Comparative analysis of skin sensitization thresholds for Essential Oils: Human, murine, and GARD®skin Dose-Response

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- New Approach Methodologies
- Skin sensitization thresholds
- Essential Oils

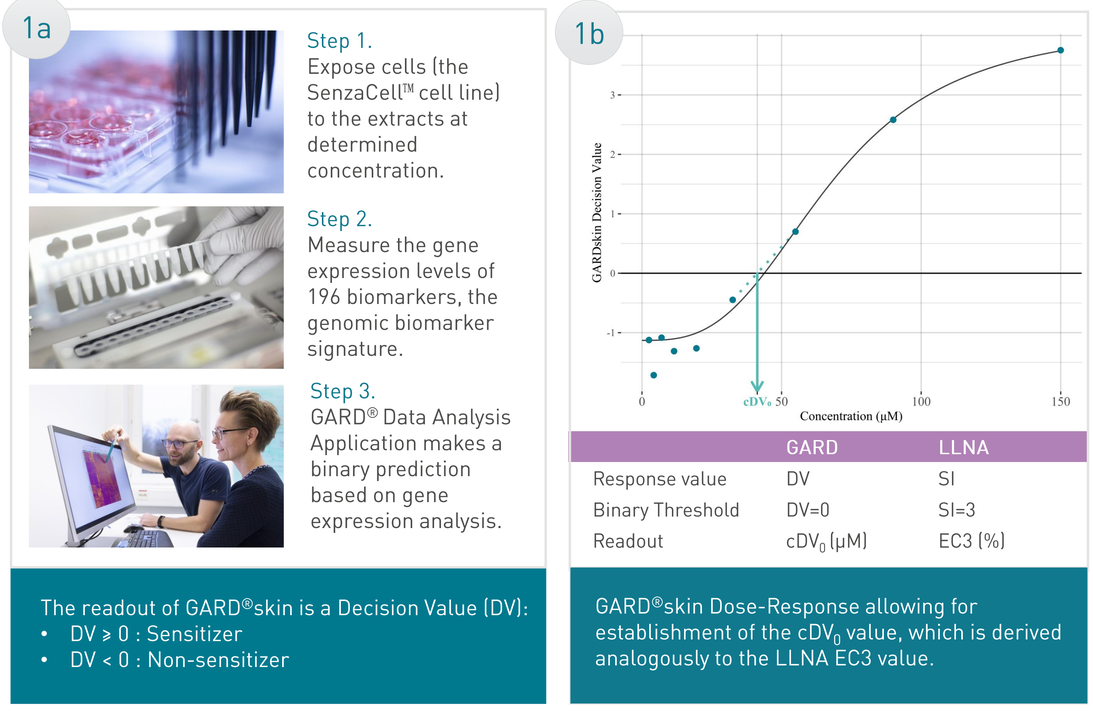
NESIL | Safe dose level | PoD for QRA

1. Introduction

Essential oils (EOs), widely used in consumer products, require robust skin sensitization hazard and potency assessment. However, dose-response thresholds for EOs remain understudied, and current classifications under CLP mixture criteria are often overly conservative. Traditional methods, such as animal testing and human patch tests, face ethical concerns, regulatory restrictions, and reliability issues. While New Approach Methodologies (NAMs) address some of these challenges, most of them are validated only for hazard identification and lack quantitative potency assessment capabilities.

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 Level (NESIL), and UN GHS/CLP classification (1A or 1B), all with high statistical significance (Gradin et al., 2021; Lee et al., 2025).



	GARD	LLNA
Response value	DV	SI
Binary Threshold	DV=0	SI=3
Readout	cDV_0 (µM)	EC3 (%)

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

Three EOs (A, B, C) were analysed using GARD®skin Dose-Response. Their skin sensitization potency threshold levels were estimated by the assay readouts (cDV_0) and predicted NESILs. NESILs were predicted using three separate methods for each EO using the following starting values: 1) the material's cDV_0, 2) the published weight of evidence NESILs for each material's primary sensitizing constituent (Api et al. 2022a, Api et al. 2022b, Api et al. 2024), and 3) the material's average EC3 value from LLNA reference data. Predicted NESIL values (µg/cm²) were calculated by multiplying the starting values by 1) the fitted parameter of the GARD®skin Dose-Response regression model transformed into base 10, 2) the inverse of the maximum relative abundance (Satyal & Poudel 2017-2022) of the primary sensitizing constituent, or 3) by 250. The predicted NESIL values were then compared to sensitization induction NOAELs (µg/cm²) from human reference data (HRIPT/HMT) for an overall weight-of-evidence analysis.

3. Results

Material A and B were classified as sensitizers in GARD®skin Dose-Response, with cDV_0 values of 18.8 and 78.4 µg/ml, and corresponding predicted NESILs of 5700 and 24000 µg/cm² (**Figure 2**). Material C was classified as non-sensitiser in GARD®skin Dose-Response, thus no cDV_0 or NESIL value was determined. **Table 1** and **Figure 3** summarize the assay readouts (cDV_0) and corresponding NESIL predictions for the three EOs, alongside the reference NESILs and NOAELs based on in-house data (constituent-based predictions, LLNA, HRIPT, HMT). The NESIL values predicted by GARD® aligned closely with those derived from reference HRIPT data and constituent predictions.

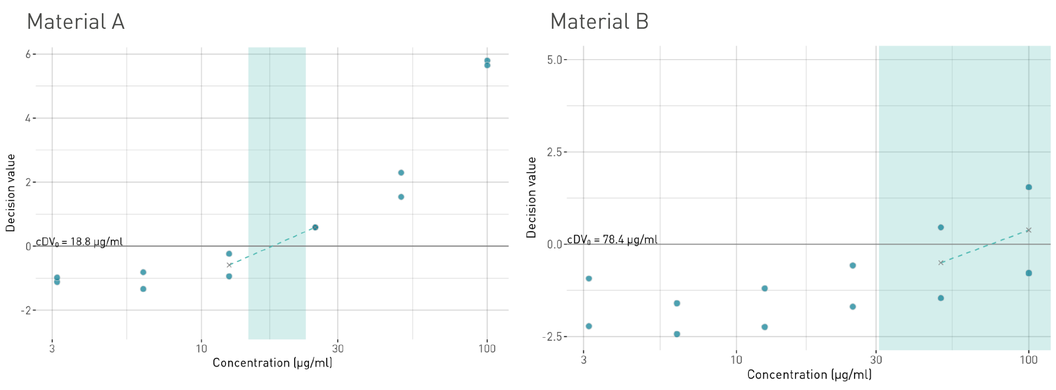


Figure 2. Dose-response curves from the GARD®skin Dose-Response assay for Material A and B, illustrating the reported DVs (y-axis) at different concentrations (x-axis). The linear interpolation between the two data points is used to estimate the concentration at DV_0. The shaded area represents a 95%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

Table 1. Summary of the GARD® readouts (cDV_0) and NESILs predictions for the three essential oils (green-shaded columns), alongside NESILs predicted from reference data.

Material	cDV_0: GARD® (µg/ml) (95% CI)	NESIL: GARD® (µg/cm²) (95% CI)	NESIL: Constituent (µg/cm²)	NESIL: LLNA (µg/cm²)	NOAEL: HRIPT (µg/cm²)	NOAEL: HMT (µg/cm²)
A	18.8 (14.6, 23.2)	5700 (3880, 8360)	6900	1800	2000	3500
B	78.4 (30.8, Inf)	24000 (16200, 34900)	29000	6700	44000	11000
C	Not Sensitizing	Not Sensitizing	20000	3900	20000	690

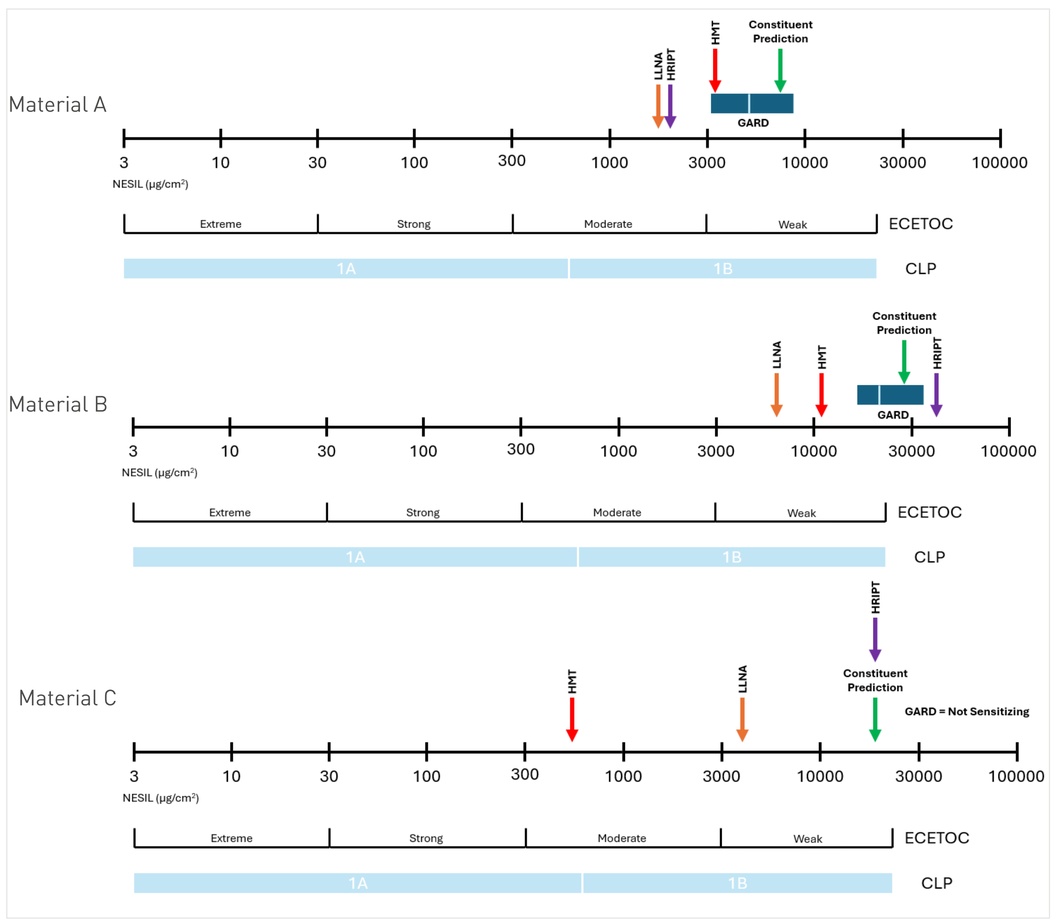


Figure 3. Comparison of NESIL predictions from GARD®skin Dose-Response with those derived from reference data. Skin sensitization potency of the three EOs are characterized by NESIL values, mapped to corresponding ECETOC potency categories and CLP classifications.

4. Discussion and Conclusions

This study demonstrates the utility of GARD®skin Dose-Response in predicting NESILs for Essential Oils, helping to bridge the gap between hazard identification and quantitative risk assessment.

The NESIL predictions from GARD®skin Dose-Response were consistent with other skin sensitization data, particularly when compared to reference human data (HRIPT) and constituent predictions. Additionally, these findings agree with previous analyses demonstrating that the assay aligns well with the murine LLNA (Gradin et al., 2021; Lee et al., 2025), and at least for the materials tested here, GARD®skin Dose-Response was a more accurate estimator of the "true" NESIL. These findings support its relevance for quantitative risk assessment of sensitization potency, especially for complex mixtures such as EOs.

While confirmatory human studies (e.g., CNIH protocols) are still recommended, the assay offers a promising non-animal approach, reducing reliance on traditional animal and human testing and advancing the application of NAMs in safety assessment. Expanding the research to more EOs is recommended to further substantiate the suitability of the assay.

- **NESIL predictions from GARD® were consistent with other skin sensitization data, particularly when compared to reference human data (HRIPT) and constituent predictions.**
- **The assay supports quantitative skin sensitization potency assessment of complex mixtures, offering a reliable non-animal alternative to traditional testing methods.**

References:
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