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Identification of an allergen by non-clinical pre-tests

(historic animal and human data and existing and future tools of alternatives to animal testing) for the characterisation of allergens

IDEA WS Sept 2014

- Well known methods to determine the potential for a material to be a sensitiser (allergen)
- CLP criteria
- Alternatives and OECD Integrated Approach to Testing and Assessment (IATA)

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Well known methods to determine sensitisation of a substance

- In Silico tools (WoE)
- Read-across and structural considerations (WoE)

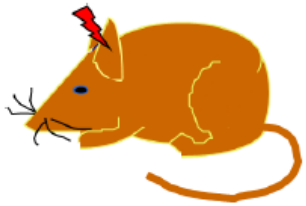
- Animal studies
  - OECD 429: LLNA
  - OCED 406: GPMT, Buehler occluded patch test
  - Other

- Human non-clinical studies
  - Human maximisation test
  - HRIPT

- In vitro studies
  - DPRA, Keratinosens, H-Clat – OECD guidelines to be publshed
  - Other

Non-human animal tests

Bühler and guinea pig maximization test (OECD TG 406)



Local lymph node assay (OECD TG 429)

Human test

Human patch test

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Sensitiser Non-sensitiser

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CLP Criteria

Human Data

Annex I: 3.4.2.2.2.1. Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

Annex I: 3.4.2.2.2.2. Human evidence for sub-category 1B can include:

(a) positive responses at > 500 µg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

HRIPT: Human Repeat Insult Patch Test; HMT: Human Maximisation Test

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CLP Criteria

Clinical data

Table 3.4.2—b Relatively high or low frequency of occurrence of skin sensitisation*

Human diagnostic patch test data	High frequency	Low/moderate frequency
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Work place studies:		
1: all or randomly selected workers	≥ 0.4 %	< 0.4 %
2: selected workers with known exposure or dermatitis	≥ 1.0 %	< 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

* Only one or two types of information may be sufficient for sub-categorisation.

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CLP Criteria

Animal data

Table 3.4.2—e Definition of significant skin sensitising effect

Test	Result
Mouse local lymph node assay (LLNA) (OECD TG 429)	Stimulation Index ≥ 3
LLNA: DA (OECD TG 442A),	Stimulation Index ≥ 1.8
LLNA: BrdU-ELISA (OECD TG 442B)	Stimulation Index ≥ 1.6
Guinea pig maximisation test (GPMT) (OECD 406)	Redness (Score ≥ 1) in ≥ 30% of the test animals
Buehler assay (OECD 406)	Redness (Score ≥ 1) in ≥ 15% of the test animals

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CLP Criteria

Animal data

deriving sub-categories

3.4.2.2.3.2. Non human data

Annex I: 3.4.2.2.3.2. Animal test results for sub-category 1A can include data with values indicated in Table 3.4.3

Table 3.4.3

Animal test results for sub-category 1A

Assay	Criteria
Local lymph node assay	EC3 value ≤ 2 %
Guinea pig maximisation test	≥ 30 % responding at ≤ 0,1 % intradermal induction dose or ≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose
Buehler assay	≥ 15 % responding at ≤ 0,2 % topical induction dose or ≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose

3.4.2.2.3.3. Animal test results for sub-category 1B can include data with values indicated in Table 3.4.4 below:

Table 3.4.4

Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2 %
Guinea pig maximisation test	≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose
Buehler assay	≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1,

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Alternatives

OECD Guidance under development

GUIDANCE DOCUMENT ON THE EVALUATION AND APPLICATION OF INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) FOR SKIN SENSITISATION

Draft 16 June 2014

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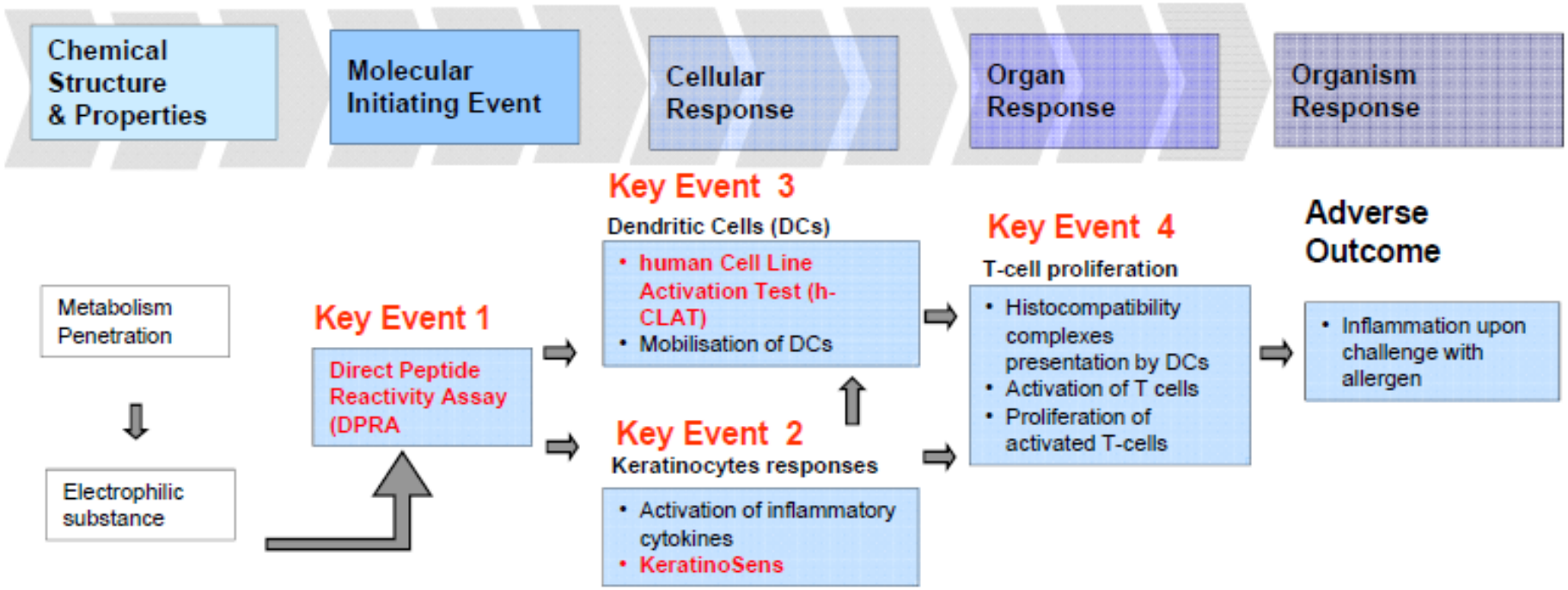
AOP

The AOP describes such key events starting from the molecular initiating event (MIE) (covalent binding of a chemical to skin proteins) through to sensitisation.



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OECD IATA

- An Integrated Approach to Testing and Assessment (IATA) is a structured approach used for hazard identification (potential), hazard characterisation (potency) and/or safety assessment (potential/potency and exposure) of a chemical or group of chemicals, which strategically integrates and weights all relevant data

- Provide a general framework for skin sensitisation IATA that enables sufficient flexibility in the use of the individual information sources to cover multiple regulatory needs within OECD member countries
- Provide generic guidance on the evaluation and application of IATA
- Provide consistent description of the information sources that can be used within an IATA for skin sensitisation
- Include a template for describing IATA so that the same documentation format for describing and evaluating IATA can be used by member countries.

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IATA Elements		Information Sources
Exposure considerations		• Dose-unit area • Use conditions • Others
	Dermal bioavailability (penetration and metabolism)	**Chemical structure** **Physico-chemical properties** • Molecular Weight • pKa • Log Kow • Evaporation rate/Vapour pressure • Melting point • N° of H bond donors/acceptors • Others **Non-testing methods** • In silico (TIMES-SS, Meteor, Nexus, OECD Toolbox) • Physiological based-pharmacokinetic (PBPK) models • Read across • Others **Testing methods** • TG 427 (Skin absorption: in vivo method) • TG 428 (Skin absorption: in vitro method) • Others **To the extent addressed by each of the test methods e.g:** • Peroxidase-peroxide system (PPRA) • Incubation with S9 fractions • Use of metabolically competent test systems

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AOP key event 1: Protein binding reactions, Reactivity and Metabolism	
	Non-testing methods • Protein binding alerts (e.g. OECD Toolbox, Derek Nexus, Toxtree)
Protein binding/Reactivity	• Others **Testing methods** • DPRA and other methods measuring peptide depletion • PPRA and other methods measuring adduct formation • Methods measuring relative reactivity rate • Others
AOP key event 2: events in Keratinocytes	
Activation of biochemical pathways	**Testing methods** • KeratinoSens™ (Keap-1 NrF2-ARE pathway) • LuSens (Keap-1 NrF2-ARE pathway) • ARE c32 assay (Keap-1 NrF2-ARE pathway)
Pathways-associated gene expression	• Sens-is • SenCeeTox • HaCaT gene signature
Release of pro-inflammatory mediators	• RhE-IL-18 • Others

AOP key Event 3: Events in Dendritic cell	
Expression of co-stimulatory and adhesion molecules	**Testing methods** • h-CLAT • MUSST • Modified MUSST • PBMDC
Pathways-associated gene expression	• GARD • VitoSens
Pathways-associated protein expression	• SensiDerm
AOP key event 4: Events in Lymphocytes	
	(Existing) animal data • TG 429 • TG 442a • TG 442b **Testing methods** • Human T cell priming/proliferation assay

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	AOP Adverse Outcome	
		(Existing) human data • Human Repeat Insult Patch Test (HRIPT) • Clinical data • Data from occupational exposure • Epidemiological data **(Existing) animal data** - TG 406 **Others**
Other supporting information		• Skin irritation • Skin corrosion • Genotoxicity • Others

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Generic Matrix for Weight of Evidence Analysis

Component	Information source	Reference (scientific literature, Test Guidelines, Methods etc.)	Study result and/or positive/negative evidence obtained	Data reliability e.g. Klimisch rating	Data relevance, including coverage / prediction of relevant parameters	Consistency with other information	Conclusive remarks (adequacy of information for given component)
Exposure information							
Dermal penetration							
Dermal metabolism							
Protein binding/reactivity							
Events in keratinocytes							
Events in dendritic cells							
Events in lymphocytes							
Adverse outcome							
Other information							
Overall conclusions	1. WoE allows a decision on the skin sensitisation potential (and possibly potency) of a substance to be made 2. WoE does not allow a decision of skin sensitisation potential (potency) of a substance to be made. Recommendation of most appropriate additional testing (could be based on other structured ITS) NB: This will also depend on the decision e.g. prioritisation, hazard identification, risk assessment						

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Compared to human		Positive predictive value	Negative predictive value	Accuracy
In vivo standard	LLNA	86 %	94 %	89 %
Individual assays	DPRA	88 %	86 %	87 %
	LuSens	85 %	81 %	83 %
	MUSST	100 %	73 %	85 %
	h-CLAT	83 %	71 %	78 %
Combinations	DPRA and LuSens	80 %	100 %	85 %
	DPRA and MUSST	100 %	69 %	81 %
	DPRA and h-CLAT	100 %	71 %	83 %
	LuSens and MUSST	100 %	67 %	80 %
	LuSens and h-CLAT	88 %	66 %	76 %
Prediction model	DPRA, LuSens and MUSST	97 %	91 %	94 %

Courtesy of BASF

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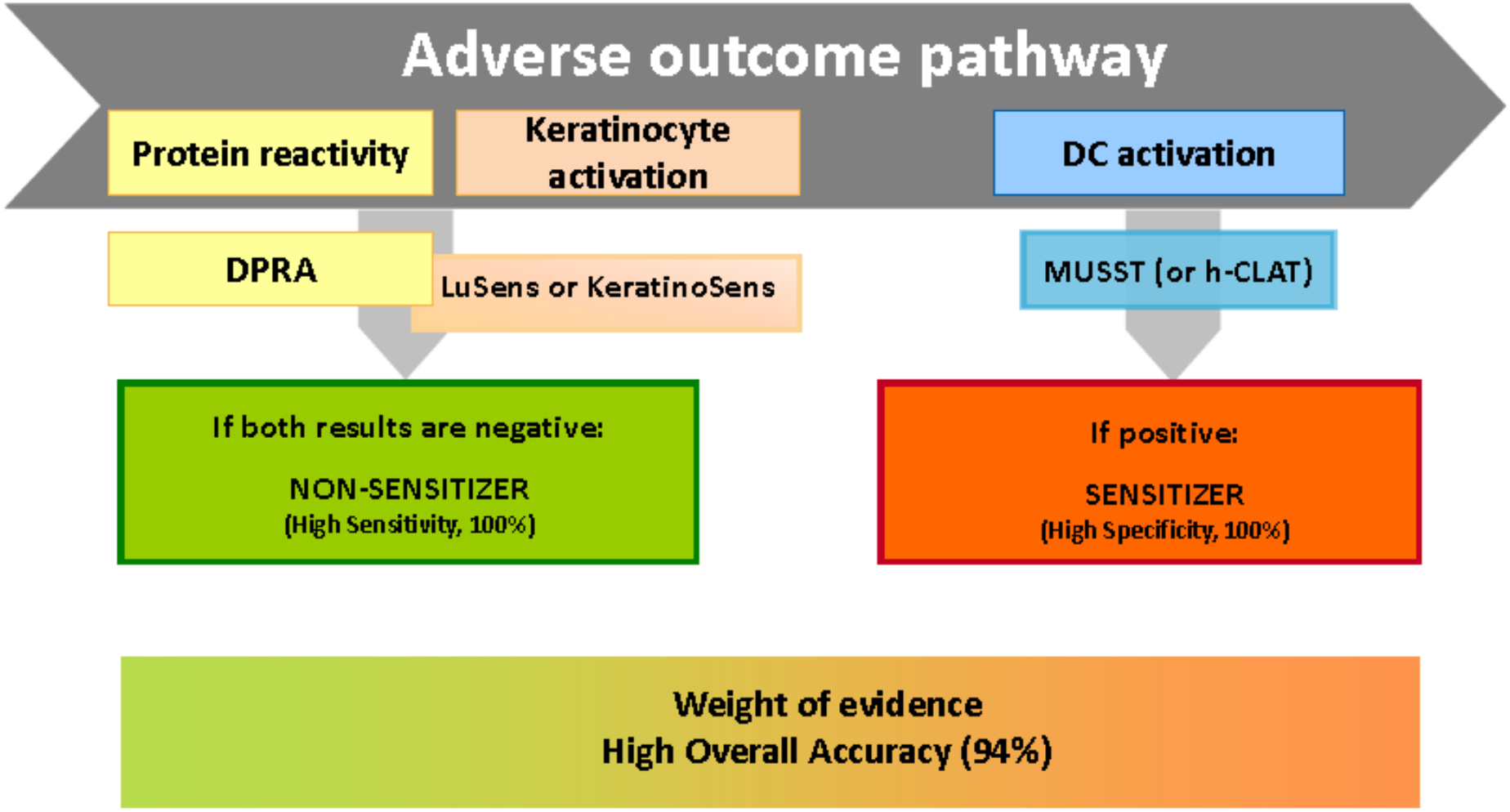

Courtesy of BASF

Conclusions

- Well know animal and human models for identifying skin sensitisers
- Regulatory framework for hazard classification
- Significant progress on in vitro methods for hazard identification
- Challenge remains potency assessment
- OECD guidance will be valuable in bringing approaches together

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