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

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Background/Aims

NGRA is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure safety without generating animal data. We have developed an NGRA framework (Figure 1) for skin allergy that is based upon ICCR principles (Dent *et al.*, 2018) and aligns with the Cosmetics Europe Skin Allergy NGRA framework (Gilmour *et al.*, 2020).

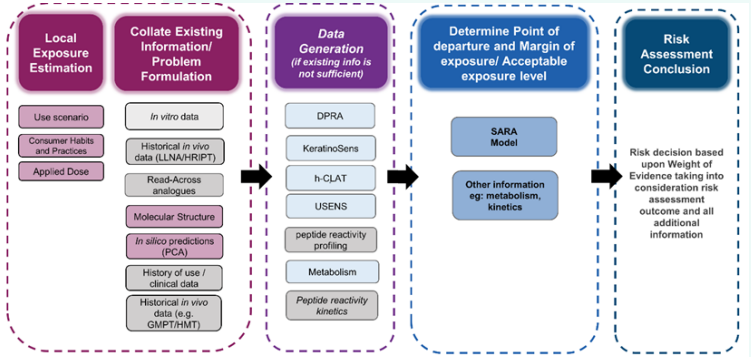


Figure 1. Coumarin skin allergy risk assessment framework.

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.

Exposure

Table 1. Applied dose exposure estimates (SCCS, 2018).

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 ($\mu g/cm^2$)	**2.73**	**75**

References. Dent, et al. "Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients." Comput. Toxicol 7 (2018). 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). Reynolds, et al. "Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment." Comput. Toxicol 9 (2019).

Problem Formulation

In silico chemistry predictions for the sensitiser potential of coumarin: TIMES-SS predicts coumarin and metabolites 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 one of the main metabolites in an investigation in human hepatocytes.

Data Generation

Table 2. In vitro assay results for coumarin and 7-OH coumarin

	DPRA (TG442C)		KeratinoSens (TG 442D)	h-CLAT (TG 442E)		U-SENS (TG 442E)
	%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

Coumarin was positive in all tests, except for DPRA where peptide depletion was too low to meet 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 was considered negative for 7-OH coumarin.

These 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 for a HRIPT population). A risk benchmarking approach is also used within the SARA model to define a MoE and assign a low risk probability for a given chemical exposure.

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).

Point of Departure (PoD)

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).

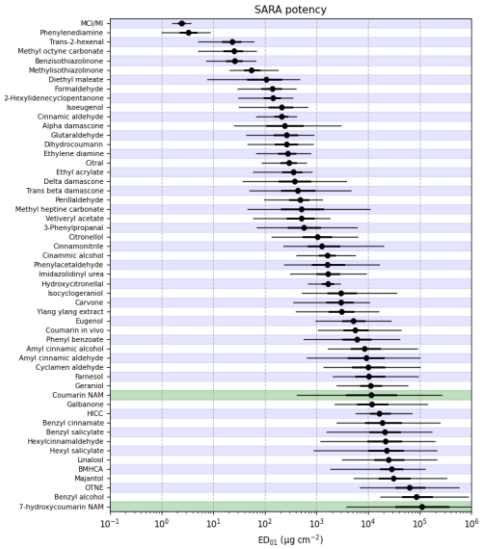
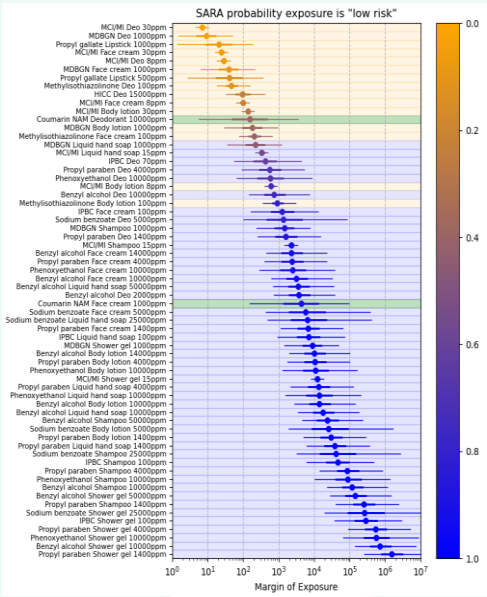


Table 3. Summaries of the probabilistic estimates of the ED01 for coumarin and 7-OH coumarin

Chemical	ED_{01} 2.5th (µg/cm²)	Expected ED_{01} (µg/cm²)	ED_{01} 95th (µg/cm²)
Coumarin	420	11,000	160,000
7-OH Coumarin	3,800	110,000	2.3e+06



Margin of Exposure (MoE)

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 DA probability that the exposure is low risk is calculated to be 0.90 for the face cream dermal exposure and 0.39 for the deodorant dermal exposure.

In conclusion, coumarin exposure at 0.1% in a face cream is low risk for skin sensitisation whereas coumarin exposure at 1% in a deodorant is high risk.