REPORT ON

17th Conference of the European Society of Toxicology *In Vitro* (ESTIV)

Lisbon - Portugal

October 16 -20, 2012

SPONSORS:

TUESDAY 16 OCTOBER 2012

13h30-16h30: Preconference workshop: The economics of alternatives

Organised jointly between ESTIV, CAAT and IVTIP.

Chairs: T. Hartung and G. Schoeters.

For the first time in the field of *in vitro* toxicology, a workshop was organised to analyse the cost benefits aspects of the use of alternatives. The speakers with different backgrounds from academia, consulting, small-medium enterprises and big companies presented different views to make this analysis.

Even looking at it from different angles, it became clear that the cost aspect can and will not be a major driver for changing toxicology towards a non-animal approach for the acute and topical endpoints addressed so far.

Cost calculations based upon the predicted requirements for testing of the REACH program, with skin sensitisation and reproductive toxicology as examples, showed that use of alternative tests is complex in the case of developmental toxicity. In the case of skin sensitisation, *in vitro* tests are not necessarily cheaper than *in vivo* tests or human volunteer tests. Costs largely depend on the suppliers and their location.

Industry seeks a balance between better science and profits. *In vitro* tests need to provide better scientific information and increased confidence, but also costs need to be considered. As shown for skin sensitisation, *in vitro* tests can mimic different steps of the biology of skin sensitisation. However, biological processes are complex and as such it is essential not to mimic complexity by *in vitro* tests, but to focus on the most important endpoints, throughput, technical simplicity and robustness.

Ensuring unlimited availability and wide implementation of *in vitro* models and computational models are needed to guarantee economical benefits. The risk to depend on test systems that are validated but later no longer commercially available should be reduced. One way of achieving this is to provide open source tissue models, such as reconstructed skin or eye models, or to provide free software for computational modelling. Wide availability, right to use and distribute as well as technical transparency are major steps towards sustainable alternative testing. *In vitro* data and information on kinetics can waive testing by supporting categorisation of chemicals and read-across approaches. Costs can be reduced by *in vitro* tests searching for similarities and dissimilarities of compounds making *in vivo* testing more efficient and targeted. This was also illustrated by the approach taken by BASF for the identification of compounds with endocrine disrupting properties and by OncoBioTek which uses *in vitro* tests as a cost-effective approach to increase drug target selection for cancer treatment in dogs and humans.

The speakers at the round table discussion came to the conclusion that the advantages of the use of alternatives are clearly animal welfare, the ability to test with low amounts of test substance, the ability to address adverse outcome pathways and the ability to assess human relevance. One of the most important economical benefits of the use of alternative tests is to reduce the time needed for development of a compound. The long period that is currently needed to bring a new compound onto the market is one of the major concerns of pharmaceutical companies for which alternative testing strategies may be of great value.

17h00-19h00: Welcome address and reception

WEDNESDAY 17 OCTOBER 2012

9h00-10h30: Session 1: Dermal toxicity

Chairs: J. Barroso and N. Alépée.

Dr. Barroso opened the session with a talk on the latest developments at the OECD level in the area of dermal irritation and corrosion. In his talk, he introduced all the regulatory *in vitro* methods that can currently be used for skin corrosion and irritation testing and described their applicability and limitations. He then explained that the existing *in vitro* OECD test guidelines (TG) in this area are presently under revision, mostly to clarify their applicability to the testing of mixtures and the applicability of the skin corrosion test methods to subcategorise corrosive chemicals according to the optional UN GHS subcategories 1A, 1B and 1C. While TG 431 (*i.e*. reconstructed human epidermis for skin corrosion) is currently only accepted for discriminating corrosive from non-corrosive chemicals, it is expected that the reconstituted human epidermis (RhE) test methods can subcategorise corrosive chemicals in at least 2 different classes (*i.e.* category 1A *versus* category 1B/1C). To verify this hypothesis, it was agreed at the OECD level that test method developers would test a set of about 80 chemicals in their methods. These data are already available and are currently being reviewed by the OECD. Dr. Barroso explained that subcategorisation of corrosive chemicals is very important for transportation purposes. Without the possibility to subcategorise corrosive chemicals *in vitro*, there is a potential risk that *in vivo* skin corrosion testing may be carried out to avoid a default *in vitro* category 1A classification, which is associated with very stringent transportation requirements. He also explained that TG 439 (*i.e.* RhE test for skin irritation) cannot be used to discriminate between mild-irritants and irritants, as required under certain regulatory frameworks. For such regulatory frameworks, an *in vivo* test or a non-validated *in vitro* test may still be required for distinguishing UN GHS category 3 from UN GHS category 2. These and other issues are currently being addressed by the OECD. The final objective is to develop an integrated testing strategy for skin corrosion and irritation based on a bottom-up or top-down approach to replace, or at least minimise, the use of animals while maintaining safety.

Dr. Alépée presented an industry view on the use of validated *in vitro* test methods according to the OECD TG 404 (*i.e.* acute dermal toxicity) testing strategy. She first presented the data L'Oréal recently acquired using EpiSkin™ and SkinEthic™ RhE on the 80 chemicals selected by the OECD to evaluate the ability of these 2 skin corrosion test methods to subcategorise corrosive chemicals into 2 classes (*i.e.* category 1A and category 1B/1C). Using the appropriate controls for direct MTT reduction, the new data show that both methods are able to differentiate category 1A from category 1B/1C, although EpiSkin™ is able to do it with higher accuracy than SkinEthic™ RhE. L'Oréal also tested many of these chemicals in the RhE skin irritation protocols and Dr. Alépée presented the results of applying bottom-up (*i.e.* start with skin irritation) or top-down (*i.e.* start with skin corrosion) testing strategies to these data. She pointed out that, by using a bottom-up approach with EpiSkin™, there is a slim possibility that a skin corrosive is underclassified as non-irritant. Therefore, L'Oréal always evaluates IL-1α release in the skin irritation protocol in order to increase its sensitivity.

Dr. Groux presented the IRR-IS® test method developed by ImmunoSearch, an EpiSkin™ based method for quantifying chemical irritation potency. This new test method is based on the quantitative analysis of genes expressed in the EpiSkin™ RhE model and is able to predict irritancy of chemicals according to UN GHS, including the differentiation of non-irritants (*i.e.* no category), mild-irritants (*i.e.* category 3) and irritants (*i.e.* category 2). The protocol of the IRR-IS® test method is similar to the protocol of EpiSkin™, but the posttreatment incubation period is reduced from 42 hours to 6 hours and the endpoint measured is gene expression of 13 selected genes instead of cytotoxicity. Data on 45 chemicals showed good overall

performance of the EpiSkinTM IRR-IS® test method (*i.e.* 82% accuracy according to UN GHS rules), with good discrimination of mild irritants (*i.e.* category 3).
Dr. Rocha presented the strategy used by Natura, a Brazilian cosmetics company, to improve the protocol for *in vitro* skin irritation testing of sticky and greasy natural botanicals. Natura used the SkinEthic™ RhE method to test certain types of materials used in their cosmetic products, which have physico-chemical properties, such as lipophilicity, sticky or buttery-like texture, waxy or creamy foam characteristics, that make them difficult to remove from the reconstructed tissue after exposure using normal washing procedures. The mechanical damage induced by more vigorous washing of these materials off the tissues and/or their incomplete removal results in overprediction of their skin irritation potential. Different alternative washing procedures were attempted, and the best results were obtained with the use of 0.1% SDS in PBS solution to remove the sticky and greasy test material *prior* to the normal washing procedure. Histological analysis of tested tissues supported that washing with 0.1% SDS improves the removal of sticky and greasy natural botanicals from the tissues, while not altering the normal responses of the validated RhE test method. The predictive capacity of the refined 0.1% SDS washing procedure was investigated by using commercially available oily and viscous compounds. While the normal washing procedure resulted in 8 out of 10 correctly predicted compounds (*i.e.* 3/3 irritants and 5/7 non-irritants), the refined washing procedure resulted in 9 out of 10 correct predictions (*i.e.* 3/3 irritants and 6/7 non-irritants) and lower variability of results. She clarified that non-ionic milder detergents, such as Tween-20, had also been tried but the results were not as good as with 0.1% SDS.
Dr. Weindl presented the development and use of *in vitro* human tissue models to study infectious and inflammatory skin diseases. Models are available for a wide range of skin disorders. Epidermal and epithelial infection models of localized candidiasis are one example. *In vitro* models have been successfully established to evaluate the effectiveness of topical anti-infectives, to characterize the role of fungal virulence factors and to study the immune responses during *Candida albicans* infections. Recently, these models have been supplemented with immune cells to investigate their role during the course of infection and to characterize the interaction between the skin barrier and accessory immune cells. Skin models were infected with *Candida albicans* and neutrophils were subsequently added to the other side of the filter. The skin models supplemented with neutrophils showed fungi invasion only in the epidermis as opposed to full dermis invasion in the absence of immune cells. Full-thickness skin appears to be promising also for modelling inflammatory diseases and testing of anti-inflammatory agents. The topical application of glucocorticoids under inflammatory conditions allows the evaluation of potential anti-inflammatory and atrophogenic mechanisms and the comparison of their efficacy to other drugs or formulations. The ongoing research in skin disease models offers new approaches for future reduction of animal testing in fundamental research and preclinical drug development

11h30-13h00: Session 2: Ocular toxicity
Chairs: P. McNamee and C. Eskes.
The ocular toxicity session aimed at providing an overview of the current regulatory status on acceptance of alternative methods for eye irritation, the identified gaps where industrial sectors are currently working on progressing the development of relevant assays as well as recent developments on novel models and biomarkers to assess specific *in vivo* ocular effects, such as persistence and stinging. This was achieved through the range of presentations provided.
Dr. Eskes initiated the session by giving an overview on the advances on regulatory acceptance of *in vitro* alternatives for eye irritation and corrosion testing and current

challenges especially in terms of combining different assays into testing strategies that may allow animal replacement.
Dr. McNamee presented the current ongoing activities from industrial sectors on the development of *in vitro* test methods for the evaluation of eye irritation and in particular the importance of understanding *in vivo* drivers for classification.
Dr. Spöler presented the latest advances in test optimisation for the *ex vivo* eye irritation test, an *in vitro* test system proposed to identify substances classified based upon severity and persistency of effects.
Dr. Yamagushi showed the performance of transepithelial electrical resistance as an indicator of eye irritation when using a human corneal epithelium sheet reconstructed in a collagen vitrigel membrane chamber.
Dr. Alépée closed the session with a discussion on using transient receptor potential vanilloid type 1 for evaluating eye stinging potential.

13h30-14h30: Student session
Chairs: M. Vinken and A. Maia.
The first presentation was brought by Astrid Reus from the TNO in Zeist-The Netherlands, who showed the promising potential of *ex vivo* human skin and 3-dimensional models for genotoxicity testing. Indeed, given the current legislation in the field of cosmetics, a positive outcome of *in vitro* genotoxicity testing can no longer be followed by *in vivo* testing, thus necessitating the establishment of more appropriate *in vitro* strategies. In this respect, an *in vivo*-like human skin model was developed, to which formulations can be applied in realistic exposure conditions, coupled with a comet assay and a micronucleus assay. This methodology can be implemented into routine safety evaluation of consumer products.
Yannick Brunner from the University of Applied Sciences Western Switzerland in Geneva-Switzerland presented his work that was targeted towards the establishment of an *in vitro* system for testing testicular toxicity, which is a ubiquitous problem. Specifically, an organotypic model was generated, including Sertoli cells, Leydig cells and early germ cells, that mimicks the *in vivo* situation for up to 2 weeks.
Along the same line, Eva Ramboer, affiliated to the Free University Brussels-Belgium, discussed a novel strategy to counteract dedifferentiation of primary hepatocytes in culture, and thus to establish a long-term liver-based *in vitro* model for testing hepatotoxicity. The new approach is based upon epigenetic modification of hepatocellular gene expression patterns by using histone deacetylase inhibitors as culture medium additives. Focus was put on drug transporters and it was found that the activity of the efflux transporters Mrp2 and Bsep is enhanced by epigenetic modification, thereby demonstrating the potential of this strategy.
The student session was closed by Henriqueta Louro from the National Institute of Health in Lisbon-Portugal. In her work, which was performed in the context of the EU joint action NANOGENOTOX, several types of manufactured nanomaterials were tested in cultures of human lymphocytes. In particular, silicon dioxide, titanium dioxide and multiwalled carbon-nanotubes were evaluated using the micronucleus assay and by counting of micronucleated binucleate and mononucleate cells. It was concluded that this setting, although promising, should be complemented with data from other cell lines and other assays in order to ensure robust genotoxicity testing.
The 4 student presentations were evaluated by a panel of ESTIV and CellTox Executive Board members (*i.e.* Leonora Buzanska, Marisa Meloni, Greet Schoeters and Mathieu Vinken). It was decided that the best student presentation was the lecture of Eva Ramboer, which was awarded with a 250€ prize offered by CellTox.

14h30-16h30: Session 3: Computational toxicology and toxicokinetics

Chairs: E. Casimiro and M. Cronin.

The computational toxicology and toxicokinetics session was focused on the use of *in silico* methods to predict information related to the toxicity and kinetics of compounds. Overall, a clear role for computational approaches in product development and regulatory toxicology was demonstrated. *In silico* models can assist in the prediction of toxicity and understanding of chemical space and properties, although further development is needed in key areas such as chronic toxicity and nanoparticles. Models need to be developed further also in the area of toxicokinetics and to allow proper use of *in vitro* data and their extrapolation to *in vivo* outcomes.

Dr. Cronin reported that there are many uses of computational approaches from screening of compounds to regulatory risk assessment. For toxicity prediction, there are a number of approaches. Read-across from a rationally formed category has seen an increase in popularity, particularly for regulatory risk assessment. Such regulatory use requires appropriate documentation and justification of the prediction.

Dr. Maher stated that there is also a great need to develop methods to predict the toxicity of nanoparticles. A new approach was presented, based upon sequential differential equations, to understand and predict the toxicity of polymeric dendrimer nanoparticles.

The application of computational approaches was also demonstrated by Dr. Detroyer to illustrate whether the chemicals tested in the US EPA ToxCast program are representative of "real-life" chemicals. In this case, a comparison was made with an inventory of chemicals used by a cosmetics manufacturer. It was concluded that ToxCast chemicals are focused on bioactive compounds (*e.g.* pharmaceutical space) and that more testing is required in the area of long chain surfactants, fatty acids, ...

There was also a strong focus on the use of predictive kinetics to provide information on toxicity. In this respect, Dr. Blaauboer confirmed that the modelling of kinetics is vital to enable *in vitro* to *in vivo* correlations. In particular, these correlations as well as understanding of the *in vitro* effect are vital to use these data successfully for risk assessment by combining physiologically-based pharmacokinetic modelling with efforts to calculate the concentration in the test system.

More detailed analysis described by Dr. Broeders demonstrated the nominal concentrations are not useful for using the results from *in vitro* assay. Instead, the free concentration should be used allowing for corrections for the loss of chemicals. This has been shown to correct for variances in different cytotoxicity assays.

Dr. Wilk-Zasadna reported good progress is being made in the validation, led by ECVAM, of *in vitro* systems (*e.g.* HepaRG) for cytochrome P450 induction.

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17h00-18h30: Session 4: Crossing the transatlantic barriers

Chairs: R. Curren and K. Sullivan.

The final session of the first day was a practical session focused on efforts presenters had made over the years or that could be made in the future to increase the acceptance and use of *in vitro* and other non-animal methods. The session was a collaboration between ESTIV and the American Society for Cellular and Computational Toxicology (ASCCT).

Troy Seidle from Humane Society International presented a "London Tube" map of current and future directions which linked worldwide efforts to reduce and replace animals, improve methods for risk assessment and integrate biology and computational science into toxicology. He presented the great strides his team had made in securing reductions in requirements for animal tests in biocides legislation in Europe and how they were applying that model to other regions. He highlighted the linkages that can be made between cosmetics, crop protection, industrial chemical, and other regulatory agencies and industry sectors in order to maximise

the application of replacement and reduction strategies. This short-term activity is undertaken while promoting a shift of regulatory testing science through projects (*e.g.* AXLR8) to eventually transform toxicology testing completely.

Rodger Curren from the Institute for *In Vitro* Sciences took up that theme to look at drivers for regulatory change, focusing on the US FDA in his presentation. He reminded the audience that regulatory sectors necessarily define the appropriate alternatives. For example, pesticides and drugs are designed to be bioactive whereas industrial chemicals are not. High-throughput screening is perhaps more useful for industrial chemicals, since there is a large untested inventory of substances. For pharmaceuticals, the frame of reference for regulators is that drugs will be given to people. The FDA also has a unique opportunity to use human data more than any other sector and has started to take advantage of this opportunity by starting a new "Regulatory Science" effort, to improve regulatory testing and assessment. They have begun a pioneering new partnership with other US agencies to create "Human On a Chip" testing methods that will allow better prediction of the human effects of pharmaceuticals.

A specific example of regulatory harmonisation was presented by Dr. Sullivan from the Physicians Committee for Responsible Medicine. She discussed the current regulatory acceptance of *in vitro* dermal absorption methods for pesticides. North American regulatory agencies do not accept *in vitro* dermal absorption studies alone. The workshop made several recommendations to harmonise testing protocols and these were presented and discussed by Dr. Sullivan and the audience.

Finally, Dr. Stoddart from People for the Ethical Treatment of Animals presented her organisation's work with companies and regulators on both sides of the Atlantic. She discussed how PETA-UK and its international affiliates influenced policy in the US and Europe for shellfish toxicity testing and vaccines potency testing, including differences and similarities between regulatory agencies and how those can be surmounted.

THURSDAY 18 OCTOBER 2012

9h00-10h30: Session 5: Innate immune responses in toxicology

Chairs: E. Roggen and M.T. Cruz.

This session was initiated by a plenary lecture addressed by Stefan Martin from Freiburg University-Germany and was focused on the innate immune and stress responses triggered by contact allergens. An overview of the initial molecular events occurring during skin sensitisation was given with special attention for the degradation of the extracellular matrix component hyaluronic acid, ATP release and production of reactive oxygen species. Those danger signals activate both TLR receptors and the NLRP3 inflammasome, which could be regarded as molecular targets for the development of new therapeutic strategies for the treatment of allergic contact dermatitis as well as for the development of *in vitro* alternatives to animal testing for contact allergen evaluation.

Samuel Constant from Epithelix-Switzerland highpointed the advantages and disadvantages of using *in vitro* human airway epithelia for identifying respiratory chemical sensitisers, thereby highlighting the importance of the genetic predisposition of the donors.

Vicki Summerfield from Unilever-UK, addressed the role of keratinocytes in chemical-induced adaptive immune responses. The results concerning a global transcriptomic analysis in the HaCaT cell line exposed to a skin allergen were presented with special focus on genes that map to components of the MAPK, NF-kB and Nrf2 pathways.

Sylvie Remy from VITO-Belgium presented a study performed in bronchial epithelial cells that aimed at identifying gene markers for the characterisation of respiratory sensitisers. The results demonstrated that the potential to identify respiratory sensitizers in the BEAS-2B cell line is rather low and that the activation of the Nrf2 pathway after stimulation with low-molecular weight chemicals is not specific for sensitisation.

Nynke Kramer from Utrecht University-The Netherlands presented an *in vitro* model to study the regulation of alkaline phosphatase induction in the liver and its release from the cells after an inflammatory insult. The main objective of this model is to improve the search for drugs and techniques to prolong the residence time of alkaline phosphatase in the bloodstream of patients with, for example, rheumatism and a high risk of systemic inflammatory response syndrome.

11h30-13h00: Session 6: Dermal sensitisation

Chairs: G. Maxwell and S. Teissier.

The session was aimed at covering the challenges of applying non-animal test methods for the prediction of skin sensitisation and photo-allergy. Overall, the session provided a comprehensive view of the field, encompassing the challenges of how best to interpret, integrate and evaluate non-animal test methods for skin sensitisation.

The plenary presentation was provided by Dr. Maxwell on the topic of "applying the skin sensitization adverse outcome pathway (AOP) to risk assessment". The presentation incorporated a summary of the current status of non-animal method development and evaluation for skin sensitisation, including how the OECD developed AOP for skin sensitisation can be used as the basis for these activities, and explored how mathematical modelling can be used to predict the human adverse immune response that will be induced following skin exposure to a sensitizing chemical.

The second presentation was given by Dr. Gomes, who presented a summary of how 5 different statistical methods were used to integrate non-animal datasets to predict skin sensitising potential, concluding that the most successful method was a "stacking meta-model" that integrated several of the statistical model predictions to generate a more accurate prediction.

The third presentation was given by Dr. Rocha, who presented an evaluation of 4 *in vitro* skin sensitisation test methods for botanical mixtures covering a LC-MS peptide reactivity test, the KeratinoSens test, the NCTC 2544 test and an epidermal equivalent test. The main question to be answered was whether each test could detect a known sensitising chemical when spiked within a botanical mixture. Based on this preliminary evaluation, the NCTC test and epidermal equivalent test were found to most promising.
The penultimate presentation was given by Dr. Galbiati, who presented her research on the development of an *in vitro* photo-allergen test using NCTC2544 cells, which are capable of differentiating chemical photo-allergens from photo-irritants. It was found that all allergens and photo-allergens produce a dose-dependent increase in IL-18 production, which was not observed with irritants and photo-irritants.
The final presentation was given by Dr. Martins, who summarised his proteomic analysis using MALDI-MS/MS of a Langerhans cell precursor-like cell line following exposure to lipopolysaccharide, the skin sensitiser DNFB and the irritant SDS to identify cellular biomarkers associated with allergic contact dermatitis.

14h00-23h00: Social events: touristic Lisbon tour and conference dinner

FRIDAY 19 OCTOBER 2012

9h00-10h30: Session 7: Carcinogenicity testing

Chairs: M. Vinken and S. Viegas.

The keynote lecture was brought by Dr. Jos Kleinjans from Maastricht University-The Netherlands. He acted as coordinator of the recently ended FP6 project carcinoGENOMICS that was focused on the development of "omics"-based *in vitro* methods for assessing the carcinogenic potential of chemicals. Specifically, sets of genotoxic and non-genotoxic carcinogens as well as non-carcinogens were tested in a number of liver-based, kidney-based on lung-based *in vitro* models. In essence, it was found that the human RPTEC/TERT1 kidney cell line and the human HepaRG liver cell line are promising tools to test genotoxicity and/or carcinogenicity *in vitro*.

The presentation of Dr. Vera Rogiers, member of the Scientific Committee on Consumer Safety (SCCS) and working at the Free University Brussels-Belgium, was dealing with mutagenicity and genotoxicity testing of cosmetic ingredients. For this purpose, a critical evaluation was done of published SCCS safety evaluations in the period 2000-2012. As such, this showed that the classical battery of 3 genotoxicity tests results in a high number of false positives. A series of parameters that could affect this outcome has been identified, including the choice of cell type for investigation. Reduction of the standard battery from 3 to 2 tests was not found to decrease the number of false positives.

In the subsequent talk by Dr. João Barroso, affiliated to Cosmetics Europe based in Brussels-Belgium, an animal-free genotoxicity testing approach for cosmetic ingredients was presented, which can serve as an alternative for *in vivo* follow-up of positive *in vitro* genotoxicity testing results. Basically, these approaches aim at the development of novel *in vitro* models that provide a better prediction of genotoxic potential. This can be achieved by selecting more relevant cells, by using 3-dimensional settings and by maintaining metabolic capacity *in vitro*.

Dr. Cordula Hirsch, working at the Swiss Federal Laboratories for Materials Science and Technology in St. Gallen-Switzerland, discussed a new platform for toxicity testing, including viability, inflammation, oxidative stress and genotoxicity, of engineered nanomaterials. In a first set of experiments, these materials were tested for recognition of interference reactions in cell-free conditions. In a second evaluation round, engineered nanomaterials were studied in 2-dimensional culture systems, with focus on the assessment of robustness, reliability and reproducibility. Preliminary results as well as challenges for future testing purposes were presented.

The session was ended by Dr. Anne von Bergh from the University of Applied Sciences-The Netherlands, who presented the prevalidation of a high-throughput reporter gene assay for the detection of genotoxicity and oxidative stress. The assay uses human osteosarcoma U2OS cells, which respond to p53 and Nrf2 activation, indicative for genotoxicity and oxidative stress, respectively. More than 80 chemicals, among which genotoxic and non-genotoxic compounds, were independently tested in 2 laboratories. The assay produced only a marginal number of false positives and was highly reproducible, thus rendering it a promising tool for early high-throughput and human-relevant genotoxicity testing.

11h30-13h00: Session 8: Reproductive and developmental toxicity

Chairs: L. Buzanska and E. Fritsche.

This session aimed at showing emerging technologies and reviewing different test models used for developmental and reproductive toxicity testing. Developmental toxicity covers a broad variety of alternative methods, embracing different target organs and distinct stages of development started from the earliest stage of stem cell embryonic body formation (*i.e.* embryonic toxicity), covered by Dr. Meganathan (Cologne-Germany), through tissue-specific

developmental toxicity including neural, bone and adipocytic differentiation, discussed by Dr. Fritsche (Düsseldorf-Germany), Dr. Sittner (Berlin-Germany) and Dr. Vinggaard (Soborg-Denmark), respectively. Dr. Durand (Lyon-France) presented a novel *ex vivo* cell culture model of rat spermatogenesis, which maintains the blood testis barrier and allows the assessment of Sertoli cells and different stages of spermatogenesis. The advantage of using zebrafish as a vertebrate model filling a gap of developmental and neurobehavioral endpoints between mammalian studies and *in vitro* cell culture models for DNT was discussed by Dr. Witters (Mol-Belgium). In the presentation by Dr. Oreskovic (USA) concerning nanomaterials in developmental toxicology, the issue of employing proper controls was considered. It was shown that gold nanoparticles, generally known to be bioinert, have been evaluated for their potential to interfere with neurite outgrowth.
In conclusion, current alternative methods for developmental and reproductive toxicity are mainly based on human stem/progenitor cell systems. The adverse effects of the chemicals are usually tested based upon the modes of action approach, giving the better inside to the molecular mechanisms underlying cellular toxicity pathways. Emerging technologies, such as global gene expression profiling, epigenetic analysis and proteomics analysis combined with bioinformatics, are used to investigate these mechanisms. Finally, the important message is that species differences are relevant and have to be seriously considered for the proper predictive value of any test method.

14h30-16h30: Session 9: Systemic toxicity
Chairs: R. Taalman and G. Schoeters.
This session clearly reflected the large potential of alternative approaches for systemic toxicity testing as well as the high expectations from the field to make progress and illustrated the success of interdisciplinary research. Rapid application of innovative approaches are needed for further break-through in the challenging field of *in vitro* toxicology.
The keynote lecture of Paul Jennings introduced the promising use of "omics" to obtain a more complete understanding of molecular processes related to cellular toxicity. The applicability of the combination of transcriptomics with proteomics and metabolomics elucidates pathways of compound-related cellular stress, as shown in human renal proximal tubular epithelial cells. Still challenged by how to deal with large and complex datasets, this rapidly expanding field of molecular toxicology is applied to a diversity of topics which were highlighted during the session and by the posters such as differentiation of human stem cells. It was also clear that emerging health and societal concerns are drivers for test development. Novel assays are being developed to test the negative outcome of chemicals on adipogenesis, thereby addressing the emerging obesity epidemic. Mixtures, which are part of the real world to which humans are exposed, are difficult to deal with in toxicity testing. Computational and empiric *in vitro* approaches intend to model and predict the response of mixtures to enable identification of chemicals that drive toxicity and to support risk assessment of multiple exposures. Safety of nanoparticles was another emerging issue. *In vitro* research has the potential to evaluate both increased efficacy and safety of the nanoparticles. Bioavailability, biocompatibility, correct dosing, *in vitro* simulation of physiological and dynamic microenvironments of nanoparticles were addressed, illustrating the particular challenges related to correct interpretation of results from testing nanoparticles. The session also highlighted the potential of new devices, such as a perfusion bioreactor cell culture of human liver cell spheroids for repeated dose testing. Functional primary human hepatocytes could be maintained over several weeks, thus allowing prolonged dosing, which is important for both safety testing as for preclinical drug development.

16h30-17h00: Closing of the conference and award sessions

SATURDAY 20 OCTOBER 2012

9h00-15h30: Postconference workshop: *in vivo* extrapolation of *in vitro* data in toxicology: state-of-the-art and challenges

Chairs: M. Vinken and B. Blaauboer.

Following the ESTIV2010 conference in Linz-Austria, a questionnaire was sent to the young ESTIV members to inquire about their expectations regarding their involvement in the ESTIV society. From this survey, it became clear that there is an outspoken interest in workshops that cover specific topics related to *in vitro* and *in silico* toxicology. Based upon this request, the ESTIV Executive Board decided to set up a postconference workshop in Lisbon-Portugal, following the ESTIV2012 conference, dealing with *in vivo* extrapolation of *in vitro* toxicological data. As such, the workshop consisted of a theoretical part (*i.e.* lectures in the morning) and a practical part (*i.e.* a computer exercise in the afternoon).

In the first lecture, Prof. Bas Blaauboer from the University of Utrecht-The Netherlands, addressed opportunities, pitfalls and challenges regarding quantitative *in vitro-in vivo* extrapolations (QIVIVE). First, the actual relevance, advantages and disadvantages as well as a historical perspective of *in vitro* toxicology and methodologies were discussed. Thereafter, strategies and difficulties in quantitatively extrapolating *in vitro* cytotoxicity data to the *in vivo* reality were outlined. Particularly, integrated QIVIVE testing schemes and PBPK models were presented. The lecture was ended with some practical case studies, including those of acrylamide and glycolether.

In a subsequent presentation, Dr. Nynke Kramer, also affiliated to the University of Utrecht-The Netherlands, focused on dose metrics in *in vitro* assays. Following a general introduction into dose metrics and *in vitro* assays in toxicology, physico-chemical (*e.g.* pKa) and assay (*e.g.* plastic well plate material) properties that determine free concentrations *in vitro* were discussed. Thereafter, a number of prominent approaches to measure as well as to model free concentrations *in vitro* were presented.

The morning session was ended by Prof. Mark Cronin from Liverpool John Moores University-United Kingdom, who discussed quantitative structure-activity relationships (QSAR), and more specifically what can be learned from the structure and physico-chemical properties of a chemical with respect to its toxicity. At the start of the lecture, an overview of *in silico* and computational toxicology was provided. Subsequently, the applicability domain of QSAR was discussed and its power was illustrated using QSAR-based prediction of LogP as an example, *in casu* in the context of skin permeability. Another case study related to the role of QSAR in metabolism prediction. Finally, some directions for future QSAR purposes were provided.

In the afternoon session, the participants were given the opportunity to gain hands-on experience with a PBPK *in silico* tool. During this exercise, they were continuously assisted by Prof. Bas Blaauboer and Dr. Nynke Kramer.

The workshop, which became fully booked soon after its advertisement on the ESTIV2012 website, was attended by 25 participants, mainly youngsters, from 12 different countries in and outside Europe. There was an active interaction between the lecturers and the participants throughout the workshop. At the end of the workshop, all participants received a certificate of attendance. An evaluation form was sent around to the participants and from this answers received, it was clear that the workshop was considered of high quality and of great practical value.