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PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.

SKIN SENSITISATION

1 February 2018

Aims of webinar series

- Update 2014-2015 webinar series
- Live and recorded webinars
- Reflects significant progress in use and acceptance of non-animal methods
- Describe methods and testing strategies that can be used to meet REACH data requirements

Webinars in this series

Perspectives on the Development, Evaluation, and Application of *in Silico* Approaches for Predicting Toxicity Recorded	Dr. Grace Patlewicz, US EPA Prof. Mark Cronin, Liverpool John Moores University
3R Approach to Acute Oral Toxicity Recorded	Dr. Kimmo Louekari, ECHA
Skin Irritation and Corrosion 25 January 2018, 4–5 pm GMT	Dr. Gertrude-Emilia Costin, Institute for In Vitro Sciences Dr. Costanza Rovida, TEAM Mastery and CAAT-Europe
Skin Sensitisation 1 February 2018, 4–5 pm GMT	Dr. Susanne Kolle, BASF SE Dr. Silvia Casati, EURL ECVAM
Serious Eye Damage and Eye Irritation 15 February 2018, 4–5 pm GMT	Dr. Kim Norman, Burt's Bees Dr. Els Adriaens, Ghent University

Speakers

- **Dr Susanne Kolle** – Susanne is a trained biotechnologist (BSc (Hons) and MSc) and obtained her PhD in biotechnology from the University of Heidelberg, Germany. Since 2009, she has headed BASF SE's Laboratory for Tissue Toxicology, primarily conducting research into alternative methods for local tolerance testing, including eye and skin irritation/corrosion and skin sensitisation. Her previous responsibilities at BASF include managing the Laboratory for the Development of Alternative Methods (2007–2010). She is also a member of expert groups in the field of local tolerance.

- **Dr Silvia Casati** – Silvia obtained a PhD in biomedical sciences from the University of Nottingham, UK. She is a senior scientific officer at the European Commission's Joint Research Centre in Ispra, Italy, which hosts the European Union Reference Laboratory for Alternatives to Animal Testing. Since 2003, she has been coordinating its activities related to the evaluation of non-animal test methods for skin sensitisation and in support of their regulatory acceptance.

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Directorate General Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Chemicals Safety and Alternative Methods Unit (F.3)
European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Chair – Emma Chynoweth – Chief Customer Officer – Chemical Watch
Christopher Faßbender – Advisor – PETA International Science Consortium

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Skin Sensitisation

- Allergic contact dermatitis is the clinical manifestation of a skin sensitisation
- Hypersensitive reaction after repeated contact to an allergen
- 15 - 20% of the population sensitised
- Most common allergic contact dermatitis:

  Nickel contact dermatitis

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Information requirements under REACH 2006

8.3. Skin sensitisation

The assessment of this endpoint shall comprise the following consecutive steps:

(1) an assessment of the available human, animal and alternative data,

(2) *In vivo* testing.

8.3. Step 2 does not need to be conducted if:

- the available information indicates that the substance should be classified for skin sensitisation or corrosivity; or
- the substance is a strong acid (pH < 2,0) or base (pH > 11,5); or
- the substance is flammable in air at room temperature.

The Murine Local Lymph Node Assay (LLNA) is the first-choice method for *in vivo* testing. Only in exceptional circumstances should another test be used. Justification for the use of another test shall be provided.

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Information requirements under REACH 2017: Update of Point 8.3 of Annex VII

8.3.1. Skin sensitisation, *in vitro/in chemico* Information from *in vitro/in chemico* test method(s) recognised according to Article 13(3), addressing each of the following key events of skin sensitisation: (a) molecular interaction with skin proteins; (b) inflammatory response in keratinocytes; (c) activation of dendritic cells.	The(se) test(s) do not need to be conducted if: — an *in vivo* study according to point 8.3.2 is available, or — the available *in vitro/in chemico* test methods are not applicable for the substance or are not adequate for classification and risk assessment according to point 8.3. If information from test method(s) addressing one or two of the key events in column 1 already allows classification and risk assessment according to point 8.3, studies addressing the other key event(s) need not be conducted.
8.3.2. Skin sensitisation, *in vivo*	An *in vivo* study shall be conducted only if *in vitro/in chemico* test methods described under point 8.3.1 are not applicable, or the results obtained from those studies are not adequate for classification and risk assessment according to point 8.3.

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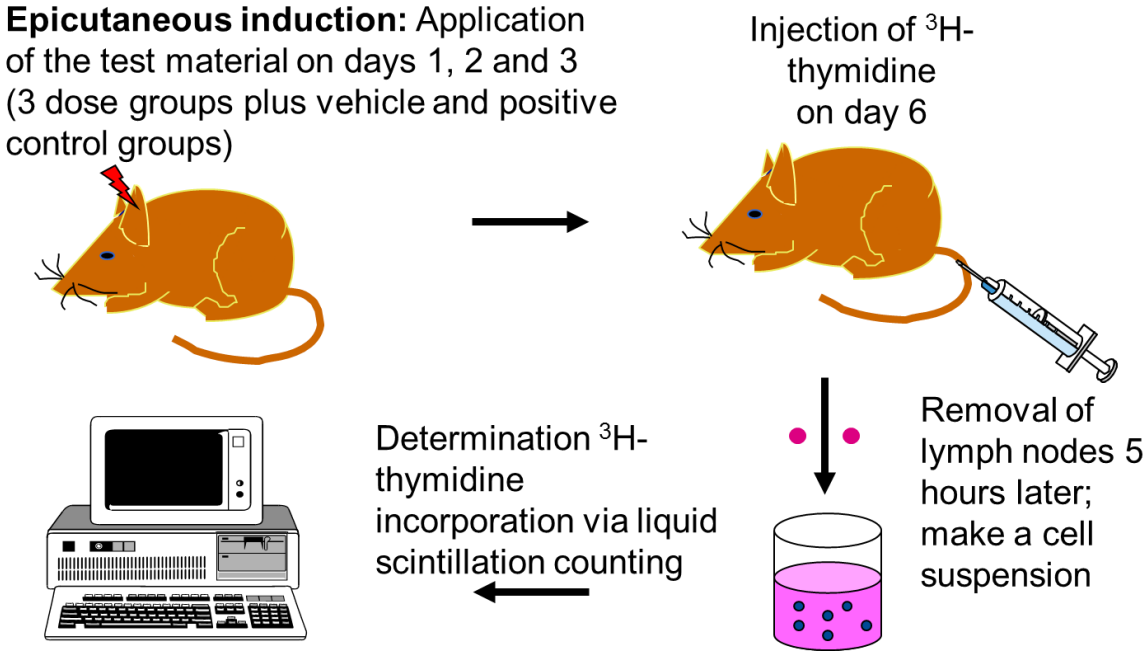
OECD TG 429: Local Lymph Node Assay

- First ever validated animal test
- Regulatorily accepted for the assessment of hazard and potency



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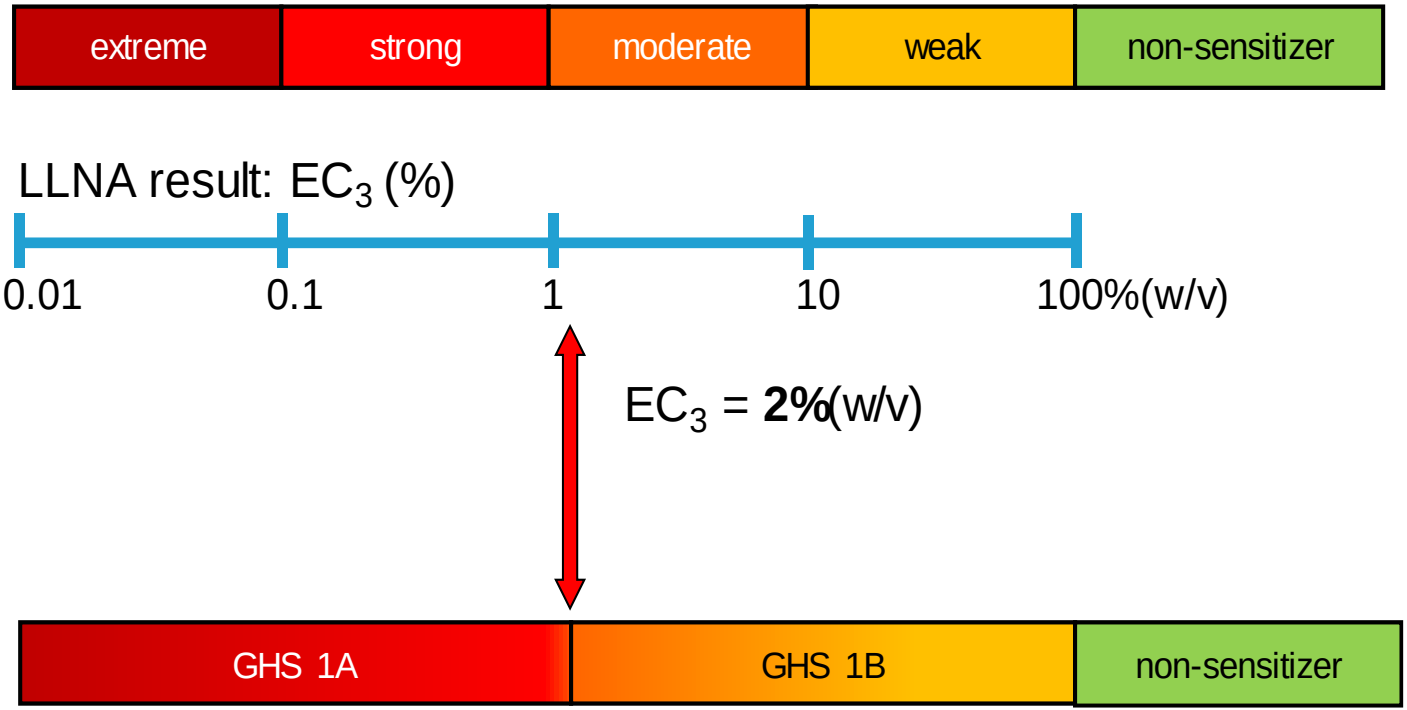
Potency classes assessed by LLNA



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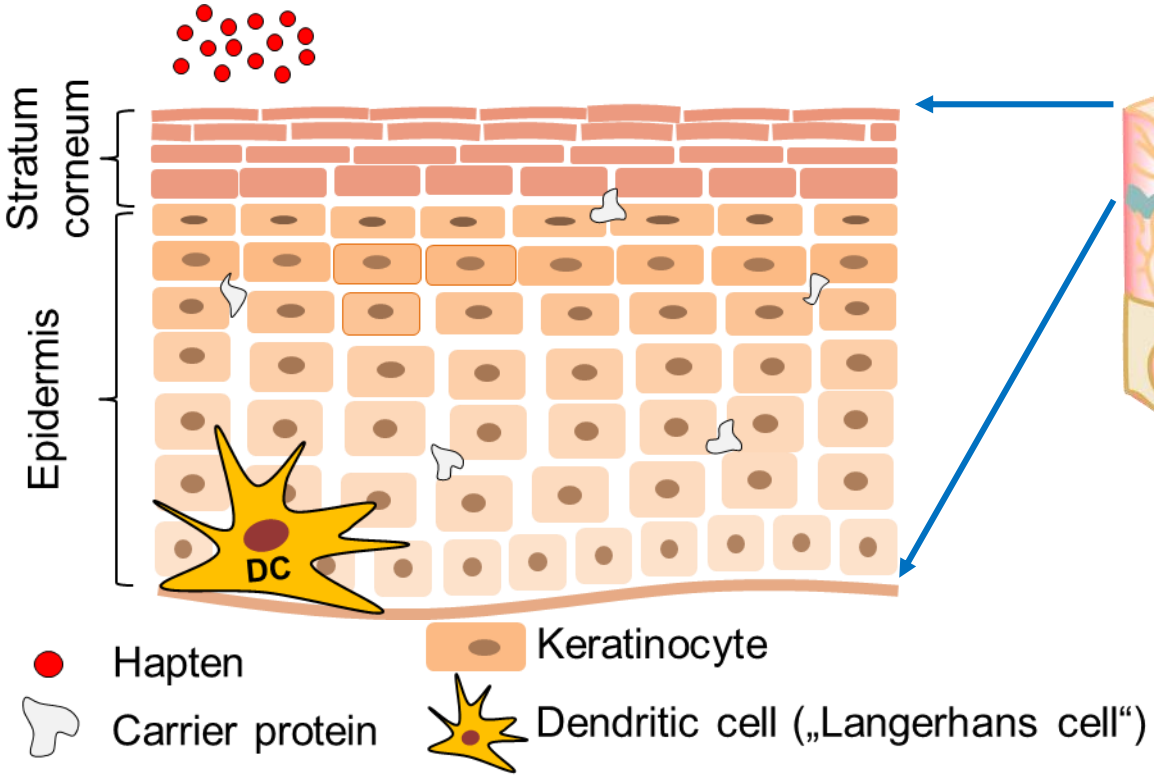
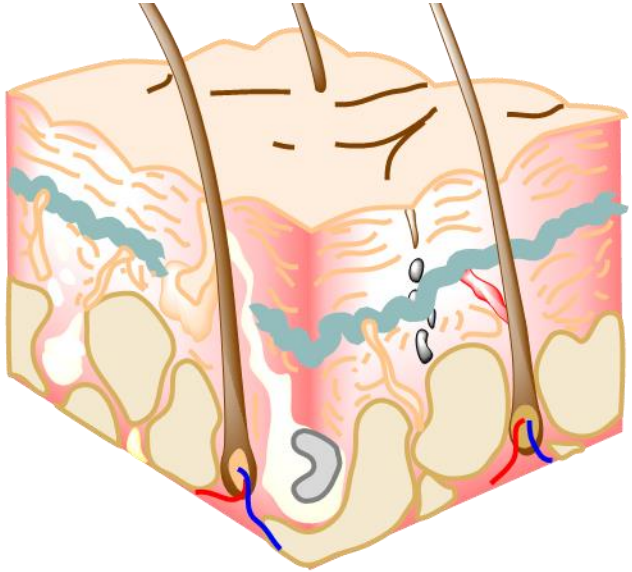
The Skin Sensitisation Mechanism





Courtesy of D. Urbisch

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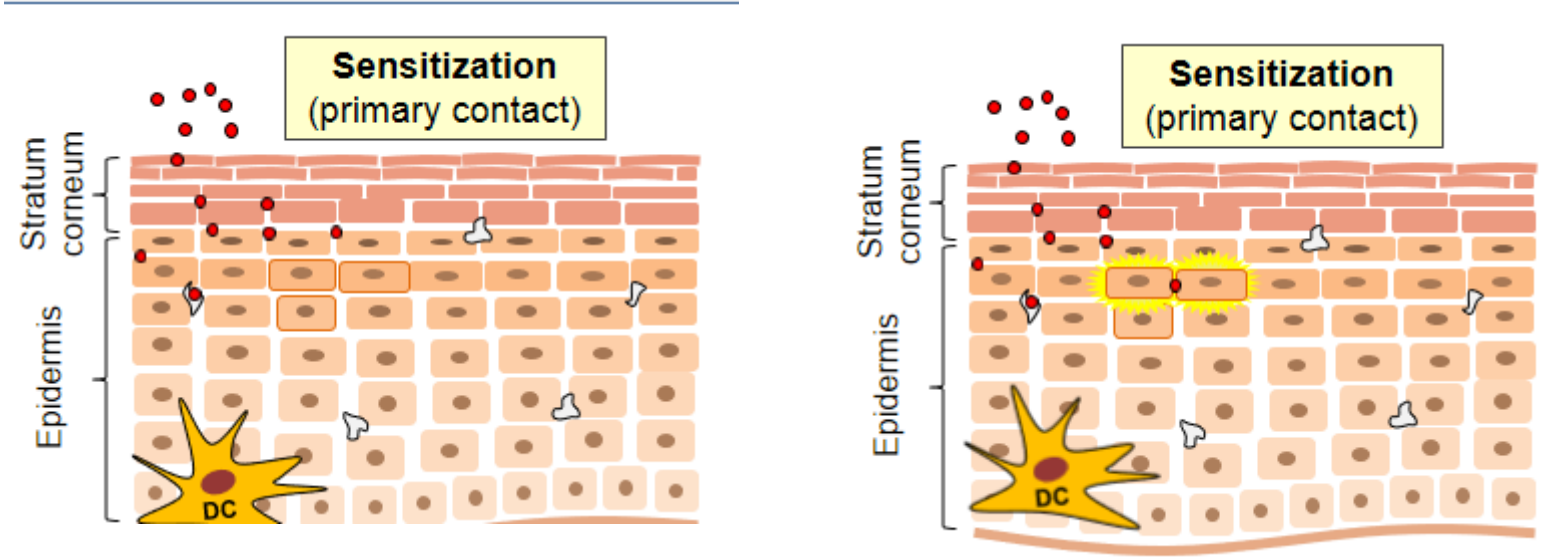
The Skin Sensitisation Mechanism: KE1 (MIE) + KE2



Courtesy of D. Urbisch

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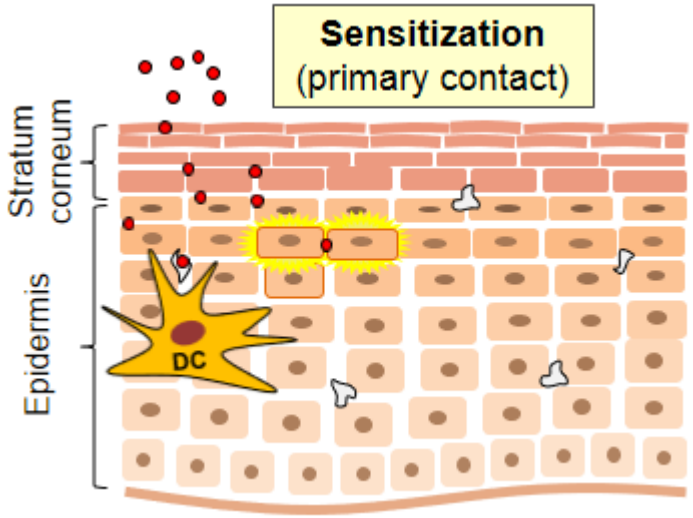
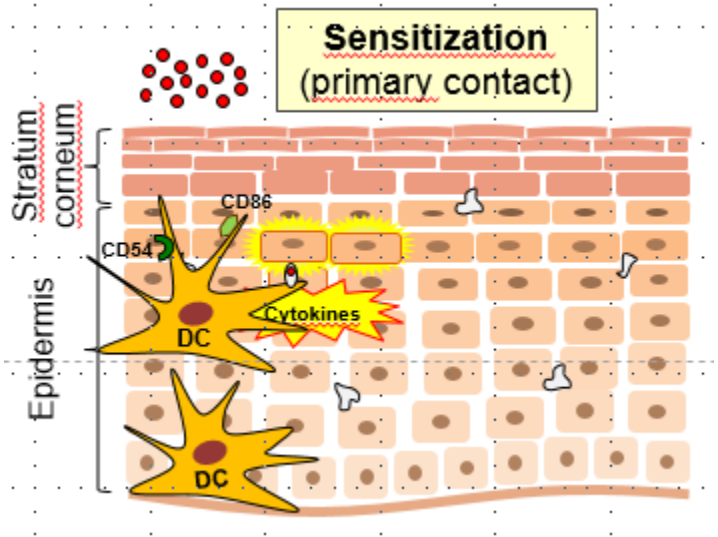
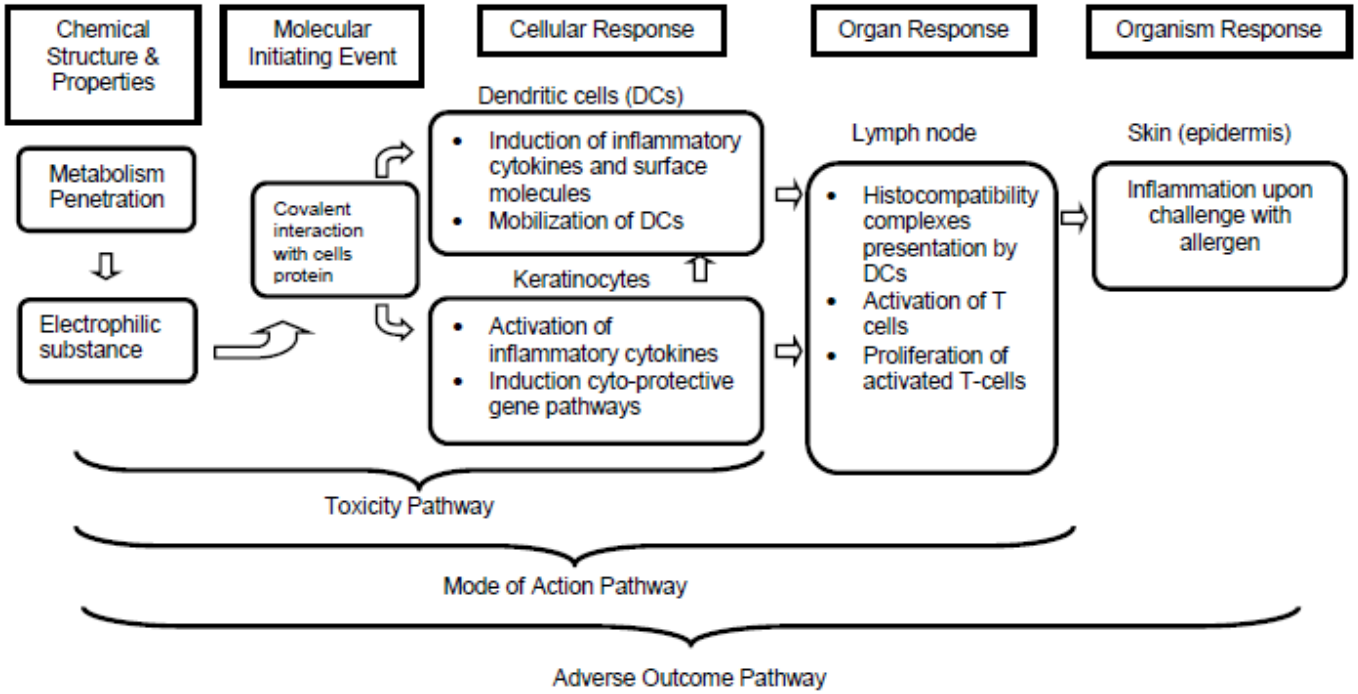
The Skin Sensitisation Mechanism: KE3





Courtesy of D. Urbisch

The **A**dverse **O**utcome **P**athway for Skin Sensitisation



The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins; Part 1: Scientific Evidence Series on Testing and Assessment No.168 ENV/JM/MONO(2012)10/PART1

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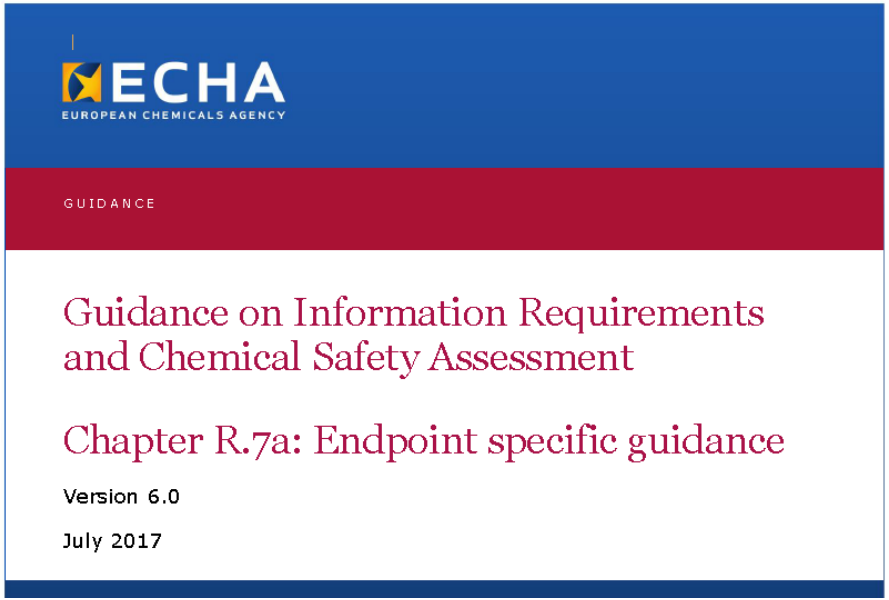
REACH Guidance on IR&CSA



- Provides guidance on how to fulfil REACH information requirements using different types of information, existing or newly generated with testing and non-testing methods
- Includes a general Integrated Testing Strategy

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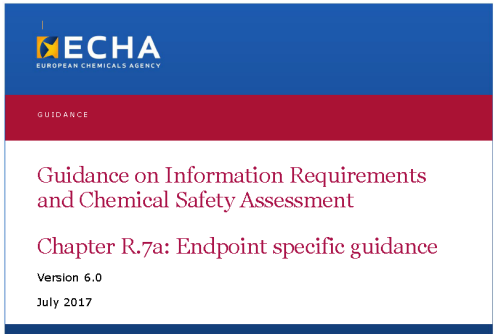
REACH Guidance on IR&CSA



The *in vitro* tests for which OECD TG are available can – and must – be used for the assessment of the skin sensitisation potential!

The animal test is, however, still needed when:

- *in vitro* are not applicable (lipophilic or highly cytotoxic substances, mixtures, ...)
- *in vitro* results are ambiguous (discordant single test results, pro-haptens, ...)

More than 50% of all substances?

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OECD Adopted Test Guidelines

Test No. 442C: ***In Chemico*** **Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA)**

http://dx.doi.org/10.1787/9789264229709-en

Test No. 442D: ***In Vitro*** **Skin Sensitisation assays addressing the AOP Key Event on keratinocytes activation**

http://dx.doi.org/10.1787/9789264229822-en

Test No. 442E: ***In Vitro*** **Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation**

http://dx.doi.org/10.1787/9789264264359-en

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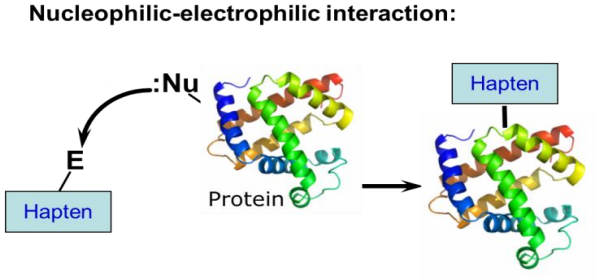
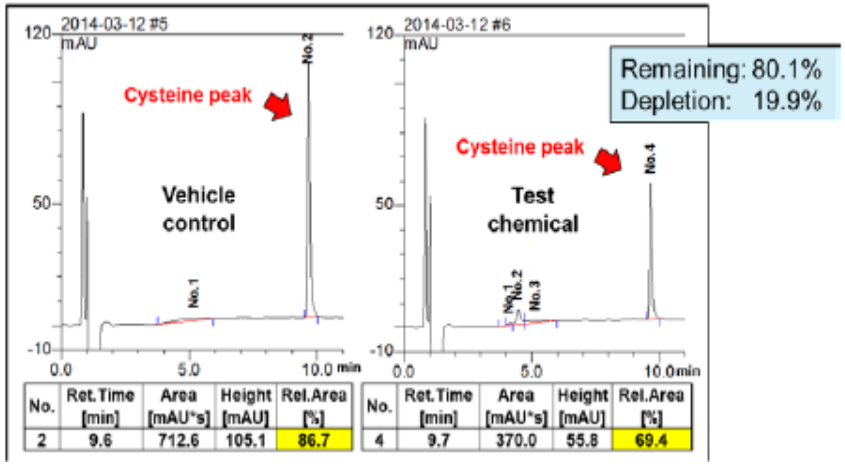
Direct Peptide Reactivity Assay (DPRA, OECD TG 442C)

- *In chemico* assay addressing the MIE of the skin sensitisation AOP, i.e. protein reactivity
- Quantifies the reaction of a chemical with synthetic peptides containing Cysteine (Ac-RFAA**C**AA-COOH) or Lysine (Ac-RFAA**K**AA-COOH)
- Chemical reactivity is expressed as peptide % depletion.
- Mean % C- and K- peptide depletion value used to discriminate between negative and positive results





No.	Ret.Time [min]	Area [mAU*s]	Height [mAU]	Rel.Area [%]
2	9.6	712.6	105.1	86.7

No.	Ret.Time [min]	Area [mAU*s]	Height [mAU]	Rel.Area [%]
4	9.7	370.0	55.8	69.4

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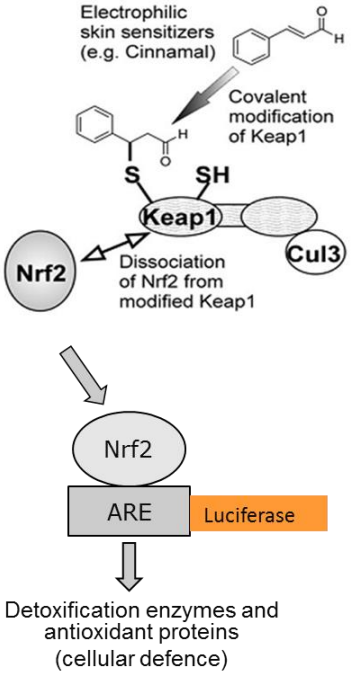
ARE-Nrf2 Luciferase Test Methods

(KeratinoSens™, LuSens, OECD TG 442D) publication of revised TG to include LuSens expected soon!



- Cell-based assays addressing the second key event of the AOP, i.e. keratinocytes activation
- Use immortalised adherent cell lines derived from human keratinocytes stably harbouring a luciferase reporter gene under the control of the antioxidant response element (ARE)
- A prediction is considered positive when luciferase expression is observed at the conditions specified in the respective protocols in 2 of 2 or 2 of 3 repetitions

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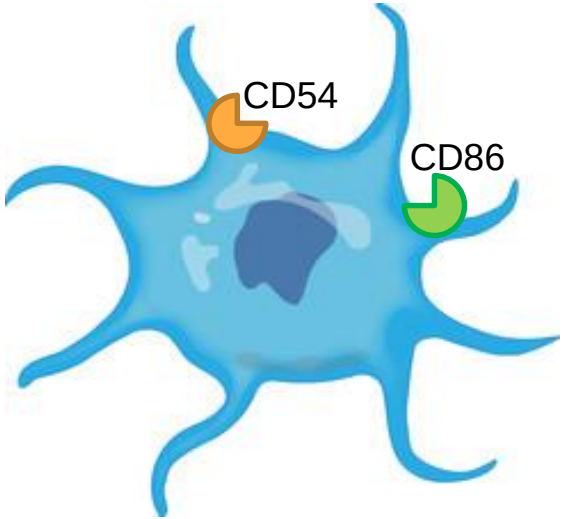
Test Methods Addressing Activation of Dendritic Cells

(human Cell Line Activation Test - h-CLAT, U937 Cell Line Activation Test - U-SENS™, Interleukin-8 Reporter Gene Assay - IL-8 Luc assay; OECD TG 442E)

h-CLAT and U-SENS™

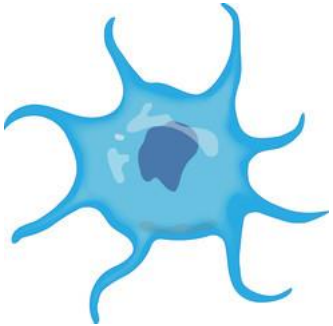


- Cell-based assays addressing the third key event of the AOP, i.e. dendritic cells (DC) activation
- Quantify changes in the expression of cell surface markers (CD54, CD86), associated with activation of monocytes and DC, in the human monocyte derived cell lines THP-1 (h-CLAT) and U937 (U-SENS™)
- A prediction is considered positive when markers expression, quantified by flow cytometry, is above a given threshold as specified in the respective protocols, in 2 of 2 or 2 of 3 independent runs

Test Methods Addressing Activation of Dendritic Cells

(human Cell Line Activation Test - h-CLAT, U937 Cell Line Activation Test - U-SENS™, Interleukin-8 Reporter Gene Assay - IL-8 Luc assay; OECD TG 442E)

IL-8 Luc Assay



- Uses THP-1-derived IL-8 reporter cell line, THP-G8, that harbours the Stable Luciferase Orange (SLO) and Stable Luciferase Red (SLR) genes under the control of the IL-8 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; used as internal control) promoters
- A prediction is considered positive when luciferase expression regulated by the IL-8 promoter is above a given threshold as specified in the protocol in at least 2 of 4 independent runs

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OECD Adopted Methods

- Detailed protocols available at: EURL ECVAM DB-ALM (ecvam-dbalm.jrc.ec.europa.eu) or JaCVAM (IL-8 Luc Assay) (http://www.jacvam.jp/en_effort/effort02.html)
- The Test Guidelines provide Positive or Negative predictions within the defined domain of applicability (e.g. not applicable to lipophilic, highly cytotoxic substances, signal interference, mixtures etc. Check individual TGs!)
- Negative predictions cannot be used on their own to conclude on the absence of skin sensitisation potential of chemicals
- Although the test guidelines provide some quantitative information this cannot be used in isolation for the purpose of sub-categorisation (GHS Cat 1A and 1B)
- Data should be "considered in the context of Integrated Approaches to Testing and Assessment (IATA)", i.e. in combination with complementary information

OECD *In Vitro* Methods – metabolic capacity

- Approximately 25% of sensitising substances are pre- or pro-haptens
- Great majority are pre-haptens
- Pre-haptens are generally correctly predicted by *in vitro* methods
- Slow oxidisers may not be correctly predicted, as in *in vivo* methods
- <10% of skin sensitisers are exclusively pro-haptens
  - Not identified by the DPRA
  - Correctly predicted by cell-based assays, with h-CLAT detecting the majority
- >90% of pre- and pro-haptens are correctly predicted by *in vitro* methods

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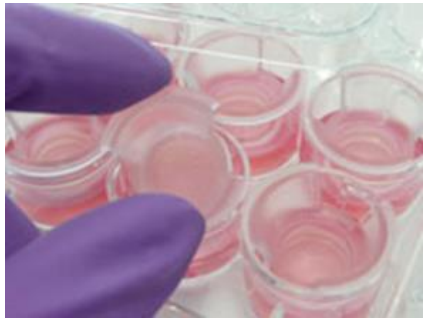
Methods in the OECD Pipeline - SENS-IS



- Uses the commercially available reconstituted human epidermis EpiSkin™
- Measures the expression of 61 genes by qRT-PCR
- Proposed to discriminate between sensitisers and non-sensitisers and to classify sensitisers into four potency classes (weak, moderate, strong and extreme)
- Under evaluation by EURL ECVAM

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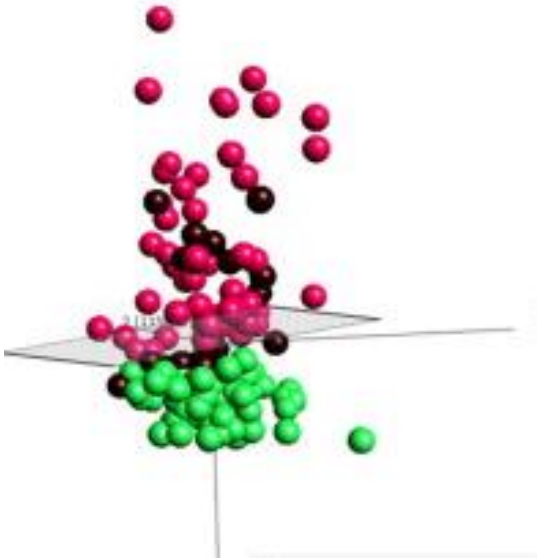
Methods in the OECD Pipeline – Genomic Allergen Rapid Detection Assay (GARD)

- Uses MUTZ-3 cells as surrogate model of human dendritic cells (DCs)
- monitors changes in the expression of 196 genes (GARD prediction signature).
- Compounds are predicted as either sensitisers or non-sensitisers by a support vector machine model
- Under evaluation by EURL ECVAM



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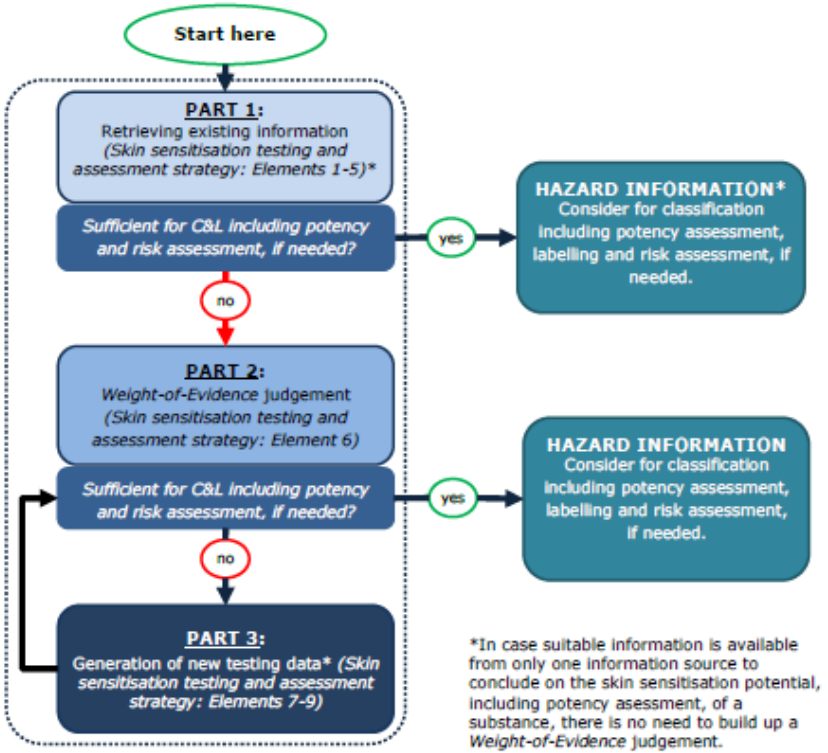
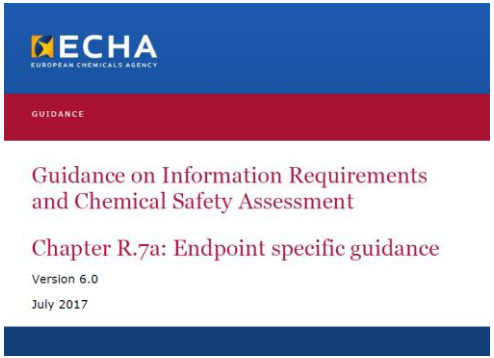
OECD TGs – Use Under REACH





https://echa.europa.eu/guidance-documents/guidance-on-reach

Assessment largely based on weight-of-evidence

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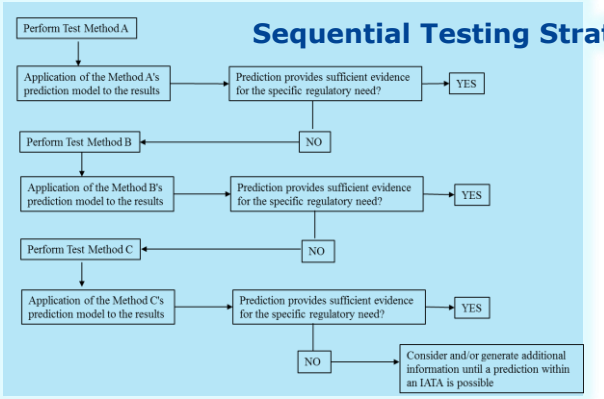
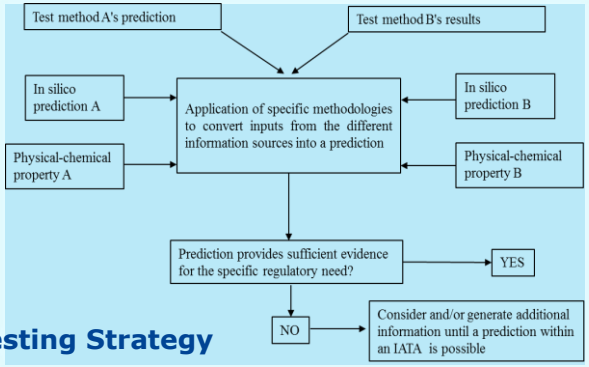
Use of Methods in Combination – Defined Approaches

A Defined Approach consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources (formalised decision-making approach)





Guidance Document No. 255

OECD Guidance Documents (GD) on Defined Approaches

GD 255 Templates for reporting

GD 256 Case studies

Six defining principles:

1. Defined endpoint
2. Defined purpose
3. Description of the underlying rationale, including mechanistic basis (e.g. AOP)
4. Description of the individual information sources used
5. Description of how the individual information sources are processed
6. Consideration of the known uncertainties

Defined Approaches – Case Studies

	Case Study	Bioavailability	Phys-chem properties	*In silico*	Protein binding /reactivity (MIE)	Events in Keratinocytes (KE2)	Events in DC (KE3)	Events in T cells	Adverse effect	Others
1	Sensitiser potency prediction Key event 1+2 (**Givaudan**)		X	TIMES SS	Cor1C420-assay	TG 442D				
2	The artificial neural network model for predicting LLNA EC3 (**Shiseido**)		X		SH Test	AREc32 assay	TG 442E			
3	ITS/DS for hazard and potency identification of skin sensitisers (**P&G**)	penetration (PBPK model)	X	TIMES SS	TG 442C	TG 442D	TG 442E U937 test	TG 429		
4	Tiered system for predicting sensitising potential and potency of a substance (STS) (**Kao Corporation**)				TG 442C		TG 442E			
5	Score-based battery system for predicting sensitising potential and potency of a substance (ITS) (**Kao Corporation**)			DEREK Nexus	TG 442C		TG 442E			
6	IATA for skin sensitisation risk assessment (**Unilever**)	penetration modified OECD TG428			modified OECD TG428					
7	Weight of evidence in vitro ITS for skin hazard identification (**BASF**)				TG 442C	TG 442D LuSens	TG 442E m-MUSST			
8	STS for hazard identification of skin sensitisers (**RIVM**)			Various	TG 442C	TG 442D HaCaT gene signature	TG 442E			
9	IATA (**Dupont**)		X	Various	TG 442C glutathione depletion assay	TG 442D	TG 442E U937	TG 429	TG 406	E.g. Skin Irr/Corr, Ames
10	Decision strategy (**L'Oréal**)		X	Various	TG 442C	TG 442D ARE-Nrf2 Assay	U-SENS™ PGE2 Assay			
11	Integrated decision strategy for skin sensitisation hazard (**ICCVAM**)		X	OECD Toolbox			TG 442E			
12	Consensus decision tree model for skin sensitisation hazard prediction (**EC JRC**)			TIMES SS Dragon						

Annex 1 to Guidance Document No. 256

- Some based fully on *in vitro* methods, some on *in silico*, some combine both
- The *in vitro* methods are mainly OECD Test Guidelines, but some are not
- Algorithms used to combine data to make a prediction vary in complexity

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Defined Approaches (OECDENV/JM/MONO(2016)29 Annex)

Case study		Purpose
I	**An Adverse Outcome Pathway-based "2 out of 3" integrated testing strategy approach to skin hazard identification (BASF)**	**Hazard**
II	Sequential Testing Strategy (STS) for hazard identification of skin sensitisers (RIVM)	Hazard
III	A non-testing Pipeline approach for skin sensitisation (G. Patlewicz)	Hazard
IV	Stacking meta-model for skin sensitisation hazard identification (L'Oréal)	Hazard
V	Integrated decision strategy for skin sensitisation hazard (ICCVAM)	Hazard
VI	Consensus of classification trees for skin sensitisation hazard prediction (EC- JRC)	Hazard
VII	**Sensitiser potency prediction based on Key event 1 + 2: Combination of kinetic peptide reactivity data and KeratinoSens® data (Givaudan)**	**Potency**
VIII	The artificial neural network model for predicting LLNA EC3 (Shiseido)	Potency
IX	Bayesian Network DIP (BN-ITS-3) for hazard and potency identification of skin sensitisers (P&G)	Potency
X	Sequential testing strategy (STS) for sensitising potency classification based on *in chemico* and *in vitro* data (Kao Corporation)	Potency
XI	Integrated testing strategy (ITS) for sensitising potency classification based on *in silico, in chemico*, and *in vitro* data (Kao Corporation)	Potency
XII	DIP for skin allergy risk assessment (SARA) (Unilever)	Potency

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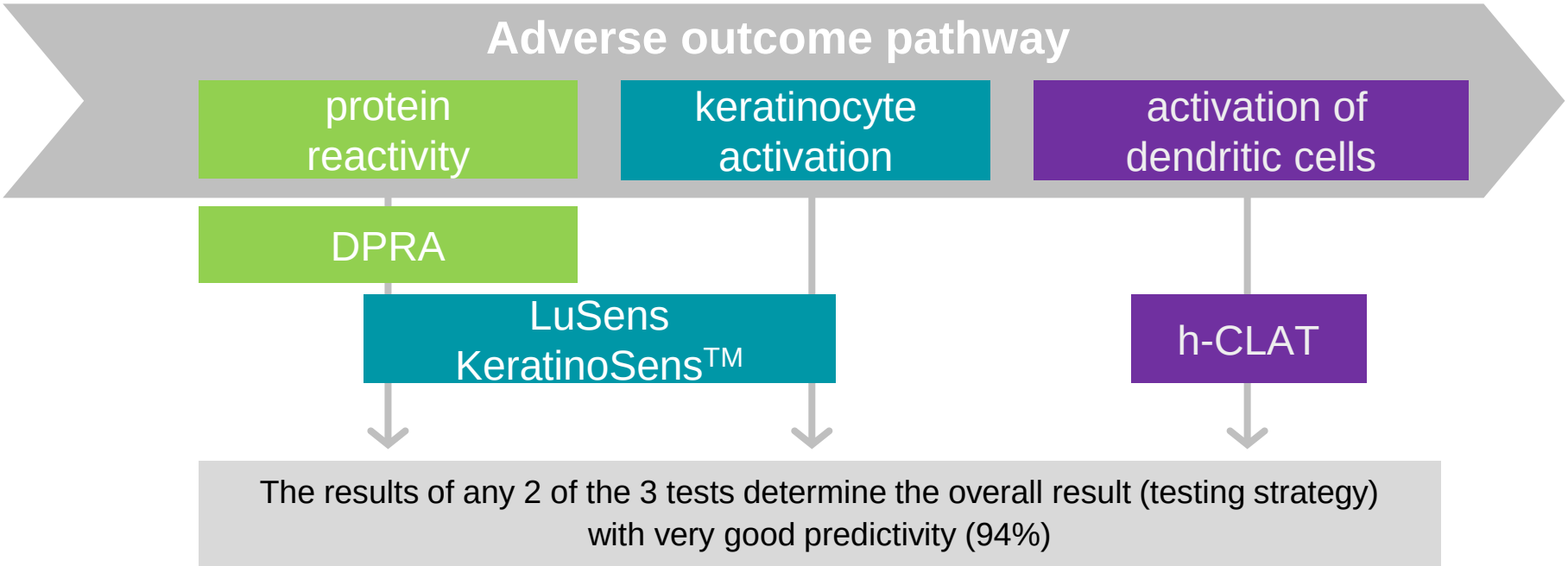
DA Case Study I: “2 out of 3“ for Hazard ID



Bauch et al., 2012

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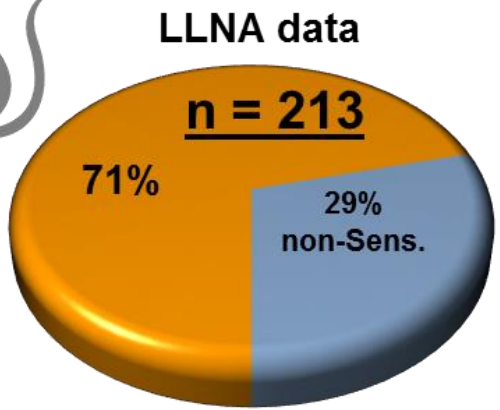
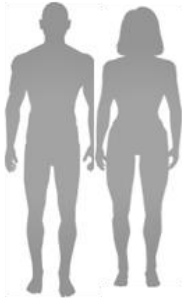
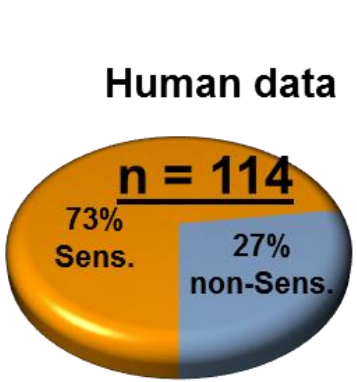
Predictive Capacity of DA Case Study I







In vitro WoE Approach	
Accuracy	**79%**
Sensitivity	82%
Specificity	72%

for comparison:
DPRA: Accuracy 75%

In vitro WoE Approach	
Accuracy	**90%**
Sensitivity	90%
Specificity	90%

for comparison:
LLNA: Accuracy 82%
DPRA: Accuracy 84%

Urbisch et al., 2015

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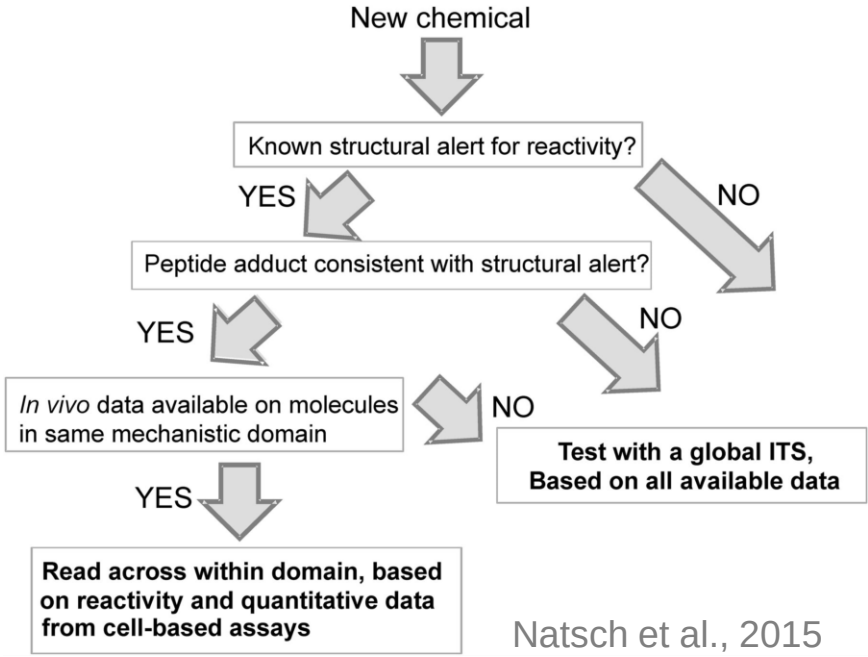
DA Case Study VII: kinetic DPRA+ KeratinoSens™ for Potency Assessment

Combination of [reaction mechanism] domain- based and global models for potency prediction

- Step 1: Hazard ID: Sensitiser if either KeratinoSens™ or covalent adduct formation
- Step 2: Attribution to mechanistic domain
- Step 3: Potency prediction
  - A) LLNA EC3 prediction via domain based regression for Michael acceptors, chemicals reacting by addition eliminations, epoxides, quinone methides and aldehydes
  - B) LLNA EC3 prediction via global regression for substances that cannot be atributed to the mecahnistic domains in 3A
  - C) human potency prediction



Natsch et al., 2015

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Predictive Capacity of DA Case Study VII

- Best potency prediction by multivariate regression model of
  - KeratinoSens ™ (luciferase induction [EC1.5] and cytotoxicity [IC50]
  - Peptide reactivity: LC-MS-based assay using the peptide Cor1-C420 [Kmax]
  - Physicochemical parameters: clogP, vapour pressure
- Prediction of “most likely LLNA EC3 value”, GHS category, or and human DSA_{05} values
- Accuracy (CLP/ GHS 1A or 1B or non-sensitizer)
  - 71% (n = 244, vs. LLNA, global model)
  - 75% (n = 244, vs. LLNA, combined global and domain models)
  - 61% (n=71, vs. human)

Natsch et al., 2015

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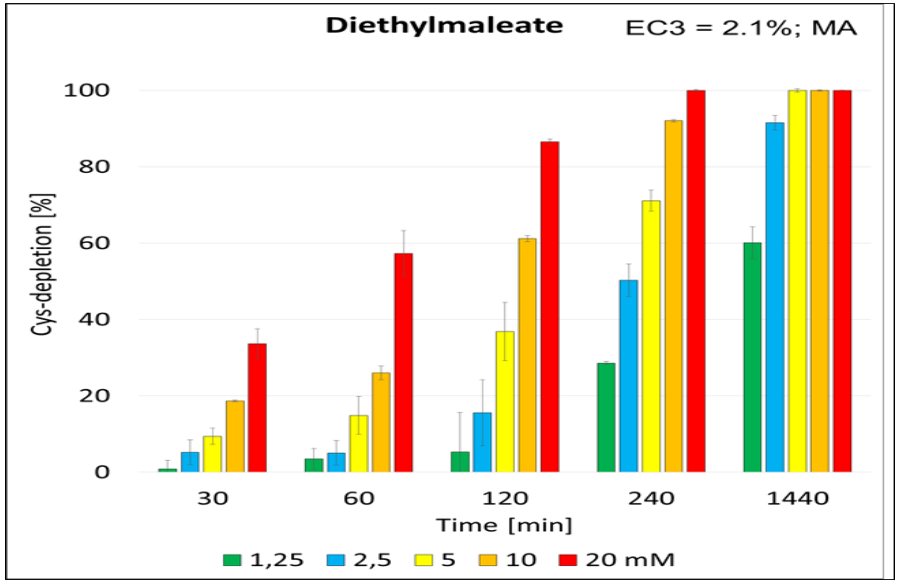
Sensitising Potency Assessment using Peptide Reactivity Data (kinetic DPRA)

- Using different reaction times and test substance concentrations → much larger dynamic range than standard DPRA
- Fluorescent read out (Cys-peptide only)
- Accuracy (CLP/ GHS 1A or 1B)
  - 92% (n = 38, LLNA)
  - 93% (n = 14, human)



Wareing et al., 2017

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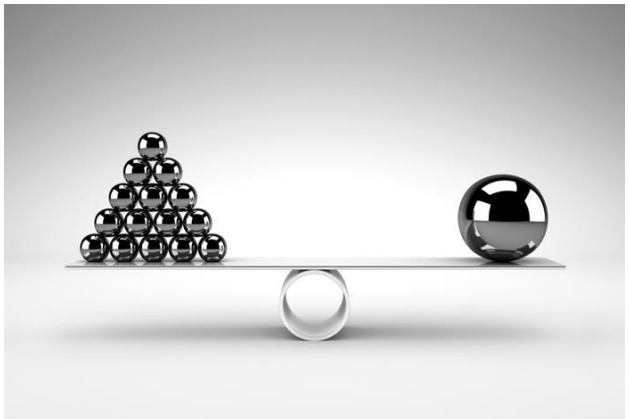
Background to Ongoing OECD Activities

Position of the International Cooperation on Alternative Test Methods (ICATM)

- An alternative approach for skin sensitisation testing that provides equivalent information to the animal test should be given equivalent regulatory recognition and status

- Defined Approaches which are shown to be scientifically valid and fit-for-purpose can be incorporated into an OECD instrument covered by MAD to guarantee equal footing with the regulatory animal tests



European Commission

OECD Project on The Development of a TG on Defined Approaches for Skin Sensitisation

Included in OECD WP in 2017- Led by European Commission, US and Canada with support from the other ICATM partners (Japan, South Korea, Brazil, and China)

Aims:

- Definition of an internationally agreed evaluation framework for DAs
- Translation of scientific valid DAs into a TG that would fall under MAD

OECD
Organisation for Economic Co-operation and Development

Special session of the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) on the project: Performance-Based Test Guideline on Defined Approaches for Skin Sensitisation - 13-15 December 2017

European Commission

Summary

- Standard information requirement for REACH updated in the light of scientific progress. Potential to produce significant sensitisation in humans has to be considered
- Information on the first three key events of the AOP should be addressed in first place with the validated and OECD adopted methods and for test items shown to be in their domain of application
- Updated ECHA guidance for the generation of data to fulfill the requirements published
- Methods adopted so far need to be used in combination to generate sufficient evidence for negative results and significant effects
- In the near future it may be possible to have one-to-one replacements for the LLNA, so far it is not
- DAs for skin sensitisation appear promising for predicting LLNA and human responses
- Ongoing OECD activities aim to give to DAs the same regulatory recognition as the animal tests

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Perspectives on the Development, Evaluation, and Application of *in Silico* Approaches for Predicting Toxicity Recorded	Dr. Grace Patlewicz, US EPA Prof. Mark Cronin, Liverpool John Moores University
3R Approach to Acute Oral Toxicity Recorded	Dr. Kimmo Louekari, ECHA
Skin Irritation and Corrosion 25 January 2018, 4–5 pm GMT	Dr. Gertrude-Emilia Costin, Institute for In Vitro Sciences Dr. Costanza Rovida, TEAM Mastery and CAAT-Europe
Skin Sensitisation 1 February 2018, 4–5 pm GMT	Dr. Susanne Kolle, BASF SE Dr. Silvia Casati, EURL ECVAM
Serious Eye Damage and Eye Irritation 15 February 2018, 4–5 pm GMT	Dr. Kim Norman, Burt's Bees Dr. Els Adriaens, Ghent University