Finnish Society of Toxicology Annual Symposium

MECHANISTIC TOXICOLOGY
THE KEY TO SUCCESSFUL RISK ASSESSMENT

16-17 May 2002, Kuopio, Finland

Thursday 16 May

9.30 Morning coffee

9.45 Opening
   Jyrki Liesivuori, President of the Finnish Society of Toxicology
   Welcome
   Matti Uusitupa, Rector of the University of Kuopio

10.00 Overall plenary: Threshold for toxicological concern in safety assessment
    Robert Kroes, IRAS, Utrecht University, Utrecht, The Netherlands

   Theme: Mechanisms of neurotoxicity
   Chair: Jyrki Liesivuori, University of Kuopio

10.45 Plenary: Molecular mechanisms of ammonia and glutamate neurotoxicity: role of nitric oxide and cGMP
    Vicente Felipo, Instituto de Investigaciones Citologicas, FVIB, Valencia, Spain

11.20 Role of glutamate in lead neurotoxicity
    Jarkko Loikkanen, University of Kuopio, Kuopio

11.40 Neuronal effects of fumonisin B1
    Helene Stockmann-Juvala, Finnish Institute of Occupational Health, Helsinki

12.00-13.30 Lunch and Posters

   Theme: Immunotoxicity
   Chair: Pekka Karhunen, University of Tampere

13.30 Plenary: Animal models of immunotoxicity studies
    Henk van Loveren, National Institute of Public Health and Environment, Bilthoven, The Netherlands

14.00 The Role of chemokines in the pathogenesis of chronic inflammatory skin diseases
    Harri Alenius, Finnish Institute of Occupational Health, Helsinki, Finland

14.30 Endotoxins and alcohol: cytokine responses by liver macrophages
    Kai Lindros, National Public Health Institute, Helsinki, Finland

15.00 Detection of inflammatory markers in human upper airways in association with bioaerosol exposure
    Marjut Roponen, National Public Health Institute, Kuopio, Finland
**COFFEE**

15.30 **FST/STY Annual Meeting**

19.00 **Dinner at Musta Lammas Restaurant**
   Satamakatu 4

**Friday 17 May**

**Theme: Reproductive toxicity**

**Chair:** Aimo Oikari, University of Jyväskylä

09.00 **Plenary: Dietary phytoestrogens - mechanisms of action and possible role in the development of hormonally dependent diseases**
   Sari Mäkelä, Karolinska Institute, Department of Medical Nutrition, Huddinge, Sweden and University of Turku, Institute of Biomedicine and Functional Foods Forum, Turku, Finland

09.40 **Effects of phytosterols on the European polecat (Mustela putorius) and the field vole (Microtus agrestis) in vivo**
   Petteri Nieminen, University of Joensuu, Department of Biology, Joensuu, Finland

10.00 **Role of p53 in teratogenicity**
   Kirsi Vähäkangas, Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland

10.30 **In utero and lactational TCDD exposure: Effects on prostate development in the mouse**
   Ulla Simanainen, National Public Health Institute, Laboratory of Toxicology, Kuopio, Finland

11.00-12.30 Lunch and Posters

**Theme: Nuclear receptors in toxicity**

**Chair:** Hannu Raunio, University of Kuopio

12.30 **Plenary: The impact of nuclear receptors on toxicology**
   Carsten Carlberg, University of Kuopio, Kuopio, Finland

13.10 **CAR and PXR receptors**
   Paavo Honkakoski, University of Kuopio, Kuopio, Finland

13.50 **Aryl hydrocarbon receptor structure and dioxin sensitivity**
   Merja Korkalainen, National Public Health Institute, Laboratory of Toxicology, Kuopio, Finland

14.30 **Concluding remarks**
Overall plenary

Threshold for toxicological concern in safety assessment

Robert Kroes
IRAS, Utrecht University, Utrecht, The Netherlands

Mechanisms of neurotoxicity

Molecular mechanisms of ammonia and glutamate neurotoxicity: role of nitric oxide and cGMP

Vicente Felipo
Instituto de Investigaciones Citologicas, FVIB, Valencia, Spain

Role of glutamate in lead neurotoxicity

J. Loikkanen¹, J. Naarala², K. Chvalova¹, K. Vähäkangas¹ and K. Savolainen³
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Lead (Pb²⁺) is a widely distributed toxic heavy metal in our environment having several adverse health effects. One of the main targets of lead toxicity is the nervous system. Lead-related neuronal disorders involve lead encephalopathy, hearing disorders and peripheral neuropathy [1]. During the development of central nervous system (CNS) the brain is particularly sensitive to the effects of lead. Therefore, children may develop toxic CNS symptoms at lower doses of lead than adults [1,2]. At present, a lot of attention has been directed towards lead-induced cognitive disorders, such as learning deficits, in children [2]. Several neurotoxic effects of lead may be due to its ability to disrupt cellular calcium homeostasis and to affect the activity of some important signal transduction enzymes (eg. protein kinase C) [2,3]. Moreover, there is evidence for the interaction of lead with the most abundant excitatory amino acid neurotransmitter, glutamate, in the brain [4].

It has been shown that lead inhibits the function of NMDA receptors. These glutamatergic receptor subtypes are important in the regulation of long-term potentiation (LTP) and synaptic plasticity which are essential in the process of learning and memory [4]. Thus, inhibition of NMDA receptors may be one factor contributing to lead-induced cognitive disorders in children [2]. Finally, glutamate may increase neurotoxicity of lead by increasing lead-induced cytotoxicity. This is associated with increased production of reactive oxygen species (ROS), increased caspase-3 activity and internucleosomal DNA fragmentation. Thus, it is evident that glutamate may increase lead-induced apoptosis through oxidative stress in neuronal cells [Loikkanen et al., unpublished results].
Neuronal effects of Fumonisin B₁

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Fumonisin B₁ (FB₁) is a toxin produced by the fungus Fusarium verticillioides. Fusarium fungi are commonly present in mould infected corn, but also in buildings with moisture problems. Hereby the occurrence of FB₁ is highly likely in buildings with water damage and mould growth. FB₁ inhibits the normal metabolism of sphingolipids in cells and it causes for example equine leukoencephalomalacia, porcine pulmonary oedema and human oesophageal cancer.

Almost nothing is known about the mechanisms whereby microbial toxins affect the CNS and because of this the objective of this study was to try to explore the mechanisms whereby FB₁ affects neuronal and glial cells.

Human U-118MG glioblastoma, human SH-SY5Y neuroblastoma, mouse GT1-7 hypothalamic and rat C6 glioblastoma cells were exposed to FB₁ at concentrations of 0.1-100 µM for 0-144 hours. The parameters studied were intracellular glutathione levels (GSH), production of reactive oxygen species (ROS), cell viability and apoptosis (DNA-ladder, caspase-3, Comet assay, p53, MDM-2, Bcl-2-family).

The results show that FB₁ does affect all cell lines, especially after long lasting exposure (72 and 144 hours). Decreased GSH levels were observed in all cells, and decreased ROS production in U-118MG and C6 cells. Also cell viability decreased in all cell lines. Signs of apoptosis could be seen in U-118MG, C6 and GT1-7 cells. Especially U-118MG cells seem to be sensitive to the treatment with FB₁.
Immunotoxicity

Animal models of immunotoxicity studies
Henk van Loveren
National Institute of Public Health and Environment, Bilthoven, The Netherlands

The role of chemokines in the pathogenesis of chronic inflammatory skin diseases
Harri Alenius
Finnish Institute of Occupational Health, Helsinki, Finland

Endotoxins and Alcohol: Cytokine responses by Liver Macrophages
Kai O. Lindros
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This presentation will describe the putative role of gut-derived endotoxin (lipopolysacharide, LPS) in the development and aggravation of alcoholic liver damage (ALD). Elevated circulating levels of LPS, as a consequence of impaired intestinal function, activates Kupffer cells, the stationary liver macrophages, via the CD14 receptor and the TOLL-like receptor(s), and this stimulates secretion of pro-inflammatory mediators. To test the role of LPS in aggravating ALD, an animal model was developed, combining chronic alcohol administration to rats by liquid diet and LPS infusion via osmotic minipumps. The continuous presence of 10-50 times elevated LPS levels only marginally increased early signs of ALD, demonstrated development of marked tolerance to LPS. Elevation of liver pro-inflammatory cytokines TNF-alpha and IL-1-beta was counteracted by increased anti-inflammatory IL-10 expression.

A single nucleotide polymorphism (C-->T(-159)) in the promoter region of the CD14 receptor had been detected and found to confer increased CD14 expression. When this SNP was investigated in a Finnish population, it was found to clearly associate with advanced ALD (alcoholic hepatitis and cirrhosis), indicating that the T allele confers a 3-4 fold increased risk of ALD.

Detection of inflammatory markers in human upper airways in association with bioaerosol exposure
Marjut Roponen
National Public Health Institute, Kuopio, Finland

An association between bioaerosol exposure and adverse health effects has been demonstrated in several epidemiological studies. However, methods to investigate people...
exposed to bioaerosols and a causal relationship between exposure and health outcomes have been limited. Nose is the first part of the respiratory tract that comes into contact with airborne pollutants. Therefore, inflammatory reactions of nasal mucosa could reflect the inflammatory potential of the inhalable agents and, in that way, provide a possible link between exposure and human responses. In this study, the nasal lavage (NAL) method was applied in three occupational environments: employees of the moisture and mould damaged school and office buildings as well as sawmill workers with intense exposure to fungal spores were studied. NAL was performed during the working (exposure) period and during the vacation. Concentrations of inflammatory mediators (nitric oxide, assessed as nitrite, interleukin(IL)-1β, IL-4, IL-6 and tumor necrosis factor alpha (TNFα)) in the NAL fluid were studied. After NAL, subjects filled a short questionnaire concerning their health during the preceding week. Our results indicate an association between inflammatory markers in the NAL fluid, reported symptoms and exposure in moisture and mould damaged indoor environment. Thus, NAL appears to be a useful tool in the evaluation of upper airway inflammation induced by bioaerosol exposure.

Reproductive toxicity

Dietary phytoestrogens - mechanisms of action and possible role in the development of hormonally dependent diseases

Sari Mäkelä
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Effects of phytosterols on the European polecat (Mustela putorius) and the field vole (Microtus agrestis) in vivo


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Plant sterols or phytosterols (PS) are the analogues of animal cholesterol in many plant species. They are added to margarines to lower elevated serum total and LDL-cholesterol levels. PS enter the ecosystem via e.g. pulp mill effluents. PS cause intersexuality and delayed sexual maturation in fish. They accumulate in endocrine glands secreting steroid hormones and can be used as their precursors. The aim of the studies was to perform a preliminary screen on the effects PS have on various endocrine and enzymatic variables of natural mammals. A carnivore (polecat) and a herbivore (field vole) were selected as experimental animals.

PS was administered orally to 32 polecats (0, 1, 5 or 50 mg kg^{-1} d^{-1}) and 31 voles (0, 5 or 50 mg kg^{-1} d^{-1}) for two weeks. PS caused no changes in the body mass or body
adiposity of the animals, but the food intake of the field voles increased at 50 mg PS kg\(^{-1}\) d\(^{-1}\). In the polecats, high PS doses caused an increase in plasma testosterone and estradiol concentrations, but the circulating ghrelin concentrations decreased. In the field voles, the hormonal responses were biphasic. At 5 mg PS kg\(^{-1}\) d\(^{-1}\) an increase in sex steroid concentrations was observed with a lower value of pooled LH concentration. The pooled leptin value decreased and ghrelin value increased. Thyroid hormones were unaffected but the pooled TSH value of the voles decreased due to PS exposure. In the polecats the liver glycogen content increased due to PS together with liver glucose-6-phosphatase activity. Liver lipase esterase activity, on the other hand, decreased. In the field voles, again, the liver glucose-6-phosphatase and glycogen phosphorylase activities were the highest at 5 mg PS kg\(^{-1}\) d\(^{-1}\). Biotransformation enzymes were quite unaffected in both species. In the polecats, serum LDL cholesterol concentrations were the highest at 50 mg PS kg\(^{-1}\) d\(^{-1}\).

The observed increase in sex steroid concentrations can be due to their increased synthesis from PS precursors. This is supported by the lower pooled LH value of the field voles at 5 mg PS kg\(^{-1}\) d\(^{-1}\). The decreased ghrelin levels of the polecats could be a direct effect of PS, as the leptin levels or body adiposity remained unaffected. Also the effects of intermediary metabolism remain unexplained and will need to be clarified in future studies. The observed increase in serum LDL cholesterol levels cannot be taken as a deleterious effect as the polecat is an animal with most of its circulating cholesterol present in HDL—the most abundant lipoprotein fraction of the species. Yet the effects appeared at relatively low doses used e.g. in cholesterol-lowering spreads. Chronic exposure studies are warranted to confirm the results.

### Role of p53 in teratogenicity

Kirsi Vähäkangas
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In utero and lactational TCDD exposure: Effects on prostate development in the mouse

Ulla Simanainen
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Prostate gland develops from the fetal urogenital sinus (UGS). On gestation day 16 in the mouse the prostate development is characterized by the formation of solid buds of basal epithelial cells that invade the mesenchyme. After birth, branching morphogenesis and canalization of the prostatic ducts occur and the basal epithelial cells differentiate into luminal epithelial cells that serve a ductal secretory function. In utero and lactational TCDD exposure impairs development of the mouse prostate in a lobe-specific manner: the ventral prostate is most sensitive, followed by anterior prostate, and dorsolateral prostate. Using aryl hydrocarbon receptor (AhR) knockout mice we found that the disruptive effects of TCDD on prostate development are AhR-dependent. Results of cross-fostering experiments in the mouse established the role of prenatal versus postnatal TCDD exposure on the impairment of prostate growth. We found that TCDD acts both prenatally
and postnataally to inhibit prostate growth. However, prenatal exposure is far more disruptive. The scanning electron microscopy of the mouse UGS epithelium on gestation day 18 (after removal of the mesenchyme by trypsin digestion) revealed decreased formation of prostatic buds from the UGS epithelium in fetuses exposed to TCDD beginning on gestation day 13. Furthermore, to increase our understanding of prenatal prostate development and provide new research directions for understanding how TCDD inhibits prostate development we used Affymetrix™ Murine Genome U74Av2 arrays to examine AhR-dependent, TCDD affected gene expression in the UGS.

**Nuclear receptors in toxicity**

**The impact of nuclear receptors in toxicology**

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Sequencing of the human genome demonstrated that the superfamily of nuclear receptors has 48 members. Characteristic for this transcription factor family is a central, highly conserved DNA binding domain and a carboxy-terminal, moderately conserved ligand binding domain. For 25 members of the family no ligand has been identified so far, they are referred to as orphan nuclear receptors. The remaining family members can be subdivided into 12 “classical” high affinity endocrine receptors (K<sub>d</sub> for ligand binding in the order of 0.1-1 nM), such as those for steroid hormones, thyroid hormone, retinoids and vitamin D, and into 11 adopted orphan receptors, which bind with relaxed specificity and reduced affinity (K<sub>d</sub> in the order of 1-1000 µM) lipophilic compounds, such as fatty acids, oxysterols, steroid metabolites and bile acids. However, common to these 23 nuclear receptors is that their ligands are derivatives of cholesterol and its precursors. Moreover, the relatively large ligand binding pocket of adopted orphan receptors (in the order of 1000 Å<sup>3</sup>) allows them to bind a series of xenobiotics. This makes them not only lipid sensors but also xenobiotic sensors. AhR is another xenobiotic receptor and transcription factor that binds with high affinity dioxins and related compound. Although AhR is not a member of the nuclear receptor family and recognizes clearly distinct binding sites within promoter regions, it shares several protein-protein interaction mechanisms with the members of the nuclear receptor superfamily. These mechanisms include heterodimerization with another family member and the ligand-dependent interaction with coactivator and corepressor proteins. In this way xenobiotics can act as regulators of local chromatin structure and have the potential to activate or repress several nuclear receptor responding genes, such as cytochrome P450s (CYPs) and lipid transporters (e.g. multidrug resistance genes).
Nuclear receptors CAR and PXR

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Nuclear receptors regulate critical physiological functions through modulation of their target gene expression in a ligand-dependent fashion. The synthesis and degradation of endogenous nuclear receptor ligands (steroid hormones, retinoids, vitamin D3, derivatives of cholesterol and fatty acids) are often mediated by cytochrome P450 (CYP) enzymes. CYP expression in turn is controlled by nuclear receptors (Honkakoski & Negishi, 2000). CYPs also metabolize many exogenous compounds such as drugs and environmental pollutants as a part of a defense mechanism against their harmful effects. A central part of this defense mechanism is the induction of CYP gene expression in response to such foreign chemicals. Recent findings indicate that the induction process is controlled mainly by activation of two nuclear receptors CAR and PXR that are closely related to vitamin D3 receptor.

Studies on the regulation of drug- and steroid-metabolizing CYP2B genes identified a drug-responsive DNA enhancer that contained several binding sites for nuclear receptors. Biochemical and co-transfection studies indicated that the enhancer was activated by an orphan receptor termed CAR, which had no known target genes at that time. Other experiments indicated that CYP2B genes are indeed the first identified targets for CAR signalling. Gene disruption studies later demonstrated that CYP2B mRNA induction is absent in CAR null mice, and CAR seems to control of mitogenic and carcinogenic processes initiated by some foreign chemicals. DNA chip technology has recently helped to identify novel CAR-responsive genes.

Studies on the ligand binding properties of orphan receptor PXR indicated that PXR could be activated by both glucocorticoids and anti-glucocorticoids such as RU846. This unusual ligand dependency and PXR tissue expression pattern closely matched those of CYP3A gene expression and inducibility. Studies on human, rat, and rabbit CYP3A gene promoters indicated that PXR could bind to and activate the CYP3A genes in an appropriate ligand and species specificity. Disruption of the PXR gene abolishes the steroid-responsiveness of the CYP3A gene expression while some drugs are still able to activate CYP3A genes through a CAR-mediated process. Previously unsuspected roles for PXR in the metabolism of bile acids and cholesterol homeostasis have also been found.

Development of high-throughput assays for PXR and CAR will provide a rapid preliminary screen to assess potential for CYP induction via a specific receptor. The overlapping ligand and DNA binding properties of CAR and PXR result in receptor cross-talk, which complicates e.g. assessment of CYP inducibility by foreign chemicals.
AH receptor structure and dioxin sensitivity

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Dioxins are ubiquitous environmental contaminants, which are very stable and tend to bioaccumulate in the food chain. TCDD and other dioxins bring about a wide variety of biochemical and toxic effects, most of which are mediated by an intracellular protein called the AH receptor (AHR). A characteristic feature of TCDD toxicity is a large variation in sensitivity among species and even strains of the same species. To the guinea pig, TCDD is the most toxic compound known with an LD50 value of about 1 µg/kg, while the resistant hamster tolerates over 1000-fold higher doses. The same kind of difference exists between two rat lines, the sensitive Long-Evans (Turku AB) and the resistant Han/Wistar (Kuopio; H/W). Cloning of H/W rat AHR revealed a critical point mutation in the C-terminal transactivation domain, which appeared to be the principal reason for TCDD resistance in H/W rats. Therefore, the AH receptors from hamster and guinea pig were cloned and sequenced to better understand the role of transactivation domain in dioxin sensitivity. At amino terminus, hamster and guinea pig AHRs proved to be highly similar to all other mammalian AHRs cloned previously. In the carboxyterminal end, however, hamster AHR differed conspicuously from all other AH receptor proteins. The glutamine-rich (Q-rich) region, demonstrated to be essential and critical for the transactivation function of AHR, was expanded and contained over twice as many glutamine residues as exist in this AHR region of guinea pig. Furthermore, there was a distinct inverse correlation across the published mammalian species between the number of number of glutamine residues and sensitivity to the acute toxicity of TCDD. These results suggest that the Q-rich subdomain in C-terminal transactivation region may be an important contributing factor to the sensitivity differences in TCDD toxicity. Moreover, the guinea pig AHR turned out to be highly homologous to the human AHR, which may be relevant for dioxin risk assessment.
Posters

PROMOTION OF NEOPLASTIC TRANSFORMATION BY 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) IN CELL TRANSFORMATION ASSAY

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3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) is a mutagenic by-product in chlorinated drinking water and it is carcinogenic in rats. The mechanisms of the tumorigenesis of MX in rats are not known. We explored the potential of MX (0.5, 1.0 and 2.0 µg/ml) to act as a promoter in a cell transformation assay in C3H 10T1/2 cells in vitro. 3-Methylcholanthrene (MC, 5 µg/ml) was used as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a positive control promoter. The cells were grown on dishes with an initiator for 3 days (initiation) and after one week washout period with a promoter for 14 days (promotion). Two to three weeks later the cells were fixed, stained and the transformation foci type I, II and III and their sum (the total number of foci) were counted. MC increased all foci types and their total number, and TPA promoted further their development. MX added as an initiator in the test (0.5, 5.0 and 10.0 µg/ml) elicited a similar foci formation as MC alone, added at the initiation phase. When the cells were exposed first to MC (initiation phase) and then to MX (the promotion phase), MX increased the total number of foci and their malignancy in a dose-dependent manner. The results suggest that MX promoted the development of malignant transformation foci initiated by MC.

EXPOSURE TO MITES IN TEXTILE FACTORIES

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In Finland, mite caused occupational diseases increased during the late 1990's. An attempt was made to assess the exposure of textile workers to mites at their work places. Samples of settled dust were collected from four different textile factories: one specializing in weaving, one manufacturing both the fabric and clothes and two factories producing clothes. Dust for mite and allergen (Der p 1) analysis was vacuumed at an approximate rate of 1 minute/m² to a glass fiber filter or on cellulose acetate/cellulose nitrate filters. Mites were counted and identified microscopically. Mite allergen (Der p 1) content of the dust was analyzed by two-site ELISA. 129 workers answered the questionnaire about their background factors, respiratory diseases and symptoms. Their serum samples were analyzed for mite specific IgE-antibodies by AlaStat® Microplate IgE system. Mites were found in 23.6% of the 157 samples studied. Most of the mites belonged to Tarsonomidae and Acaridae, and only a few house dust mites were present. Four samples contained more than 100 mites per gram dust, the suggested limit for mite sensitization. Four
allergen samples out of the 27 were positive for Der p 1, and the concentrations were very low. Four to 46% of the workers had weekly respiratory or irritation symptoms. Most of them considered this to be occupational. 13% of the workers had specific IgE antibodies to one or several house dust or storage mites.

**EFFECTS OF IN UTERO / LACTATIONAL EXPOSURE TO TCDD ON BONE IN RATS.**

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We have previously shown that TCDD treatment alters bone geometry and decreases mechanical strength in adult rats. In this study bone geometry, mineral density and mechanical strength were examined in rats exposed to TCDD in utero / lactationally. Pregnant female rats of TCDD sensitive line C were given a single oral dose of TCDD (0, 0.03, 0.1, 0.3 or 1 µg/kg) on gestational day 15, and female offspring examined on postnatal day 35. Tibial and femoral diaphysis was scanned using a peripheral quantitative computed tomography (pQCT) system. Mechanical properties of tibial and femoral shaft and femoral neck was assessed using three-point bending test and axial loading, respectively. Body weights of offspring were slightly decreased at the highest dose-level only. Tibial and femoral length, as well as diaphyseal cortical and medullary cross-sectional areas were significantly decreased. In addition, cortical mineral density and polar moment of inertia were decreased. Some of these changes were dose-dependent, but reached statistical significance only at 1 µg/kg TCDD. Biomechanical testing revealed significantly decreased bending breaking force and bending stiffness of tibial and femoral shaft, and femoral neck. These changes were only seen at the highest dose level. The results indicate that bone is a sensitive target of dioxin toxicity, and that pre- and neonatal rats are clearly more sensitive to the bone effects than adult rats. (Supported by the Academy of Finland, the Finnish Research Programme on Environmental Health, Project 42551, and the European Commission, Contract QLK4-CT-1999-01446)

**RAT THYROID GLAND TUMORS INDUCED BY 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) DO NOT EXPRESS p53 AND p21 Ki-RAS PROTEINS.**

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3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a disinfection by-product in chlorinated drinking water, induces follicular tumors in thyroid glands in Wistar rats. The mechanisms of the MX-induced thyroid gland tumorigenesis are not known. The
expressions of p53 (primary antibody CM 5) and p21 Ki-ras (primary antibody F234) proteins were evaluated by immunohistochemistry in MX-induced tumors in Wistar rats.

p53 expression was studied in 3 follicular adenomas, 29 follicular carcinomas and two C-cell carcinomas of thyroid glands. p21 Ki-ras expression was studied in 13 follicular carcinomas and one C-cell carcinoma. A weak expression of p53 protein (1-5% of tumor cells) observed in six follicular carcinomas (21%) and one C-cell carcinoma was not considered to be p53-positive. No expression of p21 Ki-ras was observed in any of the samples. These data indicate that p53 and Ki-ras proteins are not overexpressed in the MX-induced thyroid tumors in rats.

COMPETITIVE INHIBITION OF CYP2A5 AND CYP2A6 MEDIATED COUMARIN 7-HYDROXYLATION BY NAPHTHALENE AND ITS OXIDATION IN LIVER MICROSOMES

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The objects of this study were to study if naphthalene inhibits CYP2A5 and CYP2A6 mediated liver coumarin 7-hydroxylation and if these CYPs oxidize it. Recently constructed inhibitory CoMFA model of CYP2A5 predicted that IC50-values of naphthalene is 18 –115 µM while the experimental result was 65 –83 µM. The corresponding values for CYP2A6 are 18 –112 µM and 21-30 µM, respectively. Naphthalene appeared to be a competitive inhibitor both for CYP2A5 (Ki 12-26 µM) and CYP2A6 (Ki 1.2-5.6 µM) coumarin 7-hydroxylation. A one hour in vitro incubation of naphthalene with human and pyrazole treated liver microsomes produced more 1-naphthol than 2-naphthol, the formation of which were inhibited 30 – 60 % by the antibody against the purified CYP2A5. It can be concluded that CoMFA is a useful technique for studing the interaction and potency of untested chemicals with CYP2A5 and CYP2A6 and these enzymes are able to metabolise naphthalene.

CHEMICAL AND IN-VITRO CYTOTOXIC AND PROINFLAMMATORY CHARACTERIZATION OF WINTER AND SPRING PM10 IN HELSINKI

Raimo O. Salonen1, Arja I. Hälinen1, Arto S. Pennanen1, Maija-Riitta Hirvonen1, Markus Sillanpää2, Risto Hillamo2, Tarja Koskentalo3 and Päivi Aarnio3
1National Public Health Institute, Department of Environmental Health, Kuopio, Finland 2Finnish Meteorological Institute, Air Quality Research, Helsinki, Finland 3Helsinki Metropolitan Area Council, Environmental Office, Helsinki, Finland

The ambient air particulate matter (PM) pollution in Finnish cities has large contrasts in different seasons. In mid-winter, the 24-hour concentrations of PM2.5 and PM10 are usually low and the PM originates mainly from regional + long-distance transport and
local combustion sources (especially traffic), whereas in springtime, the 24-hour concentrations of PM$_{10}$ are relatively high and a large proportion of PM originates from resuspension of road dust (sand, asphalt, tyre and stud dust etc.). In Finnish epidemiological studies, the variations in winter- and springtime PM$_{10}$ and PM$_{2.5}$ concentrations have been associated with changes in cardiorespiratory functions among susceptible population groups. The objective of our present study was to compare the winter-PM$_{10}$ and spring-PM$_{10}$ in Helsinki with regard to the watersoluble ionic and elemental contents, and the in-vitro cytotoxicity and proinflammatory activity produced by the watersoluble and insoluble PM$_{10}$ fractions.

The field study was conducted at a traffic site in Helsinki between 5 March and 17 May 1999. Ambient air PM pollution was characterised by continuous monitoring of PM$_{2.5}$ and PM$_{10}$ with a beta attenuation method (Eberline FH 62 I-R). A total of 12 samplings of ambient air PM$_{10}$ were made in 3 to 7-day periods using a High-Volume Low cutoff Impactor (HVLI) at 68 m$^3$/h. The collected HVLI-PM$_{10}$ samples were extracted from the polyurethane foam (PUF) sampling substrate with 100% methanol that was subsequently evaporated in vacuum at room temperature. The extracted PM masses from different samples and periods were pooled together on the basis of the PM$_{2.5}$:PM$_{10}$ concentration ratio (Eberline data) to form larger samples representing three different types of ambient air PM pollution:

1) Winter-PM$_{10}$ during high PM$_{2.5}$:PM$_{10}$ ratio (0.77; low resuspension)
2) Spring I-PM$_{10}$ during medium PM$_{2.5}$:PM$_{10}$ ratio (0.55; medium resuspension)
3) Spring II-PM$_{10}$ during low PM$_{2.5}$:PM$_{10}$ ratio (0.36; high resuspension)

These three pooled samples were characterised chemically and biologically. Watersoluble ions and elements were analysed with ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS). The cytotoxicity (MTT test for functioning mitochondria) and pro-inflammatory activity (NO and cytokine productions) of the PM$_{10}$ samples were tested in a standard murine macrophage cell line RAW 264.7. The macrophages were exposed for 24 hours to five mass doses (30, 100, 300, 1000 and 2000 µg per $10^6$ cells) prepared from each pooled PM$_{10}$ sample. Similarly, two doses of Winter-PM$_{10}$ and Spring I-PM$_{10}$ (300 and 1000 µg per $10^6$ cells) were selected for subsequent tests on responses to the watersoluble and insoluble PM$_{10}$ fractions and on response modification by the iron-chelator, deferoxamine, and the endotoxin-chelator, polymyxin B.

There were no major differences between the three pooled PM$_{10}$ samples with regard to their watersoluble ionic and elemental contents. All three PM$_{10}$ samples induced clear-cut dose-dependent NO production in murine RAW 264.7 macrophages without major differences in potency between each other. Winter-PM$_{10}$ was a significantly less potent inducer of TNF-$\alpha$ production than Spring I-PM$_{10}$ and Spring II-PM$_{10}$. Moreover, it caused practically no IL-6 production, whereas both Spring I-PM$_{10}$ and Spring II-PM$_{10}$ produced at least partially dose-dependent responses. All three PM$_{10}$ samples induced equal reductions in cell viability. With regard to the cell viability and especially cytokine productions, the insoluble fractions of Winter-PM$_{10}$ and Spring I-PM$_{10}$ seemed to be responsible for nearly the whole responses. Polymyxin B abolished the IL-6 production induced by Spring I-PM$_{10}$ and somewhat reduced the TNF-$\alpha$ production induced by both Winter-PM$_{10}$ and Spring I-PM$_{10}$. Deferoxamine did not modify these responses. Neither polymyxin B nor deferoxamine modified the PM$_{10}$-induced reductions in cell viability.

In conclusion, wintertime PM$_{10}$ and springtime PM$_{10}$ had different proinflammatory profiles in a standard murine macrophage cell line. The cytotoxicity and proinflammatory activity of both types of PM$_{10}$ were strongly associated with the insoluble PM fraction, while
soluble constituents, like transition metals, had no major role. The proinflammatory activity of especially springtime PM$_{10}$ seemed to be partially mediated by insoluble endotoxin.

Finally, the *in-vivo* relevance of the present findings needs to be investigated in future studies.

**COMBINED EFFECTS OF ELECTROMAGNETIC FIELDS WITH CARCINOGENIC AGENTS.**

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Objective: This is a summary of several *in vivo* and *in vitro* studies that our group has conducted to study possible combined effects of electromagnetic fields (ELF and RF) with known physical or chemical carcinogenic agents.

Methods: Effects of 50-Hz magnetic field (MF) exposure on UV-induced skin tumorigenesis were studied in mice (1). Further studies with mice evaluated effects of MFs and UV exposures on epidermal ornithine decarboxylase (ODC) and polyamine levels (2), and on apoptosis in mouse skin (unpublished). Combined effects of 50-Hz MFs and UV radiation on cell cycle kinetics and growth were studied in yeast (*Saccharomyces cerevisiae*) cells (3). In addition, the effects of 50-Hz MF on ionizing-radiation-induced carcinogenesis were studied in mice (4). Combined effects of mobile phone-type radiofrequency (RF) radiation with ionizing radiation (5) or UV radiation (unpublished) have been investigated in two long-term carcinogenesis studies with mice. Samples for studying genotoxicity (micronuclei) were taken in these two mouse studies (unpublished). Effect of RF exposure on UV-induced apoptosis was studied in yeast (unpublished). An ongoing project (www.uku.fi/cemfec) evaluates combined effects of RF exposure with the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) *in vivo* and *in vitro*.

Results and discussion: Exposure to 50 Hz MFs did not promote carcinogenesis initiated by ionizing radiation (4), but seemed to enhance skin tumor development induced by repeated UV exposure (1). The latter experiment was a cocarcinogenesis rather than a promotion study, which is one possible explanation for the different results (6). 50-Hz MF exposure also seemed to increase the response of yeast cells to UV radiation (3) and enhanced the effects of UV radiation on apoptosis in mouse skin. The combined effects of UV radiation and 50-Hz MFs might be explained by the radical pair mechanism (the MF flux density was of the order of 0.1 mT in all experiments). Exposure to mobile phone-type RF radiation did not promote carcinogenesis initiated by ionizing radiation (5), and did not statistically significantly enhance skin tumors induced by repeated UV exposure. In a mutant yeast strain that shows apoptotic responses to stress, UV-induced apoptosis was significantly enhanced by pulse-modulated RF radiation similar to that emitted by GSM mobile phones, but not by unmodulated RF radiation at identical specific absorption rates (0.6 or 5 W/kg). There is little evidence that RF radiation enhances the effects of genotoxic carcinogens, but the suggestive positive findings warrant further study.
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