking biomarkers in preschool children of Crete, Greece

C.I. Vardavas [1], M.N. Tzatzarakis [2], A.M. Tsatsakis [2], D. Athanasopoulos [3], E. Balomenaki [3], M.K. Linardakis [1], A.G. Kafatos [1]

[1] *Preventive Medicine and Nutrition Clinic, Department of Social Medicine, School of Medicine, University of Crete, Greece;* [2] *Center of Toxicology Science and Research, School of Medicine, University of Crete, Greece;* [3] *Pediatrics Clinic, Chania General Hospital, Crete, Greece*

Greece is known to have an extensive smoking problem. Specifically it has been estimated that it has the highest adult smoking prevalence among all countries of the European Union and the O.E.C.D. Although Greek children are exposed involuntarily to environmental tobacco smoke there is insufficient data on the exact levels of passive smoking biomarkers such as cotinine and nicotine in the Greek population especially among children. To estimate the exact levels of early age passive smoking among preschool children in Crete in regard to parental smoking habits by estimating serum cotinine and nicotine values. All children enrolled in kindergarten in western Crete (1757 preschool children and 2809 parents), and interviewed during the 2004 Cretan health promotion programme. A sample of 81 children were randomly selected according to parental smoking status and provided blood samples for cotinine and nicotine assay. Cotinine and nicotine values in serum samples from children with smoker parents were evaluated at 1.69 ng/ml (95% confidence limits 0.93–3.06) and 0.71 ng/ml (95% confidence limits 0.62–0.80) respectively. Correspondingly the cotinine and nicotine levels from children with non-smoker parents were estimated at 0.15 ng/ml (95% confidence limits 0.09–0.28) and 0.59 ng/ml (95% confidence limits 0.49–0.69). Cotinine levels were found to be related to household smoking ($p < 0.001$) and so was the children's sex with females having higher levels than males ($p = 0.043$). Passive smoking is a serious problem among Greek preschool children with elevated cotinine levels found even in non-smoking households. Greece's high adult and adolescent smoking rates could partly be attributed to elevated child cotinine levels since Greek children are heavily exposed to secondary tobacco smoke from their parents. Our findings stress the need for immediate action so as to prevent the predisposition and early addiction to tobacco.

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P9 Toxicodynamics and Toxicokinetics

P9-01
Toxicological studies and pharmacodynamic effect of enalapril (an angiotensin-converting enzyme inhibitor) in rodents

Vera Stetinova, Vladimir Herout, Jaroslav Kvetina

Institute of Experimental Biopharmaceutics, Czech Republic

The aim of this study was to find effective antihypertensive dose of enalapril decreasing blood pressure and dose producing adverse reactions. Enalapril maleate in a dose of 20 mg/kg in intact rats significantly decreased blood pressure (determined in a sanguinary way) by 19% in male and by 16% in female 4 h after enalapril administration. After angiotensine I pre-treatment, the decrease after enalapril administration reached 80% in male and 93% in female. In toxicity studies enalapril was administered daily p.o. in a maximum administered dose (625 mg/kg) for 3 weeks to mice of both sexes (the short term repeated dose) and 6 months (chronic toxicity) in a dose of 47 and 942 mg/kg to male and female rats. Neither the mouse nor the rat studies resulted in any deaths considered to be directly attributed to the drug. Both studies have shown, that body weight gain has slightly decreased in all used doses (even though the consumption of food was not changed), heart weight has modestly decreased and kidney weight has slightly increased. In rats, the daily intake of water was increased (dose relatedly) which was followed by polyuria. Other changes were noted only after the higher doses. Apart from transient neutropenia in rats recorded after 3 months and slightly increased plasma concentration of urea after 3 and 6 months, the studied clinical chemistry parameters and haematology were not significantly changed. The autopsy showed heart dilatation in several rats of both sexes and changes in kidney parenchyma in two female rats. The histological examination revealed moderate vacuolisation of kidney-convoluted tubules in several rats. Toxicity studies were done in doses 2–50× higher than the dose used for studying antihypertensive effect of enalapril.

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P9-02
Role of phosphate transporters in the membrane transport of arsenate

RicardoVilla-Bellosta[2], Hector Giral[2], Ana Ferrer-Dufol[1], Victor Sorribas[2]

[1] *Clinic University Hospital, Zaragoza. Spain;* [2] *Laboratory of Molecular Toxicology, University of Zaragoza, Spain*

Arsenate is the most common form of the metalloid As in nature. After exposure and absorption, mainly through the intestine, it is distributed to peripherical tissues and reduced to arsenite. In spite of the extensive toxicokinetic studies of arsenate, the molecular mechanisms of its translocation through cell plasma membranes are still unknown. In this work we have studied the role of inorganic phosphate (Pi) transporters in the transport of arsenate. Our hypothesis is based on: (i) arsenate is a known inhibitor of Pi transport, and it was historically used to stop Pi transport in uptake assays; (ii) the three p*K*s of arsenate and phosphate are almost identical; (iii) one mechanism of arsenate toxicity consists on the substitution of Pi in biochemical reactions (arsenolysis).

We have performed inhibition kinetics of Pi transport by arsenate in several cell lines and in *Xenopus laevis* oocytes. Opossum kidney (OK) cells were chosen as a renal cell line and rat aortic vascular smooth muscle cells (VSMC) as a non-epithelial cell line. IC50 of arsenate in OK cells was 1 mM approximately, and >5 mM in VSMC. As a renal cell line, OK cells expresses both type II (a and c) and type III (Pit1 and Pit2) phosphate transporters, while VSMC only type III. In OK cells arsenate stimulated Pi transport at low concentrations (100 μM AsV). This hormetic effect was not observed in VSMC.

Next, we expressed the rat Na-dependent transporters NaPiIIa, NaPiIIc, Pit1 and Pit2 in *X. laevis* oocytes, by injection of *in vitro* transcribed capped-cRNA. Competition kinetics of arsenate in expressed Pi transporters matched the results in cultured cells, namely the type II a and c transporters showed a similar IC50 of about 1.5, while for the type III arsenate needed up to 5 mM to inhibit half of Pi transport in both Pit1 and Pit2. These results suggest that the affinity of the Pi transporters for arsenate are 5–100× less than for Pi, what precludes a role of type II and III Pi transporters as carriers of arsenate through cell membranes under physiological concentrations of phosphate. Other candidates are currently under study as arsenate transporters.

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P9-03

The toxicodynamics of antidiabetic glyklaside drug with subchronic administration to the rats

M. Kudrya, V. Kozar, N. Kavok, N. Ustenko

Institute of Endocrine Pathology Problems, Kharkov, Ukraine

Glyklaside (diabeton, predian, medoklaside) is an effective hypoglycemic drug for the therapy of diabetes mellitus representing the sulpha urea derivative.

The possible damage of glyclaside subchronic (30 days) administration (500 mg/kg, per os) was examined for the purpose to elicit the sensitive criteria of its potential danger during the output of substance. In dynamics some of the indicators of carbohydrate, protein and lipid metabolism, state of free radical processes and antioxidant protection, organism unspecific resistance were tested.

It was found that glyklaside produces marked decrease of glucose level and liver glycogen to 62% and 44% accordingly, some stress of protein metabolism in the form of decrease of general protein ($p < 0.05$) and disproteinemia. In the presence of relatively stability of lipid metabolism the decrease of β-lipoproteid fraction was noted ($p < 0.05$). The lipid free radical processes did not change markedly, stability of this system was mainly provided by to use the reduced glutathione ($p < 0.05$). The changes were seen with decrease of unspecific resistance and activation of oxygen depended mechanisms of neutrophiles bactericidal action.

Thus, glyklaside with subchronic administration produces transgression of general trophic processes following by disproteinemia, dislipidemia and decrease of non-specific protection factors.

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P9-04

Effect of subacute parathion administration on the pharmacokinetics and pharmacodynamics of nifedipine in rats

Ayse Gelal, Ozlem Eminoglu, Yusuf Cem Kaplan, Sule Kalkan

Dokuz Eylul University Medical Faculty, Department of Pharmacology, Izmir, Turkey

Background: Families living in agricultural areas are submitted to repeated exposure to organophosphate pesticides. In this present study, we hypothesized that repeated administration of low doses of parathion would modify the metabolism of nifedipine, which is metabolised by CYP3A and is not a substrate of P-glycoprotein, in rats. If parathion inhibits nifedipine metabolism, it would result in higher plasma levels of nifedipine and increased cardiovascular side effects.

Methods: Parathion suspension (1/100 LD_{50}, $n = 6$ or 1/25 LD_{50}, $n = 6$), or tap water (control, $n = 5$) was administered orally *via* gastric gavage (0.5 mL) to the unanesthetized male rats for 14 days. On the 15th day, the left carotid artery and right jugular vein were cannulated for measurement of cardiovascular parameters and blood sampling, respectively. Nifedipine was administered 3 mg/kg via the cannula inserted in duodenum of the rat. Serum nifedipine levels and cardiovascular effects were measured at frequent intervals. Differences in pharmacodynamic (PD) and pharmacokinetic (PK) parameters between control and parathion suspension groups were analyzed by the unpaired Mann–Whitney *U* test.

Results: Subacute parathion administration did not change PK (AUC_{0-240}, C_{max}, t_{max}, $t_{1/2}$) and PD (mean arterial pressures, heart rates) parameters of nifedipine.

Discussion: Parathion was a potent inhibitor of CYP3A2 in rat liver, in *in vitro*. In our previous study, we have shown that subchronic parathion exposure increased the blood cyclosporine concentration in rats. We had proposed that this effect could have been due to inhibition of CYP3A enzyme activity by parathion. However, increased cyclosporine concentration can also be explained by the effect of parathion on P-glycoprotein since cyclosporin is also a P-glycoprotein substrate.

Conclusions: These results suggest that repeated exposure low doses of parathion might not effect the first-pass metabolism of nifedipine, by inhibiting CYP3A in rats. The findings may contribute to the understanding of parathion drug interaction in human.

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P9-05

Induction of the nuclear receptor PXR in hepatoma cell lines

Y. Fery [1], S.O. Mueller [2], D. Schrenk [1]

[1] *Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany;* [2] *Institute of Toxicology, Molecular Toxicology, Merck KGaA, D-64293 Darmstadt, Germany*

Nuclear receptors are ligand-activated transcription factors that respond to endogenous substances, food constituents, therapeutic drugs and other xenobiotics. They play key roles in development and homeostasis as well

as in the etiology of certain diseases. They regulate metabolic pathways by alterations in gene expression of key metabolic enzymes. One of these receptors, the Pregnane X Receptor (PXR) was found to be efficiently activated by pregnanes, clinically used drugs, and by a broad spectrum of other compounds including plant constituents, pesticides, and environmental contaminants. PXR mediates the induction of several drug-metabolizing enzymes most notably of the CYP3A subfamily. Both PXR and CYP3A are co-expressed mainly in liver and intestine. As a heterodimer with Retinoid X Receptor (RXR) PXR binds to CYP3A promoter sequences activating CYP3A transcription.

To assess the ability of several xenobiotics to induce CYP3A enzymes via activation of PXR we examined the levels of PXR in human and rat hepatoma cell lines and its inducibility by the ligands dexamethasone and rifampicin. We assayed CYP3A4/1 and PXR protein levels by Western blotting and mRNA levels by real time PCR. Furthermore a PXR-dependent reporter gene assay was designed. Both cell lines expressed PXR being inducible by dexamethasone in the rat hepatoma cell line H4IIE and by rifampicin in the human hepatoma cell line HepG2. After induction of PXR we could observe a concentration-dependent increase of CYP3A1 in rat cells and of CYP3A4 in human cells. Transient transfection assays with a XREM-reporter gene correlated with the results of Western blotting and PCR. In summary, our findings show that rat and human PXR expression is inducible in hepatoma cell lines in a species-selective way similar to regulation of CYP3A enzymes.

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P9-06

Effect of grapefruit juice and naringin over the ifosfamide absorption in mouse

Eduardo Madrigal-Bujaidar [1], Laura Martino-Roaro [1], Isela Álvarez-González [1], Edilberto Pérez-Montoya [2]

[1] *Departamento de Morfología, Laboratorio de Genetica, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional Mexico;* [2] *Laboratorio de Biofarmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico*

A number of investigations have reported grapefruit juice–drug interactions, where a raise in drug bioavailability by inhibition of CYP3A4 isoenzyme and/or glycoprotein-P has been demonstrated, originating a reduction in the first-pass metabolism during intestinal absorption. The aim of this study was to evaluate the effect of grapefruit juice (GJ) and its flavonoid, naringin (NAR), on the absorption of ifosfamide (IFF), an antineoplasic which is biotransformed by CYP3A4. Earlier studies showed that GJ and NAR reduced the DNA damage produced by IFF, but their mechanism is not clear. We suggest it could be related with the variation in the bioavailability of IFF. Plasmatic levels of IFF (300 mg/kg oral route) were monitored in the following groups: (1) IFF, (2) NAR (250 mg/kg) + IFF and (3) GJ (500 mg/kg) + IFF. NAR and GJ were orally administered 1 h before the drug. Each group constituted 84 male mice NIH. Blood samples were taken by cardiac puncture in subgroups of 12 mice each at 2.5, 5, 7.5, 10, 20, 30 and 60 min. Temporal profiles of IFF were determined and the following pharmacokinetic parameters were obtained by a non-compartmental model with the program WinNonlin 4.0: maximum plasma concentration (C_{max}), the time to reach the C_{max} (t_{max}), the area under the curve ($AUC_{0-\infty}$) and the half-life ($t_{1/2}$). Statistical analysis for C_{max} was calculated with the ANOVA and Student–Newman–Keuls tests ($\alpha = 0.05$) and for AUC, t_{max} and $t_{1/2}$, with the Kruskal–Wallis and Student–Newman–Keuls tests ($\alpha = 0.05$). The results showed that NAR increased 17.8% C_{max} and GJ 22.9% in comparison with data of the IFF treated group. Furthermore, NAR reduced 53.8% the t_{max} value and GJ reduced 19.2%. When GJ was administered to mice, the value of $t_{1/2}$ diminished 29.8% in comparison with the obtained in the NAR treated group. $AUC_{0-\infty}$ results in all groups did not show a significant statistical difference. GJ and NAR clearly increased IFF absorption velocity but not the quantity absorbed.

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P9-07

Lactobacillus rhamnosus strain GG modulates toxicity and kinetics of orally administered AFB_1 in rats

Silvia Gratz [1], Martin Täubel [1], Risto O. Juvonen [2], Matti Viluksela [3], Paul C. Turner [4], Hannu Mykkänen [1], Hani El-Nezami [1]

[1] *Food and Health Research Centre, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland;* [2] *Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland;* [3] *National Public Health Institute, Laboratory of Toxicology, P.O. Box 95, FIN-70701 Kuopio, Finland;* [4] *Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, LIGHT Laboratories, University of Leeds, Leeds LS2 9JT, UK*

Aflatoxin B_1 (AFB_1) is an unavoidable food contaminant and reducing its bioavailability *in vivo* is of major interest. Our *in vitro* studies demonstrated that the probiotic *Lactobacillus rhamnosus* strain GG (GG) can bind AFB_1. In this study we assessed the effect of GG *in vivo* kinetics of orally administered AFB_1 and its toxicity. Male Han–Wistar rats (5-week old) were divided into two groups ($n = 12$ per group), one group receiving the probiotic (GG, 5×10^{10} CFU in 0.5 mL PBS) daily for 6 successive days, and the other group receiving only PBS. Immediately after the fourth dose of GG or vehicle, all animals received a single oral dose of AFB_1 (1.5 mg/kg body weight in 0.5 mL DMSO). Urine and feces were collected daily for 3 days after the AFB_1 dose, and body weight was recorded at the beginning of the study, at the day of the AFB_1 dose, and at the end of the study. Blood samples were taken at the end of the study. In the presence of GG, the fecal excretion of AFB_1 and AFM_1 was increased significantly (127% increase, $p = 0.015$ and 154% increase, $p = 0.001$ respectively) within 24 h after dosage and the cumulative excretion over 3 days was increased by 28% ($p = 0.094$) and 66% ($p = 0.013$) respectively when compared to animals dosed with AFB_1 alone. Body weight was reduced in animals receiving AFB_1 alone, whereas the body weight of animals receiving AFB_1 and GG maintained stable. Furthermore, the hepatotoxic effect of AFB_1 (increased plasma alanine transaminase activity 103.7 ± 84.9 U/L versus 41.6 ± 18.7 U/L in controls, $p = 0.053$) was prevented by GG administration (56.4 ± 34.2). In conclusion, GG administration increased the retention of AFB_1 and the AFM_1 metabolite within the gastrointestinal tract, leading to increased excretion via the fecal stream.

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P10 Biomarkers and Exposure Assessment

P10-01

Effects of female aging and metal pollution on glutathione-dependent enzymes in _Chorthippus brunneus_ nymphs

Maria Augustyniak, Agnieszka Babczyńska, Elżbieta Szulińska, Ilona Witas

Department of Animal Physiology and Ecotoxicology, University of Silesia Bankowa 9, 40-007 Katowice, Poland

The aging is connected with an increase of pro-oxidative factors and gradual weakening of antioxidant systems. Also heavy metals, present in contaminated environment, may stimulate oxidative processes and than accelerate the process. Therefore, aging in heavy metal polluted environment may be faster and can leave its impress on the offspring. On the other hand, insects chronically exposed to heavy metals might have developed some eggs and/or offspring protection mechanisms. The main aim of this study was to assess the relations between the age of female grasshoppers, living in polluted habitats, and the glutathione contents as well as glutathione-dependent enzymes in their offspring.

Our study was conducted on first instar nymphs of *Chorthippus brunneus*, the mothers of which were collected at two sites located in the vicinity of non-ferrous metals smelter in Bukowno (Pollution Index, PI = 20.5) and Szopienice (PI = 22.7). The reference site was located near Pilica (PI = 0.53).

Significant influence of females' age as well as their origin on nymph's GSH-dependent system was found, especially in insects from metal polluted sites. In case of grasshoppers from reference site (Pilica) activity of enzymes connected with glutathione (in most cases) did not depend on female age. In insects from polluted sites, significant increase of GSH-dependent enzymes was observed in offspring originated from the oldest females in comparison with nymphs from the youngest females. The strongest effects were found in individuals from Szopienice, where activity of glutathione *S*-transferase or glutathione peroxidase were almost two times higher in offspring from oldest mother in comparison with nymphs from the youngest mothers. The pattern of glutathione reductase activity was different in nymphs from all three populations. In hatchlings from Pilica GR activity did not depend on female age. In grasshoppers from Bukowno the highest activity of the enzyme was mea-

sured in offsprings of the youngest mothers. The opposite relation was observed in individuals from Szopienice. This result may be an effect of some differences in the population adaptation to heavy metal polluted sites.

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P10-02

GSH-dependent enzymes and heavy metals mapping in grasshopper associated with Nickel hyperaccumulators

Maria Augustyniak[1], Wojciech Przybyłowicz[2,4], Jolanta Mesjasz-Przybyłowicz[2], Monika Tarnawska[1], Paweł Migula[1], Elżbieta Głowacka[3], Agnieszka Babczyńska[1]

[1] *Department of Animal Physiology and Ecotoxicology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland;* [2] *iThemba LABS, P.O. Box 722, Somerset West 7129, South Africa;* [3] *Department of Zoology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland;* [4] *Faculty of Physics and Applied Informatics, AGH University of Science and Technology, Kraków, Poland*

Grasshopper *Stenoscepa* sp. is one of the insects feeding on the South African nickel hyperaccumulating plant *Berkheya coddii*. The test of choice experiments carried on the second stage larvae demonstrated that this insect prefers three out of seven offered plants. These three plants are Ni hyperaccumulators. Apparently ingested Ni (up to 7.6%) does not disturb the development of this grasshopper. This particular ability provoked us to search for symptoms of the adaptation to survive in such extreme conditions. Elemental mapping for nickel and other elements in the body of this insect was carried out. The activity of glutathione-dependent enzymes and glutathione (GSH) content in the second stage larvae tissues were also measured.

Analysis of concentration and distribution of Ni in *Stenoscepa* sp. tissues performed by micro-PIXE method showed the highest Ni level in the gut (in the peritrophic membrane areas and in the pyloric valves—over 1000 $\mu g\, g^{-1}$). In Malpighian tubules Ni content was about 700 $\mu g\, g^{-1}$, while in the brain ganglia the concentration of over 100 $\mu g\, g^{-1}$ was found.

One of the ways to survive under permanent Ni intoxication conditions is an intensified GSH synthesis. GSH concentration in tissues of the grasshoppers was very high (average value 28 $\mu g\, mg^{-1}$ protein). In comparison to larvae of other Acridities species-inhabiting areas contaminated with heavy metals in Europe this amount was about six times higher. GSH-dependent enzymes activity was at similar (or much lower) level as in the Orthoptera larvae, which grow in contact with heavy metals. Also catalase activity was 5–10 times lower in comparison to other Orthoptera. Glutathione reductase (GR) activity was unexpectedly low (at detection limit level). It is likely that the studied grasshopper belongs to a group of animals, which may use thioredoxine system for regeneration of the reduced form of GSH.

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P10-03

The effects of female age and heavy metals on DNA damage in grasshopper brains

Maria Augustyniak, Jolanta Juchimiuk, Agnieszka Babczynska, Elzbieta Szulinska, Ilona Witas

University of Silesia, Poland

Heavy metals may generate free radicals and thus cause DNA damage observed as its fragmentation. Moreover, the increase of prooxidants contents accompanies the aging process. The processes could sum up, so in contaminated areas ageing may be faster than in unpolluted sites. However, in some cases we could expect, that insects may use mechanisms, which are developed under metal exposure, to diminish aging. The purpose of this work was to study the relations between the age of female grasshoppers, living in polluted habitats and DNA damage in their offsprings.

Grasshopper females originated from two heavily metal polluted (Bukowno and Szopienice) and reference (Pilica) sites. The initial culture of insects was reared under controlled condition and every 10 days the egg-pods were collected. Five cohorts were obtained (the first one originated from the youngest females and the last one from the oldest ones). Additionally, part of the egg-pods was exposed to zinc (added to sand) during diapause. To assess genotoxic effects of zinc and female aging, the cells from brains of first instar grasshopper larvae were isolated and alkaline version of comet assay (20 V, 300 mA in 4 °C) was performed. Tail DNA, tail length and tail moment were measured as parameters of DNA strand breaks level (Komet 5.0, Kinetic Imaging, Liverpool, UK). Simultaneously the visual scoring of comet images has been applied.

Both methods of DNA damage analysis have shown that the level of DNA damage depend on grasshopper mother's age. The degree of DNA damage was higher in offsprings of young females in comparison with nymphs hatched from eggs laid by the oldest mothers. The site of origin effect was less distinct, however, some differences were observed. This may suggest that grasshoppers from Bukowno might have adapted to chronic metal exposure.

Zn-treatment (in lower dose) had a limited influence on DNA fragmentation in cells isolated from nymph's brain. This may confirm the hypothesis that Zn influence on the brain is precisely controlled; over the threshold zinc concentration defensive and/or repair mechanisms are activated.

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P10-04

Contribution of cholinergic muscarinic functions in cadmium-induced hypertension in rats

Nattaporn Yoopan[1], Orapin Wongsawatkul[2], Piyajit Watcharasit[3], Pawinee Piyachaturawat[4], Jutamaad Satayavivad[1,3]

[1] *Toxicology Graduate Programme, Faculty of Science, Mahidol University, Rama 6 Rd., Bangkok 10400, Thailand;* [2] *Department of Pharmacology, Faculty of Medicine, Srinakarinwirot University, Bangkok 10110, Thailand;* [3] *Laboratory of Pharmacology, Chulabhorn Research Institute, Vibhavadee-Rangsit Highway, Bangkok 10210, Thailand;* [4] *Department of Physiology, Faculty of Science, Mahidol University, Rama 6 Rd., Bangkok 10400, Thailand*

Cadmium (Cd) has been reported to induce hypertension in rats; however, its mechanism is not clearly understood. In this study an attempt has been made to study the responses of cholinoceptors to acetylcholine (ACh) in isolated atria and descending aortas of Cd-exposed rats. Male Sprague–Dawley rats were exposed to cadmium via drinking water at concentrations of 5, 10 and 50 ppm for 3 months. The systolic blood pressure was measured weekly. Significant increases in systolic blood pressure of about 20–30% of the control values were detected in 10 and 50 ppm Cd-treated groups. At the end of 3 months, the contents of Cd in the hearts and kidneys were determined by using graphite atomic absorption spectrometer. There were highly significant increases of Cd in these tissues in a dose-dependent manner. The isolated spontaneously beating right atria and the norepinephrine-induced vasoconstriction of the isolated descending aorta were used as the test model to study the muscarinic receptor responses to acetylcholine. In addition, the responses of these Cd-treated tissues to norepinephrine (NE) at the concentration range 10^{-9} to 10^{-5} M were also investigated. It was found that the responses to ACh (10^{-9} to 10^{-5} M) in both cardiac and vascular muscarinic receptors were significantly decreased in Cd-50 ppm exposed group. The EC_{50} values of ACh in right atria and aorta of the control and Cd-50 ppm treated groups were 2.66×10^{-6}, 8.32×10^{-6} and 1.75×10^{-7} and 3.85×10^{-7} M, respectively. However, the EC_{50} values of NE of both control and Cd-exposed groups were not significantly different. Moreover, the maximal responses to NE in both tissues were decreased. This finding suggested that alpha-1 adrenoceptor does not play a critical role in Cd-induced hypertension. The results of this study revealed that cardiac and vascular muscarinic receptors also play a significantly role in Cd-induced increased blood pressure. Further study is needed to elucidate the mechanism of Cd-induced altered responses of cardiac and vascular muscarinic receptors.

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P10-05

Metallothionein induction in mammary gland and reduced milk synthesis in mice exposed to cadmium during lactation

Jonas Tallkvist, Helena Öhrvik, Maria Nyström, Agneta Oskarsson

Cadmium (Cd) is retained in the lactating mammary gland of various species and only low levels of the element is transferred into milk. We have recently demonstrated a significant negative dose–response relationship between β-casein expression in the mammary gland and exposure levels of Cd. The present investigation was undertaken to examine whether Cd exposure during lactation induces metallothionein (MT) in the mammary gland and also to examine whether Cd exposure affects milk synthesis by measuring gene expression of whey acidic protein (WAP) as well as body weight gain of the suckling pups. Primiparous NMRI mice with normalized litter sizes ($n = 8$) were injected subcutaneously with $CdCl_2$ (2 mg Cd/kg body weight/day) or only vehicle as controls on postnatal days (PND) 8, 9 and 10. Left inguinal mammary glands (4 and 5) were dissected on PND 11 for immunohistochemistry, quantitative gene expression and histopathology. The body weights of the suckling pups were recorded on PND 2, 8 and 11. As demonstrated by the immunohistochemistry MT was mainly localized in the alveolar epithelium and MT synthesis appeared to be induced in these cells as a result of the Cd treatment. Upregulation of MT in the mammary gland of the Cd treated dams was confirmed by real-time RT-PCR. In contrast, WAP gene expression was significantly downregulated. The histopathology revealed increased fat content in the mammary tissue of the Cd treated dams and a less active feature of the alveolar epithelium as compared to the controls. The body weight

gain of the suckling pups nursed by the dams treated with Cd was significantly reduced. In conclusion, our results indicate that MT is involved in the retention of Cd in the lactating mammary gland which may explain the low transfer of Cd into milk. Furthermore, Cd exposure during lactation appears to disturb milk synthesis and development of the suckling offspring.

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P10-06

Metal exposure assessment in native fish, *Mullus barbatus*, from the Eastern Adriatic Sea

Vlatka Filipović Marijić, Biserka Raspor

Ruđer Bošković Institute, Zagreb, Croatia

Environmental contamination is steadily increasing due to diverse anthropogenic activities. In order to estimate the risk of metal exposure, reliable biomarkers as an early warning cellular mechanisms, have to be validated and applied in the field. Metal-associated stress in the organism is reflected in the induction of metallothioneins (MTs), cytosolic proteins responsible for essential metal homeostasis (Zn, Cu) and detoxification of toxic metals (Cd, Hg, Ag). Prerequisite for reliable application of MT, as a biomarker in native aquatic organisms, is to define differences between species and tissues, as well as physiological and environmental conditions that influence cellular MT and metal content. Age related MT and metal (Zn, Cu, Fe, Mn, Cd) variations have been studied in three indicator tissues of benthic fish *Mullus barbatus* from the Eastern Adriatic Sea. Liver and kidney are commonly used as indicator tissues, while intestine has been selected as primary uptake route for dietary metals. Liver cytosol is characterized by MT and essential metal association (Zn and Cu), reflecting the MT role in their homeostasis, while Cd accumulates with fish age (significant increase from 1 to 8 years old specimens; $b = 2.84$, $R^2 = 0.90$). Cadmium accumulation also occurs in kidney tissue, but slower that in liver ($b = 1.03$, $R^2 = 0.73$). Intestinal MT level is associated with Cu level ($R = 0.38$), without significant relation to age. MT levels are age-related in kidney, but not in liver and intestine. In native red mullet constitutive hepatic MT level amounts to 8.89 $\mu g\, mg^{-1}$ proteins and intestinal to 28.3 $\mu g\, mg^{-1}$ proteins. Metal distribution is tissue specific with highest cytosolic Cu and Cd levels in liver, Zn and Mn in intestine and Fe in kidney. Besides comparison of metal levels in different fish tissues related to age, comparison was performed for specimens from differently contaminated locations, i.e. the Kaštela Bay and the area around Šolta Island. For both locations hepatic MTs are comparable, but MT level in fish intestine is significantly higher in specimens from coastal urban area (Kaštela Bay) and is significantly associated with Cu ($R = 0.44$). Therefore, MT induction in intestine cytosol has been regarded as Cu associated biochemical response in native red mullet specimens dwelling above sediments in the Kaštela Bay that have already been reported as elevated in Cu, as the consequence of anthropogenic activities.

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P10-07

Inexpensive and automated predictive and mechanistic analysis of compound effects in vivo and in vitro using microarrays for discovery, lead optimization and preclinical safety assessment

Jacques Retief, Georges Natsoulis, Alexander Tolley, Brigitte Ganter, Richard Brennan, Mark Fielden, Kurt Jarnagin

Iconix Pharmaceuticals, United States

We have assembled a large toxicogenomic database, DrugMatrix®, containing microarray expression profiles from short-term repeat dose rat studies for over 630 reference drugs and toxicants. Gene expression profiles were collected from up to seven different tissues, in addition to standard hematology, clinical chemistry, histopathology and pharmacology assay data. We systematically mined the gene expression domain of this dataset using SPLP (Sparse Linear Programming), a two-class supervised classification method. More than 300 thoroughly cross-validated toxicology and pharmacology biomarkers (Drug Signatures®), each composed of an average of 47 genes, were identified. We have previously demonstrated that these signatures resolve distinct and uncorrelated end-points, and that a small number of overlapping genes (<2000) are sufficient to recreate all signatures with no appreciable loss in classification performance. Based on contextual data in DrugMatrix and relevant pathways, we can quickly obtain insight into potential mechanisms of action and toxicity. We have taken advantage of this opportunity to create an inexpensive multi-endpoint diagnostic device on an Affymetrix platform applicable within the drug discovery and development process. To overcome the expense, time and expertise needed for the interpretation and reporting of large-scale gene expression data, we have automated the evaluation of Drug Signature predictions, pathways and individual genes in a fully detailed and documented report. This higher throughput analytical platform will be able to predict numerous important toxicological and pharmacological endpoints

in vivo using liver, kidney or heart tissue. For increased throughput, the platform can also be used with in vitro expression data obtained from compound-treated rat primary hepatocytes. By addressing the major bottleneck in the use and expense of large-scale gene expression analysis in safety assessment, decision making in the drug discovery and development process will be improved.

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P10-08

Preliminar characterization of carboxylesterase activities found in plasma of wild birds

Eugenio Vilanova, Francisco Soler, Raquel Ganga, Miguel A. Sogorb

Universidad Miguel Hernández, Elche, Alicante, Spain

Organophosphorus and carbamate insecticides inhibit the carboxilesterases found in plasma. Therefore, these carboxylesterases might be used as biomarkers of exposure to these insecticides. This work initiates the characterization of the phenylacetate (PAase) and 1-nafthylacetate (NAase) hydrolysing activities in plasma of 11 different wild bird species with the aim of determinate their suitability for being used as biomarkers of exposure. PAase activity values, expressed as nmol product/30 min/mL plasma, ranged between $38{,}000 \pm 2300$ (black vulture) and $27{,}400 \pm 850$ (barn owl), while NAase values did between 6000 ± 5200 (griffon vulture) and $37{,}700 \pm 850$ (barn owl) nmol product/30 min/mL plasma. In all assayed species NAase was between 1.1 and 2.8 times higher than the corresponding PAase. PAase and NAase of chicken white stork were 1.6 and 1.7 times higher respectively than the corresponding activities of adult individuals. The nocturnal raptors eagle owl and barn owl exhibited PAase and NAase between 1.3 and 8.0 times higher than activities exhibited by diurnal raptors (montagu's harrier, common buzzard, booted eagle, Spanish imperial eagle, black kite, griffon vulture and black vulture). These data suggest that plasma PAase and NAase of the studied birds might be used as biomarkers of exposure to organophosphorus and carbamate insecticides, although further studies of inhibition of these activities are needed.

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P10-09

Studies on the impact of heavy metal cadmium on certain enzymes in a freshwater teleost fish, *Cyprinus carpio*

Mathan Ramesh

Unit of Toxicology, Department of Zoology, Bharathiar University, Coimbatore 641046, India

Pollutants of anthropogenic origin alter the water chemistry and induce stress in organisms living them. Heavy metals due to their bio-accumulative and non-biodegradable properties constitute a core group of aquatic pollutants. Cadmium is a ubiquitous relatively rare metal and is regarded as one of the most toxic heavy metals and potential for fish exposure has increased with increasing industrial use of the metal. Cadmium interferes with nearly all the metabolic processes and accumulates in soft tissues of the body. Perturbations of enzyme activity in aquatic animals may serve as early indicators of toxicity of pesticides, heavy metals and other pollutants. In the present study the toxicity of cadmium chloride on glutamate oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) activities in heart, liver and kidney of an economically important freshwater fish *Cyprinus carpio* was evaluated. During acute treatment (1.60 pp/L), all the tree enzymes enhanced in their activity in all three organs; GOT being maximum followed by GPT and LDH. The increase in GOT, GPT and LDH activities might have resulted from tissue damage and increased synthesis of particular enzymes to defend against stress. During sub lethal treatment (1.60 ppm), GOT activity elevated in all the organs, whereas GPT activity was found to be inhibited in heart; while liver and kidney showed enhanced GPT activity. LDH activity was inhibited during initial study period, which later recovered. The significant decrease in GOT activity during sub lethal treatment results from the stronger inhibitory effect of the cadmium on the enzyme activity. The intracellular action of cadmium subsequent to initial damage of the plasma membrane might have caused the inhibition of GPT activity during sub lethal toxicity. The inhibition of LDH activity during initial sub lethal treatment results from the changes in the mitochondrial membrane function. Whereas the increase of enzyme activity during sub lethal treatment indicates that glycoltic rate might be stepped up to cope up with cadmium stress. The significant changes in the enzyme activities can serve as a valuable biomarker of pollutant exposure and effects.

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P10-10

Variation of metallothioneins and antioxidant defense system state in natural and transplanted freshwater bivalves

O.V. Mishchuk, H.I. Falfushinska, O.B. Stolyar

Ternopil V. Hnatiuk National Teachers' Training University, Ternopil, Ukraine

Western Ukraine is reported to be a relatively clean ecologic territory. Negative changes in the composition of freshwater biota were, nevertheless, pointed here during last years. So the collection of baseline environmental data and the study of the effect of animals' transplantation in the sites of their absence is the important task for the assessment of possibility of utilization of biochemical parameters of freshwater bivalve as the early biomarkers of water poisoning.

The freshwater bivalves of two species (*Colletopterum piscinale* and *Anodonta cygnea*, Unionidae) were investigated in natural aquatic bodies of the basin of river Dnister characterized by different water quality and after transplantation to laboratory or another field site for up 14 days. General tissues content of selected metals (Fe, Zn, Mn, Cu, Pb, Cd) as well as their content in metallothioneins (MTs) in digestive gland and gills of clams, Cu, Zn and Mn superoxide dismutase (SOD) activity, reduced glutathione and oxidative stress markers were measured.

The accumulation of marked quality of Mn, Fe, Zn in the gills was reflected in native groups. Two major isoforms of MTs were resolved by consequent gel-permeation and ion-exchange chromatography from tissues of clams. The elevation of Zn:Cu ratio was observed in MT-1 of clams from field conditions in comparison to laboratory groups. It was accompanied with appropriate changing of UV-spectra. The oppression of the antioxidant capacity in field groups both in residents and in transplanted clams was revealed. Especially reduced Mn-SOD activity in conjunction with elevated lipid peroxidation was reflected in gills of clams from agricultural pond and from river near motorway. The groups of transplanted clams were differed from native populations by the reduced proteins' level in tissues. Any regularity of metals content in tissues in accordance to its fluctuations in water for exception Mn content in gills wasn't revealed. Consequently the measuring of Zn:Cu content ratio in MTs may be a control point of metal homeostasis in clams. Among the inspected parameters the final toxic effect of native waters was the best shown relate to oxidative stress.

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P10-11

A polymorphism in the delta-aminolevulinic acid dehydratase gene modifies plasma/whole blood lead ratio

Marcelo F. Montenegro [1], Fernando Barbosa Jr. [2], Valeria C. Sandrim [1], Raquel F. Gerlach [3], Jose E. Tanus-Santos [1]

[1] *Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes 3900, 14049-900 Ribeirao Preto, SP, Brazil;* [2] *Department of Clinical, Toxicological and Food Science Analysis, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo, Av. do Cafe s/n, 14040-903 Ribeirao Preto, SP, Brazil;* [3] *Department of Morphology, Estomatology and Physiology, Dental School of Ribeirao Preto, University of Sao Paulo, Av. do Cafe s/n, 14040-904 Ribeirao Preto, SP, Brazil*

Delta aminolevulinic acid dehydratase (ALAD) plays an important role in lead poisoning. This study was carried out to examine the effects of ALAD gene polymorphism (G177C) on %Pb-P(plasma lead)/%Pb-B(whole blood) ratio in 142 subjects environmentally exposed to lead. Genotypes for the ALAD G177C polymorphism were determined by PCR and restriction fragment length digestion. Pb-P and Pb-B were determined by inductively coupled plasma mass spectrometry and by graphite furnace atomic absorption spectrometry, respectively. The allele frequencies for ALAD1 and ALAD2 alleles were 0.897 and 0.103, respectively. We combined both ALAD 1-2 and ALAD 2-2 genotypes together (ALAD 1-2/2-2 group) and compared with the ALAD 1-1 genotype group. While no significant differences were found in Pb-B, subjects from the ALAD 1-2/2-2 genotype group presented significantly higher Pb-P concentrations and %Pb-P/%Pb-B ratios (0.89 ± 0.07 μg/l, and 1.45 ± 0.10%, respectively) when compared with subjects from the ALAD 1-1 genotype group (0.44 ± 0.05 μg/l, and 0.48 ± 0.02, respectively; both $P < 0.0001$). The higher %Pb-P/%Pb-B ratios in carriers of the ALAD-2 allele compared with noncarriers indicate that ALAD 1-2/2-2 subjects are probably at increased health risks associated with lead exposure.

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P10-12
Clinical evidence for lead-induced inhibition of nitric oxide formation

Fernando Barbosa Jr. [1], Jonas T.C. Sertorio [2], Michele M. de Castro [2], Maria Regina Torqueti Toloi [1], Jose E. Tanus-Santos [2]

[1] *Department of Clinical, Toxicological and Food Science Analysis, Faculty of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Av. do Cafe s/n, SP 14040-903, Ribeirao Preto, Brazil;* [2] *Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil-FMRP-USP, Av. Bandeirantes, 3900 Monte Alegre, CEP 14049-900 Ribeirao Preto, SP, Brazil*

Lead exposure has been associated with increased cardiovascular risk, which may result, at least in part, from lead-induced increases in oxidative stress and depressed nitric oxide (NO) availability. However, no previous clinical study has examined whether lead exposure is associated with significant effects on biomarkers of NO activity (plasma nitrites, nitrates, and cyclic guanosine 3′,5′-monophosphate; cGMP). We investigated whether there is an association between the circulating concentrations of nitrites, nitrates, and cGMP and the concentrations of lead in whole blood (B-Pb) or plasma (P-Pb) from 62 lead-exposed subjects (30 men and 32 women). P-Pb was determined by inductively coupled plasma mass spectrometry (ICP-MS) and B-Pb by graphite furnace atomic absorptions pectrometry (GF AAS). Plasma nitrite and nitrate concentrations were measured using an ozone-based chemiluminescence assay. Plasma cGMP concentrations were measured using a commercial enzyme immunoassay. We found a negative correlation between plasma nitrite and B-Pb concentrations ($r = -0.358$; $P = 0.004$), and between plasma nitrite and P-Pb concentrations ($r = -0.264$; $P = 0.038$), thus suggesting increased inhibition of NO formation with increasing B-Pb or P-Pb concentrations. However, no significant correlations were found between plasma nitrate or cGMP and B-Pb or P-Pb concentrations (all $P > 0.05$). These findings suggest a significant inhibitory effect of lead exposure on NO formation and provide clinical evidence for a biological mechanism possibly involved the association between lead exposure and increased cardiovascular risk.

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P10-13
Induction of Cytochrome P450 enzymes in Rat Liver and Rat Primary Hepatocytes by Polybrominated Diphenyl Ethers

S. Germer [1], Y. Fery [1], L. van der Ven [2], A.H. Piersma [2], A. Kamyschnikow [1], D. Schrenk [1]

[1] *Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany;* [2] *Laboratory for Toxicology, Pathology and Genetics, National Institute for Public Health and the Environment RIVM, NL-3720 BA Bilthoven, The Netherlands*

Polybrominated diphenyl ethers (PBDEs) have been used in large quantities worldwide to reduce the risk of fire, and are widely spread in the environment. The rising levels of PBDEs in human tissues over the last decades have raised concerns about possible health effects. Some PBDEs have been suspected to act as 'endocrine disrupters' and/or to affect the development of the unborn. Induction of drug metabolism may play a role in such effects by changing the body's homeostasis of certain hormones. In this study a cleaned-up commercial PBDE mixture ('Pentamix') mainly containing BDE 99 (2,2′,4,4′,5- pentabromo-DE) was applied by gavage to adult Wistar rats (0, 0.27, 0.82, 2.47, 7.4, 22.2, 66.7, 200 mg/kg b.w. per day) over 28 days. Hepatic levels of cytochrome P450 (CYP) 1A1/2, 2B1/2, and 3A1/3 and microsomal 7-ethoxyresorufin-*O*-deethylase (EROD), 7-pentoxyresorufin-*O*-dependentylase (PROD), and luciferin benzylether-debenzylase (LBD) activities were analyzed. Additionally, rat primary hepatocytes were treated with the mixture for 48 h and EROD activity was determined. EROD analysis revealed a dose dependent increase of up to 40 fold in female rat liver as well as in rat primary hepatocytes. The increase in EROD activity in male rat liver was lower than in females (up to 20 fold).

A strong increase in PROD activity was seen at a dose of 2.47 mg/kg b.w. per day, reaching a maximum of 25 fold at the highest dose in female rats and 10 fold in male rats. No significant change in LBD activity was observed. Western blot analysis indicated a dose-dependent increase in CYP1A, 2B and 3A protein through treatment with 'Pentamix'.

These findings suggest that oral treatment with a commercial PBDE mixture has a significant inducing effect on hepatic drug-metabolizing enzymes CYP1A, CYP2B and CYP3A in rat liver. This pattern of CYP induction may cause variations in hormone metabolism, thus leading to endocrine effects. Furthermore, induc-

tion of CYP2B enzymes by the mixture indicates a 'phenobarbital-like' induction through the constitutive androstane receptor (CAR) by some PBDE congeners.

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P10-14

***Crassostrea virginica* glutathione *S*-transferase activity as biomarker of environmental contamination in terminos lagoon, Campeche, Mexico**

Jaime Rendón-von Osten, Alejandro Ortiz Arana, Martin Memije-Canepa, Guillermo Villalobos, Jorge Benítez

Centro EPOMEX, Universidad Autónoma de Campeche, P.O. Box 520, Campeche, Mexico

Terminos lagoon in Campeche is one of the most productive ecosystems in Mexico, and is constituted by three fluvio lagoon system in which agricultural activity is carried out along these systems. In aquatic organisms, induction of specific GST following exposure to diverse agents such as PAHs, PCBs and organochloride pesticides has been well documented. The objective of this study was to establish baseline values of GST activity in reference and contaminated areas and their responsiveness as indicators of exposure to environmental contaminants. Twenty adult oysters of undetermined sex and selected size ($n = 254$) were handpicked in thirteen sampling stations around Terminos lagoon during March 2005. Organisms were placed in thermally insulated boxes, previously filled with local water, and transported to the laboratory. The activity of GST was determined by the Habig method adapted to microplate. Mean GST activity of oyster (*Crassostrea virginica*) was 24.76 ± 10.27 U/mg protein. It was identified significant differences among sampling sites ($F_{(12,253)} = 21.37$; $p = 0.00$) which indicate that oyster are exposed to contaminants in different grades. Oysters from a small lagoon showed the lowest GST activity (9.75 ± 2.16 U/mg protein), therefore this site was considered as reference site. According to this site, induction of GST activity ranged from 35.7% in the mouth of the lagoon to 263% which correspond to the fluvio lagoon system of Palizada. Oysters collected from the three fluvio lagoon systems showed the highest induction of GST, ranged from 185% to 263%. These inductions can be due to compounds carried out by the stream of each river which could contain traces of pesticides used in rice crops and residues of DDT used many years ago for malaria control. Oyster GST activity seems to be useful to address an environmental risk assessment.

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P10-15

Pterins—Possible markers of stress situations in pigs

K. Breineková, M. Smutná, M. Svoboda, L. Vorlová

Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Neopterin and biopterin belong to a group of unconjugated pterin derivatives. Neopterin is produced primarily by immune system cells, *i.e.* monocytes/macrophages, and, in human medicine, it is used as a reliable marker of Th-1 type of cell immunity activation. The reduced form of biopterin, *i.e.* tetrahydrobiopterin, is an important co-factor of the NO synthasis and hydroxylases of aromatic amino acids (phenylalanine hydroxylase, tryptophan hydroxylase and tyrosine hydroxylase), which play a role in catecholamine and serotonin metabolism. In veterinary medicine, however, the issue of neopterin and biopterin metabolism and their functions have neither been thoroughly studied nor understood. The aim of our studies was to identify and quantify neopterin and biopterin in the porcine serum under various stressful conditions. To analyze pterin compounds, HPLC method with fluorescent detection was used.

In the first study, transport-related stress loads in pigs were investigated. The pigs were being transported for 30 min. After that period, a statistically significant increase in pterin concentrations in all experimental pigs was observed ($p < 0.01$ for both pterin derivatives). The relationship between the two pterins and the RYR-1 genotype in pigs (NN and Nn) was also investigated. No statistically significant differences in pterin concentrations between individual groups of pigs divided according to their genotype were, however, found. In the second study, increased neopterin and biopterin concentrations in serum of calves 4 h after intramuscular administration of Fe^{3+}-dextran compared with the control group ($p < 0.01$) were detected. Twenty-four hours after Fe^{3+}-dextran administration, a statistically significant decrease in both biopterin and neopterin concentrations were recorded ($p < 0.05$ and $p < 0.01$, respectively). In the third study, pterin concentrations were investigated in pigs with a respiratory disease. Concentrations of both neopterin and biopterin were significantly increased compared with a group of healthy pigs ($p < 0.01$ for both pterin derivatives).

It follows from our results that the synthesis of pterin derivatives, *i.e.* neopterin and biopterin, is influenced by diverse stress factors. Neopterin and biopterin serum concentrations may thus provide additional information on the influence of stress factors on the porcine organism *in vivo*, and, at the same time, they extend the modest list of papers on pterins in veterinary practice.

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P10-16

Lessons learned from longitudinal studies to identify high exposure groups using OP pesticide urinary biomarkers

Elaine M. Faustman, William C. Griffith, Eric Vigoren

University of Washington, Washington, United States

We reanalyze data on a urinary biomarker, TCP a metabolite of an organophosphate pesticide (OP) chlorpyrifos in two populations to understand issues in identifying highly exposed individuals. The studies being reanalyzed are two population-based samples, one of 79 persons from Maryland over a 12-month period and the other of 90 persons from Minnesota over a 4-day period. Both data sets have longitudinal measurements of urinary metabolites so that within and between person variances can be estimated after accounting for any major temporal trends and effects of other important covariates, such as gender and age. An important feature of the between and within person variances emerged from the analysis is that the within person variance is larger than the between person variance in both populations. The large within person variance makes it difficult to identify which individuals are most highly exposed in these populations. For example, if you try to identify individuals in the upper 10% of the population, only 20–30% would be correctly identified after a single measurement and 65–75% after 20 measurements. A similar pattern of large within person variability compared to between person variability has been observed in a study of diakyl metabolites in urine of 44 children of farmworkers in the Yakima Valley of Washington over a 21 month period. This suggests that in similarly exposed populations it will be difficult to conduct epidemiological studies of diseases associated with higher exposure because of exposure misclassification. The large within person availability also suggests that future studies should include multiple measurements on the same person so that estimates of the between person variability be made to describe the population variability in exposure. Estimates of the population variability are useful for probabilistic risk assessment of cumulative exposures.

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P10-17

Urinary biomarkers of aflatoxin exposure in young children in Egypt and Guinea

Nektaria Polychronaki[1], Christopher Wild[2], Hannu Mykkänen[1], Mosaad Abdel-Wahhab[3], Abdoulaye Sylla[4], Mamadou Diallo[4], Hani El-Nezami[1], Paul Turner[2]

[1] *University of Kuopio, Finland;* [2] *University of leeds, Leeds, UK;* [3] *National Research centre, cairo, Egypt;* [4] *Institut Pasteur de Guinee, Kindia*

Chronic exposure to the fungal toxins aflatoxins (AFs) is considered to be a major risk factor in the development of primary liver cancer. In parts of Africa AF exposure is common and early life exposure could be a contributing factor towards the early onset of liver cancer in adulthood. This project assessed the level of exposure of children to aflatoxin in Egypt (predicted moderate aflatoxin exposure) and Guinea (predicted high aflatoxin exposure) by measurement of AF metabolites in urine.

Aflatoxins were extracted from urine samples of 50 Egyptian (aged 1–1.5 years) and 50 Guinean children (aged 2–4 years) by C18 cartridges and aflatoxin immunoaffinity columns and analyzed by HPLC-fluorescence. AFB1, AFB2, AFG1, AFG2 and AFM1 were detected in children's urine and the identities of the aflatoxins were confirmed by spiking with aflatoxin standards and co-chromatography using different HPLC conditions.

AF were less frequently present in Egyptian than Guinean children (38% versus 86%) with statistically significant differences in prevalence for most of the detected toxins (AFB1 (2% versus 16%, $p = 0.016$), AFB2 (10% versus 58%, $p = 0.000$), AFG1 (4% versus 2%), AFG2 (24% versus 36%, $p = 0.190$) and AFM1 (8% versus 64%, $p = 0.000$). For AFM1 the mean levels in Guinea were 18-fold higher than in Egypt.

Worldwide there is a scarcity of urinary biomarker data for AF exposure in children. The lower frequencies of both AFB1 and AFG1 in the urine mostly likely reflect conversion of the dietary AF to other metabolites in the liver. Overall AF exposure in Egypt is modest in compar-

ison to Guinea though it should be noted that more of the Egyptian children in this study were still at least partially breast feeding, and thus may be protected against exposure due to the limited passage of AF into breast milk. These data would suggest that measures to reduce AF exposure in both regions are important although the situation is particularly pressing in Guinea where exposure is more prevalent and at higher levels.

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P10-18

The verification of the "drug addictive" status in drug abuse legal cases in Greece

M.A. Savvopoulos [1], M.N. Tzatzarakis [2], M. Toutoudaki [2], M. Christaki [2], E. Pallis [2], A.M. Tsatsakis [2]

[1] *Center of Toxicological Sciences and Research, Department of Medicine, University of Crete, Heraklion, Greece;* [2] *Center of Toxicological Sciences and Research, Department of Medicine, University of Crete, Heraklion, Greece*

The Court should decide whether the defendant is a drug addict or a simple user. The law demands an expert report (i.e. medical, psychiatric, clinical, toxicological or other, e.g. segmental hair testing) to be conducted. One hundred and twenty five legal cases related to the judicial verification of drug dependence and over than 2500 expert reports conducted by the medical examiner were studied. Information from each file about the process, the decision, the police record, the indictment, the expert reports, the defendant's individuality, the crimes, the penal confrontal, etc. were classified into 11 groups. Conclusions on the kind of the expert report, the evident value of each kind of the expert reports, are presented. The following procedure is compared to the rightful procedure. Three legal cases and three expert reports were methodically examined and analytically presented. The expert report is not committing for the Court. The District Attorney is allowed to choose the kind of the expert report to be done. The kind of the report depends on the available technostructure and human resources. Evaluation of the nine common scientific criteria of addiction is presented. The chronic frames and the examination by several experts are impossible to be kept to. Hair testing is accepted in Court, although is not specially refereed in any Law.

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P10-19

Evaluation of methylmercury cytotoxicity at intestinal level

S. Torres [1], M.J. Laparra [2], V. Devesa [1], D. Vélez [1], R. Barberá [2], R. Montoro [1], R. Farre [2]

[1] *Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Aptdo 73, 46100 Burjassot, Valencia, Spain;* [2] *Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot, Spain*

Mercury toxicity is highly dependent on its chemical form. Methylmercury (MeHg), the most toxic chemical specie of the element, is a neurotoxic agent that affects the development of the nervous system, resulting in psychological disturbance, impaired hearing, loss of sight, ataxia, loss of motor control and neural debilitation. There are abundant data and advisories for MeHg levels in seafood products, the major source of MeHg exposure for humans. The aim of this work is to evaluate the effects of MeHg on the essential cell survival processes of Caco-2 cells, a widely accepted model of intestinal epithelia – the first physiological barrier for exogenous toxicants towards systemic blood circulation. Caco-2 cells are seeded onto polycarbonate filters, placed into 24-well plates and grown for 15 days until morphological and functional differentiation. Cell cultures are incubated for 24 h with MeHg at different concentrations (0.125, 0.5, and 1.5 μM). To monitor energetic cell metabolism, MTT conversion is estimated. Mitochondrial membrane potential ($\Delta\psi m$) is evaluated as an early indicator of induced apoptosis. To estimate alterations on cell biology, total RNA and DNA contents are identified histochemically by their differential stainability with pyronin Y and Hoechst 33342 fluorochromes respectively. After cell exposure, MTT assays suggest that at the concentrations assayed, MeHg has no inhibitory effects on mitochondrial enzyme function. However, $\Delta\psi m$ is altered in those cultures exposed to MeHg 1.5 μM. In addition, changes in cell cycle phases and RNA contents suggest alterations of cell signaling pathways and cell biology. Due to the physiological importance of intestinal epithelia as the main site of absorption, the understanding of MeHg effects on this epithelia could contribute to a better estimation of health-risk assessment associated with the ingestion of MeHg contaminated foods.

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P10-20

Ecotoxicological evaluation of metallothionein level in selected tissues of estuarine invertebrates

Jasenka Pavičić [1], Dušica Ivanković [1], Čedomil Lucu [1], Bojan Hamer [1], Marijana Erk [1], Majda Tušek-Žnidarič [2], Ingrid Falnoga [2]

[1] *Rudjer Bošković Institute, Zagreb, Croatia;* [2] *Josef Stefan Institute, Ljubljana, Slovenia*

Organisms living in the estuarine habitats, near to urban and industrial areas are often subjected to interactions of varying natural stressors and diverse anthropogenic impacts, possibly modulating environmental trace metals bioavailability.

Biomarker metallothionein (MT) belonging to the class of cytoplasmic stress-defense proteins, is particularly responsive to environmental trace metal exposure. Evidently, several abiotic and physiological variables may induce MT concentration in aquatic invertebrate tissues. The present study objective is to evaluate the contribution of natural stressors, salinity and temperature, on tissue-specific MT induction in two common estuarine invertebrates *Mytilus galloprovincialis* and *Carcinus aestuarii* under constant and fluctuating laboratory conditions. Spectrophotometric MT and cytosolic metal determinations (Cd, Zn, Cu) provided by AAS were expressed on wet tissue basis ($\mu g\,g^{-1}$).

The results indicate that under short-term, 8–12 days of acclimation to diluted seawater (DSW; 15‰; 10–12 °C) crabs and mussels display distinct, tissue-specific responses. The most pronounced effect of hypoosmotic stress was time-dependent elevation of branchial MT in the anterior ($23.0\,\mu g\,g^{-1}$) and posterior gills ($26.4\,\mu g\,g^{-1}$) of *C. aestuarii* exceeding by 2.4- and 2.9-fold level in control crabs (SW 38‰) showing high positive correlation with cytosolic zinc; (r) 0.71 and 0.99 respectively. The hepatopancreas control MT level ($278\,\mu g\,g^{-1}$) was slightly reduced in crabs exposed to DSW.

Under the same laboratory conditions alteration of mussel MT in both, digestive gland ($154\,\mu g\,g^{-1}$) and gills ($50\,\mu g\,g^{-1}$) varyed within 30% of corresponding SW controls. Digestive gland hyposaline-induced MT displays decreasing temporal trend in comparison to varying MT level in gills, markedly affected by temperature. The most pronounced MT increase was found in gills of mussels exposed to combined environmental variables, further of optimum ranges (DSW 11‰; 27 °C).

Additional supportive evidences for tissue-specific, time-dependent responses were obtained in mussels subjected within 15 days to low ambiental cadmium ($5\,\mu g\,Cd\,l^{-1}$) under the fluctuating salinity-temperature conditions provided by laboratory running estuarine water-supply system. Digestive gland, Cd-induced MT was significantly elevated several days prior to level in gills, surrpassing confidence interval attributed to natural stressors.

Considering Cd-mediated signal to noise ratio, related to natural stressors, branchial tissue appears to be less appropriate for biomonitoring purpose than digestive gland/hepatopancreas. Assuming complexity of estuarine situation, for conclusive evaluation on preferential tissue usage, the interactive effects of other environmental variables on tissue-specific MT modulation should be examined.

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P10-21

Environmental contamination of lead, food chain and childhood health risk assessment

V. Nikolova, R. Georgieva, Zh. Halkova

National Center of Public Health Protection, Sofia, Bulgaria

The technology of the greatest manufacturer of lead and zinc is not environmentally sound and has caused serious pollution of environmental media. The plant management introduced certain innovations in lead production in 1992. The aim of this study is to assess the early effects of lead pollution on children by determination of appropriate biomarkers of lead exposure.

The study covers children's groups aged 7–13 years ($n = 112$) from two exposed villages and one for comparison ($n = 18$), situated at 4 km, 6 km and 14 km of the plant. The biological monitoring is realized by using biomarkers of exposure and effect: lead in blood (PbB), erythrocyte protoporphyrins (EP) and hematological indices. A personal inquiry study of the children's parents for nutrition habbits was carried out.

The mean PbB values of children's groups from two exposed villages were $248 \pm 97\,\mu g/l$ ($77 \div 631\,\mu g/l$), $193 \pm 4\,\mu g/l$ ($127 \div 427\,\mu g/l$) and for comparison group respectively $149 \pm 57\,\mu g/l$ ($63 \div 285\,\mu g/l$). The analysis of the distribution of individual values showed that 23%, 38% and 24% of exposed children had PbB in concentration ranges: 150–200, 201–300 and >300 μg/l. A "concentration-effect" ($r = 0.51$, $p < 0.001$) and "concentration-response" ralationship between PbB and EP were established. Twenty-four percent of children had elevated FEP with PbB levels of $200 \div 300\,\mu g/l$ and the cases had increased to 42% at PbB values

301–650 μg/l. The mean PbB value of children having consumed certain foods from home production was significantly higher than PbB of children whose families used purchased foods ($P < 0.05$). The mean PbB value of children having consumed foods from home production was significantly higher than PbB of children consumed purchased foods ($P < 0.05$).

The high health risk was outlined by the high mean PbB values. The early biochemical changes associated with the haem biosynthesis were proven by the revealed "concentration-response" and "concentrationeffect" relationships between PbB and EP. The established changes in the red blood indices are due to a complex of reasons related to the life style and nutrition habits of the population as well as to the continuous lead exposure.

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P11 Endocrine Disruptors

P11-01
Subacute oral toxicity study of di(2-ethylhexyl)-adipate and diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407"

K. Yamasaki, K. Shiraishi, K. Miyata, S. Houshuyama

Hita Laboratory, Chemicals Evaluation and Research Institute, Oita, Japan

The Organisation for Economic Co-operation and Development (OECD) has proposed the use of uterotrophic assay, the Hershberger assay, and enhanced OECD test guideline no. 407 (enhanced TG 407) as *in vivo* screening tests to detect endocrine properties. The enhanced TG 407 for various chemicals has recently been performed in several laboratories, and its usefulness as an *in vivo* screening test to detect endocrine-mediated effects has been confirmed. Di(2-ethylhexyl)adipate is widely used as a plasticizer in an extensive array of products, and it also has been detected in ready-to-eat baby food. A prolonged gestation period in the dams and an increase in postnatal deaths among their offspring have been reported when rats were given di(2-ethylhexyl)adipate from pregnancy day 7 to weaning day 17, and urethal deformity and skeletal abnormalities have also been reported in the offspring of rats given di(2-ethylhexyl)adipate on pregnancy days 1–22. On the other hand, diethylphthalate is also used as a plasticizer, in a wide variety of products, but a number of phthalates and their metabolites are suspected of having endocrine disrupting effects. Diethylphthalate has been reported to have binding affinity for hepatic estrogen receptors, and phthalic acid di-*n*-amyl ester has been reported to have an androgenic antagonistic effect in the Hershberger assay. Therefore, we performed a 28-day repeated-dose toxicity study of di(2-ethylhexyl)adipate and diethylphthalate based on the draft protocol of the "Enhanced OECD Test Guideline 407" to investigate whether these coumpounds have endocrine-mediated properties according to this assay. Di(2-ethylhexyl)adipate and diethylphthalate were orally administered to SD rats at doses of 0, 40, 200 and 1000 mg/kg/day for at least 28 days. Disturbance of the estrous cycle and increased ovarian follicle atresia were detected in the 1000 mg/kg group given di(2-ethylhexyl)adipate, while no endocrine-mediated effects were detected based on any of parameters examined in rats given diethylphthalate.

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P11-02
Effect of malathion at subchronic exposure on insulin secretory response of rat isolated pancreatic islets

Shirin Pournourmohammadi [1,2], Seyed Nasser Ostad [1], Ebrahim Azizi [1], Mohammad Hossein Ghahremani [1], Bijan Farzami [3], Bagher Minaie [4], Bagher Larijani [5], Mohammad Abdollahi [1]

[1] *Department of Toxicology and Pharmacology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran;* [2] *Department of Toxicology and Pharmacology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran;* [3] *Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran;* [4] *Department of Histopathology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran;* [5] *Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran*

Hyperglycemia is one of the sideeffects of organophosphate (OP) poisonings and there are some evidences for a relationship between acetylcholinesterase (AChE) inhibition and OP-induced hyperglycemia. Malathion is a widely used OP with various effects on different organs. To investigate the effects of malathion on insulin secretion by isolated rat islets in pancreas, malathion was administered through food for 4 weeks at concentrations of 100, 200, and 400 ppm. Results indicated that malathion at doses of 200 and 400 ppm increase blood glucose concentrations by 44.4% and 60.6% as well as insulin concentrations by 36.6% and 143.2% of control, respectively. Although in vitro findings showed isolated islets from 4 weeks-pretreated rats with doses

of 200 and 400 ppm malathion, in the presence of basal and stimulatory glucose released less insulin secretion (%content) by 57.1% and 69% compared to control, respectively; however malathion could not change KCl-stimulated insulin secretion. Light microscopic examination revealed that malathion causes patchy degenerative changes developing from 100 to 400 ppm in pancreatic islets. Malathion administration developed hyperglycemia that was not compensated by insulin despite its increased secretion; maybe linked to ACh which is a potent secretagogue of both insulin and glucagon or detrimental effect of malathion on insulin resistance. The decrease of the glucose-stimulated insulin secretion by isolated islets can be attributed to the ability of malathion to affect the cellular metabolism possibly mitochondrial activity, suggesting that malathion can influence insulin secretory function of pancreatic islets in vivo and in vitro.

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P11-03

Effects of endocrine disruptors on human endometrial endothelial cells in vitro

Carolina Bredhult, Britt-Marie Bäcklin, Matts Olovsson

Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

Bioaccumulation and biomagnification of polluting chemicals such as PCBs, pesticides and dioxins put species at the top of the food chain at risk. Some polluting substances are classified as endocrine disrupting chemicals (EDCs). The influence of EDCs on sex steroid controlled organs and tissues is of great importance since possible effects not only can affect the exposed individual, but also its progeny.

Human endometrial endothelial cells (HEEC) from women in the proliferative and secretory phases were isolated, cultured and exposed to the endocrine disruptors *o*,*p*′-DDT, PCB 77, PCB 126, di-*n*-butyl phthalate (DBP), bisphenol A and 2,3,7,8-TCDD, and to 17β-estradiol, progesterone, 17α-ethinylestradiol and levonorgestrel. Cell proliferation was studied using immunocytochemistry for PCNA-expression and a BrdU-assay. Viability was assessed by vitalstaining with propidium iodide and Hoechst 33258.

The PCNA-immunocytochemistry provided a crude measure of the HEEC proliferation, which corroborated the results from the BrdU-assay. The proliferation increased in response to 10 nM of 17β-estradiol whereas exposure to 1 μM levonorgestrel resulted in decreased proliferation compared to the control. With the exception of *o*,*p*′-DDT and PCB 77, increased doses of the endocrine disruptors resulted in dose dependent reduction of the HEEC proliferation. In response to *o*,*p*′-DDT, only the highest dose (100 μM) resulted in lowered proliferation. Exposure to PCB 77 caused a diverging proliferation pattern since only the middle concentration (1 μM) resulted in decreased cell proliferation.

The proportion of necrotic cells was increased by DDT, PCB 77, PCB 126 and bisphenol A.

All tested endocrine disruptors were able to reduce HEEC proliferation and/or viability *in vitro*. This finding opens up for the possibility that such compounds could affect the function of human endothelial cells *in vivo*. The monthly occurring endometrial angiogenesis would then likely be affected, which specifically targets female fertility functions.

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P11-04

Use of protein profiles to characterise concentration–effect curves of mixtures of estrogenic compounds in human breast cell lines

Zheying Zhu, Alan R. Boobis, Robert J. Edwards

Imperial College, London, UK

There is considerable speculation as to the risk to human health from xenoestrogens in the environment. There are contradictory data on the shape of the dose–response curve and the appropriate model for dose addition for mixtures of xenoestrogens and/or phytoestrogens. The present study utilises a proteomic approach to address these issues. The proliferative activities of genistein, bisphenol A and endosulfan were compared with that of 17β-estradiol on human breast cell lines that vary in their oestrogen receptor (ER) α and β phenotype. It was possible to establish concentration–effect curves for all four compounds in MCF-7 cells (ERαβ). However, none of the compounds had any proliferative effect on the ERα-negative cell lines MBA-MB-231 (ERβ), MCF-10F (ERβ) or MCF-10A (no ER) cells. Binary mixtures of 17β-estradiol with each of the other compounds showed concentration additivity of proliferative activity. Protein profiles of the cell lines were determined by selective-elution ionisation-desorption time-of-flight mass spectrometry (SELDI-TOF-MS) following treatment with each pro-estrogenic compound. MCF-7 cell protein profiles indicated a common pattern of responsive proteins that varied in a concentration-dependent manner for each compound. No compound-specific responsive proteins were found and binary mixtures of

17β-estradiol with the other chemicals affected the levels in a manner predicable from their individual proliferative activity, suggesting that all four compounds share a common estrogenic mechanism of action. Further, no changes in the protein profiles of MDA-MB-231, MCF-10F or MCF-10A cells were found with any compound, indicating a key role for ERα in the response. In conclusion, the main effect of the pro-estrogenic compounds is through a common pathway involving the ERα receptor. The log concentration–effect curves were classically sigmoidal in shape. The mixtures showed concentration additivity and hence the response is predictable from the individual mixture components. We are currently seeking to identity the responsive proteins in order to elucidate the mechanisms involved and rationalise the effects of mixtures of pro-estrogenic compounds.

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P11-05
Estrogen-like activity of phosalone on MCF7 cell line

Valentina Campi, Daniele Ferrario, Maria Carfi', Cristina Croera, Laura Gribaldo

ECVAM, Institute for Health and Consumer Protection, J.R.C., Ispra, Italy

Phosalone is a member of the organophosphate family of insecticides. It is active against the red spider mite that affects apples and pears.

Phosalone shows an estrogen-like structure by QSARs analysis, and then it could interfere with endocrine system causing developmental and reproductive toxicity.

The presence of this compound in our environment as pollutant is a fact and causes an increasing concern for its possible impact not only on wildlife, but also on human health.

First of all, we investigated cell proliferation induced in human breast cancer cell line MCF7 by phosalone exposure, using MTT test: phosalone had a strong proliferative effect at the highest concentration tested (10^{-5} M). Moreover we showed that phosalone modulates the expression of estrogen receptors (ERs).

Real Time PCR and Western blot analysis performed on MCF7 treated for 24 h, showed gene and relative protein down regulation of ERα and up regulation of ERβ at the highest concentration tested.

The ERα down regulation as well as ERβ up regulation observed were dose-dependent, in line with the maximum proliferative effect at the same doses.

In conclusion these results suggest that phosalone has an estrogen-like activity on MCF7 cells.

Further investigation of genes and proteins involved in cell proliferation and cell cycle progression, such as p21 and cyclin D, will clarify the mechanisms of toxicity of the phosalone.

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P11-06
Embryonic exposure to an ERα-agonist affects reproductive organ development but not copulatory behaviour in Japanese quail

Anna Mattsson [1], Krister Halldin [2], Björn Brunström [1]

[1] *Uppsala University, Uppsala, Sweden;* [2] *Karolinska Instituet, Stockholm, Sweden*

Oestradiol is directing sexual differentiation of the reproductive organs and the brain during embryonic development. Male Japanese quail that are exposed to xenooestrogens during a critical embryonic period become demasculinized regarding their copulatory behaviour. Xenooestogens also interfere with the differentiation of the reproductive organs in both sexes.

As part of a project aiming at determining the respective roles of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) in sexual differentiation we injected fertilised Japanese quail eggs with the selective ERα agonist 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT) on incubation day 3, i.e. well before sexual differentiation of reproductive organs and copulatory behaviour is completed. Controls were injected with ethinyloestradiol (EE2), a potent agonist to both ERα and ERβ. Gross anatomy of the reproductive organs was examined in both sexes and the males were tested for copulatory behaviour when they were sexually mature.

The copulatory behaviour was significantly reduced in the EE2 group, whereas the PPT-treated males performed just as well as the controls. There was no correlation between behavioural performance and plasma concentration of testosterone. The effects on the reproductive organs were similar in the PPT-exposed as in the EE2-exposed birds. Effects included retention of the right Müllerian duct in females and retention of one or both Müllerian ducts in males, vesicles on the Müllerian ducts in both sexes, testicle weight asymmetry and reduced cloacal gland area.

Our results show that treatment with the selective ERα agonist PPT causes similar adverse effects on reproductive organ development as treatment with EE2. However, treatment with PPT does not seem to be suffi-

cient to demasculinize the copulatory behaviour in male Japanese quail. This indicates that the effect on the brain is not mediated by ERα alone. However, a difference in kinetics of PPT and EE2 in the embryos is also a possible explanation for the differential effects of the two compounds.

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P11-07
Differential effects of TCDD and ARNT on ERα and ERβ

Joëlle Rüegg, Ingemar Pongratz

Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden

Dioxins like TCDD interfere with several hormonal signalling pathways mediated, e.g. by nuclear receptors (NRs). The biological effects of dioxins are mediated by the aryl hydrocarbon receptor (AhR) and its dimerisation partner AhR nuclear translocator (ARNT). There is extensive evidence that TCDD has an anti-estrogenic effect. The effects of estrogens are mediated by two estrogen receptors (ER), ERα and ERβ, that belong to the family of ligand induced NRs. Although the two ERs are similar in structure, they differ substantially with respect to their biological functions, e.g. stimulation of ERα leads to proliferation in certain cell types, whereas induction of ERβ antagonises this effect. Recently, we have shown that ARNT can also act as co-activator of the ERs, i.e. increased amounts of ARNT enhance ER activity with a remarkable preference for ERβ. This finding suggests that the interference of dioxin with the ER function is at least partly due to a competition between ER and AhR for their common co-activator ARNT. The aim of this study was to confirm the effects of ARNT on the ERs using siRNA against ARNT. Additionally, we wanted to test whether ERα and ERβ are also differentially affected by exposure to TCDD, and if this has implication on the proliferation of cells. Our results show that lowering the ARNT levels in cells using siRNA leads to an impaired activation of the ERs with a stronger effect on ERβ. Furthermore, TCDD had a stronger anti-estrogenic effect on cells expressing ERβ than on those expressing ERα. We also looked at proliferation of HC11 cells that express both ERα and ERβ. It has been shown that PPT (ERα selective agonist) has a proliferative effect in these cells whereas DPN (ERβ selective) leads to decreased cell number. Co-treatment of cells with TCDD and DPN lead to reversion of the ERβ dependent inhibition of proliferation, and thus to enhanced proliferation, while the effects of TCDD were only minor on cells co-exposed to TCDD and PPT. These results were confirmed in an ERα positive breast cancer cell line in which ERβ expression can be induced. We conclude that TCDD affect the ERs differentially by activating the dioxin receptor and thus recruiting ARNT away from the ER pathway. We conclude further that this could lead to a marginal anti-proliferative effect of dioxin in tissues dominantly expressing ERα but to a pronounced proliferative effect in tissues dominantly expressing ERβ.

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P11-08
Developmental exposure to PCB153 and methylmercury on sex hormone levels at early and late postnatal periods in rats

T. Coccini[1], A. Bongiorno[1,2], E. Roda[2], S. Gralewicz[3], A.F. Castoldi[1], V. Felipo[4], L. Manzo[1,2]

[1] *Maugeri Foundation, Italy;* [2] *University of Pavia, Toxicology Division, Pavia, Italy;* [3] *Nofer Institute, Lods, Poland;* [4] *Fundacion Valenciana de Investigaciones Biomedicas, Valencia, Spain*

PCBs exhibit estrogenic and anti-estrogenic properties depending on the congener type. Steroid receptors levels and estrogen receptor-mediated functions as well as steroid metabolism and circulating hormone concentrations are altered after PCB treatment. In vivo studies have documented that even MeHg, which bioaccumulates in food chain together with PCBs, can disrupt endocrine function including steroid hormones.

The study aimed at determining the effects of the developmental oral exposure (from GD7 to PND21) to the *ortho*-PCB substituted (PCB 153; 1 or 5 mg/kg/day) alone or in combination with MeHg (0.5 mg/kg/day), on rat serum concentrations of testosterone (T) and 17 beta-estradiol (E2) at weaning (PND21), puberty (PND36) and adulthood (PND90), separately according to gender. Radioimmunoassay analyses were used for measuring the amount of sex hormones.

E2 level was markedly increased (15-folds) in PND21 male offspring by either MeHg or PCB153 (5 mg/kg/day) (control: 0.3 ± 0.1 pg/ml vs. MeHg: 4.1 ± 1.9, PCB153: 3.8 ± 1.2 pg/ml). Co-exposure produced an increasing effect similar in extent to that induced by either compound alone. Female E2 level was increase (50%) by PCB153 only. At PND36, PCB153 alone and combined with MeHg, diminished E2 level in both males (50–70%) and females (35–60%). PCB153 (1 and 5 mg/kg) effect on this hormone was still strongly evidenced at 90 days of age in both progenies gender

(increase of 8–10 folds in males and of 1–2 folds in females).

Serum T levels were not influenced by any of the treatment at any time points considered in both male and female offspring with the exception of a transitory deceasing effect at PND21 in females caused by the concomitant exposure to MeHg and PCB153.

The results indicate that circulating steroid hormones are affected by developmental exposure to PCB153 and/or MeHg in a different manner according to the gender and sex hormone considered as indicated by the early and long-lasting changes of E2 in males only, following perinatal exposure to PCB153.

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P11-09

Effects on bone tissue in sheep reared on pasture treated with sewage sludge

P. Monica Lind [1], Emma Colleen [2], Frida Broman [2], Carolina Wejheden [1], Rebecca Lundberg [1], Stewart Rhind [3], Jan Örberg [2]

[1] *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden;* [2] *Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden;* [3] *Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, UK*

Most laboratory animal studies concerning endocrine disrupting compounds (EDCs) involve short time exposure to fairly large amounts of single compounds. However, natural exposures involve long term exposure to small amounts of compounds of many different classes, each with different properties and effects. In order to study effects on bone tissue of environmental levels of a mixture of EDCs, ewes were maintained throughout their breeding lives on pastures treated with either sewage sludge (2.25 tonnes of dry matter (DM)/ha; twice annually), which contains a mixture of EDCs (exposed animals, $N = 10$), or with inorganic fertiliser providing equivalent amounts of nitrogen (control animals, $N = 10$). After rearing 3 or 4 crops of lambs, ewes were mated at a synchronised oestrus and slaughtered at 55 days of gestation and bones were recovered.

Bone composition and dimensions were determined by peripheral quantitative computed tomography (pQCT) on excised femur. Cortical, as well as total bone mineral content and cortical area were increased. The results in the exposed ewes were not statistically significant ($0.07 < p < 0.10$), however, the effects are similar to previous findings in experimental settings with rodents as well as observations in wildlife species.

Apparently, compounds present in sewage sludge might be able to alter the bone remodelling process in sheep resulting in net gain of cortical bone.

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P11-10

Hershberger assay: Testing of coded chemicals and supplementary molecular and biochemical investigations

A. Freyberger, H. Ellinger-Ziegelbauer, A. Brockes, F. Krötlinger

Bayer HealthCare AG, Pharmaceuticals, GDD, Toxicology, D-42096 Wuppertal, Germany

Under the auspices of OECD the Hershberger assay (HA) is being validated as an in vivo screen for compounds with (anti)androgenic potential. We participated in the final activity, the testing of coded chemicals. Test compounds included trenbolone (TREN, 1.5, 40 mg/kg), testosterone propionate (TP, 0.4 mg/kg), flutamide (FLUT, 3 mg/kg), linuron (LIN, 10, 100 mg/kg), *p*,*p*′-DDE (16, 160 mg/kg), and two negative substances, nonylphenol (NP, 160 mg/kg) and 2,4-dinitrophenol (DNP, 10 mg/kg). Compounds were administered for 10 days by oral intubation or subcutaneous injection (TP). After submission of study reports to OECD by participants uncoding revealed the following results: (A) When assessing androgenic potential in castrated rats, administration of TREN increased the weights of ventral prostate (VP), seminal vesicles (SV), glans penis, levator ani and bulbocavernosus muscles, and Cowper's glands at the high dose. A similar or stronger (VP, SV) increase of androgen-sensitive tissue weights (ASTW) was observed for TP, NP and DNP were ineffective. (B) When assessing antiandrogenic potential in TP-supplemented castrated rats, administration of LIN and *p*,*p*′-DDE decreased ASTW only at the high dose. FLUT even more effectively decreased ASTW, NP and DNP were again without effect. (C) We also performed further investigations not requested by OECD. Gene expression analysis in prostate by quantitative RT PCR for prostate specific binding protein polypeptide C3 and ornithin decarboxylase generally provided responses reflecting ASTW changes, but was slightly more sensitive for TREN at the low dose. Measurement of liver enzymes revealed strong induction of testosterone metabolizing and phase II conjugating enzymes in *p*,*p*′-DDE-treated animals. Our study accurately repro-

duced ASTW changes obtained in previous studies also under code suggesting that the Hershberger assay is a robust tool to screen for an (anti)androgenic potential. Assessment of gene expression did not consistently provide increased sensitivity. Finally, *p*,*p*′-DDE may affect ASTW by several mechanisms including enhanced testosterone metabolism. This study was performed in collaboration with OECD and was sponsored in part by CEFIC-EMSG.

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P11-11

Determination of Androgenic activity on a stably transfected human breast cell line

Mihye Jeong [1], Hyung-gab Kim [2], Are-Sun You [1], Kyung-Hun Park [1], Byung-Seok Kim [1], Jin-Hwa Kim [1], Michael S. Denison [3]

[1] *Hazardous substances Division, Department of Crop Life Safety, NIAST, RDA, South Korea;* [2] *Department of Environmental Engineering, Jinju National University, South Korea;* [3] *Environmental Toxicology, University of California, Davis, USA*

This study was carried out to establish a method for rapid screening of endocrine disrupting chemicals. To develop a stably transfected cell line for determination the androgenic activity of chemicals. We attempted transfection using reporter plasmid vectors that contain the firefly luciferase gene under hormone inducible control of androgenic responsive DNA enhancer elements.

The optimal condition for detecting the activity of dihydrotestosterone (DHT) on a recombinant humans breast carcinoma (T47D) line and the effect of temperature on the cell line was investigated.

As the results of these studies, the recombinant cells grown for 3 days in estrogen-stripped media(ESM) showed the high activity by treatment with 1 μM DHT for 24 h. The activity of DHT under 0.1 μM DHT at 33 °C was higher 4.5 times than 1 μM DHT at 37 °C.

This bioassay is useful for identifying and determining the androgenic activity of chemicals known endocrine disrupters.

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P11-12

The evaluation of xenoestrogenic potential of propylparaben in zebrafish (*Danio rerio*)

Premysl Mikula, Radka Dobsikova, Zdenka Svobodova

University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, Brno, Czech Republic

Many chemicals produced by human and abundantly used in the industry can damage processes of hormonal regulation in living organisms and act as so-called endocrine disruptors. The subgroup of these chemicals is also xenoestrogenic substances. The aim of present study was to investigate xenoestrogenic potential of propylparaben, one of the most commonly used preservatives in cosmetics and drugs. This potential was investigated in semi-static experiment in zebrafish (*Danio rerio*) by means of measuring vitellogenin. Vitellogenin synthesis in liver of zebrafish is estrogen dependent, and, hence, is normally limited to the adult female individuals. Since male and juvenile zebrafish possess gene for vitellogenin synthesis, exposure to exogenic estrogens or xenoestrogens can induce vitellogenin synthesis also in them. In our study, 20 days old juvenile zebrafish were exposed for 20 days to different concentrations of propylparaben dissolved in water and concentrations of vitellogenin in their whole body homogenates were measured by direct sandwich ELISA method. Mean vitellogenin concentrations were as follows: 442.33 ± 205.64 ng/ml for control group, 258.09 ± 96.10 ng/ml for the group exposed to 100 μg/l of propylparaben, 228.81 ± 72.51 ng/ml for the group exposed to 400 μg/l of propylparaben, 275.87 ± 62.14 ng/ml for the group exposed to 900 μg/l of tested substance and 58055.98 ± 49782.11 ng/ml for the group exposed to 100 ng/l of β-estradiol (positive control). The results of present study did not confirm estrogenic effect of propylparaben in tested concentrations; conversely antiestrogenic effect of propylparaben exposure was demonstrated pursuant to statistically significant decline of vitellogenin production ($P < 0.001$). On the other hand, our results confirmed estrogenic effect of β-estradiol exposure, since zebrafish exposure to β-estradiol elicited statistically significant induction of the vitellogenin production.

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P11-13

Biotransformation of bisphenol A and genistein in MCF7, HC11 and HepG2 cell lines used for testing endocrine disruptors

J. Bursztyka [1], E. Perdu [1], K. Pettersson [1], I. Pongratz [1], M. Fernández-Cabrera [2], N. Olea [2], L. Gamet-Payrastre [3], J.P. Cravedi [3], D. Zalko [3]

[1] *Karolinska Institutet, Institute of Environmental Medicine, Novum, SE-141 57 Huddinge, Sweden;* [2] *University of Granada, Laboratory of Medical Investigation, School of Medicine, Hospital Clinico, 18071 Granada, Spain;* [3] *INRA, UMR 1089 Xénobiotiques, BP3 31931 Toulouse Cedex 9, France*

We have investigated the biotransformation capabilities of human HepG2 hepatoma cells, MCF7 breast cancer cells and mouse HC11 mammary gland cells toward two estrogenic molecules: bisphenol A (BPA) and genistein. BPA (2,2-bis(4-hydroxyphenyl)propane) is a major chemical used in the production of polycarbonates, as a component in epoxyresin. In addition to occupational exposure BPA may leach from polycarbonates and epoxyresins used in food cans and bottles to result in possible widespread exposure of the general public to low daily doses. BPA interacts directly with the ERα and exhibits *in vitro* and *in vivo* estrogenic effects. Genistein (4′,5,7-trihydroxyisoflavone) is a phytoestrogen mainly present in soybeans and soy food. It has been found to have both weak estrogenic and anti-estrogenic effects. There is a growing body of *in vitro* and animal studies suggesting that genistein may be helpful in preventing some cancers, principally breast and prostate cancers.

For all the experiments, we used [^{3}H] BPA and [^{14}C] genistein. For each cell line and for each compound, five concentrations (5, 10, 15, 20, 25 μM) were incubated for 24 h. One concentration (5 μM) was incubated for 12, 24 and 48 h. HPLC coupled to online radioactivity detection was used for metabolic profiling. Metabolite identification was based on enzymatic hydrolysis using bacterial aryl-sulfatase and bovine liver β-glucuronidase, and on comparison with the retention times of authentic standards.

Incubations of BPA and genistein with HepG2 cell lines resulted in the formation of sulphoconjugates and, to a lesser extent of glucuronoconjugates. Higher metabolic rates were observed for genistein than for BPA.

No metabolite was detected in the experiments performed with HC11 cells, even after 48 h.

Incubations of BPA with MCF7 cells produced BPA sulfate and BPA glucuronide, whereas genistein only formed the sulphate conjugate. As for HepG2 cell lines, genistein was biotransformed at a higher rate than BPA.

Our results concerning HepG2 are in agreement with those usually described for mammals hepatocytes except that no trace of phase I compound was detected in our incubates, suggesting a low expression level of cytochrome P450-dependent enzymes. The data obtained with MCF7 demonstrate the substantial activity of xenobiotic metabolizing enzymes in these cell line.

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P11-14

Effects of gestational exposure to 2,2′,4,4′,5,5′-hexachlorobiphenyl on postnatal development and thyroidal status in rat offspring

K. Kobayashi, M. Miyagawa, R.S. Wang, M. Suda, S. Sekiguchi, T. Honma

National Institute of Safety and Health, Kawasaki, Japan

Polychlorinated biphenyls (PCBs) are a group of most widespread environmental contaminants due to their persistence and ubiquitous presence in wildlife and humans. Exposure to PCB mixtures at an early stage of development has been reported to affect endocrine glands, however, little is known about the precise toxicological properties of individual PCB congeners. This study was undertaken to determine whether in utero exposure to 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), a di-*ortho*-substituted noncoplanar congener, exert any effect on postnatal development and thyroid function in rat offspring. Pregnant Sprague–Dawley rats (Crj: CD (SD) IGS) were exposed with PCB 153 (1 or 4 mg/kg/day), or corn oil vehicle orally from gestation day 10–16, and developmental parameters in the offspring were examined. There were no compound-related changes in body weight, body length (nose-anus length), tail length or several organ weights (liver, kidney, testes, prostate, seminal vesicles and ovaries) in the offspring. Anogenital distance was unaffected by PCB 153 in both sexes. No effects on plasma thyroxine or thyroid-stimulating hormone levels in both sexes were observed. These findings suggest that in utero exposure to PCB 153 (gestation day 10–16) does not affect postnatal development and thyroidal status in rat offspring for either sex under the experimental condition of the present study.

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P11-15

Evaluation of the effects on rat sperm of Dibromochloropropane by use of mitochondrial metabolism

Katsumi Ohtani, Hisayo Kubota, Junzo Saegusa

National Institute of Occupational Safety and Health, 6-21-1, Nagao, Tama-ku, Kawasaki, Kanagawa 214-8585, Japan

Dibromochloropropane (DBCP), one of endocrine disrupters, was reported to cause health influence such as azoospermia to pesticide sprayers and pesticide manufacturers in the latter half of the former century. The authors have already reported that MTT (one of the tetrazolium salts) method with a microplate leader can be detectable for the sperm depletion by 2-bromopropane in rat. We are reporting influence to the sperm of DBCP, comparing six tetrazolium salt methods as an index of mitochondria metabolic capacity in sperm with the other methods including Computer-Assisted Sperm Analysis method (CASA). F344 male rats (12 week old) were subsequently administered with DBCP (25–100 mg/kg) dissolved in olive oil twice a week for 4 weeks (8 times). In the suspension medium, epididymal cauda was cut with scissors and then the followings were performed; (1) the absorbance measurement by the tetrazolium salt methods with a microplate leader (the tetrazolium used were six kinds, MTT, MTS, XTT, WST-1, WST-3 and WST-8, all are abbreviations) (2) measurement of a sperm motility index (SMI) value by Sperm Quality Analyzer (SQA) method, (3) measurement of sperm count by CASA. Significant decrease of absorbance was found by tetrazolium salt methods except WST-1 method in the 75 and 100 mg/kg treated groups of DBCP. Moreover, significant sperm decrease by CASA was confirmed in the same treated groups, and even SQA method recognized a fall of SMI value equally. On the other hand, no such differences were recognized by all measurement methods except WST-8 method in the 25 and 50 mg/kg treated groups of DBCP. Based upon the foregoing, most tetrazolium salt methods make it possible to get the results that are similar to CASA and SQA method. Tetrazolium salt methods with a microplate leader have characteristics superior at a point of the simpleness, mass-screening capacity, and economy, and they have an advantage because they are suitable for qualitative analysis to be able to judge sperm normality or abnormality by the naked eye. Totally, WST-3 method is thought to be the most useful as sperm analysis procedure for its sensitivity and simple operation among six kinds of tetrazolium salt methods used.

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P11-16

Distribution and cytochrome P450 induction in mothers and offspring rat organs after PCB treatment during pregnancy and lactation

A. Colombo [1], P. Bonfanti [1], B. Costa [2], S. Villa [1], F. Orsi [1], F. Comelli [2], E.A. Santagostino [1]

[1] *Dipartimento di Scienze dell'Ambiente e Territorio, Università di Milano-Bicocca, Italy;* [2] *Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Italy*

In mammals, the foetal and post-natal phase are critical periods for the exposure to PCBs because the energetic consumption during gestation and lactation moves PCBs from the adipose tissue where they have accumulated in time, reaching the offspring tissues through placenta and breast milk. While different studies investigated the effects of PCB technical mixtures, there are not studies, in which levels of the different congeners typically present in diet, are correlated to biotransformation activities by cytochrome P-450 and to the expression of membrane protein pump P-170 in tissues of both mothers and developing organisms. The ability of PCBs to remain or not in the cells might depend on the efficiency of these defence mechanisms.

A mixture of congeners (PCB 126, 138, 153 and 180) was administered daily to pregnant rats from gestation day 15 (GD15) to GD20 and until the end of lactation, postnatal day 21 (PN21), twice a week. In groups GD20, PN12, PN21 and PN60 CYP1A, 2B and P-170 expression was evaluated in specific organs. The results evidenced the induction of the two CYP isoforms in liver of PCB treated mothers (GD20, PN12, PN21) and pups during lactation (PN12, PN21); on the contrary in liver of foetus and PN60 offspring no significant induction has been evidenced for both CYP isoforms as well as for P-170 protein levels in placenta and liver of mothers, foetus and offspring exposed to PCBs. These data are supported by PCB quantitative analysis performed in liver, suggesting that placenta limits PCB transfer from mother to foetus, while the milk represents the way for PCB secretion. The failed induction of CYP-450 in PN60 implies that PCB reaching pups through breast milk are segregated in adipose tissue reducing their levels in liver. Moreover the presence of ematoencephalic barrier could explain the CYP 450 absence in brain.

In conclusion, the investigation about PCB distribution and defence mechanisms induction, during development, can allow how these molecules can be accumulated in maternal tissues and then transferred to offspring reaching the target of their toxicity on neuroendocrine system.

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P11-17

Effects of octylphenol on male reproductive tissues, epididymal sperm motility, and testicular gene expression

Daniel G. Cyr, Mary Gregory

INRS-Institut Armand-Frappier, Pointe-Claire, Que., Canada

p-tert-Octylphenol (OP) is a degradation product of alkylphenol ethoxylates, which are commonly used surfactants. OP is known to selectively bind to the estrogen receptor; however, its effects on males reported in the literature are controversial, with some studies showing dramatic effects on testicular functions while others observe nominal effects. To date however, there have not been any long-term studies to assess the effects of OP on male reproductive function. The objective of this study was to evaluate the effects of OP on various male reproductive parameters. Based on preliminary toxicokinetic studies, three doses of OP were selected (25, 50, and 125 mg/kg bw) and administered daily by gavage to male and female Sprague–Dawley rats. Rats were administered OP for 60 days, which represents 1.5 cycles of spermatogenesis. Animals were euthanized at the end of the exposure; body and sex accessory gland weights were measured. Tissues were subsequently frozen in liquid nitrogen, fixed in Bouin's, or placed in medium for sperm analysis. A tendency towards decreased body weight, relative to untreated controls, was observed in all OP-treated groups, with a statistically significant decrease in body weight at the highest dose (125 mg/kg bw). Testuicular histology indicates that in the seminferous tubules of rats at the two higher doses, there was a sloughing of germ cells into the lumen. Semen analyses were performed using a Hamilton–Thorne IVOS system. Sixteen sperm motility parameters were assessed; of these, total %motility in the intermediate dose (50 mg/kg bw) was approximately 13% lower, a statistically significant difference relative to untreated controls. Correspondingly, a tendency for an increased percentage of static cells was also observed in all OP-treated groups, with the intermediate dose (50 mg/kg bw) displaying a significantly higher percentage of static cells (28%), relative to untreated controls (15.7%). Sperm counts (millions/ml) were also slightly lower in all OP-treated groups, and significantly lower at the highest OP dose (125 mg/kg bw), relative to untreated controls. In conclusion, daily OP exposure via gavage for 60 days, at doses ranging from 25–125 mg/kg bw, did not appear to exert any major detrimental effects on male reproductive organs or motility associated parameters, and only slightly affected sperm motility and sperm counts. Analyses of gene expression profiles using the Agilent cDNA arrays indicate that the expression of 14 genes were modulated by OP treatment. The significance of these will be discussed. Together these data indicate that OP causes subtle effects on the male reproductive tract.

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P11-18

Increased caspase-3-dependent spermatogenic cell death and dysregulated adult spermatogenesis following *in utero*, lactational and direct exposure to para-nonylphenol

Leon M. McClusky [1], Christiaan de Jager [2], Maria S. Bornman [3]

[1] *Department of Physiology, University of Pretoria, South Africa;* [2] *School of Health Systems and Public Health, University of Pretoria, South Africa;* [3] *Department of Urology, University of Pretoria, South Africa*

Germ cell development in the testis occurs in the context of a complex three-dimensional relationship with the supporting intratubular Sertoli cells, which are often the targets of environmental toxicants. One such a toxicant is the alkylphenolic compound, *para*-nonylphenol (*p*-NP). We previously showed that *p*-NP reduced several testicular morphometric parameters, including sperm counts. The present study re-examined material collected in that study and set out to determine the site(s) of *p*-NP action and the processes targeted. Seven-day pregnant Sprague–Dawley rats were treated with either vehicle, 100 or 250 mg/kg *p*-NP through gestation, lactation and afterwards directly to all male ($n = 20$/group) off-spring until 10 weeks of age. At necropsy, the testes and epididymi were fixed in Bouin's fluid and embedded in paraffin wax. Paraffin sections of 10 testes from each group were dewaxed, rehydrated, and processed for terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) and cleaved caspase-3 (Cell Signaling Technology, Beverly, MA, USA) immunohis-

tochemistry (IHC) using a rabbit polyclonal at a 1:100 dilution. Both doses of *p*-NP significantly ($P < 0.02$) increased the number of TUNEL-positive germ cells. However, TUNEL-labelling was selective, and excluded labelling of basal cells with apoptotic morphology. Cleaved caspase-3 IHC strongly labelled basal cells (spermatogonia and early spermatocytes) with condensed chromatin, but not degenerate germ cells at intermediate positions in epithelium. Only the caspase-index of the 100 mg/kg *p*-NP group was significantly ($P < 0.05$) three fold greater than controls. Both doses of *p*-NP significantly ($P = 0.0011$) increased the frequencies of stages IV–VI, whereas those for stages VII–VIII were significantly ($P < 0.05$) reduced in the 250 mg/kg group, and those of stages late VIII–IX (spermiating and recently spermiated tubules) were significantly ($P < 0.01$) reduced by 1.6 fold in the 100 mg/kg group. Thus, *p*-NP, a waterborne xenoestrogen, insidiously alters spermatogenesis in male offspring.

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P11-19

Co-treatment of TCDD and estrogen alter the expression of c-fos in an osteoblastic cell line

Carolina Wejheden, Annika Hanberg, P. Monica Lind

Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Dioxin and dioxin-like compounds are endocrine disrupting environmental pollutants known to disturb estrogen signalling and to impair the homeostasis of bone tissue. Thus, these compounds may contribute to the increasing prevalence of osteoporosis in industrialized countries. Anti-estrogenic properties of dioxin and dioxin-like compounds have often been reported in the literature. However, there is a growing awareness that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) also has estrogenic effects. Furthermore, *in vivo* studies with a dioxin-like PCB suggest that dioxin-like compounds may have estrogenic or anti-estrogenic effects depending on the estrogen status. To investigate the effects of TCDD at different estrogen concentrations in the osteoblastic cell line, UMR-106, we studied the mRNA expression of c-fos after treatment with TCDD or 17β-estradiol (E2) only, and co-treatment of TCDD and E2 for 6, 12 and 24 h. c-fos is one of the two proteins forming the transcription factor AP-1, which regulates various genes such as collagenase-3. Co-treatment was performed with one concentration of TCDD (10^{-10} M) and with two concentrations of E2 (10^{-6} and 10^{-10} M). Co-exposure of TCDD together with high concentration of E2 significantly increased the mRNA expression of c-fos compared to controls after 6 and 12 h. c-fos was significantly down-regulated after 12 h exposure to 10^{-6} M E2 only, while the expression in the co-exposure (TCDD + 10^{-6} M E2) still was significantly up-regulated. This suggests that TCDD may prolong the effect of a high concentration E2 on c-fos expression in UMR-106. However, this effect was not observed after 24 h of co-exposure with the high concentration of E2. In the co-exposure with low concentration of E2 the expression of c-fos was significantly down-regulated both after 12 and 24 h.

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P11-20

A robust examination of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the developing male reproductive system

David R. Bell, Sally Clode, Alwyn Fernandes, Paul Foster, George Loizou, Alan Macnicoll, Brian G. Miller, Martin Rose, Lang Tran, Shaun White

University of Nottingham

The effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) on the developing male reproductive system, after exposure of the fetus via dosing of the pregnant mother to dose levels as low as 64 ng dioxin/kg, is one of dioxin's most potent reported effects. The UK, EU and WHO have used this endpoint as the basis of the TDI for dioxin. We set out to examine two issues (1) to determine the fetal dose of TCDD associated with a toxic endpoint in a concurrent experiment; (2) to clarify the considerable contradiction and inconsistency in the reported literature on the effect of dioxin on the developing male reproductive tract.

Time-mated Wistar rats were exposed to control vehicle, 50, 200 or 1000 ng dioxin/kg bodyweight on gestational day (GD) 15. Dams were killed on GD16 and 21 for determination of dioxin concentration. ~20 dams per group were allowed to litter, and 5 males per litter retained after weaning. F1 males were killed on post-natal day (PND) 70 or PND120 for reproductive assessment.

Dioxin at 1000 ng dioxin/kg bodyweight caused a reduction in the number of pups alive on day 1, and reduced the number of pups surviving to PND21. The male offspring in this group were lighter than control at all times although their weight gain exceeded controls, and had a delayed balano-preputial separation. The testes were slightly lighter than control in this group, and the seminology at PND70 showed a transient increase in

abnormal sperm. There was no significant effect on epididymal sperm number or testicular spermatid number at PND 70 or 120. There was no significant effect of dioxin at PND70 or 120 in the 50 or 200 ng/kg dose groups on survival, body weight, or sperm parameters, with the exception that the body weight of the 200 ng/kg F1 males was transiently lower than control for post-natal days 1–7. The fetal concentration of dioxin at GD16 was approximately 0.04, 3.3, 8.9 and 51 ng/kg tissue in the 0, 50, 200 and 1000 ng/kg groups, respectively.

This experiment demonstrates that the high dose level is the maximal tolerated dose of dioxin, as there are decreased numbers of offspring. There is no evidence for any effect on the male reproductive system even in response to these very high levels of dioxin. A follow-up sub-chronic study has been carried out and will provide further robust data as a basis for consideration of tolerable (dietary) intake levels. This work was supported by the UK Food Standards Agency (Contract no. T01034).

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P12 Environmental Pollutants

P12-01
Effects of different extracts of mistletoe leaves (*Viscum album* L.) on CCl_4-induced hepatotoxicity in rats

Tatjana Cebovic [1], Mira Popovic [2]

[1] *School of Medicine, Novi Sad, Serbia and Montenegro;* [2] *Institute of Chemistry, Faculty of Sciences, Novi Sad, Serbia and Montenegro*

Mistletoe (*Viscum album* L.) is well known as a medicine from ancient times and the earliest notes. Today it is used as a remedy. Mistletoe contains viscotoxins, phenylpropanes, lignans, flavonoids, biogenamines, polisaccharides and lectins, which have cytostatic effects and immunomodelling potential. Isolated viscotoxins show hipotensive effects. During some preliminary analysis, we concluded that mistletoe contains significant amount of flavonoids and phenolic compounds. Considering well known antioxidative properties and hepatoprotective effects of flavonoids, the effects of different extracts obtained from mistletoe leaves on some biochemical parameters in rats blood hemolysate were examined.

Mistletoe extracts were prepared using successive extractions with four solvents of increasing polarity; ether (Et_2O), chloroform ($CHCl_3$), ethylacetate (EtOAc), and *n*-butanol (nBuOH). The residue was the aqueous extract (H_2O). All five extracts were evaporated to dryness and after that dissolved in 50% ethanol to make 10% solutions to be used in the experiment.

Albino "Wistar" rats of both sexes that were used in this experiment were devided into groups of five animals in each. Some groups have been receiving pure ethanol solutions of different fractions (Et_2O, $CHCl_3$, EtOAc, nBuOH and H_2O) for 7 days, while some groups received carbon tetrachloride (CCl_4) in olive oil (1:1) on the eighth day beside the mistletoe extract. Twenty four hours after intoxication with CCl_4, animals were sacrified. Liver was removed, weighed and homogenized; also, blood samples were collected. Liver homogenate and blood samples were further used for determination of the following enzymes: xanthine oxidase (XOD), catalase (Cat), peroxidase (Px), glutathione peroxidase (GSHPx), as well as reduced glutathion content (GSH) and intensity of lipid peroxidation (LPx).

Results of the investigation showed some very interesting points concerning changes of activity of examined enzymes, both their increase/decrease of activity after administering pure extracts and after intoxication with CCl_4 (more detailed results are going to be given during presentation). We concluded that ethanol solutions of different extracts of mistletoe leaves showed some very good antioxidative and protective properties.

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P12-02
New web-based resources developed by the toxicology and environmental health information program of the USA National Library of Medicine

Jeanne Carriere Goshorn

National Library of Medicine, Bethesda, USA

Organizing toxicology information available on the web is a challenge as access to the Internet continues to expand and diverse users find their way to a wide range of resources. Reliable, timely, understandable information is important to fostering the improvement of public health in the United States and around the globe. The Toxicology and Environmental Health Information Program (TEHIP) of the National Library of Medicine (NLM), a part of the federal Health and Human Services Department in the United States, creates and disseminates scientific and consumer level health information in the area of toxicology and environmental health (http://tox.nlm.nih.gov). Two new products are especially useful in helping many different users navigate the web:

- *World Library of Toxicology* (http://worldtoxicology.nlm.nih.gov) a portal to global information resources on chemical safety, toxicology, and environmental health.
- *ToxSeek* (http://toxseek.nlm.nih.gov), a metasearch and clustering tool allowing users to search multiple websites and databases from diverse sources simultaneously, with relevance ranking and focusing capabilities.

Other TEHIP resources include:

- *TOXNET* (http://toxnet.nlm.nh.gov), a network of toxicology and environmental health databases including the Hazardous Substances Data Bank and TOXLINE.
- *Haz-Map* (http://hazmap.nlm.nih.gov), an occupational health database.
- *Household Products* (http://householdproducts.nlm.nih.gov), a database of household products, their chemical ingredients, and health and safety warnings.
- *WISER* (http://wiser.nlm.nih.gov), Wireless Information System for Emergency Responders, a resource for identifying hazardous chemicals and providing immediate and succinct health and safety information during emergencies.
- *ALTBIB* (http://toxnet.nlm.nih.gov/altbib.html) Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing, includes literature on the development and use of alternative testing methodologies for toxicity evaluation.

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P12-03

Protector effect of silymarin on hepatic metabolism in rats with partial hepatectomy or acute tetrachloride treatment

José Antonio Morales-González[1], Ricardo Perez-Pasten[1], Alvaro Hernández Caballero[2], Abril A. Arellano-Llamas[2]

[1] *Pharmacy Academic Area, Health Sciences Institute, Autonomous University of the State of Hidalgo, Mexico;* [2] *UNAM, Mexico*

Silymarin is a flavenoid possessing an antioxidant function which blocks lipid peroxidation caused by free radicals. The aim of the present study was to describe the effect of silymarin over the metabolism of regenerated liver exposed to acute tetrachloride (CCl_4) treatment, and to correlate this with changes implied in hepatic damage.

Partial hepatectomy (HP) was performed on Wistar rats (230–270 g). Later, CCl_4 (25 ml) was administered intraperitoneally and silymarin intragastrically (100 mg/kg body weight). Blood samples were taken 24 h post-surgery and glucose, albumin, cholesterol, triglycerides, and creatinine concentrations were measured. Animals were sacrificed and the remaining liver was weighted, additionally remaining tissue was used to determine the same biochemical parameters as performed in blood samples.

At 24 h post-HP, serum glucose and triglycerides (TG) presented a similar profile; their levels diminished (50% glucose and 20% TG) in comparison with the control and without significant differences with respect serum levels on administering CCl_4. Silymarin administration in HP rats provoked a 45% and 6% increase in glucose and TG levels, respectively. Co treatment CCl_4 and silymarin during cellular proliferation diminished TG levels by 65%. In the case of TG, differences were observed with CCl_4 treatment alone, diminishing by 16%. With regard to hepatic triglycerides, HP and CCl_4 significantly raised TG levels, this effect was cushioned by silymarin and only significant in the case of HP. Concerning albumin and creatinine, we observed no differences with respect to the control. HP diminished albumin by 10%. Serum cholesterol levels were decreased by 17% as compared with the control after hepatectomy and 41% on adding CCl_4; treatment with silymarin raised it by 11%. Regarding hepatic cholesterol after HP, observed changes were not significant.

In this study, silymarin partially protects against the harmful effects of HP and CCl_4. More significant results would probably be obtained on modifying the silymarin treatment dose.

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P12-04

Monitoring of organochlorine pesticides (OCP), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in human milk in Croatia since 1977

Blanka Krauthacker, Elsa Reiner, Snježana Herceg Romanić

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Numerous xenobiotics have been found in breast milk and the discovery of their presence has caused concern about the safety of breast-feeding. Due to their stability, lipophilicity, accumulation in the environment and

possible health effects special attention is being given to persistent organic pollutants (POPs). The objective of this long-term study is to evaluate trends in levels of four groups of POPs compounds: organochlorine pesticides (OCP), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in human milk collected in Croatia. OCP and PCBs are commercial products while PCDDs/Fs are undesirable by-products. Once emitted, they spread over all abiotic and biotic compartments of the environment.

Since 1977, about 800 human milk samples were analysed. Monitoring started with OCP analyses only. In 1980 the total PCBs analyses started and about 10 years later congener specific PCB analysis was introduced. PCDDs/Fs analysis is not introduced so far but our milk samples were analysed in laboratories within Europe and in the USA as the result of our participation in international projects.

DDE and PCBs were present in all analysed samples while the incidence of other compounds was lower. Levels of OCP and PCBs decreased about 60% during 1980–1990, and then continued decreasing to about 10% of the initial levels. Levels at present are $<200\ \mu g\ kg^{-1}$ milk fat for DDE and total PCBs while levels of the other compounds (including individual PCB congeners) are much lower or below detection limits. PCDDs/Fs levels were 24.2 pg I-TEQ g^{-1} milk fat in 1981/82 and 5.2 pg I-TEQ g^{-1} milk fat in 2000 showing also a downward trend. Comparison of our results with similar studies within and outside Europe showed that the levels of investigated groups of compounds in our samples are at the lower half of the concentration range. The decrease in levels is slower than in developed countries.

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P12-05

Juvenile fish—Perspective bioindicators for assessment of the aquatic environment contamination

Tomas Randak, Ondrej Slavik, Vladimir Zlabek, Jitka Kolarova, Hana Kroupova

University of South Bohemia Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

Standard contamination assessments of adult fish tissues in 2002–2005 were complemented with analyses of juvenile fish (fingerling, 0+) from selected localities of the Elbe River (Czech Republic). Adult fish are not suitable bioindicator for contamination assessment of particular locality due to their migrations. High variability of detected pollutant concentrations in fish tissues is characteristic for individual adult fish within one species from the same locality. Minimal migration activity of juvenile fish during first months of life is a presumption for more exact contamination assessment of particular locality. Juvenile (cyprinid species with spring reproductive period) and adult fish were sampled in August. Pooled samples (monospecific and multispecific) of whole body homogenate (including viscera and chymus) of juvenile fish were analyzed. Muscle samples of individual adult fish were analyzed from the same localities. Concentrations of pollutants (Hg, Cd, Pb, As, PCB, HCH, HCB, DDT, OCS) detected in various species of juvenile fish from the same locality were similar. Interspecific variability of juvenile fish contamination was significantly lower than variability of pollutant concentrations in individual muscle samples of adult fish within one species from the same locality. Juvenile fish seem to be highly suitable bioindicator for assessment of the aquatic ecosystem contamination.

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P12-06

Distribution of organochlorine compounds in sediments from the natural distribution area of Farfantepenaeus duorarum from the campeche bank and the southeast coast of Campeche, Mexico

Jaime Rendón-von Osten, Rodolfo E. Del Rio-Rodríguez, Martin Memije-Canepa

Centro EPOMEX, Universidad Autónoma de Campeche, P.O. Box 520, Campeche, Mexico

The coastal zone of Campeche and its Bank are among the most productive ecosystems; therefore the objective of this study was to determine the current state of organic pesticide pollution in the marine and coastal sediments from Campeche State circumscribed to the main coastal and offshore distribution areas of one of the most economically important shrimp species *Farfantepenaeus duorarum*. Sediment was collected in nine sampling stations offshore and eight inshore stations during the local three climatic seasons. The main compounds detected in sediments were *p*,*p*′-DDE and *p*,*p*′-DDT and the average concentrations were 0.15 and 1.16 ng g^{-1} from the coastal zone and the marine area, respectively. Almost 90% of OC's detected was DDT. During Nortes season, OCs were only detected in coastal stations with

a ΣDDT average of 1.09 ng g^{-1}. The highest concentrations (ΣDDT = 1.4 ng g^{-1}) were found mainly in the station situated close to a cane crop settlement where the runoff could contribute to the pollution. During dry and rainy seasons in three of nine marine stations were detected *p*,*p*′-DDE residues in identical average concentrations of 0.11 ng g^{-1}. In contrast, only in one coastal station OC's was detected during those seasons (C3 dry season, and C8 rainy season), although in higher concentrations (ΣDDT = 0.9 ng g^{-1}) than offshore stations (ΣDDT = 0.1 ng g^{-1}). The atmospheric deposition in North America has been demonstrated and probably the residues found in the Campeche Bank are deposited via this.

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P12-07

Alkylphenols in muscle of fish from rivers in the Czech Republic

Vladimir Zlabek [1], Tomas Randak [1], Tomas Cajka [2], Jitka Kolarova [1], Zdenka Svobodova [1], Jana Hajslova [2], Jiri Jarkovsky [3]

[1] *University of South Bohemia Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic;* [2] *Institute of Chemical Technology Prague, Dept. of Food Chemistry and Analysis, Prague, Czech Republic;* [3] *Masaryk University Brno, Centre of Biostatistics and Analyse, Faculty of Medicine and Faculty of Science, Brno, Czech Republic*

The pilot study of aquatic environment contamination with alkylphenols was performed in the Czech Republic during 2003 and 2005. Levels of alkylphenols and its effects on aquatic organisms have never been studied in the Czech Republic before. Alkylphenol polyethoxylates (APEO) are world wide manufactured and used nonionic tenzides.

The chub (*Leuciscus cephalus* L.) was selected as a suitable bioindicator for the field study. It is abundant in all monitored localities. Samples of fish muscle for analyses of alkylphenols (4-*tert*-nonylphenol, 4-*tert*-oktylphenol) were collected from 16 localities of Vltava, Elbe and Blanice rivers in the Czech Republic. In total, 218 fish specimens were analyzed. Gel permeation chromatography was used for separation of analytes. Sample extracts were analyzed by gas chromatography employing mass selective detector.

Levels of alkylphenols in the fish muscle were slightly increasing in stream longitudinal profiles of the Vltava and the Elbe rivers. The highest average values of alkylphenols in 2003 (sum of 4-*tert*-nonylphenol and 4-*tert*-oktylphenol) were registered at Valy (2.94 ± 0.83 μg kg^{-1} w.w.), Obříství (2.93 ± 0.73 μg kg^{-1} w.w.) and Zelčín (2.77 ± 0.85 μg kg^{-1} w.w.). Increased concentrations of alkyplphenols in fish from localities Podbaba (3.06 ± 2.25 μg kg^{-1} w.w.) and Vranany (3.47 ± 2.64 μg kg^{-1} w.w.) are possibly due to sewage water from Prague and its surroundings. The presented levels of biologically highly active substances in this study indicate possible risk for aquatic organisms and therefore detailed study of alkylphenols effects is needed.

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P12-08

PCB153 and methylmercury (MeHg) assessment of target tissues doses in rats after single and combined exposures: Mothers versus pups comparisons

D. Poli [1], A. Caglieri [1], M. Goldoni [1], M.V. Vettori [2], T. Coccini [3], A.F. Castoldi [3], S. Ceccatelli [4], A. Mutti [1]

[1] *Laboratory of Industrial Toxicology, University of Parma, Italy;* [2] *European Food Safety Authority, Largo Palli 5/A, Parma, Italy;* [3] *IRCCS Salvatore Maugeri Foundation, Pavia, Italy;* [4] *Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*

Due to a growing recognition of an apparent increase in the incidence of developmental disabilities, considerable attention is being focused on the effects of exposure during brain development to a variety of environmental contaminants such as PCBs and MeHg, persistent in food.

The complex problem of simultaneous exposure to different neurotoxicants (MeHg and PCB153) has been addressed in this study. Specific aims were: (1) to determine the brain target dose in mothers and pups after single and combined mother exposure, (2) to compare brain and blood levels.

Pregnant rats were orally treated from gestational day 7 to postnatal day 21 (PND21) with PCB 153 (5 mg/kg/day) and MeHg (0.5 mg/kg/day), alone or in combination. Samples of tissue and blood were collected from animals (both mothers and pups) at different timepoints (PND1, 21, 35 and 90).

The following points summarise conclusions for the assessment of target tissue dose: (i) at PND21, brain versus blood Hg levels (Brain Hg/Blood Hg) were similar in

mothers and pups, (ii) at PND35, the same ratios in pups were higher than in mothers, indicating that Hg accumulates in pups' brain. In addition, (iii) Hg clearance from pups' brains displayed a half time of 4.7 days, (iv) at PND21, brain versus serum PCB153 levels (PCB153 in brain)/(PCB153 in serum) were higher in mothers than in pups, both after single and combined exposure, (v) at PND35, the same ratios were higher in pups than in mothers, indicating that PCB was eliminated by mothers, and milk is a source of exposure for pups. Notably, PCB clearance from pups' brains displayed a half time of 29.8 days.

This study indicates that MeHg and PCB 153 show different accumulation rates in pups' brain after chronic mothers' exposure. Observed *in vivo* target doses will be related to relevant end-points, such as neurophysiological and neuro-behavioural effects.

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P12-09

Protection of α-ketoglutarate, oxaloacetate, succinate, malate, fumarate and citrate against seizures, lipid peroxidation and mitochondrial DNA damage induced by potassium cyanide

Hiro-aki Yamamoto

University of Tsukuba, Graduate School of Comprehensive Human Science, Tsukuba, Ibaraki 505-8575, Japan

The effects of α-ketoglutarate, oxaloacetate, succinate, malate, fumarate or citrate, a substrate of enzyme on the Krebs cycle on seizures induced by cyanide were examined in mice. A subcutaneous injection of potassium cyanide (7 mg/kg) produced broad-spectrum limbic and severe sustained seizures in all of the treated mice. The seizures were abolished when α-ketoglutarate (1 g/kg), oxaloacetate (1 g/kg), succinate (1 g/kg), malate (1 g/kg), fumarate (1 g/kg) or citrate (1 g/kg) was injected intraperitoneally in the animals 1 min before potassium cyanide administration. In addition, the administration of cyanide caused damage to mtDNA in brain frontal and middle cortex of mice. These effects were completely abolished by the ip preinjection of α-ketoglutarate (1 g/kg), oxaloacetate (1 g/kg), succinate (1 g/kg), malate (1 g/kg), fumarate (1 g/kg) or citrate (1 g/kg). *In vitro* exposure of cyanide (1.0, 2.0 mM) to brain homogenate inflicted damage to mtDNA in a concentration-dependent manner. The damage of mtDNA induced by 1.0 mM cyanide was attenuated by the co-treatment with α-ketoglutarate (5 mM), oxaloacetate (5 mM), succinate (5 mM), malate (5 mM), fumarate (5 mM) or citrate (5 mM). Furthermore, *in vivo* and *in vitro* exposure of cyanide elicited an increase in lipid peroxidation. However, the increased lipid peroxidation was completely inhibited by cotreatment of α-ketoglutarate, oxaloacetate, succinate, malate, fumarate or citrate. However, preinjection of maleate (1 g/kg) or malonate (1 g/kg), which is non-component on the Krebs cycle did not prevent against seizures, lipid peroxidation and mitochondrial DNA damage induced by cyanide. These results suggest that the Krebs cycle may play a key role for protection against seizures, lipid peroxidation and mitochondrial DNA damage induced by cyanide.

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P12-10

Impact of pollution on livestocks of adjoining areas of barauni industrial area in Northern India

M.C. Sharma, Pankaj Kumar

Indian Veterinary Research Institute, Izatnagar, Bareilly 243122, Uttar Pradesh, India

Barauni Industrial area of Bihar has one of the countries biggest oil refineries, Barauni Thermal Power station and Chemical Fertilizer plant that are considered to be source of industrial pollution for this area. In the present study samples were collected from Barauni block and Begusaria block as control which is about 30 km away from this industrial area. Equal numbers of samples were collected from both Barauni and Begusarai block by standard techniques which included hair ($n = 50$) and blood from cattle ($n = 27$) and buffaloes ($n = 23$) and water from nearby pounds ($n = 7$), tap water ($n = 7$), well ($n = 10$) and hand pump ($n = 5$). Water, digested blood, serum and hair samples were examined by standard procedure using atomic absorption spectrophotometer for status of toxic metals (arsenic, cadmium and lead) and trace elements (copper, cobalt, zinc, iron, molybdenum and selenium). The arsenic level in hair samples of cattle and buffaloes and pound water was found to be higher than normal and significantly ($P < 0.05$) different form samples collected from Begusarai. However, cadmium level in serum and water from all the four sources was non-significantly ($P > 0.05$) different in both the areas and within the permissible limits. Lead level in blood samples of both cattle and buffaloes of Baurani was towards higher side and significantly ($P < 0.05$) different from Begusarai. However, the levels were normal in water samples and non-significantly ($P > 0.05$) different from Begusarai area indicating industrial source

and ruling out geological source. The level of iron, selenium in serum and water was higher than normal in both the area of Barauni and Begusarai. The concentration of serum zinc, copper was significantly ($P < 0.05$) lower than normal levels suggested but serum zinc level in both these areas were non-significantly different. Serum copper concentration in both cattle and buffaloes were significantly ($P < 0.05$) lower in Barauni area compared to Begusarai block. However, concentration of cobalt and molybdenum in both serum and water was towards normal and non-significantly ($P > 0.05$) different in both areas. Thus copper deficiency due to Cu:Mo antagonism was ruled out and attributed to higher level of lead, arsenic and iron as concentration of copper in soil and fodder of these areas was above the critical limits suggested, ruling out primary cause of copper deficiency in bovines of this region. Comet assay (single cell gel electrophoresis) was done in blood samples collected for cytogenetic study which indicated mild DNA damage (single strand breaks) and oxidative DNA damage resulting from higher arsenic and lead level and lower serum copper and zinc concentration in cattle and buffaloes of these regions.

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P12-11

Effect of diazinon on development of embryos of South African clawed frog (*Xenopus laevis*) in standard and pond water

Helena Modra, Dagmar Vrskova

University of Veterinary and Pharmaceutical Sciences, Brno Czech Republic

Diazinon is an organophosphate pesticide used in agricultural and urban applications to control a variety of insects. In the Czech Republic, is diazinon used in fish farming in well-founded cases as a biocide to suppress excessive propagation of coarse daphnian zooplankton. In these cases, diazinon is applied straight into water. Diazinon decomposes to various degradation products except for to diazoxon, which is more toxic than diazinon.

We studied embryotoxic effect of diazinon in the 96 h Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX) in standard and pond water and observed concentration of diazinon and diazoxon during the test. During 5-days duration of the test, the concentration of diazinon moderately decreased in both types of water. In both types of water we found increased concentration of diazooxon after 3 days, but the 5th day its concentration moderately decreased. The 96-h LC50 and the 96-h EC50 (malformation) of diazinon was 9.84 mg l^{-1} and 5.36 mg l^{-1}, respectively in standard water and 12.64 mg l^{-1} and 6.79 mg l^{-1}, respectively in pond water. Calculated Teratogenic Index (96-h LC50 divided 96-h EC50) signifies a greater potential for all embryos to be malformed in the absence of significant embryo mortality.

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P12-12

Microbiological assessment of the organophosphorous pesticide methyl azinphos persistence in Argentinian productive soil

W. Copes [2], D. Gonzalez [1], J. Quagliano [1], Carlos Vázquez [1]

[1] *Organic Synthesis Area of the Laboratory for Research and Analysis of Chemical Contaminants, Applied Chemistry Department, Institute for Scientific and Technological Research of the Argentine Armed Forces (CITEFA), J.B. de La Salle 4397, CP 1603 Villa Martelli, Buenos Aires, Argentina;* [2] *Alto Valle Agricultural Experimental Station, Argentine National Institute of Agrarian Technology, Gral. Roca, Río Negro, Argentina*

In this contribution we developed a modification of a microbiological method based in the activated sludge degradation of pesticides for assessing its persistence. This test, originally presented by Leoni and coworkers, has the advantage of having a shorter experimentation time and can be easily standardized in the laboratory. Degradation of pesticides in soils is difficult to evaluate, because of the difficulty in isolating the degradation process from other effects which reduce chemical concentrations in soil, such as leaching, run-off, volatilization and plant absorption. It was reported that biodegradation and evaporation are the primary routes of disappearance for methyl azinphos.

Methyl azinphos was sprayed under controlled conditions in apple productive areas. Three rows were sprayed, and three points were sampled per row. One-gram soil samples were extracted with chloroform and filtered cleaned up by passing the extracts through Anakrom adsorption columns and then measured spectrophotometrically. Spray-drift was not observed, as methyl azinphos did not reach two nearby water streams.

In the laboratory, five standard solutions of the organophosphorous pesticide methyl azinphos were prepared in methylene dichloride at concentrations ranging from 5 to 50 mg/l. Then, each sample was added to a slurry made up with 50 g of a sample of soil from the field and 200 ml of tap water, by triplicate. Samples were

agitated in a rotatory shaker at 100 rpm and 25 °C and sampled at 3, 7 and 9 h. Concentration of methyl azinphos was determined by UV spectrophotometry, measuring absorbance at 235 nm. Biodegradation curves were constructed and these curves were correlated with the field results.

A positive correlation was found between laboratory and field results, indicating that this simple and accelerated laboratory method of evaluating pesticide persistence can give prompt results that can be correlated to the decay of these chemicals on the field. This fast methodology developed could allow to process a larger amount of data without the need of performing costly field tests, once being statistically standardized.

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P12-13

Determination of alkylated and methylene-bridged polycyclic aromatic hydrocarbons in environmental matrices

Albrecht Seidel [1], Heinz Frank [1], Hansruedi Glatt [2], Andrea John [1]

[1] *Biochemical Institute for Environmental Carcinogens Grosshansdorf Germany;* [2] *German Institute of Human Nutrition (DIfE) Potsdam-Rehbruecke, Department of Nutritional Toxicology, 14558 Nuthetal, Germany*

Polycyclic aromatic hydrocarbons (PAH) occur in the environment as complex mixtures and along with the parent compounds also a substantial number of alkylated and methylene-bridged PAH are found many of which are also carcinogens including methylene-bridged chrysene (4*H*-cyclopenta[*def*]chrysene, CPCHR). In contrast to the parent PAH benzylic hydroxylation and subsequent conjugation leading to sulfate esters must be considered as a possible metabolic activation pathway of these PAH. However, quantitative and isomer specific determinations of alkylated and methylene-bridged PAH are often hampered by unavailable standards.

In the present study we have synthesized the isomeric methyl (MePYR), 1,6- and 1,8-dimethylpyrenes (DiMePYR), isomeric ethyl pyrenes (EtPYR), and methylene-bridged benz[*a*]anthracene (11*H*-cyclopenta[*pqr*]benz[*a*]anthracene, CPBA) as well as CPCHR and determined their concentrations in several environmental matrices. Isomeric MePYR and DiMePYR have been determined in different cigarette smoke condensates (range 6.55–24.9 ng/cig), whereas isomeric EtPYR have not been detected. MePYR and DiMePYR are also found in used engine oil (range 360–480 μg/kg), in particulate matter of diesel engines (range 940–1750 μg/kg), in soil (range 7400–7950 μg/kg) and sediment samples (range 98–200 μg/kg) as well as in coal tar pitch (range 390–730 μg/kg). Again the isomeric EtPYR have not been detected in these matrices. CPCHR and CPBA were also identified and determined in different cigarette smoke condensates (range 1.8–2.5 ng/cig). They have also been detected in used engine oil (range 30–80 μg/kg), in particulate matter of diesel engines (range 25–57 μg/kg), in soil (range 120–190 μg/kg) and sediment samples (range 36–53 μg/kg) as well as in coal tar pitch (range 680–1110 μg/kg). The obtained data on alkylated PAH concentrations can be used for risk assessment of the complex PAH mixtures in the investigated matrices.

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P13 Exposure Monitoring

P13-01

Toxicologic effects of particulate matter loading to alveolar macrophages and enhancement of translocation of particles from lungs of mice

Deepak Kumar [1], N. Singh [2], A.P. Sahu [2]

[1] *Department of Environmental Sciences, Lucknow University, Lucknow 226007, India;* [2] *Preventive Toxicology Division, Industrial Toxicology research Centre, Lucknow 226001, India*

The lungs are organ of respiration and gaseous exchange and forcefully exposed to the undesirable particulate matter. Mounting evidence shows that alveolar macrophages and different types of epithelial cells constitute the primary targets of inhaled lung toxicants and are therefore particularly important in the induction of inflammatory responses in the lungs. Our scientific efforts have not yet reached for the stoppage of entry of these particles into the lungs and prevention of the resultant adverse health effects. Sensitive population with chronic bronchitis, asthmatic disorders and interstitial lung fibrosis conditions induce significant impairment in the alveolar clearance mechanism, exposure to the ultrafine and fine particles increases respiratory morbidity due to cardiopulmonary disorders.

In the present study PM_{10} was collected from the various residential areas of the Lucknow City and loading and toxic effect of PM_{10} to MØ of normal and asthmatic mice were undertaken. Thirty-two mice were

grouped in the four groups. Group I—Control; Group II—Asthmatic mice; Group III—Asthmatic mice + PM (3 mg); Group IV—PM. Asthmatic condition was induced in mice by sensitization through intraperitoneal route and nasal inhalation with ovalbumin. On day 30, mice of Group III and Group IV were exposed to PM_{10} through intratracheal route. The MØ from BALF of control mice showed normal features and asthmatic mice showed foamy MØ with large hyperchromatic nuclei, clear cytoplasm. While in Group-III combined effects of induced asthma and PM showed MØ having deeply stained cytoplasm, darkly stained nuclei, PM trapped in the cytoplasm and multinucleated cells. The PM loading as with heavy deposition of black particles in MØ and micronuclei in the cytoplasm were observed in Group IV. In sensitive conditions the impairment of the MØ was observed which leads to severe adverse effects in combined condition of exposure of PM_{10} and asthmatic disorders. Our earlier studies demonstrated that feeding functional food Jaggery helps in the translocation of inhaled particles form lungs in normal and sensitive conditions.

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P13-02

Effects of PM2.5 and PM10 collected during the dust storm period on the phagocytic function and cytokine secretion of rat alveolar macrophages

Xinbiao Guo, Xuelian Huang, Yu Jin

Peking University School of Public Health, China

The increased dust storms in China and other part of the world have contributed to high mass concentrations of ambient $PM_{2.5}$ and PM_{10} in recent years, which may be related with increased incidence of respiratory diseases in the affected population. Although considerable attention has been devoted to other kinds of inhalable particles, very little information has been reported about the health effects of dust storm particles. In this study we investigated effects of dust storm $PM_{2.5}$ and PM_{10} on the phagocytic function and IL-8 and TNF-α secretions of rat alveolar macrophages. $PM_{2.5}$ and PM_{10} were collected during the dust storm period in the urban area of Beijing. Alveolar macrophages were isolated from male Sprague–Dawley rats by routine methods. Cytotoxicity of $PM_{2.5}$ and PM_{10} was measured by MTT assay. Flow cytometry was applied to characterize effects of $PM_{2.5}$ and PM_{10} on the phagocytic function of alveolar macrophages. IL-8 and TNF-α secretions from cells were measured by radioimmunoassay. The viability of rat alveolar macrophages significantly decreased after incubated with $PM_{2.5}$ and PM_{10} for 8 h at the concentrations above 20 and 50 μg/ml, respectively. $PM_{2.5}$ and PM_{10} impaired phagocytic function of alveolar macrophages in a dose-dependent manner and were observed even at the concentration without apparent cytotoxicity. $PM_{2.5}$ and PM_{10} also dose-dependently increased the secretion of IL-8 and TNF-α by alveolar macrophages. In Conclusions, dust storm $PM_{2.5}$ and PM_{10} could impair the phagocytic function and induce TNF-α and IL-8 secretion of rat alveolar macrophages, which may be related to the mechanism of respiratory injury caused by dust storm particle.

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P13-03

Enhanced esterase activity and azinphosmethyl exposure association in target and non-target organisms

Cristina Monica Montagna, Ana Ferrari, Jimena Soleño, Ana Pechen de D'Angelo, Celina Martinez, Liliana Anguiano

Universidad Nacional del Comahue, Neuquem, Argentina

The organophosphate azinphosmethyl is extensively applied in the Alto Valle (Argentinean Patagonia) to control codling moths (*Cydia pomonella*) from apples and pears. This area is irrigated by fast-flowing channels providing a favourable habitat for many species including black fly larvae (*Simulium* spp.) and amphipods (*Hyalella curvispina*). These non-target species are also highly exposed to pesticides because of the air drift from spraying, the natural run off of chemicals into irrigation channels and the important discharge of the products caused by clean up of containers and sprayer tanks. Previously, we have observed differences in toxicity to azinphosmethyl between insecticide exposed and non-exposed populations of simuliids and codling moths. Thus, the objective of the present investigation was to determine the mechanism involved in the tolerance to this insecticide.

Esterase activities were individually assayed on simuliid larvae ($n = 180$), adult amphipods ($n = 93$) and diapausing larvae of codling moths ($n = 67$) from pesticide exposed and unexposed areas. All populations were collected an evaluated on 2005.

The three populations of organisms from pesticide exposed sites showed significantly higher esterase activities than the control ones. Mean esterase activities of

Simulium spp. were 2.17 ± 1.71 and 0.81 ± 0.35 μmoles of α-naphthol/min^{-1} mg^{-1} ($P < 0.001$). On the other hand, mean esterase activities of *Hyalella curvispina* were 0.27 ± 0.99 and 0.14 ± 0.69 μmoles of α-naphthol/min^{-1} mg^{-1} ($P < 0.001$). At last, diapausing larvae of *C. pomonella* from azinphosmethyl managed orchards evidenced significantly higher ($P < 0.001$) mean esterase activity (0.17 ± 0.065 μmoles of α-naphthol/min^{-1} mg^{-1}) than the populations collected from organic orchards (0.014 ± 0.0073 μmoles of α-naphthol/min^{-1} mg^{-1}). Increased esterase activity is a common mechanism of resistance to organophosphates in arthropods by sequestration of the insecticide. Therefore, we conclude that there is an association between enhanced esterase activity and insecticide exposure of simuliids, codling moths and amphipods.

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P13-04

Inflammatory potential of wood smoke and traffic derived particles

Anette Kocbach, Jan I. Herseth, Ellen Namork, Per E. Schwarze

Norwegian Institute of Public Health, Oslo, Norway

Exposure to ambient particulate matter (PM) has been associated with increased respiratory and cardiovascular morbidity and mortality. However, the biological mechanisms, as well as the particle characteristics contributing most to the adverse health effects, have not been extensively clarified. Residential wood smoke and vehicle exhaust are primary sources of PM in Norway and the relative inflammatory potential of particles from these sources was, therefore, investigated. Particles were collected from a conventional Norwegian wood stove during high-temperature combustion, and in a road tunnel during summer and winter seasons. A reference diesel sample (SRM 2975) was also included in the study. A human macrophage cell line (THP-1) was used to study the release of the cytokines IL-8, TNF-α and IL-1β after particle exposure. All particle samples increased the release of IL-8, with relative potencies; Tunnel summer > Wood ≈ Tunnel winter ≫ Diesel. A similar response pattern was observed for the TNF-α and IL-1β release, with some exceptions; Diesel did not induce a release of TNF-α, whereas Wood did not induce a release of IL-1β. These results may indicate that the role of TNF-α and IL-1β in the release of IL-8 differs between particles from wood smoke and traffic. This will be further investigated using inhibitors of TNF-α and IL-1 receptor. The four particle samples have previously been characterised with respect to sample composition (combustion vs. mineral particles), particle size, content of organic and elemental carbon, as well as PAH content. Their physicochemical characteristics were found to differ considerably. The release of IL-8, TNF-α and IL-1β did, however, not correlate with the chemical and physical characteristics of the particles. Organic extracts of the particle samples will be used in future experiments, to investigate which particle components may be responsible for the observed pro-inflammatory responses.

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P13-05

Influence of 2-week exposure to amosite and their substitutes on the selected cytotoxic parameters of bronchoalveolar lavage in rats

Silvia Cerna, Marta Hurbankova, Zuzana Kovacikova, Sona Wimmerova, Milan Beno

Slovak Medical University, Bratislava, Slovakia

Changes in selected cytotoxic parameters of bronchoalveolar lavage fluid (BALF) after 2-week exposure to asbestos-amosite (A) and its substitutes – glass fibres (GF) and refractory ceramic fibres (RCF) – were studied in male Wistar rats. Aim of this work was to evaluate the possible dose dependence of GF and RCF cytotoxic effects on lung in subcronic phase of exposure and compare them with those of amosite. Male Wistar rats weighing at the beginning of the experiment (on the day of instillation) 248.9 ± 31.3 g were treated as follows: the control animals ($n = 12$) were intratracheally instilled by saline (0.2 ml per animal); the animals exposed to fibrous dusts (6 animals in each group) were instilled by 0.5, 1, 2 or 4 mg of A, GF or RCF respectively (per animal) dissolved in 0.2 ml of saline solution. After 2-week exposure the animals were exsanguinated in anaesthesia and the bronchoalveolar lavage was performed. Viability and phagocytic activity of alveolar macrophages, activity of lactate dehydrogenase in cell-free bronchoalveolar lavage fluid (cfBALF) and activity of acid phosphatase (ACP) and cathepsin D (CATD) in cfBALF and in BALF cells as well were estimated. The negative correlation of viability to the dose of instilled A and GF was extremely significant with significantly higher slope of the regression line for A. Significant correlation between dose and the phagocytic activity was found in groups instilled by A and by GF. Positive correlations between dose and CAT D activity in cfBALF were found after exposure to all of examined fibrous dusts. The correlation was most significant after amosite exposure. Activities of ACP measured in BALF cells correlated with the dose of GF and RCF.

Activities of CAT D measured in BAL cells correlated extremely significantly with the dose of all of tested fibrous dusts (the highest after amosite exposure). The closest relation between the dose and the levels of examined parameters was found after the amosite exposure, but the dose effects of GF and RCF were not negligible.

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P13-06

Effect of synthetic prethroids on the activities of trypsin and lipase in fresh water fish *Channa punctatus*

Kamal Kumar Saxena [1], Vidhi Sirohi [1], Pankaj Kumar [1,2]

[1] *Pest and Parasite Research Laboratory, Department of Zoology, Bareilly College, Bareilly 243005, Uttar Pradesh, India;* [2] *Division of Medicine, Indian Veterinary Research Institute, Izatnagar, Bareilly 243122, Uttar Pradesh, India*

Synthetic pyrethroids are potential toxic pollutants contaminating aquatic ecosystem. Two important members of this group permethrin and α-cypermethrin are widely used as insecticides and are considered to be relatively toxic to non-target species including fish. In the present studies fresh water fish *Channa punctatus* ($n = 49$) of nearly equal age group and weight were collected from local water bodies and after acclimatization in the laboratory conditions they were exposed to various sublethal concentrations of permethrin and α-cypermethrin. They were equally divided into seven groups including one control group.

The effect of exposure to sublethal concentrations of permethrin (0.15 ppm, 0.20 ppm, 0.25 ppm) and α-cypermethrin (0.00015 ppm, 0.00020 ppm and 0.00025 ppm) was observed for 30 days on trypsin and lipase activities in muscle and liver tissue of *Channa punctatus.* Sublethal concentration of 0.30 ppm permethrin and 0.0005 ppm of α-cypermethrin were found to be lethal to *Channa punctatus*. After exposure to the various sublethal concentration of permethrin and α-cypermethrin, the supernatant of homogenized and centrifuged liver and muscles of these fish was taken for measuring the activities of these enzymes and compared with the activity of these enzymes in control (unexposed) group fish liver and muscles. The results indicated that both permethrin and α-cypermethrin caused reduction in the activities of trypsin and lipase in liver and muscle tissue of *Channa punctatus*. It was observed that changes in these enzyme levels were dependent on period of exposure and concentration of these compounds. However, the effect of α-cypermethrin on these enzyme activities in tissues of *Channa punctatus* was found to be more pronounced compared to permethrin.

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P13-07

Development of free radical scavenging system and lipid peroxidation under the influence of gestational cadmium exposure

R.A. Kireev [1,2], O.V. Slyzova [1,2], E.V. Stepanova [1,2]

[1] *Research Institute General Pathology and Pathophysiology, Moscow, Russia;* [2] *Saratov State University, Department of Biochemistry and Biophysics, Saratov, Russia*

The aim of the present investigation is to study the lipid peroxidation processes and antioxidant defense in rat pups, born from the mothers, who were exposed to the cadmium treatment during their pregnancy. Pregnant rats were divided into control (C) and cadmium (Cd) groups. Control animals received 0.9% NaCl while the Cd group received Cd as $Cd(NO_3)_2$ per os during 10 days (from 6 to 15 days gestation) in dosage of 2 mg/kg. On the 60th and the 120th postnatal days the male and female offspring were decapitated. The measured parameters were tissue, serum and erythrocytes MDA; in erythrocytes—reduced glutathione (GSH), glutathione reductase (GR), glutathione-*S*-transferase (GST); in serum—γ-glutamyltransferase activity, level ceruloplasmin (Cp) and sialic acid.

The results of the experiment showed that on the 60th day there took place a decrease of the level of Cp in serum in the males (315.1 ± 41.1 mg/l) as compared with the control group (436.8 ± 20.6 mg/l). The female rats did not display a significant difference with the control group. On the 120th day of the postnatal development the difference in the level of Cp between experimental and control groups was not detected. The activity of serum γ-glutamyltransferase was considerably higher in the experimental male group as well as in the experimental female, as compared to the control groups of corresponding sex and age. The 120th day of the postnatal development signifies an increase of the level of sialic acid only in the experimental male group (4.3 ± 0.6 mmol/l) in comparison with the control male (2.4 ± 0.2 mmol/l), while in the experimental female group and the control group the authentic differences were not registered (2.2 ± 0.08 and 2.3 ± 0.14 mmol/l corresponding). The

levels of GSH were decreased significantly in experimental male and increased significantly in experimental female animals on the 60th day of age. Cd exposure increased the activities of GR and GST in experimental female group and decreased in male as compared to control group on the 60th day. The MDA level was higher in the experimental male group as well as in the female experimental group on the 60th and 120th days of age, compared to the control animals groups. However the MDA level in liver and kidney was higher in experimental male group as compared to female one.

The data indicate that the exposure of pregnant mothers of Cd produced changes in the antioxidant defense mechanisms and metabolic processes at critical periods of development, which may have serious implications in the following period of life.

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P13-08
TLC determination of allergenic and carcinogenic dyes

Michaela Zeiner [1], Iva Rezic [2], Ilse Steffan [1]

[1] *University of Vienna, Vienna, Austria;* [2] *Faculty of Textile Technology, University of Zagreb, Zagreb, Croatia*

Forty-nine different dyes are classified as contact allergens with reference to textile related contact dermatitis. Two thirds of these are disperse dyes representing only a small fraction of the eight thousand commercially used dyes. These dyes are used for immersion or printing of acetate/polyester, polyamide, poly-acrylonitrile, PVC, and polyurethane textiles. Today these textiles having direct skin contact such as underwear, stockings, bathing suits, shirts pants, baby's and children's clothing as well as sleeping bags are gaining more and more attention (CAMAG applications notes in instrumental TLC, A-79.1 and A-64.5). Additionally carcinogenic amines deriving from azo dyes represent another problem. Therefore the dyes used as well as specified textiles have to be tested to ensure absence of any carcinogenic amine. For this purpose many different analytical procedures can be applied (CAMAG applications notes in instrumental TLC, A-79.1 and A-64.5; Öko tex standard).

In this research work a thin layer chromatographic method was developed for testing several carcinogenic and allergenic dyes being applied on different textile products. These substances may be extracted from the fabrics during every day's use and then be absorbed by the skin. The development of this analytical procedure included optimization of the thin layer chromatographic procedure (qualitative and quantitative factors). Preliminary experiments comprised the testing of different stationary and mobile phases, as well as different reagents for visualization. Afterwards optimization of the mobile phase composition was performed, which included testing different volume ratios of the chosen solvents maintaining constant all the other parameters. By this method different disperse and azo dyes classified as critical under the aspect of consumer protection were chromatographically separated and determined qualitatively and quantitatively, respectively.

The advantages of using thin layer chromatography for this analytical task are the following: ease of operation, low operating costs, high sample throughput, and visual color recognition. In comparison to the classical procedures for separation and determination of different dyes, very small sample and solvent amounts needed for the analysis made this method economically and ecologically favorable. For these reasons the method developed may be applied in different textile testing laboratories evaluating textiles treated with dyes as potentially allergenic or carcinogenic.

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P13-09
Environmental confounding factors to occupational exposure to polycyclic aromatic hydrocarbons

K. Lyubomirova [1], T. Popov [1], T. Panev [1], D. Chohadzieva [1], V. Vitcheva [2]

[1] *National Center of Public Health Protection, Sofia, Bulgaria;* [2] *Department of Toxicology and Pharmacology, Pharmaceutical Faculty, Medical University, Sofia, Bulgaria*

Carcinogenic polycyclic aromatic hydrocarbons (PAHs) are an important component of ambient air pollution. They are a health hazard to the whole population living in regions with heavy traffic, as well as to people occupationally exposed to high concentration of PAHs. They can be bound to the respirable part of PM10 and PM 2.5.

Aim: The aim of the study was to measure the occupational exposure to PAHs and to assess the influence of environmental confounding factors as tobacco smoke.

Materials and methods: Two occupationally exposed groups were selected—50 bus drivers, 50 traffic policemen and 50 clerks (control group).

Information about the residential place, smoking habits, diet, alcohol consumption, drugs and vitamins intake, etc. was obtained from a questionnaire completed by each participant. Stationary air sampling in heavy traf-

fic areas as well as personal dosimetry were performed. The analysis of the samples was done by gas chromatography followed my mass spectrometric determination. Biomarkers of exposure to PAHs (1-hydroxypyrene/1-HP) and to tobacco smoke (coutinine) were measured in the urine of the exposed groups.

Results: No statistical significance of the demographic, dietary and habitants indices was found. The results from the stationary sampling showed that the concentration of PM10 in winter varied from 36.9 to 272.73 $\mu g\,m^3$, and in the spring from 21.84 to 142.07 $\mu g\,m^3$. The concentration of PAHs varied from 13.21 to 543.1 $ng\,m^3$ and those of benzo(a)pyrene—from 0.201 to 241.97 $ng\,m^3$. The analysis of the personal dosimetry showed that the exposure of the traffic policemen was slightly higher than that of the bus drivers. The concentration of 1-HP at the end of the working shift was three times higher in the urine of the drivers compared to the policemen. The concentration of coutinine was three time higher among the bus drivers–smokers, in comparison with policemen (smokers) and five times higher that the control group (smokers). These data could explain the higher concentration of 1-HP registered among the drivers. Obviously this is because of their additional exposure to tobacco smoke. In spite of the similar smoking habits between the three investigated groups, the concentration of coutinine among the bus drivers is much higher because of the characteristics of their working place.

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P13-10

Development and in-house validation of biosensor-based transferable techniques for the low-cost toxicity quantification in agro-zootechnical foodstuffs

Chiara Frazzoli [1], Roberto Dragone [2], Alberto Mantovani [1], Agostino Macri [1], Luigi Campanella [3]

[1] *Department of Food Safety and Veterinary Public Health and WHO/FAO Collaborating Centre for Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy;* [2] *Institute for Complex Systems, Consiglio Nazionale delle Ricerche, Rome, Italy;* [3] *Department of Chemistry, University of Rome "La Sapienza", Italy*

Diseases caused by ingested agents are recognized as a key barrier to human and livestock health. Besides the traditional concept of food-borne diseases, increasing interest is directed towards long-term risks due to chemicals in foods. Both anthropic activities and chemical uses in agriculture and animal husbandry (e.g., pesticides, biocides, veterinary drugs and feed additives) have dramatically raised the levels of toxic residues and contaminants (e.g., heavy metals and persistent organic pollutants) in environment-food chains.

This study is aimed at the development, in-house validation and successive transfer of a versatile, rapid, low cost and easy-to-use analytical system for the determination of toxicants in food. The analytical system is addressed to associations of farmers, food processors, retailers and traders in Developing Countries, who want to undertake specific actions towards international quality standards.

Biosensors are deemed suitable for the purpose, combining biological specificity, reliability of chemical reactions and electronic processing sensitivity. A number of samples are collected, such as edible tissues, milk and derivatives, where several chemicals are known to accumulate. Only a minor centrifuging pre-treatment may be required.

An oxygen sensor is coupled to different biological mediators to obtain complementary analytical information. The yeast and algal biosensors are used for integral toxicity tests, taking into account any additive, synergic or compensative effect due to combined exposures. They are respectively based on the respiration of yeast cells and the photosynthetic activity of algae.

Measurements performed on centrifuged aliquots are compared with those on microwave-assisted acid digested ones to extrapolate the inorganic and organic fractions. Further, a proper modification of the oxygen sensor allows the quantification of the organic fraction due to pesticides, thank to their inhibiting action on the tyrosinase enzymatic activity. The outcomes on real matrices are compared with those found in Certified Reference Materials.

This pilot approach for the assessment of criteria for a new relative toxicity estimate is undertaken in order to contribute the implementation of low-cost approaches for the evaluation of dietary exposures to residues and contaminants.

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P13-11

Environmental monitoring of metal exposure and in vitro evaluation of genotoxic and oxidative effects induced by PM10 from an electric steel plant

Cinzia Lucia Ursini [1], Delia Cavallo [1], Raffaele Maiello [1], Aureliano Ciervo [1], Arianna Di Francesco [1], Andrea Caglieri [2], Sergio Iavicoli [1], Pietro Apostoli [3]

[1] *Department of Occupational Medicine ISPESL, National Institute of Occupational Safety and Prevention Monteporzio Catone, Rome, Italy;* [2] *ISPESL, Parma, Italy;* [3] *University of Brescia, Italy*

Carcinogenic risk for specific metal compounds (Cr(VI), Ni_3S_2, As, Cd), PAHs, POPs or type of production (iron and steel founding) has been individuated in metallurgical industry. In particular in this study we investigated the effects of occupational exposure in an important area of Italian metallurgy (steel production by electric arc furnace). We evaluated on human lung epithelial cells A549 the genotoxic and oxidative effects of inhalable particulate matter (PM10) collected in the furnace area from an Italian electric steel plant. Airborne particulate matter was collected with a flow rate of 15 l/min over 6-h period by PM20 HMA Air Sampler (cellulose filter 37-mm, 0.8 μm). The PM10 amount in the extract was measured and the metal content was analysed by ICP-MS. Early direct and oxidative DNA damage were evaluated by fpg-modified comet assay. The cells were exposed for 30 min and 2 h to 4.64 mg/ml, 9.28 mg/ml, 23.5 mg/ml of PM10 contained in the extract dissolved in DMSO. Oxidative and direct DNA damage were evaluated analysing Tail moment values from fpg-enzyme treated cells (TMenz) and enzyme untreated cells (TM) respectively and by comet percentage analysis. The monitored air sample contained 5,54 mg/m^3 of PM10. The metals present in higher concentrations were Zn (383 μg/m^3), Al (85 μg/m^3), Pb (72 μg/m^3), Mn (17 μg/m^3), Ba (10 μg/m^3), Ni (5 μg/m^3), Cu (3.4 μg/m^3), Sr (1.5 μg/m^3) and Cr (0.7 μg/m^3) and although present in much lower amount, Co, As, Mo, Cd, Sn, Sb, Hg and Fe were found. Comet test showed for both exposure times an increase of comet percentage in cells exposed in respect to control already at the lower dose with a further slight increase at the higher doses. After 30 min of exposure a small increase of TM and TMenz, indicating a slight DNA damage, was detected in cells exposed only at highest dose. While for the longer exposure time (2 h) a dose-dependent increase of TM and TMenz, indicating a dose-related direct/oxidative DNA damage to the chosen concentrations was found. The results indicate a dose and time dependent direct and oxidative early DNA damage by PM10 from steel plant, a mixture of heavy metals and PAHs that although present at low doses could have induced a synergic effect.

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P13-12

An experiment on the use of absorbent media (activated carbon and activated alumina) for controlling chemical exposures and environmental pollution

Jinky Leilanie Lu [1,2]

[1] *University of the Philippines, Manila, The Philippines;* [2] *National Institutes of Health, Affiliate College of Arts and Sciences, Manila, The Philippines*

This study tried to test the effectiveness of activated alumina and activated carbon as absorbent media for certain solvent exposures in a plastic and lamination manufacturing plant employing 400 workers and situated in a populated community. Using a controlled experiment, it was shown that activated media were 92–99% effective in reducing vapour concentrations in the ducting system. Odour concentration was also significantly reduced using the said media. Since airflow velocity in the ducting system is reduced with the insertion of the activated media filters, it is suggested that an increased motor capacity be installed with the use of these media. Environmental disasters and adverse health effects of chemical solvents can thus be reduced significantly with the use of activated alumina and activated carbon in countries like the Philippines where incineration is banned.

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P13-13

Occupational exposure monitoring to organic solvents of a group of workers from a typography

Elena Teslariu [1], Oana Teslariu [1], Corina Papaghiuc [1], Veronica Oprea [2], Brandusa Constantin [1], Cornelia Mihalache [1]

[1] *Occupational Health Department, University Med. Pharm. "Gr.T.Popa", Iasi, Romania;* [2] *Biomedical Engineering Faculty, University Med. Pharm. "Gr.T.Popa", Iasi, Romania*

In this study we followed the exposure monitoring to organic solvents of a group of workers from a typography. They use a solvent water emulsifiable mixture, that contains naphta, for the inking rollers and blankets washing. The investigated subjects have an average age of 45 ± 11 years and an average period of time at

work place of 22 ± 9 years. The exposure conditions and clinical examination were performed. Some biotoxicological parameters (phenols, hippuric acid, index sulphate) were measured, at the end of the work week, in order to evaluate the exposure effects. The evaluation of exposure conditions showed that the exposure varies and is not continuous and the collective and individual protective measures are completely inappropriate. The clinical exam showed that all investigated subjects presented pronounced dizziness and nausea and 38% of subjects presented irritations of the respiratory system and of the eyes. Toxicological parameters (phenols and index sulphate) were found pathological; 50% of subjects presented values over permissible biologic limits for conjugated phenols (50 mg/l) and 38% of subjects presented values lower than normal for index sulphate (0.85). Values found for conjugated phenols and index sulphate were 87.69 ± 9.53 mg/l and 0.78 ± 0.04 respectively. Recommendations have been made in order to eliminate occupational exposure (introduction of the special protective measures and safety guidelines) and to improve the occupational medicine services for the exposed workers.

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P14 Metals and Metalloids

P14-01
Exposure to extractable chromium from tanned leather

Michaela Zeiner [1], Iva Rezic [2], Ilse Steffan [1]

[1] *University of Vienna, Vienna, Austria;* [2] *Faculty of Textile Technology, University of Zagreb, Zagreb, Croatia*

Although synthetic fibers are nowadays widely used for a variety of textile products, leather is still a material unmatched in properties by synthetics. Leather making is an environmentally challenged process. A tanning process is indispensable in order to render to the skin matrix stability against many factors, such as microbial degradation, heat and sweat. A common way of tanning is using chromium(III) not only due to the excellent properties that it renders to the leather, but also because of the simplicity of operation. Apart from the environmental pollution caused by chromium containing wastewater allergic reactions are reported for persons working wearing leather gloves. Indeed poor uptake of chromium during tanning process has been stated, but nevertheless nocuous effects to human skin have been observed.

Therefore the chromium contents of raw leather as well as of chromium tanned leather samples were determined. First all samples were dried at 65 °C overnight and then cut in small pieces using a ceramic knife. In order to receive the total chromium amount the samples were weighed and analyzed after microwave assisted digestion of the entire sample. Digestion was performed using about 0.2 g of each sample applying a mixture of concentrated nitric acid and concentrated hydrochloric acid (5:1, v/v). To determine the quantity of soluble chromium extraction experiments were carried out using four different kinds of solvent, e.g. distilled water, artificial sweat solutions (pH 5.5 and 8.0), and artificial saliva. For each experiment 10 mL of the extraction solution were added to about 0.5 g of sample, which were then shaked at 37 °C for one or three hours, respectively.

The quantification measurements of chromium were performed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 3000 XL, concentric nebulizer). The limits of detection (LODs; 3σ) calculated for chromium in all media were in the range from 1 ppb up to 6 ppb. The total chromium amounts found were 100 μg/g, 1 μg/g and 30 mg/g for untreated cow skin, washed skin after primary treatment with sodium hydroxide and final chromium tanned leather, respectively. The results of the extraction experiments reflect these findings. Whereas only small amounts of chromium were extracted from the raw skin and the primary treated skin, up to 2 mg/g were leached from the tanned leather sample.

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P14-02
Ascorbic acid protection against arsenic induced oxidative stress

Suresh Vir Singh Rana

CCS University, India

Exposure to inorganic arsenic (iAs) is known to cause a number of toxic effects involving multiple organ systems. Species differences in the metabolism and toxicity of arsenic have also been established. Nevertheless, sex differences in arsenic toxicity are poorly known. Recent report from our laboratory has shown that arsenic depletes ascorbic acid. Deficiency of ascorbic acid inhibits cytochrome content and microsomal mixed function oxidases. Therefore, supplementing inorganic arsenic treated rats with ascorbic acid might be a protective mechanism. Since gender differences in detoxication mechanisms are known, it becomes mandatory to study ascorbic acid interaction with arsenic in both the sexes.

Protective effects of ascorbic acid on oxidative stress induced by inorganic arsenic in liver and kidney of

rat has been studied. Furthermore, gender differences observed in ascorbate protection have been reported. Male and female rats (200 ± 30 g) were simultaneously treated with arsenic trioxide (4 mg/100 g B.W.) and ascorbic acid (25 mg/100 g B.W.) on each alternate day for thirty days. Thereafter, observations on microsomal lipid peroxidation, reduced glutathione, oxidized glutathione, and glutathione-*S*-transferases were made. It was found that ascorbic acid treatments increased arsenic excretion, inhibited lipid peroxidation, improved GSH status, regulated GSSG turnover and also restored glutathione-*S*-transferases activity in liver and kidney. However, gender differences in all these observations were observed. It is concluded that ascorbic acid protection is controlled by gender dependent factors. The study is considered to be important from public health point of view.

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P14-03

Sample pretreatment of trace toxic metals prior to atomic absorption spectroscopy for evaluation of different occupational exposures

Seyed Jamaleddin Shahtaheri, Monireh Khadem, Farideh Golbabaei, Abbas Rahimi-Froushani

Tehran University of Medical Sciences, Iran

Heavy metals are important constituents widely used in different industrial processes for production of various synthetic materials. For evaluation of workers' exposure to trace toxic metals, including Pb, Hg, Cd, Cr, Co, Cu, and Ni, environmental and biological monitoring are essential processes, in which, preparation of samples is one of the most time-consuming and error-prone aspects prior to analysis. The use of solid-phase extraction (SPE) has grown and is a fertile technique of sample preparation as it provides better results than those produced by liquid-liquid extraction (LLE).

To evaluate factors influencing quantitative analysis scheme of toxic metals, solid phase extraction using minicolumns filled with different sorbents including various Chromosorbs (102, 105) and XAD resins (2, 4, and 7) was optimized with regard to sample pH, ligand concentration, loading flow rate, elution solvent, sample volume (up to 500 ml), elution volume, amount of resins, and sample matrix interferences. Trace metal ions were retained on different solid sorbents and were eluted simultaneously with 10–20 ml 1 M HNO_3 followed by simple determination of analytes by using flame atomic absorption spectrometery. Obtained recoveries of metal ions were more than 96%. The amount of the analytes detected after simultaneous preconcentration were basically in agreement with the added amounts.

The optimized procedure was also validated with three different pools of spiked urine samples and showed a good reproducibility over six consecutive days as well as six within-day experiments.

The developed method promised to be applicable for evaluation of other metal ions present in different environmental and occupational samples as suitable results were obtained for relative standard deviation (less than 10%), therefore, it is concluded that, this optimized method can be considered to be successful in simplifying sample preparation for trace residue analysis of heavy metals in different matrices for evaluation of occupational and environmental exposures.

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P14-04

Arsenic induced clastogenicity: Modulation by functional-food Jaggery

Nrashant Singh, Deepak Kumar, A.P. Sahu

Division of Preventive Toxicology, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow 226001, India

Water, a most essential component of life, contaminated with arsenic, is global human health hazard. Vast majorities of developing countries are forced to drink, only available arsenic contaminated water, resulted in multifactorial dysfunctions including mutagenicity and genotoxicity. There is no effective remedial action of chronic arsenicosis, despite it; a well-nourished diet can modulate the delayed effect of arsenic in drinking water. Functional-food Jaggery has enormous wealth of protein, vitamins and minerals and has great nutritive and additional medicinal value as reported in Indian-Ayurveda. It also has the anti-toxic and anti-carcinogenic activity.

The present research work aimed to evaluate the potential of Jaggery against the clastogenic effect induced by arsenic. Forty mice were grouped as, Group-I served as Control; Group-II arsenic as arsenic trioxide (12.9 mg/kg body weight); Group-III arsenic along with Jaggery (250 mg/mice) and Group-IV Jaggery alone. Mice were exposed to arsenic through subcutaneous route (s.c.) on days 1, 7, 14, 21 and 28 and mice were sacrificed and chromosomal preparations were made from bone-marrow cells. The cytogenic endpoints studied were on chromosomal aberrations and damaged cells. As-usual, chromosomal aberrations were more pronounced in arsenic treated mice (Group-II), while co-

administration of Jaggery with arsenic reduced the clastogenicity in Group-III. Thus, the present study demonstrated that Jaggery the natural functional food has the efficiency to encounter the clastogenic effects induced by arsenic.

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P14-05
Cardiotoxic effects of sodium selenite in rodents

Vesna M. Jacevic [1], Zoran A. Milovanovic [1], Katarina A. Jelic [2], Lidija D. Zolotarevski [2], Dragana A. Stankovic [3], Dubravko R. Bokonjic [1], Ivica M. Milosavljevic [2]

[1] *National Poison Control Center, Military Medical Academy Belgrade, Serbia and Montenegro;* [2] *Institute for Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia and Montenegro;* [3] *Department of Pathology, Clinical Hospital Centre, Zemun, Serbia and Montenegro*

The essential trace element, selenium, it can be potentially toxic. The toxicity depends on the chemical form of selenium. Sodium selenite is more toxic than sodium selenate. As an active ingredient sodium selenite is used as a novel rodenticide. It is absorbed through the gastrointestinal tract and accumulated in different tissue. Target organs are respiratory tract, CNS, cardiovascular system, gastrointestinal tract and skin. The aim of this study was to investigate pathohistological alterations in the heart after oral application of sodium selenite in rodents.

Adult Wistar rats and Swiss mice, of both sexes, were fed during 2 consecutive days with ready-to-use baits: 0.09% of sodium selenite. Animals were sacrificed on the days 1, 3, 5 and 7 of the study. Pathohistological alterations of the heart were evaluated in whole visual fields stained by haematoxylin and eosin (HE) method. The changes observed were scored by using tissue damage scoring scale (TDS): 0—normal structure, 1—mild damage, 2—moderate damage, 3—severe focal damage, 4—severe diffuse damage and 5—tissue necrosis. Statistical evaluation was performed using commercial statistical software (Stat for Windows, R.4.5, Stat Soft, Inc., USA, 1993). Comparation of data was done by one-way ANOVA + post hoc analysis (Tuckey's test). The differences with values of $p < 0.05$ were considered significant.

The pathological changes detected in all animals ranged from diffuse degeneration to a focal necrosis of myocytes and massive circulatory changes. Parenchymal degeneration, diffuse oedema and hyperemia were predominant in the heart of animals sacrificed on the day 3 of experiment. Dissolution of cytoplasm with nuclear pleomorphism in round or ovoid cardiomyocytes was seen. Thickening of the blood vessels with necrosis of endothelial cells were particularly prominent from the fifth to seventh day of study. The most interesting finding is the presence of massive, focal hemorrhages with polimorphonuclear cell infiltrations. The cytoplasm of majority myocardial cells was irregular, with fat transformation. Sodium selenite caused massive, diffuse degenerative and vascular changes associated with focal necrotic areas in male rats and mice on the day 7 of the study ($p < 0.05$).

Two-days oral ingestion of sodium selenite induced prominent pathohistological alterations in rodent heart, especially in male rats and mice.

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P14-06
Prenatal developmental toxicity of dibutyltin in cynomolgus monkeys given on consecutive three days during organogenesis

M. Ema [1], A. Arima [2], K. Fukunishi [2], M. Matsumoto [1], A. Hirose [1], E. Kamata [1], T. Ihara [2]

[1] *Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan;* [2] *Shin Nippon Biomedical Laboratories Ltd., Kagoshima 891-1394, Japan*

Dibutyltin dichloride (DBTCl) has been shown to be embryolethal and teratogenic in rodents. We previously reported that DBTCl at 2.5 and 3.8 mg/kg bw/day by nasogastric intubation during the entire period of organogenesis, days 20–50 of pregnancy, was embryolethal but not teratogenic in cynomolgus monkeys. The present study was conducted to further evaluate the developmental toxicity of DBTCl given to pregnant monkeys on consecutive three days during organogenesis. Cynomolgus monkeys were given DBTCl at 7.5 mg/kg bw/day by nasogastric intubation on days 19–21, days 24–26, days 29–31 or days 34–36 of pregnancy. The pregnancy outcome was determined on day 100 of pregnancy. No markedly maternal toxicity was observed in any group. Abortion on day 90 in one female given DBTCl on days 19–21, abortion on day 35 in one female and embryonic loss on day 35 in one female given DBTCl on days 24–26, no embryonic loss or abortion in females given DBTCl on days 29–31, and fetal death on day 90 in one female given DBTCl on days 34–36 were found. No striking changes in developmental parameters in sur-

viving fetuses, including fetal body weight, crown-rump length, tail length, or placental weight, were induced by DBTCl. No external, internal or skeletal malformations were detected in fetuses in any group. Although skeletal variations were found in fetuses, no increased incidence of fetuses with variation was noted in the DBTCl-treated groups. No effect on skeletal ossification was also observed in fetuses in the DBTCl-treated groups. These data confirmed our previous findings that DBTCl was embryolethal but not teratogenic in cynomolgus monkeys. Furthermore, the data show that the susceptibility to developmental toxicity of DBTCl varies with the developmental stages at the time of administration, and developing offspring on day 24–26 of pregnancy are highly susceptible to embryolethal effects of DBTCl in cynomolgus monkeys.

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P14-07

Analysis of toxic metals in Inorganic bovine bone for implant

N. Munguía Acevedo [1], L. Saldívar [1], C. Márquez [2], R. Palma [3], C. Piña [3]

[1] *Department of Analytical Chemistry, University of Mexico, Mexico, D.F. 04510, Mexico;* [2] *Department of Metallurgy, School of Chemistry, University of Mexico, Mexico, D.F. 04510, Mexico;* [3] *Materials Research Institute, University of Mexico, Mexico, D.F. 04510, Mexico*

An anorganic bone is a bone that has been taken out all its organic matter, it is used for surgical implants and it is denominated xenoimplant or xenogenic implant. Xenogenic implants are those obtained from individuals of different species from that who receives it. When an implant is required, all the conditions of biocompatibility must be fulfilled, which can be divided in both physical-chemical and medical-biological requirements.

In this research, the Ca/P relation and the elements Arsenic, Cadmium, Mercury and Lead were determined in treated bovine condyle bone by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Those elements are considerated highly toxic and must be measured in the inorganic bovine bone as part of the physical-chemical characteristics in order to prove that this kind of bone can be used as a xenoimplant.

Small pieces were sliced from bovine condyle bone and were subjected to cleaning with special soaps and heated, trying not to loose its mechanical properties. The samples obtained were decomposed by a microwave sample preparation system. In order to determine the Ca/P ratio and the trace amounts of Pb, Cd, As and Hg the ICP-OES technique was used. The detection and quantification limits as well as the % of recovery were determined.

In this case, the relation Ca/P value expected for the bone is $1.4 < Ca/P < 4.0$ and the obtained value was 2.08. To consider the bone an implant the trace concentrations must be lower than the next limits: 3 μg/mL for As, 5 μg/mL for Cd, 5 μg/mL for Hg and 30 μg/mL for Pb. The obtained values were 0.4 μg/mL, 2.7 μg/mL, 0.3 μg/mL and 26.4 μg/mL, respectively.

ICP-OES technique allows the multiple simultaneous analysis of Ca and P and the toxic elements with a very small uncertainty in the results. As, Cd, Hg and Pb concentrations were lower than the maximum values for anorganic bone for surgical implants established by the American Standard Testing Materials (ASTM). The relation Ca/P value is acceptable considering that the value of stoichiometric hydroxyapatite is 1.6. These results and the other biocompatibility tests of the anorganic bone, show that it can be used as a xenoimplant.

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P14-08

Genetic polymorphisms of arsenic (+3 oxidation state) methyltransferase (AS3MT) influences arsenic metabolism—Evidence from a population group in Argentina

Karin Maria Schläwicke, Karin Broberg, Gabriela Concha, Barbro Nermell, Margareta Warholm, Marie Vahter

Occupational and Environmental Medicine, Lund University, Sweden

The susceptibility to arsenic-induced diseases differs greatly between individuals. This may be due to inter-individual variation in arsenic metabolism, which affects the retention and distribution of toxic metabolites. Most likely, this variation is largely due to hereditary factors. To further elucidate the role of specific genetic factors in arsenic metabolism, we studied how polymorphisms in six arsenic-metabolizing genes affected arsenic metabolite pattern in urine of an indigenous population group in Argentina. This group had a fairly high arsenic exposure and low levels of monomethylated arsenic (MMA), of which the reduced form is one of the most toxic arsenic metabolites. The genes studied were arsenic (+3 oxidation state) methyltransferase (*AS3MT*), glutathione S-transferase omega 1 (*GSTO1*), methylene synthetase (*MTR*), methylenetetrahydrofolate reductase (*MTHFR*), glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*). We found that

one polymorphism; a G → A transition in position 35991 in *AS3MT* was associated with lower levels of MMA This polymorphism had a high frequency in this population group (allele frequency 76%), with 58% homozygous carriers. We did not find any associations between metabolite pattern and the other genes studied. This finding concludes that AS3MT plays an important role in arsenic metabolism, and polymorphisms in this gene may partly be responsible for the high inter-individual variation in arsenic metabolism and susceptibility.

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P14-09

Mercury concentrations in bluefin tunas (*Thunnus thynnus*)

Jelena Pompe Gotal, Emil Srebočan, Andreja Prevendar-Crnić

Veterinary Faculty, University of Zagreb, Zagreb, Croatia

Levels of total mercury in the muscle (29 samples) and liver tissue (15 samples) of bluefin tuna (*Thunnus thynnus*) weighing 100–300 kg were determined by cold vapor atomic absorption (AA) spectroscopy. Tunas were previously captured in the waters of Malta, towed to the farm in the Adriatic Sea and fattened defrosted herring and sardine for the period of 6–7 months. The purpose of the investigation was to determine the magnitude of mercury contamination and to ascertain whether the concentrations in muscle tissue exceeded the maximum level fixed by the European Commission Decision (1 μg/g wet wt). Total mercury concentrations in muscle tissue of tunas ranged from 0.49 to 1.809 (median 0.899 μg/g wet wt) while in liver tissue it was from 0.324 to 3.248 (median 1.165 μg/g wet wt). Total mercury concentrations in six samples of sardine ranged from 0.050 to 0.072 μg/g wet wt, while two samples of herring contained 0.020 and 0.053 μg/g wet wt. Twelve out of 29 (41%) muscle samples of tuna contain mercury above maximum level fixed by the European Commission Decision. It is generally believed that mercury levels in Mediterranean fish are higher than those of the other seas or oceans due to numerous deposits of mercury ores and metallic mercury in surrounding countries. We also have to keep in mind that tunas are highly mobile fish which swim for the whole life what enables them to enter or leave Mediterranean Sea or Atlantic Ocean.

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P14-10

Effect of selenium pre-treatment on cadmium content and enzymatic antioxidants in tissues of suckling rat

Maja Lazarus [1], Tatjana Orct [1], Maja Blanuša [1], Krista Kostial [1], Jasna Piršljin [2], Blanka Beer-Ljubić [2]

[1] *Institute for Medical Research and Occupational Health Zagreb Croatia;* [2] *Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

Neonates are a group of special concern regarding higher absorption and retention of cadmium (Cd) compared to adults. Selenium (Se) prevents the toxicity of Cd through still undefined mechanisms. Cadmium has indirect role in free radical generation. Studies have shown that free radical scavengers and antioxidants are useful in protecting against Cd toxicity. This protective effect has not yet been studied in the early postnatal period. The effect of Cd on the activity of two antioxidant enzymes, glutathione peroxidase (GSH-Px) and superoxid dismutase (SOD), was studied in the liver, kidney and brain of suckling Wistar rats. Also, the influence of Se on Cd body burden of sucklings was evaluated. Rats were orally exposed to $CdCl_2xH_2O$ and/or Na_2SeO_3 in equimolar amounts from day 6 to day 14 after birth. After 4 days of pre-treatment with Se, rats were concurrently treated for 5 days with Cd and Se (8 μmol/kg/day, each). Cadmium exposure had no effect on both SOD and GSH-Px activities in kidney and brain whereas in liver activities were increased. Selenium exposure increased both enzyme activities in all organs (except SOD in liver). Concurrent administration of Se and Cd elevated both enzyme activities in kidney and brain compared to Cd and control group up to the levels found in Se treated animals. The same treatment showed opposite action on tissue Cd content: Se significantly lowered Cd burden in the order liver > kidney > brain. It can be concluded that selenium had positive effect on reduction of Cd body burden as well as on enzyme activities in kidney and brain.

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P14-11

Effect of Thiomersal and mercuric chloride on mercury distribution in suckling rats

Tatjana Orct, Maja Lazarus, Maja Blanuša, Krista Kostial

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Thiomersal (ethylmercury) used as preservative in vaccines represents a new aspect in the field of mercury

toxicity. Given to infants during the vaccination period it might cause neurotoxic effect. Since data on the toxicokinetics of ethylmercury in the very young are scarce, we investigated the difference in distribution between two forms of mercury, ethylmercury and mercuric chloride in suckling rats.

In our experimental model organic and inorganic mercury were administered three times during the suckling period (on days 7, 9 and 11after birth) by subcutaneous injection and at a dose of 0.81 μmol Hg kg^{-1} body weight. At the end of the experiment 72 h after the last treatment, total mercury was analysed in blood and organs (liver, kidneys, brain) by the method amalgamation and atomic absorption spectrometry.

In the Thiomersal group as compared to the inorganic mercury group, concentrations of total mercury were higher in blood (23 times) and brain (1.5 times) and lower in the kidney (4 times) and liver (1.3 times).

Further studies on mercury kinetics of Thiomersal treated sucklings are in progress.

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P14-12
Heavy metal hazards of sachet water in Nigeria

I.O. Igwilo, Onyenmechi Johnson Afonne, Ejeatuluchukwu-alo Obi, Orish E. Orisakwe

Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria

Sachet water popularly known as "pure water" has become a booming business in Nigeria. In the view of the Nigerian government whose preoccupation is poverty eradication the sachet water industry (packaged ground water) is as a poverty alleviation industry for many Nigerians without jobs. Quality of drinking water depends on its original source and treatment. Chemicals in drinking water, which are toxic, may cause either acute or chronic health effects. The present study investigated the quality of different sachet water samples purchased from six cities in Eastern Nigeria namely Nnewi, Elele, Onitsha, Portharcourt, Owerri and Aba. Four samples namely "Christo, Carter, Delina glory, Neptune and Lippo were purchased from Nnewi, six samples namely Mevok, Davimor, Hejiks, Mifid, Mr. Ben and Hanek were purchased from Owerri. From Aba we bought the following samples, Evita, Cannan, Upright, Neulife, Quanta and Zonas while from Elele, Avalanche, Emgee, IDN Table water, Jeros Delight, Pilgrim and Triumph were purchased. The following samples were bought from Port Harcourt" Daisy, Saviour, Rillet, Solak, Ololo and Insight. Heavy metals—lead, cadmium, copper and nickel were analysed in all samples using Pye Unicam 969 Atomic Absorption Spectrophotometer with a detection limit of 0.001 mg/l. Other parameters analysed were nitrate, sulphate, chloride, salinity, total hardness (TH), biological oxygen demand (BOD), total dissolved solid (TDS) and pH. All samples were randomly selected and analysed in duplicates. The results show that the heavy metals were within the WHO maximum permissible levels in all the samples. However cadmium exceeded the WHO recommended level of 0.005 mg/l in the following samples "Delight, Saviour, Rillet, Solak, Mifid, Hejiks, Mr. Ben and Upright". The BOD of all the samples from Owerri ranged from 6.40 to 25.60 mg/l exceeded the permissible level. The sulphate, chloride, nitrate, salinity, TDS and pH were all within the WHO permissible levels. The present work suggest that the some sachet water sold in Nigeria may be contaminated as shown in the high BOD and cadmium levels that exceed the WHO permissible levels.

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P14-13
Assessment of two arsenic-contaminated drinking water mitigation interventions in Bangladesh

Jack Chakmeng Ng[1], Abul Hasnat Milton[2], Wayne Smith[2], Keith Dear[3], Bruce Caldwell[3], Malcolm Sim[4], Geetha Ranmuthugala[4], Kamalini M. Lokuge[2], Amjad Shraim[1], Shu-Huei Huang[1], Michael R. Moore[1]

[1] *The University of Queensland, Brisbane, Qld, Australia;* [2] *University of Newcastle, NSW, Australia;* [3] *Australian National University, ACT, Australia;* [4] *Monash University, Vic., Australia*

Arsenic is a carcinogen. In Bangladesh, there are over 10 million tube-wells of which about 50% have arsenic concentrations exceeding the WHO recommended guideline value of 10 μg/L for drinking water. This study aimed to evaluate the efficacy of two relatively inexpensive mitigation interventions, three-pitcher filters and dug-wells.

A randomised controlled field trial was conducted in Natore. Six Hundred and forty participants, 60 clusters of 47 villages were included in the trial. Two hundred and six participants were selected for the control group, 218 participants for the dug-wells, and 216 participants for the three-pitcher filters. The average arsenic in the drinking water was 128 μg/L in the three-pitcher trial. Twelve months post intervention, about 30% of the filtered water samples were >50 μg/L whereas dug-well water was <10 μg/L. Urinary arsenic speciation by HPLC-ICP-MS was utilised to assess the internal dose of

arsenic prior to and during the interventions. One month after the trial, urinary arsenic did not significantly differ between the three-pitcher group and the control group, but the dug-well group was slightly lower compared to the control group. By 12 months there were no significant differences between the treatment groups and the control group. Compliance in the dug-well group was about 20%, and dropped from an initial 84% to 20% in the three-pitcher group.

Low compliance was thought to be a major confounder. Our results raise a question about the social acceptability of these intervention technologies and their ultimate health benefit. Large scale watershed management program by utilizing the vast surface water that exists in Bangladesh is a future priority to deliver a better public health outcome.

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P14-14

Effect of copper(II)-curcumin complex and curcumin on cadmium-induced oxidative damage and essential elements status in mice

V. Eybl [1], D. Kotyzova [1], J. Koutensky [1], L. Leseticky [2]

[1] *Charles University in Prague, Faculty of Medicine in Pilsen, Czech Republic;* [2] *Charles University in Prague, Faculty of Nature Science in Prague, Czech Republic*

Cadmium (Cd) is a carcinogenic metal and serious environmental pollutant which toxic effects are associated with oxidative stress. Curcumin, a biologically active compound from turmeric, acts as a natural antioxidant and is considered to be a potent chemopreventive agent. A copper(II)-curcumin complex was synthesized as an alternative of curcumin with SOD mimicking activity. In this study the protective effect of copper(II)-complex of curcumin and curcumin was examined relating to lipid peroxidation, antioxidant defense system and trace element changes in cadmium intoxicated mice. Male CD mice were treated once daily with copper(II)-curcumin or curcumin for 3 days (25 mg/kg b.w., po, dispersed in methylcellulose). Another group served as control receiving vehiculum only. One hour after the last dose, cadmium chloride was administered (33 μmol/kg b.w., sc) to half of the animals from each group ($n = 10$). At 24th hour after Cd administration the lipid peroxidation (LP—expressed as malondialdehyde production), reduced glutathione level (GSH), catalase (CAT) and glutathione peroxidase (GPx) were estimated in liver homogenates. Cadmium and trace element concentrations were measured in the liver, kidney and brain tissue by AAS. Cd-induced increase in hepatic lipid peroxidation (122% of controls, $p < 0.05$) was significantly attenuated by both curcumin and Cu(II)-curcumin complex. The decrease of hepatic GSH level (to 82% of controls, $p < 0.01$) in Cd-intoxicated mice remained unaffected by antioxidants pretreatment. An increase of GSH level ($p < 0.05$) and GPx activity ($p < 0.01$) was measured in both curcumin and Cu(II)-curcumin only treated mice. The decrease in CAT activity (to 77% of controls, $p < 0.05$) was inhibited by curcumin only, even though Cu(II)-curcumin alone enhanced CAT activity compared to control animals (to 122%, $p < 0.05$). Cadmium distribution and Cd-induced trace element changes were not affected by curcumin or Cu(II)-curcumin pretreatment. In conclusion: regarding the studied effects in Cd-induced oxidative damage, curcumin and Cu(II)-curcumin were equally effective.

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P14-15

Comparative study of melatonin and curcumin effects on ferric nitrilotriacetate induced renal oxidative damage in rats

V. Eybl, D. Kotyzova, J. Koutensky

Charles University in Prague, Faculty of Medicine in Pilsen, Czech Republic

Ferric nitrilotriacetate (Fe-NTA), a known renal carcinogen, acts through the generation of oxidative stress. Several natural antioxidants, e.g. melatonin (ME) and curcumin (CUR) are able to counteract the effect of metal ions which participate in free radical generating reactions. Melatonin, an indole amine product of pineal gland, was referred to be a potent scavenger of free radicals. Curcumin, a major component of *Curcuma longa*, is considered to be a potent cancer chemopreventive agent. In our study the ability of these compounds to inhibit Fe-NTA induced oxidative stress and influence iron, copper and zinc content in the kidneys of rats was compared.

Experiments were performed in male rats (CD, Charles River, FRG) injected with a single dose of Fe-NTA (8 mg Fe/kg b.w., ip) alone and in combination with melatonin (10 mg/kg b.w., po) or curcumin (50 mg/kg b.w., po) administered 48 h, 24 h and 1 h before Fe-NTA and once daily for 3 days after Fe-NTA injection. The following groups of animals ($n = 9$-8 per group) were used: I control; II Fe-NTA; III Fe-NTA + ME; IV Fe-NTA + CUR. Twenty-four hours after the last dose of antioxidants the lipid peroxidation (LP—expressed

as malondialdehyde production) and GSH level were estimated in kidney homogenates and Fe, Zn and Cu concentration was measured in kidney tissue by AAS. The single injection of Fe-NTA caused a significant increase in LP ($p < 0.001$), GSH level ($p < 0.001$) and renal Fe content ($p < 0.001$) compared to control group. Both melatonin and curcumin treatment significantly reduced LP ($p < 0.01$). In Fe-NTA + ME treated group the decrease in LP was accompanied by a significant decrease of Fe and GSH level, while in Fe-NTA + CUR group the Fe concentration and GSH level remained uncorrected. Similarly the decreased zinc ($p < 0.01$) and copper level ($p < 0.05$) in the kidneys of Fe-NTA treated rats were corrected by melatonin and remained diminished in curcumin treated animals. Thus, the protective effect of melatonin and curcumin seems to exert different mechanisms of action. In the antioxidative effect of melatonin rather than in that of curcumin the interaction with iron (and possibly other metals) is probably more involved.

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P14-16
Preliminary investigation on high nickel release from coins

Michaela Zeiner [1], Iva Rezic [2], Ilse Steffan [1]

[1] *University of Vienna, Vienna, Austria;* [2] *Faculty of Textile Technology, University of Zagreb, Zagreb, Croatia*

The nickel presence in coins has been the subject of many debates. It is known that nickel can potentially contribute to allergenic reactions on human skin, and that this allergy affects more than 10% of women and several percent of men in industrialized countries. A high nickel release was confirmed for One and Two Euro coins. Their allergenic impact on humans was monitored and frequently described in recent investigations. In order to estimate the amounts of nickel and other allergenic metals present in Croatian coins being released during everyday manipulation, the preliminary skin test experiment presented here was performed. For this purpose the following coins have been chosen: 5, 10, 20 and 50 lipa, and 1 kuna coins. The compositions of the alloys used for these coins are as follows: 72.5% Cu and 27.5% Zn (5 and 10 lipa coins), 5% Ni and 95% Fe (20 and 50 lipa) and 65% Cu, 23.2% Ni and 11.8% Zn (1 kuna). Special attention was paid to the nickel containing coins.

The following experiment was performed for 45 h. This exposure time was chosen due to the EU "nickel directive" EN 1811 concerning objects designed to be in direct and prolonged contact with the skin. An exposure period of one week (minimum 40 h) was taken into account. During this period an experiment included exposure to the Croatian coins which were taped on the skin. After 45 h the coins were removed and the resulting marks were compared. The imprints of the nickel containing coins were visible, whereas the coins containing no nickel (5 and 10 lipa coins) did not cause any allergic reactions. The 20 lipa, 50 lipa and 1 kuna coins caused strong allergic reactions with erythema, infiltration and formation of vesicles.

This indicates that nickel release from Croatian coins is actually high. Therefore, its quantity should be determined in future. Such experiments should be guided according to the EN1811 tests which are relevant to the long-term salvation upon the contact with skin. They will be performed in order to estimate the real quantity of nickel being extracted from Croatian coins due to everyday manipulation.

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P14-17
Evaluation of apoptotic activity of mercury chloride by DNA diffusion assay

Vilena Kašuba, Davor Želježić, Ružica Rozgaj, Mirta Milić

Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia

Mercury, one of the most widely diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states, each of them with unique characteristics of target organ specificity. An important mechanism of mercury genotoxicity is its ability to produce free radicals that can cause DNA damage. In our study we tried to find out if those effects are capable of triggering the apoptotic pathway. We used a relatively new method in the detection of apoptosis–DNA diffusion assay. It is based on the diffusion of nucleosomal-sized DNA fragments into the agarose, which gives a hazy or undefined outline without any clear boundary to the apoptotic cell nuclei. The assay was performed on the human whole blood samples. Samples were incubated in 10, 50, 100 and 200 μM mercury chloride solution at 37 °C for 24 and 48 h. For each treatment protocol a thousand micrographs were analyzed. A significant increase in the apoptosis induction capacity of mercury chloride was observed already

after 24 h of treatment for all tested concentrations. However, no strong correlation between concentration and number of apoptotic micrographs was observed. The correlation was found after 48 h of treatment but only for doses of 50, 100 and 200 μM. Based on these results it could be suggested that apoptosis induction is not primary effect of mercury chloride on human lymphocytes. It also supports the theory of mercury's organ specificity.

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P14-18

Mercury chloride genotoxicity in human lymphocyte culture assessed by the alkaline comet assay

Mirta Milić, Ružica Rozgaj, Davor Želježić, Vilena Kašuba

Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia

Mercury is the most toxic of the heavy metals and exerts a variety of toxic effects in the body. Within the cell it can destroy the various components selectively or in total by releasing lysosomes, damaging DNA and by rupturing the cell membrane. Mercury from mercuric chloride binds to the sulfhydryl groups of the cell membrane and other proteins, causing increased membrane permeability and inhibition of ATPase-dependent transport. The genotoxicity of mercury chloride in this study was assessed by alkaline single-cell gel electrophoresis (the comet assay) in human peripheral blood lymphocytes. Four concentrations of metal salt dissolved in re-distilled water were used, 10, 50, 100 and 200 μM. Mitomicyn C (MMC) (0.5 μg/ml) was used as a positive control. An untreated control sample was also included in the experiment. Whole blood was exposed to $HgCl_2$ for 24 and 48 h at +37 °C in CO_2 incubator. All the samples were in duplicate. Three parameters were measured: tail length, tail moment and tail intensity. The data were statistically analyzed by one-way ANOVA followed by Tukey post hoc test. $P < 0.05$ was assumed significant. After 24 h of exposure to mercury chloride tail length values in samples treated with 50 and 100 μM of $HgCl_2$ were statistically different from the control sample. There were no significant differences neither in tail moment neither in tail intensity after 24 h of exposure. In 48 h of exposure statistically significant differences in tail length were found between the positive control and all other samples, and non-treated control sample and the highest mercury chloride concentration (200 μM). For the tail intensity and tail moment only the positive control sample was significantly different from all other samples.

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P14-19

Mercury chloride genotoxicity evaluated by micronucleus test in human lymphocyte culture

Ružica Rozgaj, Mirta Milić, Vilena Kašuba, Davor Želježić

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Mercury is naturally occurring in the biosphere, in addition, it is released into the environment by human activities, such as mining, combustion of fossil fuels and other industrial release. It is highly toxic element, which after inhalation or ingestion absorbs and deposits mainly in the kidney. The genotoxicity of mercury compounds have been investigated with a variety of genetic endpoints in prokaryotic and eukaryotic cells. Results point to an inhibition of DNA synthesis, DNA damage, inhibition of spindle microtubule assembly, reduction in the frequency of mitosis, endoreduplication, chromosomal damages.

This study was carried out to evaluate genotoxicity of mercury chloride in human lymphocytes using cytokinesis blocked micronucleus test. After 24 and 48 h incubation of whole blood samples with mercury chloride at concentrations of 10, 50, 100 and 200 μM, of, cultures were initiated and processed according standard protocol. Mitomycin C at concentration of 0.5 μg/ml served as positive control. One thousand binucleated cells per dose and per time were analysed. The results were analysed by chi-square. Our initial findings lead us to next conclusions: no significant differences between analysed samples were found after 24 h of exposure to mercury chloride; 48 h exposure resulted in increased frequency of micronuclei, but not in dose response way. This preliminary study was aimed to evaluate suitability of micronucleus test in mercury genotoxicity at applied doses. Further study with some improvements in design and more donors will provide more reliable results.

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P14-20

Determination of urinary arsenic and selenium among residents in Eastern Croatia

S. Ćavar [1], T. Klapec [2], M. Milić [3]

[1] *Institute of Public Health for the Osijek-Baraya County, Osijek, Croatia;* [2] *Faculty of Food Technology, Osijek, Croatia;* [3] *Department of Medical Biochemistry, Clinical Hospital Osijek, Osijek, Croatia*

Arsenic and selenium concentration in urine were investigated in two Croatian areas with different arsenic levels in drinking water. Total arsenic and selenium were determined in urine samples of adult participants (Osijek: $n = 16$, Andrijaševci: $n = 34$) using hydride generation atomic absorption spectrometry. Creatinine was determined in all urine samples with automated chemistry analyser using a colorimetric Jaffe rate method. The mean concentrations of arsenic, originating from natural geological sources, in community drinking water samples were 37.9 (Osijek) and 612 μg/L (Andrijaševci), respectively. The corresponding mean concentrations of total arsenic in first void urine samples of subjects residing in each of the localities were 28.2 and 653.7 μg/g creatinine. Mean value of total selenium in urine of residents from Osijek was higher (16.2 μg/g creatinine) than from Andrijaševci (9.9 μg/g creatinine). A positive correlation between arsenic and selenium urine concentrations was established ($r = 0.53$, $p < 0.01$). According to the results, seriously high chronic intake of arsenic in one of the localities is accompanied by lower selenium intake. Considering the ameliorating effects of selenium on arsenic toxicity, this might predispose the area residents to more adverse outcomes of excessive arsenic exposure.

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P14-21

Adaptogenic and toxicity evaluation of Sea buckthorn (*Hippophae rhamnoides*) leaf extract: A dose dependent study

Shalini Saggu, Harish Madan Divekar, Ramesh Chand Sawhney, Vanita Gupta, Pratul Kumar Banerjee, Ratan Kumar

Department of Biochemical Pharmacology, Defence Institute of Physiology and Allied Sciences (DIPAS), Lucknow Road, Timarpur, Delhi-110054, India

The effect of Sea buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae), leaf aqueous extract was examined in rats for its adaptogenic activity and toxicity. Dose dependent adaptogenic study of Sea buckthorn leaf aqueous extract was carried out at different doses administered orally, 30 min prior to cold (5 °C)-hypoxia (428 mmHg)-restraint (C-H-R) exposure. The least most effective adaptogenic dose of Sea buckthorn leaf aqueous extract was 100 mg/kg body weight. After sub-chronic (single oral dose of 1 and 2 g/kg once daily for 14 days) and chronic (single oral dose of 100 mg/kg once daily for 30 days) oral administration of the extract the biochemical and hematological parameters were studied in the serum and blood. No significant changes were observed in organ weight/body weight ratios of the vital organs studied (except liver and kidney in 1 and 2 g/kg body weight doses, respectively), and biochemical and hematological parameters of the sub-chronic and chronic drug treated animals in comparison to control rats. In acute toxicity study, LD50 of the extract was observed to be >10 g/kg when given orally. These results indicate that Sea buckthorn leaf aqueous extract possess potent adaptogenic activity with no toxicity even after chronic (30 days) administration of effective dose (100 mg/kg body weight) administration. Histological evaluation of major organs showed no toxicity after the administration of 100 mg/kg, 1 g/kg and 2 g/kg of the doses given in above mentioned time schedules.

Heavy metal toxicity of herbal preparations is a major concern. Concentrations of heavy metals (As, Pb, Hg, Cd, Zn, Cu, Cr) were measured by atomic absorption spectrometry in the aqueous extracts of Sea buckthorn leaf and compared with the maximum permissible values. The studied heavy metals concentrations in aqueous extracts of the plant, except chromium, were below the maximum permissible values. The chromium level in lyophilized aqueous extract of dried Sea buckthorn leaves was 3.0 mg/kg which is slightly higher than the WHO proposed limit of 2.0 mg/kg. The results suggested that the studied extract of Sea buckthorn is safe with reference to heavy metals and possess potent adaptogenic activity and could be used for nutraceuticals purposes.

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P14-22

Study on cattle's hair and blood lead content around Isfahan oil industry

Mehrdad Pourjafar, Raoof Rahnama, Faridreza Baharloo

University of Shahrekord Shahrekord, Charmahal&Bakhtiari, Iran

Lead is one of the heavy metals that can create poisoning and sickness in domesticated animals and human and it has various pollutant sources in industry.

This survey aimed at acquaintance the amount of dairy farms lead pollution around the Isfahan oil industry. At first, location of the dairy farms around Isfahan oil industry was identified. Then dairy farms that were in distant less than 20 km identified and divided in four groups (Group A: farms distance 1.5–2.5 km; Group B: farms distance 3.5–4.5 km; Group C: farms distance 6.5–7.5 km; Group D: farms distance 12.5–13.5 km). Control group was chosen from farms in one of the eastern Isfahan village (Group E). From 24 cows in each group blood and hair samples were collected. The lead content of samples was measured by Atomic Absorption. The lead content of hair samples were 10.40 ± 1.34 ppm, 9.21 ± 0.76 ppm, 7.90 ± 0.91 ppm, 5.93 ± 0.87 ppm and 1.96 ± 1.12 ppm, respectively, for A, B, C, D and E. The lead content of blood was 0.073 ± 0.0085 ppm, 0.065 ± 0.0077 ppm, 0.061 ± 0.0056 ppm, 0.042 ± 0.0087 ppm and 0.017 ± 0.0011 ppm, respectively, for A, B, C, D and E. The results showed that in the examined area, cow's blood lead was normal but lead content of hair had shown a subclinical lead poisoning. Between the lead content of hair and lead in all of groups a relation was found ($r = 0.926$).

These levels show that lead pollution around Isfahan oil industries can be a serious problem and must be considered.

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P14-23

Cyto and genotoxicity of natural uranium after acute or chronic exposures of normal rat kidney cells In favour of a cell transformation?

C. Thiebault, M. Carrière, S. Milgram, B. Gouget

Laboratoire Pierre Sue, CEA/CNRS UMR9956, Gif sur Yvette, France

Uranium (U) is a heavy metal used in the nuclear industry and for military applications. U compounds are toxic. Their toxicity is mediated either by their radioactivity or their chemical properties. After acute exposure, U is cytotoxic to kidney cells; however, little is known about the effect of chronic exposure and its associated genotoxicity.

In this study, cyto and genotoxicity of uranium after acute or chronic exposures has been looked for on NRK-52E renal cells. For acute exposures, cells were exposed to 0–1000 μM U-bicarbonate for 24 h. To evaluate the long-term effect of U, cells were continuously exposed to 0.1–100 μM U-bicarbonate for a few weeks.

A special emphasis was given in the research of cell death mechanism and the ratio between apoptosis and necrosis. Apoptosis induction as a function of exposure time or concentration was investigated using the DNA fragmentation method and the caspase-3 enzymatic assay. In order to distinguish between the intrinsic and the extrinsic pathways of apoptosis, caspases-8, 9, 10 assays were conducted and the mitochondrial membrane potential was measured.

In parallel, DNA damages were evaluated. Two methods were selected for their complementarities in the detection of genetic lesions. The Comet assay was used for the detection of primary lesions of DNA (single or double strand breaks for instance). The micronuclei assay permitted to detect chromosomic breaks or losses.

After acute exposure, results show that DNA damages and apoptosis increase in a dose-dependant manner: U seems to induce apoptosis by the intrinsic pathway. Inversely, DNA damages decreased in cells exposed to U for long periods. Interestingly, after chronic intoxication, cells acquired resistance to uranium. Indeed, the cytotoxicity index (CI_{50}), defined as U concentration leading to 50% cell death after 24 h of exposure, was higher in cells previously exposed to 100 μM U than in control cells (550 μM compared to 450 μM). These results suggest that U is genotoxic and that environmental exposure to low doses for long periods could lead to cell transformation.

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P14-24

Speciation governs chemical toxicity and cellular accumulation of lead on rat osteoblastic bone cells

Sarah Milgram [1], Céline Thiebault [1], Marie Carriere [1], Luc Malaval [2], Barbara Gouget [1]

[1] *Laboratoire Pierre Süe CEA-CNRS UMR9956, Gif-sur-Yvette, France;* [2] *INSERM E366/Laboratoire de Biologie du Tissu Osseux, St Etienne, France*

Lead (Pb), as other heavy metals, presents a strong chemical toxicity. After blood contamination, Pb complexed with proteins or inorganic molecules is conveyed to target organs. Skeleton is the main organ for Pb long-term fixation. Once in bones, Pb is incorporated in the matrix of hydroxyapatite by substitution with calcium and it can be released during osseous remodelling, explaining in part its toxicity.

The purpose of this study was to investigate the biological effects of Pb acute exposure on osteoblastic bone cells. A special emphasis was given to the influence of Pb speciation during exposure. ROS17/2.8 rat

osteoblast-like cells were exposed to controlled speciations of Pb. The six most relevant Pb speciations, namely the most probable forms of the toxic in contact with cells after blood contamination, were selected for cell exposures. Pb was either complexed with phosphate ($PbHPO_4$), bicarbonate ($PbCO_3$), citrate (PbCIT), cysteine (PbCYST), albumin (PbAlbumin) or left free in the exposure medium (Pb^{*}). For each chemical state, Pb toxicity was assessed using the MTT assay. Results show that $PbHCO_3$, Pb^{*} and PbCIT induce a significant toxicity to bone cells with the cytotoxicity index CI_{50} defined as Pb concentration leading to 50% cell death after 24 h exposure determined, respectively, at 25, 100 and 130 μM. A concentration-dependant cytotoxicity was shown after 24 h exposure to PbCYST, however its toxicity never exceeded 50% cell death. On the opposite, $PbHPO_4$ or PbAlbumin did not demonstrate toxicity to ROS17/2.8 osteoblast-like cells.

In order to explain this difference of sensitivity between Pb species, cellular accumulations were quantified according to concentration, then to time, at lethal or sub-lethal doses (ICP-MS analyses of digested cell pellets). A correlation between toxicity and cellular accumulation could be evidenced. Finally, Pb repartition at the cell scale was characterized by SEM-EDS and μPIXE. Precipitation phenomena could be observed inside and outside cells. These results stress the importance of a strictly controlled speciation of the metals in toxicology studies.

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P14-25

Effect of magnesium pretreatment on glutathione levels in kidney and liver of mice exposed to acute cadmium intoxication

Danijela Djukić-Ćosić [1], Milica Ninković [2], Živorad Maličević [2], Vesna Matović [1]

[1] *Institute of Toxicological Chemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia and Montenegro;* [2] *Institute of Medical Research, Military Medical Academy, Belgrade, Serbia and Montenegro*

Among numerous mechanisms proposed for Cd toxicity are oxidative stress and disturbances in metabolism and function of bioelements. Up-to-date investigations indicate that cadmium can decrease intracellular glutathione content and activities of cellular antioxidant enzymes, which lead to the accumulation of reactive oxygen species and an increase in intracellular oxidative stress.

It is well known that GSH plays a crucial role in intracellular protection against metals, e.g. Cd. Glutathione can act both as an antioxidant (scavenger of intracellular reactive oxygen species by a direct reaction, or via the GSH peroxidase/GSH system) and as a metal-chelating agent. On the other hand, investigations on interaction between Cd and bioelements indicate that excessive intake of bioelements, particularly magnesium, may antagonize cadmium effects.

The objective of the study was to determine the effect of increased oral magnesium pretreatment on reduced GSH levels in kidney and liver of mice exposed to acute Cd intoxication. Swiss albino male mice were divided into four groups: I—control group, not treated animals; II—Cd group, animals given single oral dose of 20 mg Cd/kg b.w. as aqueous solution of $CdCl_2$; III—Mg + Cd group, i.e. mice given orally 40 Mg/kg b.w. as aqueous solution of $Mg(CH_3COO)_2$ 1 h before Cd intoxication; IV–Mg group, animals given orally 40 Mg/kg b.w. as aqueous solution of $Mg(CH_3COO)_2$. The animals were sacrificed by decapitation at 4, 6, 12, 24 and 48 h and GSH content was determined in investigated organs.

The obtained results show that acute Cd intoxication induced significantly increased GSH content in kidney after 12 and 24 h. On the other hand, statistically significant decrease of GSH content was observed in liver 4, 6 and 12 h after single oral Cd administration. Beneficial effects of Mg pretreatment on GSH content was observed partly; Mg pretreatment reduced the observed changes of GSH content in liver after 6 and 12 h and in kidney after 12 h.

These results contribute to our investigations on interaction between cadmium and magnesium.

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P14-26

URANIUM (VI) toxicity after acute exposure of cultured renal cells: Citrate increases bioavailability and toxicity

M. Carrière, C. Thiebault, S. Milgram, B. Gouget

Laboratoire Pierre Süe, CEA-CNRS UMR 9956, CEA/Saclay, 91191 Gif-sur-Yvette, France

Uranium is a natural heavy metal, widely present in the environment. Its use in the nuclear industry and for military applications has raised concerns about its potential toxicity for populations and environment. Contamination can occur by ingestion of contaminated food or water, inhalation or through a wound. The metal rapidly transfers to target organs: kidneys, particularly proximal

tubular epithelium, and bones where it is stocked for longer periods.

In vitro, its effects are studied on renal and osteoblastic cell lines. As a function of the protocol of preparation, U(VI) is known to cause cell mortality or not. The differences in toxicity are assumed to come from differences in speciation, which are known to regulate bioavailability and subsequently toxicity.

In biological fluids (blood, glomerular filtrate, in vitro cell culture media), speciation modelling shows that U(VI) is mainly distributed between carbonate and citrate complexes, the proportion of these complexes depending on bicarbonate and citrate concentrations but also on Ca^{2+} concentration and the pH of the solution. Experiments made with media prepared to give various U(VI)-bicarbonate and U(VI)-citrate proportions allowed to draw a parallel between the presence of one U(VI) chemical form and the occurrence of toxicity. With such experiments, it is possible to identify U(VI) complexes responsible for toxicity and inversely complexes trapping U(VI) in a non-bioavailable chemical form. X-ray absorption spectroscopy (XAS) analyses of cells after U(VI) accumulation at a sub-lethal dose permits to propose hypotheses concerning U(VI) metabolization by cells and/or speciation modification within cell exposure media. These results are another step towards the comprehension of U(VI) biovailability in animal cultured cells.

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P14-27

Glutathione-dependent transport of heavy metal compounds by multidrug resistance proteins MRP1 and MRP2

Nicole H.P. Cnubben [1], Michiel G.J. Balvers [1], Mustafa Usta [1], Jelmer J. van Zanden [2], Peter J. van Bladeren [3], Ivonne M.C.M. Rietjens [2], Heleen M. Wortelboer [1]

[1] *TNO Quality of Life, P.O. Box 360, 3700 AJ Zeist, The Netherlands;* [2] *Wageningen University, P.O. Box 8000, 6700 EA, Wageningen, The Netherlands;* [3] *Nestlé Research Centre, P.O. Box 44, CH-1000 Lausanne 26, Switzerland*

The membrane-embedded multidrug resistance-associated protein 1 (MRP1) and MRRP2 are members of the ATP-Binding Cassette (ABC) transporter protein superfamily and mediate the unidirectional cell efflux of many xenobiotics in which process glutathione (GSH) can play a different role. Heavy metal compounds have a high affinity for GSH and as such can be transported by MRP1 and MRP2 either as the parent compound, and/or as the parent compound with co-transport of GSH and/or as its formed GS-adduct. In addition, heavy metal compounds might interact with MRP leading to inhibition of its transport activity without being transported (inhibition). In the present study, the GSH-dependent transport of three heavy metal compounds, i.e. cisplatin, mercury dichloride ($HgCl_2$) and arsenic trioxide (As_2O_3) by MRP1 and MRP2 was investigated. For this purpose, MRP1- and MRP2-transfected Madin Darby canine kidney cells (MDCKII) were exposed to non-cytotoxic levels of the heavy metal compound and the effect on intracellular GSH levels and directional GSH efflux was determined. Possible inhibition of MRP1 and MRP2 by the heavy metal compound was studied by following the transport of the model substrate calcein. Finally, the MRP-associated ATPase activity was studied in isolated *Sf*9-MRP1 or *Sf*9-MRP2 membrane vesicles to study whether the metal compound is transported via a GSH co-transport mechanism or as GS-adduct. The results show that all three heavy metal compounds cisplatin, mercury dichloride ($HgCl_2$) and arsenic trioxide (As_2O_3) interact with MRP1 and MRP2 transporter proteins and GSH, although through different mechanisms.

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P14-28

Factors influencing the metabolite pattern of urinary arsenic following exposure via drinking water

Anna-Lena Lindberg [1], Walter Goessler [2], Giovanni Leonardi [3], Mahfuzar Rahman [4], Lars-Åke Person [5], Eva-Charlotte Ekström [5], Rajiv Kumar [6], Marie Vahter [7]

[1] *Karolinska Institutet Stockholm Sweden;* [2] *University Graz, Graz, Austria;* [3] *London School of Hygiene and Tropical Medicine, London, UK;* [4] *ICDDR,B: Centre for Health and Population Research;* [5] *Uppsala Universtiy, Uppsala, Sweden;* [6] *German Cancer Research Center, Germany;* [7] *Karolinska Institutet, Stockholm, Sweden*

The metabolism of inorganic arsenic (iAs) varies considerably between populations and individuals, which may explain observed variations in health effects. The metabolism of iAs involves alternating reduction and oxidative methylation. In people exposed to iAs, e.g. via drinking water, the urinary excretion consists mainly of arsenite [As(III)], arsenate [As(V)], methylarsonate [MA(V)] and dimethylarsinate [DMA(V)]. The trivalent intermediate methylarsonite [MA(III)] is highly reactive

and toxic and unlikely to appear in urine. MA(V) in urine seems to reflect the formation of the toxic MA(III) in the body. Recent studies indicate increasing prevalence of arsenic-related health effects with increasing relative amount of MA in urine. Therefore, it is essential to evaluate factors influencing the metabolism of urinary arsenic. We have compared arsenic metabolism and influencing factors between two large case-control studies (only controls) of risk for cancer and skin lesions in relation to arsenic exposure via drinking water in Central Europe (ASHRAM study) and Bangladesh (AsMat study), respectively. Individual water and urine samples were collected in both studies for evaluation of current arsenic exposure and arsenic metabolites. There were large differences in both water and urine concentrations between the ASHRAM study (mean (S.D.): 9.8 (13.5) and 15.2 (18.2) μg/L, respectively) and the AsMat study (mean (S.D.): 113 (169) and 100 (132) μg/L, respectively). Although the individuals in the AsMat study have higher exposure and poorer nutrition than individuals in the ASHRAM study, they still have better methylation capacity (mean %MA (S.D.); 10.4 (4.6) and 17.0 (8.1)%, respectively). The main factor influencing the metabolism was exposure level. Since the exposure was very low in the ASHRAM study, factors other than exposure could be evaluated in relation to arsenic methylation, i.e. age, gender, BMI, selenium status and genetic polymorphisms of three candidate genes for involvement in the methylation of arsenic, Cyt 19, MTHFR and GSTO1. In the AsMat study, where exposure was much higher and age span was larger (5–88 years), the role of age, gender and exposure level could be evaluated in more detail, as well as the role of arsenic metabolism in the induction of skin lesions.

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P14-29

Does micronutrient status influence the uptake and accumulation of the toxic metal cadmium?

Maria Kippler[1], Eva-Charlotte Ekström[2], Anisur Rahman[1,3], Bo Lönnerdal[4], Shams El Arifeen[3], Lars-Åke Persson[2], Marie Vahter[1]

[1] *Karolinska Institutet, Stockholm, Sweden;* [2] *Uppsala University, Uppsala, Sweden;* [3] *ICDDR,B: Centre of Health and Population Research;* [4] *University of California, Davis, USA*

Cadmium (Cd) is a widespread environmental contaminant that has toxic effects on kidneys and bone, possibly also neurodevelopment, even at environmental exposure levels. Absorbed Cd has a long half-time, in the order of decades, especially in the kidneys. People are exposed to Cd mainly via food. Cereals and vegetables, particularly rice contributes significantly since Cd is easily taken up from soil. In Bangladesh, heavy doses of phosphate fertilizers have been applied over the last 50 years, and Cd is a common impurity in low-price phosphate fertilizers. Rice is eaten several times a day in Bangladesh and therefore, the exposure to Cd via rice may be high. In general, women have higher Cd body burden than men. This is probably due to increased absorption at low iron (Fe) stores that is common among women in childbearing age. There are also studies suggesting that Zn may be involved in the uptake and accumulation of Cd. We are presently investigating to what extent Fe and Zn deficiencies increase uptake and accumulation of Cd in pregnant women. The study is nested into the Maternal and Infant Nutrition Intervention in Matlab (MINIMat), a food and micronutrient supplementation trial in Matlab, Bangladesh. In this trial, 4500 women were enrolled in early pregnancy. Urinary Cd (U-Cd), reflecting life-long exposure, was measured in early pregnancy (gestational week 6–8) in 889 randomly selected women. Blood samples for measurements of serum ferritin (s-Ft) and serum zinc (s-Zn) were collected in gestational week 14 before micronutrient supplementation was started. Our preliminary results show that the women in Matlab have 0.6 μg Cd/L (median) in urine in early pregnancy, which is three to four times higher than in Swedish women of similar age. U-Cd was negatively associated with s-Ft levels. No significant direct association was found between U-Cd and s-Zn. However, Zn seems to influence the Cd–Fe interaction, as the effect of low s-Ft on U-Cd was apparent only at adequate Zn levels. Further studies on the effects of Zn status on Cd in blood are underway.

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P14-30

Urinary arsenic profile in residents of blackfoot disease-endemic area in Taiwan: Role of GSTO1 and GSTO2 polymorphisms

Wan-Fen Li, Chien-Wen Sun, Kuang-Hsi Chang, Shu-Li Wang

Division of Environmental Health and Occupational Medicine, National Health Research Institutes, Zhunan, Taiwan

Globally, millions of persons are at risk for the adverse effects of arsenic due to exposure to inorganic arsenic from drinking water. Humans metabolize and eliminate arsenic through oxidative methylation and subsequent urinary excretion. Within a given population, individuals differ in the quantity and the distribution of the various metabolites (i.e. As3+, As5+, monomethyl arsenic (MMA), dimethyl arsenic (DMA)) excreted in urine. This variation may be affected by many factors, but the existence of genetic determinants has been strongly suggested. The purpose of this project is to study the genetic basis of variability in human metabolism of arsenic. We followed up two established cohorts: one is composed of long-term residents from the black foot disease-endemic area of southwestern Taiwan and the other from a nearby area where the arsenic exposure level is considered low. We analyzed the distribution in urine of total arsenic and arsenic metabolites and found substantial differences between two cohorts. Compared with the control group, the cohort from the endemic area had higher percentage of inorganic arsenic and MMA, but lower percentage of DMA in urine. We also analyzed the association between arsenic metabolism and polymorphisms of two human glutathione transferase omega genes (GSTO1 and GSTO2), which catalyze the reduction of MMA(V), a rate-limiting reaction in the biotransformation of inorganic arsenic. No significant association was found between the GSTO1 A140D polymorphism and urinary arsenic profile, while the GSTO2 N142D polymorphism appeared to be related to elevated ratio of DMA to MMA.

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P14-31

Does metabolism of arsenic affect the toxicity during early human development?

Marie Vahter [1], Li Li [1], Anisur Rahman [2], Barbro Nermell [1], Britta Fängström [1], Eva-Charlotte Ekström [3], Shams El Arifeen [2], Lars-Åke Persson [3]

[1] *Karolinska Institutet, Stockholm, Sweden;* [2] *ICDDR,B: Centre of Health and Population Research, Dhakka;* [3] *Uppsala University, Uppsala, Sweden*

Millions of people world-wide are exposed to inorganic arsenic, a potent human carcinogen and toxicant, through drinking water (ground water), especially in many low-income countries with low availability of safe surface water. Although many severe health effects of arsenic, including various forms of cancer, are well documented, little is known about effects on fetal and infant health. Our ongoing studies are evaluating the health effect of arsenic on pregnancy outcomes and child health in Matlab, Bangladesh. The present sub-study, nested into a food and supplementation trial (the MINIMat trial), aims at elucidating the mechanisms behind the variation in arsenic metabolism (reduction and methylation reactions) and its role in the health risks for fetuses and children. We observed a high prevalence of elevated arsenic concentrations in the tube-well water, about 70% exceeding the WHO guideline value of 10 μg/L. The urinary arsenic concentrations in pregnancy ranged from 1 to 1500 μg/L, with an overall median about 80 μg/L. There was a good correlation with water arsenic concentrations, with an intercept of 20 μg/L, indicating exposure via food probably due to the prevalent use of tube-well water for irrigation of rice fields. The pattern of arsenic metabolites excreted in urine varied markedly. The 10th and 90th percentiles of the inorganic arsenic were 7% and 24%; those of methylarsenic acid (MMA), 5% and 17%; and those of dimethylarsinic acid (DMA) 62% and 85%, respectively. Most likely, a high first methylation step in combination with a low second methylation step is the most critical from a toxicological point of view. Arsenic methylation increased during pregnancy, but, contrary to the hypothesis, it was little affected by malnutrition. The methylation, in particular the second methylation step from MMA to DMA, decreased significantly at elevated exposure levels, probably due to inhibition of the involved methyltransferases and reductases. Possibly, also other methyltransferases, e.g. those involved in DNA methylation are affected by arsenic.

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P14-32

Toxic effects of Cr(VI) in liver after administration in the drinking water to Wistar rats

A. Rafael[1], A. Almeida[2], I. Parreira[3], P. Santos[2], M. Carvalho[1], A.M. Cabrita[1], M.C. Alpoim[3]

[1] *Faculdade de Medicina da Universidade de Coimbra, Portugal;* [2] *Centro de Histocompatibilidade do Centro HUC, Coimbra, Portugal;* [3] *Departamento de Bioquímica da FCT Universidade de Coimbra, Portugal*

Chromium is a lustrous, brittle, hard metal. The main human activities that increase Cr(VI) concentrations are chemical, leather and textile manufacturing, electro painting, and is used to manufacture magnetic tape, and often used as pigments for photography. These applications will mainly increase concentrations of chromium in water. Through coal combustion chromium will also end up in air and through waste disposal chromium will end up in soils. Cr(VI) is a danger to human health. Cr(VI) compounds can be toxic if orally ingested or inhaled. Apoptosis is a selective process for deletion of cells in various biological systems. This event is tightly regulated with processes playing essential roles in the homeostasis of renewable tissues. Diverse groups of molecules are involved in the apoptosis pathway. One set of mediators implicated in apoptosis belong to the asparate-specific cysteinyl proteases or caspases. Caspase-8 is supposed to be the top of the death-mediated apoptosis pathway, whereas caspase-3 belongs to the "effector" proteases in the apoptosis cascade. The aim of this study was to evaluate the effects of Cr(VI) on the liver. Expression of caspases 3 and 8 in liver rats was analyzed by the reverse transcriptase-polymerase chain reaction (RT-PCR).

Ten male Wistar rats aged 4 months were divided in two groups. One group was the group control, the other one was submitted of Cr(VI) in the drinking water in a concentration of 20 mg/ml. Food and water were at libitum. After 8 weeks, the animals were euthanized. Liver was collected, weighed and divided in two, half was fixed by immersion in 10% buffered formalin and embedded in paraffin, the other half for total RNA extraction by RT-PCR.

The results demonstrated that technique RT-PCR is sensitive enough to detect caspases 3 and 8 mRNAs and that caspase 3 and 8 participate in the apoptotic process induced by Cr(VI) in rats.

We could observe that Cr(VI) induced liver toxic effects because there is an increase of the expression of the mRNA of caspase 3 and 8.

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P14-33

Toxic effects on Wistar rat kidney of lead administrated in water

A. Rafael[1], A. Almeida[2], I. Parreira[3], P. Santos[2], M. Carvalho[1], R. Alves[4], A.M. Cabrita[1], M.C. Alpoim[3]

[1] *Faculdade de Medicina da Universidade de Coimbra, Portugal;* [2] *Centro de Histocompatibilidade do Centro HUC, Coimbra, Portugal;* [3] *Departamento de Bioquímica da FCT Universidade de Coimbra, Portugal;* [4] *Serviço de Nefrologia dos Hospitais da Universidade de Coimbra, Portugal*

Lead occurs as expected in the environment. Nevertheless, most lead concentrations that are found in the ecosystem are a result of human performance. Lead is used in building construction, lead-acid batteries, bullets and shot, and is part of solder, pewter, and fusible alloys, in car engines, such as fuel combustion, industrial processes and solid waste combustion. Lead can end up in water and soils through corrosion of leaded pipelines in a water transporting system and through corrosion of leaded paints. It cannot be broken down, it can only be converted to other forms. These will experience health effects from lead poisoning, lead is a particularly dangerous chemical, as it can accumulate in individual organisms. Apoptosis is an important cellular response that is induced by different stimuli among them lead chronic intoxication. The cell death process is irreparable following the activation of cytoplasmic cysteine proteases (caspases). The caspases are responsible for the degradation of cellular structural and repair proteins. The aim of this study was to evaluate the effects of lead chronic intoxication on rat Wistar kidney.

Ten male Wistar rats aged 4 months were divided in two groups. One group was the group control, the other one was submitted of Pb(II) in the drinking water in a concentration of 20 mg/ml. Food and water were at libitum. After 4 weeks, the animals were euthanized. Kidney was collected, weighed and divided a sample was fixed by immersion in 10% buffered formalin and embedded in paraffin and the other half for total RNA extraction.

The results demonstrated that technique RT-PCR is sensitive enough to detect caspases 3 and 8 mRNAs and

that caspase 3 and 8 participate in the apoptotic process induced by Pb(II) in rats. Histological results demonstrate a discrete fibrosis and necrosis.

We could observe that Pb(II) is toxic to kidney because there is an increase in the expression of the mRNA of caspase 3 and 8.

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P14-34

Determinants of blood lead levels in children and adults living in a former mining area in Brazil

Monica Paoliello [1], Eduardo M. De Capitani [2], Alice M. Sakuma [3], Fernanda G. Cunha [4], Bernardino R. Figueiredo [5], Maria F. Carvalho [3], Tiemi Matsuo [1], Fernanda DalMolin [1]

[1] *Universidade Estadual de Londrina Londrina, Parana Brazil;* [2] *Centro de Controle de Intoxicações, Universidade Estadual de Campinas, Brazil;* [3] *Instituto Adolfo Lutz, Sao Paolo, Brasil;* [4] *Companhia de Pesquisa em Recursos Minerais, Rio de Janeiro, Brasil;* [5] *Instituto de Geociências, Universidade Estadual de Campinas, Brazil*

The Ribeira river valley is located in the far south of the state of São Paulo and the east side of the state of Parana, Brazil. During the last 50 years, it had been under the influence of the full activity of a huge lead refinery and mining along the riverside. The plant completely stopped all kind of industrial activities at the end of 1995, and part of the worker population and their families still remain living nearby in small communities. The objective of the study was to assess the determinants of blood lead levels in those mining areas, where residual environmental contamination from the past industrial activity still remains. Blood samples of 295 children aged 7–14 years and 350 adults aged between 17 and 70 years old, were collected. A standardized questionnaire was applied, aiming to get information on food consumption habits, leisure activities, current and former occupation (adults), current and former occupation of parents (children), current and former dwelling place and conditions, and other issues. The blood lead concentrations were determined by graphite furnace atomic absorption spectrophotometry with Zeeman background correction (model SIMAA 6000, Perkin-Elmer). The samples were diluted 1:10 with 1% Triton X-100 in 0.1% nitric acid, and a mixture of ammonium dihydrogen phosphate and magnesium nitrate was used as chemical modifier. Logistic regression models were used to correlate some independent variables to blood lead levels and to assess the specific effect of each adjusted variable by the others. The dependent variable was blood lead level, categorized as 14 μg/dl or greater and lesser for adults and 10 μg/dl or greater and lesser for children. For adults, the following variables showed significant association with high blood lead levels: residential area close to lead refinery [odds ratio (OR) = 7.27 (95% confidence interval (CI) = 2.61–20.24)], former dwelling at the refinery village [OR = 5.43 (95% CI = 1.89–15.60)], male gender [OR = 18.35 (95% CI = 5.41–62.35)], smoking habits [OR = 4.24 (95% CI = 1.44–12.49)], and consumption of fruits from home backyard [OR = 3.63 (95% CI = 1.32–9.98)]. For children, the variables were: residential area close to the lead refinery [OR = 10.38 (95% CI = 4.86–23.25)], former father's occupational lead exposure [OR = 4.07 (95% CI = 1.82–9.24)], and male gender [OR = 2.60 (95% CI = 1.24–5.62)].

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P15 Toxicology *in vitro*

P15-01

Protective effect of vitamin C towards *N*-nitrosamine-induced DNA damage in the single-cell gel electrophoresis (SCGE)/HepG2 assay

Nuria Arranz, Ana I. Haza, Almudena Garcia, Paloma Morales

Facultad de Veterinaria, Universidad Complutense, Madrid, Spain

The aim of this study was to investigate the protective effect of vitamin C towards *N*-nitrosamine-induced DNA damage in the single-cell gel electrophoresis (SCGE)/HepG2 assay. To determinate the role of oxidative DNA damage in *N*-nitrosamines genotoxicity, we employed formamidopyrimidine DNA glycosylase (Fpg). None of the vitamin C concentrations tested (1–10 μM) in presence or absence of Fpg enzyme caused DNA damage per se. Combined treatment of HepG2 cells with vitamin C and *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodibutylamine (NDBA) or *N*-nitrosopiperidine (NPIP) reduced the genotoxic effects of the *N*-nitrosamines in a dose-dependent manner. The protective effect of vitamin C was higher towards NPYR-induced oxidative DNA damage than against NDMA, NDBA and NPIP. Vitamin C (10 μM) reduced the NPYR-induced oxidative DNA damage in 73–81% in absence or presence of Fpg enzyme, respectively.

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P15-02

Lithium and Wnt/β-catenin pathway: Effect on cell proliferation

V. Moronvalle-Halley, E. Boitier, G. Labbe

Sanofi-Aventis, Drug Safety Evaluation, Paris Research Center, France

Lithium (Li) has been used for 50 years for the treatment of manic depression and it has been shown to be a reversible inhibitor of glycogen synthase kinase-3 β (GSK3-β). GSK3-β is now known to be a key component of the Wnt/β-catenin signaling pathway, clearly associated with cell proliferation. We studied whether Li had an impact on the Wnt/β-catenin pathway by measuring the accumulation of the cytosolic β-catenin protein and cyclin D1 and c-myc gene expression in rat ileum cancer IEC-18 cell line. Cell proliferation was evaluated in vitro in primary culture of rat hepatocytes after Li treatment and in vivo after oral administration of 100 mg/kg and 200 mg/kg of Li to male Sprague-Dawley rats euthanatized 6 h or 24 h after acute treatment or 5 days after repeated treatment.

Li induced an increase in cytosolic β-catenin content from 3 mM in IEC-18 cells after a 24-h treatment which was not associated with any increases in cyclin D1 and c-myc gene expression. From a dose of 3 mM Li, a mitogenic effect was observed after 24 h of treatment in rat hepatocytes and was in agreement with the cytosolic accumulation of β-catenin observed in IEC-18 cells. However, after Li treatment in rat, neither an increase of mitogenic index in the five organs studied (liver, kidney, adrenals, pancreas and colon), nor changes in expression of cell proliferation genes evaluated by Toxicogenomics in liver were observed. The apparent discrepancy between the in vitro and the in vivo results may be due to the low plasmatic Li level in rats (<2 mM), as doses above 200 mg/kg/day Li were lethal. As a conclusion, we demonstrated that the GSK3-β inhibitor Li has a clear impact on β-catenin stabilization which occurs via a Wnt-dependent mechanism and results in cellular growth in in vitro models.

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P15-03

Role of the in vitro hematopoietic cultures in the European Project A-Cute-Tox

Laura Cerrato, Susana G. Gomez, Juan A. Bueren, Beatriz Albella

CIEMAT, Madrid, Spain

The European Integrated Project "Optimisation and prevalidation of an in vitro test strategy for predicting human acute toxicity" (A-Cute-Tox, LSHB-CT-2004-512051) was started up in January 2005. As a partner, our group is responsible for the characterization of the effect of xenobiotics on the hematopoietic tissue, included in WP4 (new cell systems, new endpoints).

Hematopoietic system displays a wide spectrum of cell populations whose constant proliferation and differentiation give rise to the different lineages of blood cells. The objectives of toxicology studies are the identification of potentially dangerous toxicants, so that human exposure can be prevented or controlled, and the provision of information relevant for undertaking risk–benefit analyses and for conducting clinical trials. Toxicants can cause hematotoxicity by interfering with mature blood cells, committed progenitors or stem cells functions or survival. Following cytotoxic insult (e.g. chemotherapy), neutropenia and trombocytopenia are often effects which make the hematopoietic system to be the limiting factor. CFU-GM and CFU-Meg assays will be used in A-Cute-Tox project for characterizing the toxicity on myeloid and megakaryocytic progenitors.

Up to now, CFU-GM assays, using human mononuclear cord blood cells, have been performed to characterized the effects of some of the selected compounds on the myeloid progenitors. Recently, this assay has been approved by ECVAM's Scientific Advisory Committee (ESAC). It is based on the semisolid in vitro culture of mononuclear hematopoietic cells in the presence of GM-CSF as the only hematopoietic growth factor.

One group of compounds did not show effect at the higher concentration tested, which was equal or exceeded the human peak plasma levels reported in previous published works. Isopropyl alcohol and pentachlorophenol are included in this group. The survival curves of CFU-GM exposed to different doses of atropine sulfate, caffeine, colchicine, sodium valproate and 5-fluorouracil have been already finished and their IC50 values are: 0.27 ± 0.06 mM, 0.59 ± 0.05 mM, 7.74 ± 0.80 nM, 1.34 ± 0.2 mM, 3.80 ± 1.33 μM, respectively. In vitro toxicity of these seven chemicals

on myeloid progenitors in relation to levels achieved in human plasma will be discussed.

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P15-04
Hygienic evaluation of territories based on monitoring urine toxicity of children

A.P. Eskov, R.I. Kayumov, A.E. Sokolov, A.I. Ivannikov

Firm "BMK-INVEST" ZAO, State Scientific Center of Children's Health of Russian Academy of Medical Sciences, Moscow, Russia

Opportunity of hygienic evaluation of territories has been examined on the base of monitoring urine toxicity of children. Urine is a waste product of the human body and it shows the degree of chemical contamination of the inner body under the influence of chronic chemical load. The method used to assess the urine toxicity is based on the influence of the examined solution on the motility of bull spermatozoa suspension. The influence of the examined solution on the motility of spermatozoa suspension is valued by the toxicity index I_t which is equal to the ratio of the total motility of spermatozoa suspension in the test sample per the total motility of spermatozoa suspension in the check sample. Homogeneous groups of children at the age of 2–6 years old have been examined. They attend municipal preschool educational institutions placed on environmentally favorable and unfavorable territories of two cities. The results obtained have been estimated by frequency distributions of urine toxicity indexes of the corresponding groups. The qualitative comparison of distributions has been performed by means of the average value of toxicity index. The results have demonstrated that monitoring urine toxicity of children at the age of 2–6 years old have makes it possible to determine the territory favorable for living due to its hygienic conditions.

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P15-05
Ethanol and ecstasy: Allied enemies of freshly isolated mouse hepatocytes

Helena Pontes, Maria João Marques, Fernando Remião, Félix Carvalho, Maria Lourdes Bastos

REQUIMTE, Department of Toxicology, Faculty of Pharmacy, University of Porto, 4099-030 Porto, Portugal

3,4-Methylenedioxymethamphetamine (MDMA) is frequently consumed at "rave" parties in association with ethanol. Both compounds are metabolized in the liver, leading to the formation of hepatotoxic metabolites. Thus, it was thought to be of high relevance to evaluate their putative toxicological interaction. The objective of the present study was then to evaluate, in vitro, the toxic effect of MDMA (0 μM, 800 μMand 1600 μM), using hepatocytes freshly isolated from mice repeatedly treated with 12% ethanol (8 weeks), and compare it to the results obtained in hepatocytes isolated from control animals. The ex vivo studies were performed at normothermic (37 °C) and hyperthermic (41 °C) conditions since hyperthermia has been recognized as a life-threatening problem associated with MDMA exposure. Under hyperthermic conditions, the time-dependent loss of cell viability was more evidenced in the ethanol pretreated group, comparatively to the control group. Likewise, the time-dependent variations in GSH and GSSG levels were more obvious in the ethanol group when hepatocytes were incubated at 41 °C. The obtained results strongly suggest that the consumption of ethanol may increase hepatic susceptibility towards MDMA toxicity.

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P15-06
The differentiation and biotransformation potential of oval cells isolated from untreated and CDE-treated rats of different age

Piotr Czekaj, Halina Koryciak-Komarska, Anna Wiaderkiewicz, Izabela Malinowska-Kołodziej, Ryszard Sebesta, Tomasz M. Czekaj, Maciej Tarnowski, Ryszard Wiaderkiewicz, Aleksander L. Sieroñ

Medical University of Silesia, Katowice, Poland

Population of non-differentiated liver cells is heterogenous and consists of stem cells and so called oval cells. It is believed that during liver regeneration and in some pathological conditions, these cells intensively proliferate and differentiate into cholangiocytes and hepatocytes. Due to their features, they are attractive targets for cell/gene therapy of liver disorders.

Here, we have investigated differentiating potential of oval cells isolated from livers of fetuses (16 days old), and 4 months and 20 months old Sprague-Dawley rats. Under conditions of experimental hepatocarcinogenesis adult rats, young and old, were fed ad libitum for 3 weeks with CDE diet (choline-deficient ethionine-supplemented), which injures hepatocytes and stimulates liver regeneration, a process depending on non-

differentiated cells. Liver precursor cells were isolated, cultured in vitro and differentiated to hepatocytes following induction with sodium butyrate in culture on MesenCult medium.

We have reported that even from relatively old animals (20 months), both control and CDE-treated, we could isolate and maintain a number of non-differentiated cells expressing Thy-1, CD34, CK18, CK19, πGST or αGST. In vitro differentiation of the oval cells isolated from CDE-treated rats begun earlier as compared to non-treated ones. Both, proliferation and differentiation potential of these cells, however, decreased with age. Age-dependent pattern of cytochrome P450 mRNA expressions accompanying oval cells differentiation towards hepatocytes was also observed. CYP2E1 could be a good candidate for maturing hepatocyte-precursor cell marker in aged regenerating livers.

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P15-07
Behaviour of fibrous antigorite on mesothelial Met-5A cell line

Venera Cardile [1], Laura Lombardo [1], Elena Belluso [2], Annanaria Panico [3], Silvana Capella [2]

[1] *Department of Physiological Sciences, University of Catania, Italy;* [2] *Department of Mineralogical and Petrological Sciences, University of Turin, CNR IGG-Sezione I Turin, Turin, Italy;* [3] *Department of Pharmaceutical Sciences, University of Catania, Catania, Italy*

This study investigated the cellular toxicity and examined the behaviour of fibrous antigorite on mesothelial Met-5A cell lines, widely employed in studies on the lung diseases, in particular regarding some mechanisms involved in carcinogenesis. Antigorite is a mineral with asbestiform habit found very abundant in serpentinite rocks of the Western Alps, either associated with asbestos chrysotile or tremolite as like the only mineral filling the veins of several rocks. Certain types of fibres originating from natural sources such as asbestos can cause a wide variety of respiratory diseases ranging from inflammation and fibrosis to highly malignant forms of cancers. In this study, the cellular toxicity was determined by measuring antigorite activity on cell viability and membrane integrity by performing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl test (MTT) and measuring lactic dehydrogenase (LDH) release, respectively. The possible induction of oxidative stress from antigorite was examined by both performing a fluorescent analysis of intracellular reactive oxygen species (ROS) production, and evaluating the amount of nitrite/nitrate ($NO^{\bullet}$, nitric oxide) in culture medium. Prostaglandin E_2 (PGE_2), implicated as having an important role in the pathogenesis of solid tumors through the inhibition of apoptosis, the facilitation of tumor cell invasiveness and the promotion of angiogenesis, by the enzyme-linked immunosorbent assay (ELISA) method was investigated. Fibrous antigorite at 5 μg/ml, 50 μg/ml and 100 μg/ml for 72 h showed dose-dependent cytotoxicity on Met-5A cells. All three doses of antigorite significantly enhanced the ROS production, induced the generation of $NO^{\bullet}$ and increased the amount of PGE_2. The results of this study revealed significant biochemical changes in mesothelial Met-5A cells after fibrous antigorite treatment and suggested that these changes may directly or indirectly be one of the biological events responsible for eliciting antigorite-mediated host pathological or neoplastic responses.

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P15-08
In vitro myelotoxicity of aflatoxins B1 and M1 on murine and human hemopoietic progenitors

A.F. Castoldi [1], E. Roda [2], L. Manzo [1,2], T. Coccini [1]

[1] *Toxicology Division, IRCCS Maugeri Foundation, Italy;* [2] *University of Pavia, Pavia, Italy*

Immunosuppression caused by aflatoxin B1 (AFB1) has been demonstrated in various livestock species as well as in laboratory animals. A matter of concern remains the incidental contamination of milk and dairy products with AFM1, a metabolite of AFB1 originated from hepatic metabolism.

In this study, an interspecies comparison of in vitro toxicity of AFB1 and AFM1 on human and murine burst- and colony-forming unit erythrocyte (BFU-E, CFU-E) and colony-forming unit-granulocyte/macrophage (CFU-GM) progenitors has been carried out. Human and murine bone marrow (BM) progenitors were exposed to AFB1 and AFM1 at concentrations ranging from 0.001 to 50 μg/ml, and the toxicity was detected by clonogenic assays with continuous exposure to the compounds. AFB1 concentration-dependently decreased the formation of BFU-E, CFU-E and CFU-GM colonies of both human and murine origin. AFB1 IC50 values (mean ± S.E.M.) were 0.57 ± 0.25 and 0.56 ± 0.06 μg/ml for human and murine BFU-E colonies, respectively, 4.58 ± 1.88 and 12.58 ± 2.04 μg/ml for human and murine CFU-

E colonies, and 0.76 ± 0.65 and 3.46 ± 1.52 μg/ml for human and murine for CFU-GM colonies, respectively. Relatively to human BM cells, AFM1 appeared to exert a similar inhibitory effect to that of its parent compound on the formation of all three types of colonies. The experiments examining the effects of AFM1 on murine erythroid and myeloid colony formation are still ongoing. In summary, the present in vitro results suggest a similar species sensitivity of the most immature erythroid progenitors to AFB1, while human CFU-GM and CFU-E colonies seem to be three- to four-fold more vulnerable to AFB1 myelotoxicity than those of murine origin. Thus, the higher susceptibility of human cells compared to murine cells may have relevant implications in terms of human risk assessment.

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P15-09

Hippocampal neurons undergo apoptotic and necrotic cell death after exposure to methylmercury, PCB 153 and PCB 126

R. Tofighi [1], C. Johansson [1], M. Goldoni [2], M.V. Vettori [3], A. Mutti [2], S. Ceccatelli [1]

[1] *Division of Toxicology and Neurotoxicology, Institute of Environmental Medicine, Karolinska Institutet, S-17177 Stockholm, Sweden;* [2] *Department of Clinical Medicine, Laboratory of Industrial Toxicology, University of Parma, I-43100 Parma, Italy;* [3] *European Food Safety Authority, I-43100 Parma, Italy*

The neurotoxic effects of the environmental contaminants methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are well established, although their mechanisms of action are not fully understood. Exposure to MeHg and PCB can occur simultaneously because the two contaminants are found in the same food sources, in particular fish and seafood. Several studies have shown that exposure to these toxicants impairs cognition, especially learning and memory. In the present study, we have used the mouse hippocampal neuronal cell line HT22 to evaluate the dose-dependent effects of MeHg (1–4 μM), PCB153 (50–200 μM), and PCB 126 (12.5–100 μM). The morphological changes induced by these substances suggest that both apoptosis and necrosis occur (MeHg 4 μM 20% apoptosis, 30% necrosis; PCB 153 200 μM 12% apoptosis, 38% necrosis, PCB 126 50 μM 8% apoptosis, 26% necrosis at 24 h). Caspases are not involved in the cell death process as supported by the absence of caspase activity and the lack of protective effects of the pan caspase inhibitor z-VAD-fmk. Conversely, the $Ca2^{+}$-dependent proteases calpains are activated as shown by the increase in the 150 kDa α-fodrin, a calpain breakdown product. In agreement, pre-treatment with the calpain specific inhibitor PD150606 exerts a partial protection. Lysosomal disruption is also observed after 16 h exposure by decreased uptake of the lysomotrophic vital dye acridine orange in the exposed cells, and the cathepsin D inhibitor Pepstatin A partially protects cells from the toxic effects of MeHg and PCBs. Furthermore, mitochondrial functions are impaired as shown by the significant decrease in mitochondrial Ca^{2+} uptake capacity and ATP levels. Pre-treatment with the antioxidant MnTBAP is only protective against cell death induced by MeHg, suggesting that oxidative stress does not play a major role in PCBs toxicity. Analysis of data from simultaneous exposures to MeHg and PCB 153 or PCB 126, using the Bliss' independence criterion, suggests a competitive interaction between MeHg and both PCBs.

In summary, all three toxicants induce hippocampal cell death via activation of both calpains and lysosomal proteases, possibly through disruption of mitochondrial function and intracellular calcium signaling.

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P15-10

Plasmidic vector of human neuropathy target esterase in primary cultures of bovine chromaffin cells

Encarna Quesada Jr. [1], Jose V. Castell [2], Eugenio Vilanova [1], Victoria Carrera [1]

[1] *Universidad Miguel Hernández, Instituto de Bioingeniería, Elche, Alicante, Spain;* [2] *Centro de Investigación, Hospital La Fe*

Chromaffin cells in culture show a high neuropathy target esterase (NTE) activity. It is well known that inhibition and specific modification of NTE by some organophosphorous (OPs) compounds induce a neurodegenerative neuropathy. It has been suggested that NTE is a lysophospholipase responsible for the phosphatidylcholine homeostasis but its role in the neuropathy induction is not completely clear. The cDNA of human NTE (4.4 kb) has been inserted into a plasmidic vector. Bovine chromaffin cells cultured at 50,000 cells/well were incubated with the plasmid for 2 h and after that cells were kept in the incubator. NTE activity measured after 24 h was 6.8 ± 0.5 mU/10^6 cells in untreated cells and 14.8 ± 1.5 mU/10^6 cells, 19.3 ± 2.9 mU/10^6 cells,

24.8 ± 0.9 mU/10^6 cells and 30.9 ± 1.0 mU/10^6 cells in cells incubated with 2, 4, 8 and 16 μl of plasmidic vector, respectively. Kinetic inhibition curves with mipafox of NTE activity were performed in cells treated and untreated with the plasmid. After 60 min of inhibition with the inhibitor the calculated I_{50} values were 5.5, 6.2 and 6.6 μM for cells incubated with 0, 2 and 10 μl of plasmid. Results present in this work confirm that the plasmid containing the human NTE gen is active in bovine chromaffin cells in culture and the NTE activity expressed by the plasmid show the same inhibition pattern by the neuropathic OP mipafox than NTE activity from bovine chromaffin cells.

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P15-11

In vitro cytotoxicity of patulin, deoxynivalenol, nivalenol and zearalenone on CHO-K1 cells

Mónica Fernández, Alen Simonían, Houda Berrada, Guillermina Font, María José Ruiz

University of Valencia, Faculty of Pharmacy, Laboratory of Food Chemistry and Toxicology, 46100 Burjassot, València, Spain

Mycotoxins are secondary metabolites produced by moulds which can grow on certain food under special conditions of moisture and temperature before and/or during harvest, handling, shipment and storage. Patulin is produced by some fungal species of *Penicillium* and *Aspergillus* growing on fruit, including apples, pears, and grapes. Deoxynivalenol, nivalenol, zearalenone are produced by *Fusarium* species and are found worldwide in cereals and other food. These mycotoxins are an important problem because of the economical loss and their acute and chronic effects on human and animal health.

The cytotoxicity of these mycotoxins has been compared on well established Chinese hamster ovary cells (CHO-K1). The endpoints evaluated at a concentration range of 0.05–20 μg/mL of each mycotoxin after 24 h, 48 h and 72 h exposure were cell proliferation by quantification of total protein content (PR), lysosomal function by relative neutral red (NR) uptake and mitochondrial's integrity by mitochondrial succinate dehydrogenase (MTT) activity. After 24 h, cytoplasmic membrane integrity to cytosolic lactate dehydrogenase (LDH) leakage and LDH intracellular activity were also studied. Toxicity of these mycotoxins expressed as 50% inhibitory concentration (IC_{50}) was calculated from generated dose–response curve. An increased sensitivity was observed in CHO-K1 cells when the exposure was extended from 24 h to 72 h, especially for deoxynivalenol.

Metabolic enzymes such as succinate dehydrogenase and LDH showed similar behaviour compared to basal cytotoxicity endpoints (PR, NR) for most of the mycotoxins. LDH release was detectable only with high exposure concentrations.

The results of mycotoxins in CHO-K1 cells demonstrate that a battery of in vitro assays can provide useful information of mycotoxins at cellular level and gives proof of the application of these models for the investigation of the toxic effects in environmental biomonitoring.

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P15-12

Effects of flavones on AhR-signaling pathway in rat oval stem-like WB-F344 cells

Jirina Zatloukalova [1], Pavel Krcmar [2], Katerina Chramostova [1], Alois Kozubik [1], Miroslav Machala [2], Jan Vondracek [1,2]

[1] *Institute of Biophysics, Brno 612 65, Czech Republic;* [2] *Veterinary Research Institute, Hudcova 70, Brno 621 32, Czech Republic*

Flavones are products of plant secondary metabolism possessing antioxidant, anti-inflammatory, anti-viral, anti-bacterial, cytostatic and other functions. Based on their structure and concentration, they may behave either as aryl hydrocarbon receptor (AhR) agonists or antagonists. This ligand-dependent transcription factor, localized in cytoplasm in a multiprotein complex, may bind a wide array of ligands, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other polyaromatic hydrocarbons (PAHs). After ligand-mediated activation, AhR translocates to the nucleus and activate transcription of target genes, such as Cyp1a1. In this study, we investigated effects of TCDD and two synthetic flavones, 3′-methoxy-4′-nitroflavone (3M4NF) and β-naphthoflavone (BNF), on AhR-signaling pathway in contact-inhibited rat liver epithelial WB-F344 cells. After treatment of confluent WB-F344 cells with BNF or TCDD, a model AhR-agonist, we observed activation of AhR leading to its nuclear translocation, induction of Cyp1a1 expression, accompanied with elevation of

cyclin A levels and induction of cell cycle progression and cell proliferation. Both compounds also induced activation-related degradation of AhR. 3M4NF was originally described as a pure AhR antagonist, but in WB-F344 cells it behaves as a partial agonist, inducing a weak activation of AhR and low Cyp1a1 expression. TCDD-mediated induction of Cyp1a1 expression and AhR-degradation were strongly suppressed by co-treatment of the cells with TCDD and 3M4NF. Nevertheless, after treatment with this flavone, we observed a massive proliferation of cells, even higher than in case of TCDD, especially at 10 μM concentration. We may conclude that 3M4NF is able to partially abolish TCDD-induced activation of AhR-signaling. However, 3M4NF alone caused a wide range of changes in cell cycle and proliferation, suggesting involvement of other signaling pathways activated by this flavone.

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P15-13
In vitro evaluation method for cardiovascular toxicity using whole cells isolated from the mouse heart

Tomoaki Inoue, Yuko Kanno, Tetsuro Sugimoto

Safety Assessment Department, Kamakura Research Labos., Chugai Pharmaceutical Co., Ltd., Japan

In early stages of drug discovery, cell lines and primary cultured cells are used for in vitro toxicity screening. In most cases, isolated specific cell types are used even in primary cultured cells. In test systems with isolated specific cell types, direct actions on the specific cells would be evaluated, but it would be difficult to evaluate differences of toxicities among cell types or indirect actions from other cell types. In this experiment, culture system with whole cells from adult mouse heart was investigated for evaluation of cardiovascular toxicities. The heart from adult BALB/c mice was dispersed by enzyme digestion, and whole cells were subjected to culture in 96-well plate. After 0–7 days pre-culture period, test compounds (doxorubicin, Dox as a positive reference compound) were added and incubated for 1–4 days. The cells were immunohistochemically stained with anti-von Willebrand Factor (vWF) an endothelial cell (EC) marker. The supernatants (Sup) and cell lysates (Lys) were collected and, vWF and lactate dehydrogenase (LDH) were measured. In the immunohistochemical staining, positive cells (ECs) were observed in the control cultures and selectively damaged by exposure to Dox. The vWF levels in the control cultures showed high values in the Sup, therefore, evaluation in the Lys was required. The vWF and LDH increased depending on the culture period that suggests cell proliferation. By the exposure to Dox, decreases of vWF in the Lys were more marked than LDH. These suggest that ECs are more susceptible to Dox than the other cell types. In conclusion, these results are consistent with known toxicities of Dox, and this in vitro toxicity screening system would be useful for evaluation of cardiovalcular toxicities especially in endothelial cells.

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P15-14
Evaluation of computer-based analysis for assessment of differentiation in embryo midbrain micromass culture

Iwona Wilk, Maria Minta, Jan Żmudzki

Department of Pharmacology and Toxicology, National Veterinary Research Institute, Pulawy, Poland

The scientifically validated micromass test (MM), proposed for developmental toxicity studies of xenobiotics is based on primary cultures of embryonic limb buds (LB) cells (INVITTOX 122). It is fully automated and allows to quantify the endpoints related to proliferation (IC_{50}-P) and differentiation (IC_{50}-D)—basic processes in embryogenesis in vivo. In previous version of this method (INVITTOX 114) embryonic cells from midbrain (CNS) were also considered. However, the assessment of differentiation of these cells, based on manual counting of neurospheres, is less objective and arouses anxiety. So, more information from morphometrical analysis of neurospheres is of big importance.

The aim of these studies was to improve the assessment of CNS cells differentiation. Two parameters (number of aggregates and their total area) were compared using computer-based image analysis. Two compounds, i.e. lithium chloride (Li) and aluminium chloride (Al), for which CNS is suspected to be a target tissue, were evaluated.

In house assessment of the procedure was done on LB cells cultures in which analysis of differentiation was checked by spectrophotometrical method in alcian blue uptake test. For this reason, model compound—trypan blue (TB) was used in seven concentrations ranged from 0.06 to 250 μg/ml. Results obtained indicated that total area measurement was more precise and might be very useful in morphological assessment of CNS differentiation. This is of big importance, specially when cells are

exposed to the compounds, which disturb cell–cell communication and can cause more numerous but smaller aggregates in appearance.

The assessment of developmental neurotoxicity of Li and Al using neurospheres total area measurement confirmed that these compounds might be potential neuroteratogens.

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P15-15

Cigarette smoke constituents induce CYP1A1 in cultured primary urothelial cells in a complex manner

Sabine Ploettner, Peter H. Roos, Wolfram Foellmann

Institute for Occupational Physiology at the University of Dortmund, Germany

Cigarette smoke is the most relevant non-occupational risk factor for bladder cancer. Though almost 70 carcinogens are known to be present within tobacco smoke, it is unclear which of these are primarily responsible for the bladder-specific carcinogenic effect. Recently we showed that the tobacco smoke constituent benzo[*a*]pyrene (B[*a*]P) exerted concentration-dependent genotoxic effects in primary cultured porcine urinary bladder epithelial cells (PUBEC). The effects were accompanied by a massive increase in cytochrome P450 1A1 (CYP1A1) mRNA expression.

To further investigate CYP1A1 induction on the protein level, we performed Western blot analysis and immuno-flow cytometry (FACS). PUBEC were incubated for 24 h with B[*a*]P (0.001–50 μM) or cigarette smoke condensate (CSC; 0.1–10 μg/ml). Subsequent examination of CYP1A1 induction revealed a linear, concentration-dependent increase of CYP1A1 protein between 1 and 50 μM B[*a*]P by Western blot technique. FACS analysis, however, demonstrated the presence of a sensitive subpopulation of cells already expressing CYP1A1 protein after treatment with 0.1 μM B[*a*]P and a maximum portion of induced cells ($45 \pm 2\%$) at 3–10 μM. After incubation with CSC a linear but more moderate effect than after B[*a*]P-treatment was indicated by Western blot analysis. CYP1A1 induction for the most sensitive cells was recorded for 1 μg/ml CSC. At most $17 \pm 6\%$ of the cells were induced between 5 and 10 μg/ml CSC as revealed by FACS analysis.

In relation to CYP1A1 induction our results exhibit the presence of sensitive cells within a heterogeneous population which already respond towards low B[*a*]P concentrations. The number of induced cells increased with rising B[*a*]P concentration as well as the intracellular amount of CYP1A1 protein of the induced cells.

The B[*a*]P content within 1 μg/ml CSC of the used reference cigarette was about 2.4 pM, a B[*a*]P-concentration that showed no CYP1A1 induction by Western blot or FACS. Neither B[*a*]P alone nor the sum of polycyclic aromatic hydrocarbons within the cigarette smoke condensate can explain the CYP1A1 induction in PUBEC after CSC treatment. Complex combinatory effects therefore seem be responsible for this effect.

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P15-16

Effects of *Chelidonium majus* extracts in human hepatocytes in vitro

M. Adler[1], K. Appel[2], T. Canal[3], P. Corvi Mora[3], R. Delfino[4], R. Gennaro[4], K. Gritzko[5], L. Pascolo[4], F. Ruzzier[3], C. Tiribelli[4], B. Wallner[5], J. Schulze[6]

[1] *Siegen, Germany;* [2] *VivaCell Biotechnology GmbH, Denzlingen, Germany;* [3] *Actimex Srl, Trieste, Italy;* [4] *Center for Liver Studies (CSF) and Department BBCM, University of Trieste, Italy;* [5] *GenPharmTox BioTech AG, Planegg, Germany;* [6] *University Frankfurt, Frankfurt/Main, Germany*

Chelidonium majus extracts are used mainly in the therapy of biliary and hepatic dysfunctions. In recent years, there has been a discussion whether reversible hepatitis may be a rare potential side effect in this therapy. Therefore, earlier in vitro studies of cytotoxic effects of these extracts in rat hepatocytes have found increased attention of regulatory agencies. With the aim to establish a better database, two studies in human hepatocytes have been conducted.

A study in primary human hepatocytes and the provision of the test substance were conducted under GMP/GLP conditions. The test substance was a hydroethanolic *Chelidonium* extract with a content of total alkaloids of 5.9 mg/g. For indication of cytotoxicity, MTT and neutral red assay were used. EC_{50} over 24 h was calculated as 0.83 ± 1.69 and 0.82 ± 2.49 mg/ml, which was equivalent to 4.9 μg/ml total alkaloids. In a concentration of 0.74 mg/ml 86% of the cells survived (MTT). The EC_{50} of ascorbic acid in this type of assay is in a similar range (0.8–3.5 mg/ml).

A second study was conducted in the human-derived Chang liver cell line, testing *Chelidonium* extract with 6.2 mg/g total alkaloids. The vitality of the cells was determined from MTT assay and morphological appearance. EC_{50} (24 h) was 0.96 ± 0.49 mg/ml, equivalent to 5.9 μg/ml total alkaloids. The EC_{50} of *Ginkgo biloba* extract and Paracetamol, assayed for comparison, were 0.31 and 2.49 mg/ml.

These data show a clear dose dependency of all observed effects and do not point to special hepatotoxicity of *Chelidonium majus* extracts, compared to other drugs with established therapeutic safety. They are in accordance with available toxicity data for oral application, which show no hepatotoxic effects and give no indication for extended pharmacovigilance risk limitation.

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P15-17

Competitive and additive effects of methyl-mercury and PCB153 on PC12 cells viability, lipidic peroxidation products (TBARS) and dopamine levels

M. Goldoni [1,2], M.V. Vettori [3], A. Caglieri [1], D. Poli [1,2], G. Folesani [1,2], S. Ceccatelli [4], A. Mutti [1]

[1] *Laboratory of Industrial Toxicology, Department of Clinical Medicine, Nephrology and Health Sciences, University of Parma, Via Gramsci 14, Parma, Italy;* [2] *National Institute of Occupational Safety and Prevention (ISPESL), Research Centre at the University of Parma, Via Gramsci 14, Parma, Italy;* [3] *European Food Safety Authority, Largo Palli 5/A, Parma, Italy;* [4] *Institute of Environmental Medicine, Division of Toxicology and Neurotoxicology, Karolinska Institutet, Stockholm SE-171 77, Sweden*

Methyl-mercury (Me-Hg) and PCB153 are known neurotoxic compounds which tend to accumulate in fish. Aim of this study was to characterise their combined effects in vitro on a neuronal cell line (PC12 cells).

PC12 cells were treated with Me-Hg (1e−7–1e−5 M) and PCB153 (1e−5–4e−4 M) in single and combined either synchronous or asynchronous experiments. Possible interactions were assessed relying on a mathematical model assuming the Loewe additivity law.

Some combinations (e.g., Me-Hg 5e−7 M and PCB153 1e−4 and 2e−4 M; Me-Hg 1e−6 M and PCB153 5e−5 M; Me-Hg 1e−7 M and PCB153 4e−4 M) were associated with antagonistic effects on cell viability of PC12 cells. These results were confirmed by other toxicological end points, namely lipid peroxidation products (TBARS) and intracellular dopamine (DA) levels. Combined asynchronous exposure showed that whereas Me-Hg can modulate PCB153 toxicity, the opposite seems not to be not true.

Further studies are necessary to understand the mechanistic bases of Me-Hg by PCB153 interactions. Our experimental and mathematical approach could be useful to investigate the effects of different toxic compounds in mixture.

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P15-18

Comparison of in vitro and in vivo tests in water toxicology

Jitka Kolarova, Zdenka Svobodova, Vladimir Piacka

University of South Bohemia Ceske Budejovice, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

The in vitro and in vivo toxicity tests were performed with 26 chemical substances and preparations. In vitro test were performed by NR-assay on RTG-2 cell line, in vivo tests included acute toxicity on fish *Poecilia reticulata* and crustacean *Daphnia magna*. The 20hEC50 values obtained by NR-assay were higher nearly in all cases when compared to results of toxicity tests on *P. reticulata* (48hLC50) and *D. magna* (48hEC50). Sensitivity of RTG-2 cell line to toxic agents was lower compared to those registered in fish and daphnia. Close ($r = 0.89$) and statistically highly significant correlation ($P < 0.001$) was registered between 20hEC50 values on RTG-2 and 48hEC50 values on *D. magna*. Correlation between 20hEC50 values on RTG-2 and 48hLC50 values on *P. reticulata* was found lower ($r = 0.65$) but highly significant as well. Results of the testing performed enable us to recommend utilization of NR-assay on RTG-2 cell line as a screening method for evaluation of toxicity of chemical substances and preparations from the point of view of the aquatic environment.

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P15-19

***In vitro* exposure to arsenic in human cord blood and murine bone marrow cells**
Comparison between genders and species

Daniele Ferrario, Cristina Croera, Ilaria Malerba, Laura Gribaldo

ECVAM, Institute for Health and Consumer Protection, JRC–Ispra, Italy

Chronic exposure to inorganic arsenic has been associated with several cancers such as skin, lung, bladder and kidney cancer. Myelogenous leukaemia may also occur

as well as increased risk of Hodgkin's disease in exposed workers.

Increased incidence of spontaneous abortion and infant malformation has been reported among women exposed to arsenic and teratogenic effects have been reported in arsenic-exposed animals. In fact, arsenic can easily cross the placenta, and so posing a risk to the fetus. Hematopoietic tissue is involved in the production of mature blood cells, and responds quickly to an increased demand for mature cells, making it a major target for xenobiotic toxicity.

In this study, we evaluated the effect of arsenic on the clonogenicity of blood progenitors (cord blood and bone marrow cells) belonging to different species (human and murine), different genders (male and female).

In addition, we investigated the ability of arsenic to increase the production of metallothioneins (MT), which often acts in the defense against metal-induced or oxidative cellular injury.

Our data indicated that arsenic has a relevant toxic effect on CFU-GM, at the same level both in human and in mouse, without significant difference between genders ($IC50 = 1.08 \pm 0.51$ μM in female mice and 1.37 ± 0.89 μM in male mice; $IC50 = 1.22 \pm 0.19$ μM in male human, and 1.45 ± 0.23 μM in female human).

Moreover, both in human and murine females' arsenic slightly increased the number of CFU-GM colonies at lower doses tested.

Metallothioneins were induced only at the concentration of 1.22 μM and after 24 h of exposure.

Further "*in vivo*" investigations are already being carried out to verify the toxic activity of arsenic in naturally exposed population.

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P15-20

Effect of Diosgenin on freshly isolated hepatocytes, treated with *tert*-butyl hydroperoxide

M. Kondeva [1], M. Mitcheva [1], I. Krasteva [2], S. Nikolov [2]

[1] *Laboratory of "Drug metabolism and drug toxicity", Department of "Pharmacology and Toxicology", Faculty of Pharmacy, Medical University, Sofia, Bulgaria;* [2] *Department of "Pharmacognosy and Botany", Faculty of Pharmacy, Medical University, Sofia, Bulgaria*

In this study Diosgenin, isolated from *Asparagus officinalis* L. (*Liliaceae*), was investigated for protective effect against *tert*-butyl hydroperoxide (*t*-BuOOH) toxicity in isolated rat hepatocytes. For isolation of the hepatocytes, in situ, two-stepped, collagenase perfusion was used. Diosgenin (100 μM) was administered in combination with *t*-BuOOH (75 μM). *t*-BuOOH was used as a generator of reactive oxygen radicals and a model for oxidative stress. The levels of thiobarbituric acid reactive substances (TBARS) were assayed as an index of lipid peroxidation (LPO). Lactate dehydrogenase (LDH) leakage, cell viability and reduced glutathione (GSH) depletion were used as signs of cytotoxicity. *t*-BuOOH significantly decreased hepatocytes' viability by 74%, GSH level by 58% and increased TBARS level by 447% and LDH leakage by 193% compared to the control. Our data indicated that in combination with *t*-BuOOH Diosgenin showed statistically significant protection against the toxic agent. The plant compound protected hepatocytes from *t*-BuOOH toxicity as followed: preserved cell viability by 262%, increased GSH level by 87%, decreased LDH leakage by 47% and TBARS level by 63%. We suggested that its protective effect might be due to the antioxidative activity of the saponins and a possible influence on the metabolism of *t*-BuOOH.

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P15-21

Human Ishikawa cells with distinct estrogen receptor expression display characteristic gene expression profiles

Stephanie Simon, Stefan O. Mueller

Merck KGaA, Germany

Estrogens regulate diverse physiological processes in various tissues including the mammary gland and reproductive organs. Estrogenic effects are mediated by the estrogen receptors belonging to the superfamily of ligand-inducible transcription factors. We analyzed cellular responses to estrogens at the gene expression level in a time and dose-dependent manner. For that, we selected genes involved in cell cycle control, cell growth and differentiation, apoptosis, metabolism, stress response, transcriptional regulation and signal transduction. To determine gene expression levels we used the real time PCR-based TaqMan® low density array technique (TLDA). Ishikawa cells expressing endogenous estrogen receptor (ER) alpha (Ishikawa-plus) or lacking ER (Ishikawa-minus) were treated with 17β-estradiol (E2), the anti-estrogen ICI 182,780 (ICI) and a combination of E2 and ICI. Cells were harvested after 2, 6, 24, 48 and 72 h and subjected to TLDA analysis. We were able to identify cell type-specific, estrogen-dependent gene expression profiles. The direction of regulations seems to be diametrically in ER positive vs. ER negative cell lines. The *gene regulated by estrogens in breast*

cancer 1 (GREB1) was upregulated by E2 but not by ICI in Ishikawa-plus, suggesting a role as a biomarker for estrogenicity in these cell lines. Interestingly, in Ishikawa-plus the junD proto-oncogene (JUND) that is proposed to protect cells from p53-mediated senescence and apoptosis was upregulated by E2 and downregulated by ICI. Due to the involvement of JUND and p53 in tumor progression and apoptosis, they provide putative targets for anti-estrogen treatment of estrogen-dependent cancers. Furthermore, EGF, a known mitogenic factor, was highly upregulated by E2 in Ishikawa-plus but not in Ishikawa-minus supporting crosslinks between EGF and ER signalling pathways. Additionally, our results revealed distinct time- dose and ER-dependent differential expression profiles of estrogen-regulated genes. Moreover, this technology may enable characterization of putative endocrine active compounds by gene expression profiling. In a next step, global gene expression analyses with selected samples will be performed using the Illumina® technology to confirm TLDA-based results and to identify more estrogen-regulated genes and pathways involved.

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P15-22

Effect of *Astragalus corniculatus* on enzyme-induced lipid peroxidation in liver microsomes from spontaneously hypertensive rats (SHR)

R. Simeonova [1], M. Mitcheva [1], V. Vitcheva [1], M. Kondeva [1], I. Krasteva [2], S. Nikolov [2]

[1] *Laboratory of "Drug Metabolism and Drug Toxicity", Department of "Pharmacology and Toxicology", Faculty of Pharmacy, Medical University, Sofia, Bulgaria;* [2] *Department of Pharmacognosy and Botany, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*

There are literature data, reported the antioxidant effects of the saponins, isolated from different *Astragalus* species. The aim of the following study was to evaluate the effect of purified mixture containing mostly saponins (PMS), isolated from *Astragalus corniculatus* Bieb. (*Fabaceae*), on enzyme induced lipid peroxidation in liver microsomes from SHR- Okamoto Aoki, compared to Normotensive (NTR–Wistar) rats.

The lipid peroxidation was induced by incubating rat liver microsomes with carbon tetrachloride in the presence of NADPH. The effect of PMS (100 μM) was assessed at the 20th min of incubation. TBARS, products of lipid peroxidation, were measured spectrometrically.

The results of our study showed that in pure microsomes, TBARS formation in SHR was elevated around five times, compared to NTR. After incubation of pure microsomes from both strains with PMS (100 μM), TBARS formation was increased by 25% in NTR and decreased by 25% in SHR.

In the conditions of enzyme-induced lipid peroxidation, PMS significantly decreased the formation of TBARS by 55% in NTR and by 35% in SHR.

According to the results of this experiment, we could conclude that PMS from *A. corniculatus* Bieb. (*Fabaceae)* possesses antioxidant properties both in SHR and NTR.

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P15-23

Characterization of compound-induced hepatotoxicity using gene expression profiling in rat primary hepatocytes

Gwo-Jen Day, Lyndsay Brady, Barry Eynon, Mark Fielden, Susan Fujimoto, Kurt Jarnagin, Donald Halbert

Iconix Pharmaceuticals, USA

Toxicity studies on drug candidates are traditionally conducted in animal models requiring large amounts of compound, significant time and animal resources, and are therefore not amenable to early compound screening. To address this need, we have developed a predictive in vitro toxicogenomic database by treating rat primary hepatocytes with therapeutics and prototypical toxicants. Gene expression data from this DrugMatrix® database has been collected and mined for biomarkers of in vivo biological outcomes, termed in vitro Drug Signatures®. Screening in hepatocytes using in vitro Drug Signatures can be performed in short timeframes with mg quantities of drug to predict compound-induced hepatotoxicity in vivo. In an evaluation of the database, gene expression was analyzed for the blinded Compound B after 12 and 24 h of exposure to a TC20 dose in rat primary hepatocytes. Using in vitro Drug Signatures, Compound B was predicted to cause steatosis and necrosis in vivo. This prediction was confirmed in subsequent in vivo studies. Based on the observed gene expression changes, the predicted steatosis was most likely a consequence of the fatty acid beta-oxidation inhibition coupled with the induction of oxidative stress, leading to an accumulation of hepatic triglycerides and causing mitochondrial dysfunction. These observations provide a mechanistic explanation for matches to the signatures predicting steatosis and necrosis for Compound B and demonstrate that in vitro toxicogenomics in rat primary hepatocytes is a valuable

tool for the characterization of compound-induced liver toxicity.

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P15-24
Characterisation of two dendritic cell models for in vitro sensitization testing

Inge Nelissen, Geert R. Verheyen, Daniella Ooms, Hilde Leppens, Rosette Van Den Heuvel, Hilda Witters, Greet Schoeters

Flemish Institute for Technological Research (VITO), Belgium

In recent years, a lot of attention has been paid to the development of in vitro tests which could reduce sensitization studies employing animals. In this perspective, dendritic cells derived from human blood constitute a promising cell model, as these cells play a pivotal role in the induction of skin and respiratory allergy. After contact with sensitizing compounds, dendritic cells undergo a maturation process during which cytokine production increases and surface marker expression changes. These changes represent potential endpoints for predictive in vitro sensitization tests.

To obtain a representative in vitro cell model, we raised two different dendritic cell types starting from human cord blood $CD34^+$ stem cells, and compared their maturation phenotype in response to a known cytokine maturation cocktail using flow cytometry. $CD34^+$ stem cells were cultured in the presence of GM-CSF, SCF, TNF-α and IL-4 to obtain a phenotype resembling myeloid dendritic cells ($CD11c^+$). The second culture was grown in the presence of TGF-β yielding Langerhans cell-like DC ($CD207^+$).

In both cell models, we studied changes in CD1a, HLA-DR, CD83, CD86, CD54, CD207, and E-cadherin expression after 24 and 48 h exposure to IL-1β and TNF-α. A more marked maturation effect, represented by increased CD83 and CD86 expression, was observed on the Langerhans-like cells compared to the dendritic cells. Therefore, the Langerhans cell-like model may serve as a good predictive cell model for development of an in vitro allergy test.

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P15-25
Biological effects of fullerene C60 in mouse embryonic stem cells

Tetsuji Nishimura[1], Reiji Kubota[1], Maiko Tahara[1], Megumi Nagaoka-Hamano[1], Kumiko Shimizu[1], Akihiko Hirose[2], Hiroshi Tokunaga[1]

[1] *Division of Environmental Chemistry, National Institute of Health Sciences, Tokyo, Japan;* [2] *Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan*

Fullerenes are a new class of compounds with potential application in chemical and engineering processes and in numerous photophysical electrochemical fields. Science discovering, fullerene and its derivatives have been studied on the physical and chemical characteristic including photophysical and photochemical properties. However, biological properties of fullerene itself have not been investigated for their high hydrophobicity. Recently, the research has been started on the effects for human health and for ecosystem. In this study, we have investigated on the biological effects of fullerene (C60) using mouse embryonic stem (ES) cells in order to clarify the effect on the differentiation. The fullerene solution was prepared in toluene/dimethyl sulfoxide (1:1, v/v). The fullerene solution was suspended in the culture medium with 20% fetal calf serum. Then, the culture medium was stirred in sealed condition at 37 °C overnight. Fullerene in the culture medium and in cells was extracted with toluene and detected by liquid chromatography–electrospray ionization tandem mass spectrometry. Mouse ES cells were exposed with the fullerene solution. The uptake of fullerene increased concentration-dependent qt concentration range from 2 to 20 μg/ml after exposure for 24 h. When cells were continued to culture in the culture medium without fullerene, the total amount of fullerene in all cells was constant and the growth rate of the cells was not changed for 48 h. In other words, the concentration of fullerene per cell was decreased. These results indicate fullerene was incorporated into cell by this established method. Mouse ES cells were continuously treated with fullerene for 24, 36, 48 and 72 h. By exposure of 15 μg/ml of fullerene, the growth rate slightly decreased. However, there was no significant difference at the growth rate by exposure of 10 μg/ml or less in this experiment. The evaluation of the effect on physiological function and development by the molecular–biological analysis becomes important on next step.

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P15-26

Detection of contact allergens in an U937 activation test based on CD86 expression and IL-8 secretion

François Python [1], Carsten Goebel [2], Sylvie Python [1], Fabio Protopapa [1], Pierre Aeby [1]

[1] *Experimental Product Safety, P&G Prestige and Professional, Cosmital SA, Marly, Switzerland;* [2] *Product Safety, Human Safety Assessment, P&G Prestige and Professional, Wella Service GmbH, Darmstadt, Germany*

In a previous work (recently submitted for publication), we have evaluated the activation by sensitizers and irritants of the human myeloid cell line U937 in an in vitro test protocol called the "U937 activation test". Cells were cultured in the presence of interleukin-4 (IL-4) to obtain dendritic-like cells. 16 chemicals were selected (8 sensitizers, 3 non-sensitizers and 5 oxidative hair dye precursors) and their activation was analyzed by flow cytometric detection of CD86 protein expression and by quantifying IL-1β and IL-8 gene expressions using real time reverse transcriptase-polymerase chain reaction (RT-PCR). Based on the different expression levels of the selected markers (CD86, IL-1β and IL-8) at three time points (24, 48 and 72 h), the "U937 activation test" was able to discriminate sensitizers from non-sensitizers. To further simplify the test procedure, we only analyzed the protein expression of CD86 by flow cytometry and of IL-8 by enzyme-linked immunosorbent assay (ELISA) after a 72 h exposure period to 20 chemicals. Based on the different expression levels of CD86 and IL-8, a score was calculated for each chemical to estimate its sensitizing potential. Although the role of IL-8 in the induction of skin sensitization is not yet completely elucidated, our results indicate that IL-8 protein expression is a promising endpoint to screen for potential contact allergens. In conclusion, the simplified version of the "U937 activation test" with a combination of only two markers, CD86 and IL-8, allows to discriminate sensitizers from non-sensitizers.

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P15-27

Synergistic effect of chemical insult and toll-like receptor ligands on dendritic cell activation

Ian Kimber, Marie Cumberbatch, Craig Portsmouth, Kirsty Clelland, Carolin Schramm, Gavin Maxwell, Carl Westmoreland, David A. Basketter, Rebecca Jane Dearman

Syngenta CTL, Macclesfield, Cheshire, UK

There is considerable interest in the development of in vitro methods for the identification and characterisation of contact sensitisers. Dendritic cells (DC) play a central role in cutaneous immune responses and methods are available for their culture from bone marrow precursors. We have demonstrated previously that chemical allergens, such as dinitrobenzene sulfonic acid (DNBS), or skin irritants, such as benzene sulfonic acid (BS), induce modest changes in the maturation status of murine bone marrow derived DC. In the current experiments, we have examined the influence of co-culture of murine bone marrow derived DC with chemical and selected toll-like receptor (TLR) ligands, potent activators of DC. Cells were cultured with increasing concentrations of DNBS (0.1–1 mM) or BS (10–100 mM), together with suboptimal doses of selected TLR ligands (Pam_3Cys-Ser-$(Lys)_4$ [PAM], TLR1-2 ligand; macrophage-activating lipopeptide-2 [MALP-2], TLR6-2 ligand; or flagellin; TLR5 ligand). Culture of DC for 24 h with DNBS or BS alone caused a dose dependent decline in cell viability, as assessed by dual staining for annexin V/propidium iodide (PI). However, whereas DNBS induced a higher proportion of late apoptotic (annexin V^+/PI^+) cells, BS was associated with more early apoptotic (annexin V^+/PI^-) cells, suggesting that DNBS and BS may cause cell death by different mechanisms. Synergy was observed for interleukin (IL)-1α, IL-6 and tumour necrosis factor α (TNF-α) production when DC were co-cultured with the skin irritant BS (50 mM) and MALP-2 (10 ng/ml) or PAM (50 ng/ml), but not with DNBS. In contrast, a synergistic effect on IL-6 secretion was observed when DC were cultured with sub-toxic doses of the chemical allergen DNBS (0.5 mM) and flagellin (100 ng/ml or 500 ng/ml), compared with only additive effects for BS with this TLR ligand. These results suggest that chemical allergens and skin irritants may interact differently with DC and that chemical insult may prime DC for enhanced reactivity to TLR ligands. Such differences may provide for the basis of an approach to the development of in vitro assays for skin sensitisation.

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P15-28

Pro-apoptotic effect of fly ash leachates in hepatocytes of freshwater fish (*Channa punctata* Bloch)

Mehboob Ali, Shakilur Rahman, Hasibur Rehman, Kanchan Bhatia, Rizwan A. Ansari, Sheikh Raisuddin

Department of Medical Elementology & Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110062, India

We studied the pro-apoptotic effect of fly ash leachates (FAL) in the hepatocytes of a freshwater fish, *Channa punctata* Bloch. Hepatocytes were exposed to different concentrations of '7-day' FAL for 24 and 48 h and various parameters of apoptosis were studied using standardized procedures. FAL induced apoptosis in hepatocytes as indicated by various biomarkers of apoptosis (cytological examination, DNA fragmentation and DNA laddering assays, caspases, cytochrome-*c*, lactate dehydrogenase, LDH). Cytological examination showed an unambiguous apoptotic effect of ash leachates in fish hepatocytes. Exposed hepatocytes also showed increased production of H_2O_2 and superoxide ions and increased lipid peroxidation (LPO). The observations confirm a role for reactive oxygen species (ROS) and associated oxidative stress in FAL-induced apoptosis in hepatocytes. As regards the induction of caspases, cytochrome-*c* and LDH, the major contributor appears to be the induction of various caspases. Biomarker of apoptosis has recently been used for ecotoxicological impact assessment of environmental chemicals. Our findings show that this biomarker may also be used for as complex chemical mixture as fly ash and its leachates. Also, findings may be helpful in understanding the cytological damage caused by exposure to fly ash in human subjects where it is exposed through inhalation route and is a cause of lung cancer.

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P15-29

Bovine spermatozoa: An in vitro model for reproductive toxicity?

Mariska M. Tegelenbosch-Schouten [1], Sabina Arabi [2], Donatella Balduzzi [2], Jolanda H.M. van Bilsen [1], Mary-Lène H.M.H. Brouwer [1], Anja Dijkstra [1], Andrea Galli [2], Linda J.M.L. van der Horst-Groeneveld [1], Peter T. Theunissen [1], André P.M. Wolterbeek [1], Ine D. Waalkens-Berendsen [1]

[1] *TNO Quality of Life, Zeist, The Netherlands;* [2] *Istituto Sperimentale Italiano "Lazzaro Spallanzani", Rivolta d' Adda, Italy*

As part of the EU ReProTect project, an in vitro method is developed to test chemicals for their toxic effect on *thawed* mature bovine spermatozoa using Computer Assisted Sperm Analysis (CASA). Motility parameters and membrane integrity were used to determine toxicity.

Bovine mature spermatozoa were thawed and exposed to chemicals for one hour at 37 °C. After incubation, motility parameters were determined in a dose range finding study (DRF) at concentrations of 10, 100 and 1000 μg/ml. Based on the outcome of the DRF a main test was performed using seven concentrations in a narrowed range to determine the motility IC_{50} (concentration at which the motility has decreased to 50% of the control value measured at the same time) for each compound. Positive and negative controls were included. A viability test was performed to distinguish between deactivation of sperm motility and cell death due to unspecific toxicity.

This test appeared to be a simple, rapid and reproducible method to establish quantitative assessment of cytotoxicity using endpoints motility and membrane integrity.

Swimming activity is dependent upon intact cellular structures and functions, but the measurement of inhibition of the motility of spermatozoa alone gives no information about the mode of action of chemicals. Since, for some chemicals, percentages viable cells differed from percentages motile cells, inclusion of a viability test seems useful in order to set up a prediction model. Besides, specific motility parameters (e.g. velocity) easily measured with CASA could give useful information on working mechanisms of specific chemicals. Whether this test could be used as part of high throughput in vitro tests which covers the whole male and female reproductive cycle in reproductive toxicological evaluation should be evaluated within the EU ReProTect project.

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P15-30

In vitro models using blood cells from wild birds to assess the effects induced by cadmium and lead

E. Martínez-López, D. Romero, A. Hernández-García, P. María-Mojica, P. Gómez-Ramírez, C. Tagliatti, A.J. García-Fernández

Department of Toxicology, Faculty of Veterinary Medicine, Campus de Espinardo, 30100, University of Murcia, Spain

Various reports from the European Union advocate the necessity for designing and improving risk-assessment methods capable of estimating the interactive effects of multiple exposures. There also exists the need for new tests to undergo a reduction in the use of experimental animals. Lastly, ethical, legal and other issues, such as access to a sufficient number of samples, make the use of protected wild animals for "in vivo" experimental studies difficult. Heavy metal environmental contamination is responsible of several diseases in wildlife species. Erythrocyte is target cell for lead and cadmium. However, scientific literature on erythrocytes in wildlife is scarce. The avian erythrocyte is able to maintain its functional status in vitro, being an excellent material to investigate the effects of environmental contaminants in wild birds. Once the level of lead and cadmium exposure experienced by the species studied is known, or the risk of said exposure has been estimated via monitoring studies, an attempt is made at reaching an approximation of the effects that said exposure may cause. Lead and cadmium have been related to induction of both apoptosis and necrosis in several cell lines, however is not clear the sequence and predominance of these processes.

The aim of this study was to develop new "in vitro" experimental models using red blood cells from wild birds (mallard blood—*Anas platyrhynchos*, eagle owl—*Bubo bubo* and buzzard—*Buteo buteo*) in order to assess effects of concentrations of contaminants and prepared blends, based on results obtained from monitoring studies.

Red blood cells were obtained after performing a density gradient centrifugation (57%). The fragility of red blood cell membranes allows us to use standardized techniques in order to measure cell survival via spectrophotometry (measurement of oxyhemoglobin) and flow cytometry (propidium iodide staining). Once the dose–response curves were plotted, two concentrations of each heavy metal and their combination were chosen, one with a low capacity for reducing cell viability and another with a higher capacity for causing cell death.

The effects of these concentrations were evaluated as cell death (PI staining) and apoptosis (annexin staining). Our results demonstrate the usefulness of such cells for research into programmed cell death following lead and cadmium exposure.

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P15-31

Testing of lindane cytotoxicity on Chinese Hamster Ovary (CHO-K1) cell line

I. Kmetič [1], V. Gaurina-Srček [2], K. Radošević [2], J. Kniewald [1]

[1] *Faculty of Food Technology and Biotechnology, Laboratory of Toxicology, University of Zagreb, Zagreb, Croatia;* [2] *Faculty of Food Technology and Biotechnology, Laboratory for Cell Culture Technology, Applications and Biotransformations, University of Zagreb, Zagreb, Croatia*

Organochlorine insecticide lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) has been extensively used for protection of agricultural products, livestock and trees from variety of pests. Although the use of lindane has been restricted for many years, the chemical stability and earlier widespread use caused its ubiquitous presence in the environment, what enables entering the food chain. Due to its lipophilic characteristics, lindane can accumulate in various tissues of living organisms, and can easy reach essential tissues of reproductive system. It is declarated as a highly toxic compound and identified as potential endocrine disruptor. In order to determine its possible toxic effects at ovarian cellular level, in this study *Chinese Hamster Ovary* (CHO-K1; CCL-61) cell line was used. Cells grew in monolayer at 37 °C in the atmosphere of 95% air and 5% CO_2, in the medium Dulbecco's MEM with 5% newborn calf serum. After biomass production in T-bottles, cells were separated in the early logarithmic phase of growth and seeded on multiwell plates in concentration of 2×10^4 cells/mL of cultivating medium. Cytotoxic effect of lindane at the concentration range of 20–100 μg/mL was determined by colorimetric methods after 24, 48 and 72 h. IC_{50} value was determined from the slope of % inhibition versus log dose values. Cell viability and the number of cells were measured by: *Trypan Blue* exclusion method (IC_{50} 37.2 μg/mL); *Kenacid Blue R* binding method change in total cell protein (IC_{50} 80.21 μg/mL); lyzosomal activity by *Neutral Red* method (IC_{50} 52.71 μg/mL); *MTT* assay, the ability of viable cells to convert a soluble tetrazolium salt into insoluble formazan precipitate (IC_{50} 68.64 μg/mL). Application

of in vitro assays in evaluation of the toxicity should be an important contribution in elucidating cellular and molecular mechanisms of lindane.

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P15-32

Effects of vitamin E on lindane induced cytotoxicity in Chinese Hamster Ovary cell line

I. Kmetič, B. Šimić, J. Kniewald

Faculty of Food Technology and Biotechnology, Laboratory of Toxicology, University of Zagreb, Zagreb, Croatia

Chinese Hamster Ovary (CHO K1) cell line was introduced in order to reduce the expensive and regulated tests in different animal species usually used for the safety assessment of pesticides. Lindane (γ-hexachlorocyclohexane) is an insecticide with restricted permission of usage, declared as a highly toxic compound and their residues in the environment may present also a risk for the reproductive processes.

The in vitro effects of the antioxidant α-tocopherol, vitamin E, on lindane induced cytotoxicity was studied by two different bioassays: Trypan Blue exclusion method and Kenacid Blue method. The cells were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 in the medium Dulbecco's MEM supplemented with 5% newborn calf serum. The biomass was produced in T-bottles, cells were separated in the early logarithmic phase of growth and seeded on six-multiwell plates. Initial concentration of CHO cells was 2×10^4 cells/ml/well. Target cells were preincubated with two different concentrations of vitamin E (25 and 50 μg/ml) for 24 h, and then treated up to 100 μg/ml of lindane. Cytotoxicity of lindane was determined after 72 h in the presence or absence of vitamin E. The applied concentration of lindane markedly inhibited the cell growth. When target cells were preincubated with higher dose of vitamin E (50 μg/ml), the cell viability was significantly enhanced compared to the observed results in cells exposed only to lindane. These results suggest that vitamin E may exert a protective role in cell defence against lipophilic xenobiotics such as lindane.

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P15-33

The toxic effects of irinotecan on human erythrocyte AChE *in vitro*

A. Lucić Vrdoljak [1], B. Radić [1], N. Kopjar [1], D. Želježić [1], I. Milas [2], M.J. Petek [3]

[1] *Institute for Medical Research and Occupational Health, Zagreb, Croatia;* [2] *University Hospital for Tumors, Zagreb, Croatia;* [3] *Forensic Centre "Ivan Vučetić", Ministry of Interior, Zagreb, Croatia*

The best characterised function of AChE is the hydrolysis of acetylcholine (ACh) at cholinergic synapses. Inhibition of its enzyme leads to an accumulation of ACh resulting in an over-stimulation of the whole cholinergic system. Muscular and nerve AChE are only present in the synaptic cleft and cannot be measured directly. Since erythrocyte AChE has a similar structure as the synaptic enzyme, it appears to be a suitable parameter to reflect the various reactions at the synaptic site. Therefore its measurement is of high value for therapy management, especially during the course of the intoxication with different chemicals or drugs that inhibits activity of enzyme.

Irinotecan is one of the most important new anticancer drugs developed in the last few decades. It has undergone extensive clinical investigation worldwide and demonstrated potent activity against many types of human cancer, in particular, gastrointestinal and pulmonary malignancies, but also leukemia and lymphomas, as well as central nervous system malignant gliomas. The principal dose-limiting toxicity of irinotecan is diarrhoea related to a cholinergic surge from inhibition of AChE.

In our experiments, the inhibitory power (IC_{50}) of irinotecan on AChE of human erythrocytes is determined and compared with physostigmine which is tested as reference drug. IC_{50} values were 5.0×10^{-7} for irinotecan and 2.0×10^{-8} mol/l for physostigmine. Also, a continuous decrease of catalytic activity of human erythrocyte AChE was obtained 10, 30, 60, 120, 150 and 180 min after addition doses of irinotecan recommended as monotherapy in adult patients (9.0 and 4.6 μg/ml). A higher dose of irinotecan inhibited the AChE activity with potency similar to that estimated for the physostigmine, and there was a slight reduction in the inhibition of AChE by both of doses of irinotecan with increased incubation times.

In conclusion, the inhibition of AChE activity seems to be connected with administration of irinotecan. Judging from experimental in vitro data, we believe that in addition to the measurement of AChE activity, it would

be appropriate to control acute cholinergic syndrome in patients with carcinoma.

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P15-34
Effect of hydroxyurea on rat Sertoli–germ cell coculture

Mingyang Liao, Li Zhou, Chunqi Wu

Beijing Institute of Pharmacology and Toxicology, 27 Taiping Road, Haidian District, Beijing, 100850, PR China

The present study was undertaken to evaluate the cytotoxic effects of hydroxyurea (HU) in vitro employing rat testicular Sertoli–germ cell coculture. Cytotoxic changes induced by HU (0, 10^{-3}, 10^{-2}, 10^{-1} M) in mixed cultures of Sertoli and germ cells were seen at 96 h after treatment, respectively. HU led to an increase in germ cell detachment from the Sertoli cell monolayer in a dose dependent manner. Detached cells were observed in all treated groups. The viability of detached cells was decreased in 10^{-2} M and above treated groups. Substantial leakage of the cytosolic enzyme lactate dehydrogenase (LDH) was observed in the medium after 96 h HU treatment at concentrations of 10^{-2} M and above treated groups, the extent of toxicity was greater at 10^{-1} M and at 96 h after treatment. The same, 10^{-2} M HU were added to the culture, at 24, 48, 72 and 96 h after treatment, respectively, HU exposure led to an increase in germ cell detachment, the leakage of LDH, a decrease in cell viability from the Sertoli cell monolayer in a time dependent manner compared with the control culture. The extent of toxicity was greater at 96 h after treatment. These results indicate that HU leads to cytotoxicity in Sertoli–germ cell coculture and the effect of HU exposure both in vivo and in vitro was closely resembled to testicular cell.

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P15-35
In vitro toxicity study of eye irritation potential of agrochemicals

J. Tavaszi [1], P. Budai [1], G. Hirka [2]

[1] *University of Veszprém, Georgikon Faculty of Agriculture, Keszthely, Institute of Plant Protection, Department of Hygiene, Hungary;* [2] *LAB International Hungary Corporation, Hungary*

Recently, all of chemicals have to be tested before put into circulation. In this process toxicological examinations play an important role because they can show several features of the ingredients which preclude the possibility of manufacture.

Using animals in toxicological screening is a controversial issue. To get knowledge about eye irritation, recently only the in vivo Draize-test is accepted. The Draize eye irritation test is costly in terms of time and resources, uses significant numbers of animals and may be painful to the test animals.

The HET-CAM test (using the corioallantoic membrane of the chick embryo) is an alternative test to replace the Draize Rabbit Eye Test. The chick chorioallantoic membrane (CAM), being a connective tissue sheet with a visible blood supply, has been proposed as a substrate to identify the eye irritation potential of chemicals. During the test the chemicals are placed directly onto the chorioallantoic membrane. The changes of the vascular injury (haemorrhage, lysis or coagulation) are indications of the potential of the chemical to damage mucous membranes in vivo.

In these studies, 10 agrochemicals were tested. The materials were: Dezormon (2,4-D), Perenal (haloxyfop-*R*-methyl ester), Proponit (propizochlor), Talstar (bifentrin), Talendo, Sherpa (cypermethrin), Glialka (*N*-foszfonometil-glicin izopropilaminsó), and three manure: Compo, Fito Horm, Genezis.

In our experiments, a good correlation was observed between the HET-CAM assay and in vivo data. The present form of the HET-CAM test can be proposed as a pre-screen method of eye irritation tests, therefore the number of test animals can be reduced.

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P15-36
Development of a simple *in vitro* method for the screening of metabolism-induced toxicity of drugs

Marika Mannerström [1], Tarja Toimela [1], Timo Ylikomi [1,2], Hanna Tähti [1]

[1] *Medical School, Cell Research Center, University of Tampere, Finland;* [2] *Tampere University Hospital, Tampere, Finland*

Systemically administered drugs can be extensively metabolized in the liver in vivo. In some cases, the metabolites may be more toxic than the drug itself. Therefore, when testing drug effects in vitro, not only the effects of the parent compound should be considered, but also its possible metabolites.

The aim of this study was to develop a simple screening method for the evaluation of metabolism-induced (neuro)toxicity of drugs. The method involved intact

mouse hepatocytes as a metabolizing system and the neuroblastoma cell line SH-SY5Y as target cells. The test drugs were dosed on hepatocytes grown in multiwell plates or filter cups. Carbamazepine and selegiline were chosen for test drugs, because they are known to have also toxic metabolites. After 3-h exposure, the medium containing drug metabolites produced by the hepatocytes, was harvested and the target cells were exposed to it for 24 h. Finally, the viability of the target cells was assessed by using luminescence based ATP-measurement. The drug effects on the viability of hepatocytes and SH-SY5Y cells were also tested separately.

Selegiline and carbamazepine increased the viability of SH-SY5Y cells when administered directly on them, but when first "treated" with the primary hepatocytes, selegiline and carbamazepine decreased the viability of SH-SY5Y cells. In conclusion, this in vitro method using metabolically active mouse hepatocytes and target cell cultures made it possible to detect the biotransformation-dependent toxicity of drugs.

In future studies, human hepatocytes will be used in the models to improve their value in estimating the human effects of the drugs.

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P15-37
Validation of an embryotoxicology screen using zebrafish

Anita Marguerie, Clare Buckley, Angeleen Fleming

Daniolabs, UK

Mammalian organisms have been the obligatory model of toxicology research but in recent years, there has been a significant increase in the use of non-mammalian models systems driven by the desire to reduce cost and by the need to test more compounds. Indeed, large numbers of potential drugs are nowadays identified by pharmaceutical companies thanks to high throughput screening that need to be assessed for toxic properties by equally efficient methods. The zebrafish (*Danio rerio*) provides an excellent vertebrate whole organism model for studying vertebrate development since embryos are optically transparent and the morphology and physiology of the embryo is similar to mammals. Its large clutch size, and the fact that zebrafish can readily absorb chemicals from the water render this species amenable to large scale embryotoxicology studies.

We have established an embryotoxicology screen using zebrafish and have validated this assay using an 18 compound "reference set". The embryotoxicology screen involves two types of assay: a general toxicity on the larvae to establish the maximum tolerated concentration (MTC) after the embryogenesis period, and the embryotoxicity assay per se. In the latter, embryos were incubated with three concentrations up to the MTC for two separate time periods in order to observe defects at different developmental stages. We conducted our pilot study in 3 months using 18 compounds previously selected by ECVAM (European Centre for the Validation of Alternative Methods) for the validation of new embryotoxicology assays. The ECVAM study assigned these compounds into the following classes: (1) non-embryotoxic, (2) weakly embryotoxic, and (3) strongly embryotoxic, using three test systems: stem cell lines, limb bud micromass cultures, and post-implantation rat whole embryo cultures. By in vivo morphological assessment of zebrafish embryos, we were able to predict the teratogenic potential of compounds in our model with a high degree of accuracy, especially for safe compounds; 75% correct at predicting class 2 and 3 compounds and 100% correct at predicting class 1 compounds. Our model is therefore suitable to rank compounds according to their embryotoxic/teratogenic potential. In addition, the zebrafish model allows further in depth behavioural analysis following compound exposure (e.g. assessment of locomotion, vision, and hearing)—such analysis cannot be performed in any other embryotoxicology model.

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P15-38
Automated analysis of cells by machine vision—Application in toxicity studies

Tarja Toimela[1], Hanna Tähti[1], Jussi Tarvainen[2], Juha Korpinen[2], Timo Ylikomi[1]

[1] *Medical School, Cell Research Center, 33014 University of Tampere, Finland;* [2] *Chip-Man Technologies Ltd., Biokatu 12, 33520 Tampere, Finland*

In vitro cell toxicity studies are used as part of the early phase assessment of the safety of drug candidates. For testing toxicity, there are numerous endpoint assays that monitor cell proliferation, cell death, cellular metabolism or membrane permeability. All of the current endpoint methods require the use of specific labels or reagents and the tests usually permit the analysis of only one parameter in a cell population.

A change in the dynamic behavior of cells is a powerful indicator of cellular responses not easily measured with current methods. Here we have studied the possibility of using machine vision technology (Cell-IQ) to follow and automatically analyze changes in living cells during exposure to test compounds. Basic endpoint methods, neutral red uptake, Hoechst 33258 and crystal violet stainings were conducted parallel to machine vision analysis. Cell growth curves were constructed using MCF-7 cells, and toxic responses were evaluated in HK2 cells after 0–100 μM amitriptyline exposure. Cell migration and changes in neurite lengths were followed in SH-SY5Y cells.

We demonstrated that Cell-IQ machine vision analysis yielded comparable results to endpoint measurements. Furthermore, the continuous follow-up and analysis revealed such information from the whole course of the experiment as cannot be gained from endpoint measurements with just a few time points. This made it possible to create exact profiles of cell behavior during the experiments. We also found that a decrease in cell migration and changes in cell morphology (e.g. the surface area of neurites) may be sensitive indicators of cell toxicity, seldom even noticed with traditional methods.

Thus, machine vision permits novel and sensitive ways of profiling compounds based on dynamic cell behavior. Furthermore, machine vision can be used to automatically measure the kinetics of responses in living cells without interference from labels.

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P15-39
Cytoxicity of trazodone in isolated rat hepatocytes

Akram Jamshidzadeh, Hossein Niknahad, Jafar Zarifi

Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran

Trazodone is an antidepressant agent used extensively in many countries for the treatment of depression disorders. Several cases of hepatotoxicity is reported with the use of even low therapeutic doses of trazodone, starting few days to weeks after start of the usage of the drug. However, the mechanism(s) of its hepatotoxic effect is not known. The purpose of this study was to investigate the cytotoxic mechanism of trazodone.

Cytotoxicity was studies using freshly isolated rat hepatocytes incubated in Krebs–Henseleit buffer under a flow of 95% O_2 and 5% CO_2. Cytotoxicity was assessed by trypan blue exclusion test. Glutathione and lipid peroxidation were measured by colorimetric methods and ATP of hepatocytes were measured by HPLC.

Trazodone was toxic towards hepatocytes and caused cell death with an ED50 of about 0.45 mM for 2 h. Its active metabolite *m*-chlorophenylpiperazine (mCPP) was less toxic with an ED50 of 0.75 mM for 2 h. The events before cell death were rapid GSH depletion, lipid peroxidation, and ATP depletion. Depleting hepatocytes GSH beforehand increased their cytotoxicity. Cytochrome P-450 inhibitors, piperonyl butoxide and metyrapone decreased cytotoxicity of trazodone but slightly increased cytotoxicity of mCPP. Another P-450 inhibitor troleandomycin prevented cytotoxicity of trazodone but slightly affect that of mCPP. Antioxidants and ATP suppliers slightly prevented cytotoxicity of both trazodone and mpp.

Trazodone and its metabolite mCPP are toxic towards rat hepatocytes, however, preventing its metabolism to mCPP, its main metabolite by specific P-450 inhibitors decreases its toxicity suggesting that a major part of its toxicity is mediated by its metabolite mpp and its further metabolites. Depletion of antioxidant defense system is involved in the mechanism of cytotoxicity.

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P15-40
In vitro effects of trichothecene metabolites on human hematopoietic progenitors

R. Josse, N. Hymery, Y. Sibiril, P. Batina, Dominique Parent-Massin

Food Toxicology Laboratory, EA 3880, ESMISAB, West Brittany University, Technopôle Brest-Iroise 29280 Plouzané, France

Trichothecenes mycotoxins are food contaminants, mainly produced by *Fusarium* sp. The aim of this work was to compare the in vitro effect of trichothecene metabolites (HT-2 and T-2 triol; 3AcDON and 15AcDON) on human haematopoietic progenitors $CD34^+$ cells to myelotoxic effects induced by T-2 toxin and DON.

Two aspects have been explored: direct myelotoxicity using CFU-GM clonogenic and capacity of trichothecenes metabolites to induce apoptosis of human cord blood hematopoietic progenitors ($CD34^+$ cells).

Tested metabolites present a myelotoxic effect, the IC 50 were equal to 6.5×10^{-8}, 1×10^{-9} M, for T-2 triol, HT-2, and 7.5×10^{-7}, 3.4×10^{-7} M 3AcDON and 15AcDON respectively.

According to results in Hoechst coloration, DNA fragmentation and annexin-V/PI labeling in flow cytometry T-2 triol, HT2, 3AcDON, 15AcDON induced apoptosis in human CD^{34+} progenitors. There effects were dose- and time-dependent with a significant increase of apoptotic cells 12 h after incubation at 10^{-6} M for T-2 triol and HT2 and 15 h after incubation at 6.10^{-7} M or $7.4.10^{-7}$ M for 15AcDON and 3AcDON respectively. It has been observed that in presence of a pan-caspase inhibitor the trichothecene metabolites-induced apoptosis is cancelled, suggesting the involvement of caspases in this phenomenon.

These results suggest that trichothecenes metabolites such HT-2 and T-2 triol, 3AcDON and 15AcDON, could induce myelotoxic effects and haematological troubles as well as T-2 toxin and DON in case of T-2 toxin and DON intoxication.

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P15-41

Toxicity of oxide nanoparticles and carbon nanotubes on cultured pneumocytes: Impact of size, structure and surface charge

A. Simon [1], C. Thiebault [1], C. Reynaud [2], B. Gouget [1], M. Carriere [1]

[1] *Laboratoire Pierre Süe, CEA/CNRS UMR 9956, CEA Saclay 91191 Gif-sur-Yvette, France;* [2] *Laboratoire Francis Perrin, CEA/CNRS URA 2453 CEA Saclay, 91191 Gif-sur-YVette, France*

The development of nanotechnologies leads to increased nano-objects release in the environment and potential risks to human population and ecosystems. Few studies already assessed biological effects of these highly reactive materials. The first organs that are subject to exposure are either the skin in case of direct contact or the lung if the nano-object is inhaled. In both cases, it is important to study their physico-chemical comportment (aging, aggregation, dissolution, ...), the kinetics of cellular adsorption or absorption, and the reaction of the living cell to the contact (toxicity or resistance).

This work focuses on biological effects of some oxide nanoparticles including TiO_2, Al_2O_3 and ZnO and multiwall carbon nanotubes (CNT). It was done in a view to determining the influence of size, shape and chemical composition of the nano-object. Biological studies were completed on A549 lung carcinoma human cells, taken as a model for lungs exposed by inhalation.

A particular attention was put on their stability in exposure medium and on the evolution of their size distribution before and after cells exposure. Moreover, their physico-chemical properties (surface charge and area, shape) were strictly controlled during the experiment. Finally cytotoxicity indexes (CI_{50}), i.e. concentrations of nano-object leading to 50% cell death were defined for each type of nano-objects. The first results show that under our conditions and for cells exposed to a same concentration of nano-objects, their cytotoxicity can be classified from least to most toxic as follows: $Al_2O_3 < TiO_2 <$ CNT.

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P15-42

From skin corrosion to skin irritation: Epidermal Skin Test 1000 (EST-1000)—A reliable tool for hazard identification *in vitro*

Jens J. Hoffmann, Eckhard Heisler, Jan Losse, Diemo Thomas, Horst W. Fuchs

CellSystems Biotechnologie Vertrieb GmbH, R&D, St. Katharinen, Germany

The reliability of skin corrosivity testing using the reconstructed epidermis Epidermal Skin Test (EST-1000) was shown already in different studies. The results of those blinded multicenter trials fulfilled all criteria of OECD TG 431: "In Vitro Skin Corrosion: Human Skin Model Test". The findings include the intact barrier function which was confirmed by the resistance against several solutions of detergents over a long period and reliable classification of all 12 reference compounds mentioned in the guideline. To support our data, the proper morphology of EST-1000 is demonstrated by an extensive histological and immunohistochemical characterization.

Additionally, we present first data on EST-1000 used for the more subtle task of skin irritation testing. Dermal irritation is generally defined as "the production of reversible inflammatory changes in the skin". The potential of chemicals to induce skin irritation has to be taken into account when establishing procedures for the safe handling, packing and transport of chemicals. Up to now, classification of irritants is usually performed in vivo by the Draize rabbit test. But the skin irritating potential of a substance may be predicted by in vitro systems. These are sufficiently complex to mimic human skin barrier and cell reactivity. As shown by this study, EST-1000 was able to correctly classify a large number of tested compounds in vitro according to EU classification system (R 38 or no label). With these data, we demonstrate the adaption of EST-1000 to an actual demand of haz-

ard identification. With respect to in vivo tests, this adaptability is one major advantage of in vitro skin models.

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P15-43

Matrix metalloproteinase (MMP) expression in the EpiDerm-FT skin equivalent: Relevance to dermal wound healing and blistering skin diseases

Patrick J. Hayden, Carolyn Cooney, Gina Stolper, Mitchell Klausner

MatTek Corporation, Ashland, MA, USA

Matrix metalloproteinases (MMPs) play an important role in dermal wound healing, tissue remodeling, blistering diseases, tumor invasion and metastasis. In the current work, the expression of various MMPs was evaluated in a full-thickness in vitro human skin equivalent, EpiDerm-FT™ (MatTek Corporation). Normal human epidermal keratinocytes (KC) and dermal fibroblasts (FB) were cultured to produce the highly differentiated full-thickness skin equivalents. Histologic examination of EpiDerm-FT shows a collagen dermis populated by viable FB and an epidermis of stratified KC including basal, spinous, granular and stratum corneum components. Examination of the dermal–epidermal junction by transmission electron microscopy (TEM) and immunohistochemistry revealed a well-developed basement membrane zone. Expression of MMP genes in EpiDerm FT and its separated dermal and epidermal components was evaluated by RT-PCR. Prior to addition of epidermal KC, contracted collagen matrices containing dermal FB expressed significant amounts of MMP-1, MMP-2, MMP-11 and MMP-14. Following addition of the epidermal component to produce the full-thickness skin equivalent, MMP-3, MMP-7, MMP-9 and MMP-10 expression was increased. Subsequent separation of the dermal and epidermal components revealed that the epidermal KC contributed significant amounts of MMP-1, MMP-3 and MMP-11. The dermal component contributed a relatively greater amount of MMP-2 and MMP-7. Additionally, expression of MMP-7, MMP-9, MMP-10 and MMP-11 was significantly elevated in the human skin equivalent compared to monolayer KC and FB cultures. The results demonstrate that KC-FB and matrix interactions significantly influence expression of MMPs in the in vitro human skin equivalents. EpiDerm-FT represents an important tool for elucidation of KC-FB and matrix interactions related to MMP expression, wound healing, blistering skin diseases and matrix remodeling.

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P15-44

Drug/xenobiotic-metabolizing enzyme (XME) expression in the EpiAirway *in vitro* human airway model: Utility for assessing tracheal/bronchial biotransformation of inhaled pharmaceuticals and environmental chemicals

Jennifer Bolmarcich, Gina Stolper, George R. Jackson, Mitcehll Klausner, Patrick J. Hayden

MatTek Corporation, Ashland, MA, USA

Human tracheal/bronchial epithelium contains xenobiotic metabolizing capabilities provided by a variety of phase I (oxidative) and phase II (conjugative) enzyme systems. These XMEs can play an important role in biotransformation of inhaled drugs, tobacco smoke and environmental/occupational chemicals. Biotransformation of inhaled chemicals may lead to altered drug activity or formation of toxic/mutagenic metabolites. The present work evaluated expression of XMEs in a highly differentiated in vitro model of human tracheal/bronchial epithelium (EpiAirway, AIR-100) that is cultured at the air–liquid interface to facilitate in vivo-like exposure to chemicals. RT-PCR gene expression experiments were conducted to evaluate baseline and inducible expression of CYP isoforms in EpiAirway cultures derived from four individual donors. CYP1A1 (weak), CYP1B1, CYP2A6, CYP2B6 (weak), CYP2C8 (weak), CYP2C19, CYP2D6, CYP2E1 and CYP3A5 were expressed constitutively, while CYP3A4 and CYP3A7 were not detected. 3-Methylcholanthrene (3MC) strongly increased expression of CYP1A1 and slightly increased CYP2B6 and CYP2C8 expression. Thus CYP expression in EpiAirway showed a high concordance with CYP expression reported for in vivo human bronchial epithelium. Total GST activity in EpiAirway was also evaluated by measuring conjugation of glutathione with 1-chloro-2,4-dinitrobenzene. High baseline GST activity was not further enhanced by 3MC treatment. The results demonstrate that the EpiAirway in vitro human tracheal/bronchial epithelial model possesses numerous in vivo-like XME activities and thus will likely be useful for evaluating airway metabolism of drugs, tobacco smoke and environmental/occupational chemicals.

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P15-45
Irritation testing of contraceptive and feminine-care products using epivaginal™, an in vitro human vaginal–ectocervical tissue model

Seyoum Ayehunie, Joseph Kubilus, Patrick Hayden, Chris Cannon, Sarah Lamore, Mitchell Klausner

MatTek Corporation, Ashland, MA, USA

Normal human vaginal–ectocervical (VEC) cells were cultured to reconstruct VEC tissues in vitro (designated as VEC-100). In some cultures, normal human dendritic cells were also incorporated (designated as VLC-100-FT). Both tissues mimic native in vivo tissue in that they have basal, parabasal, glycogenated intermediate, and the superficial cell layers. To test the utility of the VEC tissue, contraceptives, microbicides, anti-itch agents, and other vaginal-care products (VCP) were topically applied. To mimic heterosexual HIV infection, the VLC tissue was topically exposed to HIV-1 viruses. Quality control (QC) testing on each batch of tissue utilized Triton X-100 (1%) and water as positive and negative controls, respectively. The MTT assay was used to determine the exposure time necessary to decrease the tissue viability to 50% (ET-50) for the positive control and 18 VCP. QC testing showed the tissues to be highly reproducible; the average intra-lot coefficient of variation was <10% and ET-50s averaged 1.26 h ± 0.23 ($n = 25$ lots). The VEC tissue model discriminated between the mildness of VCP. The ET-50 values ranged between 1.7 and 2.7 h for feminine washes, 3.5–7.0 h for contraceptives, 6.9 to >18 h for anti-itch creams, and were >18 h for douches, lubricants, and anti-fungal creams. Released cytokines and gene expression levels showed that IL-1α, IL-1β, and IL-8 were associated with toxicity of VCP. Finally, the VLC tissue was infectible with macrophage-tropic and T-cell tropic HIV-1 strains as evidenced by DNA-PCR. Based on these results, it is likely that the VEC tissue model will serve as a useful, highly reproducible, non-animal tool to assess the irritation of VCP. In addition, the VLC tissue will enable studies pertaining to HIV infection, microbicides and drug absorption.

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P15-46
Long term reproducibility of EpiOcular™, a three-dimensional tissue culture model of the human corneal epithelium

John E. Sheasgreen, Joseph Kubilus, Patrick J. Hayden, Mitchell Klausner

MatTek Corporation, Ashland, MA, USA

The EpiOcular tissue model (OCL-200) is an organotypic model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. Paraffin embedded, H&E stained histology cross-sections show the structure of EpiOcular closely parallels that of the HCE; large nucleated basal cells lie beneath 5–6 stratified cell layers which progressively flatten out, ending with a highly squamous, non-keratinized layer at the tissue's apical surface. Since commercial introduction in 1995, EpiOcular has been increasingly used by many personal care and household product companies to determine the ocular irritancy of their products without using animals. Currently, validation of the EpiOcular model as a replacement for the Draize rabbit eye test is underway in the US. In addition, an ECVAM objective for 2006 is to issue a report on the use of EpiOcular for prediction of ocular irritation. For commercial and regulatory purposes, it is very important to know that the model is reproducible both within a given lot and between lots, especially over extended periods. Quality control of weekly batches of EpiOcular is performed using the MTT assay, which historically has been the in vitro endpoint of choice for European and US regulators. The time needed to reduce EpiOcular viability to 50% (ET-50) after 0.3% Triton X-100 exposure is determined. Yearly average ET-50 values have ranged from 20.6 min (2000) to 25.0 min (1998). The coefficients of variation (CV) for the negative control tissue (exposed to ultrapure H_2O) have averaged under 6% for every year since 1997. In addition, the yearly average CV for all tissues has never exceeded 6.5%. These results over the past 9 years of commercial production show EpiOcular to be a highly reproducible, stable toxicological model that is ideally suited for industrial and regulatory ocular irritancy studies.

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P15-47

Use of plasmacytoid dendritic cells in screening allergenicity of chemicals

Seyoum Ayehunie, Patrick J. Hayden, Sarah Lamore, Maureen Snell, Mitchell Klausner

MatTek Corporation, Ashland, MA, USA

A predictive test system for assessing the allergenicity potential of chemicals will have enormous significance in industries involved in cosmetic, personal hygiene and topical medication products and in the fields of dermatology and immunology. Since dendritic cells/Langerhans cells (DC/LC) are the first cells responsible for sampling skin and mucosal surfaces for changes in the antigen microenvironment, we investigated whether phenotypic and functional changes to subset of DC, plasmacytoid dendritic cells (pDC), could be used to identify allergens. To achieve this goal, normal human DC were generated from CD34+ progenitor cells and were cryopreserved. Frozen DC were thawed and the pDC fraction (CD123+/CD11c−) was collected using FACS sorting. The pDC were cultured, expanded, and pulsed with chemical allergens ($n = 12$) or irritants ($n = 7$). Results showed that exposure of pDC ($n = 3$ donors) to allergens induced an increase ($\geq$1.5-fold) in surface expression of CD86 for 11 of the 12 allergens; however, 6 of 7 irritants did not result in increased CD86 expression. Based on these findings, a prediction model was developed with a sensitivity of 92%, specificity of 86%, and an accuracy of 89%. Increased levels of released IL-6 were also detected in culture supernatants of allergen-pulsed pDC (6 of 6) but not from cultures of non-allergen-treated pDC. In conclusion, the use of CD86 expression on 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.

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P15-48

Xenobiotic metabolizing capabilities of the EpiDerm in vitro human skin equivalent: Utility for assessing dermal biotransformation of pharmaceuticals and environmental chemicals

Patrick Hayden [1], Jennifer Bolmarcich [1], Gina Stolper [1], Ting Hu [2], Marilyn Aardema [2], Rodger Curren [3], Mitchell Klausner [1]

[1] *MatTek Corporation, Ashland, MA, USA;* [2] *Procter and Gamble Company, Cincinnati, OH, USA;* [3] *Institute for In Vitro Sciences, Gaithersburg, MD, USA*

The EpiDerm in vitro human skin equivalent has shown utility as an alternative to animal tests for assessment of skin corrosivity, irritation, and photoxicity. Efforts are now underway to expand the use of the model for skin genotoxicity screening. Human skin contains xenobiotic metabolizing capabilities provided by a variety of phase I (oxidative) and phase II (conjugative) enzyme systems. These XMEs can play an important role in biotransformation of topically exposed chemicals, leading to formation of mutagenic metabolites. The present work evaluated expression of XMEs in EPI-200. Affymetrix gene expression microarrays were utilized to compare XME gene expression in EpiDerm to that of excised human skin. Numerous XMEs including cytochrome P450s (CYPs), epoxide hydrolases, flavin-containing monooxygenases, *N*-acetyltransferases, glutathione peroxidases, glutathione *S*-transferases (GST) and UDP glycosyltransferases were detected, with a high concordance between specific XMEs expressed in the ex vivo skin and the EpiDerm model. Additional RT-PCR gene expression experiments were conducted to evaluate baseline and inducible expression of CYP isoforms in EpiDerm cultures. 3-Methylcholanthrene (3MC) and β-naphthoflavone (βNF) strongly increased expression of CYP1A1 and CYP1B1, and slightly enhanced expression of CYP2C19, CYP2D6, CYP3A4 and CYP3A5. Enhanced metabolism of the CYP1A1 and CYP1B1 substrate ethoxyresorufin confirmed increased activity following treatment with 3MC or βNF. GST and UDP glycosyltransferase activity in EpiDerm was also evaluated by measuring conjugation of glutathione with 1-chloro-2,4-dinitrobenzene, and UDP with 4-methylumbellipherone, respectively. High baseline GST and UDP glycosyltransferase activity was not further enhanced by 3MC or βNF treatment. The results demonstrate that the EpiDerm in vitro human skin equivalent possesses numerous in vivo-like XME activities and thus will likely be useful for evaluating der-

mal metabolism of drugs, cosmetics and environmental chemicals.

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P15-49

In silico technology for identification of potentially toxic compounds in drug discovery

Pranas Japertas, Remigijus Didziapetris, Alanas Petrauskas

Pharma Algorithms, Toronto, Ontario, Canada

This study presents a computational analysis and derivation of endpoint-specific predictive models based on toxicity data of several types: acute toxicity (mouse and rat LD50), genotoxicity (Ames test) and organ-specific health effects (based on diverse animal and human studies). The datasets were collected from a number of public databases, reviews and original publications. The data were then 'cleaned' by removing many suspicious values. At the next step, classification and quantitative structure–activity analyses of the data for each toxicity endpoint were performed. This work is an attempt at stepwise identification of unknown effects using simple descriptors to facilitate chemical explanations of toxicity. The resulting predictive toxicity models supplement or replace various pre-defined filters of 'alert substructures' that ignore the dependence of toxicity on substituent effects. In drug discovery these tools can help prioritize in vitro measurements and estimate animal toxicity, although multiple data gaps in the training sets restrict their usefulness. A partial solution to this problem is the calculation of 95% confidence intervals (or continuous probabilities) which indicates toxicological similarity of a given compound to the training set. If a compound is not too dissimilar, 'hazard substructures' can be automatically generated, thus suggesting possible mechanistic explanations and structural modifications of a lead compound. The best solution however is to develop new predictive algorithms based on company-specific data, and the new analytical and development software tools that can help do this are described in the study.

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P15-50

REPDOSE: A database on repeated dose toxicity studies of commercial chemicals

A. Bitsch [1], S. Jacobi [2], C. Melber [1], U. Wahnschaffe [1], N. Simetska [1], I. Mangelsdorf [1]

[1] *Fraunhofer Institute of Toxicology and Experimental Medicine, Department Chemical Risk Assessment, Germany;* [2] *Degussa AG, Fine Chemicals, Chemicals Safety Management, Germany*

Within the framework of the Long-range Research Initiative of the chemical industry, we developed a database for repeated dose toxicity studies. This database focuses mainly on the analysis of possible structure–activity relationships (SARs), i.e. the influence of structural features on target organs, effects and LOELs/NOELs after repeated dosing. Moreover, it can be used as a helpful tool for a series of non-SAR, general toxicological questions.

Content of the database: The database consists of three core data sets for each chemical: (1) structural features and physico-chemical data, (2) data on study design, and (3) study results including overall NOELs/LOELs and all effects in target organs with corresponding LOELs.

Data were taken mainly from review documents or peer-reviewed risk assessments in order to get a prescreened selection of valid data. The sources of all data are evident to the user. Chemicals were chosen by structure.

Standardization and query options: Glossaries of usual chemical and toxicological termini have been developed to achieve a high degree of standardization, thereby supporting comfortable query options.

Present status: At present, the database consists of 405 chemicals investigated in 1021 studies which resulted in a total amount of more than 6000 specific effects.

Examples of application: Test queries addressing different endpoints have been performed with the database. Most chemicals affect, at least at high doses, liver, kidney or body weight. Associations between certain chemicals/chemical substructures and other less frequently affected target organs have been demonstrated as well. Further analyses refer to the influence of study duration on LOELs or show differences in sensitivity between rats and mice. These queries have shown that the database is a valuable and multifunctional tool.

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P15-51
Cytotoxic effects of resveratrol on macrophages and T cells

Blase Billack, Vijayalaxmi Radkar, Diane Hardej

College of Pharmacy and Allied Health Professions, St. John's University, Queens, NY, USA

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring phytoalexin. In mammalian systems, resveratrol has been found to exhibit antiproliferative and anti-inflammatory properties. The present study has evaluated the effect of resveratrol treatment on leukocyte proliferation, specifically transformed mouse macrophages (RAW 264.7) and tumor-derived human T cells (CEM). Whereas a low concentration (5 μM) of resveratrol stimulated the proliferation of both types of cell compared to untreated cells, higher concentrations (25 and 50 μM) were inhibitory. The effect of resveratrol on the viability of both cell types was also measured using a MTT assay. Significant reduction of cell viability was observed after 48 h at the 25 and 50 μM concentrations. Light and scanning electron microscopic examination for both cell types with 50 μM resveratrol treatment (48 h) confirmed viability studies. To identify if production of reactive oxygen species (ROS) may be involved with resveratrol toxicity, the role of the cellular antioxidant glutathione (GSH) was investigated. Macrophages were incubated with resveratrol (5–100 μM), with and without pretreatment with 50 μM buthionine sulfoximine (BSO), a GSH-depleting agent. Macrophages pretreated with BSO demonstrated significantly greater toxicity (LC_{50} = 20 μM; 48 h) when compared to macrophages treated with resveratrol alone (LC_{50} = 50 μM; 48 h), suggesting that ROS production may account for, at least in part, the cytotoxicity seen with this agent. The response of stimulated and unstimulated macrophages to resveratrol was also evaluated. To this end, macrophages were treated with or without resveratrol (0–100 μM; 48 h) in the presence or absence of lipopolysaccharide (LPS, 500 ng/ml), a potent macrophage immunogen. Macrophages were less sensitive to the toxicity of resveratrol in the presence than in the absence of LPS (LC_{50} > 50 μM). The present results suggest that, in vitro, resveratrol is toxic to T cells and unstimulated, but not immunostimulated, macrophages and that the depletion of intracellular GSH contributes to the toxicity.

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P15-52
Cytochrome P450 expression in regenerating liver of rats exposed to electromagnetic fields

R. Wiaderkiewicz [1], P. Czekaj [1], A. Wiaderkiewicz [1], J. Karpowicz [2], K. Gryz [2], K. Miranowicz [2]

[1] *Department of Histology, Medical University of Silesia, Katowice, Poland;* [2] *Central Institute for Labor Protection, Warszawa, Poland*

Liver is an organ involved in biotransformation and detoxication of many endo- and exogenous compounds. It has also a tremendous regenerative capacity. Expression of cytochrome P450 isoforms is important in determining the biotransforming potential of the liver regenerating after chemical insult, and especially the potential of undifferentiated cells. It may also have important implications in characterization of repair processes and therapies of different pathophysiological states of the liver. In the presented study, we analyzed the expression of CYP1A1, CYP2B1/2, CYP2E1 and CYP3A1 isoforms in rats exposed to occupationally permissive levels of electromagnetic fields (50 Hz, 1 mT), during liver regeneration in the conditions of experimental carcinogenesis. The liver regeneration was induced by use ad libitum CDE diet for the period of 3 weeks. The expression of analyzed CYP isoforms was assessed on mRNA level by RT-PCR method and on the level of protein by immunoblotting, with the use of commercially available primers and antibodies. Morphological analysis of the liver slices were performed by light microscopy (H–E staining) and ABC immunohistochemical method was used to visualize the markers of oval cells and hepatoblasts (α-fetoprotein, Thy-1.1, CD34, cytokeratins—CK18, CK19) and identification of cytochrome P450 isoforms.

The results show that the exposure of experimental animals to magnetic field in the conditions of performed experiment has little effect on regenerative capacity of the liver. However, it affects the expression of analyzed CYP450 isoforms what may influence the biotransforming potential of the liver during regeneration.

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P15-53

Effect of Rosmarinic acid on puromycin aminonucleoside induced nephrosis in rats

Jingwei Tian, Wanglin Jiang, Jian Hou, Haixia Wu

School of Pharmacy, Yantai University, Yantai, Shangdong, PR China

Recent studies indicate that excessive production of oxidants plays a role in the pathogenesis of glomerular injury leading to proteinuria in patients with minimal-change nephrotic syndrome (MCNS). Rosmarinic acid (RosA), frequently found as a secondary metabolite in herbs and medicinal plants, has exhibited antioxidative and anti-inflammatory activities. We studied whether RosA would be beneficial in patients with MCNS by its antioxidant activity and examined its effect on proteinuria in nephrosis induced by puromycin-aminonucleoside (PAN) in rats. Forty-eight Wistar–Kyoto rats injected with PAN were assigned to four groups: group 1, without Rosmarinic acid ($n = 12$); group 2, concomitant Rosmarinic acid injection from 1 day prior to PAN administration ($n = 12$); group 3, concomitant Rosmarinic acid injection from 1 day after PAN administration ($n = 12$); group 4, concomitant Rosmarinic acid injection from 3 days after PAN administration ($n = 12$). Daily urinary excretions of protein and 8-hydroxy-2′-deoxyguanosine (8-OHdG), a new sensitive marker of oxidative DNA damage in vivo, were measured in each group from the 1st to the 30th day after PAN injection. In group 1, proteinuria developed from the 5th day and reached the peak level on the 9th day. In groups 2–4, proteinuria did not appear until the 6th day. The excretions in urinary protein and 8-OHdG were significantly lower in groups 2–4 than group 1 on days 5, 9, and 25. In conclusion, Rosmarinic acid could delay and ameliorate the urinary protein excretion in accordance with the urinary 8-OHdG excretion in PAN-induced nephrosis.

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P15-54

***In vitro* studies on mesenchymal stem cells of a dry birch tree bark extract**

Cristina A. Dehelean [1], C. Tatu [1], Cîntă-Pînzaru Simona [2], Peev Camelia [1], Soica Codruta [1], Tanasie Gabriela [1], S. Anghel [1]

[1] *Department of Toxicology, University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Pharmacy, P-ta Eftimie Murgu, nr.2, 300041 Timisoara, Romania;* [2] *University Babes Bolyai, Cluj-Napoca, Faculty of Physics, Molecular Spectroscopy Department, Kogalniceanu 1, RO 400084 Cluj-Napoca, Romania*

The aim of this study was to evidence the therapeutic/toxic activity of a dry outer bark birch tree extract (*Betula pendula Roth*) individually or mixed with 2-hidroxypropyl-β-cyclodextrin, on mesenchymal stem cells. Vibrational FT-IR and FT-Raman spectroscopy has been performed in order to characterize the birch extract and cyclodextrin molecular species. The vibrational analysis has been correlated with the preliminary solubility test. Extract analysis was also performed by gas chromatography coupled with mass spectrometry (GC/MS), using a non-derivatization method. In vitro toxicologic analysis was performed by a standardized colorimetric MTT assay. Our results are in accordance with other in vitro tests showing the cytotoxic activity of birch tree extracts on MCF-7, A-431 and HeLa cell lines.

Also, our data proves an increase of the bioavailability for the dry extract and cyclodextrin mixture as reflected by its higher cytotoxic activity on mesenchymal stem cells, compared to the extract administered alone.

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P15-55

Effects of *S*-adenosylmethionine and tobacco smoke condensate on primary lung cells

Michaela Jambor, Felix Glahn, Tina Roder, Heidi Foth

Institute of Environmental Toxicology, Halle/Saale, Germany

One important factor in carcinogenesis is the hypermethylation of tumor suppressor genes by yet unknown factors. This leads to an epigenetic inactivation of these genes without alterations in DNA sequence. Therefore, it is necessary to find out which substances are able to influence the hypermethylation of the tumor suppressor genes. Our candidates are *S*-adenosylmethionine (SAM) and tobacco smoke condensate (total particulate matter, TPM).

SAM is the natural methylene group donor of the cells used by methyltransferases.

To investigate whether the treatment of cells with these two substances leads to a hypermethylation of the tumor suppressor genes it is important to find the right concentration for treatment. Therefore, we made the resazurin viability test to find the concentration at which more than 80% of the cells are vital. For SAM and TPM we found 50 μmol/l and 1 mg/l, respectively.

We treated primary normal human bronchial epithelial cells and primary human peripheral lung cells with these two substances. The tissue we made our cell culture from was obtained from lung cancer patients undergoing a lung resection. Parts of healthy lung and bronchial tissue were taken additionally to the tumor. For making cell culture from tissue we cut the tissue in little pieces and put them in airway epithelial cells growth (AECG)-medium. After one week the cells grow out and were taken for experiments.

To investigate the effects of SAM and TPM on methylation of tumor suppressor genes we treated the cells with these two substances for 72 h. Subsequently the cells were splitted and DNA was isolated from one part of the cells, the others were reseeded. These cells could grow up to 40% confluency and were treated for 72 h again. This treatment is repeated until the end of the culture.

The isolated DNA is treated with bisulfite in order to distinguish methylated from unmethylated DNA and a subsequent methylation specific PCR (MSP).

Genes of interest are the tumor suppressor genes RASSF1A, p16 and DAPK.

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P15-56

Comparison of toxic effects produced by natural blooms, cyanobacterial cultures strains and pure Microcystins in two fish cell lines

Silvia Pichardo [1], Angeles M. Jos [1], Jorge L. Zurita [2], Isabel M. Moreno [1], Manuel Salguero [2], Ana Ferrer-Dufol [3], Ana M. Cameán [1], Guillermo Repetto [1]

[1] *University of Seville Seville Spain;* [2] *National Institute of Toxicology and Forensic Science, Seville, Spain;* [3] *Toxicology Unit, Clinic University Hospital, Zaragoza, Spain*

Contamination of nature by cyanobacterial blooms is a worldwide problem, causing serious water pollution and public health hazard to humans and livestock. The intact cells as well as the toxins released after cellular lyses, especially Microcystins (MCs), can be responsible of the observed toxic effects in both animals and humans, being actually associated with fish kills.

In the present study, two fish cell lines, PLHC-1 derived from a hepatocellular carcinoma in the topminnow *Poeciliopis lucida*, and RTG-2 fibroblast-like cells derived from the gonads of rainbow trout *Oncorhynchus mykiss*, were observed after treatment with two cyanobacterial algae and two pure cyanobacterial toxins (MC-LR and MC-RR). The effects of different concentrations of these toxins at 24 h of exposure were investigated by morphological observation and biochemical changes (total protein content, lactate dehydrogenase leakage, neutral red uptake and MTS metabolization).

The results obtained showed that the higher toxicity was observed after exposure to the cyanobacterial strains culture. Morphological changes produced by MCs were rounding, blebbling, shrinking and reduction in the cell number. In addition, a higher sensitive was experiment by PLHC-1 cells compared to RTG-2 cells.

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P16 Natural Toxins

P16-01

Efficacy of royal jelly against fumonisin-induced oxidative stress in rats

Mosaad A. Abdel-Wahhab [1], Aziza A. El-Nekeety [1], Naglaa F. Abbas [2], Wafaa El-Kholy [3], Hassan A. Amra [1], Ahmed Ebaid [3]

[1] *Food Toxicology & Contaminants Department, National Research Center, Dokki, Cairo, Egypt;* [2] *Pathology Department, National Research Center, Dokki, Cairo, Egypt;* [3] *Zoology Department, Faculty of Science, Mansoura University, Egypt*

Fumonisins (FB) are mycotoxins produced by *Fusarium verticillioides*, frequently associated with corn. It produces species-specific and organ-specific toxicity, including teratogenicity, equine leukoencephalomalacia, porcine pulmonary edema, and hepatic or renal damage in most animal species and perturb sphingolipid metabolism. The aim of the present study was to evaluate the protective effects of royal jelly (RJ) against the oxidative stress of FB. Sixty male Sprague–Dawley rats were divided into sex treatment groups including the control group; group fed FB-contaminated diet (200 mg/kg diet) and the groups treated orally with RJ (100 mg/kg or 150 mg/kg body weight) with or without FB for three

weeks. FB alone resulted in a significant decrease in body weight gain, feed intake, GPX and SOD. Whereas it caused a significant increase in ALT, AST, triglycerides, cholesterol, HDL, LDL, createnine and uric acid levels. Animals received FB showed severe histological and histochemical changes in liver and kidney tissues. Cotreatment with FB plus RJ resulted in a significant improvement in all the tested parameters and the histological and histochemical pictures of the liver and kidney. These improvements were pronounced in animals fed FB-contaminated diet plus the high dose of RJ. It could be concluded that RJ have a protective effects against FB toxicity and this protection was dose dependent.

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P16-02

Protective effect of Tunisien phyllosilicate clay against Zearalenone-induced toxicity in mice with special reference to chromosomal aberrations

Samir Abbès [1], Jalila ben Salah-Abbès [1], Nahdi Kais [2], Ridha Oueslati [1], Omar Othman [1]

[1] *Laboratory of Immunology, Microbiology, Environmental and Cancerology, Faculty of Sciences Bizerte-7021-Zarzouna, Tunisia;* [2] *Laboratory of Mineral Physico-chemical, Faculty of Sciences Bizerte 7021-Zarzouna, Tunisia*

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin present in corn, as well as food mixture for farm animals. ZEN was associated with hyperestrogenism and several physiological alterations of the reproductive tract in several laboratory animals. The objective of the current study is to evaluate the protective role of phyllosilicate caly (PC) collected from the Tunisian environment against ZEN-induced biochemical changes and chromosomal aberration in balb/c mice. Forty-eight female balb/c mice were divided into eight treatment groups included the control group, the olive oil group, the groups treated with PC alone at three doses (400, 600 and 800 mg/kg b.w.) and the group treated with ZEN (40 mg/kg b.w.) plus PC (400 mg/kg b.w.). All animals were received their respective treatment in a single dose. Blood samples and bone marrow were collected 48 h after the dosing. The results indicated that ZEN induced severe biochemical changes in all biochemical parameters tested as well as increase the chromosomal aberration in bone marrow. PC at the different tested doses had no significant effects on all the tested parameters. The combined treatment showed power protective effects of PC against ZEN. It succeeded to restore all the parameters to the normal values of the controls and significantly decreased the number of chromosomal aberration resulted from ZEN. It could be concluded that the Tunisian Clay is effective in the prevention of ZEN and may be useful with other mycotoxins.

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P16-03

Antioxidant and radical scavenging effects of Tunisian radish (*Raphanus sativus*) extract against oxidative stress of Zearalenone in Balb/c mice

Jalila Ben Salah, Jalila Ben Salah, Samir Abbès, Chemli Rachid, Ridha Oueslati, Omar Othman

Faculty of Dentistry, Monastir, Tunisia

Zearalenone is a Fusarium mycotoxins, it was shown to be hepatotoxic, haematotoxic, immunotoxic, nephrotoxic and genotoxic. The present study was conducted to evaluate the protective role of radish extracts against ZEN-induced toxicity and free radical generation in mice. Forty-two mature male Balb/c mice were randomly assigned to eight experimental groups included the control group, olive oil group, the groups treated with radish extract alone (200, 400 and 600 mg/kg bw), the group treated with a single dose of ZEN (40 mg/kg bw) and the group treated with ZEN plus the lowest dose of radish extract. Blood, liver and kidney samples were collected from all animals 48 h after dosing. The results indicated that ZEN treatment significantly increased ALT, AST, ALP, BILT, BILD, CRE, MDA in liver and kidney, significantly decrease the hepatic and renal GSH and SOD and induced severe histological changes in liver and kidney tissues. Radish extract alone had no significant effects on serum biochemical parameters whereas, it increased GSH and SOD and decrease MDA in liver and kidney. Animals treated with ZEN plus radish extract showed a significant improvement in all the biochemical parameters tested and the antioxidant enzyme activities in both hepatic and renal tissues. Moreover, the histological picture of both organs was comparable to the control. In conclusion, our data strongly suggested that the deleterious effects of ZEN could be overcome or, at least, diminished by radish extract. Moreover, by it self did not show any toxic effects.

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P16-04

Simple liquid chromatography assay for analyzing ochratoxin a in bovine milk

Liliana González, Mónica Fernández, Jose Miguel Soriano, Juan Carlos Moltó, Jordi Mañes

University of Valencia, Burjassot (Valencia), Spain

Ochratoxin A (OTA) is a secondary fungi metabolite mainly produced by *Penicillium verrucosum*, *Aspergillus ochraceus* and *A. carbonarius*. OTA can occur in a large variety of commodities, for example, cereals, beans, spices, dried fruits, coffee, beer, wine, and, because of a carry-over effect, in milk, blood, liver, kidney, and poultry meat from animals fed with contaminated feed. Because of the persistence of OTA in the food chain, exposure to the compound is a potential human health hazard. OTA has been experimentally shown to be teratogenic, a potent renal carcinogen, immunosuppressive, an enzyme inhibitor and has been implicated in Balkan nephropathy in humans, it is listed as a possible carcinogen of group 2B by the International Agency for Research on Cancer.

The role of milk in nature is to nourish and provide immunological protection for the mammalian young. Milk is a highly complex food since it contains more than 100,000 molecular species. There are many factors that can affect milk composition such as breed variations, cow-to-cow variations, feed considerations, seasonal and geographic variations.

Intake of animal feedstuffs contaminated with OTA may cause that some residues may be found in milk. In Norway levels of OTA up to 58 ng/L have been found. Mycotoxin contamination usually occurs in trace levels. Detection and identification, therefore, require a highly sensitive technique. Monitoring is important for not only consumer protection but also producers of raw products prior to transport or processing. In order to have precise and reliable analytical methods, the objective of this work was to develop a simple and rapid methodology to determine OTA in bovine milk.

The obtained method is based in liquid–liquid extraction with methanol as extraction solvent, followed by one filtration step and extract concentration. Liquid chromatography coupled to fluorescence detection was used for OTA analysis. In this way several impurities are filtered off and OTA is quantitated with a mean recovery of $93.0 \pm 7.4\%$. The limit of detection was 0.01 ng/mL, hence it can be said that this methodology allows a simple quantitative extraction of OTA from bovine milk.

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P16-05

Relationship between endotoxin and biogenic amines levels in sardines

Ljerka Prester[1], Jelena Macan[1], Veda M. Varnai[1], Rajka Turk[1], Tatjana Orct[1], Jelena Vukusic[2], Dubravka Kipcic[2]

[1] *Institute for Medical Research and Occupational Health, Zagreb, Croatia;* [2] *Croatian National Institute of Public Health, Zagreb, Croatia*

Background: The role of endotoxin food contamination in the patophysiology of food poisoning is not well defined. This paper presents results of endotoxin and biogenic amines (histamine, tyramine and putrescine) measurements in sardines kept at room temperature during a period of 24 h.

Material and methods: Five fresh sardines were finely cut and mixed. Sardine mixture was divided into three samples, closed in transparent plastic bags, left at room temperature for 0, 12, and 24 h, and frozen afterwards. Samples were stored at $-20\,^{\circ}C$ until assayed. Endotoxin level was determined using end-point Limulus amoebocyte lysate (LAL) bioassay (Charles River Endosafe, USA). A rapid, convenient thin-layer chromatographic method was used for detecting histamine and other amines.

Results: Increase in both endotoxin and biogenic amines level in sardines kept at room temperature over period of 12 and 24 h was detected. Endotoxin levels in sardine samples left at room temperature for 0, 12, and 24 h were 0.11, 1.23, and 12.63 EU/mg, respectively. Corresponding values for histamine were <2, 8, and 100 mg/100 g. Tyramine and putrescine concentrations in sardines left for 12 and 24 h at room temperature were 20 and 78 mg/100 g, and 12 and 33 mg/100 g, respectively, and were undetectable in fresh fish (at 0 h). The highest increase was noted for endotoxin and histamine levels, which raised approximately 10 and 100 times after 12 and 24 h, respectively.

Conclusion: In this preliminary report, increase in endotoxin level followed the increase in histamine level in sardines experimentally spoiled at room temperature during 24 h. Therefore, it is suggested that endotoxin may play a role in histamine fish poisoning, acting together with other biogenic amines present in spoiled histidine-rich fish. The underlying biological mechanism needs further evaluation.

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P16-06
Apoptosis in neurotoxicity of fumonisin B1

Kai Savolainen, Helene Stockmann-Juvala, Jonne Naarala, Jarkko Loikkanen, Kirsi Vähäkangas

Finnish Institute of Occupational Health, Helsinki, Finland

The mycotoxin fumonisin B1 (FB1) is produced by the fungus *Fusarium verticillioides*, which commonly infects corn across the world. *Fusarium* species are also found in moisture-damaged buildings, and airborne exposure to mycotoxins like FB1 may therefore be possible.

The aim of this study was to investigate the role of apoptosis in the toxicity of FB1. Four different cell lines, human U-118MG glioblastoma, human SH-SY5Y neuroblastoma, rat C6 glioblastoma, and mouse GT1-7 hypothalamic cells, were exposed to graded doses (0.1–100 μM) of FB1 for 0–144 h. Activation of caspase-3 like protease was studied by a fluorometric method. DNA fragmentation was visualised by gel electrophoresis and the expression of p53 and Bcl-2 family proteins (Bax, Bcl-2, Bcl-X, Mcl-1) by immunoblotting.

At 12 h, caspase-3 like protease activity increased significantly in U-118MG cells, and in the other cell lines, except SH-SY5Y, at later time points after exposure to the highest dose of FB1 (100 μM). The results also showed that internucleosomal DNA fragmentation occurred in all cell lines; in C6 cells already at 24 h. On the contrary, the expression of p53 or pro- or antiapoptotic Bcl-2 family proteins were not affected in any of the cell lines at any time points studied. The results of this study indicate that p53 independent apoptosis plays a role in the neurotoxicity induced by FB1. However, the toxicity varies between different types of cell lines. When comparing human cell lines, our results indicate that cells of glial origin (U-118MG cells) may be more sensitive towards FB1 than those of neural origin (SH-SY5Y cells).

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P16-07
Effect of ochratoxin A on prothrombotic and proinflammatory activities of stimulated blood mononuclear cells

Maria Rosaria Carratù, Addolorata Coluccia, Pietro Borracci, Maria Rosaria Rossiello, Mario Colucci, Nicola Semeraro

Department of Pharmacology and Human Physiology, Medical School, University of Bari, Italy

The mycotoxin ochratoxin A (OTA) is an ubiquitous contaminant of human and animal food products. Apart from its known nephrotoxicity and carcinogenicity, OTA has been shown to variably affect several functions of mononuclear leukocytes. We have studied the effect of OTA on tissue factor (TF) and plasminogen activator inhibitor-2 (PAI-2) production by peripheral blood mononuclear cells (MNC) stimulated with endotoxin (1 μg/ml, 3 and 18 h at 37 °C for TF and PAI-2, respectively). TF was measured by functional (one-stage clotting time) and immunological (ELISA) assays, and by RT-PCR whereas PAI-2 was assessed by ELISA in conditioned media. OTA caused a dose-dependent reduction in TF activity and antigen (with more than 90% inhibition at the concentration of 1 μg/ml) and also reduced PAI-2 release (80% inhibition at 1 μg/ml). Inhibition of TF expression was also observed at mRNA level. The inhibitory effect disappeared if OTA was added to MNC suspensions 20–60 min after endotoxin. Moreover, OTA was much less efficient in reducing TF expression when MNC were suspended in medium containing 40 mg/ml human albumin. TF production was also impaired by OTA (1 μg/ml) when MNC were stimulated with 10^{-9} M PMA (99% inhibition), 10 ng/ml IL-1beta (84%) or 100 ng/ml TNF-alpha (55%). Finally, we determined the effect of OTA on endotoxin-induced cytokine release by MNC and found that OTA inhibited IL-6, but not IL-8 or TNF-alpha production, thus ruling out an unspecific effect of the mycotoxin on protein synthesis. Because of the important role of blood clotting activation and fibrin deposition in cell-mediated immune responses, it is suggested that the inhibitory effect on cell TF and PAI-2 expression might represent one of the mechanisms whereby OTA exerts its immunomodulatory activities.

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P16-08
Analysis of aflatoxin B1 in human hair samples

O. Sabzevari [1], I. Emrarian [1], K.H. Abdi [2], G. Abouhossein [3], M. Amirahmadi [3]

[1] *Department of Toxicology & Pharmacology, Tehran, Iran;* [2] *Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran;* [3] *Food & Drug Control Labs, MOH, Tehran, Iran*

Aflatoxins, a group of related secondary metabolites of Aspergillus fungi, are commonly found on grains and seeds. Numerous outbreaks of acute aflatoxicosis have been documented worldwide and several epidemiological studies have shown a highly significant, positive correlation between human hepatic carcinoma and dietary aflatoxin contamination. Hair analysis has been introduced as an alternative or complementary method for testing the chemicals. The aim of this study was to identify aflatoxin B1 (AFB1), in human hair by HPLC.

Fifty milligrams of hair samples, collected from healthy volunteers, was weighted and decontaminated by washing with 2 ml isopropanol, 2 ml distilled water (×3) and 2 ml isopropanol (1). The specimens were dried at room temperature and cut into small pieces. Thirty milligrams of the latter was incubated with AFB1 for 7 days at +4 °C. After the incubation period, the samples were washed with isopropanol and distilled water (×2) until no AFB1 was present in washing liquid. The hair samples were then extracted using toluene/acetonitrile for 5 days at +4 °C. The extraction phase was analysed by HPLC using fluorescence detector.

By using the above procedure, we were able to detect AFB1 in hair samples which can be employed as tool to study patients' history of exposure to aflatoxins.

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P16-09
Pathological and membrane biomarkers alteration induced by microcystins in liver, kidney and gills in tilapia fish (*Oreochromis* spp.)

Silvia Pichardo [1], Isabel M. Moreno [1], Rafael Molina [1], Angeles M. Jos [1], Rosario Moyano [2], Ana Ferrer-Dufolt [3], Ana M. Cameán [1]

[1] *University of Seville Seville Spain;* [2] *Faculty of Veterinary, University of Cordoba, Spain;* [3] *Toxicology Unit, Clinic University Hospital, Zaragoza, Spain*

Cyanobacterial blooms, which are considered a serious risk to animal and human populations, have been reported worldwide. Microcystins (MCs) are the most studied hepatotoxins produced by cyanobacteria. Toxic blooms of cyanobacteria, containing MCs, have been frequently associated with fish damage and mortality.

In this study, membrane biomarkers and pathological changes produced by these toxins on hepatic, renal and gill tissues were investigated in Tilapia fish (*Oreochromis* spp.) under laboratory conditions. Four different groups of Tilapia were exposed to MC: the first group was injected intraperitoneally (i.p.) a dose of 900 μg/kg of MCs (containing 500 μg/kg MC-LR, 200 μg/kg MC-YR and 200 μg/kg MC-RR), other two groups were injected i.p. doses of 500 μg/kg MC-LR and 500 μg/kg MC-RR, respectively, and a control group which was injected saline solution via i.p.

Acid phosphatase (ACP), as a marker enzyme of lysosomal membranes, and alkaline phosphatase (ALP), as apical membrane enzyme, were analysed after exposure to MCs. Acute i.p. administration of all the exposures assessed produces a significant increase of alkaline phosphatase, whereas enhancement of acid phosphatase was only observed after exposure to MC-LR and to the mixture of MCs. The most severe histopathological changes were observed in kidney and liver, although gills were also affected. The most significant alterations observed were steatosis and necrosis in liver and necrotic epithelial cells and nephrosis in kidney.

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P17 Pesticide Toxicology

P17-01
Optimization of DI-SPME combined with GC–MS for analyses of chlorophenoxypropionic acids

R. Krzyzanowski, B. Leszczynski

Department of Biochemistry, University of Podlasie, B. Prusa 12, Siedlce 08-110, Poland

Solid phase microextraction (SPME) is one of the most promising method in analyzing herbicide residues. However, an application of SPME method requires determination of the proper analytical conditions. In the present paper, we report on optimization of the solid phase microextraction for analysis of such chlorophenoxypropionic herbicides like: 2-(4-chloro-2-methylphenoxy)propionic acid (MCPP) and 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP).

Seventy-five micrometer CAR/PDMS fiber was used to study an effect of time and temperature on the herbicide SPME (adsorption and desorption). The optimization was done for duration of adsorption (5–30 min) and

desorption (4–10 min) and for temperature 20–70 and 140 °C–220 °C for adsorption and desorption, respectively.

The obtained results showed the highest adsorption of the MCPP after 20 min, when the concentration reached 0.0072 μg/l, at the temperature 50 °C. The best adsorption time for the 2,4-DP was after 15 min of the fiber exposition at 50 °C, when the concentration was 0.0007 μg/l. Optimum conditions for desorption of MCPP was as fallows: desorption temperature 220 °C and desorption time 6 min, instead desorption time for 2,4-DP varied between 4 and 8 min, at 220 °C.

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P17-02
Toxicity of chlorophenoxy herbicides to wheat plants and aphids

R. Krzyzanowski, B. Leszczynski, A. Gadalinska-Krzyzanowska, K. Lugowska

Department of Biochemistry, University of Podlasie, B. Prusa 12, Siedlce 08-110, Poland

Commercial preparations (Chwastox) contained (4-chloro-2-methylphenoxy)acetic acid (MCPA) salts, amines or esters are still widely used as herbicides for cereal crops in Poland. The present paper reports on concentration of the (4-chloro-2-methylphenoxy)acetic acid residues within tissues of winter wheat and their effects on the grain aphid *Sitobion avenae* (F.).

Dichloromethane extracts of the winter wheat was performed. After evaporation, and estrification with MeOH has been made, and then solid phase microextraction (SPME) was performed. Identification and quantification of the MCPA residues within the plant tissues was done using a gas chromatography–mass spectrometry (GC–MS) system with a selective ion monitoring (SIM). Grain aphid population tests were performed under laboratory conditions, using the insects that came from a stock culture kept at the University of Podlasie.

The highest concentration of MCPA was found within the wheat tissues, one hour after treatment and the MCPA residues were declined during the next 4 days. The dose applied MCPA was toxic to the grain aphid and decreased level of its population on the studied winter wheat in all used commercial formulations of Chwastox. Higher doses of chlorophenoxy herbicides killed over 50% of the grain aphid individuals. So, the SPME/GC–MS is very useful method not only for routinely determination of MCPA residues but also might be used in study of tritrofic interactions between cereal crops, weeds and insect pests.

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P17-03
Antagonism of paraoxon pulmonary toxicity by pralidoxime in rats
Assessment of efficiency–dose relationship

Houzé Pascal, Monier Calire, Risède Patricia, Baud Frédéric

INSERM U705, Paris CEDEX 10, France

The efficiency of oxime therapy in organophosphate poisoning is still a matter of debate. Using pralidoxime methylsulfate (Contrathion®) in rats, we tested various doses and routes of administration to determine the best regimen according to the pharmacokinetic parameters. This therapeutic regimen was then tested in paraoxon-poisoned rats to observe the reversibility of the effects on respiratory function and blood cholinesterase activities. Sprague–Dawley male rats were treated with pralidoxime (base: 10, 25, 50 mg kg^{-1}) using IV and IM routes. Paraoxon solution (0.215 mg kg^{-1}, i.e. 50% LD_{50}) was administered by subcutaneous route. Rats were treated with pralidoxime at 30 min post injection of paraoxon. Respiratory function was tested using whole body plethysmography and cholinesterase activities were measured in whole blood. All results are expressed as mean ± S.E.M. Statistical analysis was performed using Student's *t*-test and ANOVA tests with $p < 0.05$ as significant value. In naïve and poisoned rats, pharmacokinetic studies showed that the 50 mg kg^{-1} IM regimen of pralidoxime allowed maintenance of plasma concentrations $\geq$4 mg L^{-1}, during the longest time (>35 min post infusion). Plethysmography values after paraoxon showed an increase in total time, expiratory time and tidal volume, and a decrease in respiratory rate. In the same time, whole blood cholinesterase activities were decreased to 40% of the basal value. Thirty minutes after paraoxon administration, pralidoxime (50 mg kg^{-1}, IM) infusion induced: (1) a fast, total and prolonged cholinesterase reactivation (>180 min) and (2) a rapid (<5 min), complete but transient (<30 min) reversal of paraoxon-induced respiratory effects. Single dose of paraoxon (50% LD_{50}) induced a significant alteration of respiratory parameters and a decrease of cholinesterase activity. A single dose of pralidoxime rapidly corrected the respiratory and biological effects. The antidotal activity on paraoxon respiratory effects was related to pralidoxime concentration. A minimal concentration was needed to reactivate the target enzyme, but this concen-

tration was very transient after a dose of 50 mg kg^{-1} and the paraoxon-induced respiratory effects reappeared. Our data suggest that cholinesterase reactivation is a necessary but not sufficient condition for the antidotal effect of pralidoxime. Some mechanisms of toxicity, not related to the inhibition of cholinesterase activity, could explain these discrepancies.

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P17-04

Toxic doses of paraoxon alters respiratory pattern without causing respiratory failure in the rats

Villa Antoine, Houzé Pascal, Monier Claire, Risède Patricia, Baud Frédéric

INSERM U705, Paris CEDEX 10, France

Respiratory failure through a combination of muscarinic, nicotinic and central effects is considered the primary cause of death in acute organophosphate poisoning. However, the literature is unclear as to the mechanisms involved. The aim of this study was to assess the mechanisms of paraoxon-induced respiratory toxicity. We studied in rats poisoned subcutaneously with paraoxon, at doses near the LD_{50}, the pattern of respiration by whole body plethysmography in awake rats and the occurrence of acute respiratory failure using arterial blood gases over 4 and 3 h, respectively. Thereafter, we assessed the effects of atropine on paraoxon-induced effects on ventilation and arterial blood gases. The tested doses of paraoxon were, respectively, 10 (0.043 mg kg^{-1}), 50 (0.215 mg kg^{-1}) and 75% (0.322 mg kg^{-1}) of the subcutaneous LD_{50} of paraoxon (plethysmography study) and, 50% and 75% LD_{50} (blood gas study). The effects of atropine (10 mg kg^{-1}) were studied at the 75% dose. All results are expressed as mean ± S.E.M. Statistical analysis was performed using Student's test and ANOVA tests with $p < 0.05$ as significant value. The rats were frankly symptomatic at the two highest doses of paraoxon. At 30 min post injection and throughout the study, there was a significant decrease in respiratory rate, an increase in expiratory time with no modifications of the inspiratory time. There was a significant increase of the tidal volume for the 50% and 75% LD_{50} doses. Apnea was not detected. For the two lowest doses of paraoxon, no effect on arterial blood gases was observed. For the 75% dose, paraoxon had no effects on PaO_2, $PaCO_2$ and HCO_3^- but there was a significant decrease in pH at 30 min. Atropine reversed all the paraoxon-induced respiratory pattern alterations. We conclude that paraoxon, at doses equal to 50% and 75% of the LD_{50}, alters the pattern of respiration at rest. As evidenced by the complete and sustained effects of atropine, the respiratory effects, at these doses, only involved muscarinic receptors, with no evidence of any depressant effect on the respiratory centres and nicotinic effect as evidenced by an increase in tidal volume with the lack of modification of the minute ventilation.

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P17-05

Effects of carbamates as oxidative stressors on glutathione levels and lipid peroxidation in CHO-K1 cells

M.J. Ruiz [1], E. Maran [2], H. Berrada [1], M. Fernández [2]

[1] *Laboratorio de Toxicologia, Facultad de Farmacia, Universidad de Valencia, Spain;* [2] *Sezione di Chimica Analitica e Ambientale, Dip. Scienze Chimiche, Universita degli Studi di Trieste, Italy*

The toxic effect of four carbamates, aldicarb and its metabolites (aldicarb sulfone and aldicarb sulfoxide) and propoxur in CHO-K1 cells was investigated.

After 24 h exposure of 10 μg/ml of each carbamate in cells, intracellular reduced glutathione (GSH) content was depleted. The rank order for GSH decreased as follows: aldicarb sulfone > aldicarb and aldicarb sulfone > propoxur. On the other hand, oxidized glutathione (GSSG) level appeared almost unchanged. The GSH:GSSG ratio was significantly reduced with aldicarb, aldicarb sulfone and aldicarb sulfoxide compared to control cells.

Glutathione reductase (GR), glutathione peroxidasa (GPx) and glutathione-*S*-transferase (GST) contribute to antioxidant defence mechanisms in cells after carbamate exposure. Alteration of GSH levels was accompanied by induction of GR activity for all carbamates. The highest increase of GR activity was observed in cells exposed to aldicarb. Some differences between carbamates in GPx and GST activity were observed. GPx activity was increased after aldicarb sulfone, aldicarb sulfoxide and propoxur treatment, while GST activity was increased only after aldicarb sulfone and propoxur exposure.

Lipid peroxidation in CHO-K1 cells exposed to carbamates was studied by thiobarbituric acid reactive substances (TBARS) production. Addition of aldicarb sulfone, aldicarb sulfoxide and propoxur to cells resulted in an increase in TBARS levels compared with control cells.

For a further characterization of the nature of carbamate toxicity, cells were pre-treated before carbamate exposure with D-L-buthionine-(*S*,*R*)-sulfoximine (BSO).

Aldicarb sulfone, aldicarb sulfoxide and propoxur cytotoxicity was exacerbated by the previous depletion of cellular GSH by BSO.

Results suggest that carbamates induce GSH depletion, leading to oxidative stress. However, the induction of the antioxidant enzyme GST produced by aldicarb sulfone and propoxur in CHO-K1 cells, suggests that the enzyme provides adequate protection to mammals cells through the detoxification of these carbamates.

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P17-06

Effects of cypermethrin on hematological and biochemical profile on common carp (*Cyprinus carpio*)

J. Velíšek [1,2], T. Wlasow [4], P. Gomulka [4], Z. Svobodová [2,3], R. Dobšíková [3]

[1] *University of South Bohemia Ceske Budejovice, Czech Republic;* [2] *Research Institute of Fish Culture and Hydrobiology Vodnany, University of South Bohemia Ceske Budejovice, Czech Republic;* [3] *University of Veterinary and Pharmaceutical Science Brno, Czech Republic;* [4] *University of Warmia and Mazury Olsztyn, Poland*

The aim of this study was to assess the effect of cypermethrin [(*R*,*S*)-α-cyano-3-phenoxybenzyl (1*RS*)-cis,tra-3-(2,2-dicholorovinyl)-2,2-dimethyl cyclopropane carboxylate] on common carp (*Cyprinus carpio*). The effect was assessed on the basis of the results of haematological and biochemical examination of a control and an experimental group exposed to Alimethrin 10 EC pesticide preparation (active substance 100 g l^{-1} of cypermethrin) in a concentration of 29.1 μg l^{-1}. The experimental group showed significantly higher values ($p < 0.01$) of erythrocyte count (RBC), glucose (GLU), creatinkinase (CK), lactate (LACT) and significantly lower values ($p < 0.01$) of mean erythrocyte volume (MCV), erythrocyte haemoglobin (MCH), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), lactate dehydrogenase (LDH) and alkalic phosphatase (ALP) compared to the control group. Also, the relative and absolute count of lymphocyte was a significant ($p < 0.01$) decreased and a significant increased in both the relative and absolute count of developmental forms of neutrophile granulocytes, and both the band- and segmented neutrophile granulocytes in the experimental group. Values of haemoglobin content (Hb), haematocrit (PCV), mean colour concentration (MCHC), leukocyte count (Leuko), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), cholinesterase (ChE), calcium (Ca^{2+}) and inorganic phosphate (PHOS) were comparable in the two groups during the study.

Changes in the erythrocyte and biochemical profile after exposure to cypermethrin-based preparation may be referred to possible disruption of haematopoiesis and mild damage of liver.

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P17-07

Acute renal failure alters the kinetics of pralidoxime in rats

Maya Kayouka, Chantal Martin, Claire Monier, Patricia Risede, Jean-Michel Warnet, Frederic J. Baud, Pascal Houzé

INSERM U705; CNRS, UMR 7157; Université Paris 5, France

Pralidoxime methylsulfate (Contrathion®) (PRX) is used as an antidote to organophosphate poisoning. The efficiency of the antidote depends on its plasma concentrations that should be greater than 4 mg L^{-1}. The pharmacokinetics of PRX is characterized by complete absorption, no protein binding, no metabolism and fast renal excretion resulting in an extremely short half-life (<60 min). Therefore, the aim of this study was to assess the effect of renal failure on the kinetics of PRX. We developed a rat model with acute renal failure (ARF) induced by potassium bichromate administration. On the first day, male Sprague–Dawley rats (250–350 g) were intoxicated by potassium bichromate subcutaneously administered (15 mg kg^{-1}) and kept for 4 days in metabolic cages. On the second day, animals were treated with PRX (50 mg kg^{-1}) by the intramuscular route. Blood samples were collected during 180 min after PRX injection and urine were collected 48 h post injection. Plasma PRX concentrations were measured by liquid chromatography with electrochemical detection. Results are expressed as mean ± S.E.M. Statistic analysis were performed using to Mann–Whitney and Anova tests ARF was observed 48 h following bichromate injection. Simultaneously, PRX kinetics were significantly different between the control and the treated groups (alpha and beta half-life: increment × 2 in ARF

versus control, $p < 0.05$). No significant difference was observed regarding the C_{max} concentration between the two groups. The areas under the curve ($AUC_{0–180\ min}$) in the treated group were three times higher than in the control group (976.3 ± 29.7 mg min L^{-1} control versus 2661.0 ± 535.6 mg min L^{-1} ARF, $p < 0.05$). Distribution volumes were not modified, but PRX clearances decreased significantly ($p < 0.01$). A positive linear correlation was found between the AUCs and the creatinine values. PRX kinetic profile was strongly correlated with creatinine values. Our study showed significant differences between controls and ARF rat kinetics. Acute renal failure did not modify PRX distribution but decreased significantly its plasma elimination. We conclude that ARF significantly alters PRX elimination. From a clinical viewpoint, our study showed that dosage regimen of PRX should be adjusted in patients with severe ARF and suggest the usefulness of the measurement of plasma PRX concentrations in ARF. Future investigations will be necessary to determine whether the antidotal efficacy of PRX is modified in ARF.

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P17-08

Role of the immune system in the pathogenesis of delayed neurotoxic effect of some organophosphorous substances and means of its prophylaxis

Peter Zhminko, Mykola Prodanchuk

Medved's Institute of Ecohygiene and Toxicology, Kiev, Ukraine

The research was conducted on hens of Leggorn breed, with mass of body −1.6–1.7 kg. The delayed neurotoxic effects (DNE) were modelled with the help of organophosphorous substances—aphos (0,0-diphenyl-1-acetoxy-2,2,2-trichlor-ethylphosphonate) in the dose of 200 mg/kg and three ortocresilphosphate in the dose of 750 mg/kg, which caused expressed paresis and paralysis for hen. The hemoperfusion was realized on 7–10 days after the poisoning by aphos during the period of the first signs of the DNE, increase of the level of pathogenic circulating immune complexes (CIC) in blood and autoantibodies to antigens of neural tissue. For the hemosorption, the hard phase porous carbonic hemosorbent SCN-2k was used.

It is established that pathogenesis of DNE of the aphos and three ortocresilphosphate is conditioned by violations of autoimmune reactions of organism (decreasing of the T- and B-lymphocytes amount, NK-cells, T-suppressors, increasing of B-lymphocytes function activity, microdispersed CIC level, titre of antybodies to nervous tissues antigen).

It is shown that hemocarboperfusion inhibit the DNE development, diminish severity of clinical signs and prevents development of paresises and paralyses at the expense of elimination of pathogenic CIC, elimination of autoantibodies to neural tissue out of blood and also at the expense of the normalization of T- and B- systems of immunity.

Thus, the immune system plays a vital part in the pathogenesis of delayed neurotoxic effect of organophosphorous compounds. The hemocarboperfusion can be used as the method of prophylaxis of the delayed neuropathies caused by organophosphorous substances.

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P17-09

Dexamethasone treatment decreases the pathological effects and increases the survival rate of paraquat-intoxicated rats

Ricardo Jorge Dinis-Oliveira [1], Fernando Remião [1], José Alberto Duarte [2], Amparo Sánchez Navarro [3], Maria de Lourdes Bastos [1], Félix Carvalho [1]

[1] *REQUIMTE, Department of Toxicology, Faculty of Pharmacy, University of Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal;* [2] *Department of Sport Biology, Faculty of Sport Sciences, University of Porto, Rua Dr. Plácido Costa, 91, 4200-450 Porto, Portugal;* [3] *Faculty of Pharmacy, Avenida Campo Charro s/n. 37007, Salamanca, Spain*

Dexamethasone (DEX), a synthetic corticosteroid, has been used successfully in clinical practice in the treatment of paraquat (PQ) poisonings due to its anti-inflammatory effects, although, as recently observed, its effects related to induction of P-glycoprotein de novo synthesis, may also strongly contribute for its healing effects. The main purpose of this study was to provide a comprehensive study of the effects of DEX administration, reflected in the histological and biochemical parameters in lung, liver, kidney and spleen of acute PQ-intoxicated rats. For this approach, four groups of rats were constituted: (i) control group, (ii) DEX group, (iii) PQ group and (iv) PQ + DEX group. DEX (100 mg/kg i.p.), administered 2 h after PQ (25 mg/kg i.p.) to Wistar rats, attenuated the PQ-induced histological alterations in the lung and liver in only 24 h. This was corroborated by a significant reduction

in lipid peroxidation (LPO) and carbonyl groups content and by normalization of the myeloperoxidase (MPO) activities. On the other hand, these improvements were not observed in kidney and spleen of DEX treated rats. Conversely, an increase of LPO and carbonyl groups content and aggravation of histological damages were observed. In addition, MPO activity increased in the spleen of DEX exposed rats beyond PQ and *N*-acetyl-β-D-glucosaminidase activity, a biomarker of renal tubular proximal damage, also augmented in the urine of these rats giving credit to an aggravation of the renal lesion caused by PQ. In spite of these apparent contradictory effects, the increased survival rate indicates that DEX treatment constitutes an important and valuable therapeutic tool to be used against PQ-induced toxicity.

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P17-10
Effect of endosulfan on LDH level in tilapia

Ashok Rani, T.R. Ambili

Lady Doak College, Madurai, Tamilnadu, India

Demand for agricultural products is increasing day by day due to ever increasing human population. This inturn increases the stress on soil due to overdose of application of fertilizers and pesticides. Endosulfan, a banned pesticide in developed countries but still in use in developing countries is of importance as they reach the waterbody as run off from fields due to rain, etc. The fish forms the staple food and as a source of protein to economically weak human society. Detoxification of ingested pesticide is done by the activity of various enzymes. In the present dose and time dependent study, effect of endosulfan on LDH level is studied by spectrophotometric method using pyruvate as substrate. From the study, we find that the level is altered and that medium concentration of endosulfan produces more significant effect in kidney LDH and as the concentration and days increased, there was considerable increase in amount of LDH in muscle indicating bioaccumulation of endosulfan.

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P17-11
Pralidoxime kinetics in rats pre-treated with organic cationic transporters substrates

Maya Kayouka, Pascal Houzé, Claire Monier, Patricia Risède, Frederic J. Baud

INSERM U705, CNRS, UMR 7157; Université Paris 5, France

Pralidoxime methylsulate (PRX) is a nucleophilic agent used as antidote after intoxication with organophosphates. The efficacy of the antidote depends on its plasma concentrations that should be greater than $4\,mg\,L^{-1}$. PRX is rapidly (half life <60 min) eliminated in urine and may be handled by the kidney as a strong organic base (as thiamine or tetraethylammonium, TEA) and secreted by similar mechanisms. Strong bases are transported by organic cationic transporters (Oct1 and 2); thus, their co-administration with PRX may delay its secretion and increase its plasma levels. The aim of this study was to determine the effects of thiamine or TEA pre-treatments on PRX pharmacokinetic.

Sprague–Dawley male rats were pre-treated by thiamine or TEA ($75\,mg\,kg^{-1}$, IM). Animals were than treated with PRX ($50\,mg\,kg^{-1}$, 30 min perfusion). Aiming at determining a dose–effect relationship, thiamine pre-treatment was performed at 15, 30 and 60 min; and at different doses (0.15, 1.5, 15 and $150\,mg\,kg^{-1}$, 15 min pre-treatment). TEA was injected at different times (15, 30 min, 1, 2, 4 and 6 h) before PRX infusion. Blood samples were collected during PRX infusion and 180 min post infusion. Plasma PRX concentrations were measured by liquid chromatography. Results are expressed as mean ± S.E.M.

Thiamine induced a significant increase of PRX distribution volume and renal clearance, with a maximal effect at 15 min, with no half-life modification. Using 15 min pre-treatment, we observed a dose–effect relationship for thiamine. Only 1.5 and $15\,mg\,kg^{-1}$ of thiamine reduced significantly C_{max} and area under the curves and increased distribution volume and renal clearance. A pre-treatment with TEA induced an increase of beta half live, a decrease of renal clearance of PRX; distribution volume was not altered. A time effect relationship was also found with a maximal effect observed for 30 min pre-treatment.

Our results support that PRX might be excreted via Oct1 and 2 because TEA decreased the urinary elimination of PRX without altering its distribution. Thiamine renal excretion is more complex implying different transporters. Our work suggests that thiamine alters the distribution of PRX in tissues without modifying its elimina-

tion. It may be hypothesized that different mechanisms and/or transporters may be involved in PRX excretion.

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P17-12

The separate and combined action of Decis, Polyram and 2,4-D on the female rats reproductive system

N. Shepelskaya, M. Prodanchuk

Medved's Institute of Ecohygiene and Toxicology, Kiev, Ukraine

On the basis of food pesticides residues monitoring results the plant protection substances, which most widely contaminate in agricultural commodities of Ukraine were identified. Experimental investigations of separate and combined action of this pesticides (Decis, Polyram and 2,4-D) on the female rat reproductive function were carried out.

All studies were conducted using 500 Wistar rats (females and males). Pesticides were administered 5 days per week for 10 weeks, orally by gavage in the form of aqueous emulsions. After the scheduled period of exposure, treated females were paired with untreated males. The day on which evidence of mating was found was designated day zero of gestation. On days, 20 of gestation period females were sacrificed, and the number of corpuses luteum, of uterine implantation sites, resorptions, number of live and dead fetuses was recorded. The fetal body weights and the presence of visual developmental anomalies were recorded too.

The reproductive performance of females was evaluated based on parameters as the reproductive cycle normality, precoital interval, fertility index, percentage mating, conception rate, gestation index, number of ovarian corpuses luteum, litter size, embryonic and fetal loss.

Lowest-observed-adverse-effect levels (LOAEL) and no-observed-effect levels (NOEL) for reproductive toxicity under the conditions of separate exposure to examined pesticides were determined. Then the reproductive effects of a combination of this pesticides in the same doses and conditions were studied. Results of this experiments showed that lowest doses, which caused an adverse alteration under the conditions of separate exposure, did not affect adversely under three pesticides complex action.

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P17-13

Interaction of organophosphate and carbamate cholinesterase inhibitors with acetylcholine binding proteins

Zoran Radić, Todd T. Talley, Scott B. Hansen, Wenru Yu, Palmer Taylor

University of California at San Diego, La Jolla, CA 92093, USA

Acetylcholine binding proteins (AChBPs) are a family of invertebrate proteins that share significant structural similarity with nicotinic acetylcholine receptors (nAChRs). AChBPs assemble as stable homopentamers and display ligand recognition properties similar to those of the neuronal homopentameric a7 nAChR subtype. We have analyzed the interaction of recombinantly expressed and purified AChBPs from *Lymnaea stagnalis*, *Aplysia californica* and *Bulinus Truncatus*, with several organophosphate (OP) and carbamate (CB) cholinesterase inhibitors commonly used as pesticides or therapeutic agents. Binding of malathion, paraoxon, chlorpyrifos, chlorpyrifos oxon, echothiophate and eserine to AChBPs was measured from the concentration dependence of the pseudo-first order association rate, k_{obs}, of the AChBP ligand gallamine on the OP or CB concentration. Rates were monitored in a millisecond time frame by stopped-flow measurements of intrinsic Trp fluorescence enhancement of AChBPs. The dissociation constant was determined from semi-logarithmic plots of k_{obs} versus OP or CB concentrations and calculated by nonlinear regression. Up to millimolar malathion, paraoxon, chlorpyrifos and chlorpyrifos oxon did not show noticeable binding to AChBPs, in the absence or presence of an agonist acetylthiocholine. Eserine and echothiophate, however, bound reversibly to AChBPs at high nanomolar and low micromolar concentrations, respectively. Organophosphates have been known to reversibly interact with several types of neuronal nAChRs, suggesting the possibility of their involvement in the mechanisms of toxicity in prolonged exposure to OP and CB compounds.

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P17-14
Effect of pectin in eletrocardiographics changes induced by permethrin and deltamethrin in Wistar rats

Mônica Alessandra Teixeira dos Santos, Miguel A. Areas, Felix G.R. Reyes

UNICAMP, Campinas, São Paulo, Brazil

Pyrethroids insecticides modify neuronal and cardiac sodium channels, inducing persistent and steady-state sodium current at depolarized membrane potential. Researchers suggest that some pyrethroids have arrhythmogenic potential in mammalian heart because cardiac myocytes are also rich in sodium channels. On the other hand, pectin fiber can reduce the gastrointestinal absorption of toxic substances. In this work, the effects of pectin on permethrin and deltamethrin action were studied in Wistar rats considering eletrocardiographics parameters. Three groups of 20 male Wistar rats, 4 weeks old, were used. After the adaptation period of 7 days, the experimental groups received 47.1 mg/kg b.w./day of permethrin and 1.1 mg/kg b.w./day of deltamethrin corresponding to 1/10 of LD50. The insecticides were dissolved in corn oil and administered orally (5 mL) by gavage. Rats serving as control received 5 mL of corn oil. The animals were given commercial diet and water ad libitum. The experimental group diet had 5% (w/w) pectin. After 28 days, the animals were anesthetized with intraperitoneal injection of sodium barbiturate (50 mg/kg b.w). The animals were placed in the supine position and electrodes were placed subcutaneously for the eletrocardiographics registration in a Heart Ware Systems Equipment, considering standard ECG derivation (I, II, III, aVR, aVL, aVF). The insecticides decreased the heart beat rate and prolonged PR interval, QRS complex and QT and QTc intervals. The results showed that permethrin and deltamethrin are cardiotoxic, probably because of inhibitory effect on sodium channels which are responsible for the negative chronotropism, decreasing the atrium to ventricle electric stimulus conduction, besides the ventricular polarization and depolarization. Pectin protected the heart of the toxic effects caused by these insecticides, reducing the risk of sudden death.

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P17-15
Protecting capacity of pectin to pyrethroids orally administered in histological changes

Mônica Alessandra Teixeira dos Santos, Miguel A. Areas, Felix G.R. Reyes

UNICAMP, Campinas, São Paulo, Brazil

Pyrethroids insecticides are neurotoxins that exert potent actions on neuronal sodium channels causing symptoms of poisoning in animals. It has been observed that pyrethroids cause histological changes in animals on chronic and sub-chronic exposure. However, only few investigators studied the relationship between fiber consumption and the histological changes induced by the pyrethroids. This study investigated whether pectin protects the heart and liver tissues of rats exposed to permethrin and deltamethrin administered orally. Three groups of 20 male Wistar rats, 4 weeks old, were used. After the adaptation period of 7 days, the experimental groups received 47.1 mg/kg b.w./day of permethrin and 1.1 mg/kg b.w./day of deltamethrin corresponding to 1/10 of LD50. The insecticides were dissolved in corn oil and administered orally (5 mL) by gavage. Rats serving as control received 5 mL of corn oil. The animals were given commercial diet and water ad libitum. The experimental group diet had 5% (w/w) pectin. After 28 days, the animals were sacrificed and the heart and liver were taken for histological studies. For light microscopy, the material was embedded in paraffin and 6 μm thick sections were stained with hematoxylin and eosin. The histological analysis of the heart of all animals showed complete cells, central and irregular nucleus, characteristics of normal myocites. The muscle fibers have normal transversal striations and no changes were observed in the nucleus and intercaled discs. The liver of control rats showed complete hepatocytes with one or two regular nucleus, normal cytoplasm and Kupffer cells localized between hepatocytes and synusoids. The liver of rats exposed to deltamethrin showed inflammatory cells, infiltrations being present around the hepatic veins. Irregular hepatocytes, cytoplasmatic changes and irregular granular and condensed nucleus with indefinite nucleolus were observed. The liver of rats exposed to permetrin showed the same histological aspects as deltamethrin group, but without inflammatory infiltration. The pectin in the diet reduced all the histological changes observed in the groups fed only with commercial diet. Apparently, the reduction of cytoplasmatic changes induced by the pyrethroids was higher for deltamethrin. No changes were noted in the heart tissue. It can therefore be concluded that the presence of pectin in the diet had a

protective effect against the hepatotoxic action of the pyrethroids.

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P17-16

Evaluation of the toxic potentials of pyrethroid insecticides: Cypermethrin, deltamethrin and fenvalerate on some innate immune response parameters in mouse

A. Haratym-Maj, M. Mach, M. Tokarska-Rodak

Department of Pathology, Institute of Agricultural Medicine, Lublin, Poland

Activation of polymorphonuclear neutrophils reflects a primary immunological response to invading pathogens. Myeloperoxidase (MPO) is a leukocyte-derived enzyme released upon polymorphonuclear neutrophils activation, being an integral component of the innate immune response. MPO catalyzes the conversion of hydrogen peroxide and chloride to hypochlorous acid—the major strong oxidant produced by neutrophils. Also cytokines produced during the immune response interacts with neutrophils and exert direct and priming effects on the immune cells. The objective of this study was to determine the effects of alfa-cypermethrin, deltamethrin and fenvalerte on serum myeloperoxidase activity (MPO), IL-1 and IL-10 levels in mice. We investigated also correlations between serum MPO and IL-1 and IL-10. Titers of MPO, IL-1 and IL-10 were measured by ELISA technique. The statistical analysis revealed no correlation between the cytokine and MPO production in the studied cases. However, the increase in IL-1 and decrease in IL-10 serum concentration with simultaneous elevation of serum MPO in the deltametrin group of was noted. Our study suggests that pyrethroids may interfere with nonspecific primary host defence mechanisms of immunological response.

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P17-17

Oral toxicity of deltamethrin and fenvalerate and its distribution in mice organs evaluated by HPLC method

A. Haratym-Maj, M. Mach, G. Raszewski, M. Tokarska-Rodak

Department of Pathology, Institute of Agricultural Medicine, Lublin, Poland

Pyrethroids with their insecticidal action related to neurotoxicity, are widely used insecticides of low acute toxicity in mammals, but the consequences of long-term exposure are of concern. Although pyrethroids are reported to be rapidly metabolized in mammals, it is still unknown whether permanent small pyrethroids doses absorbed through the digestive tract, affect the function of the internal organs.

The aim of this study was to evaluate the amount of deltamethrin and fenvalerate in mouse internal organs after oral intoxication by HPLC method, and to assess the toxicity of these insecticides based on histological examination of selected internal organs. The studies were conducted on male and female Swiss mice. Animals received pyrethroids per os in following doses: deltamethrin—2 and 10 mg/kg b.m.; fenvalerate—4 and 20 mg/kg b.m. After 28 days of the experiment, the following organs were taken: lung, heart, liver, kidney, brain.

In the experimental groups, the liver was the most affected organ followed by the brain, kidneys, lungs, heart. Histopathological examination revealed vascular congestion and focal inflammation changes in liver and kidneys. In addition there are parenchymatous degeneration in kidneys were observed. Oral application of deltamethrin and fenvalerate resulted in slight histological changes in heart, spleen, lung. Also, results showed significant alterations in hematological parameters, body weight and relative weights of organs.

The tested compounds when administered for 28 days, produced hepato-nephro toxicity in mice.

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P17-18

Flow cytometric determination of granulocyte and monocyte respiratory burst activity in mice after deltamethrin poisoning

A. Haratym-Maj, M. Mach, M. Tokarska-Rodak

Department of Pathology, Institute of Agricultural Medicine, Lublin, Poland

Synthetic pyrethroids are widely used pesticides due to their high effectiveness, low mammalian toxicity and easy biodegradability. However, it is suggested that generation of reactive oxygen species has been implicated in the toxicity of pyrethroids. Phagocytic cells, like macrophages and neutrophils, are a first line of defense against invading bacteria. Oxidative burst capacity of granulocytes, an indirect indicator of bactericidal function, was measured. Deltamethrin was administered per os in two concentrations: 2 and 10 mg/kg b.m., once daily for 28 days. Peripheral blood granulocytes were stimulated by phorbol-12-myristate-13-acetate (PMA),

N-formyl-methionyl-leucyl-phenylalanine (FMLP) and *Escherichia coli*. The percentage of fluorescent granulocytes was measured by flow cytometry. The results showed that *E. coli*-, FMLP-induced oxidative burst increased in mouse poisoned with deltamethrin compared to control group. The findings of the present investigation show that deltamethrin has immunomodulatory potential.

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P17-19
GC–MS determination of organochlorine pesticides in medicinal plants harvested in Brazil

Marili V.N. Rodrigues, Pedro M. Magalhães, Felix G.R. Reyes, Susanne Rath

State University of Campinas, Campinas, Sao Paulo, Brazil

Medicinal plants or material derived from them have been widely employed in all cultures, throughout history, for the prevention and treatment of diseases. The pesticide contamination of plants as medicinal or food uses is very common, and could be due to the own cultivation in large scale, migration of neighboring cultures or the environmental contamination. Although banished of many countries, besides Brazil, due to its high toxicity and environmental persistence, the organochlorine pesticides (OCP) continue thoroughly distributed in the planet. A method using gas chromatography–mass spectrometry for the determination of nine organochlorine pesticides (hexachlorobenzene, lindane, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, 4,4′-DDT and 4,4′-DDE) in leaves of *Mikania laevigata, Maytenus ilicifolia* and *Cordia curassavica* was developed. For the OCP determination an HP 5890 Series II gas equipped with a HP 5971 MS detector was used. Separations were on a fused silica capillary column HP-5 (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The GC–MS interface temperature was 300 °C. The column temperature program was as follows: 90 °C, hold for 1 min, 12 °C min^{-1} to 150 °C, hold for 1 min, 2 °C min^{-1} to 230 °C, hold for 3 min, 10 °C min^{-1} to 275 °C, hold for 25 min. The selected ion monitoring (SIM) mode was used in quantification. The mass-spectrometer acquisition settings were: electron-impact ionization 70 eV, solvent delay 10 min, electron multiplier voltage 2000 V. Extraction of the pesticides was carried out by solid–liquid extraction with *n*-hexane:dichloromethane (4:1, v/v) in a Ultra-Turrax system. Clean-up was achieved by solid phase extraction on a column, successively packed with florisil, silica and anhydrous sodium sulfate. The pesticides were eluted with n-hexane–dichloromethane (6:4, v/v). Mean recovery rates of 70–124% were obtained. The inter-assay precision of a sample fortified with 0.20 μg g^{-1} of each pesticide was in the range of 1.0–7.3%. The quantitation limits ranged from 3.0 to 30 ng g^{-1} and were below the maximum residue limit for all the pesticides under study. The method was employed to analyze samples of *M. laevigata*, *M. ilicifolia* and *C. curassavica* from an experimental field near Paulínia, SP, Brazil. The samples presented contamination with dieldrin above the maximum residue limit (MRL) established by the European Pharmacopeia (50 ng g^{-1}).

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P17-20
Fatal poisoning by malthion following intramuscular injection and ingestion of pesticide

Anica Simic [1], Biljana Milosavljevic [1], Maja Vujovic [1], Goran Ilic [1], Slobodanka Milicevic-Misic [2], Simonida Seskar-Stojancov [2]

[1] *Institute of Forensic Medicine, Department of Toxicology, Nis, Serbia and Montenegro;* [2] *Department of Toxicology, Clinical Center of Nis, Serbia and Montenegro*

A case of unusual suicidal poisoning by insecticide malathion is reported. After 43-year-old women had injected malathion to her left upper arm and both of her hips, she ingested unknown amount of the same pesticide. At her admission to the Clinic for toxicology intact insecticide was found in blood at concentration of 34.10 μg/mL. Gas chromatography-mass spectrometry (GC-MS) techniques were used. The amount of active BuChE in blood was 4900 IU/L. During four days of hospitalization, patient had admitted total of 2380 mg of atropine. Although all symptomatic therapy had been applied, the values of active BuChE had been constantly reducing to amount of 349.3 IU/L until she died. After autopsy was performed post mortal tissue samples were sent for toxicological analysis. The quantitative analy-

sis was performed by means of gas chromatography-nitrogen phosphorus detector (GC-NPD) techniques. The highest concentrations of malathion were found in kidneys (1.54 μg/mg), and liver (0.524 μg/mg) as well.

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P17-21
Setting AOELs for pesticides: Using of equivalent effect principle at different routes of exposure

S. Sergeyev

L.I. Medved's Institute of Ecohygiene and Toxicology, Kyiv, Ukraine

It is optimum to use the Acceptable Operator Exposure Level (AOEL) for the primary routes of exposure—inhalation and dermal at risk assessment of pesticides toxic action on operator. Using of these AOELs is justified in connection with distinctions in the degree of toxic effects displays, which can cause identical pesticide doses at inhalation and dermal routes of exposure. The ineffective doses for experimental animals (mg a.i./kg b.w./day), set at multiple inhalation and dermal exposure, are used in procedure of AOELs establishment. Which criteria and procedures should be used to establish AOELs at presence of critical effects at oral administration? In this case it is suggested to use equivalent effect principle (possibility of identical effect at different routes of administration). The following is possible: ineffective oral dose is corrected by oral-inhalation coefficient (K o/i, shows in how many time toxicity of a.i. at multiple oral exposure is below/higher, than at multiple inhalation exposure) and by dermal-oral coefficient (K d/o, shows in how many time toxicity of a.i. at multiple dermal exposure is below/higher, than at multiple oral exposure). These coefficients are set with using of least effective doses at multiple inhalation, dermal exposure and least effective dose (on a critical effect) at multiple oral exposure. The least ineffective doses at inhalation, dermal exposure and proper equivalent doses at oral exposure are used for AOELs establishment. Example of establishment of least ineffective doses depending on a critical effect is represented.

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P17-22
Toxic effects of endosulfan on blood lymphocyte subsets in adult rats

A. Lafuente, T. Cabaleiro, A. Caride, A. Romero

Laboratorio de Toxicología, Facultad de Ciencias, Universidad de Vigo, Campus de Orense, Las Lagunas s/n, 32004-Orense, Spain

Endosulfan is an organochlorine insecticide, which presents important neuroendocrine toxicity, so it is important to note its possible immunotoxicity. Like other organochlorines, it accumulates in fatty tissue, and it can cross the placenta leaving the fetus and breeding after its birth through maternal milk. The objective of the present work was to evaluate possible immune alterations induced by endosulfan exposure during pregnancy and lactation in both male and female adult rats. The pesticide was administered to mothers orally. At 60th day of life offspring (males and females) of mothers exposed to endosulfan (two different doses: 0.61 and 6.12 mg/kg/day) and of mothers not treated with the insecticide (control group) were sacrificed. In these animals we studied different lymphocytes populations and subsets in peripheral blood by flow immunocitometry.

In males of mothers treated with the lowest dose induced an increment of B^+ lymphocytes percentage, but no more significantly changes were observed in the other lymphocyte populations studied. In females of mothers exposed to the dose of 0.61 mg/kg/day of endosulfan decreased B^+ lymphocytes percentage. However, in females of mothers treated with the higher dose, T^+ and $CD4^+$ lymphocytes percentage decreased. Furthermore, the percentage of $CD8^+$ lymphocytes did not change significantly in female offspring of mothers exposed to endosulfan.

These results suggest that endosulfan exposure during pregnancy and lactation could modify humoral immunity in offspring adulthood, and that females are more susceptible to this pesticide because their cellular immunity was also altered.

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P17-23

Response surface modeling in evaluation of trimedoxime, atropine and sodium bicarbonate against dichlorvos poisoning in rats

D. Stefanović [1], B. Antonijević [2], D. Bokonjić [3], Z.A. Milovanović [3], M.P. Stojiljković [4], M. Nedeljković [2]

[1] *Medicines and Medical Devices Agency of Serbia, Vojvode Stepe 458, 11 152 Belgrade, Serbia and Montenegro;* [2] *Institute of Toxicological Chemistry, School of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11 221 Belgrade, Serbia and Montenegro;* [3] *National Poison Control Centre, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia and Montenegro;* [4] *Clinical Pharmacology Research Unit, Southern Denmark University, Winsløwparken 19, 3, DK-5000 Odense C, Denmark*

The aim of this work was to evaluate the optimal dose regiments of trimedoxime (TMB-4), sodium bicarbonate ($NaHCO_3$) and atropine in dichlorvos (DDVP) poisoned rats.

Male Wistar rats were poisoned with increasing doses of DDVP (sc) and immediately thereafter treated with increased doses of trimedoxime (2.5 mg/kg, 5 mg/kg and 10 mg/kg im), sodium bicarbonate (1 mmol/kg, 2 mmol/kg, 3 mmol/kg or 6 mmol/kg ip) and atropine (0 mg/kg, 10 mg/kg im). Twenty-four-hour survival rates were recorded and further analysed by response surface method (RSM). This computerized modeling procedure enabled the precise estimation of the optimal treatment dose of oxime and bicarbonate in the mixture.

The administration of $NaHCO_3$ provided the further improvement of TMB-4 antidotal effect in DDVP intoxication. When atropine was introduced, lower doses of TMB-4 were needed to counteract toxicity caused by DDVP poisoning.According the RSM, the administration of 5.9 mg/kg TMB-4 and 3 mmol/kg $NaHCO_3$ would result in 50% survival of rats at 18.32 LD-50 DDVP. Given along with atropine, calculated doses of TMB-4 and $NaHCO_3$ necessary to protect 50% of experimental animals against 29.30 LD-50 DDVP were 5.7 mg/kg and 3 mmol/kg, respectively.

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P17-24

The extent and mortality of acute insecticide related poisoning in Upper Egypt

Khaled M. Mohamed [1], Soheir A. Mohamed [2], Nady S. Aly [1], Magda H. El-Karadawy [1]

[1] *Medico-Legal Department, Ministry of Justice, Egypt;* [2] *Forensic Medicine and Toxicology, Faculty of Medicine, South Valley University, Egypt*

Along 2 years (1/2004–3/2006), we set up a study in Upper Egypt to follow acutely poisoned cases by the most commonly used Insecticides which are organophosphates, carbamates and inorganic phosphorus compounds. The recorded cases were 77 in number, 34 of them were males and 43 were females. Their ages ranged from 12 to 73 years. Most of the cases were between the second, third and fourth decades of life. Forty-two cases were poisoned by organophosphates, 25 cases by carbamates, and 10 cases by inorganic phosphorus compounds. It was found that 68 cases were suicidal; four of them were saved (two males and two females) by rapid atropinization and gastric lavage on admission to hospital. The homicidal cases were three (two males and one female), all of them were saved. Accidental exposure by ingestion of contaminated food or drinks was also recorded in six cases (two males and four females). Severe weakness, abdominal pain, nausea and vomiting were the common manifestations of the cases, which appeared immediately after ingestion. Fatal cases showed a characteristic postmortem picture in the form of frothy discharge coming from the mouth and the nose, cyanosis of lips and fingers, generalized congestion of the internal organs and mucous membranes. In most cadavers the stomach showed severe congestion associated with multiple small ulcers. The residue of the ingested substance was found in the stomach with a characteristic odor. Samples were taken and sent to Chemical Laboratory of Medico-legal Department for analysis. Detection of Insecticides was done by color test, TLC, GC-MS.

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P17-25

Effects of acute organophosphate poisoning on thyroid hormones in rats

Salim Satar[1], Deniz Satar[2], Sinan Kirim[3], Hülya Leventerler[4]

[1] *Cukurova University School of Medicine, Department of Emergency Medicine, 01330 Balcali/Adana, Turkey;* [2] *Cukurova University School of Medicine, Department of Histology and Embriology, 01330 Balcali/Adana, Turkey;* [3] *Cukurova University School of Medicine, Department of Internal Medicine Endocrinology Division, 01330 Balcali/Adana, Turkey;* [4] *Cukurova University School of Medicine, Department of Biochemistry, 01330 Balcali/Adana, Turkey*

The aim of this study was to investigate the effects of organophosphate poisoning on thyroid hormones. In this study, male Wistar albino rats weighing 200–225 g were used. The rats were divided into four groups. Group 1 ($n = 10$) was administered 30 mg/kg lethal dose of methamidophos, whereas group 2 ($n = 7$) was treated with physiologic NaCl (SP). Group 3 ($n = 10$) was treated with 30 mg/kg of methamidophos. When cholinergic symptoms developed among the rats in group 3, they were treated with 40 mg/kg pralidoxime intraperitoneally (IP) and administered atropine IP until the cholinergic symptoms disappeared. Group 4 ($n = 7$) was treated with SP. After the cholinergic symptoms appeared among the rats in group 1, intracardiac blood samples were taken. In group 3, blood samples were taken after the cholinergic symptoms had disappeared. Then triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH), and plasma choline esterase (PCE) levels were studied by RIA. The Kruskal–Wallis test and Mann–Whitney *U*-test were used for comparison between groups. Bonferroni correction was applied when multiple comparisons were made. T3, T4, and TSH levels decreased in group 1 compared with group 2 (P, 0.01). When the results between groups 3 and 4 were compared, it was found that the T3 and T4 levels in group 3 decreased while the decreases in T3 levels were statistically significant (P, 0.01). When comparing the results of groups 1 and 3, the T4 level was lower in group 1 and the T3 level was higher in group 3 (P, 0.01). The TSH level increased in group 3 after treatment (P, 0.01). Thyroid hormones were affected after acute organophosphate poisoning. Hypothyroidism and sick euthyroid syndrome was observed during poisoning and after treatment. In serious poisoning, there may be a poor prognosis, but more extensive studies will illuminate the issue in depth.

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P17-26

Incidence of the inhibition of serum pseudocholinesterase activity in the Analytical Toxicology Laboratory determinations

R. Macovei, G. Caragea, M. Ionica

Analytical Toxicology Laboratory of the Army Centre of Medical Research, Emergency Clinical Hospital, Bucharest, Romania

Many pesticides, particularly insecticides, are neurotoxic to human and other animals because their mechanism of action is inhibition of the enzyme cholinesterase, which is essential for transmission of nerve impulses. Most pesticides in this category are organophosphate or carbamate compounds.

In the last year (2005) the Analytical Toxicology Laboratory of the Clinical Emergency Hospital Bucharest performed a number of 608 serum pseudocholinesterase activity tests, using a LabSystem apparatus with kinetic program cartridge. The quantity of reagent is reduced and the difference between acetylcholinesterase inhibitors and non-inhibitors is realized by the use of 0.5 μl of the cholinesterase activator.

Serum pseudocholinesterase (pschE) activity tests represented 8.32% from the total number of determinations due in this laboratory. The incidence on sex criterion was 43.75% tests for women and 56.25% tests for men. The inhibition of serum pschE activity represented 26.97% from total number of these determinations. For the women incidence criterion was 38.41% for inhibition of serum pschE activity and 61.59% for men also. The incidence on period of time criterion rises to a maximum in April and October (the peak months of agriculture works) for both sexes.

Organophosphate and carbamate poisoning is a health problem in developing countries and may be associated with higher mortality and morbidity. Serum pschE activity appears to be a reasonable index in the degree of clinical recovery in most cases.

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P17-27

Methodological approaches and criterions to assessment of haematoxicity of pesticides at their hygienic standardization

Valentyna Shulyak

Medved's Institute of Ecohygiene and Toxicology, Ukraine

Evaluation of the pesticides affects to the blood system with the aim of their hygienic standardization is carried out on the basis of studying of peripheral blood, as usually. These results not always adequate reflect the deep impairments in haemopoietic system. Some pesticides from different chemical classes may produce an unrecoverable depression of bone marrow's haemopoiesis. The findings of development human haemathologic diseases as a result of certain pesticides exposure are known.

Were carried out the comparison of clinical and experimental data by pesticide's influence on blood system.

Incomplete adequacy of animal's model for assessment haemopoietic system through data of analysis of peripheral blood by pesticide's exposure shown on the basis of own experimental data. The extrapolation of experimental data to human is proposed. We have elaborated the methodological approaches and criterions of assessment changes in blood system. They allow with high degree of reliability to prevent possibility to substantiate NOEL for pesticides and ADI for human as well as prevent development of haemathologic diseases.

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P17-28

The inhibitory effect of captoperil on paraquat toxicity in mitochondria isolated from the rat liver

Afshin Mohammadi-Bardbori [1,2], Mahmoud Ghazi-Khansari [1]

[1] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran University of Medical Sciences P.O. Box 13145-784, Tehran, Iran;* [2] *Faculty of Pharmacy, Shiraz University of Medical Sciences, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences P.O. Box 13145-784, Tehran, Iran*

The aim of the present study was to show the abilities of captopril as a thiol angiotensin converting enzyme inhibitor (ACEi), on paraquat-induced mitochondrial toxicity. Various concentrations of paraquat (1, 5, 10 mM) and captopril (0.08, 0.1, 1 mM) on the mitochondria isolated from rat liver with respect to time were investigated. Paraquat at concentration of 5 and 10 mM was determined to be significantly different compared to control ($p < 0.05$) and captopril at concentration of 0.08 mM (effective dose) was found not to be significantly different from control as found with MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide) assay. Lipid peroxidation was evaluated as thiobarbituric acid reactive products, mitochondrial swelling, catalase activity, GSH and GSSG concentrations were also determined. Simultaneous treatment of mitochondria with captopril (0.08 mM) and paraquat (5 mM) significantly ameliorate the mitochondria toxicity of paraquat (5 mM) alone. Our results show that captopril is effective antioxidant which its antioxidant properties is appear to be attributable to the sulphahydryl group (SH) in the compound. This effect may be due to captopril abilities to scavenge reactive oxygen species. Our results indicated that captopril can prevent oxidative stress induced by paraquat.

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P17-29

Pesticide poisoning among cut-flower farmers

Jinky Leilanie Del Prado Lu

National Institutes of Health, University of the Philippines, Manilasi, Philippines

This is a study on the adverse health effects associated with pesticide exposure among cut-flower farmers in La Trinidad, Benguet. Survey questionnaires and detailed physical and laboratory examinations were administered to 114 and 102 respondents, respectively, to determine pesticide exposure, work and safety practices, individual and family illnesses, and cholinesterase levels. Results showed that pesticide application was the most frequent activity associated with pesticide exposure, and entry was mostly ocular and dermal. Involvement of the skin was noted, with 21% of farmers having integumentary abnormalities. On physical examination, 90% or 88.2% of those examined were found to have abnormal peak expiratory flow rate (PEFR)**.** Eighty-two percent had abnormal temperature, followed by abnormal general survey findings (e.g. cardiorespiratory distress). Fifty one percent had cholinesterase levels below the mean value of 0.7 Δph/h, and 25.5% exhibited a more than 10% depression in the level of RBC cholinesterase. Certain hematological parameters were also abnormal, namely hemoglobin, hematocrit, and eosinophil count. Using Pearson's *r*, factors strongly associated with illness due to pesticides include using a contaminated piece

of fabric to wipe sweat off ($p = 0.01$) and reusing pesticide containers to store water ($p = 0.01$). Recycling of containers poses great health hazards and risks of contamination, and the current recommendation is that used containers should be buried. There is a moderate relationship between illness and average number of years of using pesticides ($p = 0.05$), and reentering a recently sprayed area ($p = 0.05$). Those with motor scale scores of equal or less than 15 indicating normal values are less likely to be sick. The greatest adverse effect of those exposed is an abnormal cholinesterase level which confirms earlier studies on the effect of pesticides on the body.

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P17-30

In vitro investigation of the immunotoxic effects exerted by organophosphorus compounds on lymphocyte proliferation

Daniela Luiza Baconi [1], Dan Balalau [1], Maria Barca [1], Mihaela Ilie [1], Monica Neagu [2], Gina Manda [2]

[1] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Bucharest, Romania;* [2] *"Victor Babes" National Institute, Immunology Department, Bucharest, Romania*

Our *in vitro* conducted study was focused on the potential immunotoxic effects of organophosphorus compounds (OPH) on the proliferation capacity of human lymphocytes. We studied chlorpyrifos (CPF), diazinon (DZN) and malathion (MLT) in the concentration range 1–1000 ng/mL. The proliferation of peripheral lymphocytes and of the lymphoblastic K562 cell line was measured by the uridine incorporation method and by the MTS reduction test. Membrane integrity, evaluated by LDH release, is not altered by OPH indicating that these compounds do not show rough cellular toxicity on normal and neoplastic lymphocytes. At 1 μg/mL DZN inhibits uridine incorporation by peripheral normal lymphocytes, nonactivated and polyclonally stimulated *ex vivo*, but experimental activation of lymphocytes cancels the inhibitory signals delivered by MLT. CPF exerts no statistically significant action on uridine incorporation. Similar results were obtained for the multiplication of the neoplastic K562 cells, evaluated by the MTS reduction test. Surprisingly, OPH tend to inhibit uridine incorporation by K562 cells at lower concentrations than 1 μg/mL. In all the investigated experimental models, cellular activation induced by OPH was also registered, mainly at lower concentrations. The observed dose-dependent biphasic effects suggest receptor-dependent mechanisms of action. Our results indicate that DZN, but not CPF, tends to restrain cell proliferation. Stimulatory effects were also noticed, without any correlation with the cell type or activation status. Accordingly, at least CPF might induce activation of the immune system with pathological consequences on hypersensitivity or autoimmune reactions.

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P17-31

Detection of organophosphorous pesticides in poisoned birds of prey

Maria Toutoudaki [1], Manolis Tzatzarakis [1], Maria Christakis [1], Maria Margariti [1], Stavros Xirouchakis [3], Panagiotis Georgiakakis [2,3], Christos Barboutis [2,3], Aristidis Tsatsakis [1]

[1] *Centre of Toxicological Sciences and Research, School of Health Sciences, Department of Medicine, University of Crete, Voutes Heraklion, 714 09 Crete, Greece;* [2] *Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Crete, Greece;* [3] *Natural History Museum of Crete, University of Crete, P.O. Box 2208, 71409 Heraklion, Crete, Greece*

Organophosporous pesticides are a very effective class of pesticides for the control of a variety of harmful insects. Their main disadvantage is their high acute toxicity and that they can be very dangerous to non target organisms.

Gastric content samples of poisoned birds of prey were presented to our laboratory by staff of the Museum of Natural History of Crete. Most of the birds where found in the island of Crete with poisoning symptoms.

The gastric content samples where liquid, hence they were centrifuged to exclude any solid matter and were passed through C18 solid phase extraction cartridges. The cartridges where preconditioned with methanol followed by distilled water. The analytes where eluted by a mixture of chloroform: methanol (9:1). The samples where screened for the following widely used organophosphates: dichlorvos, monocrotophos, phorate, dimethoate, diazinon, malathion, fenthion, parathion, ethion. They were also screened for the organochlorine endosulfan. The analysis was performed by EI GC–MS.

Out of the totally four samples analysed, belonging mostly to falco eleonorae and one to gypaetos fulvus, three samples were found positive. The gastric content sample of gyps fulvus contained 89 ng/ml malathion. The sample of MI 172 falcon eleonorae contained 57 ng/ml fenthion, and traces of endrin. MI 173, a falcon eleonorae contained 16 ng/ml fenthion. Both

organophosphates are used for the protection of very common cultivations in Crete, olive trees and vines.

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P17-32

Integrated crop management and food safety, the current status in Greece in peach cultivation

T.G. Danis [1], I.N. Tsakiris [2], M. Hourdakis [3], A.K. Alegakis [2], A.M. Tsatsakis [2]

[1] *DELCOF SA, P.O. Box 5, Anthemia Kopanos 59035, Greece;* [2] *Laboratory of Toxicology, Department of Medicine, University of Crete, Voutes 71409, Iraklion, Crete, Greece;* [3] *School of Medicine, Aristotle University of Thessaloniki, Dalipi 4-55131, Thessaloniki, Greece*

The residues of plant protection products in agricultural products is a main issue in health and food safety policy. Our research team during the last 4 years monitoring for pesticide residues in peach samples originate from Integrated Crop Management.

Monitoring was performed for the following most frequently used categories of plant protection products: (1) insecticides—methamidophos, dimethoate, chlorpyriphos-methyl, parathion-methyl, malathion, chlorpyriphos, fenthion, endosulfan, ethion, biffethrin, phosmet, phosalone, azinphos-methyl, λ-cyalothrin, deltamethrin. (2) Fungicides: chlorothalonil, captan, tebuconazole (3) Acaricides: dicofol methamidophos, dimethoate, fenthion, dicofol, endosulfan, ethion, λ-cyhalothrin and deltamethrin were not detected.

Our research points out very encouraging results for ICM in Greece and for consumers safety: (1) a total of 19 plant protection products are used in ICM cultivations. The most frequently detected plant protection product was chlorpyrifos (2) The restricted plant protection products were not detected and levels of registered plant protection products were in limits of MRLs which prove that they used only when application directives proposed their application.

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P17-33

Microbiological assessment of the organophosphorous pesticide methyl azinphos persistence in Argentinian productive soil

C. Vázquez [1], W. Copes [2], D. Gonzalez [1], J. Quagliano [1]

[1] *Organic Synthesis Area of the Laboratory for Research and Analysis of Chemical Contaminants, Applied Chemistry Department, Institute for Scientific and Technological Research of the Argentine Armed Forces (CITEFA) J.B. de La Salle 4397, CP 1603 Villa Martelli, Buenos Aires, Argentina;* [2] *Alto Valle Agricultural Experimental Station, Argentine National Institute of Agrarian Technology, Gral. Roca, Río Negro, Argentina*

In this contribution we developed a modification of a microbiological method based in the activated sludge degradation of pesticides for assessing its persistence. This test has the advantage of having a shorter experimentation time and can be easily standardized in the laboratory. Degradation of pesticides in soils is difficult to evaluate, because of the difficulty in isolating the degradation process from other effects which reduce chemical concentrations in soil, such as leaching, runoff, volatilization and plant absorption. It was reported that biodegradation and evaporation are the primary routes of disappearance for methyl azinphos.

Methyl azinphos was sprayed under controlled conditions in apple productive areas. Three rows were sprayed, and three points were sampled per row. One-gram soil samples were extracted with chloroform and filtered cleaned up by passing the extracts through Anakrom adsorption columns and then measured spectrophotometrically. Spray-drift was not observed, as methyl azinphos did not reach two nearby water streams.

In the laboratory, five standard solutions of the organophosphorous pesticide methyl azinphos were prepared in methylene dichloride at concentrations ranging from 5 to 25 mg/l. Then, each sample was added to a slurry made up with 50 g of a sample of soil from the field and 200 ml of tap water, by triplicate. Samples were agitated in a rotatory shaker at 100 rpm and 25 °C and sampled at 3 h, 6 h and 9 h. Concentration of methyl azinphos was determined by UV spectrophotometry, measuring absorbance at 250 nm. Biodegradation curves were constructed and these curves were correlated with the field results.

A positive correlation was found between laboratory and field results, indicating that this simple and accelerated laboratory method of evaluating pesticide persistence can give prompt results that can be correlated to the decay of these chemicals on the field. This fast method-

ology developed could allow to process a larger amount of data without the need of performing costly field tests, once being statistically standardized.

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P17-34

Age-dependent reproductive toxicity of dimethoate in female rats

Lyubov Ivanova, Ninel Shepelskaya, Anna Petrashenko, Igor Lepeshkin

L.I. Medved's Institute of Ecohygiene And Toxicology

Dimethoate is a systemic organophosphate insecticide used on a variety of field and orchard agricultural crops, and ornamentals. It is known that dimethoate has been shown to disrupt reproductive function in experimental animals. Even though safer alternatives to organophosphate insecticides are available, dimethoate still remains one of the most widely used insecticides in the world.

Among numerous factors risk that determine of reaction the organism for xenobiotics, the age takes one of central places. The question as the aged sensitivity of warm-blooded animals concerning to action of pesticides are little established, peculiarly in line with the reproductive system.

The study of dimethoate reproductive toxicity was conducted in adult (3-month-old) and immature (21-day-old) female Wistar rats. Dimethoate (purity, 98%) was administered for five days per week during 10 weeks before the mating, orally by gavage at dosage levels of 0, 0,1 and 0,01 mg/kg bw per day.

There was no effect on the general state, body weight gain or food consumption for any groups of animals. Investigation results have showed that the dimethoate treatment at 0,1 mg/kg induced statistically significant decreases in the mating and fertility indexes and in both in both age groups. Also dimethoate at the highest dose tested produces a minimal difference in the number of live pups decreasing in adult rats compared to juvenile animals. The analysis dates allows us doing a conclusion that the dimethoate reproductive toxicity NOEL/LOEL of 0.01 and 0.1 mg/kg/day, respectively for both groups of animals.

So there were no considerable differences in sensitivity to dimethoate-induced reproductive function damaged between adults and juveniles female rats.

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P17-35

Insight in the toxicity of malathion photodegradation product

Mojca Bavcon Kralj [1], Polonca Trebše [1], Vesna Vasić [2]

[1] *University of Nova Gorica, Laboratory for Environmental Research, Vipavska 13, Nova, Gorica, Slovenia;* [2] *Vinča Institute of Nuclear Sciences, Department of Physical Chemistry, PO Box 522, 11001 Belgrade, Serbia & Montenegro*

Organophosphorus compounds are commonly used as insecticides in agriculture. The toxic effects of organophosphorus compounds and their transformation products are based on irreversibile inhibition of acetylcholinesterase (AChE). The aim of our work was the *in vitro* investigation of the mechanism of inhibition of bovine serum AChE by malaoxon, isomalathion and diethyl maleate, i.e three main degradation products found in irradiated solutions of malathion, which can be even more toxic than the related compound.

The AcheE activity was measured by Ellman procedure, in the presence of inhibitors in the concentration range from 1×10^{-8} to 1×10^{-1} M. The experiments were performed by single exposure of 200 μg enzyme to inhibitors in final volume 0.634 ml. The increase of the incubation time up to three minutes increased the percent of inhibited activity. The sigmoid shaped inhibition curves were obtained in all cases and were analyzed by using the Hill method. IC_{50} and Hill coefficient n were determined by linear regression analysis of log [% activity/(100 − % activity)] vs. $\log C_{inhibitor}$ plots. The IC_{50} values $(2.87 \pm 0.24) \times 10^{-6}$, $(2.65 \pm 0.61) \times 10^{-6}$, $(3.01 \pm 0.36) \times 10^{-4}$ and $(5.69 \pm 0.7) \times 10^{-2}$ M were obtained for malaoxon, isomalathion, malathion and the hydrolysis product diethyl maleate, respectively. The n values fulfilled the relation $1 < n < 3$ for malaoxon (2.12 ± 0.18), malathion (1.06 ± 0.02) and diethyl maleate (1.28 ± 0.12), since for isomalathion the value 0.81 ± 0.08 was obtained. Based on the Hill coefficient one can drown the conclusion that there was the cooperative interaction between minimum two binding sites on the enzyme in all cases, since isomalathion exerted the loss of the cooperativity.

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P18 Apoptosis and Cell Cycle Control

P18-01
The effect of abnormal Savda Munziq total flavonoids on the proliferation, apoptosis and correlative gene expression in human hepatoma (HepG2) cells

Abdiryim Yusup[1,2], Halmurat Upur[2], Xirali Tursun[1], Bénédicte Berke[3], Isabelle Baudrimont[3], Nicholas Moore[3]

[1] *Institute of Xinjiang Traditional Uighur Medicine, Urumqi 830001, China;* [2] *Xinjiang Medical University, Urumqi 830054, China;* [3] *University of Victor Segalen Bordeaux 2, Bordeaux 33076, France*

Aim: Abnormal Savda Munziq (ASMq) is a traditional Uighur medicinal herbal preparation, which is commonly used for the treatment and prevention of cancer in traditional Uighur medicine. In this study, we investigated the effect of ASMq total flavonoids on the proliferation, apoptosis and correlative gene expression in human hepatoma (HepG2) cells to elucidate the mechanisms responsible for the anticancer property of ASMq.

Methods: Anti-proliferative effect was determined by MTT assay, apoptosis was determined by gel electrophoresis assay, cell cycle analysis was detected by flow cytometer, apoptosis related gene expression was detected by RT-PCR assay.

Results: ASMq total flavonoids possess inhibition effect on HepG2 cells proliferation, induction on cell apoptosis, cell cycle arrest in sub-G1 phase, and significant up-regulation of p53, p21 and Bax gene expression, down-regulation on Bcl-2 gene expression.

Conclusion: We concluded that the anticancer properties of ASMq were possibly mediated through multiple pathways, suggesting multiple ingredients, rather than single component. The acting ingredients in ASMq that exerted the anticancer effect may include total flavonoids that are abundant in ASMq. ASMq Total flavonoids may play anticancer properties by the way of anti-proliferation, induction of apoptosis and regulation of gene expression pathway.

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P18-02
The *Fusarium* toxin enniatin exerts p53-dependent cytostatic and p53-independent cytotoxic activities against human cancer cells

R. Dornetshuber[1], P. Heffeter[2], R. Kamyar[1], W. Berger[2], R. Lemmens-Gruber[1]

[1] *Department of Pharmacology and Toxicology, University of Vienna, Austria;* [2] *Institute of Cancer Research, Medical University of Vienna, Austria*

The tumor-suppressor gene p53, the "guardian of the genome", is mediating cell cycle regulating- as well as apoptosis-inducing properties. The major mechanism to induce apoptosis is believed to work transcriptionally via p53 binding to the promoter region of bax, a proapoptotic member of the bcl-2 protein family. But there also exists a less common, p53-mediated way of inducing apoptosis, believed to be independent of transcription regulation. Enniatin (ENN) is a cyclic hexadepsipeptide, produced by the genus *Fusarium*, which is well known as an inhibitor of mammalian cholesterol acyl-transferase. ENN possesses antibiotic, immunomodulatory, and ionophoric activities. Here we demonstrate that ENN exerts profound cytotoxic activity against several human tumor cells. Consequently, we further investigated the mechanisms underlying ENN-induced cell death with a focus on apoptosis- and cell cycle-regulating proteins. For this purpose, HCT116 cells with disrupted p53, p21 or bax genes by targeted homologous recombination were used to study ENN-induced cytotoxicity. In MTT assays, no significant influences of these proteins were detected, resulting for all HCT116 subclones at IC_{50} values in the low μM range at a 72 h drug exposure. In contrast, ^{3}H-thymidine incorporation revealed a significantly more efficient block of DNA synthesis by ENN in p53 wild type as compared to knock-out cells. Accordingly, PI-staining and FACS analysis demonstrated a more potent cell cycle arrest in GO/G1 phase following ENN treatment. Profound ENN-mediated inductions of both p53 and the p53-downstream cyclin-dependent kinase inhibitor p21 were detectable in p53 wild type cells by Western blot analysis. Surprisingly, p21 induction was also detectable at higher ENN concentrations in p53(−/−) cells. Bax activation by ENN was almost independent of the cellular p53 status. These results suggest that the cytotoxic effects of ENN are mediated by p53-dependent and independent mechanisms. Studies are underway to clarify the molecular mechanisms underlying the p53-independent induc-

tion of bax and consequently apoptotic cell death by ENN.

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P18-03
A quinone sensitive phenotype results from reduced *Pim3* expression in rat hepatocytes

Joel D. Parry, Jon J. Lyon, Andrew J. Smith, Timothy W. Gant

GSK R&D, United Kingdom

Quinones can exert toxicity through oxidative stress and/or sulphydral arylation of cellular macromolecules. We have observed that in rat hepatocytes exposed to quinones (e.g. 2, 3-dimethoxy-1, 4-napthoquinone, DMNQ, a pure redox cycler and menadione, MNQ, a redox cycler/arylator) transcription of *Pim3* (a member of the PIM family of serine/threonine kinases) is consistently increased. Other members of the PIM family (i.e. *Pim1* and *Pim2*) play a role in regulating anti-apoptotic signalling and *Pim3* mediates apoptosis in HepG2 cells. These data imply a role for *Pim3* in modulating the apoptotic response to quinone-mediated cytotoxicity. Using siRNA we have sought to test the hypothesis that *Pim3* is an important gene involved in regulating the hepatocytes response to redox stress.

Hepatocyte cultures were transfected with Silencer® pre-designed rat *Pim3* siRNAs or negative control 1 siRNA (Ambion) at 100 nM in WE media for 24 h. Transfection media was removed and hepatocytes exposed to concentrations of DMNQ (150 μM) or MNQ (25 μM) that are normally sub-toxic at up to 8 h of exposure. LDH leakage, activated caspase 3 activity and reduced glutathione (GSH) levels were measured along with *Pim3* transcription.

Pim3 knockdown resulted in 14% (DMNQ) and 48% (MNQ) increased LDH leakage compared with control, after 3 h exposure. *Pim3* knockdown also resulted in up to 95% (DMNQ) and 135% (MNQ) increased activation of caspase 3. No effect on GSH levels were observed with reduced transcription of *Pim3*.

These data indicate that *Pim3* knockdown sensitises hepatocytes to quinone-mediated necrotic and apoptotic cell death (as assessed by LDH leakage and activated caspase 3 activity, respectively). The response was greater with MNQ than DMNQ, suggesting that *Pim3* regulation is important in the response to cytotoxicity resulting from both sulphydral arylation and redox stress. Therefore it can be concluded that *Pim3* is important in the hepatocyte response to cellular stress resulting from sulphydral arylation and redox cycling. *Pim3* is most likely involved in mediating the apoptotic response to these chemical stressors.

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P18-04
Induction of apoptosis in murine thymocytes by arsenic

Manoj Aggarwal [1], J.K. Malik [1], A.M. Kotresh [2], P.K. Gupta [1], Prashant Maurya [1], G.S. Rao [1]

[1] *Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh, India;* [2] *Division of Animal Biochemistry, Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh, India*

Exposure to toxic metals remains a widespread problem. Human beings, livestock and wild life come in contact with these toxic metals through inhalation of air and food and water intake. Arsenic is an extremely toxic pollutant that is found in soil, water and air. The most severely affected countries include Bangladesh and India. In Bangladesh alone, 46–57 million people consume water with arsenic concentrations in excess of 10 ppb, the World Health Organization Standard. Obviously, human beings and animals are being exposed to arsenic at a concentration much higher than the permissible limit. Arsenic is reported to cause immunotoxicity, reproductive disorders, embryotoxicity, tumors of skin, bladder, liver and lung, oxidative stress, mutagenicity and apoptosis in some laboratory animal species and in *in vitro* models. Previous works in our laboratory showed immunotoxicity and apoptosis in peripheral blood lymphocytes and splenocytes of chickens receiving arsenic at 3.7 ppm in drinking water. Apoptosis of immune cells may lead to various kinds of immunological alterations leading to recurrent infections, cancers and occurrence of disease outbreaks in spite of proper vaccination. The present study was carried out to investigate the potential of arsenic to induce apoptosis in cultured murine thymocytes. As detected by flow cytometry after staining with propidium iodide, the percent apoptotic thymocytes (hypodiploid peak) was increased after 12 h exposure with arsenic. DNA gel electrophoresis showed typical ladder pattern, which was dose dependent. The results tend to suggest that arsenic may induce apoptosis in murine thymocytes, which is likely to contribute to the immunotoxic effects of arsenic.

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P18-05
Apoptotic mechanisms of ethylene glycol ether end metabolites

J. Liesivuori [1,2], S. Pennanen [1], H. Stedt [2], S. Manáková [2], K. Heiskanen [3], J. Laitinen [1], H. Savolainen [4]

[1] *Finnish Institute of Occupational Health, Kuopio, Finland;* [2] *University of Kuopio, Kuopio, Finland;* [3] *Turku Centre for Biotechnology, Turku, Finland;* [4] *Ministry for Social Affairs and Health, Tampere, Finland*

It has been firmly established that the toxicity of ethylene glycol ethers is associated with their end metabolites, the corresponding alkoxyacetic acids. The key toxicological effect seems to be the inhibition of the succinate dehydrogenase acitivity in mitochondria. It was first found in occupationally exposed workers and later confirmed in an animal model.

This finding is very important as the inhibition would disrupt mitochondrial energy metabolism thereby causing harmful effects in highly oxygen-dependent organs like brain or kidneys. In the process, mitochondria could also be destroyed liberating e.g. cytochrome *c* from them. The latter is a known initiator of the apoptotic pathway activating the caspase complex.

Thirdly, the succinate dehydrogenase also links the tricarboxylic acid cycle to the mitochondrial respiratory chain. In case of its inhibition, succinate will accumulate in the milieu. The acid seems to activate the hypoxia inducible factor-1 (HIF-1) which induces the upregulation of several growth factor, e.g. in macrophages.

In our studies, we compared the inhibitory activity of three alkoxyacetic acids and compared the results with their caspase activating capacity. The results compared favourably with empirically found toxic potential, i.e. methoxyacetic acid > ethoxyacetic acid > butoxyacetic acid. Thus, it seems that the alkoxyacetic acids are the proximate toxins. For comparison, the methoxypropionic acid does not share these effects. Further studies will show the significance of HIF-1 activation, e.g. in tumourigenesis.

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P18-06
Fumonisin B_1-induced apoptosis in rat liver: Morphometrical study

Goran Kovačević, Gordana Gregorović, Karlo Horvatin, Željko Krstanac, Ana-Marija Domijan, Davor Želježić, Maja Peraica, Mirjana Kalafatić

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Fumonisins are a family of mycotoxins produced by *Fusarium* sp. (mostly *Fusarium moniliforme* and *Fusarium proliferatum*). Exposure to fumonisins is associated with a variety of adverse health effects in domestic and experimental animals. Among more than 10 types of fumonisins, the most prevalent and the most toxic is fumonisin B_1 (FB_1). Apoptosis is a programmed cell death characterized by a cytoplasmic shrinkage and condensation of chromatin in nucleus. The aim of this study was to assess whether low doses of FB_1 induce apoptosis in rat liver at different lag time after treatment. Adult, male Wister rats (six per group) were given single oral dose of FB_1 (5 μg/kg b.w., 50 μg/kg b.w., and 500 μg/kg b.w. dissolved in sterile saline) and sacrificed 4 h, 24 h, and 48 h afterwards. Control animals were given sterile saline or Fe-NTA. Whole liver was fixed in formalin, routinely processed, and embedded with paraplast. Sections of 4 μm were stained with hematoxyllin and eosin (HE). Number of hepatocytes undergoing apoptosis was determined by counting 500 cells (computer software Lucia G 4.81) in 25–50 randomly selected microscopic fields of vision (microscope Nikon Eclipse E600). Data were presented as mean with standard deviation, median, minimum and maximum values, and geometrical mean. Possible differences in treatment and control groups were evaluated using *t*-test. Probability values of $P < 0.05$ were considered statistically significant. Microscopic examination revealed extensive changes in the rat liver. The number of hepatocytes in apoptosis and of apoptotic bodies was dose-related. Changes in histological structures were found throughout the lobules of all FB_1 treated rats, but the apoptotic cells were predominant in perilobular region. These results may be explained by the direction of blood flow in lobe from perilobular towards centrilobular part with consequent primary and more extensive damage in perilobular region. In conclusion, all applied doses of FB_1 caused changes in liver morphology irrespectively of the time of sacrifice indicating that even such low doses may be toxic for animals and humans.

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P18-07

Effect of recombinant platelet-activating factor acetylhydrolase on acetaminophen-induced experimental cell proliferation in rats

A.D. Grypioti [1,2,3], E. Fragopoulou [2,4], C.A. Demopoulos [2], M. Mykoniatis [1]

[1] *Department of Experimental Pharmacology, Medical School, National and Kapodistrian University of Athens, Greece;* [2] *Department of Chemistry, National and Kapodistrian University of Athens, Greece;* [3] *BioAnalytica, Biotechnology and Analytical Systems, Greece;* [4] *Department of Science of Dietetics-Nutrition, Harokopio University of Athens, Greece*

Aim of this study was to investigate the effects of PAF inactivator, recombinant PAF-acetylhydrolase (rPAF–AH) on post acetaminophen-treatment functional outcome of the liver in the rat. Fifty male Wistar rats were divided into two groups: the control group received a toxic dose of acetaminophen (3.5 g/kg BW) and the rPAF-AH-treated group received the same dose of acetaminophen followed by a dose of rPAF-AH (10 mg/kg BW) intraperitoneally. The animals were sacrificed at time points of 20, 24, 28, 32 and 40 after acetaminophen treatment. The hepatic injury was evaluated by determination of AST, ALT and ALP activities. Liver regeneration was estimated by [^{3}H] thymidine incorporation into hepatic DNA, liver thymidine kinase activity and hepatocyte mitotic index. The positive effects of rPAF-AH were expressed by high decrease of hepatic injury and diminution of regenerating activity. These results indicate that the use of PAF inactivator enhances liver's recovery from acetaminophen intoxication and attenuates the severity of experimental liver injury providing important means of improving liver function following acetaminophen intoxication.

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P19 Chemical Carcinogenesis

P19-01

Immunohistochemical characterization of pre-neoplastic lesions observed in a long-term clofibric acid study in the rat

C. Michel [1], C. Desdouets [2], D. Hoet [1], M.-P. Bralet [3], K.R. Isaacs [4], R.A. Roberts [5], E. Boitier [1]

[1] *Sanofi Aventis, Drug Safety Evaluation, Vitry-sur-Seine, France;* [2] *INSERM U370, Paris, France;* [3] *Hopital Universitaire Paul Brousse, Villejuif, France;* [4] *Harrogate, UK;* [5] *Astrazeneca, Safety Assessment, Cheshire, UK*

Clofibric acid (CA) is a non-genotoxic hepatocarcinogen in the rodent that belongs to the class of peroxisome proliferators that induce altered hepatocellular foci and /or neoplasms. The aim of this study was to monitor selected aspects of the phenotype of the different foci during a long-term hepatocarcinogenesis study with CA in the rat and after CA withdrawal.

Male F344 rats, some of which had received a single IP injection of diethylnitrosamine (DEN, 30 mg/kg), were dosed with 5000 ppm CA in the diet for up to eighteen months with a 10-week recovery period. Rats were necropsied on days 18, 46, 102, 264, 377, 447 (DEN + CA recovery phase), 524, and 608 (CA recovery phase). The different types of focus were evaluated by microscopic examination of hematoxylin/eosin stained sections. Immunohistochemistry was performed for glutathione-*S*-transferase pi (GST-p) to evaluate the influence of CA on DEN-induced foci, as well as Ki67 expression and caspase 3 activation for measuring cell proliferation and apoptosis, respectively. Prohibitin, which was found, by gene expression profiling, to be up-regulated in the CA-induced foci and tumors, was also evaluated immunohistochemically.

CA treatment had no influence on the incidence of tigroid foci but accelerated the onset of eosinophilic and clear cell foci, which decreased in number after CA withdrawal to return to a control level. Unlike common descriptions in the literature on the effects of CA treatment, basophilic foci were not the most representative focus type in the early timepoints, and they did not regress after CA withdrawal. Our results also confirmed that CA treatment did not promote DEN-induced GST-p foci, nor make them regress. Evaluations of cell proliferation, rates of apoptosis and prohibitin expression are currently being performed to distinguish which foci are potentially pre-neoplastic lesions. This identification will be a key step in the identification of early

molecular events responsible for the hepatocarcinogenic properties of peroxisome proliferators in rats.

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P19-02
Enhanced lung tumor response by gas-phase-depleted particle phase of cigarette mainstream smoke in A/J and Swiss SWR mice

Walter Stinn[1], Ansgar Buettner[1], Josje Arts[2], Frieke Kuper[2], Hans-Juergen Haussmann[3]

[1] *PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany;* [2] *TNO Quality of Life, Zeist, The Netherlands;* [3] *Roesrath, Germany Roesrath Germany*

Here we report on two mouse strains suggested as possible models for lung tumorigenicity. Male A/J mice and Swiss SWR mice were whole-body exposed to diluted mainstream smoke from the standard reference cigarette 2R4F or to filtered fresh air (control), 6 h per day, 5 days per week for 5 months, followed by a post-exposure period of 4 months. Smoke exposure was to whole smoke at concentrations of 120 μg or 240 μg total particulate matter (TPM)/l, to gas phase-depleted particle phase at 240 μg TPM/l, or to gas phase at a carbon monoxide concentration equivalent to 240 μg TPM/l. Lung tumor incidence and multiplicity were determined in the control and 240 μg TPM/l whole smoke groups at the end of the exposure period and in all groups at the end of the post-exposure period.

At the end of the 5-month exposure period, there was no difference in lung tumor response between smoke-exposed and control mice in both mouse strains. An enhanced lung tumor response was seen after the 4-month post-exposure period: lung tumor incidence and multiplicity in the whole smoke groups was dose-dependently higher compared to controls by a factor of up to approximately 3-fold in A/J mice and a factor of up to approximately 2-fold in Swiss SWR mice. In A/J mice the lung tumor response was similar for gas phase-depleted particle phase and whole smoke at the same TPM concentration. In Swiss SWR mice, gas phase-depleted particle phase accounted for approximately 50% of the increase in lung tumor response in the whole smoke group at the same TPM concentration. Gas phase failed to enhance lung tumor incidence and multiplicity in both mouse strains compared to controls. Microscopic examination of lung step serial sectioning in selected groups confirmed the macroscopic lung nodule assessment. Smoke exposure did not change the spontaneous lung tumor spectrum; bronchiolo-alveolar adenoma was the most prominent lung tumor type.

There appears to be a need for a post-exposure period to see an increase in lung tumor incidence and multiplicity in both mouse strains. Further research is needed to evaluate the relevance of these models with regard to the human disease.

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P19-03
IPCS framework for analysing the relevance of a cancer mode of action for humans

Alan R. Boobis[1], Samuel M. Cohen[2], Vicki Dellarco[3], Douglas McGregor[4], Carolyn Vickers[5], Deborah Willcocks[6], William Farland[7]

[1] *Imperial College London, UK;* [2] *University of Nebraska, Omaha, NE, USA;* [3] *Office of Pesticides Programs, US EPA, Washington, DC, USA;* [4] *Toxicity Evaluation Consultants, Aberdour, UK;* [5] *IPCS, WHO, Geneva, Switzerland;* [6] *NICNAS, Sydney, Australia;* [7] *ORD, US EPA, Washington, DC, USA*

Based on experience gained since the publication of the International Programme of Chemical Safety (IPCS) Mode of Action (MOA) framework in 2001 and development by the International Life Sciences Institute Risk Science Institute (ILSI RSI) of a Human Cancer Relevance Framework, IPCS have combined and extended these components to produce a unified Human Cancer Relevance Framework (IPCS HRF). This starts with the concept that for a given tumour produced by a given compound in experimental animals it is sometimes possible to establish a series of key events that are along the causal path. Involvement of the key events in the MOA is established on weight of evidence, using criteria based on those described by Bradford Hill, taking account of factors such as dose–response and temporal concordance, biological plausibility, coherence and consistency. Alternative MOAs that logically present themselves in the case must be considered. Normally, if these cannot be excluded it is difficult to proceed with the HRF. Once an MOA is established, qualitative and quantitative comparison of each key event between the experimental animal and humans enables a conclusion as to likely relevance of the MOA for human risk. Application of the HRF is not a complete risk assessment, for example there is no consideration of exposure. However, the information obtained can be invaluable in the further risk assessment of the compound, such as informing the most appropriate dose–response extrapolation approach. The IPCS HRF provides a means to harmonise assessments of the relevance of a carcinogenic response to a chemical. The framework is an analytical tool that enables greater trans-

parency, identification of key data gaps and helps ensure consistency in evaluations. Once the human relevance of an MOA has been established, when a compound is shown to produce tumours by this MOA the relevance of the response will be known. All those involved in assessing carcinogenic responses are encouraged to evaluate the use of the framework.

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P19-04

o-Toluidine adducts in human bladder DNA and hemoglobin by the local anesthetic prilocaine

Elmar Richter, Kerstin Gaber, Ulrich A. Harréus, Christoph Matthias, Norbert Kleinsasser

Ludwig-Maximilians-University of Munich, Munich, Germany

Occupational exposure to *o*-toluidine is associated with an increased risk of bladder cancer. Because *o*-toluidine is a major metabolite of prilocaine, increasing use of this local anesthetic could be a significant source of this bladder carcinogen. Therefore, the impact of prilocaine-treatment on DNA and hemoglobin (Hb) adducts from *o*-toluidine was assessed. Blood samples were obtained before and 24 h after head and neck surgery from 20 patients receiving prilocaine anesthesia (100 mg). In addition to blood samples six volunteers provided 24 h urine samples before and after s.c. injection of 100 mg prilocaine. Hb was isolated from 10 ml blood samples by ethanolic precipitation. DNA was extracted from 24 h urinary sediments using a QIamp DNA Micro Kit. After addition of d_9-*o*-toluidine, solutions of Hb and DNA were hydrolyzed in either 0.1N NaOH (Hb) or 4N HCl (DNA). *o*-Toluidine released from Hb was enriched on Bond Elut C18 cartridges and eluted with chloroform. The acidic DNA solution was washed with dichloromethane and *o*-toluidine was extracted with hexane after alkalinization. For determination of *o*-toluidine by capillary gas chromatography/mass spectrometry with negative chemical ionization, the extracts were derivatized with heptafluorobutyric anhydride. Hb adducts of *o*-toluidine were significantly increased 24 h after prilocaine-treatment (mean ± S.E.: 21.5 ± 2.6 ng/g Hb, $p < 0.0001$). Excluding one patient with high background level (40.9 ng/g) this corresponds to a 6–360-fold increase of mean *o*-toluidine Hb adduct levels from 0.54 ± 0.19 ng/g before treatment to 22.0 ± 2.7 ng/g 24 h after surgery. Neither background values nor the increase of *o*-toluidine Hb adduct levels were influenced by the smoking status. In all six healthy volunteers, background levels of *o*-toluidine-releasing DNA adducts were present in 24 h urine (0.66 ± 0.14 ng/mg DNA). Within 24 h after prilocaine treatment, adduct levels increased in only three subjects and differences did not reach significance compared to background levels (1.18 ± 0.47 ng/mg DNA). Studies are underway with longer term sampling of urine. In conclusion, prilocaine anesthesia leads to a massive increase of Hb adducts of *o*-toluidine. Together with the formation of DNA adducts in urothelia, this implies a carcinogenic risk which should be taken into account in preventive hazard minimization.

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P19-05

Use of urinary porphyrin profiles as an early warning biomarker for monomethylarsonous acid (MMA^{III}) exposure

K. Manonmanii [1], M.R. Moore [1], P.K.S. Lam [2], J.C. Ng [1]

[1] *National Research Centre for Environmental Toxicology (EnTox), University of Queensland, Brisbane, Australia;* [2] *The City University Hong Kong, Hong Kong*

Although it is well known that arsenic is toxic and that arsenic is carcinogenic, the mechanism underlying this is unknown. Work in our laboratories has established a model that produces multi-organ tumours in mice following extended exposure to arsenic in drinking water. Until recently the metabolism of arsenic was thought to be a detoxification process. Recent studies show that MMA^{III} is the toxic intermediate of arsenic metabolism. It is a more potent cytotoxin and genotoxic than As^{III} and As^{V}, and is believed to be the proximal carcinogen. The Cytochromes P450 are inhibited by arsenic although such inhibition is cytochrome-specific. The involvement of the cytochromes in carcinogenesis is now proven to be through the ability of individual cytochromes to bioactivate pro-carcinogens. Haem and porphyrin synthesis are critical in the formation of the cytochromes. Exposure to Arsenic is known to affect the activity of the enzymes of haem biosynthesis. We evaluated the use of urinary porphyrin profiles as an early warning biomarker for arsenic carcinogenicity.

Young female C57BL/6J mice were given drinking water containing 0 μg/L, 100 μg/L, 250 μg/L and 500 μg/L arsenic as MMA^{III} *ad libitum*. Twenty-four hours urine samples were collected at various time intervals for up to 48 weeks for urinary arsenic accumulation by HPLC–ICPMS and urinary porphyrin measurement by HPLC. DMA^{V} was the major metabolite excreted and it showed significant dose dependent increase at each time point and exposure dependent increase over 48

weeks. Porphyrin levels appeared to be age dependent. Total porphyrin levels among the treatment groups were in a dose-related in weeks 24 and 48. The increases after 8 weeks, 24 weeks, 32 weeks and 40 weeks of exposure were the highest compared to the control group. Level of coproporphyrin III and uroporphyrin in test groups were higher compared to control group after 24 weeks and 48 weeks, and 2 weeks, 4 weeks, 8 weeks, 16 weeks, 40 weeks and 48 weeks of exposure, respectively.

The results indicate that the urinary porphyrin concentration has the potential for use as an early warning indicator for chronic arsenic exposure prior to the onset of arsenic carcinogenesis.

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P19-06
Identification and analysis of styrene oxide adducts with amino acids in human globin

Michal Jágr, Jaroslav Mráz, Vladimír Stránský

National Institute of Public Health, Šrobárova 48, Prague 10, 10042, Czech Republic

Styrene, an important monomer used in plastics industry, is metabolized to a genotoxic intermediate styrene-7,8-oxide (SO). Minor part of SO can produce stable covalent adducts at various nucleophilic sites in the body, e.g. in DNA or proteins. In blood protein globin, the amino acids reported to form adducts with SO are Cys, His, Asp, Glu, N-terminal Val, Lys, and Tyr. Several SO adducts were detected for each amino acid because of simultanous formation of both 2-hydroxy-1-phenylethyl (21HPE) and 2-hydroxy-2-phenylethyl (22HPE) regioisomers, combined with the diastereomerism due to the chiral properties of SO. Additionally, binding of SO to His can occur via N_π or N_τ in the imidazol ring of His.

The main aim of our study was to identify major isomers of the SO adducts with Cys, Lys, and His in human globin. For reference purposes, the synthetic standards were prepared and characterized using GC/MS, HPLC/MS, and NMR. Human globin incubated with a 100-fold molar excess of SO was hydrolyzed with pronase, derivatized by a terc-butyldimethylsilyl (TBDMS) reagent, and analyzed by GC–MS. The most abundant regioisomers found were 21HPE-Cys, 21HPE-Lys, and N_τ-22HPE-His. All remaining regioisomers were also detected. The diastereomers were not resolved by GC, however, some pairs could be separated by HPLC. To develop an optimum quantitation of the SO adducts using GC/MS, several other derivatization procedures were examined. Trimethylsilylation (TMS) and acylation with chloroformates (systems ethylchloroformate–ethanol and isobutylchloroformate–heptafluorobutanol) were used. TBDMS, unlike TMS, provided single derivative (trisTBDMS) for each tested adduct, characteristic fragments in mass spectra, and good reproducibility. Chloroformates rapidly acylated the carboxy and amino groups but the hydroxy groups were resistant to acylation. Some of the expected products could not be found in the chromatograms. Thus, the TBDMS derivatives proved to be most suitable for GC/MS determination of the SO–amino acid adducts.

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P19-07
Toxicoproteomics identifies hepatocytes and Kupffer cells as Independent targets of the carcinogen *N*-nitrosomorpholine

Olga Teufelhofer, Wolfram Parzefall, Eveline Kainzbauer, Rolf Schulte-Hermann, Christopher Gerner

Internal Medicine Clinic I, Department: Institute of Cancer Research, Medical University of Vienna, Austria

Development of liver cancer is often accompanied by inflammation, which is believed to be a secondary response to primary toxic effects of chemical carcinogens on hepatocytes. Recent data have, however, demonstrated that chemical carcinogens have a direct stimulatory effect on resident macrophages of the liver, Kupffer cells, to release superoxide and some cytokines. A critical contribution of Kupffer cells as mediators of inflammation to the effects of chemical carcinogens has been suggested. The aim of the study was to get information on specific changes of protein profiles of Kupffer cells and hepatocytes in response to chemical carcinogen *N*-nitrosomorpholine (NNM) used as a model compound. Rats were treated with a single dose of NNM and whole liver samples as well as pure hepatocytes and Kupffer cells were obtained 12 h later. In addition, cells from untreated animals were treated with NNM *in vitro* for 4 h. Cells were metabolically labelled in vitro with ^{35}S-methionine and cysteine. Proteins of the cytosolic fractions from liver tissue samples and from primary cells were separated by two-dimensional gel electrophoresis (2D-PAGE) and their amounts and synthesis rates were estimated by fluorography and autoradiography. About

200 affected proteins were identified by mass spectrometry. We found that both, hepatocytes and Kupffer cells, responded to NNM *in vivo* and *in vitro* by a pronounced increase of overall protein synthesis. The alterations in protein profiles of hepatocytes and Kupffer cells after NNM were: (i) cell-type specific, (ii) quantitatively similar though qualitatively different in response to in vivo versus in vitro treatment, and (iii) largely different from those induced by inflammatory mediators IL-6 in hepatocytes and LPS in Kupffer cells. These results demonstrated that hepatocytes and Kupffer cells are massively activated by NNM *in vivo* and *in vitro* and, thus, direct independent targets for the chemical hepatocarcinogen.

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P19-08

Protective effects of amifostine on doxorubicin-induced cardiac and renal toxicity in rats

Viktorija M. Dragojevic-Simic [1], Vesna M. Jacevic [2], Dubravko R. Bokonjic [2], Silva Lj. Dobrić [3], Lidija D. Zolotarevski [4], Katarina A. Lelićic [4]

[1] *Clinical Pharmacology Center, Military Medical Academy Belgrade, Serbia and Montenegro;* [2] *National Poison Control Center, Military Medical Academy Belgrade, Serbia and Montenegro;* [3] *Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia and Montenegro;* [4] *Institute for Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia and Montenegro*

Therapeutic application of anthracycline antineoplastic agent doxorubicin (DOX) is limited due to its adverse effects on bone marrow, intestinal tract epithelium and heart. In addition, it has nephrotoxic effects in experimental rats. Amifostine (AMI) is a sulphydril containing compound which administered before some antineoplastic agents or radiation provides broad-spectrum cytoprotection of various normal tissues. However, data concerning its efficacy in preventing toxic effects of DOX is still scarce. The aim of this study was to evaluate general, cardioprotective and renoprotective effects of AMI in rats treated by large, single dose of DOX 7 days after its application. Rats were divided in six experimental groups: I—no treatments; II—AMI (300 mg/kg i.p.) 20 min before saline (1 ml/kg i.p.); III—DOX (6 mg/kg and 10 mg/kg i.p.); IV—AMI (300 mg/kg i.p.) 20 min before DOX (6 mg/kg and 10 mg/kg i.p.). Mortality, general condition and body weight of the animals were observed 7 days after treatment. Rats were sacrificed, heart and kidneys were obtained, weighed and examined by light microscopy (semiquantitative grading scales were used). Pretreatment with AMI significantly reduced mortality as well as weight loss of the rats treated with DOX in dose of 10 mg/kg. Absolute weight (g) of heart and kidneys were significantly decreased in DOX-treated animals (10 mg/kg) compared to control ones, what was not the case in the protected rats. In both groups of AMI-pretreated rats myocyte alterations were significantly less severe than those observed in animals receiving DOX alone ($p < 0.001$). The difference between the severity of the nephropathy scores obtained in both DOX-treated groups of rats comparing with those pretreated with AMI was also significant. Our results indicate that cardio- and renal protection significantly contribute to successful AMI application against DOX-induced toxicity in Wistar rats.

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P19-09

Validation of putative biomarkers for the early prediction of non-genotoxic hepatocarcinogenesis and comparison to drug signatures

Mark Fielden, Richard Brennan

Iconix Pharmaceuticals, USA

Identifying the hepatocarcinogenicity of drug candidates requires resource-intensive 2-year chronic administration bioassays in rodents. Numerous biomarkers have been proposed to facilitate early prediction of hepatocarcinogenic potential, however, their validity is not well characterized, and it is likely that supervised classification models using large scale genomic data will be required to accurately predict such a complex phenotype. The objective of this study was to use gene expression data from 119 compounds with known hepatocarcinogenic potential in order to validate a number of putative genomic biomarkers identified in the literature. While most biomarkers had little predictive accuracy, TGF-stimulated clone 22 (Tsc-22) had moderate accuracy, thus confirming previous reports. Using a supervised classification algorithm and a 119 compound data set, a Drug Signature consisting of 35 genes was derived to predict non-genotoxic hepatocarcinogenicity using liver gene expression data from 5 day repeat dose rat studies. The accuracy of the signature was then compared to Tsc-22 using an independent validation test set of 31 compounds unrelated to the training set. Tsc-22 was found to have accuracy less than 50% due to a large number of false positives. By contrast, the Drug Signature correctly classified 80% of the 31 test compounds. When incorporated into a routine toxicogenomic evaluation of candidate drugs, this signature can be used

to identify potential long term carcinogenic potential and thus reduce late stage attrition. The signature also has applications in hazard identification for production chemicals.

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P19-10
Are NADPH-oxidase knockout mice protected from diethylnitrosamine (DENA) induced liver carcinogenesis?

Wolfram Parzefall, Constanze Freiler, Olga Teufelhofer, Eveline Kainzbauer, Franziska Ferk, Rolf Schulte-Hermann

Research Unit Toxicology and Prevention, Institute of Cancer Research, Clinic of Internal Medicine, Medical University of Vienna, Austria

Nitrosamines occur in traces in food or may be generated endogenously. They are one chemical factor involved in hepatocarcinogenesis, a process mediated by genotoxic and cytotoxic events. We have previously shown that reactive oxygen species (ROS) and TNF-α production are increased after DENA treatment in wild type (wt) mouse liver but not in p47-NADPH oxidase knockout (phox-ko) mice and that DNA damage is more intense in wt mice liver [Teufelhofer et al., 2005. Carcinogenesis 26, 319–329]. Furthermore, in a recent study we pretreated wt mice with apocynin, a phox inhibitor. Under these conditions superoxide (SO) production and protein nitration after DENA was drastically decreased. We hypothesized that besides ROS also nitric oxide may be involved in the cytotoxic and inflammatory actions and might further hepatocarcinogenesis. Indeed, nitrated liver proteins were found by Western analysis at early time points after DENA in wt, but not in phox-ko mice. In addition, mRNA expression of iNOS was examined by RTPCR. mRNA levels were not changed in wt but were increased in phox-ko males only. In contrast, in female mouse livers of both strains mRNA levels remained unaltered although on a higher expression level than in males. Experiments were designed with male mice to prove the lower susceptibility of phox-ko to liver tumor formation by DENA in comparison to wt mice. However, preliminary results point to quantitative differences in tumor yields, demonstrating some but not full protection of the knock-outs. It appears therefore that other mechanisms than SO generation might participate in the cellular defense in male phox-ko livers. One particular possibility is an enhanced production of reactive nitrogen species by iNOS which is currently under investigation.

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P19-11
Antitumor medicinal combinations toxicity signs decreasing through redaction of ingredients dose in experiment

Susanna S. Tanina

Academy of Medical Science Institute of Pharmacology and Toxicology, Ukraine

One of the important directions of cancer cure is minimization of preparations toxicity signs. Our aim was creation of new preparations combinations for tumors of cerebral localizations. Creation of the regimes and combinations of antitumor medicines intend phase of new technologies experimental execution with rational approach to models choice, on which the results of activity as much as possible correlate with the clinical data. The CNS tumors objective estimation of efficiency of their treatment is rather complicated. One of most adequate models is Glioblastoma Yablonovsky, passed intra-cerebrally to young rats with mass 40.0–50.0 g (as donors were used animals with nervous system lesion symptoms). 0.1 ml of 20% suspension of tumor cells in special fluid (N 199) were injected into regio frontalis capitis of brain. Treatment began in 10 days after operation. The significance criterion is equal to 25.0% increase of life expectancy. Mean life of animals in control group is equal 21.4 ± 1.3 days. As a tested preparations where used Chlophiden (phosphorylated chloroethylamine), Brotheophin (purine antagonist) which were developed in our Institute and Vincristin, Phtorafurum and Adriamycin. Were estimated activities of preparation in mono-regimes and in schemes of polychemotherapies (I and II). At the end of experiments we received such results: after Phtorafurum and Adriamycin therapy effects were at the rate of about significance criterion, after Vincristine, effect was lower than significance criterion, Brotheophine effect did not reached 10.0% effect. The highest effect was after treatment with Clophiden (80.0% increase off lifetime). All this preparations were selected for development of therapeutic combinations in much lower doses than in mono-therapy, and that's why reduced toxicological signs (visualized), with prevention of antitumor activity. Clophiden, Brotheophin and Adriamycin formed I combination (64.0% effect); Phtorafurum, Adriamycin and Vincristin formed II combination (54.0% effect). Consequently, after this experiments were obtained dates, which indi-

cated that selected schemes of polychemotherapy, tested on experimental models of CNS with reduction of base preparations' dose, have shown high antitumor activity in comparison with some mono-chemotherapy regimes or higher it in certain cases. And, reduction of base preparations' in schemes decrease toxicity impact.

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P19-12

Skin and liver cancer induced by monomethylarsonous acid (MMA^{III}) in mice

Manonmanii Krishnamohan [1], Mohammed M. Shareef [2], Michael R. Moore [1], Alan A. Seawright [1], David J. Carey [2], Mansoor M. Ahmed [2], Jack C. Ng [1]

[1] *National Research Centre for Environmental Toxicology (EnTox), University of Queensland, Australia;* [2] *Weis Centre for Research, Geisinger Clinic, PA, USA*

Although arsenic is toxic and a proven carcinogen, the mechanism underlying carcinogenesis is unknown. Until recently the metabolism of arsenic was thought to be a detoxification process. MMA^{III} is a toxic intermediate of arsenic metabolism. Recent studies show that MMA^{III} is more cytotoxic and genotoxic than As^{III} or As^{V}. Our aim was to develop an animal model to study the role of MMA^{III} in arsenic carcinogenesis and in particular to study the development of skin cancer, the predominant presenting feature in humans.

Female C57BL/6J mice were given drinking water containing 0 μg/L and 500 μg/L arsenic as MMA^{III} *ad libitum* for 2 years. Five animals from each group were sacrificed at various times up to 24 months for arsenic and biomarker measurements. HPLC–ICPMS was used to assess excretion of As species. DMA^{V} was the major metabolite which showed a significant dose-dependent increase over 2 years of exposure.

An excess of tumors were observed in the MMA^{III}-treated animals around 18 months. MMA^{III} treatment resulted in firstly, dermatitis which progressed to trichoepithelioma. There was also an excess of liver cancer. Genome-wide expression profiling of the liver tumors induced by MMA^{III} by Affymetrix (Mouse genome 430 2.0Array) system for 45,000 probe sets, indicated the disruption of signaling pathways. The components mediating the outcome were different in treatment modalities with the same end result of increase in the expression of Bcl2 a prosurvival gene.

Our results show MMA^{III} is carcinogenic in female C57BL/6J mice and supports the contention that methylation is a toxic process, not a detoxification pathway. MMA^{III} derived metabolically from inorganic arsenic is likely to be the proximal carcinogen.

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P19-13

***XPA* A23G, *XPC* Lys939Gln, *XPD* Lys751Gln and *XPD* Asp312Asn polymorphisms, interactions with smoking and dietary factors, and risk of colorectal cancer**

Rikke Dalgaard Hansen [1,2], Mette Sørensen [2], Anne Tjønneland [2], Kim Overvad [2], Ole Raaschou-Nielsen [2], Ulla Vogel [1]

[1] *National Institute of Occupational Health, Lersø Parkalle 105, 2100 Copenhagen, Denmark;* [2] *Institute of Cancer Epidemiology, Danish Cancer Society, Strandboulevarden 49, 2100 Copenhagen, Denmark*

The nucleotide excision repair (NER) pathway is involved in removal of a wide variety of bulky DNA lesions. Previous studies on some of the genes involved in NER have shown an association between *XPA* A23G, *XPC* Lys939Gln, *XPD* Lys751Gln and *XPD* Asp312Asn polymorphisms and a lowered DNA repair capacity among carriers of the variant alleles compared to carriers of the wild type alleles. A low DNA repair capacity may influence the individual susceptibility to cancer. The four polymorphisms are associated to risk of lung cancer.

We have determined the risk of colorectal cancer in association with the four polymorphisms, and interactions between the polymorphisms and the environmental factors: smoking intensity, intake of alcohol, red meat, processed meat, fish and poultry, fruits and vegetables and dietary fibres, in relation to development of colorectal cancer. The polymorphisms were determined using blood samples from a study group of 405 colorectal cancer cases and 810 comparison persons, nested within the Danish prospective cohort, Diet, Cancer and Health, of 57,053 cohort members.

In brief, we observed no association between the *XPC* Lys939Gln, *XPA* A23G, *XPD* Lys751Gln, and *XPD* Asp312Asn polymorphisms and risk of colorectal cancer. The effect of the *XPD* $Lys^{751}Gln$ polymorphism was significantly different between genders, but not associated to risk of colorectal cancer among neither men nor women. There was a statistically significant interaction between the *XPC* Lys939Gln polymorphism and consumption of red meat, with a 3.7-fold increase in colorectal cancer risk per 100 g red meat intake among carriers of the homozygous variant. We did not see any interaction between the *XPD* Lys751Gln, *XPA* A23G,

and *XPD* Asp312Asn polymorphisms and the various environmental factors in relation to development of colorectal cancer. Our results suggest that the four polymorphisms are not of major importance in colorectal cancer carcinogenesis, and thus that low repair capacity may not be a risk factor for development of colorectal cancer.

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P20 DNA Damage and Repair

P20-01
Uptake, subcellular distribution and toxicity of arsenic species in methylating and nonmethylating human cells

E. Dopp [1], L.M. Hartmann [2], U. von Recklinghausen [1], I. Stueckradt [1], I. Pollok [1], S. Rabieh [2], A.V. Hirner [2], A.W. Rettenmeier [1]

[1] *Institute of Hygiene and Occupational Medicine, University Hospital, Hufelandstrasse 55, 45122 Essen, Germany;* [2] *Institute of Environmental Analysis, University of Duisburg-Essen, 45141 Essen, Germany*

Epidemiological studies have shown that exposure of humans to inorganic arsenic (iAs) in drinking water is associated with the occurrence of bladder cancer. The mechanisms by which arsenic induces bladder cancer remain uncertain. Following uptake, inorganic arsenic undergoes biotransformation to mono- and dimethylated metabolites. The pentavalent dimethylated arsenic compound DMA(V) is the major excreted metabolite of iAs in humans and is a suspected bladder carcinogen in rats. However, previous studies have shown that this arsenic species is poorly membrane permeable and shows no genotoxicity in vitro. In contrast, the trivalent methylated arsenic metabolites are highly cyto- and genotoxic. In the present study, we have investigated the uptake capabilities of human urothelial cells (UROtsa, nonmethylating cells) for arsenic compounds and compared it with uptake capabilities of methylating cells (human hepatocytes, HepG2). Additionally, we were interested in cytotoxic effects and in the subcellular distribution of the different arsenicals in urothelial cells. Our results show that the nonmethylating UROtsa cells are able to accumulate higher amounts of arsenic compounds than the methylating HepG2-cells. However, cytotoxic effects as well as radical generation are more pronounced in hepatocytes compared to urothelial cells. Investigation of subcellular distribution of arsenic compounds in UROtsa cells by differential centrifugation showed that nuclei and ribosomes are highly affected organelles. After 24 h exposure time, arsenicals are mainly accumulated in the ribosomes. Altogether, our results indicate that urothelial cells are able to accumulate arsenic compounds intracellularly to a higher extent than hepatocytes, but cellular effects are more pronounced in the methylating hepatocytes. Further research is needed to highlight the mechanisms behind the observed effects of arsenic-induced cellular damage.

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P20-02
Induction of excision repairable DNA lesions by lead and/or ALA?
A study by using ARA-C/CBMN test method

Aylin Üstündağ, Yalçın Duydu

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100 Tandoğan-Ankara, Turkey

As well known from the earlier studies the genotoxic potential of lead exposure was partly attributed to the formation of the highly reactive oxygen metabolites (ROM) in the blood. However, lead ions have no ability to generate ROM. Therefore, the recently published studies paid more attention to the role of ALA accumulation in lead-induced DNA damage. The aim of this study was to investigate the ability of lead and ALA to induce excision repairable DNA lesions by using ARA-C/CBMN assay. In this test method, *N*-methyl-*N*-nitrosourea (MNU) was used as a positive control which is a mutagen and known to induce excision repair. The results of the ARA-C/CBMN assay show that ALA significantly ($p < 0.01$) caused to increase the ratio of excision repairable DNA lesions. On the contrary, lead did not. In other words, ALA was more effective in inducing excision repairable DNA lesions than lead.

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P20-03
Effect in vivo of c-phycocyanin extract on hydroxyurea teratological insults

J. Vázquez [1], R. Pérez-Pasten [2], G. Chamorro [1]

[1] *Preclinical Toxicology Laboratory, Pharmacy Department, National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico;* [2] *Pharmacy Academic Area, Health Sciences Institute, Autonomous University of the State of Hidalgo, Mexico*

A number of xenobiotics can react to highly toxic electrophilic substances in embryos. These substances can

bind covalently to cellular macromolecules or free radical reactive intermediates that can react with molecular oxygen to produce ROS and interfere with embryonic and/or fetal development.

Hydroxyurea is a potent mammalian teratogen. Within 2–4 h after maternal injection it causes fast cellular death and deep embryonic DNA-synthesis inhibition. Some antioxidants have been shown to stop cellular death and malformations caused by hydroxyurea due to DNA-synthesis inhibition. Hydroxyurea was suggested to have reacted within embryos to release hydrogen peroxide and free radicals and they could be involved in early cellular death.

c-Phycocyanin is an accessory photosynthetic pigment found in blue green alga, such as *Spirulina* sp. It has several pharmacological activities related to its antioxidant and free radicals scavenger properties.

The aim of this work was to research the possible protecting effect of the c-phycocyanin extract over in vivo hydroxyurea teratogenicity, in mice.

Pregnant ICR mice (30 ± 2 g), were randomly divided into 8 groups of 10 animals each. Day 0 of gestation was determined as the day when a vaginal plug was detected. Group I was given only water; group II, an IP injection of hydroxyurea (300 mg/kg, day 8); groups III and IV, oral c-phycocyanin (200 mg/kg and 400 mg/kg, respectively, from days 0 to 9); and groups V and VI, were given c-phycocyanin (200 mg/kg and 400 mg/kg, respectively), plus hydroxyurea (300 mg/kg). The animals were killed on day 10 of gestation. Embryos were explanted, their external features recorded and their morphological development evaluated.

Results in group II showed abnormalities included phocomelia, incomplete rotation; neural tube opened and diminished yolk sac circulation. Groups III–VI did not statistically differ from group I in morphology but size and yolk circulation, which were lesser in group I. c-Phycocyanin extract protected from hydroxyurea teratological insults, perhaps because of its antioxidant and free radical scavenger properties.

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P20-04

DNA damage by organophosphate pesticides and repair profiles in human lymphocytes, in vitro

Faith M. Williams, Kathryn M. Atherton, Samantha Jamieson, Elaine Mutch

University of Newcastle, Newcastle, UK

It is recognised that the acute toxicity of organophosphate pesticides (OPs, P=S) is caused by inhibition of acetylcholinesterase by OP oxon (P=O) metabolites in the nervous system. However, several studies have also shown that various OPs can be genotoxic, but the data so far remain controversial.

This study aimed to investigate the genotoxic potential of different chemical classes of OP oxon at biologically relevant concentrations, *in vitro*. The OP oxons investigated were: dichlorvos, maloxon, dimethoate-oxon, diazoxon and paraoxon. The specific aims were to determine: (1) the 'background' DNA damage of lymphocytes from young, healthy, male individuals ($n = 30$) with no known exposure to OPs (2) the DNA repair profiles of the individuals following dosing with the OP oxons *in vitro*.

The 30 individuals were sorted into 5 groups and lymphocytes (150×10^3) were isolated from 10 ml venous blood. Each group of freshly isolated lymphocyte preparations was incubated with vehicle alone (2 μl, 'background' damage) or one of the five OP oxons (5–30 nM) for 10 min at 37 °C in a humidified atmosphere of 5%CO_2/95% air. The oxon was removed and the cells were washed with fresh culture medium. An aliquot of cells was taken immediately after replacement of the medium (0 min), and at 30 and 60 min to investigate DNA repair. The lymphocytes were subjected to alkaline COMET analysis using the 'dried down' method and confocal microscopy was used to capture the images of at least 50 cells per parameter. Komet software determined the Olive Tail Moment (OTM) as a quantitative measure of DNA damage.

The study showed a wide range in low-level 'background' DNA damage in the 30 individuals with no known exposure to OPs, due to environmental genotoxins and oxidative stress. DNA damage from exposure to the OP oxons *in vitro* was variable and more pronounced between 30 min and 60 min after removal of the dose.

These data demonstrated wide inter-individual variations in 'background' DNA damage, in the level of DNA damage induced by the OP oxons and in repair following removal of the dose. The mechanisms underlying the DNA damage are currently being investigated, but oxidative stress does not appear to be implicated since reduced glutathione was not depleted when HepG2 cells were exposed to the oxons.

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P20-05

Detection of DNA damage in A549 cells exposed to PM2,5 from an urban site at high traffic intensity

Delia Cavallo [1], Cinzia Lucia Ursini [1], Renato Cabella [2], Raffaele Maiello [1], Aureliano Ciervo [1], Arianna Di Francesco [1], Sergio Iavicoli [1]

[1] *Department of Occupational Medicine, National Institute of Occupational Safety and Prevention Monteporzio Catone, Rome, Italy;* [2] *Department of Occupational Hygiene, ISPESL, National Institute of Occupational Safety and Prevention Monteporzio Catone, Rome, Italy*

An increase of lung cancer risk in association with exposure to urban air pollutants is suggested by several studies. In particular fine particles with diameter $\leq$2,5 (PM2,5) are of the greatest health concern because when inhaled, they can be deposited in the lungs more deeply than larger particles. We investigated on lung epithelial cells A549 the genotoxic and oxidative effects of personal respirable dust samples collected in March from breathing zone of a security guard working in Rome in a site at high traffic intensity. The samples were collected in two consecutive days, each over an eight-hours period, by standard three-piece cassettes with filter (PTFE, 37 mm, 2 μm) and a respirable dust cyclone (GS-3 Cyclone) operating at flow rate of 2.75 l/min. Personal inhalation exposure to formaldehyde, benzene and toluene was monitored by passive sampling and gas-chromatography/mass spectrometry (GC/MS). Early direct and oxidative DNA damage were evaluated by the high sensitive Fpg-modified comet assay. The cells were exposed for 4 h to PM2,5 extracted from filter 1 (first sampling day) and filter 2 (second sampling day) and diluted at 0.010%, 0.015% and 0.025%. Oxidative and direct DNA damage were evaluated analysing Tail moment values from Fpg-enzyme treated cells (TMenz) and enzyme untreated cells (TM), respectively, and by comet percentage analysis. We found benzene levels of 10 $\mu g/m^3$ and 11 $\mu g/m^3$ for the first and second day, respectively, toluene levels of 18 $\mu g/m^3$ and 21 $\mu g/m^3$ and formaldehyde levels of 11 $\mu g/m^3$ and 7 $\mu g/m^3$. We found a consistent increase of comet percentage only in cells exposed to the highest concentration of extract from filter 2 and a dose-dependent increase of TM and TMenz for both extracts with highest values for filter 2. The results indicate an induction of direct and oxidative DNA damage by urban PM2,5 that seems to correlate with the pollution level indicated by the different amount of benzene and toluene. Our findings suggest to use Fpg modified comet test on A549 cells as experimental model to reproduce the exposure to PM2,5 urban airborne pollutants on target organ and to evaluate their genotoxic effects.

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P20-06

Fumonisin B_1: Oxidative status and DNA damage in rats

M. Peraica, A.-M. Domijan, D. Želježić, R. Fuchs, V. Žlender

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Fumonisins are mycotoxins that contaminate maize and wheat. The most toxic and the most frequently found fumonisin is fumonisin B_1 (FB_1) involved in several animal diseases and supposed to be involved in the etiology of some human tumors. FB_1 disturbs the metabolism of sphinganine (Sa) and sphingosine (So) increasing the ratio of their concentrations (Sa/So). FB_1 was shown to be mutagenic in several studies on cultured cells. Literature data on the studies of FB_1 genotoxicity in experimental animals are rather scarce and the mechanism of genotoxicity is not understood.

The aim of this study was to elucidate whether DNA lesions in kidney and liver cells of FB_1 treated rats are related to changes of oxidative status. Adult male Wister rats were receiving either FB_1 dissolved in sterile saline (0.9% NaCl) (0.5 mg/kg b.w./day) or solvent only (negative control animals) intraperitoneally for two or seven consecutive days and sacrificed 24 h after the last treatment. Ratio of Sa and So concentrations and parameters of oxidative status (activity of catalase, and concentration of protein carbonyls and malondialdehyde, MDA) were measured in plasma, liver and kidney homogenate, while comet assay was performed in liver and kidney.

In plasma and homogenates of liver and kidney activity of catalase, concentration of protein carbonyls and MDA were not affected after two days treatment with FB_1. At this time point the ratio of Sa and So in all tested samples was increased. After two days treatment tail length and tail intensity measured with comet assay in liver homogenate was not changed, while in kidney they where significantly different from controls. After seven days treatment all measured parameters were significantly different from controls. This study showed that in experimental animals FB_1 causes DNA lesions in kidney before affecting catalytic activity of catalase and concentration of protein carbonyls and MDA. In this time point the ratio of Sa and So significantly increases in all tissues. These results taken together confirm that oxidative stress is the consequence and not the cause of

DNA damage and that the metabolism of sphingolipids should be involved in the DNA damage caused by FB_1 rather than oxidative stress.

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P20-07

Relation of genetic polymorphism in *mEH* and *XRCC1* with risks of chronic benzene poisoning[☆]

Zhong-bin Zhang [1], Jun-xiang Wan [2], Pin Sun [2], Zhao-lin Xia [2]

[1] *Institute of Occupational Hazards, China Academy of Safety Science & Technology, China;* [2] *Department of Occupational Health, School of Public Health, Fudan University, Shanghai, 200032, China*

In order to explore the relation between genetic polymorphisms of microsomal epoxide hydrolase gene (*mEH*), X-ray repair cross-complementing group 1 gene (*XRCC1*) and risks of chronic benzene poisoning (CBP), a case–control study was conducted. One hundred and fifty-two BP patients and 152 workers occupationally exposed to benzene without poisoning manifestations were investigated. Polymerase chain reaction-restrained fragment length polymorphism technique (PCR-RFLP) was applied to detect the single nucleotide polymorphisms (SNPs) on *c.113* and *c.139* of *mEH* gene and SNPs on *c.194, c.280* and *c.399* of *XRCC1* gene. The results showed that individuals carrying *mEHc.113 T*/T + T/C genotypes had a 1.65-fold increased risk of BP compared with those carrying C/C genotype (OR = 1.65, 95% CI: 1.01–2.69, $P = 0.04$). The risk of CBP for individuals carrying of *XRCC1 194Arg*/*Trp* + *Trp*/*Trp* genotypes was 1.67-fold (ORadj = 0.60, 95% CI: 0.37–0.98, $P = 0.041$) lower compared with those carrying the *Arg*/*Arg* genotype, and the individuals carrying genotypes of *XRCC1c.280 Arg*/*His* + *His*/*His* had a 1.91-fold increased risk of BP compared with these carrying the wild allele (ORadj = 1.91, 95% CI: 1.17–3.10, $P = 0.009$). It suggests that the risk of BP for subjects carrying *mEHc.113T*/T + T/C or *XRCC1c.280Arg*/*His* + *His*/*His* genotypes may increase, while the risk of BP for subjects carrying *XRCC1 c.194Arg*/*Trp* + *Trp*/*Trp* may decrease.

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P20-08

Novel methods for detecting double DNA strand breaks

Lucy Catherine Riches, Anthony M. Lynch, Nigel J. Gooderham

Imperial College London, London, UK

The induction of DNA double strand breaks can result in chromosomal aberrations or gene mutations, if incorrectly repaired. Identification of an early biomarker of DNA double strand breaks would be very useful for mechanistic genotoxicity studies, and is therefore a primary focus of our work.

Our previous work established a significant increase in RAD52 mRNA following treatment of human cells with genotoxic agents. Here we describe a molecular beacon, which produces a green fluorescent signal upon binding complementary RAD52 mRNA. This is proposed to provide a novel method for monitoring DSB induction in human cell lines. We have carried out extensive characterisation of two molecular beacons, comprising either a DNA or 2′0-methylated RNA modified backbone, from which it has been concluded that the RNA modified backbone imparts a greater degree of target specificity for the beacon. Under cell free conditions, the limit of detection achieved with 200 nM molecular beacon has been established at between 5 nM and 10 nM complementary oligonucleotide, and in the presence of equimolar target and excess non-hybridising DNA (equivalent to that of 10,000 cells), molecular beacon fluorescence is induced by almost 20-fold. These preliminary studies have confirmed the proposed molecular beacon as a sensitive and specific probe for Rad52 mRNA detection.

Flow cytometric analysis of two human cell lines; MCL-5 and A549, exposed to 0 or 7.4 cGy irradiation indicated increased levels of Rad52 mRNA in approximately one-fifth of the irradiated cell population by 4 h. The induction of DSB by irradiation was confirmed by antibody staining for γ-H2AX foci, which indicated the desired DNA damage in over 40% of irradiated cells when compared with control cells. These preliminary studies suggest that the RAD52 molecular beacon may be useful in understanding the dynamics of double strand breaks and provide the opportunity to follow DNA damage in whole cells.

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P20-09

The influence of food constituents and genetic polymorphism of XME and DNA repair enzymes on DNA damage (DIEPHY study project)

J. Gromadzinska[1], J. Palus[1], G. Prochazka[2], D. Segerback[2], A. Seidel[3], P. Vodicka[4], P. Soucek[5], A. Naccarati[4], K. Rydzynski[1], W. Wasowicz[1], R. Nilsson[1]

[1] *Nofer Institute of Occupational Medicine, Lodz, Poland;* [2] *Karolinska Institute, Department of Biosciences and Nutrition, Huddinge, Sweden;* [3] *Biochemical Institute for Environmental Carcinogens, Grosshansdorf, Germany;* [4] *Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic;* [5] *National Institute of Public Health, Prague, Czech Republic*

Chronic exposures to mixtures of external, potentially carcinogenic pollutants can generate DNA reactive metabolites, which may have a significant influence on cancer development in humans. The balance between the defense mechanisms attempt to minimise DNA damage is potentially modifiable by nutrition, but food items can also be a source of exposure. The aim of this study was to analyse human DNA damage resulting from exposure to polycyclic aromatic hydrocarbons (PAH) as well as its prevention by the constituents present in food and beverages. The study population comprised 103 healthy, non-smoking women, aged 18–45 year, who were not occupationally exposed to PAHs.

The level of DNA single strand breaks (SSBs) was measured by the comet assay in peripheral blood lymphocytes and expressed as a tail moment, while oxidative DNA damage was determined using formamidopyrimidine glycosylase (fpg) treatment. The levels of SSBs and oxidative DNA damage were 0.190 ± 0.21 (0.02–1.27) and 0.290 ± 0.22 (0.04–1.19), respectively. Bulky DNA adducts in total white blood cells were analyzed with the ^{32}P-postlabelling assay and the mean level amounted to $0.3730 \pm .307$ adducts/10^8 dN (0.100–2.211).

High consumption of fruit and vegetable was associated with a decreased level of bulky DNA adducts. A relationship was found between a high intake of grilled, fried or smoked meat and/or fish and an increased level of lymphocyte DNA SSBs and base oxidation as well as urinary excretion of PAH metabolites. No association was noted between genetic polymorphism of the genes coding xenobiotic metabolising enzymes (CYP1A1, CYP1B1 codon 432 and 453, EPHX1 exon 3 and 4, GSTs) or DNA repair enzymes (XPD exon 23, XPC exon 15, XPG exon 15, OGG1) and the levels of DNA damage (SSBs and oxidative damage or bulky DNA adducts) in lymphocytes.

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P20-10

Redox-dependent regulation of nucleotide excision repair

S.A.S. Langie, A.M. Knaapen, J.M.J. Houben, F.C. van Kempen, J. de Hoon, R.W.L. Godschalk, F.J. van Schooten

Department of Health Risk Analysis and Toxicology, Maastricht University, The Netherlands

DNA repair plays an important role in maintaining genomic integrity and up regulation of DNA repair could thus protect cells against genotoxic assaults. Based on *in vitro* studies suggesting that nucleotide excision repair (NER) is redox-sensitive we hypothesized that oxidative stress may up-regulate or prime NER enzymes. To test this hypothesis, A549 cells were exposed to 100 μM H_2O_2 and the expression of various NER genes (XPA, XPC, ERCC1, ERCC4 and ERCC5) was determined by quantitative RT-PCR. After exposure to H_2O_2, a 2–4.5-fold increase in expression of XPA, XPC, ERCC4 and ERCC5 was observed, showing the highest expression at 4–6 h post-exposure followed by a gradual decline back to basal levels at 24 h. Surprisingly, expression of ERCC1 was 5-fold down regulated by H_2O_2 exposure with a subsequent increase in expression up to ∼2-fold above the basal level at 24 h after exposure. Phenotypical assessment of NER capacity by a modified comet-assay, showed that NER activity significantly decreased to less than 50% up to 8 h after H_2O_2 exposure, followed by overcompensation at 24 h, which parallels the effects of H_2O_2 on ERCC-1 gene expression. To study the possible role of glutathione (GSH) in this redox regulation of NER, cells were pre-incubated with 500 μM BSO for 24 h, resulting in total GSH depletion and increased intracellular oxidative stress as assessed by FACS analysis of dihydro-rhodamine-123 oxidation. In GSH-depleted cells, the down regulation of ERCC1 gene-expression by H_2O_2 was completely abolished and the up regulation of ERCC4 expression was potentiated from 2.5-fold to >10-fold. Similarly, the H_2O_2 induced decrease in phenotypically assessed NER capacity was absent in GSH-depleted cells. Overall, ERCC1 expression and NER-related damage recognition and incision were strongly correlated ($R^2 = 0.85$, $P < 0.01$), suggest-

ing a crucial role of ERCC-1 in redox status-modified NER capacity. Our data suggest that NER capacity can be modulated by oxidative stress. Considering the prominent role of DNA damage and repair in health and disease, further research on the redox-modulation of DNA repair is urgently needed.

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P20-11

Influence of *XRCC1* Arg399Gln polymorphism on basal and radiation-induced micronucleus frequencies in head and neck cancer patients and their first degree relatives

Sema Burgaz [1], Neslihan A. Kocabas [1], Erdem Coskun [1], Gonca D. Cakmak [1], Faik Cetindag [2], Osman Sunter [2], Hayriye Edinsel [2]

[1] *Gazi University, Faculty of Pharmacy, Ankara, Turkey;* [2] *Abdurrahman Yurtaslan Oncology Hospital, Dept. of Radiation Oncology, Ankara Turkey*

Studies have suggested that not only reduced DNA repair capacity (phenotype) but also genetic susceptibility (genotype) may play an important role in risk of head and neck cancer (HNC). Further support for genetic susceptibility is evidenced by aggregation of HNC patients with their first degree relatives (FDRs). For this purpose, in our study, we have investigated the relationship between the basal and radiation-induced micronucleus (MN) frequencies with *XRCC1* gene polymorphism (Arg399Gln) both for HNC patients ($n = 38$) and their FDRs ($n = 21$), as well as healthy controls ($n = 27$). X-ray repair cross-complementing group 1 (*XRCC1*) is a base excision repair protein that plays a central role in the repair of DNA base damage and strand breaks. The *XRCC1* alleles were detected using PCR-RFLP technique. For the micronucleus assay, blood samples were exposed in vitro to 2 Gy γ rays (^{60}Co) at a dose rate of 0.62 Gy/min. Mutant allele frequencies were 34.2% ($n = 26$), 38.1% ($n = 16$) and 40.7% ($n = 22$) for patients, relatives and controls, respectively. HNC patients, FDRs and controls variants had elevated but not significant basal MN levels ($p > 0.05$). For the induced MN frequencies, variants had decreased frequencies in all groups but not different significantly ($p > 0.05$). Smoking did not affect on MN frequncies and also we did not find an interaction between Arg399Gln polymorphism and smoking ($p > 0.05$). Our data seemed to be supported by other studies that found a decreased risk for the genetic variation in *XRCC1* codon 399 in HNC patients. However, further work is needed to resolve the importance of other polymorphisms of XRCC1 including codon 194, as well as other DNA repair systems, e.g. *XRCC3* and *OGG1*.

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P20-12

Rosmarinic acid inhibits acute renal injury and cyst formation in cisplatin-treated rat kidney

Jingwei Tian, Wanglin Jiang

School of Pharmacy, Yantai University, Yantai, Shangdong, PR China

Although *cis*-diamminedichloroplatinum(II) (cisplatin) is an effective anticancer agent, its clinical use is highly limited predominantly due to its adverse effects on renal functions. The present work examined the therapeutic potential of Rosmarinic acid, a free radical scavenger, for inhibiting cisplatin-induced renal injury. Rosmarinic acid was administrated intravenously at a dose of 30 mg/kg of body weight to male Wistar rats (200–220 g). After 30 min, cisplatin was injected intraperitoneally at a dose of 5 mg/kg of body weight. At the indicated times after the treatment, functions and histological changes of the kidney were analyzed. To test the therapeutic potential of Rosmarinic acid in chemotherapy, its effect on the anticancer action of cisplatin was examined in ascites cancer-bearing rats. We found that cisplatin rapidly impaired the respiratory function and DNA of mitochondria in renal proximal tubules, thereby inducing apoptosis of tubular epithelial cells within a few days and chronic renal dysfunction associated with multiple cysts 1-year after the administration. Administration of Rosmarinic acid inhibited the cisplatin-induced acute injury of mitochondria and their DNA and renal epithelial cell apoptosis as well as the occurrence of chronic renal dysfunction and multiple cyst formation. The anticancer effect of cisplatin remained unaffected by intravenous administrating of Rosmarinic acid. These results indicate that Rosmarinic acid may have therapeutic potential for inhibiting the acute and chronic injury of the kidney induced by cisplatin.

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P20-13
Effect of cigarette smoke condensate on PARP-1 activity in human lung cells

M.H. Ahmad, A. Torky, F. Glahn, H. Foth

Institute of Environmental Toxicology, Martin-Luther-University Halle, Germany

Cigarette smoke contains more than 6000 components, many of which can lead to DNA damage. Poly(ADP-ribose) polymerase-1 (PARP-1), is a nuclear enzyme that has been included in the cellular response to DNA injury. It is supposed that PARP plays a role in determining whether DNA damage lead to repair or to apoptosis.

Apoptosis of lung could be protective if it prevents the survival of cells with altered genetic programming. Alternatively, excessive apoptosis may lead to loss of lung cells and pathological lung insults such as emphysema. Therefore, this study was directed to examine the PARP-1 activity in normal human bronchial epithelial cells (NHBEC) grown directly from normal human lung tissue after exposure to cigarette smoke condensate for 24 h compared to the changes induced by other stressors such as metals.

Western blotting has revealed the expression of PARP-1 in normal human bronchial epithelial cells (NHBEC) and tumor cell line A549. PARP-1 activity in progressive generations and passages of bronchial and peripheral lung cells cultivated for 10 weeks were examined. PARP-1 activity decreased in the higher passages and the levels of PARP-1 activity varied between different cases. This reflects an adaptive response of cells to cultivation.

To study the protein activity a functional assay for measurement of PARP-1 activity in cultivated (NHBEC) has been used. Induction of DNA damage by H_2O_2 (100 μM) for 5 min in NHBEC led to formation of ADP-ribose polymers which were detected immunohistochemically by means of fluorescence detection. Treatment of NHBEC with cigarette smoke condensate 0.5 mg/L for 24 h alone has induced PARP-1 activity by factor 1.3 compared to the control (basal cellular PARP activity) and has increased H_2O_2 (100 μM) -induced PARP-1 activity from 1.4-fold to 1.8-fold. Treatment of NHBEC with copper sulfate (0.05 mM, 24 h) alone did not trigger PARP-1 activity but decreased PARP-1 activity induced by H_2O_2 (100 μM). Similarly mercury(II) chloride (0.03 mM, 24 h) exerted no effect on PARP-1 activity and decreased the activity of PARP-1 upon co-incubation with H_2O_2 (0.1 mM).

Correlation between PARP-1 activity and expression under the effect of cigarette smoke condensate is recommended.

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P21 Food Mutagens/Antimutagens

P21-01
Parsley oil protects against Zearalenone-induced alteration in reproductive function in male mice

Mosaad A. Abdel-Wahhab [1], Samir Abbes [2], Jalila Ben Salah-Abbes [2], Aziza M. Hassan [3], Redha Oueslati [2]

[1] *Food Toxicology & Contaminants Departments, National Research Center, Dokki, Cairo, Egypt;* [2] *Laboratory of Immunology, Microbiology Environmental and Cancerolgy, Faculty of Science, Bizerte, Tunisia;* [3] *Cell Biology Department, National Research Center, Dokki, Cairo, Egypt*

Zearalenone (ZEN) a nonsteroidal estrogenic mycotoxin is known to cause toxicity of testis in males. In the present study, we evaluated the chemoprevention effects of parsley oil against the cytotoxicity of ZEN. Sixty mature male mice were distributed into five treatment groups including the control group, corn oil group, ZEN-treated group (10 μg/kg b.w) and the group treated with parsley oil (0.6 ml/kg b.w) with or without ZEN. Animals within different treatment groups were divided into two subgroups (A and B). Subgroup A were used for the determination of testosterone and chromosomal analysis in germ cells and received their respective doses for two weeks whereas, subgroup B were used for determination of sperm abnormality and received their respective doses twice a day for 1 week and sacrificed after 30 days. The results indicted that ZEN treatment resulted in a significant decrease in testosterone concentration, sperm count and sperm motility. Whereas it caused a significant increase in abnormal sperms counts and total chromosomal aberrations in germ cells. Animals treated with parsley oil alone were comparable to the controls regarding all the tested parameters. The combined treatment with ZEN and parsley oil resulted in a significant improvement in all tested parameters. It could be concluded that parsley oil induced a protective action against ZEN-induced alteration in the reproductive performance at a dose as low as 0.6 mg/kg b.w.

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P21-02

Enzyme linked immunosorbant assay (ELISA) of glutathione *S*-transferase activity by in *Aspergillus* strains with emphasize to aflatoxin production

Abdolamir Allameh [1], Tahereh Ziglari [1], Mehdi Razzaghi-Abyaneh [2], Mohammad-Hossein Yadegari [1], Iraj Rasooli [3]

[1] *Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, P.O. Box 14115-111, Iran;* [2] *Department of Mycology, Pasture Institute of Iran, Tehran, Iran;* [3] *Department of Biology, Shahed University, Tehran, Iran*

Aflatoxins (AFs) are a group of structurally related mycotoxins produced by several toxigenic species of *Aspergillus*. There are reports showing that a positive correlation exists between aflatoxin formation and the glutathione *S*-transferase (GST) activity in *Aspergillus flavus*. This finding was substantiated by showing that inhibition of aflatoxins in a toxigenic *Aspergillus parasiticus* was associated with inhibition of GST activity [Allameh et al., 2002. Mycopathologia 54, 79–84 and Razzaghi et al., 2005. 159, 565–570]. To verify the role of GST in toxicity of *Aspergillus* strains attempts were are to develop an ELISA for detection of GSTs in the fungal preparations. For this purpose, first, the GST was purified from the fungal mycelia, and then polyclonal antibody was raised in rabbits and used to develop the immunoassay. For this purpose, two toxigenic strains and one non-toxigenic strain were cultured under appropriate conditions before purification of mycelial GST. Affinity purifies fungal GST was administered to young white New Zealand rabbits to raise antibody. Finally, an indirect ELISA using second antibody conjugated to horseradish peroxidase (HRP) was developed to measure the titer of antibody.

The antibody titer in sera of the rabbit immunized with the cytosolic fraction obtained from either non-toxigenic *A. parasiticus* or *Aspergillus niger* (negative controls) was much lower than that measured in sera of the rabbit administered with toxigenic strain of *A. parasiticus*. Finally, the evidences presented in this study attest to our previous findings that the activity of GST is always several folds greater in toxigenic strains as compared to their counterpart non-toxigenic strains. This data also suggest that measurement of fungal GST using sensitive methods could be useful to distinguish between toxigenic and non-toxigenic strains.

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P21-03

Antimutagenic and antioxidant potential of *Brassica compestris* (var sarason) against arsenic induced toxicity

Mukesh Kumar Sharma, Ambika Sharma, Ashok Kumar, Madhu Kumar

Department of Zoology, University of Rajasthan, Jaipur 302004, India

Health hazards caused by heavy metals have become a great concern to the population. Arsenic, a naturally occurring heavy metal, is present in food, soil, air and water. All human populations are exposed to arsenic and its compounds through occupational or environmental processes. DNA damage and genetic instability induced by the inorganic arsenicals present in water are thought to be prerequisites for the initiation of carcinogenesis. Impaired antioxidant defense mechanisms and oxidative stresses are implicated in the pathogenesis of arsenic toxicity. Many plants, which are consumed through our daily diet, possess excellent antioxidant and cancer chemopreventive properties. Mustard (*Brassica compestris*, var sarason) is one of the most popular species of family Cruciferae. Mustard seeds are widely utilized in the preparation of varieties of edible sauces, pastes and pickles. The present study was conducted to examine the antimutagenic and antioxidant action of *B. compestris* seed extract against the arsenic induced toxicity in Swiss albino mice. Arsenic treated animals showed clastogenicity in form of chromatids and chromosomal breaks, centric rings, dicentrics and acentric fragments and exchanges. Highly significant ($P < 0.001$) enhancement in micronuclei (MN) frequency and highly significant depletion in various antioxidant/detoxification enzymes was also observed following arsenic intoxication. Pre and post treatment of mustard seed extract is effective in counteracting the clastogenicity (chromatid breaks, chromosomal breaks, centric rings, dicentrics, exchanges and acentric fragments) and formation of MN frequency of the most potent form of arsenic, sodium arsenite. The antioxidant function of mustard seed extract in reducing mutagenicity may be partly due to the induction of antioxidant/detoxification enzymes, such as glutathione, glutathione-*S*-transferase, superoxide dismutase and catalase. Also, simultaneous treatment of *B. compestris* seed extract along with arsenic proved to be sufficient in reducing malondialdehyde (MDA) level. Thus, our present reports strongly suggest that increasing

the intracellular antioxidant level may have preventive or therapeutic effects in arsenic induced poisoning.

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P21-04
Inhibitory effect of alpha-bisabolol on the genotoxic damage induced by daunorubicin in mouse

Isela Alvarez-Gonzalez, Emma Uc-Artigas, Martha Moreno L., Eduardo Madrigal-Bujaidar

Laboratorio de Genética, Escuela Nacional de Ciencias Biológicas, IPN, Mexico

Chamomile (*Matricaria recutita*) is an asteraceae plant used in traditional medicine as anti-inflammatory, sedative, and spasmolitic. We have determined that its essential oil (CEO) is antigenotoxic and antioxidant, and, therefore we have considered the pertinence to evaluate the antigenotoxic potential of its main constituents. The sesquiterpene alpha-bisabolol (AB) constitutes about 50% of CEO and is known to be anti-inflammatory, and cytotoxic against various transformed cellular lines. The aim of the present study was to determine the capacity of AB to reduce the frequency of micronucleated polychromatic erythrocytes (MNPE) induced by daunorubicin (DAU) in mouse. The percentage of MNPE was determined with flow cytometry before the administration of the compounds, and at 24, 48, and 72 h postadministration. The results showed a potent genotoxic effect of DAU, no capacity of AB to increase the rate of micronuclei, but, on the contrary, we found a significant, dose-dependent inhibitory effect with the four doses tested of the chemical against the clastogenic damage induced by 3 mg/kg of DAU. At 48 h of exposure, the inhibition with 120 mg/kg of AB was 52.1%, and with 1200 mg/kg was 65%. Our study suggests that AB is a chemical involved in the biological activities of CEO.

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P21-05
Inhibitory effect of chamomile essential oil on the genotoxicity induced by daunorubicin in mouse

Isela Alvarez-Gonzalez, Emma Uc-Artigas, Martha Moreno L., Eduardo Madrigal-Bujaidar

Laboratorio de Genética, Escuela Nacional de Ciencias Biológicas, IPN, Mexico

Chamomile (*Matricaria recutita*) is an asteraceae plant used in traditional medicine because its properties as anti-inflammatory, sedative, and spasmolitic agent. Chamomile essential oil, in particular, (CEO) is also known to have anti-inflammatory and antioxidant effects, as well as to inhibit the frequency of sister-chromatid exchanges induced by daunorubicin (DAU) in mouse. The aim of the present report was to determine the capacity of CEO (a mixture of 13 metabolites) to reduce the frequency of polychromatic micronucleated erythrocytes (MNPE) induced by DAU in mouse. The percentage of MNPE was determined with flow cytometry before the administration of the compounds, and at 24 h, 48 h, and 72 h postadministration. The results showed a potent genotoxic effect of DAU, and a certain genotoxicity with the highest tested dose of CEO. However, we found that CEO at doses of 5 mg/kg, 50 mg/kg, 500 mg/kg, and 1000 mg/kg produced an inhibition of about 50% with respect to the number of micronuclei formed by DAU. Therefore, our data confirm the in vivo antigenotoxic potential of the tested mixture, and suggest the pertinence to determine the DNA inhibitory potential of specific compounds in the CEO, such as the sesquiterpene alpha-bisabolol.

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P22 Food Safety

P22-01
Determination of acrylamide in Brazilian foods by LC–MS/MS

Adriana Pavesi Arisseto[1], Maria Cecília Figueiredo Toledo[1], Yasmine Govaert[2], Joris Van Loco[2], Stéphanie Fraselle[2], Eric Weverbergh[2], Jean Marie Degroodt[2]

[1] *State University of Campinas, Campinas;* [2] *Scientific Institute of Public Health, São Paulo Brazil*

Detection of high concentrations of acrylamide in foods heated at high temperatures by the Swedish researchers in 2002 attained considerable public concern, since acrylamide was found to be carcinogenic in experimental animals and is classified as a probably human carcinogen by the IARC. Various studies on the occurrence of acrylamide in foods have been carried out in many countries, but no data on the level of acrylamide in foods from Latin America and Africa has been published. The objective of this study was to evaluate several kinds of carbohydrate-rich foods available on the Brazilian market (95 samples representing 18 product categories) for acrylamide using an in-house validated LC–MS/MS method. The level of acrylamide in the samples ranged from <LOQ to 1999 $\mu g\,kg^{-1}$ and varied considerably between single foodstuffs within food groups. The highest levels were found in

potato products (144–1999 $\mu g\,kg^{-1}$) and in instantaneous coffee (333–683 $\mu g\,kg^{-1}$), and low concentrations were detected in cassava (<LOQ – 81 $\mu g\,kg^{-1}$) and maize based products (<LOQ – 49 $\mu g\,kg^{-1}$), bread (<LOQ – 124 $\mu g\,kg^{-1}$) and beer (<LOQ). The results are comparable with those reported in other countries and reflect the influence of the asparagine and reducing sugars contents and processing conditions on the potential for acrylamide formation by different food groups. The data from the survey will be used for a preliminary dietary intake estimate of exposure of the Brazilians to acrylamide and will also contribute to data accumulation for worldwide health risk assessment.

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P22-02
Sulphites in Brazilian white wines

Rita Margarete Donato Machado [1], Maria Cecília Figueiredo Toledo [1], Eduardo Vicente [2]

[1] *State University of Campinas, Campinas;* [2] *Institute of Food Technology, São Paulo Brazil*

Sulphites are used worldwide in the wine industries as antimicrobial and antioxidant agents. In the last years, sulphites have become a concern as food additives because they have been implicated as the cause of bronchospasm in some subjects with asthma. Although direct evidence is limited, some studies suggest that wines could be important triggers for asthmatic responses. Moreover, it was reported by the Joint Expert Committee on Food Additives that the consumption of certain foods and beverages may result in intakes above the acceptable daily intake (ADI) of 0.7 mg/kg body weight, driving the Codex Committee on Food Additives and Contaminants (CCFAC) to recommend member states to monitor sulphite intake. Due to these considerations, the present work aimed on determining the actual sulphite content in a variety of white wines in order to generate data to assess the intake of sulphites in Brazil. Thirty-one samples of national white wines were collected in the market. Additionally, five brands of the most popular wines from other South American countries were also analysed. The contents of sulphites were determined by the optimized Monier–Williams distillation method. The residual sulphite levels, expressed as sulphur dioxide, ranged from 60.8 mg/L to 235.0 mg/L. To evaluate recovery, sulphite was added to the samples as sodium metabisulphite. The mean recovery was 89.4% with a coefficient of variation (CV) of 9.2%. All samples presented residual sulphite below 350 mg/L (the maximum permitted level established by the Brazilian legislation) with most of them (83%) having residual sulphite below 150 mg/L. The average content of sulphites in the national white wines was 122.0 mg/L with a CV of 32.8%. The white wines from other countries presented residual sulphite ranging from 80.5 mg/L to 143.1 mg/L. Recent estimates of sulphite intake in Brazil based on national maximum permitted level of use reported sulphite intake above 50% of the ADI for high school students. In the case of consumers of alcoholic beverages, it was observed that wine was one of the main contributors for the sulphite intake. The present findings indicate that the actual residual level of sulphite in white wines is much lower than the national maximum permitted level, stressing thus the importance of generating analytical data to be used in the calculation of food additive intakes, especially for those additives with low ADI that are widely used in food and beverages.

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P22-03
Polycyclic aromatic hydrocarbons in sugar cane juice produced with burnt and not-burnt sugar cane

Silvia A.V. Tfouni [1], Silvia H.P. Vitorino [2], Maria Cecília Figueiredo Toledo [2]

[1] *Institute of Food Technology;* [2] *State University of Campinas, Campinas, São Paulo Brazil*

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of environment contaminants formed during incomplete combustion or pyrolysis of organic matter. Many of these compounds are considered clearly carcinogenic and genotoxic. Brazil is the world's largest sugar cane producer. At harvesting season the crops are usually burnt in order to facilitate manual harvest. Studies conducted in Brazil suggest that sugar cane burning is an important source of PAHs emission and has been associated with the presence of these contaminants in burnt sugar cane and its by-products. In the present study, 10 samples of sugar cane juice produced with burnt and not-burnt sugar cane were collected at a sugar mill during the year 2004 and analysed for the presence of 5 PAHs (benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene and dibenz(*a*,*h*)anthracene). The analytical method involved extraction with cyclohexane, *N*,*N*-dimethylformamide and purification on a silica

gel chromatographic column. The analysis was carried out by HPLC with fluorescence detection (excitation wavelength 290 nm and emission wavelength 430 nm). A C18 column and a mobile phase of 75% acetonitrile and 25% water were used on the separation. PAHs were detected in all analysed samples with summed levels ranging from 0.19 to 0.61 μg/kg (sugar cane juice produced with not-burnt sugar cane) and 0.44 to 3.05 μg/kg (sugar cane juice produced with burnt sugar cane). Mean summed levels for sugar cane juice produced with not-burnt and burnt sugar cane were 0.32 and 1.32 μg/kg, respectively. Results show relatively higher levels of PAHs in cane juice from burnt sugar cane samples, confirming that the practice of burning sugar cane before harvest is a source of PAHs emission in the environment which may be transferred to the sugar cane and consequently to the sugar cane juice.

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P22-04

Toxicological effects on rats fed aflatoxin-contaminated diet with or without sorbent material

A.G. Radwan [1], M.A. Abdel-Wahab [2], S.A. Nada [2], F.A. Khalil [3]

[1] *Faculty of Medicine, Al-Azhar University, Ain Shams University, Cairo, Egypt;* [2] *National Research Center, Ain Shams University, Cairo, Egypt;* [3] *Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

Hydrated sodium calcium aluminosilicates (HSCAS); sorbent compounds obtained from natural zeolite, exerted an ability to adsorb mycotoxins with a high affinity. The efficiency of HSCAS was compared to that of the local Montmorillonite silicate in respect of the protection against aflatoxicosis in the rat as a sensitive animal model. Aflatoxin (2.5 mg/kg diet) significantly reduced blood hemoglobin, erythrocytes, leucocytes, cholesterol, triglycerides, cholinesterase, total protein, albumen, zinc and copper concentrations, but it significantly increased the concentrations of ceatinine, bilirubin, urea nitrogen, alkaline phosphatase and transaminases. Furthermore, aflatoxin administration induced degenerative changes in the hepatic and renal tissues.

Addition of HSCAS or montmorillonite to the aflatoxin-contaminated diet (0.5%, w/w) resulted in a significant improvement in the hematological and biochemical parameters, and histological picture of both liver and kidney. It is concluded that the deleterious effects of aflatoxin could be diminished by sorbents, but, themselves, sorbents have no toxic effects.

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P22-05

Assessment of the heavy metals in the food from Romania, 2005

C. Hura, B.A. Hura

Institute of Public Health, Iassy, Romania

Exposure to heavy metals is an important problem of environmental toxicology. Most of these metals are toxic to humans, animals and plants. Anthropogenic activities are the major sources of heavy metals redistribution. Man, being at the top of the food chain, is at great risk of suffering from health hazards associated with toxic metals because of bioaccumulation The aim of this study was the evaluation of the heavy metals contents in the food of the Eastern Romania area.

Material and methods: The study presents the results obtained in 2005 of some metals [Pb, Cd] in the food, 1869 samples—meat (469 samples), vegetables (750 samples), panification products (283 samples), juice (162 samples), diets (205 samples), in Romania. Trace elements concentrations were analyzed by atomic absorption spectrophotometry, using a Carl Zeiss Jena, Model AAS-1N, with flame air-acetylene.

Results: In all analysed samples these metals were found. Generally, a wide variation between individual samples was observed.

Meat: The mean metals levels in the meat products varied between 0.07 mg/kg Cd and 0.08 mg/kg Pb.
Vegetables: The mean metals levels in the vegetables varied between 0.02 mg/kg Cd and 0.07 mg/kg Pb.
Bakery products: The results of the investigations showed a variation of heavy metals between 0.02 mg/kg Cd and 0.06 mg/kg Pb.
Diets: The mean metals levels in the diets varied between 0.03 mg/kg Cd and 0.1 mg/kg Pb.

Conclusion: Determinations of these chemical contaminants in food are important in environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health and epidemiological studies.

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P22-06

SAFEFOODNET, a specific support action of the EU: Building a Network for chemical food safety for the enlarging Europe

Angelo Moretto [1], Marianna Elia [1], Claudio Colosio [2]

[1] *University of Padova, Padova, Italy;* [2] *University of Milan, Milan, Italy*

The primary goal of SAFEFOODNET, a Specific Support Action of the Framework Programme 6, is to promote Associated Candidate Countries (ACC) and New Member States (NMS) capabilities of addressing all aspects relating to chemical food safety.

SAFEFOODNET consortium, that started in January 2005, has 20 partners from 17 countries which include 4 Member States (Italy, Denmark, Germany and Belgium), 10 NMS (Hungary, Czech Republic, Slovakia, Poland, Latvia, Lithuania, Estonia, Slovenia, Malta, Cyprus), and 3 ACC (Bulgaria, Romania, Turkey).

The first step was to have a picture of the systems of chemical food safety in the participating countries. These have been achieved by the activities planned in two project working groups: one aimed at gathering knowledge regarding sources of dietary data (WP2) in individual countries, and another focusing on strategies/approaches and capabilities of monitoring food chemical contamination (WP3).

Each partner filled in on line the questionnaires both for WP2 and WP3. The questionnaires were divided in two parts: the first one consisted of the profile of the institutions; the second part was more specific and aimed at collecting details about each relevant dietary survey undertaken (WP2) and about the analysis that the institute conducted (WP3).

A web site (www.safefoodnet.net) has been developed which also includes the projects activities and participants, and information about directives on food contaminants.

The dissemination of project's results at local and international level include the organization of workshops in Slovak Republic, Latvia, Bulgaria, Malta, and the final one in Brussels where all relevant stakeholders will be invited. These activities aim at developing a set of long-term activities, to be continued after the end of the project and hopefully be expanded to other countries.

This abstract is presented on behalf of all participating institution represented by: Angerer J. (D), Benfenati E. (I), Bocsan I.S. (RO), Borg Buontempo M. (MA), Büchert A. (DK), Kambourova V. (BG), Karklina D. (LT), Kleanthous A. (CY), Kos D. (SLO), Özkaya Ş. (TR), Papocsi L. (HU), Pitta C. (CY), Roots O. (EE), Silhova Z. (CZ), Svetlikova A. (SK), Traczick A. (PL), Tuijtelaars S. (BE), Venskutonis P.R. (LV).

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P22-07

Determination of aluminum level in baby food samples by using atomic absorption spectrometer

Hande Sipahi [2], Ayse Eken [1], Ahmet Aydin [1], Gonul Sahin [2], Terken Baydar [2]

[1] *Gulhane Military Medical Academy, Ankara, Turkey;* [2] *Hacettepe University, Faculty of Pharmacy, Ankara, Turkey*

Aluminum (Al) is the most widely distributed metal in the outer crust of the earth. It is a toxic metal and accumulates especially in the lungs, liver, thyroid and brain. It has been reported that infants are more susceptible to exposure to Al than adults because of their immature systems. Infants, especially preterm neonates, display a narrow tolerance to aluminum. The aim of the present study was to determine whether there was any Al contamination in infant formulas sold commercially in Turkey in order to estimate theoretical toxic aluminum intake in comparison with the provisional tolerable weekly intake (PTWI) for Al established by FAO/WHO (2001). The proposed PTWI is 7 mg Al per kg body weight. In the present study, 63 infant formulas, follow-on formula, and baby food were randomly collected from pharmacies and supermarkets. Samples were distinguished to five groups as "milk-, cereal-, cereal plus milk-based" infant formula and starches and rice flours which are traditionally used for baby feeding. Aluminum levels were assessed by using atomic absorption spectrometer after digested in microwave unit. Aluminum level was ranged from 1.70 μg/g to 42.35 μg/g sample. The Al level in cereal-, milk-, cereal plus milk-based baby food, and starches and rice flour were found 6.43 ± 3.94 ($n = 10$) μg Al/g, 8.02 ± 8.61 ($n = 28$) μg Al/g, 7.43 ± 4.88 ($n = 11$) μg Al/g, and 3.33 ± 1.79 ($n = 5$) μg Al/g and 13.15 ± 15.39 ($n = 9$) μg Al/g sample, respectively. The susceptibility of infants and young children towards the adverse effects of toxic metals is higher than those of adults;

metal contaminations should seriously be taken into concern. It is essential to take special care throughout the entire process of manufacturing foods for infants and young children, as the fragility of them requires an increased safety. We suggest that contaminations with metals, especially aluminum should be routinely monitored in foods for babies in order to reduce food-borne hazards in infants and young children.

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P22-08
Estimating phytosterol intake from multiple food products in the diet

Sue Ohagan, Linda J. Lea

Unilever Bedford, Bedfordshire, UK

In 2000, EU approval was given for the addition of phytosterols to yellow fat spreads based on a comprehensive toxicology dataset. Results from a post-launch monitoring (PLM) programme showed that phytosterol intakes were lower than originally predicted. Based on these findings a second Novel Foods application was made to extend the approval to include other product types such as milk and yoghurt. A novel approach was required to produce a risk assessment demonstrating that these additional sources of phytosterols in the diet would not lead to safe levels being exceeded. Food consumption data for the three different product types (spreads, milk and yoghurt) were obtained from National dietary surveys where these were available in countries such as the UK and the Netherlands. Individual dietary records were then used to model the intake of phytosterols from the three different products. It was assumed that all milk, yogurt and spread consumed was replaced with phytosterol-containing products, taking into account not only the total amount of the product type consumed but also which of the products types are eaten in combination For the majority of EU countries detailed dietary survey data were not publicly available. However, it was possible to obtain consumer purchase data for these product types for some countries which could also used to calculate the intake of the different food products. As before, a worst case scenario was used to estimate phytosterol intake by assuming that all milk, yogurt and spread consumed was replaced with phytosterol-containing products. The accuracy of this data was assessed by comparing the individual intakes estimated from the National dietary surveys in UK and Netherlands with values estimated from the consumer purchase data collected in these countries. Overall, the intake modelling demonstrated that phytosterols could be added to different product types without intakes exceeding safe levels identified in toxicology studies. Novel Foods approval was granted for the use of phytosterols in milk and yoghurt type products.

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P22-09
Determination and validation of benzo(*a*)pyrene in edible oil by high-performance liquid chromatography with fluorescence detector

Ana Ranogajec, Adela Krivohlavek, Zdenko Šmit, Jasna Bošnir, Dinko Puntarić

Zagreb Institute of Public Health, Department of Ecology, Zagreb, Croatia

A sensitive and specific in house method using one-step clean up procedure based on gel permeation chromatography was developed. The method is suitable for determination of benzo(*a*)pyrene in edible oil. The main improvements compared with previous conventionale procedures are that analyte peak does not overlap with matrix peaks and that the one-step procedure of purification is rapid and simple with clearly improved analytical performance.

The quantitative analysis was performed by high-performance liquid chromatograph with fluorescence detector and confirmation was based on the GC–MS. The mean recovery rate of benzo(*a*)pyrene studied at three concentration levels ($n=9$) was 101% (R.S.D. 2.06%). No matrix effect was detected for different oils (olive, sunflower and rape oil). The limit of detection of benzo(*a*)pyrene by fluorescence was 0.1 μg/kg (R.S.D. 5.54%) and the limit of quantification was 0.2 μg/kg (R.S.D. 2.19%).

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P22-10
Assessment of nitrite contamination in baby foods, marketed in Turkey

Pinar Erkekoglu, Terken Baydar

Hacettepe University, Faculty of Pharmacy, Department of Toxicology, 90-06100, Ankara, Turkey

Nitrites (NO_2^-), which have exogenous and endogenous sources, have different physiologic effects including hematological, cardiovascular and respiratory outcomes. Nitrites are responsible for methaemoglobinemia to which infants less than 6 months are thought to be the most susceptible population. Furthermore, carcinogenicity is the most important issue, caused by the formation of nitrosamines from nitrites and nitrates in

the gastrointestinal tract. The acceptable daily intake (ADI) for nitrites suggested by FAO/WHO (1985) is up to 0.13 mg/kg/day. It was proposed that this value be changed to 0.07 mg NO_2^-/kg/day based on a more recent study. JECFA and SCF have proposed an ADI for NO_2^- of 0–0.07 mg/kg body weight per day and EPA has set an R_fD of 0.10 mg NO_2^- nitrogen per kg, body weight/day. However, these values are not applicable to infants and young children. The present study was aimed to detect whether there was any nitrite contamination in baby foods marketed in Ankara, Turkey and to estimate any possible toxicological risk in this sensitive physiological period. For this purpose, a study was designed to measure the nitrite levels in twenty collected baby foods and infant formulas, which were divided into divided into four groups (milk-, cereal-, vegetable- and fruit-based). An adapted Griess method was used to determine nitrite levels in the following samples. Nitrites reacted with Griess reagent to produce a dye that was detected at 525 nm. An extraction procedure was adapted and the method was applied to twenty baby food samples. The calibration curve was linear from 50 to 1000 ng/ml ($y = 0.067x + 0.007$, $r = 0.998$). The detection limit wasa 25 ng/ml. The coefficients of variation at 780 ng/ml concentration were 0.32% and 0.78% for within day and between days precisions, respectively. For supplementation of 1000 ng/ml of nitrite on sample which contained 780 ng/ml concentration, the average recovery value was found as 96%. We found that there were differences of nitrite concentrations between the groups. As it is largely known that babies should be fed with different kinds of food during the first year of life, it is necessary to choose the right baby food for the right month as the baby may not be able to cope with too much burden of chemicals; such as food with nitrite contamination. During manufacturing, nitrite contamination can come from several sources including water, fertilizer contamincation and from milk, fruit and vegetables within; so it is essential to take special care throughout the process of manufacturing food for infants and children.

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P22-11

Effect of processing on the degradation of ivermectin in milk and milk products

Veronica Lobato [2], Susanne Rath [1], Felix G.R. Reyes [1]

[1] *State University of Campinas, Campinas, São Paulo, Brazil;* [2] *Rural Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Ivermectin is the active compound of a veterinary drug with broad-spectrum anti-parasitic action, which is used to control internal and external parasites in bovine, swine and equine livestock. The stability to the thermal treatment, as well as the loss of the ivermectin residue in the yogurt and cheese whey preparation was evaluated in bovine milk obtained from cows ($n = 10$) treated with a subcutaneous dose of 200 μg ivermectin/kg b.w. The milk samples were analyzed before and after the following treatments: pasteurization at 62 °C for 30 min (P); heating at 80 °C for 10 min (H) and boiling at 99 °C for 1 min (B). The levels of ivermectin residue were also determined in the yogurt and whey prepared from the milk of the treated cows. High-performance liquid chromatography with fluorescence detection was used for the quantitation of ivermectin residues in the food sample matrices. After liquid–liquid extraction of ivermectin and purification of the extract, the compound was derivatized with 1-methylimidazol in *N*,*N*-dimethyl formamide to form a fluorescent derivative, which was separated by HPLC, using a Merck RP-18 LiChroCart column (125 × 4 mm, 5 μm). The column was thermostatically controlled at 30 °C. The flow rate for the mobile phase, composed of a mixture of methanol:water (96:4, v/v), was of 0.7 mL min^{-1}. The excitation and emission wavelengths of the fluorescence detector were 360 nm and 470 nm, respectively. The linearity of the method was in the range of 10–100 ng ivermectin mL^{-1}. Based on a sample of 5.0 mL, the limit of detection and quantitation for ivermectin in the samples were 0.6 ng mL^{-1} and 2 ng mL^{-1}, respectively. The average recovery, at four fortification levels, was 77.9 ± 3.2%. The inter-assay precision of the method was 13% ($n = 5$). The ivermectin presented stability to the thermal treatments of 83.3 ± 6.4% in P, 70.9 ± 15.3% in H and 63.7 ± 14.3% in B. The residue of ivermectin, in relation to the original level in the milk, verified in yogurt and in the whey were 77.6 ± 17.9% and 26.6 ± 5.6%, respectively. These results demonstrate the high stability of the drug to the principal technological processes to which the milk is submitted, pointing out the presence of ivermectin

residues in the whey, a raw material used in the food industry.

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P22-12
Effect of processing on the degradation of tetracyclines in milk and milk products

Luiz Severo da Silva Jr., Susanne Rath, Felix G.R. Reyes

State University of Campinas, Campinas, São Paulo, Brazil

Tetracyclines are considered an important group of broad spectrum antimicrobials employed in Brazil in several species of animals producing food, including bovine, swine and fish. A HPLC method for the determination of oxytetracycline (OTC), doxycycline (DC) and tetracycline (TC) residues in milk and yogurt was developed and validated. The method was used to evaluate the degradation of these antimicrobial contaminants during the thermal treatment of milk, as well as during yogurt preparation and storage. Sample extraction was carried out using 30% (v/v) trichloroacetic acid to precipitate the proteins. For the HPLC analyses a Varian C_8 analytical column (250× 4.6 mm, 5 μm) with a mobile phase: sodium acetate 0.1 mol L^{-1} + EDTA 25 mmol L^{-1} + calcium chloride 35 mmol L^{-1}: methanol (65:35, v/v) and fluorescence detection (λ excitation: 420 nm and λ emission: 530 nm) was used. Tetracycline recovery in milk and yogurt was 92–98% (OTC); 87–83% (DC) and 89–97% (TC). The quantitation limit was 27, 46 and 33 ng mL^{-1} for OTC, DC and TC, respectively. Different thermal treatments were used to evaluate the heat stability of the tetracyclines in milk: cooking stove treatment, hot plate and microwave oven. Higher degradation occurred using the hot plate, 10.9% (OTC), 27.7% (DC) and 15.0% (TC). With respect to the stability of the tetracyclines during the preparation and shelf life of yogurt, it was verified that 500 ng mL^{-1} (OTC), 1120 ng mL^{-1} (DC) and 600 ng mL^{-1} (TC) did not inhibit yogurt production, and that after 30 days under refrigeration (4 °C), residual levels of the contaminants still remained in the samples. Nonetheless, in relation to the original contamination in the yogurt, the tetracycline levels had diminished by 52%, 61% and 67%, for OTC, DC and TC, respectively. The data indicated that when conducting a risk assessment of tetracyclines in human health, the presence of these contaminants in milk and yogurt should be considered as a potential source. In addition, in order to protect consumer health, the governmental agencies should monitor the application of good practices in the use of these antimicrobials in the treatment of cows that produce milk for human consumption, as well as conducting health surveillance actions in order to control the residue levels of these substances in commercialized milk and yogurt.

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P22-13
Determination of volatile *N*-nitrosamines in sausages by HS-SPME-GC-TEA

Camila B. Dutra, Raquel Andrade, Felix G.R. Reyes, Susanne Rath

State University of Campinas, Campinas, São Paulo, Brazil

Nitrosamines are formed in certain foods by the reaction of a nitrosating agent derived from either nitrite salts or nitrogen oxide with a nitrosable amine. The majority of the nitrosamines tested have been carcinogenic in a variety of animal species. Furthermore, these compounds are toxic, mutagenic, teratogenic and act transplacentally. This work describes the development of a simple method using headspace sampling by solid-phase microextraction gas chromatography with thermal energy analyzer detector (HS-SPME-GC-TEA) for the determination of N-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) in sausages. Because the SPME efficiency is influenced by several factors such as type of fiber coating, extraction time, ionic strength of the solution and temperature, a fractional factorial design and a central composite design were developed and employed to determine the optimal experimental conditions for the nitrosamine determination in the sausages sample matrices. Fused silica fibers coated with dimethylsiloxane–divinylbenzene, dimethylsiloxane–divinylbenzene carboxen and poliacrylate were evaluated. The chromatographic separations were carried out with a HP-INOWAX megabore column (30 m × 530 μm i.d. × 1 μm). The CG operating conditions were as follows: injector temperature, 200 °C; oven temperature was held at 100 °C for 3 min, then heated to 140 °C at 40 °C min^{-1} and then to 160 °C at 5 °C min^{-1}. The TEA furnace and the GC-TEA interface temperatures were 550 °C and 250 °C, respectively. The helium carrier gas flow-rate was 5 mL min^{-1}. The validated method is simple, with adequate accuracy, selectivity, sensitivity and precision. In the analyzed hot dog sausages samples ($n = 7$) volatile nitrosamines were not detected. The results indicate that the absence of nitrosamines in the hot dog sausages possibility occurred

due to their low content of nitrite and to the action of sodium ascorbate as inhibitor of the nitrosamines formation. Two samples of sausages presented NDMA at concentrations of $43.5 \pm 6.5\ \mu g\ kg^{-1}$ ($n = 3$) and $15.0 \pm 2.2\ \mu g\ kg^{-1}$ ($n = 3$). It is recommended that action levels or tolerances be established in the Brazilian food laws and that a study of the presence of nitrosamines in the different types of sausages commercialized in Brazil be conducted in order to be able to take actions to avoid the presence of these deleterious substances.

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P22-14

Pomegranate (*Punica granatum*) rot causing microscopic fungi, their occurrence in pomegranate juice and toxigenic ability

Karine M. Grigoryan, Hasmik G. Hayrapetyan, Mariam Sarkisyan, Lusine Hakobyan

Department of Food Biology, Yerevan State University, A. Manoogian 1, Yerevan 2006, Armenia

Pomegranate is famous for its healing abilities since ancient times. It is regarding to pomegranates antioxidant activity that it is widely used in folk medicine. Taking into account the wide utilization of fruits of pomegranate both in food industry, and in medicine, its contamination with toxigenic microscopic fungi represents a great interest for researchers. Mycobiota of 50 samples of pomegranates of five different varieties, which are used as raw materials in the Juice Industry, was investigated. Chzapek agar and GYA culture medias were used for cultivation of fungi. Fifteen species (70 strains) of microscopic fungi were isolated, belonging to genuses *Penicillium*, *Aspergillus*, *Fasarium*, *Mucor*. About 80% of all isolates were representatives of the genus *Penicillium*, among which the section *Monoverticillata* was dominating with the following species: *P. implicatum*, *P. roseopurpureum*, *P. vinaceum*, *P. adametzioides*, *P. restrictum*, *P. fellutanum*. Species from the specified section were found in 76% of the investigated samples, among which, the species *P. implicatum* was the most typical and specific fungi for pomegranate fruits. *P. implicatum* was detected in 31 samples from the total of 50. Thermostability of sporous suspension of the most frequent species was investigated (by incubation in 60 °C/5 min.), to find out a possibility of contamination of juices and other products of processing of a pomegranate by these fungi. The majority of fungi were not thermostable. There were not found any viable spores of microscopic fungi in some ready juices produced in Armenia. Preliminary biotesting analysis on *Artemia salina* have shown low toxicity for the majority of tested strains of *P. implicatum*. However, among other tested species there were found toxic strains. Patulin occurrence in pomegranate juice was investigated. Results of this work can be used in HACCP plan's risk assessment for pomegranate juice processing factories.

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P22-15

Short-term oral toxicity of quercetin and pterostibene in Swiss mice

María José Ruiz, Mónica Fernández, José María Estela, Miguel Ángel Asensi, Jordi Mañes, Yolanda Picó

Faculty of Pharmacy, Dept. of Toxicology, Burjassot, Valencia, Spain

Quercetine (QUER) and pterostilbene (PTER) are flavonoids that are widely distributed in plants, especially in grapes. Many studies have been drawn toward the beneficial properties of QUER and PTER, mainly their antioxidant and anticarcinogenic effects through the diet.

Because of their new possible use as food complements, the aim of this study was to evaluate the harmful effects of high doses of QUER, PTER or the mixture of both (QUER + PTER) in Swiss mice.

Animals were divided into 10 male groups and 10 female groups, each of them containing 5 animals. QUER, PTER or (QUER + PTER) were daily oral administered in the pelleted diet for the treatment period of 28 days at a dose of 0 mg/kg/day (for control), 30 mg/kg/day, 300 mg/kg/day and 3000 mg/kg/day. These doses correspond to 100, 1000, and 10000 times, the maximum amount of PTER found in 1 kg of dark grapes; and 2, 20 and 200 times the maximum daily intake of QUER, respectively. These doses would be equivalent for an adult human being of 70 kg body weight. After 28 days, results obtained were compared to those obtained in the non-administrated flavonoid group.

All animals survived until the end of the study. Food and water consumption did not differ between mice groups treated with QUER, PTER, (QUER + PTER) and the control groups, independently on sex. However, a significant increase in body weight gain for the group of females feed at levels of 30 mg and 300 mg QUER + PTER/kg/day and a decrease for the group of females feed at levels of 3000 mg QUER + PTER/kg/day were observed. Hematologic variables (hematies amounts and hematocrite) were decreased by the administration of flavonoids compared to control groups, although all values were within histor-

ical limits for female and male Swiss mice. Biochemical variables were not affected. Histopathological examination of the organs obtained at autopsy did not reveal any alterations in clinical signs or organ weight at any doses of flavonoids.

These results support the view that repeated consumption of QUER, PTER or mixture of QUER + PTER at the concentration assayed did not cause adverse effects or mortality in mices during the experimental period.

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P22-16

Effects of temperature and time on migration of styrene monomer from polystyrene cups into milk

M. Ghazi-Khansari, M. Khaksar

Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Plastic materials excessively applied in manufacturing of disposable drinking containers. Styrene monomer is excessively used in these products, therefore the effects of these chemicals on food products is very important. This study was performed to evaluate the migration of styrene monomer to milk with respect to temperature and time. Two hundred and sixteen samples of general purpose polystyrene (GPPS) and high impact polystyrene (HIPS) were obtained from central markets with different fat milk content (3.6%, 2.5%, and 1.5%). Then milk extraction and distillation of sample were down. Detection of styrene monomer performed by high-performance liquid chromatography (HPLC) with UV–vis detector. For the reproducibility of method, within-day and day to day were performed. In this study, the average ranges of migration of styrene monomer into milk in different temperature and time in all cases were between 0.2 ± 0.05 μg/l and 8.42 ± 0.4 μg/l. The mean recovery rate of styrene monomer from spiked samples was 97.2% (range 95.8–98.6%). The analytical method was validated and was adequately reliable and sensitive. Standard curves were obtained were linear from 10 ng/ml to 100 ng/ml giving a limit of detection of 0.001 mg/kg (ppb). The amount of migration of styrene monomer from GPPS and HIPS cups to milk with fat content 3.6% was greater than fat content of 1.5% and 2.5%. Also the greatest amount of migration of styrene monomer was observed in the first 10 min of experiments. The level of migration of styrene monomer to milk essentially depends on the storage temperature and fat content and time. The method presented here is rapid, sensitive, and linear and may be useful for routine monitoring of analysis of styrene monomer in plastic materials.

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P22-17

Mycobiota of raisin from Armenian market and factors influencing its development

K. Grigoryan, L. Hakobyan, M. Sarkisyan, H. Hayrapetyan

Department of Food Biology, Yerevan State University, 2006, Armenia

Raisin is a favorable substrate for molds. The problem of contamination of raisin with toxigenic microscopic fungi is very actual, especially in developing countries. Mycological analysis of 22 samples of raisin in Armenia, prepared from white and red grapes, was conducted. Fifteen species of microscopic fungi were isolated and identified, belonging to 5 genuses: *Mucor*, *Aspergillus*, *Penicillium*, *Trichoderma* and *Alternaria*. Among isolated fungi the strains from section *A. niger* and from genus *Mucor* had a high frequency of occurrence. In some separate samples degree of contamination with spores exceeds 10 CFU/g. Strains of genus *Mucor* cause both superficial and internal contamination of raisin. The comparative analysis of fungi, contaminating raisin of Iranian and local production, has shown, that *Aspergillus carbonarius* most often is found in raisins produced in Armenia (in 62.5%), mostly prepared from red grades. In the total of 17 strains of *A. carbonarius* were isolated. In the Iranian raisin the content of sulfur dioxide exceeded 60 mg/kg, which probably has an inhibitory effect on growth of *A. carbonarius*. In samples of the Armenian raisin sulfur dioxide was not found. Influence of pH and aw on a degree of raisin contamination by fungi was studied, the positive correlation was observed.

The use of raisin in food, contaminated with *A. carbonarius*: a potential producer of ochratoxinA, represents a health hazard for the consumer.

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P22-18

Assessment of estimated daily intake of benzoates for Belgian preschool children and adolescents

M. Bilau [1], M. Bellemans [1], C. Vinkx [2], S. De Henauw [1]

[1] *Ghent University, Department of Public Health, Belgium;* [2] *Ministry of Public Health, Brussels, Belgium*

Introduction: Benzoates (E210, E211, E212 and E213) are a group of food additives important to preserve foods and protect the consumer from microbiological risks. The consumer should, however, also be protected against chemical risks from the benzoates by ensuring that the acceptable daily intake (ADI) is not exceeded. The European Commission identified benzoates as additives for which the intake has to be examined more closely. This intake assessment was done under the authority of the Belgian Health Council Task Force on Food Additives.

Material and methods: A simple distribution approach was used to estimate the daily intake of benzoates for the Belgian population. Two age groups were considered: preschool children ($N = 697$; age 2–6; 3 day food record) and adolescents ($N = 341$; age 14–18; 7 day food record). The individual food consumption data were multiplied with the maximal limits for benzoates per food group (Tier 2 approach as defined by the EC). Following food groups were considered: non-alcoholic flavoured drinks, non heat-treated dairy based desserts, low sugar jams & marmalades, chewing gum, candy(bars), (non-)emulsified sauces, liquid eggs, liquid soups and broths, prepared salads, salted/dried fish, semi preserved fish (products), cooked shrimps, mustard, olives and olive based preparations and vegetables in vinegar, brine or oil.

Results: The ADI for benzoates is 5 mg/kg bw. The median estimated daily intake is 2.08 mg/kg bw, respectively, 1.78 mg/kg bw for preschool children and adolescents. At the 95th percentile (children), respectively, the 97.5th percentile (adolescents) the ADI is exceeded slightly. In both age groups, the greatest contributor is by far the group of non-alcoholic flavoured drinks. Other important contributors at the upper end of the intake distribution are prepared salads (both age groups) and non heat-treated dairy based desserts (preschool children), respectively, candy(bars) (adolescents).

Discussion: The current exposure assessment shows that the ADI for benzoate acid is exceeded by 5%, respectively, 2.5% of the children/adolescents. However, the Tier 2 approach is known to overestimate the intake: not all food items in which benzoates are allowed, contain (the maximum level of) benzoates. A more precise estimation as defined in Tier 3 (with actual levels) is necessary.

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P22-19

Xenobiotic clenbuterol: Toxicologic implication of persisting residues in liver as edible tissue

T. Gojmerac, J. Pleadin, Z. Lipej, D. Novosel

Croatian Veterinary Institute, Department of Chemistry and Department of Pathology, Zagreb, Croatia

A β_2-adrenergic agonist clenbuterol has been misused as a growth-promoter to enhance muscle growth, reduce body lipid and optimize food conversion efficacy in food-producing animals. Long-term exposure of food-producing animals to a growth-promoting dose of clenbuterol leads to accumulation of residues in edible tissues and cases of consumer intoxication due to consumption of meat and liver contaminated with residual clenbuterol have been reported. The aim of the present study was to evaluate the clenbuterol residue depletion in pig liver as edible tissue on days 0, 3, 7, 14 and 35 of treatment discontinuation, in correlation with the severity of histopathologic changes at the same time points. A total of 18 male pigs (15 treated and 3 control), of a known breed, aged 90 days, body mass 50 kg, farm-bred, and kept under the same zoohygenic conditions were used in study. A growth-promotion dose of clenbuterol (20 μg/kg body mass per day) was administered orally to pigs for 28 days. Upon cessation of administration, on days 0, 3, 7, 14 and 35, the pigs were randomly sacrificed and the liver was collected for residual clenbuterol determination and histopathologic examination. Based on analytical assay procedure utilizing validated enzyme-linked immunosorbent assay, clenbuterol residues in pig liver declined from 30.19 ± 17.70 ng/g, 7.01 ± 1.37 ng/g, 1.32 ± 0.88 ng/g and 0.40 ± 0.11 ng/g on days 0, 3, 7 and 14, respectively, to 0.22 ± 0.04 ng/g on day 35. On day 14, the measured clenbuterol residues fell below the maximal residue level (MRL) of 0.5 ng/g, however, quantification of clenbuterol residues indicated persistence of this β_2-adrenergic agonist in the liver as edible tissue on day 35 of treatment cessation. Histopathologic studies performed at the same time points revealed vacuolar hepatocyte degeneration, with glycogen depletion from the cytoplasm. The severity of degenerative changes of the liver tissue did not follow clenbuterol depletion upon treatment cessation but remained unchanged, suggesting an irreversible effect of clenbuterol residues during the study period. Accordingly, clenbuterol reasidues in pig liver as edible tissue

appear to provoke morphological alternation that may pose a risk for consumer health.

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P22-20

Long term safety and toxicological evaluation of novel oxygen-coordinated niacin-bound chromium(III) (NBC) complex

D. Bagchi [1,2], M. Shara [1], T. Yasmin [1], A. Kincaid [1], A. Limpach [1], R. Sandstrom [1], M. Bagchi [2]

[1] *Creighton University Medical Center, Omaha, NE, United States;* [2] *InterHealth Research Center, Benicia, CA, United States*

Chromium is an essential trace element required for normal protein, fat and carbohydrate metabolism. Previously, we demonstrated the safety of NBC in a subchronic 90-day model [J. Inorg. Biochem. 99 (2005) 2161–2183]. This study examined the long term (52 weeks) safety by administering either 0 or 25 ppm [2000 μg elemental Cr(III) HED] NBC per day for 52 consecutive weeks to 36 male and 36 female Sprague–Dawley rats one-third of each group and each gender were killed at 26, 39, or 52 weeks of treatment. Body weight, feed and water intake, selected organ weights as such and as a % of liver and brain weight, hepatic and kidney lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological evaluations were conducted. At 26, 39, or 52 weeks of treatment, body weight gain was significantly reduced by 7.7%, 8.1% and 14.9% in male rats, and 5.5%, 11.4% and 9.6% in female rats, respectively, in the NBC treatment groups. No significant changes were observed in hepatic and kidney DNA fragmentation and lipid peroxidation, and hematology and clinical chemistry, between control and NBC groups at these same time points. Histopathological evaluation is currently underway. These findings, thus far, are in agreement with the subchronic studies in terms of the safety of NBC.

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P22-21

Primary aromatic amines in kitchen utensils in Slovenia

L. Perharic [1], V. Golja [1], A. Zoric [1], M. Luci [2]

[1] *Institute of Public Health of the Republic of Slovenia, Trubarjeva 2, Ljubljana, Slovenia;* [2] *Health Inspectorate of the Republic of Slovenia, Parmova 33, Ljubljana, Slovenia*

Food contact materials are all materials and articles intended to come into contact with food. They are highly regulated, and may only release substances in quantities which do not constitute a consumer health risk. Slovene monitoring of food contact materials safety is coordinated by the Health Inspectorate. The Institute of Public Health provides laboratory and risk assessment services. From European Union (EU) entry on May 1, 2004 to December 31, 2005 our laboratory analysed 383 samples of food contact materials. Of these 41 (10.7%) were not in accordance with the current legislation due to migration of chemicals including primary aromatic amines (PAA), metals, formaldehyde, semicarbazide, etc. Migration of total PAA determined by spectrophotometry in 14 samples of utensils made of black plastic ranged from 0.033–5.090 mg/kg (limit 0.02 mg/kg). Each sample underwent three trials in deionised water for 2 h at 100 °C. The migration of specific PAA such as aniline and 4,4′methylenedianiline was measured by liquid chromatography tandem mass spectrometry (limit of quantification 0.01 mg/kg). The daily amount of food utensil contact was estimated according to the size and type of utensil, and exposure expressed in aniline/kg bw/day. This amounted to 0.25–97 μg/kg/day in 60 kg adults, and 1.53–291 μg/kg/day in 10 kg children. Aniline and its derivatives act as oxidising agents and may cause methaemoglobinaemia (420 μg/kg). They are irritant, alergogenic, mutagenic and carcinogenic. In view of their genotoxicity, tolerable daily intake has not been determined. According to the United States Environmental Protection Agency, long term ingestion of aniline in drinking water at 0.2 μg/kg/day amounts to a carcinogenic effect risk of 10^{-6}. We judged exposure above this level to be unacceptable. In view of multiple other possible sources of exposure to PAA such as dyes, medicines, leather, rubber and plastics we concluded that additional exposure from kitchen utensils was undesirable. All 14 samples were analysed prior to market release. Due to unacceptable risk evaluation, the tested utensils were not

released to the Slovene market. The test results were circulated to the EU Rapid Alert System for Food and Feed.

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P22-22

Monitoring of oxytetracycline in bovine milk by high-performance liquid chromatography with UV-detector

Mir-Jamal Hosseini [1], Azam bakhtiarian [1], Roya Khosrove Khavar [2], Mortaza Pirali-Hamedani [2,3], Mahmoud Ghazi-Khansari [1], Mohsen Amini [3]

[1] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Iran;* [2] *Food and Drug Control Laboratories, Ministry of Health, Tehran, Iran;* [3] *Department of Medicinal Chemistry, School of Pharmacy, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

Oxytetracycline (OTC) is a tetracycline derivative obtained from *Streptomyces rimosus*. It inhibits protein synthesis by reversibly binding to 30S ribosomal subunits. It is used for the prophylaxis and treatment of a great number of diseases since this antibiotic possesses a broad spectrum activity against many pathogenic organisms. It is used in human therapy, veterinary medicine both as prevention or the treatment and as promotion of growth in current intense husbandry practices. The use of OTC has become a serious problem because of the possible existence of its residues in the milk which can be directly toxic or cause allergic reactions in some hypersensitive individual's. Even, low-level doses of antibiotic in milk consumed for long periods can lead to problems regarding the spread of drug-resistant microorganisms. To prevent any health problems with consumers, FAO/World Health Organization (WHO), US Food and Drug Administration (FDA), European Union (EU) have been established its maximum residue limit (MRL) for OTC 100 pbb. The purpose of the present study was to investigate residual OTC in consuming milk in Tehran using high-performance liquid chromatography (HPLC) with UV-detector. OTC residues in extracts obtained from a preliminary cleanup procedure and recoveries from spiked OTC in desire concentrations were between (80–99%) with appropriate coefficients of variation. The limit of detection (LOD) and limit of determination (LOQ) were 0.025 μg/ml and 0.05 μg/ml, respectively. Of the 100 milk samples we examined, the OTC levels were lower than 100 ppb except for one sample which was 112 ppb. It was determined that this method would be useful for routine monitoring of oxytetracycline residues in bovine dairy milk.

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P22-23

Identification of the Lebanese market food basket and evaluation of the dietary exposure to heavy metals and radionuclides in Lebanon

L. Nasreddine [1,2], N. Hwalla [3], O. El Samad [2], Y. Sibiril [1], M. Hamzé [2], D. Parent-Massin [1]

[1] *LTA EA 3880, Université de Bretagne Occidentale, Brest, France;* [2] *CLEA, CNRS Libanais, Beirut, Lebanon;* [3] *AUB, Beirut, Lebanon*

The present study is the first in Lebanon to define the market food basket of the Lebanese population by an individual dietary survey conducted in Beirut and to evaluate the dietary exposure of the average Lebanese individual to radionuclides and heavy metals Pb, Cd and Hg. The market basket was identified by selecting those foods with a mean daily intake exceeding 1 g/day. The analytical quantification of heavy metals and radionuclides in foods were performed, respectively, by inductively coupled mass spectrometry and gamma spectroscopy. The foods selected for analysis were those belonging to the market basket and those considered by the WHO as the major dietary sources of the studied heavy metals. The selected foods were prepared as normally consumed. Traces of Cs-137 were detected in 5 samples among the 36 analyzed. The analytical quantification of radionuclides permits to conclude that the level of radioactivity of the foods mostly consumed by the studied population group is in accordance with international limits and does not present any concern for public health. The highest contamination by lead (>25 μg/kg) and cadmium (>15 μg/kg) were detected in cereal-based products. The average concentration of mercury in fish was of 165 μg/kg. The average dietary exposure of the average individual to Pb, Cd, and Hg were estimated to be 18 μg/day, 12 μg/day and 3 μg/day, which correspond to 7%, 17% and 6% of the respective PTWIs. This permits to conclude that, for the average individual, there is no risk of exceeding the PTWIs.

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P22-24
Biogenic amines in Argentine wines

Edith Graciela Díaz, Carolina Santamaría, Marta Gozzi, Alejandro Ferrari Costa

Universidad Argentina de la Empresa, National Research Council, Buenos Aires, Argentina

Recent trends in food safety and the consumer's demands for healthier products are stimulating the research for trace compounds that can be harmful to human health. This is the case of biogenic amines which at high concentrations in wine can cause undesirable physiological effects in sensitive humans. Consumption of beverages rich in histamine can lead to intense headache, low blood pressure, skin rashes, facial flushing and cardiac and intestinal problems. Moreover, biogenic amines not only has oenological importance in relation to the negative impact on the aroma of the final product but also could give evidence of unsanitary conditions during the wine making process.

The aim of this work was to evaluate for the first time the profile of biogenic amines in 38 samples of red Argentine wines from different wineries of Mendoza, the most important viticulture area of the country.

Biogenic amines tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine and spermidine were analysed by reverse phase HPLC using binary gradient and photodiode array detector. The pre column derivatization reagent used was dansylchloride

The average amount of total amines was 6.46 mg/l, the highest value 14.21 mg/l and the lowest 0 mg/l. Concentration range were as follows: tryptamine 0–3.49 mg/l; phenylethylamine 0–1.71 mg/l; putrescine 0.61–14.21 mg/l; cadaverine 0–0.39 mg/l; histamine 0–5.22 mg/l; tyramine 0–5.38 mg/l and spermidine 0–0.60 mg/l. Putrescine, histamine and tyramine represented the largest percentage of total of biogenic amines and were detected in 100%, 95% and 91%, respectively, while cadaverine were only present in 32% of the wines.

From this work it is possible to conclude that the red Argentine wines analysed are below the maximum limits recommended by some countries regarding histamine levels; that the low concentrations of histamine, tyramine and phenylethyamine, suspected to cause toxicological effects, are of no concern and that small amounts of cadaverine and putrescine indicate good sanitary conditions.

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P22-25
Ochratoxin A blood concentration in healthy subjects and bladder cancer cases from Pakistan

Muhammad Aslam[2], Answer Ejaz Beg[2], Meinolf Blaszkewicz[1], Gisela H. Degen[1], Klaus Golka[1]

[1] *Department of Pharmaceutics, University of Karachi, Pakistan;* [2] *Institut für Arbeitsphysiologie an der Universität Dortmund Germany*

Background: Food contaminated with the mycotoxin ochratoxin A (OTA) is a public health issue in many countries. In Western European countries, e.g. in Germany, OTA-serum concentrations in humans in general may vary between 0.06–2.03 ng/ml. Higher OTA serum concentrations up to 100 ng/ml are reported from some areas of the former Yugoslavia known for a high prevalence of Balkan Endemic Nephropathy. Data from developing countries are scarce. Thus we aimed to investigate OTA blood concentrations in patients with bladder cancer and in non-diseased controls from Pakistan.

Methods: In 87 patients with histologically confirmed bladder cancer from a department of urology located in Karachi and 30 non-diseased local controls, OTA concentrations in whole blood were determined by HPLC and fluorescence detection. Detection limit was 0.05 ng/ml.

Results: Mean OTA concentrations observed in Pakistani bladder cancer patients were 0.325 ng/ml blood (S.D. 0.418; range 0.032–3.409 ng/ml). Mean OTA concentrations observed in non-diseased controls were 0.314 ng/ml blood (S.D. 0.291; range 0.036–1.239 ng/ml).

Conclusions: There was no difference of OTA levels between the bladder cancer group and the controls. The OTA concentrations are comparable to those reported for the general population in the European Union.

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P22-26
Evaluation of fruit consumption safety applying LC–MS

Cristina Blasco, María José Ruiz, Yolanda Picó, Guillermina Font

Faculty of Pharmacy, Burjassot, Valencia, Spain

Liquid chromatography (LC)–atmospheric pressure ionization (API)–mass spectrometry (MS) has been used to determine residues of six pesticides – carbendazim, hexythiazox, imazalil, imidacloprid, methiocarb and thiabendazole – in fruits from the Valencian Community

(Spain). Two hundred and seventy-nine samples were analyzed, of which 25 were oranges, 98 tangerines, 50 peaches, 97 nectarines and 6 plums. Samples were extracted with a conventional multiresidue extraction procedure that employs ethyl acetate as organic solvent. Recoveries of six pesticides were calculated in samples spiked at two levels, the limit of quantification (LOQ) and 10 times the LOQ. Recovery values ranging from 51% to 99% were attained. Two hundred treated samples contained pesticide residues, carbendazim was detected in 198 samples (70.1%) in a concentration range from 0.02 mg kg^{-1} to 1.44 mg kg^{-1}, hexythiazox in 10 samples (3.6%) in a concentration range from 0.02 mg kg^{-1} to 0.09 mg kg^{-1}, imazalil in 3 samples (1.1%) in a concentration range from 0.07 mg kg^{-1} to 1.8 mg kg^{-1}, imidacloprid in 15 samples (5.4%) in a concentration range from 0.02 mg kg^{-1} to 0.95 mg kg^{-1}, methiocarb in 1 sample (0.4%) at 0.05 mg kg^{-1}, and thiabendazole in 3 samples (1.1%) at a concentration range from 0.02 mg kg^{-1} to 1.30 mg kg^{-1}. Twenty-three samples contained residues of two or more pesticides. Three samples contained pesticide residues that exceeded the maximum residue levels (MRLs). The calculation of the estimated daily intakes (EDIs) from these monitoring data showed that dietary intakes were much lower than the acceptable daily intakes (ADIs) established by the Food Agriculture Organization and World Health (FAO/WHO).

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P22-27
Benzene in food

Jessica F. Greene

Exponent, Oakland, CA, USA

There is a consensus amongst regulatory agencies, including IARC and the U.S. EPA, that benzene is a human carcinogen. This conclusion is based on inhalation data in humans supported by animal evidence. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic leukemia; in animals, benzene is a multiple site carcinogen by both the inhalation and oral routes.

After long-term oral exposure to benzene, rats have developed tumors of the skin, oral and nasal cavities, liver, among others. Type and location of tumor varied with sex, type of rat and dose but the lowest tumorogenic dose in these studies was ≥25 mg/kg/day. In mice, tumors of the lymph system, lung, and mammary gland, among others, were seen. As with rats, type and location of tumor varied, but the lowest tumorogenic dose was ≥25 mg/kg/day.

Because there are no data on carcinogenicity in humans from oral exposure to benzene, EPA estimated an oral slope factor from inhalation exposure data in humans ranging from 1.5×10^{-2} to 5.5×10^{-2} $(mg/kg/day)^{-1}$. That suggests a dose of 0.2–0.7 μg/kg-day (or 14–49 μg/day for a 70 kg person) could result in cancer risk level of 1 excess case per 100,000 individuals exposed over a 70-year lifetime. There is considerable uncertainty in these estimates due to exposure concentrations, extrapolation to a different route of exposure, variation in metabolism and mechanism of action, and extrapolation to a low dose.

Concentration of benzene in food varies, although most food products have measurable levels of benzene—a recent study in the U.S. analyzed 70 foods and found that 68 of them contained benzene between 1 and 190 μg/kg. Estimates of total daily intake of benzene from food vary but are generally less than 10 μg/person/day. In the US, benzene is allowed in drinking water to a maximum level of 5 μg/L. In the UK, benzene is allowed in drinking water to a maximum level of 1 μg/L. While it is unlikely that drinking water would consistently be at the maximum permitted level, nonetheless, at a daily consumption level, this would result in 2–10 μg/person/day. This can be compared to 90–1300 μg/person/day from ambient and indoor air.

Thus, although the contribution of benzene from food may be a significant percentage of the allowable level, it is likely of the same order of magnitude of contribution from water and it is dwarfed by the contribution of benzene from air.

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P23 Genetic Toxicology

P23-01
PAH metabolites in urine and genotoxic effects in white blood cells of mastic asphalt workers exposed to fumes and aerosols of bitumen in a cross-shift study

B. Marczynski [1], M. Raulf-Heimsoth [1], R. Preuss [2], M. Kappler [1], H.U. Käfferlein [1], K. Schott [3], B. Pesch [1], T. Mensing [1], A. Spickenheuer [1], J. Angerer [2], T. Brüning [1]

[1] *Research Institute of Occupational Medicine (BGFA), Ruhr-University Bochum, Bochum, Germany;* [2] *Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg, Erlangen, Germany;* [3] *Berufsgenossenschaft der Bauwirtschaft, Munich, Germany*

A cross-shift study with 66 bitumen exposed-mastic asphalt workers and with 49 construction workers without exposure to bitumen were carried out. Exposure was assessed using urinary 1-hydroxypyrene (1-OHP) and the sum of 1-,2 + 9-,3-,4-hydroxyphenanthrene (OHPH). Genotoxic effects in white blood cells were determined with non-specific DNA adduct levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) and the formation of DNA strand breaks and alkali-labile sites. Bitumen-exposed workers had more DNA strand breaks than the reference group ($P < 0.0001$) at both time points and a significant correlation with 1-OHP and OHPH in the post-shift urines ($r_s = 0.32$; $P = 0.001$ and $r_s = 0.27$; $P = 0.004$, respectively). Paradoxically, we measured higher levels of DNA strand breaks (not significant) in both study groups before shift. 8-OxodGuo adduct levels did not correlate with DNA strand breaks. Further, 8-oxodGuo levels were associated neither with personal exposure to bitumen nor with urinary metabolite concentrations. Significantly more DNA adducts were observed after shift not only in bitumen-exposed workers but also in the reference group. Our results demonstrate that exposure to fumes and aerosols of bitumen may contribute to increased DNA strand breaks in white blood cells but not increased 8-oxodGuo concentration.

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P23-02
ECVAM genotoxicity and carcinogenicity key area: A focus on alternatives

D. Maurici, E. Sabbioni, T. Hartung, R. Corvi

European Centre for the Validation of Alternative Methods (ECVAM), IHCP, Joint Research Centre of the European Commission, Ispra (VA) 21020, Italy

The genotoxicity/carcinogenicity key area, established in 2004, focuses on the activities related to the promotion and the validation of alternative methods to refine, reduce and replace animal experiments in the area of genotoxicity and carcinogenicity. These activities are prioritised in collaboration with the ECVAM taskforce.

In the area of carcinogenicity, an inter-laboratory validation study on the cell transformation assay (CTA), which has the potential to detect both genotoxic and non-genotoxic carcinogens, was initiated. This is the first ECVAM validation study which involves laboratories from Japan, US and Europe and focuses on the SHE and Balb/c 3T3 assays.

In the area of genotoxicity, ECVAM carried out a retrospective validation of the micronucleus test (MNT) *in vitro*, based on existing data. The report on the retrospective validation of *in vitro* MNT has been submitted to the ECVAM Scientific Advisory Committee (ESAC) for peer review and an ESAC statement on the validity of the test is foreseen for October 2006.

Since considerable *in vivo* testing is still required for confirmation of the genotoxic prediction *in vitro*, it became clear that it was crucial to address issues related to the reduction and refinement of genotoxicity tests. For this purpose, a survey was prepared to be distributed to industries and CROs. The data collected will be analysed and will be considered as a basis for possible amendments of guidelines to reduce animal consumption.

The third taskforce meeting highlighted the need to establish approaches in order to clarify the "false" *in vitro* positive results and to avoid *in vivo* test confirmation. A workshop on "How to reduce false positive results in genotoxicity testing" has been organized and will take place in April 2006 at ECVAM.

ECVAM is also involved in the REACH implementation project (RIP 3.3). A drafting Working Group for the endpoint mutagenicity and carcinogenicity has been created. This working group, which also include stakeholders from different Industries and European associations, will develop technical guidance document on the data

requirements and on testing strategies for the REACH implementation.

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P23-03

Environmental exposure to Cd and Pb; effects of genotype on the induction of Human CYP2A6

Soisungwan Satarug [1,2], Michael R. Moore [1]

[1] *National Research Centre for Environmental Toxicology (EnTox), The University of Queensland, Brisbane, Queensland, Australia;* [2] *Division of Clinical Pharmacology, International Graduate Program in Biomedical Sciences, Thammasat University, Rangsit Campus, Pathumthani, Thailand*

Cadmium is an inducer of Cytochrome P450 2A6 (CYP2A6). This liver enzyme shows a high degree of inducibility and genetic variability. In the present study, we investigated the induction of CYP2A6 by cadmium in relationship to genotype and concurrent exposure to lead in a group of 193 non-smoking individuals with mean age of 32 years who were exposed to Cd and Pb from dietary sources. We used coumarin as an *in vivo* phenotypic probe specific for CYP2A6 while we used urinary excretion of Cd and Pb to reflect exposure to these metals. Geometric mean for urinary Cd excretion was 0.30 μg/day whereas the geometric mean for urinary Pb excretion was 0.95 μg/day. Of the 193 subjects examined, 32 subjects had **1A/*1A* genotype, 22 had **1B/*1B* genotype and 31 had **1A/*1B* genotype. Another 6 subjects had one active allele of the **1B* or **7* genotypes. There was no evidence for a differential effect of Pb on CYP2A6 genotype. However, differential induction of CYP2A6 by Cd was evident. The Cd-accountable variation of CYP2A6 activity in subjects having the**1A/*1A*, **1A/*1B*, **1B/*1B* and those with an allele of **1B* or **7* genotypes was 24%, 32%, 48% and 74%, respectively. CYP2A6 induction in subjects of the **1B/*1B* genotype was lower than expected because of an antagonistic effect of Pb. This was demonstrated by the inverse association between Pb and CYP2A6 activity. We concluded that the **1B* allele of the *CYP2A6* is more inducible by Cd than the **1A* allele.

Concurrent Cd and Pb exposures together with *CYP2A6* genotypes could explain differences among people in their ability to handle a variety of exogenous chemicals that are metabolized by CYP2A6. The assessment of health risk associated with exposure to environmental toxins activated by CYP2A6 requires that consideration be given to genotype in combination with environmental exposure to inducers, suppressors and inhibitors of metabolism such as Cd and Pb.

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P23-04

Relation of genetic polymorphism in *hMTH1c.83*, *hOGG1c.326* and *hMYHc.335* with risks of chronic benzene poisoning

Zhong-Bin Zhang [1], Jun-Xiang Wan [2], Pin Sun [2], Zhao-Lin Xia [2]

[1] *Institute of Occupational Hazards, China Academy of Safety Science & Technology, China;* [2] *Department of Occupational Health, School of Public Health, Fudan University, Shanghai 200032, China*

Objective: To explore the relation between genetic polymorphisms in *hMTH1*, *hOGG1* and *hMYH* and risks of chronic benzene poisoning (BP).

Methods: A case-control study was conducted. One hundred and fifty-two BP patients and 152 workers occupationally exposed to benzene without poisoning manifestations were investigated. Polymerase chain reaction-restrained fragment length polymorphism technique (PCR-RFLP) was applied to detect the single nucleotide polymorphisms (SNPs) on c.83 of *hMTH1* gene, c.326 of *hOGG1* gene and c.335 of *hMYH* gene.

Results: There were 2.51-fold (ORadj = 2.51; 95%CI: 1.14, 5.49; $P = 0.02$) and 2.49-fold (ORadj = 2.49; 95%CI: 1.52, 4.07; $P = 0.000$) increased risks of BP for individuals carrying genotypes of *hMTH1c.83Val/Met + Met/Met* or *hOGG1c.326Cys/Cys* compared with individuals carrying genotypes of *hMTH1c.83Val/Val* or *hOGG1c.326 Ser/Cys + Ser/Ser*, respectively. Compared with individuals carrying genotypes of *hOGG1c.326Cys/Cy* and *hMYHc.335His/His* at the same time, there was a 0.37-fold (ORadj = 0.37; 95%CI = 0.17, 0.81; $P = 0.01$) decreased risk of BP for these with genotypes of *hOGG1c.326Ser/Cys + Ser/Ser* and *hMYHc.335His/Gln + Gln/Gln* simultaneously. In the smoking group, there was a 0.15-fold (ORadj = 0.15; 95%CI: 0.03, 0.68; $P = 0.01$) decreased risk of BP for subjects carrying genotypes of *hMYHc.335His/Gln + Gln/Gln* compared with these carrying genotypes of *hMYHc.335His/His*.

Conclusion: Carrying genotypes of *hMTH1c.83Val/Met + Met/Met* or *hOGG1c.326Cys/Cys* may increase risk of BP, while the risk of BP may decrease when subjects carrying genotypes of *hOGG1c.326Ser/Cys + Ser/Ser* and *hMYHc.335His/Gln + Gln/Gln* at the same time.

Carrying *hMYHc.335His/His* genotype together with habit of smoking may also contribute to increased risk of BP.

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P23-05

GSTM1, GSTP1, GSTT1 and CYP1A1 genetic polymorphism and susceptibility to head and neck cancers among Polish population

Edyta Reszka [1], Piotr Czekaj [2], Jolanta Adamska, Piotr Urbaniec [4], Adam Ziólkowski [4], Urszula Mazurek [3], Wojciech Wasowicz [1]

[1] *Nofer Institute of Occupational Medicine, Lodz, Poland;* [2] *Medical University of Silesia, Katowice, Poland;* [3] *Medical University of Silesia, Sosnowiec, Poland;* [4] *Medical University of Silesia, Zabrze, Poland*

Genetically determined differences in metabolism of xenobiotics may reflect individual susceptibility to different neoplasm.

We present case-control study, which comprises of 127 diagnosed head and neck cancer patients and 151 controls individuals. Genomic DNA samples were assayed for PCR-RFLP to determinate GSTT1, GSTM1 intron 6/exon 7, GSTP1 exon 5 and CYP1A1 exon 7 genetic polymorphism.

Homozygotes for *GSTP1* Val105 allele and carriers of the *CYP1A1* Ile462Val genotype were at similar frequency in cancer and control group, while *GSTT1 null* genotype was more common in controls (30.5%) than in cases (21.3%). Homozygous deletion of *GSTM1* gene was presented significantly more frequent in patients (52.8%) compared to controls (41.1%). We also found significantly more head and neck cancer individuals, who possessed combined *GSTM1null/CYP1A1* Ile462Val genotype (8.7% versus 2.1%).

These results support association between GSTM1 and CYP1A1 genetic polymorphism and head and neck cancer susceptibility.

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P23-06

Association of smoking with cadmium induction of hepatic CYP2A6 in humans

Soisungwan Satarug [1,2], Michael R. Moore [1]

[1] *National Research Centre for Environmental Toxicology, The University of Queensland, Brisbane, Queensland, Australia;* [2] *Division of Clinical Pharmacology, International Graduate Program in Biomedical Sciences, Thammasat University, Rangsit Campus, Pathumthani, Thailand*

Cadmium and lead are metallic food contaminants of continuing worldwide concern because of their toxicity, exposure, bioaccumulation and wide dispersion in the environment. Cytochrome P450 2A6 (CYP2A6), known also as nicotine C-oxidase and coumarin 7-hydroxylase, is an inducible liver enzyme with a high degree of genetic variability. It oxidizes the tobacco alkaloid nicotine to cotinine and 3′-hydroxycotinine and metabolizes coumarin to its 7-hydroxy derivative. Here, we have quantified the effects of exposure to cadmium and lead on the variation in CYP2A6 activity and likelihood of adoption of smoking habits in relationship to *CYP2A6* genotype in a group of 290 normal Thai women and men with mean age of 32 years. Geometric mean for urinary Cd excretion of the group was 0.33 μg/day and the geometric mean for urinary Pb excretion was 0.98 μg/day. The prevalence of smokers was 82% in men having one allele of the **1B* or **7* in whom Cd induction of CYP2A6 was more pronounced than those having two **1B* alleles with a smoking prevalence of 44%. In the high-risk genotype, Cd body burden alone explained 74% of total variation in CYP2A6 activity. However, in the low-risk genotype, Cd and Pb exposures explained only 48% of the variation in CYP2A6 activity. The weaker CYP2A6 induction by Cd was due to an antagonistic effect of Pb evident from an inverse association between Pb and CYP2A6 activity. Thus, the induction of CYP2A6 by Cd increases the smoking risk by 1.9 fold in men with one allele of the **1B* or **7*, compared with those having the **1B*/**1B* genotype. We have thus demonstrated that environmental exposures to, and accumulation of Cd experienced by the subjects in the present study have effects on the expression of the human *CYP2A6* gene and on the likelihood of acquiring a smoking habit. These findings show the influence of environmental exposure to cadmium on the expression of the gene involved in nicotine clearance and links this environmental factor with smoking.

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P23-07

Mitomycin C induced sister chromatid exchange frequencies in hyperbaric oxygen exposed patients

Duydu Y.[1], Ustundag A.[1], Aydin A.[2], Eken A.[2], Dundar K.[3], Uzun G.[3]

[1] *Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandodan, Ankara 06100, Turkey;* [2] *Department of Pharmaceutical Toxicology, Gülhane Military Medical Academy, Ankara, Turkey;* [3] *Submarine Diseases and Centre of Hyperbaric Oxygen Treatment, Gülhane Military Medical Academy, Ankara, Turkey*

Hyperbaric oxygen (HBO) treatment is able to generate reactive oxygen species and induce DNA damage. The aim of this study was to evaluate the sister chromatid exchange (SCE) frequencies in lymphocytes from the patients undergoing HBO therapy (HBOT) and to determine the sensitivity of lymphocytes from these patients to SCE induction by 20 and 40 ng/ml mitomycin C (MMC). Patients were exposed to 10 consecutive daily HBO treatment according to a routine HBOT for diabetic feet. In this study, there were 12 patients at the beginning; however, it was not possible to sample all of the patients at all HBO therapy sessions; thus, towards the end of the therapy, the number of patients gradually decreased. A statistically significant induction in mean SCE/cell ($p < 0.05$, $n = 11$) was observed immediately after the first session of HBOT. The mean SCE frequency gradually decreased after 5th and 10th HBOT sessions relative to its frequency after the 1st treatment and reached baseline (pretherapy) levels 1 day after the last treatment in the sampled four patients. The mean MMC-induced SCE frequency was highest in lymphocytes sampled immediately after the first HBOT session and significantly higher than the MMC-induced SCE frequencies in cells sampled before HBOT. MMC-induced SCE frequencies remained high in lymphocytes at later stages of therapy, unlike the case with untreated cells and mean MMC-induced SCE frequencies were significantly higher ($p < 0.05$, $n = 4$) in lymphocytes sampled 1 day after the last session of HBOT than in lymphocytes sampled from these patients to the beginning of the therapy. In conclusion, HBOT induces SCE and one day after completing the therapy, lymphocytes retain increased sensitivity to the genotoxicity of MMC.

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P23-08

Genotoxicity of gliotoxin on epithelial (A549, Hep G-2, CHO), monocyte (THP-1) cell lines and human peripheral blood leukocytes using alkaline comet assay

Ivan Kosalec[1], Nevenka Kopjar[2], Snježana Ramić[2], Stjepan Pepeljnjak[1], Višnja Bačun-Družina[3], Roberto Antolović[4], Dubravko Jelić[4]

[1] *Faculty of Pharmacy and Biochemistry, Zagreb, Croatia;* [2] *Institute for Medical Research and Occupational Health, Zagreb, Croatia;* [3] *Faculty of Food Technology and Biotechnology, Zagreb, Croatia;* [4] *PLIVA Research Institute, Zagreb, Croatia*

Gliotoxin is a potent immunosuppressive and cytotoxic myctoxin from the epidithiodioxopiperazine group of secondary metabolites. Its production *in situ* by medically important molds *Asperegillus fumigatus* Fres. and *Asperegillus terreus* Thom contribute to invasive apergillosis of mammals. Our previous research found potent cytotoxic effects on epithelial, fibroblast-like, and monocyte cell lines (with IC50 from 2 to 11 μM). In this study, we analysed *in vitro* genotoxicity of gliotoxin (CAS 67-99-2) on epithelial (A549, Hep G-2, CHO), fibroblast-like (COS-7), and monocyte (THP-1) cell lines as well as on human leukocytes *ex situ* using alkaline comet assay (single cell gel electrophoresis). After 18 h-exposure of cells to the gliotoxin, comet tail length (TL, in micrometers), tail intensity (DNA%) and tail moment (TM) were recorded using fluorescence microscope and Comet Assay II software. At 2 μM, gliotoxin expresses genotoxic effects with the most differences noted in THP-1 cell line ($p < 0.001$), followed by epithelial A549, Hep G-2, CHO cell lines and leukocytes ($p < 0.05$). Gliotoxin exhibited dose-depended genotoxic effect on human leukocytes *ex situ* from concentrations 0.5–2 μM. Sub-IC50 concentration of gliotoxin (0.5 and 1 μM) showed TL (21.04 and 23.48 μm, respectively); tail intensity (3.79 and 4.84%, respectively) and TM (0.61 and 0.91, respectively) with statistically higher values than in control (TL 14.14; tail intensity 0.64%; TM 0.08). At higher concentrations of gliotoxin, from 2 to 4 μM, apoptotic effect has been noted. Our results indicate that gliotoxin was genotoxic in concentration bellow cytotoxic IC50 concentrations in epithelial cell lines, and this effect is dose-depended in human peripheral blood leukocytes.

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P23-09

Studies of the some genotoxic effects of di-*n*-buthyl phthalate (DBP) on germ and somatic cells of the laboratory male mice

E.J. Tyrkiel [1], M.M. Dobrzyńska [2], A. Hernik [2], K. Góralczyk [1], J.K. Ludwicki [1]

[1] *Department of Environmental Toxicology, National Institute of Hygiene, Poland;* [2] *Department of Radiation Protection and Radiobiology, National Institute of Hygiene, Poland*

The long-chain dialkyl phthalates (di-esters of *o*-phthalic acid), which are used mainly as plasticizers for polyvinylchloride (PCV), are a very important group of industrial chemicals. Many of them have toxic potential in gonads by induction of changes in germ cells, and also impacting fertility. Phthalate esters (PAEs) belong to a structurally diverse group of compounds that induce peroxisome proliferation in the liver in mice and rats.

In these studies, di-*n*-buthyl phthalate (DBP) have been used. Experiments were carried out on Pzh: sfiss 42–45-days-old out bread male mice which received 500 mg/kg b.w. or 2000 mg/kg b.w. of DBP for 8 weeks three times per week. After 4 weeks of the experiments, after the end exposure (8 weeks) and after next 4 weeks, the animals were killed. The liver, both testes and epididymis from each male were weighed.

The mice sperm qualities (morphology, motility and concentration) were used to determine the effects of DBP in germ cell, and DNA damages (comet assay) were used to determine the effects investigated compound in liver cells and testes.

The DBP concentration in the whole mice blood samples was detected by a high-performance liquid chromatography (HPLC). Chromatographic separation was achieved with a LiChrospher 60 RP-select B column and ultraviolet absorbance was monitored at 255 nm. The samples of the blood were collected after 4, 8 and 12 weeks.

The results obtained in the present experiments indicated that 8 weeks exposure to higher doses of DBP increased testes weight and the number of abnormal spermatozoa as well as diminished sperm motility and concentration (sperm count). DBP slightly increased the level of single strand breaks in liver cells and haploid germ cells in male mice. The mean concentrations ranged from 0.069 to 2.626 μg/ml whole blood and increased with time exposure. The DBP concentration is regarded as a direct consequence of longer exposure to this compound.

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P23-10

Two-generation reproduction toxicity studies of Di(*n*-butyl)phthalate in mice

M.M. Dobrzyńska [1], E.J. Tyrkiel [2], U. Czajka [1], K.A. Pachocki [1]

[1] *Department of Radiation Protection and Radiobiology, National Institute of Hygiene, Warsaw, Poland;* [2] *Department of Environmental Toxicology, National Institute of Hygiene, Warsaw, Poland*

Di(*n*-butyl)phthalate (DBP) is commonly used as industrial solvent and plasticizer. It has been recognised as toxic to the testes and teratogenic in animals.

Pzh:SFIS male mice were exposed by gavage to 1/16 LD_{50} or to 1/4 LD_{50} DBP daily for 8 weeks, 3 days/week. After the end of 8-weeks exposure, males were caged to two untreated females each. Majority of females was killed 18 days after finding of a vaginal plug. They were examined for the number of live and dead implantations and the number and type of gross malformations. Some of the females were allowed to deliver and rear their litter. F1 animals were weighted weekly up to the age of 8 weeks and observed for physiological markers and growth parameters. At age of 8 weeks, 5 males F1 per dose were killed to examination of testes and epididymides weight, sperm count, motility, morphology and comet assay in germ cells.

After 8-weeks exposure to DBP, the effects on the male fertility and pregnant female frequency were not observed. Exposure DBP in higher dose slightly reduced the number of total and live implantation per pregnant female and induced 10% of DLM. There were no effects on the frequency of dead implants and body weight of surviving foetuses as well as on the congenital defects and skeletal malformations. The percent of postnatal mortality was not increased. In both experimental groups, about 20% of pups aged 3, 4 and 5 weeks were growth-retarded. DBP in dose of 1/16 LD_{50} induced 2, 5-days delay in appearance of vaginal opening in the offspring of exposed males. Relative testes and epididymides weights were not changed in the offspring of males from both experimental groups. Paternal exposure to dose of 1/4 LD_{50} decreased of about 30–40% sperm count in F1 males and increased percent of malformed spermatozoa. Sperm motility and level of DNA damages in haploid germ cells were not changed.

Results confirmed mutagenic activity of DBP and possibility of transfer the effects to the offspring via the sperm.

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P23-11

Awareness of potential solutions available for regulatory genotoxicology problems

Tom Holmes

Covance Laboratories, Harrogate, UK

Ambiguous genotoxicity data, or genotoxic and carcinogenic impurities can be problematic when submitting chemicals for scrutiny by regulators. Experience has shown that one of a range of solutions might be potentially applied to such problems. One example was a new chemical for the plant protection product (PPP) market which contained two carcinogenic and genotoxic impurities. It was considered not to pose a carcinogenic hazard to consumers or operators after calculating the 'minimum risk level' doses or 'Threshold of Toxicological Concern' (involved calculating the T_{25} Bench Mark Dose) for the impurities and comparing these against predicted exposure levels for consumers and operators. In another example, two other PPP active ingredients and a veterinary active substance had ambiguous or old genotoxicity and/or carcinogenicity data packages. These cases were referred to the government's advisory committees on mutagenicity and carcinogenicity. In each case, the committees, each consisting of respected authorities in the fields of mutagenicity and carcinogenicity, were able to review all relevant data and make recommendations for further data or information that were able to clarify that these chemicals did not pose any genotoxic or hence carcinogenic risk to man. The only drawback to consideration by committee is the length of time it can take. In the future, other strategies may be available for fully assessing the genotoxic and carcinogenic potential of chemicals. The most promising strategies are target organ mutagenicity and toxicogenomics. Individual target organ mutagenicity might be used in the future as a way of ruling out practical genotoxic hazards to man. Toxicogenomics might be used to assess risk to man after comparing gene activation in animals and humans after exposure to a chemical. Other strategies, for example, for identifying apparent non-genotoxins but which are carcinogenic through a mechanism that involves indirect DNA damage may also become available.

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P23-12

The anabolic steroids trenbolone and tetrahydrogestrinone induce micronuclei in V79 cells

Susanne B. Dorn, Hermann M. Bolt, Gisela H. Degen

Institute for Occupational Physiology, University of Dortmund, Germany

Anabolic steroids are used as growth promoter in animal husbandry, but are also widely misused by athletes for doping. Besides their hormonal (androgenic) potency, the possibility of genotoxic properties raises concern. The synthetic androgen trenbolone and the structurally related tetrahydrogestrinone were studied for chromosomal genotoxicity. Trenbolone (TB) has been evaluated previously in a series of *in vitro* and *in vivo* assays. Although it apparently binds to DNA and proteins to some extent, equivocal results have been obtained for cell transformation, and in assays reflecting genotoxicity at the chromosomal level. The genotoxicity of the newly synthesized designer drug tetrahydrogestrinone (THG) has not been investigated at all.

We determined the potency for micronucleus induction in V79 cells of TB and THG in comparison to the natural androgen testosterone. The cytotoxicity of the compounds was also assessed by means of the Alamar-Blue assay. All three compounds were cytotoxic at concentrations above 30 μM. Testosterone did not cause an induction of micronuclei up to 300 μM. In contrast, TB and THG induced micronuclei in V79 cells at subcytotoxic concentrations, THG being clearly more potent: it caused almost a doubling of the micronucleus rate at 3 μM; for TB, a two-fold increase of the MN rate was detected at 23 μM. The micronuclei induced by TB and THG were predominantly kinetochor (CREST)-positive, characterising the chromosomal genotoxicity as aneugenic. Since both TB and THG contain activated conjugated double bonds, they are expected to bind to proteins, *e.g.* tubulin, and may thereby disturb functions of the spindle apparatus.

Against this background, a specific mechanism of MN induction by TB and THG is proposed, since a plot of effective concentration against lipophilicity ($-\log C$ *versus* log Pow) reveals that both compounds are more potent than expected for non-specific hydrophobic interactions.

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P23-13

Genotoxic effect of quinolone drugs at different expression periods by *in vitro* chromosomal aberration test on human lymphocytes

S.S. Murugan [1], Juerg P. Seiler [2], Anupama Mohan [1], V. Thanikaivel [1], Y. John Mathew [1], P. Balakrishna Murthy [1]

[1] *International Institute of Biotechnology and Toxicology, Padppai-601301, Kancheepuram District, Tamil Nadu, India;* [2] *ToxiConSeil, Hoelzlistrasse 38, CH-3475 Riedtwil, Switzerland*

There is not much published information on the potential influence of fluoroquinolone antibiotics on chromosomal aberration frequency, although it has been reported that gatifloxacin (Tequin®) induces chromosomal aberrations in human lymphocytes. These drugs act by inhibiting bacterial gyrase, an enzyme related to the mammalian topoisomerase II. In recent years, new generation quinolone drugs with higher activities have been introduced for better management of bacterial diseases.

We studied the ability of a third generation quinolone–gatifloxacin (Generation IIIa) to induce numerical and structural cytogenetic anomalies in cultured human lymphocytes. In addition, in order to assess the genotoxic potential of quinolones with respect to different generations, the second generation quinolones–ciprofloxacin (Generation IIa,), ofloxacin (Generation IIa) and sparfloxacin (Generation IIb) were also tested similarly. In these experiments, also the influence of the cell cycle prolongation due to topoisomerase inhibition on the expression of clastogenic events was investigated.

Lymphocytes in peripheral blood obtained from healthy adult male non-smoking donor without any recent history of illness, were used for the assay. Based on pre-tests for cytotoxicity, cultures were set up with 4 h exposure to the test compounds with and without S9. Type I cultures were washed free of drug and incubated for an additional period of 20 h. On the other hand, Type II cultures were incubated for 32 h after the end of the exposure.

Gatifloxacin induced increased frequencies in both numerical and structural aberrations, when expression period was prolonged to 32 h. This finding, together with the increased frequency of endoreduplication and polyploidy, can be considered to be caused by the mode of action as an inhibitor of topoisomerase II. The results of the other quinolones tested will be discussed in detail.

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P23-14

Ochratoxin A induced oxidative DNA damage and micronuclei as sensitive indicators of its genotoxic effects *in vitro*

Gisela H. Degen [1], Yury Lektarau [1], Ülkü Ündeger [2], Claudia Behm [1], Wolfram Foellmann [1]

[1] *Institute for Occupational Physiology at the University of Dortmund, Dortmund, Germany;* [2] *Hacettepe University, Faculty of Pharmacy, Ankara, Turkey*

Ochratoxin A (OTA), a well-known nephrotoxin and rodent carcinogen, is frequently found as food contaminant. This and the known genotoxic properties necessitate a careful risk assessment for this mycotoxin based also on information on its mode of action and dose–effect relationships. With respect to regulation of carcinogens in general and scientifically based limit values for OTA, it is important to assess dose–response characteristics and distinguish between direct and indirect modes of genotoxicity.

As part on an ongoing effort to better characterize the OTA-induced DNA damage and dose–effect relationships in cells, we have studied DNA damage both by means of the Comet Assay and by induction of micronuclei (MN) in urothelial cells and in V79 cells. For the MN assay, the cells were treated in serum-free medium for about 1.5 cell cycles with graded concentrations of OTA and with DMSO as solvent control. The Comet Assay was performed with and without addition of FPG enzyme (fpg-glycosylase known to convert oxidative DNA damage into strand breaks) in cells treated between 1 and 6 h with OTA or solvent only. Cytotoxicity of OTA was assessed in parallel cultures by neutral red uptake assay.

OTA concentrations between 0.03 and 1 μM caused a dose-dependent increase of MN in V79 cells (up to fourfold compared to the control) whilst the lowest tested concentration of 0.01 μM OTA did not induce a higher frequency of MN. Clear evidence for oxidative DNA damage was observed in the FPG-modified Comet Assay in V79 and urothelial cells treated with OTA concentrations of 0.03 μM and more, with no indication of this genotoxic effects at lower OTA levels. At higher OTA levels, a plateau was reached indicative of an indirect mode of action.

In conclusion, OTA can induce micronuclei at concentrations below those which are overtly cytotoxic. The shape of the dose–response curve at very low concentrations supports the existence of a threshold for this genotoxic effect. This observation is in accord with a study on the induction of oxidative DNA damage by

OTA which appears to be due to an indirect genotoxic mode of action.

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P23-15

The effects of thyme volatiles on the induction of DNA damage by the heterocyclic amine IQ and mitomycin C

Sevtap Aydın [1], A. Ahmet Başaran [2], Nurşen Başaran [1]

[1] *Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100 Ankara, Turkey;* [2] *Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara, Turkey*

The leafy parts of thyme and its essential oil have been commonly used in foods mainly for the flavour, aroma, and preservation and also in folk medicine for many years. In the present study the genotoxic potential of major compounds of thyme oil, *i.e.* thymol, carvacrol, and γ-terpinene and of the methanolic extracts of thyme were investigated in human lymphocytes by single-cell gel electrophoresis (COMET assay) and also the effects of these substances on the induction of DNA damage by 2-amino-3-methylimidazo [4,5-*f*]-quinoline (IQ) and mitomycin C (MMC) was evaluated. No increase in DNA strand breakage was observed at thymol and γ-terpinene concentrations below 0.1 mM, but at the higher concentration of 0.2 mM significant increases in DNA damage were seen. Thymol and γ-terpinene significantly reduced the DNA strand breakage induced by IQ and MMC at the lower concentrations studied. Carvacrol, which is an isomer of thymol, seemed to protect lymphocytes from the genotoxic effects of MMC at the non-toxic concentrations below 0.05 mM, but at the higher concentration of 0.1 mM carvacrol itself induced DNA damage. Also the constituents of the *n*-hexane and ethyl acetate fractions prepared from the concentrated aqueous methanolic extracts of *Thymus spicata* protected lymphocytes against IQ- and MMC-induced DNA damage in a concentration-dependent manner. Our results indicate that thyme and its compounds protect lymphocytes against the genotoxic effects of IQ and MMC in vitro and highlight the potential benefit as dietary supplements of thyme and its components. Although the role of diet in the etiology of cancer is complex, so additional animal and human studies should be performed to clarify the anti-mutagenic potential of thyme and its components.

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P23-16

***In vivo* genotoxicity of the synthetic pyrethroid pesticide "cypermethrin" in rat liver cells by Comet assay**

El-Hussein Naguib El-Khatib [1], Mona Abd El-Aziz [2], Yahia Badr [2], Nashwah Kamal [1]

[1] *Mammalian Toxicology Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt;* [2] *National Institute of Laser Sciences, Cairo University, Giza, Egypt*

The Comet assay (single-cell gel electrophoresis, "SCGE") is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. The assay has applications in testing different chemical and physical agents for genotoxicity and monitoring environmental contamination with genotoxins. The objective of the present study was to evaluate the genotoxic effects of the synthetic pyrethroid pesticide "cypermethrin", which is widely used in Egypt in pest-control programs in agriculture and in public health as well. Male rats were sacrificed 1, 7 or 14 days after administration of single oral dose 1/30, 1/10 or 1/5 LD_{50} of commercial formulation of cypermethrin. Single liver cell suspensions were prepared and a Comet assay was performed. With the SCGE assay, a clear induction of DNA was observed. It is generally noticed that all pesticide treatments yielded statistically significant ($p < 0.0001$) DNA damage. In conclusion, cypermethrin induced a clear significant positive dose-dependent increase in DNA damage in the rat liver cells exposed to cypermethrin as compared with controls. But the effects in the SCGE were generally decreased with time after treatments. The results of the present work suggested that Comet assay might be a suitable and sensitive endpoint in genotoxicity evaluation of pesticides, but we confirm that various tests should be used for detecting the mutagenic activity of pesticides.

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P23-17

The alkaline comet assay *in vitro* with primary rat alveolar macrophages: A suitable tool for genotoxicity testing of quartz-containing ceramic dusts

C. Ziemann [1], P. Jackson [2], R. Brown [3], O. Creutzenberg [1]

[1] *Fraunhofer ITEM, Hannover, Germany;* [2] *CERAM Research Ltd., Stoke-on-Trent, UK;* [3] *TOXSERVICES, Stretton, UK*

Inhalation of respirable crystalline silica (RCS) may lead to lung diseases, *e.g.* chronic inflammation, silicosis and cancer. In 1997, RCS has been classified as human carcinogen by the IARC, but the multifactorial aspects of RCS toxicity were acknowledged and industry and science were encouraged to characterise the properties of RCS-containing dusts from various industrial sectors by physicochemical methods and to discriminate their toxic potential by appropriate *in vitro*/*in vivo* tests. The SILICERAM project has thus been initiated as a European Collective Research Project to provide those, responsible for setting legislation, with data that will ensure definition of appropriate RCS air limits for the ceramic industry. Within the SILICERAM project, the suitability of the *in vitro* alkaline comet assay (CA) for screening of the genotoxic potential of ceramic dusts was investigated. Primary rat alveolar macrophages, pre-cultured for 24 h, were used as a model system. Mid-size DQ12 quartz was chosen as a positive control, demonstrating an almost linear, concentration-dependent (25–200 $\mu g/cm^2$) induction of DNA strand breaks (SB) after 2 h of incubation. SB formation was inhibited by co-treatment with 10 μM aluminium lactate, indicating a quartz-specific, surface-dependent clastogenic effect. In addition, DQ12 induced PGE_2 liberation and LDH release. TiO_2 Bayertitan T (rutile) was investigated as a potential negative control, but was shown to be inappropriate. Unexpectedly, TiO_2 induced a significant increase in SB formation, not counteracted by aluminium lactate. Unlike TiO_2, Al_2O_3 could subsequently be identified as an appropriate negative control for the CA. Contrived samples (representative of mineral mixtures employed in ceramic production) composed of different amounts of DQ12, China clay, and feldspar were investigated and could be ranked by their RCS content. Using the optimised CA, different ceramic dust samples collected in the tile, table- and sanitary ware, refractory and brick sector, will be screened and ranked concerning their genotoxic potential. Aluminium lactate will be used to differentiate quartz-dependent and quartz-independent effects.

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P23-18

Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese Hamster Ovary cells

Eric K. Dufour [1], Tirukalikundram Kumaravel [2], Gerhard J. Nohynek [2], David Kirkland [2], Hervé Toutain [1]

[1] *L'Oréal Research and Development Asnières-sur-Seine Cedex France;* [2] *COVANCE Laboratories Ltd, UK*

Zinc oxide (ZnO), a widely used ingredient in dermatological preparations and sunscreens, is clastogenic *in vitro*, but not *in vivo*. Given that ZnO has slightly greater clastogenic potency in the presence of UV light when compared to that in the dark, it has been suggested to be photo-clastogenic. In order to clarify whether this increased potency is a genuine photo-genotoxic effect, we investigated the clastogenicity of ZnO (mean particle size: 100 nm) in Chinese Hamster Ovary (CHO) cells in the dark (D), under pre-irradiation (PI, *i.e.* UV irradiation of cells followed by treatment with ZnO) and under simultaneous irradiation conditions (SI, standard conditions of photo-genotoxicity testing, *i.e.* ZnO treatment concurrent with UV irradiation).

The cytotoxicity of ZnO to CHO cells under the different irradiation conditions was as follows: SI > PI > D. In the dark, ZnO produced a concentration-related increase in chromosome aberrations (CA). Under both PI and SI conditions, ZnO was clastogenic at significantly lower concentrations (increased clastogenic potency, approximately two- to four-fold) when compared to effective concentrations in the dark. As ZnO was not irradiated under PI conditions, these results demonstrate an increased susceptibility of CHO cells to ZnO-mediated clastogenic effects due to UV irradiation alone. The incidence of CA under SI or PI conditions was generally higher than that in the dark (increased clastogenic activity). At similar ZnO concentrations, SI generally produced a higher incidence of CA than PI. However, comparison of the incidence of CA at ZnO concentrations of similar cytotoxicity showed nearly identical effects under PI or SI conditions. The modest increase in the clastogenic potency of ZnO following UV irradiation contrasts with the results observed with genuine photo-clastogenic agents, such as 8-MOP, which may produce an increase in clastogenic potency of >15,000-fold under SI, but not under PI conditions.

Our results provide evidence that, under conditions of *in vitro* photo-clastogenicity tests, UV irradiation of CHO cells may produce a slight increase in the genotoxic potency/activity of compounds that are clastogenic in the dark. Our data suggest that minor increases in clastogenic potency/activity under standard conditions of photo-genotoxicity testing do not necessarily represent a photo-genotoxic effect, but may occur due to an increased sensitivity of the test system subsequent to UV irradiation. Accordingly, the definition of *in vitro* photogenotoxicity for substances that are clastogenic in the dark requires urgent attention, particularly when taking into account the high rate of false positive results in *in vitro* clastogenicity tests, as well as the absence of validated *in vivo* tests that could distinguish genuine from pseudo-photo-clastogens.

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P23-19

Influence of some quinazoline derivatives on structural and functional characteristics of endoplasmic reticulum membranes and fractionated nuclear chromatin of a liver of experimental animals under *in vitro* and *in vivo* poisoning with tetrachloromethane

Peter Zhminko, Yuriy Gubskiy, Alexander Marchenko, Evgeniy Levitsky, Nadiya Litvinova, Anna Goriushko, Valentina Danilenko, Lyudmila Bobkova, Igor Belenichev, Tetyana Kurapova, Alexander Velichko, Larisa Babenko, Natalya Kurskaya

Medved's Institute of Ecohygiene and Toxicology, Kiev, Ukraine

In the conducted studies (*in vitro* and *in vivo*), the data have been received which indicate utility of research of quinazoline derivatives as potential pharmacological products with pronounced antioxidant, hepatoprotective, membranostabilizing, genomoprotective and cytoprotective properties.

Correlation between quantum–mechanical characteristics of the given compounds and their antioxidant and antiradical activity has been established. These data have enabled carrying out purposeful synthesis and search of cytoprotectors among the studied compounds.

It has been established on experimental models of white rats (Wistar strain) liver chemical lesion with tetrachloromethane (1 LD_{50}, intraperitoneally), that injection of some quinazoline derivatives (1/10 LD_{50}, per os) to the animals results in increase of their survival rate and normalization of the basic physiological parameters.

It has been established by means of biochemical and physical–chemical methods that some representatives of these compounds have the pronounced antioxidant and antiradical activity that exceeds activity of classic antioxidant butylated hydroxytoluene in a number of cases. An injection of some quinazoline derivatives to the animals, poisoned with tetrachloromethane, to a great extent promotes correction of damage of endoplasmic reticulum membranes and fractionated nuclear chromatin of hepatocytes, normalization of their physical–chemical and structural–dynamic characteristics, level of lipids peroxidation in them, and also promotes normalization of liver structural and functional characteristics.

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P23-20

Ochratoxin A causes DNA damage in rats kidney

Ana-Marija Domijan, Davor Želježić, Vilim Žlender, Radovan Fuchs, Maja Peraica

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Ochratoxin A (OTA) is product of moulds frequently found in food and feed in mild climatic zones. This mycotoxin was found to be nephrotoxic for experimental animals and the carcinogenic potential of OTA in kidney of experimental animals is well established. The results of OTA genotoxic potential on cell cultures are inconsistent and the studies on genotoxicity in experimental animals are scarce. The aim of this study was to assess the time-course of OTA-produced DNA damage by checking alkali-labile sites using the comet assay of rat kidney tissue. Rats were treated intraperitoneally with OTA (0.5 mg/kg b.w./day for 7, 14, and 21 days, respectively) and sacrificed 24 h after the last treatment. Positive controls were given methyl methanesulfonate and negative controls solvent only following the same schedule of treatment. The genotoxic potential of OTA was studied in the kidney homogenate of OTA treated and control rats using alkaline single-cell gel electrophoresis (comet assay). Concentrations of OTA in plasma and kidney homogenate were determined using high performance liquid chromatography. OTA concentrations in plasma and kidney tissue increased steadily during the treatment period. In all OTA-treated groups, the tail length, tail intensity, and tail moment in the kidney tissue were significantly higher than in controls ($P < 0.05$). The tail length and tail moment were higher after 14 days than after 7 days of treatment ($P < 0.05$), and still higher after 21 days than after 7 and 14 days ($P < 0.05$). The highest tail intensity was observed in animals treated for 21 days, and it differed significantly from animals treated for 7 and 14 days ($P < 0.05$). OTA concentration measured in

the kidney tissue strongly correlated with the tail intensity and tail moment values. These results confirm the genotoxic potential of OTA, which is in accordance with its carcinogenicity.

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P23-21

Derek for windows assessment of chromosomal aberration effects

Richard V. Williams [1], Russell T. Naven [1], Carol A. Marchant [1], Akihiko Hirose [2], Eiichi Kamata [2], Makoto Hayashi [2]

[1] *Lhasa Limited Leeds UK;* [2] *National Institute of Health Sciences*

Derek for Windows (DfW) is a knowledge-based expert system designed to assess the potential toxicity of a chemical from its structure. The knowledge base is composed of alerts, example compounds and rules which each contribute to the predictions made by the system. The alerts define chemical environments associated with toxicity and are supported by a summary of the evidence used to derive them and corresponding literature citations.

The DfW knowledge base contains over 80 alerts for genotoxicity, the majority of which relate to Ames test mutagenicity. Recent efforts have therefore focussed on the development of new alerts for the prediction of chromosomal aberrations in mammalian cells. Initial work led to the creation of approximately 100 prototype alerts derived from expert suggestions and the analysis of several collections of *in vitro* chromosomal aberration test data. In many cases, these new alerts describe chemical environments which do not lead to a corresponding mutagenic response in the Ames test. Currently, these alerts are being further developed using mechanistic information and toxicity data from the wider public domain. Examples of these new refined alerts will be presented in the context of the underlying mechanistic basis for the induction of chromosomal aberrations. An understanding of mechanism can play an important role, for example, in the interpretation of the toxicological significance of chromosomal effects observed *in vitro*.

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P23-22

A fragmental QSAR model for the prediction of AMES genotoxicity

Pranas Japertas

Pharma Algorithms, Toronto, Ontario, Canada

This study presents a computational analysis and the development of a predictive model of genotoxicity (based on the *in vitro* Ames reverse mutation test data). The dataset of approximately 8000 compounds was collected from several public databases, reviews and many original publications. The data was 'cleaned' by removing non-covalent complexes and salts of heavy metals, and compounds with incorrect structures. At the next step, partial least squares regression with fragmental descriptors (atom chains of various lengths and scaffolds) was performed. In addition to generating an estimate of the probability of a compound's genotoxicity, toxicophores can be identified in their specific chemical structural environment, providing an insight as to which parts of the molecule are responsible for the genotoxic effect. The predictive genotoxicity model can be used by researchers to supplement various pre-defined genotoxicity filters that ignore the chameleonic dependence of genotoxicity on substituent effects. The model was validated on a set of marketed drugs and on a randomly selected compound validation set ($N = 945$). The accuracy of compound classification into the 'genotoxic' or 'non-genotoxic' categories is close to 95%. Although *in vitro* bacterial reversal tests are relatively inexpensive to perform, it is much easier to test the outcome of multiple structural modifications *in silico* than to synthesize and test all compounds *in vitro*. The simplicity of *in vitro* tests means that some laboratories have accumulated test results for large numbers of compounds. These results can be converted into new predictive models which may be preferable to commercial predictors because they would have fewer data gaps in the relevant chemical space.

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P24 Toxicogenomics and Proteomics

P24-01
Toxicogenomics and biomarker discovery for the prediction of long term toxicity

Hans Gmuender, Maria Wendt, Jochen Koenig

Genedata AG, CH-4016 Basel, Switzerland

Toxicogenomics is a novel approach for predicting a compound's toxicity using gene, protein and metabolite expression information. Changes to the molecular expression profiles following exposure to a drug together with conventional clinical endpoints are used to build reference libraries of known compounds against which novel compounds can be tested. Biomarker candidates are then deduced from such libraries and characterized further to determine the causal relationship between the biomarker and toxicity in animal models and humans, respectively.

The basic steps in generating a toxicogenomics knowledge base for the evaluation of novel compounds include the collection of data from conventional toxicology, establishing quality-checked and normalized expression data, construction of a reference database with a number of well-known and well-characterized compounds, and the selection of toxicological marker genes based on statistical methods. After validating and refining the reference compendium it is used to classify novel, uncharacterized compounds. Inclusion of related functional genomics data enables the characterization of gene function and regulatory mechanisms to determine the compounds' mechanisms-of-action at various toxicological endpoints.

Genedata in collaboration with 13 pharma and 3 academic partners will apply this approach to a series of proprietary compounds that have been dropped from development after they failed conventional toxicology tests. The efforts by the PredTox consortium are actively supported by the European Commission as part of the 'Innovative Medicines for Europe' (InnoMed) programme. During the project, high throughput molecular techniques will be combined with conventional toxicology methods to build a unique toxicity profile of each compound. These profiles will be used to predict the likelihood that a drug candidate will fail during toxicological testing phases. Advanced statistical methods, including machine learning algorithms, will be applied and analysis results will be maintained and accessible to the consortium members.

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P24-02
Characteristic expression profiles induced by carcinogens in rat liver

H. Ellinger-Ziegelbauer[1], H.-J. Ahr[1], H. Gmuender[2], C. Pallez[2], J. Koenig[2]

[1] *Genedata AG, CH-4016 Basel, Switzerland;* [2] *Bayer Healthcare AG, D-42096 Wuppertal, Germany*

Application of gene expression techniques using microarrays in toxicological studies (toxicogenomics) facilitates the interpretation of a toxic compound's mode of action and may also allow the prediction of selected toxic effects based on gene expression changes. It would be of great value if the outcome of a long-term bioassay could be predicted based on gene expression changes in a short-term study. We therefore investigated whether carcinogens at doses known to induce liver tumors in the 2-year rat bioassay deregulate characteristic sets of genes in a short-term *in vivo* study in rats for up to 14 days. By global expression analysis, the characteristic early events following treatment with carcinogens were mechanistically characterized. This investigation revealed common gene expression responses in defined functional gene categories which may differentiate genotoxic from non-genotoxic carcinogens. Transcription of a small set of genes behaves similarly (co-expression), suggesting a common molecular mechanism for gene regulation (co-regulation). A subset of the co-expressed genes are known p53 targets. To generate new hypotheses different in silico-approaches were used to characterize the promoters of those genes: genome–genome comparisons ('phylogenetic footprinting'), a powerful method to deduce regulatory regions in orthologous regions from different species, and the use of libraries of experimentally derived transcription factor binding sites (TFBS) for predicting putative TFBSs based on primary genome sequence. One major part of phylogenetic footprinting is the screen for biologically relevant TFBS based on position weight matrices (PWMs) in evolutionarily conserved genomic regions. Comparing the human and rat BAX genes, we analyzed conserved upstream regions and could identify one putative well conserved p53 binding site upstream of the TSS (site I). Two additional p53 binding sites were identified in less conserved regions. The in-silico analysis suggests that besides p53 at least 16 other mammalian TFBS located in the vicinity of the p53 site might be involved in the regulation of BAX. These factors might cooperate with p53 in the

transcriptional activation caused by genotoxic hepatocarcinogens.

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P24-03

Molecular changes preceding inflammatory and vascular lesions induced by a phosphodiesterase 4 inhibitor in rats

Nicolas Daguès [1], Valérie Pawlowski [1], David Ledieu [1], Cécile Sobry [1], Gilles Hanton [1], Jean-Louis Freslon [2], Stephan Chevalier [1]

[1] *PGRD Safety Sciences Europe, Amboise, France;* [2] *UMR CNRS 6542, Faculté des Sciences et Techniques, Tours, France*

Vascular injury is a relatively common finding during the pre-clinical toxicity testing of drug candidates. The rat appears to be a sensitive species for developing vascular lesions, especially in the mesenteric tissue. Previous time-course study with a selective PDE4 inhibitor showed that the morphological changes associated with the inflammation in rat mesenteric vasculature occurred within 24 h after oral gavage. The time-course study presented here was performed to investigate the molecular changes preceding the onset of the lesion detected by histopathology.

Sprague Dawley rats (7 weeks) received a single oral dose (80 mg/kg) of a selective PDE4 inhibitor. They were euthanized 2, 4, 8, 16 or 24 h after treatment and tissues were collected for histopathological observations. Gene expression analysis and protein assays were performed using mesenteric tissue and blood samples, respectively. The Affymetrix GeneChip technology, which allows one to monitor the expression of around 8800 rat genes on an array (Rat Genome U34A), was used to select the genes whose expression was modified by the PDE4 inhibitor. Some of these gene expression changes were confirmed by real-time RT-PCR.

Histopathological examination showed treatment-related lesions at the 16 and 24 h time-points characterised by perivascular inflammation and/or vascular fibrinoid necrosis. Transcriptomic analysis showed that mRNA changes could be detected as early as 2 h post dose, *i.e.* much earlier than the detection of the lesions by histopathology. The PDE4 inhibitor altered the expression of genes involved in *inflammatory response* (Interleukin 6, Fibrinogen, Complement Component 3), *oxidative stress* (Heme Oxygenase 1), *blood coagulation* (Fibrinogen, Plasminogen Activator Inhibitor 1), *extracellular matrix remodeling* (Tissue Inhibitor of Metalloproteinase 1) and *energy metabolism*. Fibrinogen and Interleukin 6 were found to be early and sensitive protein markers of the inflammatory process associated with PDE4-inhibitor treatment.

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P24-04

Transcriptomic and proteomic analysis of adult rat testes following an acute exposure to the antiandrogen flutamide

Claire Friry-Santini, Céline Katz, Marie-Pierre Come, Philippe Kennel, Michel Samson, Mohamed Benahmed, David Rouquié, Remi Bars

Bayer Crop Science, INSERM U407, Sophia Antipolis, France

Flutamide (FM), a non-steroidal antiandrogenic model compound, is able to bind the androgen receptor (AR) and disturb the actions of endogenous androgens at the AR level in target organs, leading to testicular toxicity. Although the toxic effects of FM are well characterized using conventional toxicology, very little is known concerning changes in gene expression and protein accumulation that occur in the testes following exposure to this antiandrogen. The aim of the present study was to gain insight into the early molecular changes in testis following single acute FM exposure. Male Sprague–Dawley rats were gavaged with 150 mg/kg FM or vehicle (methylcellulose) and were sacrificed 1, 2, 8, 24 or 48 h after a single dose. At necropsy, blood samples were collected for hormone measurements (luteinizing hormone and testosterone) and testicular samples were taken for histopathology, transcriptomic, proteomic and biokinetic analyses. Gene expression profiles were monitored using Agilent full genome (44K) rat microarrays and protein accumulation profiles were performed by two-dimensional gel electrophoresis (2-DE)/MALDI-ToF analysis. Only testosterone levels were affected and no histopathological changes were observed in the testes. "Omics" analyses revealed a significant number of genes and proteins modulated by FM treatment. A good correlation between conventional toxicology data (hormone measurements) and metabolic pathways altered was obtained. Further investigations are ongoing to better characterize biological pathways affected and to understand the overall mechanism of action of the antiandrogen FM on the testis.

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P24-05
Early characterization of hepatotoxicants by focused illumina microarrays

N. Zidek, P.-J. Kramer, P.G. Hewitt

Merck KGaA, Institute of Toxicology, Darmstadt, Germany

Drug-induced hepatotoxicity is a major issue in drug development, and toxicogenomics is a potential tool for the prediction of toxicity as well as early toxicity screening. We have established a hepatotoxicity specific, bead-based oligonucleotide microarray containing 550 genes. The major aim is to establish a predictive screening system for acute hepatotoxicity by analyzing gene expression profiles of well-known hepatotoxic and non-hepatotoxic model compounds. Low and high doses of tetracycline, carbon tetrachloride (CT), 1-naphthylisothiocyanate (ANIT), erythromycin estolate, acetaminophen (AAP) or chloroform as hepatotoxicants, clofibrate, theophylline, naloxone, estradiol, quinidine or dexamethasone as non-hepatotoxic compounds, were administered as a single dose to male Sprague–Dawley rats. After 6 h, 24 h and 72 h, the livers were taken for histopathological evaluation and for analysis of gene expression. Briefly, RNA was isolated and used to generate biotinylated cRNA for hybridization onto focused illumina microarrays, and the expression data analyzed using statistical and clustering tools.

All the hepatotoxic compounds tested generated specific gene expression profiles. Among the genes significantly down regulated by all hepatotoxic compounds were a set of Phase 1 and 2 enzyme genes, indicating a decrease in xenobiotic metabolism. Such a response was expected and, therefore, confirmed the validity of our approach. Based on cross-validation analysis, even with a small set of well-characterized genes, gene expression profiling allowed the correct classification of all compounds as either hepatotoxic or non-hepatotoxic, 24 h after high dose treatment. Even during the regeneration phase, 72 h after treatment, CT, ANIT and AAP were predicted to be hepatotoxic, and only these three compounds showed significant histopathological changes. This suggests that gene expression profiles can reveal indications of hepatotoxicity earlier than histopathological evaluation. Furthermore, we identified 64 potential marker genes responsible for class prediction, which reflect typical hepatotoxicity responses. Thus, we identified genes and pathways commonly deregulated by hepatotoxicants, which may be indicative for the early characterization of hepatotoxicity and, thus, predictive of later hepatotoxic endpoints.

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P24-06
Influence of CYP2D6 polymorphism on 3,4-methylenedioxymethamphetamine ("ecstasy") cytotoxicity

H. Carmo [1], M. Brulport [2], M. Hermes [2], F. Oesch [8], R. Silva [1], L.M. Ferreira [4], P. Branco [4], D. de Boer [3], F. Remião [1], F. Carvalho [1], M.R. Schön [5], N. Krebsfaenger [6], J. Doehmer [7], M.L. Bastos [1], J.G. Hengstler [2]

[1] *REQUIMTE, Toxicology Department, Faculty of Pharmacy, University of Porto. R. Aníbal Cunha 164, 4050-047 Porto, Portugal;* [2] *Center for Toxicology, University of Leipzig. Hartelstr. 16-18, 04107 Leipzig, Germany;* [3] *Department of Clinical Chemistry, University Hospital Maastricht, The Netherlands;* [4] *REQUIMTE/CQFB, Chemistry Department, Faculty of Science and Technology, University Nova of Lisboa, Portugal;* [5] *Department of Surgery, University of Leipzig, Germany;* [6] *Schwarz Biosciences GmbH, Department of Pharmacology and Toxicology, Monheim, Germany;* [7] *GenPharmTox BioTech AG, D-Planegg/Martinsried, Germany;* [8] *Institute of Toxicology, University of Mainz, Germany*

Although considered as "safe drugs", exaggerated responses and deaths have been reported due to 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") abuse. Some individuals might be at increased risk of toxicity due to genetically altered metabolism. CYP2D6 catalyses the demethelynation of MDMA and is expressed polymorphically in humans which may be a source of variation in abuse liability and toxicity. Thus, our aim was to study the role of CYP2D6 polymorphism in MDMA toxicity. We used V79 cell lines expressing the human CYP2D6 variants *1 (wild-type), *2, *9, *10, *17 (low activity alleles), CYP3A4 and parental and mockneo controls. We observed a dramatic increase in toxicity for the cells expressing CYP2D6*1 as evaluated by the cloning efficiency assay thus raising the question whether a toxic metabolite is produced. We then performed a metabolism study in which *N*-methyl-α-methyldopamine (*N*-Me-α-MeDA) was found at a much higher concentration for the CYP2D6*1 cells. This metabolite has been implicated in the toxicity of MDMA. The data obtained after testing the cytotoxicity of *N*-Me-α-MeDA to the control cell line confirmed our hypothesis as the metabolite proved to be more than

100-fold more toxic than the parent compound MDMA under the same experimental conditions, strongly supporting that the CYP2D6 polymorphism may partially explain the interindividual variability in susceptibility to the toxic effects of this drug of abuse.

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P24-07

Human bronchial epithelial cell transcriptome: Gene expression changes following acute exposure to whole cigarette smoke *in vitro*

Heather Maunders [1], Sudhanshu Patwardhan [1], Jeremy Phillips [2], Aaron Clack [1], Audrey Richter [2]

[1] *Advanced Technologies Cambridge, 210 Cambridge Science Park, Milton Road Cambridge, England, UK;* [2] *Group R&D, British American Tobacco, Regents Park Rd., Southampton, England, UK*

Cigarette smoke is a complex mixture of over 4000 constituents. Its effects on cell biology are poorly understood, partly because whole smoke exposure *in vitro* is technically challenging. To investigate the global effect of whole smoke on cellular biological processes a 3D air–liquid interface model of tracheobronchial epithelium was grown from primary human lung epithelial cells. Cells from three different donors were exposed to air (control), 1/300 dilution (low dose) or 1/50 dilution (high dose) of whole mainstream cigarette smoke for 1 h in a purpose-designed chamber in three replicate assays. Differential gene expression profiles were then determined for 1, 6 and 24 h post exposure using Affymetrix microarrays. Genes significantly and consistently up or down regulated by smoke, compared to the air control, in all experiments were determined. At the 1/300 dose of smoke gene expression was similar to the air control at 1h, significant regulation was apparent by 6 h but had returned to control levels at 24 h. By comparison, the 1/50 dose of smoke induced significant changes in expression at all time points. The gene lists for this dose were assigned to functional categories using GOSTAT and mapped to KEGG pathways such as MAPK signalling to determine genes which may be regulating the response to whole smoke. Genes regulated in response to the stress of the toxic insult included those involved with xenobiotic metabolism, oxidant/antioxidant balance, DNA damage and repair and cell cycle regulation. Notably, there was a very marked down-regulation of the transforming growth factor-β pathway which has not been previously reported. This study provides important data on the acute effects of whole cigarette smoke on mucociliary epithelium and may be used to gain a greater understanding of smoke toxicity.

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P24-08

A simple method to extract specific signature genes and relevant algorithms of gene clustering from pangenomic microarray data sets

Carine Lambert, Catherine Spire, Dominique Bazot, Andre Guillouzo, Nancy Claude

SERVIER Drug Safety Department, France

Using the functionalities of the microarray gene expression data analysis software Resolver 4™ (Rosetta Biosoftware), a simple method was developed to identify specific signature genes that best characterize the transcriptome of rat liver cells elicited by any compound or any pair of compounds compared to other compounds present in a data set. This blind method allows to extract the most relevant signature genes from high content data sets such as those displayed by pangenomic microarrays (22,000 sequences). This approach circumvents gene expression data interpretation issues related to data overload; it also avoids interpretation bias due to oriented signature gene selection restricted to pre-defined biological processes or to arbitrary quantitative cut-offs.

Using this blind method, specific signature gene sets often delineate the most relevant mode of action pertaining to a compound or a pair of compounds. The nature of the specific signature genes confirms and complements from a qualitative standpoint the outcome of compound clustering, thus bringing a mechanistic added value to the descriptive nature of clustering results.

Using the specific gene sets extracted by this mean, several known algorithms were challenged for relevant gene clustering ability across compounds. Only 3 algorithms among 45 tested were able to discriminate parallel gene behaviours across compounds, when dealing with pangenomic gene lists. These algorithms provide a rapid way to categorize genes in a relevant manner across various treatments.

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P24-09

Toxicogenomic analysis of gene expression profiles in splenocytes from Balb/c mice orally administrated with cyclosporine A for 28 days

Hyung Soo Kim, Kyung Hee Son, Jun Ho Eom, Jin Ho Kim, Ji Soon Sin, Ji Hye Lee, Moon Sung Choi, Jong Kwon Lee, Kui Lea Park

Immunotoxicology Division, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, Republic of Korea

The recent DNA microarray technology enables to understand a large number of gene expression profiling. The technology has potential possibility to comprehend mechanism of multiple genes were related to compounds which have toxicity in biological system. The present study was performed to develop biomarker gene(s) or gene expression pattern(s) useful for immunotoxicity assessment. CsA was selected as indicator for its immunosuppressive effects. Immunological effects of CsA on murine model were confirmed by changes in parameters related to pathology, hematology, innate immunity, humoral immunity and cellular immunity. On the other hand, total RNAs were prepared from spleens of Balb/c mice which were orally dosed with CsA for 28 days. The changes in gene expression levels were analyzed using Applied Biosystems mouse genome survey microarray. There were significant changes in gene expression profiles of mice treated with CsA compared to the control group. Transcriptome profiles confirmed suppressive effects of CsA on the immune system. Numbers of genes changed significantly (>2-fold/>1.5-fold) were about 620/1400 in CsA-treated group. These genes were classified through related biological processes and signal pathways. Biostatistic analyses such PCA (principal component analysis) showed expression profiles of selected genes can distinguish not only CsA-treated groups from control group but also low dose-treated group from control group. These results suggest that expression profiles of selected genes can be used as biomarkers for immunotoxicological assessment.

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P24-10

New insights into uranium toxicity: A gene expression analysis on human kidney cells

O. Prat, F. Berenguer, V. Malard, S. Ruat, G. Steinmetz, E. Tavan, E. Quemeneur

Service de Biochimie post-génomique et Toxicologie Nucléaire, DSV/DIEP, CEA VALRHO, B.P. 17171, 30207 Bagnols-sur-Cèze, France

Industrial use of uranium and particularly of depleted uranium, has pointed out the need for revisiting its chemical impact on human health. This study compares gene expression analysis of various human cell types originating from kidney or lungs following uranium exposure. Dose-related cytotoxicity curves were obtained with increasing concentrations of uranyl salt and cytotoxicity was related to metal uptake by ICP-MS measurement. Uranium did not modulate any common gene between kidney and lungs cells lines. At the contrary, two cell lines originating from kidney presented similarly modulated genes. Their transcriptional levels were validated by RT-qPCR. Classical responses to toxicants such as cell signalling, intracellular trafficking and apoptosis were then reassessed by this method. More interestingly, numerous major histocompatibility complex genes linked to T-cell activation were highlighted, supporting the hypothesis that uranium, like some non-essential metals, could trigger an IL-15 based immune response. Some of these genes can provide new hypotheses to elucidate the mechanism of chemical action of uranium.

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P24-11

Comparison with aspirin and hepatotoxic-compounds inducing coagulopathy—Gene expression profiling in rat liver

M. Hirode, A. Ono, T. Miyagishima, T. Urushidani, T. Nagao

Toxicogenomics Project, National Institute of Biomedical Innovation, Osaka, Japan

Various serum biochemical tests are available in the diagnosis of hepato-cellular damage. The measurement of enzymes such as asparate aminotransferase (AST), alanine aminotransferase (ALT) is preferably employed, while liver insufficiency can be also approached through the measurement of serum fibrinogen and coagulation tests. In the present study, we compared gene expression profiles in rat liver treated with aspirin which

induced platelet dysfunction, and eight hepatotoxic-compounds (clofibrate, omeprazole, ethionine, thioacetamide, benzbromarone, propylthiouracil, WY-14643 and amiodarone), which induced coagulation abnormalities (decreased fibrinogen concentration, increased prothrombin time and partially activated thromboplastin time), stored in our large-scale database.

Rats were treated with three dose levels of each compound, and liver samples as well as traditional toxicological data were obtained at 24 h after 3, 7, 14 and 28-day repeated dosing. Gene expression profiling was performed using GeneChip (Affymetrix, Inc.).

In histopathological examination, aspirin and all coagulopathic-compounds showed the cytoplasmic changes of hepatocyte (e.g. eosinophilic degeneration, swelling and hypertrophy, etc.), whereas elevation of AST and/or ALT was not observed in any compounds in biochemical examination.

At first, 667 up-regulated and 631 down-regulated probe sets related to coagulation abnormality were statistically extracted from the 8 hepatotoxic-compounds. These genes were found to be involved in processes such as cellular stress, lipid metabolism and coagulation system. Moreover, deficiency of vitamin K was suggested to affect the ability of coagulation factors.

On the other hand, we extracted 380 up-regulated and 321 down-regulated probe sets from the aspirin-treated rat liver, and most of them did not accord with those found in 8 coagulopathic-compounds.

It is concluded that these probe sets related to coagulation abnormalities could differentiate drug-induced hepatotoxicity with various mechanisms.

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P24-12
Evaluation of the effects of Xenetix® and Dotarem® on hepatic and renal gene expression in rats

Sébastien Leuillet, Aurélie de Sousa, Bruno Bonnemain, Roy Forster, Philippe Ancian, Philippe Bourrinet, Jean-François Le Bigot

CIT, Evreux, France

Xenetix® and Dotarem® are leading contrast agents for X-ray and magnetic resonance imaging, respectively. The present study was undertaken to evaluate the effects of these compounds on hepatic and renal gene expression in rats. Male Wistar Han rats were treated by a single intravenous injection of Dotarem® at 2.5, 5 or 10 mmol/kg (1.5, 3 and 6-fold the maximum human dose after adjustment for body surface area) or Xenetix® at 2, 4 and 10 g iodine/kg (1, 2 and 5-fold the maximum human dose after adjustment for body surface area). Liver and kidneys were sampled 4 and 48 h after treatment. Blood biochemistry investigations were performed on blood samples taken at necropsy. A microscopic examination was carried out for liver and kidney samples preserved in buffered formalin. RNA was extracted from the snap frozen tissues using a combined Trizol/RNeasy® method. Transcriptomes were studied with Affymetrix GeneChip® Rat Genome 230 2.0 arrays. Treated groups were compared to their corresponding control at each time-point. Transcripts with at least a 2-fold modulation factor and a *p*-value lower than 0.05 were retained. Concerning the *in vivo* part of the study, effects of the compounds were low in intensity, consistent with those described in the literature and included increased blood urea levels in high dose groups on day 1 and liver and kidney vacuolations at microscopic examination on both days. In animals given Dotarem®, 109 genes (85/31 on day 1/3) were modulated in the kidney and 86 (62/26 on day 1/3) in the liver. Some of these modulated genes were implicated in oxydoreductase activity. After treatment with Xenetix®, gene expression profiling showed that 201 genes (169/22 on day 1/3) were differentially expressed in the kidney and 97 (66/34 on day 1/3) in the liver. The principal ontologies involved were symporter activity and cell migration in the kidney and cation homeostatis in the liver. The effect of treatment with Dotarem® or Xenetix® on the whole kidney and liver transcriptomes appeared to be immediate, with more pronounced effects in the kidney. However, these changes were rapidly reversible, with a return to basal levels on day 3. These results are consistent with the low toxicity in non-clinical studies and with the good tolerance profile of these products in clinical use.

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P24-13
Reproducibility of microarray analysis in toxicogenomics studies

Philippe Ancian, Sébastien Leuillet, Rachida El Gana, Sandrine Arthaud, Cecile Fisch, Stéphane de Jouffrey, Roy Forster, Jean-François Le Bigot

CIT, Evreux, France

Microarray analysis offers new vistas for the investigation of mechanisms in toxicology and toxicant profiling. Nevertheless, the technical and biological factors influencing reproducibility must be understood and controlled. In our laboratory, we have established specific procedures in order to minimise sources of variation such as post-necropsy RNA degradation, sequence of tissue

sampling at necropsy, conditions of RNA extraction etc. The reproducibility achieved using these procedures was evaluated as part of an *in vivo* study on gene expression in rats treated with methapyrilene. Male Sprague–Dawley rats were treated with 0, 30 or 100 mg/kg/day methapyrilene and whole transcriptome analysis was performed in animals sacrificed 3 h after treatment on Days 1, 3 and 7. Each treatment group included three animals; one total RNA extract was prepared from a single snap-frozen liver sample from each animal, and was analysed once with an Affymetrix Genechip® Rat Genome 230 2.0 array. Inter-animal and inter-group variability was evaluated by enumeration of transcripts exceeding a two-fold modulation between samples. This threshold was selected on the basis of spike-in experiments using varying quantities of (exogenous) bacterial RNA. Based on a sample of 20,000 detected genes, less than 2% showed between-animal variation (within the same group) which exceed two-fold. This result is very close to the technical variability inherent in such a platform. For the same sample of transcripts, comparison of control groups (vehicle on Days 1 and 3) showed between-group variability to be two logs lower (0.02%, $p < 0.05$ and at least two-fold changes). These results indicate that gene expression data can be generated from *in vivo* experiments with a high level of confidence regarding the transcripts identified.

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P24-14

The selenoprotein thioredoxin reductase is involved in expression of several genes associated with differentiation, adhesion and migration

Ivan Nalvarte, Giannis Spyrou

Department of Biosciences and Nutrition at Novum, Karolinska Institute, Huddinge, Sweden

The anti-cancerous benefits of dietary selenium are well known and both low molecular weight selenocompounds and selenoproteins are thought be implicated in this effect. The ubiquitously found, NADPH-dependent, mammalian selenoprotein thioredoxin reductase (TrxR) plays a major role in the antioxidant defense and is involved in other actions mediated by redox control. Although TrxR has clear beneficial effects, it has been shown to be overexpressed in many tumors and is thought to be a major player in tumorgenesis. Recently, we reported that human embryonal kidney (HEK-293) cells overexpressing TrxR show a decreased rate of proliferation, but increased rate of motility and a surprising distinct differentiating characteristic. We explored the gene expression of these cells using microarray and real-time PCR analysis and found several differentially expressed genes known to be associated with differentiation, cell adhesion and migration. Furthermore, when inducing cell differentiation in a nueroblastoma (SH-SY5Y) cell line with all-trans retinoic acid (ATRA), we could surprisingly see an elevated expression of TrxR and also a similar genetic expression pattern as seen for the TrxR overexpressing cell lines. These data suggest a role for the selenium-containing TrxR in tumorgenesis and metastasis and puts forward TrxR as a major target for cancer therapy.

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P24-15

Application of SELDI-TOF mass spectrometry for the identification of differentially expressed proteins by formaldehyde exposure

Young-mi Jung [1], Heekyoung Chung [1], Byung-Don Han [2], Hyeon-Yeong Kim [3], Byung-Hoon Lee [4], Gu Kong [1]

[1] *Department of Pathology, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea;* [2] *Komabiotech Research Center, Seoul 157-203, Republic of Korea;* [3] *Industrial Safety and Health Research Institute, KOSHA, Daejeon, Republic of Korea;* [4] *College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea*

Biomarkers are becoming increasingly important in toxicology and human health because established biomarkers can be utilized in monitoring human exposure to chemicals. Recently, it is generally accepted that a major contributory factor to sick building syndrome (SBS) is the increased use of chemicals such as volatile organic compounds and formaldehyde during either building construction or furnishing materials and consumer products. As formaldehyde is known to have positive correlation with SBS, we profiled differentially expressed proteins by utilizing SELDI-TOF-MS technology in formaldehyde-exposed cell culture and animal model systems. Protein profiling of formaldehyde-treated human tracheal cells, Hs 680, with an anion exchanger chip, Q-10, revealed sixteen differentially expressed proteins which were downregulated by formaldehyde. Sera isolated from formaldehyde-inhaled rats were also subjected to SELDI-TOF-MS on a Q-10 chip, and majority of the differentially expressed proteins were upregulated upon formaldehyde treatment. Intriguingly, a number of quite strongly upregulated pro-

teins were concentrated at the mass range from 30,000 to 36,000 Da. Furthermore, we identified three potential biomarkers which included two downregulated proteins of 14,600 Da and 16,700 Da in Hs 680 cells, and an upregulated protein of 33,800 Da in rat sera. By partial protein sequencing, these proteins were identified as ribosomal phosphoprotein, large, P2, calmodulin and apolipoprotein E, respectively. Taken together, we provide experimental basis for understanding the toxic mechanism of formaldehyde as well as potential biomarker candidates by applying the powerful SELDI-TOF-MS technology.

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P24-16

Comprehensive analysis of differential gene expression profiles on diclofenac-induced acute mouse liver injury and recovery

Heekyoung Chung [1], Hyun-Jun Kim [1], Ki-Seok Jang [1], Mingoo Kim [2], Jungeun Yang [3], Ju Han Kim [2], Yong-Sung Lee [3], Gu Kong [1]

[1] *Department of Pathology, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea;* [2] *Department of Biochemistry, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea;* [3] *Seoul National University Biomedical Informatics (SNUBI), Seoul National University College of Medicine, Seoul 110-799, Republic of Korea*

Microarray analysis of RNA from diclofenac-administered mouse livers was performed to establish a global gene expression profile during injury and recovery stages at two different doses. A single dose of diclofenac at 9.5 or 0.95 mg/kg body weight was given orally, and the liver samples were obtained after 6, 24, and 72 h. Histopathologic studies enabled the classification of the diclofenac effect into injury (6 and 24 h) and recovery (72 h) stages. By using the Applied Biosystems Mouse Genome Survey Microarray, a total of 7370 out of 33,012 (22.3%) genes were found to be statistically reliable at $p < 0.05$ by two-way ANOVA, and 602 (1.8%) probes at false discovery rate <5% by Significance Analysis of Microarray. Among the statistically reliable clones by both analytical methods, 49 genes were differentially expressed with more than a 1.625-fold difference (which equals 0.7 in log 2 scale) at one or more treatment conditions. Forty and two genes were identified as injury- and recovery-specific genes, respectively, showing that most of the transcriptomic changes were seen during the injury stage. Furthermore, multiple genes involved in oxidative stress, eicosanoid synthesis, apoptosis, and ATP synthesis showed variable transcript levels upon acute diclofenac administration.

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P24-17

Comprehensive analysis of differential gene expression profiles on D-galactosamine-induced acute mouse liver injury and regeneration

Heekyoung Chung [1], Hyun-Jun Kim [1], Ki-Seok Jang [1], Mingoo Kim [2], Jungeun Yang [3], Ju Han Kim [2], Yong-Sung Lee [3], Gu Kong [1]

[1] *Department of Pathology, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea;* [2] *Seoul National University Biomedical Informatics (SNUBI), Seoul National University College of Medicine, Seoul 110-799, Republic of Korea;* [3] *Department of Biochemistry, College of Medicine, Hanyang University, Seoul 133-791, Republic of Korea*

Microarray analysis of RNA from D-galactosamine-administered mouse livers was performed to establish a global gene expression profile during injury and regeneration stages at two different doses. A single dose of D-galactosamine (Gal-N) at 266 or 26.6 mg/kg body weight was given intraperitoneally, and the liver samples were obtained after 6, 24, and 72 h. Histopathologic studies enabled the classification of the D-galactosamine effect into injury (6 and 24 h) and regeneration (72 h) stages. By using the Applied Biosystems Mouse Genome Survey Microarray, a total of 7267 out of 33,012 (22.0%) genes were found to be statistically reliable at $p < 0.05$ by two-way ANOVA and 1469 (4.4%) probes at false discovery rate <5% by Significance Analysis of Microarray. Among the statistically reliable clones by both analytical methods, 401 genes were differentially expressed with more than a 1.625-fold difference (which equals 0.7 in log 2 scale) at one or more Gal-N treatment conditions and with less than 1.625-fold difference at all three vehicle-treated conditions. Three hundred thirty-seven genes and 15 genes were identified as injury- and regeneration-specific genes, respectively, showing that most of the transcriptomic changes were seen during the injury stage. Furthermore, multiple genes involved in oxidative stress, eicosanoid synthesis, apoptosis, and ATP synthesis showed variable transcript levels upon acute D-galactosamine administration.

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P24-18

Quantitative interpretation of dose- and time-dependent microarray data using a gene ontology systems biology approach

Elaine M. Faustman, Xiaozhong Yu, William C. Griffith, Kristina Hanspers, Hansel Ong, Melinda A. Vredevoogd, James F. Dillman III

University of Washington, Washington, USA

Although microarray technology has emerged as a powerful tool to explore expression levels of thousands of genes or even complete genomes after exposure to toxicants, the functional interpretation of microarray datasets still represents a time-consuming and challenging task. Some of the challenges of this task are the large volume of data generated on expression levels for tens of thousands of genes and the problems of performing large numbers of statistical tests that may lead to large number of false positive results for changes in gene expression. Gene ontology (GO) and pathway mapping have both been shown to be powerful approaches to generate a global view of biological processes and cellular components impacted by toxicants. However, current methods only allow for comparisons across singles genes. In addition, the resulting annotations are presented in extensive gene lists with minimal or limited quantitative information, data that is crucial in the application of toxicogenomic data for risk assessment. To facilitate quantitative interpretation of dose- or time-dependent genomic data, we propose to use average expression values of genes associated with specific functional categories derived from the GO database. We developed an extended program (GO-Quant) to extract quantitative gene expression values and to calculate the average intensity or ratio for those significantly altered by functional gene category based on MAPPFinder results. To demonstrate its application, we applied this approach to a previously published dose- and time-dependent toxicogenomic data set. Our results indicate that the above systems approach can describe quantitatively the degree to which functional gene systems change across dose or time. Additionally, this approach provides a robust measurement to illustrate our results compared to single gene assessments and enables the user to calculate the corresponding response for each specific functional GO term, important for risk assessment and evaluation of mode of action for toxicity. This work was supported by NIEHS (U10 ES 11387) Toxicogenomics grant, USEPA-NIEHS UW Center for Child Environmental Health Risks Research (EPA R826886 and NIEHS 1PO1ES09601), NIEHS grant R01-ES10613, Pacific Northwest Center for Human Health and Oceans funded by NIEHS (P50 ES012762) and NSF (OCE-0434087), UW NIEHS Center for Ecogenetics and Environmental Health (5 P30 ES07033) and Low Dose Program of the Department of Energy (DE-FG02-03ER63674).

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P25 Transgenic Models/Knockouts

P25-01

An exploratory inhalation toxicity study with cigarette mainstream smoke in two transgenic mouse strains, rasH2 and p53+/−

Patrick Vanscheeuwijck, Kris Meurrens, Josje Arts, Evert Duistermaat, Hans Muijser, Frieke Kuper, Piter Terpstra

Philip Morris Research Laboratories, Leuven, Belgium

Two mouse models for carcinogenicity testing proposed by the ILSI/HESI program were exposed to cigarette mainstream smoke (MS): the transgenic mouse expressing the human c-Ha-ras proto-oncogene (rasH2) and the heterozygous tumor suppressor p53 knockout mouse (p53). Female mice (16/group) were exposed to air (sham) or MS from the reference cigarette 2R4F for 6 months (3 h/d [low] or 6 h/d [high], 5 d/wk) at a concentration of 240 μg total particulate matter/l. Satellite groups (8–16/group) were kept for 3 months post-exposure. Additional groups of six mice were initiated by i.p. administration of urethane (250 mg/kg) before exposure. Body weight gain and lung nodule incidence and multiplicity were assessed as endpoints. Lungs were made transparent (Tellyesniczky fixative) for macroscopic evaluation and a limited number of sections were taken for histopathological analysis of the nodules. Body weight gain of MS-exposed mice was reduced in rasH2 mice only (10%; end of exposure period). There were no statistically significant MS-mediated increases in the incidence and multiplicity of lung nodules in either strain. Incidence is given for sham, low dose and high dose groups, respectively. At the end of the exposure period, incidence was 3/16, 3/16 and 5/16 in rasH2 mice and 2/16, 0/16 and 5/16 in p53 mice. At the end of the post-exposure period, incidence was 2/15, – and 3/16 in rasH2 mice and 5/16, 1/8 and 2/16 in p53 mice. Treatment with urethane resulted in a high nodule incidence with no statistically significant differences versus sham for both mouse strains. In rasH2 mice, incidence was 5/6, 6/6 and 3/6 at the end of the exposure period and 6/6, – and 4/6 at the end of the post-exposure period. In p53 mice, incidence was 0/6, 3/6 and 0/6 at the end of

the exposure period and 2/6, 3/8 and 0/6 at the end of the post-exposure period. Histopathological evaluation showed the presence of bronchiolar-alveolar adenomas and carcinomas in rasH2 mice only, irrespective of urethane injection. Under the conditions of this study, the mouse models for carcinogenicity testing, rasH2 and p53, were not suitable for detecting MS-induced lung tumors.

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P25-02

MMTV/c-Neu transgenic mice as a model to study antitumor activity against breast cancer in vivo

Min Kyung Nan, Kim Yeo Woon, Kim Ki Yoon, Kim Ji Hye, Kim Dae-Kee, Sheen Yhun Yhong

College of Pharmacy, Ewha Womans University, Seoul, Republic of Korea

Histone deacetylase inhibitors (HDACIs) are emerging as a promising new treatment strategy in malignancy.

The purpose of this study was to evaluate the antiproliferative activity of a novel HDAC inhibitor, IN-2001 in vitro in ER-positive and ER-negative human breast cancer cell lines and to assess its anti-tumor activity and toxicity in vivo in MMTV/c-Neu transgenic mice. Our data showed that IN-2001 treatment inhibited ER-positive and ER-negative breast cancer cell growth in a dose-dependent manner and induced accumulation of acetylated histone H4. IN-2001 induced cell cycle arrest at G_0/G_1 and/or G_2/M phase through induction of the cell cycle regulator $p21^{WAF1}$ and $p27^{KIP1}$ in breast cancer cells. In addition, IN-2001 induced apoptosis, which is related with the activation of caspase cascade and increase of ratio between Bax/Bcl-2. IN-2001 had pronounced anti-tumor activity in vivo when administered to MMTV/c-Neu transgenic mice at a dose of 15 mg/kg by i.p. injection daily for 5 days compared to control mice. The body weights of IN-2001-treat animals did not differ significantly from controls, confirming the observation that IN-2001 did not cause serious toxicity. We have found out that IN-2001 decreased tumor weight possibly through apoptosis. Furthermore, we have found out that IN-2001 increased the gene expression of estrogen receptor and $p21^{WAF1}$ in a time-dependent manner in tumors as well as uterus of MMTV/c-Neu transgenic mice. Because acetylated histones are generally associated with transcriptionally active chromatin whereas deacetylated histones are often found in conjunction with an inactive chromatin state, we next studied whether HDAC inhibition could alter chromatin structure at the ER gene locus. IN-2001 led to re-expression of ER mRNA as detected by RT-PCR in ER-negative MDA-MB-231 breast cancer cells. When MMTV/c-Neu mice were treated by intraperitoneal injection daily for 1–3 days with vehicle or IN-2001 (30 mg/kg), the mammary tumors that develop in these mice was ER negative and IN-2001 increased expression of ER in normal ovary and tumor tissues.

The present studies confirm the potent anti-tumor activity of IN-2001 against breast cancer in vitro and in vivo, strongly supporting HDAC inhibitors as a molecular target for anticancer therapy in breast cancer. Furthermore, it is possible that activation of the silenced ER by HDAC inhibition could open a new avenue for management of a subset of advanced breast cancer with hormonal resistance.

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P25-03

Target organs in transgenic mutation assays in comparison to target organs in carcinogenicity studies

Ulrich Wahnschaffe, Annette Bitsch, Janet Kielhorn, Inge Mangelsdorf

Abteilung Chemikalienbewertung, Fraunhofer Institut für Toxikologie und Experimentelle Medizin, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany

Transgenic rodent mutation assays (TMA) allow the detection of mutagenic effects in any target organ. Therefore, they may be useful in predicting target organs in carcinogenicity studies. For 23 chemicals, where several organs have been investigated in TMA with Muta™ or Big Blue® mice or rats and for 41 chemicals tested with TMA in the liver, the results were compared with results of carcinogenicity studies in the same species with the same route of application.

Target organs in carcinogenesis studies could be confirmed in most cases by the results of the TMA either in multiple target organs or in the liver. In addition, for several chemicals mutations were detected in non-target organs. On the other hand, there were some compounds with positive results in the carcinogenicity studies and negative results in the TMA. Some of these compounds were predominantly clastogens (which are difficult to detect in the TMA), some were non-genotoxic carcinogens, and in some cases the study design of the transgenic assay was not optimal. For a few compounds, both TMA and carcinogenicity studies were negative. However, there is a lack of studies using TMA with non-carcinogenic compounds.

In conclusion, the results suggest that TMA are useful for the prediction of target organs for carcinogenesis,

but a positive response does not necessarily mean that tumours will be induced by that chemical in that organ. TMA are useful tools for mechanistic studies and may be suitable for proving a non-genotoxic mode of action of a carcinogen.

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P26 Others

P26-01
Challenges associated with toxicity testing of traditional medicines in South Africa using nonhuman primate models

Jürgen Seier [1], Mongezi Mdhluli [1], Motlalepula Matsabisa [2]

[1] *Primate Unit, Medical Research Council of South Africa;* [2] *Indigenous Knowledge Systems, MRC, Cape Town, Western Cape, South Africa*

Traditional medicines are used by about 80% of the South African population and substitute for conventional pharmaceuticals in many cases. Apart from strong cultural reasons, many people have limited access to other medication due to economic circumstances. In response, African institutions are increasingly striving to provide scientific evidence for the efficacy and safety of traditional medicines. According to some local opinion, safeguarding against toxic effects is the least that should be done, even in the absence of scientifically proven efficacy.

The Primate Unit of the MRC is being increasingly approached by local commercial and non-commercial organisations to conduct toxicity tests on traditional medicines. These usually consist of a blend of dried and milled plant materials, which are administered orally. Having to work with such materials during testing on animals provides challenges, that are quite distinct from administering pure compounds, and makes adherence to internationally standards on toxicity testing at times impossible. The main reasons are that usually no *in vitro* and pharmacokinetic data can be established, poor palatability, the materials can not be gavaged, high therapeutic doses frequently limit the quantity that can administered, and often no actual toxic doses can be predicted. Additionally, there is invariably limited funding available.

Our presentation illustrates how the Primate Unit approaches toxicity testing of whole plant materials in a vervet monkey model, and how we deal with the challenges of above mentioned limitations. Our approach includes a special diet that serves as an excellent vehicle for even higher concentrations, improving palatability, a 90-day subchronic repeat-dose testing regimen, and monitoring consumption by determining daily food intake.

With our methods, we have been able to demonstrate toxicity at moderate and high doses, in two out of six traditional medicines tested by us so far.

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P26-02
Pyrazinamide and disulfiram effects on male rat's reproductive function

V.M. Kovalenko, L.B. Bondarenko, T.F. Byshovetz, G.M. Shayakhmetova, A.K. Voronina, O.S. Voloshina, N.A. Saprykina

Institute of Pharmacology and Toxicology, Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

In spite of broad utilization of pyrazinamide in tuberculosis and AIDS treatment schemes, its effects on reproductive function and posterity remains insufficiently investigated and present results are discrepant.

Effects of pyrazinamide (500, 1000 and 2000 mg/kg b.w.) and disulfiram (30 mg/kg) on liver and testis biochemical parameters and reproductive toxicity indices have been studied in experiments on Wistar rats.

It was shown that pyrazinamide caused dose dependent increasing of *p*-nitrophenol hydroxylase activity (from 1.4 to 2.3 times), cytochrome P-450 (CYP P450) contents (from 1.3 to 1.8 times) and NADPH-dependent lipid peroxidation (to 86%) in liver microsomal fraction with simultaneous decrease of DNA (to 30%), RNA (to 34%), total histones (to 49%), phospholipids (to 31%), esterified cholesterol contents (to 47%) in testis. Ratio DNA/histones was increased from 1.2 to 1.4 times. Use of CYP 450 2E1 inhibitor disulfiram prevented *p*-nitrophenol hydroxylase activation, the increase of total CYP 450 2E1 and the rate of NADPH-dependent formation of TBA-products. Administration of disulfiram caused normalization of DNA, RNA, histones contents and ratio DNA/histones. On phospholipids and esterified cholesterol contents in testis, effects of pyrazinamide and disulfiram had opposite directions. Pyrazinamide administration produced spermatozoid number decreasing in epididimys in dependence of dose. Decrease of spermatogonia number and compensatory increase of 12th meiosis stages with synchronous dystrophic changes of spermatogenic epithelia also were registered at these conditions. Disulfiram introduction with pyrazinamide produced three times decreased mei-

otic activity of spermatocytes, but did not influence on degenerative changes of spermatogenic cells. While pyrazinamide caused dose dependent increase of preimplantational embryos death and postnatal fetal death in different terms, disulfiram introduction lowered negative effect of pyrazinamide on parameters of preimplantational embryos death (11.6%) and postnatal fetal death (33%) with simultaneous increase of postnatal vitality.

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P26-03

Evaluation of cardiac mast cells in rats exposure to tabun low-level

Vesna M. Jačević [1], Milos P. Stojiljković [2], Zoran A. Milovanović [1], Lidija D. Zolotarevski [1], Katarina A. Jelić [1], Dubravko R. Bokonjić [1]

[1] *National Poison Control Center, Military Medical Academy Belgrade, Serbia and Montenegro;* [2] *Clinical Pharmacology Research Group, Southern Denmark University, Odense, Denmark*

Tabun belongs to divers group of organophosphorus compounds (OPCs), including OP insecticides (OPIs) and other nerve agents, such as a sarin, soman and VX. Prolonged administration of sublethal doses of organophosphorus cholinesterase inhibitors results in adaptation of their toxicity. The stimulus for the investigations came from the notion that those individuals that are engaged in the manufacture or use of the OPIs could receive and survive repeated exposure to small doses. Our previous experiments have showed that peripheral mechanisms are responsible for the occurrence of tolerance to tabun low-dose exposure in rats. The goal of this study was to investigate the influence of 4-week-long tabun low-level exposure on the type, localization and total number of mast cells (MCs) in the rat's heart.

Male Wistar rats were used throughout the experiment. Animals were exposed to 0.3, 0.4, 0.6, 0.8 and 1.0 LD_{50} (150 μg/kg) of tabun *sc* daily over a 4-week period. On the days 28 of the study, MCs were counted in whole visual fields of the rat's heart, stained by Giemsa method.

In the control groups of rats, the majority of the MCs were large, ovoid and hypergranular and showed diffuse spreading in all parts of the diaphragm tissue. Hypogranular MCs and degranulated MCs showed degranulation; degranulated MCs were discovered only on the external wall of the blood vessels. Hypogranular MCs could be seen only in the control group and in the one treated with 0.3 LD_{50} of tabun. In the groups of rats treated with 0.3, 0.4, 0.6, 0.8 and 1.0 LD_{50} of tabun, a total number of the hypergranular MCs was increased by 50, 298, 335, 450 and 620%, respectively. At the same time, use of the same doses of tabun was accompanied by a much faster increase in the number of degranulated MCs—by 60, 362, 518, 609 and 816%, respectively. During the entire study period, in all tabun-treated groups, a total number of different types of heart MCs were increased by 8.9, 67, 189, 201 and 270%, in comparison with the control group.

These results imply that the diffuse and dose-dependent tissue accumulation of the heart MCs and especially their massive degranulation and liberation of the active mediators could be an important reaction to tabun subacute low-dose exposure in rats. The dynamics of this phenomenon and its biological significance are yet to be investigated.

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P26-04

Effects of subcutaneous escalating doses of isoproterenol on the ECG of the dog

Gilles C. Hanton, Pascal Bonnet, Christophe Loiret, Guilaine Bazin

Pfizer Global Research and Development, Amboise Cedex, France

The aim of this study was to evaluate the effects of isoproterenol on the ECG of the dog, in particular on the QT interval, and to assess in this way, the influence of the orthosympathetic system on cardiac repolarisation.

Three Beagle dogs received subcutaneous injections of isoproterenol–HCl at the doses of 0.005, 0.0025 and 0.01 mg/kg, on Days 1, 2 and 3, respectively. Shortly after administration of any dose, the compound produced a marked, dose-related increase in heart rate, associated with an increase in P amplitude and decreases in the amplitudes of sinus arrhythmia, PR interval and uncorrected QT interval. When QTc was calculated, there were only mild treatment-related effects. However, the treatment produced a decrease in plasma potassium and when QT was corrected simultaneously for heart rate and potassium plasma levels, the resulting QTc_K values were clearly shortened by isoproterenol treatment. These changes were consistent with the known direct effects of β-adrenergic stimulation in shortening cardiac repolarisation. The treatment also produced a notching and/or flattening of the T wave, which was related to an increased heterogeneity of cardiomyocytes repolarisation across the ventricular wall and to the treatment-related decrease in plasma potassium. An increase in cardiac troponin I occurred in one dog, 3 h after treatment with 0.01 mg/kg and was probably due to cardiac

hypoxia associated with exaggerated pharmacological effects of isoproterenol.

In conclusion, the subcutaneous administration of isoproterenol to dogs at doses of 0.0025, 0.005 and 0.010 mg/kg, produced a number of changes associated with β-adrenergic stimulation, in particular a dose-related sinus tachycardia, a decrease in QT corrected simultaneously for heart rate and plasma potassium and a notching of the T wave. These effects confirm the major role of the orthosympathetic system on cardiac repolarisation, in particular in shortening the cardiac action potential independently of heart rate.

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P26-05
Effects of a single subcutaneous high dose of isoproterenol on the ECG of the dog

Gilles C. Hanton, Pascal Bonnet, Christophe Loiret, Guilaine Bazin

Pfizer Global Research and Development, Amboise Cedex, France

The purpose of this study was to evaluate the effects of a high dose of isoproterenol on the ECG of the dog, in particular on QT interval. Three Beagle dogs received a single subcutaneous injection of isoproterenol–HCl at the dose of 0.05 mg/kg. The treatment produced sinus tachycardia within 15 min after administration, which was associated with an increase in P amplitude and decreases in the amplitudes of sinus arrhythmia, PR interval and uncorrected QT interval. QTc was only mildly increased. However, the treatment produced a decrease in plasma potassium, peaking 1 h post-treatment and when QT was corrected simultaneously for heart rate and potassium plasma levels, these QTc_K values were clearly shortened by isoproterenol treatment. These changes are consistent with the known effects of β-adrenergic agonists in shortening cardiac repolarisation.

A few hours after treatment, ventricular tachycardia or frequent ventricular complexes were seen in the ECGs of all dogs. These arrhythmic events probably resulted from cardiac hypoxia as indicated by the increases in cardiac troponin I and in creatine kinase observed from 3 h after treatment. Isoproterenol also produced a notching of the T wave, which was probably related to an increase in heterogeneity of repolarisation of the different layers of cardiomyocytes across the ventricular wall and to the decrease in plasma potassium.

In conclusion, the single subcutaneous administration of isoproterenol to dogs at the dose of 0.05 mg/kg produced a decrease in plasma potassium, marked sinus tachycardia shortly after treatment followed by ventricular arrhythmia. The treatment was also associated with evidence of decreased duration and increased transmural heterogeneity of cardiac repolarisation. These data indicate that the dog is a highly sensitive model to the cardiovascular effects of isoproterenol.

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P26-06
NLM's world library of toxicology, chemical safety, and environmental health

Philip Wexler

National Library of Medicine, Bethesda, USA

The world library of toxicology (WLT) is a project of the Toxicology and Environmental Health Information Program (TEHIP) of the Division of Specialized Information Services at the National Library of Medicine (NLM), part of the U.S. National Institutes of Health. It is being designed as a portal to global information resources in the broad areas of toxicology, chemical safety and environmental health, including ancillary subjects such as occupational safety and health, risk analysis, and radiation. WLT presents credible sources of scientific and consumer information for a variety of international audiences—research, academic, government, corporate, and non-profit.

A clickable world map leads to information on continents which, in turn, lead to country-specific information by clicking on national maps. Country information includes links to governmental and non-governmental organizations, universities, professional societies, poison information/control centers, key publications, legislations, general information about the country, and listings of the country's International MEDLARS Center and IFCS National Focal Point. Links to NLM's TOXLINE bibliographic database are included as is contact information for country correspondents, who create and update country pages.

An international review panel consisting of representative from the International Union of Toxicology (IUTOX) and the U.S. Medical Library Association has been assembled to monitor the accuracy and completeness of the WLT. To further help ensure quality, reliability, and credibility, the WLT has achieved certification from the HON Code of Conduct for medical and health websites (http://www.hon.ch/HONcode/Conduct.html).

By opening channels of information and communication, the WLT will serve as an aid in reducing duplicative and overlapping research among countries, and barriers between country-to-country transfer of information.

Wherever possible, links will be presented to websites in both English and the country's native language. Developing countries will be particular beneficiaries of the WLT, enabling them to become informed about the bounty of research, activities and organizational structures in the developed world while allowing them to contribute information representative of their own countries.

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P26-07

Echographic recording of uterine, umbilical and fetal cerebral blood flow in pregnant rats

Sophie Serrière [1], François Tranquart [1], Léandre Pourcelot [1], Gilles C. Hanton [2]

[1] *Faculté de Médecine, Université de Tours, INSERM U619, France;* [2] *Pfizer Global Research and Development Amboise Cedex France*

Changes in uterine and fetal blood flows produced by drugs during pregnancy may be associated with adverse effects on the offsprings. It is therefore helpful to have a non-invasive method for recording these haemodynamic changes during embryo-fetal toxicity studies in rodents. Therefore, Doppler sonography was established in pregnant rats to evaluate normal blood flow changes in uterine and fetal arteries throughout gestation. These experiments were conducted under gaseous anesthesia (isoflurane 2%) in Sprague Dawley rats (250 g at the beginning of the experiment). Results were expressed as medians [quartile 1; quartile 3].

Color coded Doppler were performed in eight rats, daily from gestational day 3.5 (GD3.5) to 20.5 (GD20.5) to measure peak systolic (PSV) and end diastolic (EDV) velocities as well as resistance index (RI = PSV − EDV/PSV) of uterine flow. A marked statistically significant increase in PSV and EDV occurred from GD9.5. On GD9.5, median PSV was 11 cm/s [10; 11.5] and increased to 54.75 cm/s [45; 55.5] on GD11.5. EDV increased from 3.5 cm/s [3; 4] to 19.25 cm/s [15; 20] between GD9.5 and GD11.5. This level remained stable till GD20.5 (PSV = 48.5 cm/s [40.5; 53]; EDV = 20 cm/s [17.75; 21.50]) but with marked inter-individual variation. No major variations of RI were noted throughout the gestation. RI was 0.66 [0.64; 0.67] on GD3.5 and 0.59 [0.56; 0.60] on GD20.5.

In addition, in four rats, blood flows in umbilical and cerebral fetal arteries were investigated using the same technique and were detected from GD15.5 and GD18.5, respectively. On GD18.5, cerebral and umbilical RI were 0.78 [0.67; 0.81] and 0.90 [0.87; 0.94], respectively. On GD18.5, the cerebral PSV was 6 [5.60; 7.50] and cerebral EDV was 3 [2.70; 3.90]. For the umbilical artery, the PSV measured was 10 [9.60; 10.70] and the EDV was 4.30 [4; 4.50]. These values remained stable over the following days.

In conclusion, color coded Doppler could be used in pregnant rats to assess drug effects on fetal and maternal blood flows. In this way, Doppler echography may help in understanding the mechanism of drug induced embryo-fetal toxicity or malformations, when related to haemodynamic changes.

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P26-08

Stability studies of ketazolam in human samples with forensic interest

Paula Melo [1,2], Rui Rangel [2], M. Lourdes Bastos [3]

[1] *Master Student of Forensic Sciences, University of Porto, Portugal;* [2] *Laboratory of Forensic Toxicology, National Institute of Legal Medicine, Porto, Portugal;* [3] *REQUIMTE, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto R. Aníbal Cunha, 164, 4099 Porto, Portugal*

Ketazolam is a benzodiazepine used in therapeutics with interest in clinical and forensic toxicology. It is extensively metabolized in the human body giving rise to other active benzodiazepines.

Biological samples for toxicological analysis frequently remain at room temperature for some hours during transport, reception and registration, at 4 °C during some days until drug screening and analysis confirmation, and at negative temperatures for several months or years for legal purposes and reanalysis. Thus, it is crucial to know the stability of drugs involved in fatal intoxications when stored at different temperatures and periods of time in order to assure correct interpretation of the toxicological results. Our study was performed in human blood, bile, and vitreous humour collected at autopsy. Pooled samples were spiked with ketazolam standard solutions, and stored at room temperature, 4 °C, −20 °C or −80 °C.

Ketazolam was quantified by HPLC-DAD after SPE extraction, immediately after spiking and at several times during 6 months.

After 1 week at room temperature and after 4 weeks at 4 °C, the drug levels were lower than the limit of quantification (<0.082 mg/L). In contrast, the drug showed good stability at −20 °C and −80 °C during 6 months, with only a 7% loss in vitreous humour, 10% in bile, and 13% in blood.

A parallel study was performed in order to characterize and quantify compounds formed due to the degradation of ketazolam. Diazepam was identified in all samples stored at room temperature and at 4 °C. In blood, after 6 months of storage, the levels of diazepam were almost similar to the added concentration of ketazolam (0.162 mg/L of diazepam versus 0.20 mg/L of added ketazolam). Interestingly, the preservation of blood with NaF 1% had little effect on the degradation of ketazolam.

The main conclusion of this study is that ketazolam is very unstable at positive temperatures and in forensic situations involving ketazolam, the benzodiazepines resulting from its degradation must be searched for and quantified, and care should be taken in the interpretation of the toxicological results.

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P26-09

A review of background findings in Cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins

Marie-France Perron, Elodie Drevon-Gaillot, Christian Clément, Georges Porret-Blanc, Roger Burnett

MDS Pharma Services, France

This review was performed to assess variations in background observations in Cynomolgus macaques (*Macaca fascicularis*) originating from three breeding centers located in Mauritius, The Philippines and Vietnam. The data and tissue samples from 90 Cynomolgus monkeys (approximately evenly distributed between the three sources) comprising the control groups from 11 regulatory toxicology studies were used for this investigation. Clinical data – body weight, organ weights, haematology and serum biochemistry – were analyzed. Samples of stomach, colon, kidney, heart, liver, spleen and lung were examined microscopically and graded to characterize the degree of lymphoplasmacytic cell infiltration.

The main microscopic origin-related variations concerned the digestive tract, where the lymphoplasmacytic cell infiltration grade was significantly lower ($p \leq 0.001$) in Cynomolgus monkeys from Mauritius when compared with those from Asia. Generally, only the antral mucosa of the stomach was infiltrated in Cynomolgus monkeys from The Philippines, whereas both the fundic and antral regions were infiltrated in those from Vietnam. The digestive tract infiltration grade was strongly correlated with the mean white blood cell count in monkeys from all three sources. Spiral-shaped bacteria were observed in the stomach of monkeys from all three sources but their presence did not correlate with the severity of the gastric infiltrate. *Helicobacter heilmannii*-type bacteria were almost always seen in the fundus, *Helicobacter pylori*-type bacteria were only occasionally seen in the antral region.

The incidences of other microscopic findings, such as urothelial cytoplasmic inclusions or *Balantidium coli* in the caecum, also varied according to the source of the monkeys. Some variations in relative organ weights, haematology and serum biochemistry were also related to the origin of the monkeys, but these did not correlate with the microscopic findings.

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P26-10

The effect of glycerol on microscopic structure of heart: An experimental model in rat

M.R. Panjehshahin, F. Dehghani

Pharmacology Department, Shiraz Medical School, Shiraz, Iran

Experimentally, the injected glycerol could injure the skeletal muscle same as trauma. There is one question whether glycerol can effect on microscopic structure of heart. For this purpose, 30 male Sprague–Dawley rats were taken. The experimental group was administrated with a single dose of glycerol (10 ml/g) intramuscularly. Normal saline was administrated in control group. After 48 h, anesthetized rats were scarified and heart fixed with formalin (10%). Specimens were processed and stained with hematoxyline-Eosine. The result indicated that there was not any microscopic changes in heart cell but large quantity of fibroblast and fibrocyte distributed in stroma, in addition, blood vessels were congestive and became larger than the control group. Although, the finding should be supported by more specific method.

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P26-11

Impact of maternal diets and early environmental exposure on weight maintenance and development of the offspring

André Wolterbeek, Simone Eussen, Renate Jansink, Linda van de Horst, Marlies Otto, Ine Waalkens, Berensen Anja Dijkstra, Florence Salmon, Heleen Wortelboer, Wilrike Pasman, Henk Hendriks, Ivana Bobeldijk, John Groten, Didima de Groot

TNO Quality of Life, Zeist, The Netherlands

Alterations in the nutritional status of the mother during gestation and early lactation might have effects on

the development of the offspring in later life. In order to study (1) the impact of maternal diets on body weight of the offspring (predisposition for obesity) and (2) the effects of maternal diets in combination with environmental exposure on the (neuro)development of the offspring, three groups of female Wistar rats ($n = 28$–32) were kept on a control diet, a western style type diet (based on sweetened condensed milk) or a restricted diet (ca. 60% cellulose) during 6 weeks premating, mating, gestation and lactation. On postnatal day 1 (PN 1), the F1-pups were cross-fostered to dams of the same group or to dams of the other two dietary groups to obtain nine different groups and fed the various diets up to PN 70. To investigate environmental influences, the vulnerability for chemically induced neurotoxicity was studied. Hereto, F1-pups were treated subcutaneously with MAM (PN day 2–5) or MeHg (PN 2–21). Results showed that: (1) food consumption, energy intake and body weights of the dams differed significantly between the groups, (2) no significant effects on birth weight were observed and (3) pup growth was influenced, showing 'catch-up' growth of the restricted pups fostered to dams on the western style type diet and, vice versa, decreased growth of the western style type diet pups fostered to dams on a deficient diet. The effects on body weights of F1 animals were exacerbated by exposure to MAM or MeHg and this effect gradually increased with age. It was concluded that maternal diet manipulation may have (transitory) effects on the offspring which, in turn, may be responsible for increased vulnerability of the offspring for (subtle) environmental influences causing life-long (increasing) changes in metabolism, as suggested here by changes in body weight.

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P26-12

Regional blood flow changes induced by a phosphodiesterase 4 inhibitor in rats

Céline Korkmaz [1], Véronique Maupoil [1], Valérie Pawlowski [2], Cécile Sobry [2], Stephan Chevalier [2], Jean-Louis Freslon [1]

[1] *CNRS UMR 6542,Tours, France;* [2] *Pfizer Global Research and Development Amboise Cedex France*

Phosphodiesterase 4 (PDE4) inhibitors are drugs of major interest in the treatment of inflammatory diseases. Chronic oral administration of supra pharmacological doses in rats induces inflammation and necrosis in many organs, including mesenteric arteries. It is hypothesized that vascular lesions may be related to excessive vasodilatation and increased local blood flow (BF). However, the relationship between vascular injuries and arterial BF changes remains to be determined. In this study, the BF changes are determined after the administration of a PDE4 inhibitor at a dose inducing vascular lesions.

Male Sprague–Dawley rats (10–12 weeks) received orally 160 mg/kg of PDE4 inhibitor or vehicle. Four hours post dosing, fluorescent microspheres were injected to determine regional BF, cardiac output (CO) and total peripheral resistance (TRP). After euthanasia, kidneys, lungs, spleen, heart, brain, stomach, testicles, epidydimes, liver, muscle and mesenteric bed divided in three parts: (1) irrigating the duodenum (Mes-duo), (2) jejunum (Mes-jej) and (3) ileum (Mes-ile) were dissected. Both tissue and blood samples were digested with KOH and the fluorescence intensity was measured (490 and 530 nm) after fluorophore extraction with Cellosolve. Mean arterial pressure (MAP) and heart rate (HR) were also measured.

The oral administration of the PDE4 inhibitor significantly decreased MAP (−13%) whereas HR was not altered. The treatment significantly increased CO (+83%) and decreased TRP (0.61 ± 0.12 to 0.28 ± 0.07 mmHg min kg/ml; −83%). BF was increased in the kidney (6.9 ± 0.5 to 11.7 ± 1.7 ml/(min g); +70%), Mes-duo (2.2 ± 0.2 to 4.4 ± 0.9 ml/(min g); +100%), Mes-jej (1.7 ± 0.1 to 3.5 ± 0.8 ml/(min g); +106%), Mes-ile (1.1 ± 0.1 to 3.2 ± 1.2 ml/(min g); +191%), lungs (0.9 ± 0.1 to 1.7 ± 0.3 ml/(min g); +89%) and spleen (2.4 ± 0.1 to 4.6 ± 1.5 ml/(min g); +92%) whereas BF in other organs was not affected by the treatment.

This significant increase of mesenteric vascular blood flow was indicative of a vasodilatation 4 h after dosing the animals with the PDE4 inhibitor. This supported the hypothesis that drug-induced vascular lesions in rat could be due to abnormal parietal conditions of the mesenteric vasculature.

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P26-13

External telemetry investigation of ECG parameters for group housed non-rodent toxicity studies

Anne-Marie Bétat, Aurélie Baleydier, Guillaume Froget, Pierre Lainée, Roy Forster, Jean-François Le Bigot

CIT, Evreux, France

ICH guidance on Safety Pharmacology emphasizes the value of investigating the time-course and dose-relationship of any drug-induced effects, and indicates a preference for the use of conscious animals wherever

possible. Using external telemetry, electrocardiography parameters can be obtained from conscious animals without the need for surgery and in this way safety pharmacology investigations can be conveniently included in early toxicology studies. In the present work, we show that the use of external telemetry is also compatible with group-housing. Four dogs housed by pairs were trained to wear jackets and accept electrodes and a battery backpack. They were orally dosed in a randomised crossover design with either vehicle (methylcellulose), diltiazem (DTZ: 5 mg/kg) and sparfloxacin (SPA: 10 mg/kg). Antennae and hardware were placed close to the study room and signals were acquired and analysed by two dedicated software (IOX and ECG Auto, EMKA Technologies). Recordings lasted from 1 h before to 8 h after dosing. The results demonstrated the expected effects of each drug with the following maximal effects: HR increase ($+43 \pm 33\%$) and PR interval prolongation ($+37 \pm 3\%$) after DTZ, prolonged QT interval ($+16 \pm 5\%$) and Fridericia corrected QT interval ($+7 \pm 6\%$) after SPA. Similar studies have been obtained in individually housed monkeys treated with vehicle, quinidine and astemizole and with group-housed monkeys monitored over a 24 h period. Our work shows that data for safety pharmacology end-points can be readily obtained from early toxicology studies in group-housed dogs and primates using external telemetry approaches. Such data can complement safety pharmacology study results, often across a wider range of dose-levels, permitting greater confidence in safety margins.

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P26-14

Validation of a HPLC-ECD method for the quantification of the highly reactive metabolite of *ecstasy*, *N*-methyl-α-methyldopamine, in human serum

Görkem Mergen [1], Renata Silva [2], Luísa M. Ferreira [3], Paula S. Branco [3], Fernando Remião [2], Félix Carvalho [2], Maria de Lourdes Bastos [2], Tülin Söylemezoğlu [1], Helena Carmo [2]

[1] *Institute of Forensic Medicine, Ankara University, 06590 Dikimevi, Ankara, Turkey;* [2] *REQUIMTE, Toxicology Department, Faculty of Pharmacy, University of Porto. R. Aníbal Cunha 164, 4050-047 Porto, Portugal;* [3] *REQUIMTE/CQFB, Chemistry Department, Faculty of Science and Technology, University Nova of Lisboa, Portugal*

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") oxidative metabolism in humans consists mainly of demethylenation to the highly reactive catechol metabolites *N*-methyl-α-methyldopamine (*N*-Me-α-MeDA) and α-methyldopamine. We validated a method for the quantification of the potentially toxic *N*-Me-α-MeDA metabolite in human serum based on extraction by activated alumina (Al_2O_3) and analysis of extracts by high-performance liquid chromatography with electrochemical detection. For chromatographic separation, a reverse phase C18 (5 μm) column and a mobile phase consisting of a mixture of 15% methanol and 85% 50 mM citric acid, 0.46 mM 1-octanesulfonic acid solution (pH 3.0) were used. An isocratic elution was performed at a flow rate of 1.0 mL/min and at room temperature. A coloumetric detector equipped with a guard cell and dual analytical cells were used. The electrochemical potential settings were: guard cell, +0.50 V; electrode 1, −0.075 V; and electrode 2, +0.4V. A current of 1–5 μA full-scale was used for electrode 2. Calibration curves were obtained by plotting the peak area ratio of *N*-methyl-α-methyldopamine to internal standard (ISTD) 3,4-dihydroxybenzyl amine (DHBA), versus the known concentration of each reference substance added to drug-free human plasma. The curves were constructed from five replicate measurements of six concentrations ranging from 100 nM to 600 nM performed on the same day, and a linear response of the signal detector versus analyte/ISTD concentration ratio was achieved. The analytic method was tested for inter and intra-day reproducibility and the obtained values were between 4.4–2.2% and 1.8–4.5%, respectively. Recovery from plasma was 75% and the calculated limits of detection and quantification were 3.48 nM and 10.54 nM, respectively.

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P26-15

The effect of pollen on serum parameters of rats

Guldeniz Selmanoglu [1], Sibel Hayretdağ [2], Durdane Kolankaya [3], Aslı Özkök Tüylü [3], Kadriye Sorkun [3]

[1] *Hacettepe University Ankara, Turkey;* [2] *Çanakkale University, Faculty of Art and Science, Department of Biology, Çanakkale, Turkey;* [3] *Hacettepe University, Faculty of Science, Department of Biology, 06532 Beytepe, Turkey*

The pollen collected by honeybees contains generally 40% proportion of protein, essential amino acids, low amounts of fat, and high levels of minerals. The objective

of this study was to investigate any positive effects or possible side effects of the use of pollen. In this study, mature male rats were fed to three kinds of pollen (*Trifolium* spp., *Raphanus* spp., and *Cistus* spp.) in the amount of 60 mg/per animal/per day for a 30-day period. At the end of the treatment, biochemical parameters and serum enzyme activities were analysed and weights of liver and kidney were recorded. Also, the liver and the kidney tissues of rats were examined at light microscopic level.

There were decreases in serum cholesterol and HDL levels of rats fed pollen of *Trifolium* spp. and *Cistus* spp. There was no change in serum enzyme level of rats at pollen groups. No change in relative weights of liver and kidney of rats in pollen groups was determined. Histopathological change in the liver and kidney of rats given pollen was not observed. The liver and kidney tissues of pollen treated rats were similar to those of control rat.

According to the results of this study, although serum cholesterol and HDL levels decreased, we cannot suggest that bee pollen did not caused adverse and benefit effects, because of duration of pollen treatment was only 30 days.

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P26-16

Hepatoprotective activity of *Berberis integerrima* Bge extract in rats treated with CCl_4: In vitro and in vivo studies

Akram Jamshidzadeh, Hossien Niknahad

Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran

Berberis integerrima is an edible plant employed in the Asian Traditional Medicine, particularly its fruits being used as a tonic remedy for liver and heart. In this investigation, freshly isolated rat hepatocytes and rats were used as the in vitro and in vivo models to evaluate the hepatoprotective activity of aqueous extract of dried powdered fruits of *Berberis integerrima.* CCl4 was selected as hepatotoxin. Silymarin was the reference hepatoprotective agent. In the in vitro study, Trypan Blue Exclusion test was the criteria for cell viability. Freshly isolated rat hepatocytes (1×10^6 cells/ml) were treated with CCl4 (10 mM/ml) and various concentrations of extract (50, 150, 300, 400, 500, 800 μg/ml) for 3 h. The *Berberis integerrima* extract with concentrations of 300, 400 and 500 μg/ml protected the cells against CCl_4-induced cytotoxicity, but concentration of 800 μg/ml increased the CCl_4-induced cytotoxicity.

In the in vivo study, serum aminotransaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities and histopathological examination were the criteria for evidences of liver injury. CCl_4/olive oil (1:1, 3 ml/kg, i.p.) caused the increase in AST, ALT, ALP and centrilobular hydropic degeneration of hepatocytes. Pretreatment and treatment of animals with a single oral dose of extract (30 mg/kg), for 3 days before and 12 h after CCl_4 injection showed that the *Berberis integerrima* extract could protect the liver against CCl_4-induced damages.

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P26-17

Effects of subchronic exposure to camphor on male reproductive tract in rats

Akram Jamshidzadeh, Javad Sajedianfard

Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran

Camphor is extensively use in pharmaceutical preparations and skin care products. The effect of camphor on mail reproductive system is controversial. In this study, the effects of camphor on reproductive tract were studied in Sprague–Dawley male rats. Several groups of adult rats were treated (i.p.) with 5, 10 and 20 mg/kg of camphor solution for 30 days. After this period, rats were scarified and their testis, seminal vesicle, epidydimis as well as afferent duct were removed for histological examination using hematoxylin–eosin staining. The results indicated a decrease in the body weight and testis size and weight with all of the experimental doses. Testicular sperm number and motility were also decreased in all of the treated rats. Higher doses (10 and 20 mg/kg) of camphor caused morphological changes and a toxic effect on sperm and their motility, as well. Therefore, it is concluded that camphor with even the lowest dose used in this study can cause damage to male reproductive system.

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P26-18

Safety and therapeutic efficacy of UC-II alone or in combination with Glucosamine + Chondroitin in arthritic dogs

R.C. Gupta[1], M. D'Altilio[1], A. Curtsinger[1], T.D. Canerdy[1], J.T. Goad[1], M. Bagchi[2], D. Bagchi[2]

[1] *Murray State University, Hopkinsville/Murray, KY, USA;* [2] *InterHealth Research Center, Benicia, CA, USA*

Arthritis in large breed dogs is as common as in humans. Arthritis is closely associated with obesity. Obese arthritic dogs were treated with selected combinations of three supplements including glycosylated type-II collagen (UC-II), Glucosamine HCl (GLU), and Chondroitin sulfate (CH). Dogs were daily supplemented with either placebo (Gr-I), 10 mg UC-II (Gr-II), 2000 mg GLU + 1600 mg CH (Gr-III), or UC-II + GLU + CH (Gr-IV) for 120 days, followed by a 30-day withdrawal. Dogs were examined on a monthly basis for overall pain, pain upon limb manipulation, and exercise-associated lameness. Gr-I exhibited no improvement. Gr-II showed significant reductions in pain within 30–60 day of treatment. Maximum pain reductions were noted after 120 day (overall pain, 62%; pain upon limb manipulation, 91%; and exercise-associated lameness, 78%). Gr-III dogs showed some pain alleviation, while Gr-IV exhibited marked reductions (57%, 53%, and 60%, respectively). 30-Day withdrawal led to pain relapse in all dogs. Serum samples were analyzed for markers of liver function (bilirubin and ALT), renal and heart function (BUN, creatinine and CK). Body weight and temperature were measured. Serum chemistry, body weight and temperature remain unchanged. Thus, daily treatment of arthritic dogs with UC-II alone or in combination with GLU + CH significantly ameliorates the signs of arthritis, and supplements used in the present study are safe and well tolerated without any side effects.

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P26-19

Drug analyses of necrophagous insects and human tissues

Marija Definis-Gojanovic, Davorka Sutlovic, Bozo Kokan, Bogomir Milosevic

University Hospital Split, Split, Croatia

Necrophagous insects may provide useful information concerning the time, place and cause of death. In addition, they can serve as reliable alternate specimens for toxicological analyses in the cases when human tissues and fluids, normally taken during autopsies, are absent due to decomposition of the corpses.

This paper reports the results of drug analyses of the developmental stages of two flies species (Calliphoridae and Sarcophagidae), collected from the body of middle-age man who had committed suicide approximately 3 weeks prior his corpse was found. Analyses of multiple samples (human tissues and maggots) were performed using a gas chromatography–mass spectrography, and amphetamine was detected in all samples.

While the qualitative relationship of the results was without any doubt, the quantitative results were less clear and further research in this area is suggested.

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P26-20

Efficacy of different medical herbal preparations as hepatoprotective agents

Mohey M. Ellithy, Somaia A. Nada

Pharmacology Department, National Research Centre, P.O. 12622 Dokki, Cairo, Egypt

In folk medicine, there are many herbal preparations have been used for their hepatoprotective activities. One of the most common recipes contain from equal parts (w/w) of decoction of (10% concentration): *Peumus boldus* (leaves), *Cichorium intybus* (root) and *Nigella sativa* (seed) (Recipe 1). *Glycyrrhazia rhizome* (root) was replaced with *Cichorium intybus* (Recipe 2) or added (Recipe 3). Three groups of normal albino rat were orally administrated 1.5 ml/100 g of Recipe 1 (group 1), Recipe 2 (group 2) or Recipe 3 (group 3) and the control group (group 4) was given 1.5 ml/100 g distilled water daily for 30 successive days. Results for normal groups revealed that Recipe 1, 2 and 3 were decreased plasma γ-glutamyl transferase (GGT): −6.1%, −26.7%, −31.5%; ALT: −3.8%, −13.2%, 17.6%; AST: −5.9%, −6.8%, −21.5%; triglycerides: 1.8%, 0%, −13%; cholesterol: −2.4%, −1.2%, −1.9% and sleeping time: 0.5%, 1.4%, 0.9%, respectively, versus control values.

Second set of experiment, four groups of carbon tetrachloride-hepatic damaged rats were given the three recipes with the same above oral doses before carbon tetrachloride for 2 weeks, then followed by another 2 weeks after induction of the hepatic damage. The results indicated that significant decrease in GGT: −70%, −74.5%, −82.0%; ALT: −30.1%, −36.8%, −49.0%; AST: −9.9%, −33.3%, −43.8%; triglycerides: −11.8%, −10.5%, −17.0%; cholesterol: −17.4%, −16.4%, −24.4% and sleeping time: −24.0%, −25.1%, −37.9%, respectively, versus carbon tetrachloride-hepatic dam-

aged rats .In conclusion: the modified Recipe 3 was found to be more potent than Recipe 1 or 2.

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P26-21

HPLC method for methadone quantification in biological samples

Anne-Marie Ciobanu, Daniela Luiza Baconi, Maria Barca, Dan Balalau, Mihaela Ilie, Ivonne Pencu

University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

The use of methadone in substitution treatment of opiate addict patients is well documented. Clinical success in the methadone substitution treatment depends on the methadone suitable doses to maintain blood levels in the pharmacologically effective range. Many study highlighted the insufficient methadone dose as a major cause of the treatment failure. Several evidences exist supporting a relationship between methadone dosage and plasma methadone concentration in addicted patients during substitution therapy.

We present a sensitive reversed-phase HPLC method for methadone quantification in plasma. Several HPLC columns have been tested and a RP-18, 125 cm × 0.46 cm (i.d.) packed with 5 μm diameter particles has been selected. As a mobile phase, 0.1 trifluoroacetic acid–methanol (60:40, v/v), has been used. Methadone was detected at 210 nm. Linear relationships between peak area and methadone concentrations were obtained in the range of 0.0275–0.22 μg/mL. The method has been validated for determination of plasma concentration of methadone. A liquid–liquid extraction procedure has been applied. The method is precise and accurate (mean recovery percent 92.34%), with a LOQ of 2.5 ng/mL.

The developed method was applied to monitoring methadone plasma levels in heroin addicts patients, during methadone substitution treatment conducted at Sf. Stelian Center for Evaluation and Treatment of Addictions, Bucharest. Considerable inter-individual variations of methadone plasma concentrations have been demonstrated.

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P26-22

Toxic affects of HCG on the distribution pattern of mature rat uterine surface glycoconjugates during implantation period

Aliabadi Elham, Talaii khozani Tahereh, Mesbah ardekani seyed Fakhredin, Makoolati Zohreh

Department of Anatomy, Medical School, Shiraz University of Medical Science, Iran

Introduction: Unovulatory period is one of the most causes of infertility .One of the treatment to such cases is hormone therapy. These hormones may have toxic affect on the molecular organization of uterine surface e.g. glycoconjugates. Glycoconjugates are one of the most important molecules of the uterine surface which play an important role in embryo implantation. In this study, the toxic effects of one of these gonadotropin hormones, Human Chorionic Gonadotropin (HCG), on glycoconjugates of uterine surface (apical membrane, Golgi zone and basement membrane of rat endometrial cells and uterine glands) during implantation period were studied.

Material and method: Sixteen mature female rat was selected and divided to two groups (experimental and control groups). Experimental rats injected with 10 IU HCG intraperitoneally in estrus phase and mated with proven fertile male rats. The observation of vaginal plug was considered as 0.5 day of pregnancy. The rats were sacrificed at 5.5 day of pregnancy (time of implantation) and their uteruses were removed. Control rats become pregnant without any injection. The pregnant uterine tissues were fixed in bouin solution and embedded in wax. Lectin histochemistry was done with use of WGA, DBA, PNA, ConA, SBA and UEA lectins.

Results: The intensity of the reaction to WGA in apical membrane, Golgi zone and uterine gland and the reaction of uterine gland to DBA and UEA were significantly low in experimental groups.

Conclusion: HCG can induce its indirectly toxic effects on implantation rate via changes in uterine surface glycoconjugates.

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CEC1 Assessment of Immunological Health in Occupational Exposed Workers

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Human immunotoxicology: Consequences and mechanisms

Emanuela Corsini

University of Milan

The immune system has evolved to protect the host against invasive microorganisms such as bacteria and viruses, and against malignant cells.

At least two specific properties make the immune system vulnerable to chemical or physical insults: (1) the immune system develops rather late in life (thymus development lasts at least until puberty), and approximately 1% of all leukocytes are renewed daily, and (2) each pathogen attack, as well as immune surveillance, demands a delicate control of the balance between activation, silencing and regulation of immune reactivity.

Immunotoxicology studies the effects of xenobiotics on the immune system and, an immunotoxic compound can be defined as a compound that can alter one or more immune functions resulting in an adverse effect for the host. In particular, two main immunotoxic effects can be identified:

1. immunosuppression, which may result in repeated, more severe, or prolonged infections as well as the development of cancer;
2. immunoenhancement, which, as adverse effect, may lead to immune-mediated diseases such as hypersensitivity reactions and autoimmune diseases. The two most frequent manifestation of chemical-induced allergy are contact hypersensitivity and respiratory sensitization, both of which can have serious impact on quality of life and represent a common occupational health problem. Hypersensitivity reactions are often considered to be increased at such a rate to become a major health problem in relation to environmental chemical exposure.

The clinical date accumulated so far demonstrate that immunotoxicity is associated with significant morbidity and even mortality, making immunotoxicity of considerable importance to the toxicologist, who has the responsibility of identifying and characterizing the immunotoxic potential of chemicals and estimating the risk they pose to human health. The purpose of this lecture is to overview the current evidence about the importance of the characterization of xenobiotics-immunotoxic potential in humans and, to describe the molecular mechanisms of action underlying immunotoxicity.

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Epidemiological assessment of immunotoxic effects in workers

M. Gulumian [1,2]

[1] *Department of Toxicology, NIOH, PO Box 4788, Johannesburg 2000, South Africa;* [2] *Department of Haematology and Molecular Medicine, Wits University, South Africa*

Occupational disease surveillance programme at the NIOH for the period 1996–2003 has indicated that diseases linked with immunological dysfunction in workers are associated with exposure to biological and organic and non-organic agents. The latter included wheat and grain products, latex, isocyanates, platinum, chrome and vanadium compounds, which produced allergic asthma, rhinitis, allergic alveolitis and eczema.

A number of cross-sectional epidemiological studies of different industries including platinum mining industry, maize and soybean milling industries as well as latex allergy in health workers have been conducted in South Africa. A concomitant questionnaire and also airborne concentration of the immunogenic material were also performed to assess exposure of workers to these agents. The immunotoxicity of a number of agents in these environments have subsequently been confirmed using appropriate screening tests for immune function such as skin prick test, the radioallergosorbent assay (RAST) and patch tests.

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Immunotoxicological profile and pesticides exposure in farmers

Tatjana Vergieva [1], Cristo Zaikov [1], Daniela Baltadgieva [10], Pavel Theoharov [2], Mircho Vukov [9], Henk Van Loveren [3], Ludovic G.P.M. van Amelsvoort [4], Peter Steerenberg [3], Claudio Colosio [5], Silvia Fustinoni [7], Emanuela Corsini [6], Jyrki Liesivuori [8], Sirpa Pennonen [8]

[1] *National Centre of Public Health Protection, Sofia, Bulgaria;* [2] *National Centre of Parasitic and Infectious Diseases, Lab Virusology, Sofia, Bulgaria;* [3] *National Institute of Public Health and the Environment, Bilthoven;* [4] *Department of Epidemiology, Maastricht University, The Netherlands;* [5] *International Centre for Pesticides and Health Risk Prevention, University Hospital L. Sacco, Milan, Italy;* [6] *Laboratory of Toxicology, Department of Pharmacological Sciences, University of Milan, Milan, Italy;* [7] *Department of Occupational and Environmental Health, University of Milan and Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy;* [8] *Finnish Institute of Occupational Health, Kuopio, Finland;* [9] *National Centre for Health Information, Sofia, Bulgaria;* [10] *Central Immunological Laboratory, Medical University, Sofia, Bulgaria*

Pesticides are designed to interfere with living species, and are inevitably characterized by variable levels of toxicity. A number of data is at present available suggesting that the immune system may be a target of the toxic effect of pesticides.

As part of an EU project, EUROPIT (QLRT-2001-00211), the results of a study aimed to evaluate the capacity of mixed exposure to pesticides to cause immune changes in occupationally exposed workers will be presented and discussed. The immune system investigation was carried out through the application of a battery of tests adequate to collect information on cellular, serum, and functional immune parameters.

Overall, our study suggests that intermittent and low dose exposure to pesticides do not meaningfully influence the immunologic system and does not pose any significant health risk to the exposed subjects. We cannot, however, forget that, like in any other study on agricultural workers, the size of the studied groups is relatively small, and this limit could affect the possibility of identifying slight and subtle changes; on the other hand, alterations in immune status and function, which may be tolerated well in normal healthy adults, could have more serious consequences for those who are chronically sick, malnourished, or whose immune system has yet to develop or is in decline, underlying the importance to monitor pesticide exposure to the general population and define, when necessary, appropriate preventive measures.

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Perspectives on immunotoxicity testing in humans with special reference to individual susceptibility

H. van Loveren

National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Immunotoxicity is defined as the adverse effect of chemical exposure on the immune system, leading to malfunction of one or more components or imbalance of regulatory mechanisms. As such, immunotoxicity may lead to reduced resistance to infections or certain tumors, while it may also have consequences on the expression of allergy or autoimmunity.

Immunotoxicity testing has been abundantly performed in laboratory animals, and numerous assays have been developed. Test that are considered highly predictive for adverse consequences of exposure to immunotoxicants are so called immune function tests, for instance the analysis of antibody responses after immunization with a T-cell dependent antigen.

In studies of adverse effects of PCB's, antibody responses to pediatric vaccines have been used as a read out of immunotoxic effects in children. Especially effects on vaccination responses to measles appeared to be sensitive. Whereas suppressed antibody responses to vaccination will not likely have any impact on protection delivered by vaccination, a decreased response does indicate a functional deficit, and may have consequences for resistance to infectious agents for which there is no vaccination carried out. The same background concentrations of PCB's that caused a decreased vaccination response induced a significant increase of middle ear infections in these children.

In a study of adverse effects on the immune system of Ultraviolet radiation, antibody responses to Hepatitis B vaccination in young adult health care workers were investigated. No effects of UV on the average antibody titers were seen, even if the dose of UV was clearly capable of inducing local immunosuppression. However, it appeared that is subgroups of individuals, depending on their genetic background, suppressive effects of UV could be shown. It is known that UV exposure reduces resistance to Herpes simplex virus, and also effects on Human Papilloma Virus have been claimed.

Both studies indicate that differences in antibody responses may potentially be used as indicators of immunotoxicity.

Pesticides have been shown to exert immunotoxic effects in laboratory animals, but the relevance for pesticide exposure in humans has not been resolved. In an EU sponsored study effects of exposure to diethylendbisdithiocarbamates were investigated in agricultural workers in Finland, Bulgaria, Italy, and the Netherlands. The methods used to investigate the effects of pesticides on the immune system were able to reveal subtle alterations in the status of the immune system, but functional effects on immune responses (i.e. antibody response to vaccination to Hepatitis B) were not observed. Nor were effects on specific IgE antibodies to common allergens, or in fact on clinical allergy detected. Genetic polymorphisms of selected genes among studied populations were not found to affects the outcome of exposure and immunological markers. Exposure under various work place conditions in Europe does not affect the immune system in a biologically significant way.

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CEC2 Biomarkers as Predictive and Mechanistic Tools in Toxicology

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Types of Biomarker and challenges for new biomarkers

John Timbrell

Pharmacy Department, King's College London, UK

For chemicals there are three types of biomarkers: of exposure, of response, of susceptibility. All three biomarkers can be used for evaluation of toxicity of chemicals and risk assessment, because these require: (a) a *knowledge* of the dose of the chemical to which the animals or patient is exposed; (b) a means of detecting and quantifying the *pathological response*; (c) an understanding of the *factors* which affect the occurrence of the pathological response. Biomarkers of exposure can be divided into markers of *internal* dose (eg.metabolites), and of *effective* dose (eg. adducts. For example S-phenyl N-acetylcysteine, a urinary metabolite of benzene, is used as a biomarker of internal dose of the solvent whereas a N7 guanine adduct is a marker of the effective dose of aflatoxin B1. In contrast, biomarkers of response cover a much greater variety of types of parameter. Eg. simple non-invasive markers (eg. body weight), invasive markers (eg. pathological change in tissue), changes in enzymes or in specific endogenous metabolites. Some of these are well known and have long been used experimentally and clinically, such as serum enzymes (eg. LDH, ALT, AST) for detection of tissue damage, or serum levels of metabolites (eg. urea and creatinine) for evaluation of organ function. However there are many organs for which there are few if any biomarkers. For example testicular damage is difficult to detect except with invasive histopathology. We have investigated a potential biomarker, urinary creatine which will be discussed. The detection and utilization of tissue/organ specific metabolites or enzymes is therefore one important avenue for the development of new biomarkers. Similarly for particular types of toxicity specific biomarkers need to be discovered, developed and validated. For example there are no established, validated biomarkers for the non-invasive detection of fatty liver, a common toxic response. However, urinary taurine is increased by chemicals causing fatty liver and will be discussed. Biomarkers of susceptibility reflect variability in the organism, eg. polymorphisms in enzymes involved in the metabolic activation of a chemicals or DNA repair enzymes.There are great challenges for the development of novel biomarkers. The new 'omics technologies offer great promise for this. However, markers proposed must be validated and shown to be specific and robust.

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Use of genomics for mechanistic studies and the development of biomarkers

Jonathan G. Moggs

Syngenta, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ

The recent sequencing of mammalian genomes has driven the development of toxicogenomic technologies, including microarray–based gene expression profiling, which allow the expression levels of thousands of genes to be measured simultaneously. Thus, the application of toxicogenomics has great potential for providing insights into the molecular mechanisms of xenobiotic action. A major challenge in applying toxicogenomics to mechanism-based toxicology is the need to define how xenobiotic-induced changes in gene expression relate to conventional toxicological endpoints. Gene Ontology Mapping, Pathway Mapping and Phenotypic Anchoring of xenobiotic-induced gene expression changes to cellular pathways and biological processes represent key steps in defining these relationships. The utility of applying bioinformatic tools to toxicogenomic data will be illustrated using examples in which they have provided

novel insights into the molecular mechanisms induced by xenoestrogens and non-genotoxic carcinogens in rodents. The potential application of toxicogenomics for the identification of biomarkers for exposure, biological effect and differences in susceptibility to xenobiotics will also be discussed.

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Use of proteomics in development of biomarkers and early markers of toxicity

Stephen Pennington, Neil Kitteringham, Rosalind Jenkins, Tony Hunt, Sally Webb, Christie Hunter

UCD Conway Institute

Many biomarker discovery strategies share a common requirement for the rapid identification and quantification of proteins – however biomarkers are often likely to represent proteins with diverse physicochemical properties. This means that proteomic strategies for biomarker discovery require a range of integrated strategies. Two-dimensional gel electrophoresis (2-DE) and multi-dimensional liquid chromatography (LC) based methods have emerged as powerful approaches for discovery based protein expression profiling but each has significant limitations. Because the liver is the primary site for metabolism of drugs, environmental pollutants and carcinogens, it is important to understand changes in protein expression in response to exposure to these molecules. The cytochrome P450 protein superfamily of mono-oxygenases is the major phase I enzyme system responsible for metabolism of these molecules in the liver. There are four major families of P450s involved in drug metabolism, comprising ∼25 different isoforms, with different substrate specificities and which are inducible by different drugs or chemicals. Because of the role of cytochrome P450 enzymes in determining drug disposition and toxicity, a detailed knowledge of the various protein isoform induction of new chemical entities is a regulatory requirement for pharmaceutical companies engaged in development of novel drug therapies. To understand changes in expression of the P450 proteins and other liver proteins in response to drug treatment, isolated liver microsomal proteins from control and phenobarbitolinduced mice were labelled with stable isotope reagents and analyzed by mass spectrometry – this analysis enabled the simultaneousquantitative analysis of 16 P450 isoforms.

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Use of metabonomics in development of biomarkers/mechanistic studies

Catherine J. Waterfield

Central Toxicology Laboratory, Syngenta, Alderley Park, Cheshire, SK10 4TJ, UK

^{1}H NMR spectroscopy has been used as an open system to study the temporal changes in the biochemical composition of urine in response to adverse toxic events for almost two decades. Early metabon(l)omic studies used pattern recognition methods to help identify sources of variation in urinary metabolite excretion that arise following the administration of biochemicals or drugs to animals. The aim was to identify individual biomarkers or patterns of biomarkers that could be used to predict or monitor for adverse effects particularly tissue/organ specific toxicity and mechanisms of toxicity. This has resulted in a number of endogenous molecules being proposed as individual biomarkers or components of a pattern of biomarkers for renal, hepatic and testicular injury, for example. However many of the markers highlighted are similar for a range of toxicological effects and disease models and therefore not unique for any particular toxicity. Many may reflect non-specific effects related to toxicity/disease, such as weight loss, energy status and gut microflora. These effects can be demonstrated, for example, by using "pairfed" feeding studies. Understanding these non-toxin related changes has enabled the science to evolve and more subtle patterns of markers to be identified for a particular type of toxicity such as peroxisome proliferation, vascular lesions and metabolic disorders. In addition to the identification of individual markers, multiple markers have been used to improve the predictivity of any toxicological effect. The subsequent validation of markers of toxicity that are identified is very time consuming and the transfer of them across toxicology species and then to the clinic is challenging. Studies to understand a biochemical mechanism of toxicity, identification of a marker for drug induced phospholipid accumulation and markers of peroxisome proliferation that could be transferred to the clinic will be used to highlight some of the challenges in this field.

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CEC3 Dealing and Debating about GMOs

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An introduction to GMO

Dusica Vujaklija

Department of Molecular Biology, Rudjer Boskovic Institute, Bijenicka 54, Zagreb, Croatia

Are genetically modified organisms (GMOs) created recently? The answer depends entirely on different points of view; how genetic modification occurred? For thousands years, humans have been selecting and cross-breeding, fashioning plants and animals or more recently microorganisms by altering DNA randomly for the benefits of the mankind. The basis of all these was finally modification of genetic traits. However, the public interest has been focused on the GMOs that have been obtained by genetic engineering also known as recombinant DNA technology, gene cloning or new genetics. This technology comprises methods that could alter or join DNA molecules from different species in vitro and subsequently introduce the hybrid DNA into a host cell creating GMO. The techniques used in gene manipulation are relatively new, their development was stimulated by exciting scientific discoveries from the middle of the last century–from elucidation of the principle of the inheritance, development of the microbial genetics to the discovery of the DNA structure. This initiated intensive research on DNA, genes and genetic code in more detail. However, major milestones in the development of genetic engineering were isolation of the DNA ligase in 1967 and of the first restriction enzyme in 1970. Soon after, in 1972 the first recombinant DNA molecules were generated at Stanford University. The scientists initiated first discussion on the safety of manipulating DNA from the different species and that resulted in a document "Guidelines for Research involving Recombinant DNA". Recombinant human insulin became the first manufactured or commercial recombinant pharmaceutical in 1982, approved by FDA. The use of recombinant technology soon proved to be very important to achieve specific goals in basic, applied science and medicine. Genetic manipulation was of utmost importance for basic research on gene structure and function; it was useful for production of desired proteins, pharmaceuticals and medical diagnosis. However, application of recombinant DNA for generation of transgenic plants and animals and the use of transgenic food re-opened the debate about safety, potential benefits and risk and over the future of genetically modified foods.

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Lactococcus lactis - traditional and GMO strains

Peter R. Jensen, Brian J. Koebmann, Christian Solem

Technical University of Denmark, Denmark

Our work concentrates on lactic acid bacteria, often with *Lactococcus lactis* as a model organism for both fundamental and applied research. The fundamental research is focused on control and regulation of carbon and energy metabolism, nucleotide metabolism, DNA replication, bacteriophages and stress physiology. Our projects are often linked to actual problems occurring in the dairy fermentation industry, where *Lactococcus* is used extensively for cheese production. In many of our activities we use targeted genetic engineering to modulate the activity of enzymes in the cells and study how the physiology and metabolism is affected and the induced changes in cellular make-up for the mutants are monitored using various 'omics techniques.

These strains are made by "self-cloning" i.e. they do not contain heterologous genes from other organisms but have merely been adjusted for the activity of one or more native enzymes by replacing short stretches of non-coding DNA (promoters). Yet, the strains are considered GMOs as far as the European legislation is concerned and must be marked accordingly. In my talk I will compare the method of self-cloning with the traditional methods for strain improvement.

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GMO in Croatia, Legislation and Testing Facilities

Hrvoje Fulgosi, Dušica Vujaklija

Department of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, 10 000 Zagreb, Croatia

Genetically-modified organisms, especially including crops used for food production, are those that have bioengineered genes in their genomes for a variety of reasons including herbicide resistance, pest resistance, enhanced vitamin content and more. Due to the recent enactment of regulations in some countries requiring the disclosure of GMO content in foods, sophisticated systems of the DNA analysis from processed foods and their ingredients have been developed for detection and quantification of GMO content, even when the gene(s) is not expressed.

Croatia has signed the Cartagena Protocol on Global Biosafety on September 8, 2001 and Croatian Parliament has ratified the Protocol on May 24, 2002. At present, the

GMO matters are regulated through the GMO Act. Act is jointly enforced by the Ministry of Health, Ministry of Environmental Protection, Ministry of Agriculture and Forestry, and the Ministry of Science, Education and Sports. Currently, only one certified testing laboratory serves the GMO testing needs of Croatia.

Due to the intended Croatian approach toward the EU, it is likely to expect that Croatian lawmakers will strictly follow recommendations of the European Council.

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The european thematic network on the safety assessment of genetically modified food crops (ENTRANSFOOD)

Harry A. Kuiper

RIKILT- Institute of Food Safety, Wageningen University and Research Center, P.O. Box 230, NL-6700 AE Wageningen, The Netherlands

Despite intensive research efforts assessing the safety of genetically modified (GM) crops, European consumers remain sceptical. ENTRANSFOOD provided a platform for participants from a wide range of different stakeholders recruited from academia, research centres, biotech and breeding companies, food industries, food retailers, regulatory agencies, and consumer groups across Europe. Forty-five Research Centres participated in 5 RTD projects, and 62 experts in 5 Working Groups (www.entransfood.com).

Main objectives of the Thematic Network were:

- to provide detailed guidance on how to perform the safety assessment of GM food crops;
- to develop strategies for the detection and assessment of unexpected effects possibly due to the genetic modification process;
- to assess the risks of transfer of recombinant DNA from GM crops to microbes or human cells;
- to examine the fate of GM raw materials and processed products throughout food production chains (traceability);
- to examine new strategies for the detection of GM raw materials, processed products and food ingredients;
- to examine societal aspects and consumers attitudes towards the introduction of foods derived from GM food crops;
- to establish a communication platform of producers of GM foods, scientists involved in food safety research and in societal aspects of GM food introduction, regulatory authorities, retailers and consumer groups.

Results of ENTRANSFOOD have been published in a Special issue of Food and Chemical Toxicology, 42, issue 7, July 2004, and are summarised in this paper.

Detailed Stepwise Procedure for the Safety Assessment of Foods derived from GM crops

The safety evaluation of food/feed derived from GM crops should be carried out in a comparative manner, i.e. taking traditionally bred food crops as a reference, assuming that these crops are safe to eat based on a long history of use (Substantial Equivalence Concept or Comparative Safety Assessment).

The safety assessment procedure for food and feed derived from GM crops should be conducted in four steps: (i) the description of the parent (recipient) crop; (ii) the description of the transformation process; (iii) the safety and allergenicity assessment of the gene products and metabolites; and (iv) the combined safety and nutritional assessment of the whole plant. The outcome of the safety evaluation is then combined with the intake assessment.

Newly expressed gene products (proteins and metabolites) should be thoroughly investigated using classical approaches for defined chemical substances. Repeated dose studies with recombinant proteins or derived substances are recommended to identify potential adverse long-term effects unless there is sufficient information to confirm the lack of toxicity or pharmacological activity of the recombinant proteins and metabolites.

Any significant differences in agronomic, physiological, and compositional characteristics between the GM crop and the conventional counter part are subject to further testing to assess potential health implications. Selected compositional parameters are representative of the main metabolic pathways in the plant and reflect potential consequences from the introduced trait; the assessment focuses on those that might affect human health, such as key nutrients, anti-nutrients, and allergens.

If the composition of a GM food crop is modified substantially or if there are any uncertainties on the equivalence of its composition to a traditional counterpart, the whole food derived from a GM crop should be tested, and dietary sub-chronic rat studies are recommended.

Current approaches for the assessment of the allergenic potential of novel gene products have been designed by FAO/WHO (2001) and adopted by the Codex Alimentarius Commission (2003). A weight of evidence approach is proposed that classifies newly introduced proteins according to their allergenic poten-

tial. None of the single tests are conclusive and therefore all the available information must be taken into account.

Identification and Assessment of Unexpected Effects due to the Genetic Modification Process

The potential occurrence of unanticipated alterations in the composition of GM food crops as result of the genetic modification process is one of the key elements of the safety assessment procedure. Unintended effects are known to occur in GM food crops, but are not unique for GM organisms, it happens frequently in conventional plant breeding via point mutations as well as chromosomal recombination mechanisms.

For spotting alterations in the composition of a GM organism compared to the parent, a *targeted* approach is used, i.e. measurements of *single known* compounds like macro and micro-nutrients, antinutrients and toxins. The targeted approach has its limitations with respect to a limited and 'biased' selection of compounds, while detection of unknown toxicants or anti-nutrients is not possible.

In order to increase the chances to detect unintended effects, profiling methods have been suggested as a tool for characterisation of changes in the composition of GM plants. The *non-targeted* approach using DNA/RNA micro array technology, proteomics and hyphenated analytical techniques allows profiling of possible changes in the physiology and metabolism of the modified host organism at different cellular integration levels. These techniques are further developed in the GMOCARE project.

The Risk of Gene Transfer from GM Crops

ENTRANSFOOD has evaluated the risks of horizontal gene transfer of recombinant DNA in foods derived from GM crops to microbes or human cells, and the impact of such a transfer event. Gene transfer amongst different organisms is common in nature and has been a driving force in evolution. The risks from gene transfer of GM crops that are currently commercial are deemed negligible.

The risk of use of antibiotic resistance markers for selection of transformed plant cells should for instance be judged on a case-by –case basis, considering their frequency of occurrence in bacterial populations and the extent of clinical use of the antibiotics to which resistance is conferred. On this basis ENTRANSFOOD has proposed a classification of antibiotic marker genes. Markers such as the *nptII* gene and the hygromycin resistance gene can be used without the risk of compromising human or animal health, while the use of antibiotic resistance genes conferring resistance to antibiotics relevant for human or animal therapy should be avoided.

Furthermore recommendations have been formulated concerning detection, traceability and labelling, and also societal aspects of the introduction of GM Foods have been discussed. Regarding the latter it was concluded that if public confidence in science and technology is to be regained, it is important to explicitly incorporate public concerns into the risk analysis process, through developing new and influential methods of stakeholder involvement (including consumers) and consultation.

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CEC4 Genotoxicity and Cell Cycle Control

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DNA repair systems

Bernd Kaina

Department of Toxicology, University of Mainz, Obere Zahlbacher Str. 67, D-55131 Mainz, Germany

DNA repair represents the cell's main shield of protection on genomic level against harmful xenobiotics, food born carcinogens, endogenous carcinogenic species, ionizing radiation (IR), ultraviolet (UV) light and even therapeutic drugs such as anticancer agents. Non-repaired DNA damage has harmful consequences causing inhibition of transcription, replication, faulty cell functioning, chromosomal rearrangements, immortalization and malignant transformation. Several more or less complex repair systems have been evolved: damage reversal by ABH and MGMT proteins, base excision repair, nucleotide excision repair, crosslink recombination repair, mismatch repair and single- and double-strand break repair. Replication blocking lesions can also be tolerated due to error-prone polymerases at the expense of an increased frequency of mutations. In order to recognize DNA damage, sensor systems have been evolved that regulate downstream repair processes and, if this fails, activate cell death functions. These include ATM and ATR kinases, DNA-PK_{CS} and presumably also mismatch repair proteins MSH2 and MSH6 forming the DNA damage binding complex MutSα. The network of interaction of DNA damage signalling, repair and DNA damage response of mammalian cells will be discussed, notably in the context of genotoxicity and apoptosis induced by alkylating agents, IR and UV light.

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Poly(ADP-Ribosyl)ation – An immediate response to DNA damage

Alexander Buerkle

University of Konstanz

One of the most drastic post-translational modification of proteins in eukaryotic cells is poly(ADP-ribosyl)ation, catalysed by a family enzymes termed poly(ADP-ribose) polymerases (PARPs). In the human genome, 18 different genes have been identified that all encode PARP family members. Poly(ADP-ribose) metabolism plays a role in a wide range of biological structures and processes, including DNA repair and maintenance of genomic stability, transcriptional regulation, centromere function and mitotic spindle formation, centrosomal function, structure and function of vault particles, telomere dynamics, trafficking of endosomal vesicles, apoptosis and necrosis. In this presentation, the most recent advances DNA damage-related poly(ADP-ribosyl)ation will be summarized.

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Chromosomal passengers control chromosome segregation and prevent aneuploidy

William C. Earnshaw

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Scotland, UK

Mitotic chromosome segregation is a fundamental cellular process that carries considerable risks for the organism. If errors are made in the attachment of chromosomes to the mitotic spindle, the result can be aneuploidy, or an unequal distribution of the chromosomes. Consequences of aneuploidy include birth defects and cancer. This lecture will discuss what aneuploidy is and how cells avoid it. Particular attention will be paid to the mechanisms that cells use to correct mistakes made by chromosomes when they attach to the mitotic spindle. Correction of these mistakes is carried out by the chromosomal passenger complex of INCENP, Aurora B and survivin, which has essential regulatory roles at centromeres and the central spindle in mitosis. Functional studies show that the passenger complex is required for regulation of kinetochore attachments to the spindle, stability of a bipolar spindle, and cytokinesis. Thus, the chromosomal passenger complex has an essential role in the coordination of chromosomal and cytoskeletal events during mitosis.

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Deregulating the chromosome replication cycle: When epigenetics meets mutagenesis

S. Kirchner

Pharma Research Nonclinical-Development–Safety PRBN-T, F. Hoffmann-La Roche Ltd. CH-4070 Basel, Switzerland

Identification of mutagenic liabilities is a decision relevant part of safety evaluation in early phases of drug discovery and development. Damage to the genetic material can occur in many ways. The process of chromosome replication is very complex and so there is no shortage of targets and things that can go wrong.

Recently increasing evidence evolved suggesting that non-covalent drug/DNA interactions and especially processes associated with chromosome replication may have been underestimated in their role for expressing chromosomal damage. The comparison of Ames test and chromosome aberration results from our in-house screening data base reveals a substantial number of compounds showing only induction of chromosomal damage but no response for bacterial gene mutations. The obvious lack of structural alerts for DNA reactivity implies the involvement of non-DNA primary targets for the observed mutagenicity in mammalian cells. Not surprisingly, the number of agents causing chromosomal damage via indirect rather than direct mechanisms increased in parallel with an emerging new generation of drug candidates that target DNA- and cell cycle associated processes secondarily interfering with the chromosomal integrity. Response patterns are often quite complex and are characteristic of pleiotropic activity on the genetic integrity.

The molecular description and understanding of the chromosome replication cycle has improved rapidly over the last years and elucidated a number of targets and potentially mutational pathways (quite often suggesting aneugenic activity). Interference with key players regulating mitosis, cell proliferation and DNA/chromosomal structure as the aurora and cyclin dependent kinases or HDACs were demonstrated to serve as targets for the induction of numerical and structural chromosome damage. Insufficient target selectivity may be particularly important for the expression and modulation of observed chromosome damage and might also contribute to the mechanistic explanation of so called "high toxicity clastogens" (causing DNA breaks by excessive cytotoxic activity). Our clastogenicity screening data show that a much broader range of agents than would have been predicted based on chemical structure exhibit the ability to induce chromosomal damage and support the involvement of "new" targets and mutational pathways (e.g. including inhibition of different kinases, histone deacetylase or nuclear receptors). The proof for an indirect mode of action, lowest effective concentrations and thresholded dose responses are highly important factors for the risk assessment of such potential drug candidates.

A selection of possible non-DNA targets and mechanisms will be discussed based on in-house MNT in vitro data in early phases of drug discovery.

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CEC5 Clinical Toxicology on its Way to Evidence Based Medicine

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Changes in paradigms – position papers of poisons control centres and clinical toxicologists on gastric decontamination techniques (pre-absorption)

A. Ferrer-Dufol

Clinical Toxicology Unit, University Clinic Hospital, Zaragoza, Spain

Gastrointestinal decontamination techniques have been commonly used to treat poisoned patients to limit absorption of toxic compounds following acute oral exposure. The bases of this therapeutic tool are that poisons that are not absorbed cannot cause systemic toxicity. Over the past fifteen years a broad controversy has arisen on the subject following the results of clinically relevant studies that have failed to show a clear-cut benefit related to gastric emptying, activated charcoal and cathartics. In 1997 Position Statements were produced by an international group of clinical toxicologists chosen by the American Academy of Clinical Toxicology and the European Association of Poisons Centres and Clinical Toxicologists. These Statements, reviewed some years later, indicate that these techniques should not be employed routinely in the managements of poisoned patients. Following that recommendations the use of gastric emptying has been very much reduced and the use of charcoal has risen during the last ten years. The purpose of this lecture is to discuss the commonly used techniques of gastrointestinal decontamination (emesis, gastric lavage, activated charcoal, conventional cathartics and whole bowel irrigation) and present the data that can help to set a rational approach to guide treatment decisions when confronting with an individual poisoned case.

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Recent developments in antidotal therapy

Yesim Tuncok

Dokuz Eylul University School of Medicine

Recent advances in pharmacology and molecular biology have resulted in the development of new and safer antidotes for the management of poisonings. The progress in immunotoxicotherapy may lead to an era when the clinical toxicologist has a vast array of antibody fragments available for use with specific toxic agents. There is also some evidence that humans can be immunized against the effects of certain drugs and

toxins (e.g. nicotine) and while this would reduce the effectiveness of therapeutic medications, it might prove effective in minimizing the effects of poisons that they are likely to encounter as occupational hazards. This lecture addresses antidote stocking, efforts of evidence-based antidote use and new validated effective antidotes. Although the prudent use of an antidote can prevent death and shorten hospitalization in treatment of poisoned patient, the promiscuous use of antidotes may be harmful to the patient and cause an inordinate expense. Unfortunately, it is known that antidotes are insufficiently stocked in health care facilities over the world related to limited resources. As a result of the IPCS/EC project on antidotes, the WHO List of Essential Drugs has been updated and broadened to include a wider range of antidotes and other substances used in the treatment of poisoning, and it is hoped that this will make national health authorities less reluctant to facilitate the importation of these substances. The preparation of internationally evaluated monographs on each of these substances should also be helpful in this respect. The exchanges of information that take place at international meetings are also of great benefit to all those involved. Raising awareness of the importance of antidotes by education, regular review of antidote storage, distribution plans, and appropriate legislation might provide solution. Clinical uses of antidotes tend to vary widely because the data are insufficient to delineate clearly their most safe and effective method of use. It is recommended that a medical toxicologist or poison control center be involved in most cases where antidote administration is considered. Well-designed, randomised controlled clinical trials should be performed to provide reliable data on the clinical features of poisonings and the therapeutic response to antidotes.

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Satellite Symposium Dermal Exposure to Chemicals and Health Risk Assessment

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Evaluation of published QSARs for percutaneous penetration

Tialda Bouwman, Jos G.M. Bessems, Mark T.D. Cronin, Han J.M. Van de Sandt

TNO

The new EU chemicals legislation (REACh) favours the use of (quantitative) structure-activity relationships ((Q)SARs) for evaluation of chemicals. Successful use of QSARs requires characterisation in terms of quality and applicability to predict for certain endpoints.

OECD's Expert Group on QSARs is preparing guidance to facilitate the use of QSARs. Part of this guidance will be based on the characterisation of QSARs in terms of having a defined endpoint, the transparency of the model, its statistical fit and external predictivity, a mechanistic basis and a number of other criteria.

For the characterisation of (Q)SARs for skin penetration the study was performed in four phases:

- Review of (Q)SARs for skin penetration
- Short-listing of promising (Q)SARs
- Additional analyses on selected (Q)SARs, including external predictions
- Considerations on the development of high quality QSARs

Literature review found approximately forty publications with (Q)SARs for skin penetration. Of these, fourteen QSARs were further analysed: twelve for K_p, one for J_{max}, and one for percentage dermal absorption. These (Q)SAR analyses were assessed in depth according to the OECD principles for validation. The most informative statistic was for making predictions for compounds not in the original data set. Many of the models had poor external predictivity with only the models by Patel et al. for K_p and Magnusson et al. for J_{max} providing reasonable predictions for liquids tested neatly.

The study showed that (Q)SARs for skin penetration must be used with care, and values obtained may not be truly applicable for risk assessment; characterisation of (Q)SARs is complex and requires expertise whilst any prediction must be used in a context-dependent framework.

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Significance of metabolism occuring locally in the skin, for dermally absorbed chemicals

Faith M. Williams

University of Newcastle, Newcastle, UK

Dermally absorbed chemicals can be locally metabolised in the skin during absorption but *in vivo* it is difficult to distinguish this metabolism from liver metabolism. Conversion is low and may not affect the bioavailability of the parent absorbed material but may be important for local toxicity. Generally levels of metabolising enzymes in the skin are low compared to the major site of metabolism the liver. Studies with subcellular fractions have showed the presence of metabolising enzymes but with loss of localisation. Flow though diffusion systems with tissue

culture medium as receptor fluid have maintained the viability of skin and supported metabolism but dilution of the metabolites in the receptor fluid has limited detection. Isolated skin in culture has been used to measure metabolism which was not detected in them flow through system. This presentation will discuss recent studies of the expression of xenobiotics metabolising enzymes in the skin and the role of CYPs, alcohol dehydrogenases and esterases in local metabolism. Local markers of toxicity such as DNA damage, protein and DNA adducts and markers of inflammatory response can be related to metabolism.

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Biomonitoring as a tool in the human health risk assessment of dermal exposure

Peter J. Boogaard

Shell Health Services, Shell International BV, The Hague, The Netherlands

The skin is not only an important target organ for local toxicity but also a significant portal of entry for many chemicals. With continuously decreasing exposure limits for inhalation exposure, dermal exposure (DE) becomes relatively more important for systemic toxicity. Health risk assessments (HRAs), however, are often hampered by the absence of reliable DE data. In addition, when external DE is assessed, the extent of dermal penetration often remains unknown. Nevertheless, DE assessment is a standard feature in HRA under the current and future (REACh) EU chemicals legislation, in which the standard approach is to estimate DE with the EASE model. The more sophisticated model RISKOFDERM has also significant limitations as it was validated for only a limited number of chemicals and scenarios. Biomonitoring should be the preferred tool to assess exposure in HRAs since it directly reflects the concentration of a substance or its active metabolite(s) in the receptor compartment, which is most relevant for HRA. Biomonitoring assesses total uptake and with additional data (e.g. air monitoring data) it allows differentiation between inhalation, dermal and oral exposure. In several HRA scenarios RISKOFDERM (like EASE) estimates DE at least an order of magnitude higher than actual total exposure as measured by biomonitoring, which may, for instance, be caused by RISKOFDERM reading across from less volatile analogues. The usefulness of biomonitoring to correct and validate dermal (and other) exposure models will be discussed. Furthermore, recommendations for development of technical guidance regarding the inclusion of biomonitoring data in HRA will be made.

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Novel approaches and strategies for determining percutaneous absorption in humans in vivo

Sanja Kezic

Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Reliable data on percutaneous penetration (PP) are a prerequisite for appropriate health risk assessment (HRA) of dermal exposure. The most relevant PP data, which can directly be used in HRA, come from human volunteer studies. In addition, these data are needed for evaluation of alternative methods such as in vitro assays and mathematical modelling. However, few in vivo volunteer studies have been conducted which are usually explained by ethical and practical constraints as, traditionally, in vivo measurement techniques are either invasive, or require extensive sample preparation or analysis time. Furthermore, the derived PP parameters often differ from those obtained by in vitro assays and predictive modelling thus hampering comparison of data. The development of more sensitive detection techniques enables studying of PP at the exposure levels far below the values associated with adverse health effects. Moreover, advanced data analysis, based on, e.g. mathematical deconvolution or pharmacologically-based pharmacokinetic modelling (PBPK), allow deduction of all relevant parameters, such as permeability coefficient, K_p, and maximal flux, enabling direct comparison with for instance in vitro data. The rise of new technologies, such as FTIR and ATR-IR spectroscopy, is a significant step forward in non-invasive assessment of PP in vivo in humans. Another advantage of these techniques is that dermal absorption kinetics can be determined on real-time scale.

In the presentation, different approaches to assess the topical bioavailability of chemicals after dermal exposure will be discussed including mathematical deconvolution, 'dermatopharmacokinetic' approach, and PBPK. Furthermore, suitability and limitations of novel techniques for measuring of PP including microdialysis and spectrophotometric methods will be reviewed.

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Evaluation of in vivo animal and in vitro models for percutaneous absorption using human in vivo data

Ivone Jakasa

Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Reliable data on percutaneous penetration (PP) of chemicals are increasingly needed. Due to practical and ethical considerations it is impossible to measure dermal permeation in humans in vivo for the many thousands of industrial chemicals in use today. Alternative approaches to predict PP comprise in vivo animal models, in vitro assays and quantitative structure–activity relationships (QSARs). However, the main obstacle in the wider use of these predictive models for health risk assessment is the lack of their validation. Human data on PP which form a golden standard for evaluation of these alternative methods are scarce. In addition, only a limited number of human in vivo studies have been designed for actual in vitro–in vivo comparisons, and data were often generated under different experimental conditions.

In the presentation, the design of parallel in vivo–in vitro studies with the emphasis on the data analysis and experimental conditions will be addressed. Using data on PP of 2-butoxyethanol, assessed both in vivo and in vitro in human and rat skin, factors influencing the results will be discussed. Furthermore, the importance of the volunteer experiments in generating relevant data for human risk assessment as well as the development and implementation of biological monitoring in occupational settings will be highlighted.

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