In vitro sensitization tests for cosmetic ingredients

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In-Cosmetics
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IDEA TESTS Group

- Created in 1999 by Benoit Latouche
- Specialized in cosmetic products evaluation
- 3 entities:
  - Clinical tests
  - In vitro test & microbiology
  - Regulatory advice & safety evaluation

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Regulatory context

- Article 3 of the European Regulation (EC) No 1223/2009

- This absence of risk is evaluated from the toxicological profile of the ingredients, based on:
  - predictive data (comparison, read across, (Q)SAR)
  - the toxicological data obtained from tests and/or historical data (human, animals)
  - assessment of the impact (application site, application mode, quantity, frequency, etc.)

Calcul of the « Margin of Safety » MOS
Animal testing ban

- September 2004: Animals testing ban in Europe for cosmetic finished products
- June 2007: REACH
- March 11th, 2013: Animals testing ban in Europe for cosmetic ingredients

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**Article 18**

**Animal testing**

1. Without prejudice to the general obligations deriving from Article 3, the following shall be prohibited:

(a) the placing on the market of cosmetic products where the final formulation, in order to meet the requirements of this Regulation, has been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with due regard to the development of validation within the OECD;

(b) the placing on the market of cosmetic products containing ingredients or combinations of ingredients which, in order to meet the requirements of this Regulation, have been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with due regard to the development of validation within the OECD;
Parameters to assess

- Main end-points to evaluate
  - Acute toxicity (dermal and/or oral): LD_{50}
  - Chronic toxicity (90 days): NOAEL
  - Ocular irritation
  - Skin irritation
  - Phototoxicity
  - Genotoxicity
  - Skin absorption
  - Skin sensitization
Finished cosmetic products

- HRIPT: Human Repeat Insult Patch Test
- Marzulli, F.N., Maibach, H.I.
- 50 volunteers minimum
- 10 applications
- 6 weeks
HRIPT

Induction phase

Rest phase

Challenge phase

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Existing *in vivo* tests: ingredients

- **Ingredients: animal testing**
  - GPMT test OECD 406 (EC B.6)
    - Guinea Pig Maximization Test (SMK - Magnusson & Kligman)
    - Guinea pigs
  - Buehler test OECD 406 (EC B.6)
    - Guinea pigs
  - LLNA test OECD 429, 442A, 442B (EC B.42, B.42B and B.42C)
    - Local Lymph Node Assay
    - Mices
GPMT & Buehler

- GPMT OECD 406 (EC B.6)
  - 1969
  - 5 controls + 1 treated
  - 2 induction phases: 1 intradermal + 1 topical at 1 week interval
  - Rest phase of 10 days
  - Challenge phase

- Buehler test OECD 406 (EC B.6)
  - 3 or 9 inductions only topical (on 1 or 3 weeks)
  - Rest phase of 15 days
  - Challenge phase
  - ≈ HRIPT
LLNA OECD 429, 442A, 442B (EC B.42, B.42B and B.42C)
- 4 mice per batch (CBA/Ca or CBA/J, at 8-12 weeks)
- Application site: the ear
- 3 expositions spaced by 24h
- Parameters assessed: weight of draining lymph node, lymphocytes proliferation
- Stimulation index, dose response = determination of non effect level
in vitro sensitization tests

- European research program FP6, Sens-it-iv project – www.sens-it-iv.eu
- From October 2005 to 2010
- 14 million euros
- Development based on the AOP concept: Adverse Outcome Pathway

Chemical element → Macro-molecular interaction → Cellular response → Organs response → Organism response
in vitro sensitization tests

- DPRA, KeratinoSens, MUSST, h-CLAT, LuSens, VitoSens, GARD, Sens-IS, SenCeetox, IL18, etc...
- 4 main tests validated or under validation

- DPRA: OECD 442C – Feb. 2015
- KeratinoSens: OECD 442D – Feb. 2015
- h-CLAT: Draft OECD – July 2014
- MUSST

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in vitro sensitization tests

Diagram showing the process from Chemical element to Organism response:
- Chemical element
  - Macromolecular interaction
  - Cellular response
  - Organs response
- DPRA
- KeratinoSens
- LuSens
- LLNA
- HRIPT
- SMK
- Buehler
- h-CLAT
- MUSST
- Sens-IS
- IL18 release

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in vitro sensitization tests

**Sensitization**
- DPRA
- KeratinoSens

**Elicitation**
- h-CLAT
- MUSST
- HRIPT
- GPMT
- Buehler

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DPRA: Direct Peptide Reactivity Assay

- Developed by Procter & Gamble and Strasbourg University (G.F. Gerberick & J.P. Lepoittevin)

- Measurement of the protein reactivity by following the depletion of two synthetic nucleophilic peptides containing one cysteine or one lysine: Ac-RFAA\textsubscript{C}AA-COOH and Ac-RFAA\textsubscript{K}AA-COOH
Quantification by HPLC at 220 nm of the remaining concentration of the peptides after 24h of contact with the test item

- Threshold at 6.38% or 13.89% if co-elution with lysine
DPRA

- Sensitivity 80%
- Specificity 77%
- Recommandation EURL-ECVAM: November 2013
- Guideline OECD 442C: February 2015

- Limits of the tests
  - Do not detect the pro-haptens nor pre-haptens
  - Problem with non soluble test items
  - Co-elution (detection also at 258 nm or mass-spectrometry detection)
  - Tests items that react with other amino-acid than cysteine or lysine
  - Dimerisation of cysteines
KeratinoSens

- Regulation pathway of Keap1-Nrf2-ARE dependent genes on keratinocytes HaCaT by induction of luciferase
- Givaudan (Andreas Natsch)

The Nrf2-Keap1-ARE Toxicity Pathway as a Cellular Sensor for Skin Sensitizers—Functional Relevance and a Hypothesis on Innate Reactions to Skin Sensitizers

Andreas Natsch

Bioscience Department, Fragrance Research, Givaudan Schweiz AG, 8600 Dübendorf, Switzerland

- Luminometry

ARE: Antioxydant Response Element
KeratinoSens

Keap1

Nrf2

Maf

ARE
EpRE
Luciferase
AKR1C2

Sensitization induction

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KeratinoSens

- Sensitivity 79%
- Specificity 72%
- Recommendation EURL-ECVAM: February 2014
- Guideline OECD 442D: February 2015

- Limits of the tests
  - Do not detect the pro-haptens nor pre-haptens
  - Problem with non soluble test items
  - Test items that react preferably with lysines
  - Do not easily detect the weak sensitizers
h-CLAT - Human cell line activation test

- Kao & Shiseido (Ashikaga et al., Toxicol in vitro, 2006, 767-773)

- THP-1 cells: monocytic human cell line leukemia cell line), dendritic cells like

- Respond to skin sensitizers by expression of dendritic cell activation markers:
  - CD54: adhesion molecule
  - CD86: co-stimulation molecule for T-cells activation
h-CLAT

- Incubation of the test items with THP-1 cells during 24h at 8 concentrations
- Measurement of the CD54 and CD86 expression by flow cytometry
- Need for a minimum of cell stress: concentration leading to at least 25% of mortality
- Sensitizer if: CD54 ≥ 150% and/or CD86 ≥ 200%
- Sensitivity 81%
- Specificity 77%
- Draft for an OECD Guideline: July 2014
- Recommandation EURLECVAM: February 2015
MUSST «myeloid U937 skin sensitization test»

- Based on regulation of the CD86 expression on the surface of human monocytic cell (myeloïd cell U937)
- L’Oréal
- Flow cytometry
- Sensitivity 86%
- Specificity 67%

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Other tests

- IL18 on keratinocytes or reconstructed epidermis
  - Keratinocytes NCTC 2544
  - Epidermes EpiCS (CellSystems) or EpiDerm (MaTek)
  - Interleukine 18 assessment in culture supernatant after 24 hours of incubation
  - Positive response if the level increase is ≥ 5 times
  - Response type: yes or no
  - Advantage:
    - Easy to set-up
    - Convenient for non soluble compounds
Other tests

☐ Genomic approach

■ SenCeetox
  ☐ Developed by Ceetox company (US)
  ☐ On keratinocytes HaCat or reconstructed epidermis (EpiDerm or SkinEthic)
  ☐ Multiparametric approach
  ☐ Expression of 11 genes related to the Nrf2/Keap1/ARE or AhR/ARNT/XRE signaling pathways, direct reactivity, cell viability
Other tests

Genomic approach

- Sens-IS
  - Developed by Immunosearch company (Grasse - France)
  - Quantification par RT-PCR de 62 biomarqueurs
  - Sensitivity : 91%
  - Specificity : 83%
ITS: Integrated Testing Strategy

- **In silico**
  - Comparison, Read Across, QSAR, ...

- **In chemico**
  - DPRA

- **In vitro**
  - KeratinoSens
  - h-CLAT
  - Other....

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ITS: time scale

EURL ECVAM Strategy for Replacement of Animal Testing for Skin Sensitisation Hazard Identification and Classification - 2013
ITS: current approach

DPRA + KeratinoSens

+/-

Positive

-/-

Negative

-/+ or +/-

h-CLAT

+/

Positive

-/

Negative