Integrated Approaches to Testing and Assessment : concepts & case studies

Dr Gavin Maxwell

Safety Science Advocacy Lead

Unilever Safety & Environmental Assurance Centre (SEAC)

Unilever Policy & Approach
Safe & Sustainable Products without Animal Testing

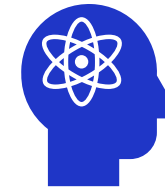**We say use science.
Not animals.**

What we believe

- **Every Unilever product must be safe for people and our environment**

- **Animal testing is not needed to assess ingredient & product safety** – there are a wide range of non-animal alternatives grounded in modern science and new technology
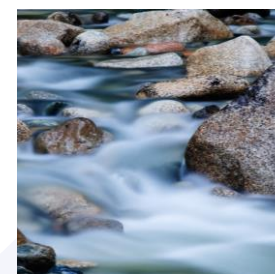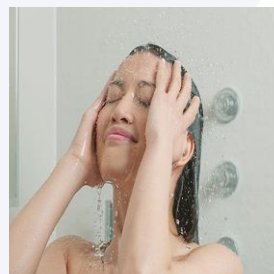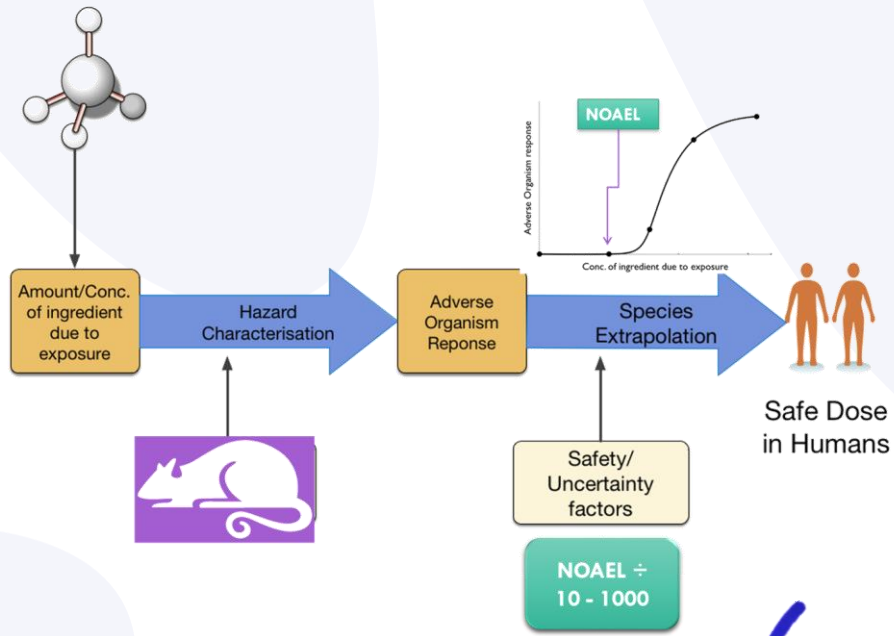
How we do it
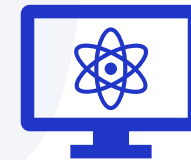
40+ years of developing non-animal safety science

70+ collaborations

600+ publications

'Traditional' Risk Assessment

Amount/Conc. of ingredient due to exposure → Hazard Characterisation → Adverse Organism Reponse → Species Extrapolation → Safe Dose in Humans

NOAEL

Safety/ Uncertainty factors

NOAEL ÷ 10 - 1000

2007

2021

'Next Generation' Risk Assessment

Exposure models (PBK, free/total concentration)

Exposure estimation: Plasma C_{max}

Point of departure derived from concentration-response data

Pathway characterisation: Point of Departure

Cellular stress assays

Transcriptomics

Receptor binding

Calculation of Margin of Safety (MoS) distribution

e.g. Margin of safety is the fold difference between the Cmax and the *in vitro* POD

A paradigm shift is underway as NAM use becomes widespread & chemical safety assessment frameworks evolve to embed NGRA

New Approach Methodologies (NAMs)

Defined Approaches (DAs)
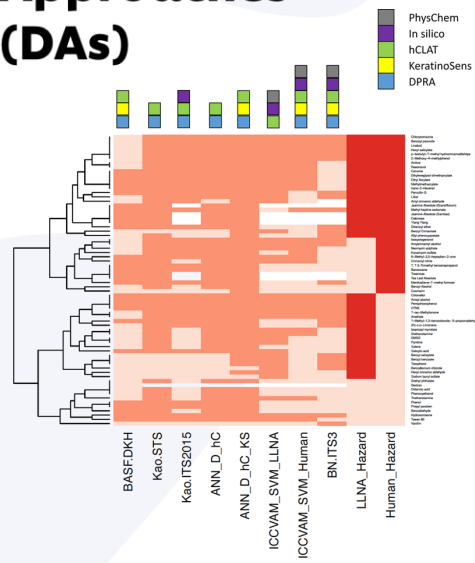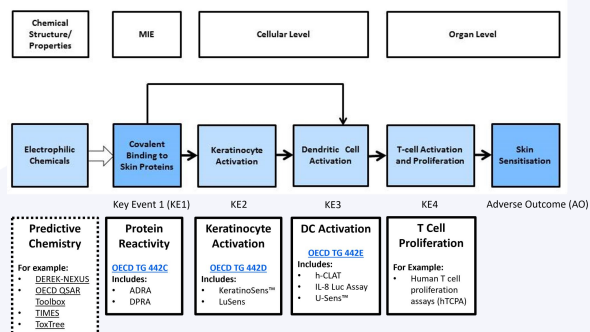
Integrated Approaches to Testing & Assessment (IATA)

Exposure models (PBK, free/total concentration)

Exposure estimation: Plasma C_{max}

Calculation of Margin of Safety (MoS) distribution

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Amount/Conc. of ingredient due to exposure

Hazard Characterisation

Adverse Organism Reponse

Species Extrapolation

Safe Dose in Humans

Safety/ Uncertainty factors

NOAEL ÷ 10 - 1000

Skin Sensitisation: road to replacement

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

Chemical Structure/ Properties	MIE	Cellular Level	Organ Level

2012
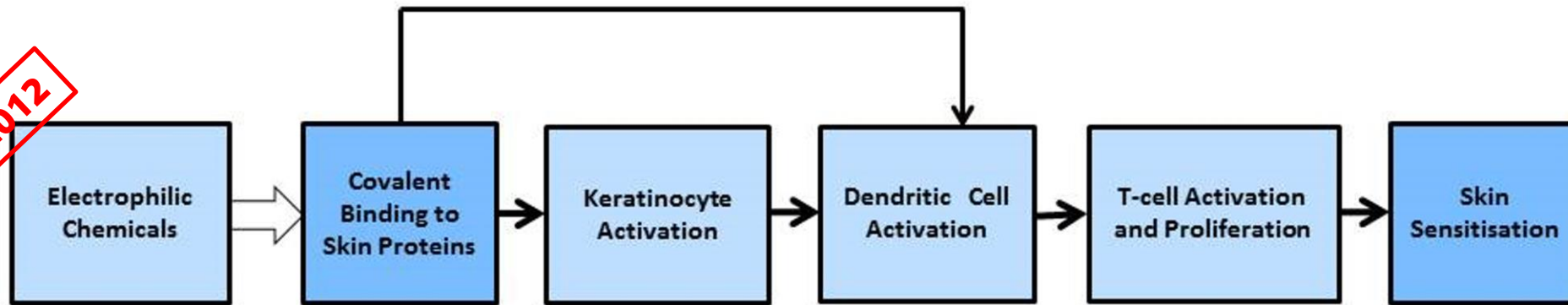
Electrophilic Chemicals	Covalent Binding to Skin Proteins	Keratinocyte Activation	Dendritic Cell Activation	T-cell Activation and Proliferation	Skin Sensitisation
	Key Event 1 (KE1)	KE2	KE3	KE4	Adverse Outcome (AO)

Predictive Chemistry	Protein Reactivity	Keratinocyte Activation	DC Activation	T Cell Proliferation	Skin Sensitisation
For example: • DEREK-NEXUS • OECD QSAR Toolbox • TIMES • ToxTree	OECD TG 442C Includes: • ADRA • DPRA	OECD TG 442D Includes: • KeratinoSens™ • LuSens	OECD TG 442E Includes: • h-CLAT • IL-8 Luc Assay • U-Sens™	For Example: • Human T cell proliferation assays (hTCPA)	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

Skin Sensitisation Defined Approaches (DAs)

- **Defined Approach:** fixed Data Interpretation Procedure (DIP) used to interpret a specific combination of information sources

2016

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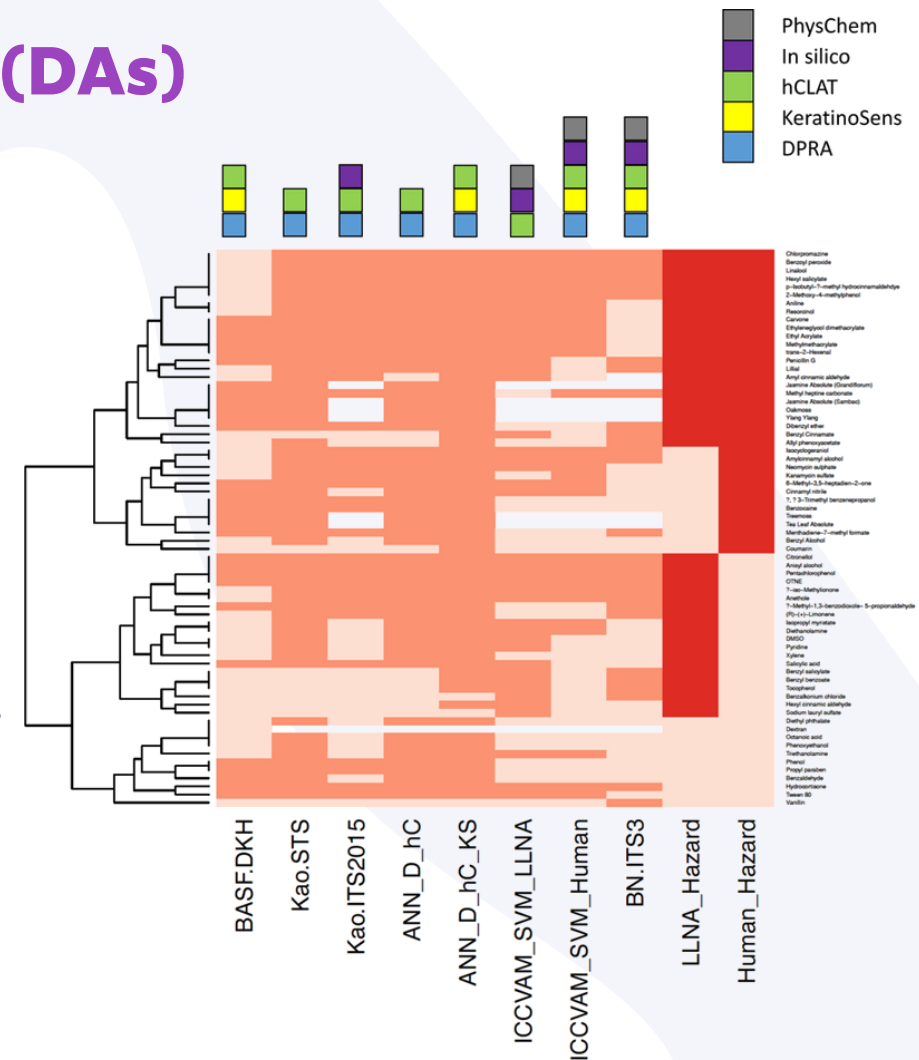
OECD

2018

- **Subset of Skin Allergy DAs evaluated by NICEATM in partnership using Cosmetics Europe database**
  - Hoffman et al. 2018. *Crit. Rev. Toxicol.* **48.** 344-358
  - Kleinstreuer et al. 2018. *Crit. Rev. Toxicol.* **48. 359-374** – see Figure 1, right

NIH NIEHS

Cosmetics Europe
the personal care association

Legend:
- PhysChem
- In silico
- hCLAT
- KeratinoSens
- DPRA

Columns: BASF.DKH, Kao.STS, Kao.ITS2015, ANN_D_hC, ANN_D_hC_KS, ICCVAM_SVM_LLNA, ICCVAM_SVM_Human, BN.ITS3, LLNA_Hazard, Human_Hazard

2021

Guideline No. 497: Defined Approaches on Skin Sensitisation

A Defined Approach (DA) consists of a selection of information sources (e.g in silico predictions, in chemico, in vitro data) used in a specific combination, and resulting data are interpreted using a fixed data interpretation procedure (DIP) (e.g. a mathematical, rule-based model). DAs use methods in combination and are intended to overcome some limitations of the individual, stand-alone methods. The first three DAs included in this Guideline use combinations of OECD validated in chemico and in vitro test data, in some cases along with in silico information, to come to a rules-based conclusion on potential dermal sensitisation hazard. The DAs included in this Guideline have shown to either provide the same level of information or be more informative than the murine Local Lymph Node Assay (LLNA; OECD TG 429) for hazard identification (i.e. sensitiser versus non-sensitiser). In addition, two of the DAs provide information for sensitisation potency categorisation that is equivalent to the potency categorisation information provided by the LLNA. ∧ Less

Published on June 22, 2021   Also available in: French

In series: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects   (view more titles)

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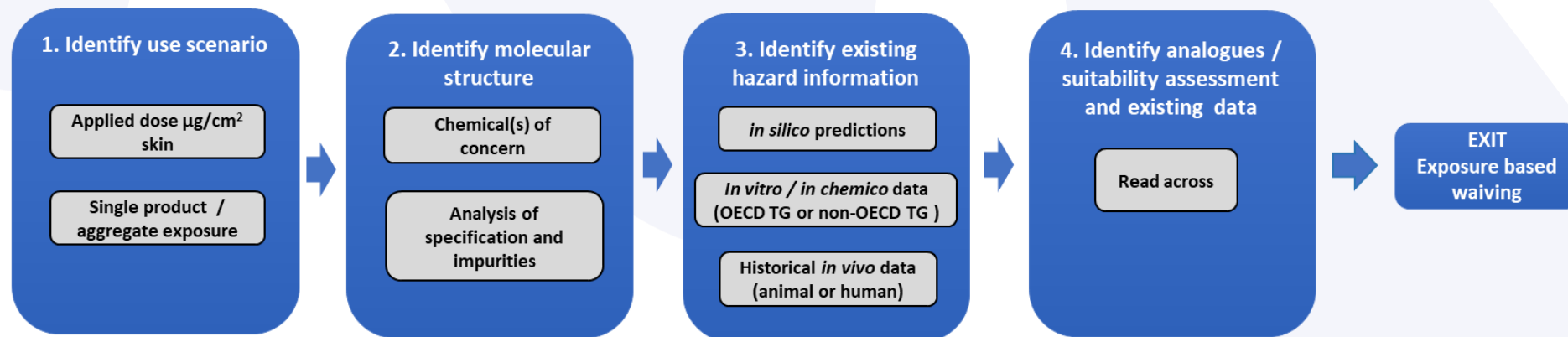
Guideline No. 497: Defined Approaches on Skin Sensitisation | en | OECD
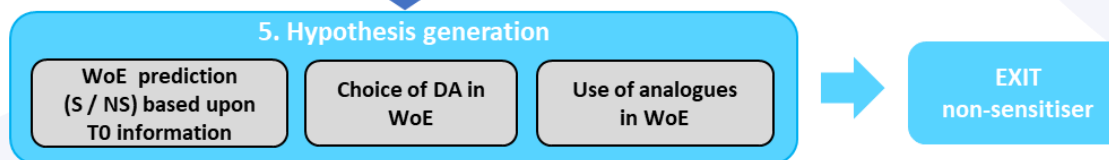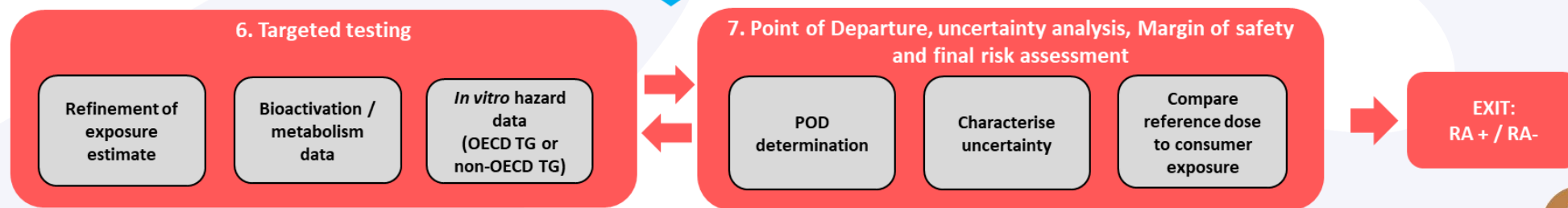
NGRA for Skin Sensitisation IATA framework

2020

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-

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

Unilever

Cosmetics Europe
the personal care association

OECD IATA case study project

- **OECD Series on Testing and Assessment No. 368**: 'Case Study on the Use of Integrated Approaches for Testing and Assessment for skin sensitisation: Demonstrating the Next Generation Risk Assessment Framework using Geraniol'

- These collated IATA explore:
  - applicability of **tiered NGRA framework to assess skin allergy risk** from consumer exposure to geraniol at 0.1% in face cream

  - **direct comparison of five skin allergy DA**:
    - Integrated Testing Strategy (ITS) v1
    - Artificial Neural Network (ANN)
    - Sequential Testing Strategy (STS)
    - Bayesian Network ITS (BN-ITS)
    - **Skin Allergy Risk Assessment (SARA)**

- Geraniol case studies were prepared & submitted to OECD IATA case study project by **Cosmetics Europe Long Range Science Strategy (CE LRSS) Skin Tolerance taskforce** following EU SCCS & EPAA NAM User Forum discussions

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)32

Unclassified

English - Or. English
1 September 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case Study on the Use of Integrated Approaches for Testing and Assessment for skin sensitisation: Demonstrating the Next Generation Risk Assessment Framework using Geraniol

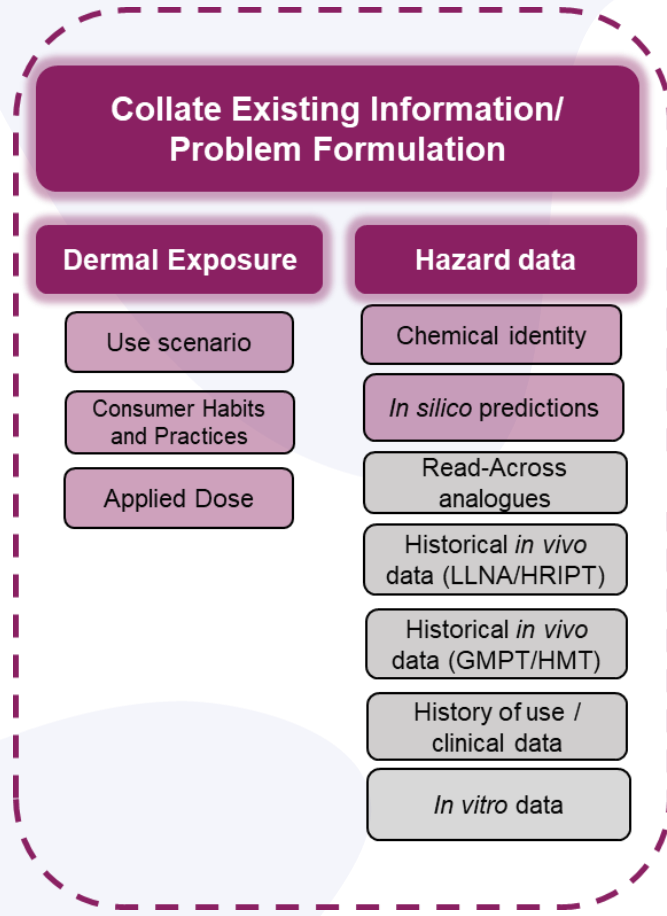Series on Testing and Assessment
No. 368

NGRA for Skin Allergy: use of Geraniol at 0.02% in face cream

For the purposes of the case study, *in vivo* data and read-across were not used, and and the exposure was too high to apply exposure-based waiving.
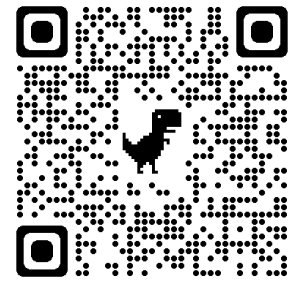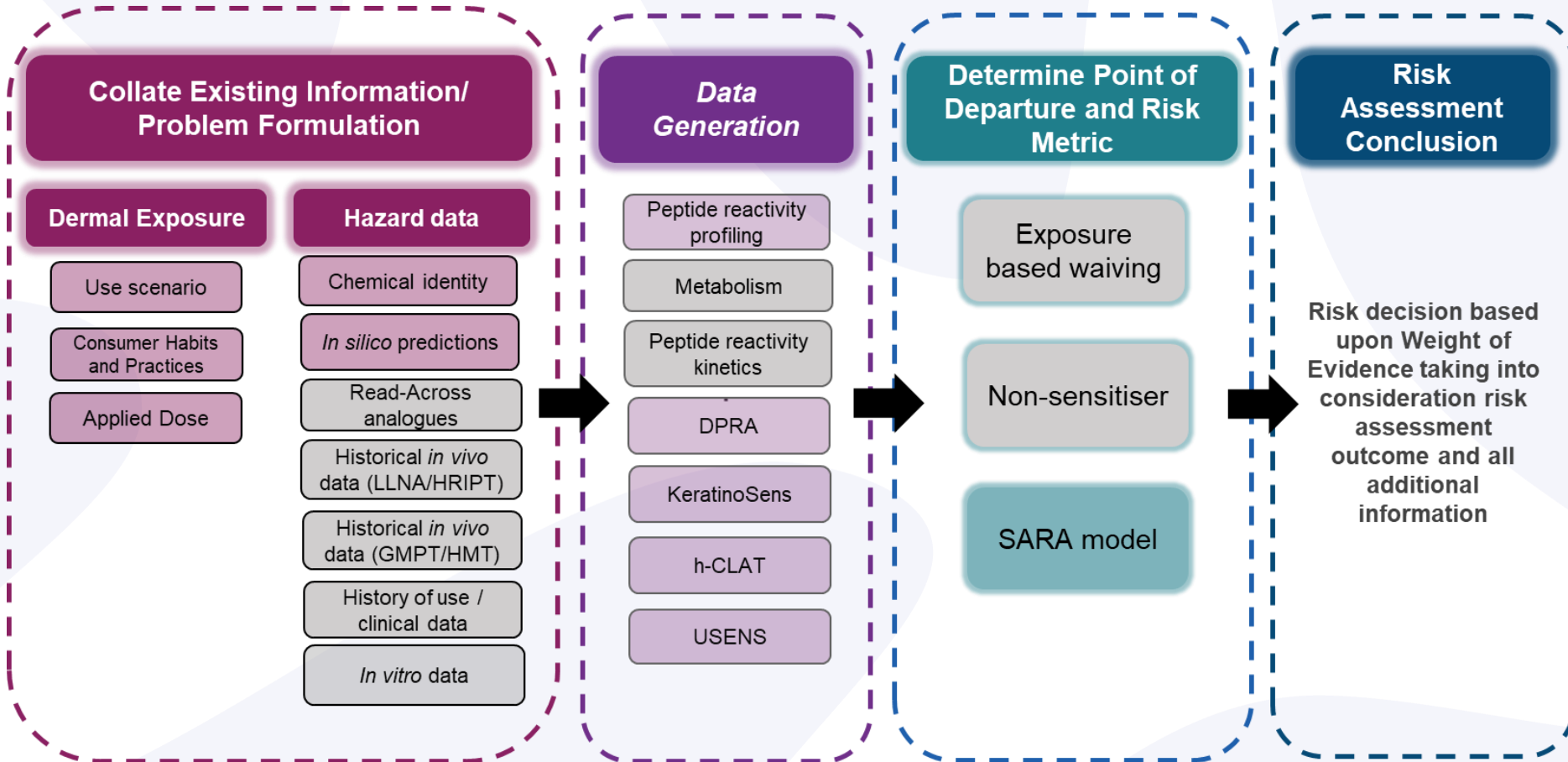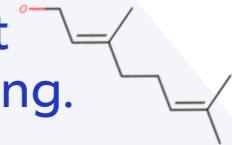
Collate Existing Information & Problem Formulation

Product type	Face cream
Product used per day (90th percentile) (g/day)	1.54
Ingredient inclusion level (%)	0.02
Skin surface area face (cm²)	565
Leave-on or Rinse-off	Leave-on
Local dermal exposure (µg/cm²)	0.544

Collate Existing Information/ Problem Formulation

Dermal Exposure
- Use scenario
- Consumer Habits and Practices
- Applied Dose

Hazard data
- Chemical identity
- *In silico* predictions
- Read-Across analogues
- Historical *in vivo* data (LLNA/HRIPT)
- Historical *in vivo* data (GMPT/HMT)
- History of use / clinical data
- *In vitro* data

DEREK NEXUS v.2.4	**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 α,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 predicted to become activated via auto-oxidation to reactive molecules Schiff base adducts.** Confidence in this prediction is high based upon chemical prediction consensus from all applied *in silico* tools.

- Peptide reactivity profiling, DPRA, KeratinoSens™, h-CLAT and U-SENS™ data should be generated to test this hypothesis & allow a sensitiser potency prediction to be made

Skin Allergy Bioactivity
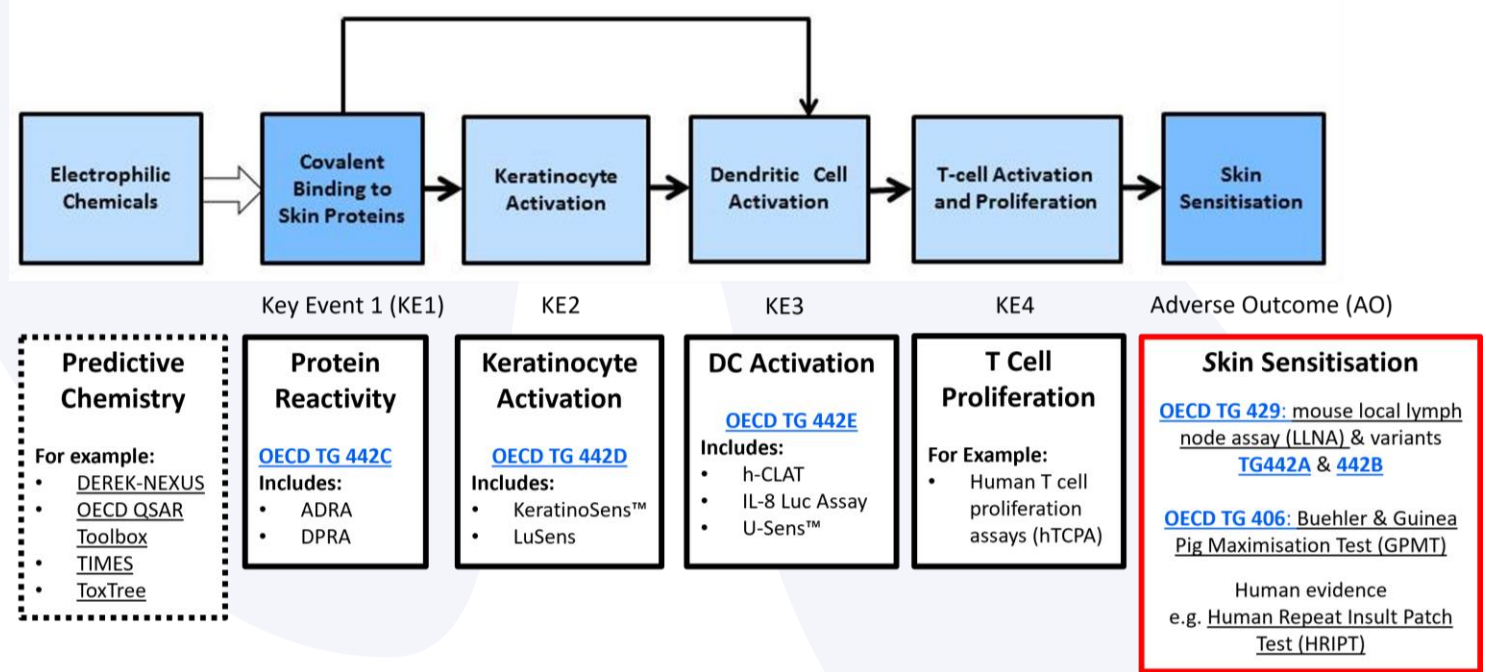

Data Generation

- Peptide reactivity profiling
- Metabolism
- Peptide reactivity kinetics
- DPRA
- KeratinoSens
- h-CLAT
- USENS

Reactivity Profiling	DPRA	KeratinoSens™	H-CLAT	U-SENS™
Cys (no adducts, 73.7 ± 0.8%)	**Negative**	**Positive**	**Positive**	**Positive**
Lys (no adducts, 3.5 ± 0.6%)	Cys depletion 0%	$EC_{1.5}$ 110 µM	CD86 EC_{150} 123 µg ml^{-1}	CD86 EC_{150} 53.6 µg ml^{-1}
His (no adducts, -11.1 ± 8.0%)	Lys depletion 10%	EC_3 >2000 µM	CD54 EC_{200} - µg ml^{-1}	CV$_{70}$ 113.9 µg ml^{-1}
Arg (double Schiff base, 15.2 ± 0.2%)		IC_{50} 875 µM	CV$_{75}$ 140 µg ml^{-1}	
Tyr (no adducts, 8.2 ± 3.7%)				
N-term (acylation, Schiff base, 40.2 ± 1.1%)				
Ala (no adducts, -2.1 ± 17.0%)				

- **Geraniol confirmed to be reactive** (Schiff base formation following autoxidation) **with sensitiser potential also detected in KeratinoSens™, h-CLAT & U-SENS™**

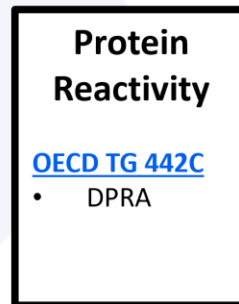# Skin Allergy Risk Assessment (SARA) Defined Approach

- **SARA DA is Bayesian probabilistic model that estimates human sensitiser potency** using data covering AOP KEs 1-3, adverse outcome & risk benchmarks

Ongoing collaboration with NICEATM to expand SARA database and adapt DA to predict GHS categories & human PoD
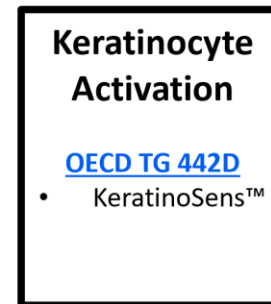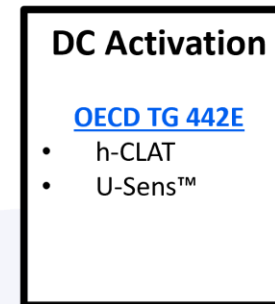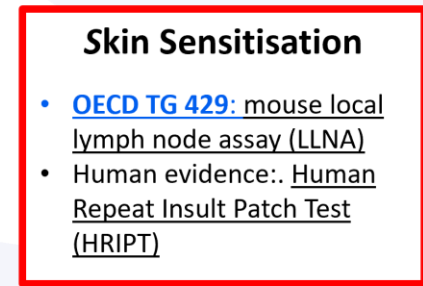
- SARA-ICE DA under OECD DASS EG evaluation

Determine Point of Departure and Risk Metric

- Exposure based waiving
- Non-sensitiser
- SARA model

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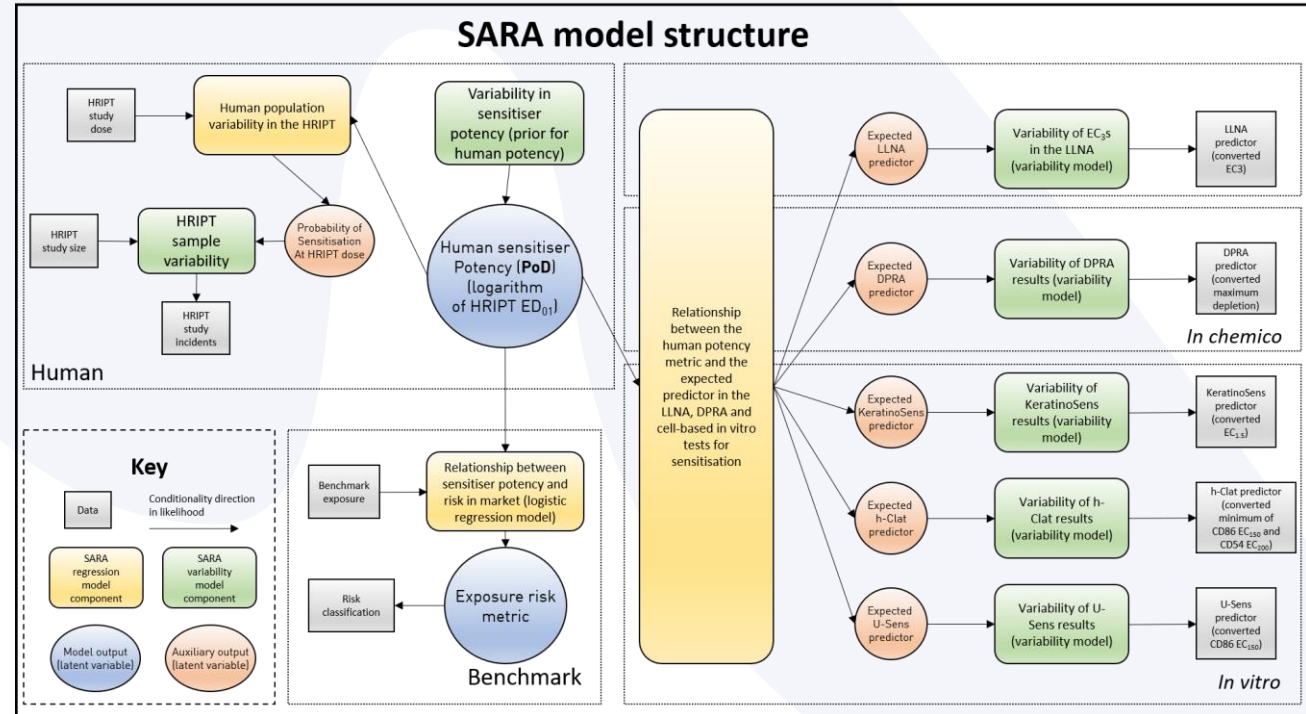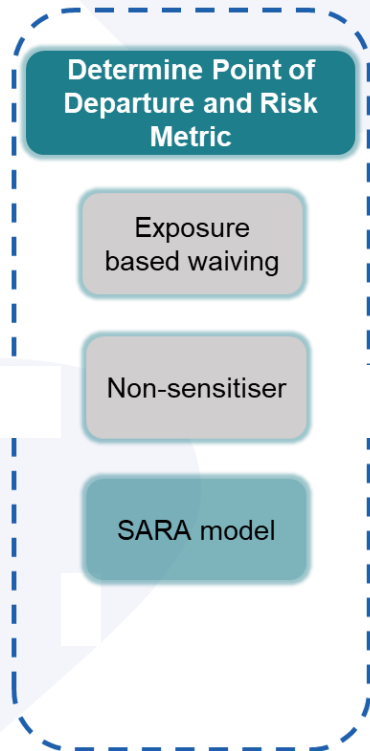
Key Event 1 (KE1)	KE2	KE3	Adverse Outcome (AO)
Protein Reactivity	**Keratinocyte Activation**	**DC Activation**	*Skin Sensitisation*
OECD TG 442C	**OECD TG 442D**	**OECD TG 442E**	• **OECD TG 429**: mouse local lymph node assay (LLNA)
• DPRA	• KeratinoSens™	• h-CLAT	• Human evidence:. Human Repeat Insult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

Reynolds et al. (2022). Reg. Tox. Pharmacol.**134**. 105219

NGRA Skin Allergy: SARA DA human PoD prediction for Geraniol

Determine Point of Departure and Risk Metric

Exposure based waiving

Non-sensitiser

SARA model

- **DPRA, KeratinoSens™, hCLAT and USens™ data were used as SARA DA inputs 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 based upon LLNA data is reported to be of **moderate potency**).

SARA potency

MCl/MI
Phenylenediamine
Benzoquinone
Trans-2-hexenal
Methyl octyne carbonate
Benzisothiazolinone
Metol
Methylisothiazolinone
Lauryl gallate
Diethyl maleate
MDBGN
Formaldehyde in vivo
Formaldehyde in vivo & NAM
2-Hexylidenecyclopentanone
2-Phenylpropanal
Isoeugenol
3-Phenylenediamine
Cinnamic aldehyde
Dihydrocoumarin
Propyl gallate
Glutaraldehyde
Alpha damascone
Ethylene diamine
Citral
Benzylidene acetone
PBC
Ethyl acrylate
Delta damascone
Trans beta damascone
Perillaldehyde
Methyl heptine carbonate
Formaldehyde NAM (no DPRA)
Vetiveryl acetate
Formaldehyde NAM
3-Phenylpropanal
Citronellol
Cinnamonitrile
3-Aminophenol
Phenylacetaldehyde
Cinammic alcohol
Hydroxycitronellal
Imidazolidinyl urea
Farnesal
Carvone
Ylang ylang extract
Isocyclogeraniol
4-Allylanisole
Geraniol NAM (no DPRA)
Sodium lauryl sulphate
Geraniol NAM
Eugenol
Limonene
Phenyl benzoate
Salicylic acid
Benzyl benzoate
Coumarin
Amyl cinnamic alcohol
Geraniol in vivo & NAM
Amyl cinnamic aldehyde
Cyclamen aldehyde
Farnesol
Methyl salicylate
Galbanone
Geraniol in vivo
Benzyl cinnamate
HCC
Benzyl salicylate
Hexylcinnamaldehyde
Dimethyl isophthalate
Hexyl salicylate
Linalool
Propyl paraben
Vanillin
BMHCA
Majantol
6-Methylcoumarin
4-Methoxyacetophenone
p-Aminobenzoic acid
OTNE
Phenoxyethanol
Isopropanol
Sodium benzoate
Butanol
Glycerol
Benzyl alcohol
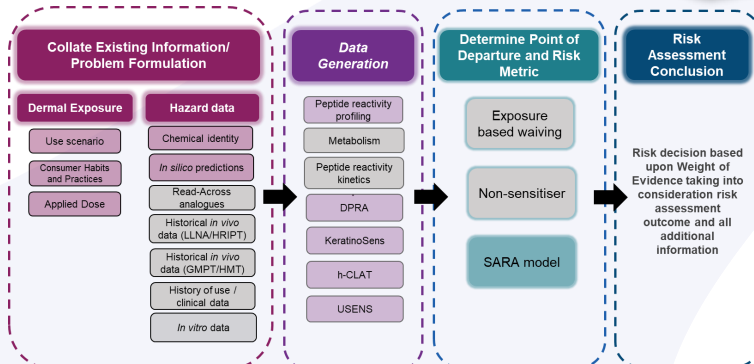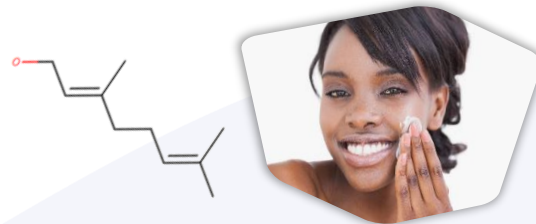Propylene glycol

ED_{01} (μg cm^{-2})

NGRA Skin Allergy: Geraniol case study conclusion

Risk Assessment Conclusion

Risk decision based upon Weight of Evidence taking into consideration risk assessment outcome and all additional information

- **Margin of Exposure (MoE) calculated using SARA DA** from ED_{01} for geraniol & dermal exposure for 0.02% geraniol in a face cream:
  - **ranks with low-risk benchmarks**
  - **95% probability this exposure is low risk**

SARA probability exposure is "low risk"

Workflow diagram:
- Collate Existing Information/ Problem Formulation
  - Dermal Exposure: Use scenario, Consumer Habits and Practices, Applied Dose
  - Hazard data: Chemical identity, *In silico* predictions, Read-Across analogues, Historical *in vivo* data (LLNA/HRIPT), Historical *in vivo* data (GMPT/HMT), History of use / clinical data, *In vitro* data
- *Data Generation*: Peptide reactivity profiling, Metabolism, Peptide reactivity kinetics, DPRA, KeratinoSens, h-CLAT, USENS
- Determine Point of Departure and Risk Metric: Exposure based waiving, Non-sensitiser, SARA model
- Risk Assessment Conclusion: Risk decision based upon Weight of Evidence taking into consideration risk assessment outcome and all additional information

OECD IATA project website: the definitive repository of case studies exploring use of NAM for chemical safety assessment
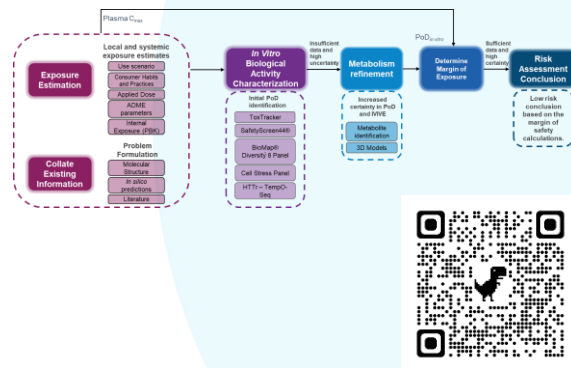
Conclusions:

1. **A paradigm shift is underway** as use of NAMs & NGRA for chemical safety assessment become widespread

2. **We can accelerate this transition** through increased scientific dialogue, collaboration & training

3. **OECD IATA activities help** through fostering knowledge exchange & harmonising best practice
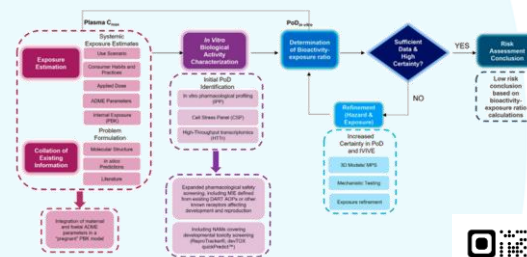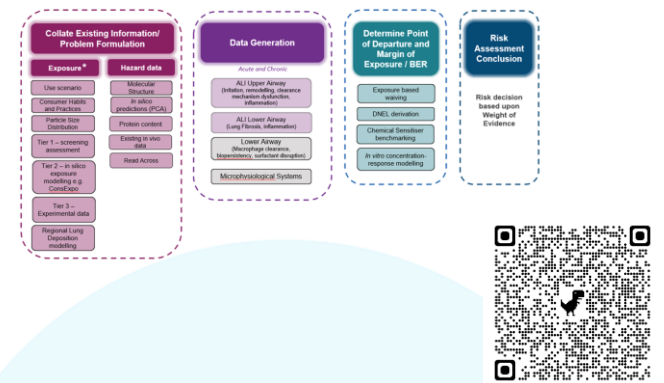
Next Steps:

Systemic

Baltazar et al (2020) *Toxicol Sci*, 176, 236-252

Developmental & Repro.

Rajagopal et al (2022) *Frontiers in Toxicology*, doi: 10.3389/ftox.2022.838466

Inhalation

Dr Maria Baltazar : Development of a NGRA framework for inhalation safety of consumer products, SOT 2022

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Collaborators:

TT21c.org: