# Making Contact Allergy History: NAMs for Skin Sensitization and Photosafety

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LA STATALE

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# EUROTOX' strategic agenda

# The role of EUROTOX in the Future of Toxicology

- Enhancing the basic (and advanced) training in toxicology
  - Conduct/support of educational courses – ERT
  - Support IUTOX educational activities - RASS
- Provide continued education for toxicologists
  - Annual EUROTOX congress: CECs (All proposals must be submitted using the online portal, deadline is November 15, 2026).
  - Specialty Sections
  - Webinars
- Early Career Forum (ECF): is an autonomous network established in March 2024 to support early-stage researchers, PhD candidates, and professionals within 10 years of their highest degree who are passionate about toxicology
- EUROTOX Ambassadors
- EUROTOX Early Career Exchange Program (ECEP)

# Want to know more about EUROTOX ?




**https://www.eurotox.com**

# Conflict of Interest Statement

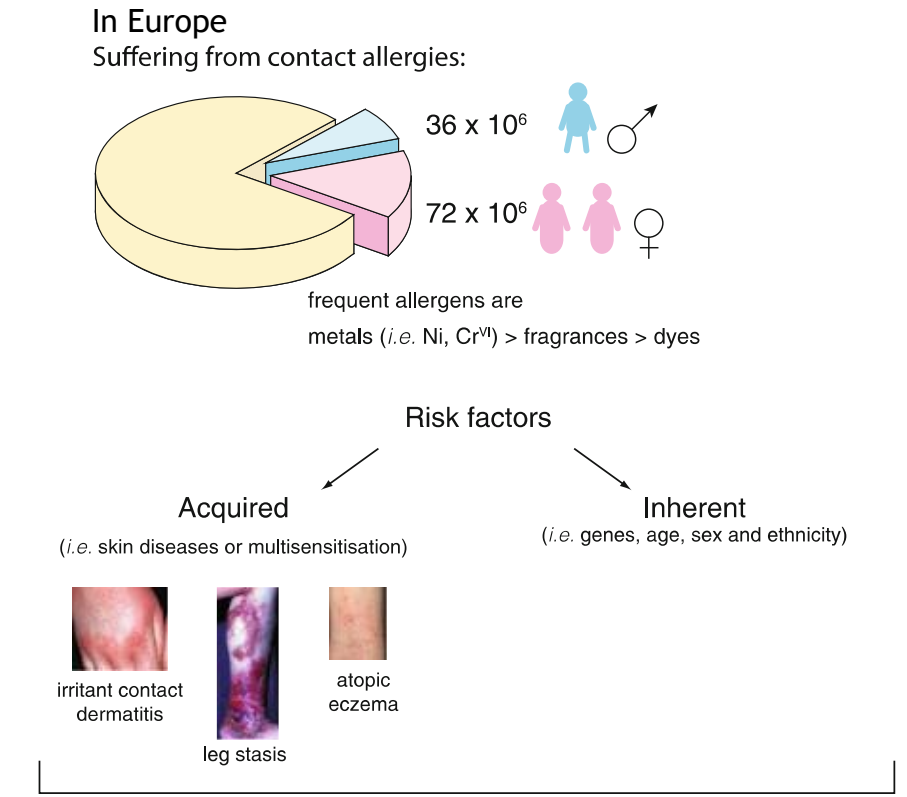
The author declares no conflict of interest.

# LAYOUT OF THE PRESENTATION

- Framing the problem
- Hypersensitivity: definition, hazard identification and characterization
- In vitro methods: the AOP
- Examples: Key Event 2
- Phototoxicity and photosensitization
- Conclusions

# CHEMICAL ALLERGY: background

- It is estimated that 20.1% of the European population suffers from contact allergies (e.g. Alinaghi et al., 2019).
- Currently, there are more than 4000 substances identified as contact allergens, most commonly nickel, fragrances and preservatives.
- **Toxicologists have the responsibility of identifying and characterizing the sensitizing potential of chemicals and, estimating the risk they pose to human health.**
- Regulatory authorities worldwide require testing for allergic contact dermatitis (ACD) and appropriate hazard labeling to minimize exposures.




Prevention of dermatitis in the occupational setting by surveillance, introduction of good working practices (*e.g.* hair dressers) and legislation (*e.g.* European nickel directive and limits for chromium in cement).

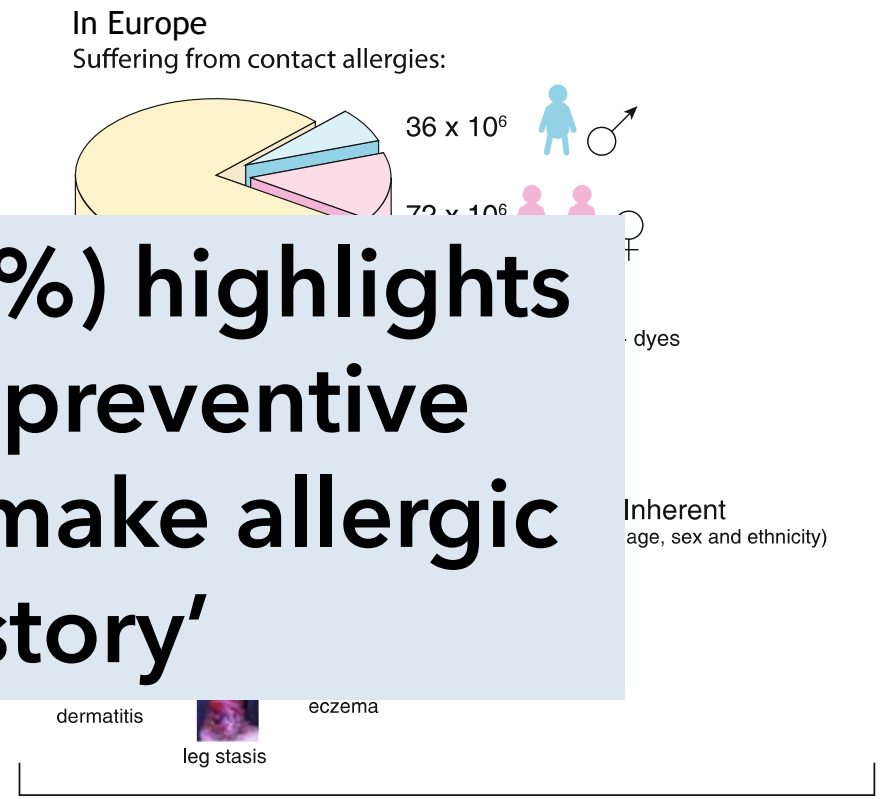
Cell. Mol. Life Sci. (2012) 69:763–781 (open access)

# CHEMICAL ALLERGY: background

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and

**The still high prevalence (~20%) highlights the need for more effective preventive strategies, including QRA, to make allergic contact dermatitis 'history'**

- **Toxi**
**char**
**and, estimating the risk they pose to human health.**
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# HYPERSENSITIVITY: definition

**Definition:** excessive humoral or cellular response to an antigen that can lead to tissue damage.

**Hypersensitivity reactions are the result of normally beneficial immune responses acting inappropriately.**

**Gell and Coombs's classification (old):**
Type 1: IgE mediated (immediate type)
Type 2: IgM, IgG, cytolysis of cells
Type 3: IgM, IgG Immune complex mediated
Type 4: T-cell mediated (delayed-type)

**Modern classification**:
1. primarily antibody-mediated reactions
2. cell-mediated reactions
3. pseudo-allergic reaction

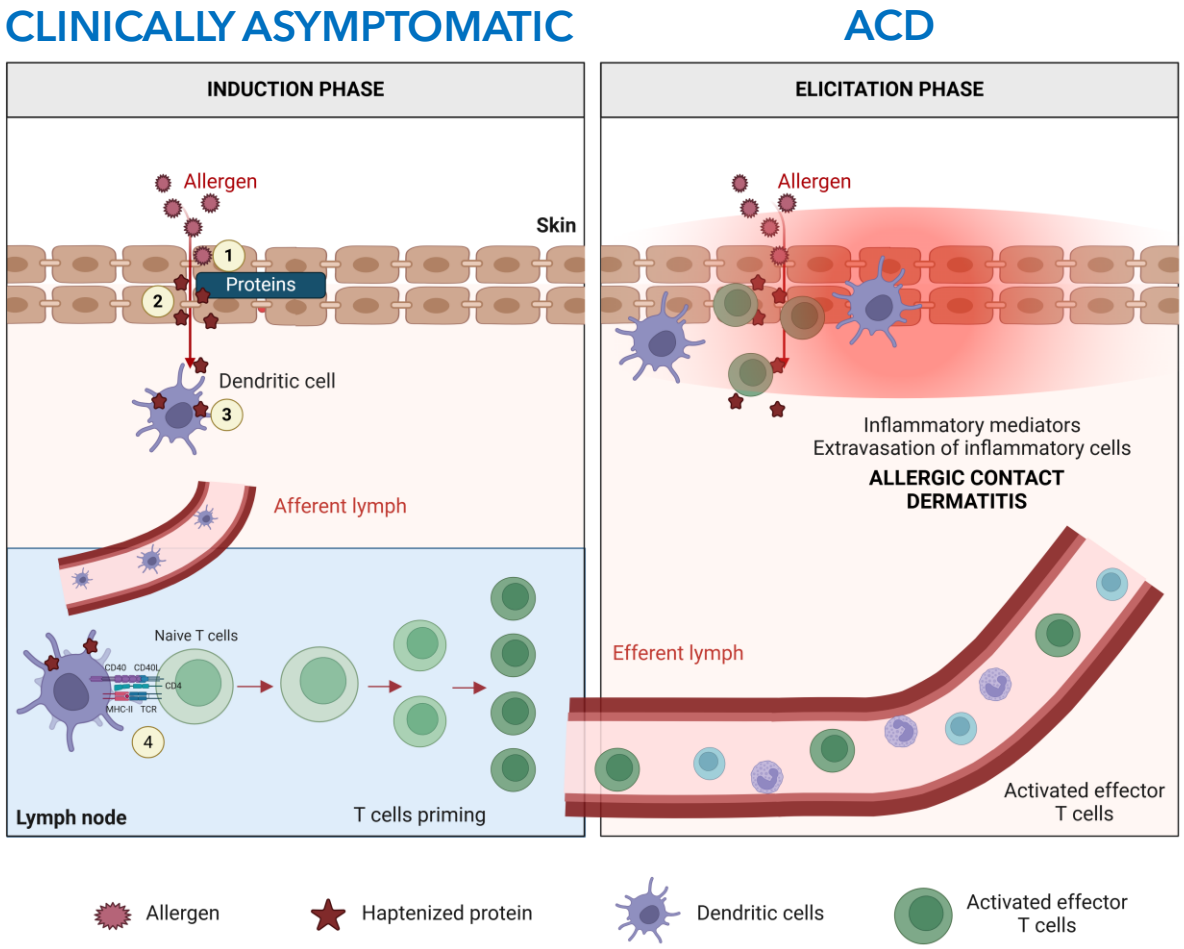
**Chemical allergy** refers to immune-mediated adverse health effects caused by exposures to low molecular weight chemicals. **Allergic Contact Dermatitis is a delayed type hypersensitivity reaction caused mainly by the generation of $CD8^+$ Tc1/Tc17 and $CD4^+$ Th1/Th17 effector T cells as a result of repeated exposure of an allergen primarily on skin** (Martin, 2015).

# ALLERGIC CONTACT DERMATITIS

**Key events**

Absorption (metabolism)

1. Protein binding (haptenation)
2. Local trauma - epidermal inflammation (danger signals)
3. Antigen processing: Langerhans cells/dermal DCs maturation and migration
4. Antigen presentation to Th cells and the generation of memory T cells (immunogenicity)




There are a variety of **characteristics** that determine whether a chemical can function as a contact sensitizer (or allergen):

- ability to penetrate into the skin
- reactivity with protein
- epidermal and dermal inflammation
- dendritic cell activation, migration to lymph nodes and recognition as antigenic by T cells.

**Allergic contact dermatitis is characterized by redness, itching, and inflammation of the skin upon re-exposure to the sensitizing substance.**

# HAZARD IDENTIFICATION AND RISK CHARACTERIZATION OF THE SKIN SENSITIZATION POTENTIAL OF CHEMICALS








# HYPERSENSITIVITY: animal models

- Well established methods for contact and respiratory hypersensitivity

- Current models and assays as inadequate predictors for system hypersensitivity reactions (Gell and Coombs: type II and III)

➢ Guinea Pig Tests
- Maximization Test
- Occlusive Patch Test
- Respiratory Challenge
- Systemic Anaphylaxix

➢ Mouse Tests
- Local lymph node assay
- Mouse Ear Swelling Test

The effective evaluation of skin sensitising substances requires not only the identification of that toxicological hazard, but also determination of relative sensitising potency.

# GHS Classification Categories for Skin Sensitizers

| Category | Classification Criteria | LLNA EC3 | Human Evidence (HRIPT or HMT) | GPMT Response | BT Response |
|---|---|---|---|---|---|
| 1<br>Skin sensitizer | Evidence that skin sensitization occurs in a substantial number of people, or positive results from an appropriate animal test | NA | NA | NA | NA |
| IA<br>Strong skin sensitizer | High frequency of occurrence in humans, and/or high potency in animals. May consider severity. | ≤2% | Positive[1] response at ≤500 μg/cm$^2$ | ≥30% responders at ≤0.1% intradermal induction dose or ≥60% responders at >0.1% to ≤1% intradermal induction dose | ≥15% responders at ≤0.2% topical induction dose or ≥60% responders at >0.2% to ≤20% topical induction dose |
| IB<br>Other skin sensitizer | Low to moderate frequency of occurrence in humans, and/or low to moderate potency in animals. May consider severity. | >2% | Positive[2] response at >500 μg/cm$^2$ | ≥30% to <60% responders at >0.1% to ≤1% intradermal induction dose or ≥30% responders at >1% intradermal induction dose | ≥15% to < 60% responders at >0.2% to ≤20% topical induction dose or ≥15% at >20% topical induction dose |

https://www.researchgate.net/publication/265451010

# LAYOUT OF THE PRESENTATION

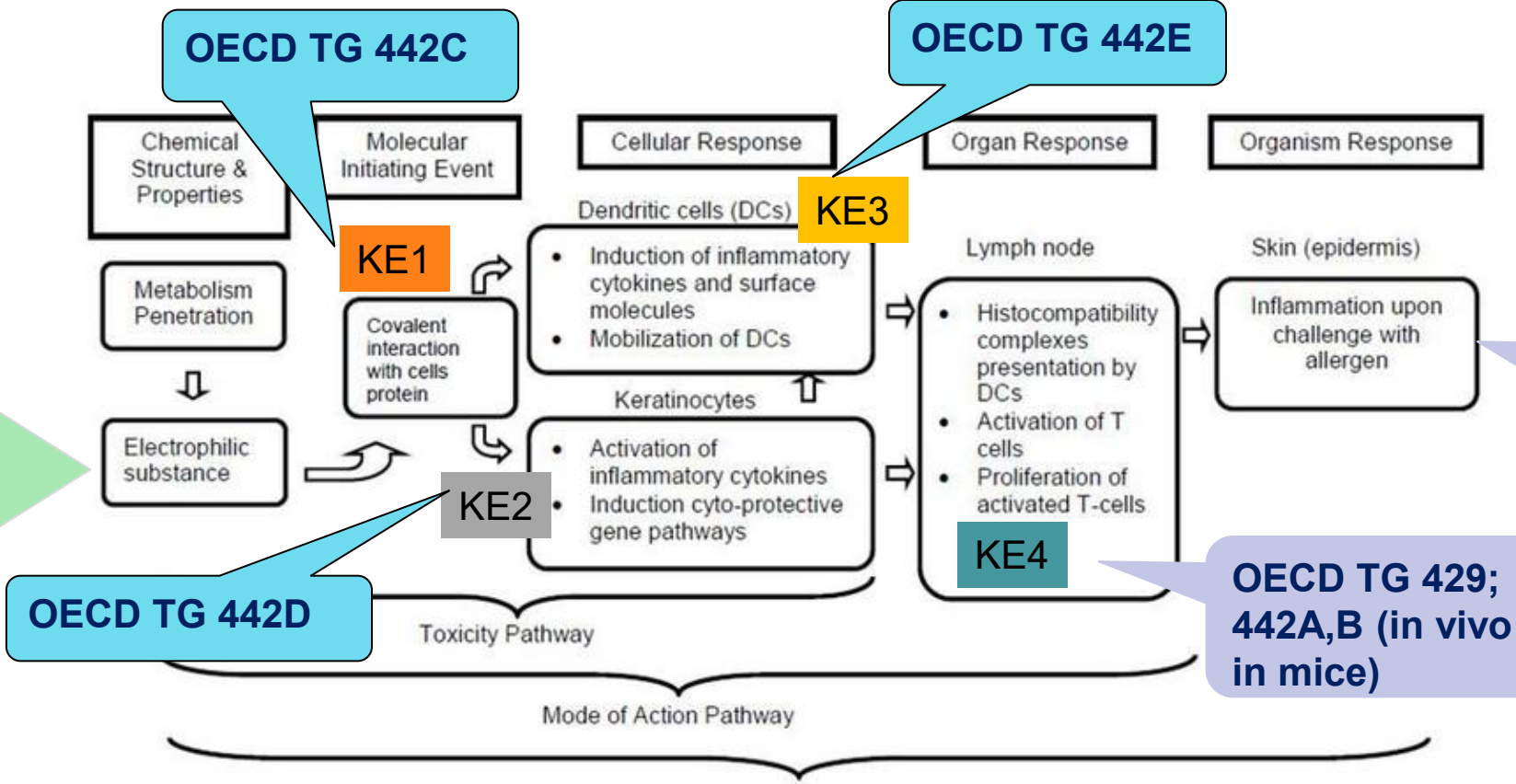
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**3 September 2014**

OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

Several in silico QSAR models are available:

- DEREK
- ToxTree
- TIMES-SS
- OECD Toolbox




OECD TG 406 (in vivo in Guinea Pigs) HRIPT

OECD TG 429; 442A,B (in vivo in mice)

Adverse Outcome Pathway and associated assays

| Key event 1 | Key event 2 | Key event 3 | Key event 4 | Adverse outcome |
|---|---|---|---|---|
| Protein binding | Keratinocyte activation | Activation of dendritic cells | T-cell proliferation | Allergic contact dermatitis |
| DPRA/ADRA | KeratinoSens/ LuSens/ α–Sens | H-CLAT | LLNA | Guinea pig models |
| Kinetic DPRA | Epi2sensA | U-SENS | | |
| | SENS-IS | IL-8 Luc assay | | |
| | RhE IL-18 assay | GARDSkin | | |

**HRIPT**, human repeated insult patch test

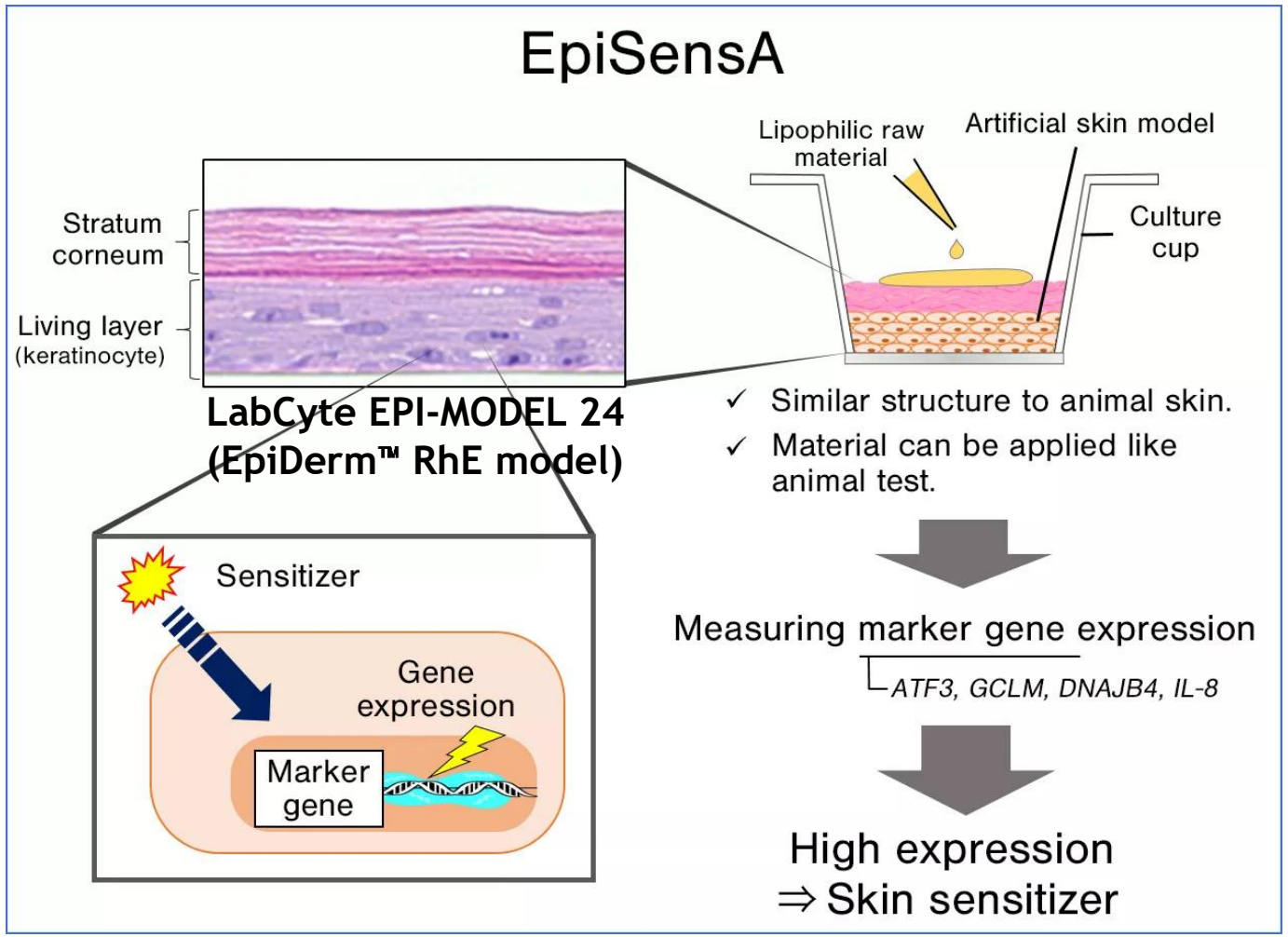
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## **Key Event 2:** Keratinocyte activation

Covalent binding triggers the activation of keratinocytes in the skin.

# EPIDERMAL SENSITIZATION ASSAY (EpiSensA and Epi2SensA)




https://www.kao.com/global/en/innovation/research-development/fundamental/safety-science/sensitization-evaluation/

The method is based on measurement of 4 mechanistically relevant genes:

- glutamate-cysteine ligase, modifier subunit (GCLM);
- DnaJ (Hsp40) homolog, subfamily B, member 4 (DNAJB4);
- encoding activating transcription factor 3 (ATF3);
- interleukin-8 (IL-8) following 6 h of treatment.

When the mean maximum fold induction of at least one out of the four marker genes exceeded the respective cut-off value (ATF3, >15-fold; GCLM, >2-fold; DNAJB4, >2-fold; and IL-8, >4-fold), the chemical is judged as positive.

**For a panel of 136 chemicals, the EpiSensA provided 88% sensitivity, specificity 66 and 82% accuracy compared with LLNA results (Mizumachi et al., 2023).**

# KEY EVENT N° 2 - applicability domain

The applicability domain of EpiSensA is broad and includes challenging substance types like lipophilic (fat-soluble) chemicals, pre-haptens, and pro-haptens.

**Advantages**

- Pre/Pro-Haptens: Successfully detects substances that require metabolic or chemical activation before triggering an immune response.
- Lipophilic Substances: Effectively tests hydrophobic and fat-soluble materials that often fail in other Key Event 2 keratinocyte assays.
- Complex Mixtures: Capable of evaluating certain finished products and mixtures with unknown or variable compositions.

# KEY EVENT N° 2 - applicability domain

**Technical and Solubility Limitations**

- Solubility Requirement: The test chemical must be fully soluble or capable of forming a stable dispersion at 0.0122% in an approved assay vehicle.
- Alternative Vehicles: Solvents outside the standard protocol may be used if supported by a valid scientific rationale.
- Assay Interferences (RT-qPCR & Biocompatibility): GAPDH Interference: Chemicals that directly modify or suppress the expression of GAPDH (the internal reference/housekeeping gene) cannot be accurately evaluated because it prevents reliable normalization of the marker genes.
- Cytotoxicity Assay Clashes: Test substances that inherently interfere with LDH (Lactate Dehydrogenase) or MTT cell viability assay reagents will distort the mandatory cytotoxicity assessments.

# WHAT WE HAVE ACHIEVED

## FROM HAZARD ASSESSMENT TO QRA USING NAMs

1. We have validated methods for hazard identification: YES/NO
2. We have Defined Approach (**DA, OECD TG 497**) for sub-classification according to GHS: 1A and1B
3. Derivation of a Point of Departure for Quantitative Risk Assessment: approaches to derive the PoD are now been added to OECD TG 497 (SARA-ICE DA; regression model)

**No additional animal testing needed (pending regulatory acceptance of the QRA)**

# Dermal Sensitisation Thresholds (DST)

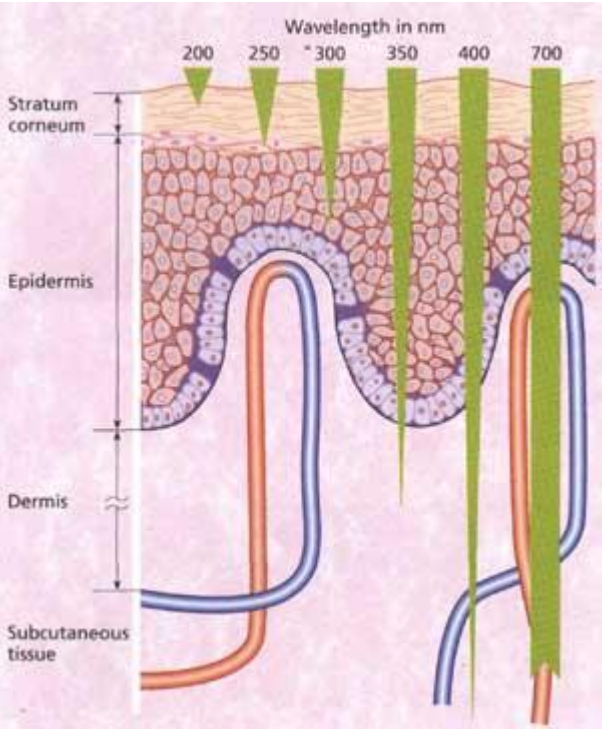
- The Dermal Sensitisation Thresholds (DST) are Thresholds of Toxicological Concern, which can be used to justify exposure-based waiving when conducting a skin sensitisation risk assessment based on the LLNA data (Chilton et al., 2022).
- The chemical reactivity is assigned using an in silico expert system (Derek Nexus). Derek Nexus is based on a dataset of 1152 chemicals.
- Updated thresholds (estimated NESIL):
  - a non-reactive DST of 710 µg/cm2
  - a reactive (non-HPC) DST of 73 µg/cm2
  - High potency category DST (direct Michael acceptors, Schiff base electrophiles, acyl transfer agents) of 1.0 µg/cm2
- **Not accepted by SCCS**

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# PHOTOTOXICITY

- **Phototoxicity** is a toxic response, which can manifest as:
  - photoirritation (narrowly defined as phototoxicity),
  - photoallergy,
  - photogenotoxicity
- Phototoxicity is elicited after the initial exposure of skin to certain chemicals and subsequent exposure to UV light, or that is induced by skin irradiation after systemic administration (oral, intravenous) of a chemical substance.




UVC (200-290 nm)
UVB (290-320 nm)
UVA (320-400 nm)

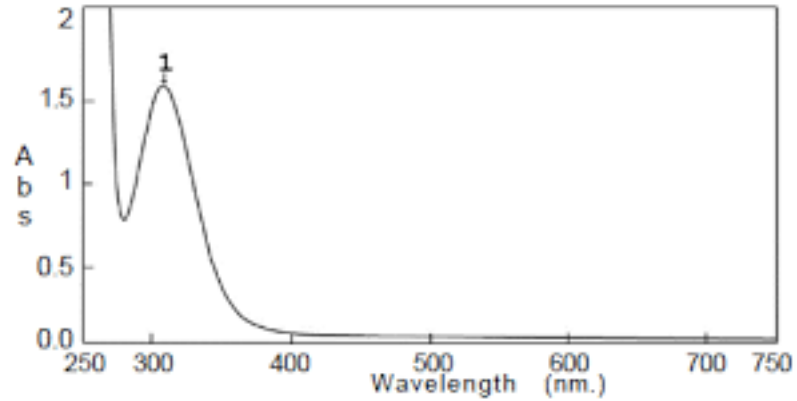
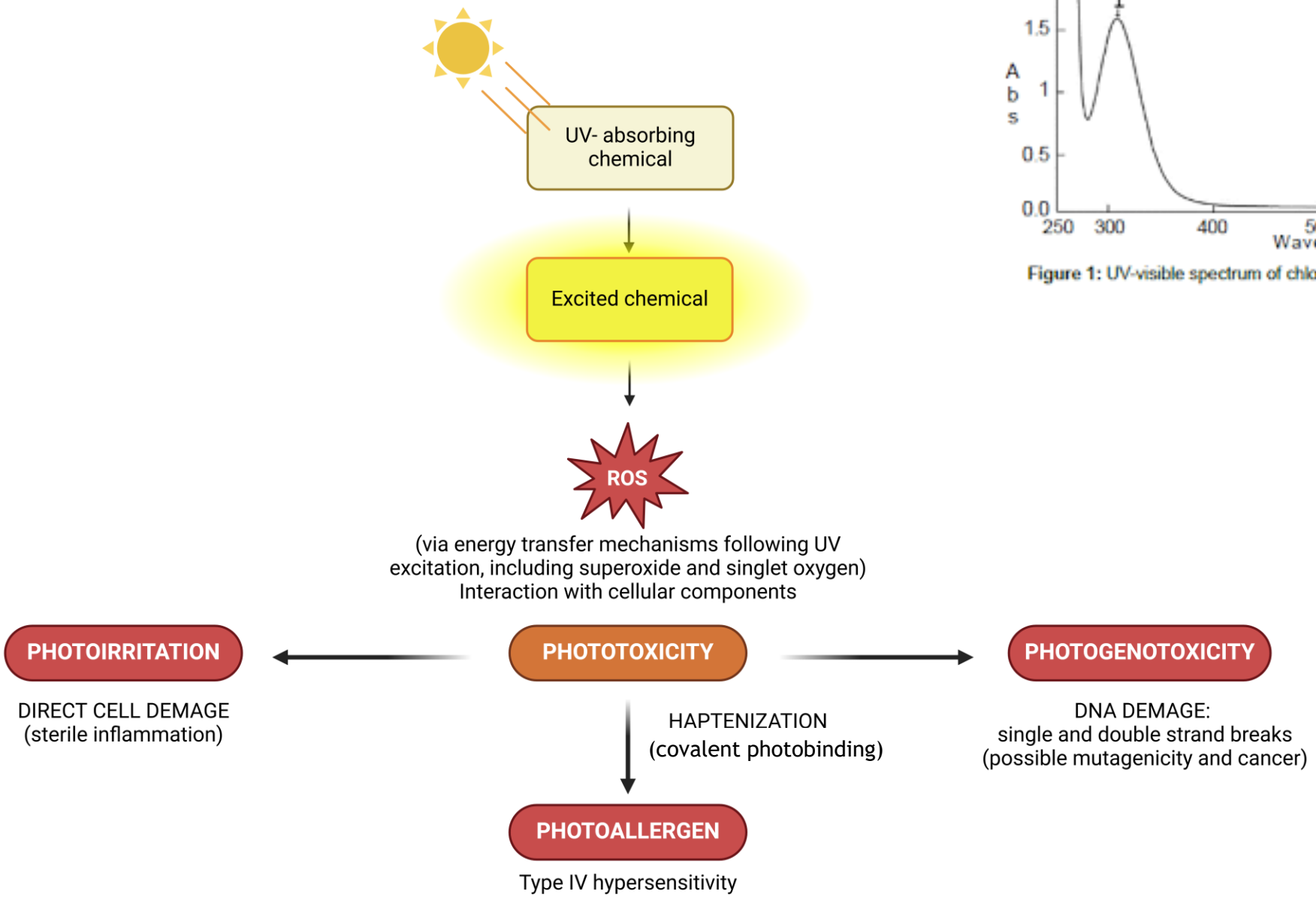



**Figure 1:** UV-visible spectrum of chlorpromazine in standard solution.

UVA (320-400 nm)



| CHARACTERSITICS | PHOTOIRRITATION | PHOTOALLERGY |
|---|---|---|
| Onset of reaction | Minutes to hours | 1-3 days |
| Incidence | More common | Less common |
| Required exposure to chemical | single | More than one |
| Mechanism | ROS cause change in skin molecules leading to cell damage and death (sterile inflammation) | UV-activated chemical binds to proteins to form a new antigen leading to immune system activation and formation of contact dermatitis skin lesions |
| Immunologically mediated | No | yes |
| Localization of lesions | Confined to the sun-exposed skin area | Outbreak is not limited to sun-exposed skin, but can spread to the whole-body area |
| Duration of the reaction | Usually lasts 2-4 days | Usually lasts 10 days |
| Clinical manifestation | Exaggerated sunburn with blistering, desquamation and hyperpigmentation | Dermatitis |

# PREVALENCE OF PHOTOALLERGY

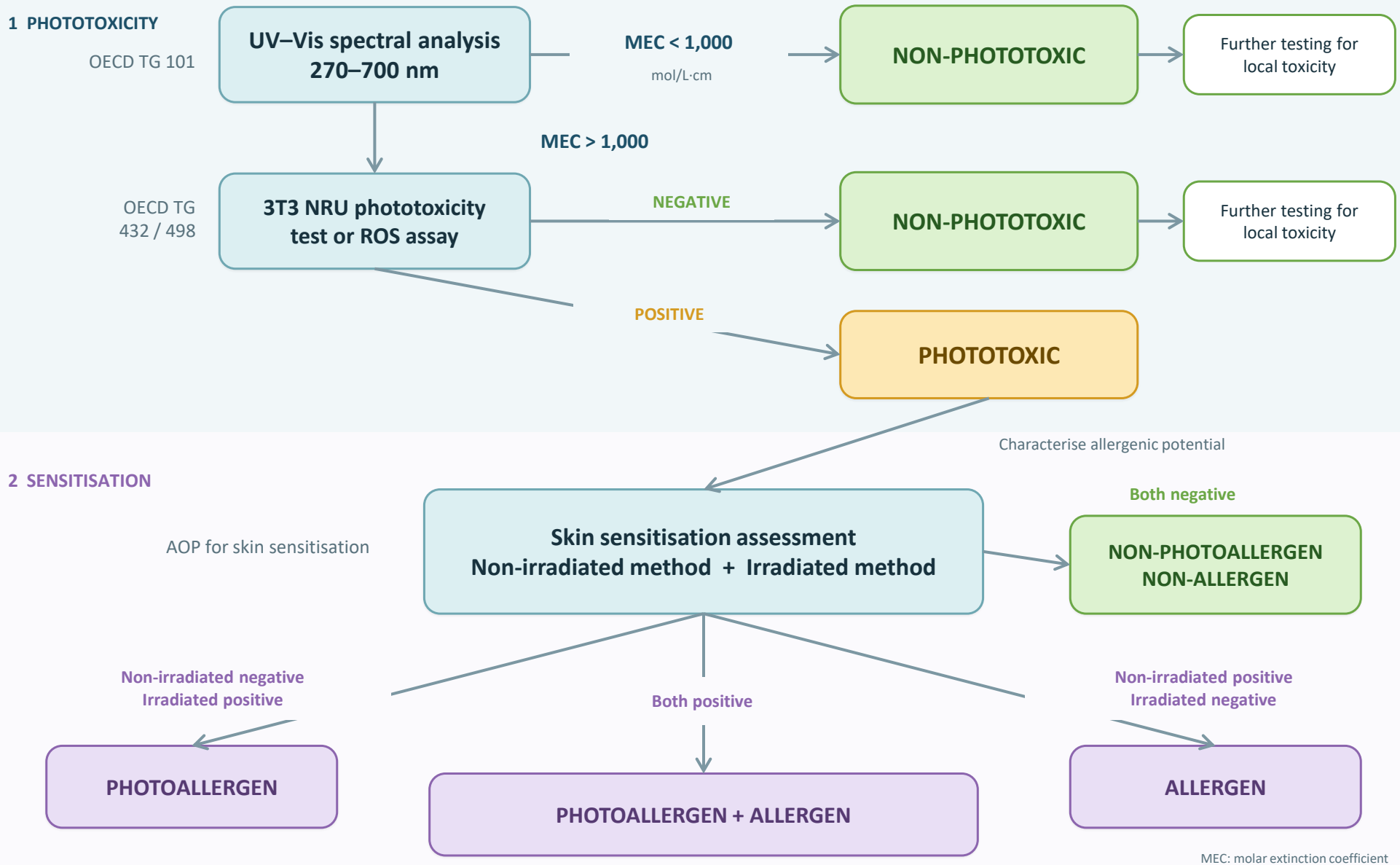
- Photoallergy is an uncommon, immune-mediated skin reaction to ultraviolet (UV) light that occurs when a chemical or drug absorbs light and triggers a delayed hypersensitivity response.

**Common Triggers**

- **Sunscreens:** Ingredients such as benzophenones are among the most frequently identified photoallergens (Ekstein  and Hylwa, 2023).
- **Topical and Systemic Agents:** Certain non-steroidal anti-inflammatory drugs (NSAIDs), antimicrobials, and specific fragrances act as responsible haptens binding to skin proteins under UV exposure. [Lozzi et al., 2020]

# Decision pathway for phototoxicity and photoallergenicity

Sequential screening of light absorption, phototoxicity and skin sensitisation



# EXAMPLES OF PUBLISHED IN VITRO METHODS FOR THE IDENTIFICATION OF PHOTOSENSITIZERS

| Test Procedure | Dataset | Compared to | Accuracy (%) | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|
| **Protein reactivity** | | | | | | |
| Photobinding assay | 7 | Human/animal | – | – | – | Barratt and Brown (1985) |
| Photobinding/photooxidation assay | 24 | Human/animal | – | – | – | Lovell and Jones (2000) |
| Photo-mDPRA | 14 | Human/animal | – | – | – | de Ávila et al. (2017b) |
| Photo-ADRA | 9 | – | – | – | – | Patel et al. (2019) |
| **Keratinocyte activation** | | | | | | |
| ***Cytokine production analysis*** | | | | | | |
| NCTC 2544 cells-associated IL-18 photoassay | 15 | Human | 100 | – | – | Galbiati et al. (2014) |
| PhotoSENSIL-18 | 16 | Human/animal | – | 89 | – | Nguyen et al. (2023) |
| ***Activation of Keap1-Nrf2-ARE via*** | | | | | | |
| Photo-KeratinoSens™ | 9 | Human/animal | 56 | 50 | 67 | Tsujita-Inoue et al. (2016) |
| Photo-ARE assay* | 30 | Human/animal | 77 | 77 | 85 | |
| **DC activation-based models** | | | | | | |
| ***Phenotyping of cell surface markers*** | | | | | | |
| THP-1 skin photosensitisation assay | 18 | Human/animal | – | – | – | Hoya et al. (2009) |
| Photo-PBMDC assay | 09 | – | – | – | – | Karschuk et al. (2010) |
| ***Cytokine secretion analysis*** | | | | | | |
| THP-1 cells-associated IL-8 photoassay | 15 | Human/animal | 93 | 86 | – | Martínez et al. (2013) |
| ***Changes in cell-surface thiols and amines*** | | | | | | |
| Photo-SH/$NH_2$ test | 33 | Human/animal | 70 | 83 | 53 | Oeda et al. (2016) |
| ***Gene expression analysis*** | | | | | | |
| GARD™skin | 4 | – | – | – | – | Forreryd et al. (2021) |
| GARD™skin Dose-Response | 12 | Human/animal | – | – | – | Lindberg et al. (2023) |

Abbreviations: ADRA, amino acid derivative reactivity assay; DPRA, direct peptide reactivity assay; mDPRA, micro-direct peptide reactivity assay; GARD, Genomic allergen rapid detection; PBMDC, peripheral blood monocyte-derived dendritic cells; SH/$NH_2$, cell-surface thiols/amines.

* Even though the cell system used is based on the human MCF7 breast carcinoma cell line, this assay was included in the table because it is a photo-KeratinoSens™ "me-too" method.

*Regulatory Toxicology and Pharmacology 145 (2023) 105499*

**There are currently no validated methods for skin photosensitization prediction.**

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# 1. CONCLUSIONS

- Several in vitro methods to assess contact hypersensitivity are also available. Ten methods for contact hypersensitivity have been successfully validated, and their integration (DA) allow appropriate classification and labeling, and estimation of the PoD for QRA.

- Acceptance of the evaluation of skin sensitizing potency to support effective quantitative risk assessment using non-animal tests is still under discussion (QRA).

- Accepted NAMs are available for phototoxicity (photoirritation).

- The acceptance of methods for photosensitization is still pending.

# 2. CONCLUSIONS

**A reduction of ACD can be achieved by**:

- correct detection of skin sensitizers;
- characterization of potency;
- understanding whether prolonged exposure to an allergen below its threshold can cause sensitization;
- understanding of human skin exposure;
- application of adequate risk assessment and management strategies.

Only improved risk assessment, better education of risk assessors, better education of consumers on the proper use of cosmetic products, better marketing surveillance by authorities to control proper cosmetic product safety evaluation, could help to ***'make contact allergy history***'.

# THANK YOU FOR YOUR ATTENTION

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