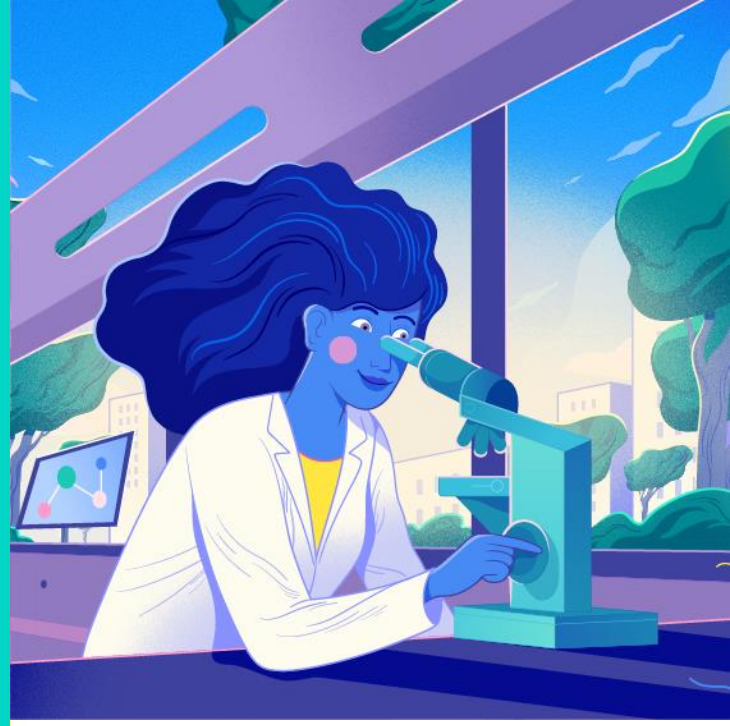
Next Generation Risk Assessment (NGRA) for Skin Allergy: experimental data & uncertainty modelling



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Symposium 1: Experimental data and uncertainty modelling, BTS Congress 2021

bts driving excellence in toxicology

Virtual CONGRESS 12–15 April 2021

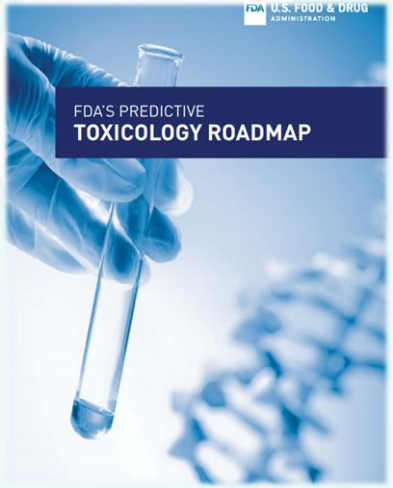
Declaration

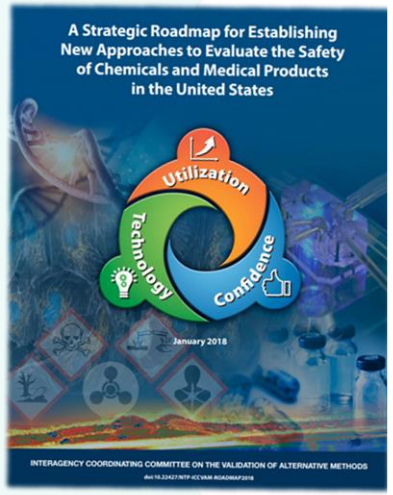
- Conflicts of interest
  - Gavin Maxwell is a full time employee of Unilever PLC
- Honoraria and sponsorship
  - None

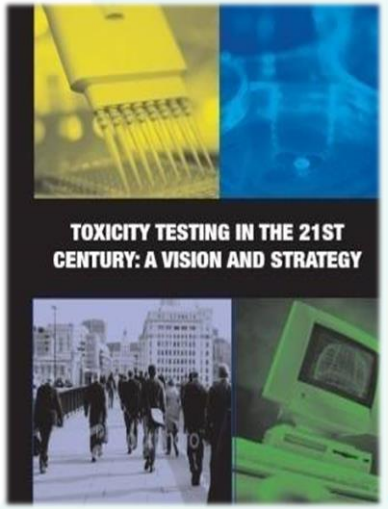
Assessing ingredient & product safety without animal testing

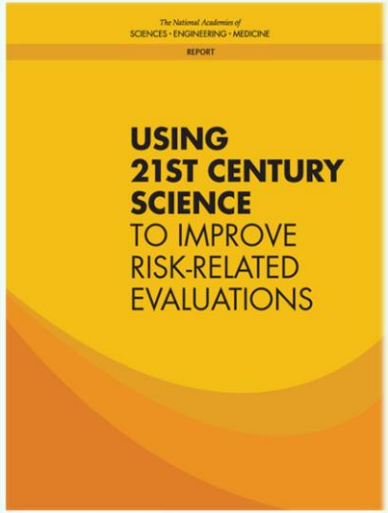
Next Generation Risk Assessment (NGRA)

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









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Principles of Next Generation Risk Assessment from ICCR Non-animal approaches in Cosmetic Risk Assessment

International Cooperation on Cosmetics Regulation

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

Dent *et al* (2018), Computational Toxicology, **7**, 20-26: https://doi.org/10.1016/j.comtox.2018.06.001

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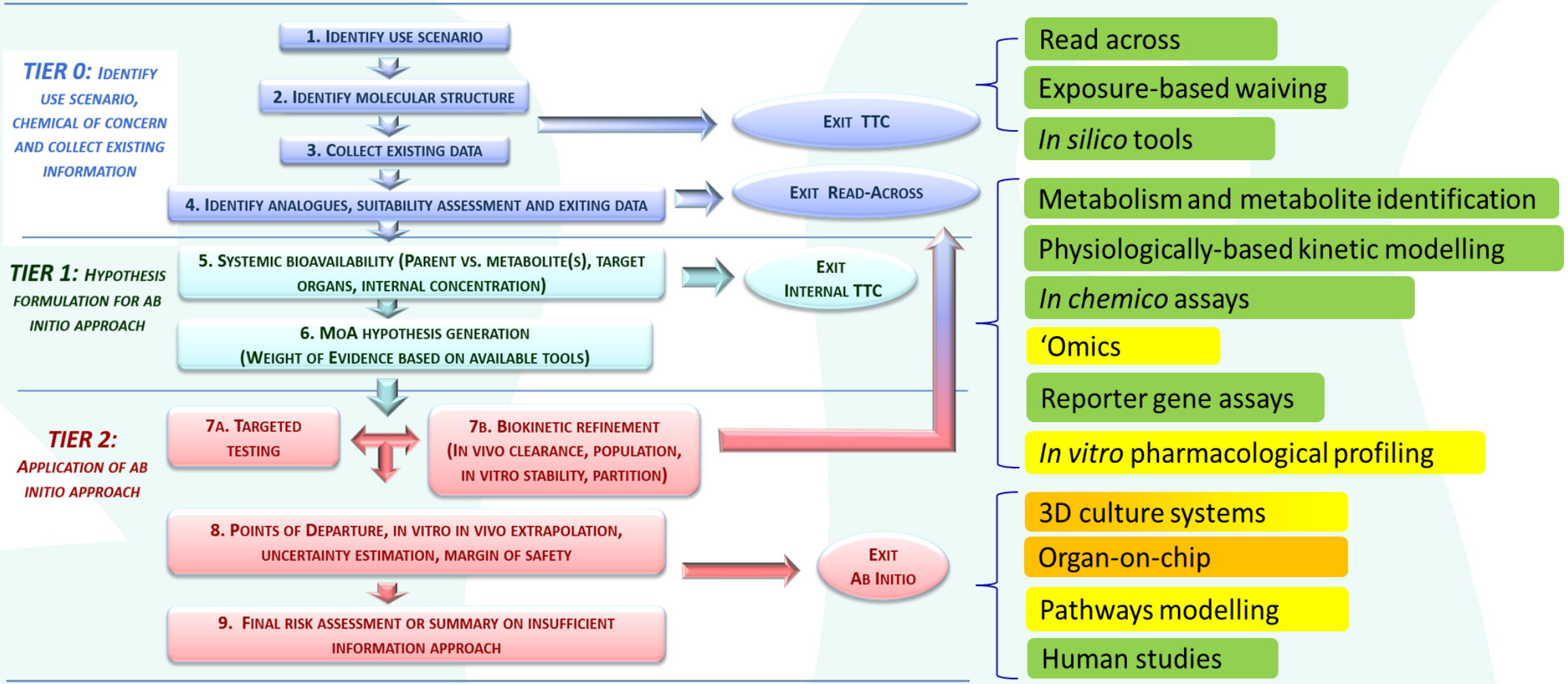
NGRA frameworks: Systemic toxicity example



Amaral et al. (2018) https://www.iccr-cosmetics.org//downloads/topics/iccr_integrated_strategies_for_safety_assessment_of_cosmetic_ingredients_part_2.pdf

Berggren *et al*, (2017),Computational Toxicology, **4**, 31-44: https://doi.org/10.1016/j.comtox.2017.10.001

NGRA framework: Skin Allergy example

Cosmetics Europe
the personal care association

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

1. Identify use scenario
- Applied dose µg/cm² skin
- Single product / aggregate exposure

2. Identify molecular structure
- Chemical(s) of concern
- Analysis of specification and impurities

3. Identify existing hazard information
- *in silico* predictions
- *In vitro / in chemico* data (OECD TG or non-OECD TG)
- Historical *in vivo* data (animal or human)

4. Identify analogues / suitability assessment and existing data
- Read across

→ **EXIT** Exposure based waiving

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

5. Hypothesis generation
- WoE prediction (S / NS) based upon T0 information
- Choice of DA in WoE
- Use of analogues in WoE

→ **EXIT** non-sensitiser

Tier 2
Risk assessment

6. Targeted testing
- Refinement of exposure estimate
- Bioactivation / metabolism data
- *In vitro* hazard data (OECD TG or non-OECD TG)

7. Point of Departure, uncertainty analysis, Margin of safety and final risk assessment
- POD determination
- Characterise uncertainty
- Compare reference dose to consumer exposure

→ **EXIT:** RA + / RA-

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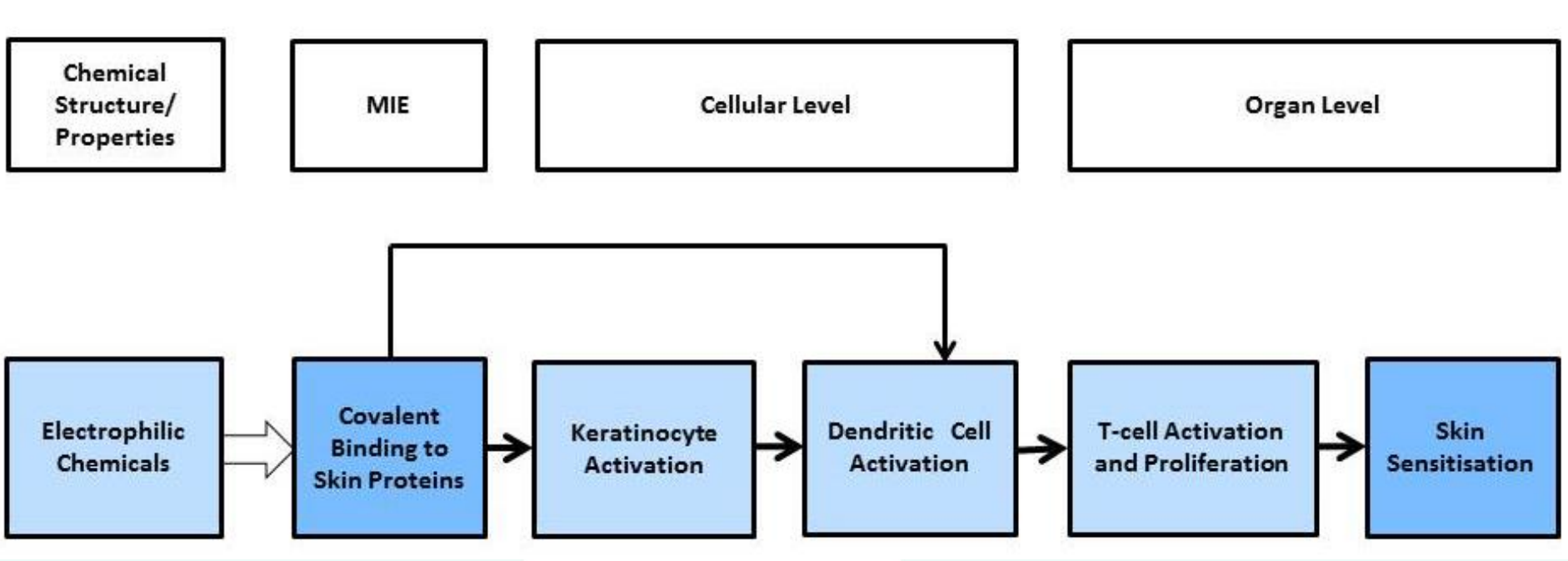
Gilmour et al, (2020),Regulatory Toxicology and Pharmacology, **116:** https://doi.org/10.1016/j.yrtph.2020.104721

Covalent Protein Binding leading to Skin Sensitisation AOP https://aopwiki.org/aops/40



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

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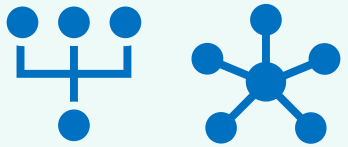
Skin Allergy Defined Approaches (DAs): state of the science

- **Defined Approach:** fixed Data Interpretation Procedure (DIP) used to interpret data generated with a defined set of information sources, that can either be used alone or together with other information sources, to satisfy a regulatory need

- **Twelve Skin Allergy DAs were reviewed by OECD to develop a DA reporting template:**
  - No. 255: Reporting of Defined Approaches to be used within Integrated Approaches to Testing and Assessment
  - No. 256: Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation

- **OECD DASS working group:** developing an OECD DA test guideline based (currently draft) based upon a performance-based evaluation of the simplest 'Group 1' DAs
  - GL DASS_22Sep2019v2.pdf (oecd.org)
  - DAGL supporting document_23 Sep2019.pdf (oecd.org)



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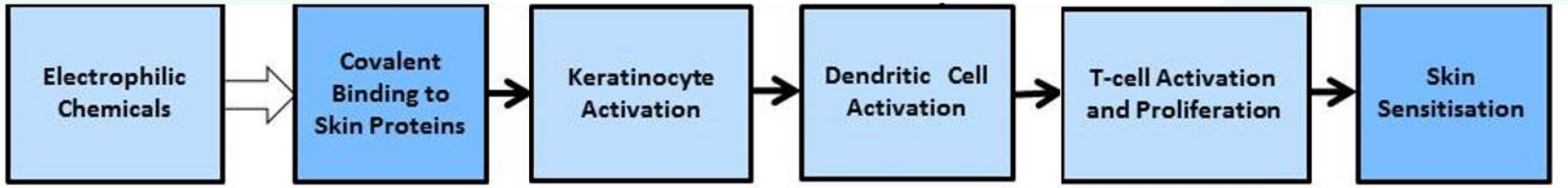
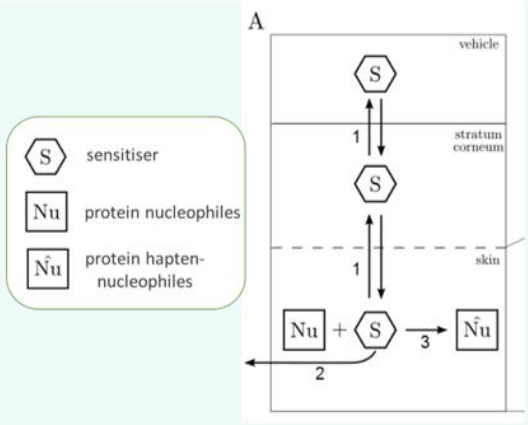
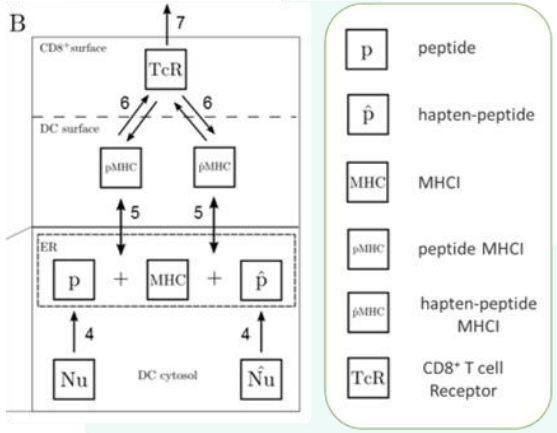
Toxicokinetic-toxicodynamic (TKTD)/qAOP defined approach





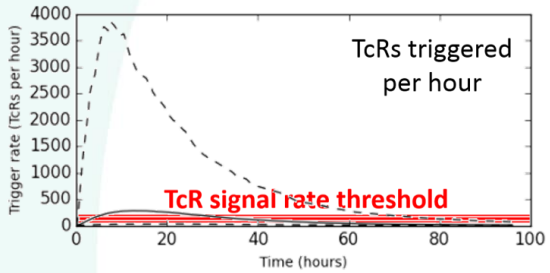
Modelling covalent binding to skin proteins

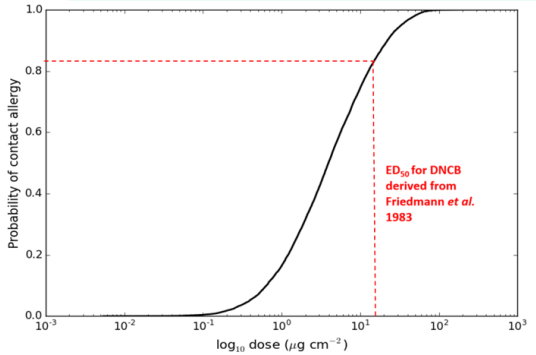
1. diffusion and partitioning into the stratum corneum and skin
2. sensitiser clearance by dermal capillaries
3. covalent modification of protein nucleophiles by hapten.



Modelling T cell activation & proliferation

4. proteasome processing of protein nucleophiles to form small peptides and transport to the endoplasmic reticulum (ER)
5. binding of peptides and hapten-peptide complexes to Class I MHC and transport to plasma membrane
6. binding of pMHC and hapten-pMHC to CD8⁺ T cell receptors
7. activation of naïve specific CD8⁺ T cells

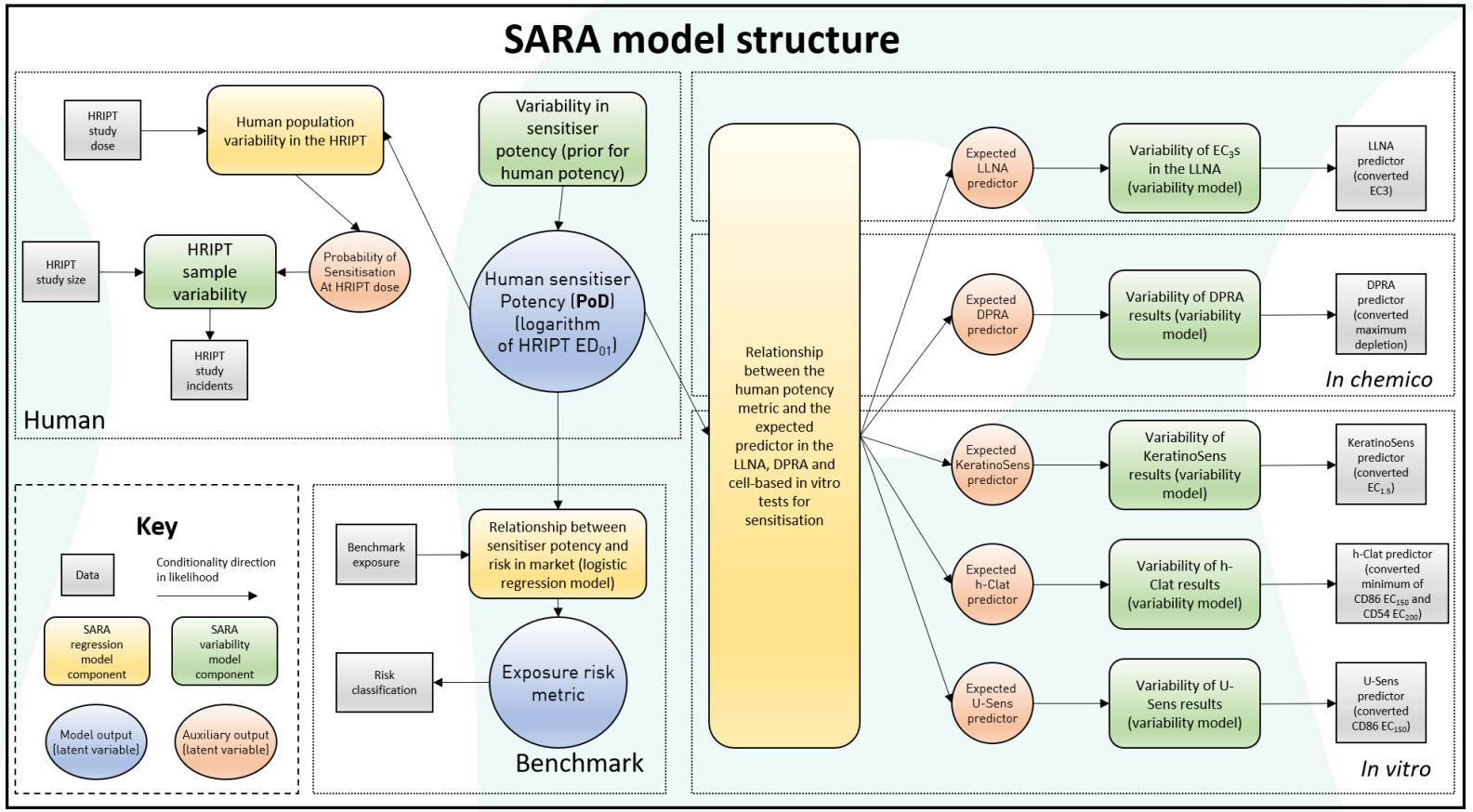




Objective: model should be simplest representation of the AOP capable of reproducing the induction of contact allergy to DNCB to enable prediction of a safe level of skin exposure (MacKay *et al*. 2013. http://altweb.jhsph.edu/altex/30_4/MacKay.pdf)

Skin Allergy Risk Assessment (SARA) Defined Approach

- Bayesian probabilistic model, which estimates human sensitiser potency for use in risk assessment decision-making
- uses a database of public experimental data covering AOP KEs 1-3 and AO
- original publication: Reynolds et al. 2019: https://doi.org/10.1016/j.comtox.2018.10.004

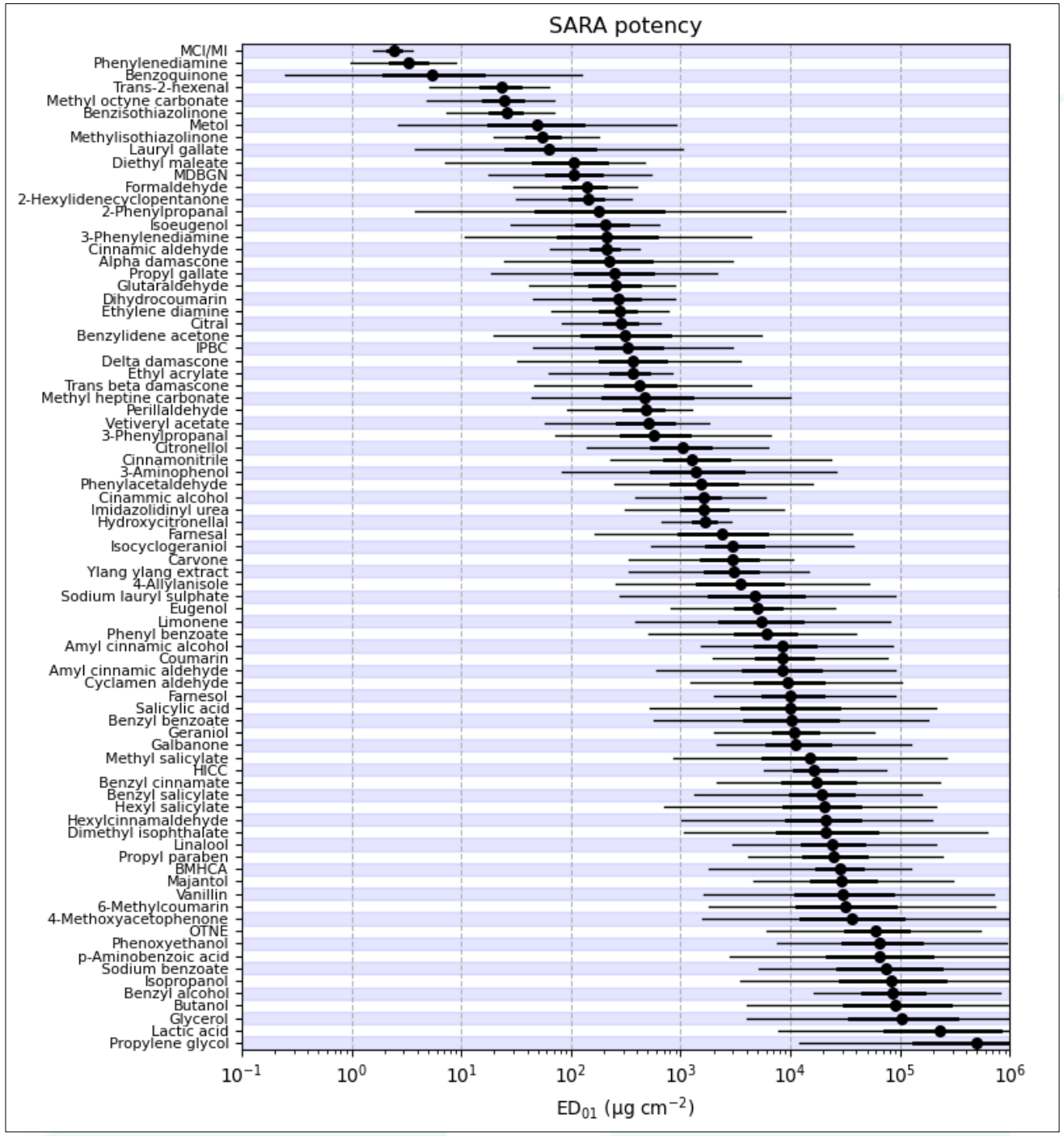


Key Event 1 (KE1)	KE2	KE3	Adverse Outcome (AO)
Protein Reactivity OECD TG 442C • DPRA	**Keratinocyte Activation** OECD TG 442D • KeratinoSens™	**DC Activation** OECD TG 442E • h-CLAT • U-Sens™	***Skin Sensitisation*** • OECD TG 429: mouse local lymph node assay (LLNA) • Human evidence:. Human Repeat Insult Patch Test (HRIPT)

SARA Defined Approach

Since the 2019 publication we've:

- redefined the point of departure (PoD) metric to a dose with a 1% chance of human skin sensitisation (termed ED01)
- expanded the SARA dataset from 30 → 81 chemicals
- expanded to account for variability in the DPRA, KeratinoSens™, h-CLAT and U-Sens™ (note: variability in the historical in vivo data was a feature of the 2019 model)
- expansion to incorporate benchmark exposure information



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

- Traditional risk assessment approaches for skin allergy use safety factors to rescale PoDs to market-equivalent safe doses for comparison against consumer exposure estimates.
- For NGRA, publicly available benchmark exposure information can be used to establish that an exposure is low risk and can be considered safe.
- To apply this concept, we established 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.

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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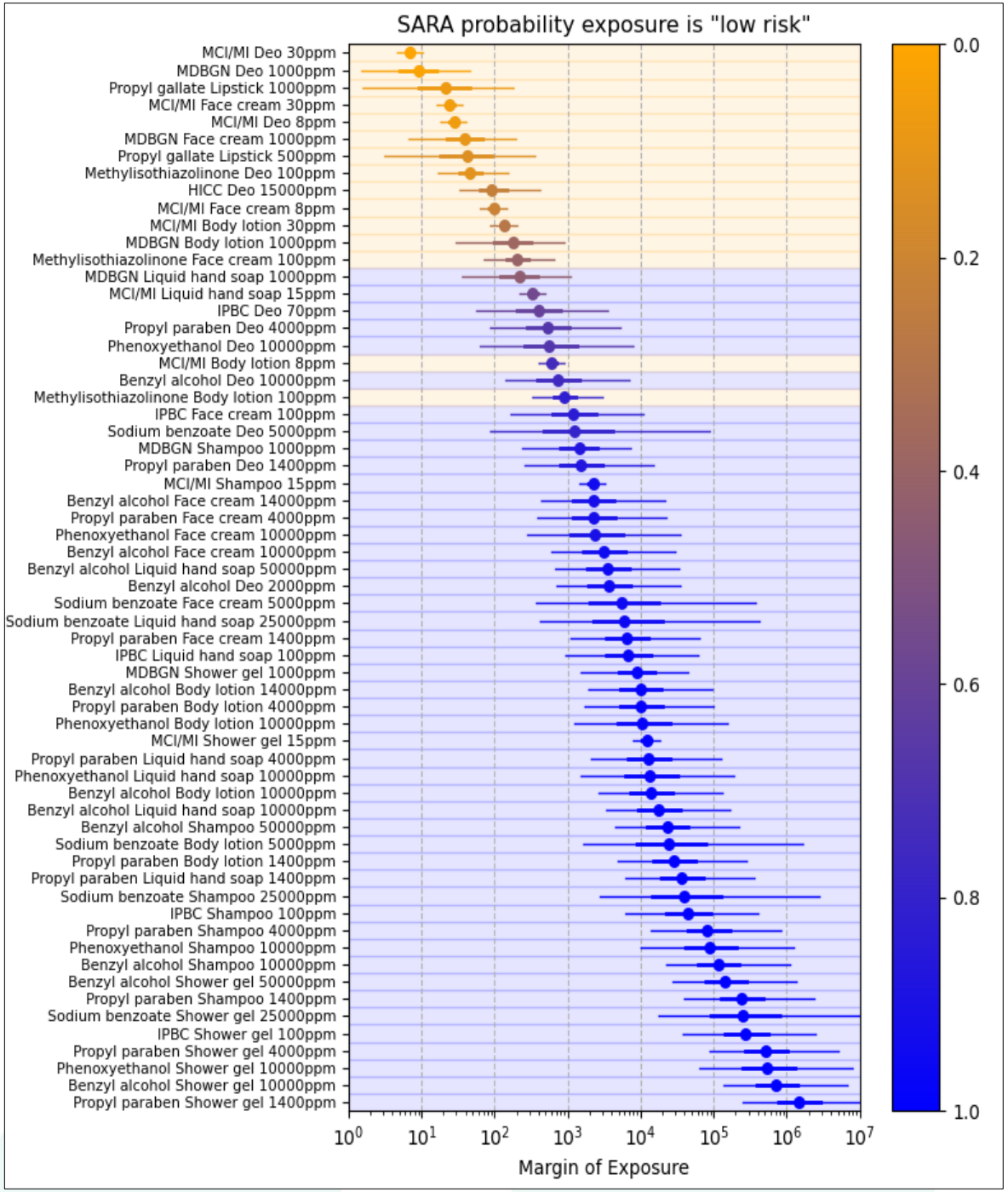
Expansion of SARA model to use benchmark exposure information

- The SARA model was expanded to incorporate benchmark exposure information as an additional input alongside historic *in vivo* and NAM data.

- After fitting the model, and given some exposure scenario of interest, the model can then be run in 'forward mode' to calculate the *SARA risk metric*, defined as the probability that the exposure is low risk for human skin sensitisation induction.



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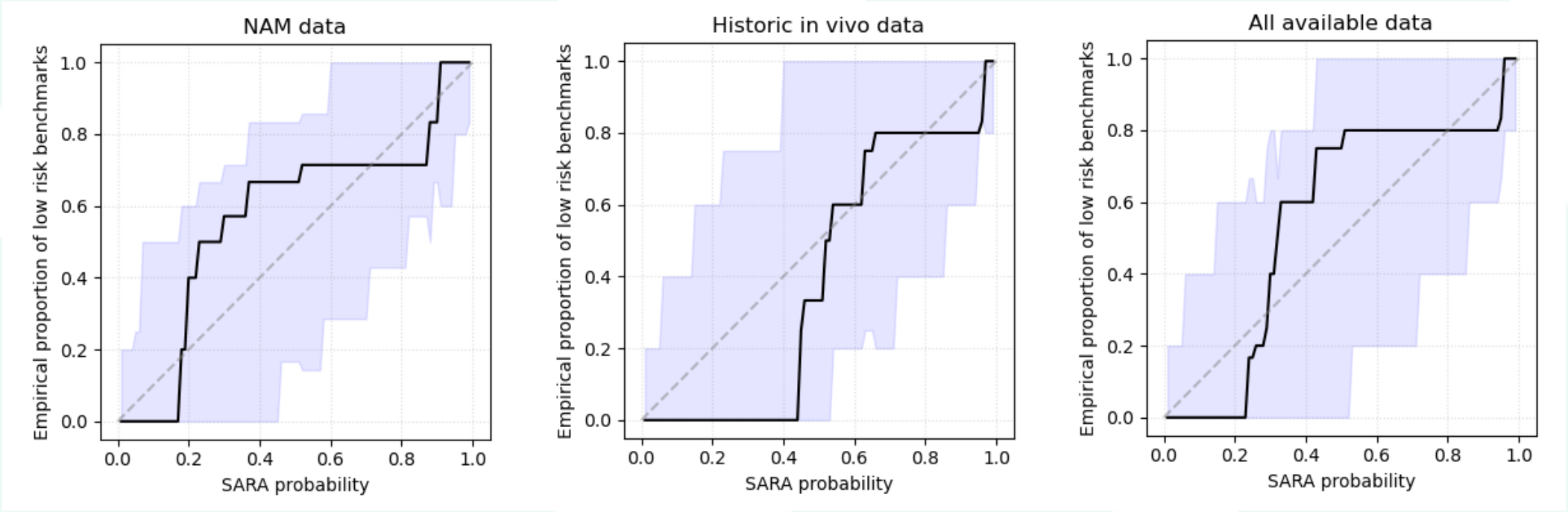
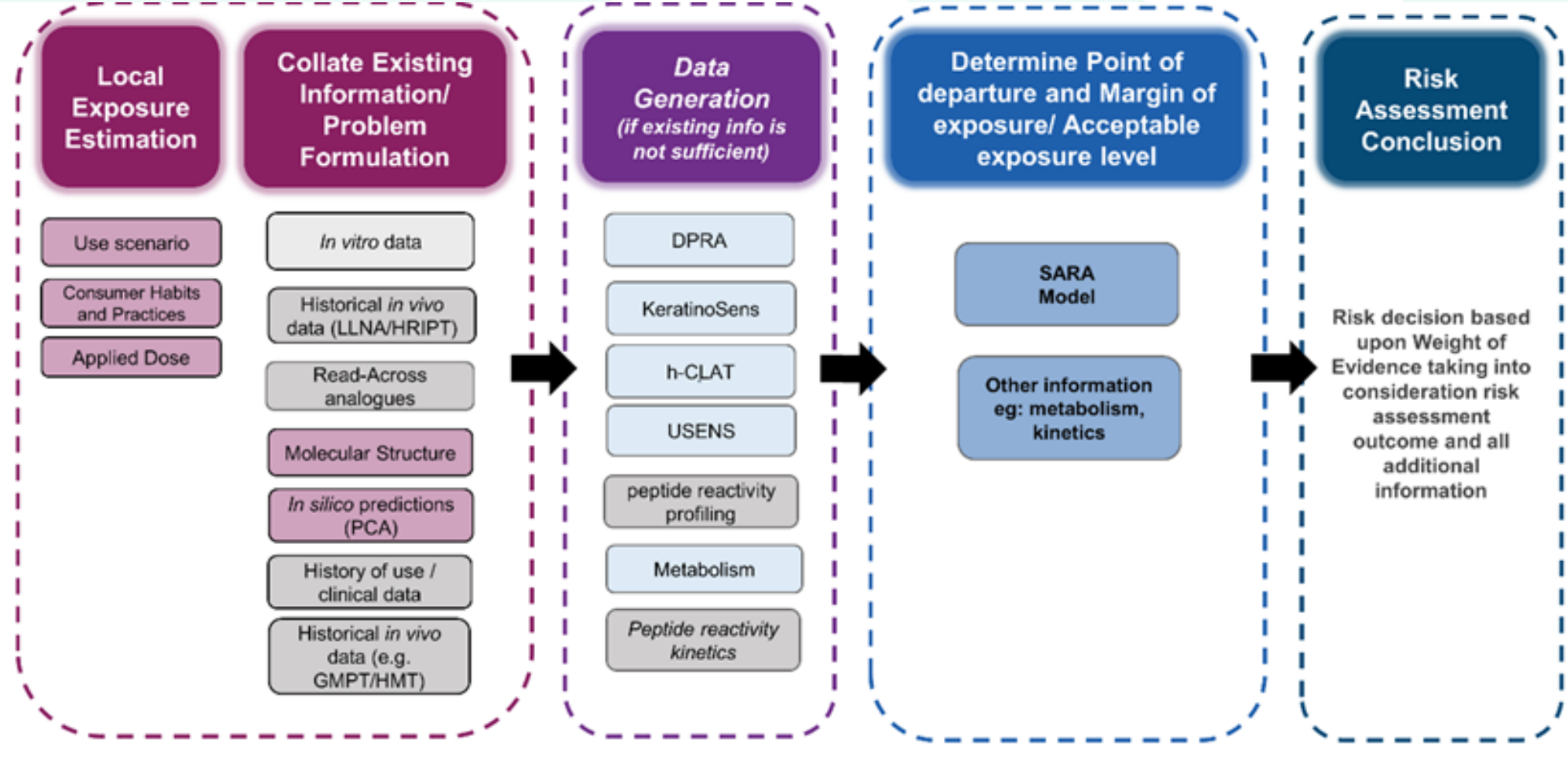
Assessment of calibration of SARA risk metric

- Reliable use of the SARA risk metric within a risk assessment requires that it be *calibrated* - understood in terms of frequencies of correct decisions.
- Benchmark exposures were used within a cross-validation exercise to assess calibration of the SARA risk metric.
- For all SARA probabilities, the frequency of truly low risk exposures was found to be within the expected range irrespective of whether predictions were trained on *in chemico/in vitro* NAM data, historic in vivo, or a combination of both.

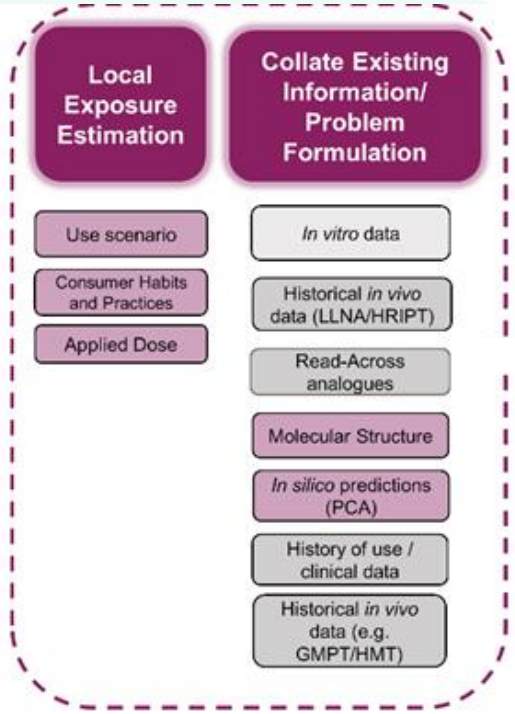


Application of NGRA framework for Skin Allergy

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 deodorant. 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.



Local exposure + Collate Existing Information/ Problem Formulation



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)	2.73	75

- *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 potential. 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.

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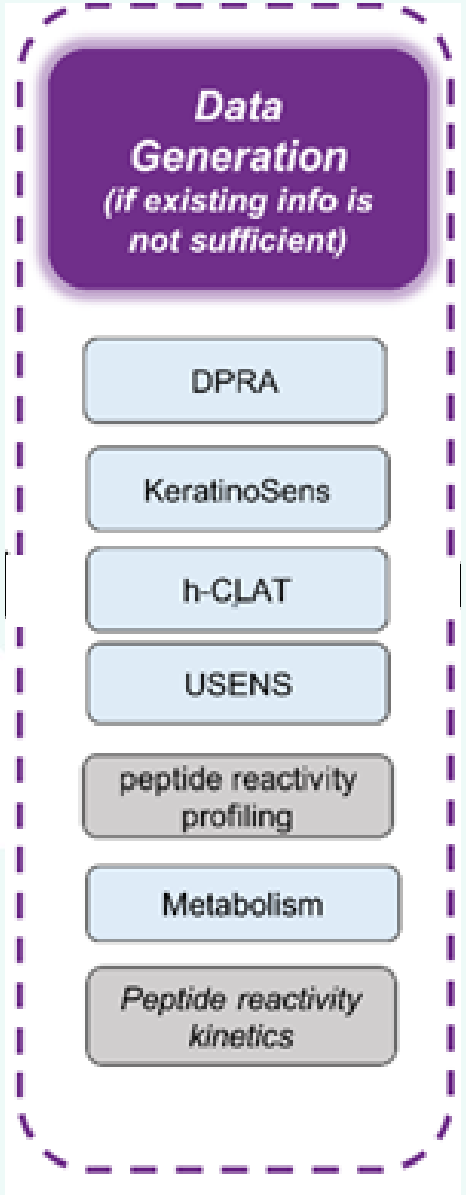
Data Generation



	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.

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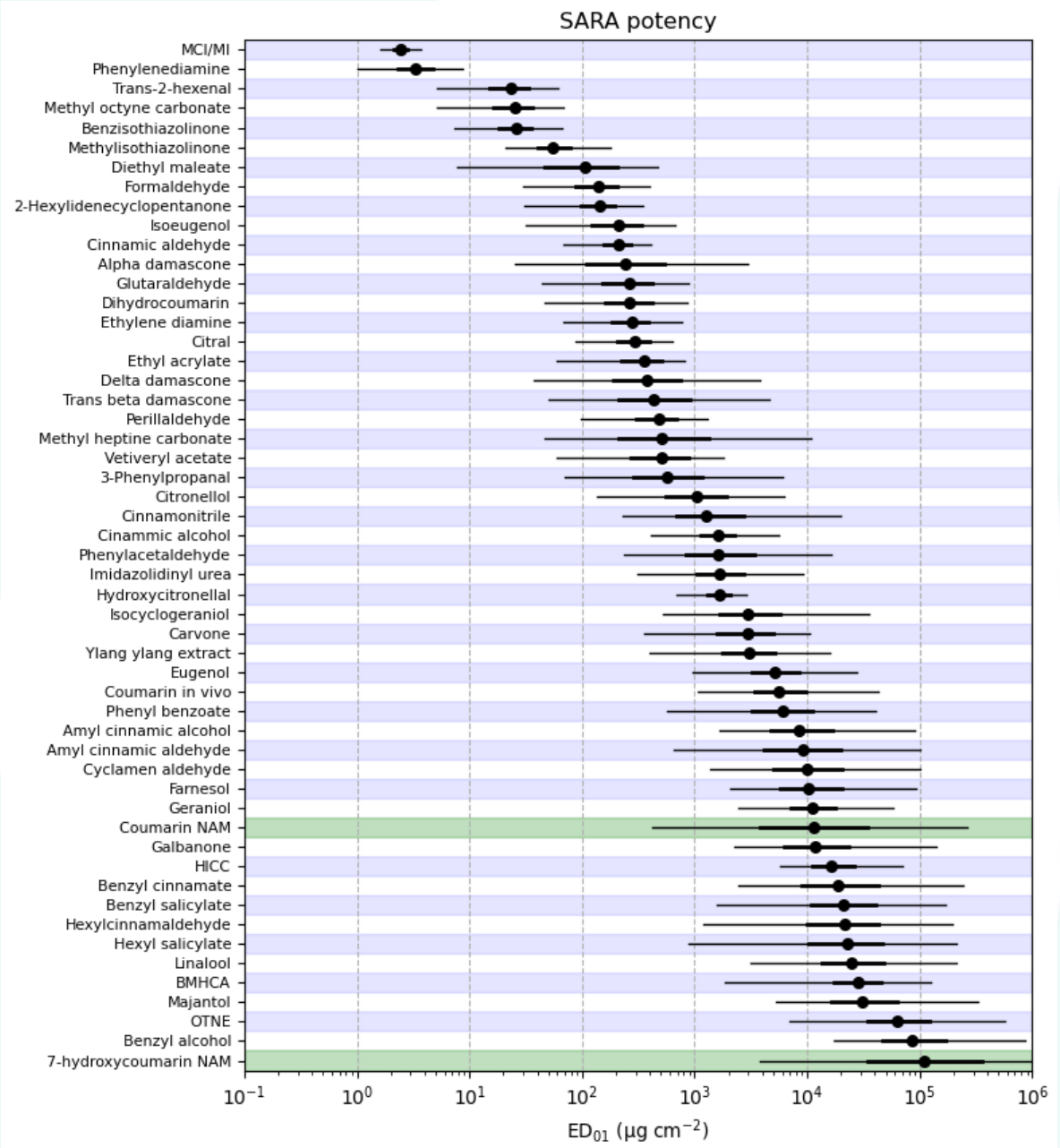
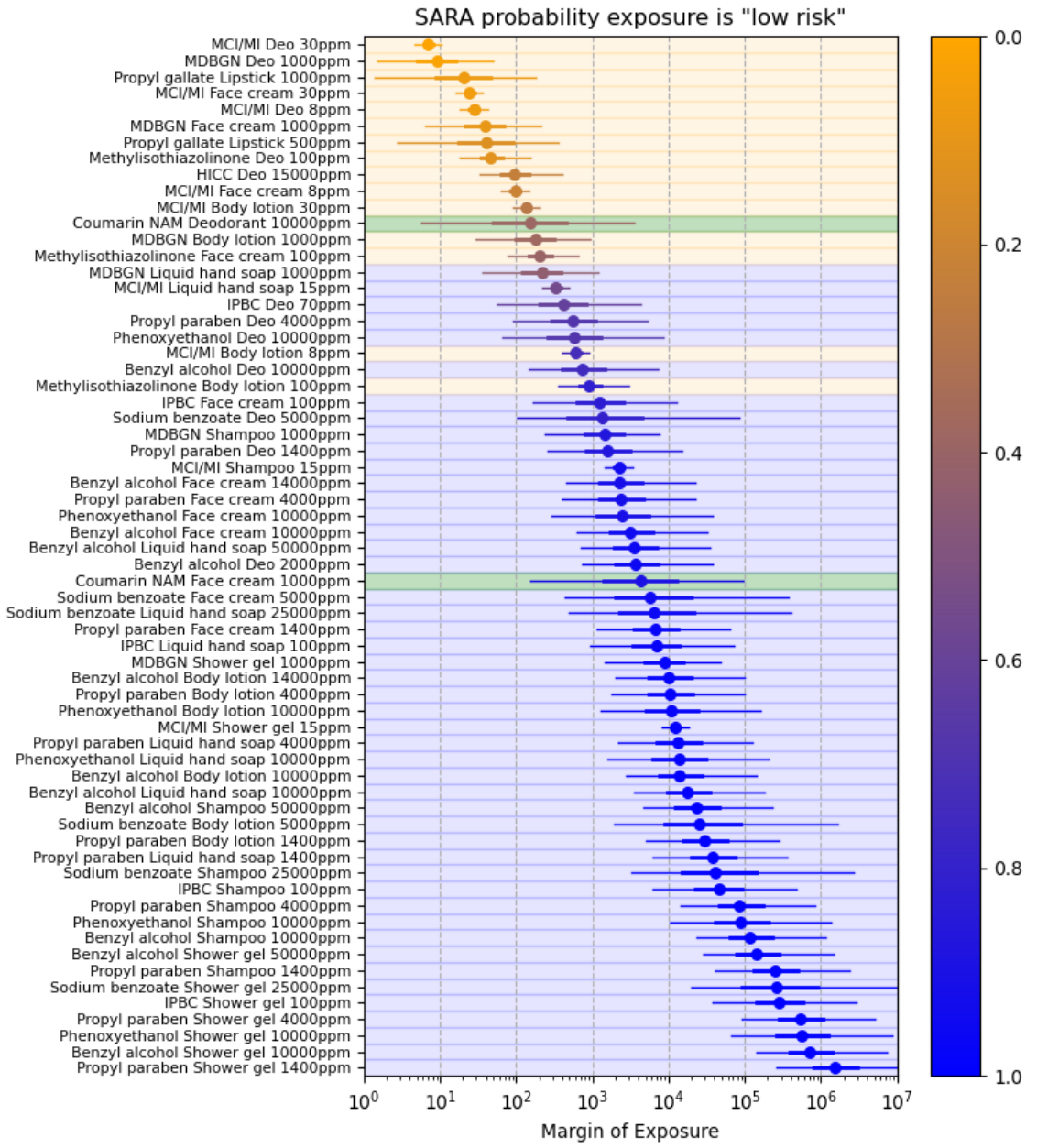
Determine Point of departure using SARA DA



- The generated DPRA, KeratinoSens™, hCLAT and USens™ 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 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} 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.

Determine MoE/Acceptable Exposure Level + NGRA conclusion



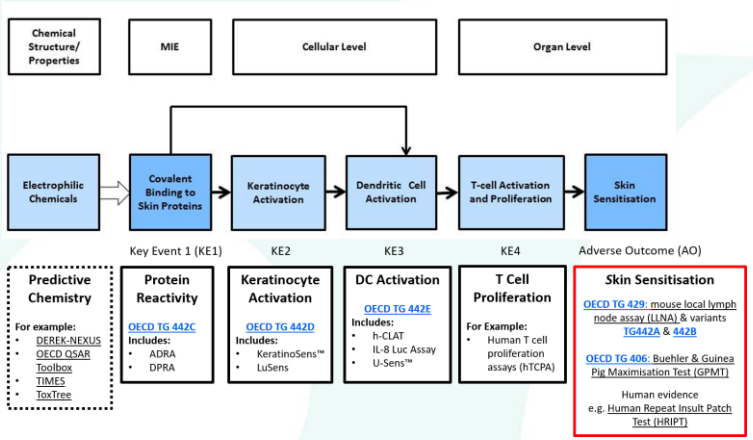
- The MoE was calculated from the ED_{01} for coumarin and the dermal exposures for each product type using SARA DA
- The MoE for face cream exposure ranks with the low-risk benchmarks whilst the MoE for the deodorant exposure ranks with the high-risk benchmarks.
- The SARA DA probability that the exposure is low risk is calculated to be 0.90 for the face cream dermal exposure and 0.39 for the deodorant dermal exposure.
- 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

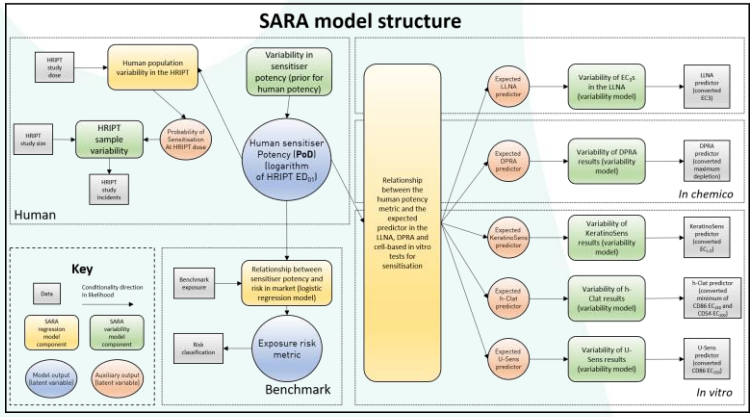
Conclusions

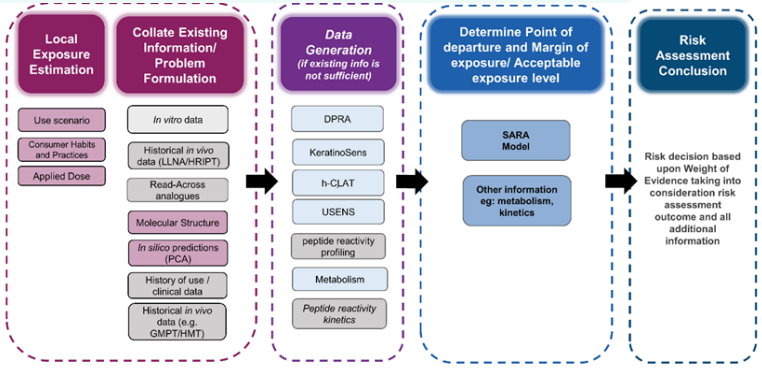
- Significant progress has been made in the last decade to apply non-animal experimental data to NGRA for Skin Allergy using Defined Approaches
- Bayesian DAs enable experimental data variability to be modelled and uncertainty in PoDs & risk metrics to be factored into decision-making

Next Steps:

- Updated SARA DA/database & Skin Allergy Risk Benchmarks to be peer reviewed (manuscripts in preparation)
- Work ongoing to explore new SARA inputs and expand the database, including risk benchmarks







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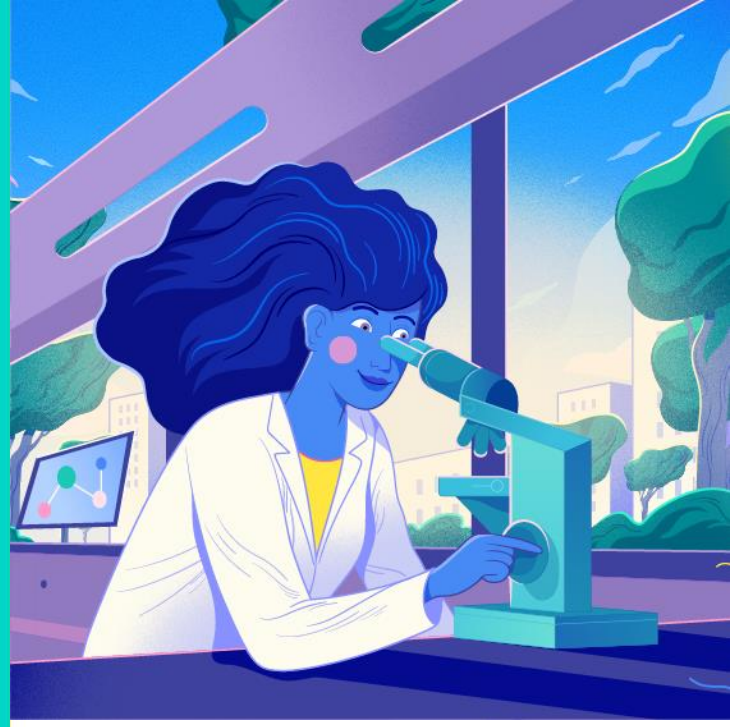

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