

The SENS-IS assay: an *in vitro* alternative to HRIPT for sensitization potential evaluation of formulations



Small epidemiological reminders

- The prevalence of contact allergies is steadily increasing worldwide
- In Europe more than 20% of the population is affected for at least one allergen
- Redness, pruritus, itching, fissures and edema
- Main allergens in cosmetics:
 - Nickel, cobalt
 - Perfume (Cf list of 57 allergens requiring labeling)
 - Preservatives (MIT, parabens, formaldehyde, imidazolidinyl urea, etc.)
 - Excipients (lanolin, propylene glycol, cocamidopropylene, etc.)
 - Hair colors
 - Nail polish (paratoluene sulfonamide)
 - Solar filters

Short regulatory update

- European Regulation 1223/2009

Article 3

Safety

A cosmetic product made available on the market shall be safe for human health when used under normal or reasonably foreseeable condition of use, taking account, in particular, of the following:

- SCCS notes of guidance for testing of cosmetic ingredients and their safety evaluation – 11th Revision, March 2021

3. SAFETY EVALUATION OF COSMETIC INGREDIENTS

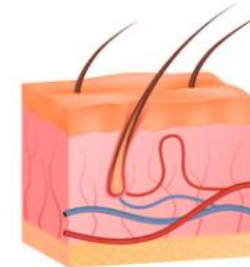
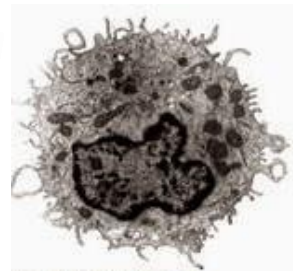
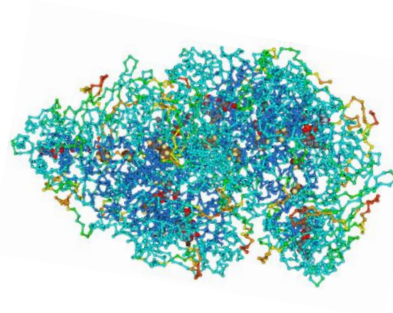
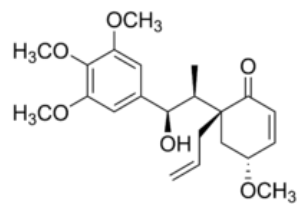
3-1 SAFETY EVALUATION OF COSMETIC INGREDIENTS AS APPLIED BY THE SCCS

- The safety of cosmetic products is based on the safety of the ingredients

SCCS: Scientific Committee on Consumer Safety

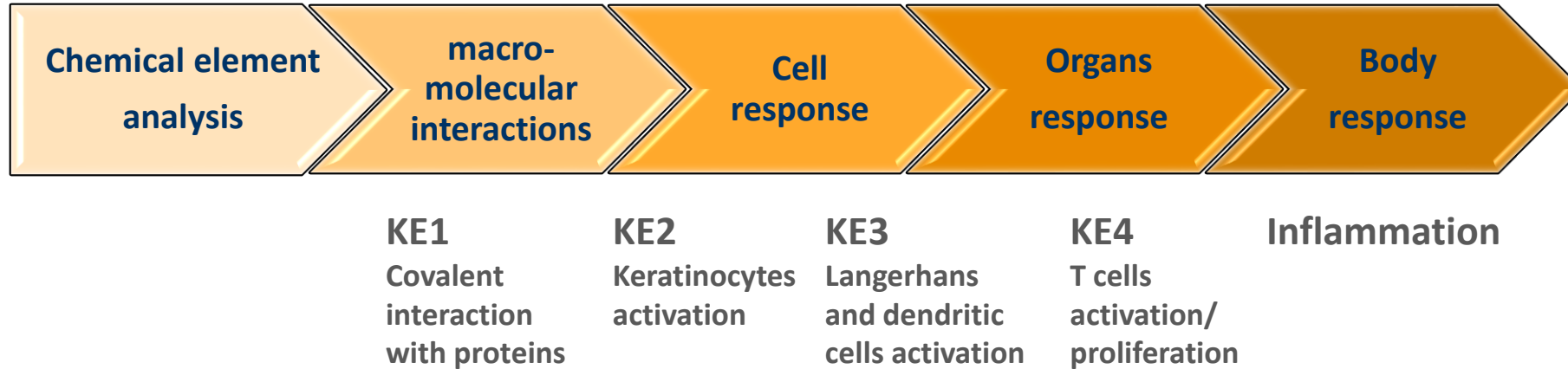
in vitro sensitization tests: quick review

Approach based on the AOP concept = Adverse Outcome Pathway



in vitro sensitization tests: quick review

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in vitro sensitization tests: quick review

Approach based on the AOP concept = Adverse Outcome Pathway



KE1
Covalent interaction with proteins

KE2
Keratinocytes activation

KE3
Langerhans and dendritic cells activation

KE4
T cells activation/proliferation

Inflammation

DPRA

ADRA

kDPRA

KeratinoSens®

LuSens

Sens-IS

IL18 release

LLNA

HRIPT

GPMT

Buehler

h-CLAT

U-SENS

IL-8 Luc

GARD

VitoSens

KE : Key Event

- Validated**
- Under validation**
- Not validated**
- Animal**
- Human**

Integrated Testing Strategy (ITS)

To cover the different « Key Events » of the AOP according to the Guideline OECD 497

In silico

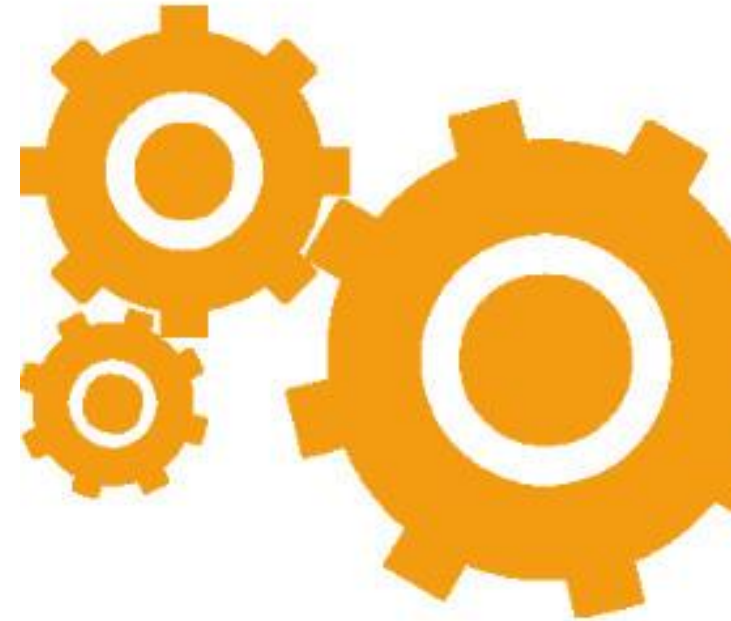
Comparison
Read Across
QSAR,...

In chemico

DPRA
KE1

In vitro

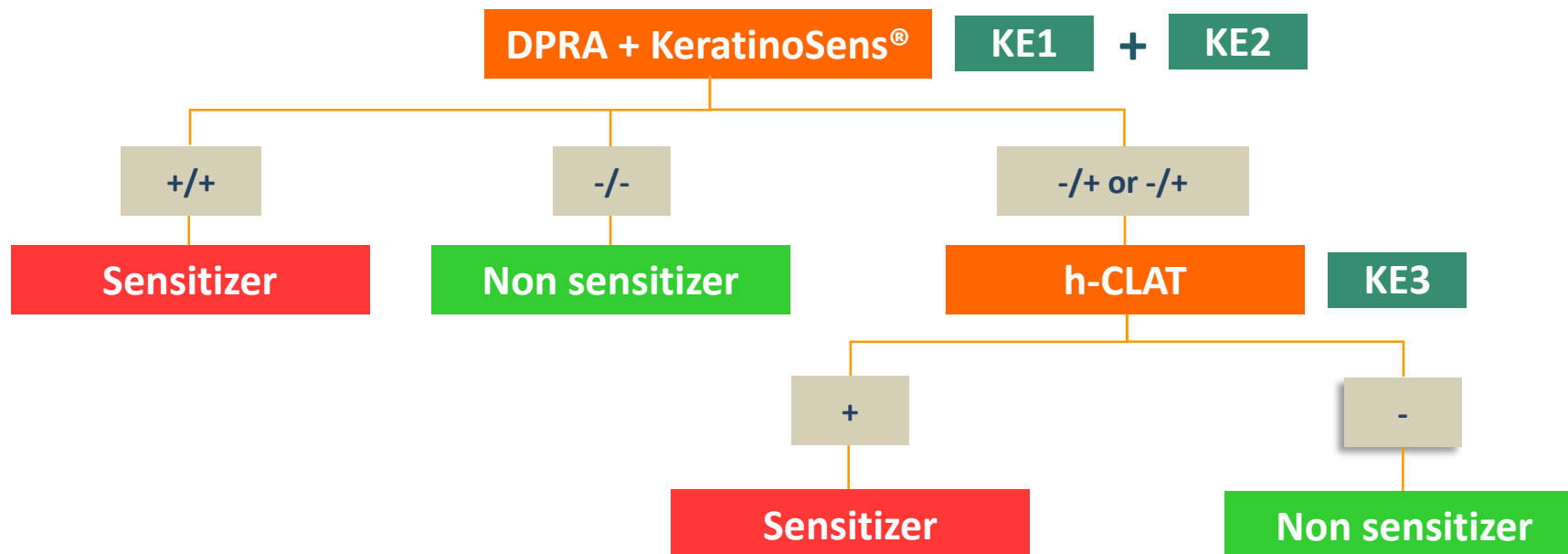
KeratinoSens
KE2
h-CLAT
KE3



Integrated Testing Strategy (ITS)

Strategy "2 out of 3" = KE1 + KE2 + KE3

- Hazard identification, approach WoE
- Accuracy 90% (vs human data)



in vitro sensitization tests

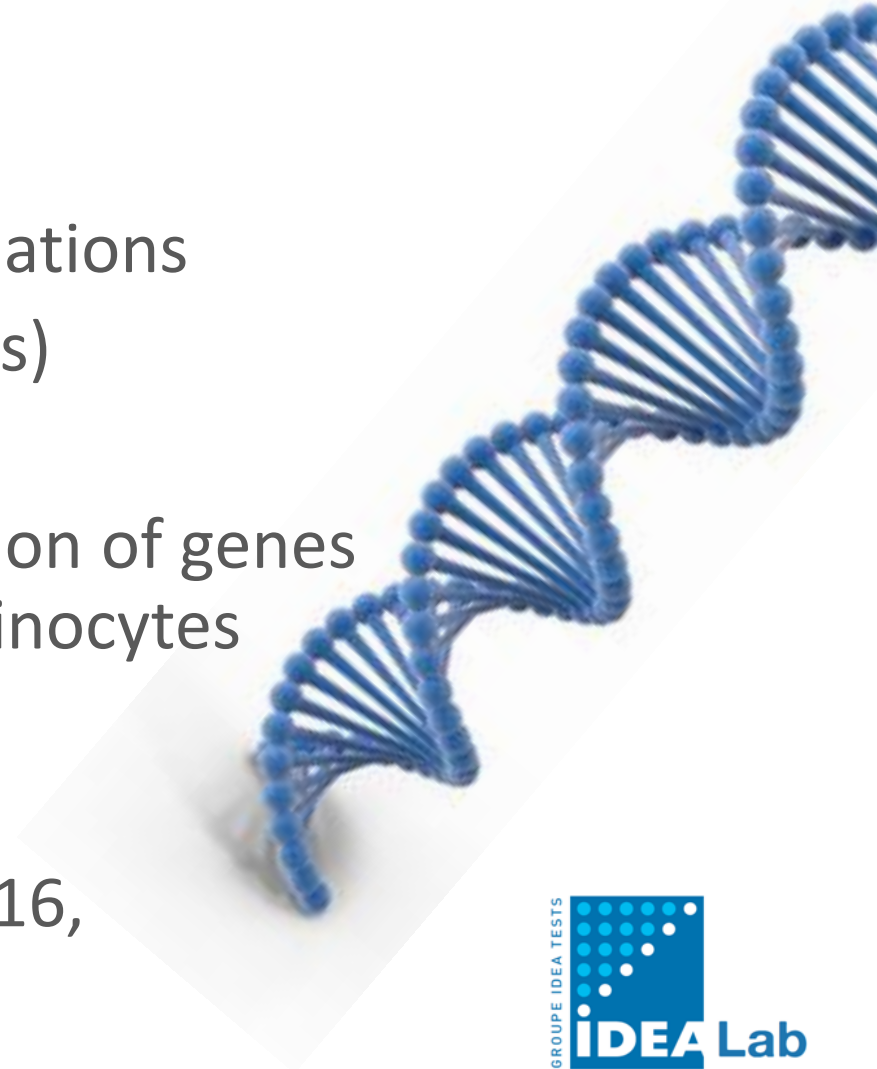
What do we do if one of these tests is not suitable (e.g. DPRA)?

What test do we have for poorly soluble or non soluble samples?

Is it possible to cover more than one KE at a time?

The SENS-IS: a genomic approach

- Developed by Immunosearch (Grasse)
- Reconstructed epidermis (Episkin models)
- Suitable for poorly soluble compounds and formulations
- Quantification by RT-qPCR of 62 biomarkers (genes)
- Accuracy: 96% (vs human data)
- Can address the 3 Key Events through the expression of genes involved in the control of sensor proteins, in keratinocytes activation and in dendritic cells activation
- External validation submitted to ECVAM in July 2016, currently being assessed by ESAC



SENS-IS: protocol

Quantification of the expression of 62 genes classified in 3 groups:

Irritation (24)

ARE anti-oxidant (17)

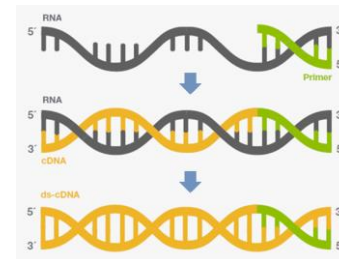
SENS-IS sensitization (21)



Treatment
15 min of contact + 6 h
@ 37 °C (post-incubation)



RNA extraction



Reverse transcription
cDNA



RTqPCR amplification from
the 62 genes primers

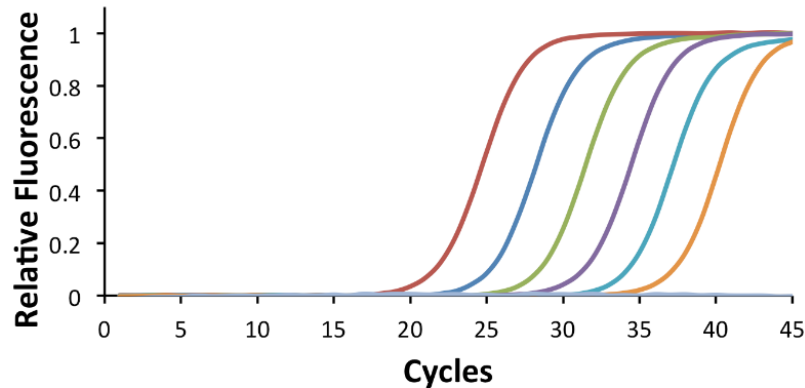
- SLS 5% (CAS Number: 151-21-3): irritation positive control and sensitization negative control
- TNBS 1% (CAS Number: 2508-19-2): sensitization positive control
- PBS, DMSO and olive oil: sensitization negative control

SENS-IS: result

Analysis



Conclusions



The number of cycles measured for each gene is proportional to the amount of DNA present at the origin

The amount of DNA originally present is indicative of gene expression level

Positive if

7/17 genes in ARE group and/or
7/21 genes in SENS-IS group
are significantly expressed

Potency evaluation

Positive @ 0.1% and/or 1% → Cat. 1 Strong to Extreme

Positive @ 10% and/or 50% → Cat. 2 Weak to Moderate

Negative @ all concentrations → Not classified - not sensitizer

Our testing battery for ingredients

OECD 442C **KE1**

DPRA Direct Peptide Reactivity Assay

OECD 442D **KE2**

KeratinoSens Keap1/Nrf2 pathway

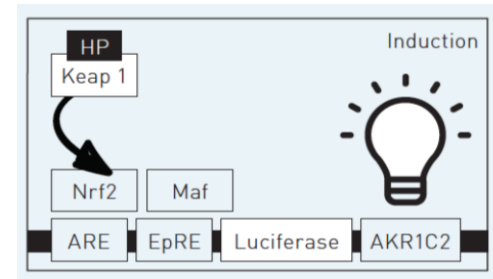
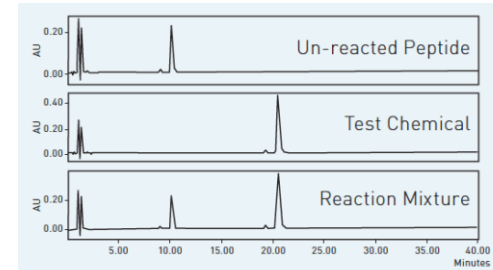
OECD 442E **KE3**

h-CLAT CD54 and CD86 activation

SENS-IS **KE1** **KE2** **KE3**

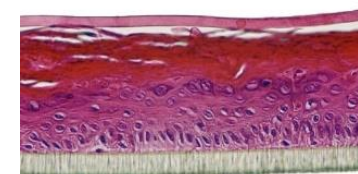
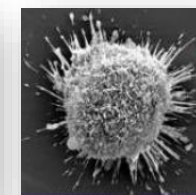
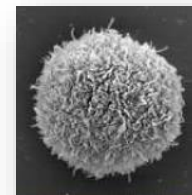
Genomic approach on HRE

- Non soluble compounds
- **Formulations**



Non activated

Activated



Sensitization evaluation for formulations

What solution for formulation?



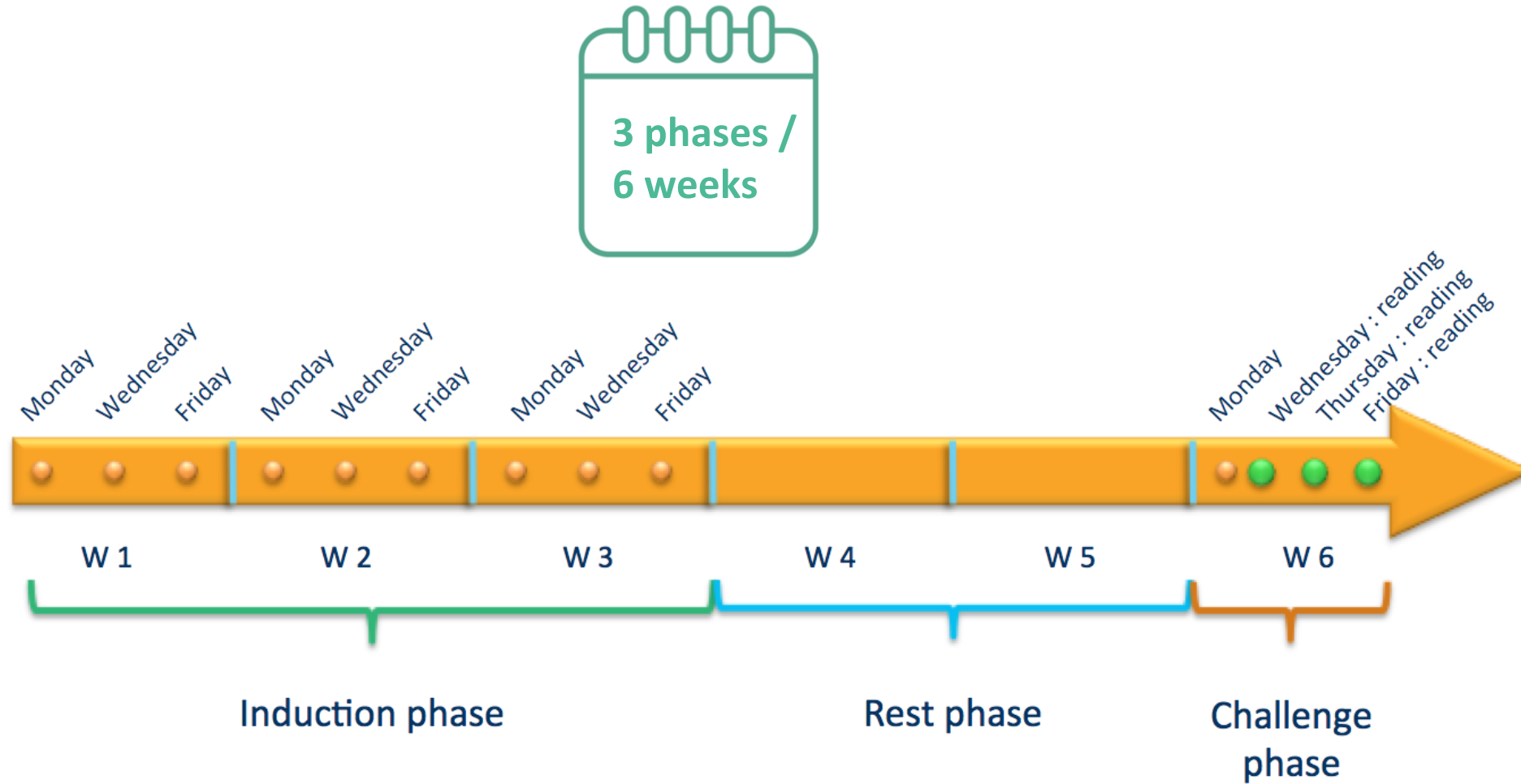
Sensitization evaluation for formulations: the HRIPT

Human Repeat Insult Patch Test

- Healthy » volunteers (Men or Women)
- Age: 18-70
- Number: 50 / 100 / 200
- +/- Sensitive skin
- Back or arm



Sensitization evaluation for formulations: the HRIPT



Sensitization evaluation for formulations: the HRIPT

- **In France:** to confirm the absence of delayed contact allergic reaction after precise analysis of the formula (TCFS «Test Clinique Final de Sécurité»)
- **In the rest of the world:** to assess the sensitization potential
- **USA:** very used, carried out on 200 subjects

- **In Europe:** the EU commission is more and more reserved for ethical reasons

 need for an alternative

The SENS-IS for formulations

Toxicology in Vitro 62 (2020) 104644

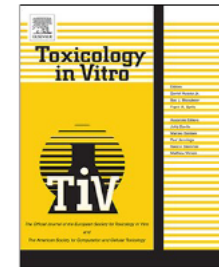


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Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



In vitro measurement of skin sensitization hazard of mixtures and finished products: Results obtained with the SENS-IS assays

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The SENS-IS for formulations

- Rapid summary:
 - Adapted protocol: incubation 15 min (+ 6 h post treatment) and 6 h
 - The sensitization potential for a same sensitizer varies from one type of formulation to another
 - The presence of irritating ingredients increases the sensitizing potential
 - A mixture of sensitizers at individual non-sensitizing concentrations may show a sensitizing potential

→ **Using sensitizing ingredient at non-sensitizing concentrations does not guaranty the absence of sensitizing potential of a formulation**

- Tests on 25 commercial formulations
 - 4 are slightly positive @ 6h00 (2 rinse-off)
 - 1 is clearly positive @15 min and 6h00, rinse-off

The SENS-IS for formulations

- SGS IDEA Lab tests
 - 6 commercial formulations
 - incubation 15 min (+ 6 h post treatment) and 6 h

	Product type	Rince-off	SENS-IS		Conclusion	Mean Irritation genes	MTT viability on HCE
			15min	6h			
Product 1	Gentle cleanser	Yes	No (1)	No (5)	NS	5	99%
Product 2	Lotion	No	No (4)	No (4)	NS	4	97%
Product 3	Cream	No	No (3)	No (5)	NS	3	100%
Product 4	Lotion	No	No (4)	No (5)	NS	6	100%
Product 5	Oily skin cleanser	Yes	No (6)	Yes (12)	S	13	19%
Product 6	Cream	No	No (3)	No (2)	NS	2	-

(x): mean of SENS-IS genes activated

The SENS-IS for formulations



An alternative to HRIPT

- The samples are tested pure
- 2 contact times:
 - 15 min (+ 6h post treatment) for rinse-off products
 - 6 hours for leave-on products.
- Two series on two RHE batches are performed
- 1 RHE per series

Conclusion



An alternative to HRIPT

- Powerful: precise information on sensitization potential and irritation
- Flexible protocol: different contact times
- Adapted to formulation development
- Rapid turn around time: 4 weeks
- **An ethical solution !**

Thank you !

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