Abstract

This talk contrasts two Next Generation Risk Assessment (NGRA) case studies as examples of how to structure Integrated Approaches to Testing and Assessment (IATA) in the presence and absence of a specific mechanism/relevant AOP. Two examples are discussed: 1) An example of application of the AOP on 'Covalent Protein Binding leading to Skin Sensitization' (https://aopwiki.org/aops/40) through the development of defined approaches (DA) and IATA to structure a NGRA for Skin Sensitization assessing inclusion of 0.1% of Coumarin in a face cream (Reynolds et al. 2021); 2) A NGRA for use of the preservative ingredient phenoxyethanol in body lotion at 1% inclusion level demonstrating a non-animal systemic toxicity IATA for a chemical with 'no defined biological target or pathway' where a decision can be reached without a specific mechanism or AOP being identified (ENV/CBC/MONO(2021)35).

Structuring Integrated Approaches to Testing and Assessment (IATA) in the presence and absence of a specific mechanism/relevant AOP

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Conflict of Interest Statement

I am employed by Unilever and this presentation covers many approaches which have either been developed by Unilever or using Unilever funding for the purpose of assessing the safety of Unilever products.

Abbreviations

- **BER**: Bioactivity Exposure Ratio
- **DA:** Defined Approach
- **IATA:** Integrated Approaches to Testing and Assessment
- **MoE/S**: Margin of Exposure/Safety
- **NAM**: New Approach Methodology
- **NGRA**: Next Generation Risk Assessment
- **NOTEL:** No Observed Transcriptional Effect Level
- **PAA:** Phenoxyacetic acid
- **PE:** Phenoxyethanol
- **PoD**: Point of Departure
- **SARA Model:** Skin Allergy Risk Assessment Model

Objective

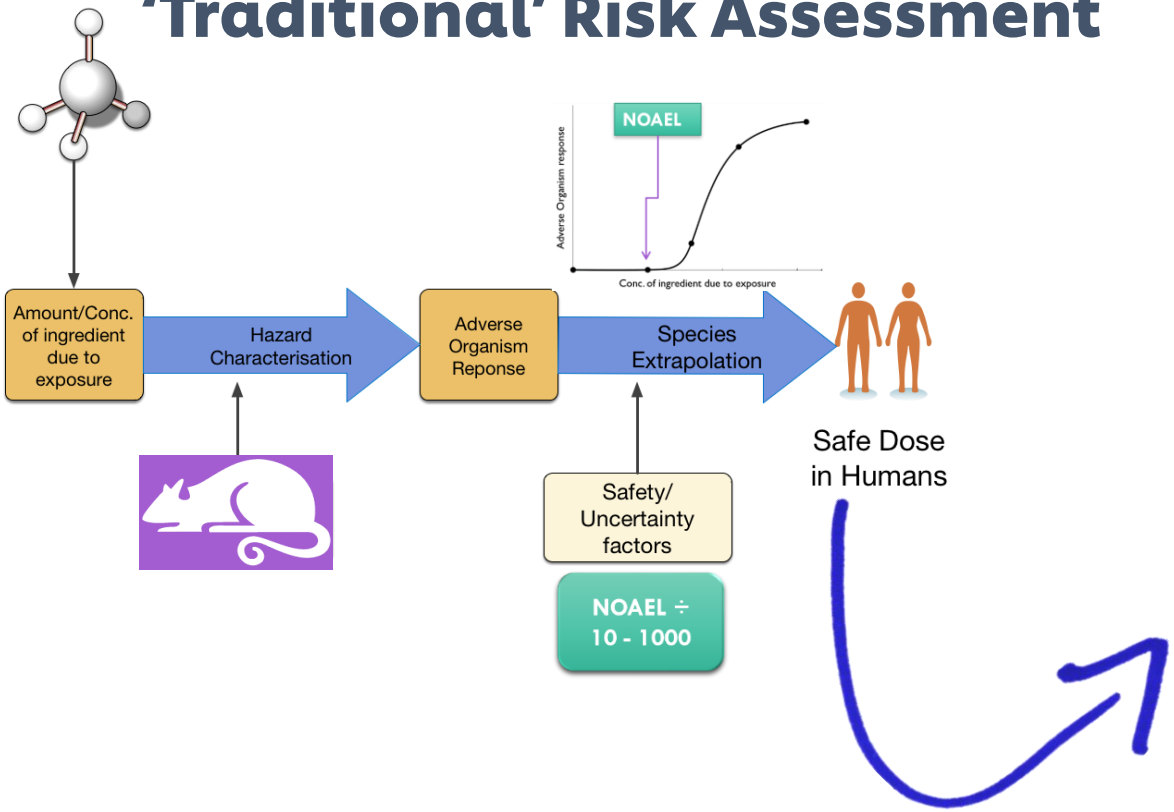
Demonstrate how to structure Integrated Approaches to Testing and Assessment (IATA) in the presence and absence of a specific mechanism/relevant AOP using Next Generation Risk Assessment (NGRA) case studies as examples.

Next Generation Risk Assessment (NGRA)

NGRA is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates New Approach Methodologies (NAMs) to assure safety without the use of animal testing.

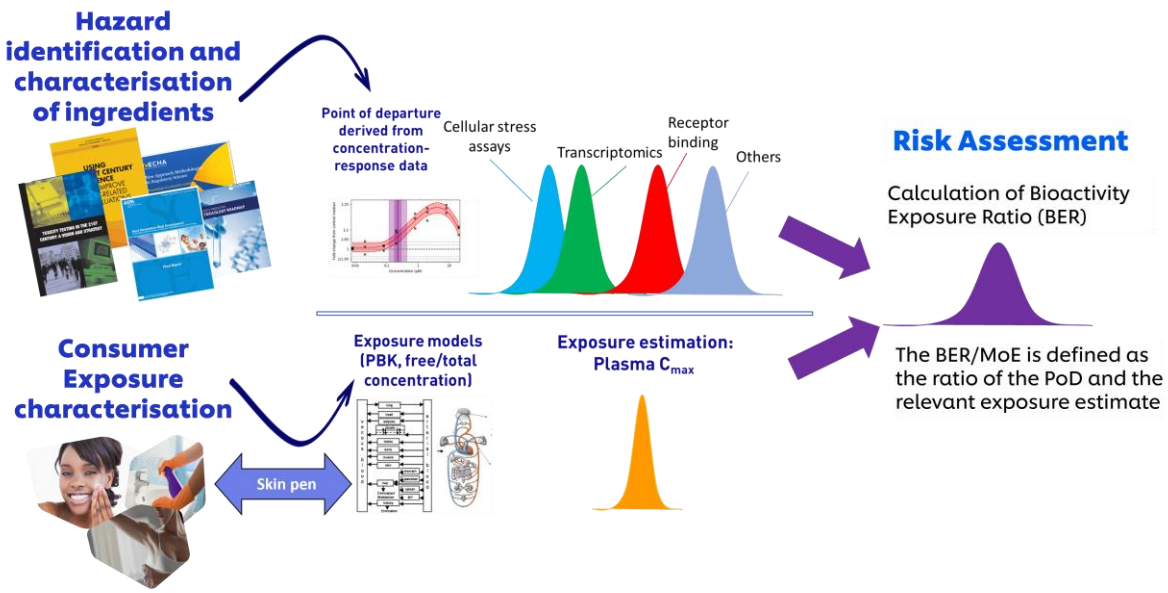
2007 2021

'Traditional' Risk Assessment



'Next Generation' Risk Assessment

'Protection not prediction'



Next Generation Risk Assessment (NGRA)

ICCR Principles of NGRA (Dent et al., 2018)

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

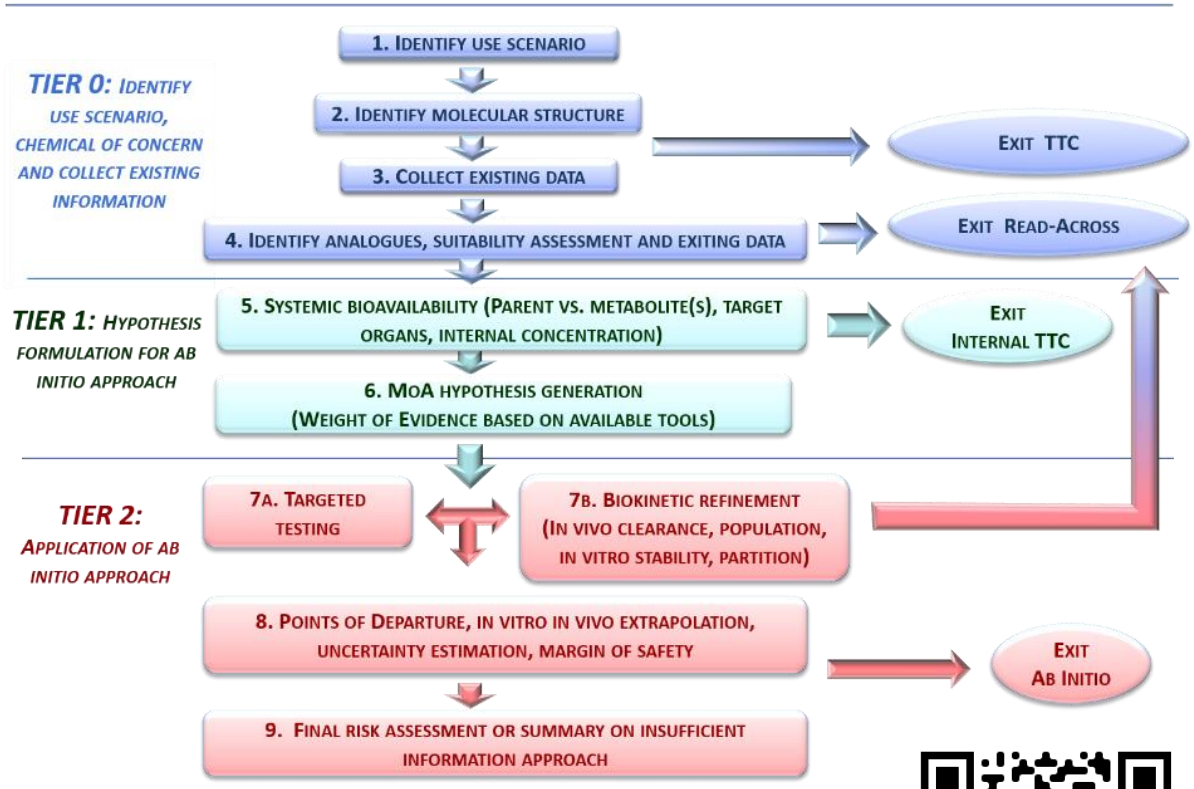
Principles describe how a NGRA should be conducted:

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

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

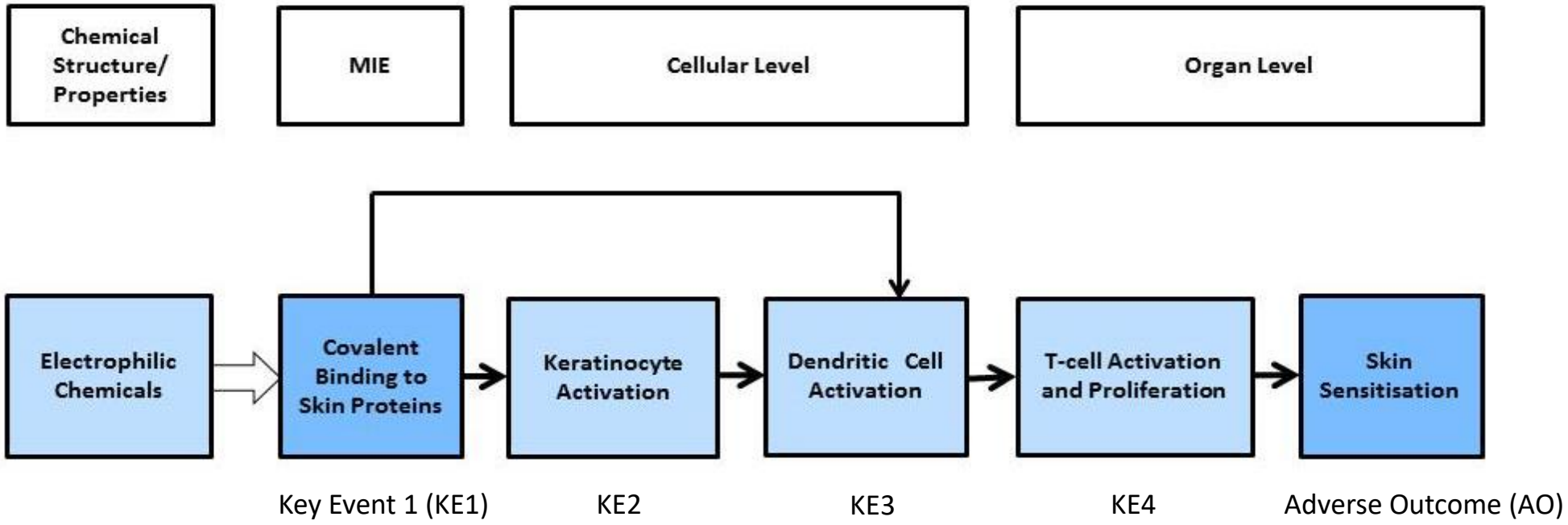


SEURAT-1 Workflow for the safety assessment of chemicals without animal testing (Berggren et al., 2017) (CC BY license http://creativecommons.org/licenses/by/4.0/)

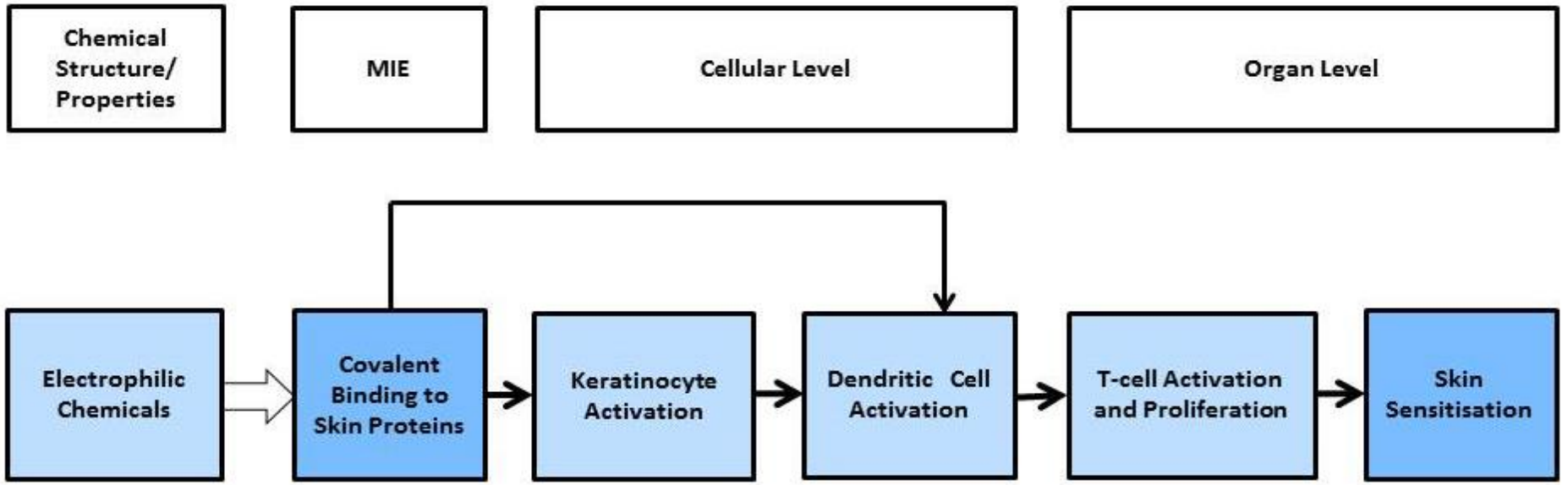
Case Study 1: Skin Allergy

Objective 1: Demonstrate how to structure Integrated Approaches to Testing and Assessment (IATA) in the **presence** of a relevant AOP using a Next Generation Risk Assessment (NGRA) case study

Skin Sensitisation AOP



Skin Sensitisation AOP



Predictive Chemistry

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

Protein Reactivity

OECD TG 442C

Includes:
- ADRA
- DPRA
- kDPRA

Keratinocyte Activation

OECD TG 442D

Includes:
- KeratinoSens™
- LuSens

DC Activation

OECD TG 442E

Includes:
- h-CLAT
- IL-8 Luc Assay
- U-Sens™
- GARD™skin

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

Defined Approaches and Integrated Approaches to Testing and Assessment

- Individual NAMs cannot be used as stand-alone replacements of animal data to conclude on skin sensitisation potential of chemicals or to provide information for potency for point of departure-based risk assessment or sub-categorisation (1A and 1B) according to UN GHS.

- Data generated with NAMs addressing multiple KEs of the skin sensitisation AOP can be used in combination in defined approaches (DAs) as well as with other weight of evidence information sources within integrated approaches to testing and assessment (IATA).

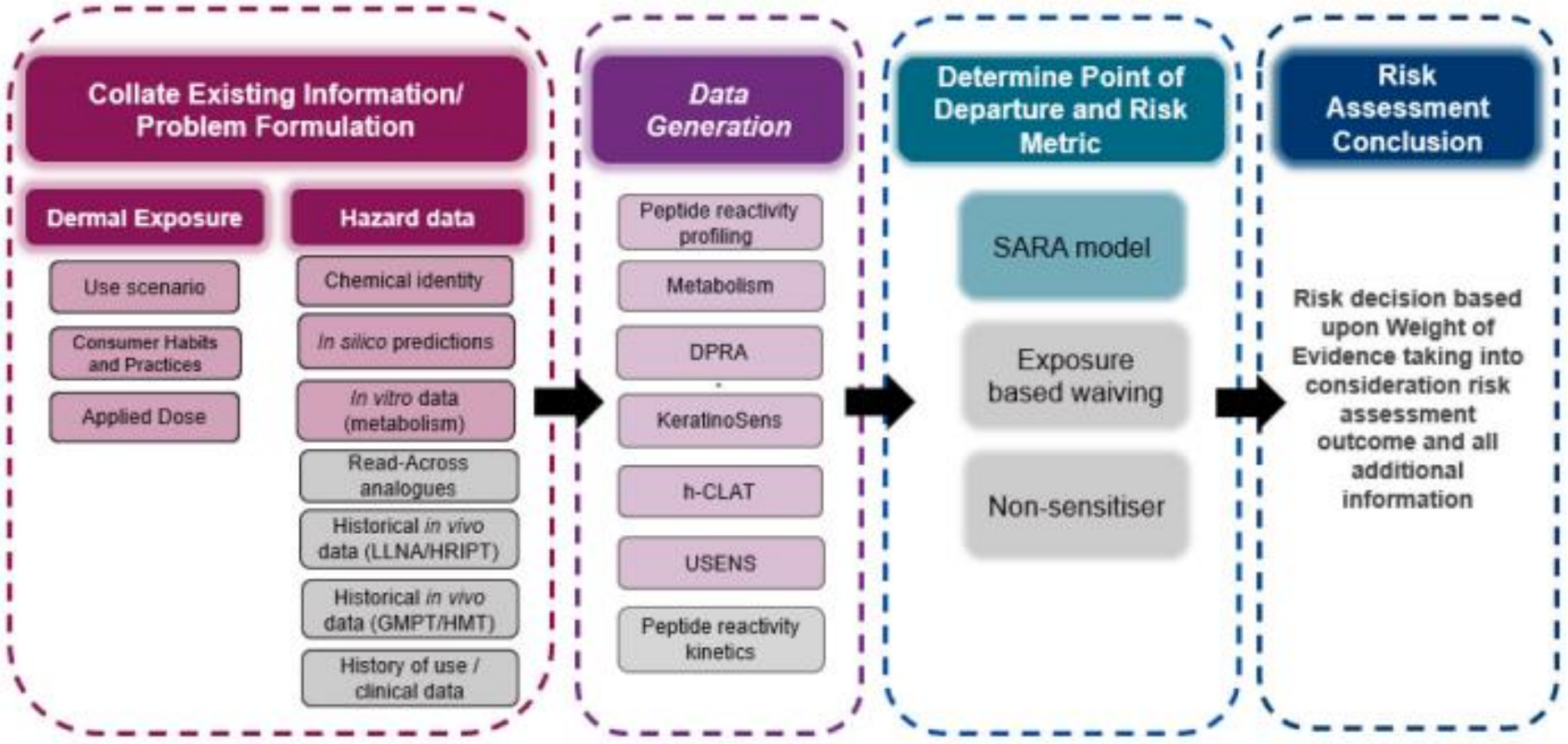
Defined Approaches and Integrated Approaches to Testing and Assessment

Guideline No. 497 Guideline on Defined Approaches for 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)…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.'*

Case Study on the Use of Integrated Approaches for Testing and Assessment for skin sensitisation: Demonstrating the Next Generation Risk Assessment Framework using Geraniol (OECD Series on Testing and Assessment No. 368). *This illustrative IATA aims to demonstrate the applicability of a tiered NGRA framework to assess the potential risk from consumer exposure to geraniol at 0.1% via a face cream.*

Application of NGRA framework for Skin Allergy

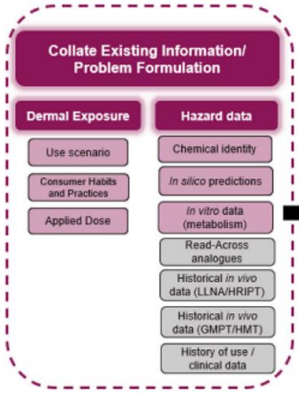
This NGRA framework is applied to a hypothetical skin allergy assessment of a consumer product at two exposures - 0.1% coumarin in a face cream and 1% in a non-spray deodorant.



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 G et al., 2021

Local Exposure

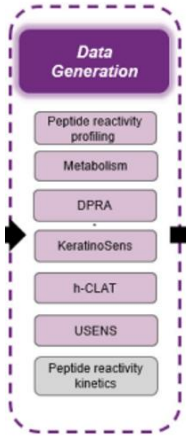


Product type	Face cream	Deodorant
Product used per day (90th percentile) (g/day)	1.54	1.5
Ingredient inclusion level (%)	0.1	1
Skin surface area (face / axilla) (cm^2)	565	200
Leave-on or Rinse-off	Leave-on	Leave-on
Local dermal exposure (µg/cm^2)	**2.7**	**75**

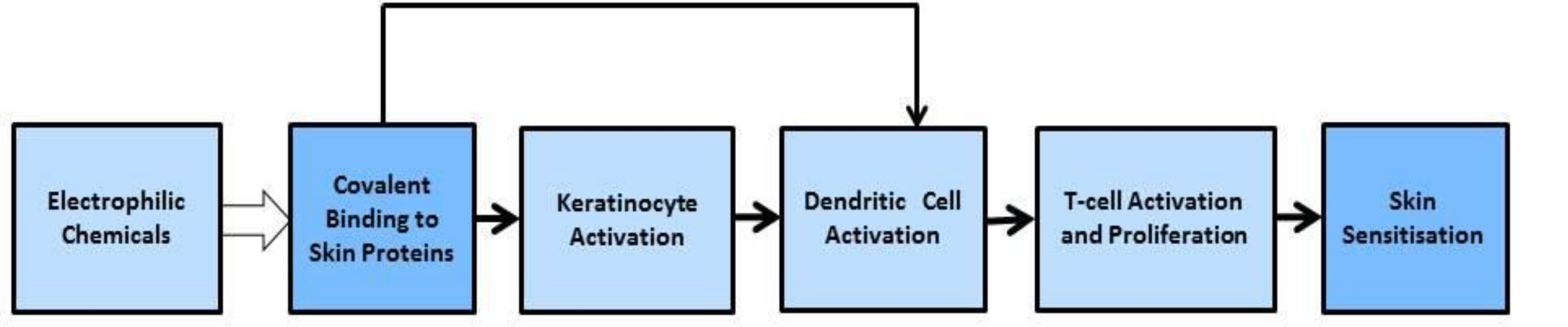
Existing Information

In silico predictive chemistry: **TIMES-SS** reported coumarin to be a **non-sensitiser** / **DEREK** Nexus predicted coumarin to be a **weak sensitiser** / **ToxTree** and **OECD QSAR Toolbox** predicted coumarin to have skin sensitisation potential (**protein binding alerts**).

Data Generation



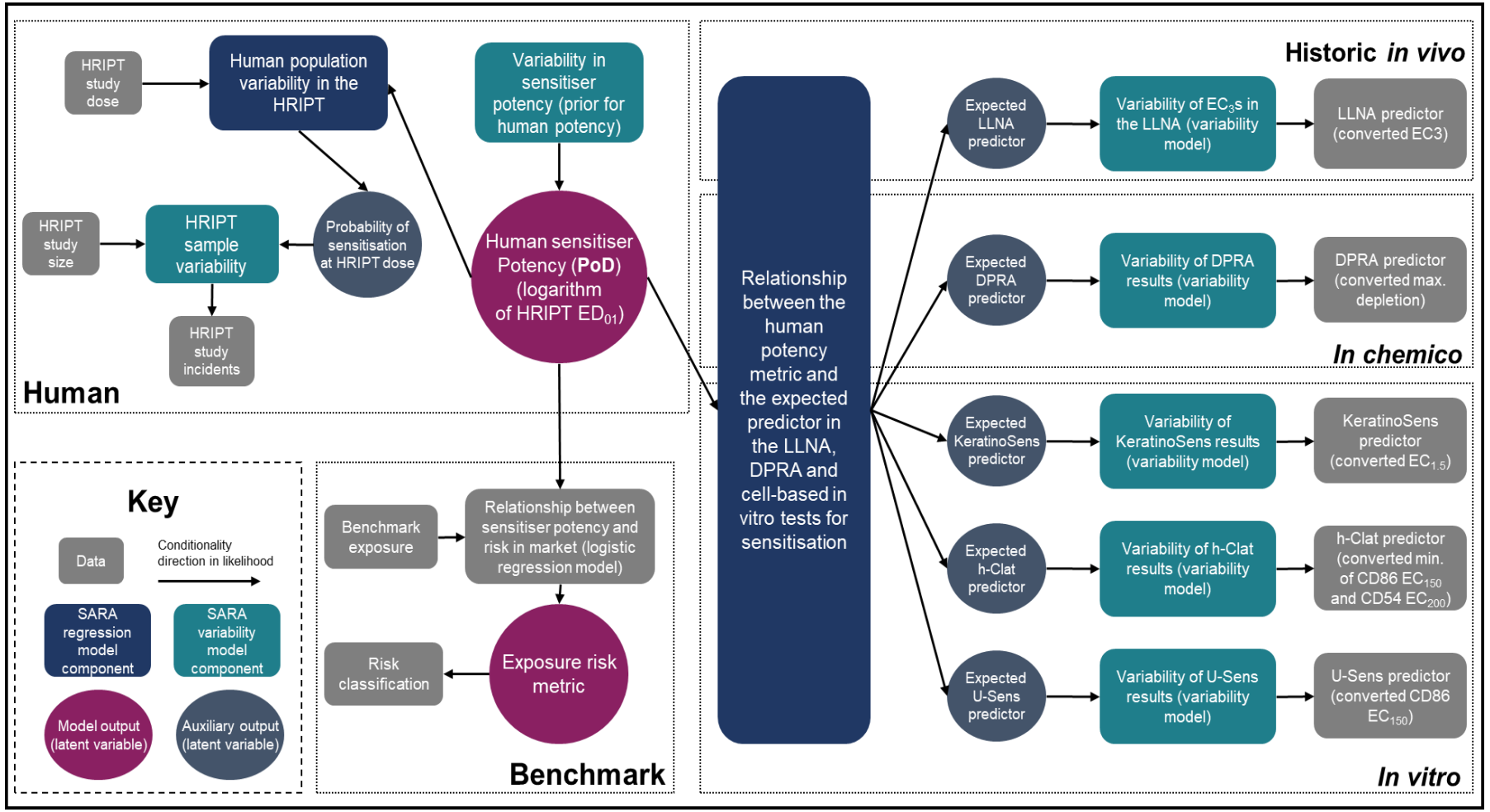
	DPRA (TG442C)		KeratinoSens™ (TG 442D)	h-CLAT (TG 442E)		U-SENS™ (TG 442E)
	%cys depl.	%lys depl.	EC1.5 (µM)	CD86 (EC200 µg/mL)	CD54 (EC150 µg/mL)	CD86 (EC150 µg/mL)
Coumarin	1.3	0	187.5	<178	>637	95.5



SARA Model: A Defined Approach

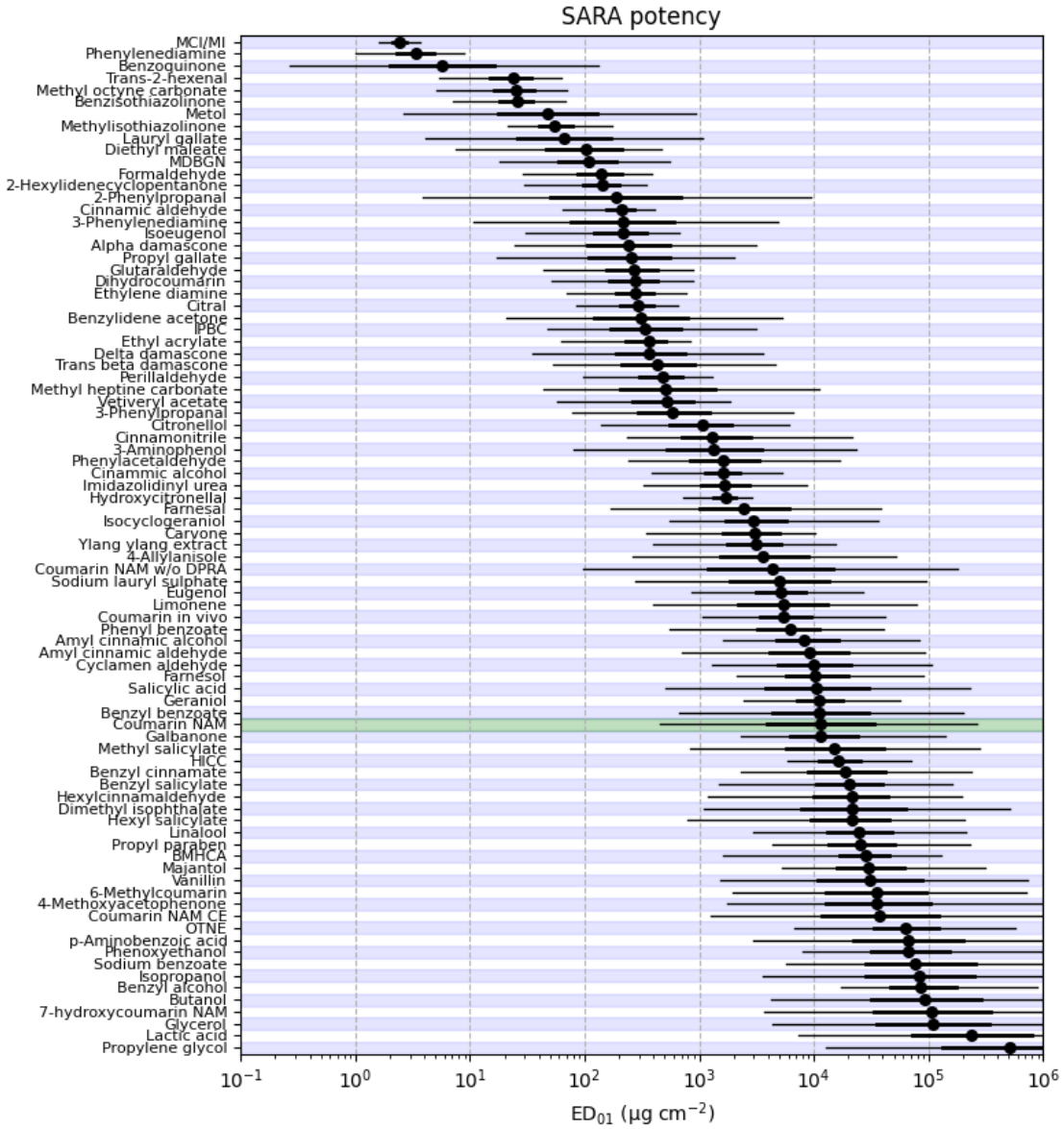
The SARA Model uses Bayesian statistics to infer a probability that a consumer exposure to a chemical can be considered low risk (*SARA risk metric*).

The SARA Model uses a database of 81 chemicals with NAM data covering AOP KEs 1-3, and historic LLNA and HRIPT data for the AOP AO, and accounts for variability within each data type.



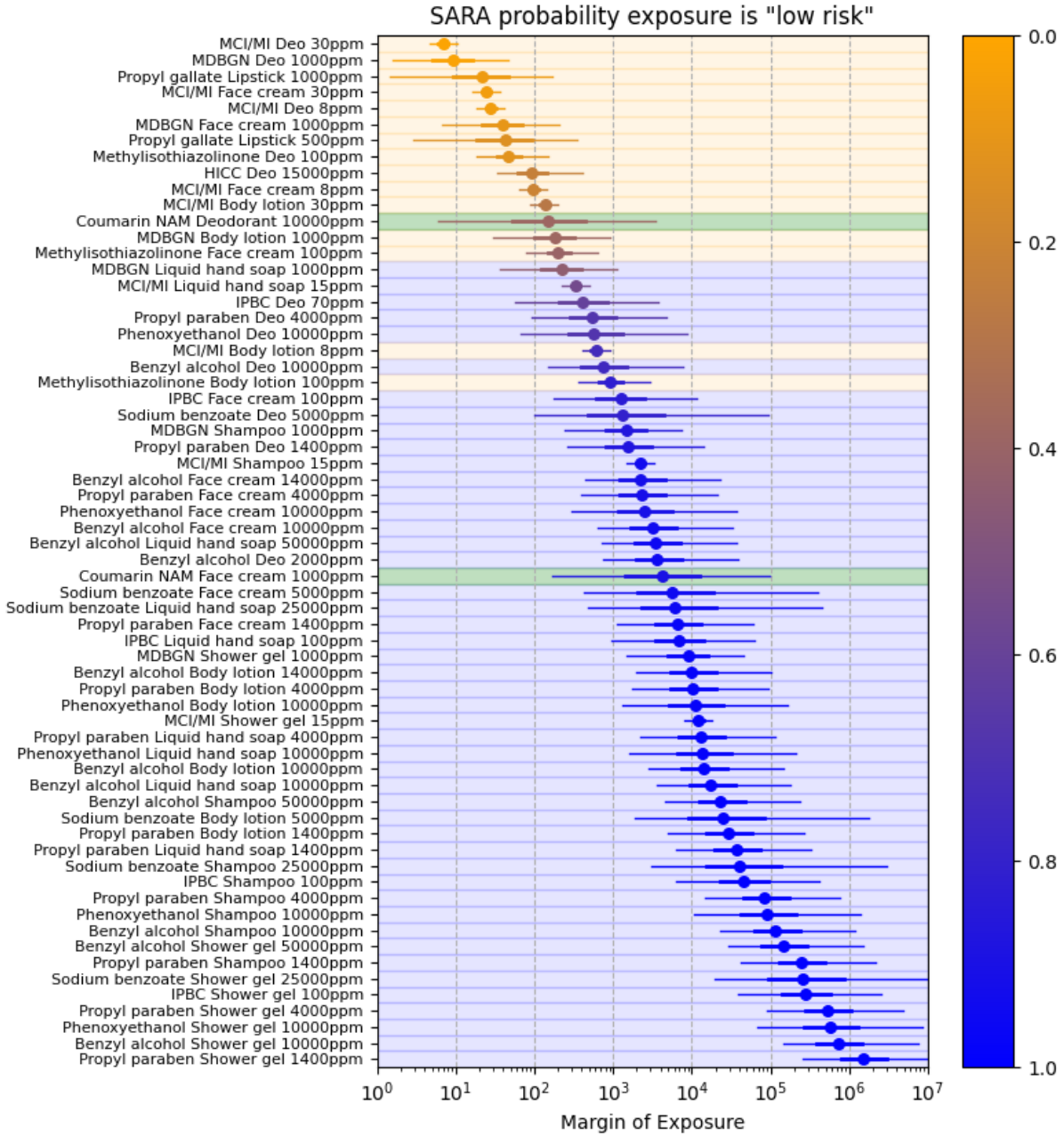
Reynolds J et al., 2022

Determine Point of Departure (PoD) using SARA Model



- 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, with all NAM data, the expected SARA Model derived ED_{01} is 11,000µgcm^{-2}**

Determine Margin of Exposure (MoE)

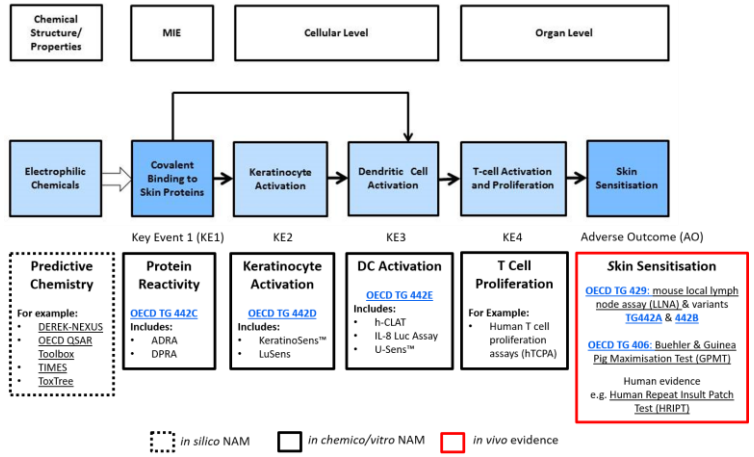


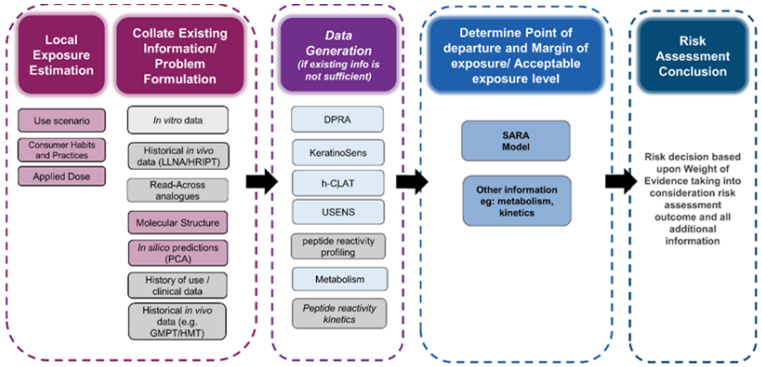
- The **SARA risk metric is 0.90 for the face cream dermal exposure** and **0.39 for the deodorant dermal exposure**.

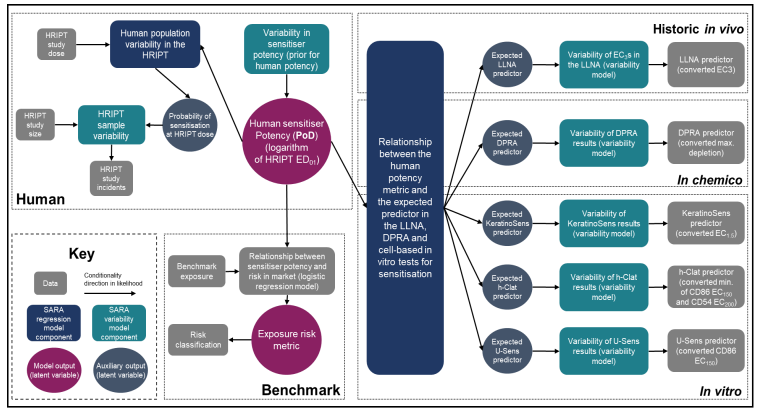
- For the face cream exposure, the SARA model predicted low risk as being the most likely classification. For the deodorant risk assessment, the high risk classification was more certain.

Learnings

- Key events defined for AOPs can be used to structure NAM assay data
- OECD Test Guidelines are now available for many NAMs aligned to the skin sensitisation AOP
- NAMs have been combined into defined approaches and IATA to increase their applicability for use in NGRA
- Bayesian DAs enable experimental data variability to be modelled and uncertainty in PoDs & risk metrics to be factored into decision-making





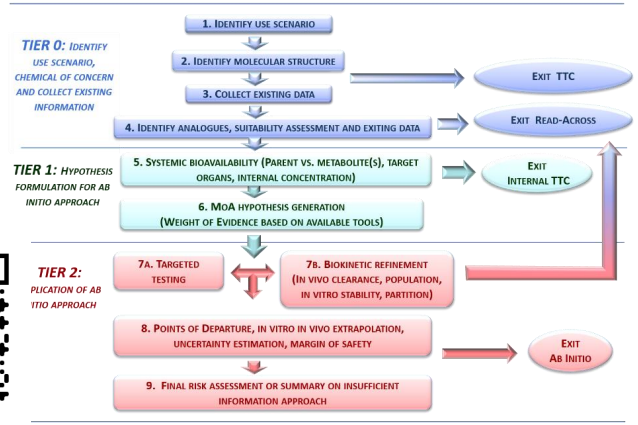


Case Study 2: Phenoxyethanol

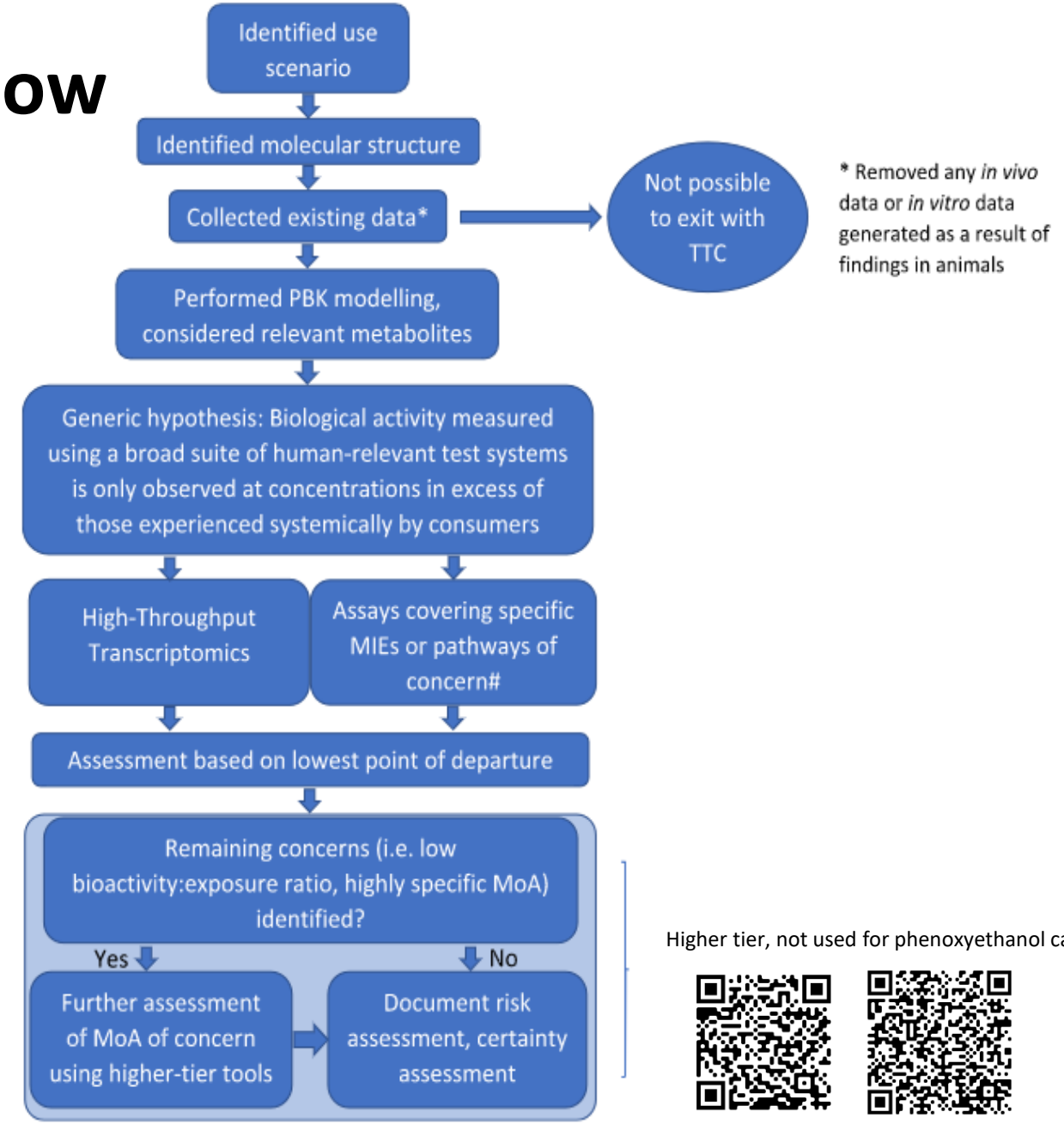
Ab initio Systemic Toxicity NGRA

Demonstrate how to structure Integrated Approaches to Testing and Assessment (IATA) in the **absence** of a specific mechanism/relevant AOP using Next Generation Risk Assessment (NGRA) case studies as examples.

A Tiered NGRA Workflow



SEURAT-1 Workflow for the safety assessment of chemicals without animal testing (Berggren et al., 2017) (CC BY license http://creativecommons.org/licenses/by/4.0/)

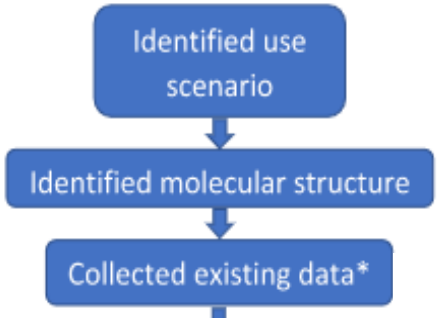


* Removed any *in vivo* data or *in vitro* data generated as a result of findings in animals

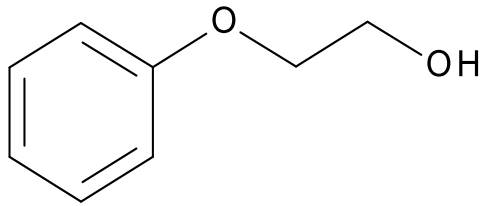
Higher tier, not used for phenoxyethanol case study

Phenoxyethanol NGRA Workflow, based upon SEURAT-1 workflow, published in Dent et al., 2021. Full report OECD Series on Testing and Assessment, No. 349

A Tiered NGRA Workflow



The **use scenario** defined for the case study is 1% in a body lotion. The applied exposure for a 95th percentile consumer is 1.23 mg/kg bw/day (Troutman et al, 2015). Based on this exposure it is not possible to apply the TTC.



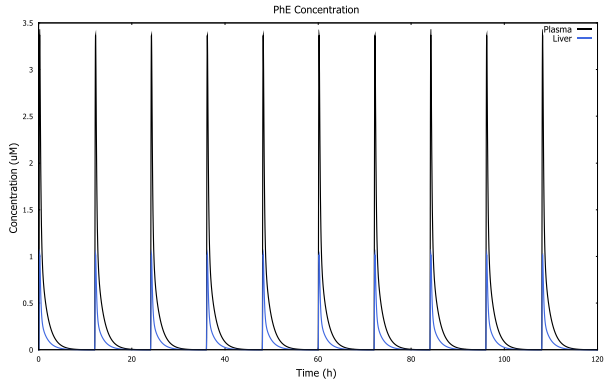
Phenoxyethanol, CAS: 122-99-6
SMILES: c1ccc(cc1)OCCO
Molecular Weight: 138.16

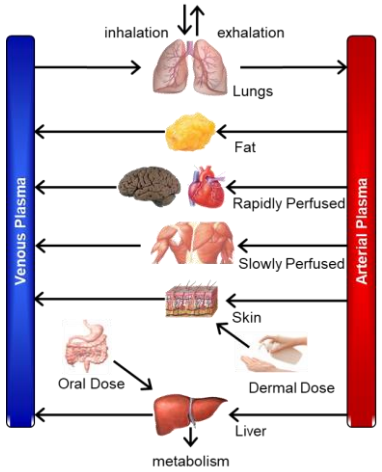
In silico tools to identify possible MoA

- **Derek Nexus (v 5.0.2 Lhasa Ltd)**
  - inactive (negative) in the Ames assay
- **OECD QSAR Toolbox v. 4.1**
  - *in vivo* mutagenicity (micronucleus) in rodents: alert for H-acceptor-path3-H-acceptor
- **CERAPP and CoMPARA**
  - no binding predicted
- **COSMOS profilers**
  - potential binding to Thyroid Hormone Receptor (THR)
- **MIE Atlas** (Allen *et al.* (2018) Tox Sci doi: 10.1093/toxsci/kfy144)
  - no alerts

Core NAM Toolbox

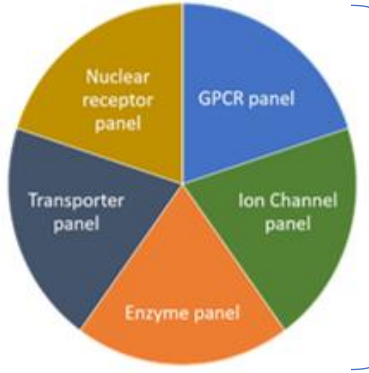
Physiologically Based Kinetics





Moxon et al 2019. Toxicol in vitro 63:104746

In vitro pharmacological profiling



44 targets

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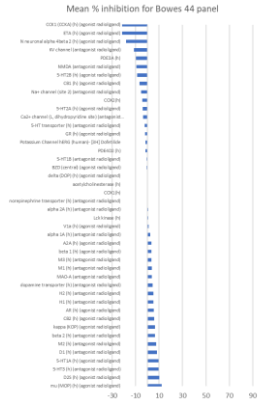
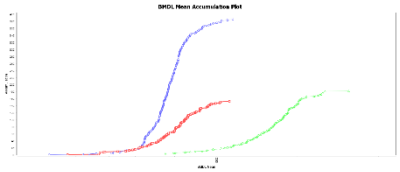
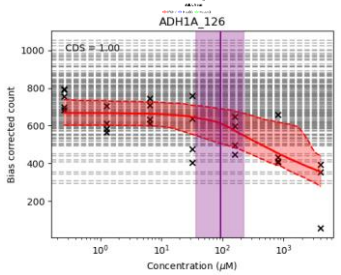


Bowes et al 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- 4 cell models: HepG2, MCF7, HepaRG and aProximate cells
- Dose-response analysis using BMDExpress2 and BIFROST model

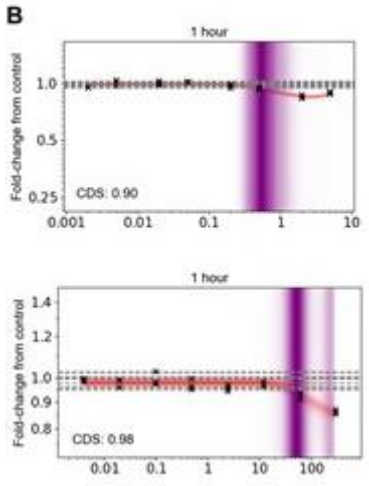




Reynolds et al 2020. Comp Tox 16: 100138
Baltazar et al, 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

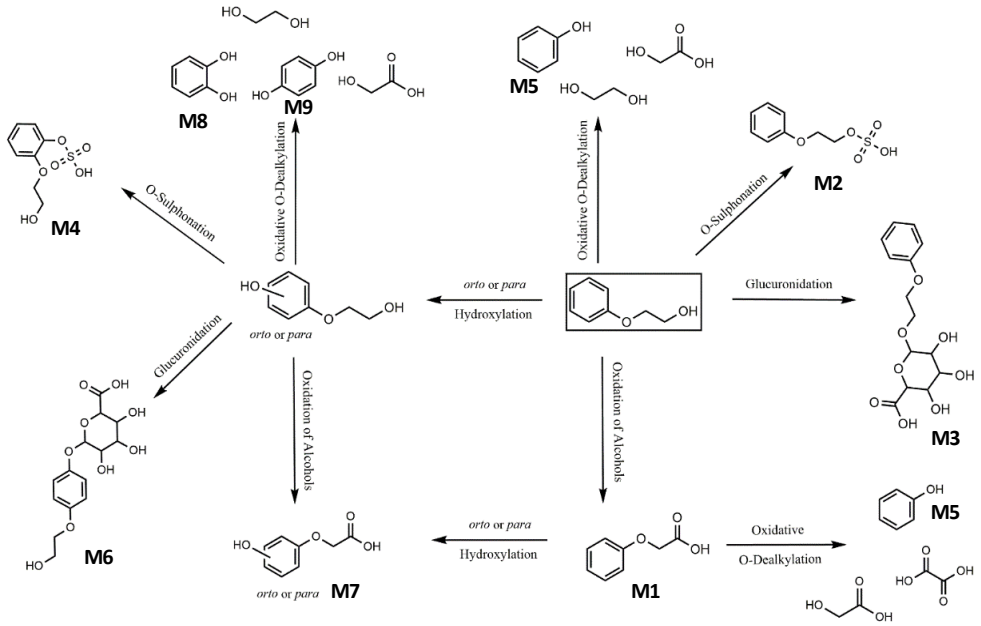


Hatherell et al 2020. Toxicol Sci 176(1): 11-33

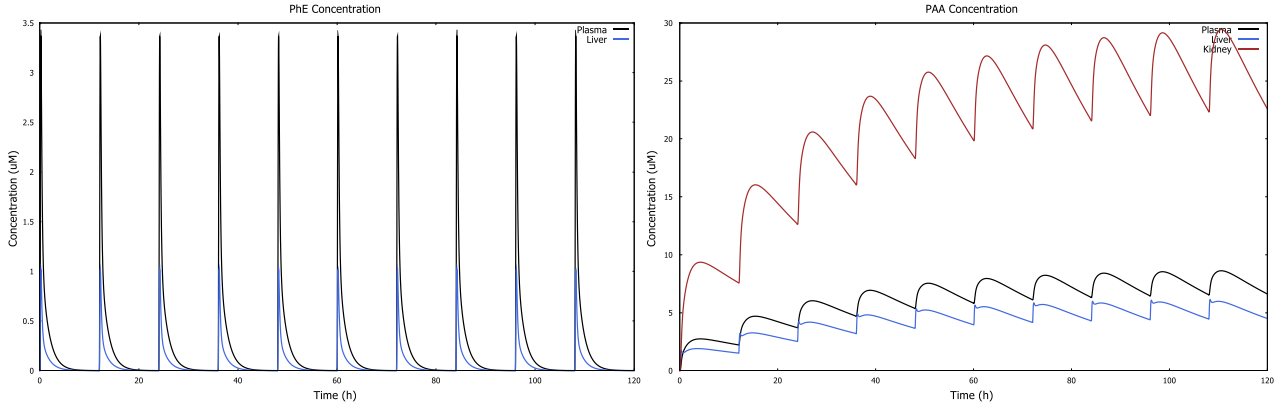
Performed PBK modelling, considered relevant metabolites

A Tiered NGRA Workflow

In silico metabolite predictions

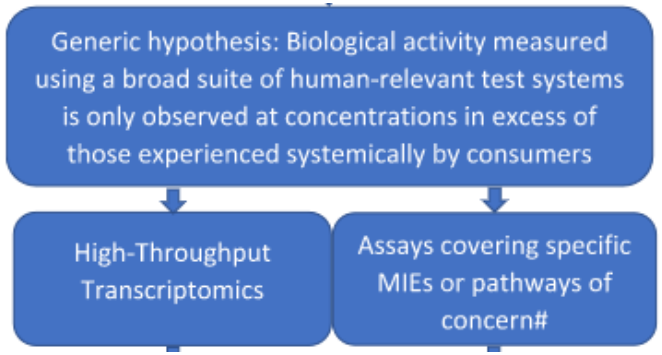


PBK modelling (parent and metabolite)



	Blood	Blood	Blood	Blood	Kidney	Kidney
	PhE C_{max}	PhE AUC_{24}	PAA C_{max}	PAA AUC_{24}	PAA C_{max}	PAA AUC_{24}
	µM	µmol*h/L	µM	µmol*h/L	µM	µmol*h/L
Average	3.7	7.3	10.5	230	36	789
SD	1.4	4.2	4.9	115	17	401
5th %ile	1.8	3.3	4.5	93	15	312
Median	3.6	6.2	9.3	206	32	699
95th %ile	6.2	15	20	453	69	1569

A Tiered NGRA Workflow



HTTr: Tempo-Seq Technology

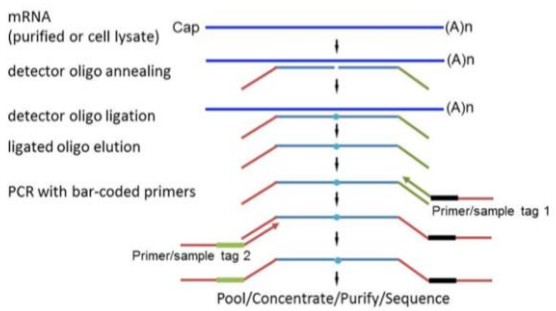
1. Defining a safe operating exposure for systemic toxicity using a **NOTEL** (No Observed Transcriptional Effect Level)
2. Defining compound similarity grouping (Read Across)

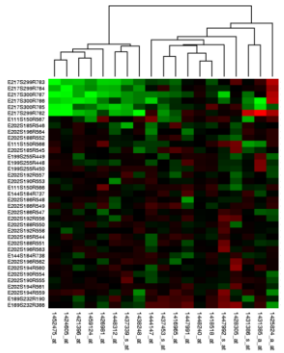
Cell lines (chosen to express a range of relevant receptors)

MCF-7 – human breast adenocarcinoma cell line

HepG2 – human liver carcinoma

HepaRG – terminally differentiated hepatic cells, retain many characteristics of primary human hepatocytes + as spheroids





Filtering criteria (Farmahin et al., 2017) resulted in fewer than the recommended 20 pathways for NOTEL calculations for each cell line. A very conservative approach of modelling the pathways with the lowest BMDs was used:

Gene Tests	HepaRG	MCF-7	HepG2
BMD_{10} of pathway with the lowest BMD_{10} (µM)	552.90	760.33	232.00
$BMDL_{10}$	220.92	512.84	171.25
$BMDU_{10}$	911.72	1648.51	557.20

HepG2 had fewest genes affected and only one pathway showing significant response to treatment (signal transduction)

In HepaRG cells, cytochrome p450 genes CYP2B6 and CYP2A6 showed the greatest fold changes

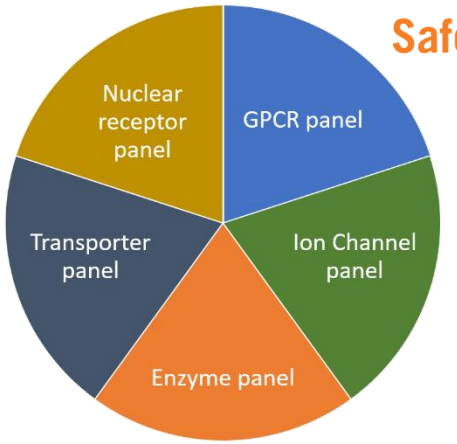
A Tiered NGRA Workflow

Generic hypothesis: Biological activity measured using a broad suite of human-relevant test systems is only observed at concentrations in excess of those experienced systemically by consumers

- High-Throughput Transcriptomics
- Assays covering specific MIEs or pathways of concern#

In Vitro Pharmacological Profiling

SafetyScreen44™ Panel



All binding and enzymatic assay results were negative at 10 µM

No receptor/target-led pharmacological effect

Mean % inhibition for Bowes 44 panel

A Tiered NGRA Workflow

In Vitro Bioactivity: Cell Stress Panel

Hatherell et al., 2020 *Tox Sci* doi: 10.1093/toxsci/kfaa054

Generic hypothesis: Biological activity measured using a broad suite of human-relevant test systems is only observed at concentrations in excess of those experienced systemically by consumers

- High-Throughput Transcriptomics
- Assays covering specific MIEs or pathways of concern#

~40 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

Stress pathways

- Mitochondrial Toxicity
- Oxidative Stress
- DNA damage
- Inflammation
- ER Stress
- Metal Stress
- Osmotic Stress
- Heat Shock
- Hypoxia
- Cell Health

Step 1

Selection of stress pathways

Mitochondrial Toxicity , Oxidative Stress, DNA damage, Inflammation, ER Stress, Metal Stress, Heat Shock, Hypoxia, Cell Health

Selection of biomarkers, probes or antibodies and optimisation of high-content imaging

Selection of cell line, exposure scenario and timepoints

HepG2 cell line, single exposure, 1h, 6h and 24h

Step 2

Selection of chemicals according to different classes and exposure scenarios (based on typical use of compound)

Non-stress inducers
- Caffeine (beverages, cosmetics)
- Coumarin (food, cosmetics)
- Niacinamide (food, cosmetics)
- Phenoxyethanol (cosmetics)

Stress inducers
- CDDO-Me (drug)
- Sulforaphane (food)
- DEM (industrial chemical)
- tBHQ (antioxidant)
- Doxorubicin (drug)
- Diclofenac (drug)
- Triclosan (antimicrobial)
- Troglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (drug)

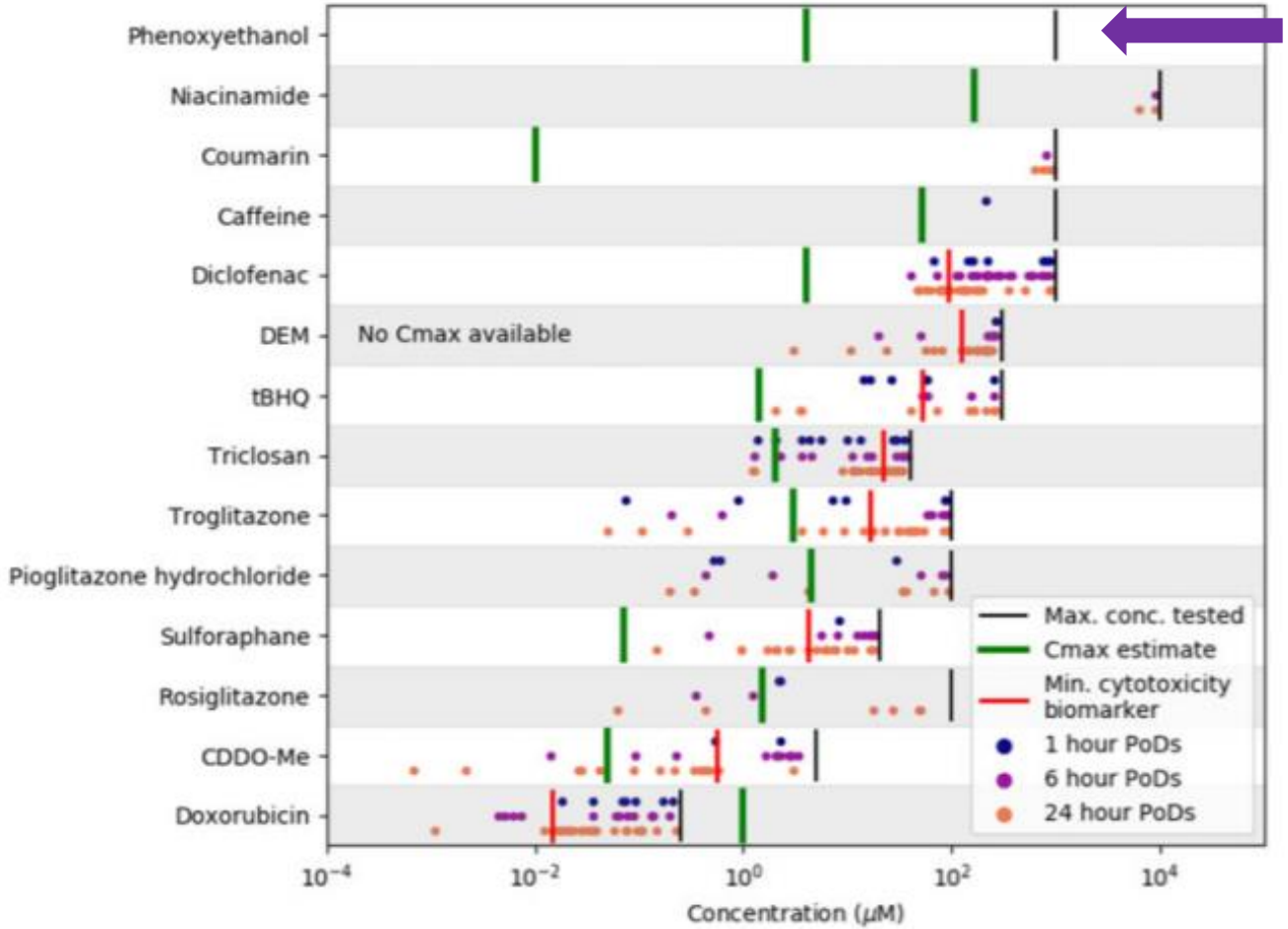
Step 3

Selection of *in vitro* concentrations based upon realistic human exposures

Information on human exposure obtained from human clinical trials or PBK modelling

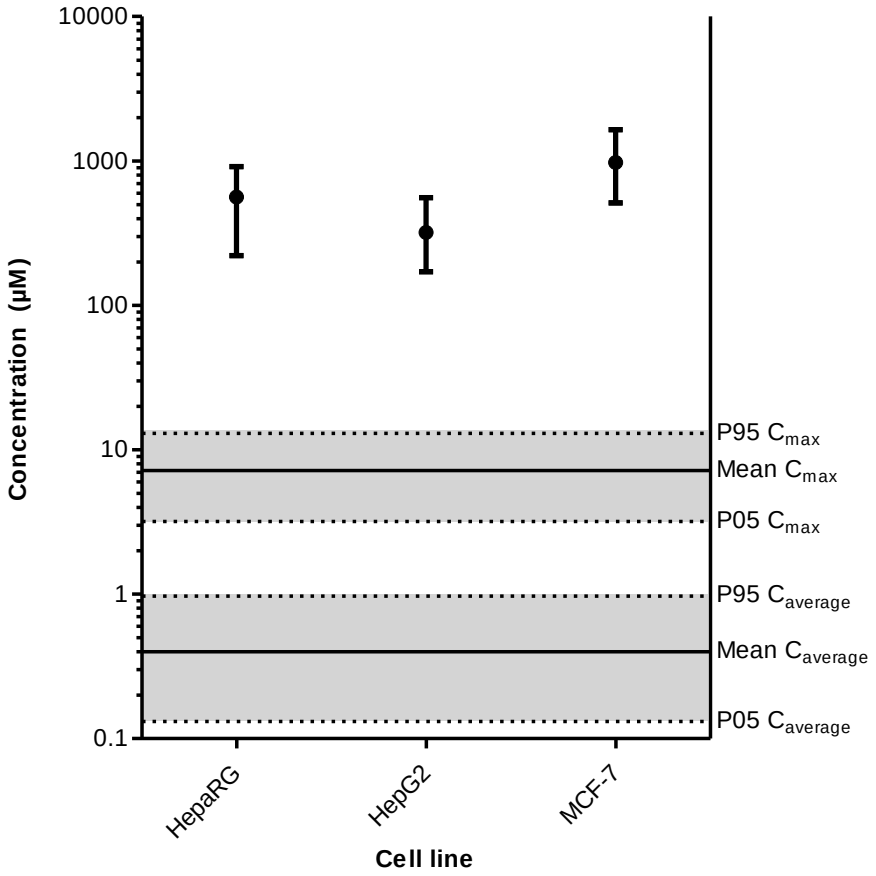
Selection of 8 *in vitro* concentrations (upper bound limited by ~20% cytotoxicity

Key
- Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective).



A Tiered NGRA Workflow

Assessment based on lowest point of departure



Chemical	Scenario	Human Exposure		PoD		MoE/BER	
		AUC_{24}	C_{max}	AUC_{24}	C_{max}	AUC_{24}	C_{max}
		µmol*h/L	µM	µmol*h/L	µM		
Phenoxyethanol	Worst case	15	6.2	3215	171	214	28
Phenoxyethanol	Mean	7.3	3.7	4381	232	600	63
Phenoxyethanol	Best case	3.3	1.8	10708	557	3245	309

Worst case = BMDL/P95 Exposure; Mean = BMD/Mean Exposure; Best case = BMDU/P5 Exposure

Comparison of 24-hour pathway NOTELs for phenoxyethanol in 3 cell lines with exposure predicted by population PBK modelling. Dot represents BMD10, error bars show 5th and 95th percentile BMD (BMDL10 and BMDU10 respectively). The lowest pathway BMDL10 (HepG2) was 27 and 248 times higher than the 95th percentile C_{max} and $C_{average}$ values respectively.

Conclusion/Key Uncertainties

How protective is the assessment?

	Exposure (following use of ingredient at 1% in body lotion)	PoD	MoS/BER
'Traditional' Risk Assessment*	1.23 mg/kg/day	357 mg/kg/day	290
NGRA based on C_{max} and NOTEL	6.2 µM	171 µM	28
NGRA based on AUC_{24} and NOTEL	15 µmol*h/L	3215 µmol*h/L	214

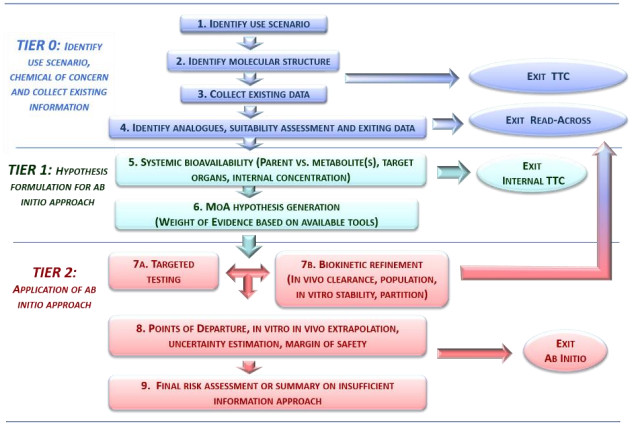
Key Uncertainties

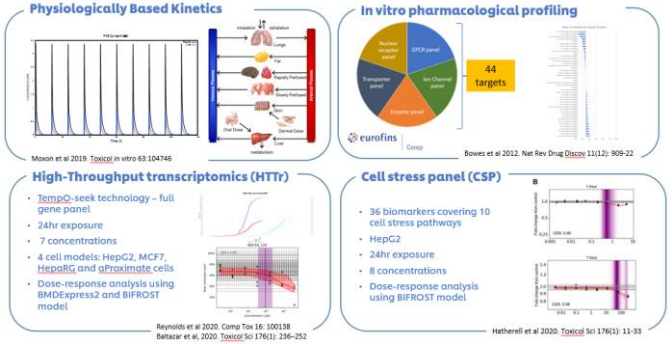
- Range of Biomarkers assessed (when do you have enough data?)
- In vitro kinetics
- Duration of studies (is 24hrs adequate?)
- Point of departure (limited number of cell lines)

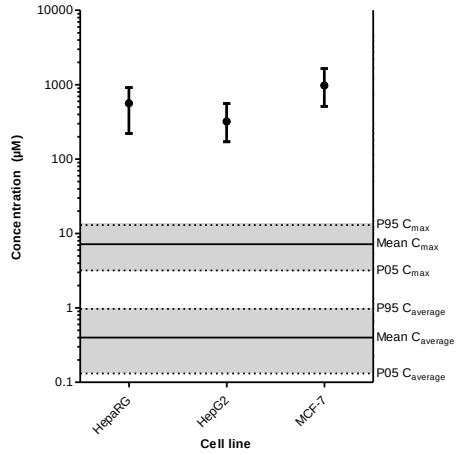
*"This case study illustrates an ab initio risk assessment of a cosmetic ingredient based on the tools and approaches currently available, and provides a **possible approach** to evaluating major metabolite. Although the calculated MoEs were above 1, which indicated that in vitro bioactivity was not seen at consumer-relevant concentrations, there were **several uncertainties** in the risk assessment which **need to be addressed** in future work."*

Learnings

- Next generation risk assessment (NGRA) can be conducted without a known chemical mechanism of action or AOP
- ‘Protection not prediction’ describes a hypothesis whereby if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects
- A tiered and iterative workflow approach can be used with a core NAM toolbox supplemented by additional tools to assess specific hypothesis

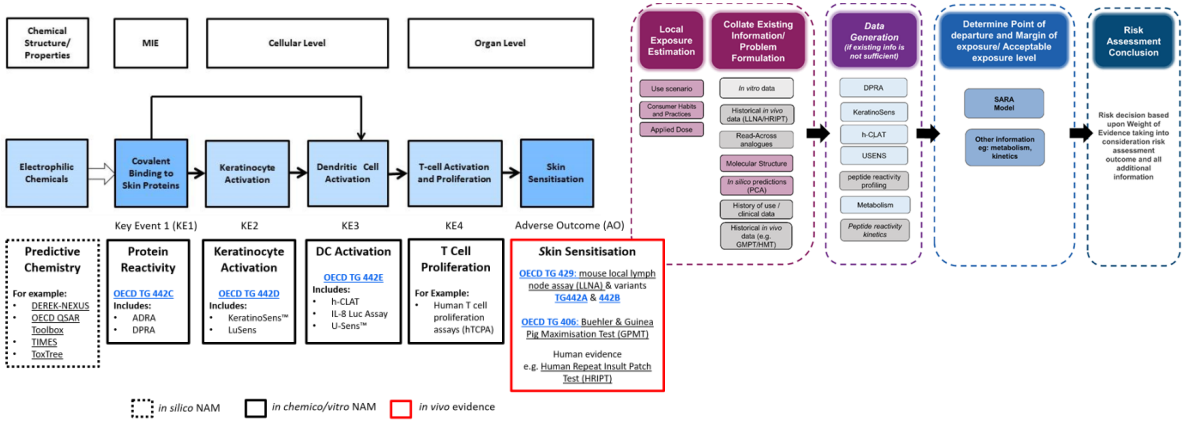


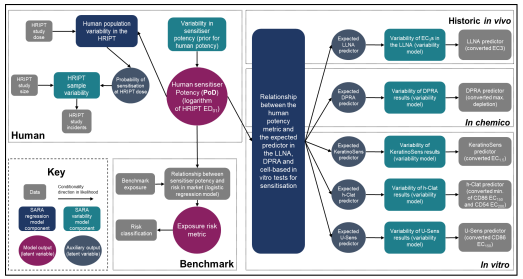


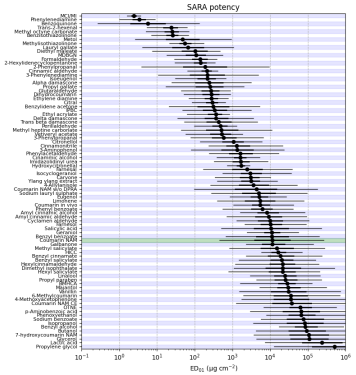


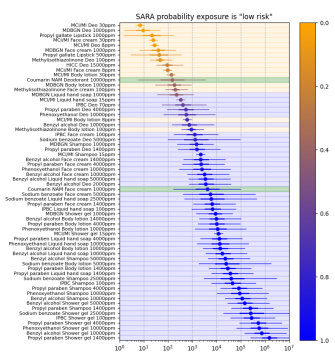
Overall Summary

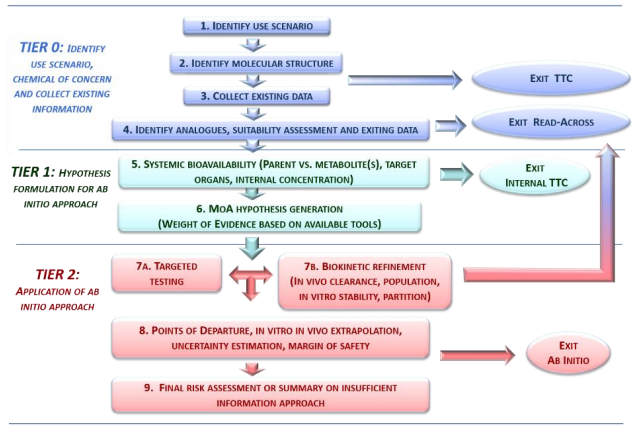
- Next generation risk assessment (NGRA) can be conducted with or without a known chemical mechanism of action or AOP
- Concepts such as tiered and iterative frameworks, PoDs and BER/MoEs, are translated across NGRA with or without AOPs

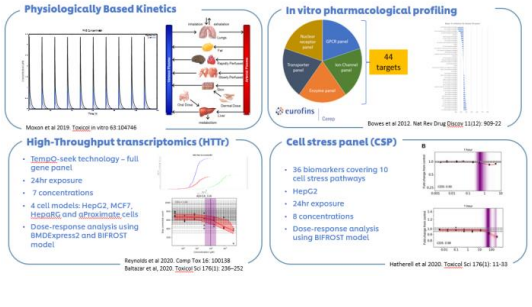


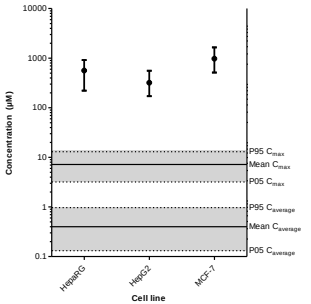












Thank you

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LRSS website: https://www.lrsscosmeticseurope.eu/

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