GARD™skin Dose-Response

In vitro quantitative potency assessment

GARDskin Dose-Response is an *in vitro* test for quantitative skin sensitizing potency assessment of chemicals, adapted from GARDskin.

GARDskin Dose-Response provides an estimated threshold concentration for a test substance to induce skin sensitizing effects; a lower concentration equals a higher potency and vice versa.

This threshold concentration can be used for skin sensitizing potency ranking of candidate ingredients and early decision-making during product development.

Additionally, readouts can be extrapolated to correlating LLNA EC3 values traditionally used to measure skin sensitizing potency of chemicals. Furthermore, the readout can also be extrapolated to human potency and/or GHS/CLP classification, all with high statistical significance.

Non-animal test data that can be extrapolated to:

- **LLNA EC3 value**
- **Human potency**
- **GHS/CLP classification**

Features and Benefits

Test system
- SenzaCells™: Human dendritic-like cell line.

Solvent
- Standard: DMSO and H_2O.
- Other available solvents: Acetone, DMF, Isopropanol, Ethanol, Glycerol, Olive oil, Sesame oil.

What it measures
- GARDskin response values in a titrated range of multiple concentrations.

Readout
- Hazard: skin sensitizer or non-sensitizer.
- Quantitative potency: cDV_0.*
- cDV_0 can be further extrapolated to:
  - LLNA EC3 value.
  - Human potency.
  - GHS/CLP classification 1A or 1B.

High performance
- The performance of GARDskin Dose-Response is associated with GARDskin, which demonstrated a 94% accuracy for skin sensitizing hazard prediction.**
- GARDskin Dose-Response readouts have shown significant correlation with the LLNA EC3 values and human potency.

Short turnaround time
- Test time: 2 weeks.
- Final report: Additional 2-4 weeks.***

Low sample requirement
- Only 0.5g (solid) or 1ml (liquid).

* cDV_0 is the threshold concentration for the test substance to induce a positive response in GARDskin.
** Validation study, OECD Test Guideline Program (TGP no. 4.106). Johansson H. et al. Toxicological Sciences 2019.
*** For non-GLP studies.

How it works

The GARDskin Dose-Response investigates the GARDskin response values in a titrated range of multiple concentrations in a dose-response manner to find the threshold concentration required to induce a positive decision value.

This threshold concentration, cDV_0, is used to estimate the inherent sensitizing potency of a chemical. A low value indicates a high inherent skin sensitizing potency and a high value indicates a low inherent skin sensitizing potency.

cDV_0 can also be extrapolated to the traditional EC3 value from Local Lymph Node Assay, the human skin sensitizing potency, and/or GHS/CLP classification 1A or 1B depending on the need.

All the GARD assays are based on the same technology platform. Read more about the GARD technology platform and assay development principles on www.senzagen.com/science.

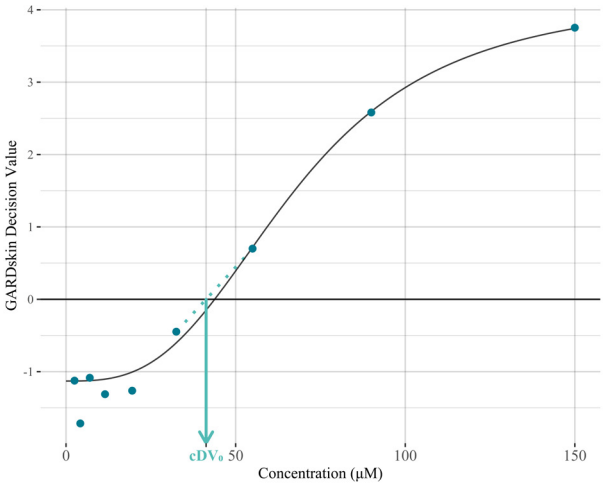
In-house validation study

Method

An in-house validation study of GARDskin Dose-Response was conducted to investigate the dose-response relationship between GARDskin classifications and test chemical concentration. Data was generated on 29 reference chemicals by performing the GARDskin assay in a titrated range of concentrations. The experimental setup is illustrated in Figure 1.

Results

The results confirmed that cDV_0 informs on the skin sensitizing potency and can be used to directly monitor sensitizing potency of chemicals. While non-sensitizers exhibit an expected lack-of-response, cDV_0 values from skin sensitizers were associated with GHS classification labels, as well as significantly correlated to both human and LLNA potency reference data (Figure 2): rLLNA = 0.81, $p = 9.1 \times 10^{-5}$; rHuman = 0.74, $p = 1.5 \times 10^{-3}$.



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

Figure 1. The experimental setup of GARDskin Dose-Response allowing for establishment of the cDV_0 value, which is derived analogously to the LLNA EC3 value.

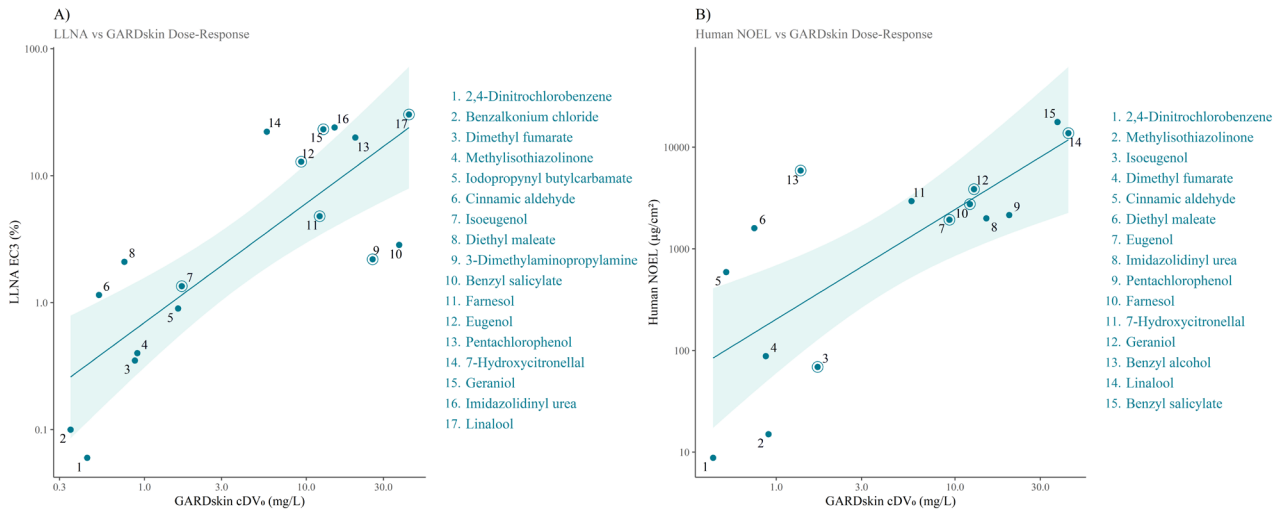


Figure 2. Scatter plots displaying the relationship between estimated cDV_0 values and A) LLNA EC3 values and B) human NOEL values. The fitted lines represent linear regression models fitted to the data, and the shaded areas describe the 95% confidence intervals of the fits. Encircled datapoints indicate pre- and pro-haptens.