



# Considerations for the Development of an Integrated Testing Strategy for Skin Sensitization

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## Summary

*Skin sensitization is a crucial endpoint for the safety assessment of cosmetic ingredients. A major challenge for the cosmetic industry is to develop a non-animal strategy for the risk assessment of the skin sensitization potential of new ingredients before the 2013 ban. Beiersdorf thus started a large effort to evaluate available non-animal tools, including physico-chemical properties, structural alerts, read-across of historical data, in silico methods, and in vitro assays. More than 50 chemicals were thus evaluated with a combination of physico-chemical property predictions and in vitro methods such as cellular stress and dendritic cell activation (e.g., peripheral blood monocyte-derived dendritic cell-assay). Overall results were excellent, the best ones being obtained for Schiff base forming chemicals. Further progress should be achievable through the incorporation of the threshold concepts and metabolism data. An integrated testing strategy combining these different approaches should allow replacement of animal tests for skin sensitization risk assessment.*

*Keywords: skin sensitization, cosmetics, in vitro assay, risk assessment*

## 1 Introduction

Non-animal test methods for skin sensitization have to consider the complex interactions of chemicals with the different parts of the skin immune system (Cumberbatch et al., 1992). A few chemicals act as prohaptens and can be converted to a hapten by oxidation, skin metabolism, or ultraviolet radiation (Karschuk et al., 2010). The hapten (parent or converted chemical) may penetrate the skin and interact with skin proteins and immature dendritic cells (DCs). These immature DCs internalize and process haptenated carrier-proteins and, once activated, migrate from the skin to the draining lymph node, terminate maturation, and present fragments of the haptenated carrier-proteins to T-cells, leading to an antigen-specific immune response (Aeby et al., 2010).

A large international research, development, and evaluation effort by various companies and organizations (e.g., COLIPA) has already lead to the proposal or publication of different approaches that may be included in toolboxes of non-animal test methods for characterizing skin sensitizer potency. A careful combination of some of these methods should allow risk assessment decisions to be made without the need for new animal test data in the near future (Aeby et al., 2010).

The Beiersdorf Toolbox described here consists of *in silico* and *in vitro* methods supported by physico-chemical and toxicological tools and reviews of historical data:

1. *Indications based on physico-chemical properties:* determination of chemical domains, presence or absence of structural alerts (structure activity relationships = SAR), prediction of chemical reactivity

2. *In silico tools:* DEREK, TOPCAT, MultiCase
3. *Read-across based on similar chemicals with available experimental historical data:* animal data, human data, *in vitro* data
4. *Exposure:* depending on product applications; determination of bioavailability, adsorption, penetration, transformation of chemical (metabolism, oxidation, ultraviolet radiation)
5. *In chemico or in vitro methods:*
  - a. Biophysical properties, protein binding: Direct Peptide Reactivity Assay (DPRA; Gerberick et al., 2007)
  - b. Cellular stress: KeratinoSens (Natsch et al., 2011)
  - c. Dendritic cell activation: Peripheral Blood Monocyte-derived Dendritic Cell (PBMDc) assay (Reuter et al., 2011).

The approach being evaluated at Beiersdorf for the determination of a predicted EC3 (pEC3) and of a “No Expected Sensitization Induction Level” (NESIL) includes, as a first step, an evaluation of the hapten or prohapten properties of the chemical (probable modifications by oxidation, metabolism, or ultraviolet radiation). The expected exposure is calculated with a combination of bioavailability, chemical domain, and reactivity data, as well as physico-chemical information (e.g., logP). An initial hazard prediction and the definition of an expected potency (pEC3 and NESIL) range are then attempted. Historical data, *in silico* tools, and read across can be used as additional support.

The second step is expected to lead to the narrowing and confirmation of the calculated pEC3/NESIL range. Suitable *in vitro* assays selected according to the results of the first step include bioavailability (adsorption, penetration, transforma-



tion) and DC activation tests (PBMDc assay, Keratinosens). Once the *in vitro* data have been produced and interpreted, a more precise prediction of hazard and expected potency (pEC3 and NESIL “range”) is possible and is followed by a safety assessment.

The results obtained with a combination of chemical domain determination (Schiff base formers), the PBMDc assay, and the DPRA with a test set of 54 chemicals are presented below.

## 2 Results

**PBMDc Assay** (see Reuter et al., 2011):

Briefly, peripheral blood monocytes were isolated from healthy donors by Ficoll gradient density centrifugation, differentiated into monocyte-derived dendritic cells by IL-4 and GM-CSF for 5 days and incubated with test substances. Following 48 h incubation, the expression of CD86 and cell cytotoxicity with 7-AAD are measured by FACS.

**Tab. 1: Predictivity of PBMDc assay in comparison to murine local lymph node data (LLNA)- experimental or literature data**

14 substances are classified as non-sensitizers in both assays, 4 substances as sensitizers in the PBMDc assay but non-sensitizers in the LLNA. 28 substances are sensitizers in both assays. 8 are non-sensitizers in the PBMDc assay but sensitizers in the LLNA. The PBMDc assay resulted in a sensitivity and a specificity of 78 % each.

PBMDc Assay Prediction			
LLNA	Non-Sensitizer	Sensitizer	Total
Non-Sensitizer	14	4	18
Sensitizer	8	28	36
Total	22	32	54

**Tab. 2: Predictivity of DPRA in comparison to murine local lymph node data (LLNA)-experimental or literature data**

15 substances are classified as non-sensitizers in both assays, 3 substances as sensitizers in the DPRA but non-sensitizers in the LLNA. 27 substances are sensitizers in both assays. 9 are non-sensitizers in the DPRA assay but sensitizers in the LLNA. The DPRA resulted in a sensitivity of 75% and in a specificity of 83%.

DPRA Prediction			
LLNA	Non-Sensitizer	Sensitizer	Total
Non-Sensitizer	15	3	18
Sensitizer	9	27	36
Total	24	30	54

25 Beiersdorf substances and 29 external chemicals were tested in the PBMDc assay, which resulted in a sensitivity and a specificity of 78%, respectively (Tab. 1).

**DPRA** (see Gerberick et al., 2007):

Briefly, cysteine and lysine peptides are exposed to a test substance for 24 h and analyzed according to the peptide peak depletion using HPLC-UV technique.

The same 54 substances were tested in the DPRA which resulted in a sensitivity of 75% and a specificity of 83% (Tab. 2).

The 54 substances were classified according to Local Lymph Node Assay results (LLNA) into sensitizers and non-sensitizers and categorized into different reactivity domains. Four of these chemicals were categorized in the Michael Acceptor as well as in the SN2 chemical domain (Tab. 3).

The impact of combining chemical domain determination with different *in vitro* sensitization assays such as the DPRA and the PBMDc assays is best exemplified by the results obtained for Schiff base formers: The PBMDc assay avoided false nega-

**Tab. 3: A total of 54 chemicals classified by LLNA as sensitizers or non-sensitizers were categorized in different reactivity domains**

4 chemicals were categorized in the Michael acceptor as well as in the SN2 chemical domain.

Chemical domain	Total	Sensitizer	Non-Sensitizer
Michael acceptors	24	19	5
S <sub>N</sub> Ar electrophiles	1	1	0
S <sub>N</sub> 2 electrophiles	5	4	1
Schiff base formers	8	5	3
Acylation agents	4	3	1
Non-reactive	16	7	9

**Tab. 4: False negative and false positive results obtained for the Schiff base formers (n=8) with the DPRA and PBMDc assay**

Test of Schiff base formers	False negative	False positive
DPRA	38%	0%
PBMDc	0%	25%



tive results (100% specificity) and the DPRA resulted in 100% sensitivity (Tab. 4).

### 3 Conclusions

The PBMDC assay (performed with cells obtained from a healthy donor population in contrast to other tests using heavily mutated and aberrant cell lines) shows a good predictivity (78%) for the chosen chemical test set (a combination of 29 well-known and characterized chemicals (training set) and 25 Beiersdorf substances). The use of our two step strategy (chemical domains plus *in vitro* sensitization data) indicates that the PBMDC assay avoided false-negative results for Schiff base generating chemicals and allowed 100% sensitivity in the DPRA.

To ensure reliable quantitative risk assessment evaluations, it will be crucial to generate a large data pool with the envisaged *in chemico/in vitro* assays (DPRA, PBMDC, KeratinoSens) and to find more relevant relationships between physicochemistry (e.g., chemical domain and reactivity) and biology.

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