Abstracts

Symposium

„Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013“

in Honor of Prof. Dr. med. Horst Spielmann

Gesellschaft für
Dermopharmazie

Chair: Prof. Dr. med. Ellen Fritsche, Düsseldorf

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Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Introduction

Ban of cosmetics testing in animals and roadmap for toxicity testing in the EU "Horizon 2020" Program

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The deadline for the 2013 marketing ban in the Cosmetics Directive/Regulation has entered into force on 11 March 2013. This completes a 20 year long process on phasing out animal testing for the purpose of cosmetic safety assessment. Promising progress has been made in advancing alternative methods to animal testing over the last years, but full replacement is not yet possible and will not be possible for some time. The Commission nevertheless believes that the most appropriate way forward is to let the marketing ban enter into force and to turn the challenges that the 2013 marketing ban is posing into an opportunity, in particular by

- ensuring a coherent implementation of the 2013 marketing ban and monitoring its impacts;
- continuing the support for research, development and validation of new alternative methods for human safety testing; and
- making alternative methods an integral part of the EU's trade agenda and international cooperation.

To successfully meet the requirements of the EU cosmetics ban, the EU DG Research and Innovation (R&I) of the EU Commission has funded research on alternatives for local toxicity testing with 238 Mio €. Due to this effort Europe has internationally taken the lead in toxicology. However, so far only in vitro safety tests for local toxicity have been accepted for regulatory purposes, while advanced non-animal toxicity test for all of the other toxicity endpoints still have to be developed. Therefore, the DG R&I should in the upcoming 5-year program "Horizon 2020" provide sufficient funding for funding to replace all safety tests in animal tests by advanced non-animal toxicity tests.

To meet this challenge, the EU FP7 project AXLR8 has proposed "to accelerate the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development". The proposal is based on a concept proposed by the US Academy of Science in 2007 entitled "Toxicity Testing in the 21st Century", in which they suggested to identify "adverse outcome pathways" (AOPs) in human cells and tissues to develop new toxicity tests. The AOP approach has successfully been applied to replace animal tests for skin irritation by an integrated testing strategy (ITS) based on in vitro tests. The AXLR8 Scientific Committee has, therefore, proposes a "roadmap for toxicity testing under Horizon2020" to the DG R&I of the EU Commission. To implement this proposal in a "public private partnership" (PPP) between EU Commission and industry, annual funding of 50-100 Mio€ will be required to achieve the goal of replacing the "classical toxicity tests in animals" by non-animal tests using human cells and tissues.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 1: Dermatotoxicological risk assessment without animal testing

Toxicological Safety Assessment of Cosmetics without Animal Testing

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The bans of the European Cosmetics Directive constitute a big, currently unsolvable challenge for the cosmetic industry. Despite very substantial, long standing efforts science has not yet reached a state that allows comprehensive safety assessment without reliance on animal toxicity data. The cosmetic industry will not compromise on safety. Hence, there will be a loss of existing ingredients and a sharply reduced ability for the industry to innovate with new ingredients, if safety questions cannot be answered based on existing data. The remarkable contributions of the industry to the evolvement on non-animal based test methods and safety assessment strategies have substantially addressed the acute toxicity endpoints. Accepted test systems are already or will be in place in the near future. Of the repeated-dose endpoints the program on alternative approaches to skin sensitization shows promising progress and is targeted to deliver later this decade. A substantial problem lies in the field of systemic toxicity which currently constitutes the main roadblock to safety assessment without animal data. Here, the cosmetic industry is heavily engaged in the SEURAT-1 program, a joint initiative with the European Commission that targets to path the way towards novel toxicology approaches.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 1: Dermatotoxicological risk assessment without animal testing

Tierversuchsfreie toxikologische Prüfmethoden für REACH

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Tierversuchsfreie Methoden für toxikologische Prüfung unter REACH werden fast ausschließlich für die Endpunkte der Mutagenität und der lokalen Toxizität an Haut und Auge eingesetzt. Für die Prüfung der lokalen Toxizität von Chemikalien sind Methoden zur Untersuchung der Hautreiz- und Hautätzwirkung vollständig regulatorisch anerkannt, für die Augenreizwirkungen können zumindest starke Reizwirkungen mit regulatorisch anerkannten Methoden geprüft werden und für die hautsensibilisierende Wirkung ist zur Zeit keine tierversuchsfreie Methode regulatorisch anerkannt. Dennoch ist es auch heute schon möglich alle drei Endpunkte ohne Tierversuche zu prüfen und die Prüfergebnisse für REACH zu verwenden. Dies soll im Folgenden erläutert werden. Daten zu den drei Endpunkten der lokalen Toxizität müssen nach den Anhängen der EU-Verordnung 1907/2006 REACH für alle Chemikalien, die unter REACH registriert werden, erfasst und ggf. neu ermittelt werden, falls aussagekräftige Daten noch nicht vorliegen. In der REACH-Verordnung sind die toxikologischen Informationsanforderungen nach dem Produktionsvolumen und Gefährdungspotenzial der Chemikalien gestaffelt. Pro Substanz werden für die Prüfung der hautsensibilisierenden Wirkung 30 Meerschweinchen oder 20 Mäuse, für die Prüfung der Hautätz- und Hautreizwirkung drei Kaninchen und für die Augenreizwirkung ebenfalls drei Kaninchen eingesetzt.

Zur Prüfung der Hautätz- und Hautreizwirkung war bis vor wenigen Jahren ausschließlich der Test am Kaninchen (OECD Prüfvorschrift Nr. 404) gesetzlich anerkannt. Ende des Jahres 2004 wurde zunächst die Prüfung der Ätzwirkung an der Haut und seit dem Jahr 2010 auch die Prüfung der Reizwirkung an der Haut an rekonstruierten humanen Hautmodellen regulatorisch akzeptiert (OECD Prüfvorschrift Nr. 431 bzw. Nr. 439). Seit 2010 ersetzen beide Methoden die Prüfung der Hautätz- und Hautreizwirkung am Kaninchen vollständig.

Starke Augenreizwirkungen können mit dem Bovine Corneal Opacity and Permeability Test (BCOP, OECD Prüfrichtlinie Nr. 437) geprüft werden. Die hierfür erforderliche Test-Apparatur, das Opacitometer, haben wir entwickelt und validiert. Für die Prüfung schwacher Augenreizung ist nach wie vor ein Tierversuch mit Kaninchen gefordert. Um jedoch auch zur Prüfung der Augenreizwirkung vollständig auf Tierversuche verzichten zu können, haben wir für den Nachweis nicht-starker Augenreizwirkungen ein rekonstruiertes Hornhautmodell (EpiOcular™) intern evaluiert. Auch diese Methode wird derzeit von ECVAM validiert. Aus beiden Methoden haben wir eine Teststrategie entwickelt und validiert, die wir bereits seit zwei Jahren als vollständigen Ersatz des Draize Tests zur Prüfung der Augenreizwirkung am Kaninchen nutzen.

Zur Ermittlung, ob Stoffe beim Kontakt mit der Haut Allergien auslösen können, ist der so genannte Local Lymph Node Assay (LLNA; OECD Prüfvorschrift Nr. 429) mit Mäusen als Methode der Wahl vorgeschrieben. Der LLNA stellt eine Refinement-Reduction-Methode im Sinne des 3R-Konzeptes dar, kommt jedoch nicht ohne Tiere aus. Tests an Meerschweinchen (OECD Prüfvorschrift Nr. 406), sind nach REACH mit besonderer Begründung ebenfalls erlaubt. Hautsensibilisierung ist ein komplexer Prozess, der nicht durch eine einzige in vitro Methode hinreichend abgebildet werden kann. Ausgehend von der Erfassung der ausschlaggebenden Mechanismen, die in den Zellen der Haut ablaufen, wenn Kontaktallergien entstehen, haben wir eine Batterie von drei Testsystemen vorgeschlagen: Den Direct Peptide Reactivity Assay, der die Reaktion von allergisierenden Stoffen mit Proteinen in der Haut widerspiegelt; den LuSens (oder KeratinoSens™) Reportergen Assays, die, wie erwähnt, die Reaktion der hornbildenden Zellen mit den sensibilisierenden Stoffen abbilden; den Monozyten-Aktivierungs-Assays MUSST und h-CLAT, die die Aktivierung der lokal in der Haut befindlichen Immunzellen beinhalten. Diese tierversuchsfreie Prüfstrategie haben wir umfassend evaluiert und nachgewiesen, dass sie dem bislang gesetzlich vorgeschriebenen Tierversuch (dem Local Lymph Node Assay, LLNA) überlegen ist. Allergische Wirkungen von Fremdstoffen am Menschen werden zu 94% korrekt vorhergesagt, eine außerordentlich hohe Prädiktivität, die über der des LLNA liegt (89%).

Wir haben bereits im Herbst 2009 unser eigenes Labor für regulatorische Haut- und Schleimhautprüfungen vollständig auf tierversuchsfreie Verfahren umgestellt. Obwohl die Prüfung der Augenreizwirkung und die Hautsensibilisierungs-Prüfstrategie noch nicht offiziell anerkannt sind, verwenden wir sie bereits heute für anstehende REACH-Dossiers. Mit den drei tierversuchsfreien Teststrategien können – rechtzeitig zur nächsten REACH Phase bis 2018 – 20 bis 36 Tiere pro Substanz eingespart werden.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 1: Dermatotoxicological risk assessment without animal testing

OECD update on local skin and eye toxicity – a never ending story?

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Upon adoption of the 7th Amendment of the Cosmetics Directive 76/768/EEC ten years ago in 2003, of the two proposed deadlines for phasing-out of safety testing in animals, it seemed realistic to achieve the goal of a complete replacement for assessments of local skin and eye toxicity by a battery of in silico and in vitro methods by 2009, while all experts agreed the deadline of 2013 for animal free testing of repeated dose toxicity, reproductive toxicity and toxicokinetics cannot realistically be met. Since also in other regulatory areas there is an international commitment to reduce and, wherever possible, replace toxicity testing with animals, many OECD projects are dealing with refining, reducing and replacing animal based methods. Several of the recent past and current OECD projects are focused on local toxicity of skin and eye. The presentation will cover an update on these activities.

In the area of skin toxicity, project leaders in collaboration with the OECD Expert Group "Skin Irritation and Corrosion" have between 2010 and 2012 revised Test Guidelines (TG) 430 and TG 431 to include Performance Standards for similar "me-too" methods, which was in particular necessary for the Reconstructed human Epidermis (RhE) Corrosion Test (TG 431). In addition TG 431 was revised to cover now the performance of various epidermis models to sub-categorize corrosive test chemicals into potency classes. Furthermore, TG 439 (RhE Skin Irritation Test) was revised to increase the understanding of common elements and differences of the protocols used with the four currently accepted RhE models (EpiSkin, EpiDerm, SkinEthic, and LabCyte). The revised Test Guidelines mentioned have been adopted in April 2013. The currently final task of the OECD Expert Group is the finalization of a German project, the "OECD Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Skin Corrosion and Irritation" which explains the available in silico, in vitro and in vivo tests and proposes an integrated strategy for their use, leaving the Draize skin test as limited a least resource.

In eye toxicity, project leaders in collaboration with the OECD Expert Group "Eye Irritation" have between 2011 and 2012 revised TG 437 (BCOP) and TG 438 (ICE) to enlarge their applicability from the limited positive identification of chemicals causing serious eye damage to the identification of chemicals not requiring the classification of eye irritation (absence of hazard potential). Both revised Test Guidelines mentioned have been adopted in April 2013, while a new Test Guideline on the "Cytosensor Microphysiometer" has not been adopted by the OECD. A new Draft Test Guideline, the "Short Term Exposure for Eye Hazard Potential" (STE Test) is currently published for national comments, and may be adopted in the future. Given the so far limited applicability of all in vitro "eye toxicity" methods adopted by the OECD, there is a strong call for

development of a Guidance Document (analogue to the Guidance Document for Skin Irritation / Corrosion mentioned above).

Thus, although expected 10 years ago, we are not “done” today with fully replacing the need of animal tests for topical toxicity testing, in particular for eye irritation / eye corrosion. The conservative position in in accepting alternative approaches in this area is due to the fact that protecting the eye from damages is regarded of higher value than protecting the skin from damages. However, new meta-analyses of Draize eye test data show for the first time that even the over-sensitive Draize eye test is producing false negative predictions.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 1: Dermatotoxicological risk assessment without animal testing

Skin metabolism in in vitro methods: Impact on skin sensitization and genotoxicity testing using aromatic amine hair dyes as an example of xenobiotics

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Skin is the first site of contact for aromatic amine hair dyes and characterisation of their skin metabolism is relevant to understand local and systemic effects occurring following skin exposure. We have compared the metabolism of key aromatic amine structures used in oxidative hair dyes in human keratinocytes (cell line HaCaT) with that observed in living human skin ex-vivo. N-acetylated derivatives were the major metabolites detected following exposure of HaCaT cells and living human skin, respectively, indicating that N-acetylation was the predominant phase II pathway. In the rat model, topical application was generally associated with a relatively high degree of N-acetylation compared to oral administration where sulfation and glucuronidation were more important. Results from human studies with realistic exposure to hair dye products containing p-phenylenediamine (PPD) and m-aminophenol indicate that N-acetylation of the hair dye precursors as well as the reaction products occurred under realistic use conditions. The predominant role of the enzyme N-acetyltransferase 1 (NAT-1) in aromatic amine skin metabolism is in line with the findings that the majority of the different aromatic amine structures used in oxidative hair dyes exhibit substantial N-acetylation rates when incubated with the recombinant human enzyme. On the other hand, activation of oxidative metabolism was not found in living human skin and could only occur when HaCaT cells were exposed to very high concentrations of PPD. In conclusion, NAT-1 dependent dermal N-acetylation of mononuclear aromatic amine hair dyes is considered to represent a relevant 'first-pass' metabolism effect in the skin by reducing the amount of the parent aromatic amine prior entering the systemic circulation. For the endpoints skin sensitization and genotoxicity in vitro data in vivo data indicate that N-acetylation represents a "first pass" detoxification effect in the skin considered relevant for risk assessment following topical exposure.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 1: Dermatotoxicological risk assessment without animal testing

In-vitro-Testing of Nanomaterials

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A growing exposure to nanomaterials either intended (e.g. drugs, cosmetics) or accidentally (e.g. environmental compounds, workplace exposure) asks for hazard analysis taking the important uptake pathways – gut, lung, skin – into consideration. Yet, the influence of damages and diseases on uptake of the nanomaterials has been neglected for long. With respect to the skin, nanomaterials allow to improve the notoriously poor drug penetration to the site of disease.

Ethics and legislation ask for non-animal testing, several OECD adopted in vitro protocols for endpoints of cutaneous toxicity are established. These protocols, however, are not validated for the testing of nanomaterials. Nanoparticles widely vary in size, lipophilicity/hydrophilicity, surface polarity, rigidity and aggregate formation. These properties influence particles´ cytotoxicity and the penetration into the skin. Moreover, nanoparticles can form a protein corona when making contact with biological fluids. Major progress in biotechnology allows the development of disease models based on reconstructed human skin. The constructs can be used for penetration testing of nanomaterials. The presentation focusses on the evaluation of surface-coated dendrimers, core-multishell nanotransporters and mPEG-coated polyglycerols.

Most advanced nanoparticles are developed, characterized and optimized for drug delivery in skin diseases. Joining regional forces the Collaborative Research Centre 1112 will allow to set-up a broad test-platform for the in-depth characterization of the nanoparticles including safety/toxicity and the interaction with normal and diseased human skin by e.g. human cell-based disease models.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

Reconstructed human skin as disease models

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Tissue engineering has been used to generate human skin equivalents that represent epidermis or epidermis plus dermis (full-thickness skin) in vitro. Several test methods using commercially available reconstructed human epidermis have been validated and adopted by the OECD. Beyond this, advances in biotechnology enabled generation of in vitro models for human skin diseases, in particular to promote dermatological drug research while reducing animal experimentation. Models are available for a wide range of skin disorders including infectious and inflammatory diseases, keratinization disorders and cancer. More complex tissue models have been developed by supplementation with immune cells, such as neutrophils and dendritic cells. This approach allows a detailed dissection of the interaction between the skin barrier and immune cells during the course of infection and inflammation. The availability of human-based skin disease models offers new opportunities for future reduction of animal testing in fundamental research and preclinical drug development. Hopefully, the progress obtained in the field of reconstruction of diseased skin may be a role model in the set-up for models of diseases for other organs.

Dermatotoxicological and other Safety Testing Methods without Animals – State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

Long-term culture of scaffold-dependent skin models

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Skin is one of the few human tissues that allows a high degree of in vitro reconstruction. Accordingly, 3D organotypic cultures (OTCs) or also called raft cultures are extensively used since a long time. OTCs were primarily established by growing human keratinocytes air-exposed on de-epidermized dermis or dermal equivalents made up of type I collagen gels and containing mouse 3T3 cells or human dermal fibroblasts. Although these models provided important insights into the regulation of epidermal differentiation, epithelial-mesen-chymal interactions, and wound healing processes, their limited lifespan remained a major drawback. Because of the restricted survival – these cultures commonly do not survive >4 weeks which equals about one epidermal regeneration cycle - it was suggested that explant and „air-lift" cultures promote differentiation, but not retention of stem cells (de Luca et al. 2006). Inhibiting degradation of the matrix already disproved this hypothesis and demonstrated the importance of an optimal matrix for long-term epidermal regeneration.

Based on a non-woven meshwork of a modified hyaluronic acid meshwork fibers (Hyalograft®) the integrated fibroblasts established an authentic dermal matrix, thus promoting the transition from a wound healing- to a homeostatic-type epidermis with the potential for epidermal long-term (>12 weeks) regeneration. Still suffering from hydrolytic degradation, next generation scaffold-reinforced OTCs were fabricated with inert cellulose-scaffolds, thereby providing a stable matrix. With this setting dermal fibroblasts build a functional stromal tissue thereby preparing the ground for a proper stem cell niche which i) supports long-term epidermal stem cell maintenance and regular epithelial regeneration for >16 weeks, ii) allows for serial epidermal transplantation, iii. study wound healing in a physiological environment of tissue homeostasis, and iv. allowing for reconstruction of skin aging models.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

3D KSS model representing accelerated skin aging

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In previous studies we and others have shown that (i) photoaged human skin, in comparison with intrinsically aged skin, contains increased amounts of the most frequent large scale deletion of human mitochochondrial (mt) DNA, ie. the common deletion (CD) and (ii) that repetitive UV exposure leads to an accumulation of the CD in cultured primary human skin fibroblasts in-vitro as well as in-vivo in human skin. In order to assess whether the accumulation of the CD in skin fibroblasts is causally related to skin aging, we next developed a 3-dimensional dermis model, in which primary human skin fibroblasts from patients with the mitochondriopathy Kearns Sayre Syndrome (KSS) were seeded into a collagen matrix. These cells constitutively carry large amounts of the photoaging-associated CD and thus functional consequences can be studied without the need for UV irradiation, which might cause multiple biological effects independent of mtDNA mutagenesis. We observed that KSS cell containing dermal equivalents (KSS DE) contained increased amounts of the CD over a 6 week culture period, when compared to DE which had been generated with normal human skin fibroblasts matched for donor age, skin site and cell passage number (NHF DE). Interestingly, KSS DE showed an increased expression of "senescence associated secretory phenotype (SASP)-associated genes" including VEGF, MMP-1, IL-8 and IL-6, which are typically found to be increased in vivo in photoaged human skin. By focusing on MMP-1 we next showed that expression of this SASP-like phenotype was of functional relevance for dermal photoaging. Accordingly, in contrast to NHF DE, in KSS DE increased MMP-1 activity was detected which was associated with an increased breakdown and rarefication of collagen fibers and a concomitant increase in collagen fiber fragments, ie structural alterations which are a hallmark and strongly reminiscent of photoaged human skin. Importantly, this phenotype developed within 1 – 6 weeks and thus in a greatly accelerated fashion, as compared to photoaging of human skin, which usually takes decades to develop.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 2: Susceptibility factors and disease models in reconstructed human skin

3D wound healing models

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3D organotypic skin equivalents for long-term culture enable demtotoxicological testing methods including wound healing studies. Punch biopsies and couter are not suitable to produce standardized injuries in 3D skin models. A comparison of substances that enhance wound healing is difficult. Using ultrapulsed fractionated CO2 laser multiple standardized injuries with defined dimensions can be set in 3D skin models. Investigation of wound healing on histological and molecular level is possible. In further studies 3D skin models could be used to examine the influence of different lasersystems on epidermal and dermal structures in vitro. This new laser technology is useful to find biomarkers for wound healing in skin as well as to investigate cellular events associated with abnormal wound healing (chronic, nonhealing wounds) and also to test wound healing potential of new approaches in this field.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 3: New challenges for toxicological safety testing

Human-on-a-Chip

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Current in vitro and animal tests for drug development are failing to emulate the systemic organ complexity of the human body and, therefore, to accurately predict drug safety and efficacy. Microfluidic culture devices combining micro-tissues of the ten (at least) most important human organs in a human-like arrangement at homeostatic steady state are supposed to become a translational solution for that testing dilemma. The presentation highlights the most recent developments in that so called “Human-on-a-chip” development arena. In particular, it focusses on a universal microfluidic chip platform the area of a microscopic slide, consisting of an on-chip micro-pump and, in a first design, capable of interconnecting two different organ equivalents. The micro-pump ensures stable long-term circulation media through the tissue culture compartments at variable flow rates, adjustable to the physiological mechanical stresses of the respective tissues. The tissue culture compartments and the connecting channels are optically accessible, thus supporting live tissue imaging. Co-cultures of human liver and skin equivalents, on the one hand, and liver equivalents and neuronal tissues, on the other hand, have proven the ability to culture these tissues over weeks at steady state. Furthermore, the connecting channels could be covered with human endothelia mimicking blood transport vessels. The system layout and chip design support repeated substance exposure for safety or efficacy test assay development. Toxicity assays have been performed using the co-cultures mentioned above. Finally, rapid prototyping tools allow for the addition of up to ten further tissue culture spaces to the MOC platform. Platform performance will be analysed against the existing chip-based co-culture systems. Opportunities and challenges are discussed against the background of other developments in the field.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 3: New challenges for toxicological safety testing

Embryotoxicity Testing with the High-Throughput EST

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At Roche, the Embryonic Stem Cell Test (EST) is routinely employed to detect potential teratogenic/ embryotoxic liabilities during lead optimization of early pharmaceutical drug candidates. Modifications to the assay design and prediction model considerably improved reproducibility of data and improved the prediction for pharmaceuticals. A recently updated retrospective analysis of all proprietary compounds tested in the EST showed a >90% concordance to rodent embryo-fetal development studies, whilst maintaining a low false positive rate. In our hands, the EST has now shown sufficient robustness and predictivity to allow for the assay to become a major decision-maker during lead optimization. As a consequence, the throughput and turnaround times of the EST needed to be adjusted to allow for parallel and regular testing of lead compounds on a weekly basis. This was achieved by developing a novel culturing tool, the Hanging Drop Culture Plate (HDCP) that allows for automated generation and differentiation of high quality embryonic bodies. With this bottleneck solved, a specially designed, fully automated platform combining compound handling, dose-response plates, cytotoxicity and differentiation test was developed. The updated historical EST analysis with case-studies will be presented as well as the results of the validation of the first fully automated HDCP-EST assay, and ongoing efforts to implement the next generation liquid handling platform for the EST.

Dermatotoxicological and other Safety Testing Methods without Animals - State November 2013

Session 3: New challenges for toxicological safety testing

Applicability of rat precision-cut lung slices for nanomaterial toxicity testing

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and Sandra Vogel, Alexandra Aumann, Annemarie Hess, Susanne N. Kolle, Lan Ma-Hock, Wendel Wohlleben, Martina Dammann, Volker Strauss, Silke Treumann, Sibylle Gröters, Karin Wiench, Bennard van Ravenzwaay, Robert Landsiedel, BASF SE, Ludwigshafen

The applicability of rat precision-cut lung slices (PCLuS) to predict nanomaterial respiratory toxicity was investigated. Sixteen OECD reference nanomaterials (NMs) (TiO2, ZnO, CeO2, SiO2, Ag, multi-walled carbon nanotubes (MWCNT)) were evaluated thereby covering dissolving and non-dissolving materials just as fibrous NMs. The addressed in vitro effects reflect the spectrum of early events that, to date, have been recognized for NM toxicity: total protein, reduction in mitochondrial activity, caspase-3/-7 activation, glutathione depletion/increase, cytokine induction. Additionally, the lung slices were submitted to histopathological evaluation. Ion shedding NMs (ZnO and Ag) induced severe tissue destruction detected by loss of total protein. Two anatase TiO2 NMs, the CeO2 NMs, and two MWCNTs caused significant (determined by trend analysis) cytotoxicity in the WST-1 assay. At non-cytotoxic concentrations, different TiO2 NMs and one MWCNT increased GSH levels, presumably a defence response to reactive oxygen species, and these substances further induced a variety of cytokines. One of the SiO2 NMs increased caspase-3/-7 activities at non-cytotoxic levels, and one rutile TiO2 only induced cytokines. Hence, PCLuS can detect different early effects of NM toxicity. Investigating these effects is, however, not sufficient to predict apical effects found in vivo. Reproducibility of test substance measurements was not fully satisfactory, especially in the GSH and cytokine assays. Effects were frequently observed in negative controls pointing to tissue slice vulnerability even though these prepared and handled with utmost care. Qualitative comparisons of in vivo to in vitro effects reveal some concordances for the metal oxide NM, but less so for the MWCNT. The highest effective dosages, however, exceeded those reported for in vivo rat short-term inhalation studies. For NM testing, the PCLuS system requires test protocol optimization, and study results should be considered preliminary.

Dermatotoxicological and other Safety Testing Methods without Animals – State November 2013

Session 3: New challenges for toxicological safety testing

3D in vitro methods for developmental neurotoxicity testing

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As intelligence is one of the most important capitals for society, protection of developing brains against any harm is desirable. The current standard method for testing of compounds for developmental neurotoxicity (DNT) is the rat in vivo test. To study DNT in the rat it is very expensive, time consuming and the predictivity for humans is questionable. Therefore, we have developed an in vitro method for DNT testing which is based on human neurospheres. Such neurospheres are three dimensional (3D) cell culture models consisting of neural progenitor cells (NPCs) which proliferate in culture and - under differentiating conditions – migrate and differentiate into neurons and glia cells. Thereby, they mimic the very basic processes of brain development in vitro: proliferation, migration and differentiation of premature brain cells.

We tested the effects of a variety of compounds on proliferation, migration, differentiation and viability of NPCs. Moreover, we compared data of human neurospheres to results we gained in rodent NPCs. This comparison allows an inter-species comparison of toxicodynamics. In the case of dissimilarities in effects of compounds across species we investigate the underlying signaling pathways driving compound-dependent disturbance of neurodevelopmental processes. Our results indicate that neurospheres can distinguish between positive and negative test compounds and that a variety of pathways are differentially expressed across species causing species-specific sensitivities towards different substances. Moreover, we can utilize such 3D systems for DNT testing in a medium-throughput system. More data is needed to understand to which extent such 3D systems reflect processes of human developing brains in vivo.

In summary, human and rodent neurospheres might be suitable 3D in vitro systems for assessing toxic potentials of compounds on neurodevelopment or identify compounds which are neuroprotective.