GARD™skin and GARD™potency: a proof of concept study to investigate the applicability domain for agrochemical formulations.

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INTRODUCTION

Abstract

In vitro methods for detection of delayed dermal sensitization have been formally validated for regulatory use in the last two decades as an alternative to the animal use. Some methods have reached regulatory acceptance as OECD test guidelines. The Genomic Allergen Rapid Detection (GARD™) is a genomic based assay platform which is currently being assessed for inclusion in the OECD test guideline program. GARD is available in the two variants, GARDskin and GARDpotency, addresses Key Event 3 (dendritic cell activation) of the skin sensitization Adverse Outcome Pathway (AOP), and provides reliably potency information for several chemical classes.

Understanding of the applicability domain of test methods is pivotal in providing confidence in assay outcomes, facilitating regulatory uptake in specific industry sectors. The purpose of this work is to verify the applicability domain of GARDskin and GARDpotency, for the product class of agrochemical formulations.

For this proof of concept, 20 agrochemical formulations were tested using GARDskin. When GARDskin was positive, GARDpotency assay was used to determine the severity of sensitization potential. Tests were conducted according to the assay developer Standard Operating Procedures. The selected agrochemical formulations were liquid (11 water based; and 9 organic solvent based) with a balanced distribution (11 not classified; 7 GHS cat 1B; 2 GHS cat 1A, which is rare for agrochemical formulations). GARD results (available for 18 formulations at this time) were compared with *in vivo* data (mouse LLNA) already available for registration purpose, in order to verify concordance (GHS hazard and potency categories). For hazard, GARDskin was able to correctly identify 7/10 not classified (true negatives) and 7/8 GHS1B/1A (true positives), with 1 false negative and 3 false positives. The accuracy, sensitivity, and specificity for prediction of hazard were 77.8% (14/18), 87.5% (7/8) and 70.0% (7/10) , when using available LLNA results as classification reference. Additionally, GARDpotency was able to correctly identify 5 GHS cat 1B and 1 GHS cat 1A out of 7 correctly predicted sensitizer (underprediction from 1A to 1B occurred in 1 case).

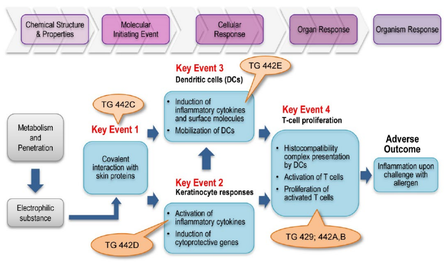
In conclusion, GARDskin and GARDpotency, showed a satisfactory performance in this initial proof of concept.

Background

Evaluation of skin sensitization potential is required for agrochemicals (pesticides active substances) and agrochemical formulations (end-use products) in many global geographies (recently reviewed by Strickland et al, 2018). More recently, higher attention is being paid to implementation of alternative in vitro approaches for skin sensitization by US, European and Brazilian regulators for the product class of agrochemical formulations.

Traditionally, *in vivo* tests described in OECD test guidelines (TG) 406 and 429 (Magnusson&Kligman or Buehler assays; local lymph node assay/LLNA) have been used. Much work has been done to elucidate the key events (KE) in the adverse outcome pathway (AOP) of skin sensitization (**Figure 1**), leading to the adoption of 5 *in vitro* alternative approaches covered under OECD TG 442 (c-d-e).

Figure 1. The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins (Adapted from Strickland et al. 2018)



In terms of agrochemical formulation, limited verification of applicability domain for OECD *in vitro* tests for skin sensitization has been published in the peer reviewed literature (Settivari et al., 2015). Despite established limitations in prediction of human outcome, the LLNA is still viewed as the gold standard and a global requirement for skin sensitization testing.

The GARD assay (OECD TGP 4.106) is a novel toxicogenomics-based *in vitro* testing platform that brings novel elements to the field of regulatory toxicology by monitoring transcriptional patterns of biomarker signatures in a human dendritic-like cell line (SenzaCells™) and provides machine-learning assisted classifications. For the endpoint of skin sensitization, the GARD™skin (Johansson et al. 2019) and the GARD™potency assay (Gradin et al. 2020) can be used for hazard identification and GHS potency sub-categorization, respectively. These assays utilize identical protocols but monitors separate biomarker signatures of genes involved in immunologically associated pathways relevant to several KE in the AOP to arrive at mechanistically based classifications (**Figure 3**).

The purpose of this work is to verify the applicability domain of GARDskin and GARDpotency, for the product class of agrochemical formulations.

RESEARCH

Methodology

- Test Items were assessed for their skin sensitizing properties in the GARD testing platform according to Standard Operating Procedures summarized in **Figure 2**.
- For calculation of input concentrations, MWs of formulations were approximated to 400g/mol, as described in Settivari et al. 2015.
- The GARD tiered approach was applied for assessment of skin sensitizing hazard and potency of each formulation (**Figure 4**).

Figure 2. Overview of the GARD testing procedure.

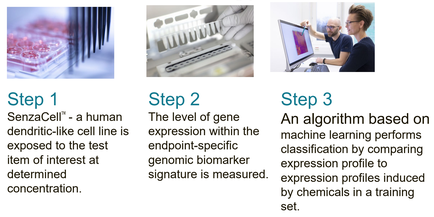


Figure 3. The GARDskin biomarker signature.
GARDskin biomarker signature monitors numerous genes and pathways associated with various KE in the AOP for skin sensitization to arrive at mechanistically relevant classifications. Figure shows a selection of the 200 genes in the signature mapped to the AOP for skin sensitization.

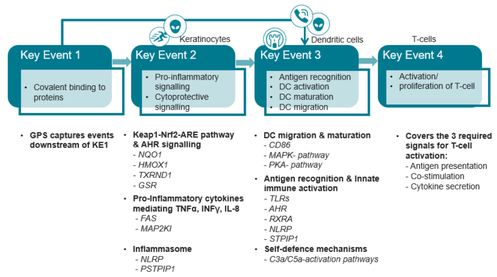


Figure 4. The GARD tiered approach.
In the first tier, all test items are being evaluated in the GARDskin assay and classified as either skin sensitizers or non-sensitizers (no Cat). In the second tier, test items identified as skin sensitizers in the first tier are further classified based on their relative skin sensitizing potency into the sub-categories 1A and 1B in the GARDpotency assay.

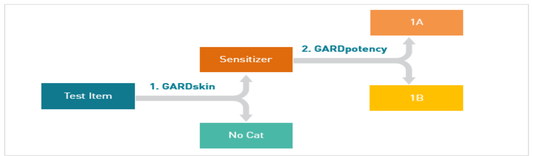


Table 4: detailed research database

Identification			Existing *in vivo* test outcome				*In vitro* test result (GARD)				
Code	Formulation group	Formulation type	GHS Classification	Test	Result	EC3	Cell Cytotox	Prediction	GARDSkin Predictions	Potency	GARDPotency Predictions
COR-4	Liquid – solvent based	EC	Cat 1A	LLNA	Positive	1	Yes	Sensitizer	True Positive	1B	underp- (1A-1B)
COR-34	Liquid – solvent based	EC	Cat 1A	GP	Positive	-	Yes	Sensitizer	True Positive	1A	correct (1A)
COR-3	Liquid – solvent based	OD	Cat 1B	LLNA	Positive	12.8	Yes	Sensitizer	True Positive	1B	correct (1B)
COR-7	Liquid – solvent based	EW	Cat 1B	LLNA	Positive	42.3	Yes	Sensitizer	True Positive	1B	correct (1B)
COR-9	Liquid – solvent based	OD	Cat 1B	LLNA	Positive	38.6	Yes	Sensitizer	True Positive	1B	correct (1B)
COR-10	Liquid – water based	SL	Cat 1B	LLNA	Positive	25.27	No	Non-Sensitizer	False Negative	Not conducted	N/A
COR-16	Liquid – water based	SL	Cat 1B	LLNA	Positive	29	No	Sensitizer	True Positive	1B	correct (1B)
COR-31	Liquid – solvent based	ME	Cat 1B	LLNA	Positive	52.3	Yes	Sensitizer	True Positive	1B	correct (1B)
COR-1	Liquid – water based	SL	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-2	Liquid – water based	SC	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-5	Liquid – water based	SL	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-6	Liquid – water based	SL	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-11	Liquid – water based	SL	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-12	Liquid – water based	SC	Not classified	LLNA	Negative	-	No	Sensitizer	False positive	(1B)	N/A
COR-13	Liquid – solvent based	EW	Not classified	LLNA	Negative	-	Yes	Sensitizer	False positive	(1B)	N/A
COR-14	Liquid – water based	SC	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A
COR-15	Liquid – water based	SC	Not classified	LLNA	Negative	-	2 out of 3	Sensitizer	False positive	(1A)	N/A
COR-18	Liquid – water based	SC	Not classified	LLNA	Negative	-	No	Non-Sensitizer	True Negative	Not conducted	N/A

Materials

- 20 liquid formulations, provided by Corteva:
  - Balanced selection for formulation types
  - Balanced selection between not classified and classified
  - GHS Cat 1A is rare for agrochemical formulations

Table 1: selected test materials

	Formulation Type*	GHS Not Classified	GHS Cat 1B	GHS Cat 1A	TOTAL
Liquid Water based (11)	SL	4	2	-	11
	SC	5	-	-	
Liquid Solvent based (9)	OD	-	2	-	7 (+2 ongoing)
	EW	1	1 (+1 ongoing)		
	EC	+1 (ongoing)	-	2	
	ME	-	1	-	
	TOTAL	10 (+1 ongoing)	6(+1 ongoing)	2	

Interim Results

Interim results available for 18/20* formulations (**Table 4**):

GARDskin performance results:
- Sensitizers: 7/8 correctly predicted + 1 False negative
- Non-sensitizers: 7/10 correctly predicted + 3 False positives.

GARDpotency results:
- GHS 1B: 5/5 correctly predicted
- GHS 1A: 1/2 correctly predicted + 1 underpredicted as 1B

A detailed summary of the predictive performance of GARDskin and GARDpotency for the evaluated agrochemical formulations can be found in **Table 2, 3 and 4**

*(1 additional solid formulation (WG: water dispersible granules) was tested and correctly predicted (Negative))

Table 2: Contingency 2x2 (GardSkin) and 3x3 tables (Tiered approach)

		Test assay (GARD)		
		Negatives	Positives	
Reference assay (in vivo)	Negatives	7	3	10
	Positives	1	7	8
		8	10	18

		Test assay (GARD)		
		NC	Cat 1B	Cat 1N
Reference assay (in vivo)	NC	7	2	1
	Cat 1B	1	5	-
	Cat 1A	-	1	1

Table 3: Cooper's statistics (GardSkin)

Accuracy	Sensitivity	Specificity	TP/FN	TN/FP	Sample size	Positives predictive value	Negative predictive value
%	%	%	n/n	n/n	n	%	%
77.8	87.5	70.0	7/1	7/3	18	70.0	87.5

CONCLUSION

Conclusion

The GARD assays were investigated for their ability to correctly detect sensitization potential of complex mixtures, such as agrochemical formulations (end-use products).

- GARDskin and GARDpotency, showed a satisfactory performance in this initial proof of concept.
- The accuracy, sensitivity, and specificity for prediction of hazard were 77.8% (14/18), 87.5% (7/8) and 70.0% (7/10), when using available LLNA results as classification reference.
- Where the GARDskin correctly predicted hazard category, the GARDpotency of GHS potency was correct in 6/7 cases, with 1 underpredicted formulation.

Recommendation

Further work needs to be undertaken to characterize:

- potential reasons for mis-predictions
- predictivity for solids formulation

Implication

Overall, the GARD assay has the potential to be another vital tool in the reduction of animal testing while ensuring human safety for agrochemical formulations.

While further testing is needed to determine the limitations of the assay for different types of formulations (and actives substances, here not addressed), these data suggest the GARD assay has good accuracy, sensitivity, and specificity values compared to currently used in vivo tests.

References

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