Session I-19: Toxicity testing strategies – progress in skin sensitization testing: A COLIPA supported session

Session I-19: Oral presentations

I-19.704

Considerations for the development of an integrated testing strategy for skin sensitization

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Skin sensitization is a crucial endpoint for the safety assessment of cosmetic ingredients with significant social and economic impact. Currently, the mouse local lymph node assay (LLNA) is the standard stand-alone test method which allows the acquisition of potency data needed for risk assessment. A major challenge for the cosmetic industry is the development of skin sensitization safety assessment strategies for ingredients with non-animal data due to the 2013 ban. Therefore Beiersdorf undertakes considerable efforts to evaluate available tools, which include in silico methods, chemical reactivity predictions based on physico-chemical properties, indications for structural alerts (structure-activity relationship: SAR) and read-across of historical data. All of these should be integrated into quantitative risk assessment (QRA) concepts based on weight of evidence as well as in vitro methods. Moreover threshold concepts and metabolism studies have to be incorporated. Besides the QSAR and read across approaches a defined battery of in vitro assays has to be applied in order to replace the LLNA as a stand-alone method in skin sensitization risk assessment based on established exposure scenarios. This battery should include in vitro assays representing biophysical (e.g. direct peptide reactivity assay) and physiological approaches such as cellular stress (e.g. KeratinoSens), dendritic cell activation (e.g. peripheral blood mononuclear dendritic cell-assay) and T-cell activation (e.g. T-cell assay). The combination of the above mentioned QRA concepts and the in vitro assay battery will enable an integrated testing strategy aimed at replacing animal testing for skin sensitization risk assessment.

I-19.705

Development of in vitro skin sensitization assay system at Shiseido

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Shiseido has developed several in vitro skin sensitization methods since the 1990’s. The h-CLAT is a method for detection of augmentation of CD86 and CD54 expression in THP-1 cells by test chemicals, while the SH test detects changes of cell-surface thiols on THP-1 cells exposed to test chemicals. In this study, we attempted to predict the published EC3 values of chemicals in LLNA from h-CLAT, SH test and cytotoxicity data by means of nonlinear analysis using a combinatorial approach. We used in vitro biomarkers (CV75, h-CLAT and SH test) as input layers and the LLNA thresholds, including EC3 values for LLNA-
positive chemicals and set maximum concentrations for LL-NA-negative chemicals, as the output layer in artificial neural network analysis. Model evaluation was implemented using the leave-some-out cross-validation method. In brief, we divided the dataset used in input layers and the output layer into 6 disjointed subsets (about 10% of all datasets). In the leave-some-out cross-validation method, we assessed whether the model derived from nine datasets predicted the remaining dataset.

We found a good correlation between in vitro model predictions and reported LLNA EC3 values. We confirmed that h-CLAT and SH test results were correlated with reported LLNA threshold values, and found that these in vitro data can be used in combination with artificial neural network analysis to build an in vitro prediction model for risk assessment of skin sensitization. Shiseido will continue research aiming at the practical use of this system.

1-19-706

Non-animal test battery optimized for detecting skin sensitizing potential

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As the mechanism of skin sensitization is complex and hard to reproduce in one in vitro system, the combination of several in vitro methods is useful in identifying the skin sensitization hazard adequately for a wide variety of chemicals. We have been developing an in vitro assay, the human Cell LineActivation Test (h-CLAT), which emulates dendritic cell activation. In this study, we investigated a battery system: the combination of h-CLAT, the direct peptide reactivity assay (DPRA), an in vitro assay, and the in silico system, DEREK. As a first step, the integrated testing strategy (ITS) was investigated. Final score calculated from the scores of each robust data set from each ITS component was used for the evaluation. ITS demonstrated a higher accuracy (85%) compared to DPRA, h-CLAT or DEREK alone. Secondly, the tiered approach using h-CLAT and DPRA were investigated as a practical system. The optimized tiered approach indicated the possibility of not only detecting the hazard but also of classifying the potency of chemicals. The predictivity for the potency classification was 72.3% while the “under prediction” rate was relatively low. Our results brought the non-animal testing system one step closer to replacing animal testing. Finally, we have been developing a novel in vitro test, EpiSensA, using a reconstructed epidermis model, which is expected to solve some current problems (e.g. lipophilic chemical evaluation). By adding EpiSensA to the tiered approach, the non-animal testing system will be used as an alternate to animal testing and be leveraged in risk assessments.

1-19-707

Towards an integrated testing strategy for skin sensitization: Development, refinement and combination of non-animal methods

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Contact sensitizers are reactive molecules that have the ability to modify skin proteins to form an antigen which will be recognized by specific T cells activated during the sensitization process. In addition to the haptenation mechanism, contact sensitizers induce several phenotypic and functional changes of dendritic cells (DC) either directly or indirectly through intercellular signaling pathways implicating keratinocytes, fibroblasts and other skin cells. This rather complex and still not fully unraveled maturation process of DC induced by contact sensitizers allows them to migrate to the lymph node, present antigen and efficiently prime hapten-specific T cells.

Due to the complexity of the sensitization process it is now commonly agreed that alternative hazard identification and risk assessment could only be addressed by combining a battery of methods. We present here our current approach based on a set of >150 chemicals and raw materials aiming to combine in
silico and in vitro tools from chemical reactivity assay to DC-based assay into an integrated testing strategy for the evaluation of skin sensitization. Through this exercise, we will share the limits and gaps of such an approach, in terms of applicability domains, heterogeneity of in vivo reference data and of requirements for statistical significance. Moreover we will give a broad overview of ongoing prospective initiatives in assay development and method evaluation in order to fill these gaps and to adapt our testing strategy in the most appropriate manner to face physicochemical diversity of cosmetic ingredients and to meet the needs for risk assessment of such ingredients.

Assuring consumer safety without animal testing is a considerable challenge, however we remain confident it is ultimately achievable. A substantial research programme was initiated by Unilever in 2004 to critically evaluate the feasibility of a new conceptual approach for consumer safety risk assessment (Fentem et al., 2008). Here we demonstrate significant progress in developing a non-animal risk assessment approach for skin sensitisation.

In collaboration with Entelos Inc. we previously developed a computational model of skin sensitisation using the published literature (Maxwell et al., 2008). Insights from this modelling exercise have allowed us to focus our subsequent non-animal test method development activities upon the identified toxicity pathways, namely skin bioavailability (Davies et al., 2011), protein binding (Aleksic et al., 2009), skin inflammation/dendritic cell (DC) maturation and T cell proliferation. Guided by our previous work (Maxwell et al., 2008), we are now developing a pragmatic, mechanistic model of skin sensitisation capable of integrating these non-animal datasets (e.g. peptide reactivity) to allow risk assessment decision-making without animal test data. The aim is for the model to predict the dynamics of the emerging sensitiser-specific T cell response. Therefore, we are also further characterising the induction and maintenance of the human immune response to skin sensitisers.

References