FEDERAL INSTITUTE
FOR RISK ASSESSMENT

Background on skin sensitization hazard identification:

Aspects in the development of « in-vitro based alternatives for sensitization testing »

Dr. Matthias Peiser

Department of Product Safety
Federal Institute for Risk Assessment (BfR)

Outline

I. New scientific aspects of sensitization

II. Current developments in in vitro testing - what`s in the pipeline?

III. When will alternative in vitro tests be available?

I. New scientific aspects of sensitization

Allergic contact dermatitis (ACD)

Cellular/molecular events sensitization and elicitation phases

Discrimination irritation vs. sensitization

Facts on Skin Sensitization/Allergic contact dermatitis and testing

- 15-20 % population sensitized ≥1 substance
- >3.000 substances known elicitors
- Animal experiments skin for sensitization/-irritation (EU-25, 2005):

  ca 60,000 animals

  [for skin sensitization 22,184 guinea pigs and 21,350 mice]

- 7th Amendment of the EU Cosmetic Guideline (**11.03.2013** „deadline“)
- validations for toxicological safety within the REACH guideline
- Test systems published base on detection of alterations of the phenotype and cytokine-/chemokine-release of dendritic cells

Allergic contact dermatitis: “Breakdown of skin immune tolerance to haptens“

Cells involved in ACD:

- **T-helper memory cells (differentiate)**
- **Suppressive T cells (loose control)**
- **Cytotoxic T cells (damage)**

Figure 12-28 Immunobiology, 6/e. (© Garland Science 2005)

	Type IV		
Immune reactant	T_H1 cells	T_H2 cells	CTL
Antigen	Soluble antigen	Soluble antigen	Cell-associated antigen
Effector mechanism	Macrophage activation	IgE production, Eosinophil activation, Mastocytosis	Cytotoxicity
	IFN-γ, T_H1, chemokines, cytokines, cytotoxins	IL-4, IL-5, T_H2, eotaxin, cytotoxins, inflammatory mediators	CTL
Example of hypersensitivity reaction	Contact dermatitis tuberculin reactior	Chronic asthma, chronic allergic rhinitis	Contact dermatitis

Figure 12-2 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

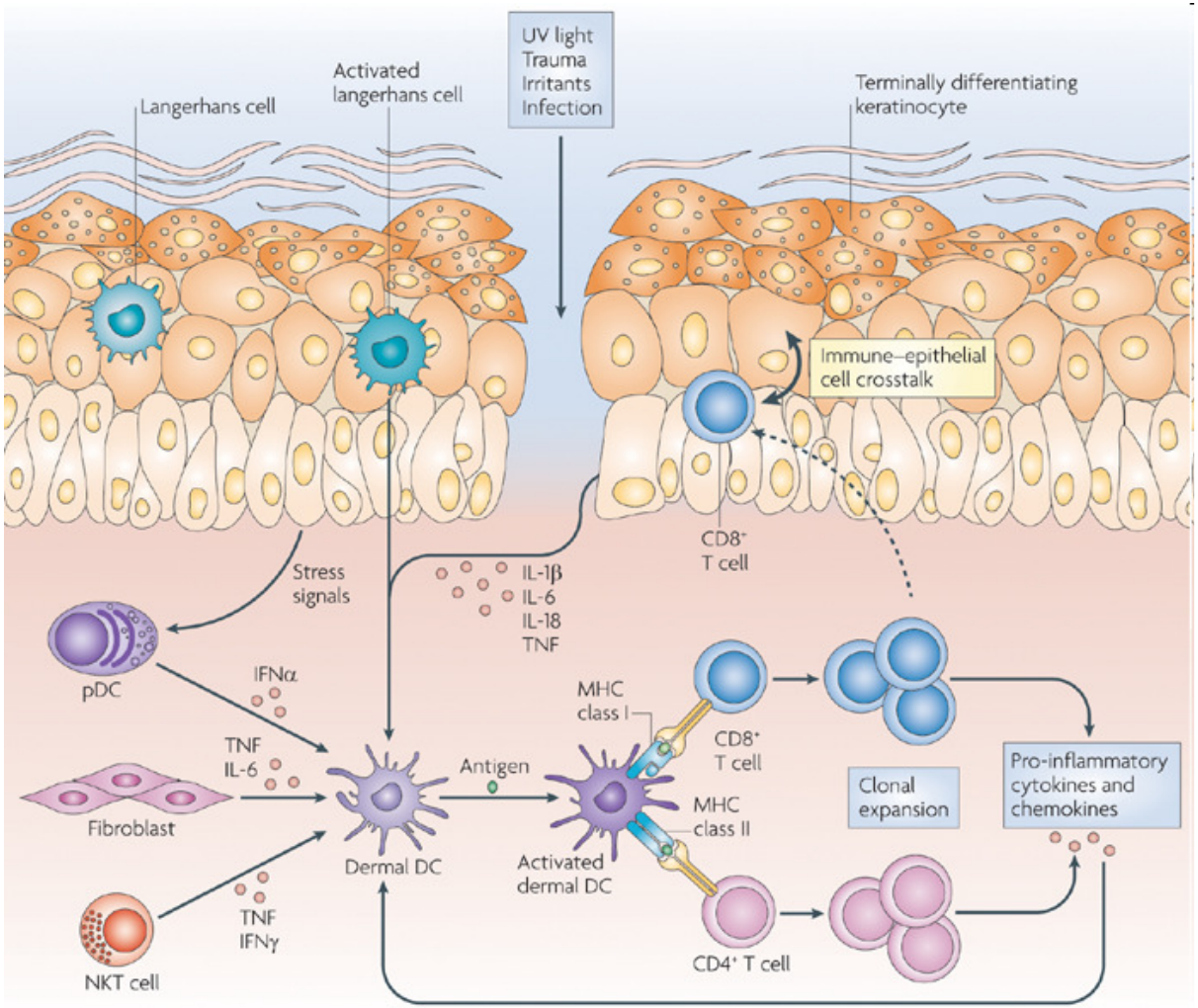
Immune reactions in skin: Cellular and molecular events

After barrier disruption:

→ Access of stress signals, PAMPs & DAMPs

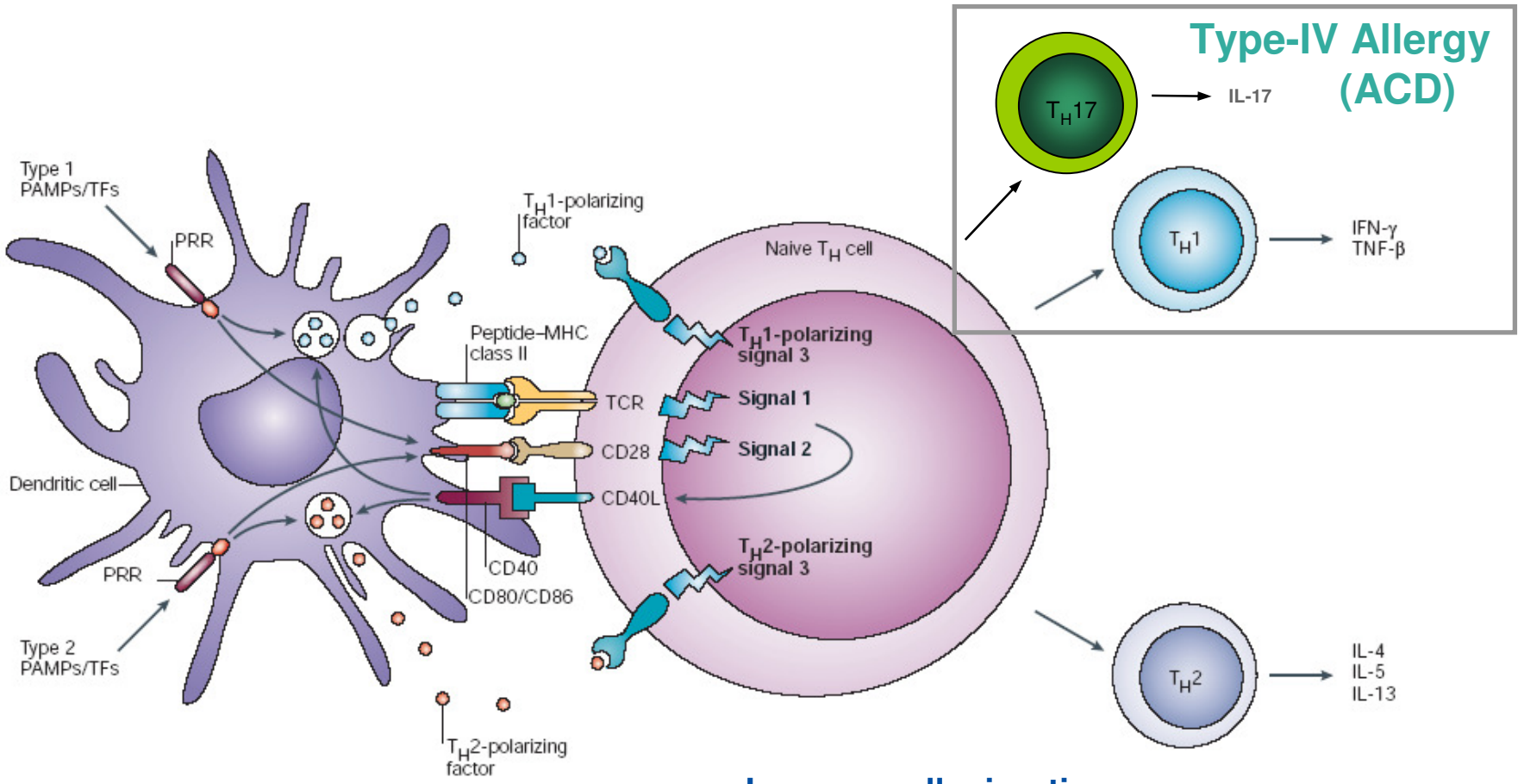
→ Irritants activate keratinocytes

→Sensitizer activate dendritic cells (and keratinocytes?)



Nestle et al. (10/2009), Nat. Rev. Immunol.

Sensitization: Cells of adaptive Immune system, Allergen/Antigen presentation

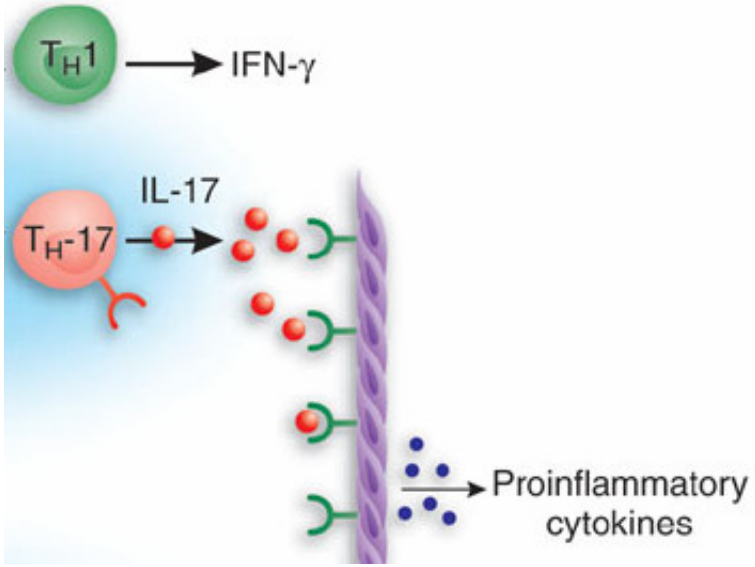


Immune cell migration
Antigen/allergen
presentation in lymph node
Proinflammatory cytokines
Differentiation of T cells
Tissue damage (cell death)

Type-I Allergy

Kapsenberg, M.L. (2003), Nat. Rev. Immunol. 3: 984-993, modified

Biomarker for activated cells in sensitization



- Cell surface molecules: CD86, HLA-DR..
- Cytokines/chemokines: IL-8, IL-12, IFNγ..
- Transcription factors: T-bet, GATA-3..

Expression of transcription factors in polarized T cells

Transcription factors	T cell type	
Runx3	CTL	($CD3^+CD8^+$)
Th-POK	T_H	($CD3^+CD4^+$)
T-bet, HLX		T_H1
GATA-3, MAF		T_H2
RORγt and RORα		T_H17
FOXP3	T_{Reg}	

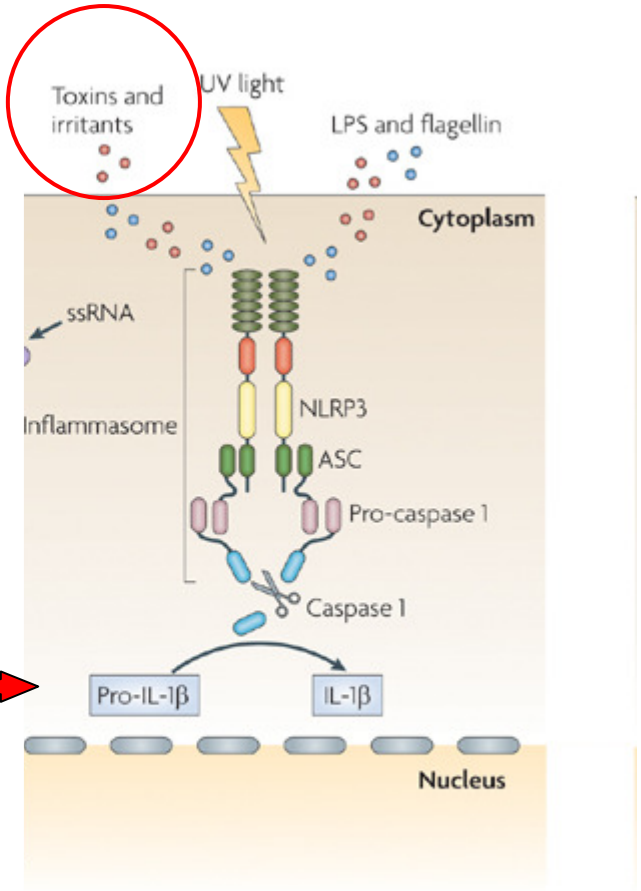
FOXP3, forkhead box P3; GATA-3, GATA-binding protein 3; HLX, H2.0-like homeobox 1; ROR, retinoic-acid-receptor-related orphan receptor; Th-POK, BTB/POZ domain-containing zinc finger transcription factor

Irritation: Keratinocytes and Inflammasome

KCs detect danger-associated molecular pattern (DAMPs): irritants and toxins via Toll-like receptors (TLRs) and inflammasome machinery

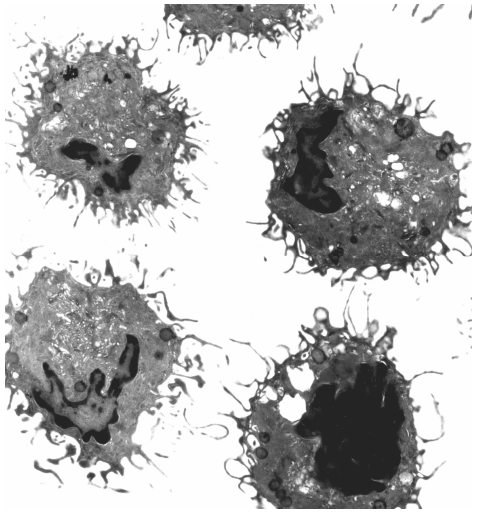
No immune cell migration
No differentiation of T cells
No antigen/allergen presentation in lymph node

Proinflammatory cytokines
Tissue damage (cell death)



Nature Reviews | Immunology

Discrimination **Irritation** vs. **Sensitization:** **Predictive biomarkers**



Skin irritating potential compound:

IL-1α, IL-8, IL-18, IL-6, IL-10, TNFα, LIF

Skin sensitizing potential compound:

Sensitization phase: CD86, MHC-II, CD54, CD58, IL-1β, IL-18, IL-12, IL-6, TNFα, ATP, PGE2, LTB4 ROS, histamine, PGE2, histamine, CCL2, IgM (liver)

Elicitation phase: IL-1β, IL-18, IL-6, IL-10, TNFα, ATP, IFNγ, IL-17

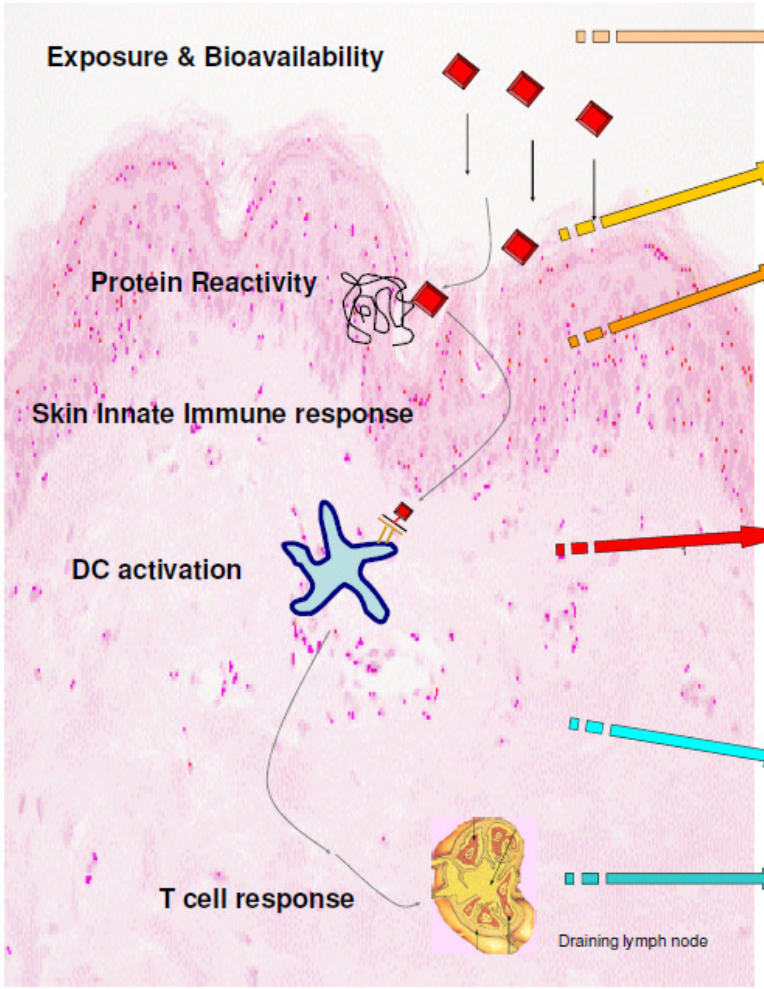
Resolution phase: IL-10, TGFβ

II. Current developments in in vitro testing - what`s in the pipeline?

COLIPA
Sens-it-iv
BfR

Testing of textile dyes

COLIPA Skin Tolerance Task Force: Portfolio of research projects



In silico **mathematical model for calculating the epidermal bioavailability of contact allergens**
GB. Kasting, S. Farahmand and MA Miller, Univ. Cincinnati, USA; G.F. Gerberick and P. Kern, P&G

Next generation peptide reactivity assay
J.P. Lepoittevin, Univ. Louis Pasteur Strasbourg, France; G.F. Gerberick, P&G

Direct tissue profiling and imagery by mass spectrometry
M.R. Clench and M.N. Woodroofe, Sheffield Hallam University, UK

Characterisation of the metabolic capacity of human skin & current *in vitro* model systems
E. Fritsche, Univ. Düsseldorf, Germany; R. Edwards, College London and C. Pease, Unilever, UK

Comparison of contact allergen-induced gene expression changes in human peripheral blood mononuclear cell- derived dendritic cells and DC-surrogate cell lines
C. A. Ryan, L. A. Gildea, L. Foertsch, G. F. Gerberick, P. Aeby and C. Goebel, P&G

Implication of signal transduction pathways in DC maturation, a potential predictive model for allergic contact dermatitis
S. Trompezinski, L. Foggia, M. Haftek, P. Courtellemont, M. Serres, Hôpital E. Herriot, Lyon, France and M. Tailhardat, LVMH Recherche, France

Identification and exploitation of molecular mechanisms underlying the homing of dendritic cells to lymph nodes following exposure to chemical sensitizers
J. E. Pease, Imperial College London, London, UK

Multi-parameter dendritic cell biosensor system to identify contact allergens
A. Takashima, University of Toledo Ohio, USA

Construction of a multi-cell human skin model to investigate the allergenic potential of novel chemicals
Amir Ghaem Maghami, Queen's Medical Centre, Nottingham, UK

Development of T cell priming assays using human PBLs depleted in CD25+ Treg cells for the sensitive *in vitro* screening of contact allergens
M. Vocanson, JF. Nicolas, INSERM U851, Lyon, France and M. Tailhardat, LVMH

Skin Metabolism Project

E. Fritsche[1], R. Edwards[2], C. Goebel[3], P. Aeby[3], S. Pfuhler[3], P. Carmichael[4], C. Pease[4]

[1]Dusseldorf University, Germany
[2]Imperial College London, UK
[3]The Procter & Gamble Company
[4]Unilever

Aiming to populate a table indicating metabolic capability in different systems:

	Human Skin	Keratinocytes	3D Skin Models	Dendritic cells

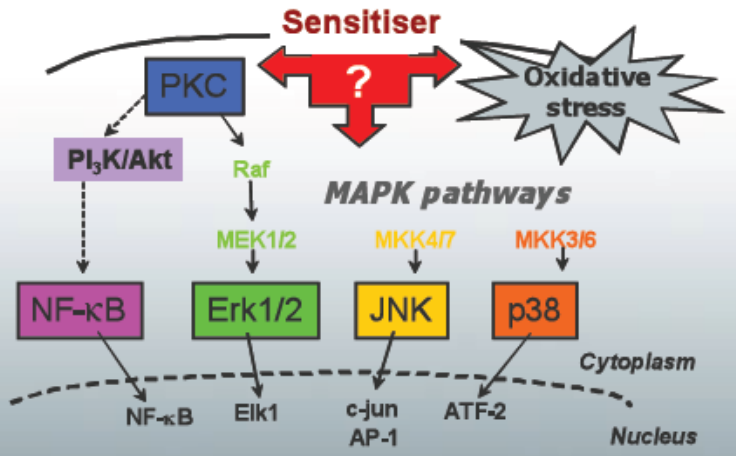
In vitro testing at COLIPA

Implication of signal transduction pathways in DC maturation, a potential predictive model for allergic contact dermatitis

S. Trompezinski#, L. Foggia*#, M. Haftek*, M. Tailhardat***

*P. Courtellemont**, M. Serres*;* *Hôpital E. Herriot, Lyon, France,**LVMH Recherche, Saint Jean de Braye, France, #COLIPA, Bruxelles, Belgique

To better characterize early events involved in the activation of human DCs induced by sensitizers versus irritant, in order to develop *in vitro* alternative methods for the prediction of contact dermatitis.



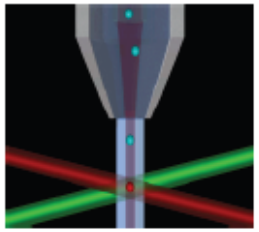
In vitro testing at COLIPA

Comparison of Contact Allergen-induced Gene Expression Changes in Human Peripheral Blood Mononuclear Cell- Derived Dendritic Cells and DC-Surrogate Cell Lines

C. A. Ryan, S. Python, P. Aeby and G. F. Gerberick

The Procter & Gamble Company

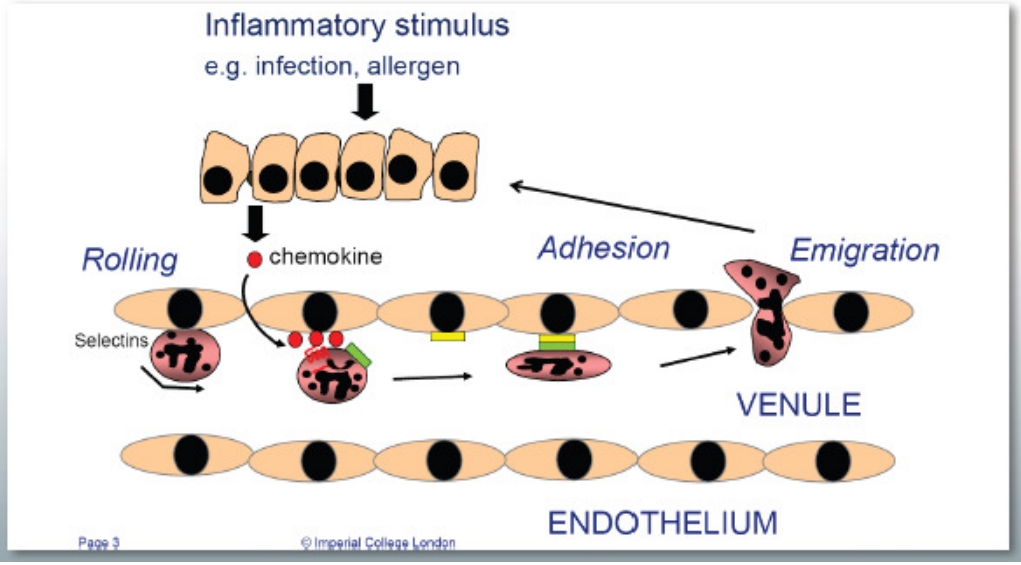
Luminex® xMAP® Technology



In vitro testing at COLIPA

Identification and exploitation of molecular mechanisms underlying the homing of dendritic cells to lymph nodes following exposure to chemical sensitizers

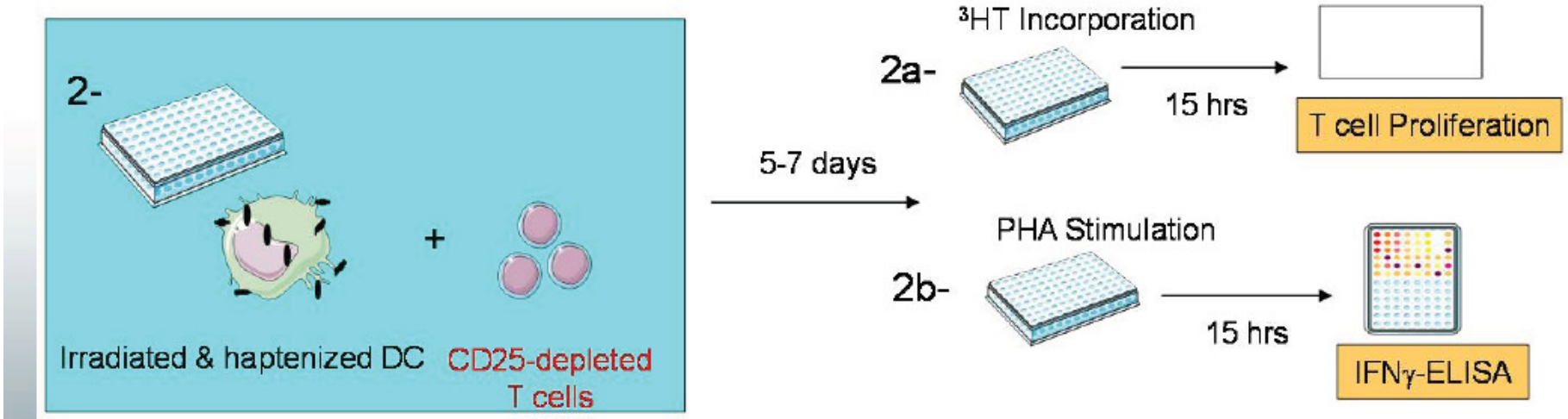
James E. Pease, Imperial College London, London, UK



In vitro testing at COLIPA

Development of T cell priming assays using human PBLs depleted in CD25+ Treg cells for the sensitive *in vitro* screening of contact allergens

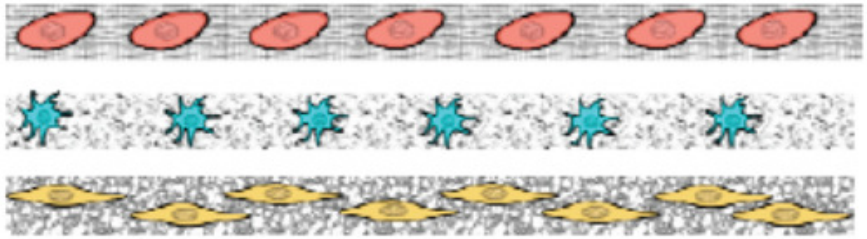
Marc VOCANSON & JF. Nicolas, INSERM U851, Lyon, France



In vitro testing at COLIPA

Construction of an immunocompetent three dimensional human skin model to investigate the allergenic potential of novel chemicals

1. Model construction. Cells embedded in discs of electrospun PLA scaffold



1. Keratinocytes (+Red cell tracker)

2. Dendritic cells (+Blue cell tracker)

3. Fibroblasts transfected with NF-κB GFP reporter construct (+Orange cell tracker)

Amir Ghaem Maghami[1], Paddy Tighe[1], Farouk Shakib[1], John Haycock[2] & Sheila MacNeil[2]

[1]University of Nottingham, United Kingdom, [2]University of Sheffield, United Kingdom

In vitro testing at Sens-it-iv:
Novel Testing Strategies for In Vitro Assessment of Allergens

WP2B: Finding the most in vivo-like EC and EC markers: IL-8, IL-6, CD47, CD54 and CXCL5

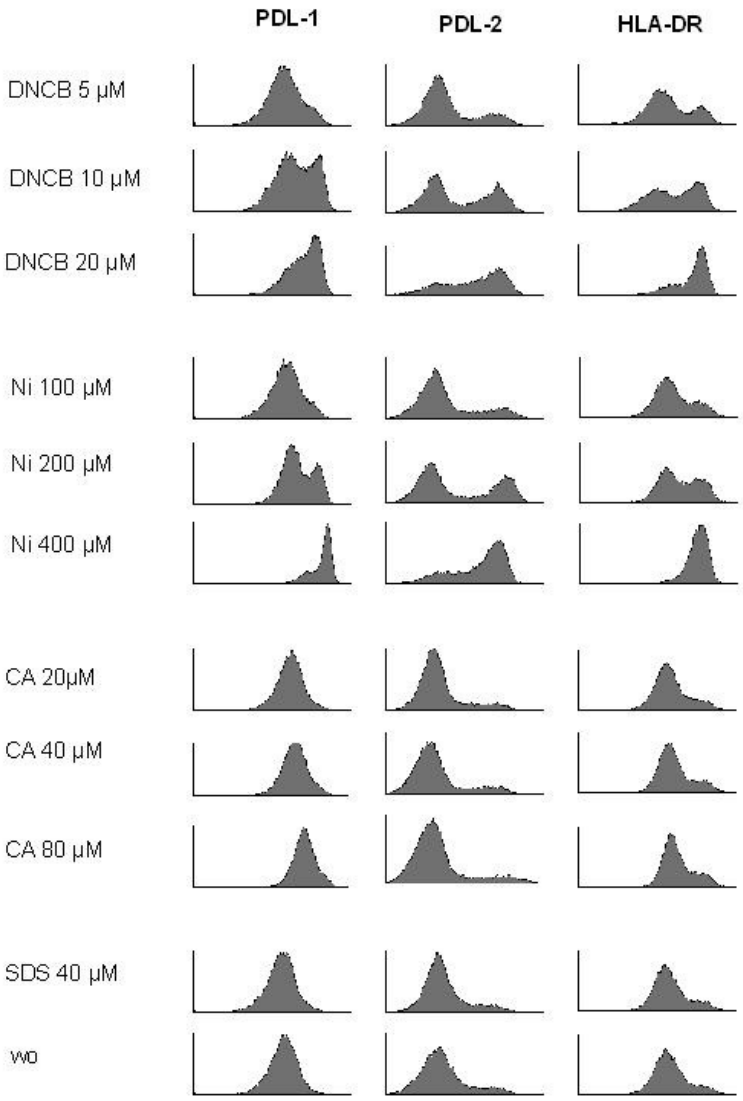
WP2D: Finding the in vitro conditions supporting the most in vivo-like EC-DC interactions: MUTZ-3 in coculture with Calu-3 in an airlifted two-layer system transwell system, Episkin epidermis and skin epidermal equivalents

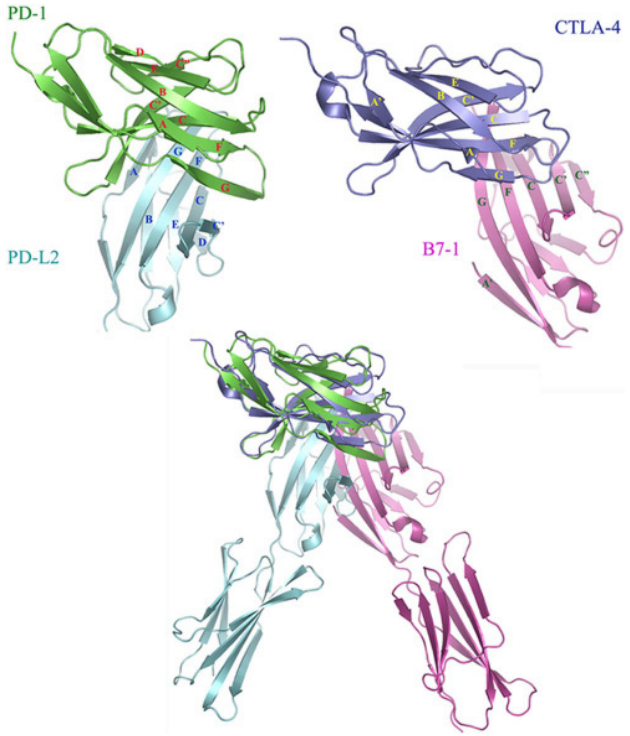
***WP3*: Establishing and implementing tools for addressing DC – T-cell interactions:** T cell based assay capable of identifing contact allergens

WP8:In vitro assay development: MUTZ-3, U937 and THP-1 and biomarkers CD86 and IL-8

FEDERAL INSTITUTE
FOR RISK ASSESSMENT

In vitro testing at BfR: New predictive biomarkers "Inhibitory molecules" on DC



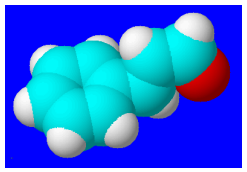


Hitzler, Peiser, unpublished

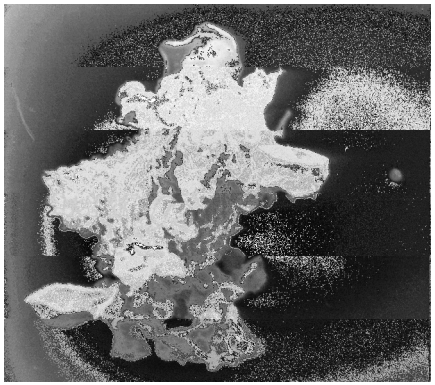
In vitro testing at BfR: BMBF-Project

„Contact Allergen Activated T-Cell (CAATC)-Assay“ using dendritic cells from skin: characterization of the sensitizing potency of chemicals via dendritic cell-induced expression of lineage specific T cell transcription factors“

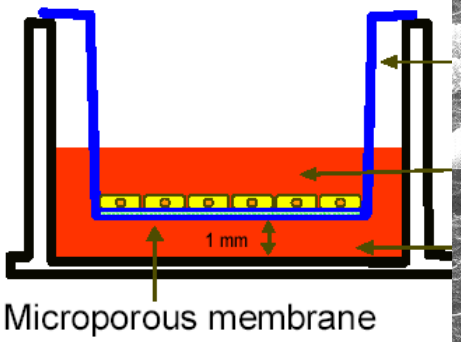
Contact allergen

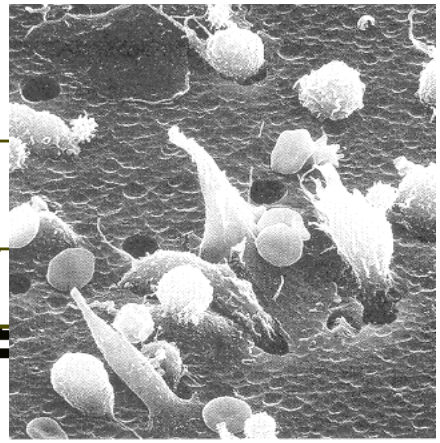


Stimulation



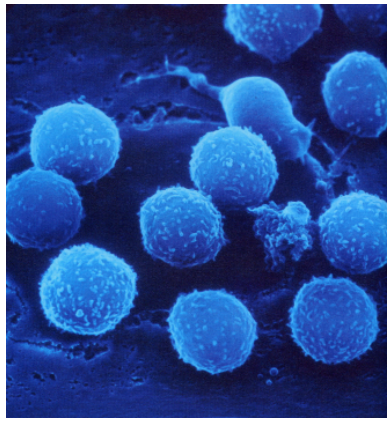
Dendritic Cell





Co-culture

T-helper cell (T_H)



CAATC Analysis

Transcr. factors

Cytokines

Endpoints:

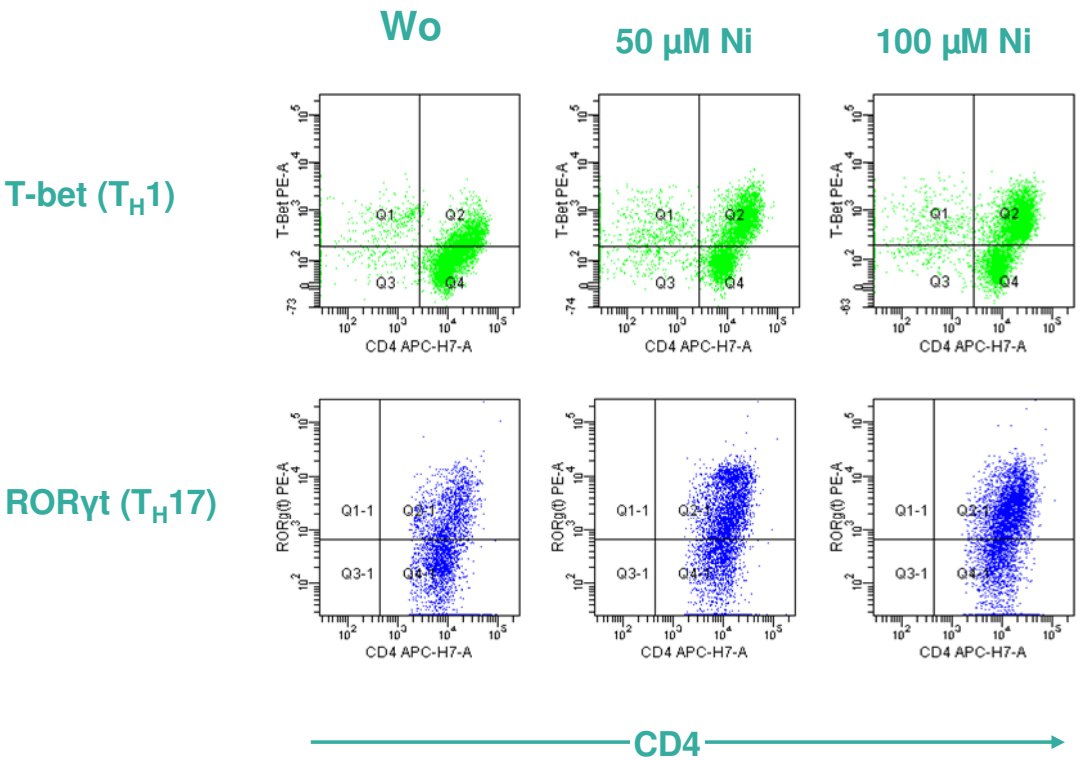
Ratio

% pos. cells

ng/ml

In vitro testing at BfR: BMBF-Project

Biomarker in T cells: Transcription factors specific for sensitization



Hitzler, Peiser, unpublished

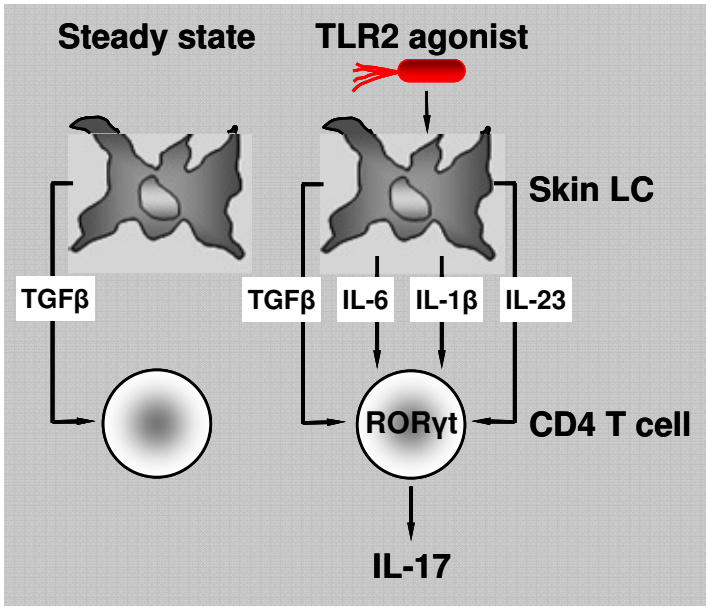
Summary CAATC-Assay

- Animal-free in vitro-test for skin sensitization
- Implementation of barrier disrupture and “danger signals“ as found in vivo
- New approach:

functional activated, migrated dendritic cells

T_H-derived cytokines and TFs quantified

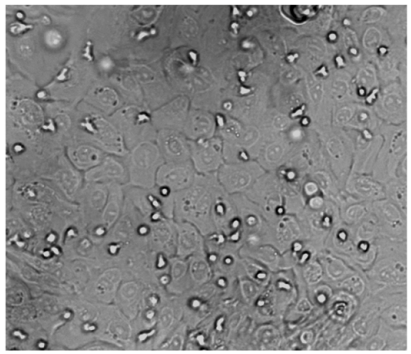
- predictive markers (T-bet, RORγt, IFNγ, IL-17, IL-22)
- Integration in a in vitro testing battery strategy



Aliahmadi, E., … Peiser, M. (2009). Eur. J. Immunol. 39, 1221-1230.

The in vitro LCSA: Loose-fit coculture-based sensitization assay

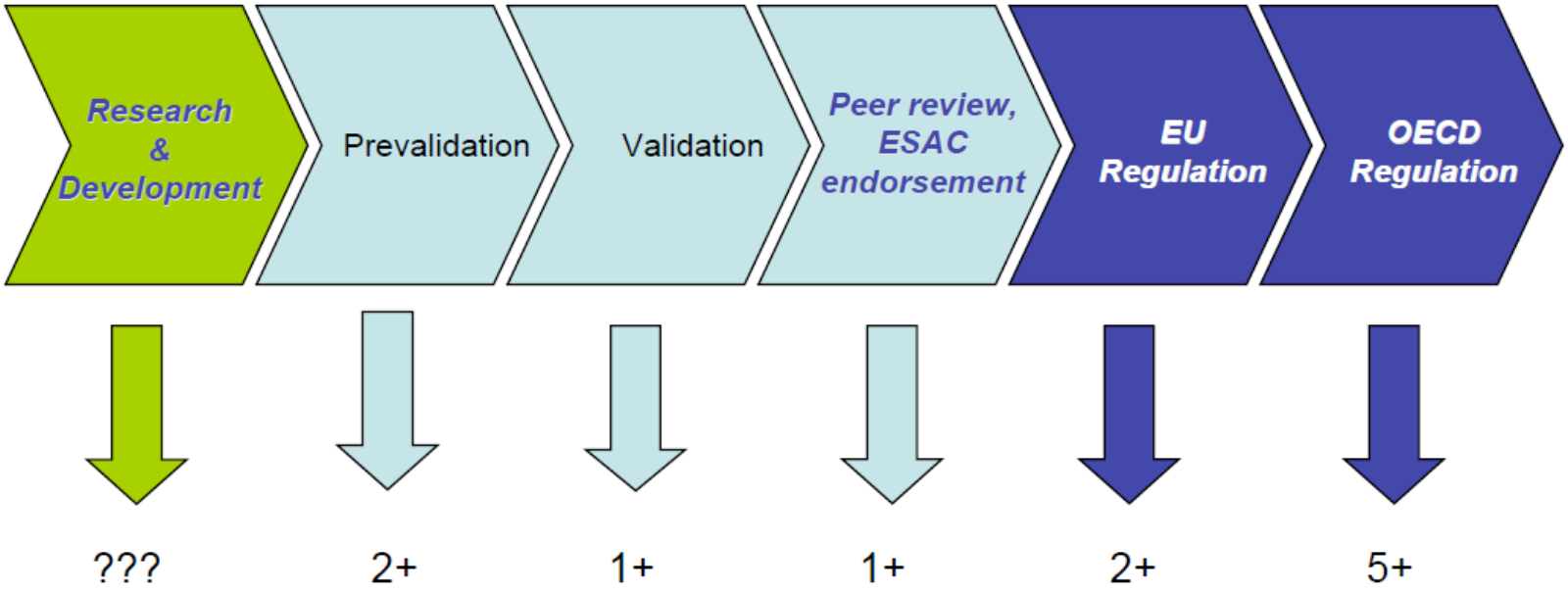
Coculture of keratinocytes and dendritic cells (Schreiner, M. et al., 2007).



Comparison of in vitro LCSA- with LLNA-results

Substance	**LCSA**		**LLNA**	
	½-max. CD86 [µM]	category	category	EC3%
Group A, accordance between LCSA and LLNA				
DNCB	3	extreme	extreme	0,04
PPD (Brand. Base)	16	strong	strong	0,10*
PTD	16	strong	strong	0,31
TNBS	40	strong	strong	0,30
Cinnamal (CA)	60	moderate	moderate	3,10
salicylic acid (SA)	negative	negative	negative	
phenol	negative	negative	negative	
Group B, no accordance between LCSA and LLNA				
Isoeugenol	140	weak	moderate	1,80
HCA (Hexylcinnamaldehyde)	3**	*(extreme)*	moderate	8,40
Sodium lauryl sulfate (SLS)	negative	negative	*(moderate)*	3,7
TMA	negative***	*(negative)*	strong	0,6
Nickel	300	positive****	*(negative)*	

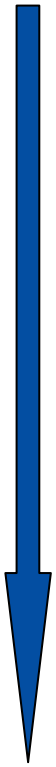
III. When will alternative in vitro tests be available?



Manou, I. et al. (2005). Safety data requirements for the purpose of the Cosmetics Directive. Altern. Lab: Anim. 33, Suppl. 1: 21-26.

Timeline of an animal test:
Murine Local Lymph node Assay on the way to a test guideline

18 years to regulatory acceptance

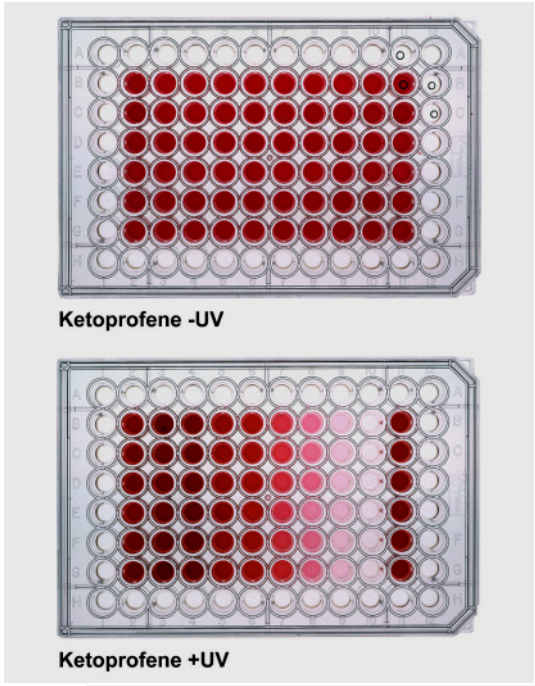


1984	LLNA conceived
1986	First paper
1987-1990	Interlaboratory development
1992	Publication of a standard protocol
1989-1997	Interlaborytory validation studies
1990-1996	Comparison with guinea pig database
1992-1996	Comparison with human data
1997-1998	Regulatory review
2002	Adopted as OECD TG 429

Timeline of an in vitro test: Example of a fast procedure
3T3 NRU PT on the way to a test guideline

10-14 years to regulatory acceptance

1985	3T3 NRU cytotoxicity assay published
1994	3T3 NRU phototoxicity test published
1992-1994	Prevalidation studies
1995-1997	Validation study
2004	Adopted as OECD Test guideline 432
2008	Council Regulation on REACH Test Methods

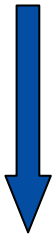


3T3 Neutral Red Uptake Phototoxicity Test

M. Liebsch, ZEBET, BfR

Timeline for potential alternative in vitro assays (hypothetical)

5 years to regulatory acceptance



2 years ago	In vitro assay conceived
Now	First paper
-2013 (REACH deadline)	Interlaboratory development
	Publication of a standard protocol
	Interlaborytory validation studies
	Comparison with guinea pig/LLNA database
	Comparison with human data
	Regulatory review
	Adopted as OECD testguideline

→ More than 10 years difference of possible adoption to deadline in REACH

What is in the pipeline?

OECD: - 2 modified versions of traditional LLNA with non-radioactive protocols
- Update TG429
- 2 updates for "*In vitro* skin Corrosion", TG430/431
- New TG *in vitro* test for skin irritation

→ No *in vitro* assay for sensitization (in 2009)

ECVAM - Replacement in vitro tests; in validation process

COLIPA: Prevalidation of Direct binding petide reactivity assay
h-CLAT
MUSST

FEDERAL INSTITUTE
FOR RISK ASSESSMENT

Thank You
For Your Attention

Matthias Peiser

Federal Institute for Risk Assessment

Thielallee 88-92 • 14195 Berlin • Germany

Phone +49-30-8412-3414 • Fax +49-30-8412-3870

Matthias.peiser@bfr.bund.de • www.bfr.bund.de