Next generation risk assessment approaches for skin sensitisation:

A case study with coumarin

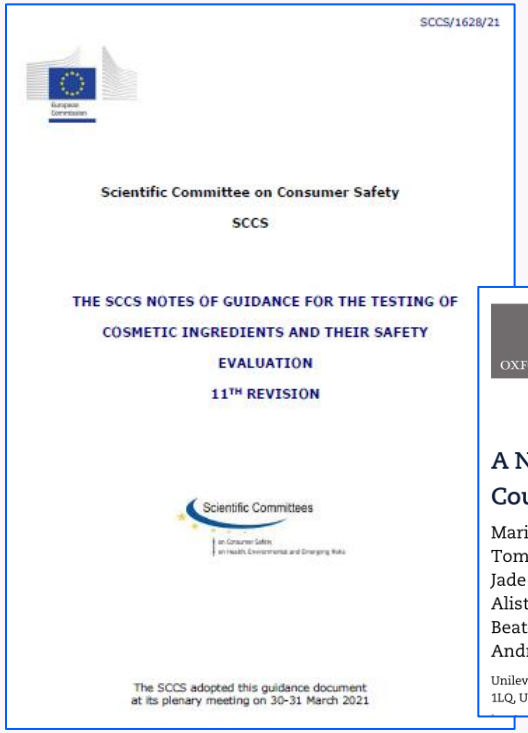
Maria Baltazar, SEAC Science Leader

SEAC UNILEVER

Non-animal Safety Science → Next Generation Risk Assessment

2007

2021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Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 11TH REVISION

Scientific Committees

The SCCS adopted this guidance document at its plenary meeting on 30-31 March 2021


TOXICOLOGICAL SCIENCES, 2020, 1–17

OXFORD SOT Society of Toxicology academic.oup.com/toxsci

doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

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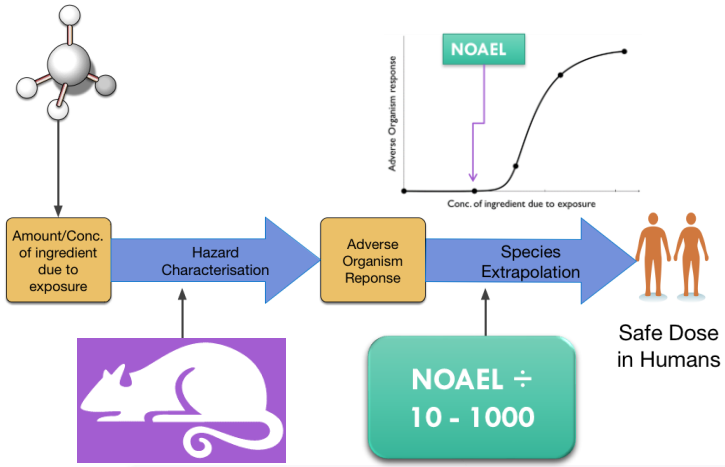
2021

'Traditional' Risk Assessment

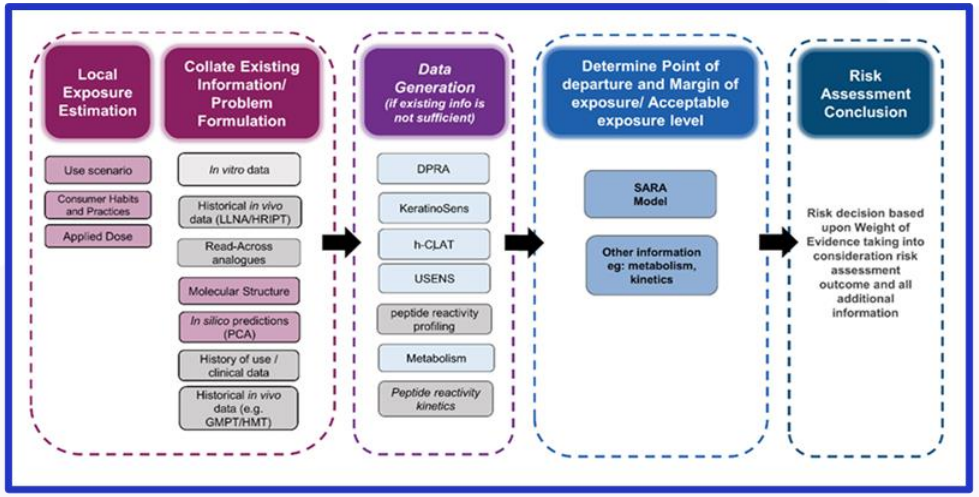


'Next Generation' Risk Assessment

based on advances in human biology and in vitro/computational modelling

Our NGRA approaches

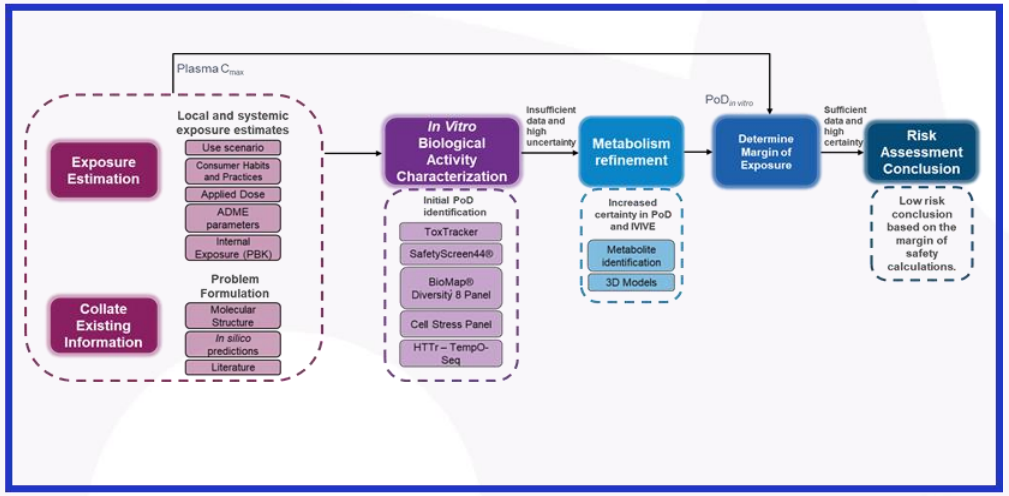
Skin Sensitisation



Reynolds et al (2021) Reg Tox Pharmacol, **127**, 105075
Gilmour et al (2022) Regulatory Toxicology and Pharmacology **13**

Systemic safety

[Link to webinar:P2.01 (widen.net)]



Baltazar *et al.*, (2020) *Tox Sci* , Volume 176, Issue 1, Pages 236–252

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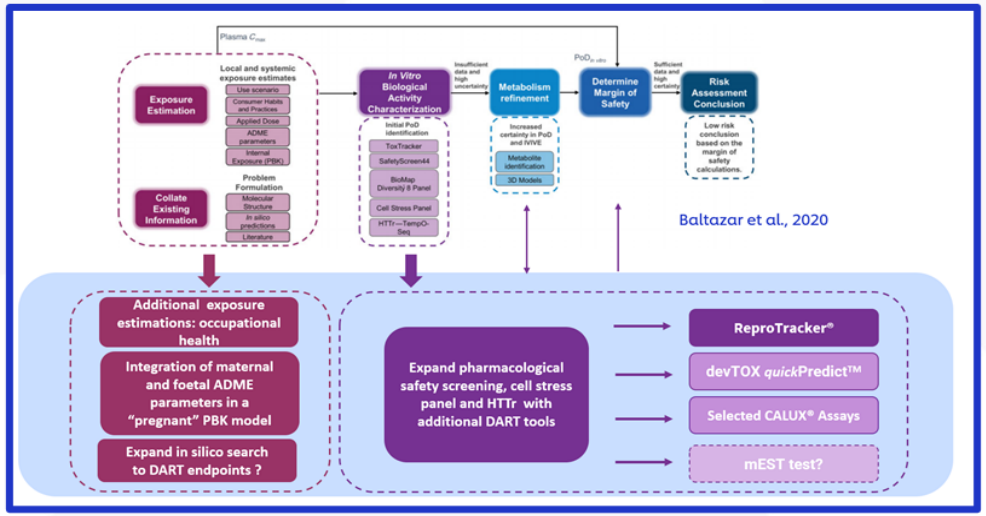
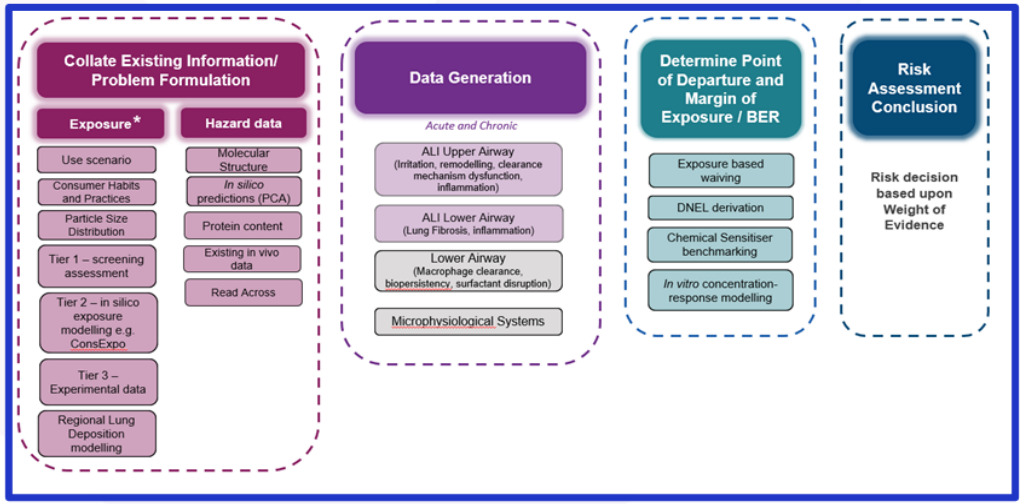


Rajagopal et al (2022). Front. Toxicol., 07 March 2022

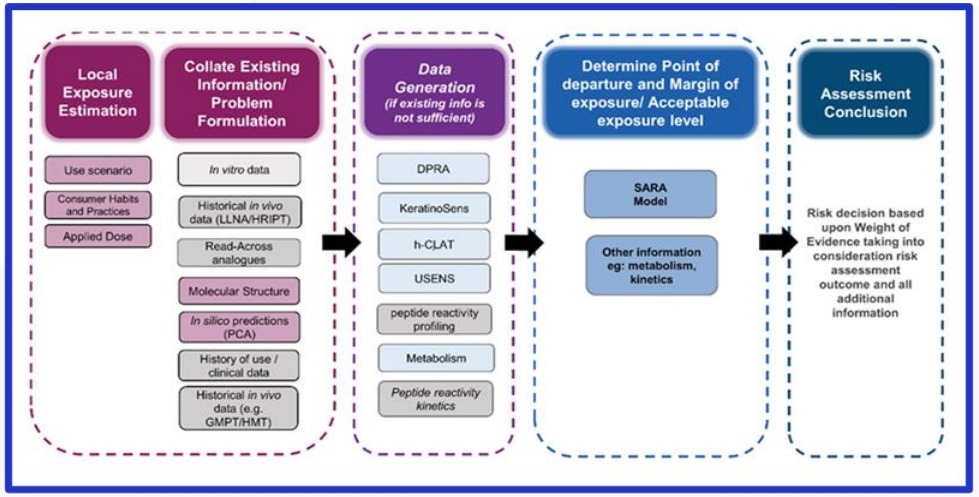
Inhalation



IVAMSS Webinar: Inhalation Toxicity: *In Vitro* to Human Risk Assessment
https://www.toxicology.org/groups/ss/IVSS/Events.asp

Our NGRA approaches

Skin Sensitisation



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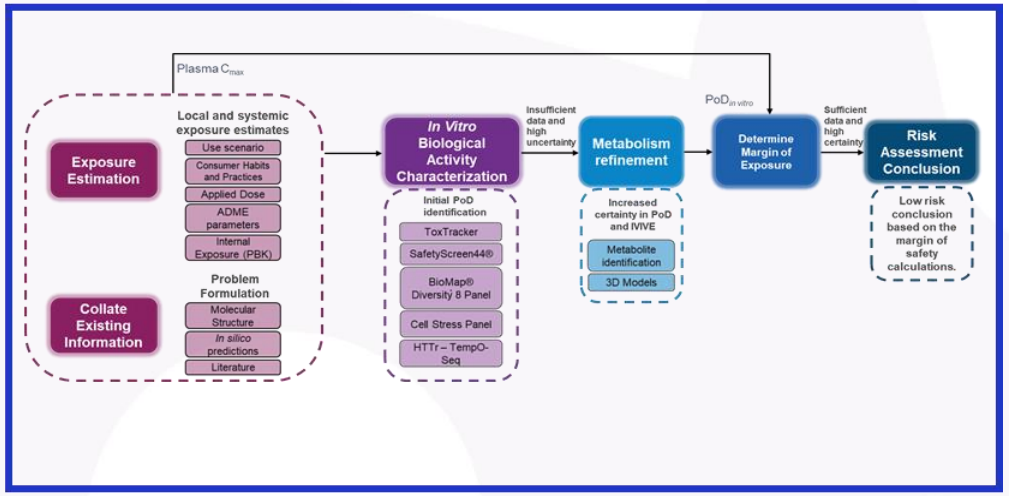

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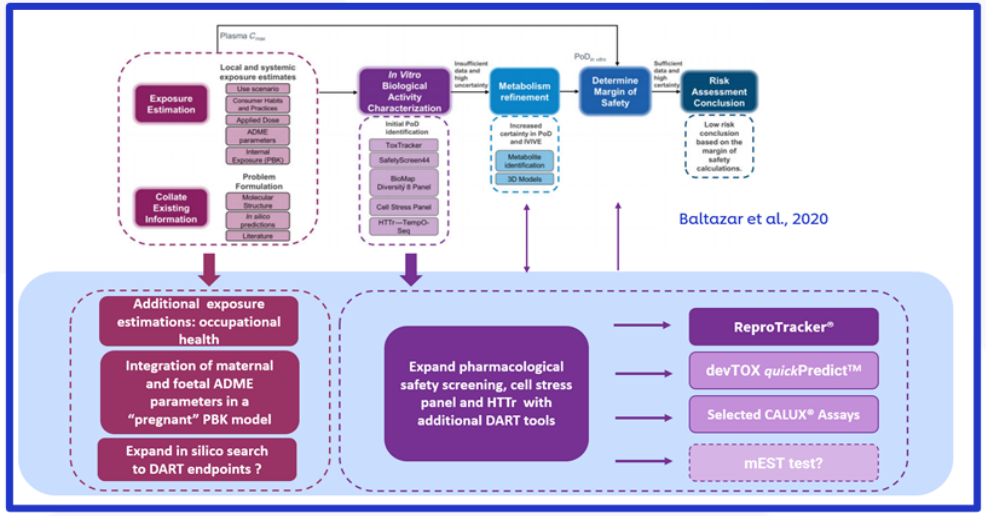

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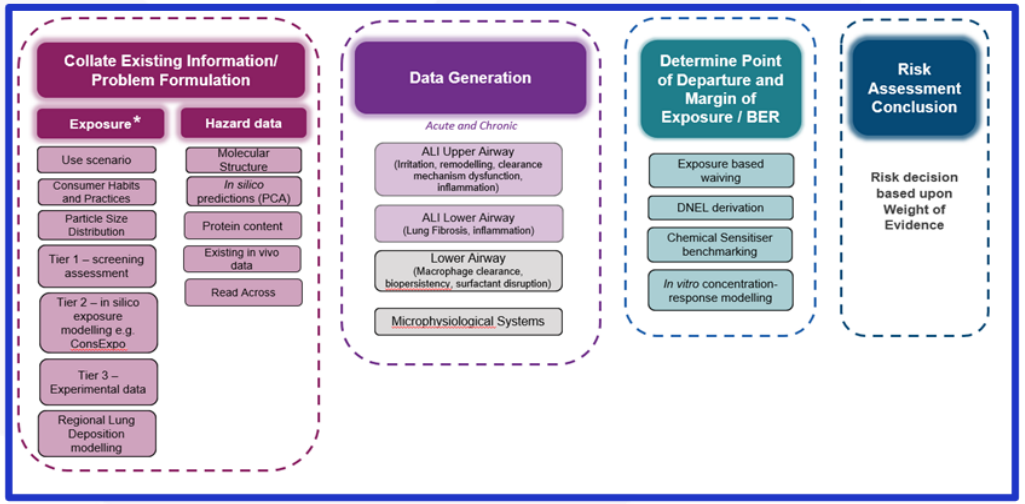

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https://www.toxicology.org/groups/ss/IVSS/Events.asp

Skin allergy risk assessment evolution

Increased mechanistic understanding

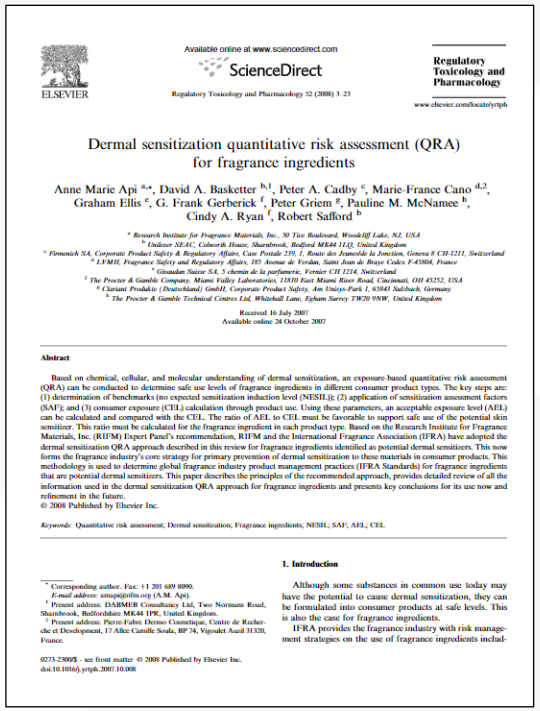
GPMT / Buehler → LLNA → Non-animal test methods

More accurate exposure assessments

Rinse off / Leave on → Individual Products → Aggregate exposure models; new exposure metrics

Increased transparency in risk assessment

Risk benchmarking → QRA → Computational / informatics approaches



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Regulatory Toxicology and Pharmacology 52 (2008) 3–23
www.elsevier.com/locate/yrtph

Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients

Anne Marie Api [a,*], David A. Basketter [b,1], Peter A. Cadby [c], Marie-France Cano [d,2], Graham Ellis [e], G. Frank Gerberick [f], Peter Griem [g], Pauline M. McNamee [h], Cindy A. Ryan [f], Robert Safford [b]

Received 16 July 2007
Available online: 28 October 2007

Abstract

Based on chemical, cellular, and molecular understanding of dermal sensitization, an exposure-based quantitative risk assessment (QRA) can be conducted to determine safe use levels of fragrance ingredients in different consumer product types. The key steps are: (1) determination of benchmarks (no expected sensitization induction level (NESIL)); (2) application of sensitization assessment factors (SAF); and (3) consumer exposure (CEL) calculation through product use. Using these parameters, an acceptable exposure level (AEL) can be calculated and compared with the CEL. The ratio of AEL to CEL must be favorable to support safe use of the potential skin sensitizer. This ratio must be calculated for the fragrance ingredient in each product type. Based on the Research Institute for Fragrance Materials, Inc. (RIFM) Expert Panel's recommendation, RIFM and the International Fragrance Association (IFRA) have adopted the dermal sensitization QRA approach described in this review for fragrance ingredients identified as potential dermal sensitizers. This now forms the fragrance industry's core strategy for primary prevention of dermal sensitization to these materials in consumer products. This methodology is used to determine global fragrance industry product management practices (IFRA Standards) for fragrance ingredients that are potential dermal sensitizers. This paper describes the principles of the recommended approach, provides detailed review of all the information used in the dermal sensitization QRA approach for fragrance ingredients and presents key conclusions for its use now and refinement in the future.
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Keywords: Quantitative risk assessment; Dermal sensitization; Fragrance ingredients; NESIL; SAF; AEL; CEL

1. Introduction

Although some substances in common use today may have the potential to cause dermal sensitization, they can be formulated into consumer products at safe levels. This is also the case for fragrance ingredients.

IFRA provides the fragrance industry with risk management strategies on the use of fragrance ingredients includ-

Regulatory Toxicology and Pharmacology 118 (2020) 104808
Contents lists available at ScienceDirect
Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph

Updating exposure assessment for skin sensitization quantitative risk assessment for fragrance materials

Anne Marie Api [a,*], David Basketter [b], James Bridges [c], Peter Cadby [d], Graham Ellis [e], Nicola Gilmour [f], Helmut Greim [g], Peter Griem [h], Petra Kern [i], Alain Khaiat [j], John O'Brien [k], Thomas Rustemeyer [l], Cindy Ryan [m], Bob Safford [n], Benjamin Smith [o,p], Matthias Vey [q], Ian R. White [r]

ARTICLE INFO

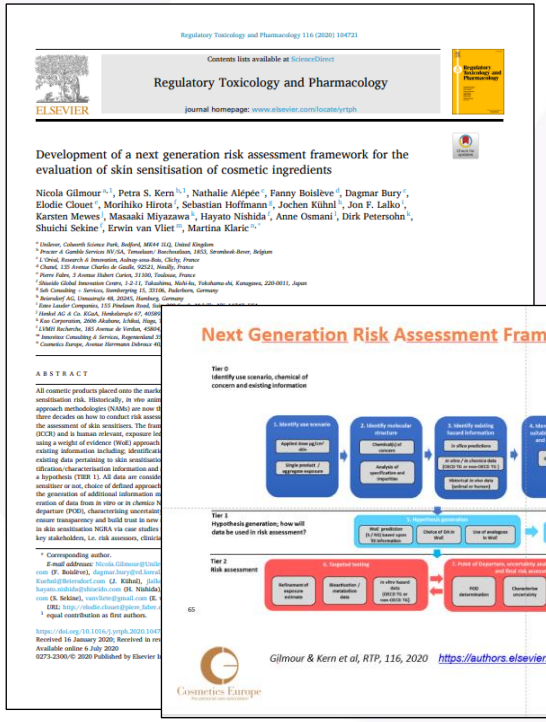
Keywords:
Quantitative risk assessment (QRA)
Fragrance materials
Aggregate exposure
Skin sensitization

ABSTRACT

In 2008, a proposal for assessing the risk of induction of skin sensitization to fragrance materials Quantitative Risk Assessment 1 (QRA1) was published. This was implemented for setting maximum limits for fragrance materials in consumer products. However, there was no formal validation or empirical verification after implementation. Additionally, concerns remained that QRA1 did not incorporate aggregate exposure from multiple product use and included assumptions, e.g. safety assessment factors (SAFs), that had not been critically reviewed. Accordingly, a review was undertaken, including detailed re-evaluation of each SAF together with development of an approach for estimating aggregate exposure of the skin to a potential fragrance allergen. This revision of QRA1, termed QRA2, provides an improved method for establishing safe levels for sensitizing fragrance materials in multiple products to limit the risk of induction of contact allergy. The use of alternative non-animal methods is not within the scope of this paper. Ultimately, only longitudinal clinical studies can verify the utility of QRA2 as a tool for the prevention of contact allergy to fragrance materials.

1. Introduction

Contact allergy to fragrance materials[1] is a topic of considerable interest for consumers, clinicians, industry and regulatory authorities. Mixtures of fragrance materials are used in a wide variety of consumer products at varying levels, leading to a wide range of exposures. Some of these fragrance materials have been identified as contact allergens and



Regulatory Toxicology and Pharmacology 116 (2020) 104721
Contents lists available at ScienceDirect
Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph

Development of a next generation risk assessment framework for the evaluation of skin sensitisation of cosmetic ingredients

Nicola Gilmour [a,1], Petra S. Kern [b,1], Nathalie Alépée [c], Fanny Boislève [d], Dagmar Bury [e], Elodie Clouet [a], Morihiko Hirota [f], Sebastian Hoffmann [g], Jochen Kühnl [h], Jon F. Lalko [i], Karsten Mewes [j], Masaaki Miyazawa [k], Hayato Nishida [l], Anne Osmani [l], Dirk Petersohn [b], Shuichi Sekine [f], Erwin van Vliet [m], Martina Klaric [b,*]

ABSTRACT

All cosmetic products placed onto the marke... sensitisation risk. Historically, in vivo anim... approach methodologies (NAMs) are now ... three decades on how to conduct risk assess... the assessment of skin sensitisers. The fram... (ECCR) and is human relevant, exposure led... using a weight of evidence (WoE) approach... existing information including: identificati... existing data pertaining to skin sensitisati... tification/characterisation information and ... a hypothesis (TIER 1). All data are conside... sensitiser or not, choice of defined approach... the generation of additional information ... ensure transparency and build trust in new ... in skin sensitisation NGRA via case studies ... key stakeholders, i.e. risk assessors, clinici...

Next Generation Risk Assessment Framework for Skin Sensitisation

Tier 0
Identify use scenario, chemical of concern and existing information

1. Identify use scenario
2. Identify molecular structure
3. Identify existing hazard information
4. Identify analogues / suitability assessment and existing data

Tier 1
Hypothesis generation; how will data be used in risk assessment?

Tier 2
Risk assessment

SCCS 11th NoG 2021

Gilmour & Kern et al, RTP, 116, 2020 https://authors.elsevier.com/a/1bRLv%7E81JLAV

Section 4
Health effects

Guideline No. 497
Guideline on Defined Approaches for Skin Sensitisation

ne 2021

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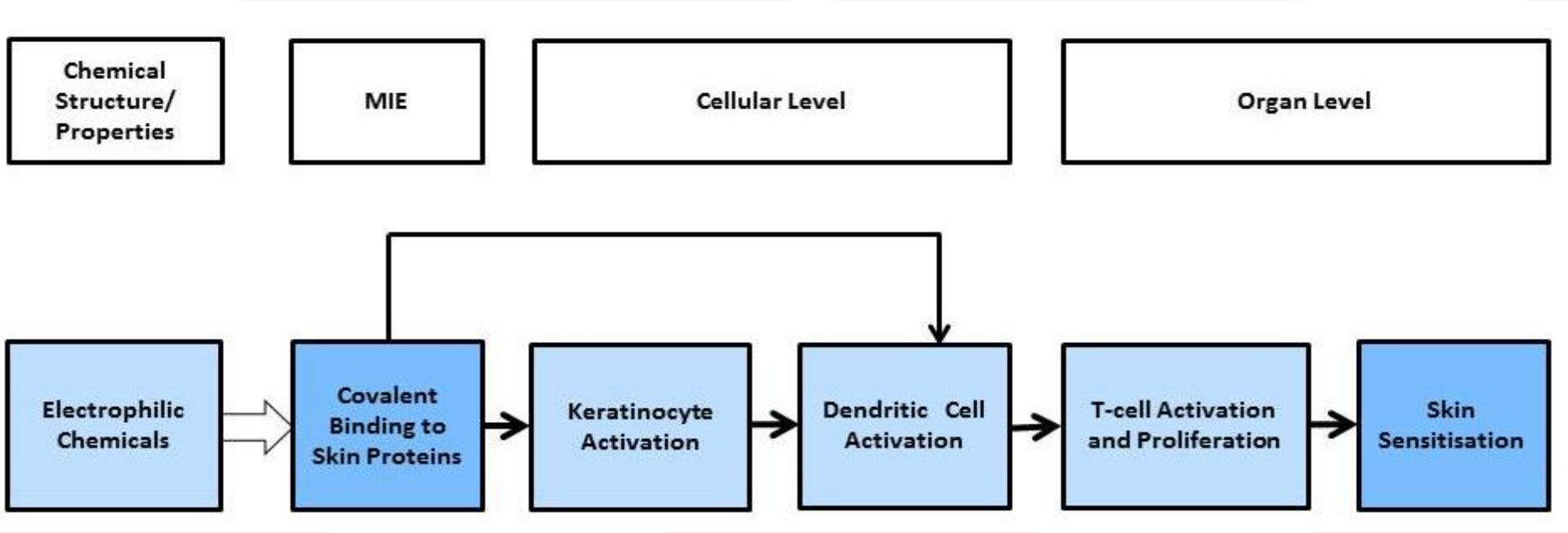
Success in skin allergy NGRA- Use of AOPs to develop NAMS



Key Event 1 (KE1) | KE2 | KE3 | KE4 | Adverse Outcome (AO)

Predictive Chemistry

For example:
- DEREK-NEXUS
- OECD QSAR Toolbox
- TIMES
- ToxTree

Protein Reactivity

OECD TG 442C
Includes:
- ADRA
- DPRA

Keratinocyte Activation

OECD TG 442D
Includes:
- KeratinoSens™
- LuSens

DC Activation

OECD TG 442E
Includes:
- h-CLAT
- IL-8 Luc Assay
- U-Sens™

T Cell Proliferation

For Example:
- Human T cell proliferation assays (hTCPA)

Skin Sensitisation

OECD TG 429: mouse local lymph node assay (LLNA) & variants TG442A & 442B

OECD TG 406: Buehler & Guinea Pig Maximisation Test (GPMT)

Human evidence
e.g. Human Repeat Insult Patch Test (HRIPT)

in silico NAM | *in chemico/vitro* NAM | *in vivo* evidence

SARA Model – a defined approach to provide potency and risk information based upon New Approach Methodologies (NAMs)

- **SARA model is a Bayesian statistical approach** which can make potency and risk predictions using any combination of **historical *in vivo* (LLNA, HRIPT) or NAM (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 for the risk assessment.

- 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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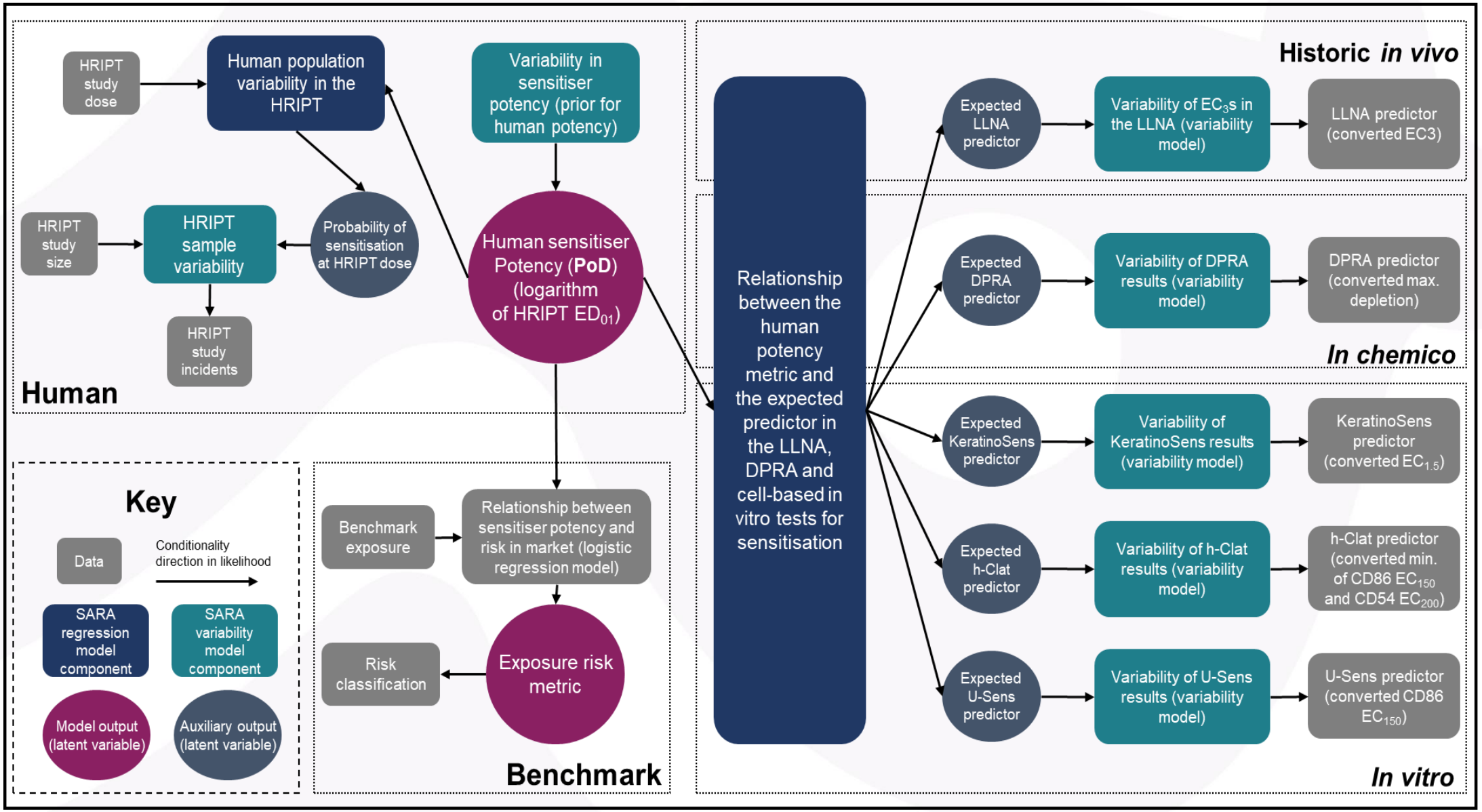
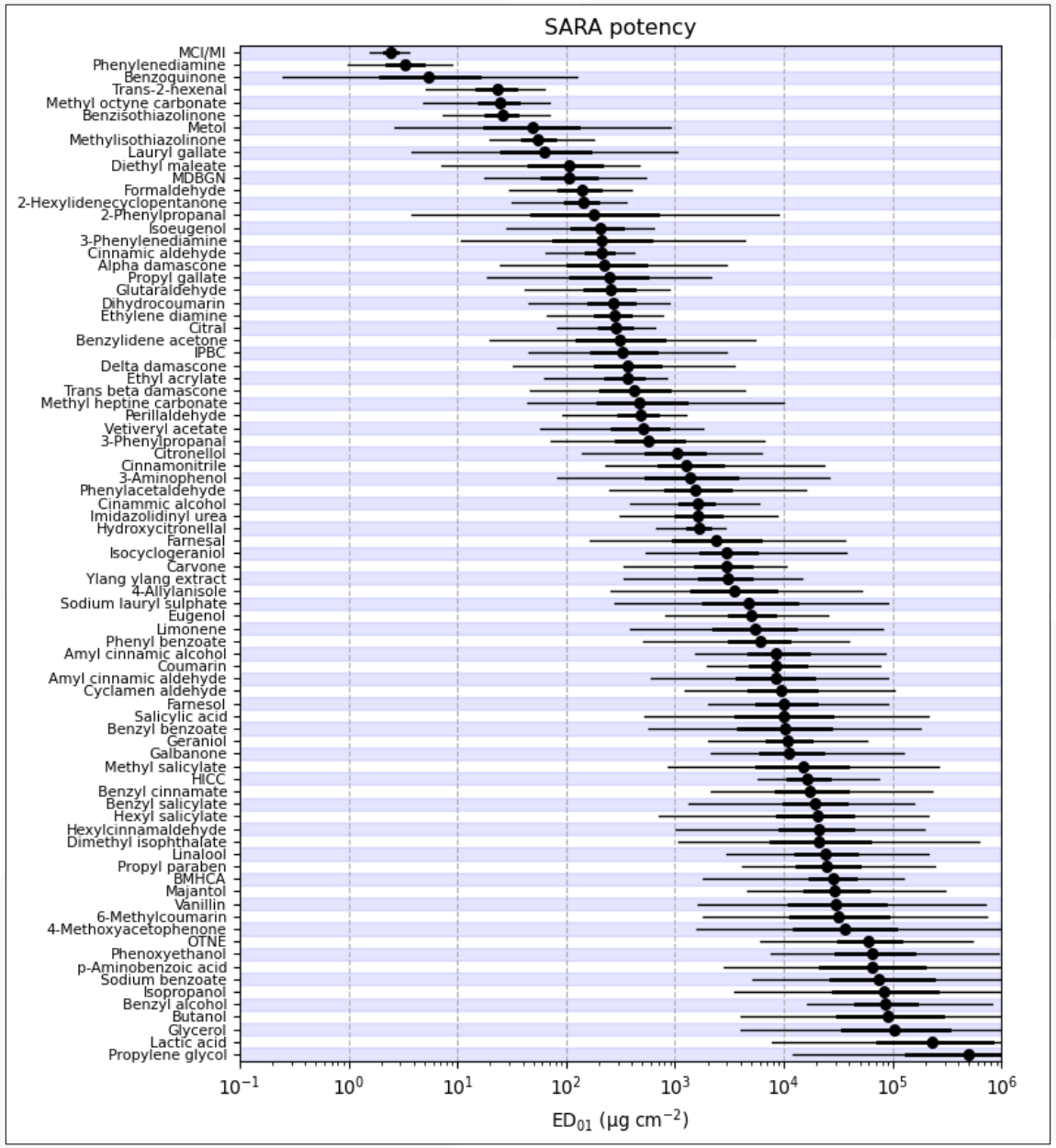
THE BASIC PRINCIPLES OF THE SARA MODEL



Potency across the SARA database - PoDs



This graph gives the ED_{01} and quantified uncertainty (the dot with the 50% and 95% confidence intervals denoted by the thick and thin lines either side)

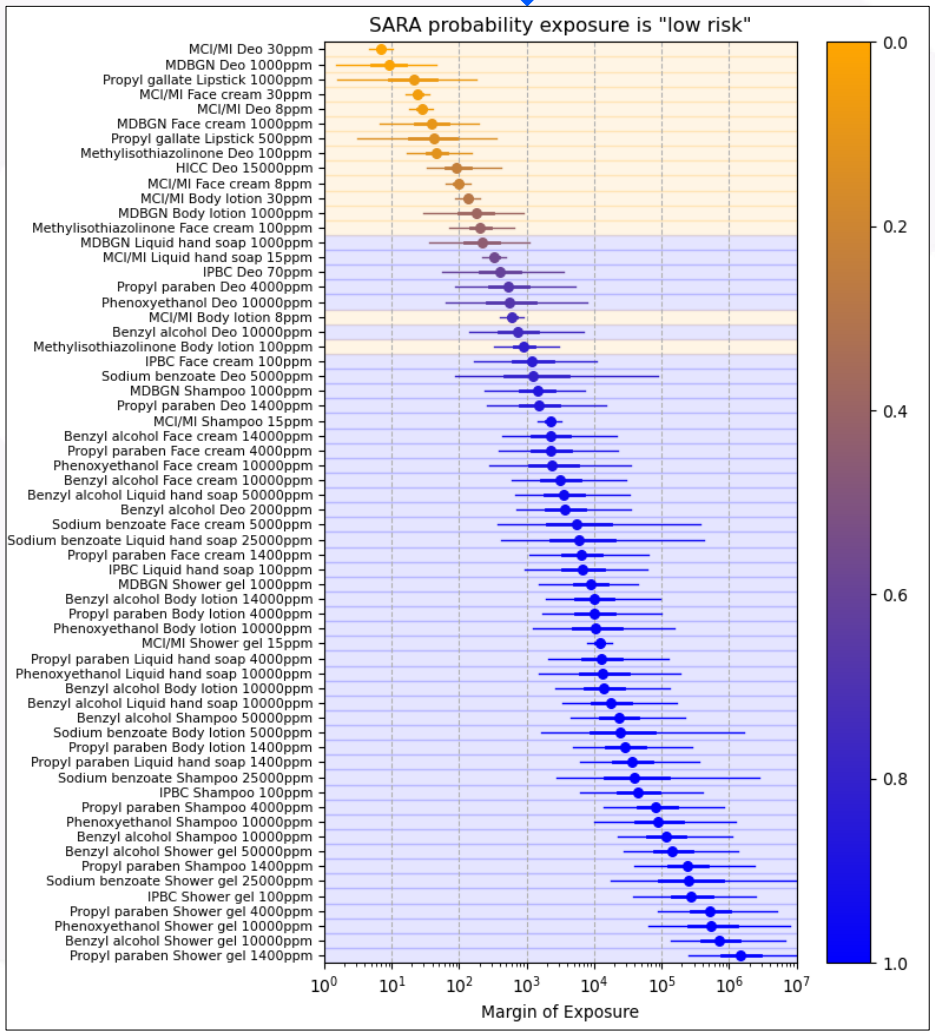
Use of consumer exposure information and clinical evidence to develop skin allergy risk benchmarks

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 calculation (PoD/Exposure)

Example

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



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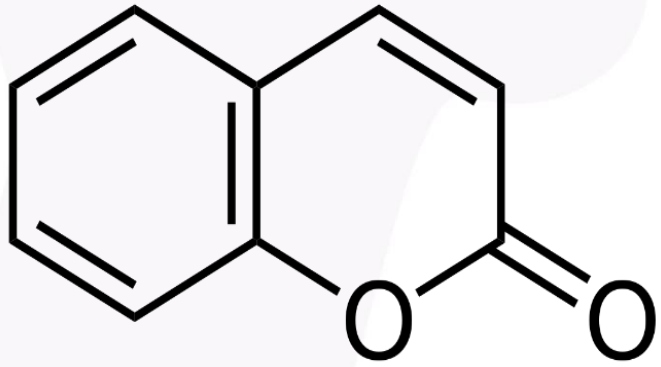
A case study approach – human health safety assessment required for...

0.1% COUMARIN IN FACE CREAM AND 1% IN ROLL-ON DEODORANT (NEW FRAGRANCE)

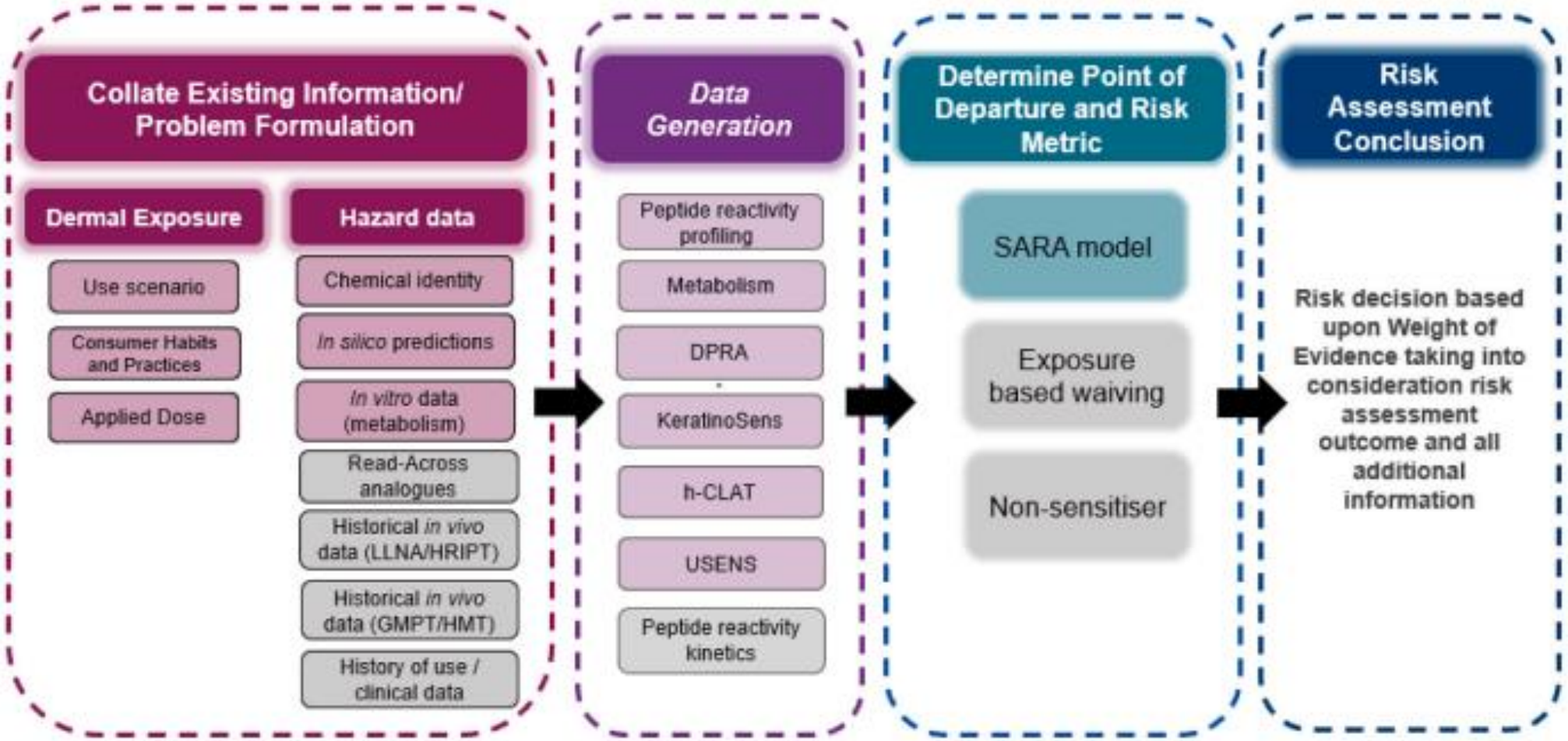
- For the purposes of the case study, *in vivo* data and read-across were not used, and the use of dermal sensitisation threshold (DST) was not appropriate.



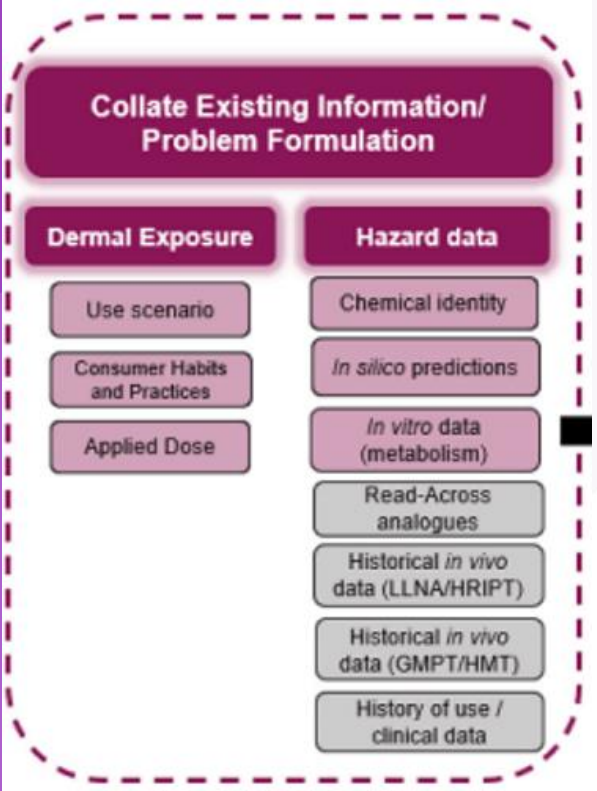
Reynolds et al (2021) Reg Tox Pharmacol, **127**, 105075

Application of NGRA framework for Skin Allergy

This NGRA 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 non-spray deodorant.

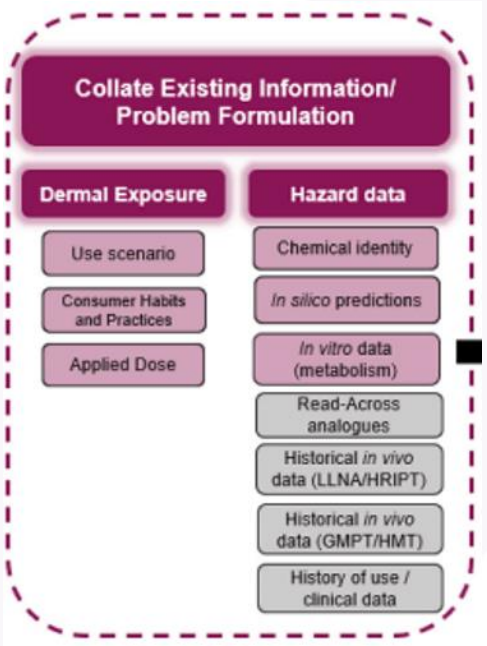


Local Exposure



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 (µg/cm^2)	**2.7**	**75**

Existing Information – In silico predictions



Chemicals	ToxTree Protein binding	OECD Toolbox*	TIMES-SS	Derek Nexus
Coumarin	MA and AC	MA and AC	Non-Sensitiser	Weak sensitiser
Metabolites				
Hydroxycoumarins (5-OH, 6-OH, 7-OH, 8-OH)	5-OH: MA and AC 6-OH: MA and AC 7-OH: MA and AC 8-OH: MA and AC	5-OH: AC and MA 6-OH: AC and MA 7-OH: AC and MA 8-OH: AC and MA	5-OH: NS 6-OH: NS 7-OH: NS 8-OH: NS	5-OH: Strong 6-OH: Strong 7-OH: Strong 8-OH: Strong
o-Hydroxyphenylacetaldehyde	Schiff base, MA and SN2	Schiff base	Strong sensitiser	Moderate sensitiser
o-Hydroxyphenylacetic acid	MA and SN2	No alerts	NS	Moderate sensitiser
(3R,4R)-3,4-Dihydroxy-2-chromanone; (Coumarin 3,4 epoxide as precursor)	MA, AC and SN2	AC, SN2	Strong sensitiser	Moderate sensitiser

MA: Michael acceptor; AC: acyl transfer; NS: non-sensitiser

Problem Formulation

Hypothesis: Coumarin has the potential to be a sensitiser and can act as either a hapten or pro-hapten.

Generate DPRA, KeratinoSens™, h-CLAT, U-SENS™ and peptide reactivity profiling data for coumarin to evaluate its skin sensitisation potential, potency, and a risk prediction for the given exposure scenarios.

To address areas of uncertainty regarding pro-haptens:

1. Replicate the testing strategy for coumarin for the major metabolite, 7-OH coumarin
2. *Ex vivo* skin cultures were used to determine if the 7-OH coumarin formation pathway was relevant/significant in human skin
3. Reactive metabolites were investigated through an experiment designed to trap reactive chemicals with GSH

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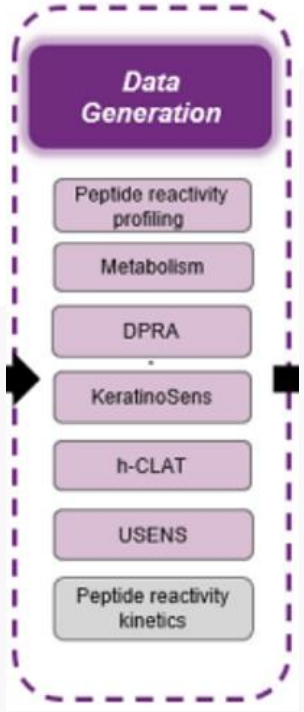
Results from NAM Data Generation for coumarin



	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

- **Coumarin was positive in all tests, except for DPRA** where peptide depletion was too low to meet positive threshold. No adducts were observed in the peptide profiling assay.

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Results from NAM Data Generation for 7-OH coumarin and metabolism data

	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)
7-OH Coumarin	0*	0	>2000	>566	>566	182

- 7-OH coumarin **was negative in KeratinoSens™ & h-CLAT, positive in USENS™, inconclusive in DPRA.**
- *Ex vivo* skin data show that the formation of **7-OH coumarin is not a major pathway in human skin**. The formation of reactive metabolites such as coumarin-3,4-epoxide could not be excluded nor confirmed in *ex vivo* skin.
- **Risk assessment based on parent, coumarin, is more appropriate**

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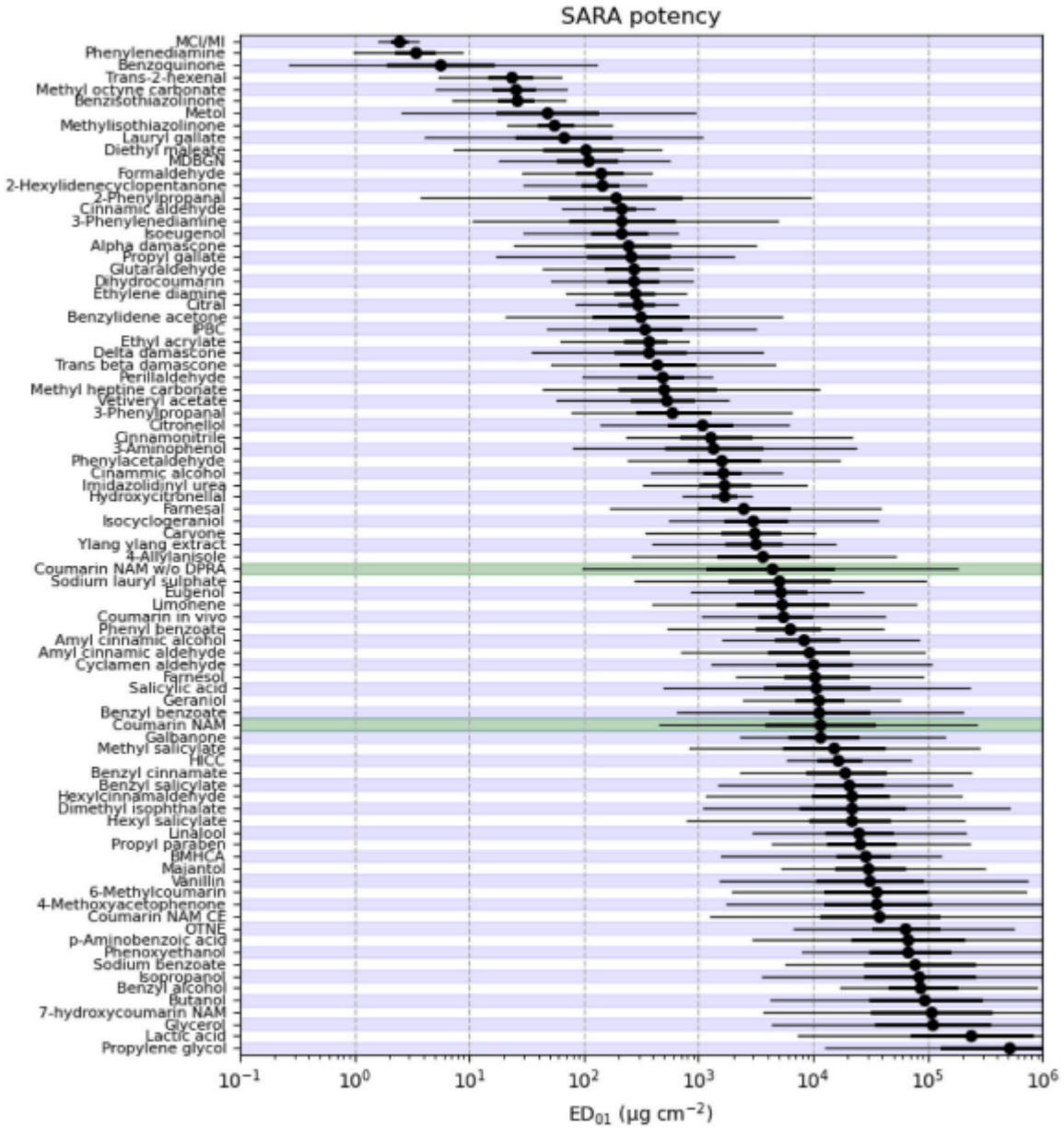
Determine Point of Departure (PoD) using SARA Model for coumarin



- SARA Model to define a human relevant PoD (ED_{01} i.e the 1% sensitising dose for a HRIPT population)
- For coumarin, with all NAM data, the expected SARA Model derived ED_{01} is 11,000 µg/cm^2,
- Coumarin ED_{01} 4200 µg/cm^2 for NAM data excluding DPRA.

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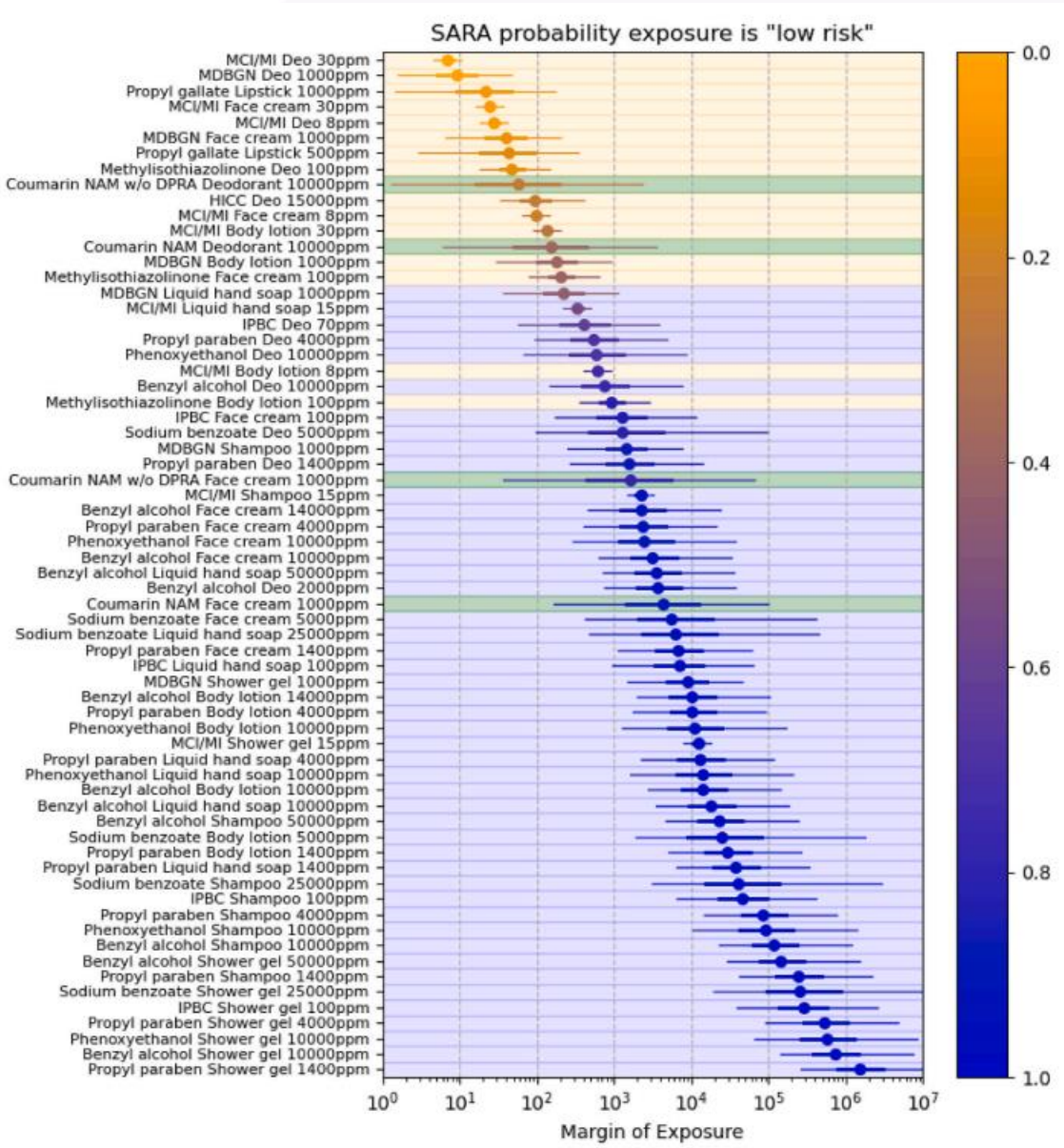
Risk assessment: Determine Margin of Exposure (MoE) and probability of risk



- Margin exposure is calculated for both exposure scenarios
- A probability of low risk is calculated based on the benchmark data
- For the **face cream exposure** – **Low Risk** outcome is most likely
- For the **deodorant exposure- High risk** outcome is most likely

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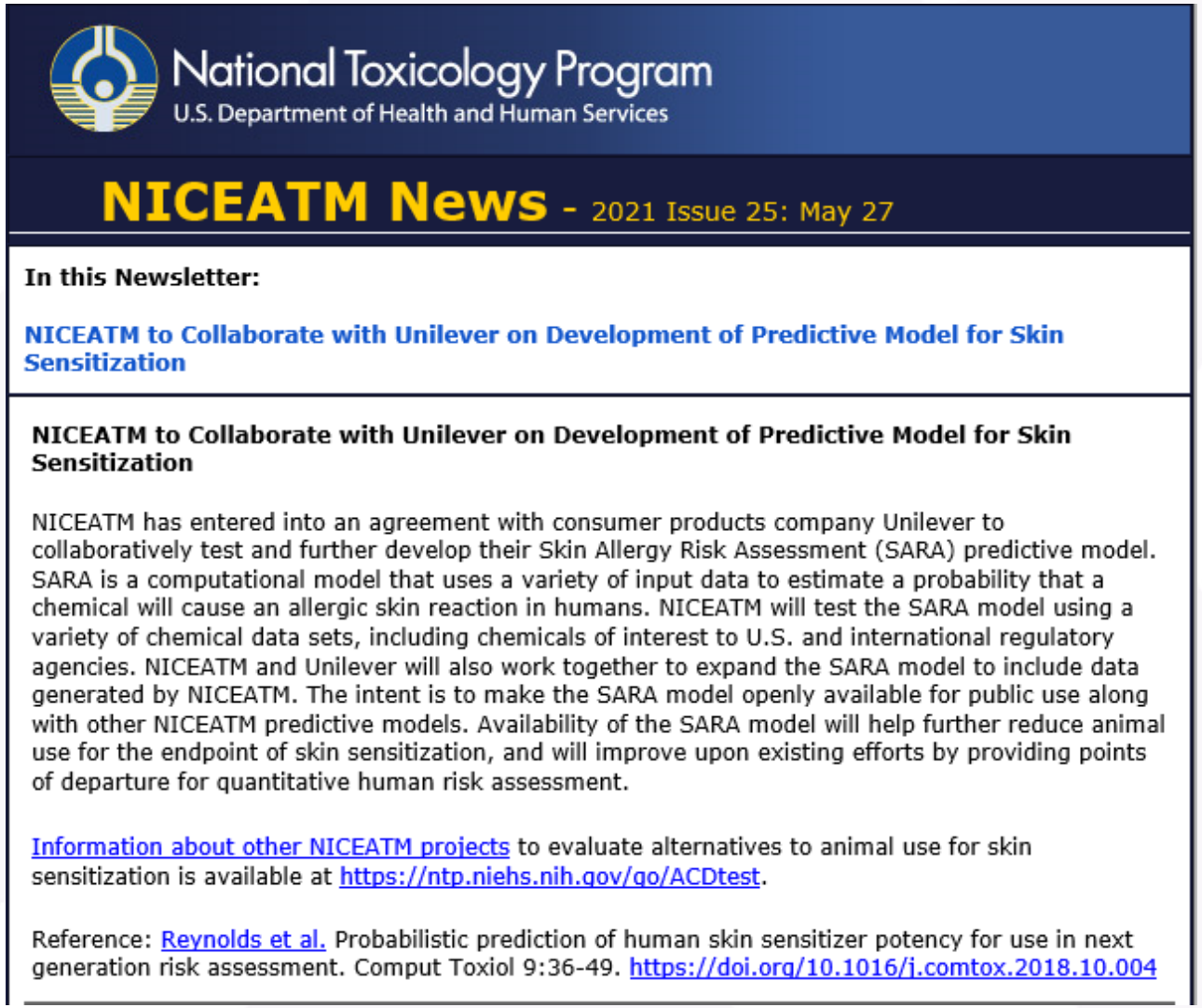

National Toxicology Program
U.S. Department of Health and Human Services

NICEATM News - 2021 Issue 25: May 27

In this Newsletter:

NICEATM to Collaborate with Unilever on Development of Predictive Model for Skin Sensitization

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NICEATM has entered into an agreement with consumer products company Unilever to collaboratively test and further develop their Skin Allergy Risk Assessment (SARA) predictive model. SARA is a computational model that uses a variety of input data to estimate a probability that a chemical will cause an allergic skin reaction in humans. NICEATM will test the SARA model using a variety of chemical data sets, including chemicals of interest to U.S. and international regulatory agencies. NICEATM and Unilever will also work together to expand the SARA model to include data generated by NICEATM. The intent is to make the SARA model openly available for public use along with other NICEATM predictive models. Availability of the SARA model will help further reduce animal use for the endpoint of skin sensitization, and will improve upon existing efforts by providing points of departure for quantitative human risk assessment.

Information about other NICEATM projects to evaluate alternatives to animal use for skin sensitization is available at https://ntp.niehs.nih.gov/go/ACDtest.

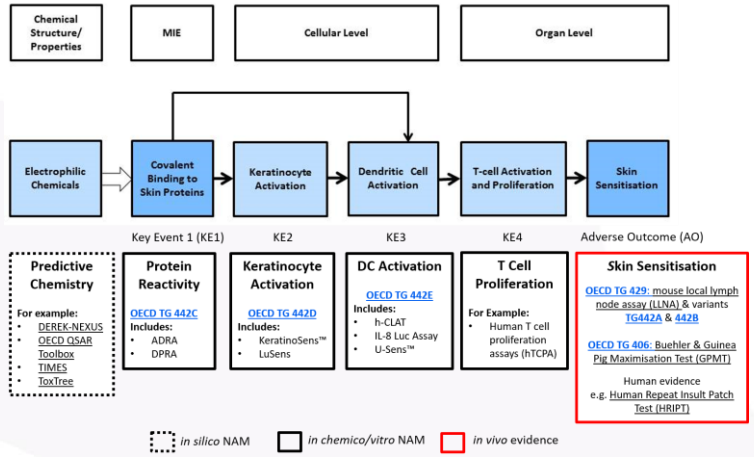
Reference: Reynolds et al. Probabilistic prediction of human skin sensitizer potency for use in next generation risk assessment. Comput Toxiol 9:36-49. https://doi.org/10.1016/j.comtox.2018.10.004

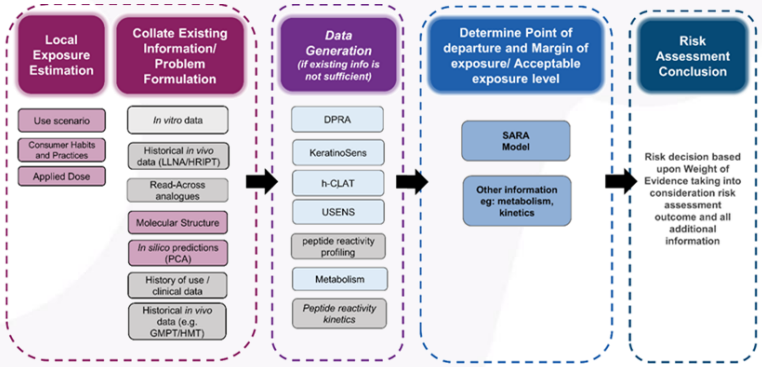
- The goal of the project is to develop a version of the SARA model for different skin sensitisation hazard and risk assessment regulatory use-cases.
- The project has a capability build phase and an evaluation phase.
- SARA-ICE model will be included in the OECD workplan for OECD DASS TG497

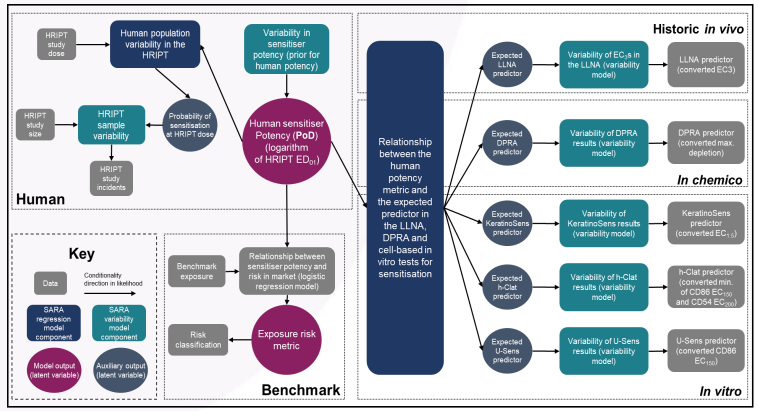
Unilever

Conclusions & Next Steps

- Significant progress has been made in the last decade to apply non-animal experimental data using Defined Approaches & tiered frameworks.
- Bayesian DAs enable experimental data variability to be modelled and uncertainty in PoDs & risk metrics to be factored into decision-making.
- Recently published NGRA framework SARA case studies: Reynolds *et al.*, 2021 and Gilmour *et al,* 2022.
- NICEATM collaboration established to evaluate SARA, expand the approach and make it publicly available.
- In-house work ongoing to explore new SARA inputs & expand the database, including risk benchmarks.







Acknowledgements

Georgia Reynolds
Joe Reynolds
Nicola Gilmour
Nora Aptula
Katarzyna Przybylak
Sam Windebank
Gavin Maxwell
Sandrine Spriggs
Richard Cubberley

Unilever