Assessing the Utility of the Genomic Allergen Rapid Detection (GARDskin) Assay to Detect Dermal Sensitization Potential in UVCBs and Formulated Lubricant Products

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Summary

- GARD®skin is considered to provide useful information in an overall weight of evidence assessment for difficult to test materials (mixtures, UVCBs) with challenging physical chemical properties.
- The accuracy for prediction of skin sensitization hazard ranged from 66% for formulated lubricants/greases to 100% for synthetic base oils compared to expected outcomes based on reference data.

Introduction

Advances in new approach methods and their combinations into defined approaches can provide clarity and confidence in concluding on skin sensitization potential. However, challenges remain in utilizing these approaches for difficult to test materials such as those with challenging physical chemical properties (low water solubility, hydrophobic substances) or complex compositions like Unknown or Variable Composition Complex reaction products or Biological Materials (UVCBs) and formulated mixtures. The previously developed available non-animal test methods for skin sensitization based on key-events of the adverse outcome pathway (AOP) have clearly defined requirements for test material properties that impact feasibility or confound reliance on negative results particularly for difficult to test materials and impedes the application of defined approaches to conclude on skin sensitization hazard. The study presented here aimed at investigating the skin sensitization hazard potential in the recently validated GARD®skin assay (OECD TG 442E) since it offered advantages such as a broader applicability domain, availability of additional validated test solvents for poorly soluble materials and provides mechanistically relevant information on key events from across the skin sensitization AOP. The difficult to test materials in this case study had previous data from other skin sensitization methodology with equivocal results on skin sensitizing potential.

Methods

The GARD®skin assay [OECD TG 442E] combines genomic and machine learning for hazard assessment of skin sensitizers. Test items were adequately solubilized in one of the following solvents, Ethanol (0.1% final), DMSO (0.25% or 0.1% final), or Xylenes (0.1% final). SenzaCells were incubated in triplicate under standard conditions with the test items at a max concentration of 500uM for those test materials with a known molecular weight or 100 ppm (w/v) for those without a known molecular weight. Following cell stimulations, RNA was isolated and endpoint measurements were performed using the GARD®skin genomic profile signature. Gene expression measurements were used as input values for the GARD®skin classification algorithm based on a support vector machine prediction model. The model produces outcome result sin the form of a decision value (DV), with positive DVs classifying a test material as a skin sensitizer and negative DVs classifying a test material as a non-sensitizer. Additional results from other in-vitro, in-vivo or non-test methods were collected for the test materials and assessed in a weight of evidence approach to produce a comparative outcome for the GARD®skin results.

Results

Mean Decision values for the test articles are presented in **Figure 1**. All positive and negative control test materials performed as expected. All of the RNA samples for the test materials passed the quality control check. A detailed summary of available data for each test material is provided in **Table 1** along with the outcome of a traditional weight of evidence assessment. Due to the limitations described below, applications of the currently available Defined Approaches as described in OECD TG 497 were not applicable for any of the test materials. Results indicated as "Low Confidence" suggest that the results were considered with lower weight due to the test material falling outside the applicability domain in the case of QSARs or presented technical/predictive limitations such as precipitation in the DPRA, or negative results where Log Kow > 3.5 in the h-CLAT assay. Overall, the GARD®skin accurately reflected the result of the weight of evidence assessment in 13 out of 16 test materials. GARD®skin had the highest accuracy for synthetic base oils, followed by lubricant additives and formulated products.

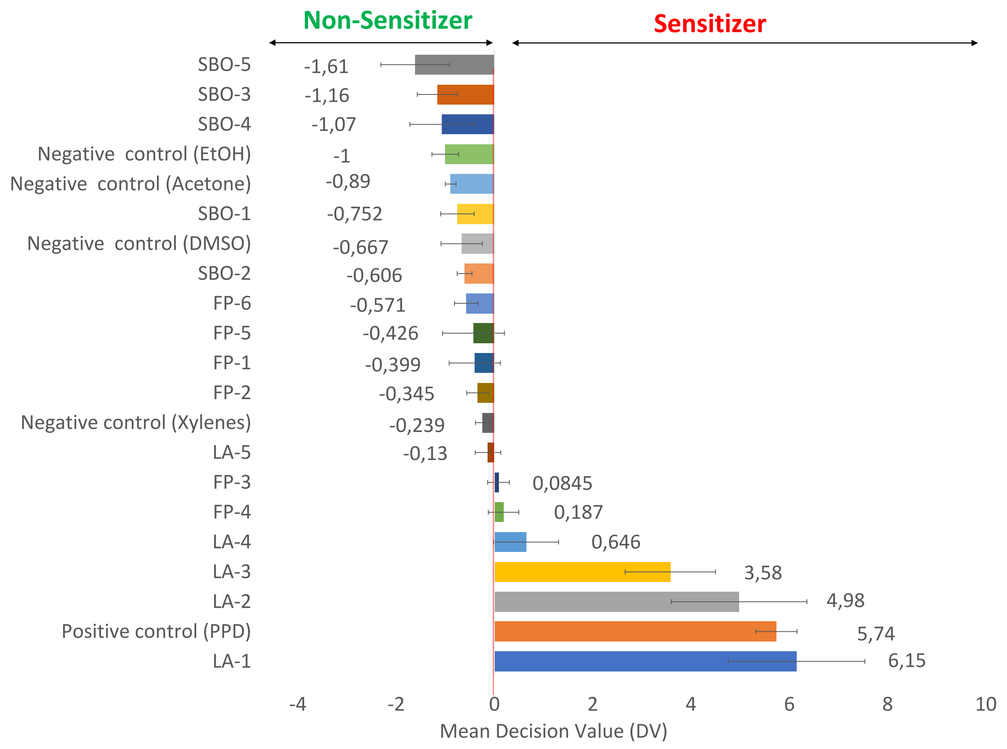

Figure 1: Mean Decision Values for Test Articles. Error bars represent standard deviation of technical replicates, N=3 for each test article as well as xylenes and acetones negative controls. N=4 for DMSO negative control and N=7 for Ethanol negative control and PPD positive control.

Conclusion

The present study aimed at evaluating the skin sensitization potential of a set of difficult to test items including UVCBs and formulated mixtures using the GARD®skin assay to inform a weight of evidence approach including conflicting or inconclusive data.

The weight of evidence conclusions for several of the test materials were confounded by conflicting and or results considered low confidence due to technical or predictive limitations in the in-vitro and in-vivo assays. In several cases, borderline positive results obtained in the in-vivo LLNA assay were considered of low confidence due to lack of dose response, irritation or single animal driven results, or results that just met the SI cut-off at the maximum feasible concentration of 100%. In all of these cases, the test materials were hydrophobic possessing high Log KOW values and low water solubility which has also recently been identified as a potential confounding factor in the LLNA (Natsch et al. 2023).

The three test materials that were incorrectly predicted compared to a traditional weight of evidence assessment were LA-2, FP-1, and FP-3. LA-2 produced positive indications of skin sensitization in guinea pigs induced with 50% concentration and challenged with 50% (5/10 first challenge; 7/10 re-challenge), however produced no evidence of skin sensitization in humans. Guinea pigs have historically been considered to be overly sensitive to calcium sulfonate chemistries and it is possible that irritation is a confounding factor. This material was considered not to have sensitizing potential based on a traditional weight of evidence. However the magnitude of response in the GARD®skin (DV = 4.98) is not borderline and would benefit from additional analysis to determine potency and if cytotoxicity is playing a role in this case. FP-1 produced evidence of skin sensitization in a standard LLNA-BrdU, with an EC 1.6 = 38.9 % and no confounding irritation. It is not readily apparent what could be contributing to the False Negative result in this case. FP-3 produce no indication of skin sensitization in a standard LLNA-BrdU assay; however the test material was only tested up to 25% this assay. The GARD®skin DV for this test material is borderline (DV = 0.0845) and would benefit from additional analysis. Further work is aimed at characterizing the specific test materials producing false positives in the GARD®skin assay. The GARD®skin Dose-Response assay could provide additional information about the dose response of LA-2 and FP-3.

References

OECD 2022, Test No. 442E: In Vitro Skin Sensitization, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing
OECD 2021, Test No. 497: Defined Approaches on Skin Sensitization, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing
Natsch et al. (2023) Reduced specificity for the local lymph node assay for lipophilic chemicals: Implications for the validation of new approach methods for skin sensitization. Reg Tox Pharm 138

Table 1: Detailed data for test articles included in this study.

Test Material	Use Category	Confounding Factors	Existing *In-Vivo* Data			Other *in-vitro/Non-test* Results		WoE Conclusion	GARD®Skin Results		
			Test	Result	EC[1] (%)	Method	Result		Cell Cytotox	Prediction	GARD®Skin Performance
SBO-1	Group V Base Oil	UVCB Log KOW >3.5 Low Wat. Sol.	Buehler	Negative	-	QSAR	Negative	Non-Sensitizer	No	Non-Sensitizer	True Negative
SBO-2	Group V Base Oil	UVCB Log KOW >3.5 Low Wat. Sol	-	-	-	QSAR	Negative	Non-Sensitizer	No	Non-Sensitizer	True Negative
SBO-3	Group V Base Oil	Log KOW >3.5 Low Wat. Sol.	LLNA-[3]HtdR	Positive/Low Confidence[2]	43	QSAR	Negative	Non-Sensitizer	No	Non-Sensitizer	True Negative
SBO-4	Group V Base Oil	Log KOW >3.5 Low Wat. Sol.	LLNA-BrdU	Positive/Low Confidence[3]	15.4	DPRA h-CLAT QSAR	Inconclusive Negative/Low Confidence Negative/Low Confidence	Non-Sensitizer	No	Non-Sensitizer	True Negative
SBO-5	Group V Base Oil	UVCB Log KOW >3.5 Low Wat. Sol.	LLNA-[3]HtdR	Positive/Low Confidence[4]	NC	QSAR	Negative/Low Confidence	Non-Sensitizer	No	Non-Sensitizer	True Negative
LA-1	Aminic Anti-wear	UVCB Low Wat. Sol. Log KOW >3.5 Irritant	LLNA-BrdU	Positive	10	Keratinosens h-CLAT QSAR	Negative Positive Positive/Low Confidence	Sensitizer Category 1B	Yes	Sensitizer	True Positive
LA-2	Calcium Sulfonate	UVCB Irritant	Buehler HRIPT	Positive Negative	-	QSAR	Negative/Low Confidence	Non-Sensitizer	Yes	Sensitizer	False Positive
LA-3	Calcium Sulfonate	UVCB	-	-	-	-	-	Sensitizer Category 1	No	Sensitizer	True Positive
LA-4	Calcium Sulfonate	UVCB	HRIPT	Positive	-	QSAR	Negative/Low Confidence	Sensitizer Category 1	No	Sensitizer	True Positive
LA-5	Calcium Sulfonate	UVCB	GPMT HRIPT	Positive Negative	-	QSAR	Negative/Low Confidence	Non-Sensitizer	No	Non-Sensitizer	True Negative
FP-1	Industrial Gear Oil	Mixture Low Wat. Sol.	LLNA-BrdU	Positive	38.9	-	-	Sensitizer Category 1B	No	Non-Sensitizer	False Negative
FP-2	Pass Vehicle Grease	Mixture Low Wat. Sol.	LLNA-BrdU GPMT	Positive/Low Confidence[5] Negative	100%	-	-	Non-Sensitizer	No	Non-Sensitizer	True Negative
FP-3	Industrial Grease	Mixture Low Wat. Sol.	LLNA-BrdU	Negative[6]	-	-	-	Non-Sensitizer	No	Sensitizer	False Positive
FP-4	Heat Transfer Grease	Mixture Low Wat. Sol.	LLNA-BrdU	Positive	12.5	-	-	Sensitizer Category 1B	No	Sensitizer	True Positive
FP-5	Multi-Purpose Grease	Mixture Low Wat. Sol.	LLNA-BrdU	Negative	-	-	-	Non-Sensitizer	No	Non-Sensitizer	True Negative
FP6	Multi-Purpose Grease	Mixture Low Wat. Sol.	LLNA-BrdU	Positive/Low Confidence[7]	85%	-	-	Non-Sensitizer	No	Non-Sensitizer	True Negative

[1] EC value dependent on LLNA method: BrdU = EC 1.6; HtdR = EC 3.0
[2] Positive results driven by one animal at the highest concentration tested
[3] Excessive irritation observed at positive dose (highest concentration tested)
[4] No consistent dose response, 50% concentration SI > 100% concentration
[5] SI = 1.6 at maximum feasible concentration (100%)
[6] Only tested up to 25% concentration
[7] SI =1.9 at maximum feasible concentration (100%)

HRIPT = Human Repeat Insult Patch Test
GPMT = Guinea Pig Maximization Test
LLNA = Local Lymph Node Assay
DPRA = Direct Peptide Reactivity Assay
h-CLAT = Human Cell Line Activation Test

SBO = Synthetic Base Oil
LA = Lubricant Additive
FP = Formulated Product
NC = not calculated
WoE = weight of evidence
In the case of UVCBs, QSAR was performed on representative constituents

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