

## Conclusions

- 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.

## Introduction

Several non-animal methods for identifying skin sensitizers have been developed with acceptable prediction performance. However, advancement of alternative methods for skin sensitizing potency assessment is still missing although a highly sought-after endpoint.

The GARDskin assay is a genomics-based *in vitro* assay for hazard assessment of skin sensitizers, currently progressing towards regulatory acceptance (Figure 1).

Here, we introduce GARDskin Dose-Response (DR), in which test chemicals are evaluated by the GARDskin assay in an extended range of concentrations, in order to investigate the dose-response relationship between GARDskin classifications and test chemical concentration.

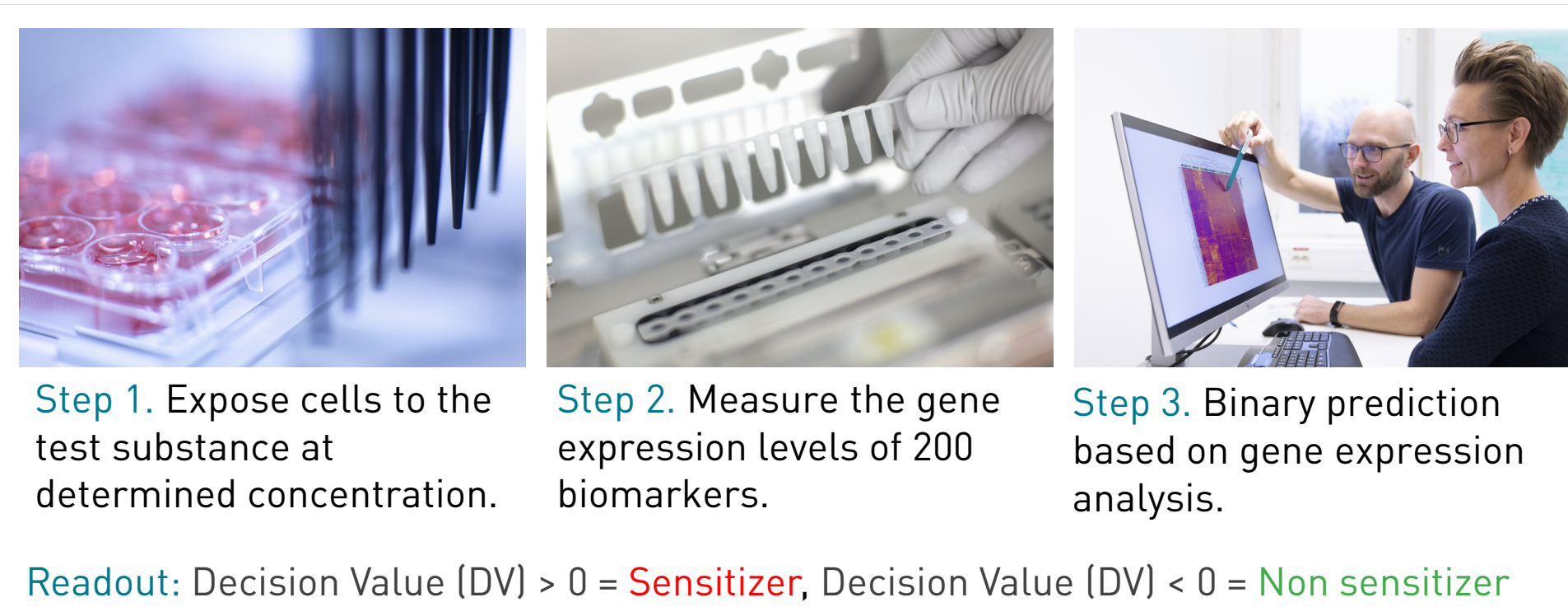


Figure 1. The GARDskin assay in three steps

## Method

The protocol of GARDskin DR investigates the lowest dose required to elicit a response in the GARDskin assay and is designed as an analog to LLNA (Figure 2). The protocol was applied to a set of reference test chemicals (n=29), and the corresponding dose-response curves are presented in Figure 3.

### The protocol of GARDskin Dose-Response in short

- Perform the GARDskin assay in a titrated range of concentrations (n ≥ 6).
- Top concentration: GARD input concentration (500µM/RV90).
- Apply standard GARDskin pipeline to generate decision values (DVs).
- Visual inspection of dose-response curve possible by plotting DVs vs input concentrations.
- Use linear interpolation to estimate  $cDV_0$ : lowest concentration required to induce a positive classification (DV ≥ 0).

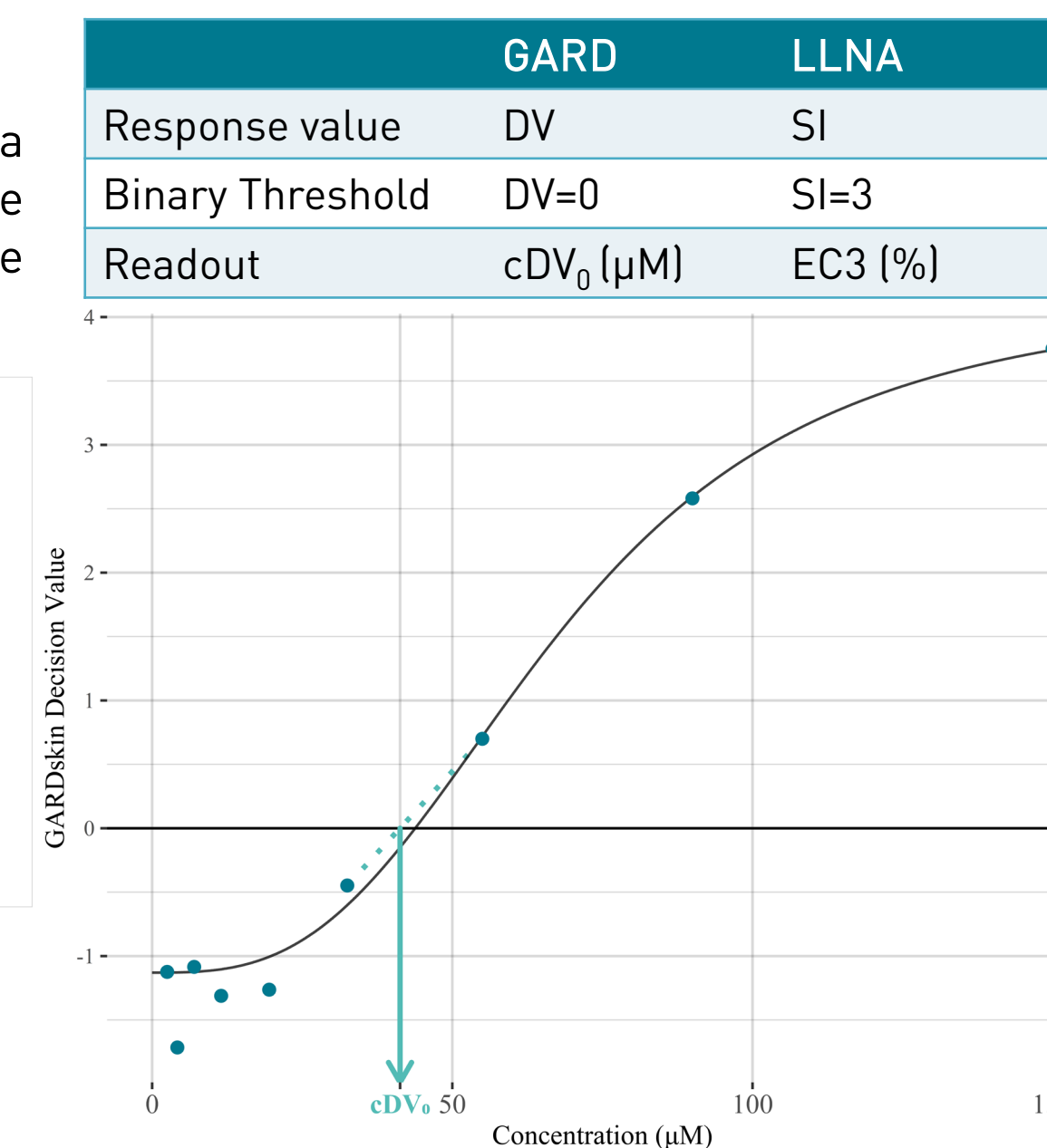


Figure 2. GARDskin DR experimental setup allowing for establishment of the  $cDV_0$ -value, which is derived analogously to the LLNA EC3-value.

## Results

As seen in Figure 3, strong sensitizers triggered response signals above the binary threshold (DV=0) at lower concentrations compared to weak sensitizers, while non-sensitizers did not demonstrate a dose-response behavior at concentrations below the maximum dose allowed in the assay. The relationship between  $cDV_0$ -values and existing metrics of sensitizing potency was further explored (Figure 4).

### Summary of results for all assayed chemicals (n=29): strong correlation between GARDskin $cDV_0$ concentration and skin sensitizing potency

- Two Non-sensitizers displayed a dose-response relationship:
  - Phenol:** LLNA (NS), HP (6)
  - Octanoic acid:** LLNA (NS), HP (6)
  - Both induced positive  $cDV_0$  above maximum dose, hence correctly classified as Non-sensitizers.
- All but two of the skin sensitizers displayed clear dose-response relationships:
  - Anethole:** LLNA (S), HP (5)
  - Kanamycin Sulphate:** LLNA (NS), (HP 4)
  - Testing limited by low solubility; more solvents will be explored.

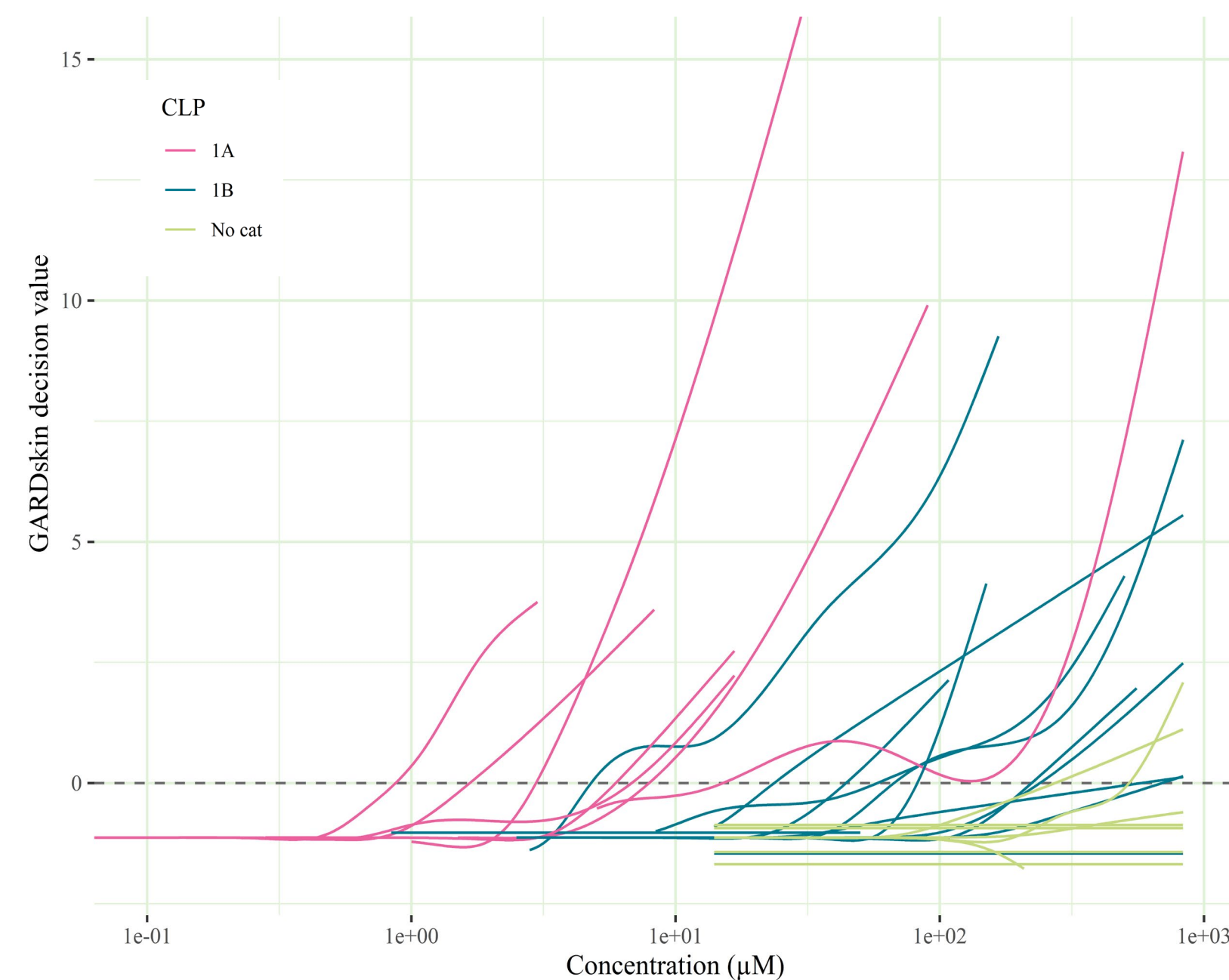


Figure 3. Individual dose-response measurements of a reference set of test chemicals. Response-curves are colored by the CLP category of the test chemical from which data points originate. Individual  $cDV_0$ -values are derived from linear interpolations of the concentrations required to generate response-values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

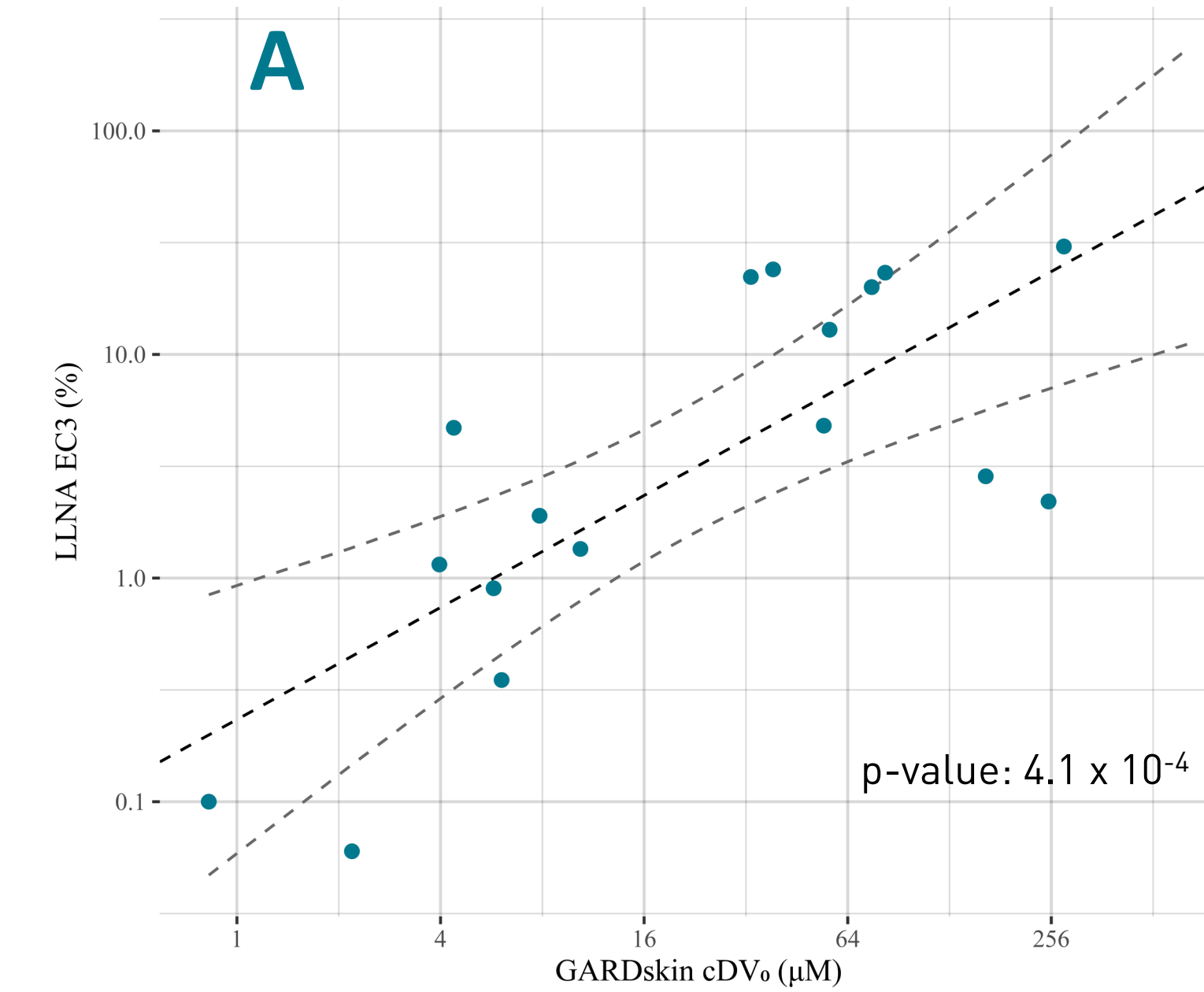
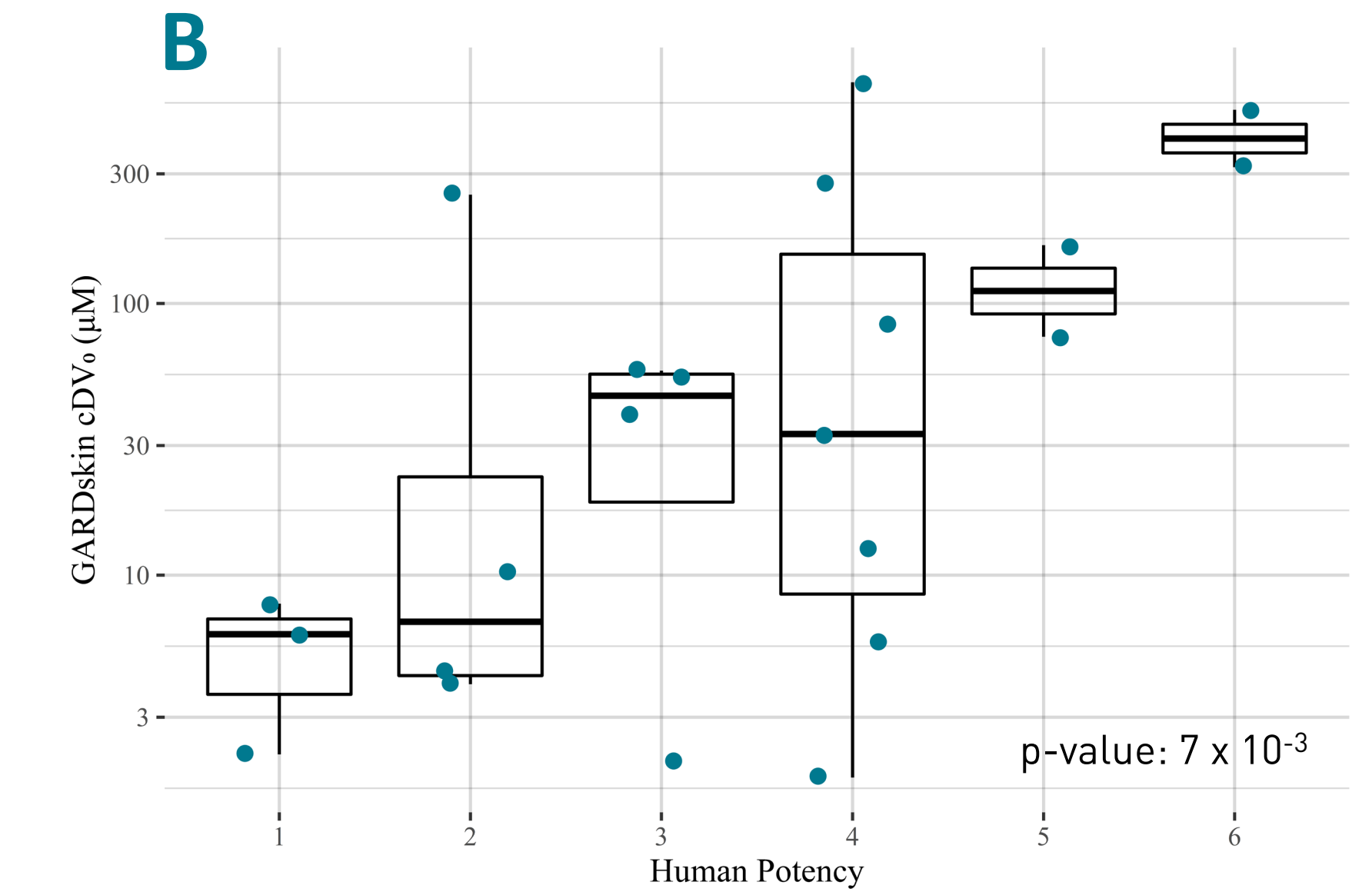


Figure 4. GARDskin DR  $cDV_0$ -values correlate with A. LLNA EC3-values and B. Human Potency categories. Both correlations are statistically significant.



### GARDskin $cDV_0$ evaluated against LLNA EC3 values

- $cDV_0$  values for sensitizers were compared to LLNA EC3 (%) values from the NICEATM database.
- Significant correlation to LLNA EC3 values.
  - Pearson correlation coefficient: 0.74
  - P-value:  $4.1 \times 10^{-4}$

### Example:

- Chemical X tested in GARDskin dose-response.
- GARDskin measured  $DV_0$ : 20µM
- Extrapolated LLNA EC3 (%): 2.8% (Moderate, ≥1 - <10) [95% confidence interval: lower = 1.44, upper: 5.5].

### GARDskin $cDV_0$ evaluated against Human Potency

- $cDV_0$  values for sensitizers were compared to Human potency categories (Basketter).
- Significant correlation to Human potency categories.
  - p-value:  $7 \times 10^{-3}$

### Interesting observations on assay sensitivity:

- Majority of strong sensitizers (HP1,HP2) detected at GARD input concentrations < 10 µM.
- Almost all sensitizers (irrespective of potency) detected at GARD input concentrations < 100 µM.

## Discussion

Statistically significant correlations between  $cDV_0$ -values and LLNA EC3-values, as well as the human potency categories (Basketter et al., 2014) was confirmed, thus enabling direct extrapolation between the different measurements.

Subsequently, GARDskin DR measurements may be readily implemented in any existing risk assessment strategy, based on e.g. LLNA or otherwise complementary assays for skin sensitizing potency.

## Contacts

Henrik Johansson, PhD  
[henrik.johansson@senzagen.com](mailto:henrik.johansson@senzagen.com)

Andy Forreryd, PhD  
[Andy.Forreryd@senzagen.com](mailto:Andy.Forreryd@senzagen.com)

Joshua Schmidt, PhD  
[joshua.schmidt@senzagen.com](mailto:joshua.schmidt@senzagen.com)

