Next Generation Risk Assessment (NGRA) for Skin Allergy: Use of Coumarin in Cosmetic Products, *Ab Initio* Case Study

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Introduction

NGRA is an exposure-led, hypothesis-driven approach which integrates new approach methodologies (NAMs) to ensure safety without generating animal data. We have developed an NGRA framework (Figure 1) for skin allergy that aligns with the Cosmetics Europe Skin Allergy NGRA framework (Gilmour N *et al.*, 2020).

This framework is applied to a hypothetical skin allergy assessment of a consumer product at two exposures - 0.1% coumarin in a face cream and 1% in a deodorant. For the purposes of the case study, animal data, clinical data and read-across were not used, and the use of dermal sensitisation threshold (DST) was not appropriate. The full case study has been submitted to Regulatory Toxicology and Pharmacology (Reynolds G *et al.*, submitted).

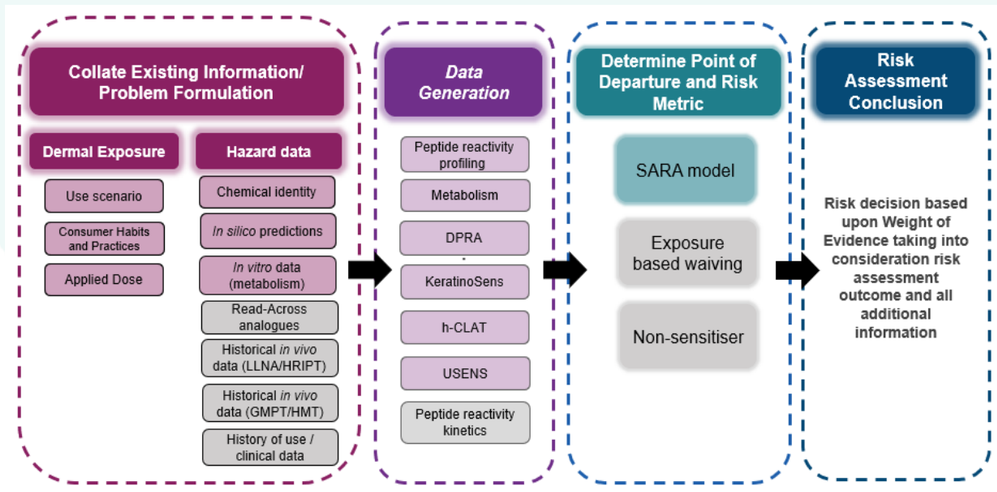

Figure 1. Skin allergy risk assessment framework. Grey boxes represent approaches which were not used for this NGRA case study.

Local Exposure Estimation & Problem Formulation

Table 1. Applied dose exposure estimates (SCCS, 2021).

Product type	Face cream	Deodorant
Product used per day (90th percentile) (g/day)	1.54	1.5
Ingredient inclusion level (%)	0.1	1
Skin surface area (face / axilla) (cm^2)	565	200
Leave-on or Rinse-off	Leave-on	Leave-on
Local dermal exposure ($\mu g/cm^2$)	**2.7**	**75**

Published consumer habits and practices information (SCCS, 2021) was used to estimate local dermal exposure, which was ultimately used to calculate the MoE within the SARA model.

In silico chemistry predictions for the sensitiser potential of coumarin: TIMES-SS predicts coumarin and metabolites as non-sensitisers; Derek Nexus, ToxTree and OECD QSAR Toolbox all predict sensitiser. ToxTree and OECD QSAR Toolbox predicted a Michael Acceptor mechanism. Both direct and indirect (pro-hapten) mechanisms were indicated.

Meteor Nexus identified hydroxylation as the main route of biotransformation. Most metabolites were predicted to bind to protein, a flag for skin sensitization. 7-OH coumarin was identified as a major metabolite in human hepatocytes (Baltazar M *et al., 2020*).

Data Generation

Data was generated in DPRA, KeratinoSens™, h-CLAT, U-SENS™ assays for coumarin and 7-OH coumarin. Coumarin was positive in all tests, except for DPRA where peptide depletion below the positive threshold. 7-OH coumarin was negative in KeratinoSens & h-CLAT, positive in USENS & inconclusive in DPRA.

Peptide reactivity profiling confirmed no significant depletion of any peptides, and so considered negative for 7-OH coumarin.

In addition, studies on cultured *ex vivo* skin, suggest that the biotransformation pathway of coumarin might be significantly different in skin, with very limited production of 7-OH coumarin when coumarin was topically applied.

Table 2. Results of OECD TG in vitro assays for coumarin and 7-OH coumarin

	DPRA (TG442C)		**KeratinoSens** (TG442D)	**h-CLAT** (TG442E)		**U-SENS** (TG442E)
	%cys depl.	%lys depl.	EC1.5 (µM)	CD86 (EC200 µg/mL)	CD54 (EC150 µg/mL)	CD86 (EC150 µg/mL)
Coumarin	1.3	0	187.5	<178	>637	95.5
7-OH Coumarin	0*	0	>2000	>566	>566	182

Determine Point of Departure (PoD)

The generated data were used as inputs into the SARA model (Reynolds *et al*, 2019) - a Bayesian statistical model used to define a human relevant PoD (ED_{01} i.e., the 1% sensitising dose in a consumer population). A risk benchmarking approach is also used within the SARA model to define a MoE and assign a 'risk metric' i.e., a low-risk probability for a given chemical exposure (Reynolds J & Gilmour N, submitted).

For coumarin, the expected SARA model derived ED_{01} is 11,000µgcm^{-2}, whilst for 7-OH coumarin the expected ED_{01} is 110,000µgcm^{-2} (Figure 2) i.e., 7-OH coumarin is predicted to be 10-fold less potent than coumarin). Therefore, a risk assessment based on coumarin potency data only would be conservative.

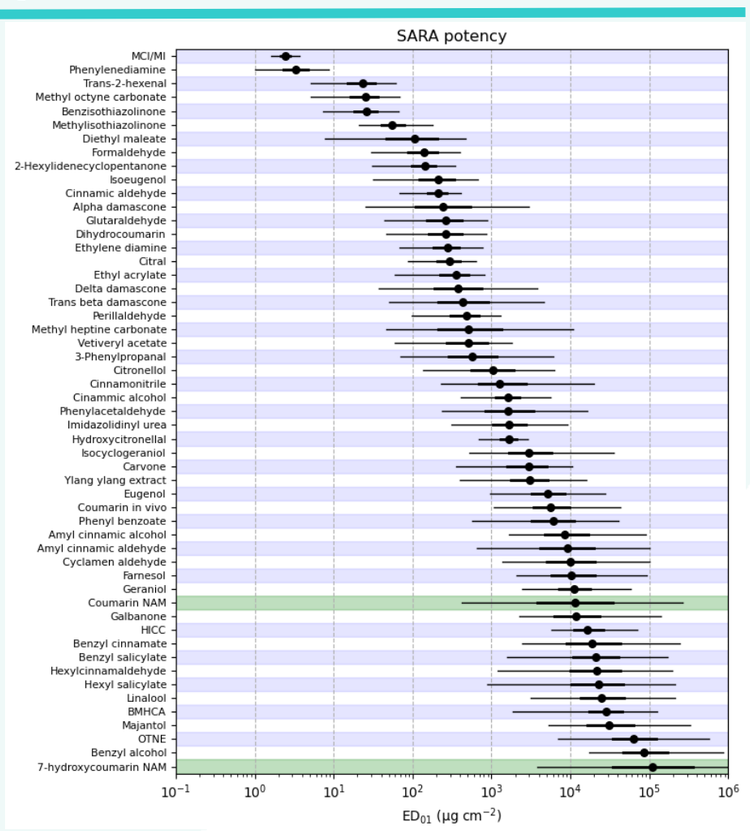

Figure 2 . Ranking of chemicals within the SARA database by median ED_{01} (central 95% and 50% credible intervals).

Table 3. Summary of the probabilistic estimates of the ED01 for coumarin and 7-OH coumarin

Chemical	ED_{01} 2.5th (µg/cm^2)	Expected ED_{01} (µg/cm^2)	ED_{01} 95th (µg/cm^2)
Coumarin	420	11,000	160,000
7-OH Coumarin	3,800	110,000	2.3e+06

Margin of Exposure (MoE) & Risk Metric

The MoE was calculated from the ED_{01} for coumarin and the dermal exposures for each product type. Results were summarised using 95% and 50% credible intervals (Figure 3).

The MoE for face cream exposure ranks with the low-risk benchmarks whilst the MoE for the deodorant exposure ranks with the high-risk benchmarks. The SARA risk metric i.e., the probability that the exposure is low risk, is calculated to be 0.90 for 0.1% in face cream and 0.39 for 1% in deodorant.

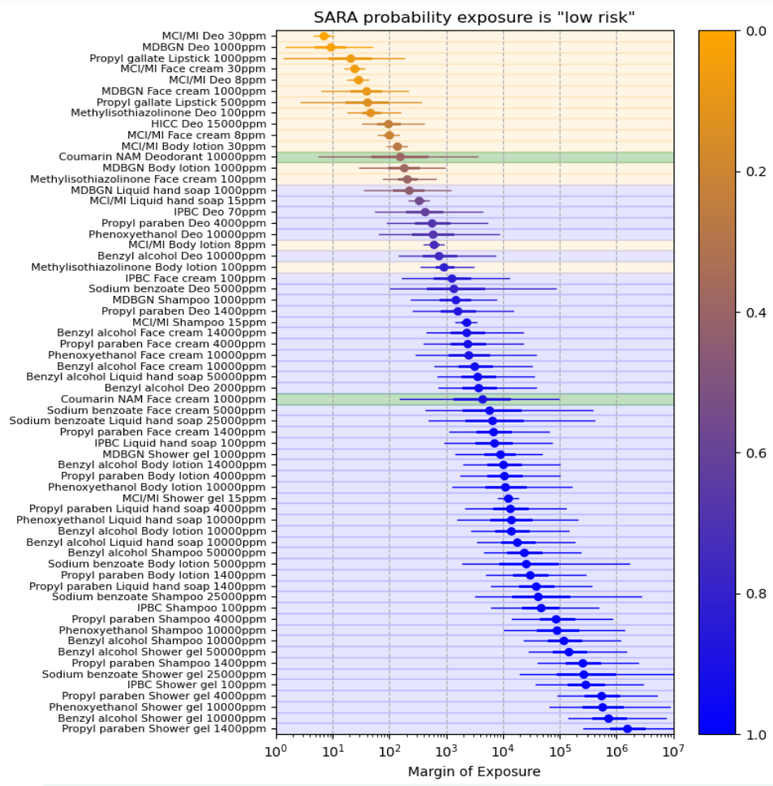

Figure 3. Distribution for the MoE between the ED_{01} for coumarin and the estimated dermal exposure for face cream and deodorant products. Line colours indicate the SARA inferred probability that the exposure is low risk. Background colours indicate the assigned risk classification for each benchmark exposure within the model (blue: low risk, yellow: high risk).

Risk Assessment Conclusions & Discussion

The data generated reinforced the hypothesis that coumarin is likely to be a pro-hapten and that 7-OH coumarin is not a relevant metabolite for the skin sensitisation risk assessment. For coumarin exposure at 0.1% in a face cream, the SARA Model predicted the most likely classification was low risk. For the 1% coumarin deodorant risk assessment, the most likely classification was high risk.

References

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