IL TASSELLO MANCANTE NELLA SENSIBILIZZAZIONE: LA COMPRENSIONE DELLA POTENZA DEGLI ALLERGENI

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IL TASSELLO MANCANTE NELLA SENSIBILIZZAZIONE

- Le due più frequenti manifestazioni di allergie indotte da sostanze chimiche sono **l'ipersensibilità da contatto** e la **sensibilizzazione delle vie aeree**, le quali possono avere un serio impatto sulla qualità della vita e rappresentano un comune problema in ambito occupazionale.

DERMATITE ALLERGICA DA CONTATTO (ACD)

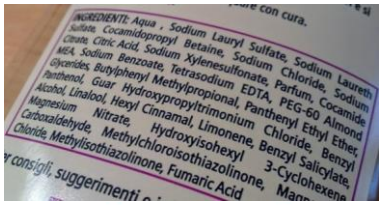
- La **dermatite allergica da contatto** (ACD) è una reazione di ipersensibilità ritardata mediata dalla risposta del sistema immunitario, in seguito ad attivazione delle cellule T.

Fattori di rischio più importanti:

Predisposizione genetica
Esposizione lavorativa
Età
Sesso

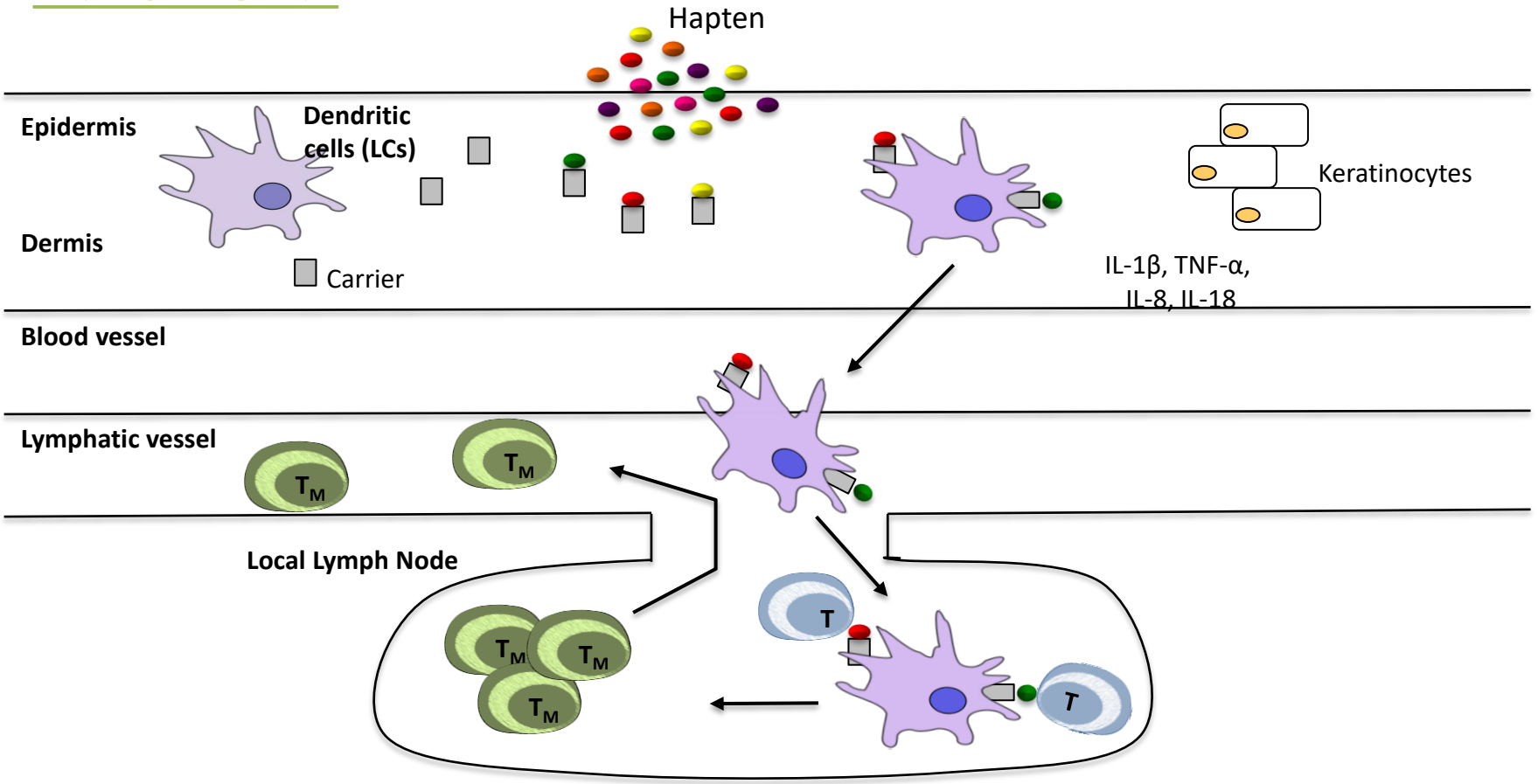
Allergeni da contatto comuni:

Farmaci (antibiotici)
Metalli (i.e. *Nickel*)
Conservanti(i.e. *Kathon CG*)
Fragranze



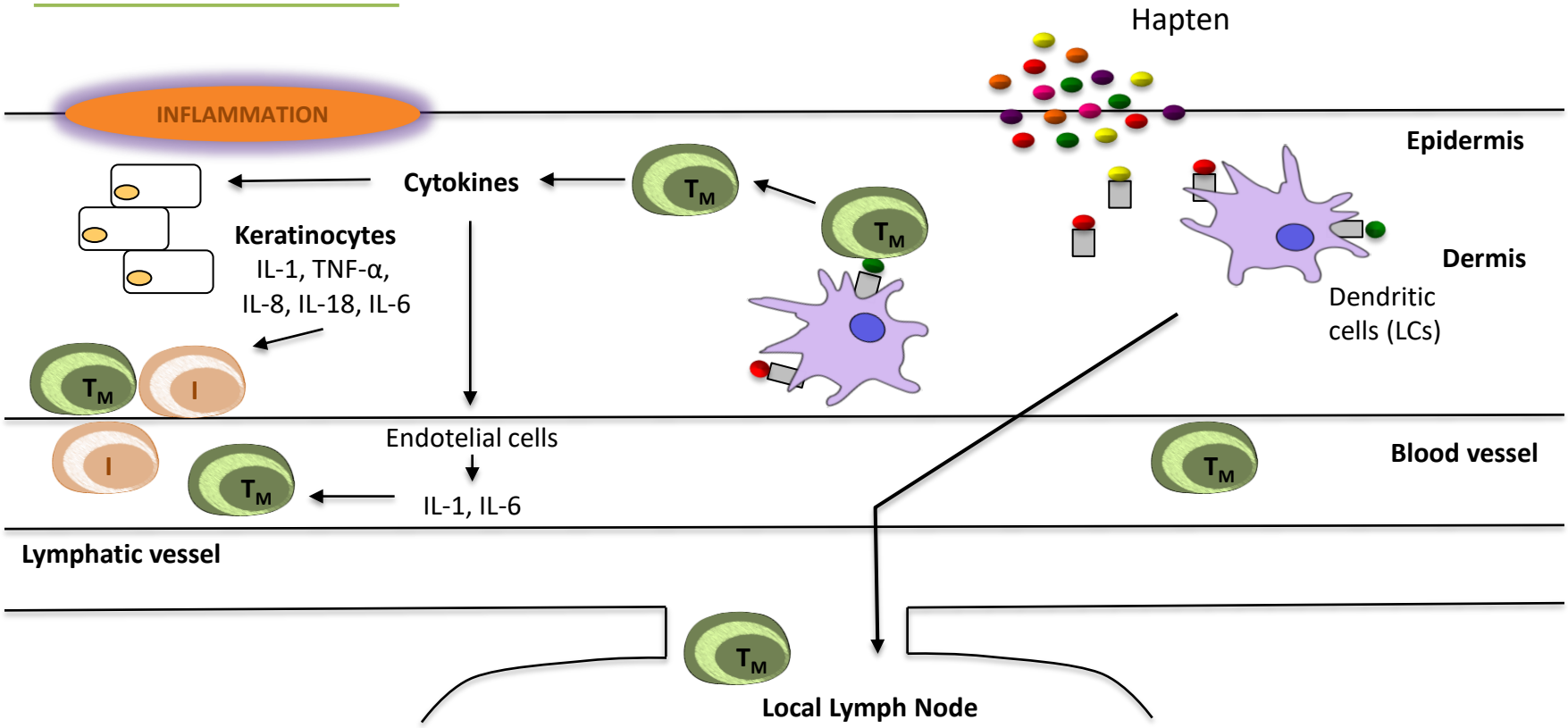
SENSIBILIZZAZIONE

INDUZIONE

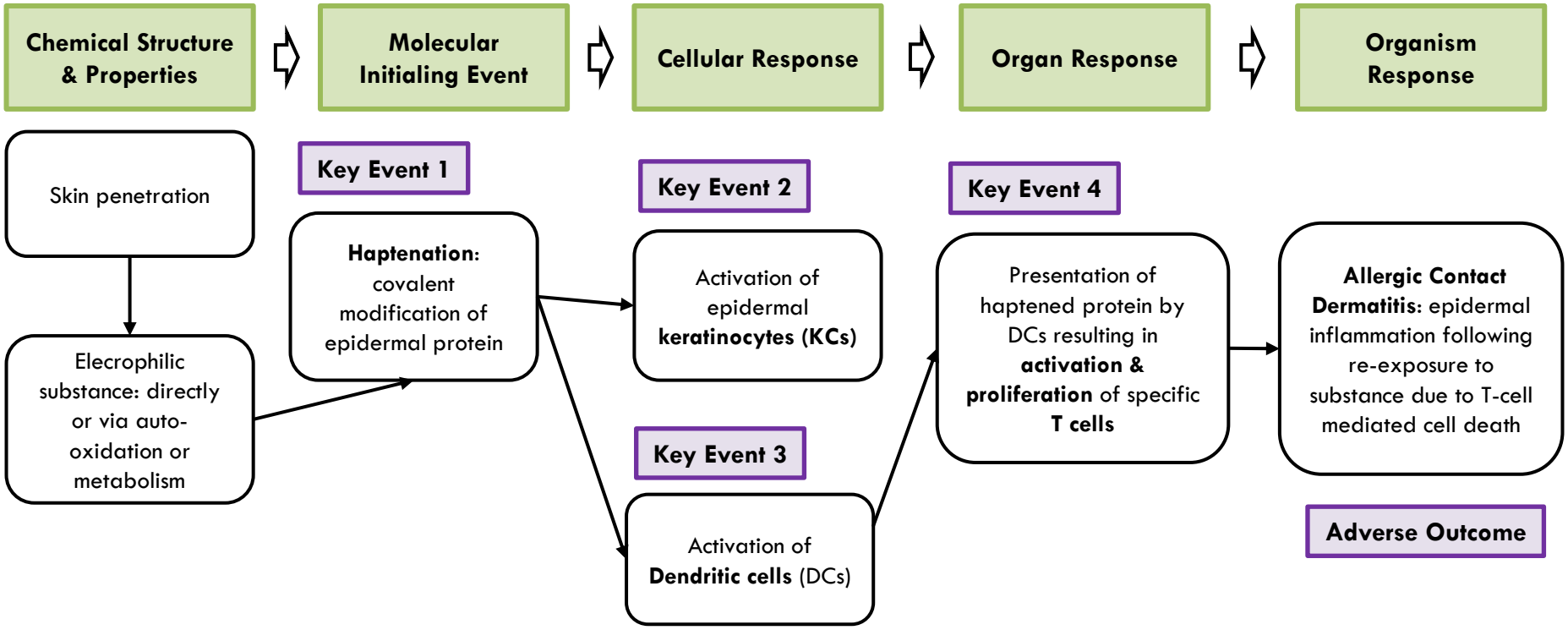


SENSIBILIZZAZIONE

ELICITAZIONE

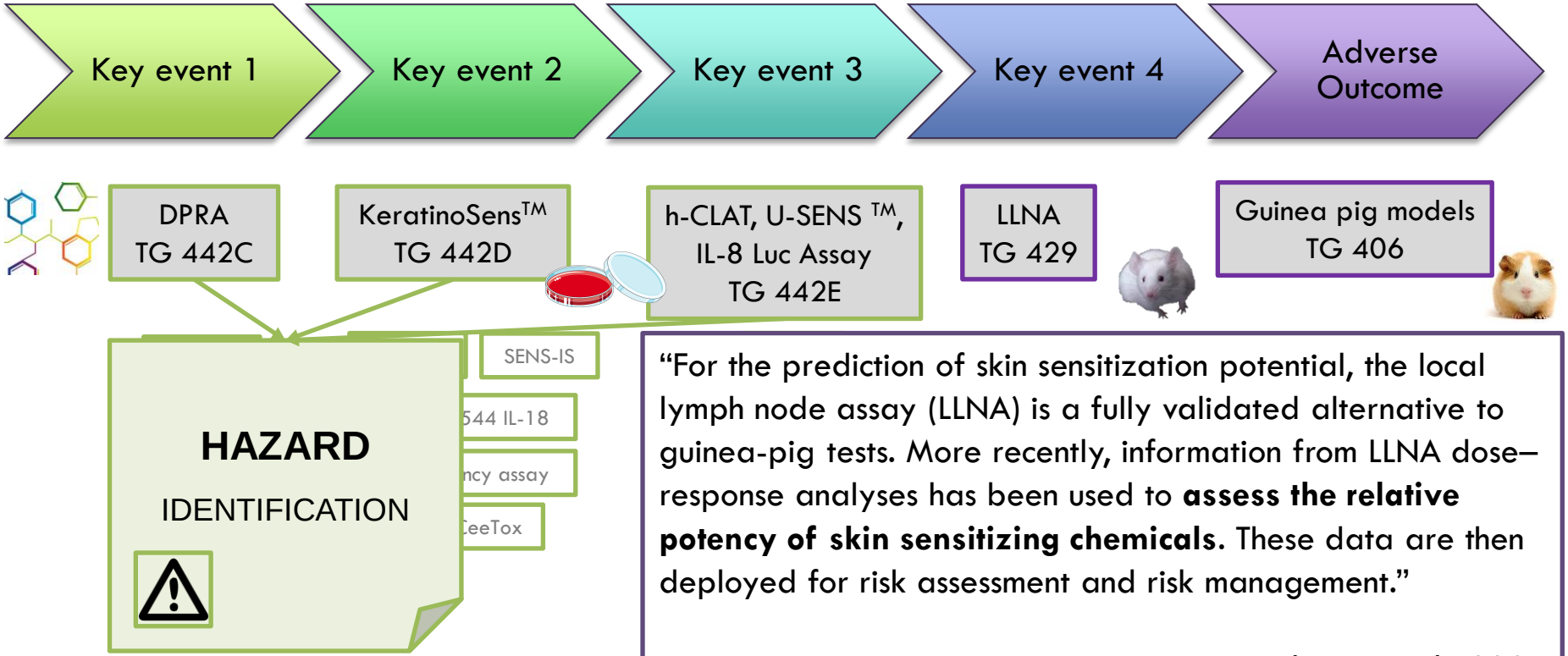


AOP e SENSIBILIZZAZIONE CUTANEA



Modified version of flowchart OECD report: The Adverse Outcome Pathway for Skin Sensitization Initiated by covalent binding to proteins Part 1: scientific evidence series on testing and assessment No.168 ENV/JM/MONO(2012)10/PART1

ADVERSE OUTCOME PATHWAY e METODI ASSOCIATI



"For the prediction of skin sensitization potential, the local lymph node assay (LLNA) is a fully validated alternative to guinea-pig tests. More recently, information from LLNA dose–response analyses has been used to **assess the relative potency of skin sensitizing chemicals.** These data are then deployed for risk assessment and risk management."

Basketter et al., 2007

IL TASSELLO MANCANTE NELLA COMPRENSIONE DELLA SENSIBILIZZAZIONE:

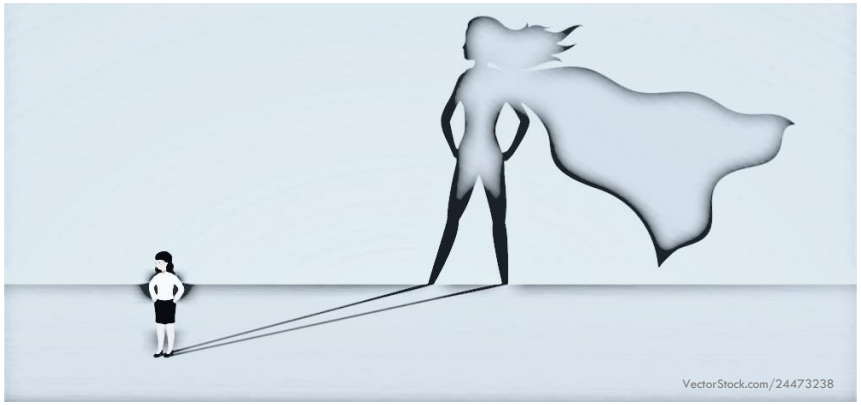
LA POTENZA DEGLI ALLERGENI

LA POTENZA DI UN ALLERGENE

- Refers to the intrinsic property of a sensitizing chemical

(ICCVAM LLNA Potency Evaluation Report, 2001)

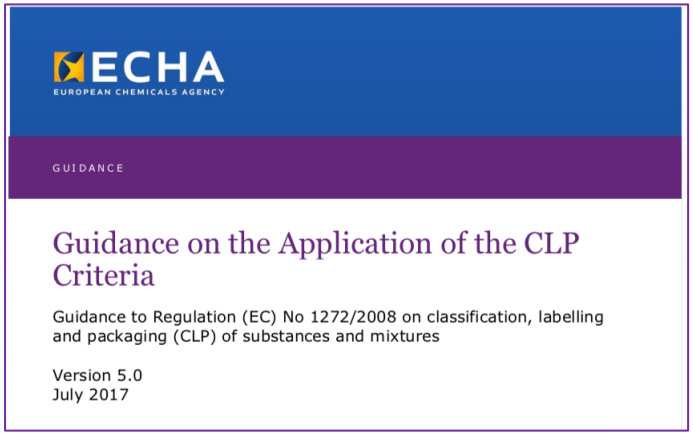
- Potency is inversely proportional to the amount of chemical required to initiate the pathway leading ultimately to an adverse event. That is, the **lower the level of exposure required to induce an effect,** the **more potent the chemical.**



- It is important to emphasize that it is **the risk for the induction of skin sensitization** (rather then the risk of eliciting a reaction in a previously sensitized subject) that is the **primary purpose of the safety evaluation process.**

Kimber et al., 2016

PERCHE' E' IMPORTANTE DEFINIRE LA POTENZA


ECHA
EUROPEAN CHEMICALS AGENCY

GUIDANCE

Guidance on the Application of the CLP Criteria

Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures

Version 5.0
July 2017

A lack of potency information [...] may result in a lower level of protection of humans.

ECHA Chapter R7.a
Endpoint specific guidance
version 6.0 July 2017

Categoria 1
- 1A
- 1B

The currently adopted test methods, when used in isolation, are not able to fulfill all regulatory **requirements on the skin sensitisation potential and potency** of chemicals comparable to that provided by the regulatory animal tests.


Arch Toxicol (2018) 92:611–617
https://doi.org/10.1007/s00204-017-2097-4

CrossMark

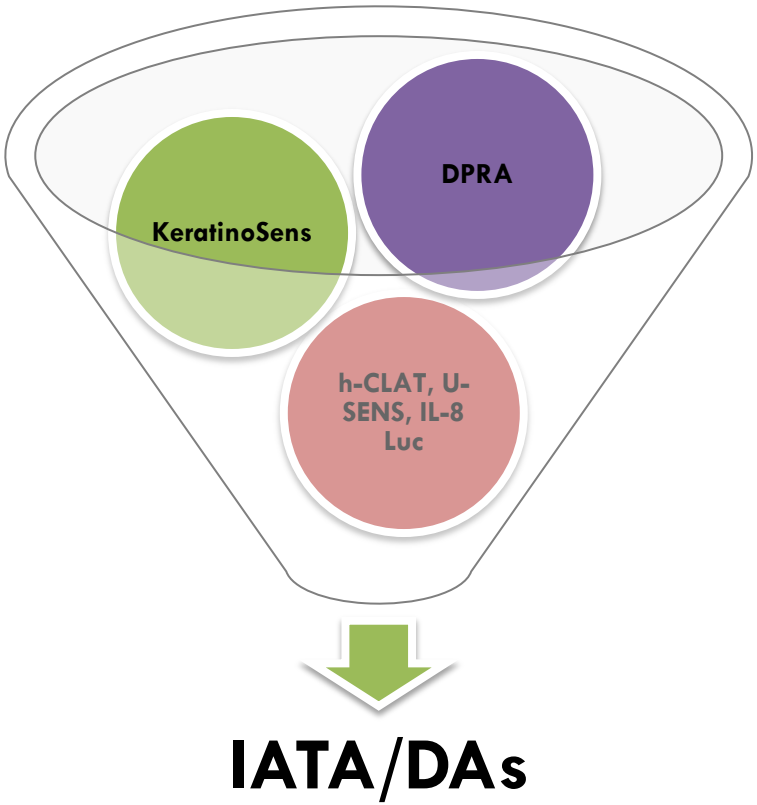
REGULATORY TOXICOLOGY

Standardisation of defined approaches for skin sensitisation testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods

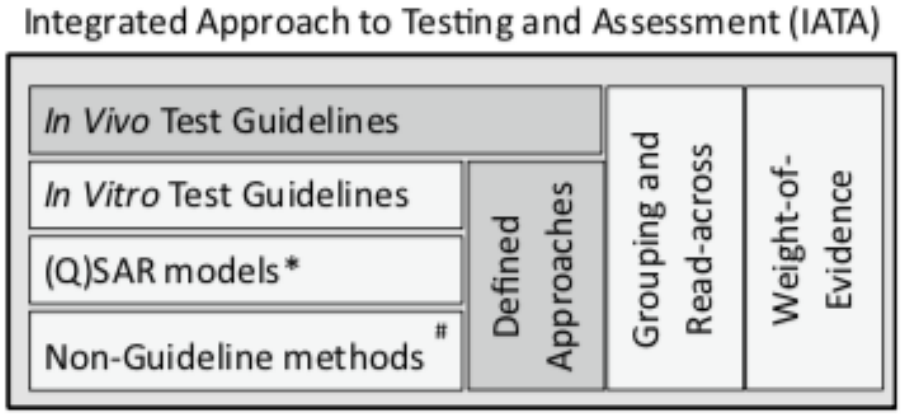
S. Casati[1] · K. Aschberger[1] · J. Barroso[1] · W. Casey[2] · I. Delgado[3] · T. S. Kim[4] · N. Kleinstreuer[2] · H. Kojima[5] · J. K. Lee[4] · A. Lowit[6] · H. K. Park[4] · M. J. Régimbald-Krnel[7] · J. Strickland[8] · M. Whelan[1] · Y. Yang[9] · Valérie Zuang[1]

Casati et al., Arch Toxicol, 2018

IATA/DAs PER DEFINIRE LA POTENZA



[…] Data generated with the DPRA, the KeratinoSens™ and the three methods addressing dendritic cell activation (h-CLAT, U-SENS™ and IL-8 Luc Assay) should be considered in IATA, in combination with other relevant complementary information if available, e.g., physical–chemical properties, information on other key events of the skin sensitisation AOP as well as non-testing methods, including read-across from chemical analogues.



Casati et al., Arch Toxicol, 2018

DAs PER DEFINIRE LA POTENZA

	Defined approaches under consideration	Prediction
1	An Adverse Outcome Pathway-based "2 out of 3" integrated testing strategy approach to skin hazard identification (BASF)	Hazard identification
2	A non-testing pipeline approach for skin sensitisation (US EPA)	Hazard identification
3	Stacking meta-model for skin sensitisation hazard identification (L'Oréal)	Hazard identification
4	Integrated decision strategy for skin sensitisation hazard (ICCVAM)	Hazard identification
5	Consensus of classification trees for skin sensitisation hazard prediction (EC- JRC)	Hazard identification
6	Sensitizer potency prediction based on Key event 1 + 2: Combination of kinetic peptide reactivity data and KeratinoSens® data (Givaudan)	Potency category
7	The artificial neural network model for predicting LLNA EC3 (Shiseido)	Potency category
8	Sequential testing strategy (STS) for sensitising potency classification based on in chemico and in vitro data (Kao Corp)	Potency category
9	Integrated testing strategy (ITS) for sensitising potency classification based on in silico, in chemico, and in vitro data (Kao Corporation)	Potency category
10	DIP for skin allergy risk assessment (SARA) (Unilever)	Potency category
11	Decision tree integrated testing strategy with an in silico model and in chemico/in vitro data using exclusion criteria (Derek Nexus)	Potency category
12	Computational approaches for prediction skin sensitisation (US/UNC)	Potency category

OECD Defined approaches for skin sensitization, Jan 2019

LE LIMITAZIONI DELLE DAs

Technical limitations

– e.g. not suitable for chemicals that are insoluble, highly cytotoxic, pre-/pro-haptens, metals, etc.

Applicability domain of the DAs should be well defined

– Defined differently for QSARs and in vitro methods

Quality assurance of in silico data

– Transparency and reproducibility to meet the standards of MAD

TG 442C DPRA	TG 442 D KeratinoSens™	TG 442E h-CLAT
Metals are outside the applicability of the DPRA since they react with proteins with mechanisms different than covalent binding. Evaluation of the reactivity of the electrophile is limited to cysteine and lysine. Test chemicals with selective reactivity towards other nucleophiles may not be detected by the assay. Test chemicals must be stable under the test conditions (e.g. DPRA uses highly alkaline conditions for lysine reactivity). Peptide depletion due to adduct formation, dimerization or oxidation of the peptide cannot be differentiated from peptide depletion. Test chemicals having the same retention time as the cysteine and/or the lysine peptides may provide inconclusive results. Due to the defined molar ratio of the test chemical and peptide, the current method cannot be used for the testing of complex mixtures of unknown composition (it is technically applicable to mixtures of known composition) or for substances of unknown or variable composition, complex reaction products or biological materials (i.e UVCB substances) due to the defined molar ratios of test chemicals and peptides.	The test method is not applicable to the testing of chemicals which are not soluble or do not form a stable dispersion. Highly cytotoxic chemicals cannot always be reliably assessed. Test chemicals that strongly interfere with the luciferase enzyme (e.g. phytoestrogens) cannot be reliably tested. Chemical stressors other than electrophilic chemicals may activate the Keap1-Nrf2-ARE pathway leading to false positive predictions. Substances with an exclusive reactivity towards lysine-residues are likely to give negative results, e.g. acyl transfer agents.	The test method is not applicable to the testing of chemicals which are not soluble or do not form a stable dispersion. Highly cytotoxic chemicals cannot always be reliably assessed. Strong fluorescent test chemicals emitting at the same wavelength as FITC or as propidium iodide (PI) may interfere with the flow-cytometry light-signal acquisition. Test chemicals with a logP of greater than 3.5 and tend to produce false negative results in the h-CLAT. Test substances present as insoluble (but stably dispersed) particles may interfere with the cell viability assessment using flow cytometry.

Draft OECD Guideline Defined Approaches for Skin Sensitisation September 2019

QUANTITATIVE RISK ASSESSMENT E POTENZA

Determine potential (hazard) to induce sensitization

Dose response information:
- Define no expected skin senstization induction level (**NESIL**)
- Apply sensitization assessment factors (**SAFs**)

Exposure:
Acceptable exposure level (**AEL**)

AEL = NESIL / SAFs

RISK CHARACTERIZATION

" […] more dialogue between clinicians with **expertise in skin sensitization and toxicologists** seeking to provide effective risk assessment to prevent human health issues. […] remaining **uncertainties regarding the induction and regulation of skin sensitization** in humans, and the opportunities and challenges associated with the refinement and improvement of risk assessment methodologies."

Gilmour et al., 2018

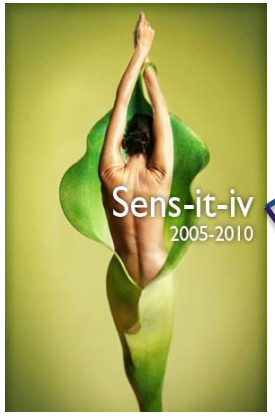
Api 2007; Kimber et al., 2018

MOLECULAR EVENTS AND POTENCY

The identification of mechanisms influencing the vigor of T cell responses, that can explain the strength of ACD reactions to weak, moderate, strong, and extreme sensitizers is a challenge still to be solved and this will require **a better understanding of the molecular events that trigger cell activation following exposure to contact allergens.**

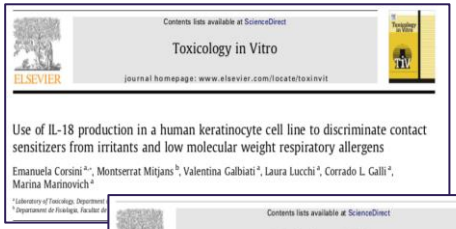
- RhE IL-18 Assay (Reconstituted human epidermis)
- Dendritic Cell Activation Assay

RhE IL-18 Assay (Reconstituted human epidermis)



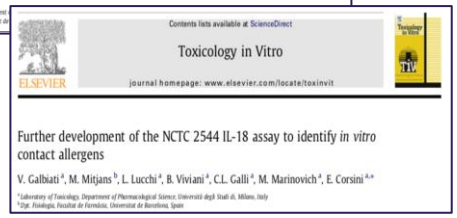
NCTC2544 **IL-18 assay**

Identification and discrimination between contact allergens and irritants and respiratory allergens


Toxicology in Vitro

Use of IL-18 production in a human keratinocyte cell line to discriminate contact sensitizers from irritants and low molecular weight respiratory allergens

Emanuela Corsini, Montserrat Mitjans, Valentina Galbiati, Laura Lucchi, Corrado L. Galli, Marina Marinovich


Toxicology in Vitro

Further development of the NCTC 2544 IL-18 assay to identify *in vitro* contact allergens

V. Galbiati, M. Mitjans, L. Lucchi, B. Viviani, C.L. Galli, M. Marinovich, E. Corsini

EE **potency assay**

Reconstructed epidermal models (EE) using primary KCs - Prof. Sue Gibbs, VUMC (The Netherlands)


Toxicology in Vitro

A potential *in vitro* epidermal equivalent assay to determine sensitizer potency

Guilherme G. dos Santos, Sander W. Spiekstra, Shakun C. Sampat-Sardjoepersad, Judith Reinders, Rik J. Scheper, Susan Gibbs


Toxicology and Applied Pharmacology

An epidermal equivalent assay for identification and ranking potency of contact sensitizers

Susan Gibbs, Emanuela Corsini, Sander W. Spiekstra, Valentina Galbiati, Horst W. Fuchs, George DeGeorge, Matthew Troese, Patrick Hayden, Wei Deng, Erwin Roggen

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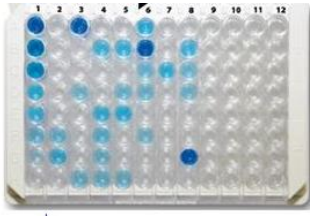
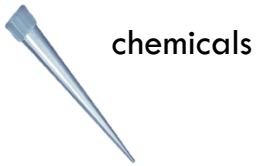
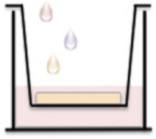


RhE IL-18 Assay



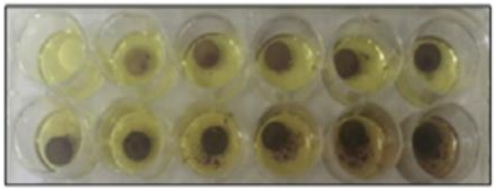
chemicals



Reconstituted human Epidermis
(**RhE**) - EpiDerm™ - MatTek (Bratislava)

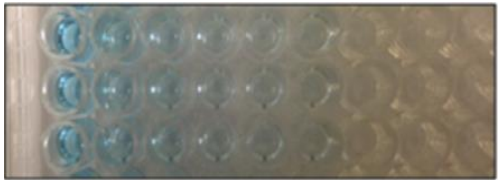
24h

37°C, 5% CO_2, 95% r.h.



MTT assay

EC50 value

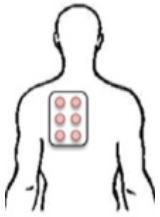


IL-18 ELISA

IL-18 SI2

LLNA EC3 value

ALLERGEN POTENCY



Human NOEL

Galbiati et al., Toxicol Lett, 2017

RhE IL-18 Assay - POTENCY

To estimate the *in vivo* induction sensitization level, curves were created using reference skin sensitizers of different potency



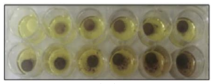



Table 2
Reference contact sensitizers used to create the regression curves for the estimation of the *in vivo* EC3 and human NOEL.

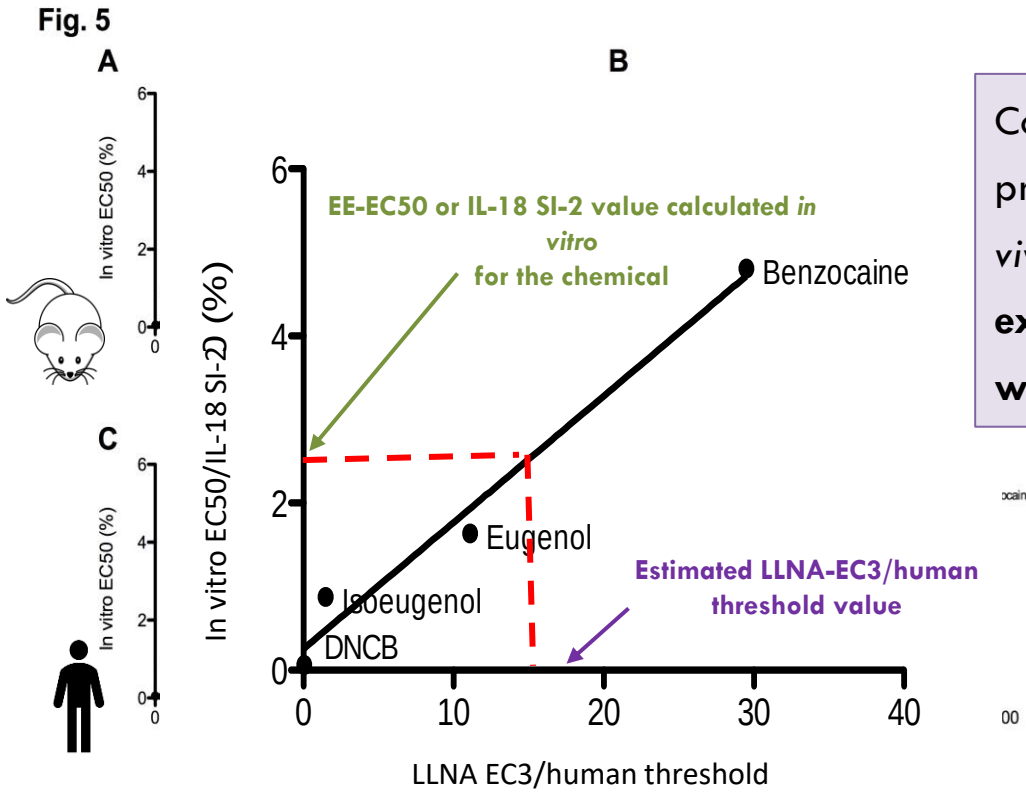
Chemical	CAS #	LLNA EC3 (%)	Human NOEL (μg/cm^2)	*In vitro* EC50 (% and μg/cm^2)		*In vitro* IL-18 SI-2 (% and μg/cm^2)	
DNCB	97-00-7	0.08	8.8	0.05	25	0.03	15
Isoeugenol	97-54-1	1.2	250	0.88	440	0.21	105
Cinnamal	104-55-2	3	591	1.1	550	0.5	250
Benzocaine	94-09-7	22	2000	4.81	2405	3.12	1560

In vivo values were obtained from ICCVAM database (NIH Publication No. 11-7709), Research Institute for Fragrance Materials (RIFM) database, Griem et al. (2003), Api et al. (2008), Natsch et al. (2013), Basketter et al. (2014), Urbisch et al. (2015).
In vitro EC50 and IL-18 SI2 values are the arithmetic means obtained in two independent experiments, and were calculated from RhE exposed to the selected compounds as described in Section 2.

Linear regression curves were created by plotting *in vivo* LLNA EC3 or human NOEL values against *in vitro* EC50 or IL-18 SI2 arithmetic mean values

Galbiati et al., Toxicol Lett, 2017

RhE IL-18 Assay - POTENCY



Comparing the *in vitro* predicted values with *in vivo* available data, an **excellent correspondence was observed.**

A $R^2 > 0.90$ with a $p < 0.05$ was obtained for all combinations

Linear regression curves we ... an NOEL values against *in vitro* EC50 or IL-18 SI2 arithmetic mean values

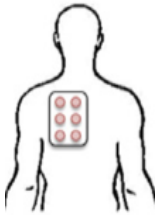
Galbiati et al., Toxicol Lett, 2017

RhE IL-18 Assay - POTENCY

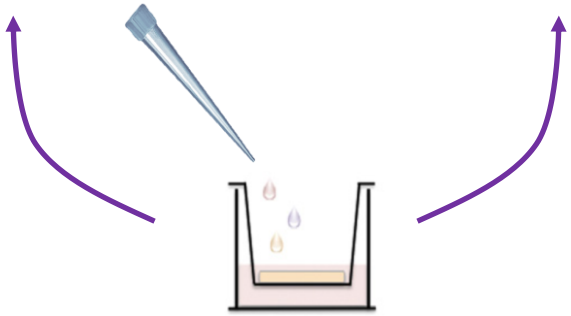
- The predicted LLNA EC3 (%) and human NOEL (µg/cm^2) values for the tested chemicals were then calculated based on the arithmetic means of in vitro EC50 or IL-18 SI2 values (n=2)

The predicted LLNA EC3 values were very close to the actual values, and **each compound remained within the same LLNA class**.

LLNA EC3 value



Human NOEL

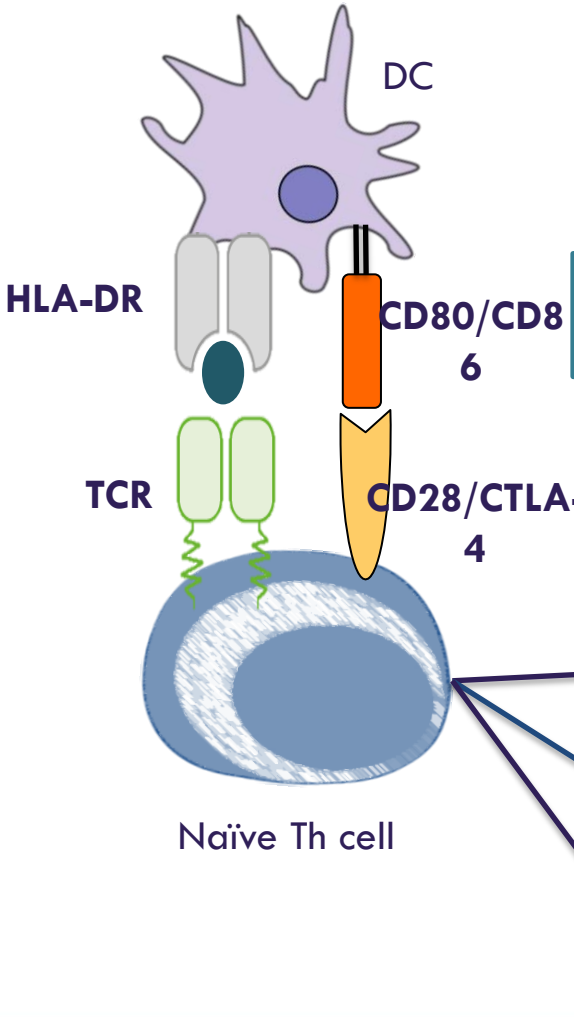


Galbiati et al., Toxicol Lett, 2017

DENDRITIC CELL ACTIVATION ASSAY

Signal 1

Stimulation by up-regulating HLA-DR



Signal 2

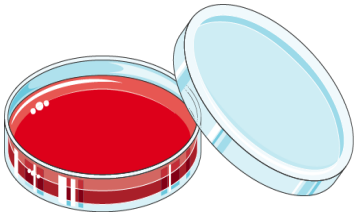
Co-stimulation by up-regulating CD80 and CD86

Signal 3

Polarization by inducing the release of cytokines

SIGNALS REQUIRED FOR T CELL ACTIVATION

DENDRITIC CELL ACTIVATION ASSAY

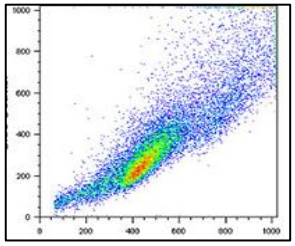


THP-1

+ Contact allergens

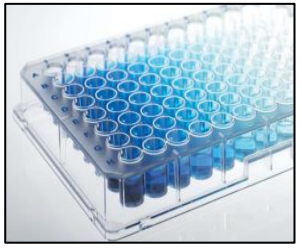
24, 48, 72 h

CELL SURFACE MARKERS EXPRESSION



CD80
CD86
HLA-DR

CYTOKINES RELEASE

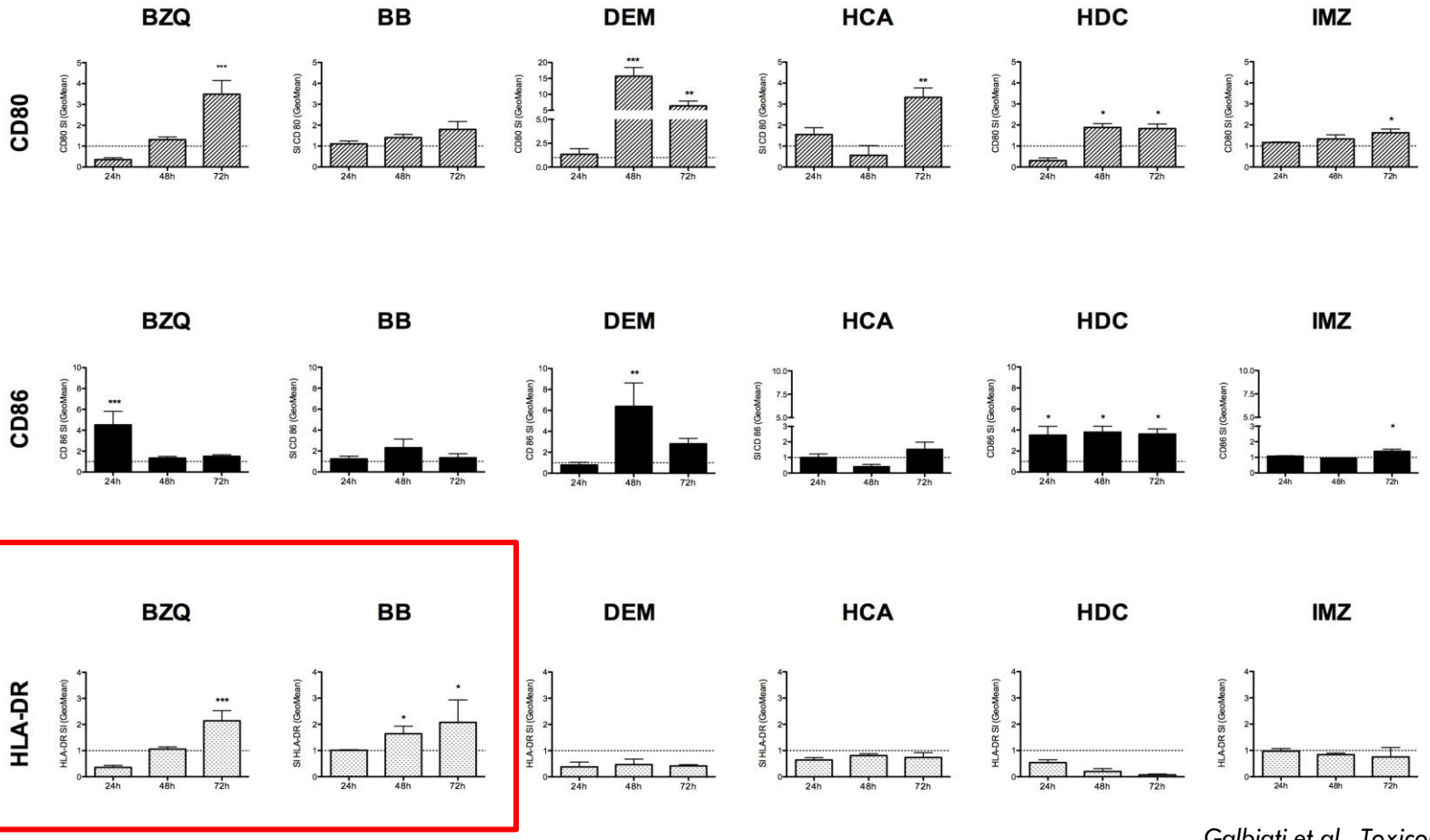


IL-10
IL-8
IL-12p40
IL-18

Galbiati et al., Toxicol Lett, 2020

DENDRITIC CELL ACTIVATION ASSAY

Fig.2



Galbiati et al., Toxicol Lett, 2020

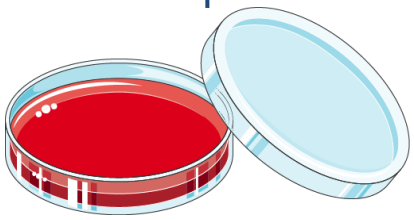
DENDRITIC CELL ACTIVATION ASSAY

DCs DIFFERENTIATION

iDCs

rh IL-4
rh-GM-CSF

5 days



THP-1

Berges et al., 2005

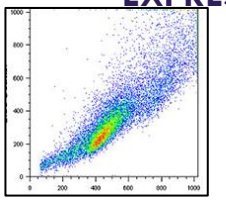
DCs MATURATION

Factors

24, 48, 72 h

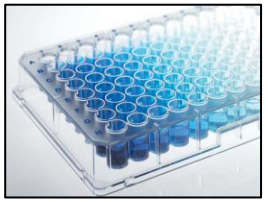
mDCs

CELL SURFACE MARKERS EXPRESSION



CD80
CD86
HLA-DR

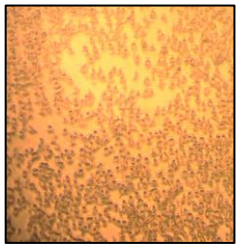
CYTOKINES RELEASE



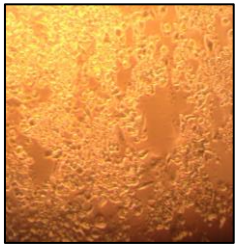
IL-10, IL-8
IL-12p40, IL-18, IL-6

THP-1

iDCs



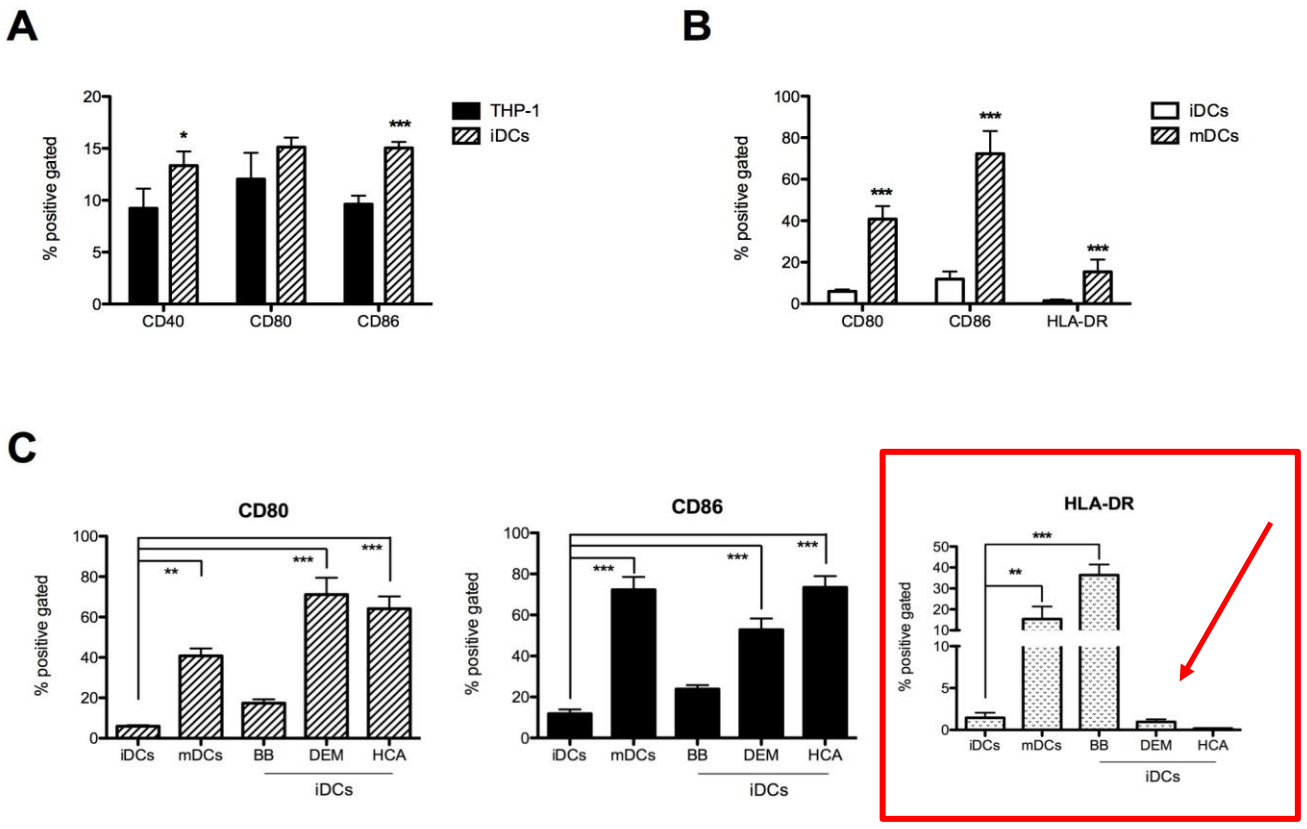
mDCs



Galbiati et al., Toxicol Lett, 2020

DENDRITIC CELL ACTIVATION ASSAY

Fig. 6

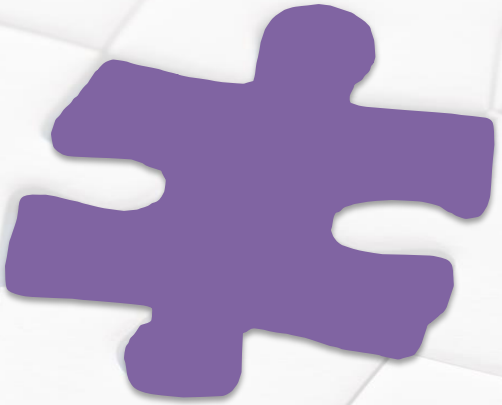


Galbiati et al., Toxicol Lett, 2020

CONCLUSIONI

IL TASSELLO MANCANTE NELLA SENSIBILIZZAZIONE

- ✓ In *vivo* data
- ✓ In *vitro* data
- ✓ In *chemico* data
- ✓ In *silico* data
- ✓ QRA
- ✓ IATA
- ✓ DAs



LA COMPRENSIONE DELLA POTENZA DEGLI ALLERGENI

RINGRAZIAMENTI

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GRAZIE PER L'ATTENZIONE!