Improved Confidence of Quantitative Sensitizing Potency Assessment for Point of Departure Using GARD®skin Dose-Response

Frédéric Amaral[1], Andy Forreryd[2], Robin Gradin[2], Fleur Tourneix[1], Ulrika Mattson[2], Johan Andersson[2], Nathalie Alépée[1], Henrik Johansson[2] | [1]L'Oréal Advanced Research & Innovation, Aulnay sous Bois, France; [2]SenzaGen AB, Lund, Sweden.

- **New Approach Methodology**
- **Quantitative Potency Assessment**
- **PoD for Skin Sensitization**

Predicting LLNA EC3 | Human NOEL

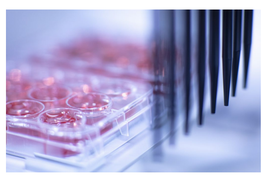
Abstract ID #661

1. 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 depends on the ability to accurately identify and characterize their skin sensitizing potential.

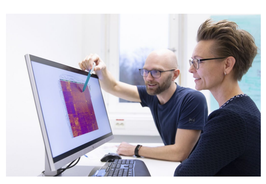
Comprehensive efforts have been made in the scientific community to develop New Approach Methodologies (NAMs) capable of replacing *in vivo* assays. However, there is still an apparent lack of new approaches that can effectively and quantifiably characterize the skin sensitizing potency, thereby generating data which can be used as a Point of Departure (PoD) in Next-Generation Risk Assessment (NGRA) strategies.

To this end, the GARD®skin Dose-Response method was recently introduced as an adaptation of the conventional GARD®skin OECD TG 442E method (**Figure 1**). GARD®skin Dose-Response is conducted by performing the GARD®skin assay in an extended range of concentrations and provides a quantitative estimation of sensitizing potency, referred to as cDV_0, which corresponds to the lowest required dose able to generate a positive response in the GARD®skin assay (**Figure 2**).



Step 1.
Expose cells (the SenzaCell™ cell line) to the extracts at determined concentration.

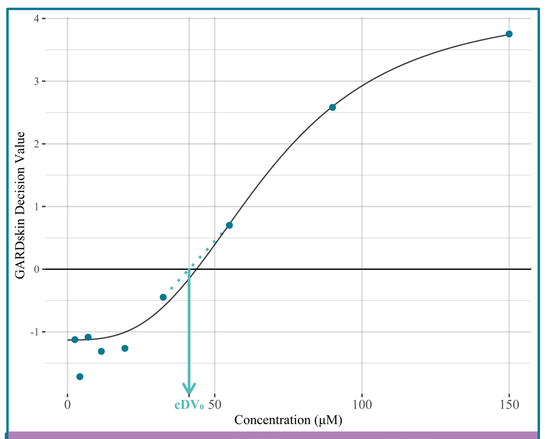
Step 2.
Measure the gene expression levels of 196 biomarkers, the genomic biomarker signature.



Step 3.
GARD® Data Analysis Application makes a binary prediction based on gene expression analysis.

The readout of GARD®skin is a Decision Value (DV):
- DV ⩾ 0 : Sensitizer
- DV < 0 : Non-sensitizer

Figure 1. GARD®skin in three steps. See OECD TG 442E for the full protocol.



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

GARD®skin Dose-Response allowing for establishment of the cDV_0 value, which is derived analogously to the LLNA EC3 value.

Figure 2. The experiment setup of GARD®skin Dose-Response.

2. Methods

GARD®skin Dose-Response was used to generate data on a set of 25 chemical skin sensitizers which have robust human NOEL and LLNA EC3 potency reference values. The potency metrices were merged and converted into a composite Potency Value (PV; µg/cm²), established as a consensus value from both LLNA EC3 values and human NOEL, by fitting a robust errors-in-variables model between LLNA EC3 and human NOEL and by orthogonally projecting the datapoints onto the fitted line (**Figure 3**).

Generated cDV_0 data was fitted to a range of regression models. The performances of the fits were evaluated using repeated cross validation, with 50 repeats and 10 folds. Classification accuracy was based on the absolute geometric mean fold change.

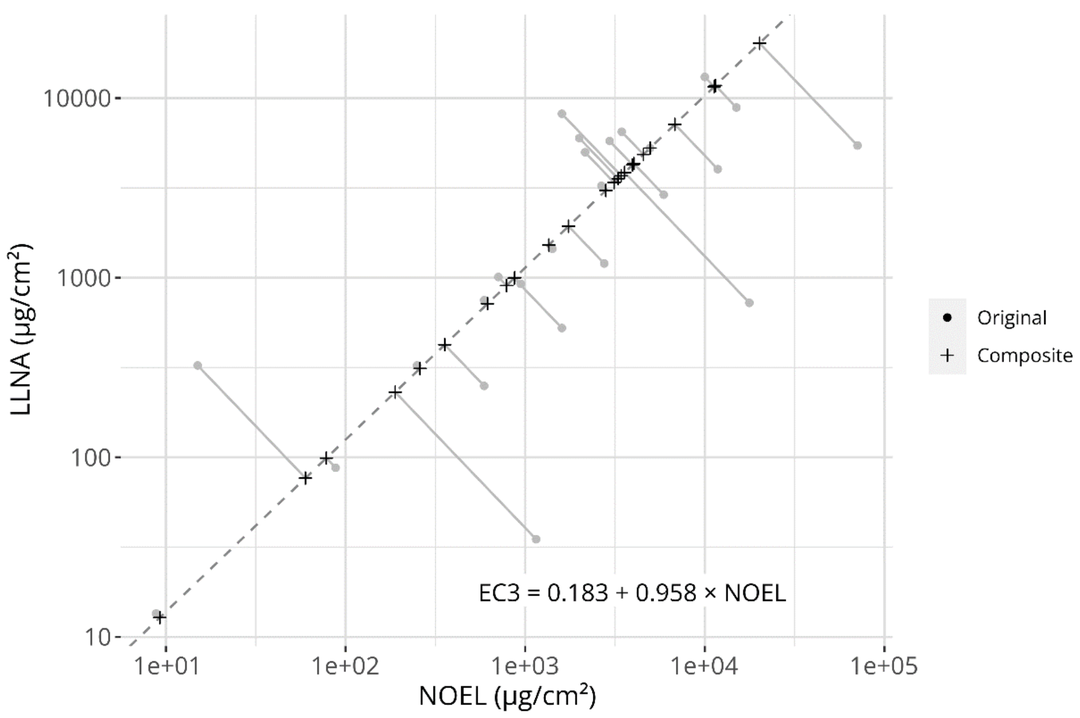


Figure 3. Visualization of the creation of the composite potency values.
The grey circular points describe the original reference values (i.e., LLNA EC3 values and human NOEL values). The dashed line describes a Passing-Bablok regression model fitted to the potency references, and its equation is described in the figure. Plus-signs describe the projection of the original reference values onto the fitted model. The grey linear segments visualize the projection. Axes are log-transformed.

3. Results

Obtained cDV_0-values for the assayed chemicals are presented in **Figure 4**. Based on generated data, it was shown that cDV_0 strongly and significantly correlates with composite PVs generated from reference data (Pearson correlation: 0.770, $p = 6.68 \times 10^{-6}$). Regression models were fitted using a standard linear regression model and a robust regression model that down-weights the influence of potentially deviating observations in reference data.

In addition, both model types were fitted with and without a parameter for a slope. When the slope was not estimated, a constant value of 1 was assumed. The lowest mean fold-change error (MFCE) was obtained from the robust model without an estimated slope parameter, with a MFCE of 2.75 when predicting LLNA EC3 and 3.22 when predicting human NOEL.

Interestingly, an optimized model fitted to a composite PV seems to perform better when predicting both LLNA EC3 and human NOEL, compared to each counterpart fitted to one or the other, respectively (LLNA: MFCE 2.82, NOEL: MFCE 3.24).

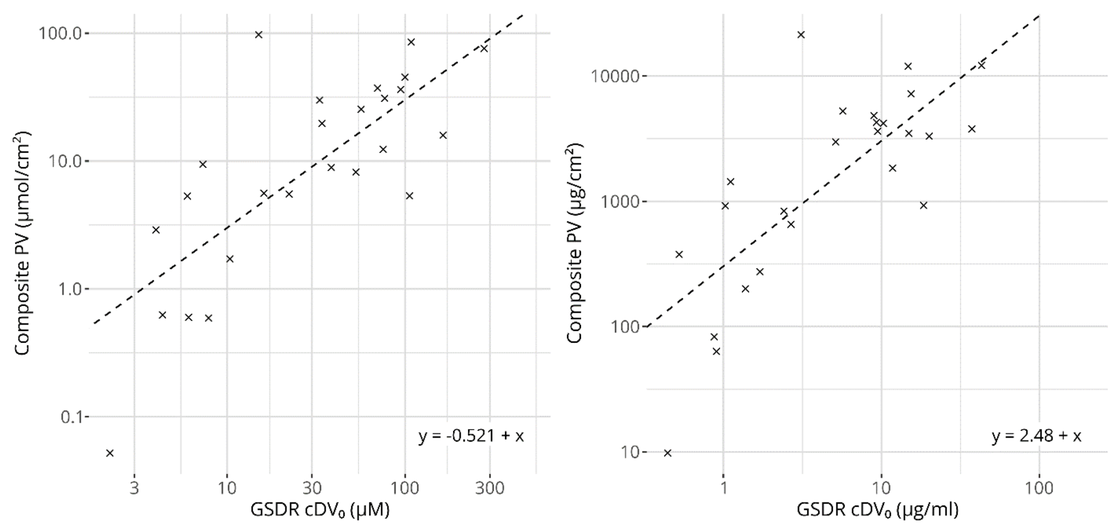


Figure 4. Scatter plots comparing GARD®skin Dose-Response (GSDR) cDV_0 values with composite potency values. Concentration units are expressed in molar-based (left) and mass-based (right) concentrations. The dashed lines describe the best performing regression models for predicting potency from cDV_0 values. The equations of the lines are described in the figures. All axes are log-transformed.

4. Discussion and Conclusions

Herein presented work demonstrates how GARD®skin Dose-Response outputs of cDV_0 can be converted to a composite PV, using an optimized regression model fitted to reference data. The proposed model can predict sensitizing potency in an intuitive unit (µg/cm²), which can readily be interpreted as both LLNA EC3 and human NOEL.

Of particular interest, two distinct sets of reference values are available for constructing separate and specialized prediction models, i.e., predicting LLNA EC3 and human NOEL values, respectively. The main assumption for the creation of the composite PV scale was that both LLNA EC3 and human NOEL values describe the same underlying phenomenon, i.e., sensitizing potency, and that both metrics are associated with errors. While it may be argued that the human NOEL is the more relevant endpoint, as we are attempting to predict sensitization in humans, the precision of the LLNA EC3 values are likely higher as their values have been estimated for the deliberate purpose of establishing a PoD value.

It was hypothesized that one could leverage both information sources against each other to create a composite potency value, to which a model could be fitted. Importantly, models fitted to the composite potency values were better or at least as proficient in predicting the original references potency values as compared with models fitted to the references separately. This does not seem unreasonable as a latent signal may be better described via the aggregation of several noisy measurements.

The consistently best performing models corresponded to those without slope estimates, i.e., the models assuming that the slope equals 1. The biological implication of a fixed slope of 1 could further indicate that the system used for evaluation of potency is relevant, as the relative difference of skin sensitizing potencies is sustained. This is also convenient as it leads to very simple prediction models. As the final models were fitted to log transformed potency values and only contained estimates for the intercepts, in practice, they can be applied by multiplying an untransformed cDV_0 value with a constant.

In conclusion, the readouts from GARD®skin Dose-Response derive a quantitative continuous potency estimate of skin sensitizers that may be used directly as a PoD for a seamless integration into downstream NGRA.

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