Proposing alerts for 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 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)). This study reviewed an EURL ECVAM dataset containing 271 substances for which information was available in the LLNA and for one or more of the three non-animal test methods. The chemical structures of the substances were inspected and each assigned to a reaction mechanistic domain. Fifty three substances were expected to require activation. The performance of the test methods and their combinations were explored to gauge whether any one combination was better at identifying sensitisers or non sensitisers for both the dataset as a whole and for those substances requiring activation. Plausible reaction pathways were considered for each of the substances from which three structural alerts were hypothesised: autoxidation to hydroperoxides, aromatic ortho and para-diamino or di phenol derivatives, and aromatic meta-diamino/hydroxy derivatives. For each alert, the available non-animal test data was compared with the LLNA results to understand whether one or other test method was more predictive for these specific substances. Nine substances were identified as likely to undergo autoxidation resulting in the formation of hydroperoxides. The performance of the 3 methods for these substances was very mixed with no clear pattern. This was anticipated since the test results are very dependent on the actual test sample and similar mixed findings have been found with LLNA data. 14 substances that fell within the scope of being an aromatic ortho and para-diamino or diphenol derivative were identified. They all were categorised as pre and/or pro-Michael acceptors. All were correctly identified as sensitisers by any of the test methods. There were 8 substances within the Aromatic meta: diamines, aminophenols, di-phenols, and aromatic monoamines alert. This alert covered aromatic meta amino/hydroxy derivatives and aromatic monoamines. The h-CLAT was found to perform better than either of the other test methods. The ability to extract structural alerts information based on reaction domain and type of activation can be helpful in directing which key event and its associated non-animal test method might be most effective in predicting skin sensitisation potential.

Aims

Aim 1: Performance of the 3 test methods

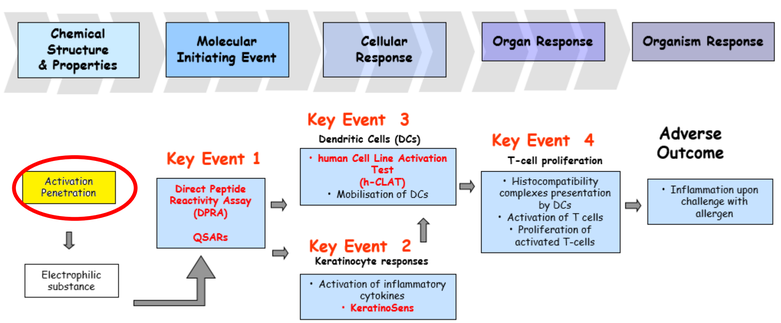
- Evaluate the performance of the test systems for the entire EURL ECVAM dataset of 271 substances and for the 53 substances requiring activation

Aim 2: Alerts for pro/pre haptens

- Investigate the feasibility of deriving specific alerts for pre- and pro- haptens and whether a test method or combination is better suited in identifying its sensitisation potential

Background

Within the skin sensitisation AOP (OECD, 2012), 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.



Performance of the non-animal test methods

	DRPA (D)	HCLAT (H)	KeratinoSens (K)	D/H	D/K	H/K	Majority vote
Sensitivity	82%	80%	76%	95%	93%	97%	87%
Specificity	75%	68%	76%	57%	55%	42%	73%
Accuracy	80%	76%	73%	84%	82%	78%	83%
Balanced Accuracy	79%	74%	73%	76%	74%	69%	80%
Accuracy for random chemicals assuming 25% incidence of sensitisers	77%	71%	71%	66%	64%	56%	76%
Probability that classed S is a true S	90%	86%	76%	84%	83%	76%	88%
Probability that classed as NS is a true NS	61%	58%	69%	84%	75%	86%	71%

For practical purposes that substances classed by the assays as S are true S would be the main concern for developers of new chemicals. They would need to have maximum confidence that they are not discarding an otherwise promising substance prematurely because of an incorrect positive prediction. For this purpose, the DRPA (90%) alone is best at predicting the sensitisation outcome (assuming a LLNA benchmark).
For the purposes of risk assessment, the main concern would be the probability that substances classed by the assays as NS are true NS, For this purpose the DRPA/H-CLAT combination would be best.

Performance of the 3 non-animal test methods for pre/pro haptens

	DRPA (D)	HCLAT (H)	KeratinoSens (K)	D/H	D/K	H/K	Majority vote
Sensitivity	69%	87%	71%	100%	83%	96%	79%
Specificity	0%	0%	20%	0%	0%	0%	0%
Accuracy	67%	79%	64%	96%	80%	86%	76%
Balanced Accuracy	34%	43%	45%	50%	41%	48%	40%
Probability that classed S is a true S	95%	87%	86%	96%	96%	89%	95%
Probability that classed as NS is a true NS	0%	0%	9%	ND	0%	0%	0%

In many cases, the small number of TN and FN in the pro/pre subgroup means that it is not possible to draw any conclusions. However, based on this exercise the DRPA appears the best for identifying true S and is equivalent to the majority vote approach.

Alerts for pre/pro haptens

There were 54 substances in the dataset that required activation.

Name	LLNA	DPRA	KeratinoSens	hCLAT
(Z)-1-(1-Methoxypropoxy) hex-3-ene	0		1	
Abietic acid	1	1	1	0
Cassyrane	1		0	
Citronellol	0		0	1
Farnesol	1		1	1
Isocyclogeraniol	0		0	
Cydrane	1		0	
d-Limonene	1	1	0	1
Linalool	1	0	0	1

There were 8 substances within the Aromatic meta: diamines, aminophenols, di-phenols, and aromatic monoamines alert. This alert comprised aromatic meta amino/hydroxy derivatives and aromatic monoamines. The h-CLAT was found to perform better than either of the other test methods.

Nine substances of these were found to be activated by formation to hydroperoxides. The performance of the non-animal test methods was very mixed for this set – this is due to the test results being highly dependent on the test sample.

Name	LLNA	DPRA	KeratinoSens	hCLAT	Chemical-based reason
1,3-phenylenediamine	1		1		pre/pro-MA
3-Aminophenol	1	0	0	1	pro-MA
4-Chloroaniline	1			1	pro/pre-MA or SB
5-Amino-2-methylphenol	1	1	1		pro/pre-MA
Aniline	1	0	0	1	pre/pro-MA or pseudo SB
N,N-Dibutylaniline	1			0	Pro/Pre-SB
Resorcinol	1	0	0	1	pro-MA
Benzocaine	0	1	1	1	pro/pre-SB

Name	LLNA	DPRA	Keratino Sens	hCLAT	Chemical-based reason
1,4-Phenylenediamine	1	1	1	1	pre-MA
2,5-Diaminotoluene sulphate (PTD)	1	1	1	1	pre-MA
2-Aminophenol	1	1	1	1	pre-MA
2-Methoxy-4-methylphenol	1	0	0	1	pro/pre-MA
2-Nitro-1,4-phenylendiamine	1	1	1	1	pro/pre-MA
3-Methylcatechol	1	1	1		pre-MA
4-(Methylamino)phenol sulfate (Metol)	1	1	1		pre/pro-MA
4-(N-Ethyl-N-2-methan-sulphonamido-ethyl)-2-methyl-1,4-phenylenediamine (CD3)	1	1	1	1	pre/pro-MA
4-Amino-m-cresol	1	1	1		pro/pre-MA
5-Amino-2-methylphenol	1	1	1		pro/pre-MA
Hydroquinone	1	1	1	1	pre-MA
Lauryl gallate	1	1	1	1	pre-MA
Propyl gallate	1	1	1	1	pre-MA
Bandrowski's Base (N,N-bis(4-aminophenyl)-2,5-diamino-1,4-quinone-diimine)	1	1	1	1	pro/pre-MA

14 substances fell within the scope of being an aromatic ortho and para-diamino or diphenol derivative were identified. They all were categorised as pre and/or pro-Michael acceptors. All but 2-methoxy-4-methylphenol were correctly identified as sensitisers by any of the test methods.

Conclusions

Aim 1: Performance of the 3 test methods

- In practical cases, the DRPA or the DRPA/h-CLAT gave rise to the best performance.

Aim 2: Alerts for pro/pre haptens

- Specific alerts for pre- and pro- haptens could be extracted from the dataset. Predictions of high confidence could be made for aromatic diamino or di phenol derivatives.

References

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