Next Generation Risk Assessment (NGRA) using NAMs for skin sensitization: Reproducibility and precision of the GARDskin Dose-Response assay for PoD determination of fragrance chemicals.

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Conclusions

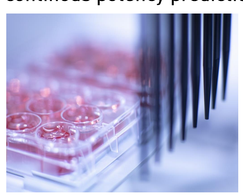
- GARD®skin Dose-Response can be used for continous predictions of skin sensitizing potency.
- The continous readout from the assay is reproducible and the assay predicts LLNA EC3 and human NESIL values with high correlation to reference benchmark data.
- The assay provides a nice tool for the fragrance industry to predict the NESIL value which can be used for conducting the quantitative risk assessment for generating the IFRA standard.

Introduction

A suite of New Approach Methods (NAMs) for hazard assessment of skin sensitizers have been adopted into OECD TG 442, and when combined into defined approaches (DA), they provide data supporting hazard classifications and GHS potency subcategorizations. However, more granular potency information, preferably on a continuous scale, is needed to derive a point-of-departure (PoD) for Next Generation Risk Assessment (NGRA) of new chemical entities, which still represents a missing element in the application of NAMs for sensitization assessments.

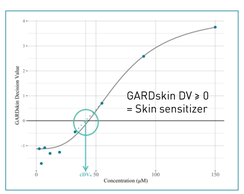
Recently, a modified version of the validated protocol of GARDskin (OECD TG 442E) was proposed. This protocol incorporates dose-response measurements for the purpose of deriving continuous potency predictions.

The aim of the following study was to evaluate the accuracy and reproducibility of the continous potency predictions from the GARDskin Dose-Response (DR) assay.



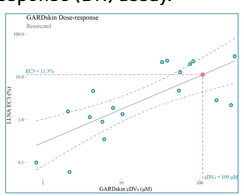
Step 1

Perform cellar stimulations in a titrated range of concentrations (n ⩾ 6). Apply the GARDskin protocol to generate decision values (DV)s.



Step 2

Generate a dose-response curve (DV vs conc). Estimate cDV_0 : the lowest concentration required to induce a positive classification (DV⩾ 0).



Step 3

Use the cDV_0 value as input into linear regression models to predict LLNA EC3 and human NOEL. The models exploit the observed linear relationship between cDV_0 and above-mentioned potency metrics.

Figure 1: The GARD®skin Dose-Response assay – conceptual overview.
The GARDskin DR model is based on the validated protocols of GARDskin, the first harmonised method that generates and interprets genomic data for a regulatory endpoint.

Methods

The GARD®skin Dose-Response protocol

The GARDskin DR protocol is based on the validated protocols of GARDskin as outlined in OECD TG 442E[1]. In short, for each test item, cellular stimulations were performed in an extended range of concentrations (⩾6), to investigate the dose-response relationship between GARDskin classifications (Decision Values, DVs) and test item concentrations. From the resulting dose-response curve, a cDV_0 value was identified, corresponding to the lowest concentration required to exceed the binary classification threshold in GARDskin (DV ⩾ 0). Resulting cDV_0 concentrations were used to predict LLNA EC3, and human NESIL values, using regression models developed to exploit the significant linear relationship between cDV_0 and above-mentioned potency metrics[2].

Study design

To ensure integrity of the study, identities of all test items (n=36) were kept blinded to the study director during the conduct of the study. For 11 of the test materials, repeated measurements were performed in separate experiments (n=3) to estimate reproducibility. Potency predictions by GARDskin DR were compared to continous LLNA EC3 values and human NESIL-values, obtained primarily from the RIFM Database (www.RIFM.org), and from OECD TG 497.

Results

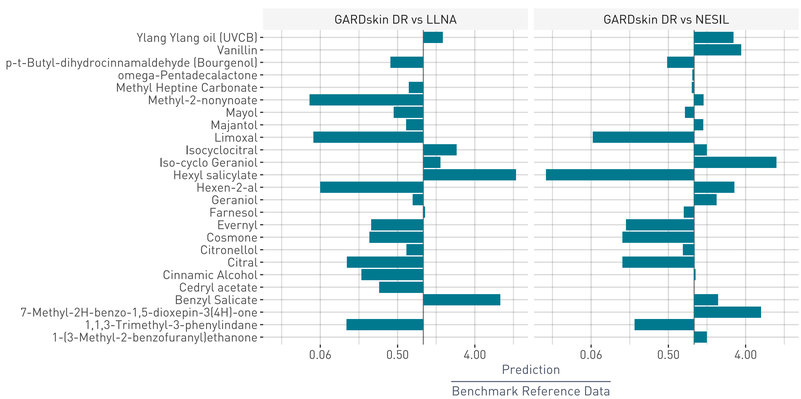


Figure 2. GARDskin Dose-Response predicted LLNA EC3 & Human NESIL values compared to reference benchmark data.
Figure shows log fold-changes between predicted values and benchmark reference data. Median fold-misprediction factors between predicted values and reference values were 3.3 and 2.0 for LLNA EC3 and human NESIL, respectively. Negative classifications were not included in the figure. For some of the Test Items, reference benchmark values were available only for one of the potency metrics. These test items were subsequently also excluded from the respective comparison.

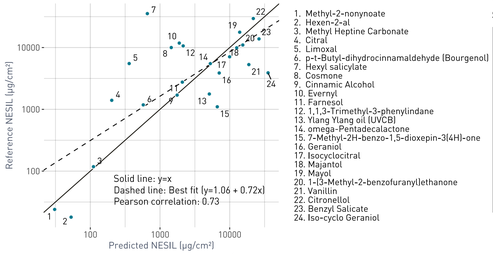


Table 1. Test items either classified as non-sensitizers, or lacked a reference NESIL value.

Test Item	GARDskin DR classification	Benchmark reference
Herbac	NC	NC
Ylanganate	NC	NC
Ethyl vanillin	NC	NC/1B
Benzyl benzoate	NC	NC
Anisyl alcohol	NC	NC/1B
Benzyl alcohol	NC	1B
Hexyl 2-methylbutyrate	NC	1B
Citrone B	NC	1B
Eucalyptol Cosmos	NC	1B
Cedryl acetate	SS	No NESIL
Pyridine	NC	No NESIL
Methyl salicylate	NC	No NESIL

NC: No category, SS: Skin Sensitizer. 1B: weak sensitizer (UN GHS/CLP). Negative classifications were either correct, or associated with borderline/weak sensitizers. No false positive classifications.

Figure 3. GARDskin Dose-Response predicted Human NESIL vs benchmark reference NESIL.
GARDskin DR predicted human NESIL values correlated well with reference NESIL values, with a Pearson correlation of 0.73.

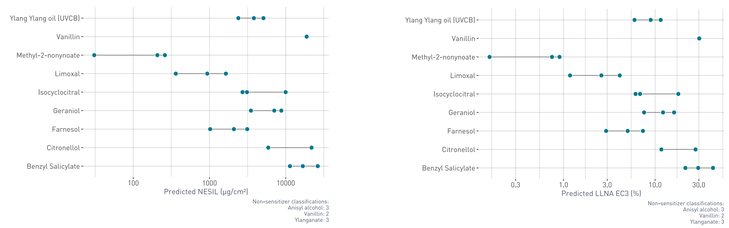


Figure 4. GARDskin Dose-Response reproducibility of predictions from repeated experiments (n=3).
Predicted NESIL and LLNA EC3 values from replicate measurements were highly reproducible, with a median range of fold-changes between replicate of 2.5.

Discussion

The results from this study represent a major step towards establishment of the GARDskin DR assay as a relevant source of information to derive a PoD for NGRA:

- GARDskin DR Predicted LLNA EC3 & human NESIL values correlated well with available reference benchmark data for most compound (median fold-misprediction factors 3.3 and 2.0).
- The continuous potency predictions from GARDskin DR were reproducible (median range of fold-changes between replicates from the three experiments of 2.5).

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)

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