A mechanistic reinterpretation of the AOP for skin sensitisation

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Non-Animal Prediction: the 21st Century Consensus

Because of the biological complexity of the skin sensitisation process no single *in chemico* or *in vitro* assay will be an appropriate replacement for an animal-based assay such as LLNA or GPMT…
…to ensure a mechanistic basis and cover the complexity, multiple methods should be integrated into a testing strategy, in accordance with the adverse outcome pathway that describes all key events in skin sensitisation
We need an ITS based on the KEs of the AOP…but
Is that what we really need?

A Chemistry Perspective

The potential of a chemical to sensitise depends on its ability, when applied to the skin, to covalently modify the appropriate proteins **AND ON NOTHING ELSE** (Roberts and Aptula 2008)[1].

Implication

All assays, including the LLNA, can be regarded as measures of how good the chemical is at modifying the appropriate proteins *in cutaneo*[2].

Non-Animal Approaches

If we want to predict what a chemical would do without animal tests, we can try to: use chemistry mechanistic insights or…
develop a non-animal "surrogate LLNA"

Why can't we do it all by Chemistry?

We don't know which are the appropriate proteins and how they are distributed *in cutaneo*
Metabolic activation (pro-haptens)
Abiotic activation (pre-haptens)

ITS – KE Assays

DPRA (direct peptide reactivity assay) ➡ To model protein binding (KE1)

Keratinosens™ assay (ARE-Nrf2) ➡ To model activation of keratinocytes (KE2)

h-CLAT assay ➡ To model activation of dendritic cells (KE3)

Really they all model the chemical's ability to achieve KE1, the Molecular Initiating Event MIE

What are These Assays Really Doing?

If the MIE occurs to a sufficient extent, all downstream steps can be taken for granted and sensitization results

All 3 assays measure ability to covalently modify protein

None of the assays completely covers the diversity and range of chemistry leading to protein binding

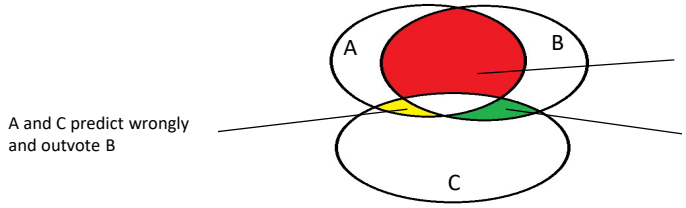
If their inapplicability domains aren't identical, combinations of assays can beat single assays

Majority voting gives wrong answers when inapplicability domains overlap

The greater the overlap of inapplicability domains, the more false predictions

The lower the overlap of inapplicability domains, the fewer false predictions

Ovals represent positive inapplicability domains, where sensitisers are wrongly predicted negative, for assays A, B and C



DPRA and Keratinosens™ are like A and B; h-CLAT is like C. The rule – positive in either DPRA or h-CLAT = predicted sensitiser, detects more sensitisers than the majority voting by DPRA, Keratinosens™ and h-CLAT on a 2 out of 3 basis.

There is no INTRINSIC reason why a single assay could not be sufficient, if its positive and negative inapplicability domains are small enough

THE GARD® ASSAY

Genomic **A**llergen **R**apid **D**etection assay[3]

Gene expression changes induced by sensitising chemicals in a human myeloid cell line - surrogate for dendritic cells

How does this compare?

DataSet 1. GARD predictions (+/-) vs LLNA data, 129 chemicals, provided by SenzaGen. 94 are also in DS2…

DataSet 2. LLNA with predictions (+/-) from DPRA, Keratinosens™ and h-CLAT 264 chemicals, from Asturiol et al (2016)[4]

Comparison[5] of GARD alone with binary DPRA + h-CLAT and with the 2 out of 3 strategy (DPRA, ARE-Nrf2, h-CLAT)

	GARD, DataSet2	GARD DataSet1	DPRA + h-CLAT	2 out of 3	Best single assay, other than GARD	
Sensitivity	90	93	97	86	DPRA	82
Specificity	84	79	52	76	DPRA	75
Accuracy	88	89	86	83	DPRA	80
PPV	94	91	86	89	DPRA	90
NPV	75	84	84	69	ARE-Nrf2	70

The inapplicability domains of GARD™ appear to be smaller than those of the other assays. This is probably because it captures better a chemical's ability to access the relevant proteins and, for sensitisers not directly reactive, to be activated to reactive species.

Conclusions

A single assay, GARD™, predicts sensitisation potential and absence of sensitisation potential better than any of, or combinations of, the OECD guideline assays DPRA, Keratinosens™ (ARE-Nrf2) and h-CLAT

We do not really need an ITS covering all KE's of the AOP

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References
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4. Asturiol D, Casati S, Worth A. (2016). Consensus of classification trees for skin sensitisation hazard prediction. *Tox. In Vitro*. 36: 197-209
5. Roberts DW. (2018). Is a combination of assays really needed for non-animal prediction of skin sensitization potential? Performance of the GARD™ (Genomic Allergen Rapid Detection) assay in comparison with OECD guideline assays alone and in combination. *Regul. Toxicol. Pharmacol.* 98: 155-160.

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