Can currently available non-animal methods detect pre and pro-haptens?

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Abstract

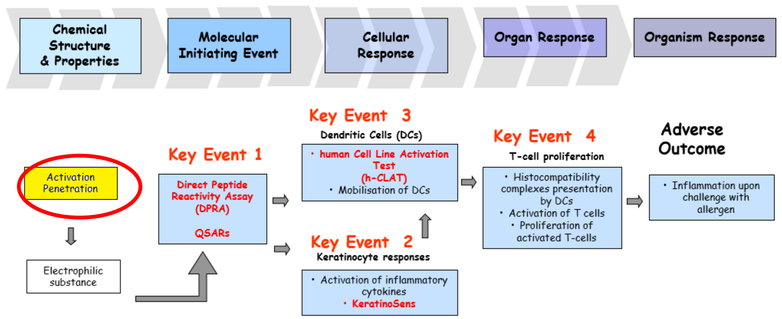
Predictive testing to identify and characterise substances for their skin sensitisation potential has historically been based on animal tests such as the Local Lymph Node Assay (LLNA). In recent years, regulations in the cosmetics and chemicals sectors has provided a strong impetus to develop and evaluate non-animal alternative methods. The AOP for skin sensitisation provides a framework to anchor non-animal test methods to key events in the pathway to help identify what tests can be combined together to generate the potency information required for risk assessment. The 3 test methods that have undergone extensive development and validation are the direct peptide reactivity assay (DPRA), the KeratinoSens™ and the human Cell Line Activation Test (h-CLAT). Whilst these methods have been shown to perform relatively well in predicting LLNA results (accuracy ~ 80%), a particular concern that has been raised is their ability to predict chemicals that need to be activated to act as sensitisers (either abiotically on the skin (pre-hapten) or metabolically in the skin (pro-hapten)). The DPRA is a cell free system, whereas the other two methods make use of cells that do not fully represent the *in vivo* metabolic situation. Based on previously published datasets of LLNA data, it has been found that approximately 25% of sensitisers are pre- and/or pro-haptens. This study, undertaken as part of an expert JRC workshop, reviewed an EURL ECVAM dataset of 127 substances for which information was available in the LLNA and the three non-animal test methods and found that 21% of sensitisers needed to be activated. The majority of these sensitisers were pre-haptens and were generally correctly identified by 1 or more of the 3 test methods. Only 6 substances were categorised exclusively as pro-haptens but these were correctly identified by one of the cell based assays with the h-CLAT detecting the majority. The analysis showed that skin metabolism is not likely to be a major consideration for assessing skin sensitisation potential and that sensitisers requiring activation can still be identified correctly using one or more of the non-animal test methods currently available.

Aims

- To determine the incidence of indirect acting sensitisers using LLNA data as a benchmark
- To determine whether the available non animal methods – DRPA, KeratinoSens™ and h-CLAT were able to correctly predict indirectly acting sensitisers

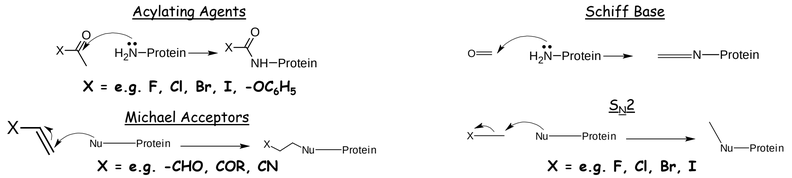
Pre and Pro-Haptens

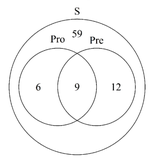
Within the skin sensitisation AOP, chemicals that cause sensitisation indirectly are known as pre or pro haptens. Pre-haptens are activated abiotically outside of the skin mainly by autoxidation. Pro-haptens are activated in the skin mainly by metabolic mechanisms.



Reaction mechanistic domain assignment

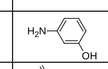
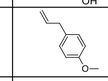
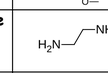
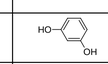
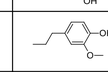
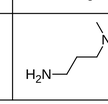
The first step of the analysis involved assigning reaction domains for each of the 127 chemicals. The reaction chemistry principles as outlined by Roberts and Aptula (2008) who proposed the following domains – Michael acceptors, Schiff base formers, Acylating agents, SNAR and SN2. An assignment of the domain included whether the substance was directly or indirectly acting.





For the dataset of 127 substances, 27 (21%) needed activation to induce sensitisation. The distribution of the sensitisers within the dataset was explored to provide a perspective of the relative proportion of pre to pro-haptens.

Performance of pro-haptens

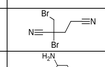
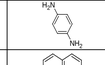
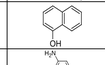
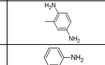
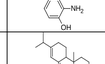
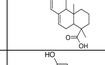
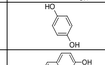
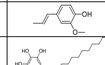
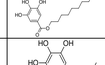
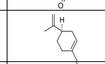
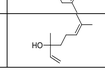
Name	Structure	LLNA	Human data	DPRA	KeratinoSens	hCLAT	Reaction Mechanistic domain
3-Aminophenol		1		0	0	1	pro-MA
4-Allylanisole		1		1	0	1	pro-MA
Ethylenediamine (free base)		1	1	0	1	1	pro-SB
Resorcinol		1	1	0	0	1	pro-MA
Dihydroeugenol		1		0	1	1	pro-MA
3-Dimethylamino propylamine		1	1	0	1	1	pro-SB

Only the h-CLAT correctly predicts all 6 pro-haptens

References

Roberts and Aptula J Appl Toxicol. 2008 28(3):377-87.

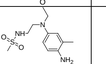
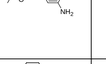
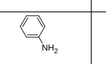
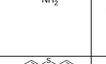
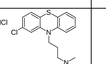
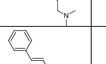
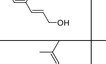
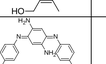
Performance of pre-haptens

Name	Structure	DPRA	KeratinoSens	hCLAT	Reaction mechanistic domain
1,2-Dibromo-2,4-dicyanobutane (MDGN)		1	1	1	pre-MA/SN2
1,4-Phenylenediamine		1	1	1	pre-MA
1-Naphthol		1	1	1	pre-MA
2,5-Diaminotoluene sulphate (PTD)		1	1	1	pre-MA
2-Aminophenol		1	1	1	pre-MA
Abietic acid		1	1	0	pre
Hydroquinone		1	1	1	pre-MA
Isoeugenol		1	1	0	pre-MA
Lauryl gallate		1	1	1	pre-MA
Propyl gallate		1	1	1	pre-MA
d-Limonene		1	0	1	pre
Linalool		0	0	1	pre

DRPA identifies 11 out of 12 pre-haptens correctly.

All nine compounds were correctly identified as sensitisers by the h-CLAT.

Performance of pre/pro-haptens

Name	Structure	DPRA	Keratino Sens	hCLAT
2-Methoxy-4-methylphenol		0	0	1
2-Nitro-1,4-phenylendiamine		1	1	1
4-(N-Ethyl-N-2-methan-sulphonamido-ethyl)-2-methyl-1,4-phenylenediamine (CD3)		1	1	1
Aniline		0	0	1
Chlorpromazine hydrochloride		0	0	1
Cinnamyl Alcohol		1	1	1
Geraniol		0	1	1
Bandrowski's Base (N,N-bis(4-aminophenyl)-2,5-diamino-1,4-quinone-diimine)		1	1	1
Eugenol		1	0	1

Conclusions

- The vast majority of indirect sensitisers were pre-haptens which were generally correctly identified by the DRPA
- Pro-haptens represented a small subset of sensitising chemicals and were identified correctly by the h-CLAT
- Indirect sensitisers that generate negative outcomes in non-animal assays such as DRPA and h-CLAT should be accepted for decision making without further testing *in vivo* unless there is a compelling scientific argument that a substance is likely to be an exclusively metabolically activated pro-hapten