safety assessments. The mechanisms that trigger skin sensitisation are complex, and various steps are involved. Therefore, a single *in vitro* method may not be able to accurately assess this endpoint. Non-animal methods are being developed and validated and can be used as testing strategies that ensure a reliable prediction of skin sensitisation potential. In this study, the predictivity of three *in vitro* assays, one *in chemico* and one *in silico* method, addressing three different steps in the development of skin sensitisation, was assessed by using 54 test substances of known sensitising potential. The predictivity of single tests and combinations of these assays were compared. These data were used to develop an *in vitro* testing scheme and prediction model for the detection of skin sensitisers based on the protein reactivity and dendritic cell activation.

Predicting Eye Irritation of Agrochemical Formulations According to Different Classification Schemes by *In Vitro* Methods

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The bovine corneal opacity and permeability (BCOP) test has been adopted by OECD for the identification of ocular corrosive and severe irritants (GHS category 1) for single component substances and multi-component formulations. Further, human reconstructed tissue models have been suggested for incorporation into a tiered test strategy to ultimately replace the Draize rabbit eye irritation test (OECD Test Guideline [TG] 405), and we have previously shown the suitability of the EpiOcular Eye Irritation Test (EIT) to be used for the prediction of ocular non-irritants (GHS no category). The purpose of this study was to evaluate whether the BCOP, including corneal histology and the EIT, could be used to predict eye irritancy of agrochemical formulations according to different classification schemes including UN GHS, EPA and Brazilian systems. We have compared data on opacity, permeability and corneal histology in the BCOP assay and relative tissue viability in the EIT, for 50 agrochemical formulations, with available *in vivo* eye irritation data. Use of the OECD TG evaluation scheme for opacity and permeability in the BCOP did not prove predictive with respect to severe eye irritation potential for the 50 agrochemical formulations assessed here, while corneal histology grades and the EpiOcular tissue viabilities were useful predictors of eye irritancy potencies. Further, we describe here the statistical evaluation based on the experimental *in vitro* data to predict eye irritancy for the different classification schemes.

Understanding the Lengthy Process to Replace the Draize Test

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The urgency of the transition to replace animal tests in the safety assessment of cosmetics, pharmaceuticals and chemicals was triggered by societal resistance to animal testing and the scientific dispute concerning the value of animal testing. Since the 1980s, the European Union (EU) has developed policies to stimulate the development of innovative methods to replace animal studies. However, for regulatory testing, these policies have not been very effective, since only a few regulatory safety tests in animals (among which the Draize test, skin sentisation test) have been (partly) replaced by innovative methods. The few 'successful' replacement processes were laborious and took decades. In order to understand why transitions toward the replacement of animal tests in the regulatory safety assessment take so much time, these transitions need to be systematically studied, taking into account all stakeholders

involved in the development and acceptance of innovative methods. We used a framework, called the combined Technological Innovation System–Multi Level Perspective (TIS–MLP) framework, to systematically study transitions, and thereby elucidate the mechanisms that complicate them. We focused on the lengthy transition toward innovative methods for the Draize test. Eye irritation testing can be considered a pioneer in the development and validation of innovative methods to replace animal tests. Several innovative methods have been developed since the early 1980s, and major multi-laboratory validation studies were undertaken as early as the 1990s. It took until 2004 before a thorough review was carried out to advance the validation of innovative methods. Finally, two tests were approved by the OECD in 2009, to partially replace the Draize test — the Bovine Corneal Opacity and Permeability assay (BCOP) and the Isolated Chicken Eye (ICE) test. Both of these tests were already published in 1985, well over 20 years before regulatory acceptance. This study elucidates why this transition was so lengthy. Based on the combined TIS–MLP analysis it can be concluded that, despite the EU policy to stimulate the development of innovative methods and societal resistance, there was initially a lack of resources to further develop innovative methods. The EU ban on the use of animal tests for cosmetics was the key to solving this issue. Without this pressure on manufacturers, there was a lack of incentives for them to invest in innovative methods. In a later stage, the innovation process was impeded due to a lack of guidance with respect to the validation process and unrealistic validation endpoints. In none of the six validation studies, were innovative methods approved. Years of successful use of the Draize test made it the 'gold standard' in the validation studies. The performance of the innovative methods was judged in relation to the Draize test, not taking into account the actual flaws of that test. Due to the pressure of the EU ban on animal tests for cosmetics, it was considered necessary to change the validation strategy. Finally, only a review of the six validation studies finally convinced the OECD to accept the ICE and BCOP in 2009.

Immortalisation of Primary Human Alveolar Epithelial Cells: Development of a New *In Vitro* Model of the Air–Blood Barrier

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The aim of this project is the establishment of a human *in vitro* cell model of the air–blood barrier, with prolonged or infinite life span simultaneously retaining the characteristics and barrier properties of primary alveolar type I (ATI) cells. Up to now, there is no cell line available that reflects these crucial features. Primary human alveolar epithelial cells (hAEpC) are an appropriate tool to mimic the air–blood barrier *in vitro*. However, their limited availability, as well as their time- and cost intensive isolation procedures, in addition to ethical concerns, limit their availability and applicability. According to the Three Rs principles (Replacement, Reduction, Refinement) for minimising the use of animal testing, the development of a novel *in vitro* model of the air–blood barrier is therefore of utmost importance. The model is based on the immortalisation of hAEpC via lentiviral vectors, in combination with a set of more than 33 genes with immortalising capability. After transfection and subsequent passaging, promising cell lines undergo characterisation regarding their epithelial origin, the expression of lung cell-specific markers and their barrier properties. The lines are analysed by morphological studies, immunofluorescence staining techniques, real-time PCR and transepithelial electrical resistance (TEER) measurement. Furthermore, the culture conditions are optimised and transport studies with model substances are carried out. Instead of using only classical transformation genes like hTERT or SV40LTAg, so-called 'mild' proliferators were used to prevent de-differentiation of the transformed cells. Thereby, seven human cell lines with prolonged lifespan could be generated by the lentiviral transfection approach and are currently under investigation regarding their cellular identity and retention of *in vivo*-like characteristics. Three cell lines showed