NTP
National Toxicology Program

NTP Approaches to Assessment of Dermal Hypersensitivity

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NIEHS, DNTP

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Skin Sensitization

"Allergic Contact Dermatitis"

Accounts for 10-15% of all occupational disease *(Anderson et al. 2010)*

Major testing requirement for cosmetics, pesticides, industrial chemicals, etc.

Skin Sensitization Process

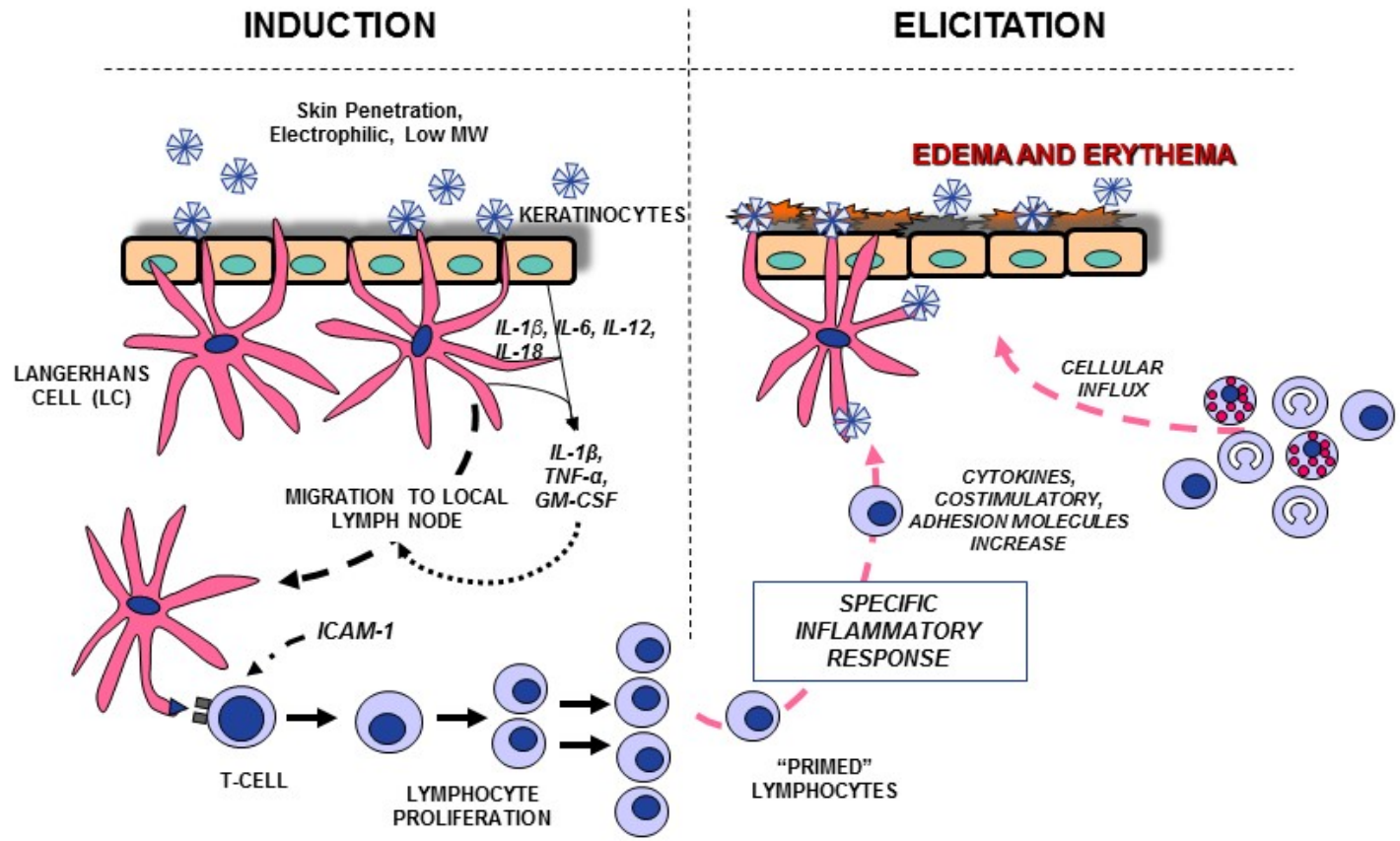


*Illustration by D. Sailstad

In Vivo Tests for Assessment of Dermal Sensitization

Guinea Pig MaximizationTest

- Intradermal and topical sensitization
- Topical challenge
- Measure erythema response 24 - 48 hours post challenge

Buehler

- Topical sensitization with closed patch
- Topical challenge distal to sensitization with closed patch
- Measure erythema response following removal of patch

Local Lymph Node Assay

- Topical treatment on dorsal surface of the ear
- Inject with radiolabel or fluorochrome
- Measure cell proliferation in the lymph nodes associated with the site of application

U.S. Regulatory Requirements/Considerations

	Reference Animal Method	Classification Criteria
Pesticides, Industrial chemicals	LLNA	NS S Hazard
Household Products	LLNA	NS S SS Potency
Dermatological Products	GPMT	Potency*

Daniel et al. 2017 in preparation

*preference

Accuracy of Animal Tests Against Human Data

GPMT / Buehler

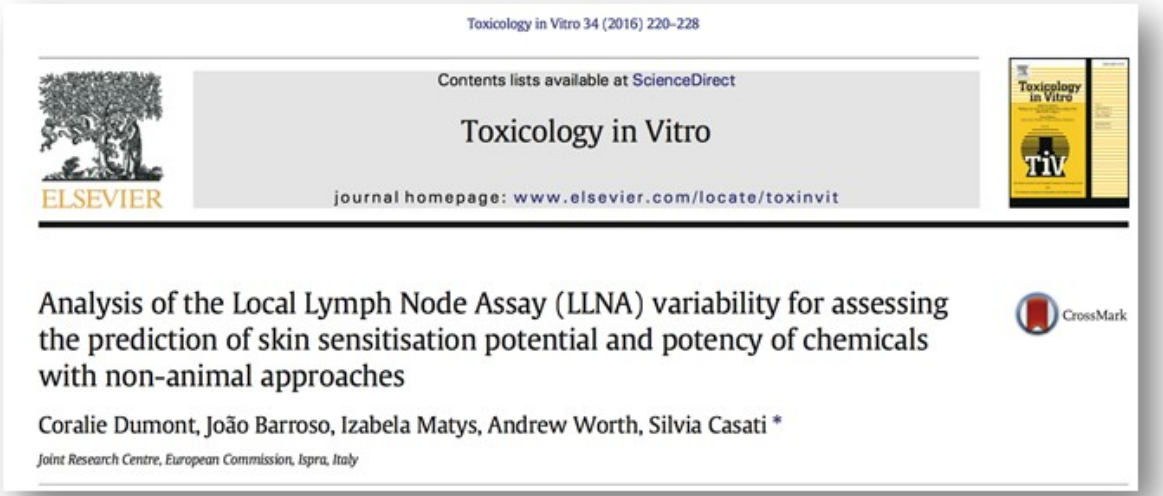
Hazard	Potency (GHS)
~72%	~60%

LLNA

Hazard	Potency (GHS)
72%-82%	54% - 60%

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). 1999. NIH Publication No. 99-4494
ICCVAM. 2010. NIH Publication No. 11-7709
Urbisch et al. 2015. Reg Tox Pharm 71:337-351.
Hoffmann et al. 2017 in preparation

Reproducibility of LLNA Data



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Analysis of the Local Lymph Node Assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches

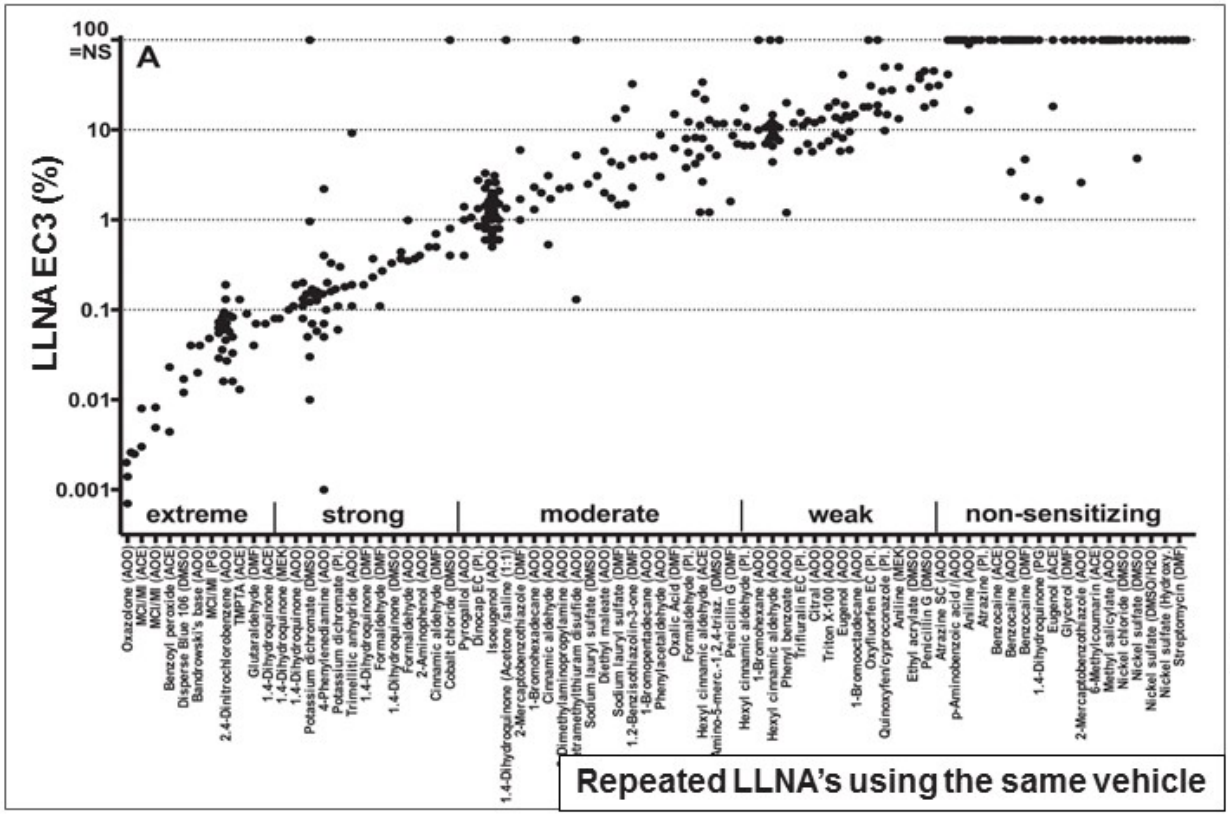
Coralie Dumont, João Barroso, Izabela Matys, Andrew Worth, Silvia Casati *

Joint Research Centre, European Commission, Ispra, Italy

How concordant are multiple LLNA outcomes for a single chemical?

- ~78% for hazard
- ~62% for potency classification

LLNA Variability

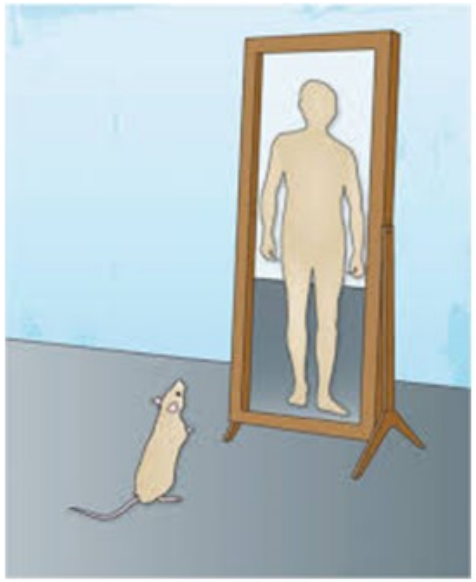


[From: S. Hoffmann, Altex 32(4), 2015]

Comparison of LLNA and Human Data

Accuracy:

- **75%** for Hazard (NS/S)
- **60%** for Potency 3-class (NS, Weak/ Moderate, Strong/ Extreme)
- **47%** for Potency 5-class (NS, Weak, Moderate, Strong, Extreme)



Provides a benchmark for comparison with new approaches

Basketter et al. 2014

Key Events in the Skin Sensitization Process

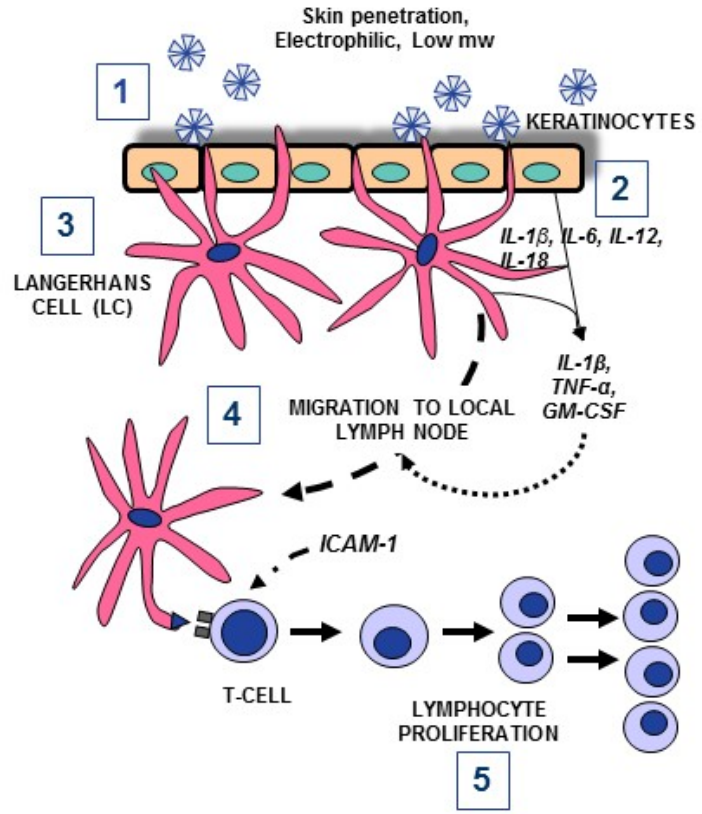
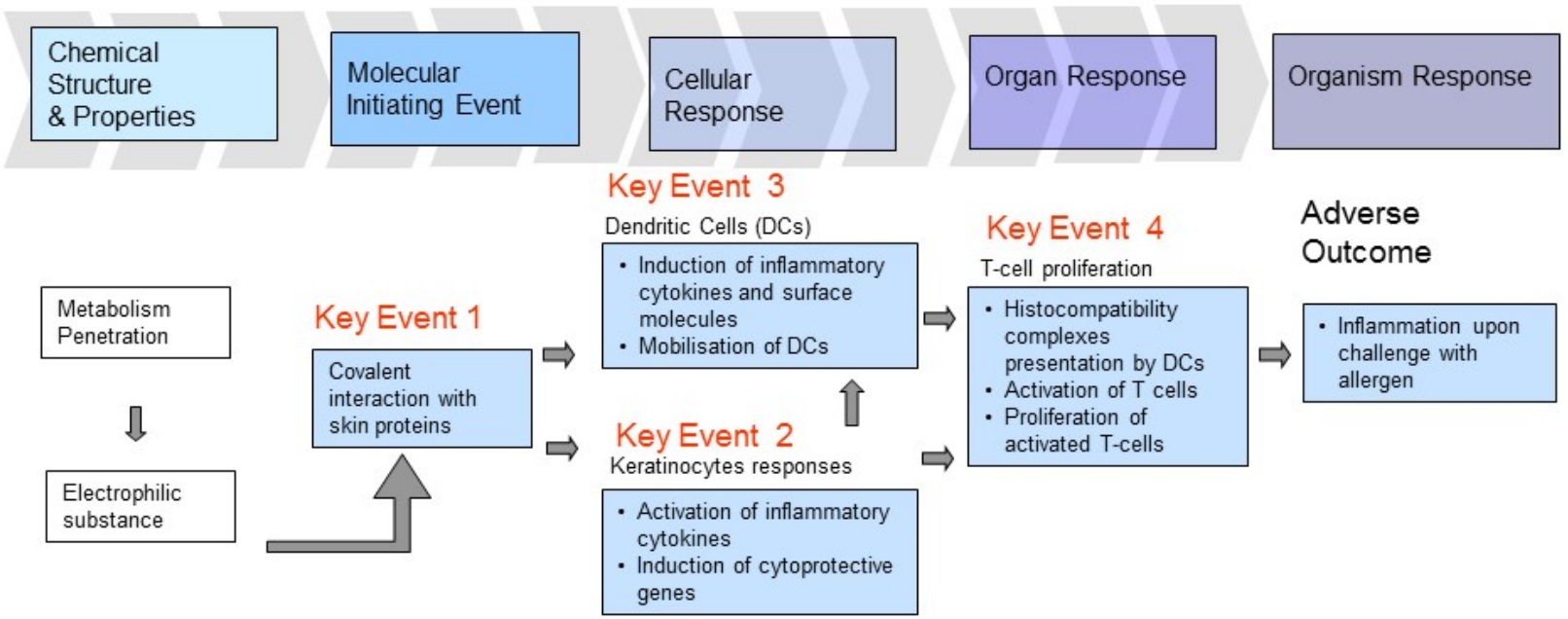
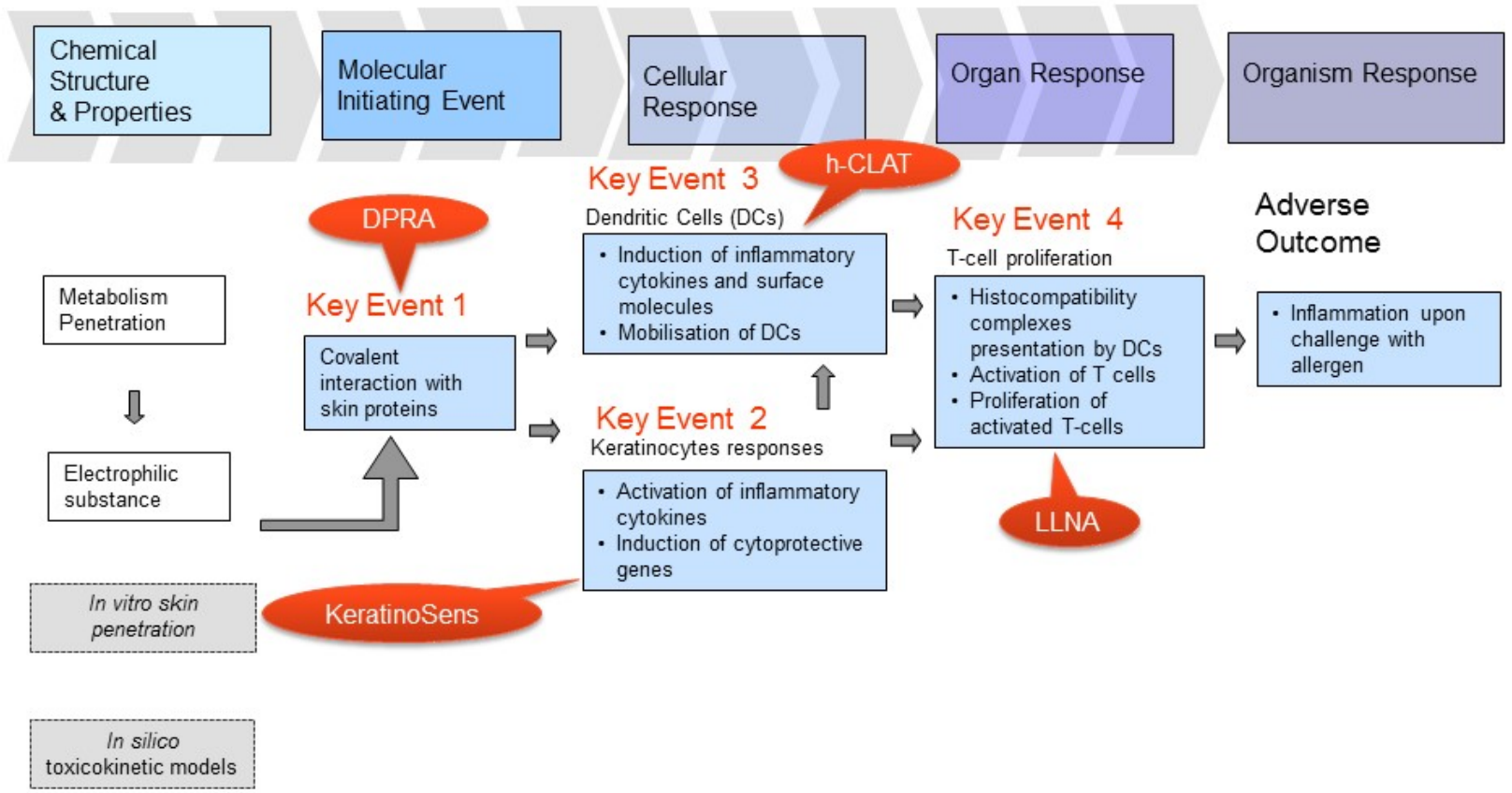


*Illustration by D. Sailstad

OECD AOP for Skin Sensitization

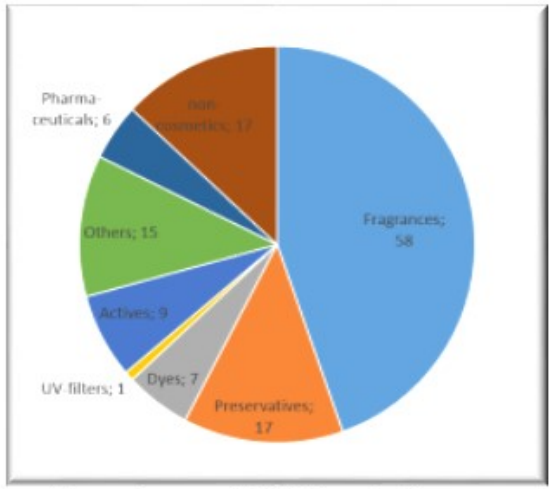


OECD AOP for Skin Sensitization



Global Skin Sensitization Project

- Objective: analysis of available non-animal approaches
  - OECD submitted case studies
- Collaboration with Cosmetics Europe
  - 128 substance dataset
  - LLNA and human data
  - Curation/generation of *in vitro* data
    - DPRA, KeratinoSens, hCLAT, U-SENS
    - PPRA, SENS-IS (underway)



Spectrum of 128 substances (largely cosmetic ingredients)

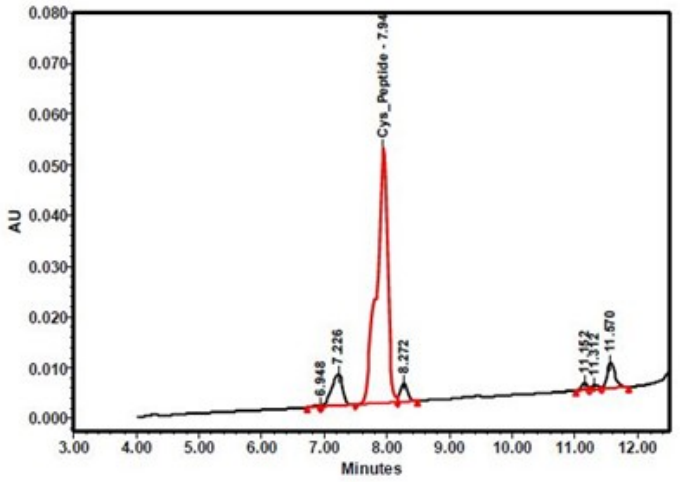
- Analyze five OECD-submitted defined approaches (i.e., code packages); open source and transparent (R, Python)
- Evaluate performance against the LLNA and human hazard/potency categories

In Vitro Models for Assessing Dermal Sensitization

- ***In vitro* assays**
  - **Direct peptide reactivity assay**
    - Assesses the ability of a substance to form a hapten-protein complex
  - **KeratinoSens**
    - Assesses the ability of a substance to activate cytokines and induce cytoprotective genes in keratinocytes
  - **h-CLAT**
    - Assesses the ability of a substance to activate and mobilize dendritic cells in the skin

DPRA

- Assesses protein reactivity of a test substance
- Uses two heptapeptides
  - One with cysteine (Cys) and one with lysine (Lys) as the reactive center
  - Incubate with test substance and measure disappearance of peptides with HPLC
  - Average depletion (Ave.Lys.Cys) > 6.38% = sensitizer
  - OECD Test Guideline 442C (2015)



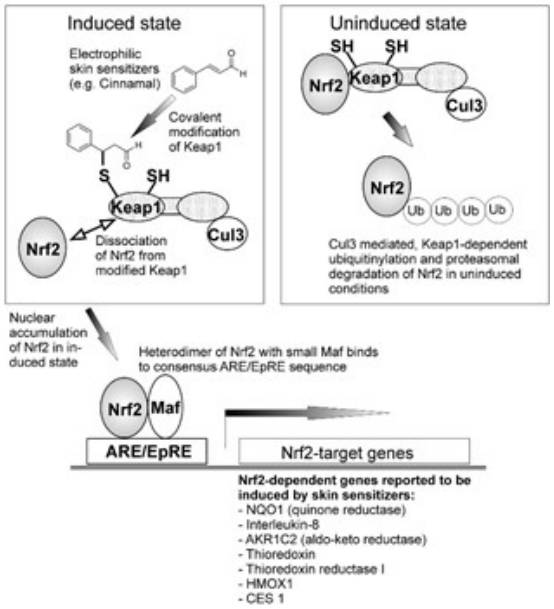
Measurements

%Cys depletion
%Lys depletion
%Ave.Lys.Cys depletion

Graphic from EC JRC. 2012. Direct Peptide Reactivity (DPRA) Validation Study Report.

KeratinoSens™

- Assesses the activation of the AKR1C2-ARE element, an indication of keratinocyte activation, in KeratinoSens cells (derived from HaCaT keratinocytes)
  - Caused by electrophilic agents, which tend to be skin sensitizers
  - Measures fold-induction of luciferase activity; induction >1.5-fold in 2/3 experiments = sensitizer
  - OECD Test Guideline 442D (2015)



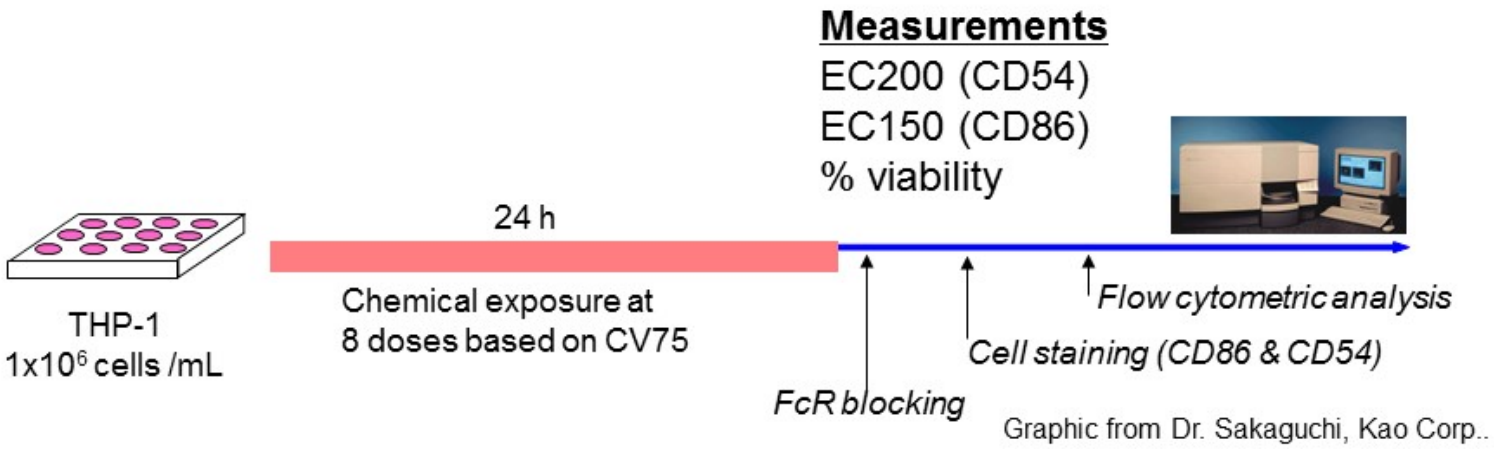
Measurements

Fold induction
EC1.5
% viability

Graphic from Natsch 2010. Tox Sci 113:284-292.

h-CLAT

- Measures 2 cell surface markers, CD86 and CD54, on dendritic cell surrogates (THP-1 cells)
  - Assesses the maturation process of dendritic cells as they transform from antigen processing cells to antigen presenting cells
  - CD86 relative fluorescence intensity (RFI) ≥150% and/or CD54 RFI ≥ 200% at any dose, in at least 2/3 experiments, then substance is a sensitizer
  - OECD Test Guideline 442E (2016)



Graphic from Dr. Sakaguchi, Kao Corp..

Individual Assays Compared to the LLNA

	hCLAT vs LLNA	DPRA vs LLNA	Keratino vs LLNA
	NEG POS	NEG POS	NEG POS
NEG	16 15	22 32	22 31
POS	17 79	11 62	11 64
Sensitivity %:	**84.0**	**66.0**	**67.4**
Specificity %:	**48.5**	**66.7**	**66.7**
Accuracy %:	**74.8**	**66.1**	**67.2**
	n=127	n=127	n=128

Hoffman et al 2017, in preparation

Individual Assays Compared to Human

	hCLAT vs Human	DPRA vs Human	Keratino vs Human
	NEG POS	NEG POS	NEG POS
NEG	20 11	29 25	31 22
POS	19 77	10 63	8 67
Sensitivity %:	**87.5**	**71.6**	**75.3**
Specificity %:	**51.3**	**74.4**	**79.5**
Accuracy %:	**76.4**	**72.4**	**76.6**
	≥ LLNA		**≥ LLNA**
	n=127	n=127	n=128

Hoffman et al 2017, in preparation

Defined Approach Evaluation

- Most non-animal testing strategies evaluated so far perform **better** than the LLNA at predicting human skin sensitization **hazard and potency**
- Combining multiple *in vitro* assays and *in silico* methods or physico chemical properties increases the ability to predict sensitizers

Combining *in vitro* assays and other approaches increases the ability to predict sensitizers

Table 6. LOOCV results for seven highest performing SVM models

No.	Model (Accuracy[a])	Sensitivity (%)	Specificity (%)	Accuracy (%)
1	DPRA + KeratinoSens + h-CLAT + Toolbox + Lys + Cys + Avg.Lys.Cys + 6 properties (95%)	89	91	89
5	KeratinoSens + h-CLAT + Toolbox + Avg.Lys.Cys + 6 properties (95%)	92	79	88
7	h-CLAT + Toolbox + 6 properties (97%)	85 (84)	94 (48)	88 (75)
8	KeratinoSens + Toolbox + Avg.Lys.Cys + 6 properties (94%)	84 (67)	91 (67)	86 (67)
9	KeratinoSens + h-CLAT + Avg.Lys.Cys + 6 properties (92%)	89	73	84
10	h-CLAT + Toolbox + Avg.Lys.Cys + 6 properties (92%)	90	88	89
11	KeratinoSens + h-CLAT + Toolbox + 6 properties (92%)	89	79	86

Avg.Lys.Cys, average depletion for lysine and cysteine; Cys, average % cysteine; DPRA, direct peptide reactivity assay; h-CLAT, human cell line activation test; LOOCV, leave-one-out cross-validation; Lys, average % lysine depletion; Toolbox, read-across using QSAR Toolbox; SVM, support vector machine.

[a]Average accuracy of the training and test sets for predicting the reference LLNA outcomes.

(Individual assay compared to the LLNA)

From Strickland et al., J. Appl. Toxicol. 2016; 36: 1150–1162

Expanding Coverage of Chemical Space

- **Most chemicals used in the validation of non-animal test methods have been cosmetics ingredients**

- NTP is supporting testing of other types of chemicals in three alternative test methods: DPRA, LuSens, hCLAT
  - Expanded chemical space includes: pesticides, agrochemical formulations, dermal excipients, personal care product ingredients, "challenge" chemicals

- Have compiled chemical nominations from multiple ICCVAM agencies
  - EPA: Office of Pesticides, Office of Pollution Prevention and Toxics, Office of Research and Development
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - NTP

Expanding Coverage of Chemical Space

- Total of 266 chemicals nominated
- NTP has procured 135 chemicals for initial testing phase (mostly nominations from the EPA)
- Testing began in late 2017
- Additional testing (~100 chemicals) to follow in mid-2018
- Coordinating with Dow to test formulations already assessed in DPRA and KeratinoSens™ in the hCLAT assay

Expanding Coverage of Chemical Space

- Combine with *in silico* data and physico chemical properties when available
- Evaluate the dataset using methods previously developed by NICEATM (Strickland et al 2016)
- Evaluate predictive performance of non-animal defined approaches submitted to OECD (Kleinstreuer et al. 2018) in comparison to LLNA data
- Characterize applicability domain of in vitro test methods and non-animal defined approaches
- Work with ICCVAM agencies to adopt non-animal defined approaches where appropriate

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