Every year EUROTOX is proud to present young scientists with the EUROTOX Gerhard-Zbinden award for drug-oriented toxicological research, the ECETOC award for toxicological research into mechanisms and risk assessment, the SITOX award for multi-national collaborative research results of a collaboration of a minimum of four researchers from different European countries, and the Bo Holmstedt Poster Award for describing a feasible method for the solution of a toxicological problem under maximum respect of the 3R-principle (Reduce, Refine, Replace animal testing).

This year’s winners are (pictured left to right): Dr. Kathleen Boheme (Gerhard-Zbinden), Dr. Katherina Sewald (ECETOC), Dr. Charles Persoz (Bo Holmstedt), and Dr. Christine Götz (SITOX).

**Abstract**

1. **A novel in vitro system for the toxicological evaluation of genotoxic compounds**
   **Kathleen Boehme**, Yasmin Dietz, Philip G. Hewitt, Stefan O. Mueller Merck KGaA, Institut of Toxicology, Darmstadt, Germany

Genotoxicity is an important issue during pharmaceutical development and chemical risk assessment. In vivo carcinogenicity studies are animal, time and cost-intensive and in vitro tests have relatively low specificity. Furthermore, economic and animal welfare aspects, in particular within the scope of REACH, indicate the importance of new in vitro tools. Previous studies have proven the suitability of toxicogenomic approaches for the predictive classification of genotoxicants in vivo, but in vitro prediction is still a challenge. We used the human cell line, HepG2, for global gene expression profiling of direct-acting genotoxicants. Transcript levels were quantified after treatment with Actinomycin D, Methyl methanesulfonate and Etoposide for 6 h, 24 h and 48 h using Illumina BeadChips. We identified a subset of genes regulated unidirectionally by all compounds, involving p53-dependent apoptosis, chromatin remodelling and oxidative stress pathways. HepG2 cells have limited metabolic capability. Therefore, to expand our model system to include genotoxic compounds that require activation, we established a combined system of HepG2 and ametabolic
activation system (MAS – S9-fraction, microsomes of human and rat donors, and different NADPH sources). To prove the functionality of the HepG2-MAS we determined cytotoxicity, p53 protein induction and micronuclei formation as characteristic endpoints for pro-mutagenic model compounds. The genes identified are potential candidates for the mechanistic characterization of compounds with an unknown mode of action and for the identification of new genotoxicants. Combined with other genotoxicity endpoints as well as the inclusion of metabolic activation might enable a holistic screening system for a broad range of potentially genotoxic compounds.

**Abstract 2**

**Respiratory toxicology and immunotoxicology in human precision cut lung slices (PCLS)**

*Katherine Sewald1,°Ø, Simone Switalla1, LanWang1, Olaf Pfennig2, Christine Förster2, Norbert Krug1, Armin Braun1 1 Fraunhofer ITEM, Immunology, Allergology and Immunotoxicology, Hannover, Germany, 2 KRH-Klinikum Nordstadt, Hannover, Germany*

**Question:** Occupational asthma is one of the most common lung diseases in developed countries. Risk assessment for potentially sensitising chemicals is performed in animal models. With an increasing public demand to limit the number of animals used in respiratory research and to reduce the distress to the animals, several models have been developed. Human PCLS are an ex vivo model where all relevant cell types are present in their natural position. We use PCLS to test for modifications of local immune responses assessing a variety of immunological endpoints.

**Methods:** Human PCLS were prepared from lung lobes of cancer patients. Tissue was exposed to LPS, dexamethasone, respiratory and contact allergens. Viability of PCLS was determined with WST-1, LDH and LIVE/DEAD staining for confocal microscopy. Cytokine contents were detected with Luminex technology and ELISA.

**Results:** Employing LPS and dexamethasone we were able to show that the inflammatory response in PCLS resembles the in vivo situation very closely. Repeated stimulation with LPS (intra- and inter-assay variances <20%) showed that human PCLS might also be suitable to characterize respiratory inflammation induced by chemicals. PCLS were exposed to 20 chemicals and EC50 were calculated. We currently investigate cytokine patterns (e.g. IL-1, TNF, IL-8) for the differentiation between respiratory and contact allergens. Indeed, IL-8 production is increased after stimulation with TMA whereas DNCB failed to induce the release of IL-8 to the same extent.

**Conclusion:** It suggests that the combination of cytokine production with cytotoxic data may represent a promising in vitro model for the screening of allergens.

**Abstract 3**

**Metabolic capacities of in vitro alternatives for chemical testing in skin: Insights from the Colipa skin metabolism project**

*Christine Götz1,°Ø, Karsten Ruwiedel 2, Roland Pfeiffer 2, Ulrike Hübenthal2, Robert Edwards3, Paul Carmichael4, Pierre Aeby5, Carsten Goebel 6, Camilla Pease4, Ellen Fritsche1, 1 IUF gGmbH, Mol. Tox., Düsseldorf, Germany, 2 IUF gGmbH, Mol. Tox., Düsseldorf, Germany, 3 Imperial College, Hammersmith Campus, London, United Kingdom, 4 Unilever, Safety & Environmental Assurance Centre, Sharnbrook, Bedford, United Kingdom, 5 Procter and Gamble, Cosmital, Marl, Switzerland, 6 Procter and Gamble, Darmstadt, Germany*

www.eurotox.com
Human skin represents a large contact site for all kinds of potentially harmful substances. The relevance of testing chemicals for skin irritation, sensitization and genotoxicity in cosmetics is unquestioned, but using animals for that purpose will be prohibited in the EU from 2009 on by the 7th Amendment to the EU Cosmetics Directive. Skin models as alternative testing methods exist, but little is known about the differences in their xenobiotic metabolism capacities compared to human skin. Therefore, the aim of this study is to characterize enzymatic activity of human skin compared to the following skin cell-derived in vitro models: keratinocyte-based cell lines, primary keratinocytes and a three-dimensional epidermal model. Skin was processed to yield cytosolic and microsomal extracts, while the epidermal model was examined in intact form as well as in cytosolic and microsomal preparations. Phase I detoxification enzymes assayed in our project include cytochrome P450 (CYP) and cyclooxygenases, while phase II activity tests are carried out for GST, NAT and UGT. Obtained results of undetectable basal CYP activity were consistent in skin and all models. Production of PGE2 metabolites was measurable in cell lines and epidermal model and will be checked for skin extracts. Preliminary results of phase II detoxification enzymes indicate a good correlation of human skin and skin models in the detoxification of chemicals. Our findings will help evaluating the potential of these in vitro models to serve as alternative toxicological screening methods for human skin. Acknowledgment: This work was funded by The European Cosmetics Association (COLIPA).

Abstract

**An in vitro model to assess the impact on respiratory cells of air pollutants**

Charles Persoz, Christopher Leleu, Sophie Achard, Isabelle Momas, Nathalie Seta Université Paris Descartes, Santé Publique et Environnement, Paris, France

Background: Many epidemiological studies revealed the existence of an association between air pollution (aldehydes, VOCs, etc.) and respiratory disorders. To study such a relationship, it is necessary to have toxic elements to clarify the impact of these pollutants on biological models targets. Toxicology has only current models too often away from the real conditions of exposure. The objective of this project is to develop a toxicological tool suitable to study the cellular impact of pollutants air delivered at low doses and to compare it at more classical in vitro methods. To implement this approach, we were interested in a major pollutant of indoor air: the formaldehyde (CHOH).

Material and methods: In a Vitrocell® exposure chamber, allowing direct contact between the atmosphere and to study the target cells, cells human alveolar epithelial (A549), grown at confluence were exposed for 30 and 60 min at different levels CHO (100–800_g/m3). After 24 h post-incubation, the mitochondrial activity (XTT) and the potential energy (ATP) of cells were evaluated added at the evaluation of the inflammatory response by assay of the chemoattractant Interleukin-8.

Results: No toxicity was measured for 100_g/m3 of CHO, whatever the exposure time, then that from 30 min exposure to 200_g/m3, a reduction of 30% of measured is observed. We can observe a low increase of IL-8 release at the highest concentrations. These results presented here show an in vitro approach, well suited for a reproducible application for constant low concentration of air mixture directly on to the air interface of a cell culture.