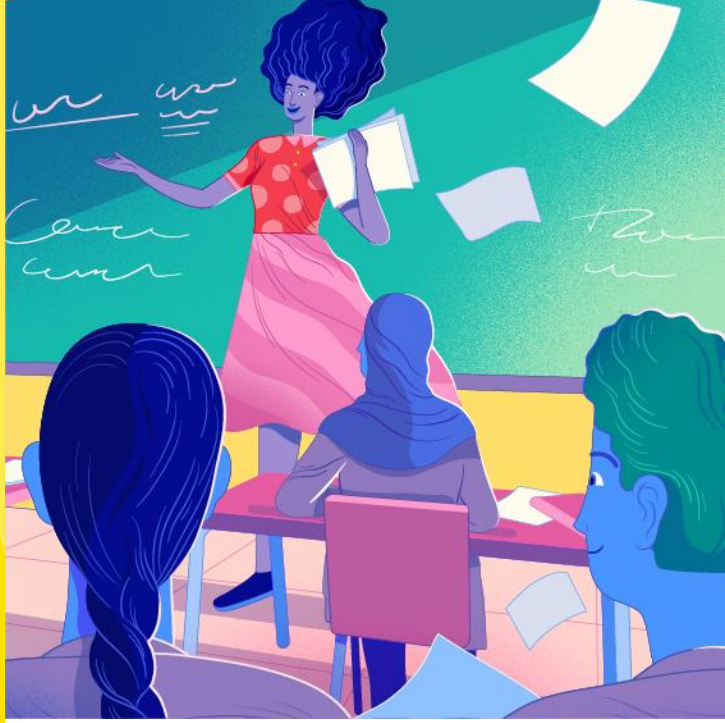
Application of a next generation risk assessment framework for skin sensitisation using new approach methodologies (NAMs): geraniol case study



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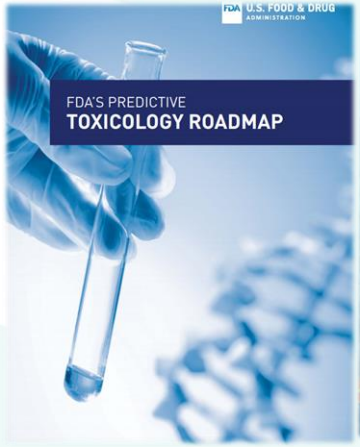
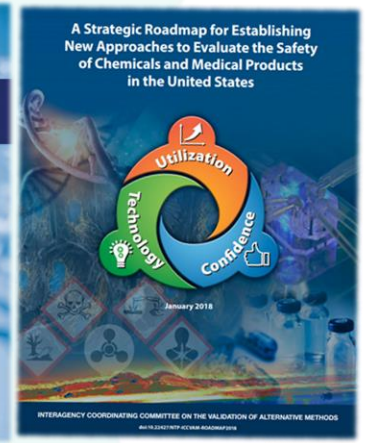
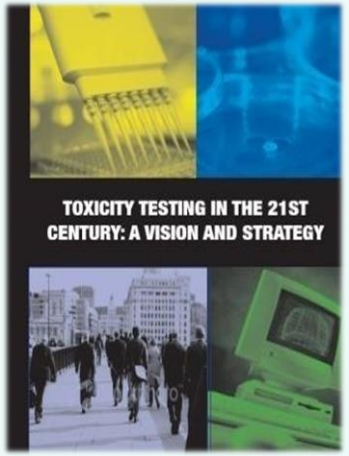
Assessing ingredient & product safety without animal testing

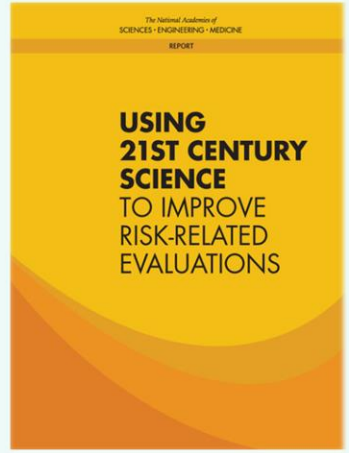
Next Generation Risk Assessment (NGRA)

Is it safe to include x% of chemical y in product z?









Outline:

1. Next Generation Risk Assessment (NGRA) framework for skin allergy
2. Skin Allergy Risk Assessment (SARA) Model
3. Case study: 0.02% (200 ppm) geraniol in a face cream

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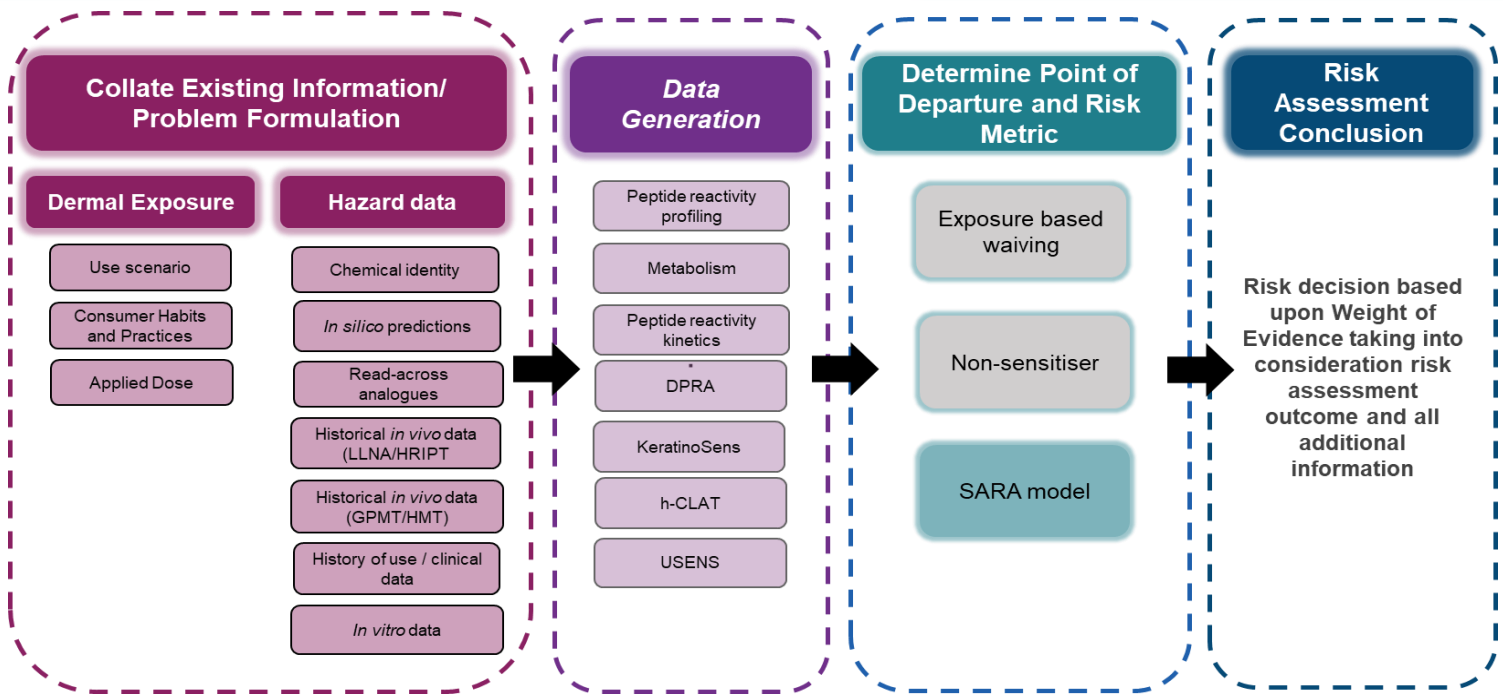
Next generation risk assessment (NGRA) framework for skin allergy



- Our NGRA framework for skin allergy is based upon the **International Cooperation on Cosmetics Regulation (ICCR) principles**[1] and the previously published **NGRA frameworks for systemic tox {SEURAT-1}**[2] and **skin allergy {Cosmetic Europe}**[3].
- Designed to use a WoE based upon all available information, accommodates range of consumer product exposure scenarios and provides a quantitative point of departure (PoD) and risk metric:

  → **Skin Allergy Risk Assessment (SARA) Model**

[1]Dent et al. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Comput. Toxicol. 7, 20–26, 2018.

[2]Berggren et al. Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods. Comput. Toxicol. 4, 31–44, 2017.

[3]Gilmour et al.. Development of a next generation risk assessment framework for the evaluation of skin sensitisation of cosmetic ingredients. Regul. Toxicol. Pharmacol. 116, 2020.

Skin Allergy Risk Assessment (SARA) model

SARA Model Inputs

- Historical Local Lymph Node Assay (LLNA)
- Historical Human Repeated Insult Patch Test (HRIPT)
- *In vitro* data: DPRA (OECD TG442C), KeratinoSens™ (OECD TG 442D), h-CLAT (OECD TG 442E), U-SENS™ (OECD TG 442E)

SARA Model Outputs

- Point of Departure (PoD) termed the ED_{01} – the expected dose at which there is a 1% chance of skin sensitisation in a human (HRIPT) population
- Risk metric – p(low risk)

- **Defined approach (DA) to provide potency and risk information based upon NAMs**
- **A Bayesian statistical approach** which can make potency and risk predictions using any combination of **historical *in vivo* (LLNA, HRIPT) or NAMs (DPRA, KeratinoSens™, h-CLAT and U-SENS™) –** curated database of 81 chemicals
- **Skin sensitiser potency is expressed as the ED_{01},** the dose estimated to induce sensitisation in 1% of a HRIPT population. This is the **Point of Departure (PoD)** for the risk assessment.
- **Risk metric**: SARA model also makes use of **benchmark exposures to infer a probability that a consumer exposure to a chemical is 'low risk'**

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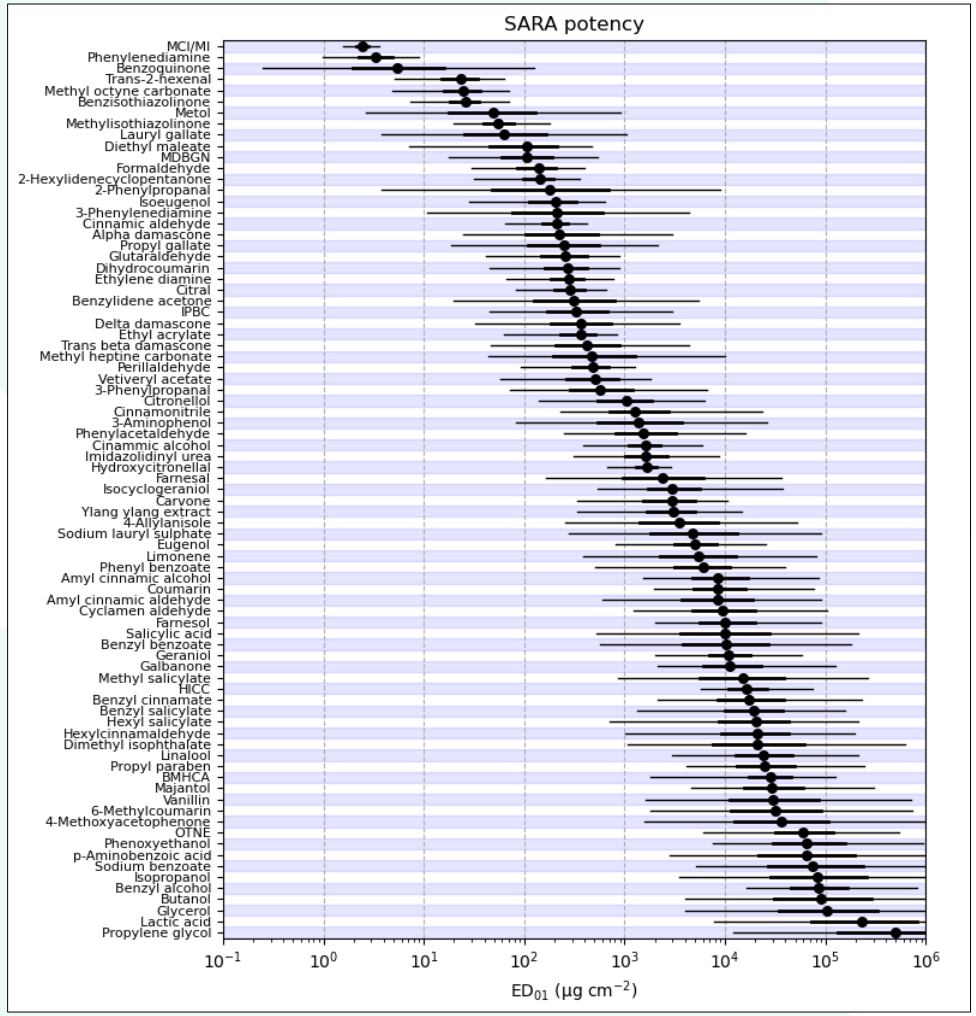
Skin Allergy Risk Assessment (SARA) Model – a Defined Approach (DA)

Potency across the SARA database – Point of Departures (PoDs)

- ED_{01} (the expected dose at which there is a 1% chance of skin sensitisation in a human (HRIPT) population)
- Quantified uncertainty (the dot with the 50% and 95% confidence intervals denoted by the thick and thin lines either side)



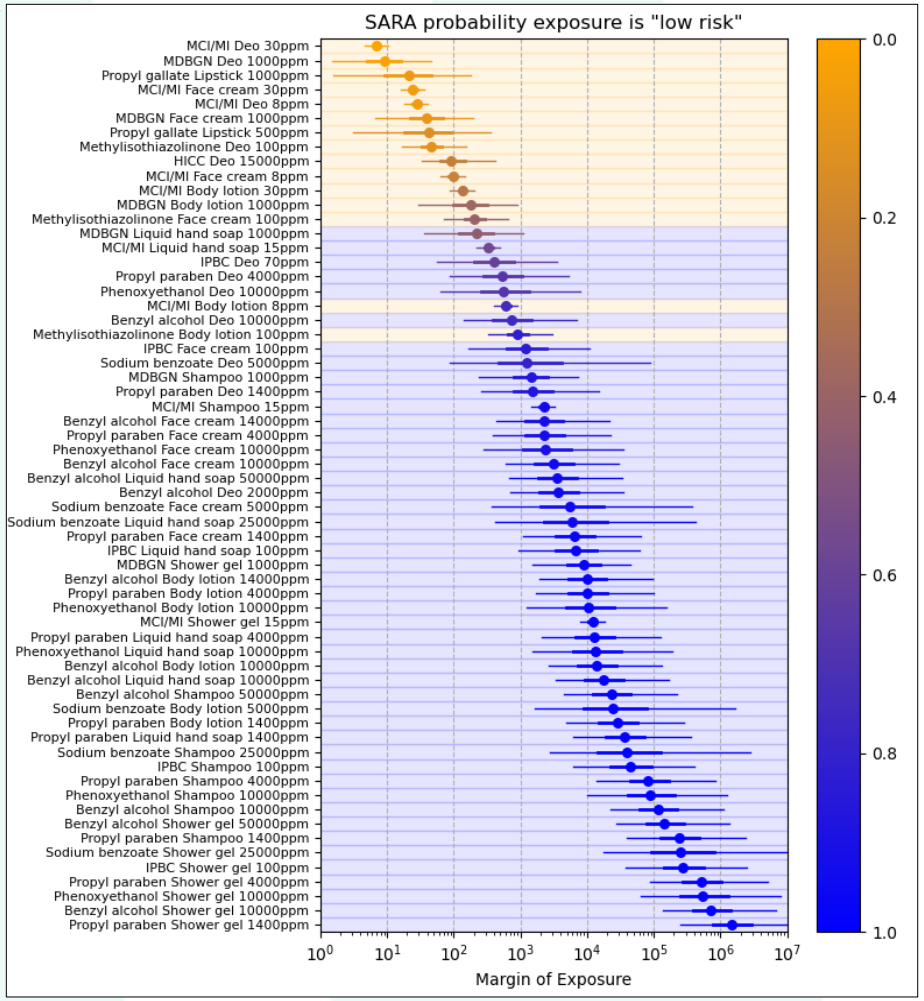
Use of consumer exposure information and clinical evidence to develop skin allergy risk benchmarks

Material	Product type	Use level (ppm)	Consumer exposure to benchmark product (ng cm^{-2})	Induction risk
MCI/MI*	Deo	30	350	HIGH
		7.5	87.8	HIGH
	Face cream	30	100	HIGH
		7.5	25	HIGH
	Body lotion	30	18	HIGH
		7.5	4	HIGH
	Liquid hand soap	15	7.3	LOW
	Shampoo	15	1.1	LOW
	Shower gel	15	0.2	LOW

*Methylchloroisothiazolinone/methylisothiazolinone

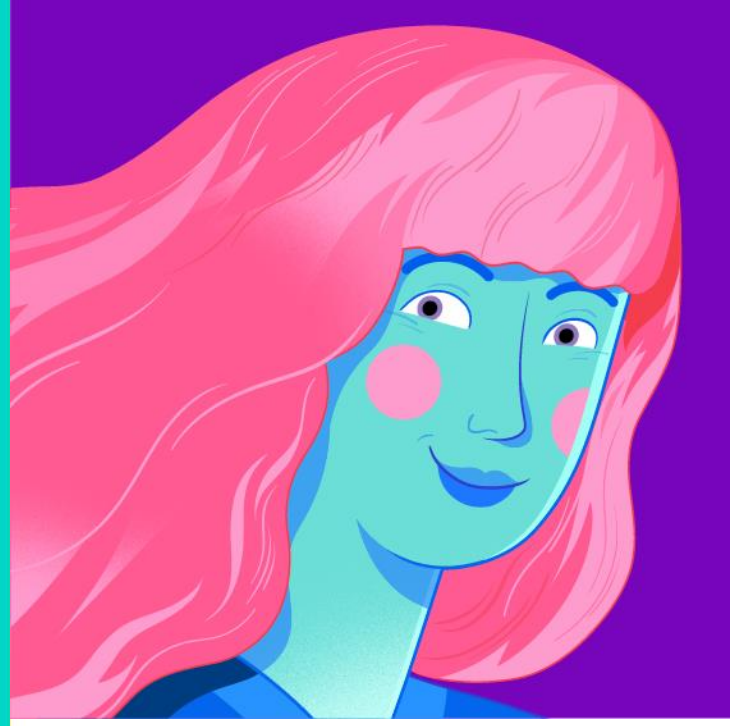
62 low or high risk benchmark exposures using 10 human skin allergens (e.g. MCI/MI) with an established history of use in 7 cosmetic product types.

Margin of exposure (MoE) (PoD/Exposure)



Skin Allergy Risk Assessment (SARA) Model Case Study

0.02% (200ppm) geraniol in a face cream



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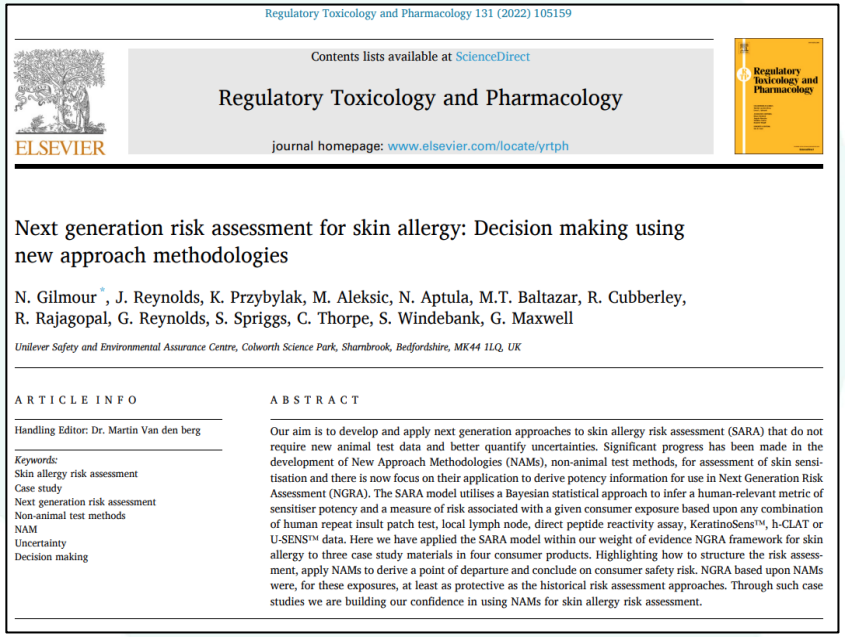
Application of the NGRA framework for Skin Allergy



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Next generation risk assessment for skin allergy: Decision making using new approach methodologies

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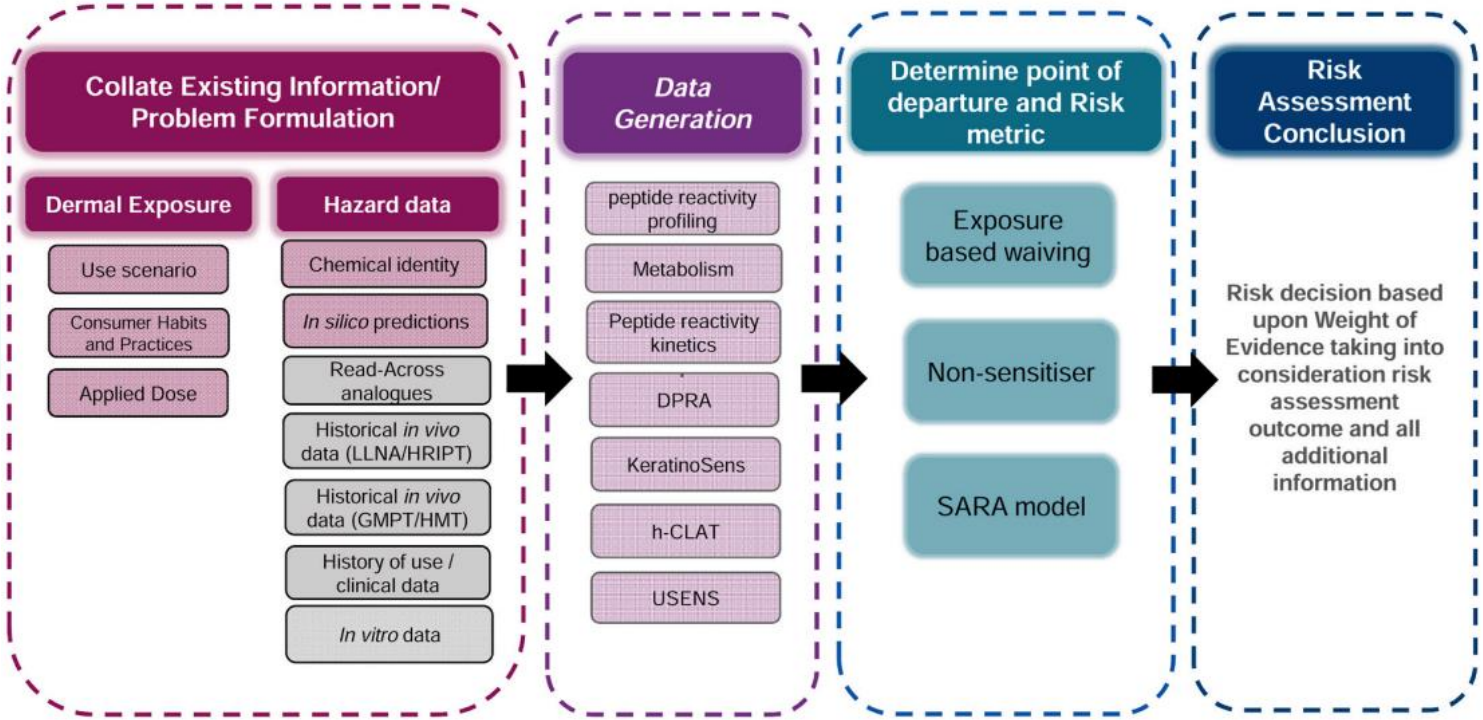
ABSTRACT

Our aim is to develop and apply next generation approaches to skin allergy risk assessment (SARA) that do not require new animal test data and better quantify uncertainties. Significant progress has been made in the development of New Approach Methodologies (NAMs), non-animal test methods, for assessment of skin sensitisation and there is now focus on their application to derive potency information for use in Next Generation Risk Assessment (NGRA). The SARA model utilises a Bayesian statistical approach to infer a human-relevant metric of sensitiser potency and a measure of risk associated with a given consumer exposure based upon any combination of human repeat insult patch test, local lymph node, direct peptide reactivity assay, KeratinoSens™, h-CLAT or U-SENS™ data. Here we have applied the SARA model within our weight of evidence NGRA framework for skin allergy to three case study materials in four consumer products. Highlighting how to structure the risk assessment, apply NAMs to derive a point of departure and conclude on consumer safety risk. NGRA based upon NAMs were, for these exposures, at least as protective as the historical risk assessment approaches. Through such case studies we are building our confidence in using NAMs for skin allergy risk assessment.



- Our NGRA framework is applied to a hypothetical skin allergy assessment of a consumer product:
  → **0.02% (200ppm) geraniol in a face cream.**

- For the purposes of the case study, **historical *in vivo* data** and **read-across** were not used, and the use of **dermal sensitisation threshold** was not appropriate.

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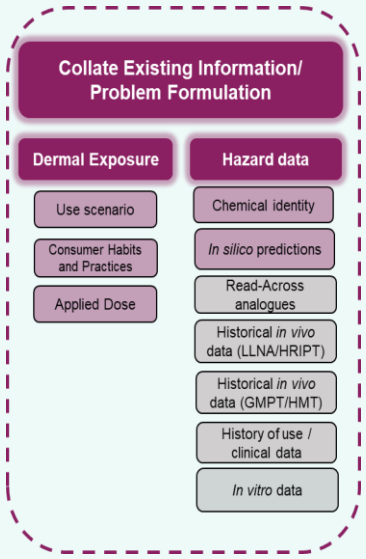
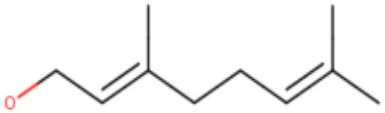
Local exposure + Collate Existing Information/ Problem Formulation



Geraniol

CAS 106-24-1



Product type	Face cream
Product used per day (90th percentile) (g/day)	1.54
Ingredient inclusion level (%)	0.02
Skin surface area face (cm^2)	565
Leave-on or Rinse-off	Leave-on
Local dermal exposure ($\mu g/cm^2$)	0.544

DEREK NEXUS	Alert – terpenoid EC3 model – 20% (weak)
TIMES-SS v.2.30.1.11 **Skin Sensitisation model with autoxidation**	Parent – Non sensitiser (in domain) Metabolites – Strong sensitiser- after autoxidation to disubstituted a,b-unsaturated aldehydes, Weak sensitiser after autooxidation to hydroperoxides
ToxTree v.3.1.0	Alert for Schiff base formation
OECD QSAR Toolbox v.4.4	Protein binding by OECD Parent - No alert found Skin Metabolites (2) - Direct Acting Schiff Base Formers >> Di-substituted alpha, beta-unsaturated aldehydes

- Geraniol is a reactive chemical and likely to be a skin sensitiser due to activation to a chemical capable of forming a Schiff base.
- Confidence in this prediction is high based upon chemical prediction consensus from all applied *in silico* tools.
- Data generation needs:
  - Assuming an abiotic activation mechanism (autoxidation), peptide reactivity profiling data should be generated to test this hypothesis. An estimation of potency is required to enable risk assessment for this exposure.
  - To enable a potency prediction using the SARA model DPRA, KeratinoSens™, h-CLAT and U-SENS™ data should also be generated.

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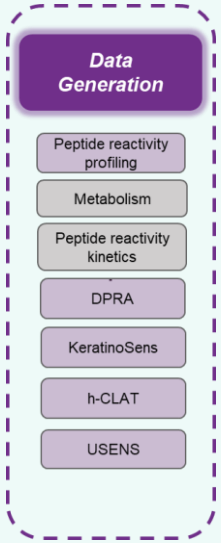
Data Generation



Reactivity Profiling (Aleksic et al., 2009*)	DPRA (OECD TG442C**)	KeratinoSens™ (OECD TG 442D**)	h-CLAT (OECD TG 442E**)	U-SENS™ (OECD TG 442E**)
Cys (no adducts, 73.7%) Lys (no adducts, 3.5%) His (no adducts, -11.1%) **Arg (double Schiff base, 15.2%)** Tyr (no adducts, 8.2%) **N-term (acylation, Schiff base, 40.2%)** Ala (no adducts, -2.1%)	**Negative** Cys depletion 0% Lys depletion 10%	**Positive** $EC_{1.5}$ 110 µM EC_3 >2000 µM IC_{50} 875 µM	**Positive** CD86 EC_{150} 123 µg ml^{-1} CD54 EC_{200} - µg ml^{-1} CV_{75} 140 µg ml^{-1}	**Positive** CD86 EC_{150} 53.6 µg ml^{-1} CV_{70} 113.9 µg ml^{-1}

- Geraniol was confirmed to be a **reactive chemical (Schiff base following autoxidation)** by peptide profiling where adducts consistent with formation of Schiff bases following oxidative activation were observed with the Arginine and N-terminus peptide.
- Geraniol demonstrated minimal depletion of Cys and Lys in the DPRA, which is consistent with the reactivity profiling data. Positive responses were evident in the KeratinoSens™, h-CLAT and U-SENS™.
- Thus, geraniol is a **skin sensitiser via Schiff base formation**.
- **Next step:** determination of the PoD, i.e. the human potency (ED_{01}) → SARA model

*Aleksic et al.. Reactivity profiling: covalent modification of single nucleophile peptides for skin sensitization risk assessment. Toxicol. Sci. 108, 401–411, 2009.

**DPRA, KeratinoSens™, h-CLAT and USENS™ data were sourced from the Cosmetics Europe database (Hoffmann et al. Non-animal methods to predict skin sensitization (I): the Cosmetics Europe database, Crit. Rev. Toxicol. 48, 344–358, 2018).

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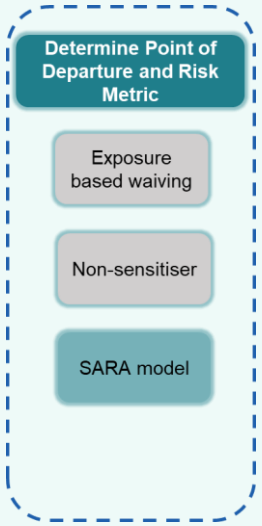
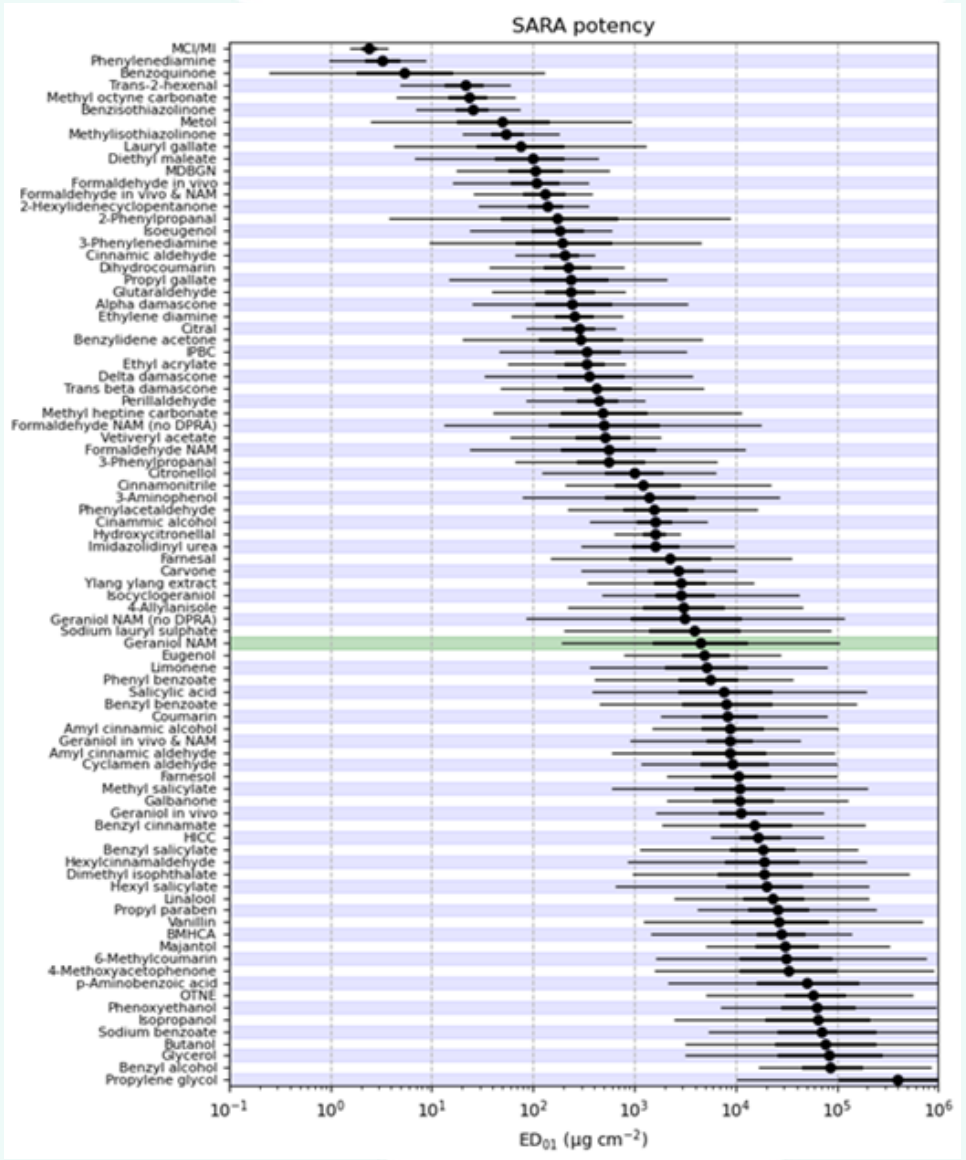
Determine Point of departure using SARA DA





- The generated DPRA, KeratinoSens™, h-CLAT and U-SENS™ data were used as inputs into the SARA model to define a **human relevant PoD (ED_{01} i.e the 1% sensitising dose for a HRIPT population)**.

- For geraniol (NAM data only), the expected **ED_{01} is 4,500 µg cm^{-2}** (2.5th percentile: 180 µg cm^{-2}, 97.5th percentile: 96,000 µg cm^{-2}).

- Geraniol ranks with eugenol, which at least based upon LLNA data is reported to be of moderate potency

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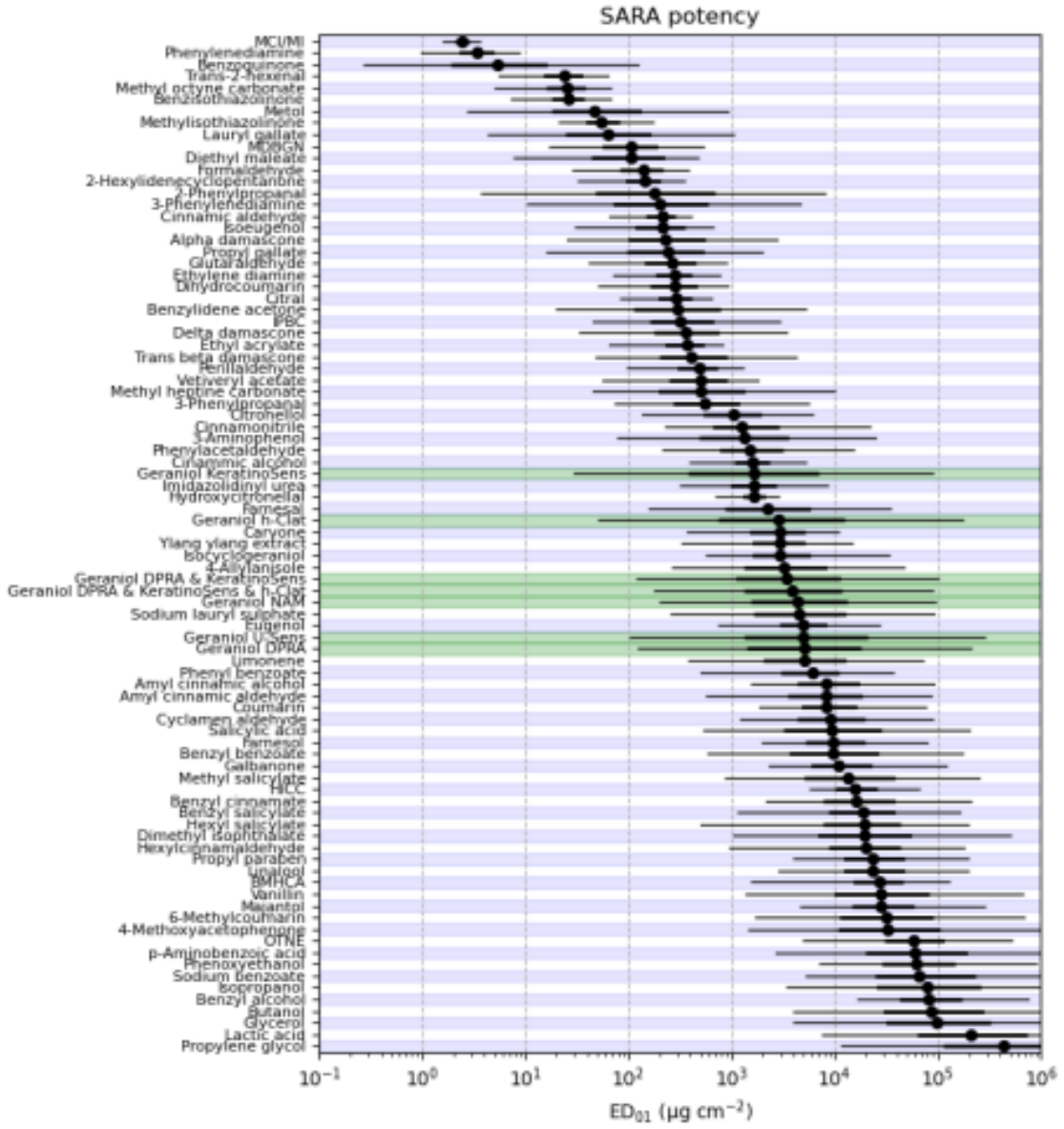
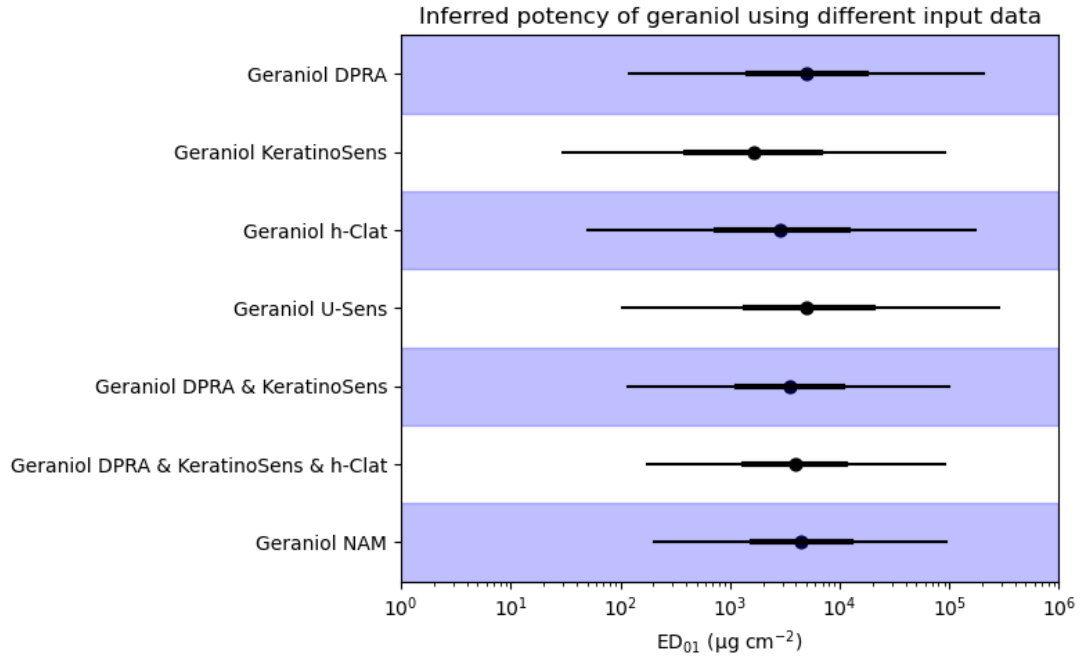
SARA model: partial datasets



- The SARA model can make predictions based upon **any combination** of the DPRA, KeratinoSens™, h-CLAT and U-SENS™ data.
- Predictions made using just KeratinoSens™ or h-CLAT data yielded a marginally higher expected potency (lower ED_{01}) compared with the predictions made using just DPRA or U-SENS™ data.
- Combining data increases the precision in the estimate of potency (reduced uncertainty).



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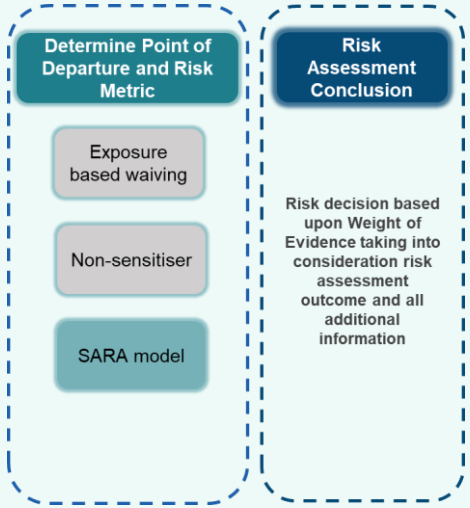
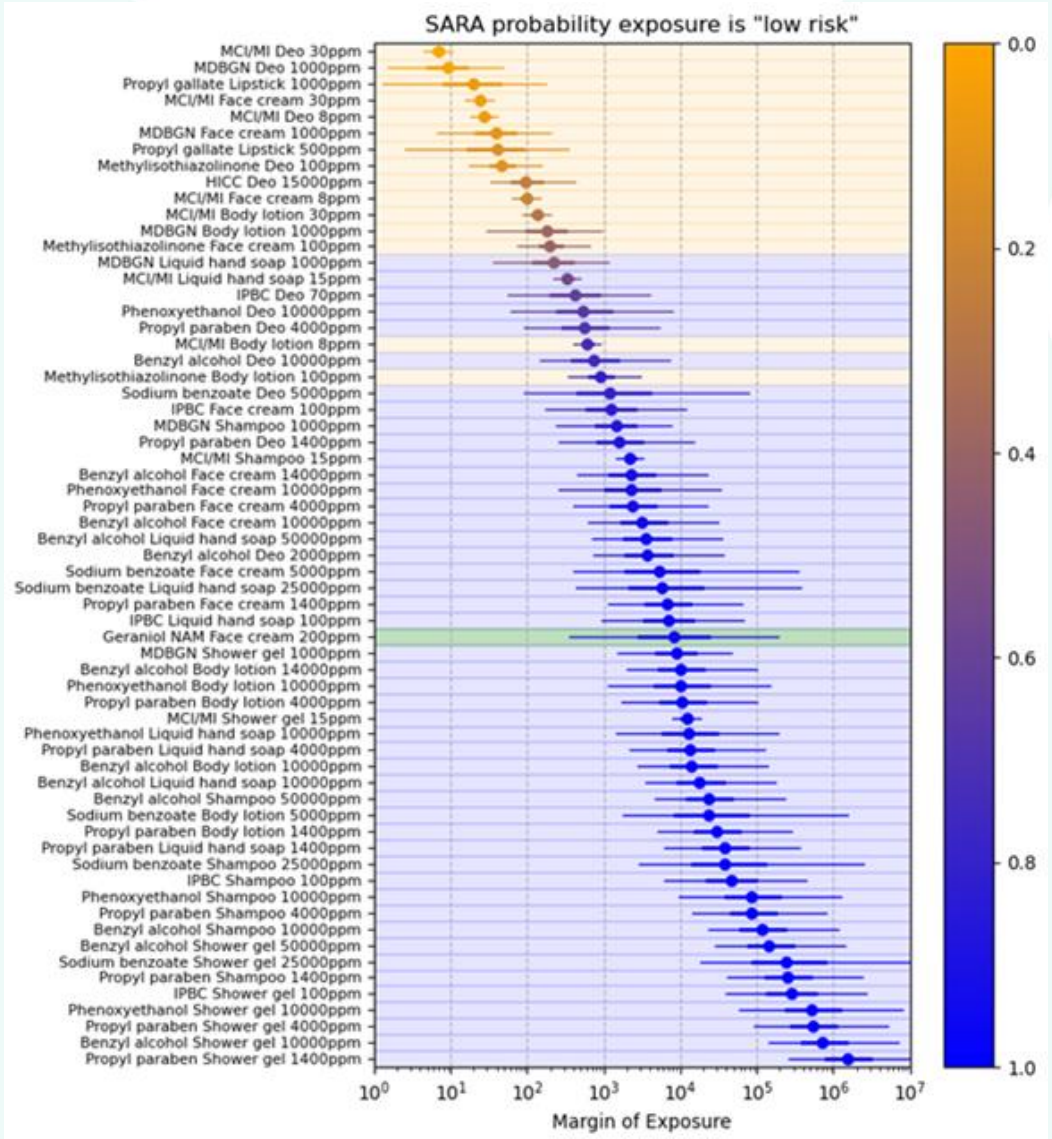
Determine MoE/Acceptable Exposure Level + NGRA conclusion





- The MoE was calculated from the ED_{01} for geraniol and the dermal exposure for 0.02% geraniol in a face cream using SARA DA
- The MoE for 0.02% geraniol face cream exposure ranks with the low-risk benchmarks.
- The SARA DA probability that this exposure is low risk is calculated to be 0.95. Thus, there is a 95% probability that this exposure is low risk.
- Geraniol used at 0.02% (200 ppm) in a face cream is low risk for induction of skin sensitisation

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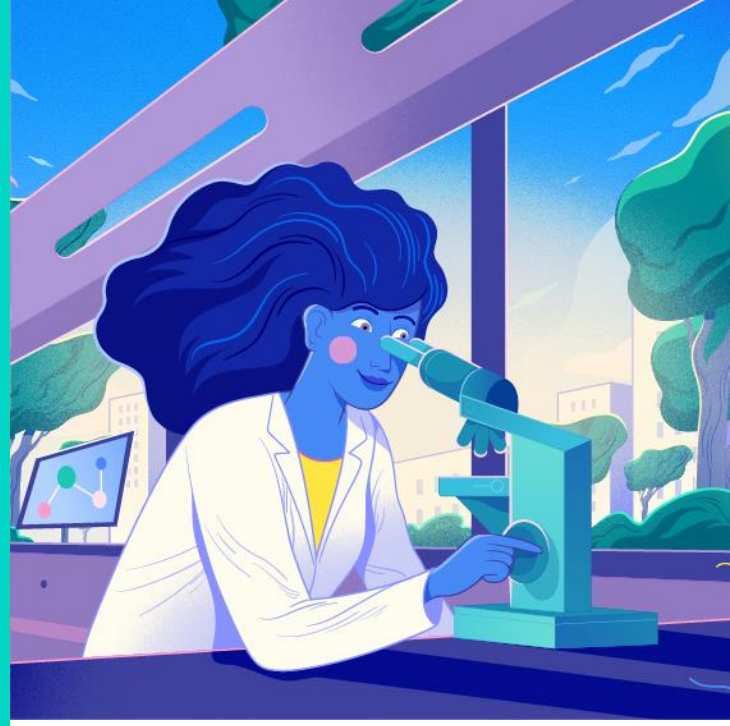

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