The applicability of GARD®skin for assessing skin sensitization potential of hydrophobic esters during product development

- New Approach Methodology
- Skin Sensitization
- Hydrophobic Esters

Case study on "difficult-to-test" samples

Marie Tintin[1], Tim Lindberg[2], Lisa Theorin[2], Ulrika Mattson[2], Robin Gradin[2]
[1] Cargill R&D Centre Europe, Vilvoorde, Belgium, [2] SenzaGen AB, Lund, Sweden.

Abstract ID #996

1. Introduction

The field of skin sensitization assessment is rapidly evolving and the recent advancements in New Approach Methodologies (NAMs) have made it possible for the industry to perform *in vitro* skin sensitization testing with good predictivity across a large chemical space.

OECD has issued several guidelines to assess skin sensitization, from a mechanistic standpoint based on the key-events (KEs) of the adverse outcome pathways (AOPs), and from a strategic standpoint by defining integrated approaches to testing and assessment (IATA). To replace animal testing, the Defined Approaches (DA) - "2 out of 3" strategy is applied where at least two different in chemico and/or in vitro tests covering multiple KEs in the AOP need to be performed.

However, challenges remain for "difficult-to-test" chemicals, those with challenging physical/chemical properties and/or of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), which are often outside the applicability domain of conventional cell-based assays.

GARD®skin (OECD TG 442E) is a genomic-based assay with demonstrated applicability to "difficult-to-test" substances, including hydrophobic chemicals, natural extracts and UVCBs. This case study highlights the successful use of GARD®skin in evaluating the skin sensitization potential of two hydrophobic esters, which are typically challenging to test. When combined with *in silico* methods, this approach enables reliable and ethical skin sensitization assessments of "difficult-to-test" substances.

2. Methods

The GARD®skin assay combines genomic and machine learning for hazard assessment of skin sensitizers. A summary of the standard procedure for the GARD®skin assay is illustrated in **Figure 1**.

Test items Substance A and Substance B were adequately solubilized in Acetone and Ethanol respectively. Since both substances were insoluble in DMSO and H2O (standard solvents), a pre-evaluation study was necessary to evaluate alternative solvents.

SenzaCells were incubated in triplicate under standard conditions with the test items at a max concentration of 500µM. 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 produced outcome results in the form of a decision value (DV), with a positive DV classifying a test substance as a skin sensitizer and a negative DV classifying a test substance as a non-sensitizer.

Additional results from other *in vitro, in silico* test methods were collected for the test items and evaluated using a Weight-of-Evidence (WoE) approach to compare with the GARD®skin results.

Step 1.
Expose cells (the SenzaCell® cell line) to the extracts at determined concentration.

Step 2.
Measure the gene expression levels of 196 biomarkers, the genomic biomarker signature.

Step 3.
GARD® Data Analysis Application makes a binary prediction based on gene expression analysis.

The read-out of GARD®skin is a Decision Value (DV):
- DV ≥ 0 : Sensitizer;
- DV < 0 : Non-sensitizer

Figure 1. The GARD®skin assay in three steps. See OECD TG 442E for the full protocol.

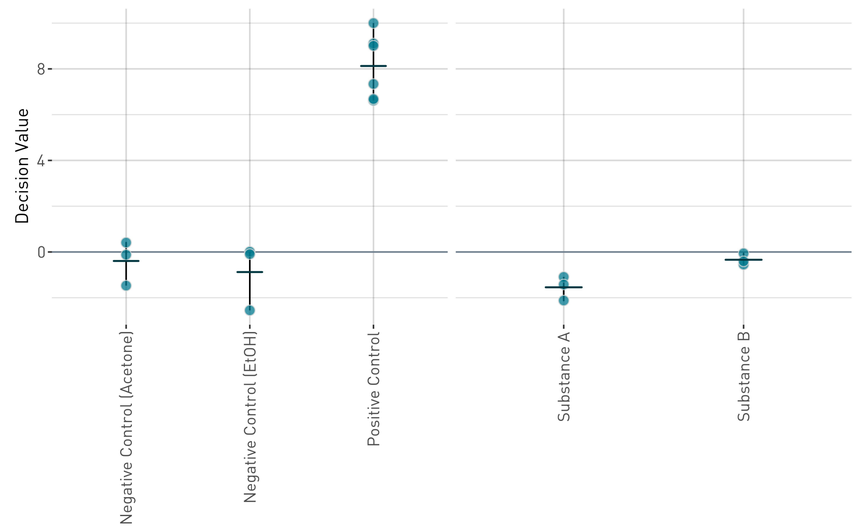

Figure 2. GARD®skin results for Test Substance A and B. Both substances are classified as non-sensitizers.

3. Results

To apply the DA "2 out of 3" described in OECD TG 497, a common approach is to conduct DPRA and h-CLAT as they cover the KE1 and KE3. **Table 1** summarizes the available data from different studies for the test substances, along with the outcome of a traditional Weight-of-Evidence assessment (WoE).

In this study, Substance A yielded a negative result in the DPRA assay, indicating that it is not a skin sensitizer. The h-CLAT method could not be applied to Substance A as it falls outside the applicability domain of the test. For Substance B, both the DPRA and h-CLAT assays produced inconclusive results. Therefore, GARD®skin was chosen as an alternative NAM to support the WoE assessment. The GARD®skin results classified both substances as non-sensitizers. (**Figure 2**)

In addition, read-across from structural analogues showed negative results for both substances, supporting the classification of both substances as non-sensitizers.

For Substance B, data is available from only one testing method (GARD®skin), therefore, a HRIPT was performed to further support the WoE, confirming the negative classification.

Table 1. Test results and classification based on Weight-of-Evidence assessment.

Substance	Studies conducted	Results	Conclusion of the WoE
Substance A	DPRA	Negative	Non-skin sensitizer
	h-CLAT	Out of the applicability domain	
	GARD®skin	Negative	
	Read-across with surrogates	Negative	
Substance B	DPRA	Inconclusive	Non-skin sensitizer
	h-CLAT	Inconclusive	
	GARD®skin	Negative	
	Read-across with surrogates	Negative	
	HRIPT	Negative	

4. Discussion and Conclusions

The aim of this study was to assess the skin sensitization potential of two ester substances of biological origin, substance A and B, using the GARD®skin assay. These substances are very hydrophobic (Log Kow > 8, water solubility < 1mg/L at 20°C), and fall outside of the applicability domain of the conventional *in vitro* assays. Additionally, it is known that fatty acid esters are prone to be over representative of false-positive outcomes in the Local Lymph Node Assay (LLNA).

Thanks to the additional pre-evaluation study and to the specific design of the GARD®skin assay, we were able to predict skin sensitizing hazard for these difficult-to-test substances. The conclusion of the testing has been confirmed by a read-across with surrogates (structural similarity > 80%), and an *in vivo* HRIPT study.

In conclusion, the inclusion of GARD®skin in the OECD test guidelines has expanded the applicability of NAMs for skin sensitization assessment.

This study demonstrates the applicability of the GARD®skin assay to assess skin sensitizing hazard of hydrophobic esters, which provides an ethical alternative to animal methods for safety assessment during product development.

> In conclusion, the inclusion of GARD®skin in the OECD test guidelines has expanded the applicability of NAMs for skin sensitization assessment.
>
> This study demonstrates the applicability of the GARD®skin assay to assess skin sensitizing hazard of hydrophobic esters, which provides an ethical alternative to animal methods for safety assessment during product development.

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
OECD 2017, Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation, OECD Series on Testing and Assessment, No. 256, OECD Publishing

Contact: Tim Lindberg, PhD, tim.lindberg@senzagen.com