Use of Non-animal Skin Sensitization Test Methods for US EPA Classification: Current Approaches and Future Opportunities

Hans Raabe, M.S.
V.P., Chief Operating Officer

27 May 2020

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

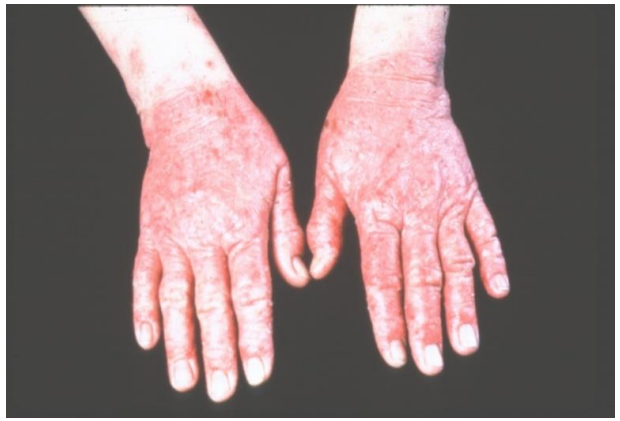
Overview

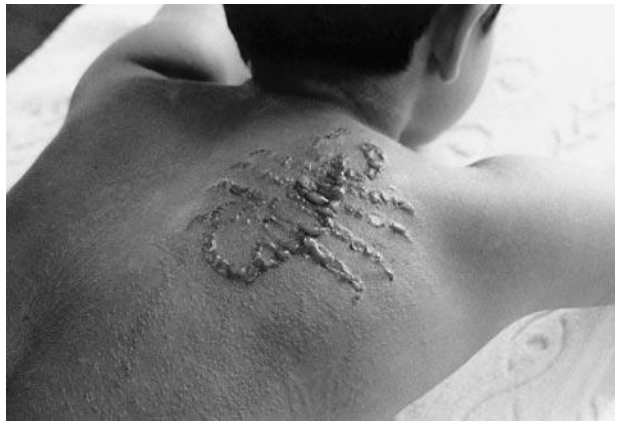
- Brief Introduction to Skin Sensitization
- Adverse Outcome Pathway (AOP)

- Current Regulatory-accepted Test Methods
  - Key Event 1: Direct Peptide Reactivity Assay (DPRA)
  - Key Event 2: KeratinoSens and LuSens Assays
  - Key Event 3: Human Cell Line Activation Test (h-CLAT)
  - Example issues presented as case studies
- EPA Recommended Testing Strategies / Defined Approaches

- Future Opportunities

Sensitization Elicitation: Allergic Contact Dermatitis





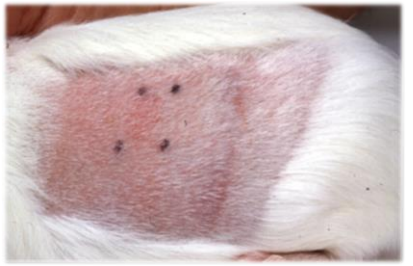
Common allergens and sources of exposure

Allergens	*Source*
Epoxy resin system(ERS)	Adhesives, paints
Formaldehyde	Pesticides, biocides
Fragrance mix	Toiletries, cosmetics
Neomycinsulfate	Creams, deodorants
Nickel sulfate	Costume jewelry, tools

Skin Sensitization Testing Methods

In Vivo

- Guinea Pig Maximization Test (GPMT) and Buehler Test

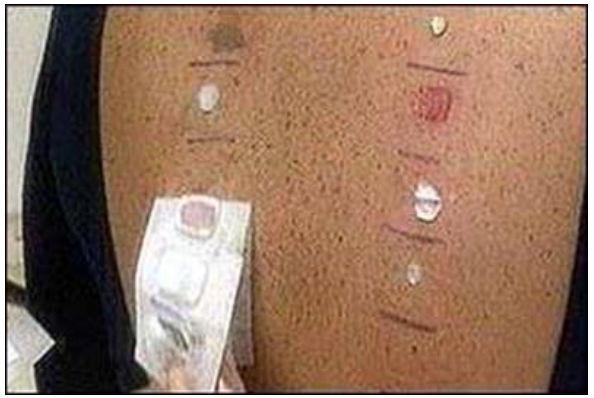


Guinea Pig Maximization Test

- Local Lymph Node Assay (LLNA)

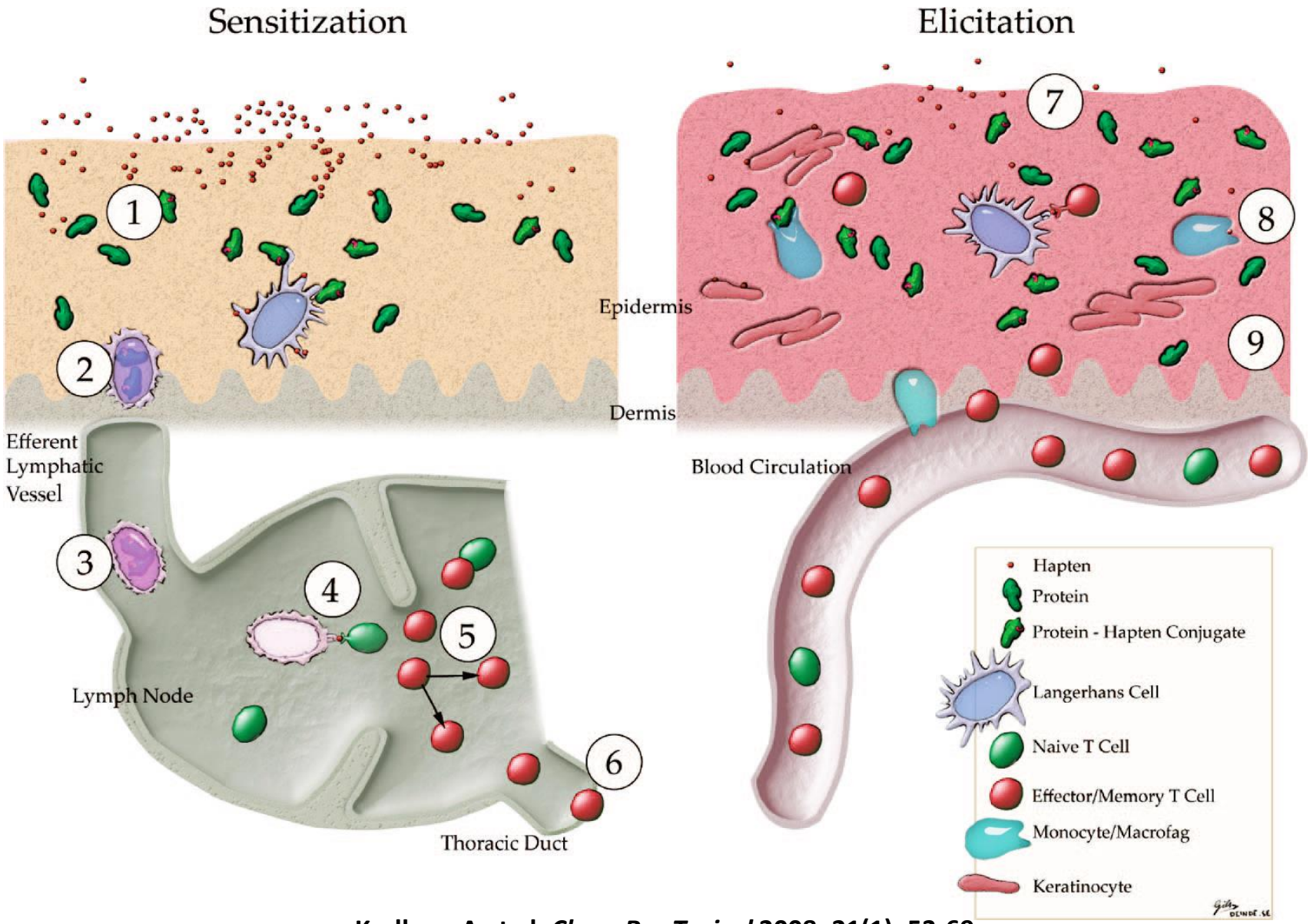
Local Lymph Node Assay

- Human Patch Testing



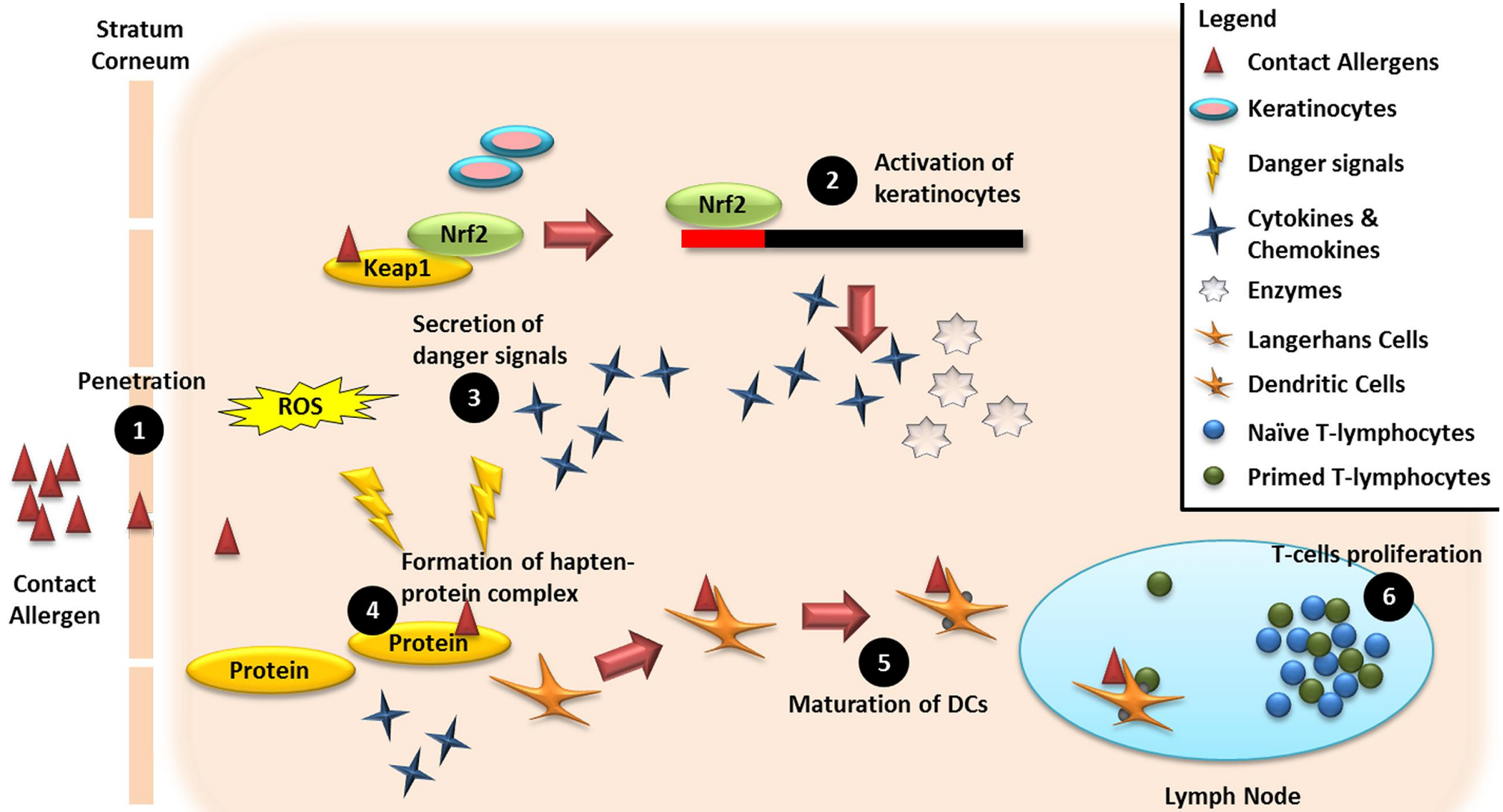
Human Patch Testing

Sensitization Induction and Elicitation



Karlberg A et al. *Chem Res Toxicol* 2008; 21(1): 53-69

Mechanistic overview supporting endpoint development



Wong et al,. *Frontiers in Pharmacology* 2015 ,(6) 94 1-13

Skin Sensitization: Adverse Outcome Pathway (AOP)

Chemical element → Macromolecular interaction → Cellular response → Organs response → Organism response

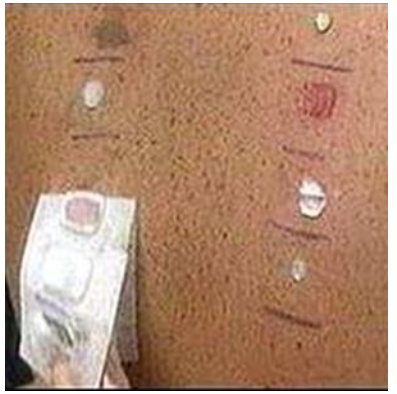
- **Key Event 1** Covalent interaction with proteins
- **Key Event 2** Keratinocytes activation
- **Key Event 3** Langerhans and dendritic cell activation
- **Key Event 4** T-cells activation/proliferation
- Allergic contact dermatitis (inflammation)

LLNA | **GPMT** | **Clinical**

DPRA
ADRA
PPRA
kDPRA

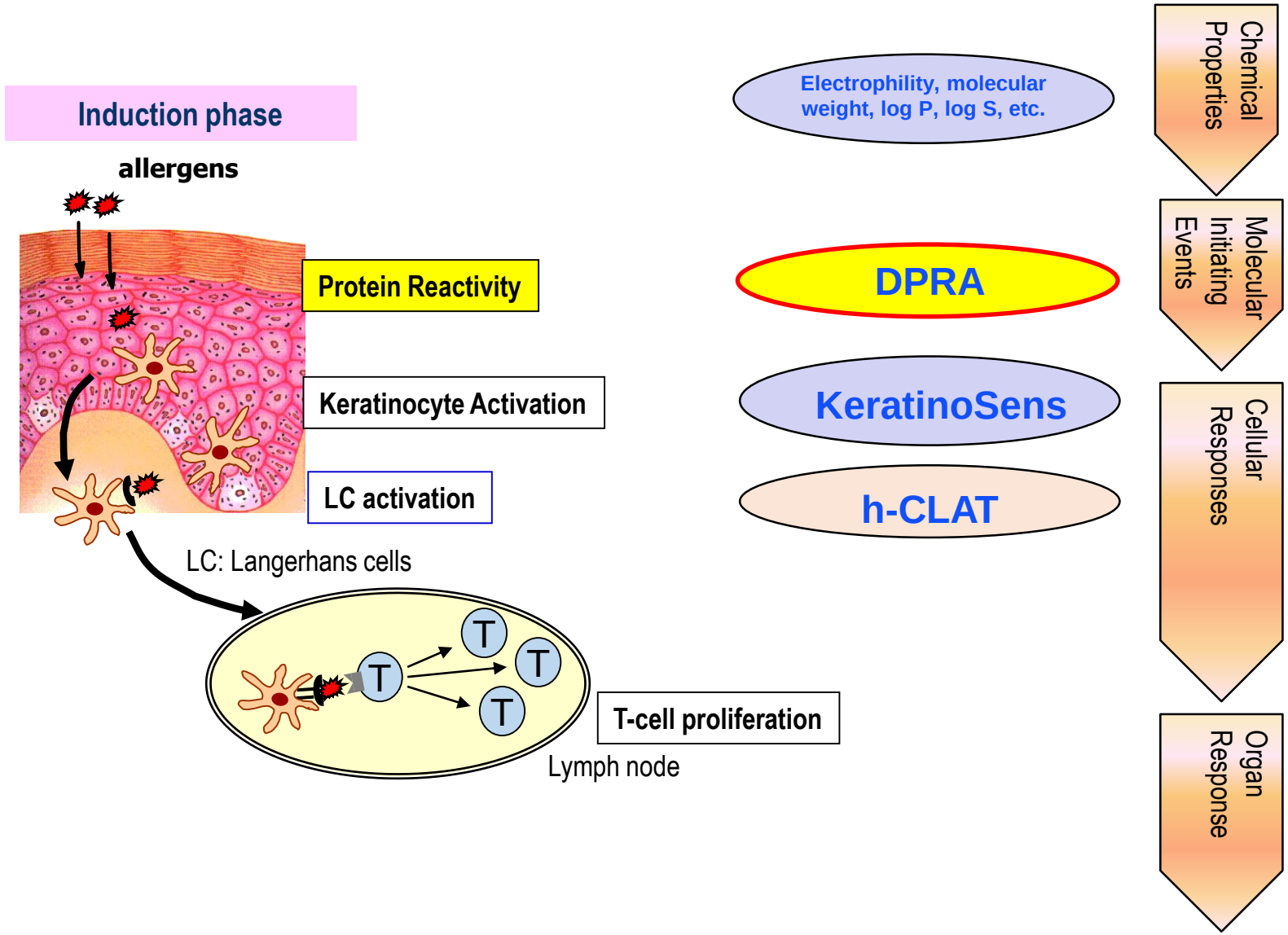
KeratinoSens
LuSens
SENS-IS
EpiSensA

h-CLAT
U-SENS
IL-8-Luc Assay
VITOSENS
GARD skin



AOP adopted by OECD, 2012

AOP – Allergic Contact Dermatitis



Direct Peptide Reactivity Assay (DPRA)

(OECD TG 442C)

Key event 1

TOXICOLOGICAL SCIENCES 81, 332–343 (2004)
doi:10.1093/toxsci/kfh213
Advance Access publication July 14, 2004

Development of a Peptide Reactivity Assay for Screening Contact Allergens

G. Frank Gerberick,*[1] Jeff D. Vassallo,* Ruth E. Bailey,* Joel G. Chaney,* Steve W. Morrall,* and Jean-Pierre Lepoittevin†

*The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45253-8707, and †Université Louis Pasteur, Laboratorie de Dermatochimie, UMR 7123, Strasbourg, France

Received April 26, 2004; accepted June 22, 2004

OECD

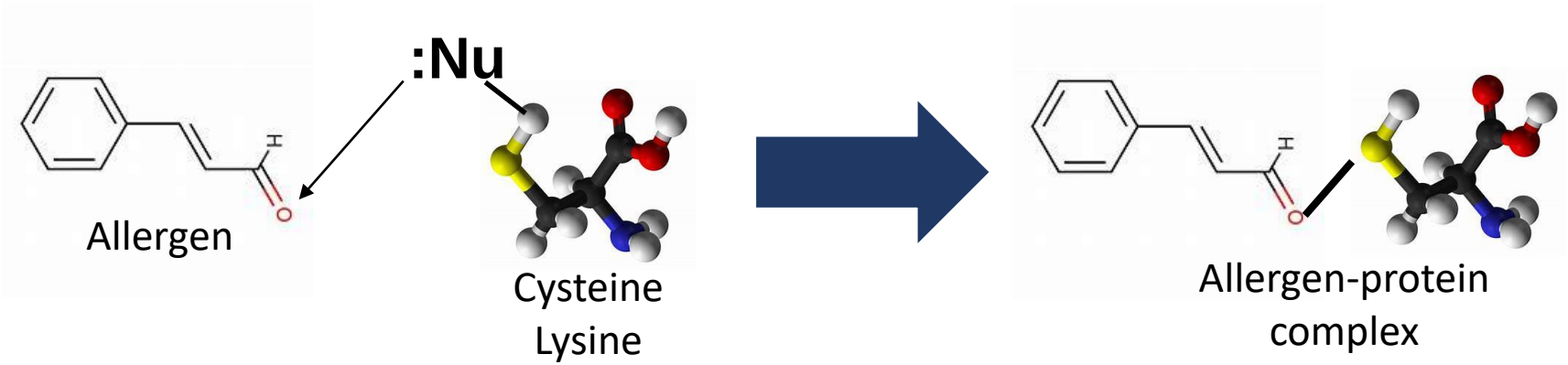
Direct Peptide Reactivity Assay (DPRA)

(OECD TG 442C) **Key event 1**

Addresses the process of haptenation (covalent binding of low-molecular weight substances (haptens) to skin proteins)

Molecular Initiating Event (MIE)

Measures peptide reactivity of test chemicals by quantifying the depletion of synthetic peptides containing either *lysine* or *cysteine*



Direct Peptide Reactivity Assay (DPRA)
(OECD TG 442C)

Synthetic cysteine and lysine-containing peptides

Ac-RFAA**C**AA-COOH (0.667 mM in pH 7.5 buffer)

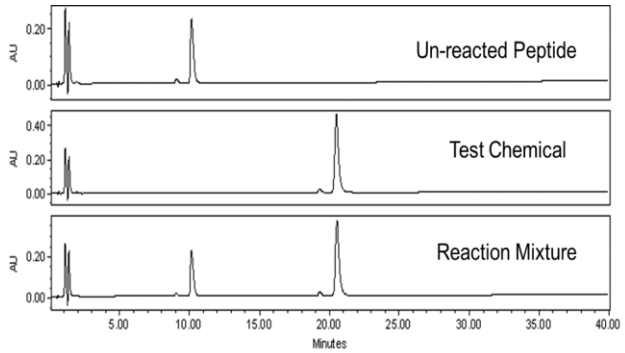
Ac-RFAA**K**AA-COOH (0.667 mM in pH 10.2 buffer)

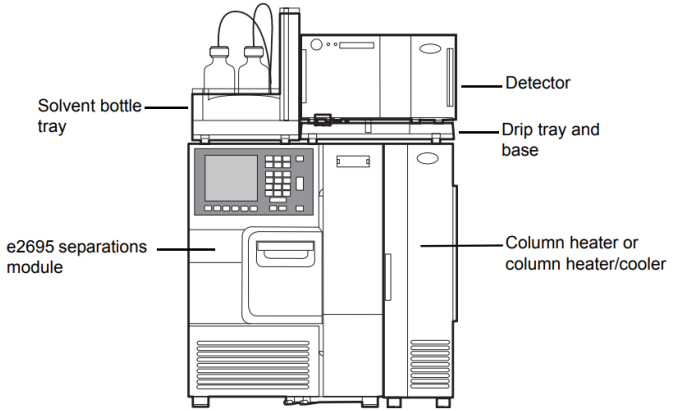
Controls: **Positive control** (cinnamic aldehyde)

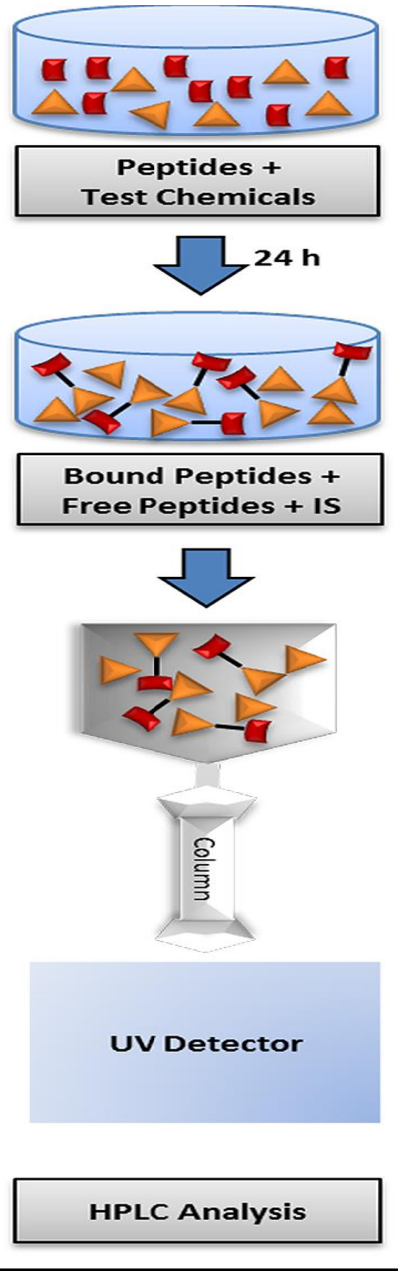
Negative control (peptide solutions)

Mix 1:10 and 1:50 for cysteine and lysine peptides for 24h.

Measure relative peptide concentration by HPLC with gradient elution and UV detection at 220nm







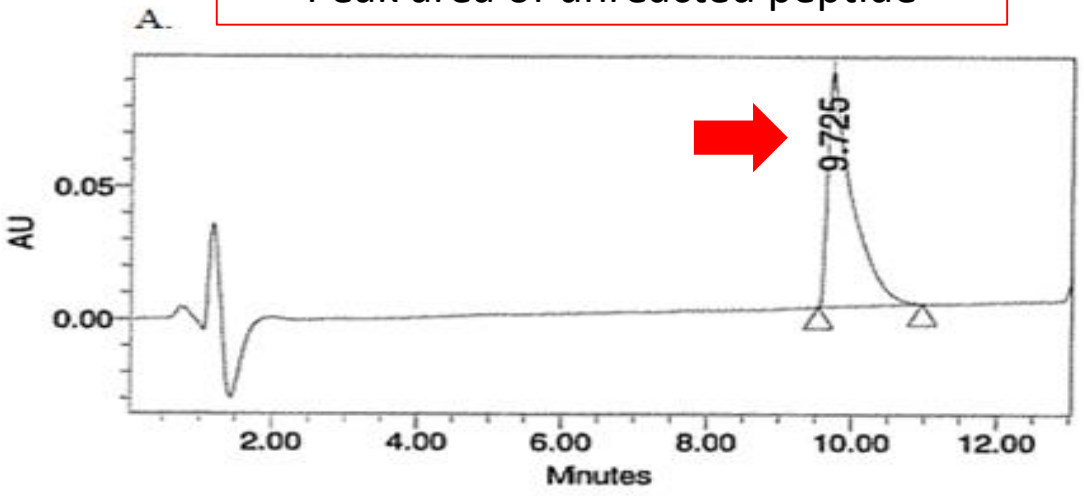
Wong et al,. *Frontiers in Pharmacology* 2015 ,(6) 94 1-13

Direct Peptide Reactivity Assay (DPRA)

(OECD TG 442C)

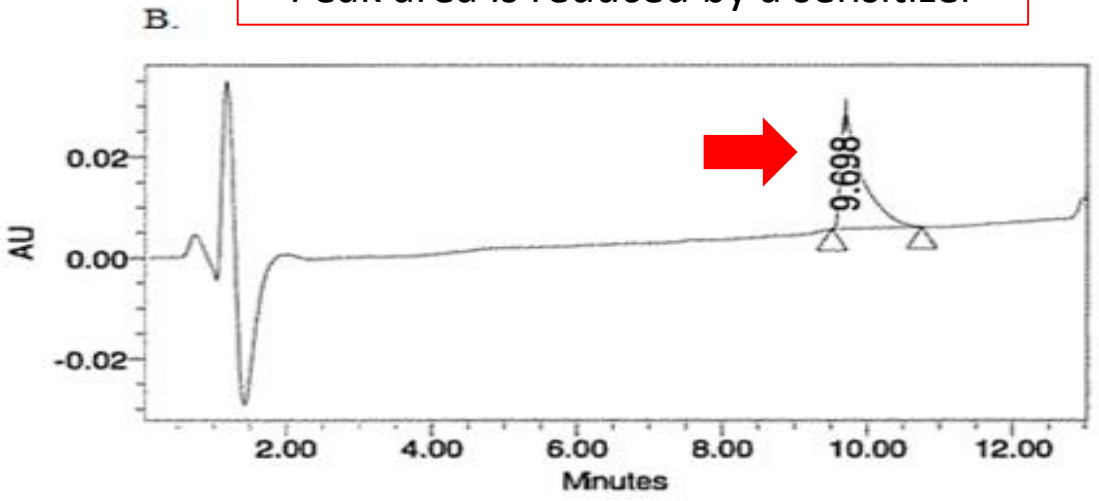
Key event 1

Peak area of unreacted peptide



	SampleName	Vial	Injection Volume (ul)	RT	Area
1	Control	22	6.00	9.725	2059227

Peak area is reduced by a sensitizer



	SampleName	Vial	Injection Volume (ul)	RT	Area
1	Cinnamic aldehyde (ref)	10	6.00	9.698	465116

Direct Peptide Reactivity Assay (DPRA)

Prediction model

There are 2 prediction models that can be used for the DPRA

Most commonly used to make a prediction

Mean of Cysteine and Lysine % Depletion	Reactivity	Prediction	
0% ≤ Mean % depletion ≤ 6.38%	Minimal	Non-sensitizer	NO
6.38% < Mean % depletion ≤ 22.62%	Low	Sensitizer	YES
22.62% < Mean% depletion ≤ 42.47%	Moderate	Sensitizer	
42.47% < Mean % depletion ≤ 100%	High	Sensitizer	

Used when lysine data is inconclusive

Mean of Cysteine % Depletion	Reactivity	Prediction	
0% ≤ Mean % depletion ≤ 13.89%	Minimal	Non-sensitizer	NO
13.89% ≤ Mean % depletion ≤ 23.09%	Low	Sensitizer	YES
23.09% ≤ Mean % depletion ≤ 98.24%	Moderate	Sensitizer	
98.24% ≤ Mean % depletion ≤ 100%	High	Sensitizer	

Direct Peptide Reactivity Assay (DPRA)

Limitations

- A test chemical should be soluble in an appropriate solvent up to 100 mM
  - In case of insolubility, test chemicals may be used at lower soluble concentrations, however, negative results may be inconclusive
- Limited dynamic range due to lack of kinetic data
- No discrimination of adduct formation from side reactions such as peptide oxidation/dimerisation – potential over-prediction?
- Lack of metabolic activity – pro-hapten predictions?
- Not applicable to metal compounds, or substances of unknown or variable composition or complex reaction products or biological materials

DPRA Case Studies

Data Interpretation

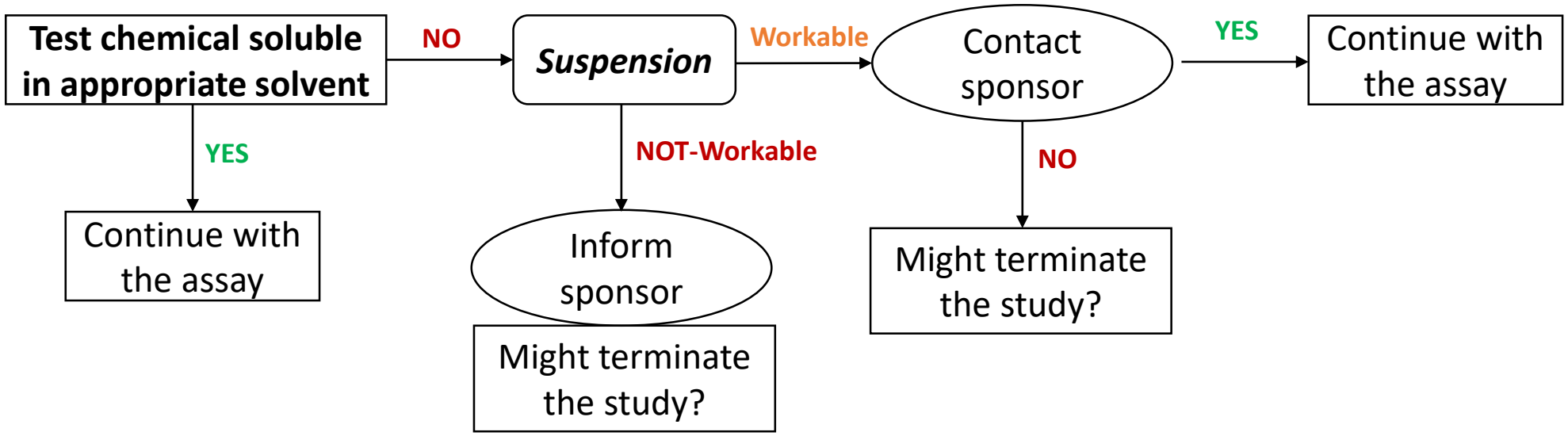
Situation: To decide weather to use **Mean Cysteine and Lysine peptide depletion** (%) model or **Cysteine only peptide depletion** (%) model to predict skin sensitization potential of the test article.

IIVS Test Article Number	Sponsor's Designation	Mean Peptide Depletion (%)		Mean Peptide Depletion (%) of Cysteine and Lysine	Reactivity (Cysteine only)	Reactivity (Cysteine and Lysine)	Potential Sensitizer?	
		Cysteine	Lysine				Based on Cysteine only prediction model	Based on mean of Cysteine & Lysine prediction model
19AIXX	Article D	12.17	3.23	7.70	Minimal	Low	Non-Sensitizer	Sensitizer
Positive Control	Cinnamic Aldehyde	75.87	65.30	**6.38 is the cutoff!**				

- **Option 1:** Repeat the study
- **Option 2:** Use Cysteine only peptide depletion (%) prediction model
- **Option 3:** Perform other skin sensitization tests to predict the skin sensitization potential

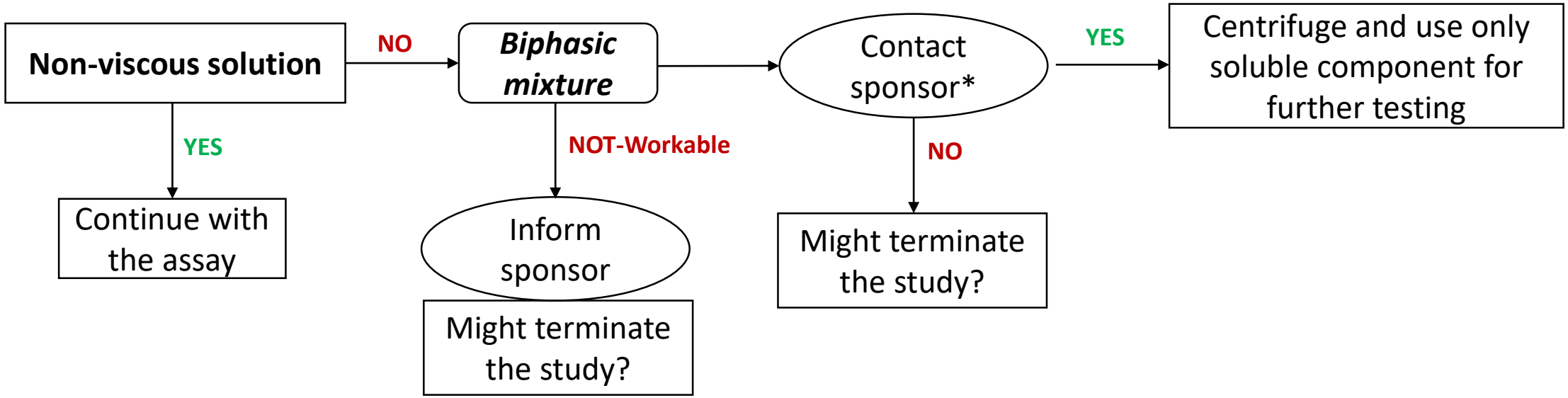
Solubility

- In an ideal situation, a test chemical has to form a non-viscous solution or non-viscous suspension in either of the preferred solvents for DPRA.
- Following is an approach we use if the test chemical does not go into the solution after vortexing, sonicating and heating.



Reaction Mixture (Test Article + Peptide)

Precipitates or biphasic mixture observed after mixing peptide solution with the test chemical



*Recommend to centrifuge at low speed for 5 min (OECD DPRA TG 442C)

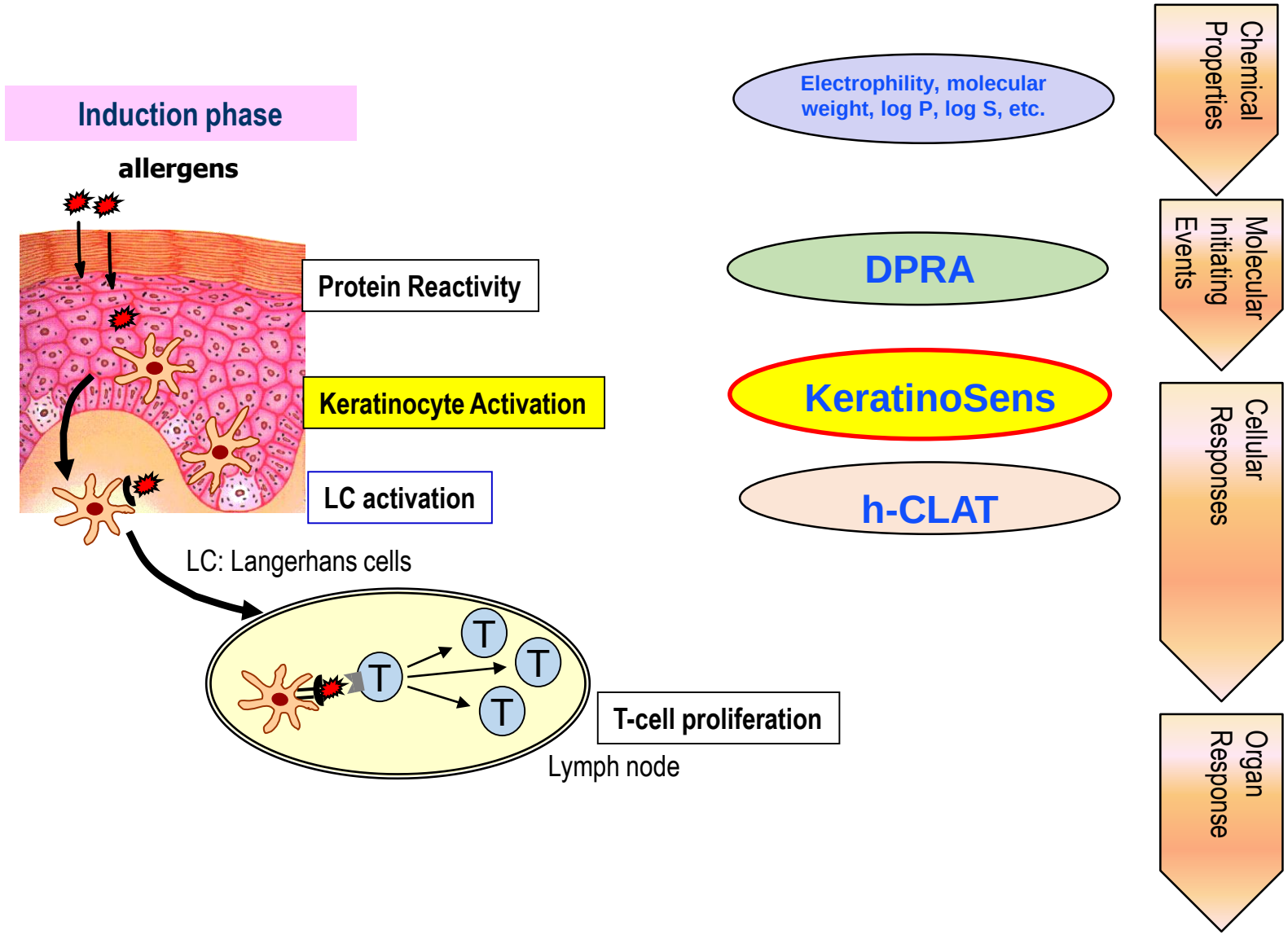
Data Interpretation

Situation: Since the test material (formulation) was tested neat and reaction mixture (test article + peptide) formed precipitates, reaction mixture was centrifuged (low speed for 5 min) and only supernatant was assayed

IIVS Test Article Number	Sponsor Designation	Mean Peptide Depletion (%)		Mean Peptide Depletion (%) of Cysteine and Lysine	Reactivity (Cysteine only)	Reactivity (Cysteine and Lysine)	Potential Sensitizer?	
		Cysteine	Lysine				Based on Cysteine only prediction model	Based on mean of Cysteine & Lysine prediction model
19AHXX (neat)	1	32.62	59.07	45.85	Moderate	High	Sensitizer	Sensitizer
19AHXX (neat)	2	41.27	76.67	58.97	Moderate	High	Sensitizer	Sensitizer
Positive Control	Cinnamic Aldehyde	72.95	49.09					

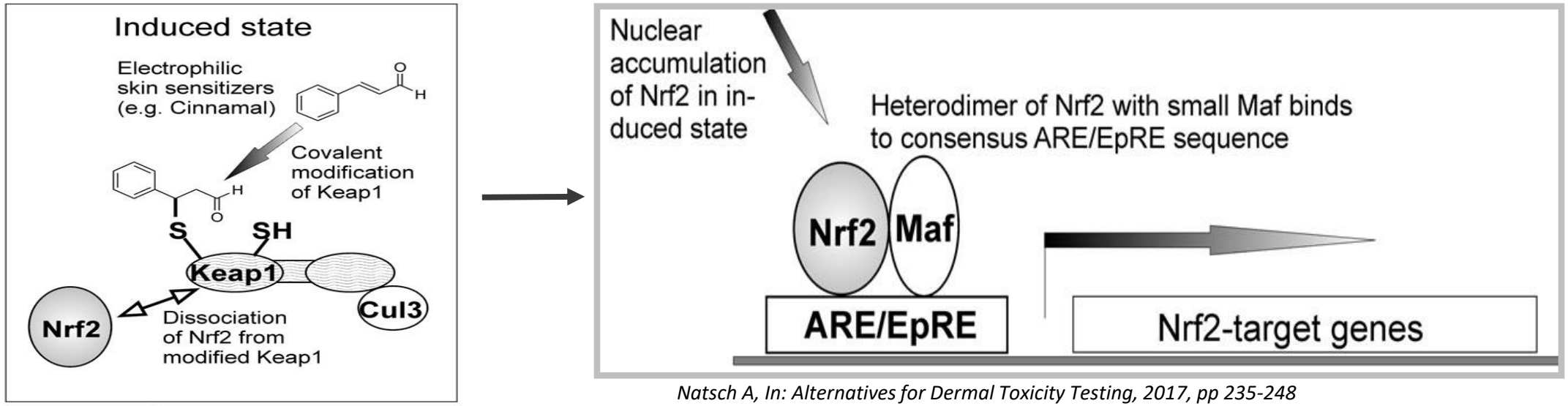
- **Option 1:** Use lower concentrations (10%, 20%, 50%) to get a dose-dependent effect that can be extrapolated
- **Option 2:** Perform other skin sensitization tests
- **Option 3:** Use WoE based on other information

AOP – Allergic Contact Dermatitis



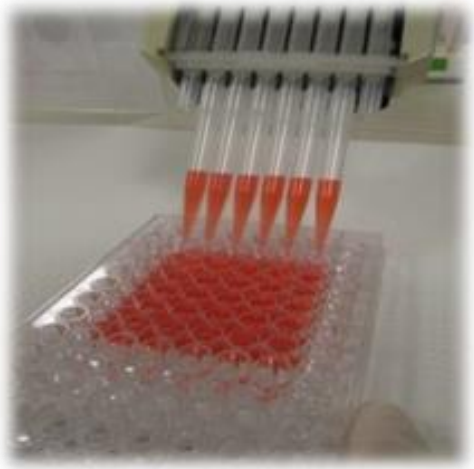
KeratinoSens™ Assay
(OECD TG442D) Key event 2

- Addresses keratinocyte responses by activation of antioxidant/electrophile response element dependent pathway (Keap1-Nrf2-ARE)
- The repressor protein Keap1 reacts with electrophiles, allowing dissociation of the transcription factor Nrf2 to translocate to the nucleus and induce the antioxidant response element (ARE)
- Reporter construct with a copy of the ARE-element of the human AKRIC2 gene upstream of a luciferase gene

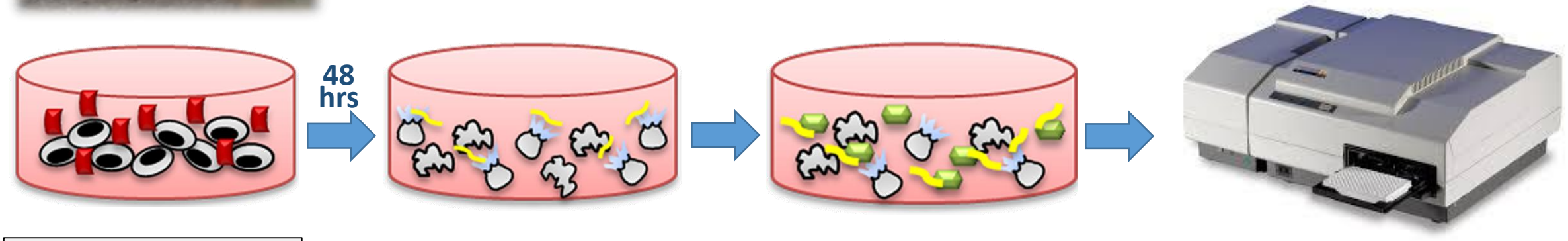


Natsch A, In: Alternatives for Dermal Toxicity Testing, 2017, pp 235-248

KeratinoSens™ Assay
(OECD TG442D) Key event 2



- HaCaT (immortalized keratinocyte cell line)
- 48 hour incubation with test material (12 concentrations)
- Addition of Promega lysis buffer and luciferase substrate
- Quantitative gene induction by luciferase activity



HaCAT Luciferase Reporter Cells + Test Chemicals

Addition of Lysis Buffer

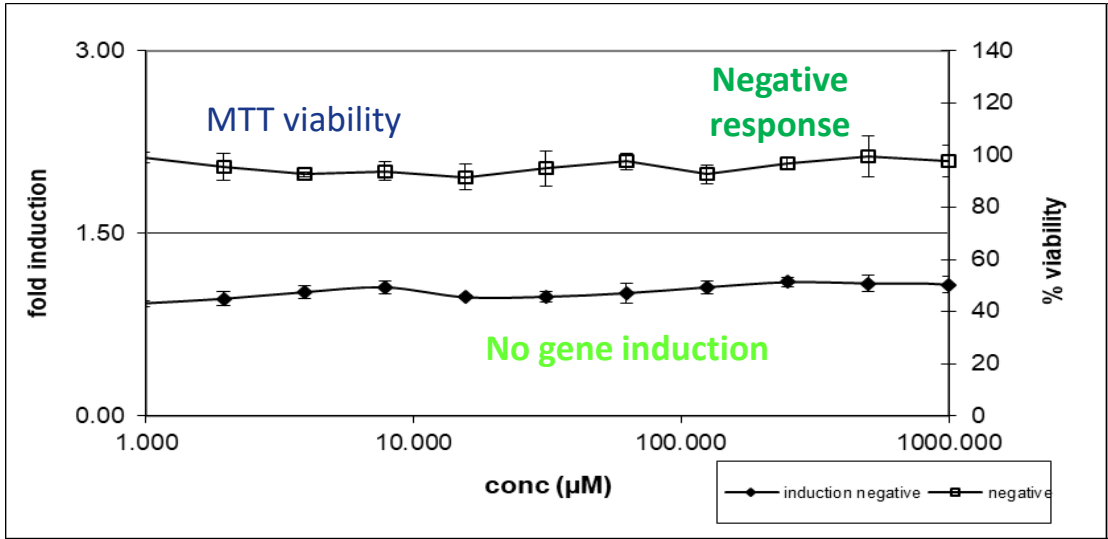
Luciferase Substrates

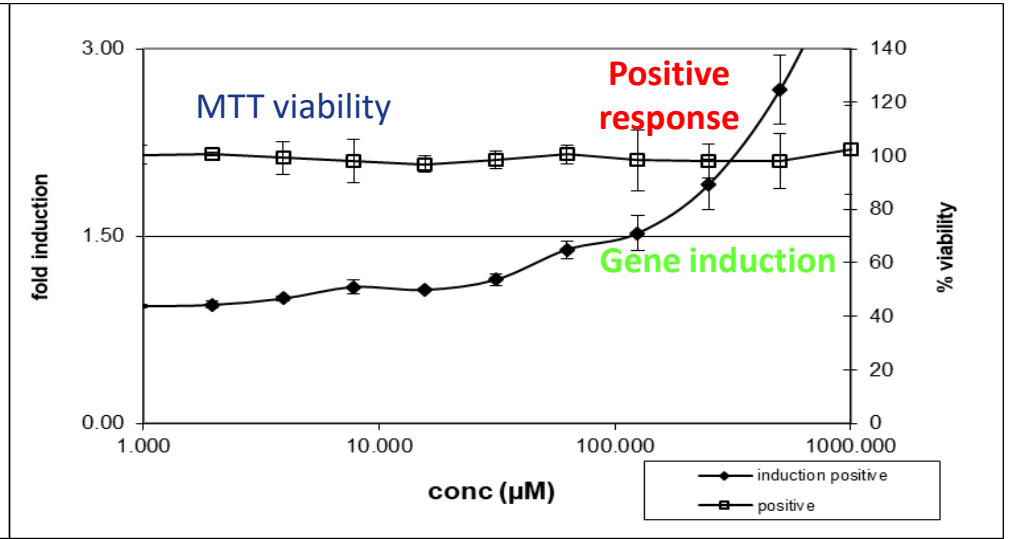
Luminescence Detector

Wong et al., 2015

KeratinoSens™ Assay
(OECD TG442D) Key event 2

- Measures luciferase gene induction and cytotoxicity compared to solvent control wells
  - > 1.5 fold gene induction; ≥ 70% viability; apparent dose response
- Controls
  - Negative/Solvent: DMSO
  - Positive: Cinnamic Aldehyde





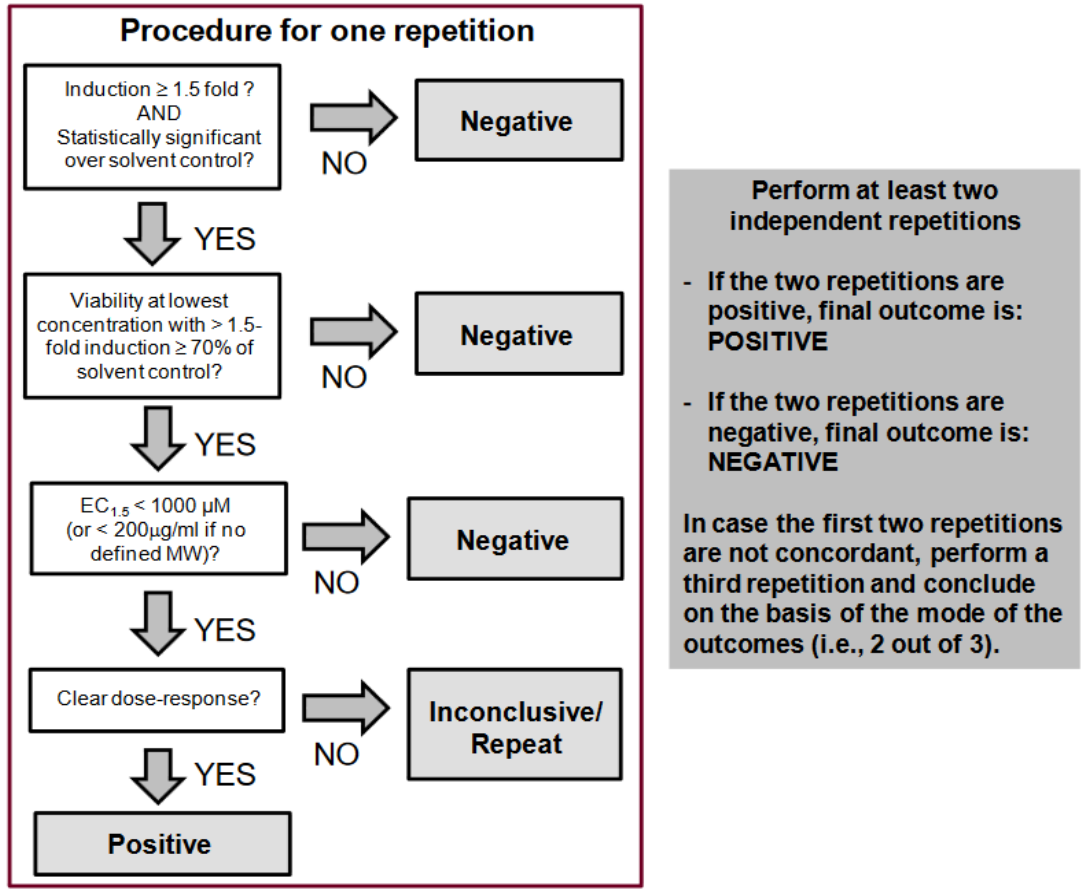
KeratinoSens™ Assay

Prediction Model

- Concordant results from at least two independent trials are required to predict skin sensitization potential of a test article
- Viability must be ≥ 70% at the lowest concentration that elicited an induction greater than 1.5-fold
- A positive prediction should display an $EC_{1.5}$ value less than 1000 µM
- If there is not a clear dose response, the prediction may be inconclusive

Figure 1. Prediction model used in the KeratinoSens™ test method.

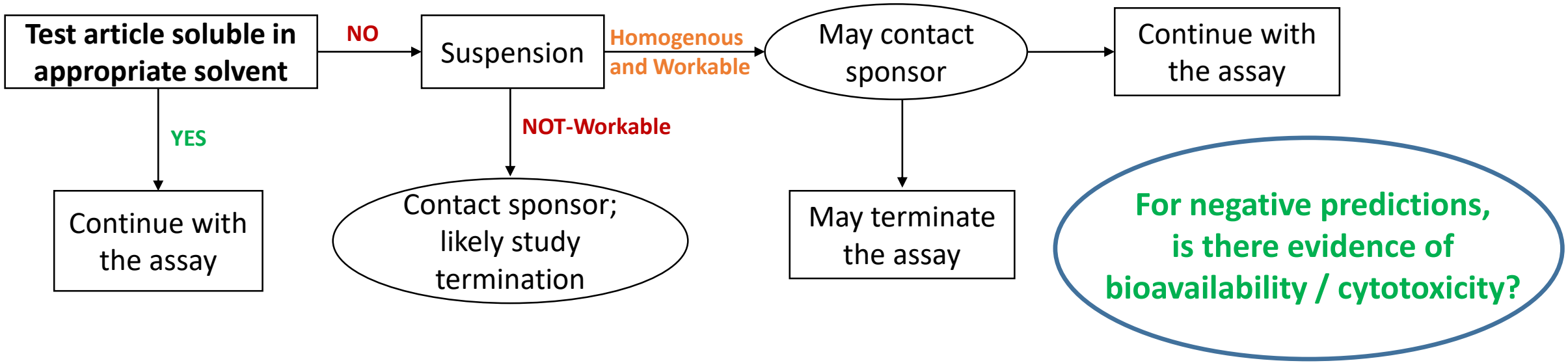
A KeratinoSens™ prediction should be considered in the framework of a Defined Approach or of an IATA and in accordance with the provisions of paragraphs 7 and 8 of the general introduction



KeratinoSens™ Case Studies

Solubility

Ideally, a test article forms a non-viscous solution or homogenous non-viscous suspension in a preferred solvent

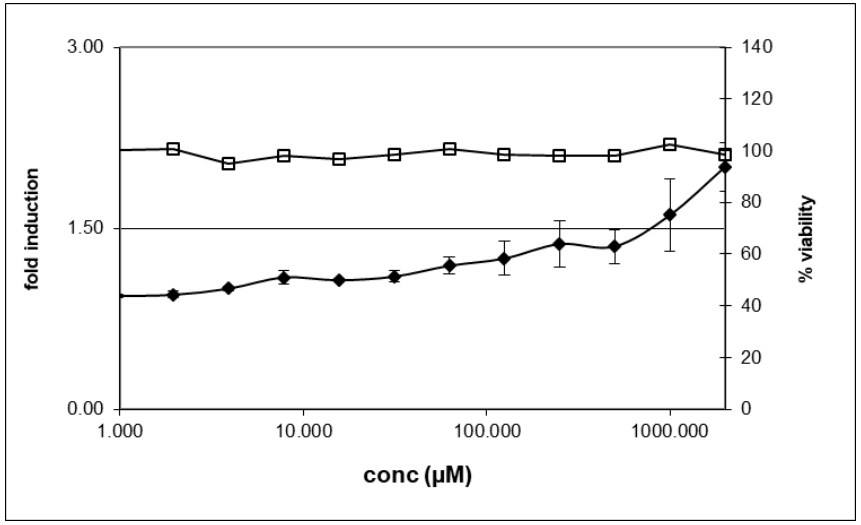


Data Interpretation

Situation: Test article prediction was positive in the first trial and negative in the second

Option: Conduct a third trial

IIVS Test Article Number	Sponsor's Designation	Trial	$EC_{1.5}$ (µM)	IC_{30} (µM)	Sensitization Potential
20AAXX	1	B1	968.8	>2000	Sensitizer
		B2	>2000	>2000	Non-Sensitizer
		B3	923.1	>2000	Sensitizer



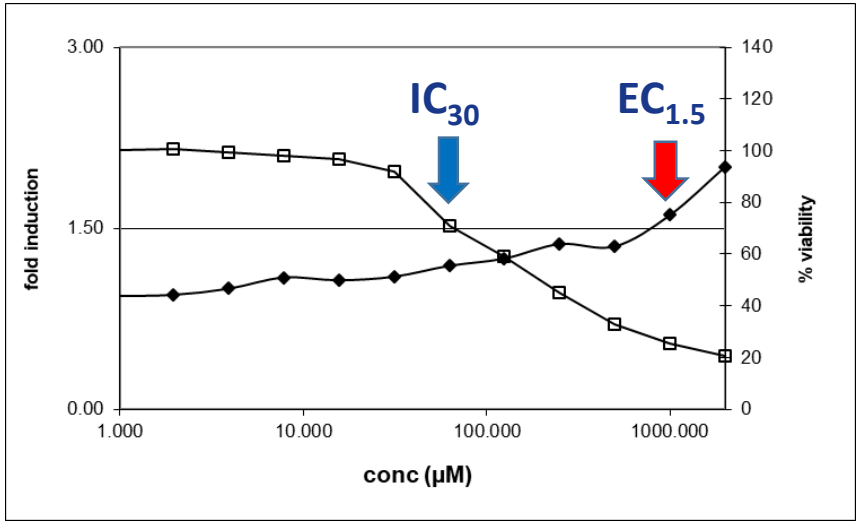
Conclusion: Test article was considered a potential sensitizer

Data Interpretation

Situation: Test article had an $EC_{1.5}$ value less than 1000 µM in the first two trials and an IC_{30} value of less than 1000 µM

Evaluation: Determine if the IC_{30} value occurs at a concentration less than the $EC_{1.5}$

IIVS Test Article Number	Sponsor's Designation	Trial	$EC_{1.5}$ (µM)	IC_{30} (µM)	Sensitization Potential
20AAXX	2	B1	968.8	85.3	Non-Sensitizer
		B2	923.1	92.4	Non-Sensitizer



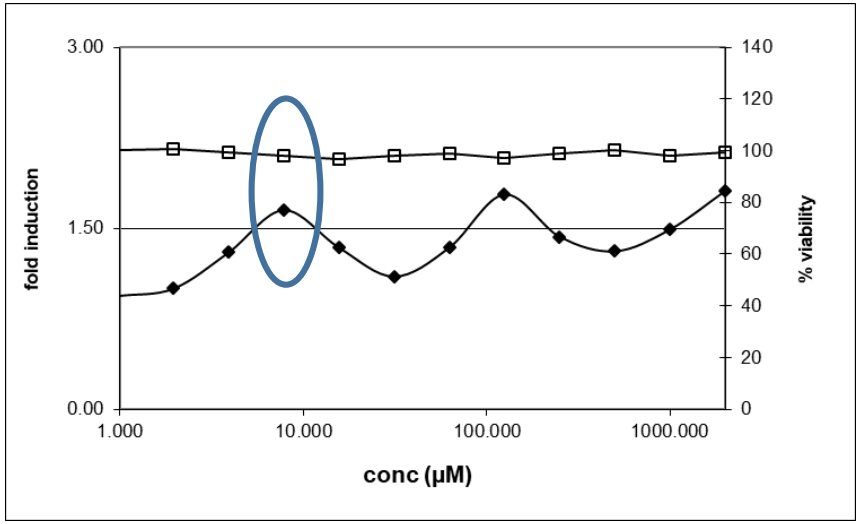
Conclusion: Test article was not considered a potential sensitizer

Data Interpretation

Situation: Test article crosses the induction cut off of 1.5 multiple times

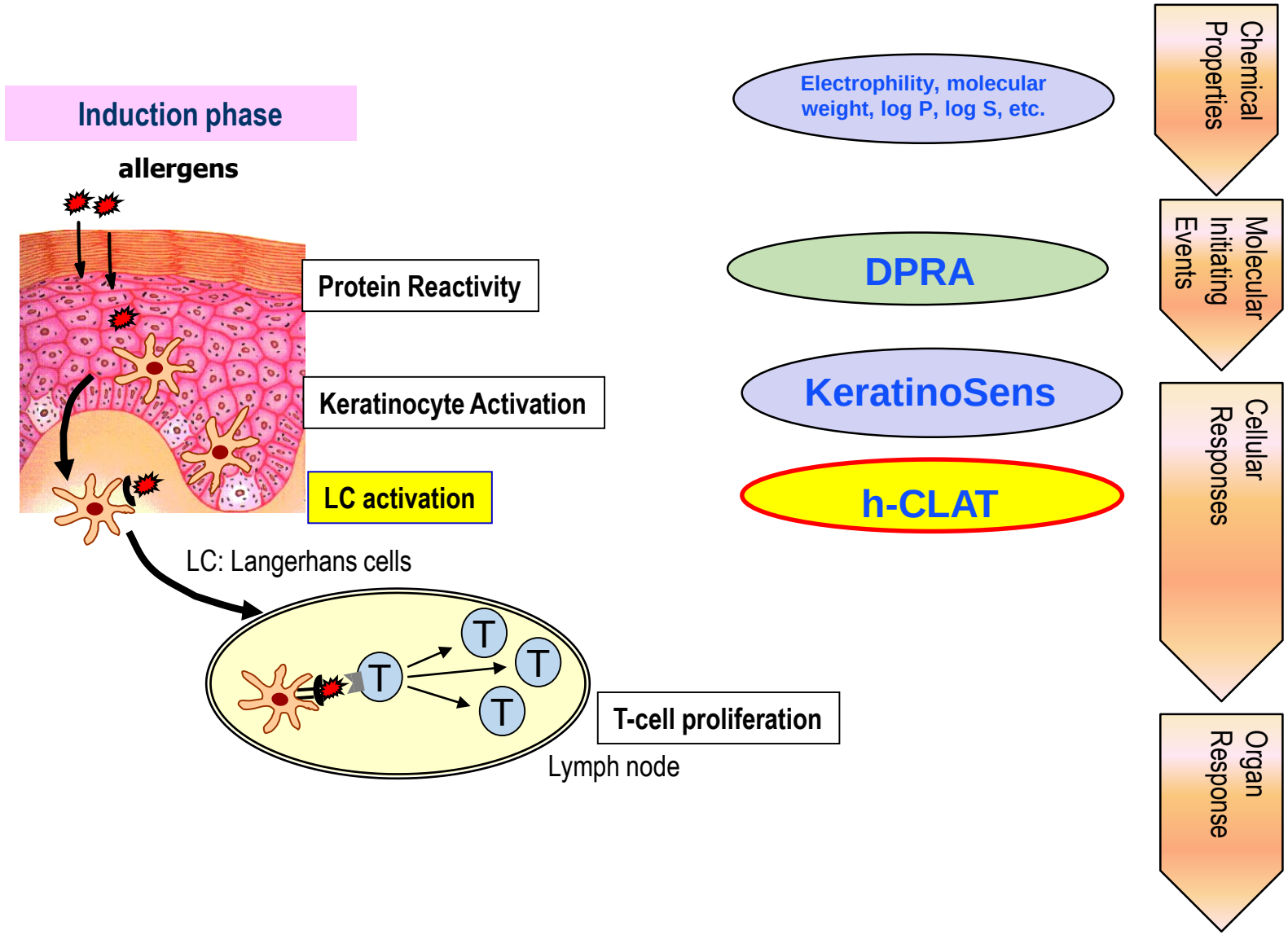
Evaluation: Determine the $EC_{1.5}$ value as the lowest dose that the test article elicits a statistically significant induction value greater than 1.5-fold

IIVS Test Article Number	Sponsor's Designation	Trial	$EC_{1.5}$ (µM)	IC_{30} (µM)	Sensitization Potential
20AAXX	3	B1	8.3	>2000	Sensitizer
		B2	7.1	>2000	Sensitizer



Conclusion: Test article was considered a potential sensitizer

AOP – Allergic Contact Dermatitis

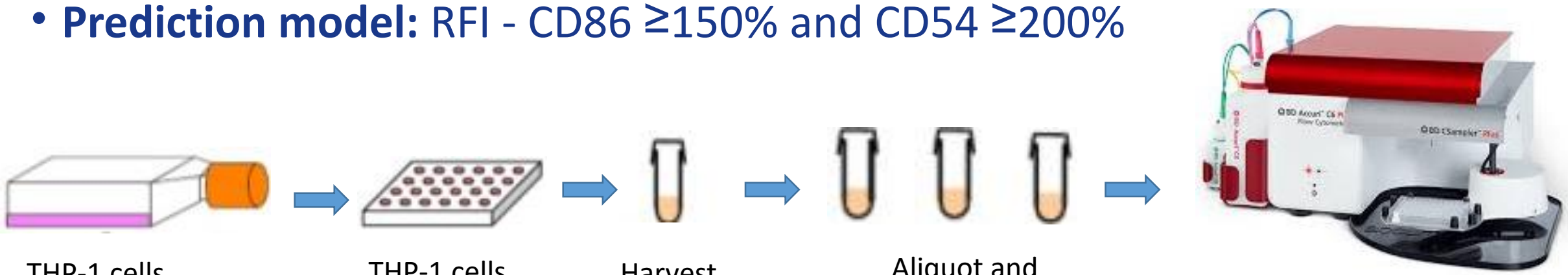


Human Cell Line Activation Test (h-CLAT)

(OECD TG 442E)

Key event 3

- **Test system:** THP-1 cells: an immortalized human monocytic leukemia cell line, used as a surrogate for DC
- Measures modulation of the expression of dendritic cell surface phenotypic biomarkers (CD86 and CD54) by flow cytometry
- **Prediction model:** RFI - CD86 ≥150% and CD54 ≥200%



THP-1 cells

THP-1 cells + Test chemical

Harvest cells

Aliquot and stain cells with anti-CD86 and anti-CD54

Analyze by flow cytometry

Human Cell Line Activation Test (h-CLAT)

ORIGINAL ARTICLE

Coupling of Contact Sensitizers to Thiol Groups is a Key Event for the Activation of Monocytes and Monocyte-Derived Dendritic Cells

Detlef Becker, Elke Valk, Sabine Zahn, Pia Brand, and Jürgen Knop
Department of Dermatology, University of Mainz, Germany

J Invest Dermatol 120:233-238, 2003

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.34, No.2, 139-150, 2009

Original Article

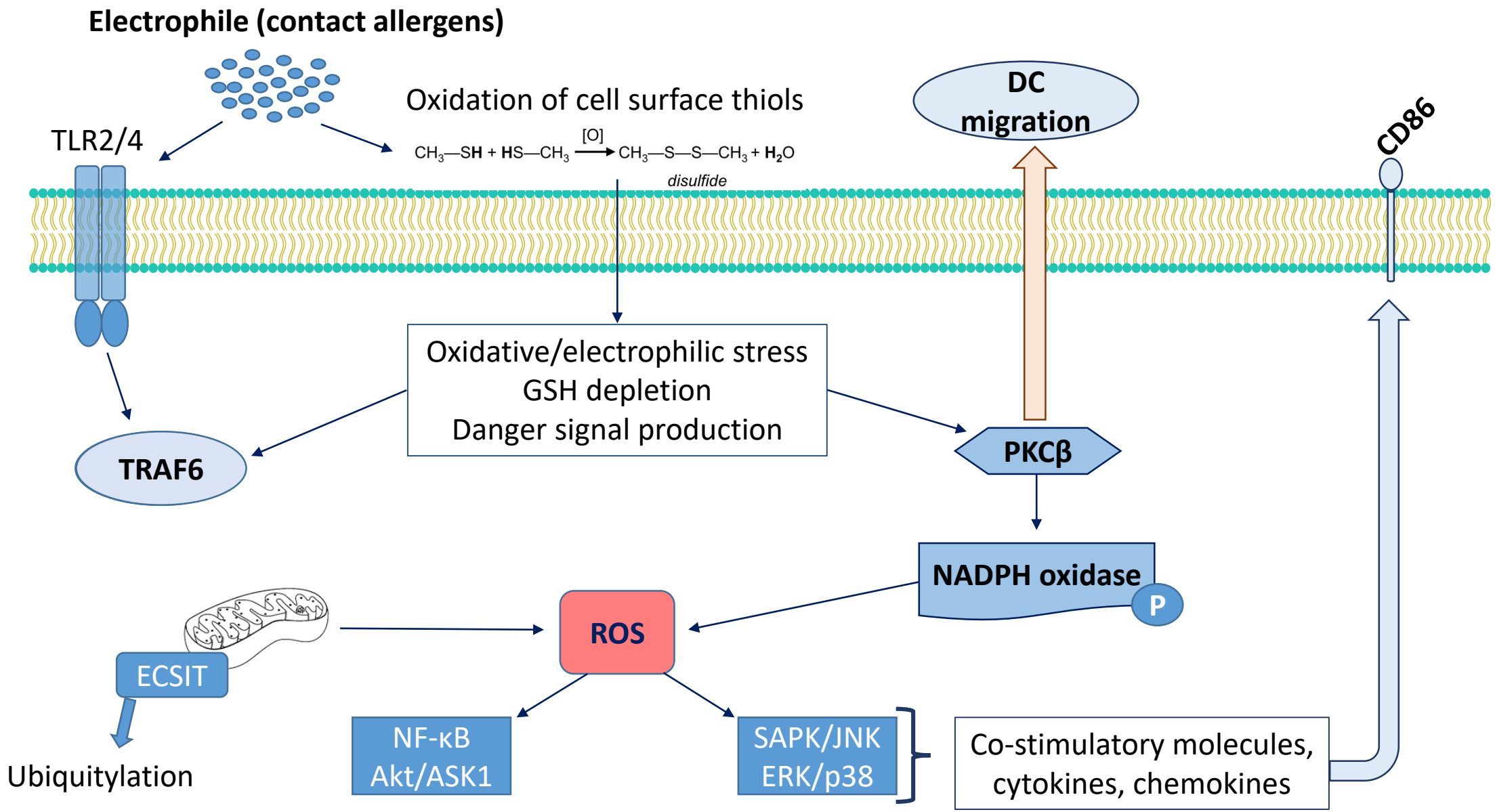
Modification of cell-surface thiols elicits activation of human monocytic cell line THP-1: Possible involvement in effect of haptens 2,4-dinitrochlorobenzene and nickel sulfate

Morihiko Hirota[1], Mie Suzuki[1], Shigenobu Hagino[1], Saori Kagatani[2], Yoshinori Sasaki[2], Setsuya Aiba[2] and Hiroshi Itagaki[1]

[1]Quality Assessment Center, Shiseido Co., Ltd., 2-12-1 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236-8643, Japan
[2]Department of Dermatology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan

(Received September 26, 2008; Accepted December 8, 2008)

Principle of the h-CLAT Test Method



Galbiati, V., Papale, A., Kummer, E. and Corsini, E., 2016. In vitro models to evaluate drug-induced hypersensitivity: potential test based on activation of dendritic cells. *Frontiers in pharmacology*, *7*, p.204.

Human Cell Line Activation Test (h-CLAT)

Limitations

- Bioavailability: Not applicable to poorly soluble compounds, but stable suspensions/dispersions acceptable
- Risk of false negatives with chemicals with log K_{ow}>3.5
- Limited metabolic activity – pro-hapten predictions?

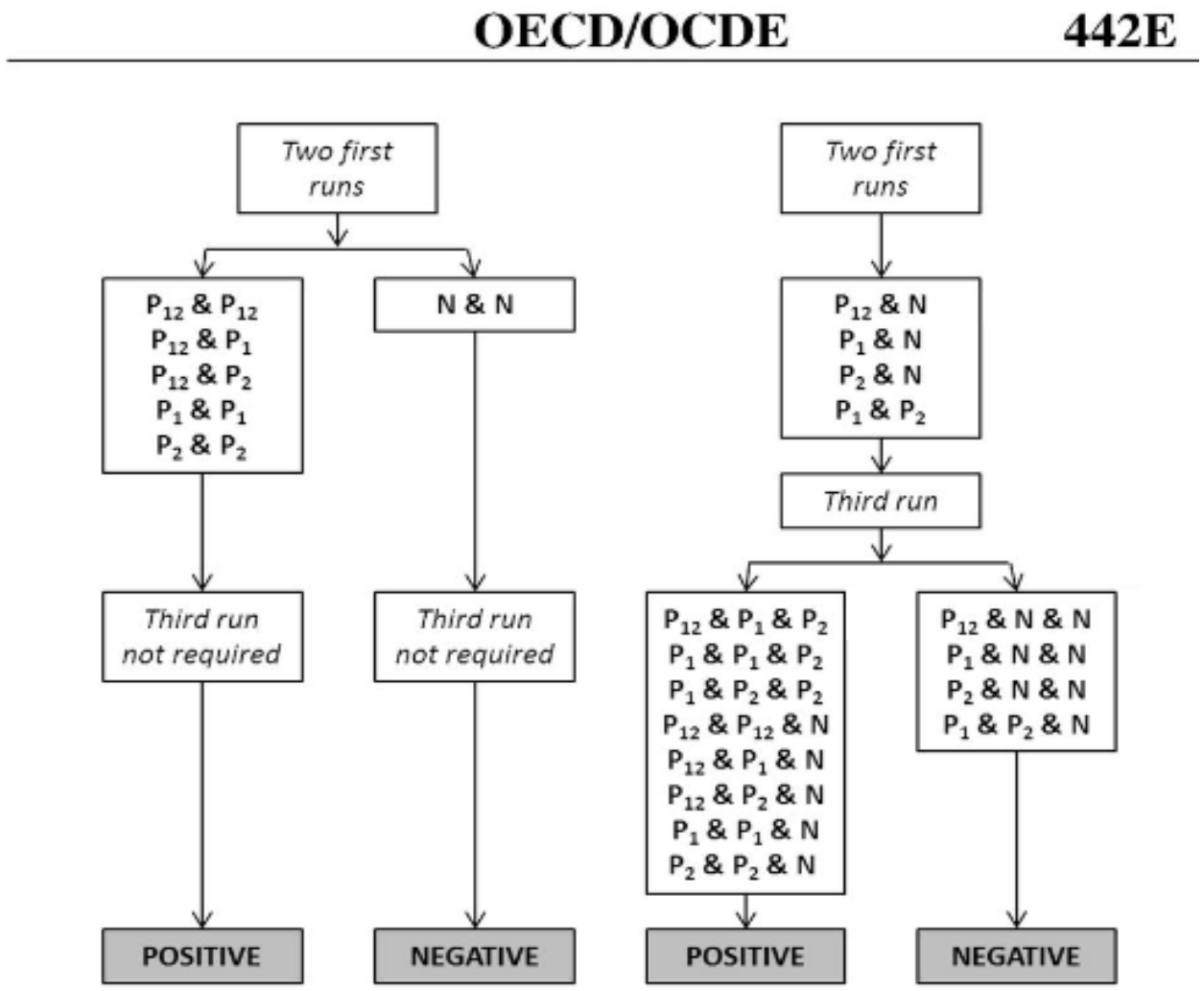
- Test chemical fluorescence at the FITC wavelength

h-CLAT Case Studies

Human Cell Line Activation Test (h-CLAT)
Prediction Model

- Prediction model for determining skin sensitization potential
- Concordant results from two runs are required to predict the skin sensitization potential of a test chemical

P_1= positive induction of CD54
P_2= positive induction of CD86



Data Interpretation

Situation: Test chemical resulted in a positive response based on CD86 RFI in first run and a positive response based on CD54 RFI in second run.

Option: Run a third run

IIVS Test Article Number	Sponsor's Designation	CV75 (µg/mL)	Trial	CD54	CD86	Sensitization Potential
19AEXX	1	>1000	B1	NO	YES	Sensitizer
			B2	YES	NO	Sensitizer
			B3	YES	NO	Sensitizer

Conclusion: Test chemical was predicted to be a skin sensitizer

Data Interpretation

Situation: Test chemical resulted in a negative response in first run and resulted in a positive response based on CD54 RFI in second run.

Option: Run a third run

IIVS Test Article Number	Sponsor's Designation	CV75 (µg/mL)	Trial	CD54	CD86	Sensitization Potential
19AAXX	1	31.8	B1	NO	NO	Non-Sensitizer
			B2	YES	NO	Sensitizer
			B3	NO	NO	Non-Sensitizer

Conclusion: Test chemical was predicted to be a non-sensitizer

Applicability Domain: DPRA, KeratinoSens and h-CLAT

Special Considerations: DPRA, KeratinoSens and h-CLAT

- **Testing mixtures**
  - Higher concentrations and dose ranges may need to be tested to account for low concentration of a sensitizer in a complex mixture
  - Assay optimization testing for mixtures is done with spiked samples

- **Addition of metabolism to correctly predict pro-haptens**
  - *In chemico* and *in vitro* assays can include a metabolism component.

    Ex. PPRA uses a peroxidase/peroxide rxn for certain pre-haptens
  - Human liver microsomes have shown to be a useful addition to the assays for chemicals requiring enzymatic activation

Testing Mixtures and Formulations

Evaluating the impact of complex matrices on the ability to detect sensitizers spiked into the matrix

Application of the KeratinoSens Assay for Prediction of Dermal Sensitization Hazard for Botanical Cosmetic Ingredients

D. Gan[1], K. Norman[2], N. Barnes[2], H. Raabe[2], C. Gomez[1], and J. Harbell[1]
[1]Mary Kay Inc. Dallas, TX, [2]IIVS, Gaithersburg, MD

Presented at the 52nd Meeting of the Society for Toxicology, San Antonio, TX, March 12, 2013

Using *In Vitro* Assays, the Direct Peptide Reactivity Assay (DPRA), KeratinoSens™ Assay (KS), and Human Cell Line Activation Test (h-CLAT) to Assess Skin Sensitization Potential of Electronic Cigarette Liquids

Abstract #3374

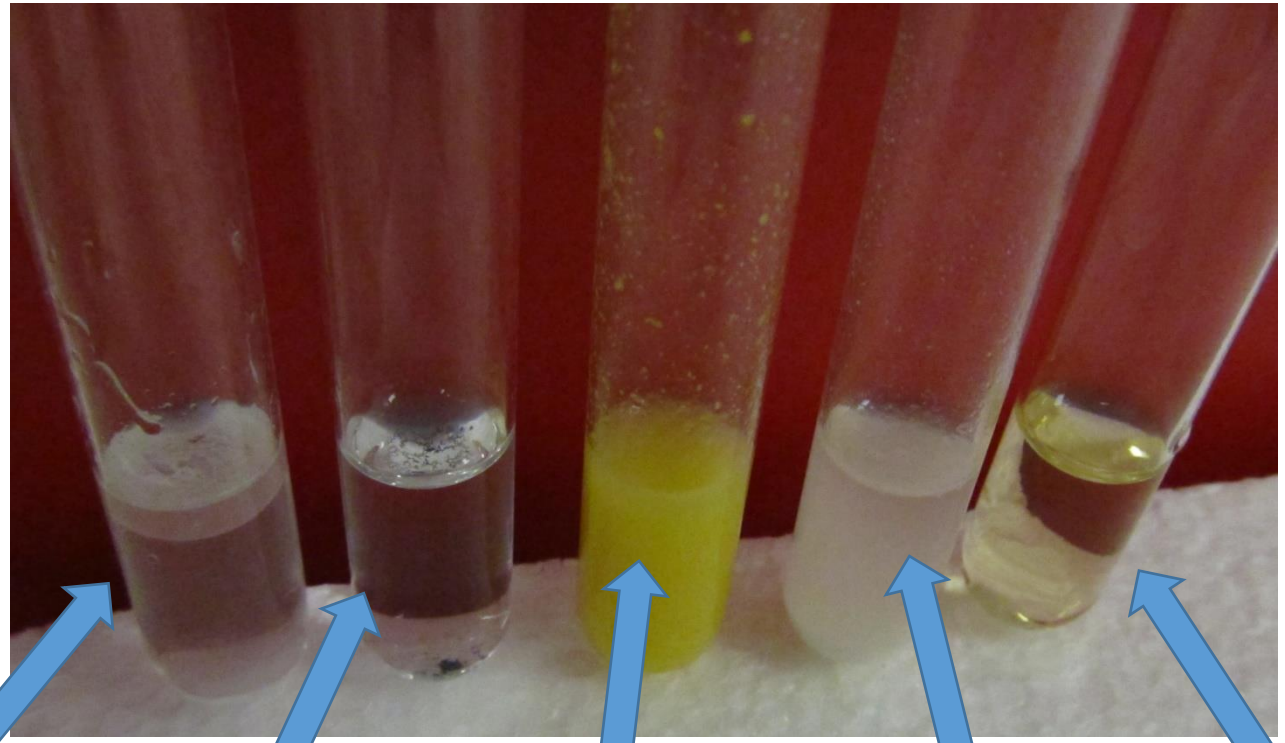
RAI Services Company

R. D. Leverette[1], B. Bombick[1], K. Fowler[1], D. Breheny[2], M. Gaça[2], A. Miller[3], G. Mun[3], K. Norman[3], A. Gamson[3], M. Lamm[3], R. Pham[3], N. Sadowski[3], V. Diersen[3], D. Sheehan[3]: [1]RAI Services Company, Winston-Salem, NC USA; [2]British American Tobacco (Investments) Ltd., Southampton, UK; [3]Institute for In Vitro Sciences, Inc., Gaithersburg, MD USA

Common Solubility Observations

KEY = Bioavailability

Is chemical available to cells?



Regulatory Acceptance

OECD Test Guidelines

442C
Adopted:
18 June 2019

***In Chemico* Skin Sensitisation**

Assays addressing the Adverse Outcome Pathway key event on covalent binding to proteins

442D
Adopted:
25June 2018

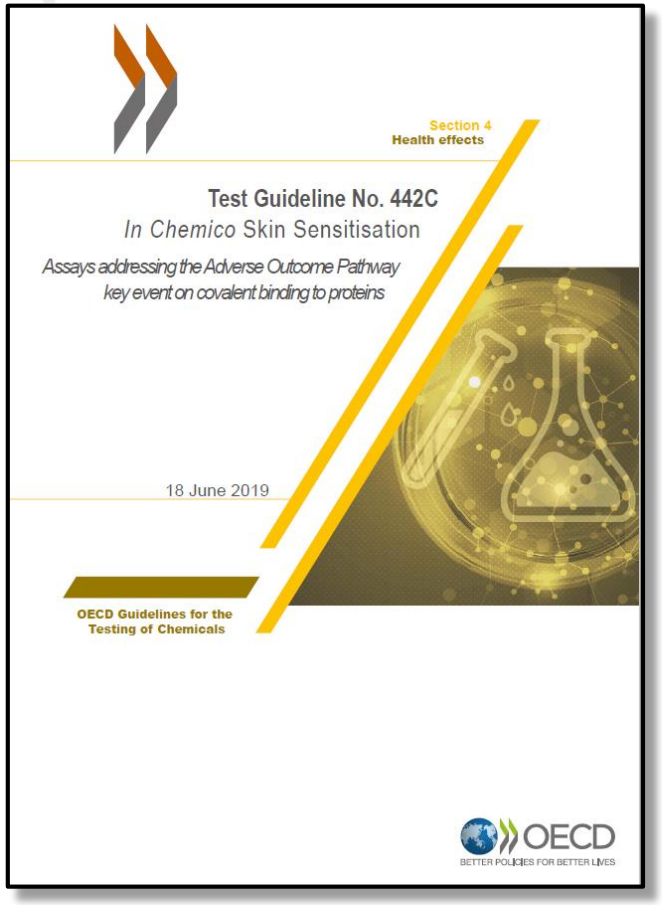
KEY EVENT BASED TEST GUIDELINE 442D

In vitro skin sensitisation assays addressing the AOP key event on keratinocyte activation

442E
Adopted:
25 June 2018

KEY EVENT-BASED TEST GUIDELINE

In vitro skin sensitisation assays addressing the key event on activation of dendritic cells on the adverse outcome pathway for skin sensitisation



OECD Guidance Document No. **256** (2016) - on the reporting of Defined Approaches to be used within IATA for skin sensitisation

Skin sensitization DA/IATA-OECD Guidance Document No. 256 (2016)

	Case study	Purpose
1	**An Adverse Outcome Pathway-based "2 out of 3" integrated testing strategy approach to skin hazard identification (BASF)**	**Hazard identification**
2	Sequential Testing Strategy (STS) for hazard identification of skin sensitisers (RIVM)	**Hazard identification**
3	A non-testing pipeline approach for skin sensitisation (G. Patlewicz)	**Hazard identification**
4	Stacking meta-model for skin sensitisation hazard identification (L'Oréal)	**Hazard identification**
5	Integrated decision strategy for skin sensitisation hazard (ICCVAM)	**Hazard identification**
6	Consensus of classification trees for skin sensitisation hazard prediction (EC- JRC)	**Hazard identification**
7	Sensitizer potency prediction based on Key event 1 + 2: Combination of kinetic peptide reactivity data and KeratinoSens® data (Givaudan)	**Potency prediction**
8	The artificial neural network model for predicting LLNA EC3 (Shiseido)	**Potency prediction**
9	Bayesian Network DIP (BN-ITS-3) for hazard and potency identification of skin sensitizers (P&G)	**Potency prediction**
10	**Sequential testing strategy (STS) for sensitising potency classification based on in chemico and in vitro data (Kao Corp)**	**Potency prediction**
11	Integrated testing strategy (ITS) for sensitising potency classification based on in silico, in chemico, and in vitro data (Kao Corporation)	**Potency prediction**
12	DIP for skin allergy risk assessment (SARA) (Unilever)	**Potency prediction**

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

CRITICAL REVIEWS IN TOXICOLOGY, 2018
https://doi.org/10.1080/10408444.2018.1429385

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Non-animal methods to predict skin sensitization (I): the Cosmetics Europe database*

Sebastian Hoffmann[a], Nicole Kleinstreuer[b], Nathalie Alépée[c], David Allen[d], Anne Marie Api[e], Takao Ashikaga[f]†, Elodie Clouet[g], Magalie Cluzel[h], Bertrand Desprez[i], Nichola Gellatly[j]‡, Carsten Goebel[k], Petra S. Kern[l], Martina Klaric[i], Jochen Kühnl[m], Jon F. Lalko[e]§, Silvia Martinozzi-Teissier[c], Karsten Mewes[n], Masaaki Miyazawa[o], Rahul Parakhia[e], Erwin van Vliet[p], Qingda Zang[d] and Dirk Petersohn[n]

[a]seh consulting + services, Paderborn, Germany; [b]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, USA; [c]L'Oréal Research and Innovation, Aulnay-sous-Bois, France; [d]ILS, Research Triangle Park, NC, USA; [e]The Research Institute for Fragrance Materials (RIFM), Woodcliff Lake, NJ, USA; [f]Shiseido Global Innovation Center, Hayabuchi, Kanagawa, Japan; [g]Pierre Fabre, Toulouse, France; [h]LVMH, St Jean de Braye, France; [i]Cosmetics Europe, Brussels, Belgium; [j]Unilever, Bedford, United Kingdom; [k]Coty, Darmstadt, Germany; [l]Procter and Gamble Services Company NV, Strombeek-Bever, Belgium; [m]Beiersdorf AG, Hamburg, Germany; [n]Henkel AG and Co. KG, Düsseldorf, Germany; [o]Kao Corporation, Tochigi, Japan; [p]Services and Consultations on Alternative Methods (SeCAM), Magliaso, Switzerland

CRITICAL REVIEWS IN TOXICOLOGY, 2018
https://doi.org/10.1080/10408444.2018.1429386

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Check for updates

Non-animal methods to predict skin sensitization (II): an assessment of defined approaches**

Nicole C. Kleinstreuer[a], Sebastian Hoffmann[b], Nathalie Alépée[c], David Allen[d], Takao Ashikaga[e]*, Warren Casey[a], Elodie Clouet[f], Magalie Cluzel[g], Bertrand Desprez[h], Nichola Gellatly[i], Carsten Göbel[j], Petra S. Kern[k], Martina Klaric[h], Jochen Kühnl[l], Silvia Martinozzi-Teissier[c], Karsten Mewes[m], Masaaki Miyazawa[n], Judy Strickland[d], Erwin van Vliet[o], Qingda Zang[d] and Dirk Petersohn[m]

[a]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, USA; [b]SEH Consulting + Services, Paderborn, Germany; [c]L'Oréal Research & Innovation, Aulnay-sous-Bois, France; [d]ILS, Research Triangle Park, NC, USA; [e]Shiseido, Yokohama-shi, Kanagawa, Japan; [f]Pierre Fabre, Toulouse, France; [g]LVMH, St Jean de Braye, France; [h]Cosmetics Europe, Brussels, Belgium; [i]Unilever, London, UK; [j]Coty, Darmstadt, Germany; [k]Procter & Gamble Services Company NV, Strombeek-Bever, Belgium; [l]Beiersdorf AG, Hamburg, Germany; [m]Henkel AG & Co. KGaA, Düsseldorf, Germany; [n]Kao Corporation, Haga, Tochigi, Japan; [o]Services & Consultations on Alternative Methods (SeCAM), Magliaso, Switzerland

Cosmetics Europe
the personal care association

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

National Toxicology Program
U.S. Department of Health and Human Services

*LLNA is ~70-80% reproducible for hazard

Predicting LLNA Hazard							
Defined Approach:	BASF 2/3 (DKH)	Kao STS	Kao ITS	ICCVAM SVM (LLNA)	Shiseido ANN (D_hC)	Shiseido ANN (D_hC_KS)	P&G BN ITS-3
N	127	126	120	120	126	126	119
Accuracy (%)*	70.1	77.8	79.2	88.3	76.2	81.0	83.2
Sensitivity (%)	72.3	92.6	85.6	93.3	90.4	97.9	83.2
Specificity (%)	63.6	34.4	60.0	73.3	34.4	31.3	83.3
BA (%)	68.0	63.5	72.8	83.3	62.4	64.6	83.3

Kleinstreuer et al. 2018 Crit Rev Tox

Hazard Identification

LLNA (benchmark): **74.2%**

Human

	NS	S
NS	20	13
S	20	75

n=128

BASF ‘2 of 3’ DKH

	NS	S
NS	29	18
S	11	69

n=127

Accuracy [%] 77.2

Kao STS

	NS	S
NS	16	2
S	23	85

n=126

80.2

Kao ITS

	NS	S
NS	26	5
S	13	76

n=120

85.0

ICCVAM SVM

	NS	S
NS	28	11
S	11	70

n-=120

81.7

Shiseido ANN

	NS	S
NS	12	0
S	27	87

n=126

78.6

P&G BN ITS-3

	NS	S
NS	25	15
S	14	65

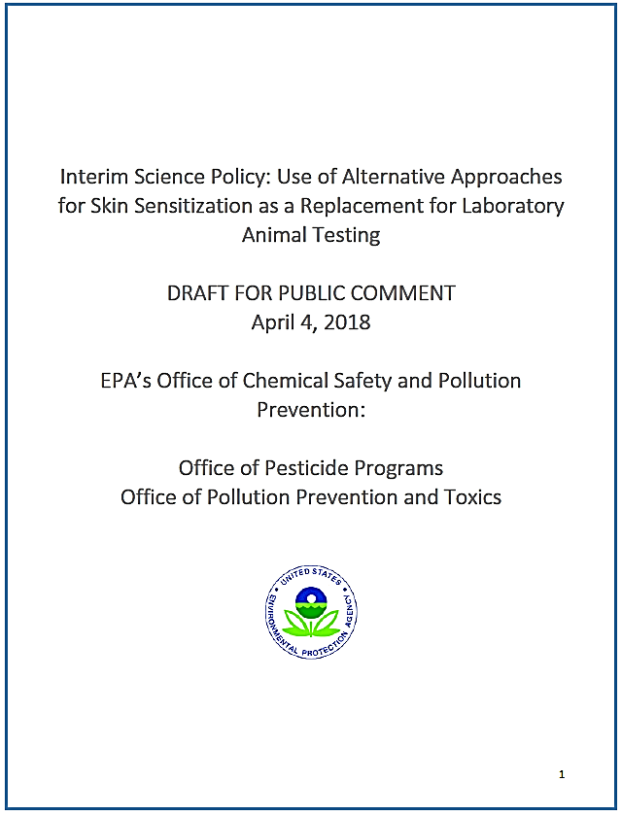
n=119

75.6

US Regulatory Progress

US EPA *Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing*

- Joint policy between Office of Pesticide Programs (OPP) and Office of Pollution Prevention and Toxics (OPPT)
- Applies to pesticide active ingredients, inerts, and single chemicals regulated under amended TSCA
- Two DAs currently accepted: “AOP 2 out of 3” and “KE 3/1 STS”
- Includes assays covered by the respective KE-based OECD TGs
- Policy to be updated to accept more DAs as the OECD GL work develops


Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

DRAFT FOR PUBLIC COMMENT
April 4, 2018

EPA’s Office of Chemical Safety and Pollution Prevention:

Office of Pesticide Programs
Office of Pollution Prevention and Toxics

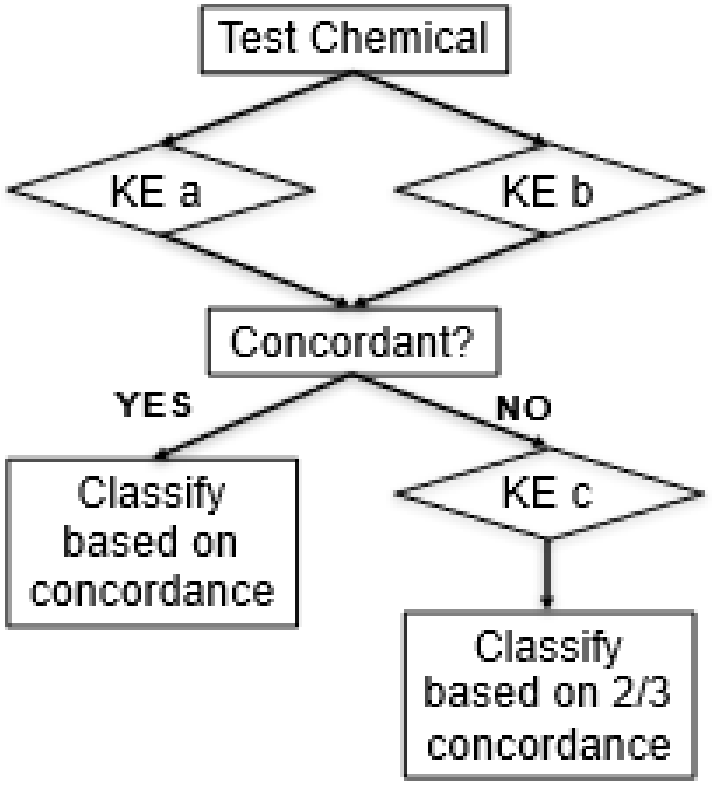
1

https://www.epa.gov/pesticides/epa-releases-draft-policy-reduce-animal-testing-skin-sensitization

Defined Approach

"2 out of 3"

AOP "2 out of 3" - Hazard Identification



N=213 (151 sensitizers, 64 non-sensitizers)

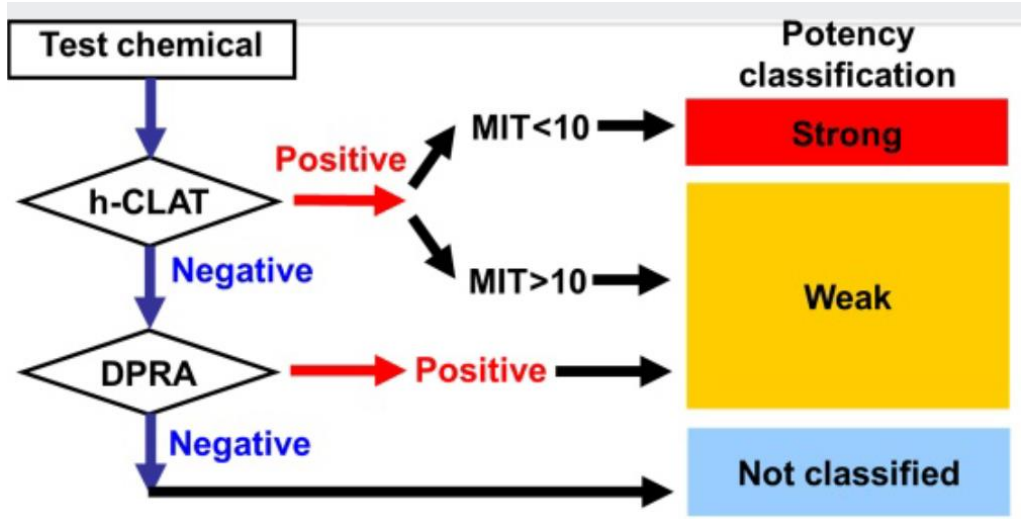
Test strategy compared to **human data**

Sensitivity	**90%**
Specificity	**100%**
Accuracy	**91%**

Bauch et al. *Regul. Toxicol.* Pharmacol *2012, (63) 489-504*

Defined Approach
KE 3/1 STS

KE 3/1 STS - Potency identification



Test strategy compared to **LLNA data**

Over prediction	11%
Under prediction	18%
Accuracy	**71%**

N=101 (76 sensitizers, 25 non-sensitizers)

Nukada et al. *Toxicology in Vitro* 2013, (27) 609-618

Skin Sensitization: Future Opportunities

What further info do we need from non-animal test methods?

Skin kinetics Potency Complex mixtures/formulations log K_{ow} >3

Emerging Opportunities

kinetic DPRA

SENS-IS

EpiSensA

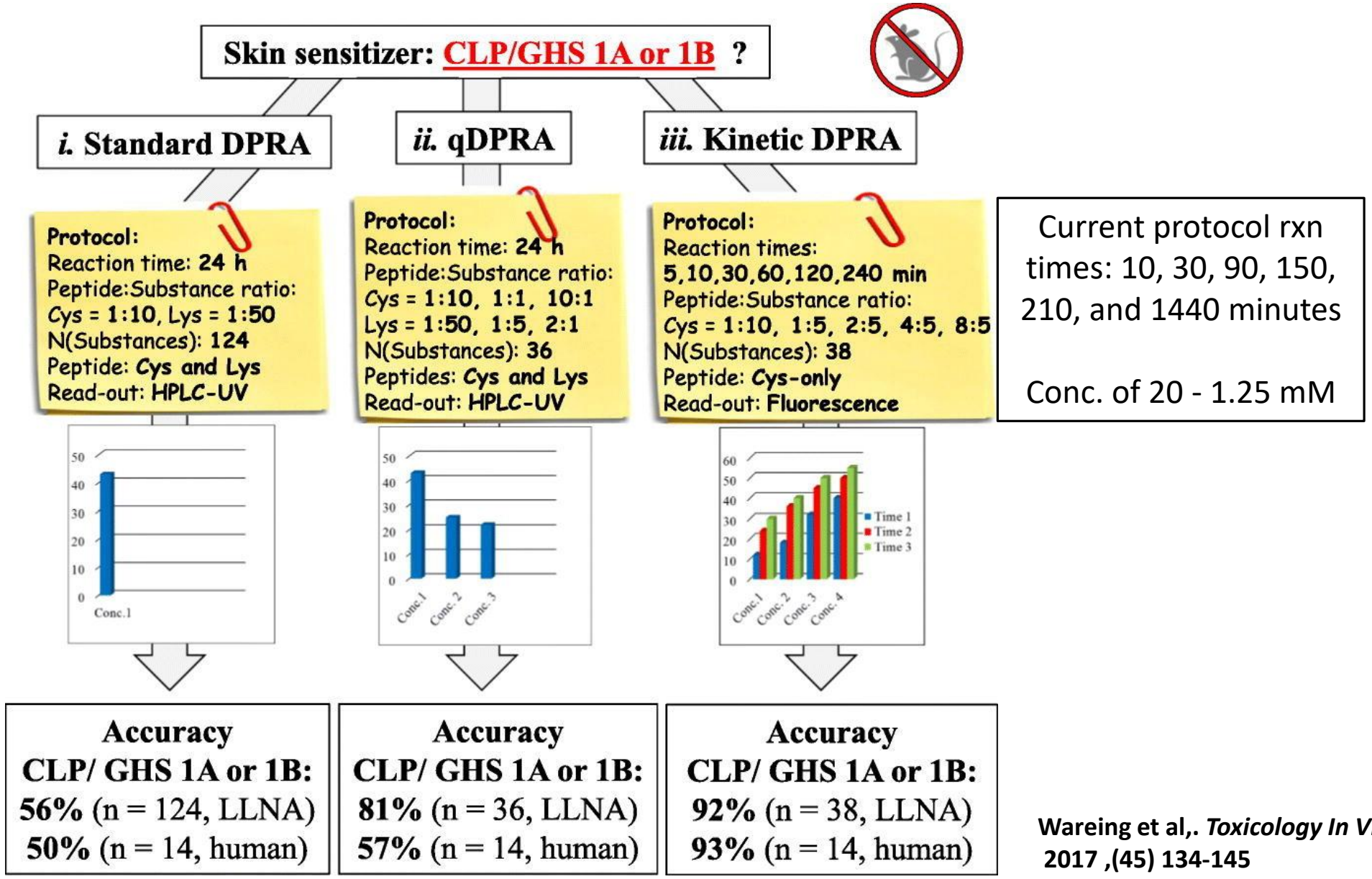
GARD™skin

Kinetic DPRA (kDPRA)

- *In chemico* method
- Can determine **potency** of chemicals

GHS 1A/1B

Kinetic DPRA (kDPRA)



Current protocol rxn times: 10, 30, 90, 150, 210, and 1440 minutes

Conc. of 20 - 1.25 mM

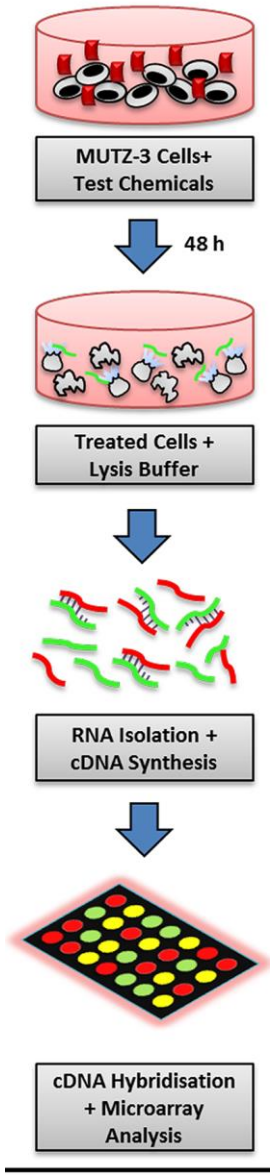
Wareing et al,. *Toxicology In Vitro* 2017 ,(45) 134-145

Genomic Allergen Rapid Detection (GARD™skin)

Cell based –MUTZ-3 cells
The readout of the assay is based on differentially regulated transcriptional changes of selected genomic biomarkers, referred to as the GARD prediction signature (GPS).
Probes over 200 genes

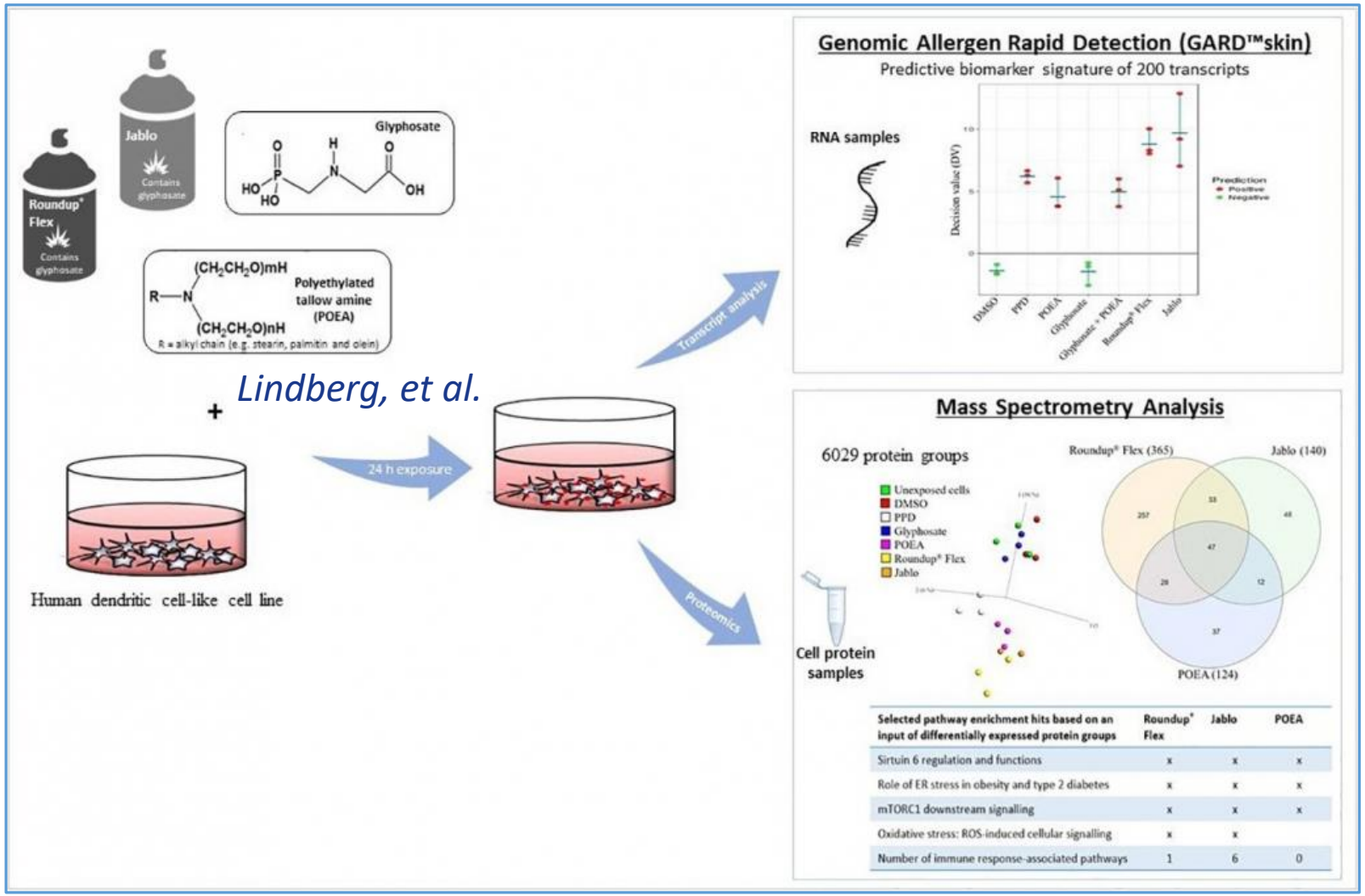
Prediction model

Classifications of unknown compounds as sensitizers or non-sensitizers are performed with a support vector machine (SVM) model, trained on the 38 reference chemicals used for GARD development



GARD™skin

An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations



Selected pathway enrichment hits based on an input of differentially expressed protein groups	Roundup® Flex	Jablo	POEA
Sirtuin 6 regulation and functions	x	x	x
Role of ER stress in obesity and type 2 diabetes	x	x	x
mTORC1 downstream signalling	x	x	x
Oxidative stress: ROS-induced cellular signalling	x	x	
Number of immune response-associated pathways	1	6	0

Lindberg, et al., Journal of Proteomics, 6 Feb 2020, https://doi.org/10.1016/j.jprot.2020.103647

SENS-IS and EpiSensA

RhE-based gene expression platforms

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]

[a] ImmunoSearch, Grasse, France
[b] Agence nationale de sécurité du médicament, Vendargues, France
[c] CNRS, Institute of Molecular and Cellular Pharmacology, Sophia Antipolis, France
[d] University of Nice Sophia Antipolis, Nice, France
[e] Independant Consultant, Marly, Switzerland

ELSEVIER

Toxicology in Vitro

Volume 27, Issue 8, December 2013, Pages 2213-2224

Development of a new *in vitro* skin sensitization assay (Epidermal Sensitization Assay; EpiSensA) using reconstructed human epidermis

Kazutoshi Saito, Yuko Nukada, Osamu Takenouchi, Masaaki Miyazawa, Hitoshi Sakaguchi, Naohiro Nishiyama

Kao Corporation, Safety Science Research Laboratories, 2606 Akabane, Ichikai-Machi, Haga-Gun, Tochigi 321-3497, Japan

Received 6 January 2013, Accepted 22 August 2013, Available online 30 August 2013.

Check for updates

Show less

https://doi.org/10.1016/j.tiv.2013.08.007

Get rights and content

Highlights

- Gene expression profile was examined in RhE model.
- Five cellular stress related genes were significantly upregulated by DNFB and OXA.
- Predictive performance of ATF3 gene displayed 100% accuracy to animal testing.

SENS-IS Assay

- **Test system:** RhE model - Reconstructed human Epidermis (3D)
  - Includes skin kinetics – minimize solubility issues
- **Analysis platform:** Gene expression measurements (RT-PCR)
- **Prediction model:**
  - **Irritation**
    - Positive if at least 15/24 skin irritation genes are significantly induced
  - **Sensitization**
    - Positive if 7/17 genes in ARE group and/or 7/21 genes on the SENS-IS gene group are significantly induced

      (provided that <20 Irritation genes are over-expressed)

SENS-IS Assay: Advantages

- Applicable to low solubility compounds
- Ideal for topical application of complex formulations
- May support predictions using weight/surface area based data
- May be applicable to mixtures and finished products

In comparison to		
	Human	LLNA
n	130	150
Sensitivity	95.8%	97.7%
Specificity	96.5%	95.2%
PPV	97%	96.6%
NPV	95%	96.7%
Accuracy	96%	96.6%

150 test chemicals were evaluated at ImmunoSearch in at least two independent experiments. Cooper statistics values (Sensitivity, Specificity and Accuracy) were calculated for the SENS-IS assay using human (see human column) and LLNA (see LLNA column) data from the literature as references.
"n": Number of results included in the calculation (depending on available reference data); "PPV »: Positive Prediction Value; ≪NPV≫: Negative Prediction Value.

(Cottrez F et al., *Toxicology in Vitro,* Volume 62, February 2020, 104644, Online 2019)

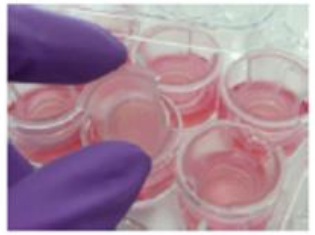
SENS-IS Assay

Assay steps:

1-Chemical application on Episkin

2-Washing

3-Post-incubation and sampling



4-Tissue lysing and cDNA preparation

5-RT-PCR quantification

6-Results analysis

- **Validation assay** by analysis of:
- negative control (Olive oil, PBS, DMSO)
- irritant control (5% SLS)
- two sensitizer controls (50% HCA, 1% TNBS)

Irritation : positive response if at least 15/24 genes are significantly induced

Sensitization : a molecule is classified as positive if at least:
- 7/17 genes in ARE genes group ***and/or***
- 7/21genes in SENS-IS genes group are significantly induced

Potency assessment :
-positive up to 0.1% : extreme
-positive up to 1% : strong
-positive up to 10% : moderate
-positive up to 50% : weak

Thank You
Questions ?

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

For further information contact:
Rishil J. Kathawala: rkathawala@iivs.org
Victoria (Tori) Diersen: vdiersen@iivs.org
Hans Raabe: hraabe@iivs.org