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

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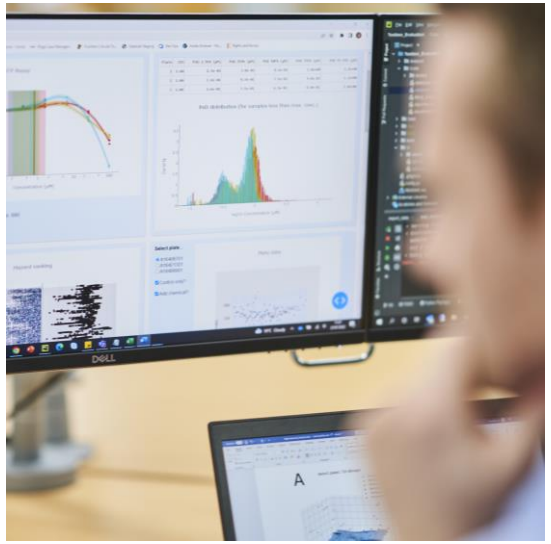

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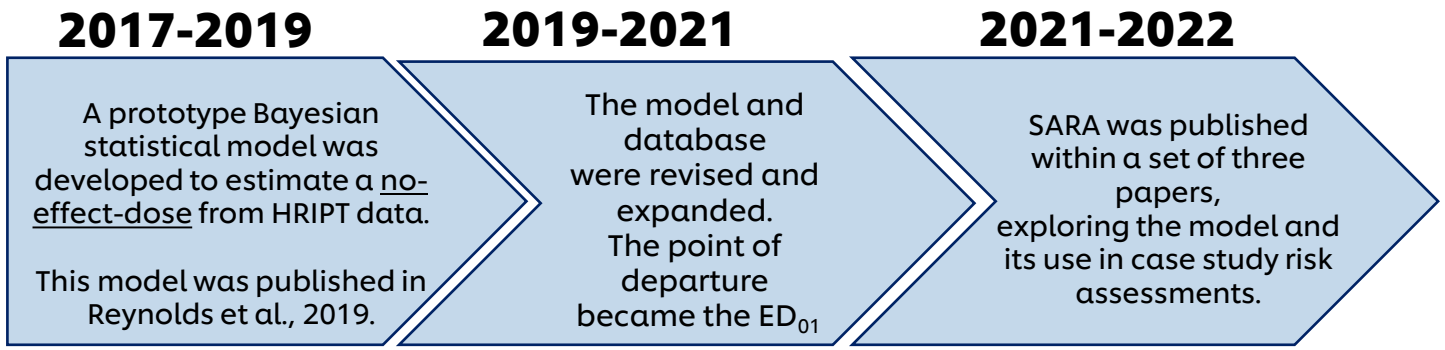
Skin Allergy Risk Assessment (SARA) Model

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

1. **Point of Departure**: An ED_{01}, 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

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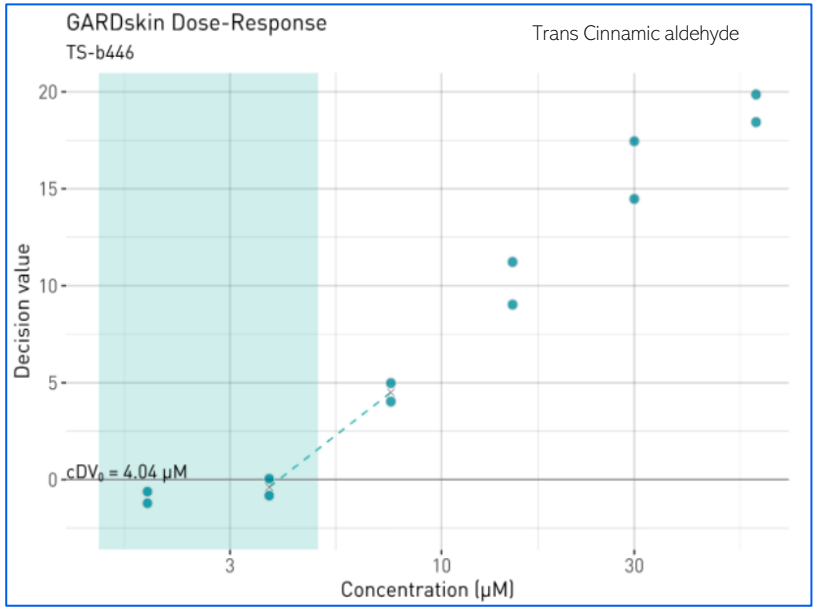
GARDskin Dose Response – viable input assay for the SARA Model?

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 (cDV_0)** 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: cDV_0 correlates with ED_{01}



- **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 (DV_0)

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Chemical selection

- Range of potencies
- Benchmark chemicals
- 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 *in vivo*
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 cDV_0 Values

cDV_0 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

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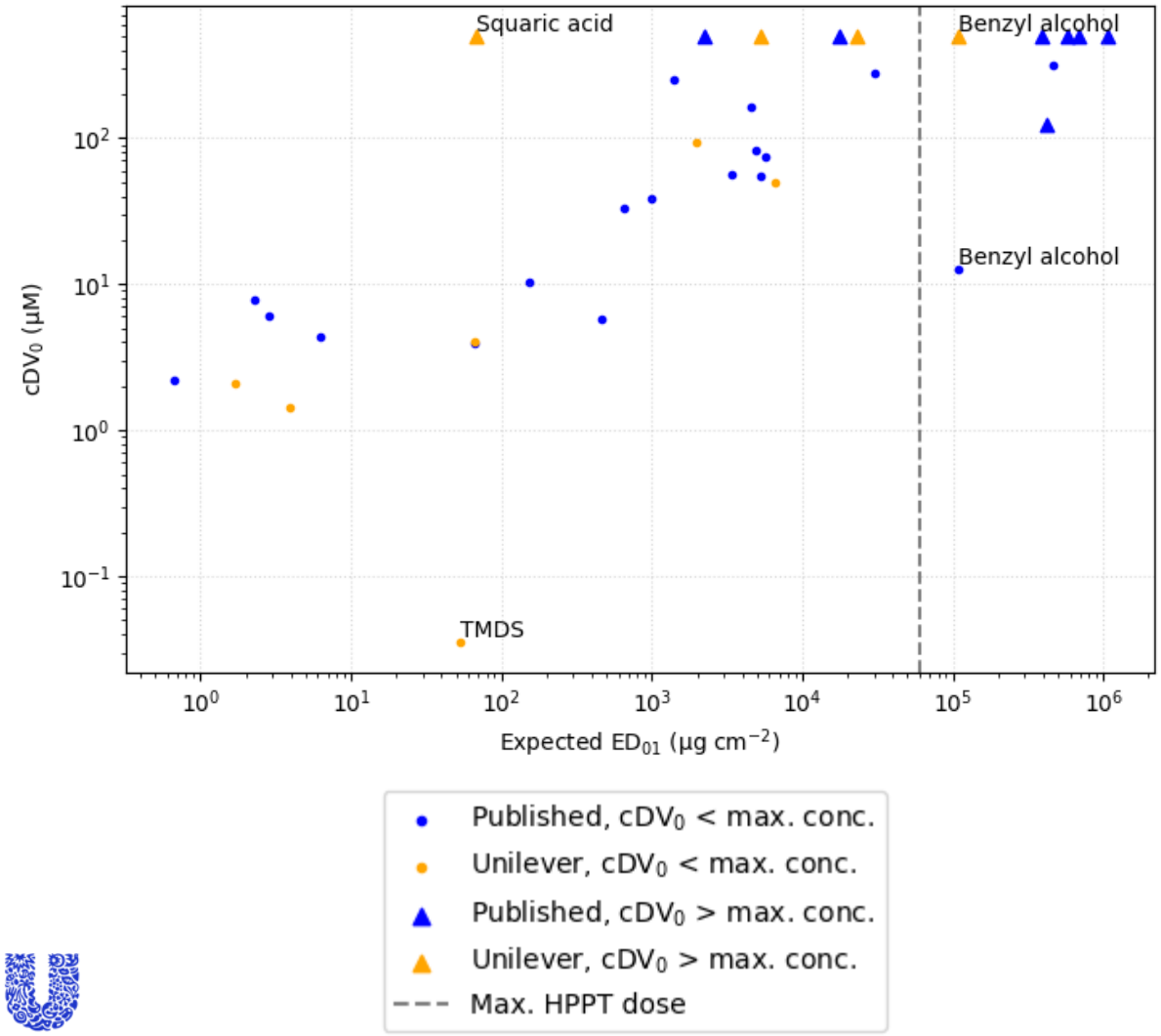
If no cDV_0 value was reported, data was treated as censored by the maximum concentration tested.

Chemical	CAS	LLNA EC3 (%)	NOEL (µg/cm²)
2,4-Dinitrochlorobenzene	97-00-7	0.06	8.8
Benzalkonium chloride	8001-54-5	0.1	ND
Dimethyl fumarate	624-49-7	0.35	88
Methylisothiazolinone	2682-20-4	0.4	15
Iodopropynyl butylcarbamate	55406-53-6	0.9	ND
Cinnamic aldehyde	104-55-2	1.15	591
Isoeugenol	97-54-1	1.35	69
2-Hydroxyethyl acrylate	818-61-1	1.56	ND
Diethyl maleate	141-05-9	2.1	1600
3-Dimethylaminopropylamine	109-55-7	2.2	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
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
Vanillin	121-33-5	NS	1181

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

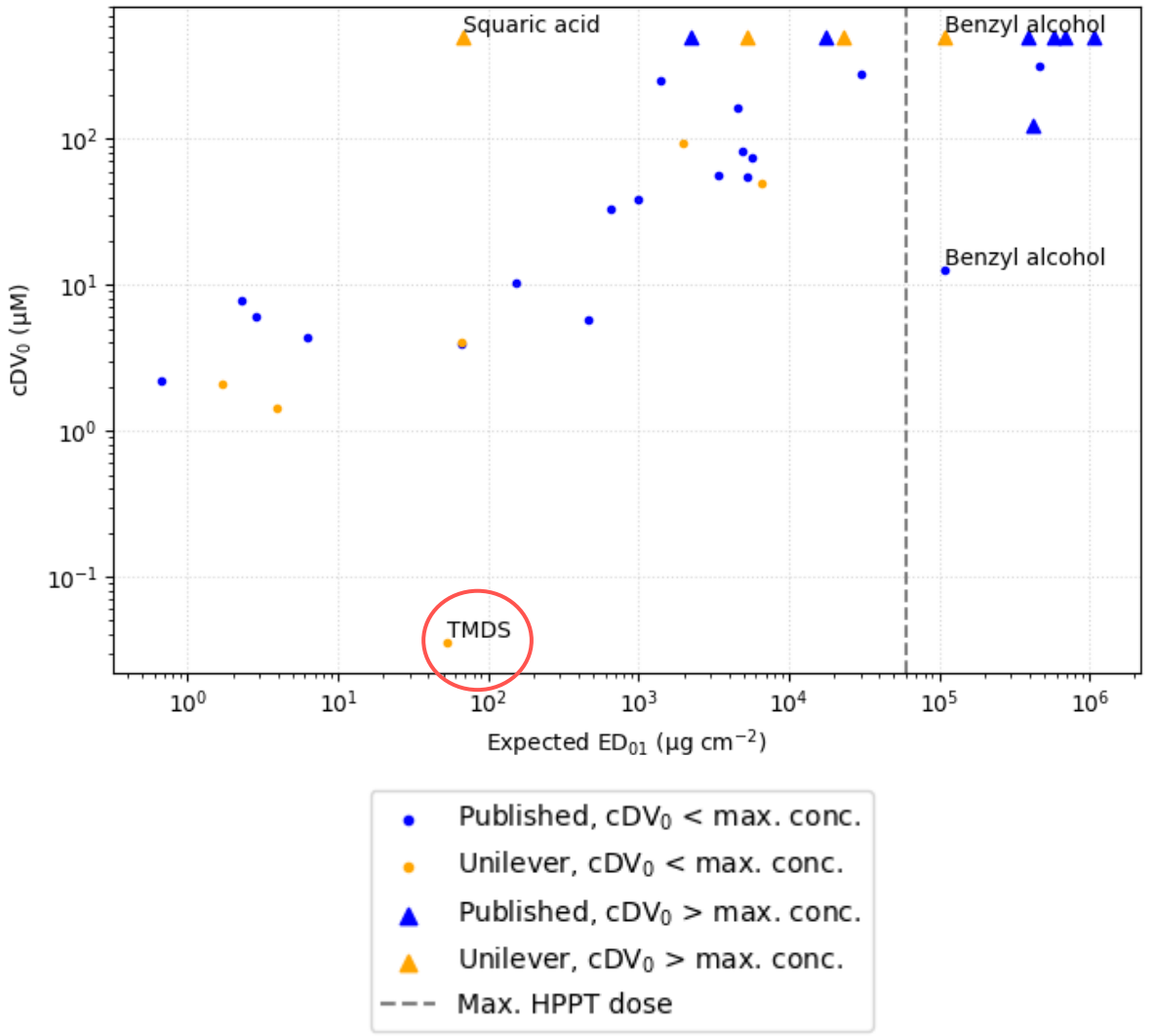
Results



- Good correlation generally between cDV_0 and expected ED_{01}
  - Correlation strongest for more potent sensitisers
  - Squaric acid and TDMS are outliers
- GARDskin cDV_0 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 cDV_0 in the published results.

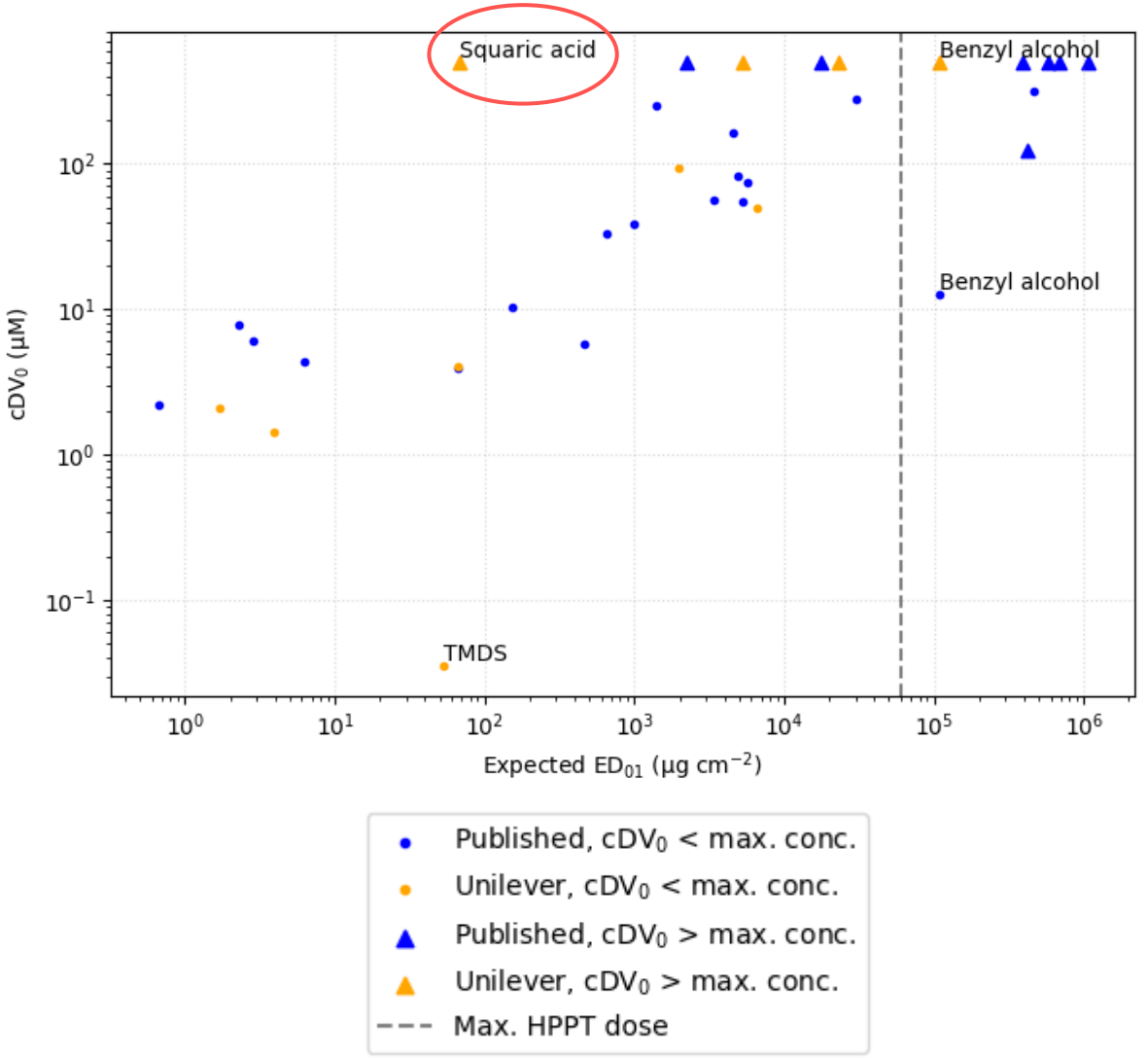
Outliers: Tetramethyl thiuram disulfide (TDMS/Thiram)

- 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 cDV_0 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



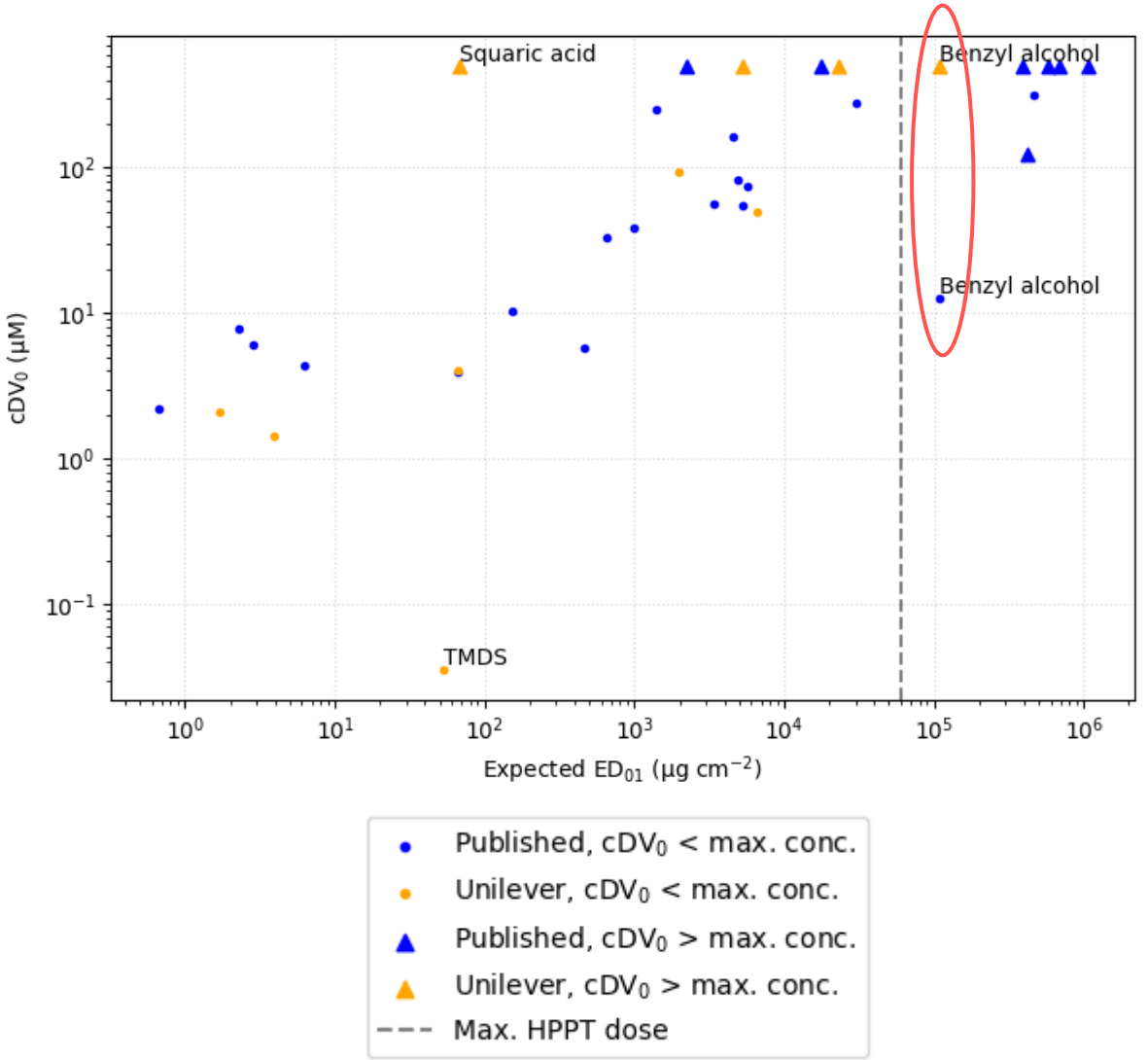
Outliers: Squaric acid

- Squaric acid is a topical strong sensitizer used to treat alopecia areata (AA) by triggering allergic contact dermatitis and redirecting the inflammatory response
- The cDV_0 > 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



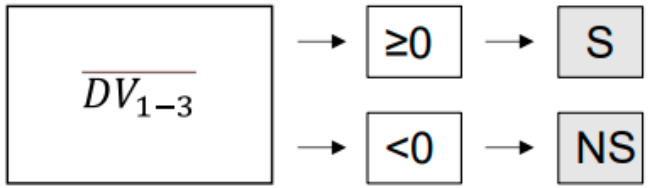
Outliers: Benzyl alcohol

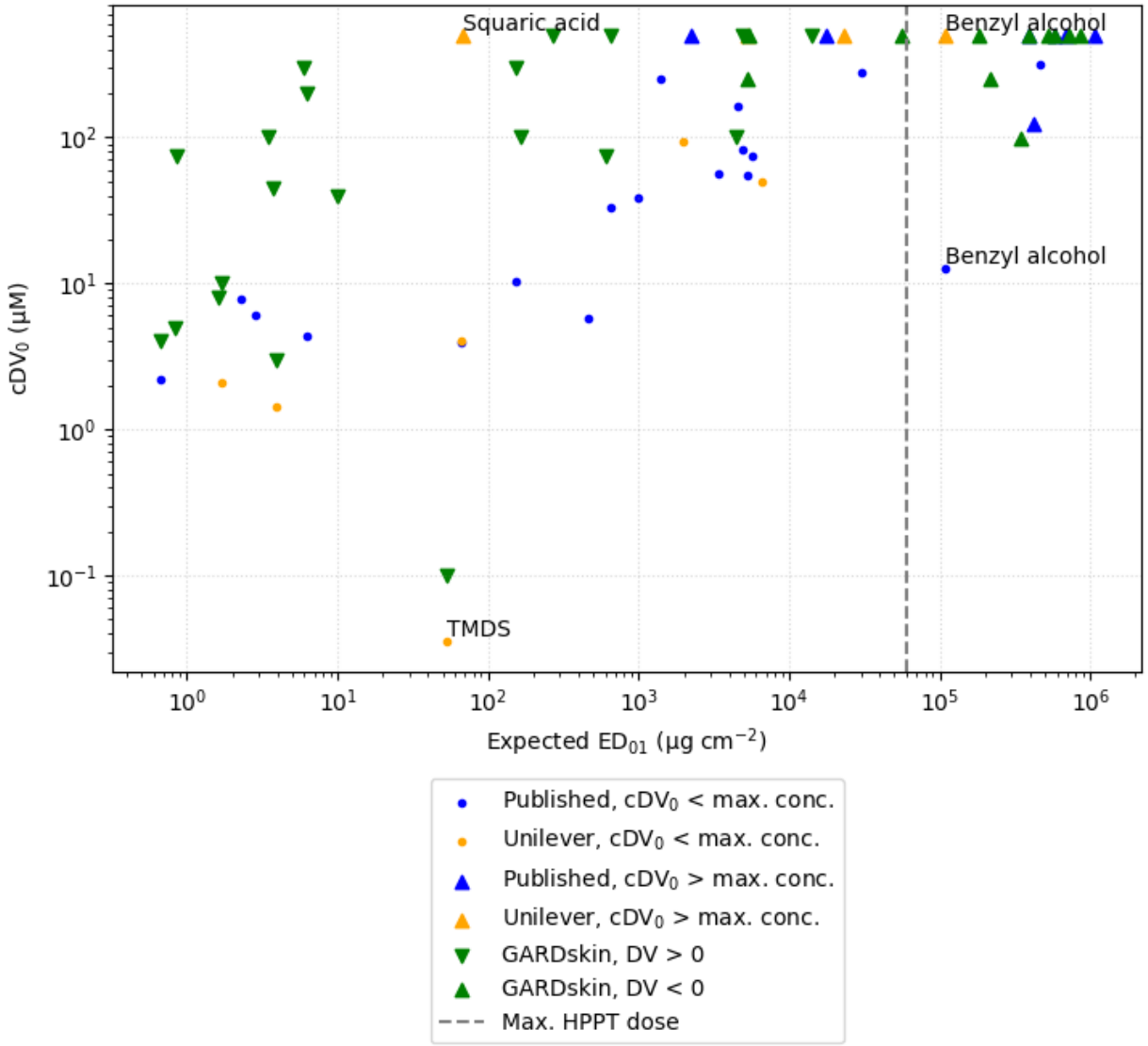
- Benzyl alcohol, a common cosmetic ingredient, was selected as it was shown to be surprisingly potent in GARDskin dose-response in published data (Gradin *et al.*, 2021)
- Repeat testing demonstrated a cDV_0 of greater than the maximum concentration tested, in line with expected result



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 cDV_0 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

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