Skin Sensitization Testing of Mixtures Without Animals

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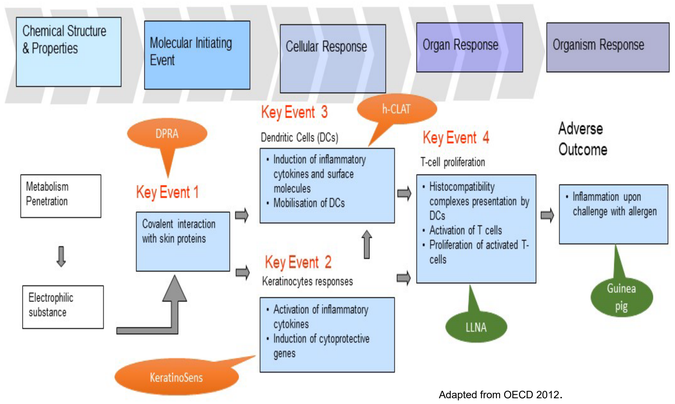
Introduction

- The assessment of skin sensitization potential is included in international regulatory safety evaluations of pesticides.
- Although several non-animal test methods based on key events of the skin sensitization adverse outcome pathway (AOP; see figure below) are internationally accepted, no single assay is recommended as a complete replacement for existing animal tests.
- Defined approaches (DAs), which integrate data from multiple methods, have been accepted to replace animal use for skin sensitization testing (OECD 2021).
- However, these DAs have been evaluated using mono-constituent substances rather than mixtures or formulations (i.e., end-use products, multi-constituent substances with defined compositions).
- To fill this data gap, we tested 27 agrochemical formulations using three non-animal methods included in OECD test guidelines (direct peptide reactivity assay [DPRA], KeratinoSens™, and human cell line activation test [h-CLAT]) to support the evaluation of three DAs for skin sensitization hazard and potency classification.
  - Using hazard classifications based on historical in vivo local lymph node assay and guinea pig assay data, these included:
    - 12 sensitizers, including 1 GHS category 1A and 11 GHS category 1B.
    - 15 non-sensitizers.

Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins

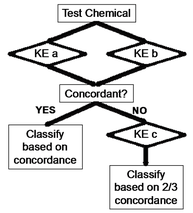


Adapted from OECD 2012.

- In vitro (orange) and in vivo (green) test methods map to various key events in the skin sensitization AOP.

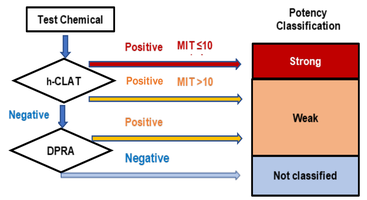
Defined Approaches for Skin Sensitization

2 out of 3



- Uses two of three concordant outcomes from the first three key events (KE) of the AOP in any order (here, labeled a, b, or c) (Bauch et al. 2012) to provide a hazard classification.
- Uses the DPRA, KeratinoSens, and the h-CLAT.
- The 2 out of 3 DA does not categorize substances for GHS potency.

KE 3/1 STS



- Uses h-CLAT (KE3) and DPRA (KE1) for hazard and potency prediction (Nukada et al. 2013).
- A chemical with a positive result in h-CLAT is classified as a strong (GHS 1A) or weak (GHS 1B) sensitizer based on the minimum induction threshold, the lowest concentration that produces a positive result for either the CD54 or CD86 marker.
- Negative h-CLAT results require testing in DPRA.

ITSv2

Score	h-CLAT MIT (µg/ml)	DPRA Mean Cysteine and Lysine Depletion (%)	DPRA Cysteine Depletion (%)	QSAR Toolbox Hazard Prediction
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	Negative	<6.38	<13.89	Negative

- Applies scores to h-CLAT (KE3), DPRA (KE1) (mean depletion is preferred when available), and a hazard prediction from QSAR Toolbox (modified from Takenouchi et al. 2015).
- Scores are summed, and a total score of 0-1 predicts a non-sensitizer result, 2-5 predicts GHS 1B sensitizer, and 6-7 predicts GHS 1A sensitizer.

Table 1. Performance of Non-animal Methods and Defined Approaches for Skin Sensitization Hazard

Performance Statistic	DPRA (n=25)	Keratino Sens (n=27)	h-CLAT (n=27)	2 out of 3 (n=26)	STS (n=27)	ITSv2 (n=24)
Accuracy (%)	64 (16/25)	81 (22/27)	52 (14/27)	73 (19/26)	52 (14/27)	54 (13/24)
Sensitivity (%)	45 (5/11)	75 (9/12)	92 (11/12)	75 (9/12)	92 (11/12)	91 (10/11)
Specificity (%)	79 (11/14)	87 (13/15)	20 (3/15)	71 (10/14)	20 (3/15)	23 (3/13)
Balanced Accuracy (%)	62	81	56	73	56	57

- Balanced accuracy for the DAs for predicting skin sensitization hazard in vivo ranged from 56% to 73%.
- Of the individual in chemico and in vitro test methods, KeratinoSens had the highest performance for predicting in vivo hazard outcomes (balanced accuracy = 81% vs. 62% for DPRA and 56% for h-CLAT) and had higher balanced accuracy than any of the DAs.

Table 2. Performance of Defined Approaches for GHS Potency Categorization

Performance Statistic	STS (n=27)			ITSv2 (n=24)		
	Not Classified (n=15)	1B (n=11)	1A (n=1)	Not Classified (n=13)	1B (n=10)	1A (n=1)
Concordance (%)	20 (3/15)	91 (10/11)	100 (1/1)	23 (3/13)	70 (7/10)	100 (1/1)
Underpredicted (%)	NA	9 (1/11)	0 (0/1)	NA	10 (1/10)	0 (0/1)
Overpredicted (%)	80 (12/15)	0 (0/11)	NA	77 (10/13)	20 (2/10)	NA

- Overall concordance with in vivo data was 52% for the STS and 46% for the ITSv2. Thus, the STS had the better performance for GHS potency classification.
- The GHS 1A substance was not underpredicted by any DA; however, both DAs overpredicted a high proportion of the non-sensitizers.
- A recently accepted international guideline on DAs for skin sensitization (OECD 2021), which included the ITSv2 but not the STS, prescribes that only high-confidence predictions should be used. Here, results for three formulations were inconclusive and thus not included in the analysis.

Conclusions

- Non-animal test methods have promising utility for evaluating the skin sensitization potential of agrochemical formulations.
  - Of the individual test methods evaluated for this project, KeratinoSens had the highest performance for predicting in vivo hazard outcomes and had higher balanced accuracy than any of the DAs (**Table 1**).
  - The DAs had overall concordance rates of 46-52% (**Table 2**) for GHS potency classification.
- Further investigation will be required to determine whether DAs can outperform individual assays such as KeratinoSens for predicting in vivo sensitization hazard of pesticide formulations in general.
- Ongoing analyses are applying the borderline evaluation from OECD Guideline 497 on defined approaches for skin sensitization to determine the effect on predictive performance of the 2 out of 3 DA.

References

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A summary of NICEATM and ICCVAM activities at the ASCCT 10th Annual Meeting is available on the National Toxicology Program website at http://ntp.niehs.nih.gov/go/ascct-2021.