PS 950 Smoking-Induced microRNA Changes in Human Sperm.

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Recent work has suggested that some of the constituents of cigarette smoke, along with other environmental chemicals, can have adverse effects not just on the exposed individuals, but also on their progeny. Although the mechanisms underlying multigenerational toxicity are not well understood, a number of studies have implicated heritable microRNA-mediated epigenetic modifications. Using microarray profiling and pathway analysis, we have shown that cigarette smoke induces specific differences in the spermatozoa microRNA content of human smokers compared with non-smokers, and that these altered microRNAs appear to predominantly mediate pathways vital for healthy sperm and normal embryo development, particularly cell death and apoptosis. MicroRNA-mediated perturbation of such pathways may explain how harmful phenotypes can be induced in the progeny of smokers. Consequently, we have also been developing an *in vitro* system for investigating the potential roles of microRNAs in toxicology. By differentiating embryonic stem cells into embryoid bodies we have been able to generate and subsequently isolate sets of clonal primordial germ cell lines. These cell lines can be produced in the presence and absence of environmental chemicals (for example 17β-estradiol and di-butyl-phthalate), and exhibit chemically-induced differential microRNA expression. This model (including both the embryoid bodies and the resulting primordial germ cell lines) shows promise for further investigating the mechanisms of microRNA-mediated toxicity induced by environmental chemicals, including cigarette smoke.
All work was approved by the Leicestershire, Rutland and Northamptonshire Ethics (Institutional Ethics) Committee and written informed consent to partake in the study was obtained from all volunteers.

PS 951 New Data from Old Studies: Development of qRT-PCR Signature Assays for Assessment of Mode-of-Action from Formalin Fixed Paraffin Embedded Archival Tissues.

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Archival formalin fixed paraffin embedded (FFPE) tissue samples collected over decades from toxicological studies represent a rich source of biological materials with detailed pathological evaluations from the testing of numerous substances. Until recently, the application of 'omics technologies to toxicology studies has been primarily limited to fresh frozen tissue samples collected at necropsy. Archival FFPE tissues represent a rich and largely untapped resource of invaluable information. There is a clear need to develop methods to apply 'omics based technologies to FFPE tissue banks. ILS and ILS Genomics have developed a systematic approach using bioinformatics and laboratory testing for the de novo design of gene specific qRT-PCR assays for use on FFPE archival tissue samples. For these studies, mouse liver carcinogen furan was administered by oral gavage at 0.0, 2.0, 4.0 and 8.0 mg/kg with 5 animals per group for three weeks. Animals were necropsied at 4 hrs after the final administration, liver sections were flash frozen in liquid nitrogen and stored at -80°C. Other liver sections were fixed in formalin for 18-24 hrs, transferred to ethanol for 2 days and then processed into FFPE tissue blocks. Total RNA was extracted from paired frozen and FFPE tissues (10 um sections) for subsequent qRT-PCR analysis. Custom designed qRT-PCR assays using TaqMan technology with amplicons of approximately 70 bp were tested to demonstrate equal primer efficiencies between frozen and FFPE tissue RNA isolations. Using this strategy we have validated a set of 8 genes and 2 lncRNAs (Chek1, Cyp4a14, Egr1, Ephx2, Cdkn1a, Xrcc1, Dppa5a, Gsta1 lncRNA-p21 and lncRNA-Chr.9: 78107225-78118850) and normalization gene Actb to quantify mRNA levels in liver RNA isolated from FFPE compared to frozen tissues. These studies demonstrate that quantitative analysis of mRNA levels from FFPE tissue samples can be done using highly specific mRNA qRT-PCR assays.

PS 952 Identification of Novel Biomarkers for Drug-Induced Renal Papillary Necrosis in Rats by Toxicoproteomic Techniques.

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Renal papillary necrosis (RPN) is a type of kidney injury that is often observed in diabetics and patients taking nonsteroidal anti-inflammatory or anticancer drugs. However, no prognostic biomarkers (BMs) for RPN in humans have been identified. Here, we searched for novel BMs suitable for early RPN detection using toxicoproteomic techniques. Urine from rats with RPN induced by a single injection of 2-bromoethylamine hydrobromide (BEA; 0, 3, 10, 30, and 100 mg/kg) was pooled into 4 groups. Urinary proteins in each group were analyzed by 2-dimensional LC-MS/MS coupled with isobaric tags (iTRAQ) to identify BM candidates. The urinary levels of BM candidates were quantified in individual rats after BEA treatment to assess the ability of these markers to detect RPN in comparison with FDA- and/or EMA-approved BMs for assessing preclinical kidney injury. The identified BM candidates were also measured in the urine of rats with glomerular- or proximal tubular (PT)-injury induced by puromycin, cisplatin, or gentamicin to verify the site specificity of the kidney lesions. In BEA-treated rats, 75 proteins with >2-fold increases in urinary concentrations compared with those in control rats were identified. Among these proteins, the selectivity and sensitivity of Es2, fetuin-A, and fibrinogen for RPN were evaluated. Es2 levels in urine were elevated rapidly after BEA treatment compared to those of serum BUN, creatinine, urinary clusterin, KIM-1, and RPA-1, and were also increased in glomerular- or PT-injury rats, suggesting that Es2 is a highly sensitive, but nonselective for RPN. In contrast, fetuin-A and fibrinogen were selective for RPN, as no marked changes in their urinary concentrations were observed in glomerular or PT-injury rats, but had low sensitivity for RPN detection. In conclusion, we identified three BM candidates for RPN with kidney lesion-site specificity and different sensitivities.

PS 953 Determination of Formaldehyde Specific DNA-Protein-Crosslinks.

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Formaldehyde (FA) is classified as a known human and animal carcinogen. It is a ubiquitous environmental pollutant and is used in a number of consumer products or industrial applications. FA is also endogenously produced as part of normal cellular metabolism. FA is a genotoxic agent, causing a number of effects on cells including DNA monoadducts, DNA-DNA crosslinks and DNA-protein-crosslinks (DPC). DPCs are believed to be one of the critical lesions involved in FA induced carcinogenesis and is thought to provide a key initiating step in the Mode-of-Action. Currently, there are no available methods to distinguish between endogenous and exogenous FA induced DPCs. To investigate the possible link between inhaled FA and the formation of both endogenous and exogenous DPCs, several analytical techniques are being developed. O^6-Alkylguanine-DNA alkytransferase (AGT) is a DNA repair enzyme that is known to form DPCs with FA and other crosslinking agents at the active Cys145 residue. Using AGT as a model protein, a series of experiments were undertaken to understand the formation, stability and degradation of the DNA-protein-crosslink at the reactive cysteine and the N^2 position of deoxyguanosine. Digestion conditions for both DNA and protein cleavage were investigated to determine approaches that would allow for the isolation and identification of either cysteine-CH_2-dG or AGT peptide-CH_2-dG crosslinks using sensitive and selective Liquid Chromatography – Mass Spectrometry. Further experiments investigating the ability to distinguish between crosslinks formed by both [$^{13}CD_2$]-FA and unlabeled FA were accomplished. Further validation and development of these approaches may allow for accurate and quantitative determination of endogenous and exogenous FA specific DPCs in cell culture and animal models. This information will be critical in advancing the understanding of the risks associated with inhaled FA and its role as a human carcinogen.

PS 954 Pulmonary Toxicity and Global Gene Expression Profile in Response to Crystalline Silica Exposure in Rats.

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The ability to detect target organ toxicity as well as to determine the molecular mechanisms underlying such toxicity by employing surrogate biospecimens that can be obtained either by a non-invasive or minimally invasive procedure has significant advantage in toxicology. Pulmonary toxicity and global gene expression profiles in the lungs, blood and bronchoalveolar lavage (BAL) cells were determined in rats 44-weeks following inhalation exposure to crystalline silica (15 mg/m3, 6-hours/day, 5 days). A significant elevation in lactate dehydrogenase activity and albumin content in the BAL fluid as well as histological alterations, mainly type II pneumocyte hyperplasia and fibrosis, observed in the lungs suggested silica-induced pulmonary toxicity in the rats. A significant increase in the number of neutrophils and elevated monocyte chemotactic protein 1 in the BAL fluid indicated silica-induced pulmonary inflammation in the rats. Determination of global gene expression profiles in the lungs, BAL cells, and blood of the silica exposed rats identified 175, 273, and 59 significantly differentially expressed genes (SDEGs) (FDR $p<0.05$ and >1.5 fold change in expression), respectively, compared with the corre-

sponding control samples. Bioinformatics analysis of the SDEGs demonstrated a remarkable similarity in the biological functions, molecular networks and canonical pathways that were significantly affected by silica exposure in the lungs, BAL cells and blood of the rats. Induction of inflammation was identified as the major molecular mechanism underlying the silica-induced pulmonary toxicity. These findings demonstrated the potential application of global gene expression profiling of blood and BAL cells as a valuable minimally invasive approach to study silica-induced pulmonary toxicity.

PS 955 Comparison of a New Skin Penetration System Containing an Automated Toxicokinetic Modul with Franz Diffusion Cells.

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Critical endpoints in in vitro testing of cosmetic ingredients are the determination of the bioavailability of test substances in different skin layers and the examination of the toxicokinetic profile. Skin penetration studies are so far performed in Franz diffusion cells using pig skin. Unfortunately with these cells an automated toxicokinetic determination of percutaneously penetrated substance is not receivable.
To perform toxicokinetic studies, we developed a new Vitrocell systems skin penetration system (SPS) with eight parallel running diffusion cells, which is able to take samples from the receptor fluid automatically. To substitute the Franz diffusion cells with the SPS it is important to compare both systems in terms of performance and reproducibility. Therefore we compared the penetration of caffeine through full thickness pig skin in Franz diffusion cells with manual sampling from the receptor fluid with the prototype of the SPS that provides automated sampling from the receptor fluid.
We could show toxicokinetic profiles for manual and automated samples with comparable lag times and recovery rates. Furthermore we could even show lower standard deviations using the SPS.
In conclusion, the new SPS is highly comparable to the Franz diffusion cell with the additional advantage to allow the automated detection of toxicokinetic profiles from the receptor fluid.

PS 956 Risk Assessment for Cosmetic Ingredients Using Alternative Methods—Skin Sensitization.

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Development of non-animal safety evaluation methods for chemicals is necessary from the viewpoint of animal welfare and to meet the 7th amendment of the European cosmetics directive. As an in vitro skin sensitization test, we have established two methods. One was the human cell line activation test (h-CLAT) detecting augmentation of CD86 and CD54 expression in THP-1 cells exposed by skin sensitizers. The other was the SH test taking advantage of changing cell-surface thiols on THP-1 cells induced by skin sensitizers. Recently the Antioxidant Response Element (ARE) assay measuring oxidative stress caused by skin sensitizers have been attracted attention. However, hazard assessment of skin sensitization has been unable to predict by only one test in vitro yet. Furthermore, non-animal methodologies in risk assessment how much a chemical has skin sensitization potency has just started in earnest. This study attempted to verify how to combine tests in vitro or in silico to assess chemical skin sensitizers. EC3 values from LLNA were accumulated by non-linear analysis using each endpoint from in vitro skin sensitization test and then some descriptors suggesting correlation to LLNA threshold values were selected. Correlation between the descriptors and EC3 values were analyzed by Artificial Neural Network (QwikNet Ver. 2.23). Molecular orbital of the three-dimensional chemical structures was also used in silico. As a result, the model obtained from tests in vitro or in silico reveals good correlation to in vivo data, thus the combination of in vitro and in silico method could be a sophisticated non-animal testing for risk assessment in skin sensitization.

PS 957 Application of a Modified Keratinosens Assay to Predict Sensitization Hazard for Botanical Extracts.

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An essential step in the safety review of cosmetic/personal care ingredients is hazard assessment, including dermal sensitization potential. In vitro methods to identify allergic (haptenic) potential are based on electrophilic interaction with marker peptides or cellular target systems. These assays use a specific molar ratio of the test chemical to the test system. Botanical extract ingredients, as mixtures, preclude specific molar ratio determination. Often, the botanical extract portion is a small fraction of the complete ingredient. To assess these mixtures, the KeratinoSens assay was selected because it operates over a wide dose range and sets cytotoxicity limits on doses used to measure marker gene expression (Emter et al, 2010). Induction of a luciferase gene, under the control of the antioxidant response element (ARE), was measured. Cytotoxicity was assessed by both NRU and MTT assays. Concentrations up to 1 mg/mL (of complete ingredient) were tested and a test dose was considered positive if the fold induction of luciferase was 1.5x (EC1.5) and viability ≥ 70% relative to the solvent controls. The goal of the study was to measure the activity of 3 known sensitizers (gluteraldehyde (GA) [strong], dimethyl maleate (DM) [moderate] and cinnamic aldehyde (CA) [moderate] spiked into four different botanical ingredients (with different excipient solvent systems). Activity was measured, relative to the EC1.5 of the neat sensitizer, as a function of sensitizer concentration and ingredient composition. Three independent trials were performed. No appreciable cytotoxicity was observed. The recovery of the GA spike required at least a 3-fold increase in concentration relative to the chemical alone and one extract reduced the activity below detection. The DM and CA showed activity at about the same effective concentrations as the neat chemicals although the DM showed reduced activity in one extract as well. These data suggest that the KeratinoSens assay has the potential to identify electrophile allergens within a botanical extract ingredient matrix.

PS 958 Development of a Highly Reproducible Three-Dimensional Chinese Skin Reconstructed Model for Evaluating Drug and Cosmetic Skin Irritation.

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In vitro models to study irritation, corrosivity and phototoxicity are important tools for research and development in the pharmaceuticals and cosmetic industries. Human skin is the best possible model for such in vitro studies. The commercially reconstructed human epidermis models are similar to the morphology, lipid composition and biomarkers of native human tissue and have been approved by European Centre for the Validation of Alternative Methods (ECVAM) for the validation of cosmetics. The models are available in many countries but not in China. Here, we describe the development of a constructed three-dimensional (3D) model using Chinese human skin, which consists of a "dermis" with fibroblasts embedded in rat collagen matrix and an "epidermis" comprised of differentiated keratinocytes. The fibroblasts and keratinocytes were first separated from foreskins of Chinese adults after incubating in dispase and collagenase solutions. Then, rat type I collagen was constructed onto the polycarbonate membrane of a culture insert. After gels solidified at room temperature, a collagen matrix with Chinese dermal fibroblasts was constructed above the acellular collagen layer. Keratinocytes were added to the surface of the contracted collagen gels and allowed to attach to the matrix to generate a confluent cellular monolayer. The reconstructed tissues were raised to an air-liquid interface to enable complete stratification and differentiation. After exposure and incubation, MTT assay was performed for cell viability. Nine well-known irritants or corrosive chemicals caused cell viability rates less than 50%. Cosmetics both from Western nations and China were also tested in the model. We found the Western cosmetics did not influence the cell viability compared to some of Chinese cosmetics which dramatically decreased cell viability 1 hour after exposure. Our data indicate that the reconstructed 3D Chinese skin model is highly reproducible and sensitive to assess skin irritation to chemicals and cosmetics.

PS 959 Incorporation of Reconstructed Human Epidermal Tissues into a Corporate Toxicology Laboratory: Use of *In Vitro* Test Data for Diverse Safety and Risk Assessment Applications.

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The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource providing toxicology support to 3M businesses. An objective in the STL is to routinely incorporate *in vitro* test methods, with a recent focus on the use of reconstructed human epidermis tissues. The first model utilized has been EpiDerm™, which is currently validated for classification of chemicals for dermal irritation in OECD 439, and also dermal corrosion in OECD 431. Following the process outlined in the standard protocols for demonstration of technical proficiency, both test methods have been validated and used to assist with classification of chemistries for consumer and industrial use, utilizing the standardized MTT based cell viability assay. In addition, custom protocols have been performed using the tissues in time course irritation studies to evaluate the release of the inflammatory marker cytokine

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