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Case study: Skin Sensitisation

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Skin sensitisation



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A skin sensitiser refers to a substance that will lead to an allergic response following repeated skin contact

United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS)

Skin sensitisation is an allergic reaction that occurs after repeated exposure to an allergen

ACD/ Delayed / Cell mediated / Chemical induced Type IV hypersensitivity

Cutaneous hypersensitivity

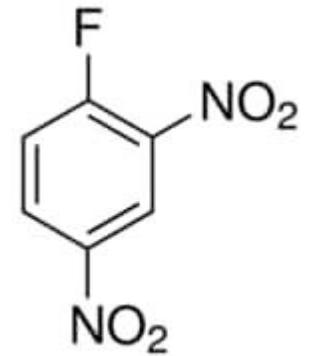
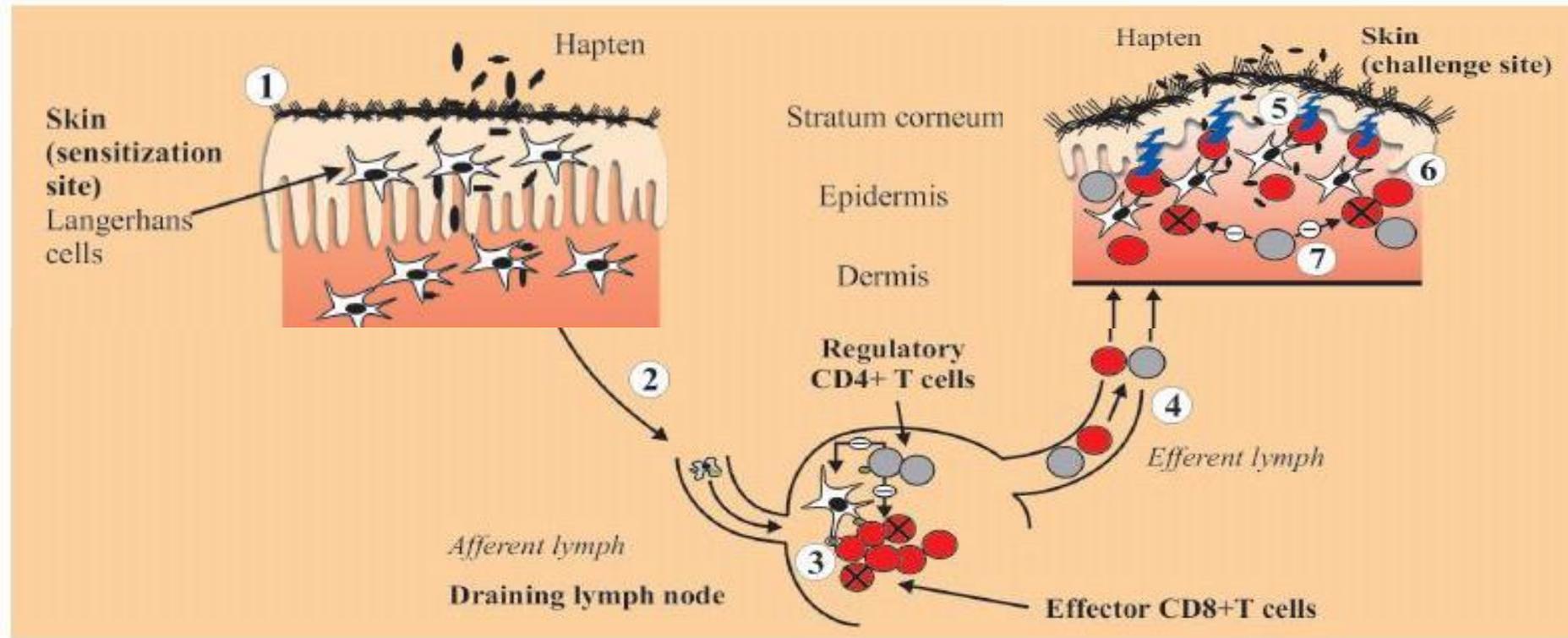


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DNFB treatment

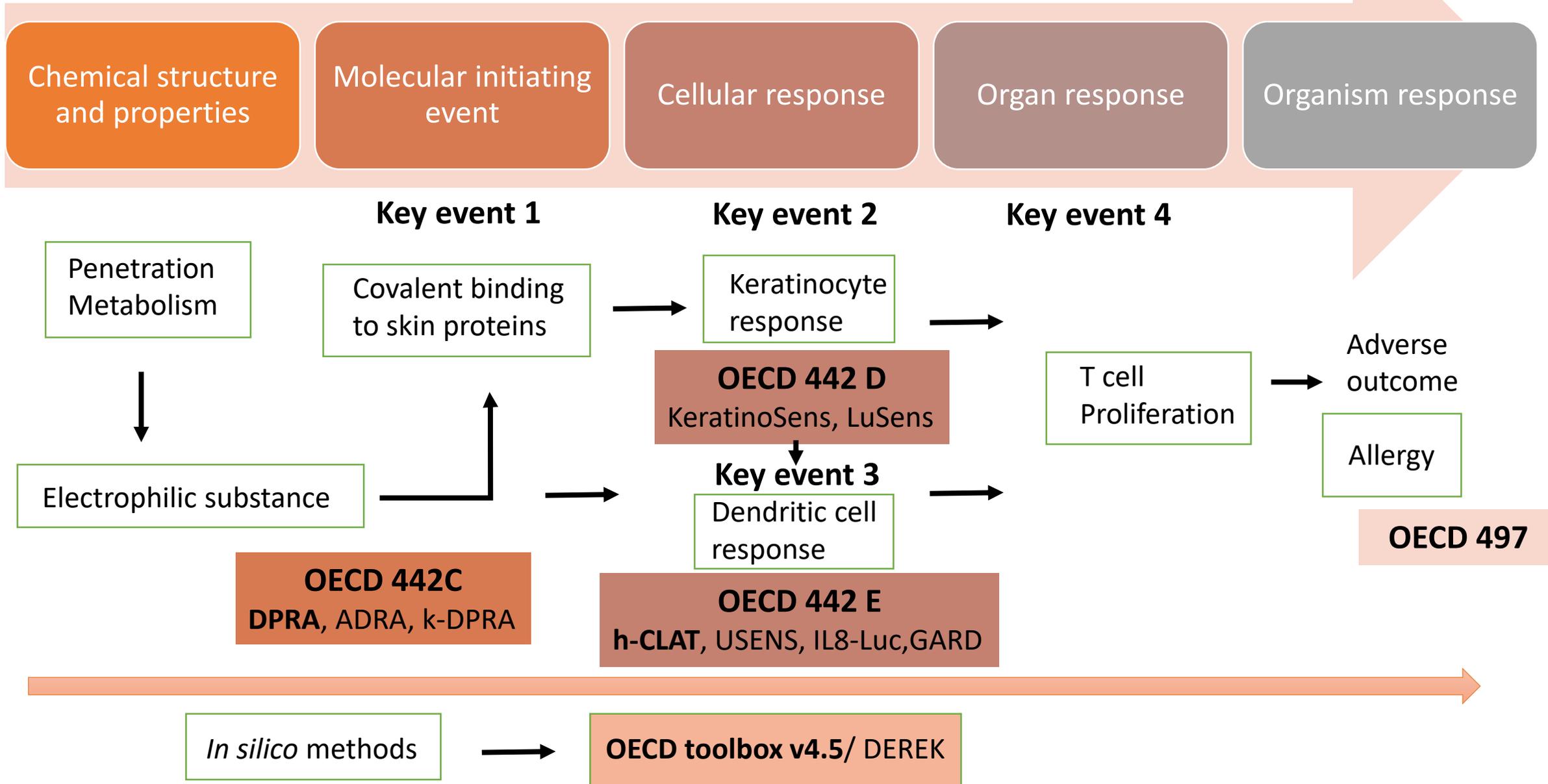
Repeated DNFB treatment



2,4-Dinitrofluorobenzene

Schematic of allergic reaction or sensitisation

Adverse outcome pathway skin sensitisation

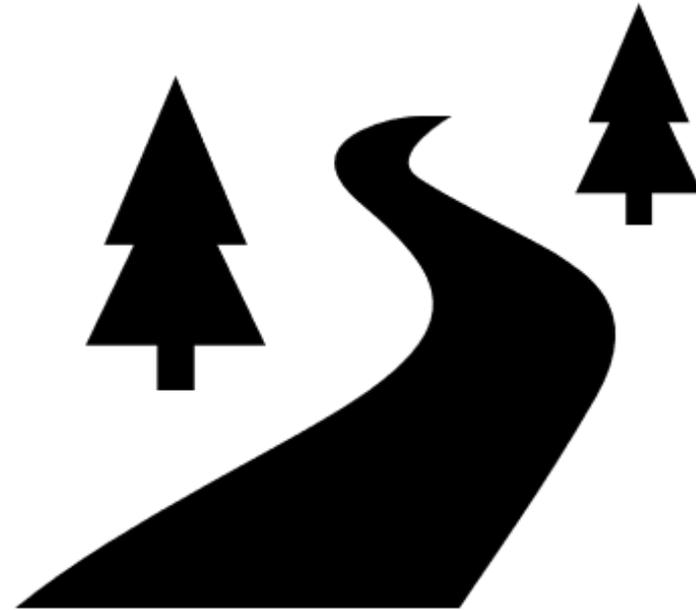
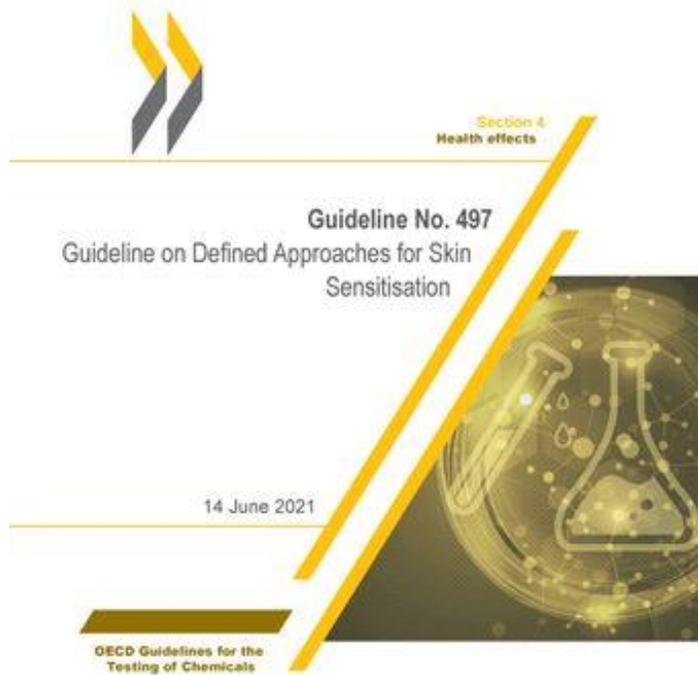


- **None** of these test methods are considered **sufficient stand-alone replacements** of animal data to conclude on skin sensitisation potential of chemicals or to provide information for potency sub-categorisation according to the UN GHS (sub-categories 1A and 1B)

No single formally validated and regulatory adopted alternative method, hence to get meaningful outcome **combination** of methods are employed

Integrated testing approach

OECD 497- Defined approach



- How to proceed ?

Interpretation of *in vitro* results

➤ ‘2 out of 3’ weight of evidence approach

DPRA, KeratinoSens, h-CLAT
(KE1, KE2, KE3)

➤ ITSv1 DA

DPRA, h-CLAT, *In Silico*- DEREK NEXUS

➤ ITSv2 DA

DPRA, h-CLAT, *In silico*- OECD toolbox v4.5



Assessing skin sensitization hazard in mice and men using non-animal test methods

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Results of 2 out of 3 determines the classification

OECD 497

DPRA

Key event 1 - Covalent

Cysteine heptapeptide

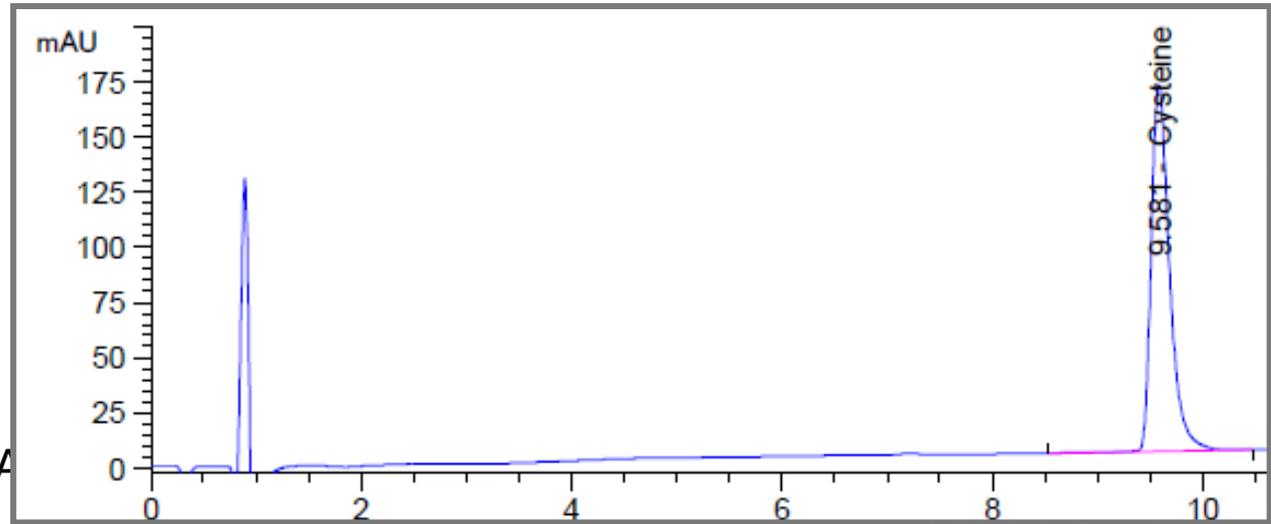


Lysine

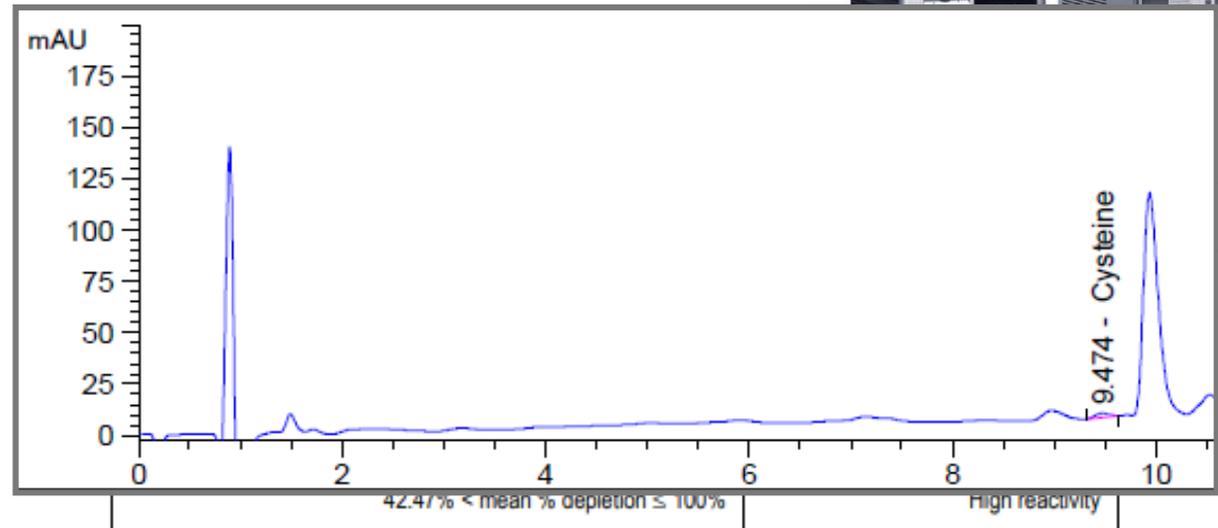


+

Test compound in appropriate solvent



24 hr incubation



DPRA Prediction ²
Negative
Positive

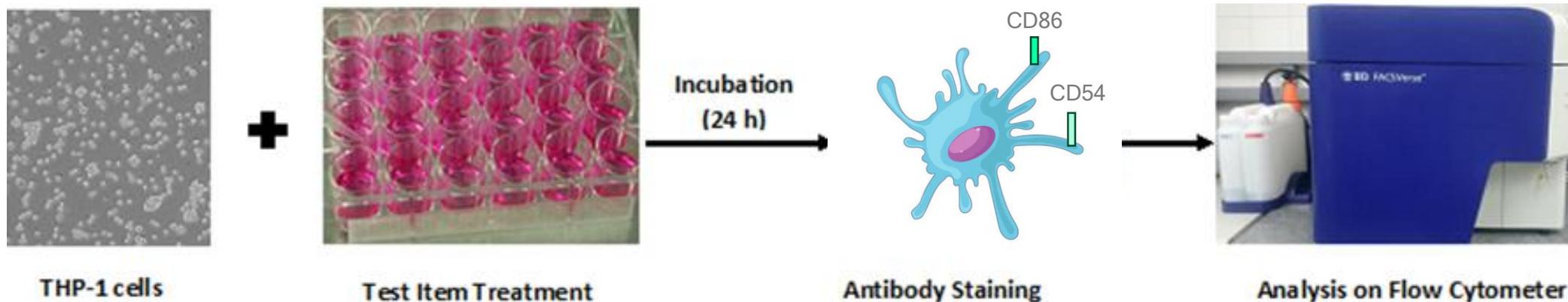
h-CLAT

Key event 3 - dendritic cell activation



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RFI

CD 86 > 150
and/or
CD 54 > 200

Sensitiser

RFI- Relative fluorescence intensity

OECD 497 : DA-Scoring pattern



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Score	h-CLAT MIT* µg/mL	DPRA mean Cysteine and Lysine% depletion	DPRA Cysteine % depletion	<i>In silico</i> (ITSv1: DEREK; ITSv2: OECD TB)
3	≤10	≥42.47	≥98.24	
2	>10, ≤150	≥22.62, <42.47	≥23.09, <98.24	
1	>150, ≤5000	≥6.38, <22.62	≥13.89, <23.09	Positive
0	not calculated	<6.38	<13.89	Negative

Potency	Total Battery Score	UN GHS
UN GHS 1A	6-7	1A – Strong sensitizer
UN GHS 1B	2-5	1B – Moderate to Weak
Not classified	0-1	Not classified – Non sensitizers

Score based classification

* Minimum induction threshold (MIT) was determined as the smaller of either EC150 or EC200.

Defined approaches included in OECD 497

DA/Method	Information Sources	Capability (Hazard and/or Potency)	Hazard Performance vs. LLNA	Hazard Performance vs. Human	Potency Performance vs. LLNA (Accuracy)	Potency Performance vs. Human (Accuracy)
2o3 DA	DPRA, KeratinoSens™, h-CLAT	Hazard	84% BA, 82% Sens, 85% Spec	88% BA, 89% Sens, 88% Spec	-	-
Comparable outcome	ITSv1 DA	Hazard, Potency	81% BA, 92% Sens, 70% Spec	69% BA, 93% Sens, 44% Spec	70% NC, 71% 1B, 74% 1A	44% NC, 77% 1B, 65% 1A
	ITSv2 DA	Hazard, Potency	80% BA, 93% Sens, 67% Spec	69% BA, 94% Sens, 44% Spec	67% NC, 72% 1B, 72% 1A	44% NC, 80% 1B, 67% 1A
LLNA (provided for comparison)	<i>in vivo</i>	Hazard, Potency	-	58% BA, 94% Sens, 22% Spec	-	25% NC, 74% 1B, 56% 1A

BA Balanced accuracy, Avg of Sensitivity of TP rate/ Specificity TN rate

Due to imbalanced nature of reference data, Specificity measurements are more uncertain than the Sensitivity



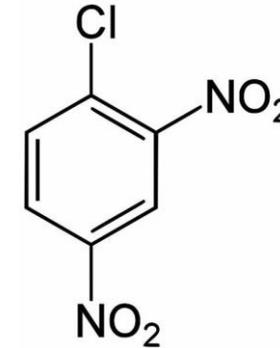
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Case studies

2,4-Dinitrochlorobenzene

CAS No: 97-00-7



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DPRA results (average depletion (%))			
% Cys-peptide depletion	% Lys-peptide depletion	% Avg depletion	Prediction
100.00	30.02	65.0	Positive*

hCLAT results		
EC150 (µg/mL)	EC200 (µg/mL)	Prediction
2	3	Positive [#]

KeratinoSens results			
I _{max}	EC _{1.5}	IC ₅₀	Prediction
11.48	2.30	7.48	Positive [^]

* = Average depletion >6.38, therefore positive

= EC150/EC200 calculated, therefore positive

[^] = I_{max} >1.5 therefore positive

DA scoring (as per OECD 497)		
DPRA	Observed % peptide depletion	Score
	≥42.47	3
hCLAT	Observed MIT	Score
	≤10	3
ITSv2: OECD TB	Prediction	Score
	Positive	1
Total Score		7
Potency (based on Score)		UN GHS 1A
Sensitisation Class		Strong Sensitiser

2-Mercaptobenzothiazole

CAS No: 149-30-4

DPRA results (average depletion (%))			
% Cys-peptide depletion	% Lys-peptide depletion	% Avg depletion	Prediction
64.62	0.0	32.3	Positive*

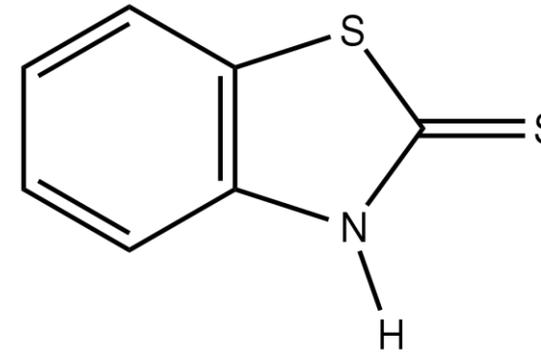
hCLAT results		
EC150 (µg/mL)	EC200 (µg/mL)	Prediction
-	48	Positive [#]

KeratinoSens results			
I _{max}	EC _{1.5}	IC ₅₀	Prediction
5.39	134.99	695.30	Positive [^]

* = Average depletion >6.38, therefore positive

= EC150/EC200 calculated, therefore positive

[^] = I_{max} >1.5 therefore positive



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DA scoring (as per OECD 497)		
DPRA	Observed % peptide depletion	Score
	≥22.62, <42.47	2
hCLAT	Observed MIT	Score
	>10, ≤150	2
ITSv2: OECD TB	Prediction	Score
	Positive	1
Total Score		5
Potency (based on Score)		UN GHS 1B
Sensitisation Class	Moderate/Weak Sensitisers	

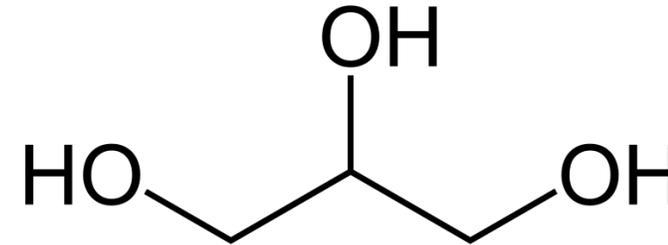
Glycerol

CAS No: 56-81-5



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DPRA results (average depletion (%))			
% Cys-peptide depletion	% Lys-peptide depletion	% avg depletion	Prediction
6.0	2.5	4.3	Negative*

hCLAT results		
EC150 (µg/mL)	EC200 (µg/mL)	Prediction
>5000	>5000	Negative [#]

KeratinoSens results			
I _{max}	EC _{1.5}	IC ₅₀	Prediction
1.05	>1000	1139.5	Negative [^]

* = Average depletion <6.38, therefore negative

[#] = EC150/EC200 not calculated, reported value is the highest conc. tested in the assay, therefore negative

[^] = I_{max} <1.5 therefore negative

DA scoring (as per OECD 497)		
DPRA	Observed % peptide depletion	Score
	<6.38	0
hCLAT	Observed MIT	Score
	NC	0
ITSv2: OECD TB	Prediction	Score
	Negative	0
Total Score		0
Potency (based on Score)		Not classified
Sensitisation Class		Non-sensitiser

Pesticides



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- Chemicals- to kill or control pests, including insects, weeds, and fungi
Insecticides, herbicides, fungicides, rodenticides, etc.

Absorbed through the skin, lungs, and digestive system

- Skin -direct contact or via clothing and equipment
- Symptoms: Itchy, red, and swollen skin, rash, blisters, etc
- Pesticides can act as allergens and cause skin sensitisation after repeated exposure

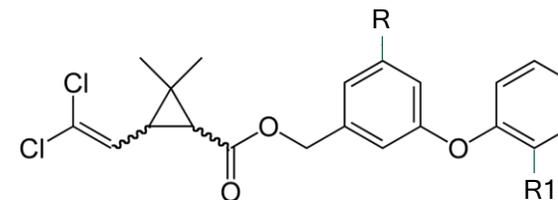
Model pesticide



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Permethrin-XYZ



DPRA results (average depletion (%))			
% Cys-peptide depletion	% Lys-peptide depletion	% avg depletion	Prediction
26.0	20.0	23.0	Sensitiser*

hCLAT results		
EC150 (µg/mL)	EC200 (µg/mL)	Prediction
45	45	Positive [#]

KeratinoSens results			
I _{max}	EC _{1.5}	IC ₅₀	Prediction
6	5	100	Positive [^]

* = Average depletion >6.38, therefore positive

[#] = EC150/EC200 calculated and MIT = 45ug/ml, therefore positive

[^] = I_{max} >1.5 therefore positive

DA scoring (as per OECD 497)		
DPRA	Observed % peptide depletion	Score
	≥22.62, <42.47	2
hCLAT	Observed MIT	Score
	>10, ≤150	2
ITSv2: OECD TB	Prediction	Score
	Positive	1
Total Score		5
Potency (based on Score)		UN GHS 1B
Sensitisation Class		Moderate/Weak Sensitisers

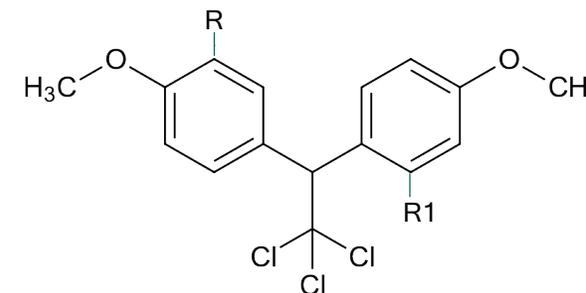
Model pesticide



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Methoxychlor-X



DPRA results (average depletion (%))			
% Cys-peptide depletion	% Lys-peptide depletion	% avg depletion	Prediction
82.0	18.0	50.0	Sensitiser*

hCLAT results		
EC150 (µg/mL)	EC200 (µg/mL)	Prediction
4	4	Positive [#]

KeratinoSens results			
I _{max}	EC _{1.5}	IC ₅₀	Prediction
5	4	32	Positive [^]

* = Average depletion >6.38, therefore positive

[#] = EC150/EC200 calculated and MIT = 4ug/ml, therefore positive

[^] = I_{max} >1.5 therefore positive

DA scoring (as per OECD 497)		
DPRA	Observed % peptide depletion	Score
	>42.47	3
hCLAT	Observed MIT	Score
	≤10	3
ITSv2: OECD TB	Prediction	Score
	Positive	1
Total Score		7
Potency (based on Score)		UN GHS 1A
Sensitisation Class	Strong Sensitisers	

Take home message



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In vitro methods provide an alternative to animal testing in skin sensitization

Defined approach is based on the combination of *in silico* and *in vitro* predictions

OECD 497 provides a defined approach to classify the compound of skin sensitization hazard as well as for the potency

Challenges



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Prevention and control of skin sensitisation from pesticides

- **Avoidance:** Avoid direct contact with pesticides, wear protective clothing and equipment, follow safety instructions, etc.
- **Substitution:** Use less toxic pesticides or alternative pest control methods
- **Education:** Train workers on pesticide safety, including proper handling, storage, and disposal
- **Surveillance:** Monitor workers for skin sensitisation and provide medical care if necessary

References



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1. OECD (2022), *Test No. 442C: In Chemico Skin Sensitisation: Assays addressing the Adverse Outcome Pathway key event on covalent binding to proteins*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264229709-en>.
2. OECD (2022), *Test No. 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264229822-en>.
3. OECD (2022), *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*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264264359-en>.
4. *Assessment of the different skin sensitization potentials of irritants and allergens as single substances and in combination using the KeratinoSens assay*, Anna M. A. De Rentiis, Mario Pink, Nisha Verma, Simone Schmitz-Spanke, *Contact Dermatitis*, 2021;84:317–325, <https://doi.org/10.1111/cod.13762>
5. OECD (2021), *Guideline No. 497: Defined Approaches on Skin Sensitisation*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/b92879a4-en>.
6. OECD (2017), *Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation*, OECD Series on Testing and Assessment, No. 256, OECD Publishing, Paris, <https://doi.org/10.1787/9789264279285-en>.

Interesting links

1. [SaferSkin™ | Edelweiss Connect](#)
2. [QSAR Toolbox](#)
3. [Pred-Skin 3.0](#)



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Let's work together to ensure the safe and responsible use of pesticides to protect both human health and the environment

In vitro services offered by JRF



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In Vitro

- Skin Corrosion Test
- Skin Irritation Test
- Eye Irritation test
- 3T3 NRU Phototoxicity Test
- Skin Sensitisation Tests
- Photosensitisation
- ADME

Ex Vivo

- Bovine Corneal Opacity and Permeability Test
- Dermal Absorption

In Silico

- 2D/3D QSAR
- EDSP
- PBPK

DPRA, kDPRA
KeratinoSens
h-CLAT
In silico



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Thank you!



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