GARD – Genomic Allergen Rapid Detection

GARDskin Dose-Response: Performance on the Reference Chemical Potency List

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IDEA RCPL (Reference Chemical Potency List) 2nd Workshop

September 22, 2023

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Overview of todays presentation

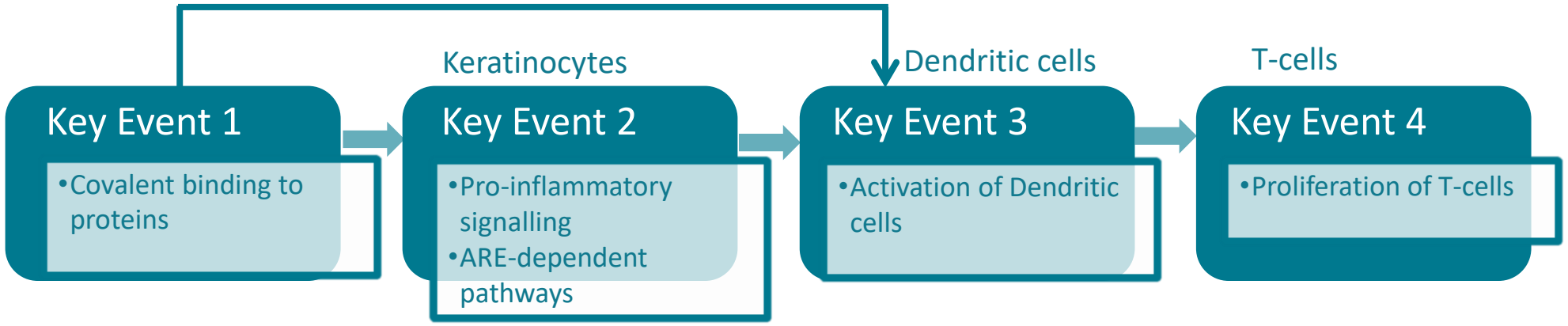
The presentation includes the following topics:

- Short introduction to the GARDskin technology (OECD TG 442E).
- GARDskin Dose-Response assay: adaptation of the validated GARDskin protocol to allow for continuous potency predictions.
- Performance of the GARDskin Dose-Response assay on the Reference Chemical Potency List

Introduction – Testing for Skin Sensitization

OECD Test Guidelines are mapped to the AOP

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



OECD 442 C
- DPRA
- ADRA
- kDPRA

OECD 442 D
- KeratinoSens™
- LuSens

OECD 442 E
- h-CLAT
- U-SENS
- IL-8 LUC
- GARD (Genomic Allergen Rapid Detection)

GARDskin provides a unique and mechanistically different method to monitor KE3 in the AOP.

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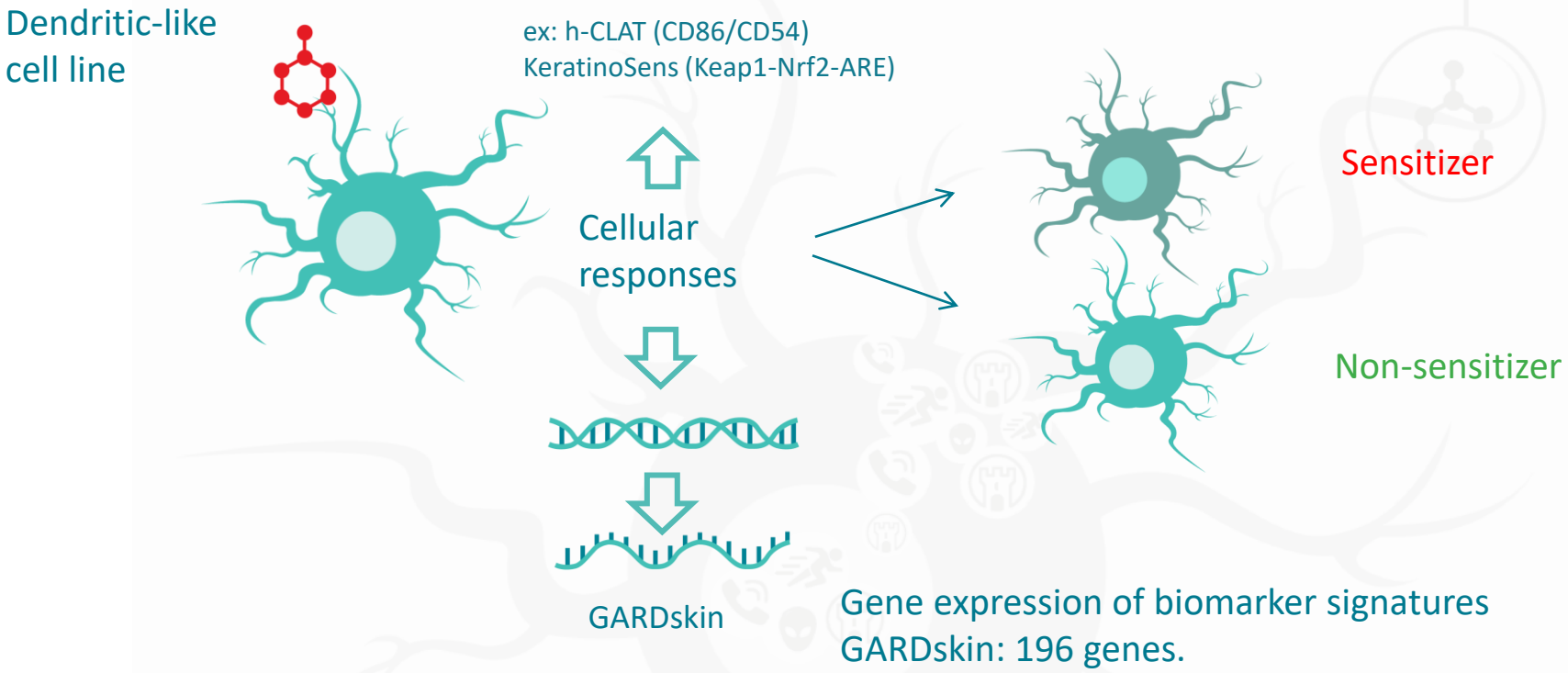
The GARD technology platform – how it works

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.

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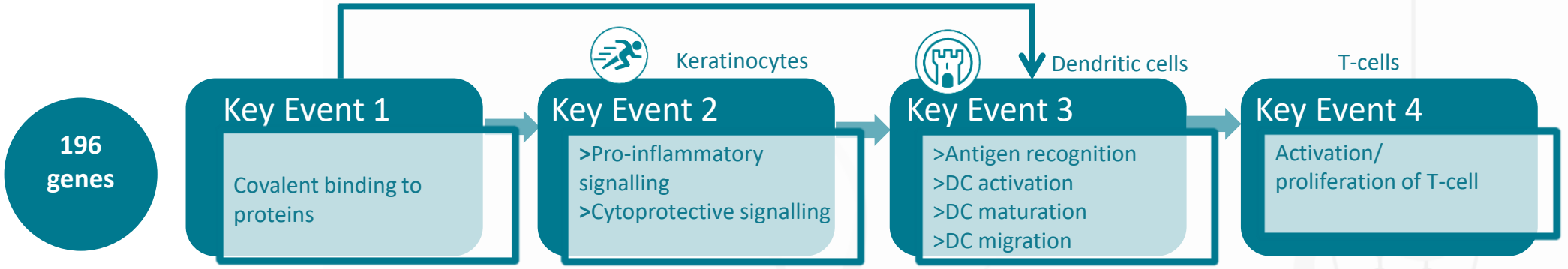
The GARD technology platform – how it works

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

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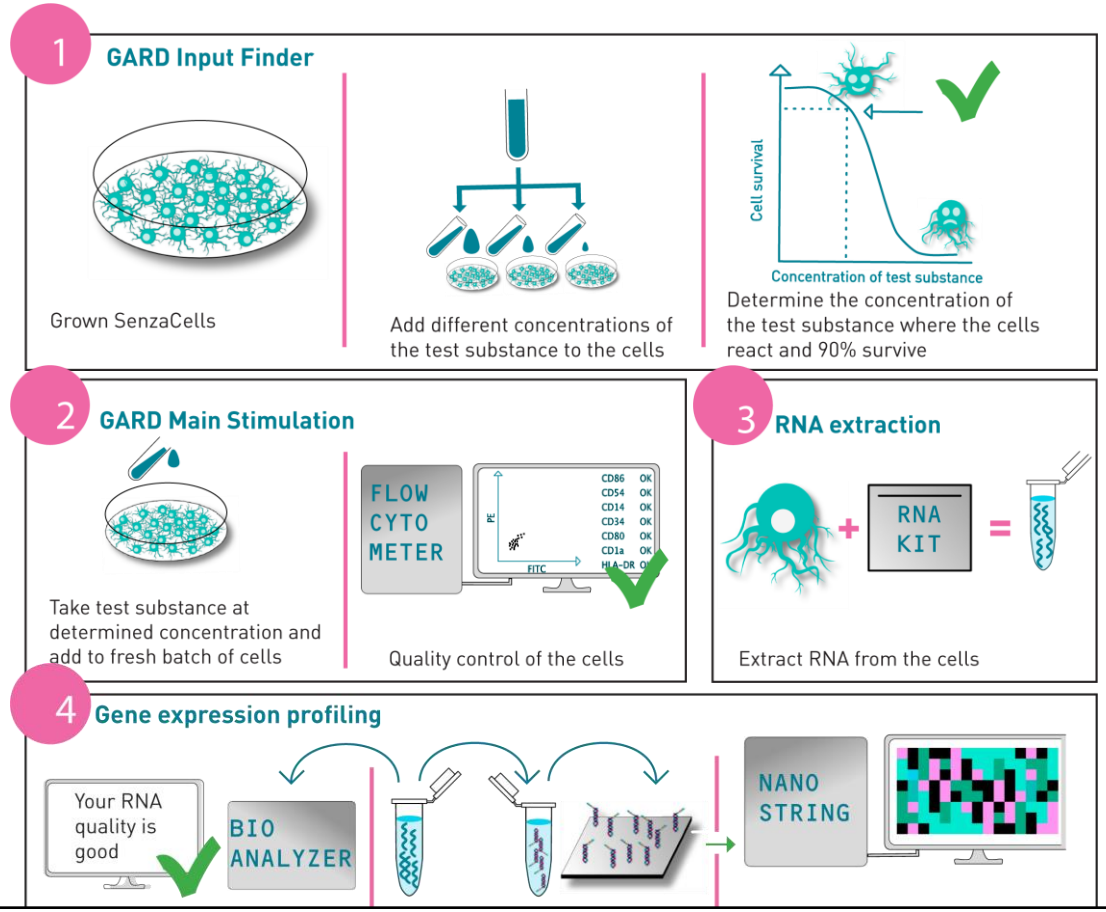
How to GARD® your products in 6 Steps



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.

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The OECD approval of GARDskin

Machine learning and omics arrive in the field of regulatory toxicology

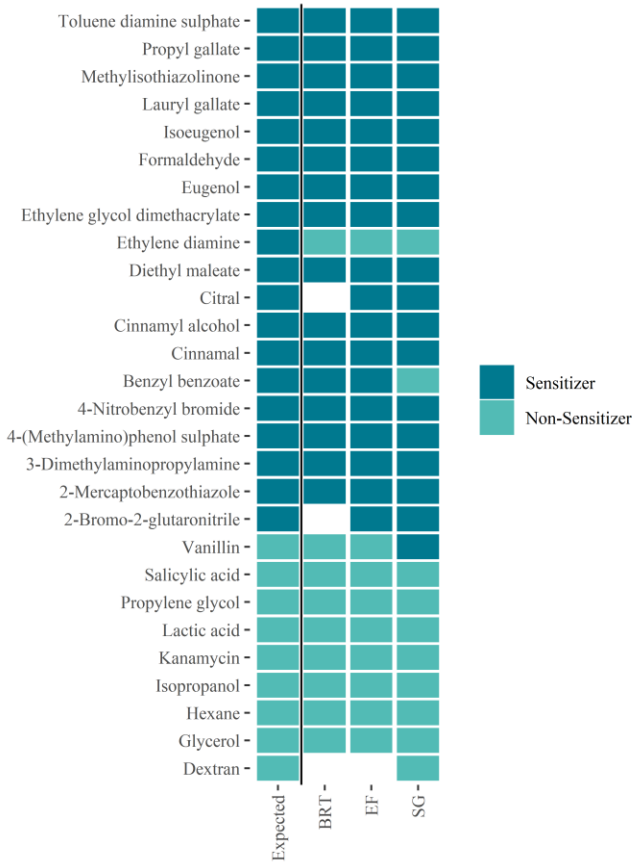
Ring trial setup

- BRT (US)
- SenzaGen (SE)
- Eurofins (DE)

GARDskin Validation phase

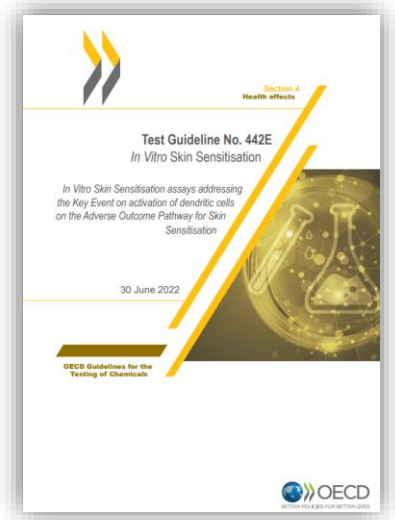
- N=28 Blinded compounds
- N=28 Blinded compounds
- N=28 Blinded compounds

GARDskin predictions



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

KE 3 in the AOP for skin sensitization: DCs activation



Performance statistics:

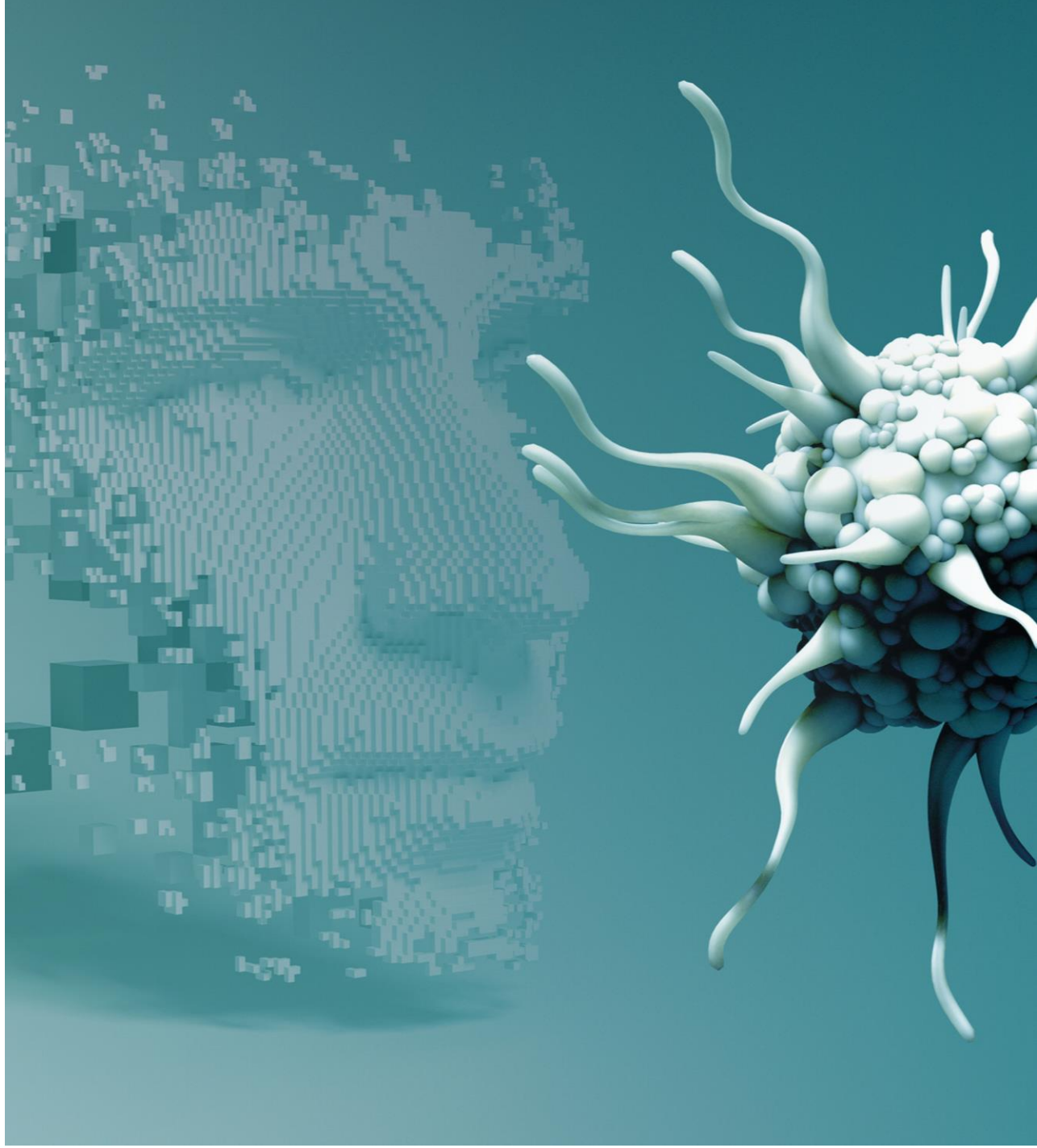
GARDskin accuracy:	94%
WLR	82.1- 88.9%
BLR	92%

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.***

GARDskin Dose-Response

Adaptation of the validated GARDskin protocol to allow for continuous potency predictions

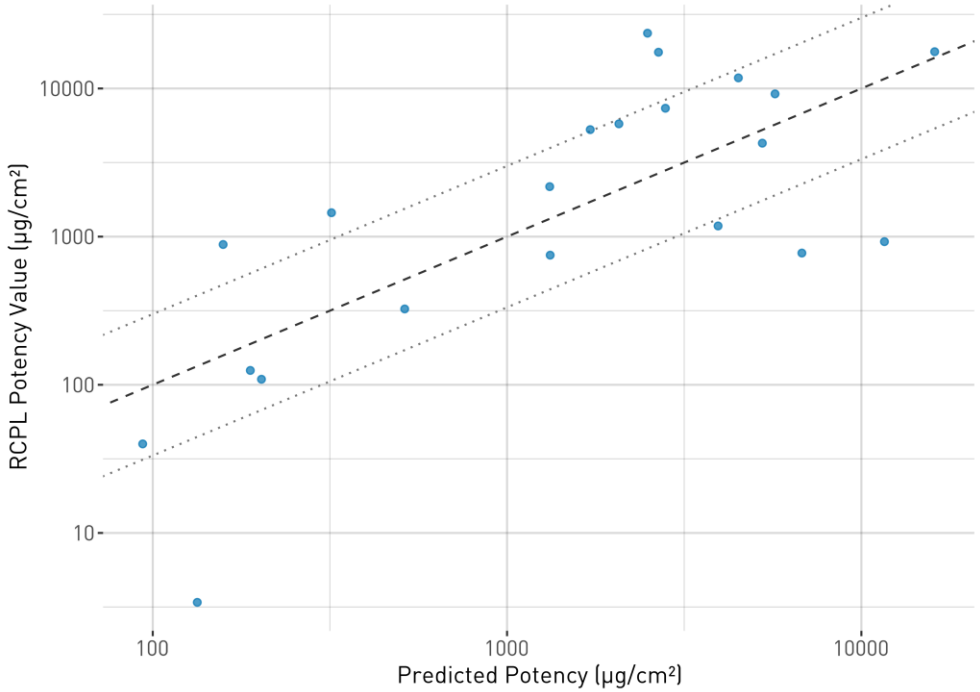


GARDskin Dose-Response

In vitro assessment of skin sensitizing potency

- Based on the validated protocol of GARDskin but produces a quantitative readout on a continous scale significantly associated with sensitizing potency.
- GARDskin Dose-Response allows for continous potency predictions in the unit ug/cm^2.
- Prediction model and data processing pipeline is fully automated and incorporated into the cloud-based GARD Data Analysis Application (GDAA). No risk for manual errors.

Spoiler alert: These are the potency predictions for the chemicals in the RCPL-list.

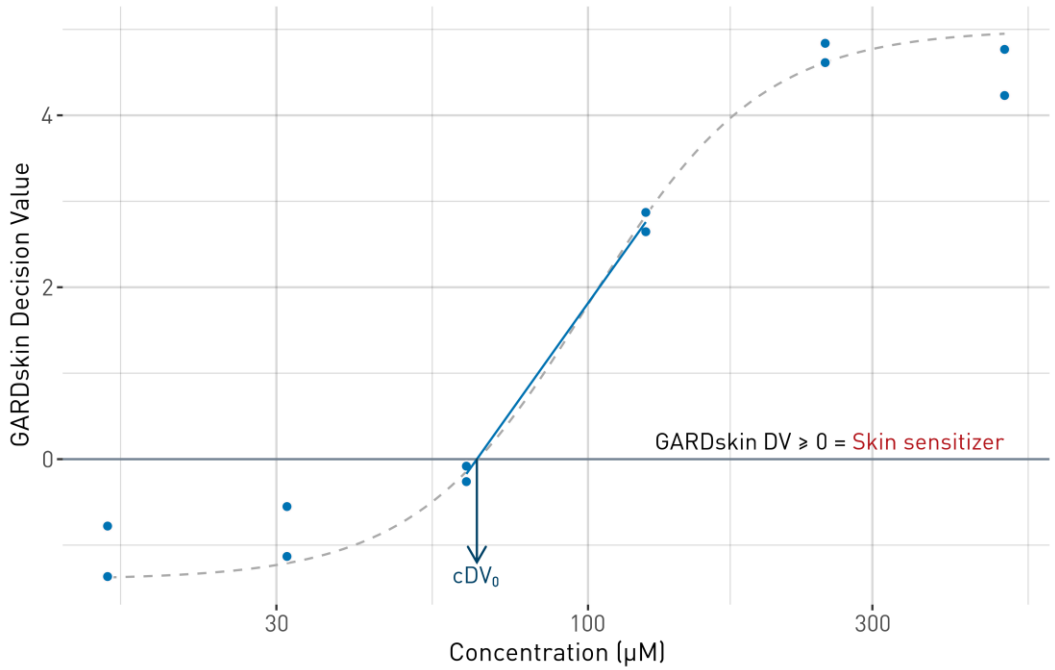


GARDskin Dose-Response

How does it work in practice

- Perform the GARDskin assay at multiple concentrations.
- Use the standard GARDskin protocol to generate decision values (DVs) for each concentration.
- Visually inspect the dose-response data by plotting decision values versus concentrations.
- Estimate cDV_0: The lowest concentration expected to induce a positive classification (DV ≥ 0).

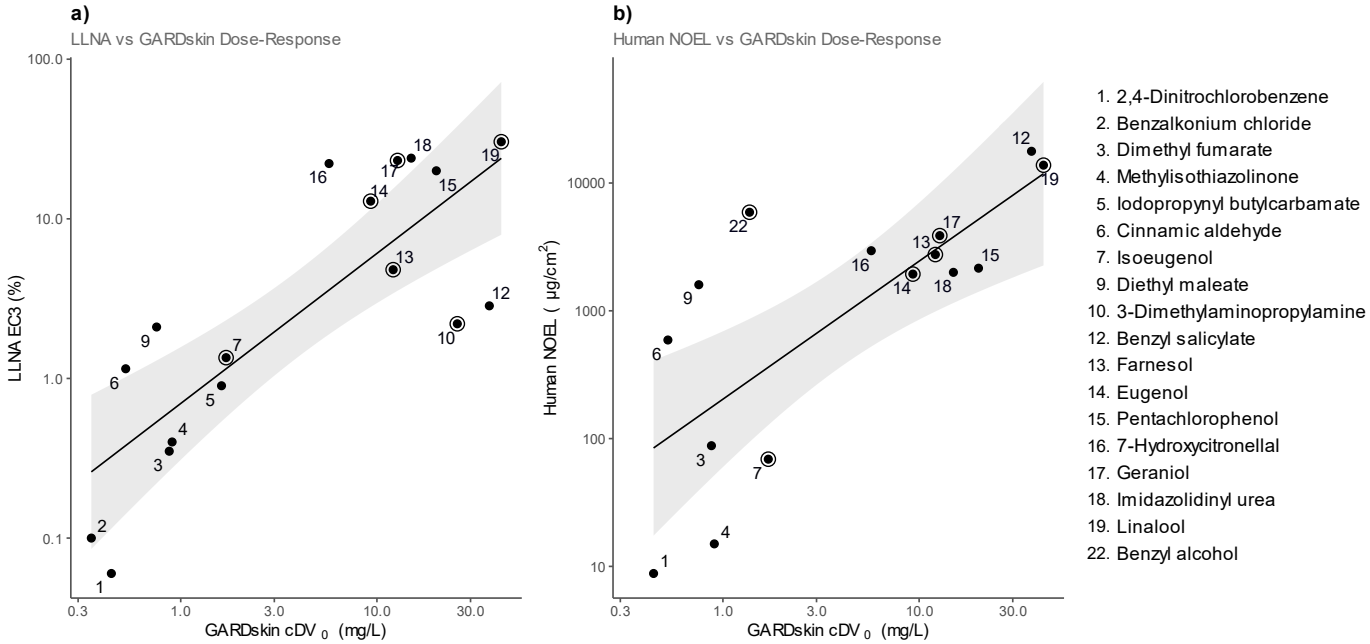
	GARD	LLNA
Response value	DV	SI
Binary threshold	DV = 0	SI = 3
Readout	cDV_0	EC3



GARDskin Dose-Response

Generated cDV_0 values can be used to inform on skin sensitizing potency

- Experimentally derived cDV_0 values correlate strongly with skin sensitizing potency (LLNA EC3 and human NESIL):
  - Linear correlation: 0.81 ($p = 9.1 \times 10^{-5}$)
  - Rank correlation: 0.74 ($p = 1.5 \times 10^{-3}$)

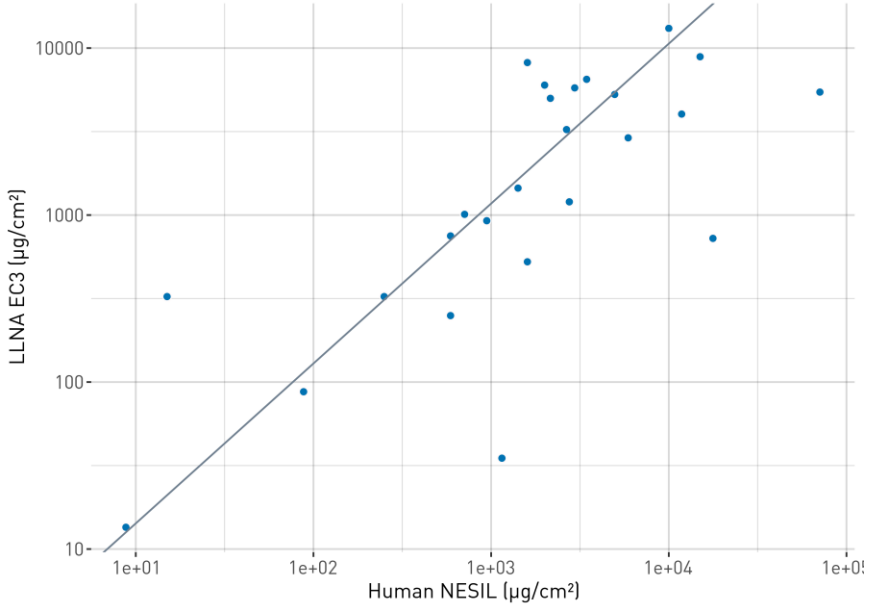


Methodology described in Gradin, R., Forreryd, A., Mattson, U., Jerre, A., Johansson, H. (2021) Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. Nature Scientific Reports

GARDskin Dose-Response

Composite potency score

- Should the model predict human NESIL or LLNA EC3?
- It was considered redundant to fit models separately to LLNA EC3 and human NESIL.
- Both references inform on the same phenomenon i.e., skin sensitizing potency, but neither is perfect and associated with measurement errors.
- The composite score was designed to account for main shared variance. Unit is continous: ug/cm^2.



Example	LLNA EC3 (µg/cm^2)	Human NESIL (µg/cm^2)	Composite (µg/cm^2)
DNCB	13.5	8.8	
Cinnamic aldehyde	250	591	
Citral	1450	1420	
…			

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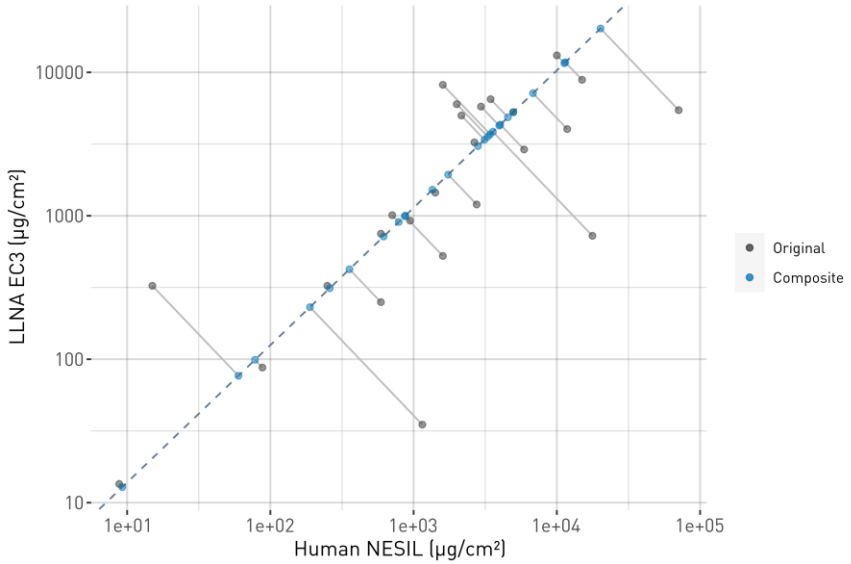

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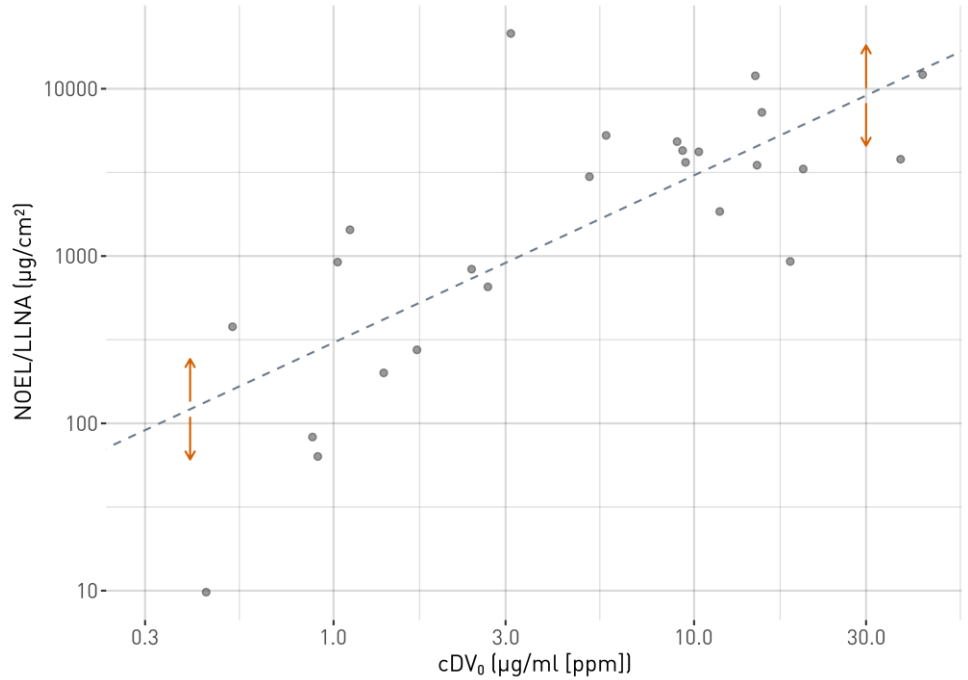
DNCB	13.5	8.8	9.8
Cinnamic aldehyde	250	591	378
Citral	1450	1420	1440
…	…	…	…

GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency

- 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 $\mu g/cm^2$ = cDV_0 in $\mu g/ml \times \theta$
- The model can be used to predict potency on a continuous scale for test materials of unknown sensitizing potential.

Composite score created from LLNA EC3 and Human NESIL



GARDskin Dose-Response

How to derive continuous potency predictions

Step 1: Dose-Response testing.

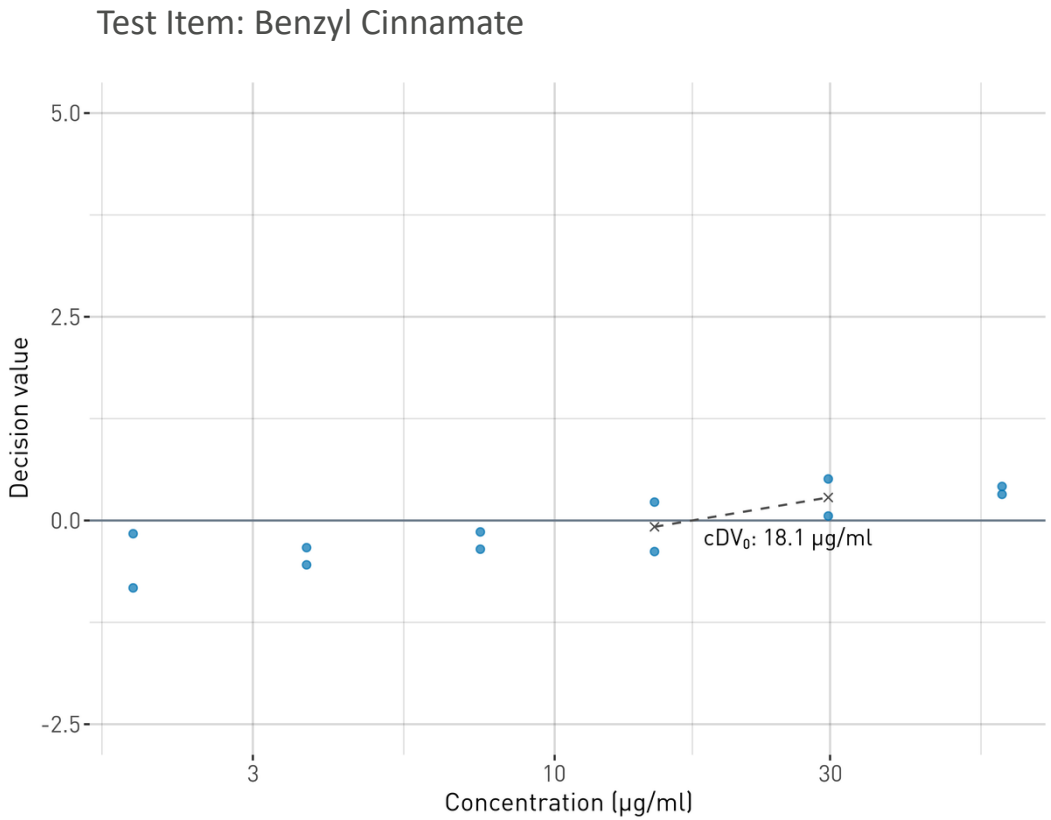
- Generation of a dose response curve by plotting DV vs concentration.
- Identification of a cDV_0 value using linear interpolation.

Step 2: Continous 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)

Step 3: Provide an estimate of uncertainty in predictions

- A 95% confidence interval for the predicted NESIL value is calculated to provide an estimate of uncertainty in prediction.



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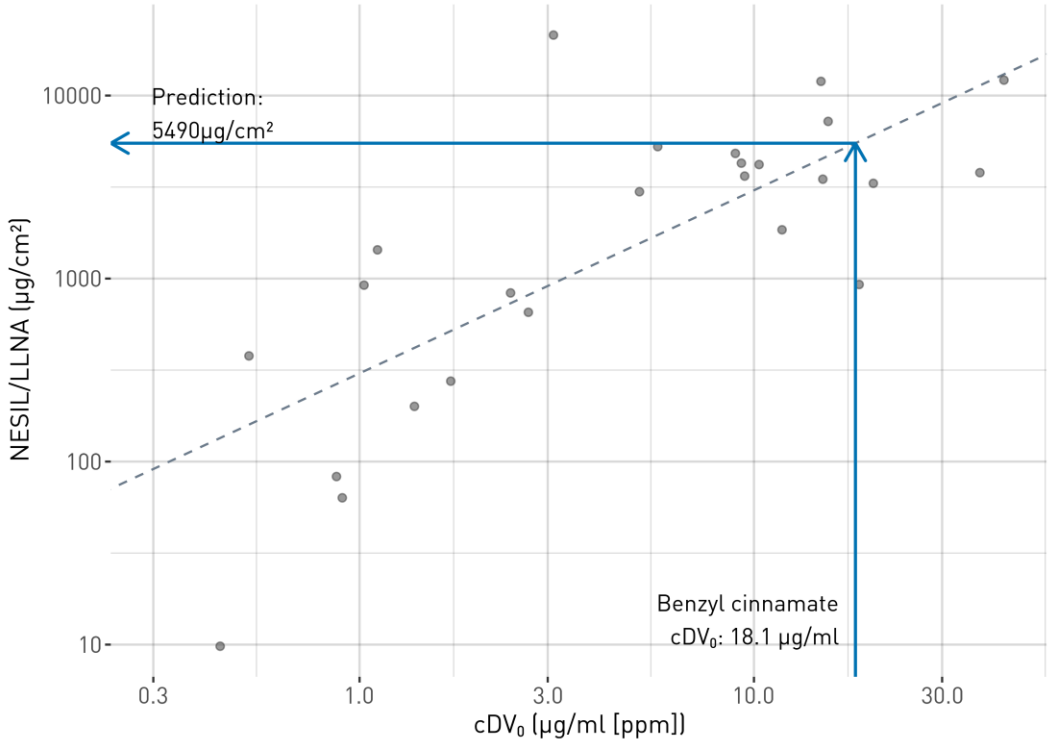
Test Item: Benzyl Cinnamate



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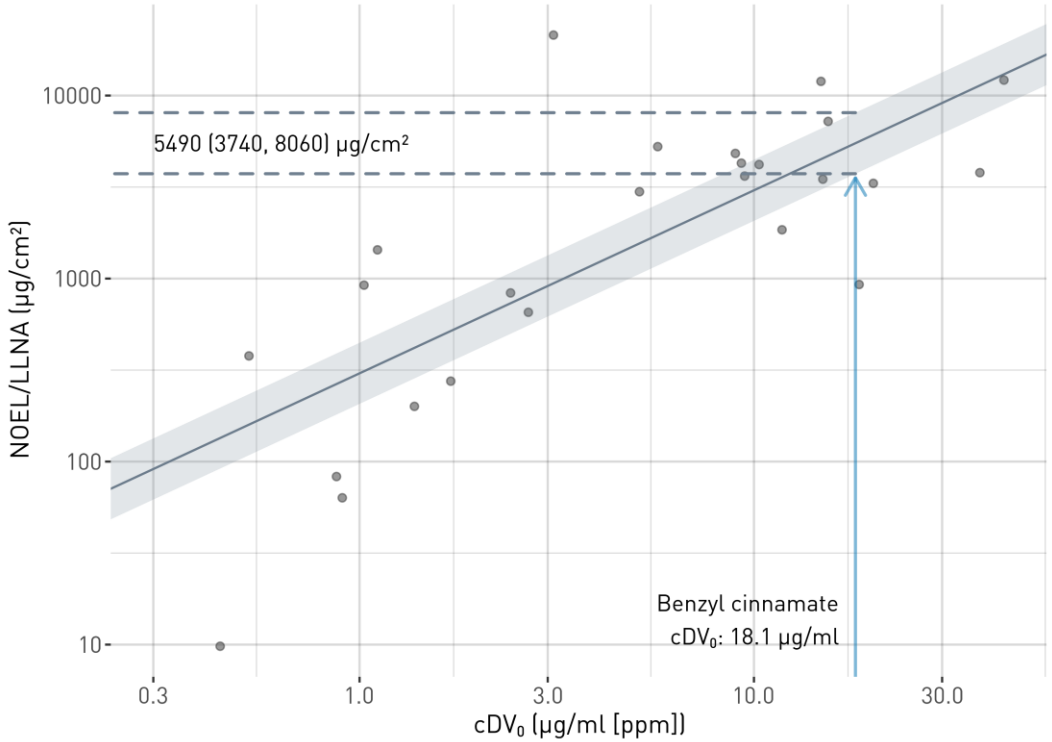


GARDskin Dose-Response

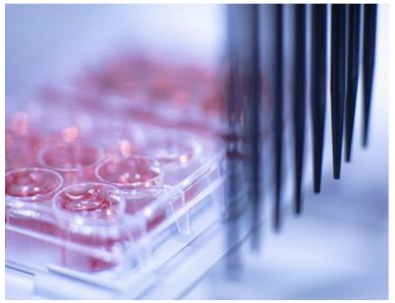
Cross-sector collaboration to evaluate reproducibility and performance

Background

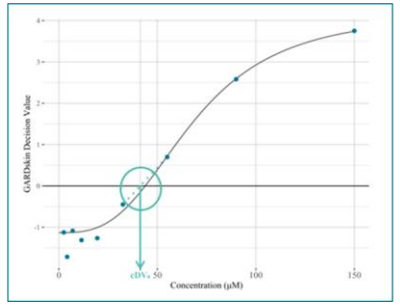
- Collaboration SenzaGen, IFF and RIFM
- Pre-validation exercise to evaluate reproducibility and performance of the GARDskin Dose-Response assay.

Methods

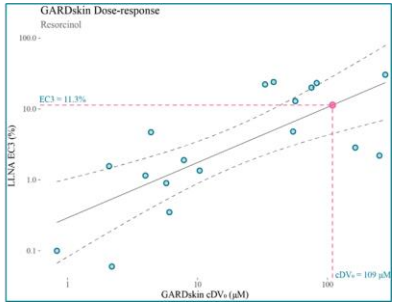
- Materials were tested in blinded studies according to GARDskin Dose-Response protocols.
- The identified cDV_0 values were used to predict potency (LLNA EC3/Human NESIL).
- Following decoding of sample IDs, results were compared to available reference data, mainly from the comprehensive database available at RIFM.



Step 1
Perform cellular stimulations (6 x conc).



Step 2
Generate a dose-response curve and identify cDV_0.



Step 3
Predict EC3/NESIL using the established regression models.

GARDskin Dose-Response

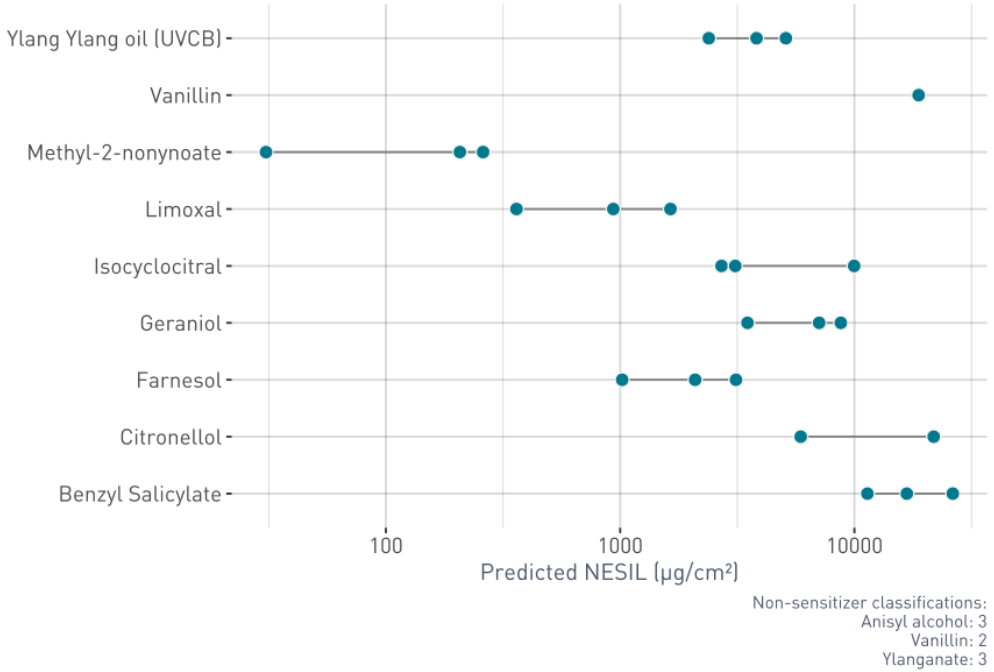
Cross-sector collaboration to evaluate reproducibility and performance

Results

- Repeated measurements of 11 materials in three independent runs (blinded).
- The predicted potency values from GARDskin Dose-Response were reproducible between experiments with a typical variation of 1.8-fold-changes.*

Conclusions

- Gold standard LLNA: Typical variation observed from multiple runs is 2.4-fold-changes.#
- GARDskin Dose-Response provides reproducible continous potency predictions.



*Based on residual standard deviations.

#Calculated from the Cosmetic Europe database published in Hoffmann et al. (2018)

GARDskin Dose-Response

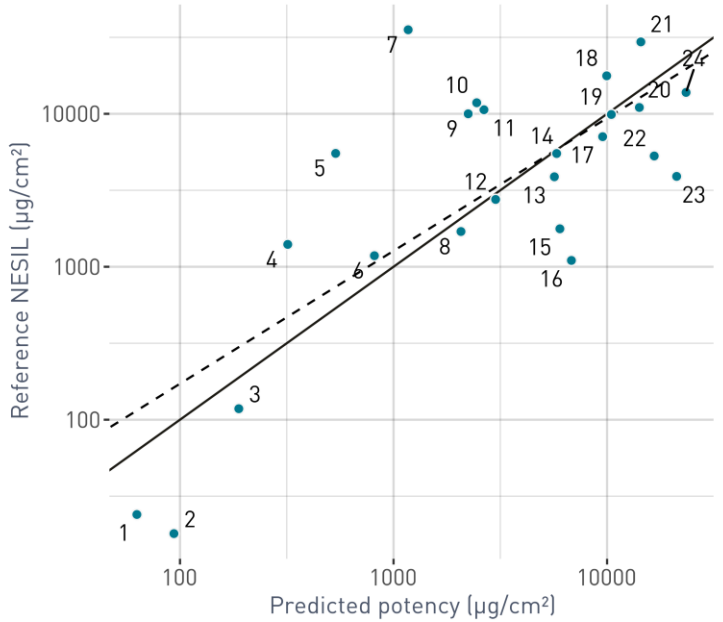
Cross-sector collaboration to evaluate reproducibility and performance

Results

- GARDskin Dose-Response data available for a total of 24 fragrance materials.
- The GARDskin Dose-Response predicted potency values correlated well with Human NESIL values (r=0.75).

Conclusions

- GARDskin Dose-Response provides reproducible and accurate potency predictions with high correlation to human NESIL values.

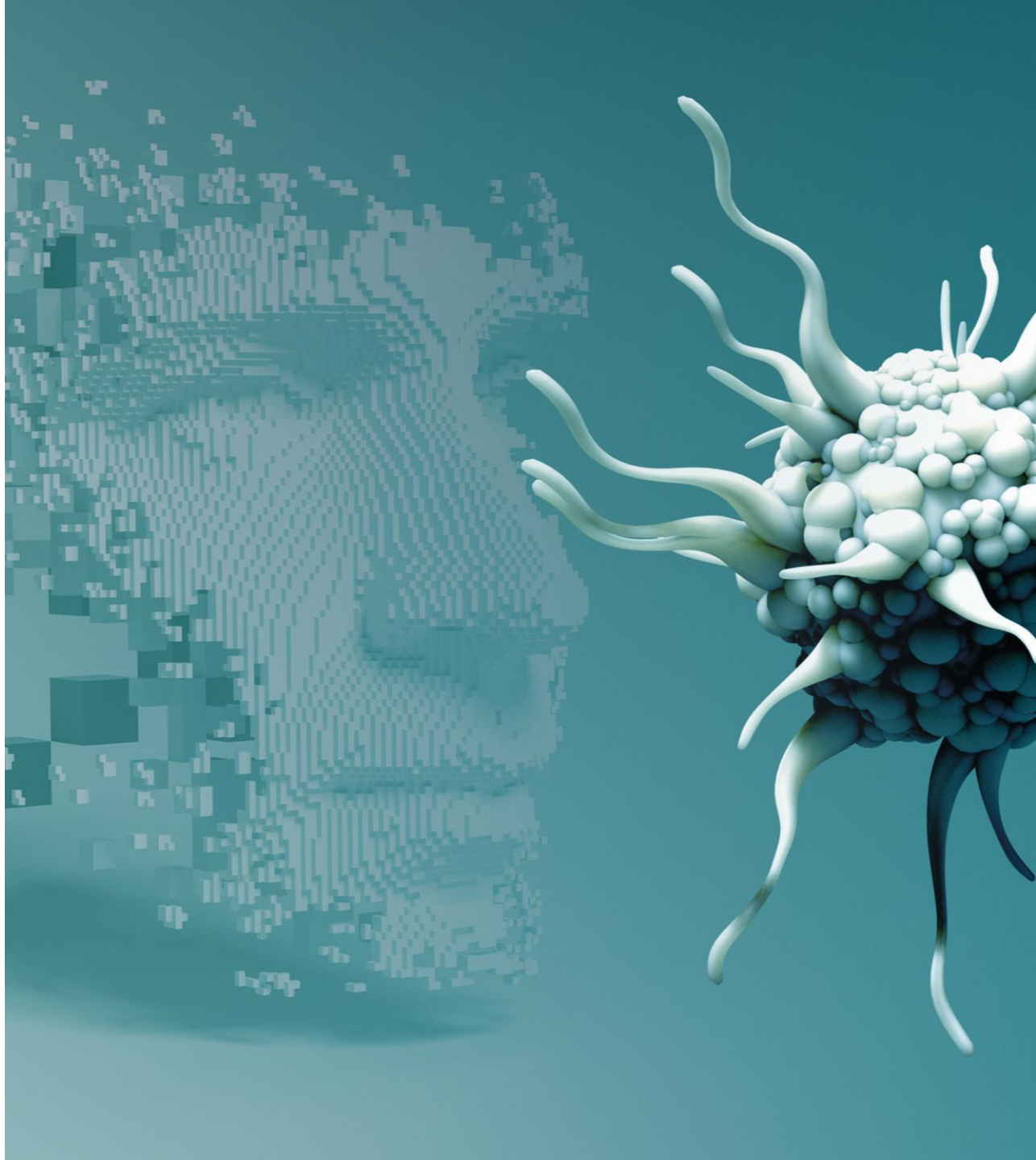


1. Methyl-2-nonynoate
2. Hexen-2-al
3. Methyl Heptine Carbonate
4. Citral
5. Limoxal
6. p-t-Butyl-dihydrocinnamaldehyde (Bourgenol)
7. Hexyl salicylate
8. Cinnamic Alcohol
9. Cosmone
10. Evernyl
11. 1,1,3-Trimethyl-3-phenylindane
12. Farnesol
13. Geraniol
14. omega-Pentadecalactone
15. Ylang Ylang oil (UVCB)
16. 7-Methyl-2H-benzo-1,5-dioxepin-3(4H)-one
17. Isocyclocitral
18. Mayol
19. Majantol
20. 1-(3-Methyl-2-benzofuranyl)ethanone
21. Citronellol
22. Vanillin
23. Iso-cyclo Geraniol
24. Benzyl Salicate

Data have been collected for an extended dataset (n=100). Manuscript is being prepared together with RIFM and IFF.

GARDskin Dose-Response

Evaluation based on Reference Chemical Potency List (RCPL)

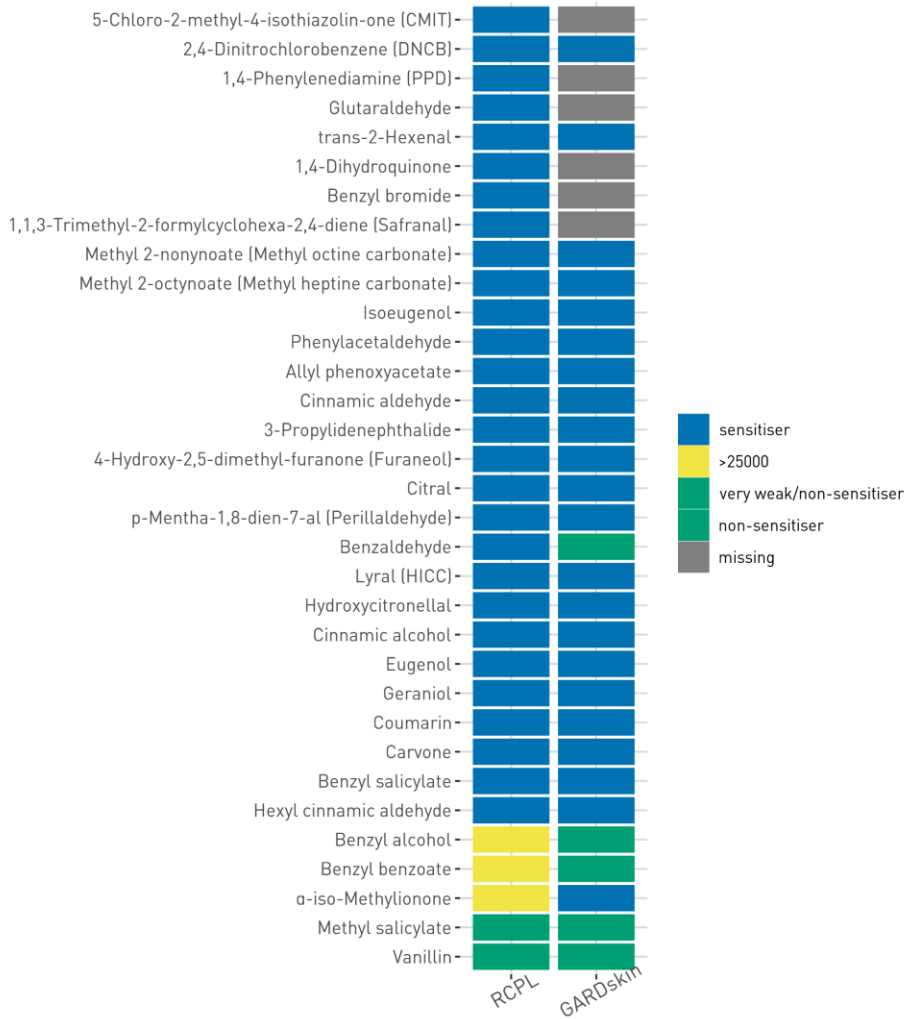


Reference Chemical Potency List

Binary outcomes and data coverage in GARDskin Dose-Response

- Data available for 27/33 materials in the RCPL list. High integrity of data, most obtained in blinded studies.
- 21/22 Sensitizers were correctly classified.
- 2/2 Non-Sensitizers were correctly classified.
- 1/3 Very weak sensitizers (<25,000) was classified as a sensitizer.

Overall, the binary classifications were consistent with the expected reference categories.

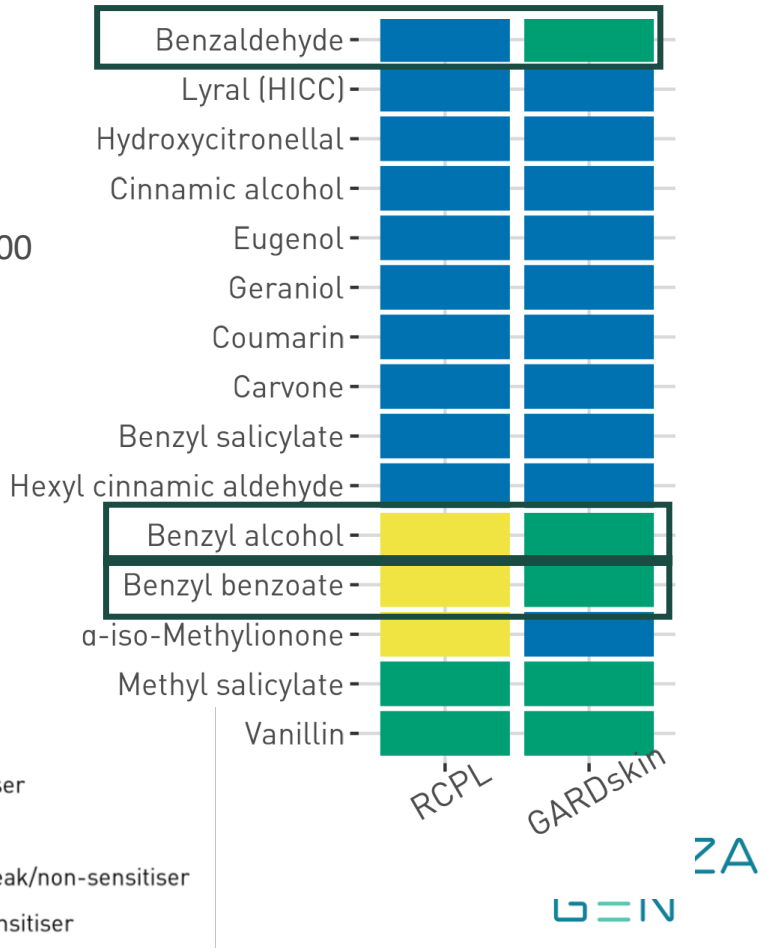


Reference Chemical Potency List

Binary outcomes and data coverage in GARDskin Dose-Response

- The binary disagreement consisted of 3 chemicals: benzyl benzoate, benzyl alcohol and benzaldehyde.
  - **Benzyl benzoate** has been assayed in GARDskin at multiple instances. Borderline chemical. Negative in DASS.
  - **Benzyl alcohol** has positive instances in the GARDskin assay but appears to be more stably classified as a non-sensitizer.
  - **Benzaldehyde** has consistently been classified as a non-sensitizer in the GARDskin assay.
    - LLNA is negative at 25% (>6250 µg/cm^2).
    - DPRA is negative. KeratinoSens & h-CLAT is positive.

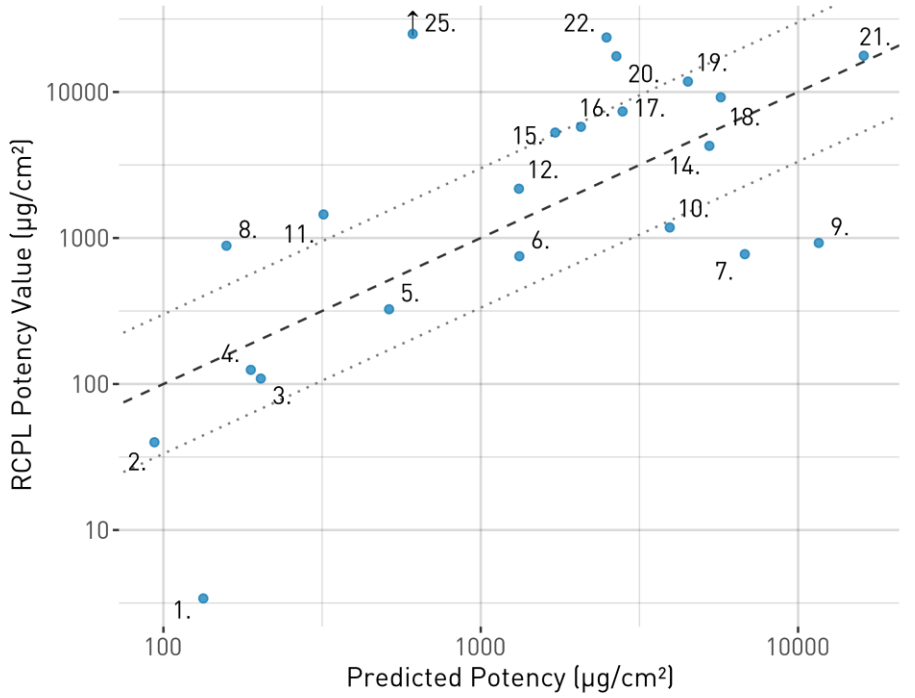
RCPL Potency Value: >25,000



Reference Chemical Potency List

GARDskin Dose-Response's prediction of potency values

- GARDskin Dose-Response data available for a total of 22 compounds.
    - Pearson correlation: 0.74 (p = 0.000136)
- **>35%** (8/22) of predictions are within 2-fold changes.
- **>50%** (12/22) of predictions are within 3-fold changes
- Only 3 materials with fold-change > 10



27. Vanillin
26. Methyl salicylate
25. α-iso-Methylionone
24. Benzyl benzoate
23. Benzyl alcohol
22. Hexyl cinnamic aldehyde
21. Benzyl salicylate
20. Carvone
19. Coumarin
18. Geraniol
17. Eugenol
16. Cinnamic alcohol
15. Hydroxycitronellal
14. Lyral (HICC)
13. Benzaldehyde
12. p-Mentha-1,8-dien-7-al (Perillaldehyde)
11. Citral
10. 4-Hydroxy-2,5-dimethyl-furanone (Furaneol)
9. 3-Propylidenephthalide
8. Cinnamic aldehyde
7. Allyl phenoxyacetate
6. Phenylacetaldehyde
5. Isoeugenol
4. Methyl 2-octynoate (Methyl heptine carbonate)
3. Methyl 2-nonynoate (Methyl octine carbonate)
2. trans-2-Hexenal
1. 2,4-Dinitrochlorobenzene (DNCB)

Dashed line: y = x
Dotted lines: 3 Fold difference
Green labels: Classified as non-sensitisers

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Reference Chemical Potency List

GARDskin Dose-Response's prediction of potency values

- Considering the entire dataset 44% (12/27) were either correct or within 2-fold change.
  - Green: 44% Correct or < 2-fold changes
  - Yellow: 70% < 5-fold-changes
  - Orange: 89% <10-fold-changes
  - Red: 11% > 10-fold-changes

Chemical	CAS	PV	Predicted
5-Chloro-2-methyl-4-isothiazolin-one (CMIT)	26172-55-4	2.3	NA
2,4-Dinitrochlorobenzene (DNCB)	97-00-7	3.4	133.6
1,4-Phenylenediamine (PPD)	106-50-3	3.9	NA
Glutaraldehyde	111-30-8	20.0	NA
trans-2-Hexenal	6728-26-3	39.9	93.67
1,4-Dihydroquinone	123-31-9	47.5	NA
Benzyl bromide	100-39-0	50.0	NA
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)	116-26-7	106	NA
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	109	202.7
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	125	188.5
Isoeugenol	97-54-1	325	514.2
Phenylacetaldehyde	122-78-1	750	1323
Allyl phenoxyacetate	7493-74-5	775	6793
Cinnamic aldehyde	104-55-2	885	158.0
3-Propylidenephthalide	17369-59-4	925	11620
4-Hydroxy-2,5-dimethyl-furanone (Furaneol)	3658-77-3	1181	3941
Citral	5392-40-5	1450	319.5
p-Mentha-1,8-dien-7-al (Perillaldehyde)	2111-75-3	2175	1319
Benzaldehyde	100-52-7	4094	NS
Lyral (HICC)	31906-04-4	4275	5254
Hydroxycitronellal	107-75-5	5275	1717
Cinnamic alcohol	104-54-1	5775	2068
Eugenol	97-53-0	7357	2798
Geraniol	106-24-1	9197	5706
Coumarin	91-64-5	11792	4496
Carvone	6485-40-1	17573	2675
Benzyl salicylate	118-58-1	17715	16100
Hexyl cinnamic aldehyde	101-86-0	23620	2491
Benzyl alcohol	100-51-6	>25000	NS
Benzyl benzoate	120-51-4	>25000	NS
α-iso-Methylionone	127-51-5	>25000	610.4
Methyl salicylate	119-36-8	non-sensitiser	NS
Vanillin	121-33-5	non-sensitiser	NS

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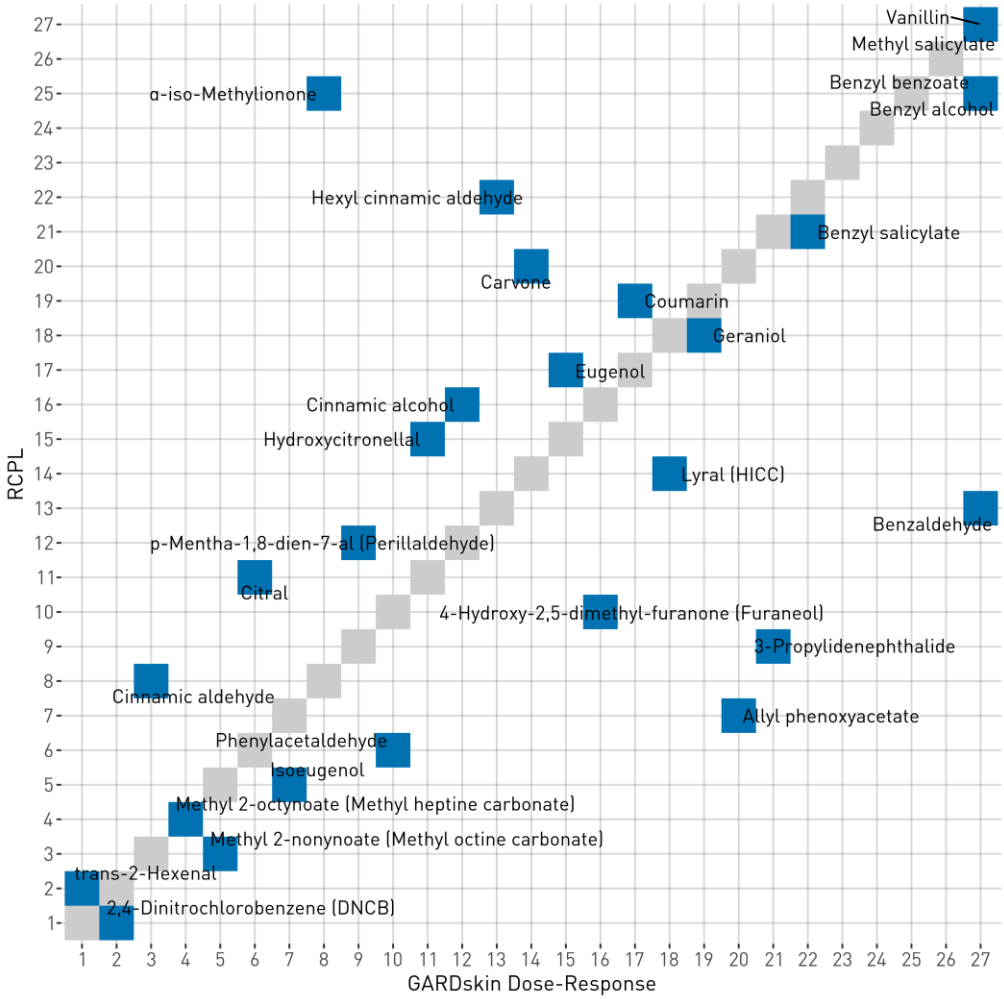
Reference Chemical Potency List

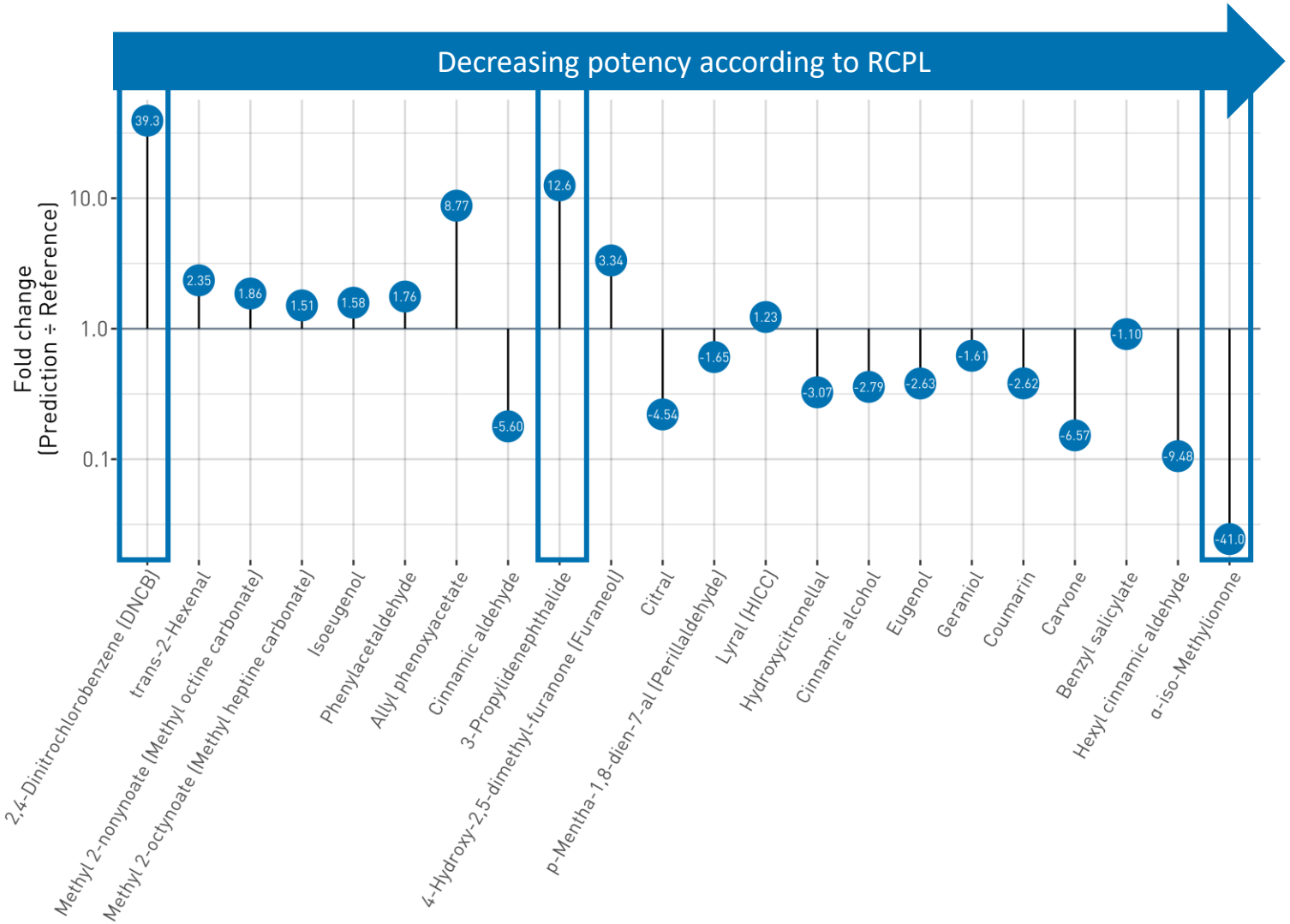
GARDskin Dose-Response's prediction of potency values

- The rank-correlation provides a measure of the similarity of the ranking between GARDskin Dose-response and RCPL-list:
    - Rank correlation: 0.69 (p = 0.000071)



Reference Chemical Potency List

GARDskin Dose-Response's prediction of potency values



Unsigned fold-changes (errors)

- Geometric mean: 3.72
- Median: 2.71

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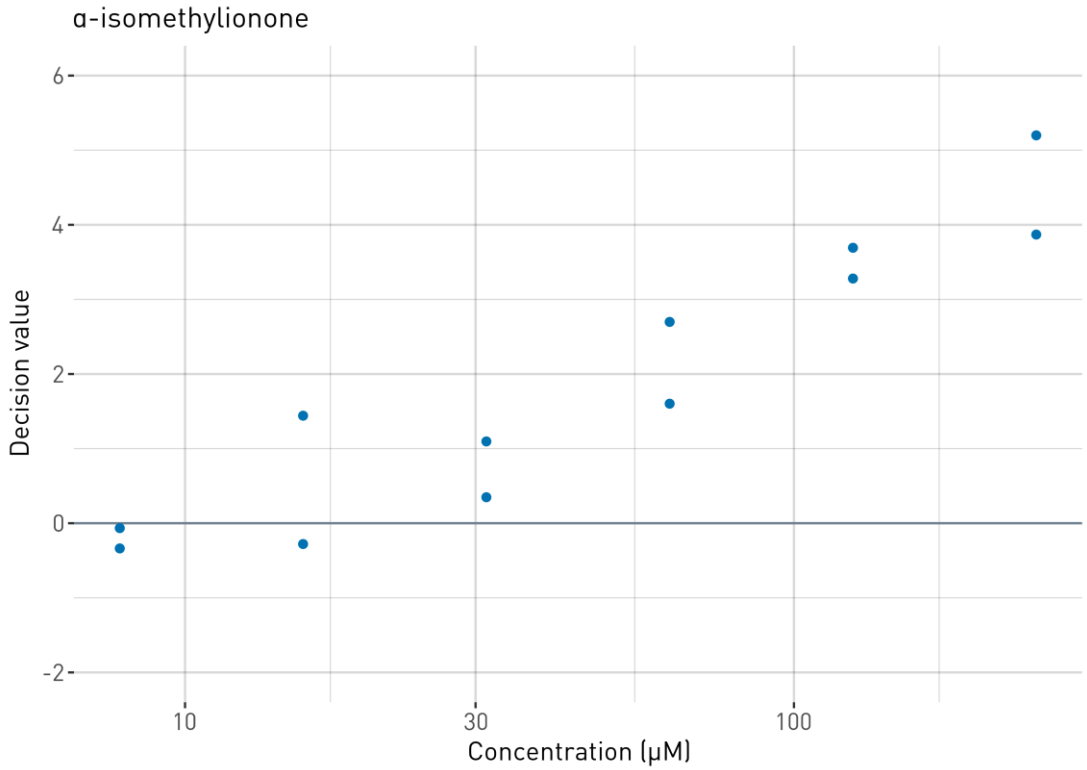
Reference Chemical Potency List

Largest discrepancies (FC > 10): α-isomethylionone

Potency overpredicted with at least 41.0-fold changes
Predicted: 610 µg/cm^2 (moderate)
Expected: >25,000 µg/cm^2

Reference data
Predicted Michael acceptor but no observed protein reactivity.
Positive in LLNA with EC3 of 5450 µg/cm^2 (21.8%).
Negative in DPRA & KeratinoSens;
Positive in h-CLAT (ITS score of 2) & U-Sens
Positive in Derek & OECD TB



Only a single experiment available: Some uncertainty in the replicate measurement for the second lowest concentration → may shift the prediction towards a higher concentration

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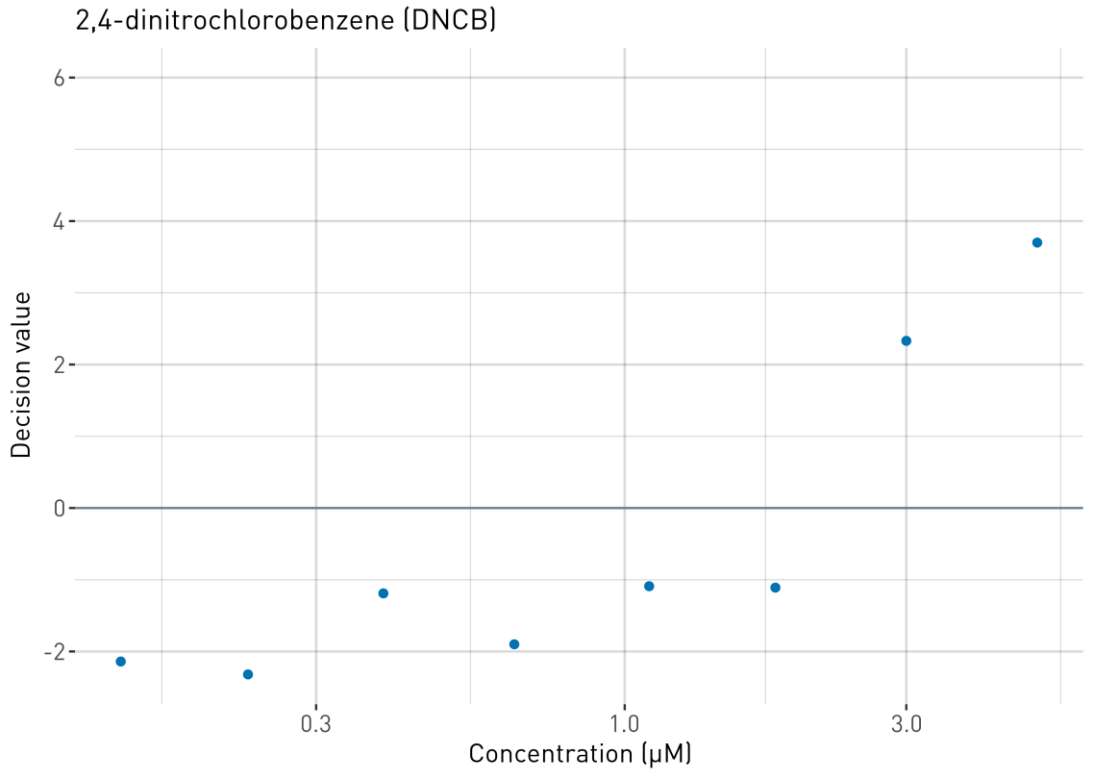
Reference Chemical Potency List

Largest discrepancies (FC > 10): DNCB

Potency underpredicted with 39.3-fold changes
Predicted: 134 µg/cm^2 (moderate)
Expected: 3.4 µg/cm^2

Reference data
Positive in LLNA with EC3 of 13.5 µg/cm^2 (0.054%).
Positive in DPRA, KeratinoSens, and h-CLAT.
Positive in Derek & OECD TB



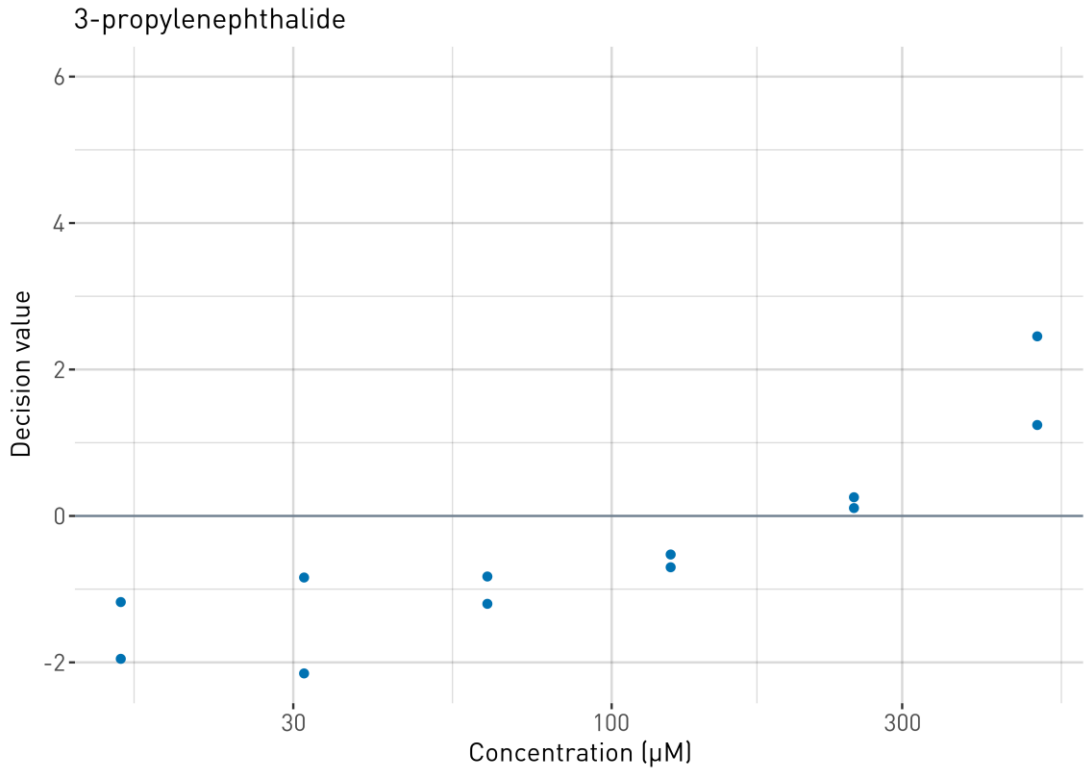
Reference Chemical Potency List

Largest discrepancies (FC > 10): 3-propylenephtalide

Potency underpredicted with 12.6-fold changes
Predicted: 11 600 µg/cm^2 (very weak)
Expected: 925 µg/cm^2

Clear dose-response pattern in GARDskin Dose-Response.

Reference data
LLNA and NESIL values very similar.
Human LOEL (HMT): 2760 µg/cm^2.
Positive in h-CLAT (ITS score of 2) and DPRA (ITS score of 1);
Negative in KeratinoSens.



Summary & conclusions

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- Continous potency predictions from the GARDskin Dose-Response assay correlated well with PVs for chemicals in the RCPL list (pearson correlation: 0.74).
- The average misprediction from GARDskin Dose-Response was 3.7-fold (geometric mean) and 44% of predictions were either correct or within 2-fold changes from reference value.
- Overall, very similar potency rankings with GARDskin Dose-Response and RCPL potency list (spearman: 0.69)
- Balanced predictions across the potency interval, no systematic bias for under- or over-predictions.
- Mispredictions appears not to be attributable to a certain chemical reactivity domains or to indirect acting haptens.

Thank you for listening!

Prediction table

Chemical	CAS	RCPL (µg/cm²)	GSDR (µg/cm²)	FC	Log_2 FC
5-Chloro-2-methyl-4-isothiazolin-one (CMIT)	26172-55-4	2.3	-	-	-
2,4-Dinitrochlorobenzene (DNCB)	97-00-7	3.4	134	39.3	5.3
1,4-Phenylenediamine (PPD)	106-50-3	3.9	-	-	-
Glutaraldehyde	111-30-8	20	-	-	-
trans-2-Hexenal	6728-26-3	39.9	93.7	2.35	1.23
1,4-Dihydroquinone	123-31-9	47.5	-	-	-
Benzyl bromide	100-39-0	50	-	-	-
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)	116-26-7	106	-	-	-
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	109	203	1.86	0.895
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	125	189	1.51	0.593
Isoeugenol	97-54-1	325	514	1.58	0.662
Phenylacetaldehyde	122-78-1	750	1320	1.76	0.819
Allyl phenoxyacetate	7493-74-5	775	6790	8.77	3.13
Cinnamic aldehyde	104-55-2	885	158	0.179	-2.49
3-Propylidenephthalide	17369-59-4	925	11600	12.6	3.65
4-Hydroxy-2,5-dimethyl-furanone (Furaneol)	3658-77-3	1181	3940	3.34	1.74
Citral	5392-40-5	1450	319	0.22	-2.18

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Prediction table

Chemical	CAS	RCPL (µg/cm²)	GSDR (µg/cm²)	FC	Log_2 FC
p-Mentha-1,8-dien-7-al (Perillaldehyde)	2111-75-3	2175	1320	0.607	-0.721
Benzaldehyde	100-52-7	4094	NS	-	-
Lyral (HICC)	31906-04-4	4275	5250	1.23	0.297
Hydroxycitronellal	107-75-5	5275	1720	0.325	-1.62
Cinnamic alcohol	104-54-1	5775	2070	0.358	-1.48
Eugenol	97-53-0	7357	2800	0.38	-1.39
Geraniol	106-24-1	9197	5710	0.62	-0.689
Coumarin	91-64-5	11792	4500	0.381	-1.39
Carvone	6485-40-1	17573	2680	0.152	-2.72
Benzyl salicylate	118-58-1	17715	16100	0.909	-0.138
Hexyl cinnamic aldehyde	101-86-0	23620	2490	0.105	-3.25
Benzyl alcohol	100-51-6	>25000	NS	-	-
Benzyl benzoate	120-51-4	>25000	NS	-	-
α-iso-Methylionone	127-51-5	>25000	610	0.0244	-5.36
Methyl salicylate	119-36-8	very weak/non-sensitiser	NS	-	-
Vanillin	121-33-5	very weak/non-sensitiser	NS	-	-

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