

EVALUATION OF SENS-IS[®], AN EPISKIN[®] BASED MODEL FOR IDENTIFYING CHEMICAL SENSITIZERS OF FRAGRANCE INGREDIENTS

A Del Bufalo¹ • F Tourneix¹ • S Martinozzi-Teissier¹ • F Cottrez² • H Groux² • J. Cotovio¹

¹L'Oréal Research, Aulnay sous Bois, France - ²ImmunoSearch, Grasse, France

INTRODUCTION

In the context of the 2013 ban given by EU Cosmetics Directive, the ability to identify and classify the skin sensitization potential of chemicals without animal is of high importance for the cosmetic industry. A range of different *in vitro* chemistry-based and cell-based methods have been developed and we are currently evaluating some of them for their applicability to cosmetic ingredients and their physicochemical diversity. Although these assays appear to be promising for hazard identification, potency assessment is still limited.

ImmunoSearch has developed SENS-IS[®], a new method, based on the quantitative analysis of specific biomarkers expressed in 3D reconstructed epidermis (Episkin[®]). This new assay provides a possible way to encompass the limitations of monolayer culture models (lack of skin bioavailability properties, different metabolism of the models compared to skin, inability to test water insoluble chemicals) and might therefore allow a better assessment of the sensitization potency of cosmetic ingredients.

AIM OF STUDY

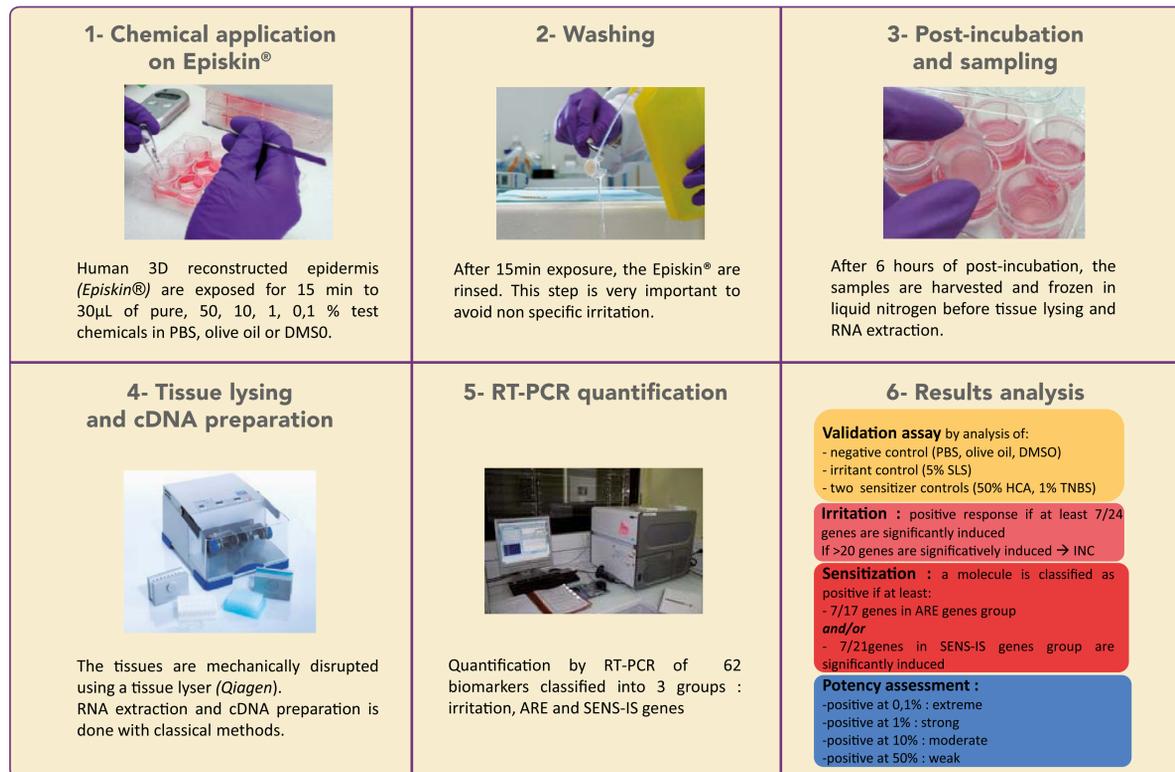
In a previous study on about 100 raw materials (RM), SENS-IS[®] showed good performances to predict hazard (S vs NS) but potency prediction performances were insufficient, particularly for weak/moderate sensitizers.

Therefore, the aim of this study was to evaluate the predictive capacities of the SENS-IS[®] method on a panel of cosmetic ingredients composed fragrance ingredients, which are mainly volatiles weak/moderate sensitizers.

For this purpose we assessed in a blinded manner a set of 22 fragrance ingredients with *in vivo* data based on LLNA and clinical data.

MATERIAL AND METHODS

SENS-IS Assay



RESULTS

Name	CAS number	Potency predicted by Sens'IS	LLNA EC(3) value	LLNA potency classification	Basketter & al.* Human classification
Methyl 2-octynoate	111-12-6	Strong	0,5	Strong sensitizer	2
Cinnamaldehyde	104-55-2	Moderate/ Strong	3,0	Moderate sensitizer	2
Cinnamyl alcohol	104-54-1	Strong	21,0	Weak sensitizer	3
Isoeugenol	97-54-1	Moderate	1,2	Moderate sensitizer	2
Citral	5392-40-5	Moderate	13,0	Weak sensitizer	3
Benzyl alcohol	100-51-6	Moderate		Non sensitizer	ND
Geraniol	106-24-1	Moderate	26,0	Weak sensitizer	4
Hydroxycitronellal	107-75-5	Weak/Moderate	33,0	Weak sensitizer	4
Eugenol	97-53-0	Weak/ Moderate	13,0	Weak sensitizer	3
Benzyl salicylate	118-58-1	Weak	2,9	Moderate sensitizer	5
Anisyl alcohol	105-13-5	Weak	5,9	Moderate sensitizer	5
Hexylcinnamaldehyde	101-86-0	Weak	11,0	Weak sensitizer	5
Amyl cinnamal	122-40-7	Weak	11,0	Weak sensitizer	4
Benzyl benzoate	120-51-4	Weak	17,0	Weak sensitizer	5
Lylal	31906-04-4	Weak	17,0	Weak sensitizer	2
Lilial	80-54-6	Weak	19,0	Weak sensitizer	4
Linalool	78-70-6	Weak	30,0	Weak sensitizer	4
R(+)-Limonene	5989-27-5	Weak	69,0	Weak sensitizer	ND
Coumarin	91-64-5	Weak		Non sensitizer	3
Citronellol	106-22-9	None/ weak	43,5	Weak sensitizer	5
Farnesol	4602-84-0	Non sensitizer	4,8	Moderate sensitizer	3
Benzyl cinnamate	103-41-3	Non sensitizer	18,4	Weak sensitizer	ND

* Basketter & al., *Dermatitis*, 2014, « Categorization according to human skin sensitizing potency »

Figure 2: SENS-IS[®] predictions for the 22 fragrance ingredients and corresponding *in vivo* data (LLNA/ clinical)

- 3 RM are predicted strong sensitizers by SENS-IS[®]: methyl 2-octynoate, cinnamaldehyde and cinnamyl alcohol. For two of them (cinnamaldehyde, cinnamyl alcohol) the method overestimates the LLNA results. However cinnamaldehyde is a strong sensitizer in human.
- 6 RM are predicted moderate sensitizers by SENS-IS[®]: Isoeugenol, citral, benzyl alcohol, geraniol, hydroxycitronellal and eugenol. 5 of them are overestimated compared to LLNA data. However eugenol and citral are moderate sensitizers in human.
- 11 RM are predicted weak sensitizers by SENS-IS[®]: benzyl salicylate, anisyl alcohol, hexylcinnamaldehyde, amyl cinnamal, benzyl benzoate, lylal, lilial, linalool, R(+)-limonene, coumarin and citronellol. Coumarin is overestimated compared to LLNA, two of them (benzyl salicylate, amyl cinnamal) are underestimated compared to LLNA even if they are classified as very weak sensitizers in human.
- Finally, 2 RM are predicted non sensitizer by SENS-IS[®]: farnesol and benzyl cinnamate. Both are underestimated compared to LLNA data.

Legend:

LLNA	colour code	Human
Extreme/ strong	Red	1/2
Moderate	Orange	3
Weak	Yellow	4
Very weak	Light yellow	5
Non sensitizer	Green	6

CONCLUSION

We present here the results of the evaluation of 22 fragrance ingredients in the SENS-IS[®] method from ImmunoSearch.

No interference was identified with the volatility of the RM which attests the technical feasibility to test fragrance ingredients in the SENS-IS[®] method.

The overall prediction capacities for the potency [compared to *in vivo* data (LLNA and/or clinical)] were satisfactory (15/22=68%).

This benchmark study gives us *in vitro* tools to perform risk assessment analysis on future mixtures containing those ingredients.