GARDskin Dose-Response

Skin sensitisation potency measurement and risk assessment without a requirement for animals

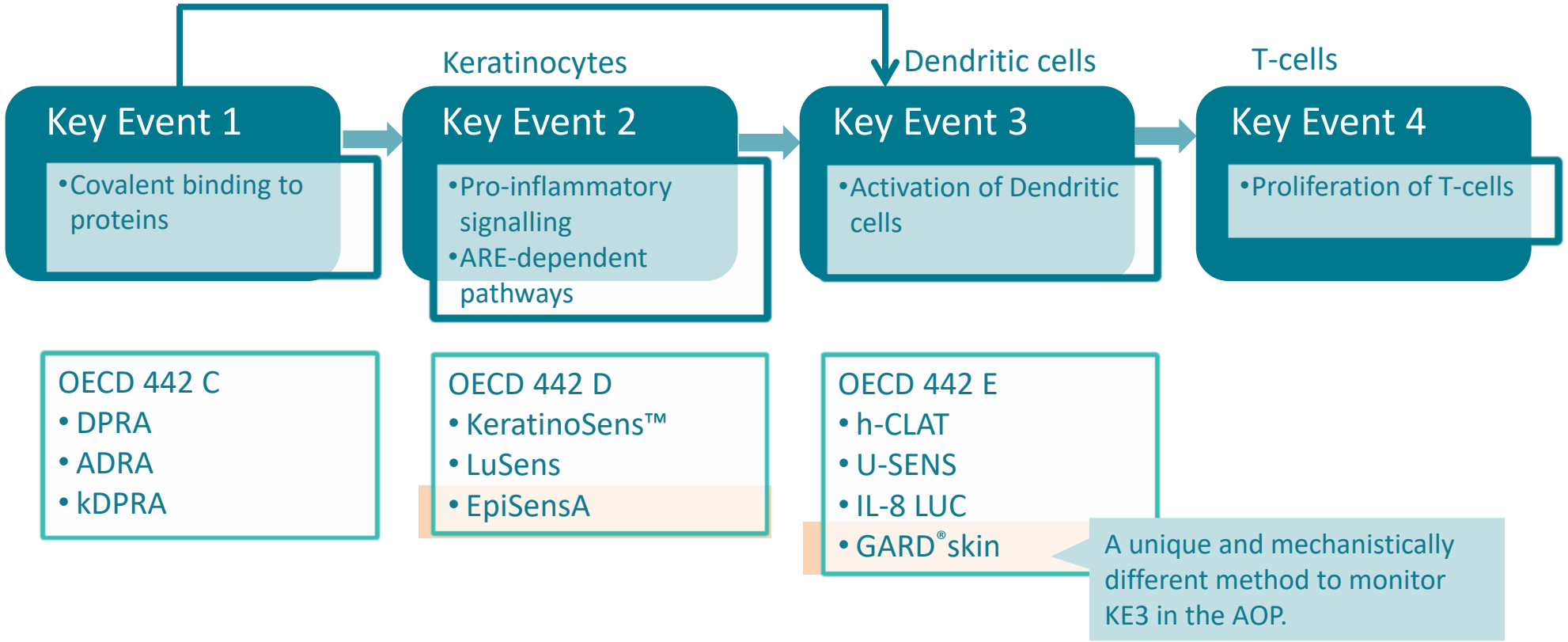
IDEA Workshop 2025

Andy Forreryd, SenzaGen AB

Introduction – Testing for skin sensitization

OECD Test Guidelines are mapped to the AOP

AOP - Adverse Outcome Pathway
NAM - New Approach Methods (KE 1-3)



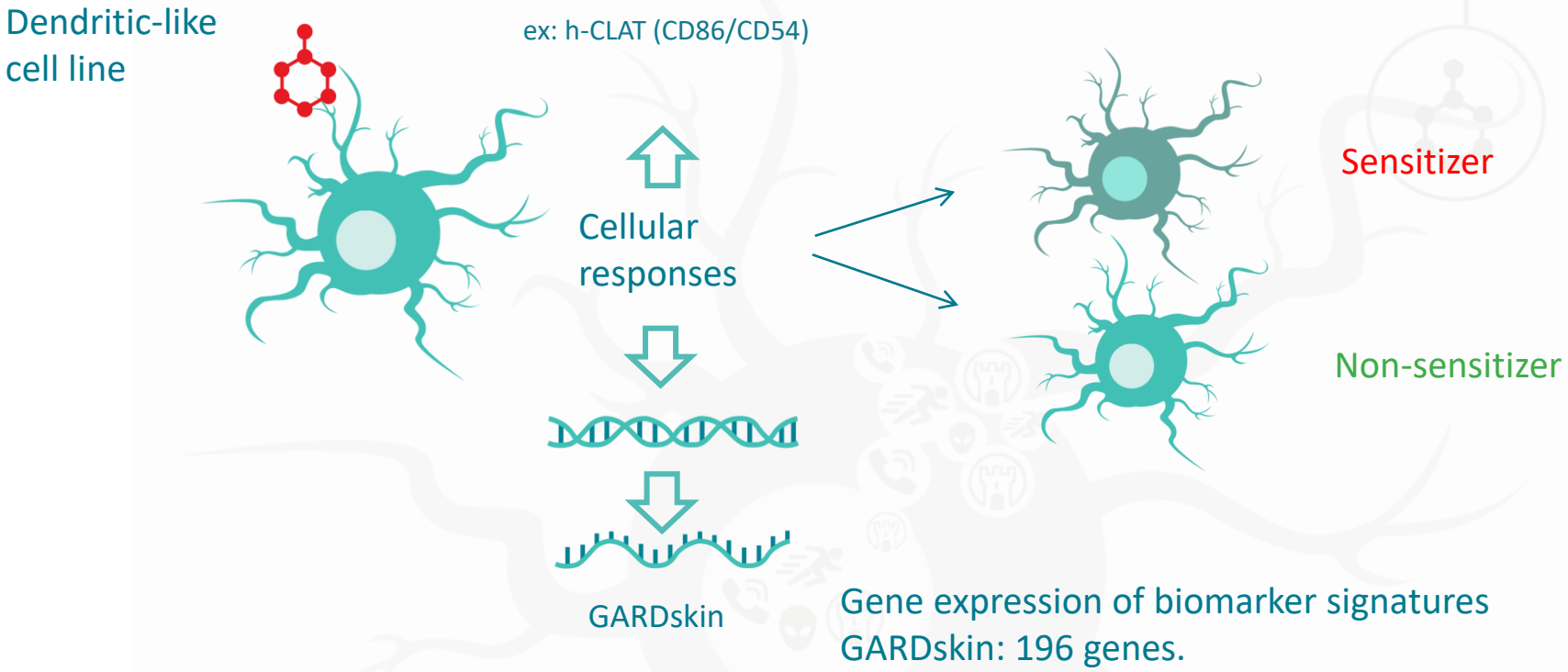
GARDskin was the first assay included in an OECD Test Guideline to combine genomics and machine learning for addressing a regulatory endpoint

SENZA GEN

The GARD technology platform – How it works

Transcriptomic read-out of the biological response

Biological system: Dendritic-like cell line (KE3)
Readout: Gene expression (genes and toxicity pathways)



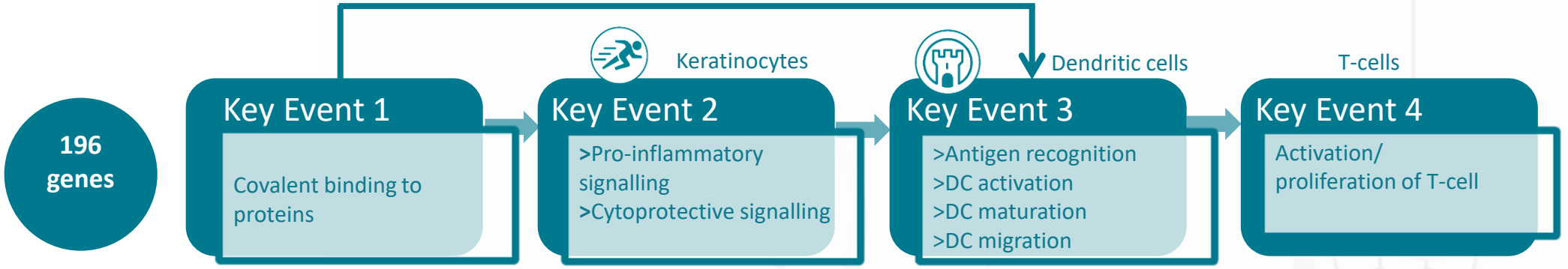
Full transparency: Identities of genes being measured available in peer-reviewed scientific literature.

See for example: Johansson et al. (2011) A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests. BMC Genomics.

SENZA GEN

The GARD technology platform – How it works

Genes cover mechanistically relevant toxicity pathways



Captures events downstream of **KE1**

Metabolic activity & identifies pre/pro haptens
ALDH
NAT-1
CYP - Cytochrome p-450

> Keap1-Nrf2-ARE pathway & AHR signalling
NQO1
HMOX1
Thioredoxin reductase I

> Pro-Inflammatory cytokines mediating e.g. TNFα, INFγ, IL-8
FAS
MAP2KI
COX20
> Inflammasome
NLRP
PSTPIP1

> DC migration & maturation
CD86
MAPK- activation
PKA- and GPCR- mediated signalling

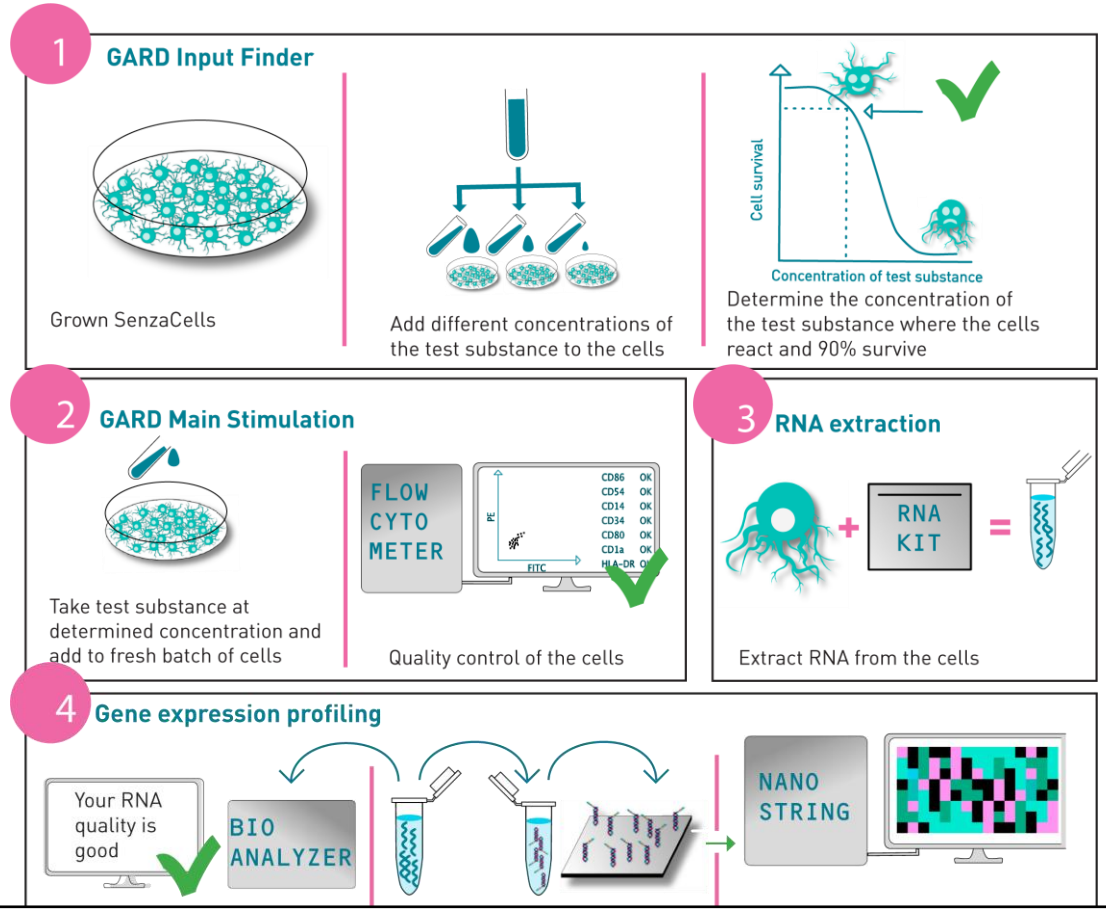
> Antigen recognition & Innate immune activation
TLR-4
TLR-6
RXRA – retinoic X receptor
NLRP
PSTPIP1
> Self-defence mechanisms
C3a/C5a-activation pathways

Covers the 3 Key steps for T-cell activation:
Antigen presentation
Co-stimulation
Cytokine secretion

Genes and pathways in the prediction signature are aligned with multiple key events in the AOP

SENZA GEN

How to GARD® your products in 6 Steps



Importantly: All genes contribute to a final classification, but with different weights

Prediction algorithm:

$$DV = b + \sum_{i=1}^{n} w_i x_i$$

n: number of variables (n for GARDskin:196)
b: constant (SVM intercept)
W_i: weight for variable i
X_i: Normalized gene expression data for variable i

Prediction model:
Mean DV ≥ 0 : Skin sensitiser (UN GHS category 1)
Mean DV < 0 : Non-sensitiser.

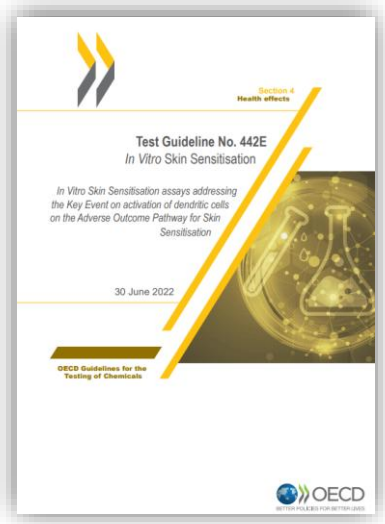
SENZA GEN

The OECD approval of GARDskin

Extending the applicability domain of NAM-based assays in OECD TG 442

OECD Test Guideline No. 442 E - *In Vitro* Skin Sensitisation

KE 3 in the AOP for skin sensitization: DCs activation



Performance statistics:

GARDskin accuracy:	**94%**
WLR	**82.1- 88.9%**
BLR	**92%**

Breaking new ground: first harmonised method integrating genomic data for a regulatory endpoint.

Validation study published in peer-reviewed scientific journal:

GARDskin: Published in Johansson et al. (2019), Validation of the GARD™skin assay for assessment of chemical skin sensitizers - ring trial results of predictive performance and reproducibility. ***Toxicological Sciences.***

Hydrophobic compounds

Lubrizol: Forreryd, A et al. (2022). Exploration of the GARD™ skin applicability domain: Indirectly acting haptens, hydrophobic substances and UVCBs.

Fragrances & Fragrance formulations

RIFM/IFF: GARDskin dose-response assay and its application in conducting quantitative risk assessment (QRA) for fragrance materials using a NGRA framework.

Metals and metal salts

Johnson & Matthey: Forreryd, A. et al. (2022). The GARD™skin assay: Investigation of the applicability domain for metals.

Complex mixtures

Corteva: Corvaro, M., et al. (2024). GARD™skin and GARD™potency: a proof-of-concept study to investigate the applicability domain for agrochemical formulations.

Polymeric materials

DSM: *In vitro* assessment of skin sensitizing potential of process-related impurities in polymeric materials during product development. Poster.

UVCBs & Natural Complex Substances

Exxon: Assessing the Utility of the GARDskin Assay to Detect Dermal Sensitization Potential in UVCBs and Formulated Lubricant Products.

Surfactants

Work in progress – to be updated.

SENZA GEN

GARD

Genomic Allergen Rapid Detection

GARDskin Dose-Response: Quantitative assessment of skin sensitizing potency.

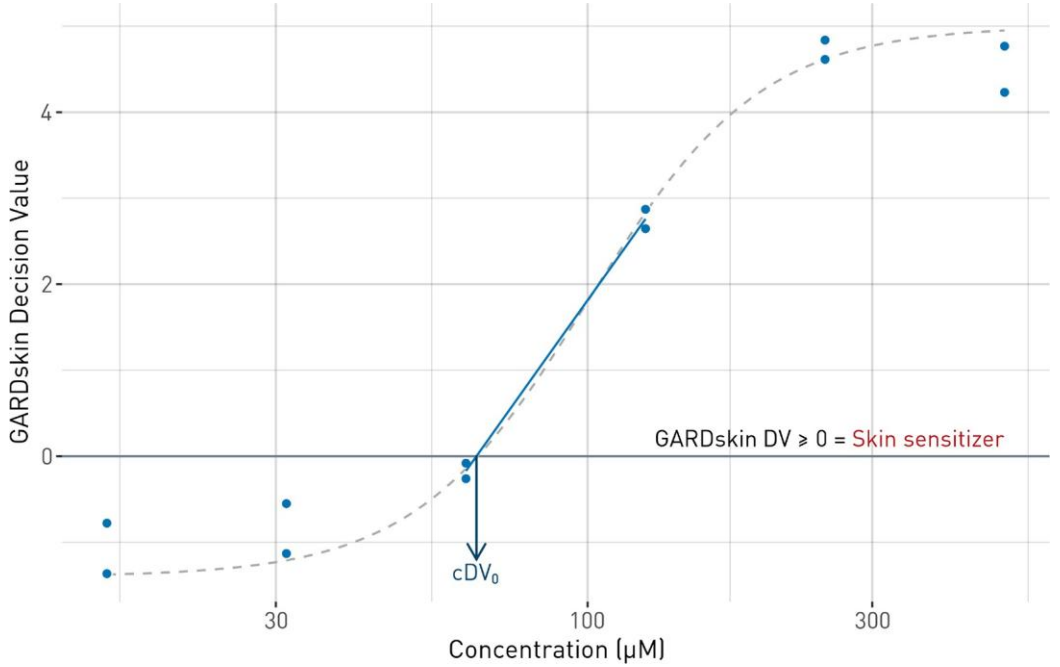
GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency

- Perform the GARDskin assay in a titrated range of concentrations (n ≥ 6).
- Apply standard GARDskin protocol to generate a decision value (DV) for each concentration.
- Estimate cDV_0: lowest concentration required to induce a positive classification (DV≥ 0).

→ The GARDskin cDV_0 value correlates significantly with both human NESIL values and LLNA EC3.

→ A simple linear regression model allows for continous potency predictions of a NESIL-value in dose per unit area (ug/cm^2) and can be used directly as a PoD for risk assessment.



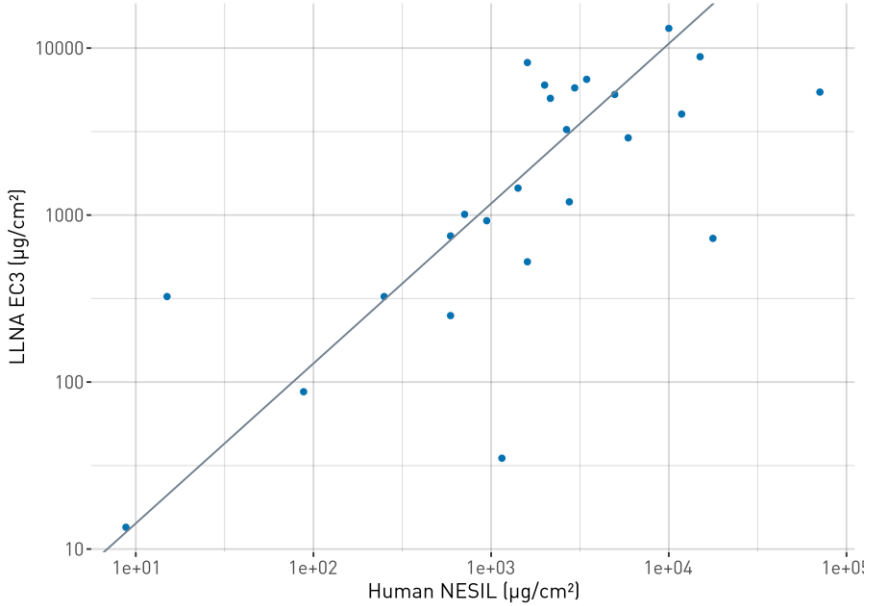
Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*
Methodology described in Gradin, R et al. (2021) Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. *Nature Scientific Reports*

GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency

The GARDskin cDV_0 value correlates significantly with both human NESIL values and LLNA EC3.

- Intrinsic limitations exist in both human NESIL and LLNA reference data.
- Both references inform on the same phenomenon i.e., skin sensitizing potency, but neither is perfect and associated with measurement errors.
- It was considered redundant to fit models separately to LLNA EC3 and human NESIL.



→ A composite potency score integrate information from both LLNA and human data into a single value equivalent to a predicted NESIL (in the unit ug/cm²).

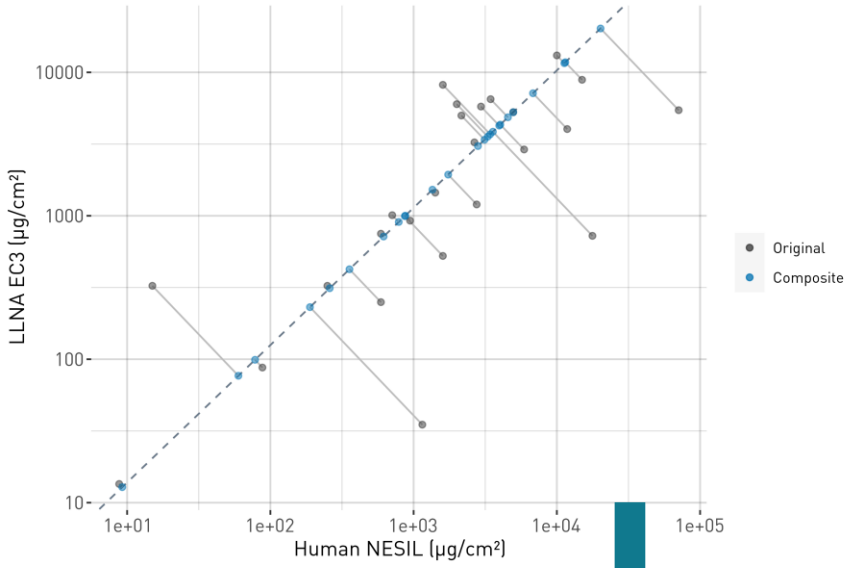
Example	LLNA EC3 (µg/cm²)	Human NESIL (µg/cm²)	Composite (µg/cm²)
DNCB	13.5	8.8	
Cinnamic aldehyde	250	591	
Citral	1450	1420	
…			

GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency

The GARDskin cDV_0 value correlates significantly with both human NESIL values and LLNA EC3.

- Intrinsic limitations exist in both human NESIL and LLNA reference data.
- Both references inform on the same phenomenon i.e., skin sensitizing potency, but neither is perfect and associated with measurement errors.
- It was considered redundant to fit models separately to LLNA EC3 and human NESIL.



→ A composite potency score integrate information from both LLNA and human data into a single value equivalent to a predicted NESIL (in the unit ug/cm²).

Example	LLNA EC3 (µg/cm²)	Human NESIL (µg/cm²)	Composite (µg/cm²)
DNCB	13.5	8.8	9.8
Cinnamic aldehyde	250	591	378
Citral	1450	1420	1440
…			

Linear projection to generate composit values

SENZA GEN

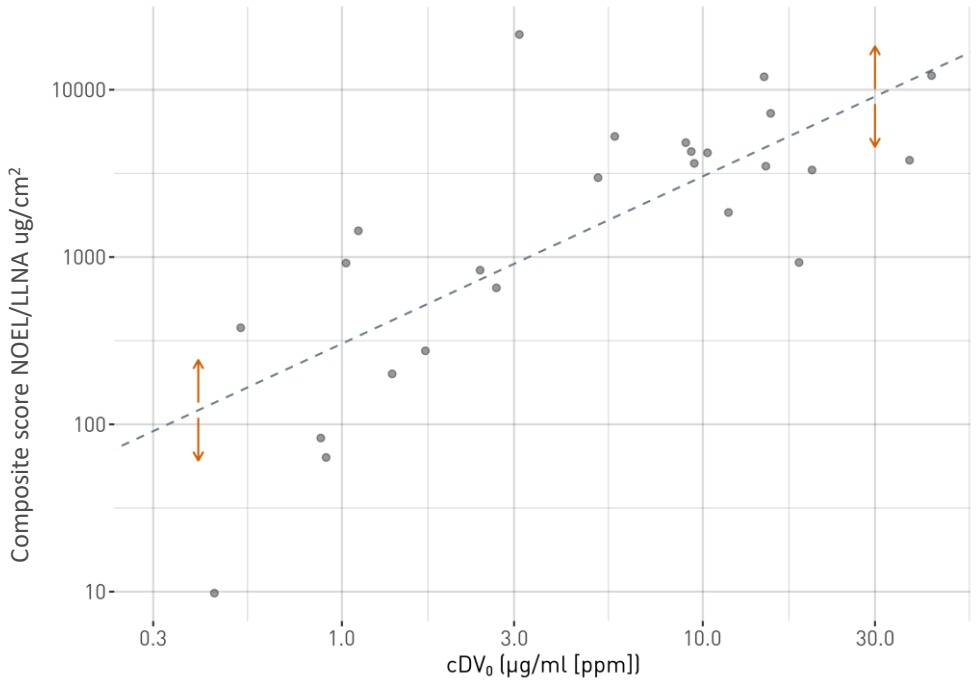
GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency

A simple linear regression model allows for continous potency predictions of a NESIL-value in dose per unit area (ug/cm^2).

- The correlation between cDV_0 and potency is described by a linear regression model.
- The regression model is simple and only contains 1 parameter:

  Prediction in µg/cm^2 = cDV_0 in µg/ml × θ
- The model use cDV_0 as input to predict the skin sensitizing potency of an unknown chemical in dose per unit area (ug/cm^2).



Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*

GARDskin Dose-Response

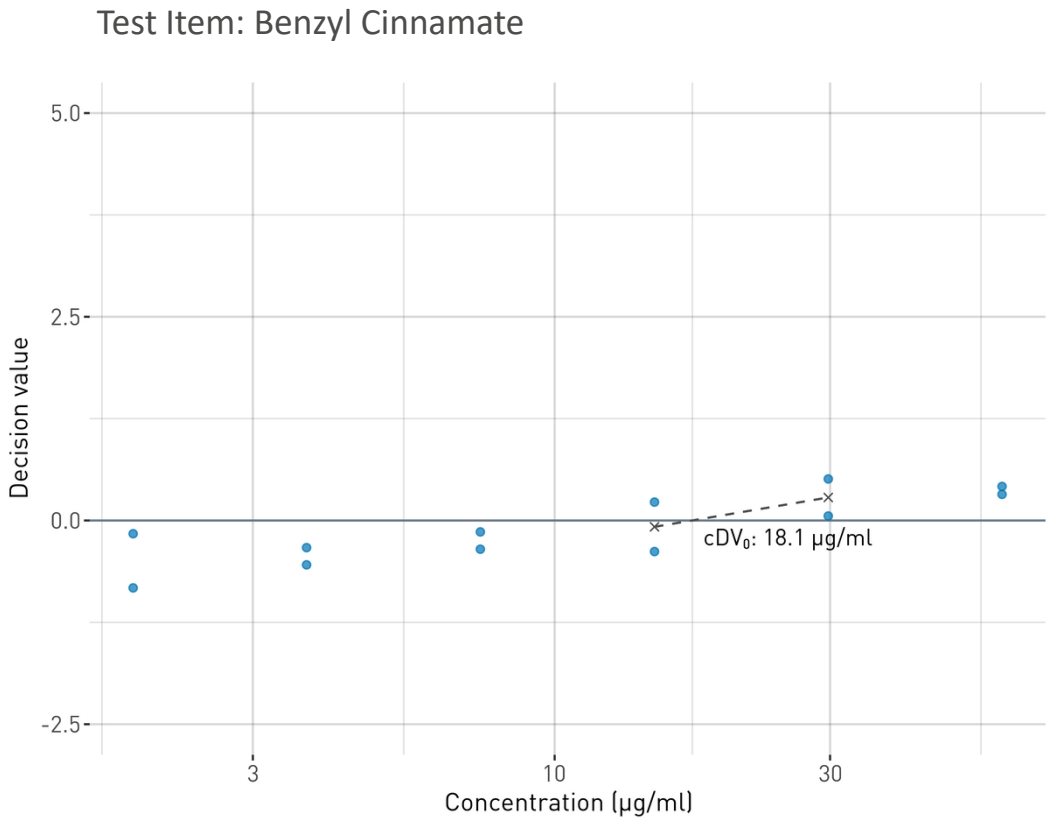
How to derive continuous potency predictions

Step 1: Dose-Response testing.

- Generation of a dose response curve by plotting DV vs concentration.
- Identification of a cDV_0 value using linear interpolation.

Step 2: Continuous potency predictions.

- Correlation between cDV_0 and potency is described by a linear regression model.
- The cDV_0 value is used as input into the regression model to derive a potency prediction in the unit ug/cm^2 (LLNA EC3/Human NESIL)



Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*

Methodology described in Gradin, R et al. (2021) Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. *Nature Scientific Reports*

SENZA GEN

GARDskin Dose-Response

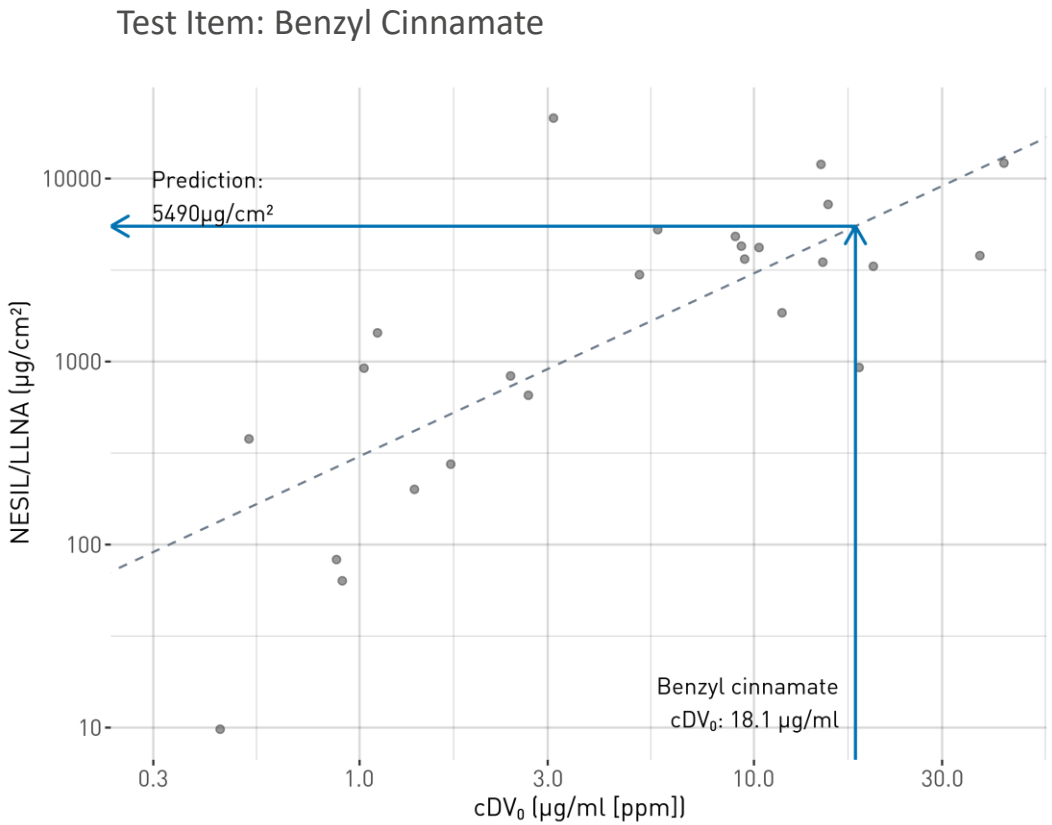
How to derive continuous potency predictions

Step 1: Dose-Response testing.

- Generation of a dose response curve by plotting DV vs concentration.
- Identification of a cDV_0 value using linear interpolation.

Step 2: Continuous potency predictions.

- Correlation between cDV_0 and potency is described by a linear regression model.
- The cDV_0 value is used as input into the regression model to derive a potency prediction in the unit ug/cm^2 (LLNA EC3/Human NESIL)



Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*

Methodology described in Gradin, R et al. (2021) Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. *Nature Scientific Reports*

SENZA GEN

GARDskin Dose-Response

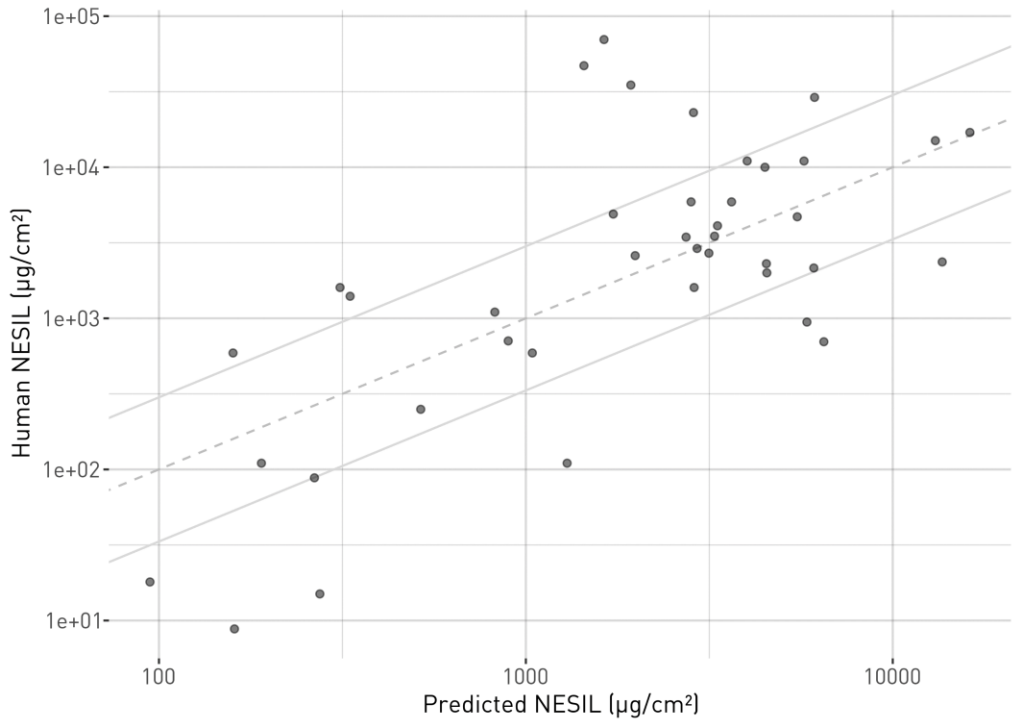
Performance for potency predictions

Background

- The performance and reproducibility of GARDskin DR have been evaluated in collaboration with industry (3 papers, ~200 chemicals).

Results – performance

- GARDskin DR predicted human NESIL values correlated well with human refence data (n=40, r=0.69).
- GARDskin DR predicts human NESILs **equally well or better** than the LLNA **(**LLNA vs Human: r= 0.6).



Note: It is challenging to find reliable reference data. The performance values above refer to a subset of data for which highly curated reference data from both LLNA and human studies were available, as documented in OECD TG 497, Annex 2.

Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*
Joint publication with IFF & RIFM: Donthamsetty & Forreryd et al. (2024) GARDskin Dose-Response assay and its application in conducting Quantitative Risk Assessment (QRA) for fragrance materials using a Next Generation Risk Assessment (NGRA) framework. *RTP*
Joint publication with IFF & RIFM: Lee et al. (2025) Determining a Point of Departure for skin sensitization potency and Quantitative Risk Assessment of fragrance ingredients using the GARDskin Dose-Response assay. *ALTEX*

GARDskin Dose-Response

Performance for potency predictions

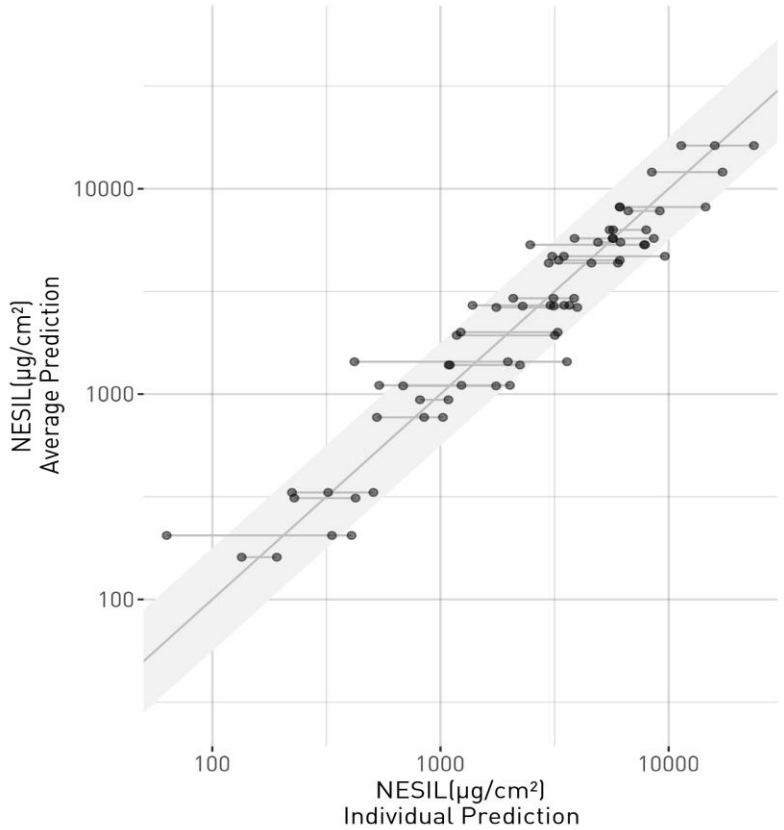
Results - reproducibility

- GARDskin DR data available for repeated measurements (n=27).
- The predicted human NESIL values were reproducible, with an expected variation between runs of 1.78-fold-changes.

Conclusion

- GARDskin DR provides accurate and reproducible potency predictions with high correlation to human NESIL values and LLNA EC3.

→The predicted potency value is equivalent to a human NESIL value (ug/cm^2) and can be used directly as a PoD for QRA (see for example Donthamsetty et al. 2024)



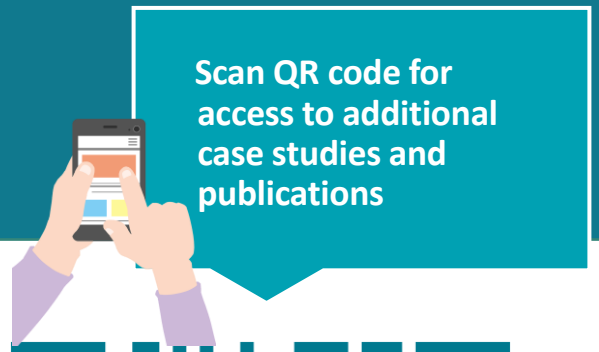
Joint publication with L'Oréal: Gradin et al. (2024) *In Vitro* Prediction of Skin-Sensitizing Potency Using the GARDskin Dose–Response Assay: A Simple Regression Approach, *Toxics*
Joint publication with IFF & RIFM: Donthamsetty & Forreryd et al. (2024) GARDskin Dose-Response assay and its application in conducting Quantitative Risk Assessment (QRA) for fragrance materials using a Next Generation Risk Assessment (NGRA) framework. *RTP*
Joint publication with IFF & RIFM: Lee et al. (2025) Determining a Point of Departure for skin sensitization potency and Quantitative Risk Assessment of fragrance ingredients using the GARDskin Dose-Response assay. *ALTEX*

Summary & conclusions

- **The GARDskin Dose-Response assay is built on the validated framework of GARDskin (OECD TG 442E).**
- **GARDskin Dose-Response provides accurate and reproducible potency predictions that can be used to establish a safe concentration of a sensitizer in a formulated product.**
- **Data from the previous IDEA WS meeting demonstrated high performance for the chemicals in the original RCPL list:**

→ Continuous potency predictions from the GARDskin Dose-Response assay correlated well with PVs for chemicals in the RCPL list (pearson correlation: 0.74).

→ Overall, very similar potency rankings with GARDskin Dose-Response and RCPL potency list (spearman: 0.69)



Collaboration L'oreal: Development and refinement of GARDskin Dose-Response.

Collaboration RIFM & IFF: Use in NGRA for risk assessments and to determine the maximum concentration of a sensitizer in various consumer products.

Collaboration Takasago: Use during development of novel fragrance materials to establish a safe dose for confirmatory HRIPT.

Collaboration BIC skin care: Use during development of novel temporary ink tattoos to formulate safe and effective products.

Collaboration DoTerra: Use of GARDskin Dose-Response to predict skin sensitization threshold concentrations for Natural Complex Substances.