

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 in 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. Stepwise procedure for conducting the assay is displayed in Figure 1. 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.



- Step 1**
Expose Cells (SenzaCells™) to the test sample at determined concentration.
- Step 2**
Measure the gene expression levels of 200 biomarkers, the genomic biomarker signature.
- Step 3**
GARD[®] Data Analysis Application makes a binary prediction based on gene expression analysis.

Figure 1: The GARD[®]skin assay in three steps

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.

Performance statistics for this set of difficult to test materials is presented in Table 2. 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.

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

| Test Material | Use Category | Confounding Factors | Existing <i>In-Vivo</i> Data | | Other <i>in-vitro</i> /Non-test Results | | GARD [®] skin Results | | | | |
|---------------|----------------------|---|------------------------------|--|---|-------------------------------|--|---------------------------|--------------|----------------|------------------------------------|
| | | | Test | Result | EC ¹ (%) | Method | Result | WoE Conclusion | 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 | Keratinsens 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

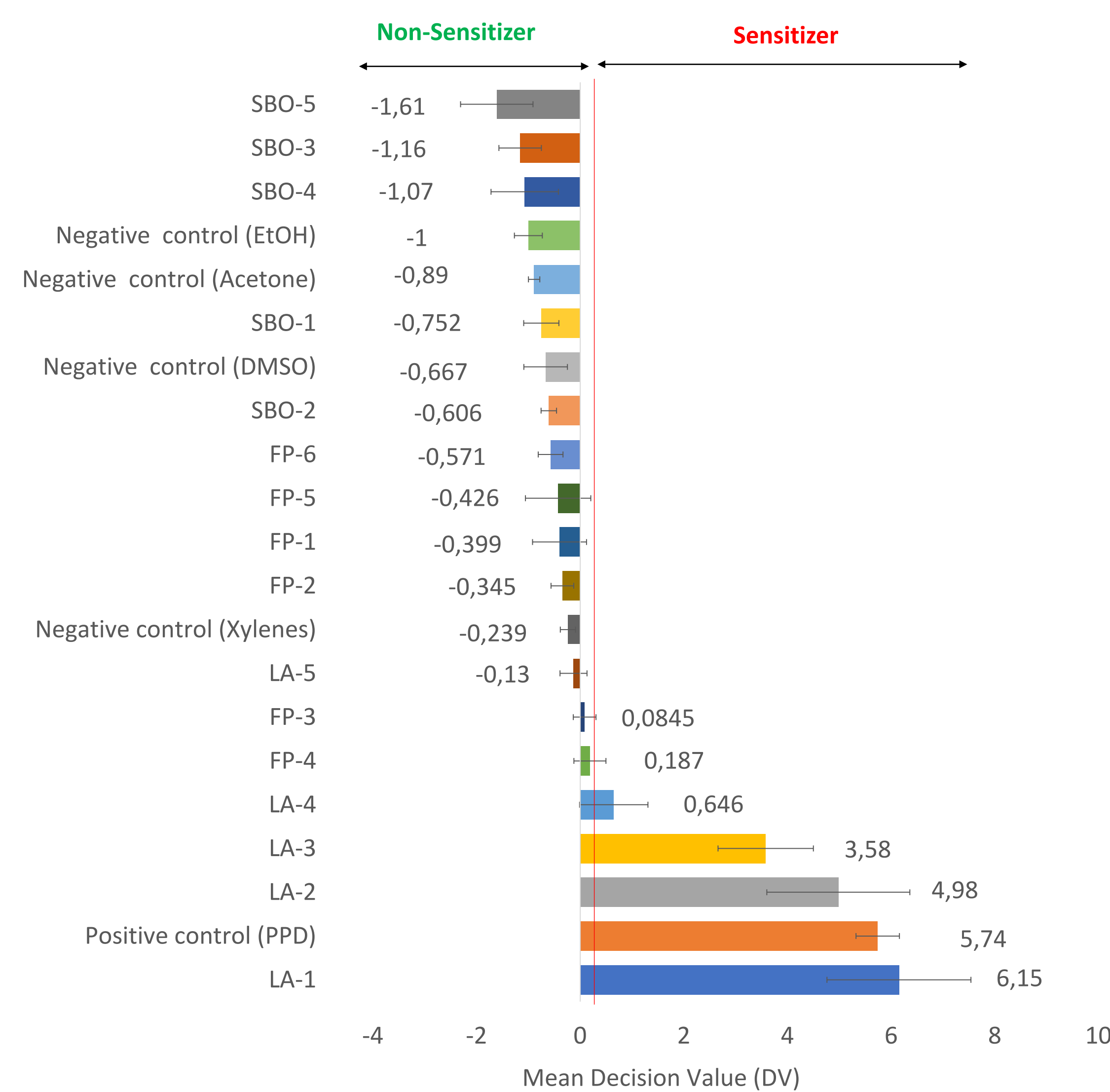


Figure 2: Mean Decision Values for Test Articles. Error bars represent standard deviation of technical replicates, N=3 for each test article as well as xylenes and acetones negative controls. N=4 for DMSO negative control and N=7 for Ethanol negative control and PPD positive control.

Table 2. GARD[®]skin performance Statistics

| | Synthetic Base Oils | | Lubricant Additives | | Formulated Products | | |
|--------------------------|---------------------|---|---------------------|---|---------------------|---|---|
| | NS | S | NS | S | NS | S | |
| GARDskin classifications | NS | 5 | 0 | 1 | 0 | 3 | 1 |
| | S | 0 | 0 | 1 | 3 | 1 | 1 |
| Accuracy | 100% | | 80% | | 66% | | |
| Sensitivity | - | | 100% | | 50% | | |
| Specificity | 100% | | 50% | | 75% | | |
| Balanced Accuracy | - | | 75% | | 63% | | |

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

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