In vitro skin sensitization potency assessment using GARD®skin Dose-Response: A case study on natural extracts-based skin-binding dyes and dye precursors

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- New Approach Methodologies
- Skin sensitization potency
- Skin-binding dyes

NESIL | Safe dose level

1. Introduction

Naturally occurring skin dyes and dye precursors are widely used in skin-binding colouring products, evolving from unrefined fruit extracts to more refined formulations in various ink applications. Evaluating their skin sensitization potential and establishing safe use levels for potential sensitizers are critical for product safety, which has traditionally relied on animal testing and/or human patch testing. While New Approach Methodologies (NAMs) have been developed to replace in vivo assays, a need remains for methods that can effectively and quantitatively characterize skin sensitizing potency.

GARD®skin (OECD TG 442E) is an *in vitro* assay that identifies chemical skin sensitizers based on the transcriptional profiling of a 196-gene biomarker signature in the dendritic-like SenzaCell® cell line. Predictions are made using a machine-learning algorithm, which classify test chemicals as sensitizers or non-sensitizers based on the assay's readout, Decision Values (DVs). GARD®skin Dose-Response (OECD TGP 4.106) extends this approach by evaluating test chemicals across a concentration range to establish a dose-response relationship between DVs and test chemical concentration. Sensitizing potency is quantified using cDV_0, the lowest dose required to elicit a positive response in GARD®skin. (**Figure 1**)

Depending on the need, the readout can be used to predict LLNA EC3 values, No Expected Sensitization Induction Levels (NESILs), and UN GHS/CLP classification (1A or 1B), all with high statistical significance. (Gradin et al., 2021; Lee et al., 2025)

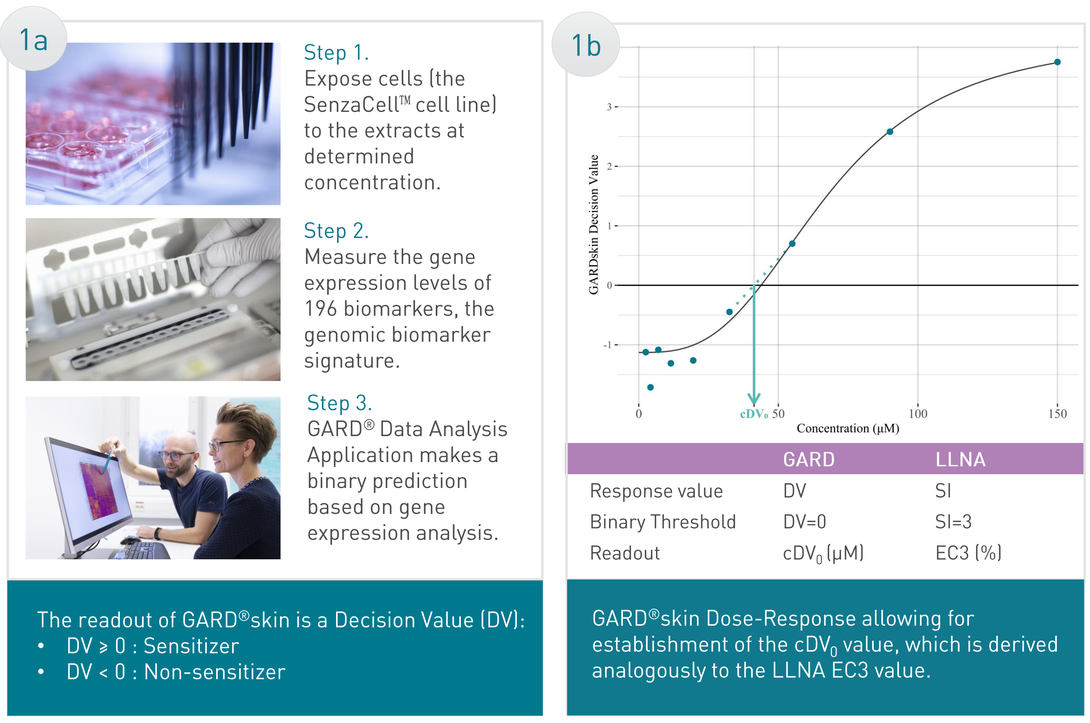

Figure 1. The experiment setup of GARD®skin Dose-Response. **1a.** shows the standard GARD®skin assay in three steps. **1b.** Illustrates the dose-response curve generated from GARD®skin testing of a test chemical in a titrated range of concentrations.

2. Methods

GARD®skin Dose-Response was applied to quantitatively assess the skin sensitization potency of seven candidate skin dyes and dye precursors. All were natural extracts-based analogue molecules with different functional groups.

Assay readouts (cDV_0) were used to predict LLNA EC3 values and NESILs, enabling further potency characterization of the candidates and supporting quantitative risk assessment and informed decision-making during product development.

3. Results

Of the seven tested natural extracts, one (Test item 7) did not induce a positive response at any of the assayed concentrations and was classified as a non-sensitizer. The remaining six extracts demonstrated varying degrees of sensitization potency, with mean cDV_0 values ranging from 3.82 µM to 34.3 µM (**Figure 2**).

Predicted LLNA EC3 values for these six extracts ranged from 0.603% to 5.94%, and NESIL estimates between 258 to 2840 µg/cm² (**Table 1**).

Table 1. Summary of the GARD® skin Dose-Response readouts (cDV_0) and corresponding potency predictions for the test items.

Test item	cDV_0 (µM)	Predicted EC3 (%)	Predicted NESIL (µg/cm²)
1	23	3.32	1570
2	3.82	0.603	258
3	34.3	4.55	2190
4	26.6	4.72	2240
5	25.1	4.89	2320
6	29.4	5.94	2840
7	Not sensitizing	Not sensitizing	Not sensitizing

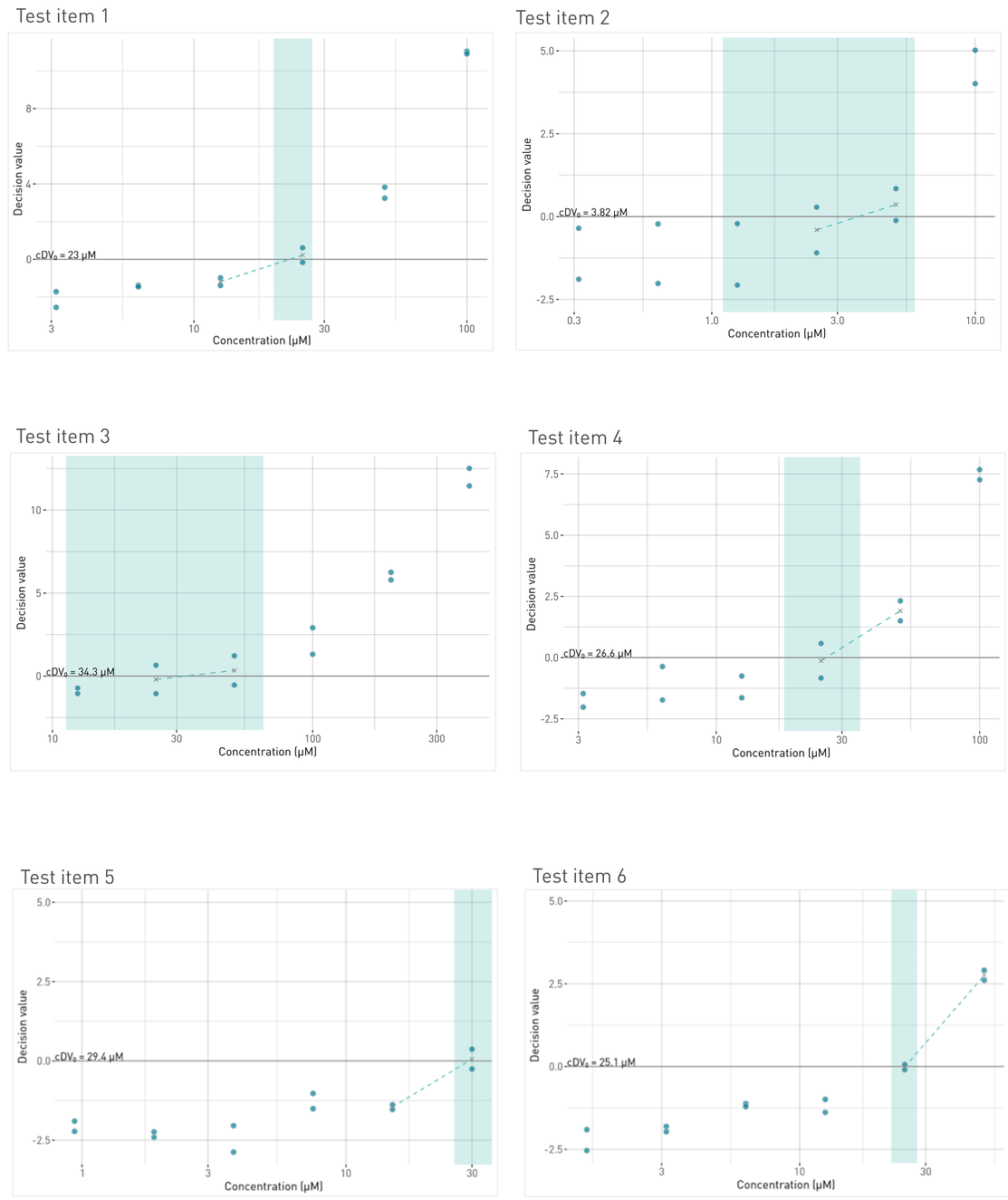

Figure 2. Dose-response curves from the GARD®skin Dose-Response assay for identified skin sensitizers (Test item 1-6), illustrating the reported DVs (y-axis) at different concentrations (x-axis). The linear interpolation between the two data points is used to estimate the concentration at DV_0. The shaded area represents a 95% confidence interval for the cDV_0 calculation.

4. Discussion and Conclusions

This case study demonstrates the utility of GARD®skin Dose-Response for quantitatively assessing the skin sensitization potency of candidate skin dyes and dye precursors used in temporary inks. Six of the seven candidates were identified as potential skin sensitizers. The assay also provides ready-to-use NESIL values, supporting the establishment of safe dose levels for identified skin sensitizers.

Overall, the study highlights the value of GARD®skin Dose-Response as a non-animal alternative for skin sensitization potency testing, offering an efficient tool to support potency ranking during product development and quantitative risk assessment.

- **GARD® skin Dose-Response provides ready-to-use NESIL values, supporting the establishment of safe dose levels for skin sensitizers.**
- **The assay is a non-animal alternative for skin sensitization potency testing, enabling potency ranking of candidate products and quantitative risk assessment.**

References:
OECD 2022, Test Guideline No. 442E: In Vitro Skin Sensitization, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.
Gradin et al, 2021, Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. Nature Scientific Reports.
Lee et al., 2025, Determining a Point of Departure for Skin Sensitization Potency and Quantitative Risk Assessment of Fragrance Ingredients Using the GARDskin Dose-Response Assay. ALTEX 2025,

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