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Two-tiered keratinocyte assay: IL-18 production by NCTC2544 cells to determine the skin sensitizing capacity and an epidermal equivalent assay to determine sensitizer potency

Marc Teunis, Emanuela Corsini, Mieke Smits, Charlotte Bernhard Madsen, Tobias Eltze, Janine Ezendam, Valentina Galbiati, Eric Gremmer, Cyrille Krul, Annette Landin, Robert Landsiedel, Raymond Pieters, Tina Rasmussen, Judith Reinders, Erwin Roggen, Sander Spiekstra, Susan Gibbs

At present, the identification of potentially sensitizing chemicals is carried out using animal models. However, it should be very important, both from ethical and economic point of view, to discriminate allergy and irritation events, and to classify sensitizers according to their potency, without the use of animals.

The aim of the EU FP6 Integrated Project *Sens-it-iv* was to develop and optimize an integrated testing strategy consisting of *in vitro*, human cell based assays which will closely mimic sensitization mechanisms *in vivo*. These assays should be an alternative approach to the LLNA.

The NCTC2544 IL-18 assay can be used to identify the sensitizing capacity of a chemical (NCTC assay, tier 1) while the Epidermal Equivalent potency assay is used to quantify the potency of the sensitizing agent (EE assay, tier 2). These assays combined, may offer a unique opportunity to provide an alternative method to the LLNA. Both assays are based on the use of human keratinocytes, which have been shown, over the last two decades, to play a key role in all phases of skin sensitization.

First, 4 known chemicals were tested during a transferability study in which 6 laboratories participated. Three sensitizers (DNCB, resorcinol, PPD) and 1 non sensitizer (lactic acid) were tested in tier 1. DNCB (extreme) and resorcinol (moderate) were ranked according to their potency in tier 2. These assays were successfully transferred to laboratories that did not perform both assays previously.

Second, the actual pre-validation was performed with 29 coded chemicals for tier 1 and 13 coded chemicals for tier 2. Currently, all chemicals have been tested and data is collected. After all data has been processed, evaluation of the proposed prediction model will be assessed.