Skin Allergy Risk Assessment (SARA) Model: GARDskin dose-response as a possible input



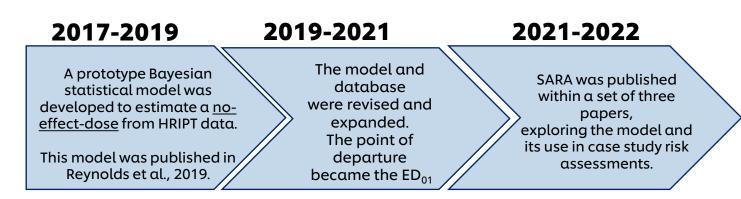
Georgia Reynolds SEAC, Unilever georgia.reynolds@unilever.com



Skin Allergy Risk Assessment (SARA) Model

The SARA Model is used within an NGRA framework to estimate:

- Point of Departure: An ED₀₁, i.e. 1% sensitising dose in a human population for a chemical of interest based upon chemical specific (primarily NAM) data
- 2. Risk Metric: A probability that a consumer exposure to a chemical is 'low risk', conditional on the available data and the model



| SARA Model | |
|----------------------------------|---|
| Database | 428 chemicals |
| Assay Inputs | LLNA (historical), KeratinoSens™, USENS, hCLAT, DPRA, kDPRA, Reactivity classification (NR, RAut, R, HPC), Human data (HRIPT & HMT) |
| Risk Benchmarking | Binary + confidence chemical exposure risk |
| Model PoD | ED ₀₁ (1% sensitising dose for a HRIPT exposure scenario) |
| Probability of Sensitiser | S/NS |
| Model Risk Metric | Probability exposure is low risk/probability exposure is high risk. Low risk/high risk/inconclusive calls |
| Production Model | Faster, approximated production model |



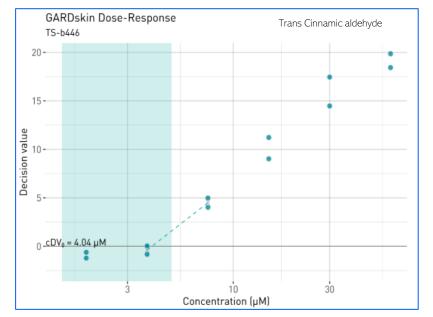
GARDskin Dose Response - viable input assay for the SARA Model?

GARDskin Dose-Response is an *in vitro* test for quantitative skin sensitizing potency assessment of chemicals, adapted from GARDskin, using the same 196 transcripts (OECD TG 442E).

The assay provides an **estimated threshold concentration** (cDV₀) for a test substance to induce skin sensitizing effects; the lower concentration the higher the expected potency and vice versa.

The SARA Model assumes correlation between assay inputs e.g. KeratinoSens EC1.5 and the ED_{01} .

Hypothesis: cDV₀ correlates with ED₀₁



- **6 test concentrations:** Starting from the identified highest concentration, 5 additional stimulation concentrations are selected
- **Data output:** cDV0 calculated using linear interpolation between the two concentrations with DVs on respective side of the decision boundary (DV₀)



Chemical selection

- Range of potencies
- \circ Benchmark chemicals
- $\circ~$ Chemicals with conflicting existing data

| Chemical | CAS | Rationale | |
|--------------------------------------|------------|--|--|
| Cinnamic alcohol | 104-54-1 | Potency benchmark for cinnamic aldehyde (weak) | |
| Cinnamic aldehyde | 14371-10-9 | Potency benchmark for cinnamic alcohol (strong) | |
| Tetramethyl thiuram disulfide (TDMS) | 137-26-8 | Very potent in SARA NAM data but weak <i>in vivo</i> | |
| Benzyl alcohol | 100-51-6 | Surprisingly potent in GARDskin DR - outlier in published data | |
| Benzaldehyde | 100-52-7 | Should be more potent than benzyl alcohol | |
| Anisyl alcohol | 105-13-5 | Surprisingly inactive in other NAMs, but positive in GARDskin | |
| Squaric acid | 2892-51-5 | Other NAMs underestimate the classification | |
| Hexyl cinnamic aldehyde | 101-86-0 | Positive control for LLNA (weak) | |
| Lauryl gallate | 1166-52-5 | Very potent in GARDskin at low concentrations | |
| 1-Fluoro-2,4-dinitrobenzene | 70-34-8 | Preliminary analysis showed good correlation with SARA, look to repeat | |



Comparator cDV₀ Values

cDV₀ values sourced from 29 chemicals of varying potency listed in Table 1 of the Gradin *et al.*, 2021 publication.

Quantitative assessment of sensitizing potency using a dose–response adaptation of GARDskin

Robin Gradin, Andy Forreryd, Ulrika Mattson, Anders Jerre & Henrik Johansson 🖂

Scientific Reports 11, Article number: 18904 (2021) Cite this article

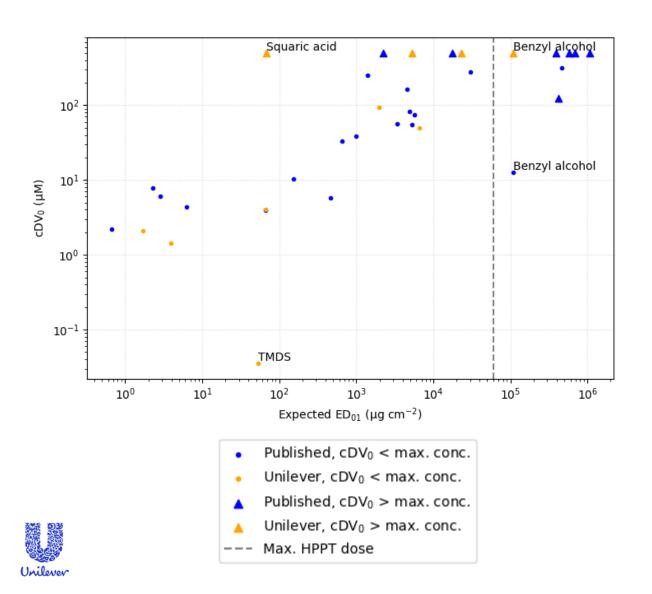
If no cDV₀ value was reported, data was treated as censored by the maximum concentration tested.

| 2.4-binitrochlorobenzene97-0-70.068.8Benzalkonium chloride6001-54-500.1NDDimethyl fumarate624-49-70.3588Methylisothiazolinone2682-20-40.415lodopropynyl butylcarbamate55406-53-60.9NDCinnamic aldehyde104-55-21.15591Isoeugenol97-54-11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.11003-Dimethylamiopropylamine109-55-72.25510Taran-Anethole4180-23-802.75510Benzyl salicylate18-58-12.81.7,17Farnesol4602-84-04.82755Eugenol97-53-01.21.93Pentachlorophenol16-24-12.2325Ceraniol106-24-12.2326Inidazolidinylurea3293-64-92.4200Linadol78-70-6NS3041.3793Benzyl salifate69-72-71.2NSBenzyl cohol130-07-75.8NS306Salicylic acid69-72-71.2NS306Salicylic acid69-72-71.2NS306Salicylic acid69-72-71.2NS306Salicylic acid69-72-71.2NS306Salicylic acid69-72-71.2NS306Salicylic acid69-81-5NSNS306 <td< th=""><th>Chemical</th><th>CAS</th><th>LLNA EC3 (%)</th><th>NOEL (µg/cm²)</th></td<> | Chemical | CAS | LLNA EC3 (%) | NOEL (µg/cm²) |
|---|-----------------------------|------------|--------------|---------------|
| Dimethyl fumarate624-49-70.3588Methyl isothiazolinone2682-20-40.415Iodopropynyl butylcarbamate55406-53-60.9NDCinnamic aldehyde104-55-21.15591Isoeugenol97-54-11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-82.75510Benzyl salicylate118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol106-24-12.322857T-Hydroxycitronellal106-24-12.323875Imidazolidinyl urea39236-65-9242000Inaloal1050-51-9NS1874Benzocaine94-09-7NS200Salicylic acid69-72-712.2NSSylene130-20-795.8NSLinalool105-16NS3926Salicylic acid69-72-712.2NSSylene130-20-795.8NSLibutanol71-63NSNSSylene130-20-7NSNSShencaine69-72-7NSNSShencaine71-63NSNSShencaine61-72-7NSNSShencaine71-63NSNSShencaine< | 2,4-Dinitrochlorobenzene | 97-00-7 | | |
| Methylisothiazolinone2682-20-40.415lodopropynyl butylcarbamate55406-53-60.9NDCinnamic aldehyde104-55-21.15591lsoeugenol97-54-11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-802.75510Eugenol118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-5202155Imidazolidinyl urea106-24-123.22953Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate90-97NS1874Benzocaine94-09-7NS2000Salicylic acid69-72-712.2NSXylene1330-20-795.8NSNSJathylenol133-20-795.8NSJathylenol56-81-5NSNSJeytenol56-81-5NSNSJeytenol56-81-5NSNSPhenol184-07-2NSNS | Benzalkonium chloride | 8001-54-5 | 0.1 | ND |
| Nodopropynyl butylcarbamate55406-53-60.9NDCinnamic aldehyde104-55-21.15591Isoeugenol97-54.11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDBenzyl adicylate118-58-12.8517,717Benzyl adicylate118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-5202155Geraniol106-24-123.22953Imidacolidinyl urea39236-46-9242000Linalool7650-51-9NS304Benzyl alcohol7050-51-9NS306Salicylic acid69-72-7NS2000Salicylic acid100-51-6NS5906Salicylic acid1330-20-795.8NSYapene1330-20-795.8NSNSSalicylic acid56-81-5NSNSOttanoic acid124-07-2NSNSPhenol188-95-2NSNS | Dimethyl fumarate | 624-49-7 | 0.35 | 88 |
| Cinnamic aldehyde104-55-21.15591Isoeugenol97-54-11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDBenzyl salicylate4180-23-802.75510Benzyl salicylate118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-01.21938Pentachlorophenol87-86-52.02155Corranol106-24-12.22953Imidazolidnjurea92364-92.42000Linalool78-70-63.0413,793Ramaycin sulfate94-09-7NS200Salicylic acid69-72-71.2NSSylene130-20-79.5.8NSSqlicylic acid71-36-3NSNSCitycerol56-15-9NSNSSalicylic acid130-20-79.5.8NSSylene130-20-79.5.8NSSalicylic acid69-72-71.2.2NSSylene130-20-79.5.8NSSulfordi130-20-7NSNSSylene130-20-7NSNSSylene130-20-7NSNSSylene130-20-7NSNSSylene130-20-7NSNSSylene130-20-7NSNSSylene130-20-7NSNS <t< td=""><td>Methylisothiazolinone</td><td>2682-20-4</td><td>0.4</td><td>15</td></t<> | Methylisothiazolinone | 2682-20-4 | 0.4 | 15 |
| Isoeugenol97-54-11.35692-Hydroxyethyl acrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimehylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-82.75510Benzyl salicylate118-58-12.8517.717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-5202155Geraniol107-75-52.22953Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate94-09-7NS200Benzyl alcohol100-51-6NS5906Salicylic acid69-72-712.2NSAylene1330-20-79.58NSSalicylic acid6-71-3NSNSCityperol56-81-5NSNSHatanol71-36-3NSNSHatanol6-72-712.2NSHatanol71-36-3NSNSHatanol6-81-5NSNSHatanol6-81-5NSNSHatanol124-0-2NSNSOttanoic acid124-0-2NSNSPhenol188-95-2NSNS | Iodopropynyl butylcarbamate | 55406-53-6 | 0.9 | ND |
| 2-Hydroxyethylacrylate818-61-11.56NDDiethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-802.75510Benzyl salicylate118-58-12.8517.717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol76-86-52021557-Hydroxycitronellal106-24-123.22953Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413.793Kanamycin sulfate9409-7NS1874Benzocaine640-2712.2NSSalicylic acid69-72-712.2NSXylene1330-20-795.8NSCitanoi71-36-3NSNSCitanoic acid66-81-5NSNSPhenol108-95-2NSNS | Cinnamic aldehyde | 104-55-2 | 1.15 | 591 |
| Diethyl maleate141-05-92.116003-Dimethylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-82.75510Benzyl salicylate118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-52021557-Hydroxycitronellal107-75-522.22953Geraniol106-24-123.23875Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate70560-51-9NS1874Benzocaine94-09-7NS2000Salicylic acid69-72-712.2NSXylene1330-20-795.8NS1-Butanol71-36-3NSNSOttanoic acid66-81-5NSNSPhenol124-07-2NSNS | Isoeugenol | 97-54-1 | 1.35 | 69 |
| 3-Dimethylaminopropylamine109-55-72.2NDtrans-Anethole4180-23-802.75510Benzyl salicylate118-58-12.8517.717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-52021557-Hydroxycitronellal107-75-52.2.22953Geraniol106-24-123.23875Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413.793Kanamycin sulfate70560-51-9NS364Benzocaine94-09-7NS200Benzyl alcohol100-51-6NS5906Salicylic acid69-72-712.2NSYelpenol1330-20-795.8NS1-Butanol71-36-3NSNS1-Butanol71-36-3NSNSOctanoic acid124-07-2NSNSPenol108-95-2NSNS | 2-Hydroxyethyl acrylate | 818-61-1 | 1.56 | ND |
| trans-Anethole 4180-23-8 2.7 5510 Benzyl salicylate 118-58-1 2.85 17,717 Farnesol 4602-84-0 4.8 2755 Eugenol 97-53-0 12.9 1938 Pentachlorophenol 87-86-5 20 2155 7-Hydroxycitronellal 107-75-5 22.2 2953 Geraniol 106-24-1 23.2 3875 Imidazolidinyl urea 39236-46-9 24 2000 Linalool 78-70-6 30.4 13,793 Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Salicylic acid 69-72-7 NS 2000 Salicylic acid 100-51-6 NS 5906 Salicylic acid 130-20-7 95.8 NS Yelene 1330-20-7 95.8 NS Sulgerol 1330-20-7 95.8 NS Sulgerol 56-81-5 NS NS Glycerol | Diethyl maleate | 141-05-9 | 2.1 | 1600 |
| Benzyl salicylate118-58-12.8517,717Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-52021557-Hydroxycitronellal107-75-522.22953Geraniol106-24-123.23875Inidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate94-09-7NS2000Benzyl alcohol100-51-6NS2000Salicylic acid69-72-712.2NSXylene1330-20-795.8NS1-Butanol71-36-3NSNSGlycerol56-81-5NSNSOctanoic acid124-07-2NSNSPenol108-52-2NSNS | 3-Dimethylaminopropylamine | 109-55-7 | 2.2 | ND |
| Farnesol4602-84-04.82755Eugenol97-53-012.91938Pentachlorophenol87-86-52021557-Hydroxycitronellal107-75-522.22953Geraniol106-24-123.23875Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413.793Kanamycin sulfate70560-51-9NS1874Benzocaine94-09-7NS2000Benzyl alcohol100-51-6NS5906Salicylic acid69-72-712.2NSYelne1330-20-795.8NS1-Butanol71-36-3NSNSOtyperol56-81-5NSNSOttanoic acid124-07-2NSNSPenol108-95-2NSNS | trans-Anethole | 4180-23-8 | 2.7 | 5510 |
| Eugenol 97-53-0 12.9 1938 Pentachlorophenol 87-86-5 20 2155 7-Hydroxycitronellal 107-75-5 22.2 2953 Geraniol 106-24-1 23.2 3875 Imidazolidinyl urea 39236-46-9 24 2000 Linalool 78-70-6 30.4 13,793 Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Salicylic acid 69-72-7 NS 2000 Sylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 68-15 NS NS | Benzyl salicylate | 118-58-1 | 2.85 | 17,717 |
| Pentachlorophenol87-86-52021557-Hydroxycitronellal107-75-522.22953Geraniol106-24-123.23875Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate70560-51-9NS1874Benzocaine94-09-7NS2000Benzyl alcohol100-51-6NS5906Salicylic acid69-72-712.2NSXylene1330-20-795.8NS1-Butanol71-36-3NSNSGlycerol56-81-5NSNSOctanoic acid124-07-2NSNSPhenol108-95-2NSNS | Farnesol | 4602-84-0 | 4.8 | 2755 |
| 7-Hydroxycitronellal107-75-522.22953Geraniol106-24-123.23875Imidazolidinyl urea39236-46-9242000Linalool78-70-630.413,793Kanamycin sulfate70560-51-9NS1874Benzocaine94-09-7NS2000Benzyl alcohol100-51-6NS2000Salicylic acid69-72-712.2NSXylene1330-20-795.8NSI-Butanol71-36-3NSNSGlycerol56-81-5NSNSOctanoic acid124-07-2NSNSPhenolNSNSNS | Eugenol | 97-53-0 | 12.9 | 1938 |
| Geraniol 106-24-1 23.2 3875 Imidazolidinyl urea 39236-46-9 24 2000 Linalool 78-70-6 30.4 13,793 Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Benzyl alcohol 100-51-6 NS 5906 Salicylic acid 69-72-7 12.2 NS Yylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Pentachlorophenol | 87-86-5 | 20 | 2155 |
| Imidazolidinyl urea 39236-46-9 24 2000 Linalool 78-70-6 30.4 13,793 Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Benzyl alcohol 100-51-6 NS 5906 Salicylic acid 69-72-7 12.2 NS Yylene 1330-20-7 95.8 NS NS Glycerol 71-36-3 NS NS NS Octanoic acid 124-07-2 NS NS NS Phenol 108-95-2 NS NS NS | 7-Hydroxycitronellal | 107-75-5 | 22.2 | 2953 |
| Linalool 78-70-6 30.4 13,793 Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Benzyl alcohol 100-51-6 NS 5906 Salicylic acid 69-72-7 12.2 NS Xylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Geraniol | 106-24-1 | 23.2 | 3875 |
| Kanamycin sulfate 70560-51-9 NS 1874 Benzocaine 94-09-7 NS 2000 Benzyl alcohol 100-51-6 NS 5906 Salicylic acid 69-72-7 12.2 NS Xylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Clycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Imidazolidinyl urea | 39236-46-9 | 24 | 2000 |
| Benzocaine94-09-7NS2000Benzyl alcohol100-51-6NS5906Salicylic acid69-72-712.2NSXylene1330-20-795.8NS1-Butanol71-36-3NSNSGlycerol56-81-5NSNSOctanoic acid124-07-2NSNSPhenol108-95-2NSNS | Linalool | 78-70-6 | 30.4 | 13,793 |
| Benzyl alcohol 100-51-6 NS 5906 Salicylic acid 69-72-7 12.2 NS Xylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Kanamycin sulfate | 70560-51-9 | NS | 1874 |
| Salicylic acid 69-72-7 12.2 NS Xylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Benzocaine | 94-09-7 | NS | 2000 |
| Xylene 1330-20-7 95.8 NS 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Benzyl alcohol | 100-51-6 | NS | 5906 |
| 1-Butanol 71-36-3 NS NS Glycerol 56-81-5 NS NS Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | Salicylic acid | 69-72-7 | 12.2 | NS |
| Glycerol56-81-5NSNSOctanoic acid124-07-2NSNSPhenol108-95-2NSNS | Xylene | 1330-20-7 | 95.8 | NS |
| Octanoic acid 124-07-2 NS NS Phenol 108-95-2 NS NS | 1-Butanol | 71-36-3 | NS | NS |
| Phenol 108-95-2 NS NS | Glycerol | 56-81-5 | NS | NS |
| | Octanoic acid | 124-07-2 | NS | NS |
| Vanillin 121-33-5 NS 1181 | Phenol | 108-95-2 | NS | NS |
| | Vanillin | 121-33-5 | NS | 1181 |



ND: Data insufficient for defining a NOEL NS: Non-sensitizer

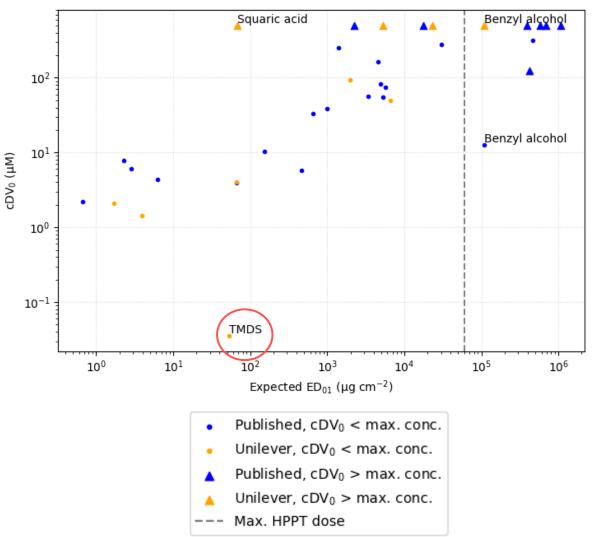
Results



- $\circ~$ Good correlation generally between cDV_0 and expected ED_{01}
 - Correlation strongest for more potent sensitisers
 - Squaric acid and TDMS are outliers
- GARDskin cDV₀ starts to return negative results for weak sensitisers (four triangles to the left of the vertical dashed line).
- Chemicals expected to be non-sensitisers (right of vertical line), also largely negative in GARD.
 - Benzyl alcohol has a surprising cDV₀ in the published results.

Outliers: Tetramethyl thiuram disulfide (TDMS/Thiram)

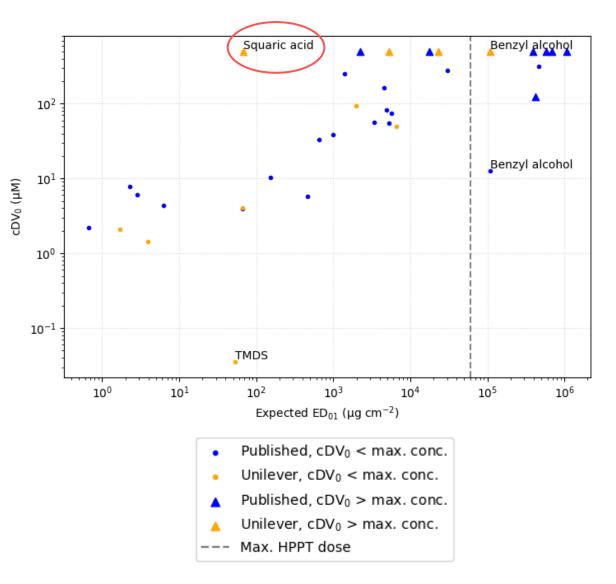
- TDMS (Thiram) is an organosulfur compound used as a bactericide, fungicide and ectoparasiticide to prevent disease in seeds and crops
- Thiram has an extremely low cDV₀ relative to its *in vivo* potency estimates (LLNA EC3 5.2%, HPPT 15000µg/cm² 4/25 sensitised)
- Other NAMs also show similar disagreement with the Thiram *in vivo* data – not a GARD-specific outlier





Outliers: Squaric acid

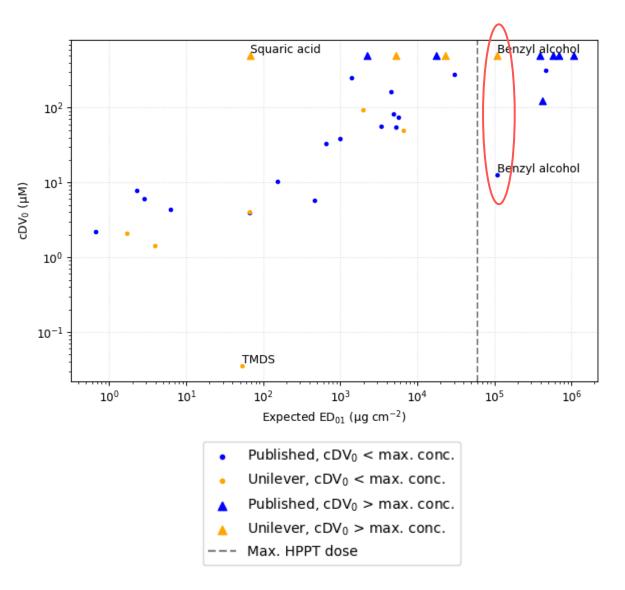
- Squaric acid is a topical strong sensitizer used to treat alopecia areata (AA) by triggering allergic contact dermatitis and redirecting the inflammatory response
- The cDV₀ > maximum dose tested
- Known skin sensitiser from its clinical use, LLNA EC3 <2.5%
- Similarly to thiram, human potency of squaric acid is not reflected in other NAMs – not a GARD-specific outlier





Outliers: Benzyl alcohol

- Benzyl alcohol, a common cosmetic ingredient, was selected as it was shown to be surprisingly potent in GARDskin dose-response in published data (Gradin *et al.*, 2021)
- Repeat testing demonstrated a cDV₀ of greater than the maximum concentration tested, in line with expected result



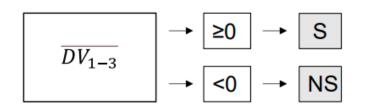


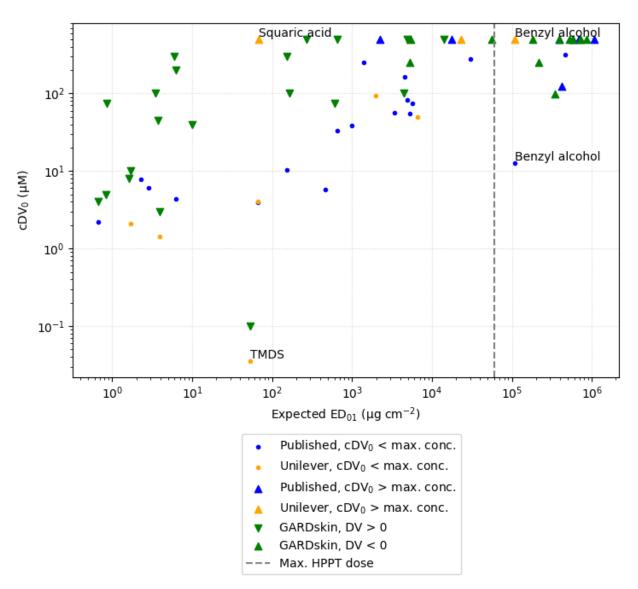
Use of censored data and GARDskin (OECD TG 442E)

 As it is possible to use censored data in SARA, use of data from GARDskin (OECD TG 442E) is also possible

Less informative, but still useful in a weight of evidence potency assessment

- 1 test concentration: cx. at which there is 90% viability, 500 µM, or highest soluble concentration
- **Data output:** decision value (DV) output of the prediction algorithm







Conclusions

- Potency estimates from GARDskin Dose-Response are, baring a small number of outliers, consistent with those obtained with the SARA Model
- This initial look at the GARD cDV₀ value suggests it could be a useful input into the SARA model
 - More reproducibility data required to adequately model variability as per the other SARA model inputs
- SARA is a weight of evidence model which allows it to utilise a breadth of data and minimise impact of outliers



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Thank You

Email: georgia.reynolds@unilever.com



seac.unilever.com

