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In Vitro Skin Sensitization – the 2 out of 3 Approach

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07 Nov 2018

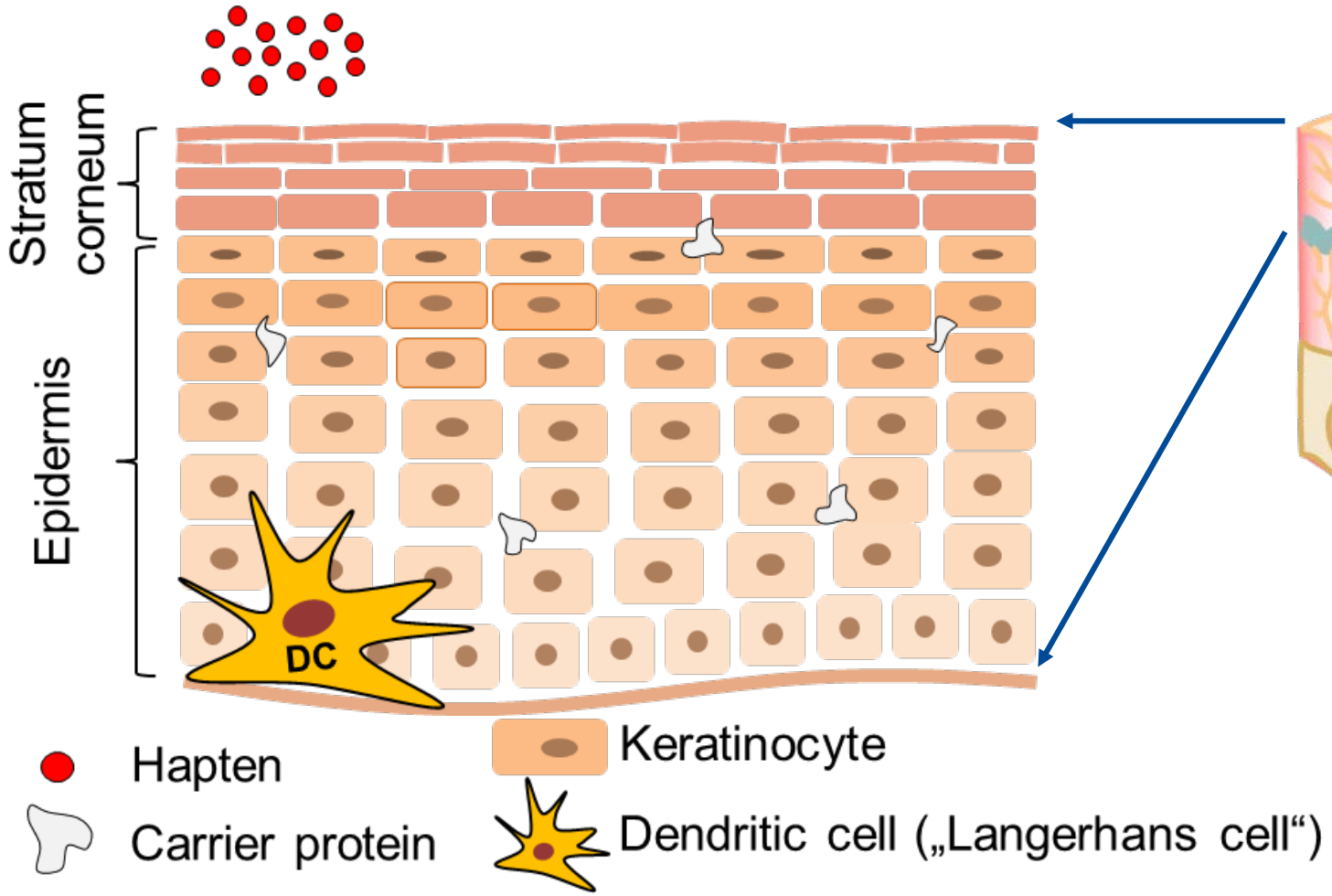
Skin Sensitization

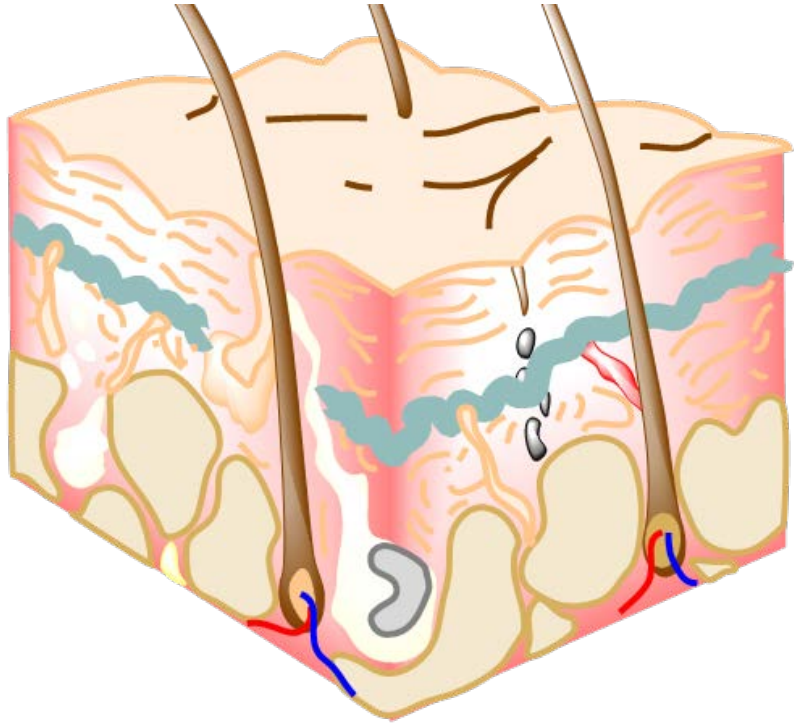
- Allergic contact dermatitis is the clinical manifestation of a skin sensitization
- Hypersensitive reaction after repeated contact to an allergen
- 15 - 20% of the population sensitized
- Most common allergic contact dermatitis: nickel contact dermatitis

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.

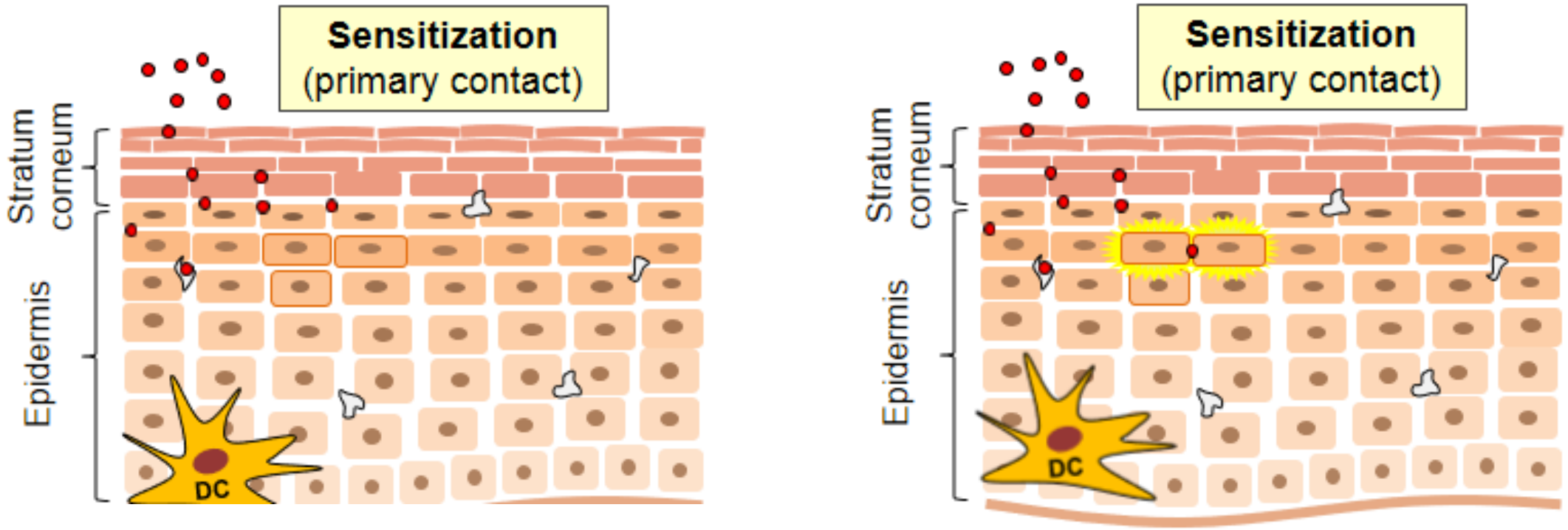
The Skin Sensitization Mechanism





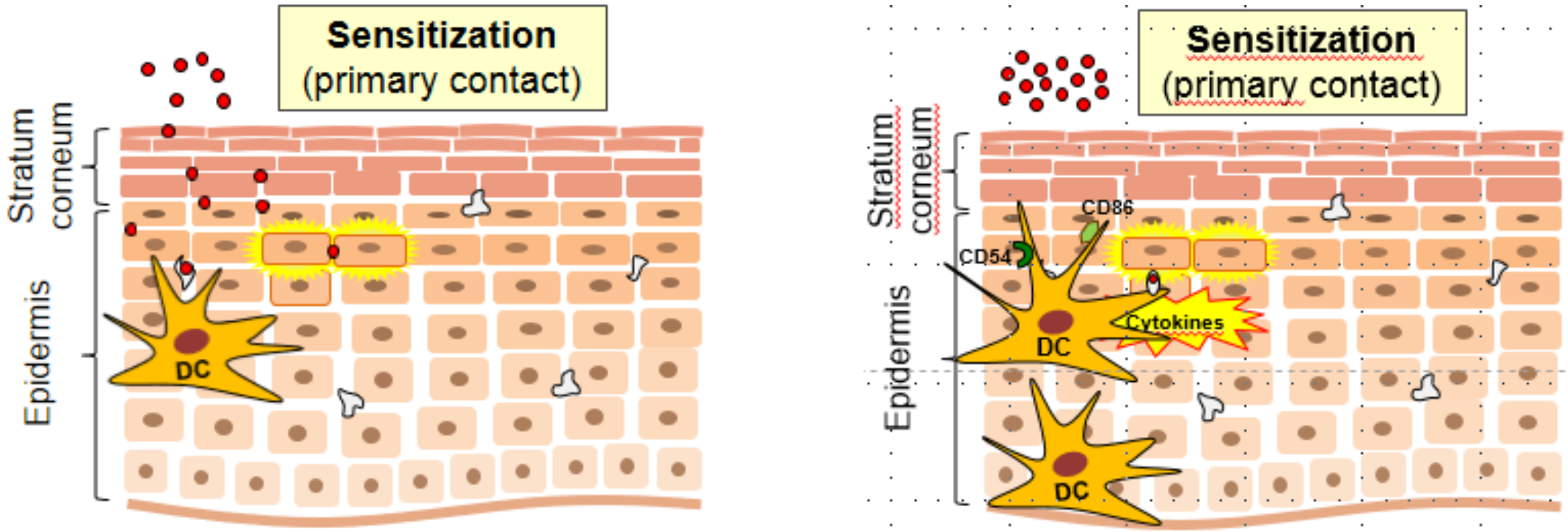
Courtesy of D. Urbisch

The Skin Sensitization Mechanism: KE1 (MIE) + KE2



Courtesy of D. Urbisch

The Skin Sensitization Mechanism: KE3



Courtesy of D. Urbisch

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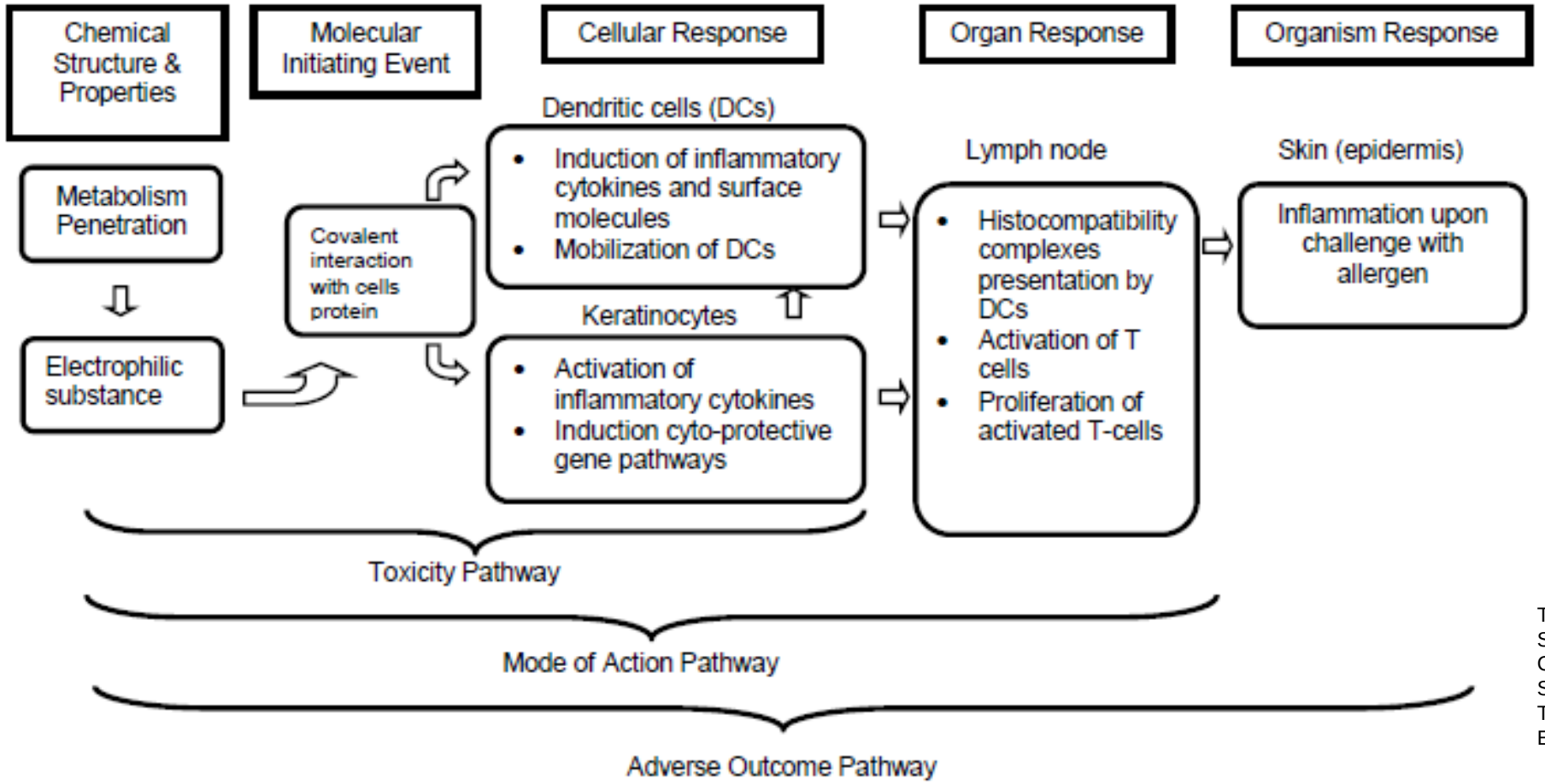
The Adverse Outcome Pathway for Skin Sensitization



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

OECD Adopted Test Guidelines

Test No. 442C: In Chemico Skin Sensitisation

Direct Peptide Reactivity Assay (DPRA)

Test No. 442D: In Vitro Skin Sensitisation

ARE-Nrf2 Luciferase Test Method

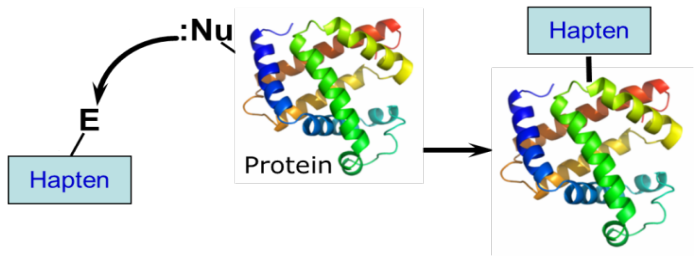
Test No. 442E: In Vitro Skin Sensitisation

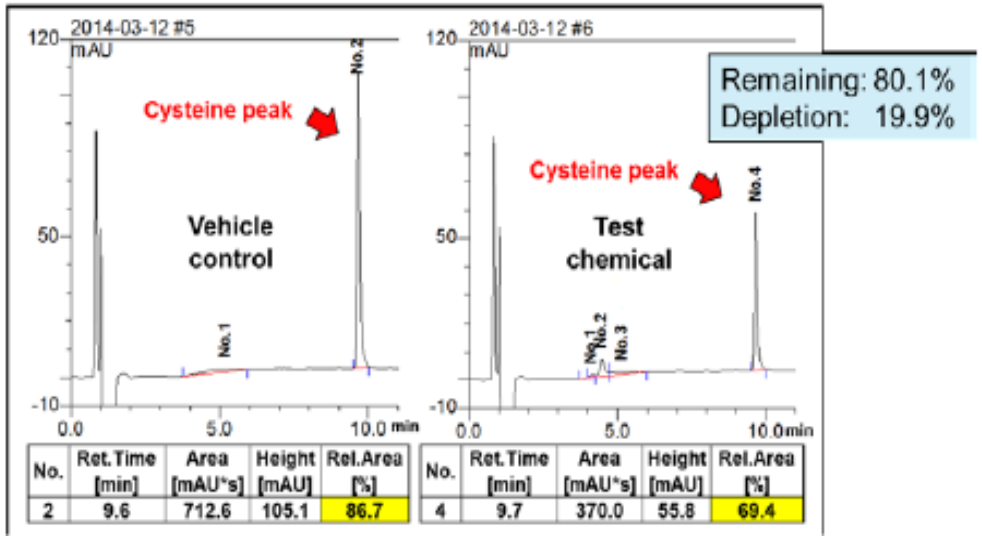
In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation

Direct Peptide Reactivity Assay (OECD TG 442C)

- In chemico assay addressing the MIE of the skin sensitization 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 of % C- and K- peptide depletion values used to discriminate between negative and positive results

Nucleophilic-electrophilic interaction:





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

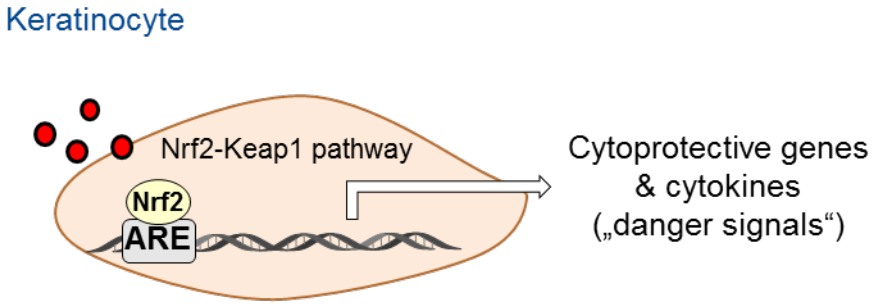
Direct Peptide Reactivity Assay (OECD TG 442C)

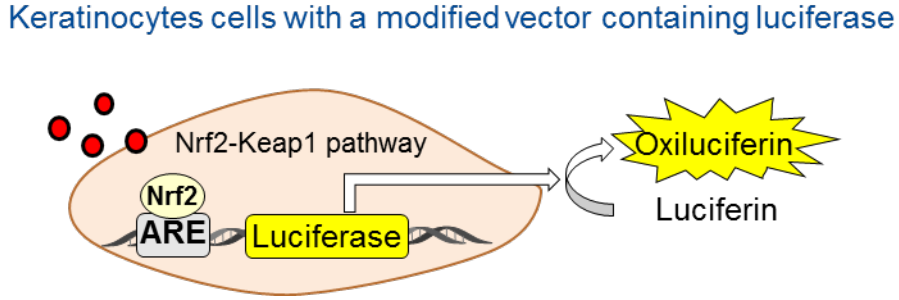
- Not applicable for the testing of **metal** compounds
- Test chemicals that are **not soluble at 100 mM** may still be tested at lower soluble concentrations, BUT no firm conclusion on the lack of reactivity should be drawn from a negative result
- Technically applicable to the testing of **mixtures** of known composition (use of apparent molecular weight)
- According to the TG the current prediction model cannot be used for **complex mixtures of unknown composition or for substances of unknown or variable composition, complex reaction products or biological materials** (i.e. UVCB substances)
- **Polymers** are tested according to their predominant molecular weight or molecular weight of monomer; in addition tested undiluted
  - ▶ Test also undiluted / max. concentration of test substance
- Method does not encompass a **metabolic system** but majority of pre-haptens and pro-haptens are sufficiently well identified

ARE-Nrf2 Luciferase Test Methods (OECD TG 442D)

- Cell-based assays addresssing the second key event in skin sensitization AOP, i.e. keratinocyte activation

- Uses immortalised adherent cell lines (KeratinoSens™, LuSens) derived from human keratinocytes stably harbouring a luciferase reporter gene under the control of the antioxidant response element (ARE)



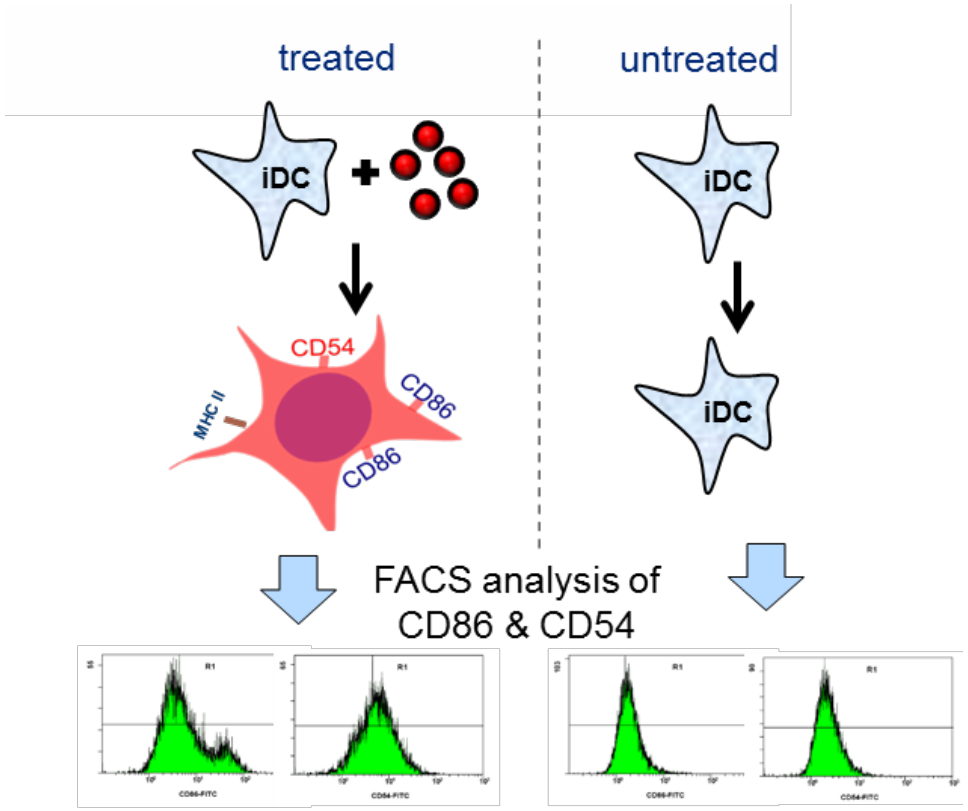


ARE-Nrf2 Luciferase Test Methods (OECD TG 442D)

- Applicable to test chemicals **soluble** or that form a **stable dispersion** either in water or DMSO (no longer a LogP limitation with the 2018 revision of TG 442D)
- If above does not apply up to 2000 µM a negative result should be considered as inconclusive
- May underpredict test chemcials exclusive reactive towards **lysine** residues
- Limited **metabolic capability** but majority of pre-haptens and pro-haptens are sufficiently well identified
- **Chemical stressors** may lead to false positive
- Test chemicals (e.g. phytoestrogens) **interfering with the luciferase** enzyme and hence luminescence determination
- Substances acting as **acylating agents** may be under-predicted

Test Methods Addressing Activation of Dendritic Cells (OECD TG 442E)

- Cell-based assay addressing third key event of the skin sensitization AOP
- Human cell line activation test (h-CLAT): Quantification of changes in the expression of cell surface markers associated with the process of activation of monocytes and DC (i.e. CD86 and CD54) in the human monocytic leukaemia cell line THP-1



CD54 (= ICAM-1) → DC migration and T cell activation

CD86 (= B7-2) → Co-stimulation during T cell activation

Test Methods Addressing Activation of Dendritic Cells (OECD TG 442E)

- Use only! THP-1 cells from ATCC (TIB-202™)
- Negative results for test substances with log K_{OW} > 3.5 and no cytotoxicity discussed in the ongoing revision are interpreted as “inconclusive. However, a positive result will be accepted.
- Limited information on multi-constituent substances/mixtures is available but test is technically applicable
- Applicable to test chemicals soluble or that form a stable dispersion
- Limited **metabolic capability** of the cell line but majority of pre-haptens and pro-haptens are sufficiently well identified
- **Fluorescent** substances interfering with the flow cytometric detection

OECD *In Vitro* Methods – metabolic capacity

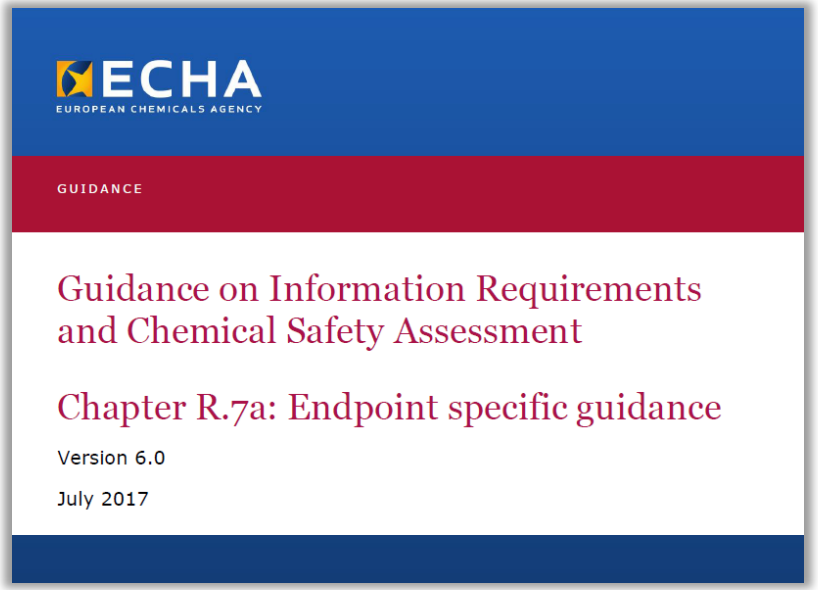
- Approximately 25% of sensitizing substances are pre- or pro-haptens
- Great majority are pre-haptens
- Pre-haptens are generally correctly predicted by *in vitro* methods
- Slow oxidisers may not be correctly predicted, as in *in vivo* methods
- <10% of skin sensitizers are exclusively pro-haptens
  - 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

Patlewicz et al., 2016

OECD Adopted Methods

- Detailed protocols available e.g. at: EURL ECVAM DB-ALM (ecvam-dbalm.jrc.ec.europa.eu)
- The Test Guidelines provide positive or negative predictions within the defined domain of applicability of an assay
- Negative predictions cannot be used on their own to conclude on the absence of skin sensitization 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

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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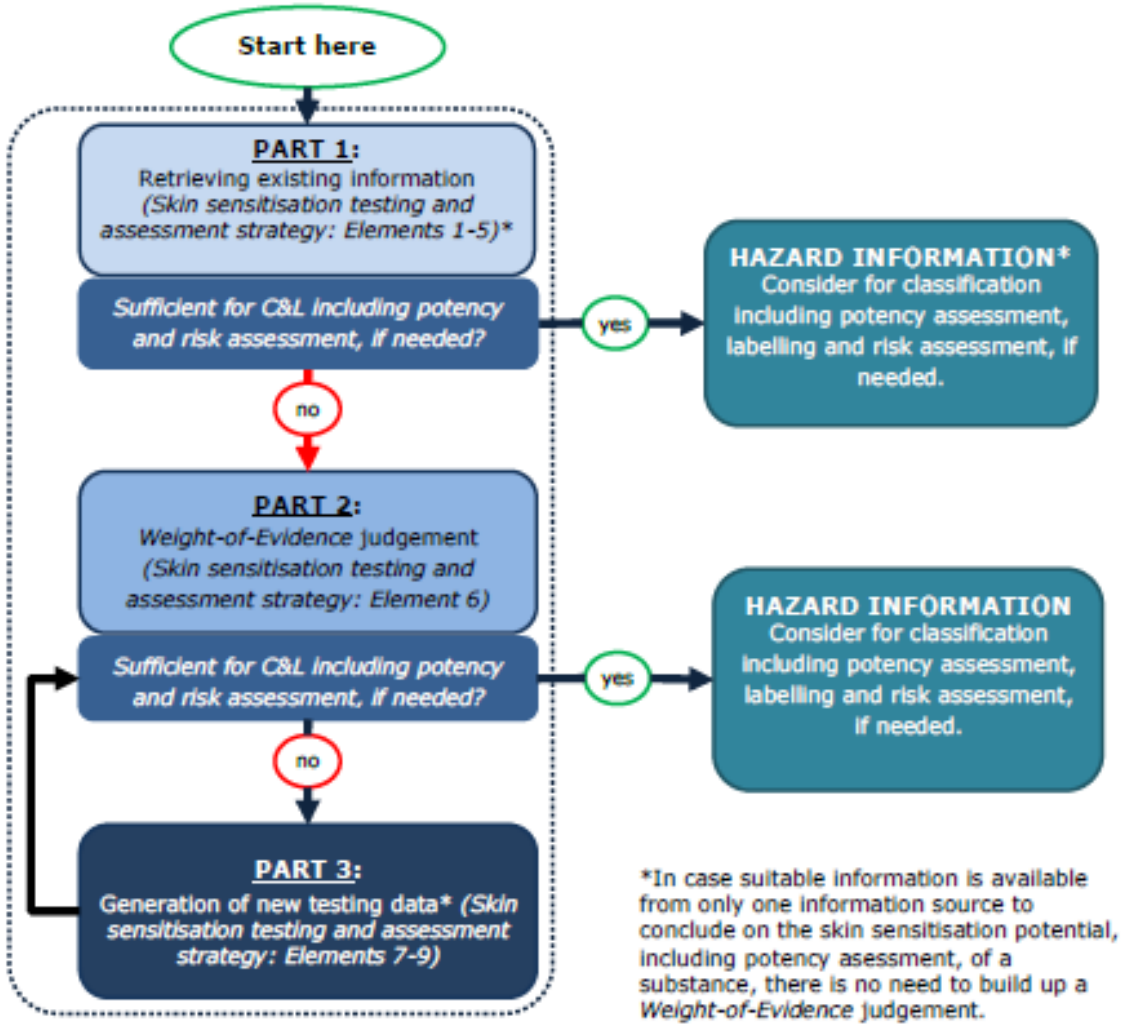
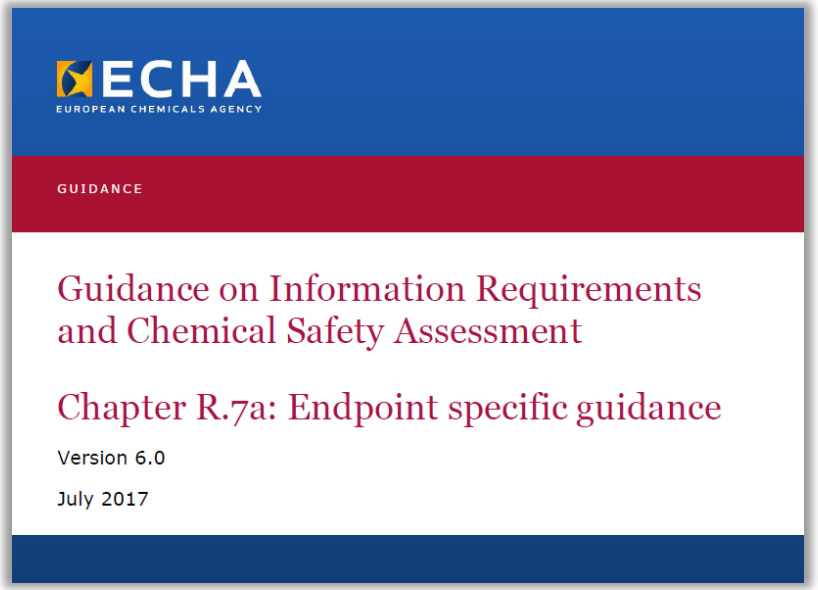
OECD TGs – Use Under REACH





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

Assessment largely based on weight-of-evidence

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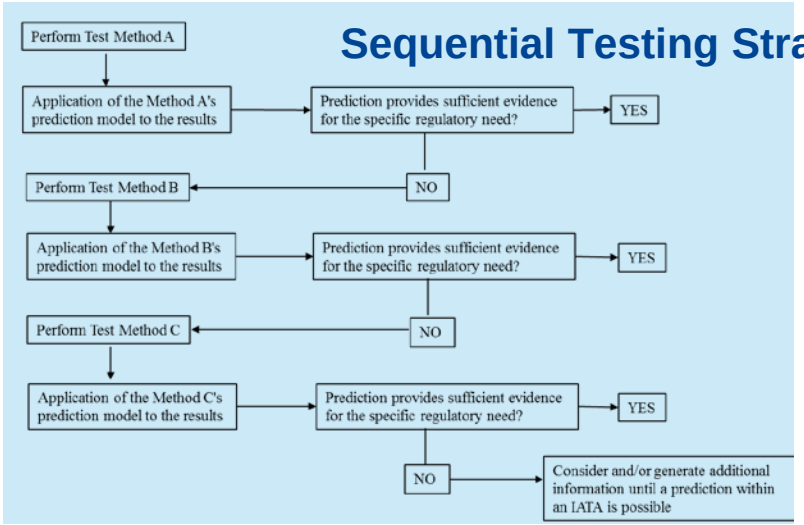
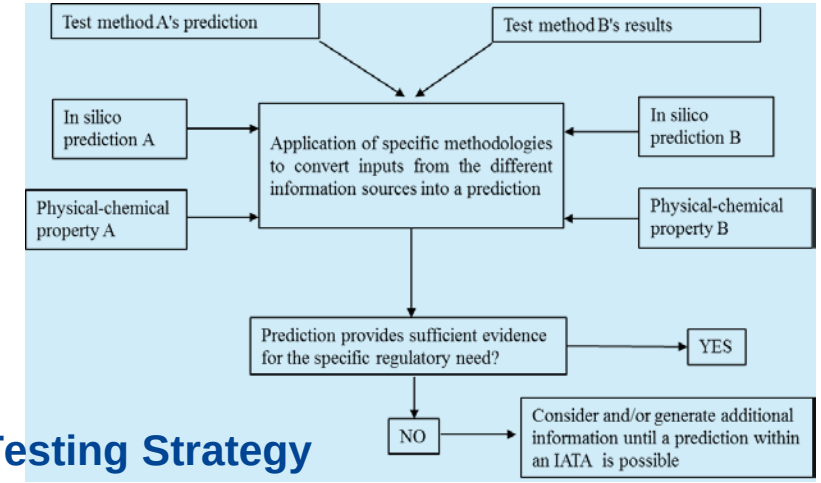
Use of Methods in Combination – Defined Approaches

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)

Sequential Testing Strategy



Integrated Testing Strategy



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

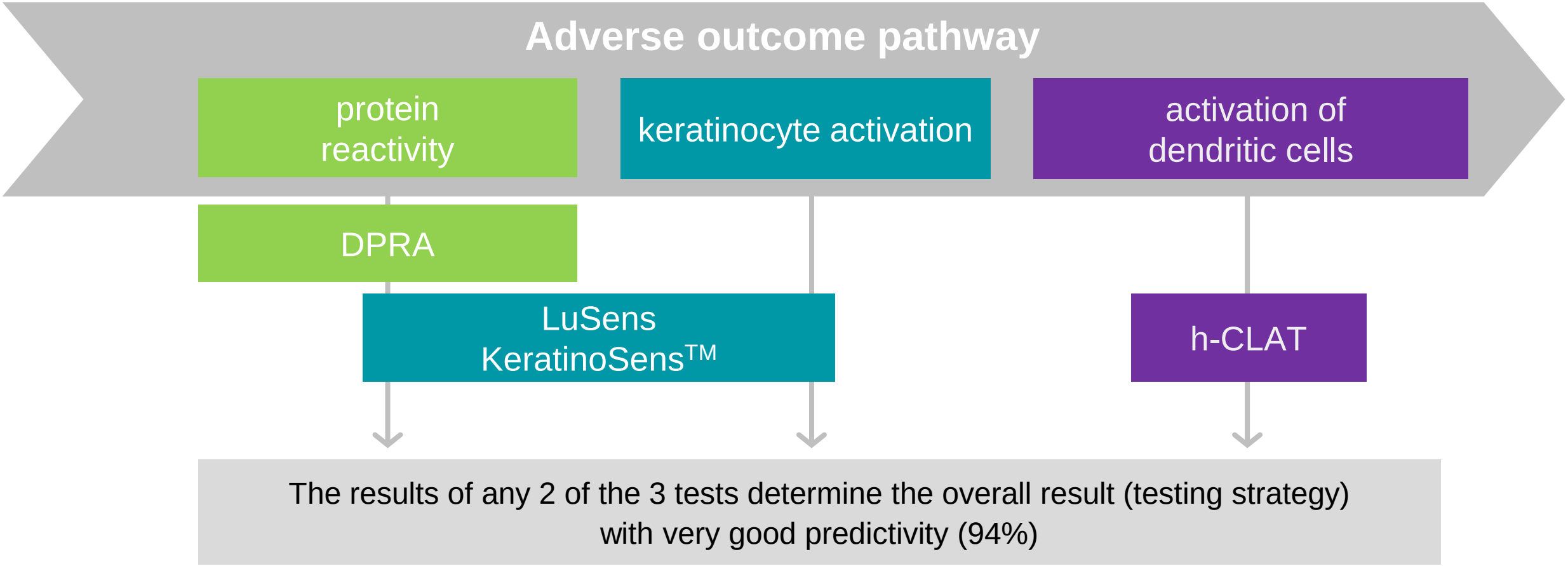
Defined Approaches – Case Studies

					MIE	KE2	KE3			
	Case Study	Bioavailability	Phys-chem properties	*In silico*	Protein binding /reactivity	Events in Keratinocytes	Events in DC	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

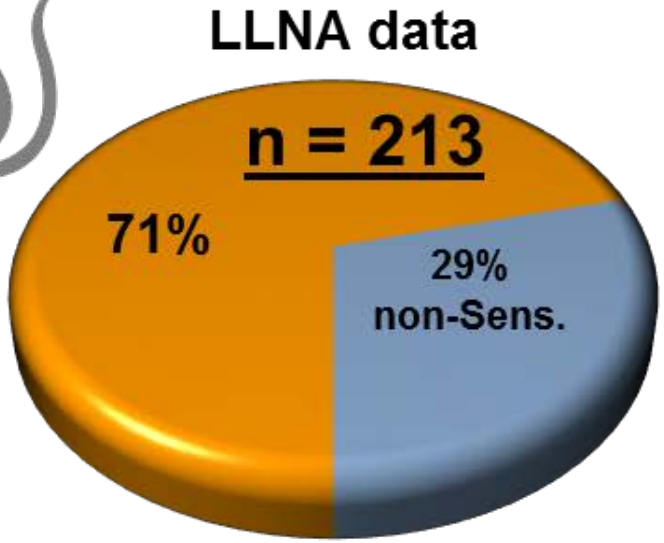
- Some based fully on *in vitro* methods, some on *in silico*, some combine both
- The *in vitro* methods are mainly OECD Test Guidelines, but some are not
- Algorithms used to combine data to make a prediction vary in complexity

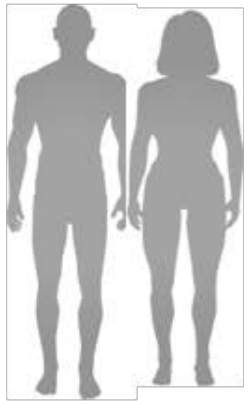
DA Case Study I: “2 out of 3“ for Hazard ID

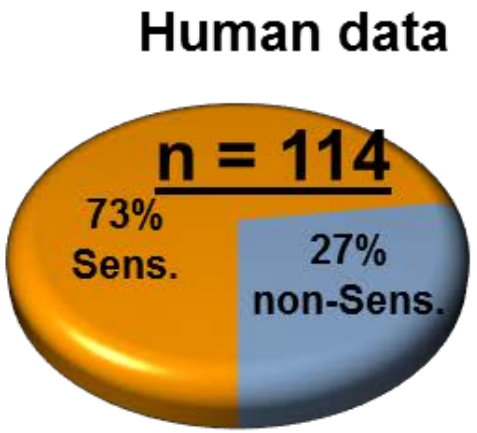


Bauch et al., 2012

Predictive Capacity of 2 out of 3 Approach







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

Predictive Capacity of 2 out of 3 Approach

	Urbisch et al., 2015 **vs. LLNA**	Urbisch et al., 2015 **vs. human**	Kleinstreuer et al., 2018 **vs. LLNA**	Kleinstreuer et al., 2018 **vs. human**
n	213	114	127	127
Accuracy [%]	79	90	70	77
Sensitivity [%]	82	90	72	79
Specificity [%]	72	90	64	73

- The 2 out of 3 approach is an AOP based hazard identification DA providing mechanistic data
- The 2 out of 3 approach achieves slightly better predicitivites than the LLNA compared to human data
- Technical limitations of individual test methods apply

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

- Definition of an internationally agreed evaluation framework for DAs
- Translation of scientific valid DAs into a TG that would fall under MAD
- First draft **Guideline on Defined Approaches for Skin Sensitization** is available and open for comments until 16 Nov 2018

Summary

- Standard information requirement for REACH updated in the light of scientific progress. Potential to produce significant sensitization 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
- 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 sensitization appear promising for predicting LLNA and human responses
- Ongoing OECD activities aim to give to DAs the same regulatory recognition as the animal tests

Some References

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ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance available at http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

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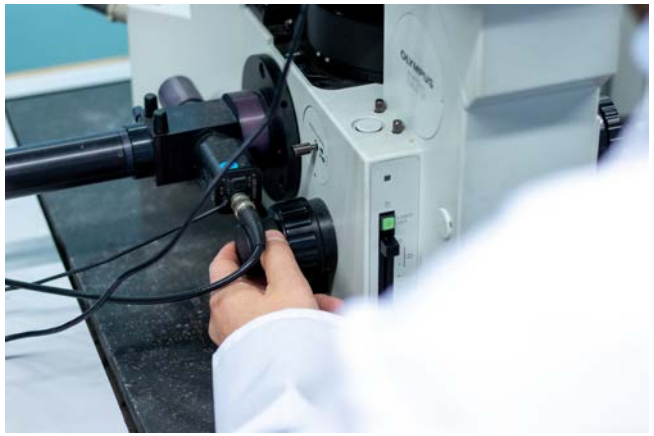

BASF I TRAINING

SKIN SENSITIZATION

Thursday 22nd - Friday 23rd of November 2018

Ludwigshafen, Germany

ALTERTOX academy

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