Practical application of the GARDskin Dose-Response assay to derive a No Expected Sensitization Induction Level (NESIL) value for confirmatory human patch studies to determine safe use level for novel fragrance ingredients

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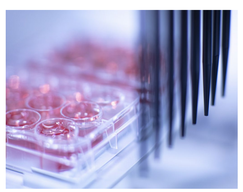
Summary

- Combining the results from the GARD®skin Dose-Response assay with other NAMs enables weight-of-evidence based approaches to determine safe use levels of novel fragrance ingredients.
- Based on the results from the weight-of-evidence approach, confirmatory human patch test and HRIPT are conducted. HRIPT is performed at the top concentration of 11250ug/cm^2. Both results are negative, confirming the predicted NESIL-value from GARD®.

Introduction

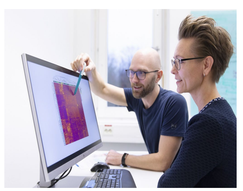
Skin sensitization, which is clinically manifested as Allergic Contact Dermatitis, is one of the required testing endpoints imposed by ECHA and the US EPA for registration of novel fragrances to ensure safety in occupational and consumer product exposures. Testing of this endpoint is conventionally performed using *in vivo* assays, such as the LLNA, but recent developments has shifted the paradigm towards the use of New Approach Methodologies (NAMs) in a battery approach of 2 out of 3 consensus. However, lack of validated potency approaches puts the NAMs at a disadvantage compared to conventional *in vivo* assays.

The GARD®skin assay (OECD TG 442E), which is based on a human dendritic-like cell line, and combining genomics and machine learning, is a next-generation NAM for hazard classification of skin sensitizers. To meet the need for informative potency information, the newly developed GARD®skin Dose-Response assay generates a dose-response curve to identify the lowest exposure concentration of the test compounds required to elicit a positive classification in the assay, the so-called cDV$_0$-value. The study presented here aimed at investigating the skin sensitizing potency of one novel fragrance ingredient, Fragrance A, using the GARD®skin Dose-Response assay. The compound has previous data from other NAMs with equivocal results on skin sensitizing potential.



Step 1
Expose Cells (SenzaCell™) to the test sample 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.

Figure 1: The GARD®skin assay in three steps

Methods

The GARD®skin assay (OECD TG 442E) [1] combines genomics and machine learning for hazard assessment of skin sensitizers. In short, compounds are assayed by stimulation of a dendritic cell-like cell line, SenzaCell™, with a single input concentration determined by max solubility or cytotoxicity (<500µM). Following RNA purification and gene expression measurements, the gene levels are used as input values for the GARD®skin classification algorithm, which is based on a Support Vector Machine prediction model. The model generates a decision value (DV) to give the final classification of the test compound. Positive DVs classifies the compound as a skin sensitizer and a negative DV classifies the compound as a non-sensitizer. Stepwise procedure for conducting the assay is illustrated in Figure 1.

The GARD®skin Dose-Response assay [2] is based on the conventional GARD®skin protocols, but instead of assaying only at one concentration, a titrated range of at least six concentrations is used to generate a dose-response curve. The dose-response curve is then used to identify the lowest concentration required to elicit a positive response in the classification (DV≥ 0). This concentration is termed the cDV$_0$–value. The cDV$_0$–value is significantly correlated with LLNA EC3 and human NESIL values, which in turn can be used as a point of departure for quantitative risk assessment. Here, novel GARD®skin Dose-Response data on one fragrance ingredient, Fragrance A, was used in a weight-of-evidence approach together with previous data generated using the DPRA assay and KeratinoSens assay.

Results

Fragrance A were identified with a cDV$_0$ value of 475 µM (Figure 2), which corresponded to a predicted EC3 value of 57.5%, a NESIL value of 37800 µg/cm^2 and a human potency category of HP5 (0.654). Results are summarized in Table 1.

Previous data from DPRA suggests Fragrance A not to be a skin sensitizer while KeratinoSens data indicates this compound to have skin sensitizing potential.

Based on the data from GARD®skin Dose-Response, further human patch test and HRIPT are conducted for confirmatory purposes. HRIPT testing is performed at the top dose of 30% (corresponding to 11250ug/cm^2). Both results are negative.

Table 1: GARD®skin Dose-Response readout measurements of Fragrance A

Test Item	cDV$_0$ (µM)	Predicted EC3 (%)	Predicted NESIL(µg/cm^2)	Predicted HP Category
Fragrance A	475 (274, Inf[III])[I]	57.5 (15.5, 100)[I]	37800 (4490, 318000)[I]	5 (0.654)[II]

I) 95 % confidence intervals for respective endpoint.
II) Probability associated with HP categories (see section 8.8 for details)
III) Inf: Upper confidence interval could not be defined.

References:

[1] OECD 2022, Test No. 442E: In Vitro Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing

[2] Gradin et al. 2021, Nature Scientific Reports, 11 (18904)

Conclusion

The study aimed at investigating the skin sensitizing potency of a novel fragrance ingredient, Fragrance A, using three NAMs, the DPRA, KeratinoSens and GARD®skin Dose-Response assays.

To move away from traditional safety testing, which includes animal studies, there is a paradigm shift towards the use of multiple NAMs in a weight-of-evidence approach when risk assessment of novel fragrance ingredients are conducted. However, the lack of established potency assays puts the alternative methods at a disadvantage as compared to the *in vivo* counterparts.

In this case, equivocal results were seen from different methods, where the DPRA assay suggesting the ingredient to be a non-skin sensitizer while KeratinoSens classify it as a skin sensitizer. GARD®skin Dose-Response suggests the test compound to be a skin sensitizer, with a predicted cDV$_0$–value of 475 µM, which is used to predict a NESIL-value of 37800 µg/cm^2 and a human potency category of HP5 (0.654). Based on the data from GARD®skin Dose-Response, confirmatory human patch test and HRIPT testing are conducted. HRIPT is performed at the top dose of 30% (corresponding to 11250ug/cm^2). Both results are negative, confirming the predicted NESIL-value generated from GARD®skin Dose-Response.

In conclusion the data presented here show how the use of the GARD®skin Dose-Response assay in combination with other NAMs can be used as a replacement of animal studies for quantitative risk assessment of novel fragrance materials.

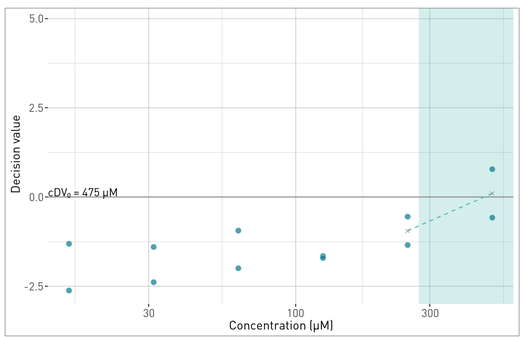


Figure 2: GARD®skin Dose-Response result illustrating the Decision Values (y-axis) for fragrance A at different concentrations (x-axis). The linear interpolation between the two data points is used to estimate the concentration at DV$_0$. The shaded area represents a 95 % confidence interval of the cDV$_0$ calculation.

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