Quantitative sensitizing potency assessment using GARD™skin dose-response

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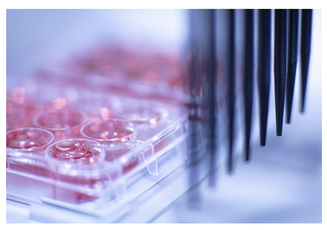
Henrik Johansson[1], Robin Gradin[1], Andy Forreryd[1], Joshua Schmidt[2] I [1]SenzaGen AB, 22381 Lund, Sweden; [2]SenzaGen Inc, Raleigh, NC

Introduction

Skin sensitizers are chemicals that possess the ability to induce hypersensitivity reactions in humans, giving rise to a condition termed allergic contact dermatitis. The capacity to limit hazardous exposure to such chemicals depend on the ability to accurately identify and characterize their skin sensitizing potential. Comprehensive efforts have been made in the scientific community to develop New Approach Methodologies (NAMs) capable of replacing *in vivo* assays. However, there is still an apparent lack of new approaches that can effectively and quantifiably characterize the skin sensitizing potency.

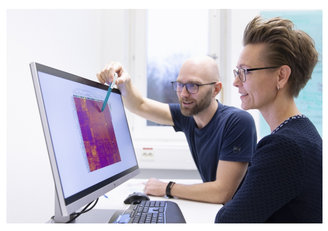
The GARDskin assay (OECD TGP 4.106) is a next-generation *in vitro* assay for skin sensitizing hazard assessment, currently progressing towards regulatory acceptance. The assay evaluates a genomic biomarker prediction signature in a dendritic cell-like cell line following test chemical exposure, to provide machine-learning assisted hazard classification of skin sensitizers (Figure 1).

Here, we introduce GARDskin Dose-Response, an adaptation from the GARDskin assay, is a novel methodology for quantitative assessment of skin sensitizing potency. We further demonstrate how the generated results can be used for downstream GHS classification, prediction of corresponding Local Lymph Node Assay (LLNA) EC3 values and quantitative risk assessment.



Step 1. Expose cells to the test sample at determined concentration.

Step 2. Measure the gene expression levels of 200 biomarkers.



Step 3. Binary prediction based on gene expression analysis.

Readout: Decision Value (DV) > 0 = Sensitizer, Decision Value (DV) < 0 = Non sensitizer

Figure 1. The GARDskin assay in three steps

Methods

GARDskin Dose-Response is conducted by performing the GARDskin assay in a titrated range of concentrations and provides a quantitative estimation of sensitizing potency, referred to as cDV_0, which corresponds to the least required dose able to generate a positive response in the GARDskin assay.

Thus, the GARDskin Dose-Response may be viewed as an *in vitro* analogue to the LLNA, as illustrated in Figure 2. In this study, GARDskin Dose-Response data was generated on 29 reference chemicals and used for investigation of the dose-response relationship between GARDskin classifications and test chemical concentration.

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

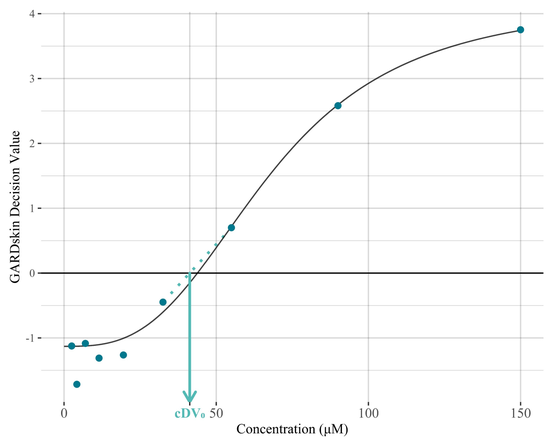


Figure 2. The experimental setup of GARDskin Dose-Response allowing for establishment of the cDV_0-value, which is derived analogously to the LLNA EC3-value.

Results

The GARDskin Dose-Response study results confirmed that cDV_0 informs on the sensitizing potency. While non-sensitizers exhibit an expected lack-of-response, cDV_0-values from skin sensitizers were associated with GHS classification labels (Figure 3), as well as strongly and significantly correlated to both human and LLNA potency reference data (rLLNA = 0.76, $p = 4.5x10^{-4}$; rHuman = 0.77, $p = 8.6x10^{-4}$) (Figure 4).

Following these findings, a draft protocol for routine testing was established, based on a titration range consisting of 6 concentrations in biological duplicate samples. The functionality of the protocols was demonstrated using Resorcinol and Formaldehyde as test chemicals, with chemical-specific dose-response relationships visualized in Figure 5.

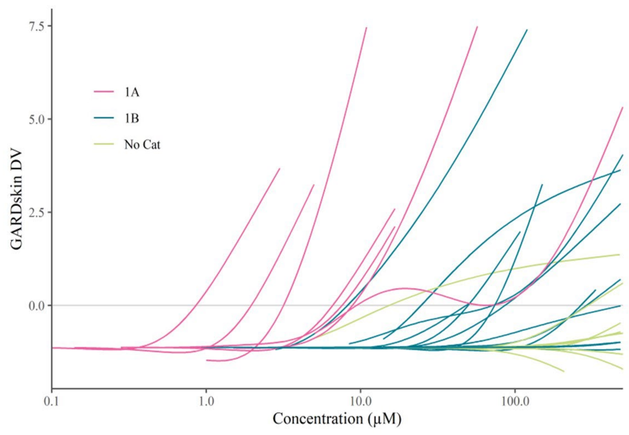


Figure 3. Individual dose-response measurements of a reference set of test chemicals. Response-curves are colored by the CLP category of the test chemical from which data points originate. Individual cDV_0-values are derived from linear interpolations of the concentrations required to generate response-values above the binary threshold (DV=0), indicated by a dashed line.

Discussion

Having identified chemical specific cDV_0-values for each test chemical, it is evident that GARDskin Dose-Response data may be directly utilized and interpreted as relative potency-characteristics. Indeed, Formaldehyde is determined to be a more potent skin sensitizer, compared to resorcinol, according to expectations.

For classification purposes, comparisons with reference data are required. Using 10 µM as a tentative cut-off for 1A/1B classification, as derived from the reference set of chemicals in Figure 3, Formaldehyde and Resorcinol are appropriately classified as 1A and 1B, respectively.

Furthermore, using the linear regression fitted by the reference set of chemicals as a tentative prediction model for LLNA extrapolation, Formaldehyde and Resorcinol are predicted to have LLNA EC3-values of 1.64% and 11.6%, respectively (Figure 5), corresponding well with expectations from historical data. Thus, GARDskin Dose-Response can be implemented in established models for quantitative risk assessment and act as a replacement for LLNA.

Conclusion

- As an adaptation from the GARDskin assay, GARDskin Dose-Response is suitable for quantitative skin sensitizing potency assessment of chemicals.
- The experimental readout, referred to as cDV_0, corresponds to the lowest dose required to elicit a positive response in GARDskin. As such, experimental protocols are analogous to the LLNA, in which the cDV_0 corresponds to the EC3-value.
- The cDV_0 may be used to directly monitor sensitizing potency, or further used to extrapolate LLNA EC3-values, estimation of Human Potency categories, or CLP 1A/1B classifications.

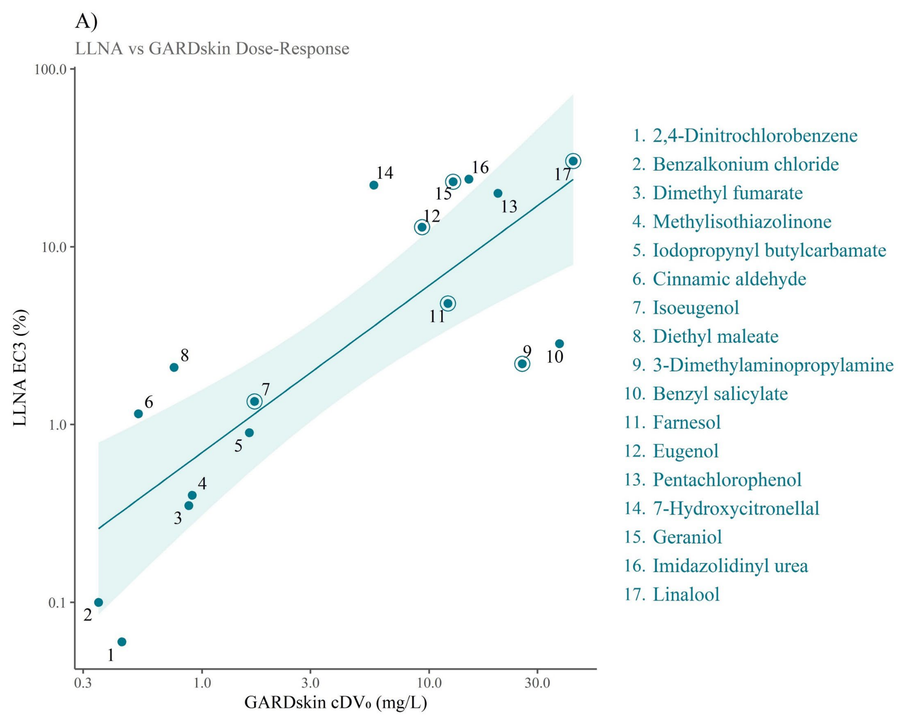


Figure 4. GARDskin Dose-Response cDV_0-values correlate with A. LLNA EC3-values and B. Human potency categories. Both correlations are statistically significant.

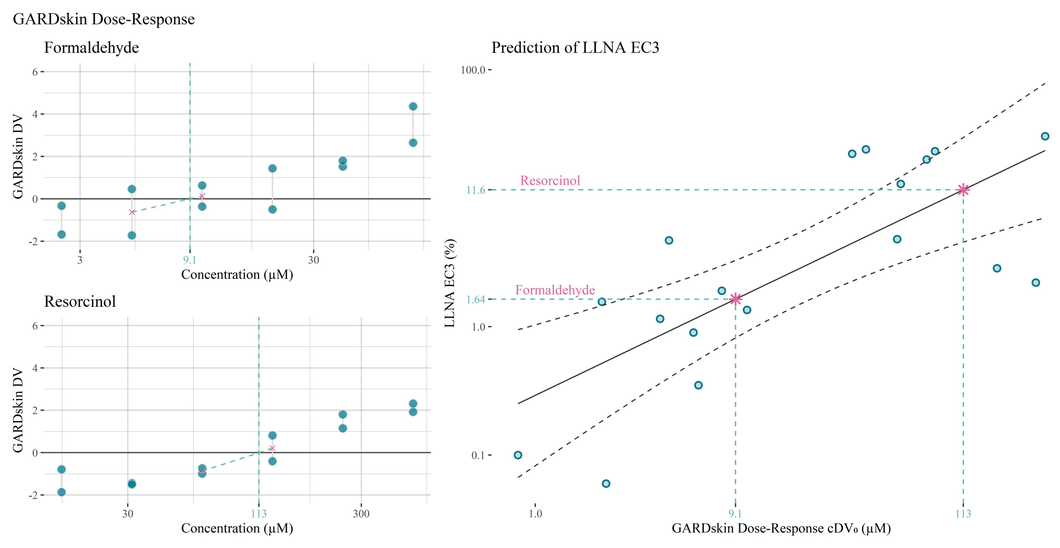


Figure 5. Demonstration of GARDskin Dose-Response protocols, using Formaldehyde and Resorcinol as illustrative examples of test chemical handling and analysis. GARDskin data is collected in a titrated range of 6 concentrations, each with 2 biological replicates. The cDV_0-value is established using linear interpolation of the mean. Downstream interpretation of data allows for relative potency comparison, GHS classification, LLNA EC3 predictions and implementation in established strategies for quantitative risk assessment.

Contact information: Henrik Johansson, PhD, Email: henrik.johansson@senzagen.com