SKIN SENSITISATION TESTS

- WE HAVE HAD GOOD PREDICTIVE TESTS FOR OVER HALF A CENTURY
- WE UNDERSTAND KEY EVENTS OF THE TOXICOLOGICAL MECHANISM
- WE DEVELOPED THE FIRST FORMALLY VALIDATED ALTERNATIVE (LLNA)
- WE HAVE A PROCESS FOR QUANTITATIVE RISK ASSESSMENT (QRA)
- WE HAVE RAPID AND RELIABLE FEEDBACK FROM DERMATOLOGISTS
- ….AND WE NOW HAVE A PORTFOLIO OF IN VITRO ALTERNATIVES, VALIDATED AND MOVING INTO USE

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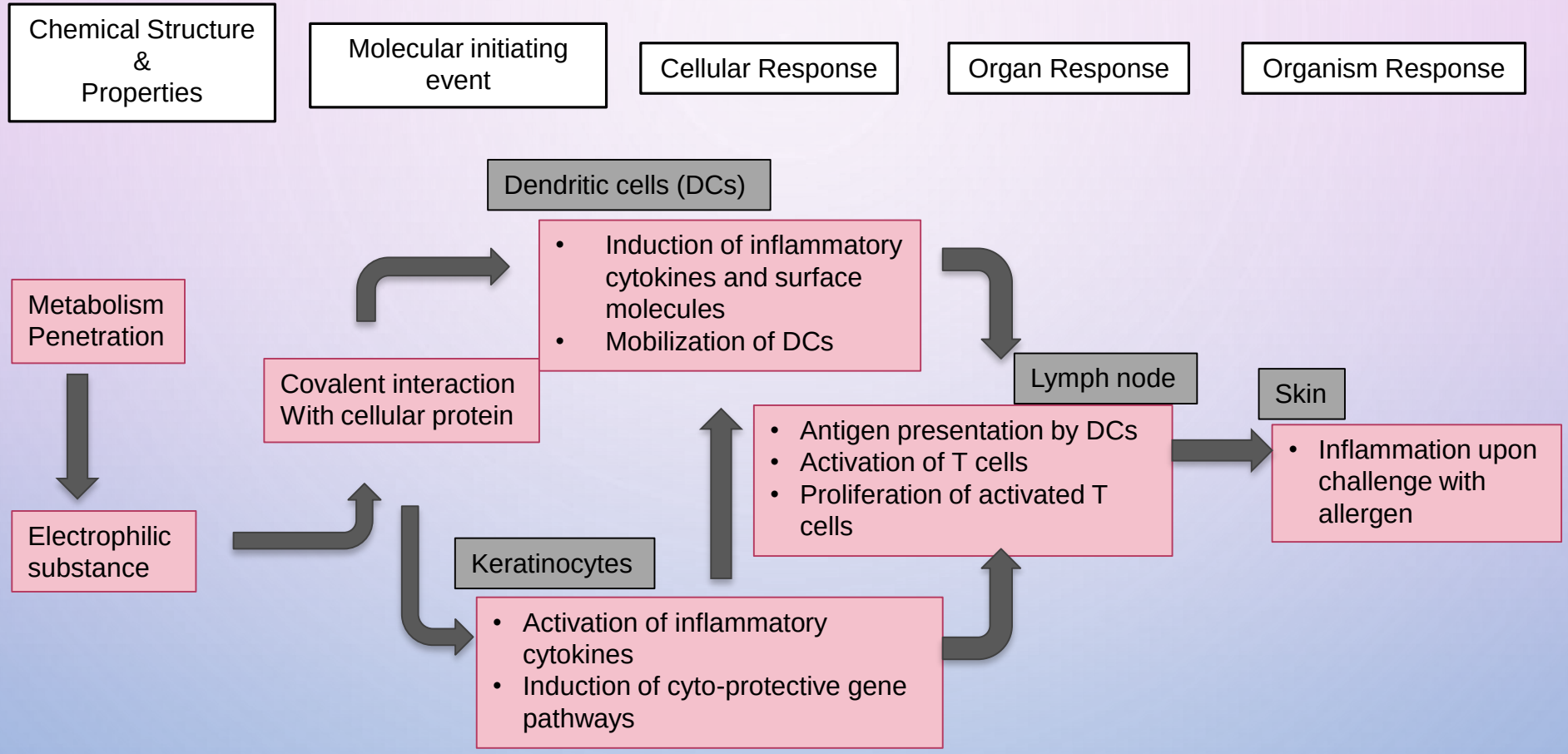
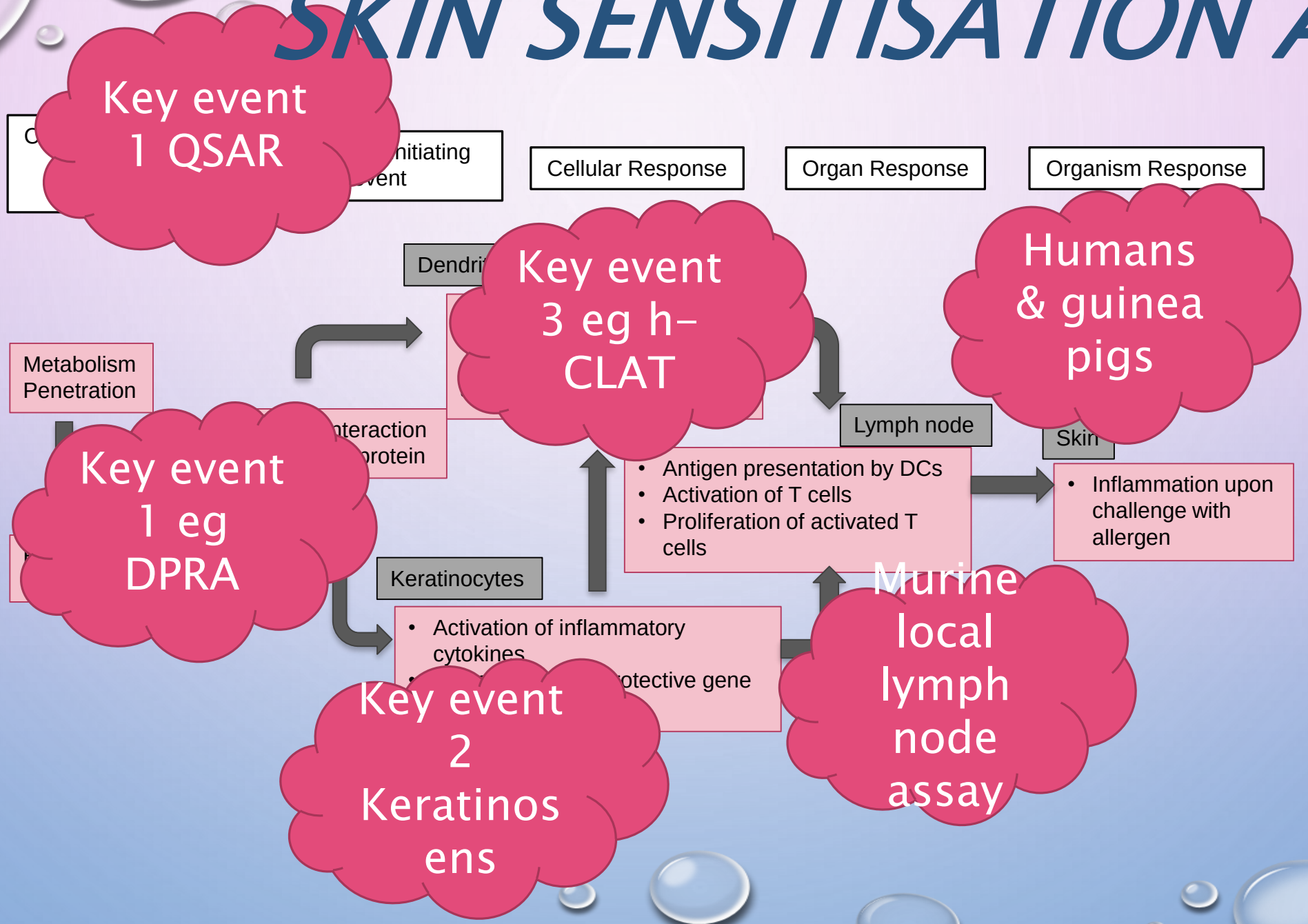
85%

SKIN SENSITISATION AOP



SKIN SENSITISATION AOP



IN VITRO METHODS

- IN SILICO (e.g. TIMES-SS; DEREK; OECD TOOLBOX; TOPKAT)
- IN CHEMICO REACTIVITY (KE1) (e.g. DPRA; PPRA UNDER VALIDATION)
- IN VITRO (KE2) (e.g. KERATINOSENS; LUSENS UNDER VALIDATION)
- IN VITRO (KE3) (e.g. h-CLAT; IL-8 LUC & U-SENS UNDER VALIDATION)
- IN VITRO (KE4) (COSMETICS EUROPE T-CELL WORKSHOP IN JUNE)

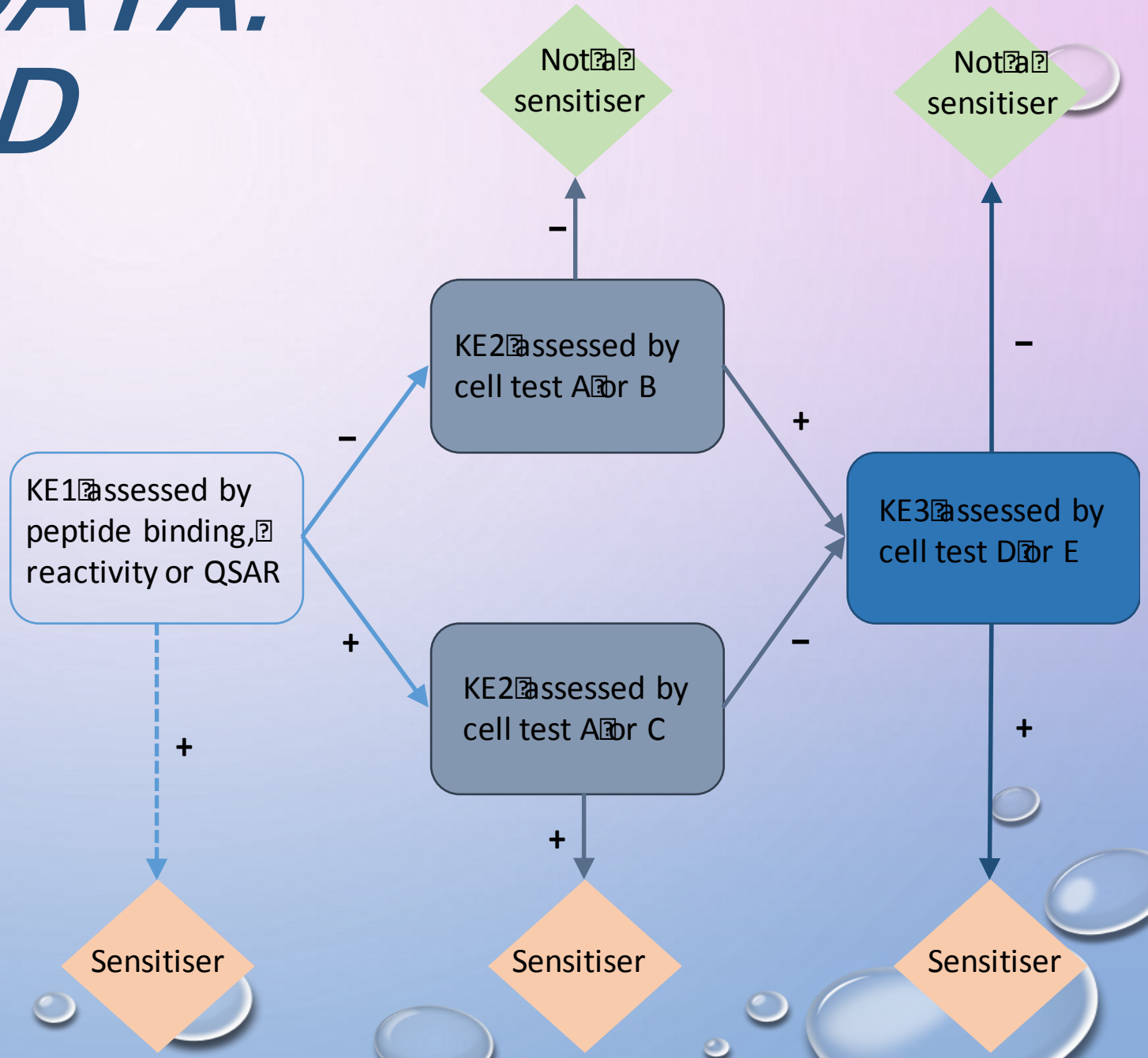
IN VITRO METHODS: DATA ANALYSIS

- ESSENTIALLY ALL NON-ANIMAL METHODS GENERATE DATA WHICH FEEDS INTO A YES/NO PREDICTION MODEL
- NONE OF THE IN VITRO METHODS ARE IDENTIFIED AS STANDALONE OPTIONS
- THERE IS NO CREDIBLE WAY TO COMBINE YES/NO OUTPUTS TO DELIVER ANYTHING OTHER THAN BASIC HAZARD IDENTIFICATION

- THE APPROACH TO THE COMBINATION OF VITRO DATA HAS BEEN MUCH DEBATED, BUT NOT YET AGREED
- CURRENTLY, DEMOCRACY RULES AND CAN DELIVER PREDICTIVE ACCURACY AS GOOD AS THE IN VIVO TESTS
- MORE COMPLEX, e.g. NEURAL NETWORKS, BAYESIAN METHODS ARE ALSO CONSIDERED

IN VITRO DATA: HAZARD

Basketter et al (2015) Alternatives for skin sensitisation testing and assessment. Regulatory Toxicol Pharmacol, 73, 660–6



This is a recent example of the performance of a simple ITS with in vitro inputs (KE1 + KE2, then KE3 if necessary).

It shows performance equal to the LLNA and better accuracy with comparison to human data.

It also demonstrates effective inter-industry collaboration across 3 continents.

Regulatory Toxicology and Pharmacology 71 (2015) 337–351

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Assessing skin sensitization hazard in mice and men using non-animal test methods

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ARTICLE INFO

Article history:
Received 22 September 2014
Available online 23 December 2014

Keywords:
Skin sensitization
Allergic contact dermatitis (ACD)
Adverse outcome pathway (AOP)
DPRA
KeratinoSens™
LuSens
h-CLAT
(modified) MUSST
Database
Integrated testing strategy (ITS)

ABSTRACT

Sensitization, the prerequisite event in the development of allergic contact dermatitis, is a key parameter in both hazard and risk assessments. The pathways involved have recently been formally described in the OECD adverse outcome pathway (AOP) for skin sensitization. One single non-animal test method will not be sufficient to fully address this AOP and in many cases the use of a battery of tests will be necessary. A number of methods are now fully developed and validated. In order to facilitate acceptance of these methods by both the regulatory and scientific communities, results of the single test methods (DPRA, KeratinoSens™, LuSens, h-CLAT, (m)MUSST) as well for a the simple '2 out of 3' ITS for 213 substances have been compiled and qualitatively compared to both animal and human data. The dataset was also used to define different mechanistic domains by probable protein-binding mechanisms. In general, the non-animal test methods exhibited good predictivities when compared to local lymph node assay (LLNA) data and even better predictivities when compared to human data. The '2 out of 3' prediction model achieved accuracies of 90% or 79% when compared to human or LLNA data, respectively and thereby even slightly exceeded that of the LLNA.

THE SENS-IS TEST PROTOCOL FOR *IN VITRO* DETECTION OF SENSITIZERS IS BASED ON A RECONSTRUCTED HUMAN SKIN MODEL (EPISKIN) AS THE TEST SYSTEM AND ON ANALYSIS OF THE EXPRESSION OF A LARGE PANEL OF GENES. ITS EXCELLENT PERFORMANCE WAS INITIALLY DEMONSTRATED WITH A LIMITED SET OF TEST CHEMICALS. FURTHER STUDIES WERE ORGANIZED TO CONFIRM THESE PRELIMINARY RESULTS AND TO OBTAIN A DETAILED STATISTICAL ANALYSIS OF THE PREDICTIVE CAPACITY OF THE ASSAY. A RING STUDY WAS THUS ORGANIZED AND PERFORMED WITHIN THREE LABORATORIES, USING A TEST SET OF 19 BLIND CODED CHEMICALS. DATA ANALYSIS INDICATED THAT THE ASSAY IS ROBUST, EASILY TRANSFERABLE AND OFFERS HIGH PREDICTIVITY AND EXCELLENT WITHIN AND BETWEEN LABORATORY REPRODUCIBILITY. TO FURTHER EVALUATE THE PREDICTIVITY OF THE TEST PROTOCOL, A COMPREHENSIVE TEST SET OF 150 CHEMICALS WAS ANALYZED. DATA ANALYSIS CONFIRMED THE EXCELLENT CAPACITY OF THE SENS-IS ASSAY FOR PREDICTING BOTH HAZARD AND POTENCY CHARACTERISTICS, CONFIRMING THAT THIS ASSAY SHOULD BE CONSIDERED AS A SERIOUS ALTERNATIVE TO *IN VIVO* SENSITIZATION TESTS.

ELSEVIER

Toxicology in Vitro

Volume 29, Issue 4, June 2015, Pages 787–802

Genes specifically modulated in sensitized skins allow the detection of sensitizers in a reconstructed human skin model. Development of the SENS-IS assay

Françoise Cottrez, Elodie Boitel, Claude Auriault, Pierre Aeby, Hervé Groux

Received 24 November 2014, Accepted 17 February 2015, Available online 24 February 2015

ELSEVIER

Toxicology in Vitro

Volume 32, April 2016, Pages 248–260

SENS-IS, a 3D reconstituted epidermis based model for quantifying chemical sensitization potency: Reproducibility and predictivity results from an inter-laboratory study

Françoise Cottrez[a], Elodie Boitel[a], Jean-Claude Ourlin[b], Jean-Luc Peiffer[b], Isabelle Fabre[b], Imène-Sarah Henaoui[c, d], Bernard Mari[c, d], Ambre Vallauri[c, d], Agnes Paquet[c, d], Pascal Barbry[c, d], Claude Auriault[a], Pierre Aeby[e], Hervé Groux[a]

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90%

Example of a test which bypassed validation and went direct to market.

Genomic Allergen Rapid Detection is based on exposure of MUTZ-3 cells.

GARD Skin

The animal free allergy test for classification of skin sensitizers by the use of a biomarker consisting of 200 genes.

GARD Air

The animal free allergy test for classification of respiratory sensitizers by the use of a biomarker consisting of 389 genes.

The GARD test - How it is done

By exposing the test substance to cells from the human immune system, specific genetic changes occur. These can be viewed as on/off-switches of the immune response and the potential risk of the test substances´ ability to induce allergy can be predicted.

The **GARD** test gives a 90% accuracy in predicting sensitization ability in the Human Immune System.
The test is fit for purpose and a suitable method for screening and safety testing for skin and respiratory sensitization.

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Example of a test which bypassed validation and went direct to market.

Genomic Allergen Rapid Detection is based on exposure of MUTZ-3 cells.

10 papers from the Swedish team (in the peer reviewed literature) underpin this work (which originated from Sens-it-iv FP6)

The GARD test - How is it done

Support Vector Machine Model

SENSITIZER

NON-SENSITIZER

By exposing the test substance to cells from the human immune system, specific genetic changes occur. These can be viewed as on/off-switches of the immune response and the potential risk of the test substances´ ability to induce allergy can be predicted.

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GHS 1a
• 1 Extreme
• 2 Strong
GHS 1b
• 3 Moderate
• 4 Weak
GHS NC
• 5 Very weak
• 6 Non-sensitiser

IN VITRO DATA: POTENCY

- CAN WE USE SKIN SENSITISERS IN PRODUCTS?
- YES, eg. VIRTUALLY ALL COSMETICS CONTAIN THEM
- SAFETY IS A MATTER OF DOSE/RISK ASSESSMENT
- QRA IS CENTERED ON MEASURING POTENCY

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Basketter DA and Safford RJ (2016) Skin sensitisation quantitative risk assessment; a review of underlying assumptions. Regul Toxicol Pharmacol, 74, 105–116