The use of the GARD®skin Dose-Response assay to assess skin sensitizing potency in developing novel fragrance ingredients

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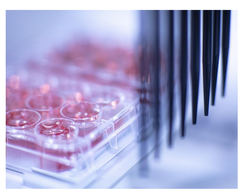
Summary

- The GARD®skin Dose-Response assay, in combination with other NAMs, can be used in a weight-of-evidence approach to determine the safe use of novel fragrance ingredients
- Based on the results from the weight-of-evidence approach, confirmatory HRIPT testing will be conducted with a concentration of 562.5 µg/cm² for Fragrance 1 and 15000 µg/cm² for Fragrance 2.

Introduction

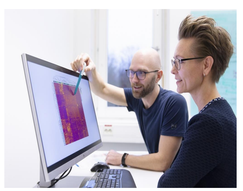
Skin sensitization, which is clinically manifested as Allergic Contact Dermatitis, is one of the required testing endpoints imposed by ECHA and the US EPA for registration of novel fragrances to ensure safety in occupational and consumer product exposures. Testing of this endpoint is conventionally performed 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 two novel fragrance materials, Fragrance 1 and Fragrance 2, in the GARD®skin Dose-Response assay. Both fragrance materials had previous data from other NAMs with equivocal results on skin sensitizing potential.



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

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 GARDskin 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 two fragrance ingredients, Fragrance 1 and Fragrance 2, was used in a weight-of-evidence approach together with previous data generated using the kDPRA assay and KeratinoSens assay.

Results

Fragrance 1 were identified with a cDV_0 value of 18.4 µM (see Figure 2A), which corresponded to a predicted EC3 value of 1.93% and a NESIL value of 659 µg/cm². Results are summarized in Table 1. kDPRA peptide depletion after 24h were 20.4% which did not categorize the test compounds as a category 1A. Additionally, KeratinoSens data predicted this compound to have skin sensitizing potential. Further, Fragrance 2 had a cDV_0 value of 296 µM (see Figure 2B) corresponding to a predicted EC3 value of 27.8% and a NESIL value of 16600 µg/cm². Results are summarized in Table 1. kDPRA data for Fragrance 2 did not categorize it as a subcategory 1A while the KeratinoSens data showed the test item not to have skin sensitizing potential.

Based on these data further HRIPT testing will be conducted, with a concentration of 562.5 µg/cm² for Fragrance 1 and 15000 µg/cm² for Fragrance 2.

Table 1: GARD®skin Dose-Response readout measurements of Fragrance 1 and 2

Test Item	cDV_0 (µM)	Predicted EC3 (%)	Predicted NESIL(µg/cm²)	Predicted HP Category
Fragrance 1	18.4 (9.33, 31.8)[I]	1.93 (1.03, 3.61)[I]	659 (263, 1650)[I]	4 (0.274)[II]
Fragrance 2	296 (123, 362)[I]	27.8 (8.69, 88.7)[I]	16600 (2580, 107000)[I]	5 (0.511)[II]

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 present study aimed at investigating the skin sensitizing potency of two novel fragrances, Fragrance 1 and 2, using three NAMs, the kDPRA, 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.

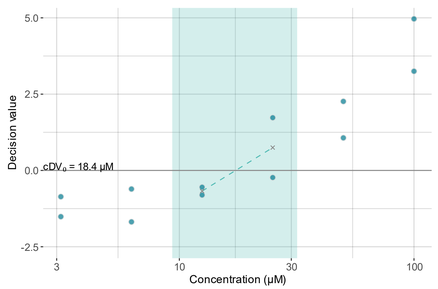


Figure 2A: GARD®skin Dose-Response result of Fragrance 1

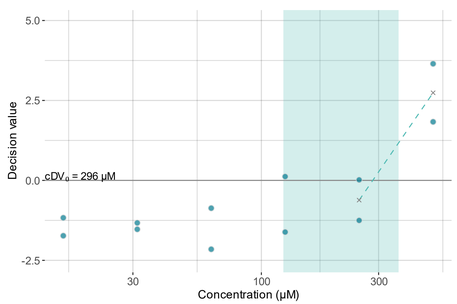


Figure 2B: GARD®skin Dose-Response result of Fragrance 2

Fragrance 1 showed similar results across the three NAMs, with the GARD®skin Dose-Response assay predicting the cDV_0–value to 18.4 µM, which in turn was used to predict a NESIL-value of 659 µg/cm².

For Fragrance 2, equivocal results were seen, where the kDPRA assay predicting the ingredient not to be a category 1A skin sensitizer while KeratinoSens predicted it as a non-skin sensitizer. GARD®skin Dose-Response predicted the cDV_0–value to 296 µM, which was used to predict a NESIL-value of 16600 µg/cm². Combining the results from all three NAM assays, a confirmatory HRIPT testing concentration was determined for both ingredients, 562.5 µg/cm² and 15000 µg/cm² for Fragrance 1 and Fragrance 2, respectively.

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.

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