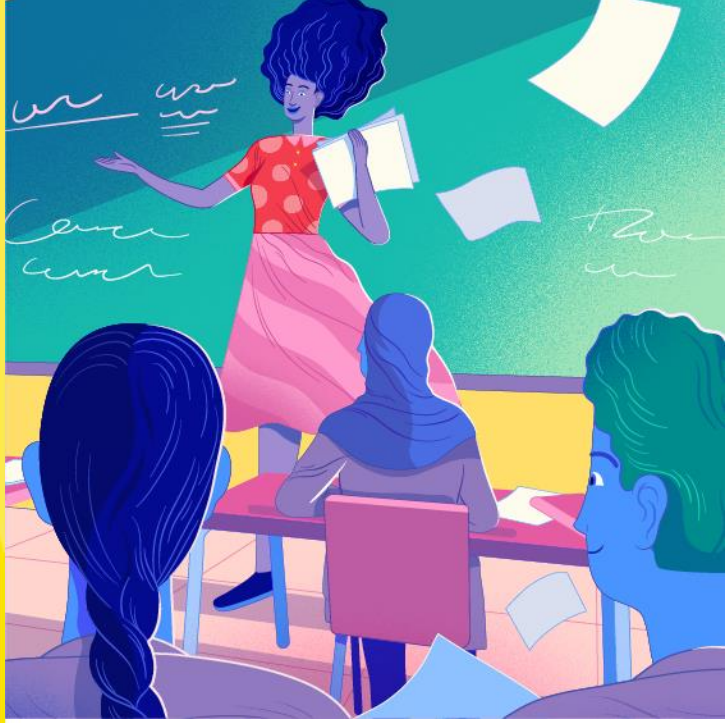
Non-animal safety assessment of cosmetic ingredients



Paul Russell & Renato de Ávila

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2.5bn consumers reached

48k suppliers

190 countries

60% sales in emerging markets

12 billion euro brands

1.3bn people helped to improve health and hygiene

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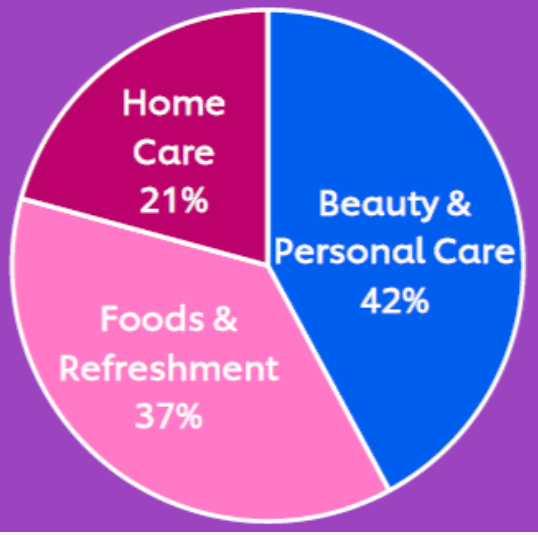

90% local leaders

14 of the top 50 global consumer brands

Lifebuoy, sunsilk, Dove, Knorr, LUX, vim, Brooke Bond, Sunlight, Rexona, closeup, Comfort, Lipton, POND'S, Pepsodent

Unilever

Safety and Environmental Assurance Centre (SEAC)

SEAC is a team of industry-leading safety and environmental sustainability scientists. They use the latest techniques, deep scientific expertise and an evidence-based approach to ensure that our products are safe for consumers and workers and better for the environment.

Working with teams across Unilever, from the beginning to the end of a product's life, SEAC scientists ensure that safety and sustainability are built into everything we make and do. In addition to this, SEAC scientists work with leading experts around the globe to constantly advance the science we use to assess our product innovations of the future.

> "Our leading-edge approach has one clear purpose: to continue to develop, apply and let others know about the research we do to guarantee that our products are safe, without the need for animal testing."
>
> **Julia Fentem, Head of SEAC**

https://www.unilever.com/brands/innovation/safety-and-environment/

Unilever

AXE MAGNUM OMO Dove Knorr LUX HELLMANN'S SURF Rexona Lipton sunsilk CO-CREATIONS WALL'S

Outline

PART ONE

- Introduction to non-animal safety assessment
- History of safe use (HoSU) for botanical ingredients

PART TWO

- Next Generation Risk Assessment (NGRA) – concepts and tools

PART THREE – NGRA Case studies

- Local effects – skin sensitisation
- Systemic effects

AFSA – Animal Free Safety Assessment Collaboration

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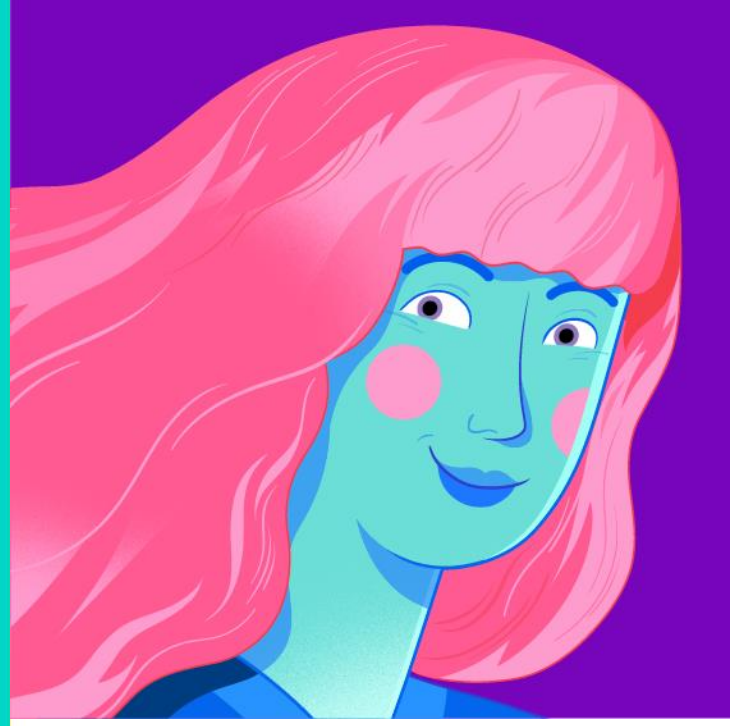
PART ONE

Introduction to non-animal safety assessment



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Can we use a new ingredient safely?

- Can we safely use x% of ingredient y in product z?

Risk = Hazard x Exposure

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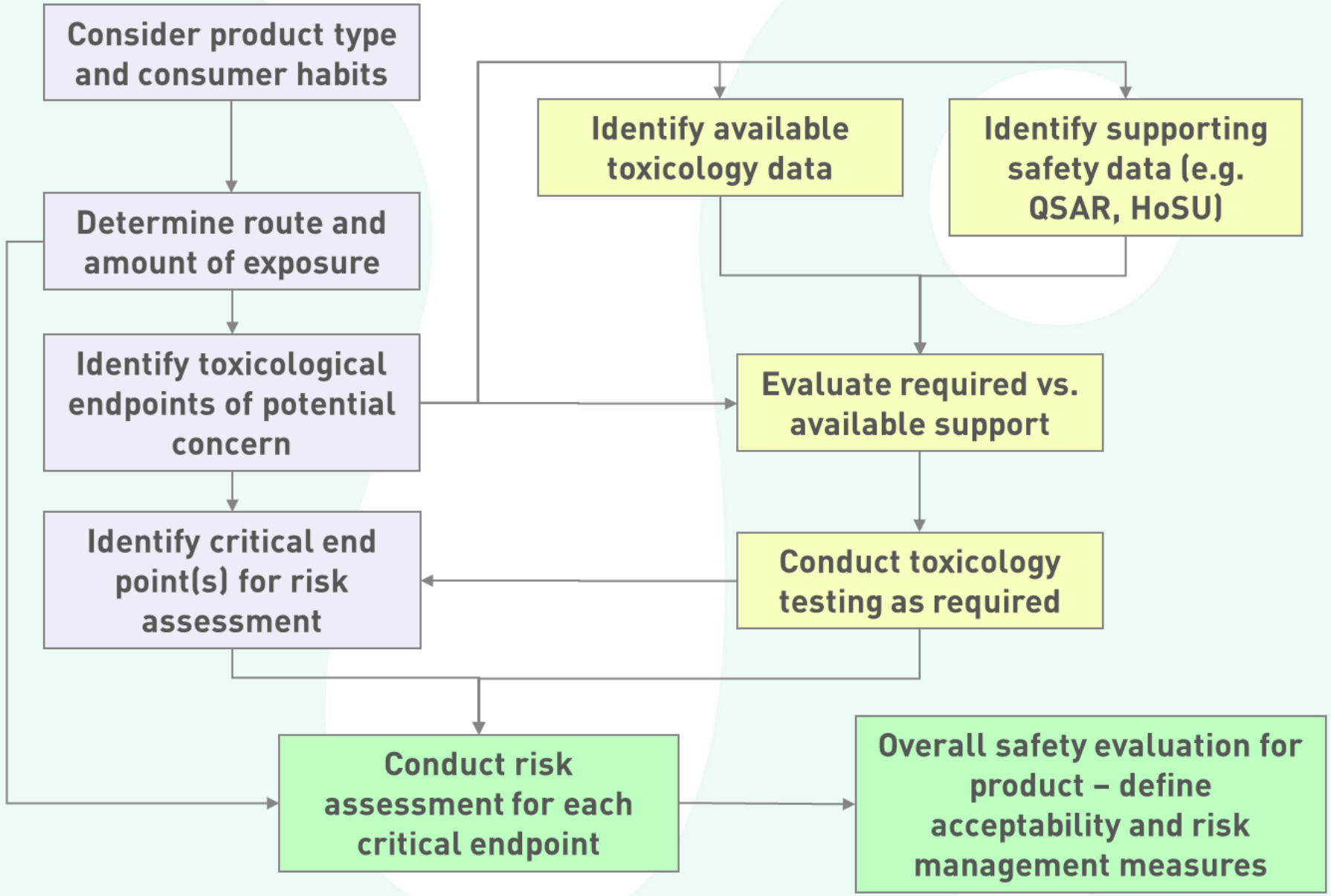
Exposure-driven Safety Assessment



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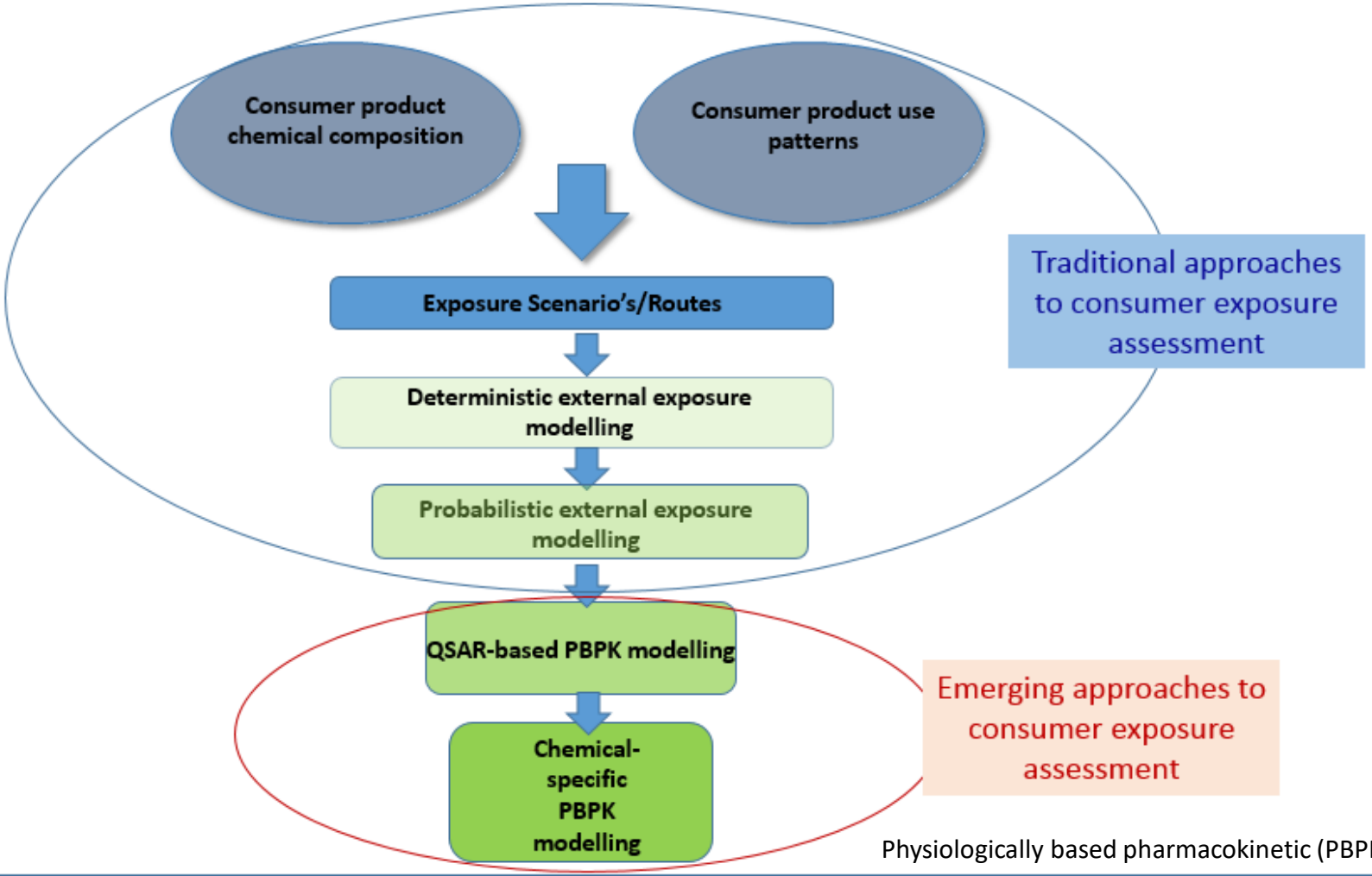
Exposure Science overview

Exposure assessment: Drives the risk assessment process. This quantifies the dose (amount) of a material that is externally applied during consumer use of the product, which is then compared to the relevant dose at which toxicological effects are expected to establish the safety risk.

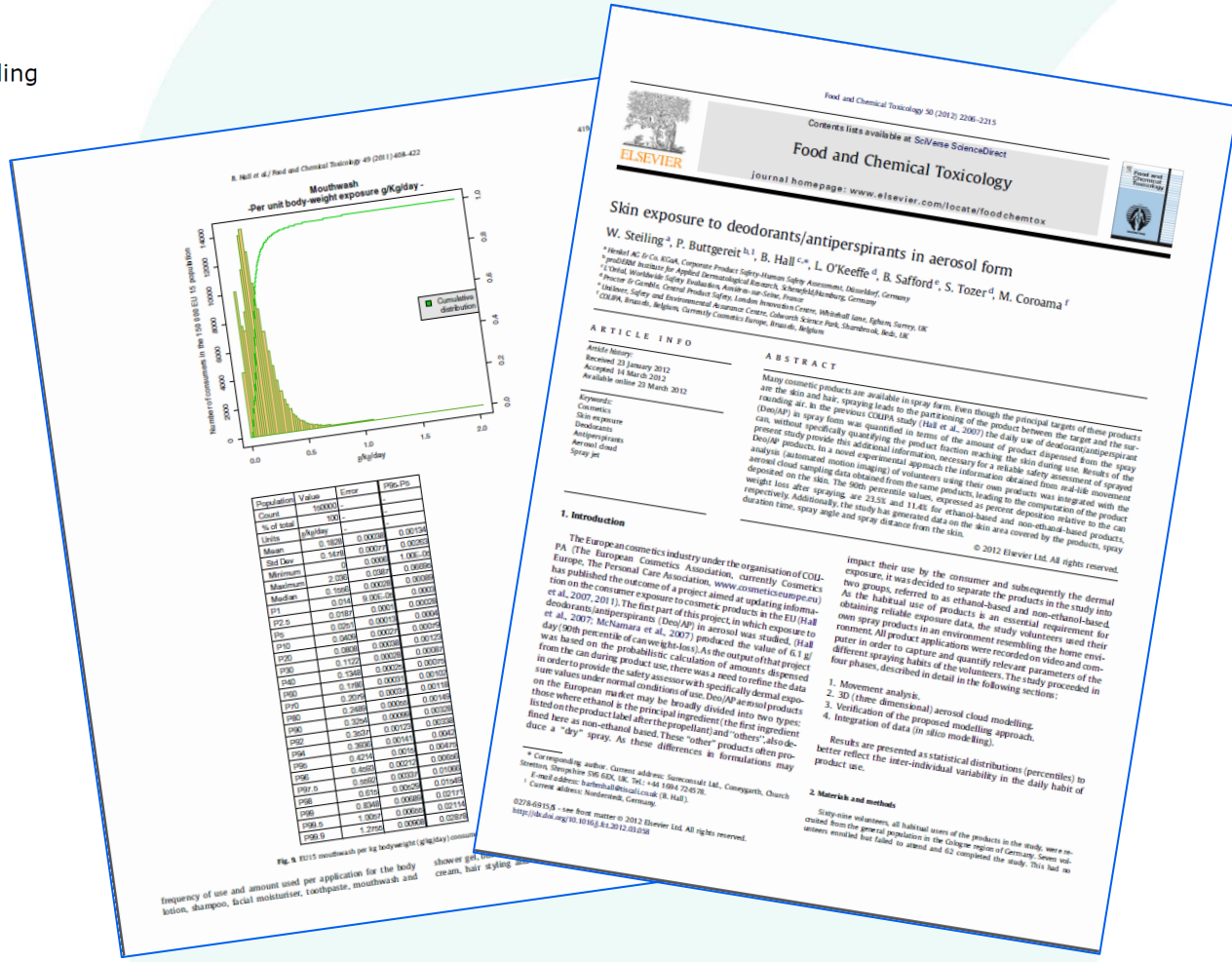


Physiologically based pharmacokinetic (PBPK)

Habits and practices

Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNFP/0321/00; Hall *et al.*, 2007, 2011).

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap [2]	20.00 g	-	0.01	0.20 [3]	3.33
Hair care					
Shampoo	10.46 g	150.49	0.01	0.11	1.51
Hair conditioner [2]	3.92 g	-	0.01	0.04	0.60
Hair styling products	4.00 g	57.40	0.1	0.40	5.74



B. Hall et al. / Food and Chemical Toxicology 49 (2011) 408–422

Creme GLOBAL

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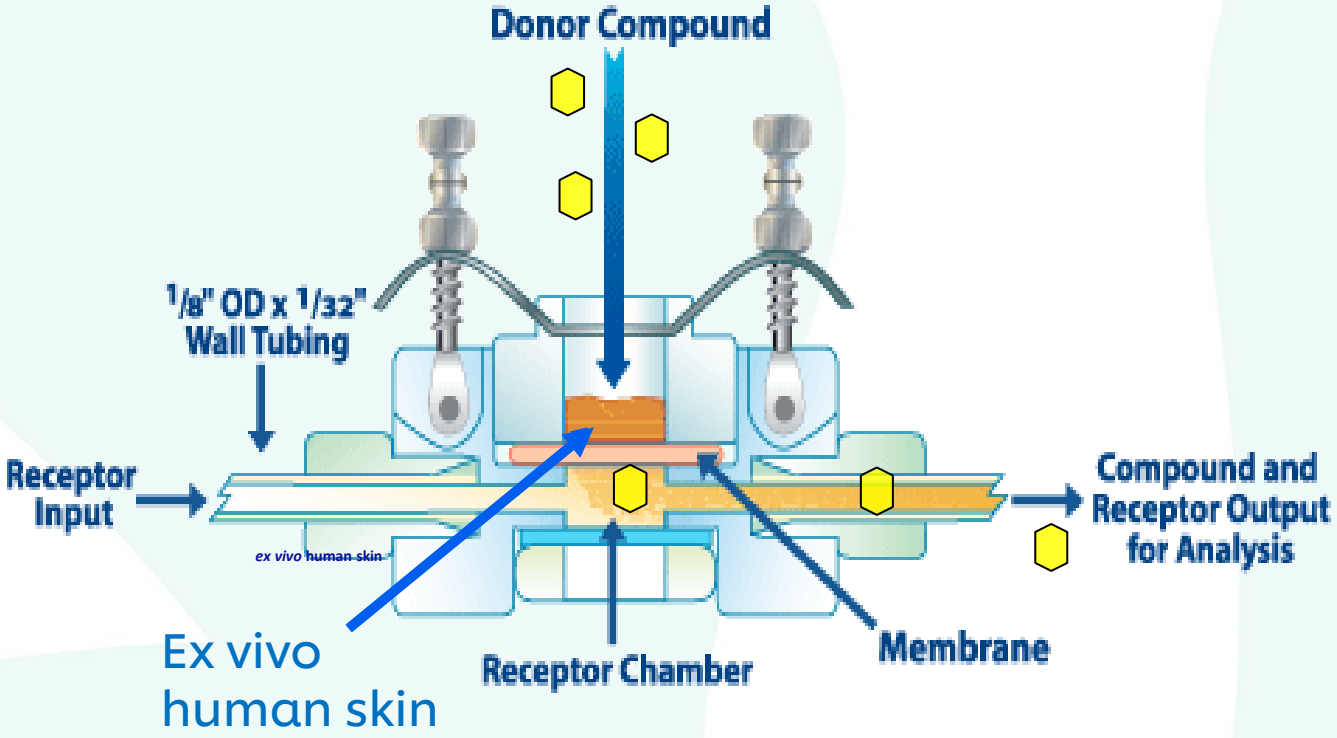
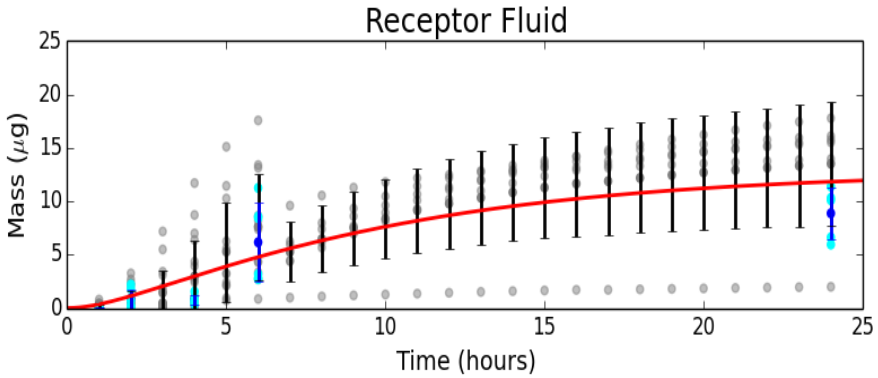
Skin penetration measurements





OECD/OCDE **428**

Adopted :
13 April 2004

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Skin Absorption: *in vitro* Method

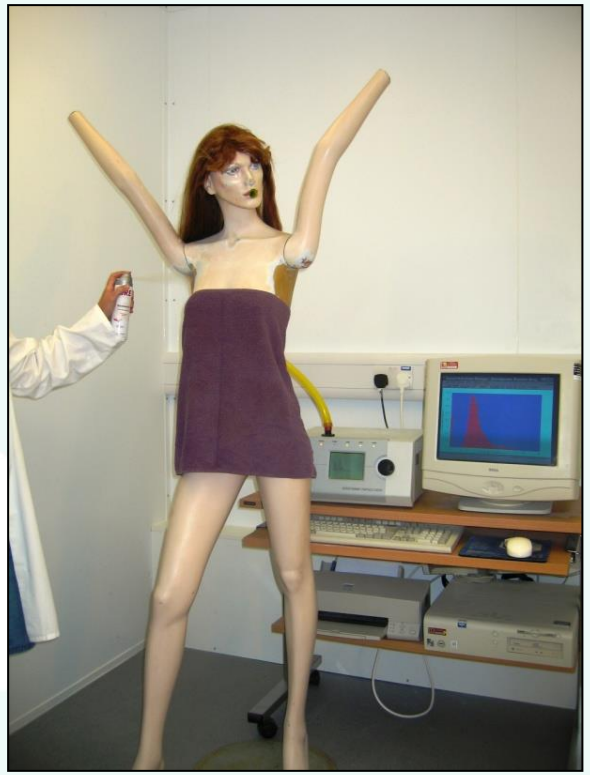
INTRODUCTION

1. This test guideline has been designed to provide information on absorption of a test substance applied to excised skin. It can either be combined with the OECD Test Guideline for Skin Absorption: *In vivo* Method (1), or be conducted separately. It is recommended that the OECD Guidance Document for the Conduct of Skin Absorption Studies (2) be consulted to assist in the design of studies based on this Test Guideline. The OECD Guidance Document has been prepared to facilitate the selection of appropriate *in vitro* procedures for use in specific circumstances, to ensure the reliability of results obtained by this method.

INITIAL CONSIDERATIONS

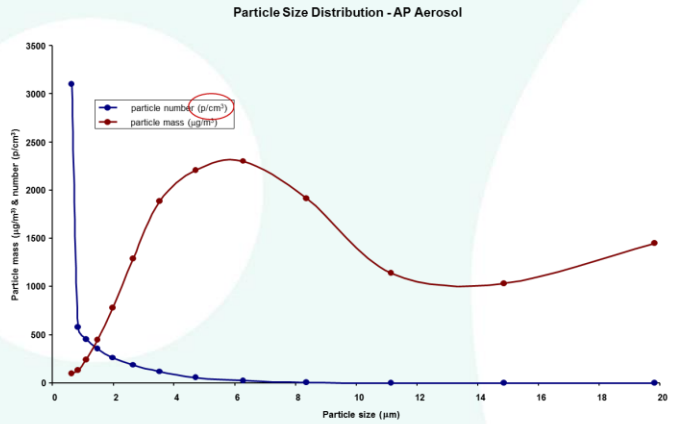
2. The methods for measuring skin absorption and dermal delivery can be divided into two categories: *in vivo* and *in vitro*. *In vivo* methods on skin absorption are well established and provide pharmacokinetic information in a range of animal species. An *in vivo* method is separately described in another OECD guideline (1). *In vitro* methods have also been used for many years to measure skin absorption. Although formal validation studies of the *in vitro* methods covered by this Test Guideline have not been performed, OECD experts agreed in 1999 that there was sufficient data evaluated to support the *in vitro* Test Guideline (3). Further details that substantiate this support, including a significant number of

Inhalation Exposure



- Simulated use studies can be conducted to measure lung exposure
- Usually concerned with aerosol or pump spray products. Other products can be tested under simulated use conditions
- Can measure inhalation of volatile and non-volatile components using aerodynamic particle sizer

Simulated use study output



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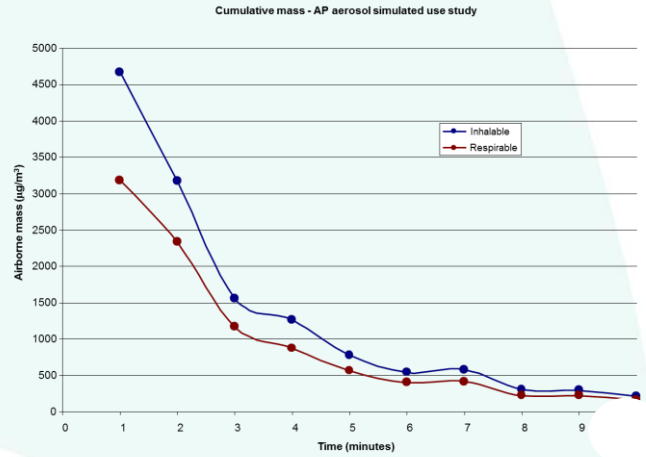
Breathing zone aerosol



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Maximising use of existing information

- All available safety data (of suitable quality)
  - public domain, historical in-house data, supplier data etc
  - chemistry data, *in vitro* data, clinical data, epidemiological data, animal toxicology data etc
- Exposure-based waiving approaches
- History of safe use
- Read across
- Use of existing *in vitro* data and approaches

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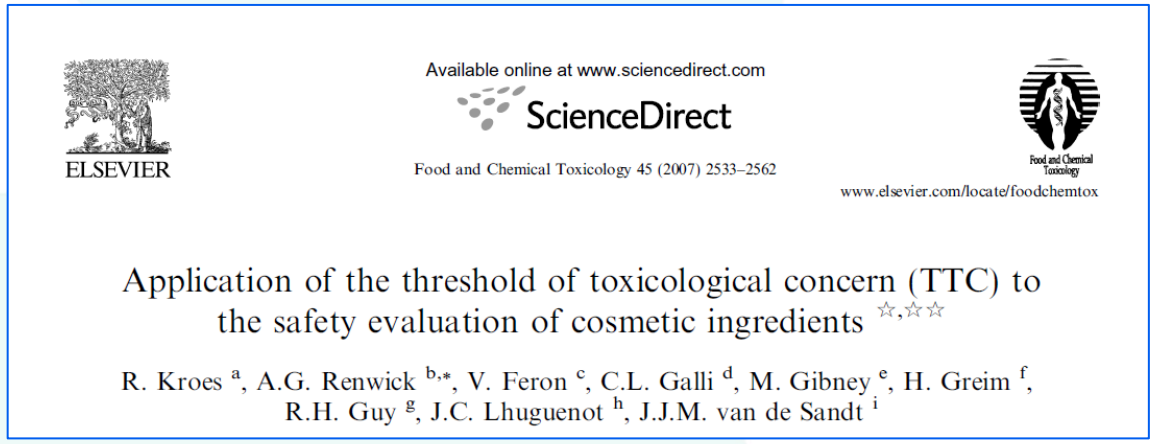
Exposure-based waiving approaches

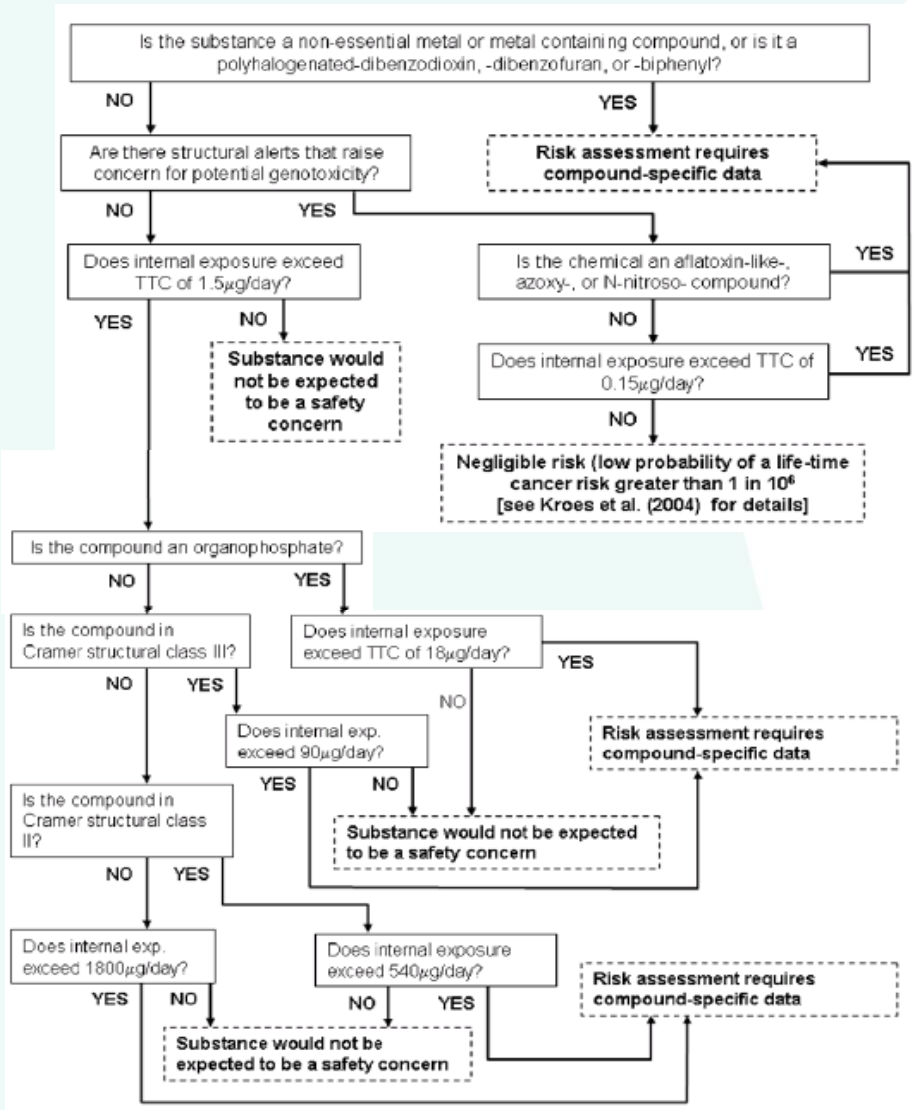
- If no data are available then in some instances exposure based waiving approaches such as the Toxicological Threshold of Concern (TTC) can be employed
- TTC – a pragmatic approach to derive an exposure level at which there is no appreciable risk to human health
- The TTC levels were determined applying a 100-fold extrapolation factor to the 5th percentile NOAEL for chemicals in each Cramer class derived from chronic studies.



NOAEL – No Observed Adverse Effect Level

Toxicological Threshold of Concern (TTC)

Dataset (number of chemicals)	Human Exposure threshold values (number of chemicals)					
	ug/day			ug/kg bw/day		
	Cramer Class I	Cramer Class II	Cramer Class III	Cramer Class I	Cramer Class II	Cramer Class III
Cosmos (552)	2500 (219)	- (40)	470 (293)	42 (219)	- (40)	7.9 (293)
Munro - 1996 (613)	1800 (137)	540 (28)	90 (448)	30 (137)	9 (28)	1.5 (448)
Munro - 2016 (606)	2900 (141)	640 (30)	90 (435)	49 (141)	11 (30)	1.5 (435)
Federated (963)	2700 (243)	370 (49)	140 (671)	46 (243)	6.2 (49)	2.3 (671)



Kroes et al, 2007, *Food Chem. Toxicol.*, **45**, 2533-2562

Other types of exposure-based waving

Regulatory Toxicology and Pharmacology 51 (2008) 195–200

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The Dermal Sensitisation Threshold—A TTC approach for allergic contact dermatitis

R.J. Safford *

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedford MK44 1LQ, UK

ARTICLE INFO

Article history:
Received 14 December 2007
Available online 18 March 2008

Keywords:
Threshold
Threshold of toxicological concern
TTC
Contact sensitisation
Allergic contact dermatitis

ABSTRACT

The Threshold of Toxicological Concern (TTC) is a useful concept that is becoming of increasing
an addition to the arsenal of tools used for characterising the toxicological risk of human e
chemicals. Traditionally used for low level indirect additives, flavours and contaminants in
TTC obviates the need for toxicological testing of chemicals where human exposure is low. Prop
recently been made for the use of the TTC for low level ingredients in cosmetic and personal
ucts. However, use of the TTC is only protective for systemic toxicity endpoints, and cannot
local endpoints such as contact sensitisation. In this paper a probabilistic analysis of available
ion data, similar to that used in the development of the TTC, is presented. The incidence of se
the world of chemicals was estimated using the EINCS (European List of Notified Chemical S
data set, and a distribution for sensitisation potency was established using a recently publish
lation of Local Lymph Node Assay data. From the analysis of these data sets it is concluded tha
Sensitisation Threshold (DST) can be established below which there is no appreciable risk of se
even for an untested ingredient. Use of a DST would preclude the need for sensitisation testin
dients where dermal exposure is sufficiently low.

Food and Chemical Toxicology 47 (2009) 1287–1295

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Exposure based waiving: The application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products

P. Carthew *, C. Clapp, S. Gutsell

Safety and Environmental Assurance Centre, Unilever Research, Colworth Science Park, Sharnbrook, Bedford MK44 1LQ, UK

ARTICLE INFO

Article history:
Received 19 December 2008
Accepted 27 February 2009

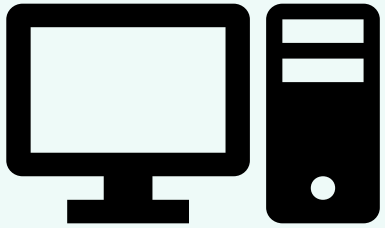
Keywords:
Exposure based waiving
Inhalation
Respiratory tract
Threshold of toxicological concern
Intelligent testing strategy
REACH

ABSTRACT

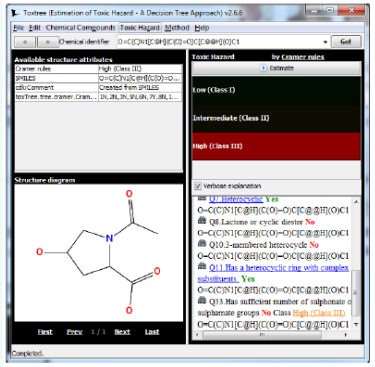
The inhalation toxicology studies available in the public domain have been reviewed to establish a database for inhalation toxicology and derive thresholds of toxicological concern (TTC) for effects in the respiratory tract and systemically for Cramer class 1 and 3 chemicals. These TTCs can be used as the basis for developing an exposure based waiving (EBW) approach to evaluating the potential for adverse effects from exposure to ingredients in aerosol products, used by consumers. The measurement of consumer exposure in simulated product use is key to the application of an exposure based waiving approach to evaluating potential consumer risk. The detailed exposure evaluation for aerosol ingredients with defined use scenarios, in conjunction with an evaluation of the potential structure activity relationship for toxicity and the TTCs for inhalation exposure could be used to waive undertaking inhalation toxicology studies under REACH. Not all classes of chemicals are suitable for such an approach, but for chemicals with a predictable low potential toxicity, and very low levels of exposure, this approach, could reduce the amount of inhalation toxicology studies required for the implementation of the European REACH legislation. Such an approach is consistent with the concept of developing 'intelligent testing strategies' for REACH.

Computational approaches

In silico tools

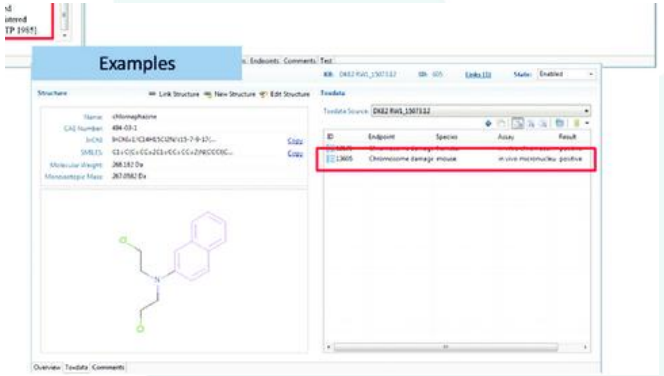


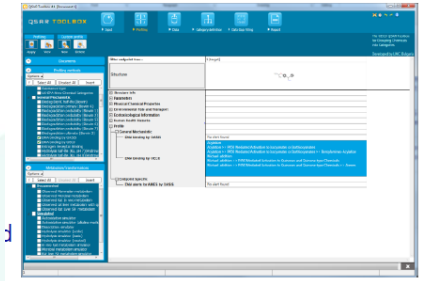
ToxTree



Derek nexus

OECD QSAR TOOLBOX





In silico models to predict Molecular initiating events (MIEs)

OXFORD
SOT Society of Toxicology
www.toxsci.oxfordjournals.org
ToxSci 20 Years

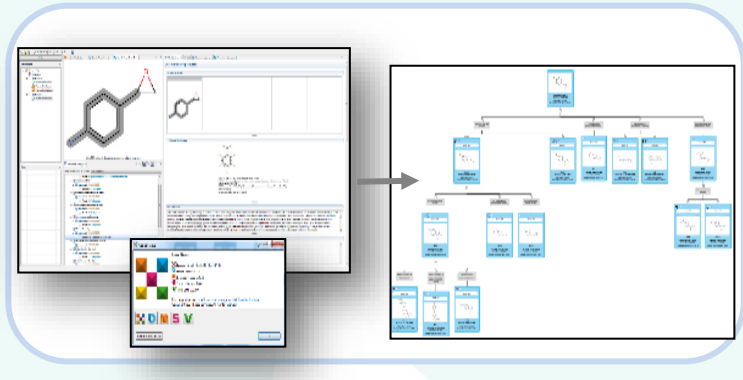
TOXICOLOGICAL SCIENCES, 165(1), 2018, 213–223

doi: 10.1093/toxsci/kfy144
Advance Access Publication Date: July 18, 2018
Research Article

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events

Timothy E. H. Allen,* Jonathan M. Goodman,*,[1] Steve Gutsell,† and Paul J. Russell†

Meteor nexus



Metabolic fate predictions

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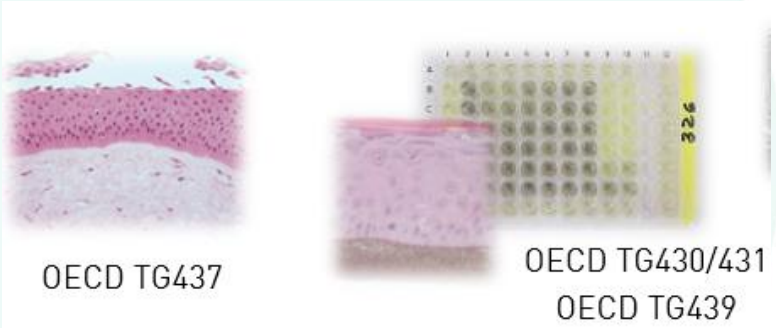
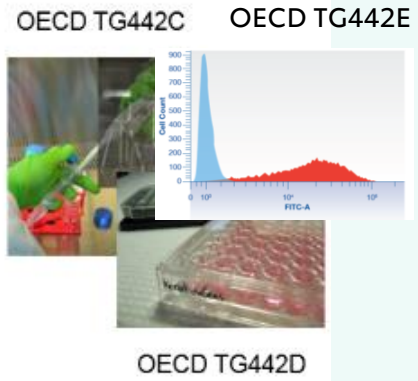
In vitro approaches

OECD test methods



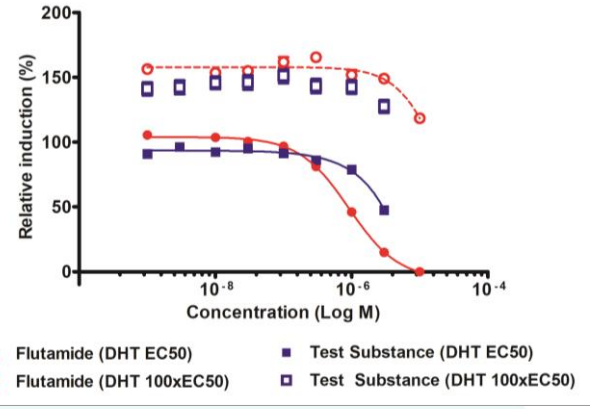
Skin and eye irritation



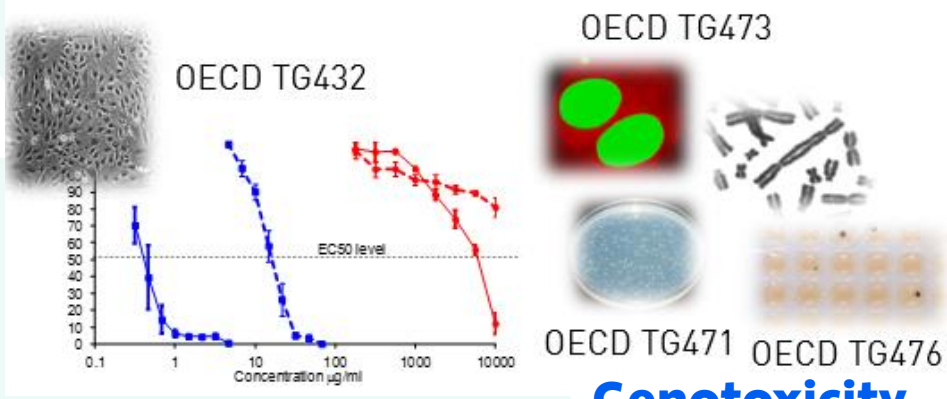
Skin sensitisation

Receptor-binding assays

e.g. AR-CALUX® assay to measure androgen receptor activity



Dent et al (2019), Toxicological Science, 167, 375-384



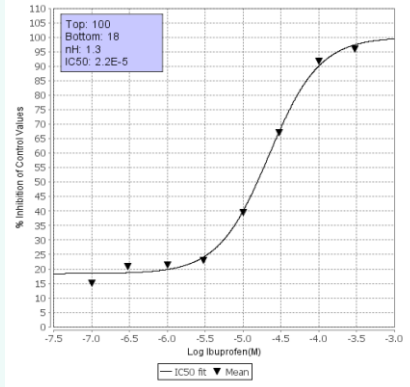
Phototoxicity

Genotoxicity

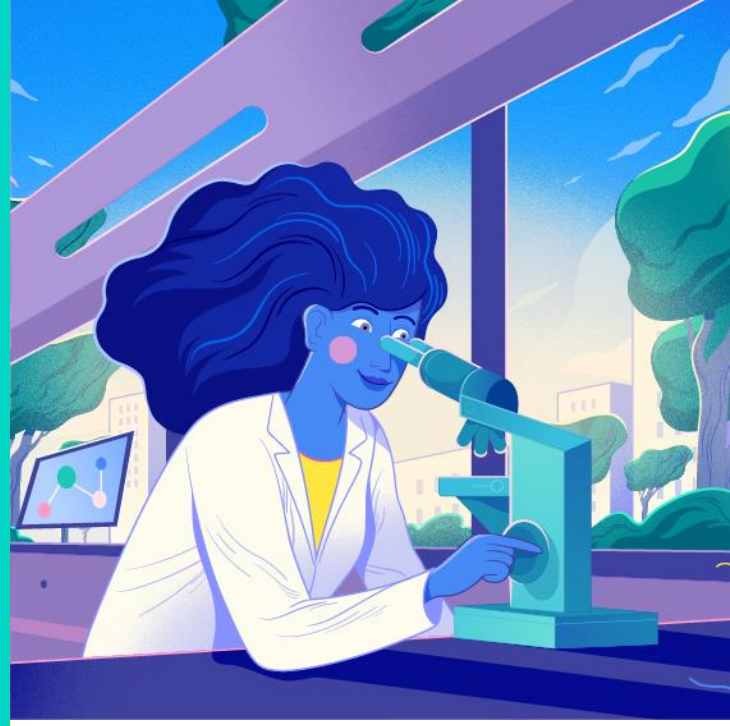

PERSPECTIVES

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Ibuprofen – Cox-1.



History of Safe Use (HoSU)



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Do you have a favourite?

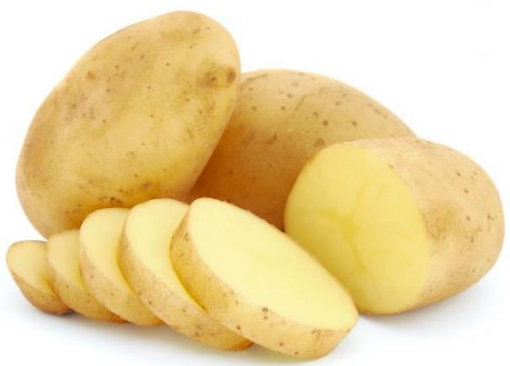
'Everything is poison, there is poison in everything. Only the dose makes a thing not a poison.' Paracelsus

Amygdalin
(0.6g/kg seeds)

1.1 kg apple seeds

Formaldehyde
(0.06g/kg)

116 kg pears



Solanine
(0.2g/kg)

79 kg potatoes

Cucurbitacin E
(0.25-7 g/kg,
high in bitter courgettes)

119 kg courgettes

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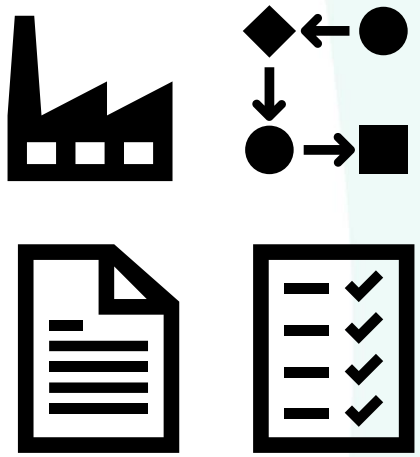
Naturally challenging

Raw Material Identification

e.g. Which Ginseng? American, Korean, Chinese, Indian….

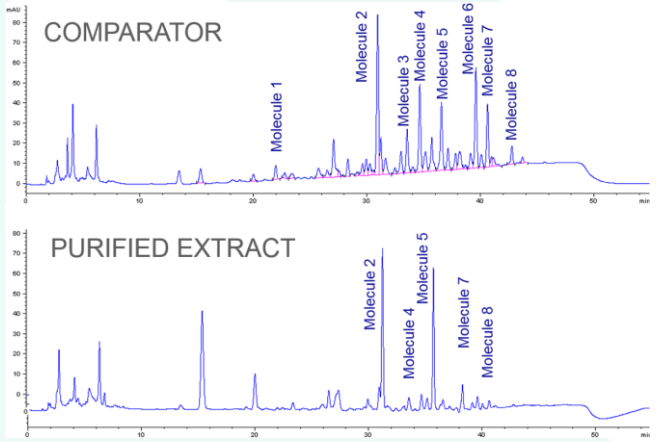
Specification control

- Processing
- Marker compounds
- Mass balance?



Chemical analysis

- Fingerprinting
- Targeted quantitation



Control of sample variation: Natural plant variation, geographical, seasonal, age…

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‘History of Safe Use’ Risk Assessment

- Risk assessment of botanical materials (herbals, traditional Chinese medicines, Ayurvedics etc) which have a long history of use in certain parts of world.

- ‘History of Safe Use’ (HoSU) is widely used for safety assessment of food ingredients (e.g. novel foods and foods derived from genetically modified organisms) and the principles can be extended for cosmetic products.

- History of safe use assessments need to be robust, transparent and evidence based.
  - Identification of suitable comparator with a history of prior use
  - Evidence for toxicological concern (and lack of concern) of the comparator.
  - The similarity of the botanical of interest with the comparator.

Useful references:

History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms; Constable, A et al, Food and Chemical Toxicology; 45 (12) (2007); 2513-2525.

A multi-criteria decision analysis model to assess the safety of botanicals utilizing data on history of use; Neely, T *et al;* Toxicology International; 18 (2011); 20-29.

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Evidence of History of Use (Exposure)

- Origin of ingredient
- Similarity of ingredient specification
- Preparation and processing similarity
- Similarity of population to be exposed especially products aimed at babies/children - comparator should have similar history of exposure
- Number of people exposed
- Pattern of use/frequency of application
- Bioavailability/Skin penetration

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Evidence for Concern (Hazard)

Toxicology data

- High Concern: Reproductive or developmental toxicity, mutagenicity, neurotoxicity or any organ toxicity, data showing skin sensitization (type IV allergy), type I allergy, skin carcinogenicity, phototoxicity effects

Chemical components of concern

- High concern: known skin sensitisers, photoallergens, proteins….
- Biological effects/mechanism of action
- Evidence of adverse effects in man (Information from literature review or existing clinical data)

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Useful Data Sources

- Food Standards Agency: https://www.food.gov.uk/
- European Food Safety Authority (EFSA) - http://www.efsa.europa.eu/ , https://www.efsa.europa.eu/en/topics/topic/dietary-reference-values
- World Health Organization - https://www.who.int/foodsafety/areas_work/nutrition/en/
- Health Canada - http://recherche-search.gc.ca/rGs/s_r?st=s&langs=eng&st1rt=0&num=10&cdn=health
- JECFA - Monographs & Evaluations - https://www.who.int/foodsafety/publications/monographs/en/
- U.S. Food and Drug Administration - https://www.fda.gov/food
- Natural Medicines Comprehensive Database - www.NaturalMedicines.com/login
- European Medicines Agency - https://www.ema.europa.eu/en/committees/committee-herbal-medicinal-products-hmpc
- PubMed - https://www.ncbi.nlm.nih.gov/pubmed?tool=cdl&otool=cdlotool
- Toxicology Data Network (TOXNET) - https://toxnet.nlm.nih.gov/
- Personal Care Products Council - http://online.personalcarecouncil.org/jsp/Home.jsp
- Chemical Safety Information from Intergovernmental Organizations - http://www.inchem.org/

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Case Study: Green tea in skin cream

- Green tea (*Camelia sinensis*)
- Traditionally drunk as a hot beverage – some history of topical use
- Large amount of historical oral consumption information
- The primary chemical components are polyphenols
- Safety assessment was needed for inclusion of green tea extract in a leave-on skin product
- History of Safe Use (HoSU) approach used

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Information Gathering

Criteria	Response for green tea	Evidence
Origin of ingredient	Identical to traditional/comparator	*Camelia sinensis* leaves used. Harvested in SA Asia for tea production
Similarity of specification	Almost the same	Fingerprint and quantitative assessment of components confirms similar specification
Preparation and processing	Almost the same	Aqueous extract – prepared by boiling dried leaves
Populations	Use encompasses population intended to expose e.g. healthy adult females	Evidence of topical use of green and black tea
No. of people exposed	Thousands	Evidence of topical use reported in open literature
Duration of exposure	20 years +	Evidence of topical use reported in open literature
Pattern/frequency of use	Ingested and topically applied on a daily basis	Evidence from Natural Medicines Database
Bioavailability	Not known	-
Toxicological data	Some data showing green tea extracts to cause skin sensitisation when applied topically	Literature search (numerous references)
Chemical components of concern	Catechins	Literature search (numerous references)
Biological effects/mechanism of action	Catechins may have anti-inflammatory activity	Evidence from Natural Medicines Database
Evidence of adverse effects in man	Some evidence of irritation when used at high concentrations in topical applications	Literature search (numerous references)

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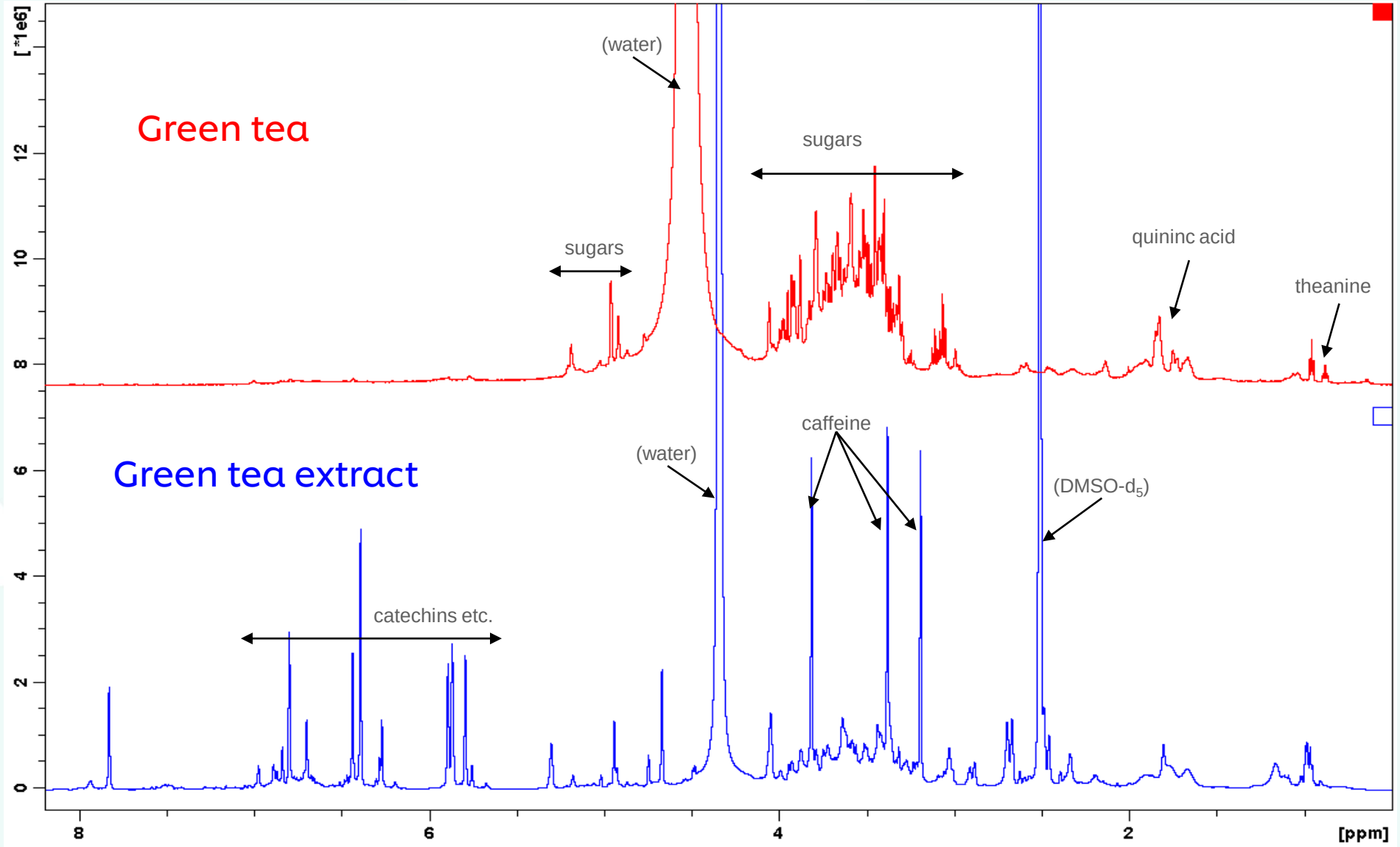
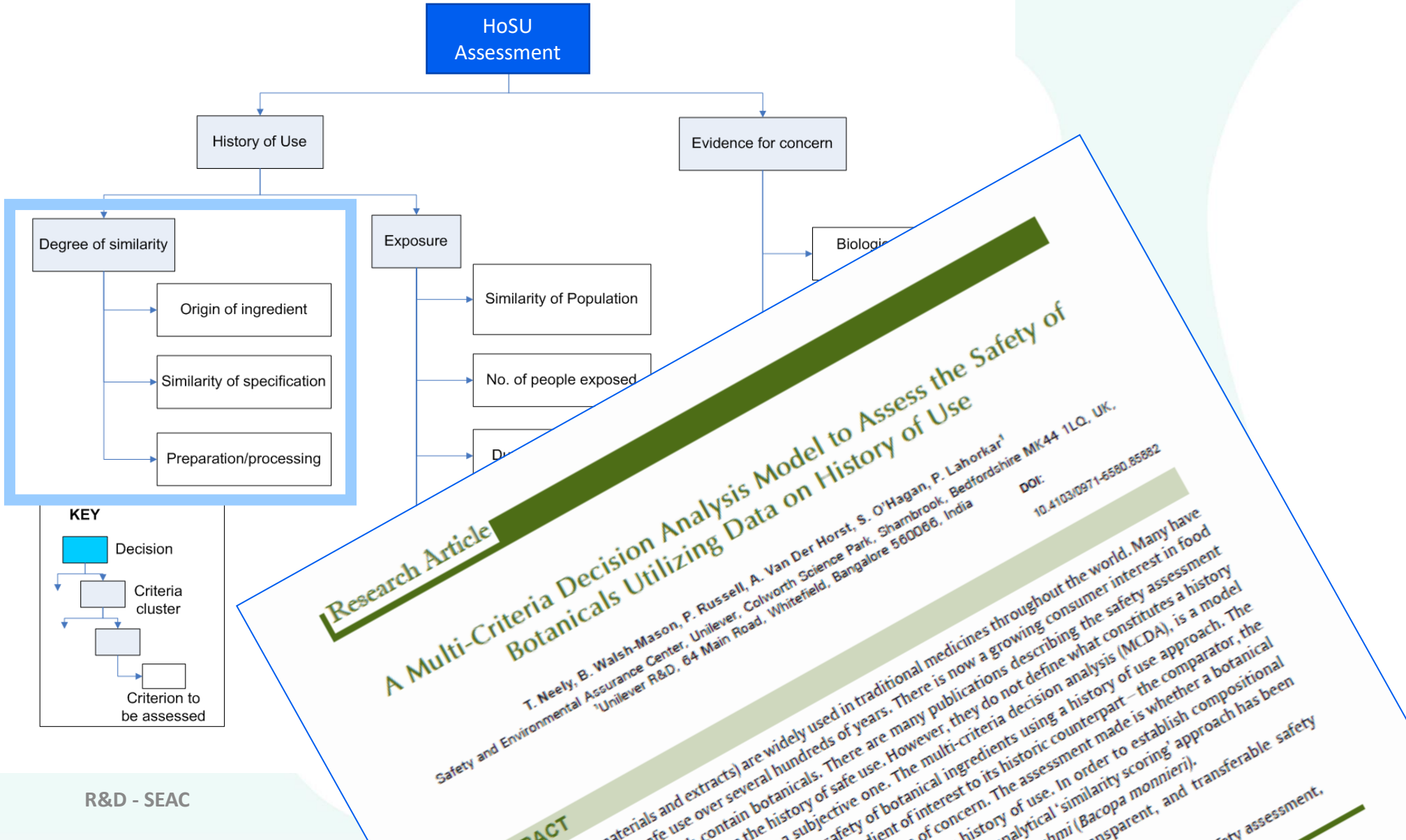
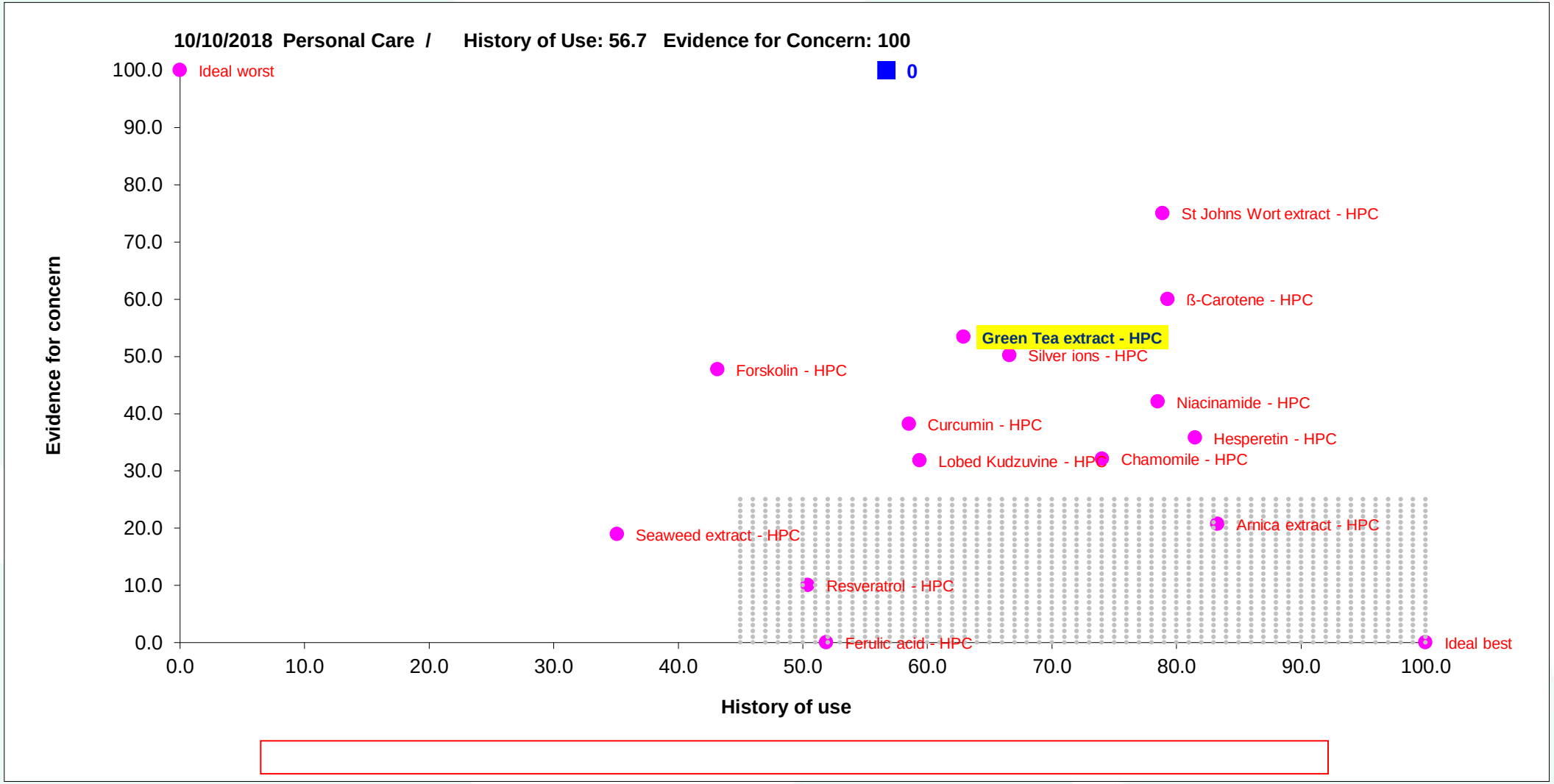
Green Tea – Composition analysis



History of Safe Use (HoSU)



Benchmarking the output – Unilever HoSU model



Risk assessment outcome – Green Tea Extract

- Not supported for the desired use scenario based on high evidence of concern
  - High catechin levels associated with skin sensitisation

- Further hazard and exposure data would be required to refine the assessment
  - In vitro assays to assess sensitisation hazard
  - Skin penetration measurement/prediction

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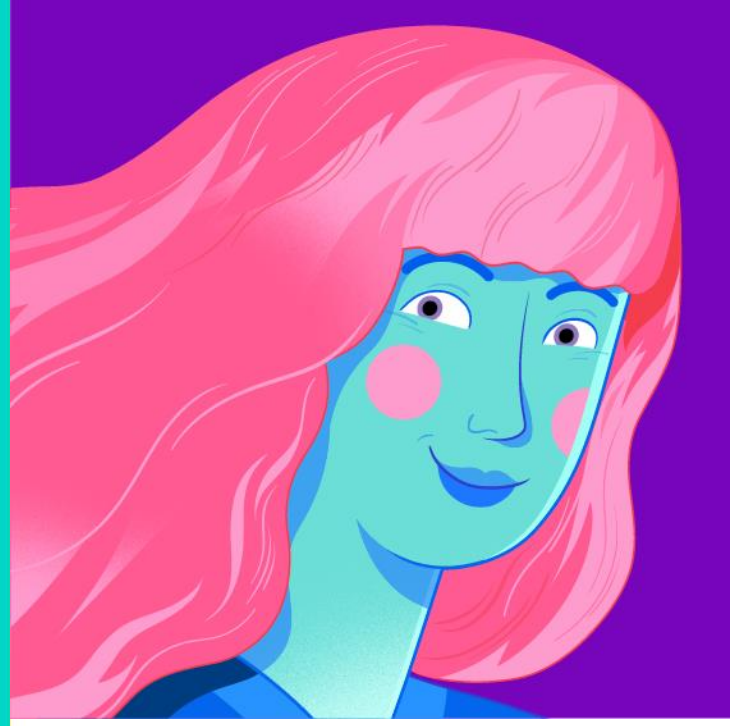
PART TWO

Next Generation Risk Assessment (NGRA): concepts and tools



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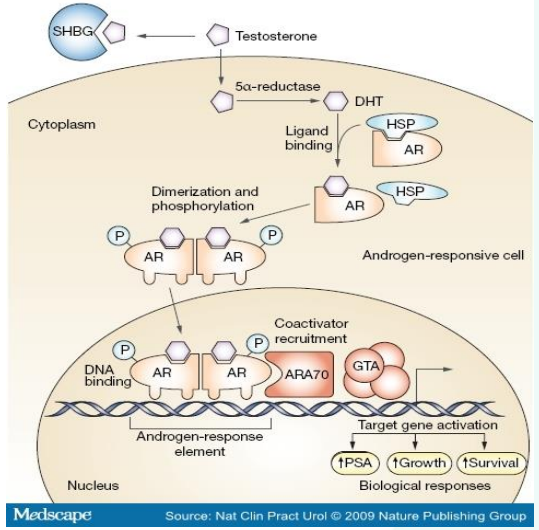
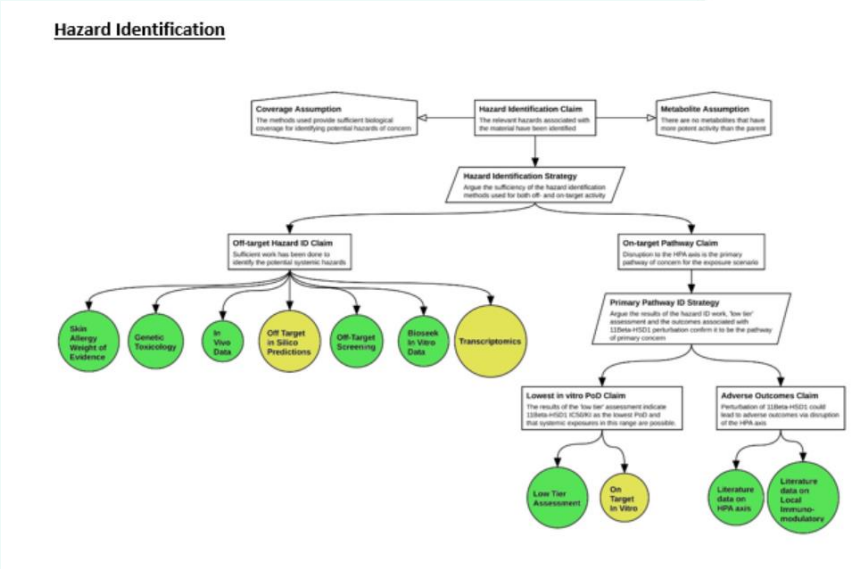
Next Generation Risk assessment (NGRA)

What is NGRA?

- Using new tools and approaches (NAMs – New Approach Methods) to build a risk assessment to enable decisions to be made
- An exposure-led risk assessment solution to biological pathway-indicated hazard concerns



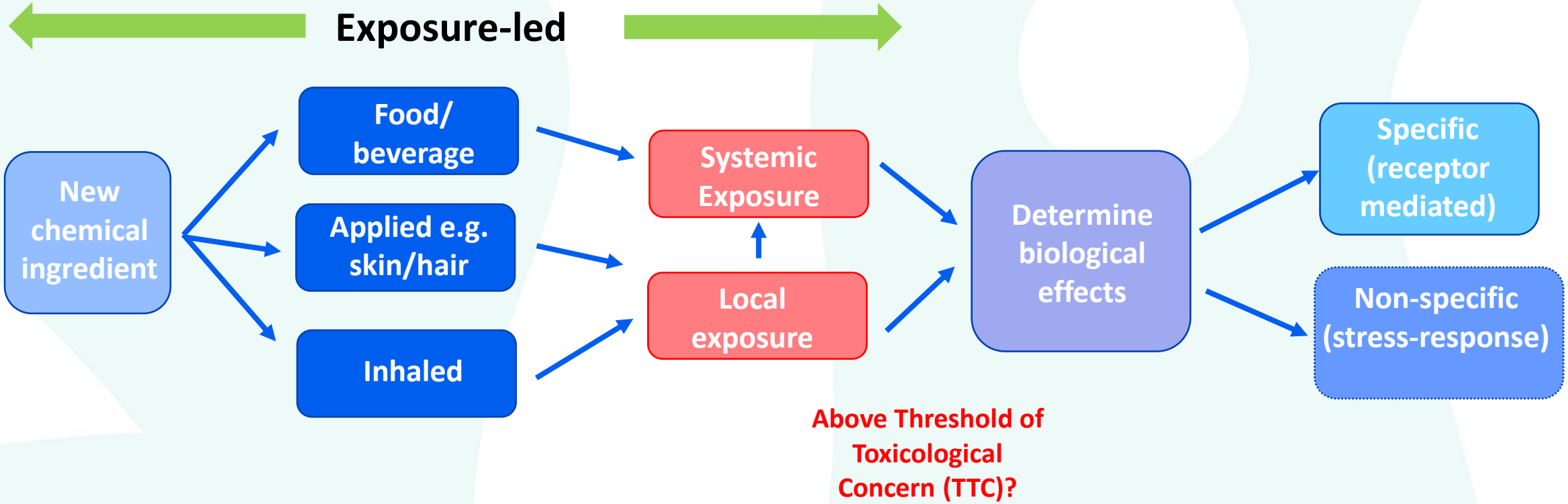


Exposure led

Mechanistic

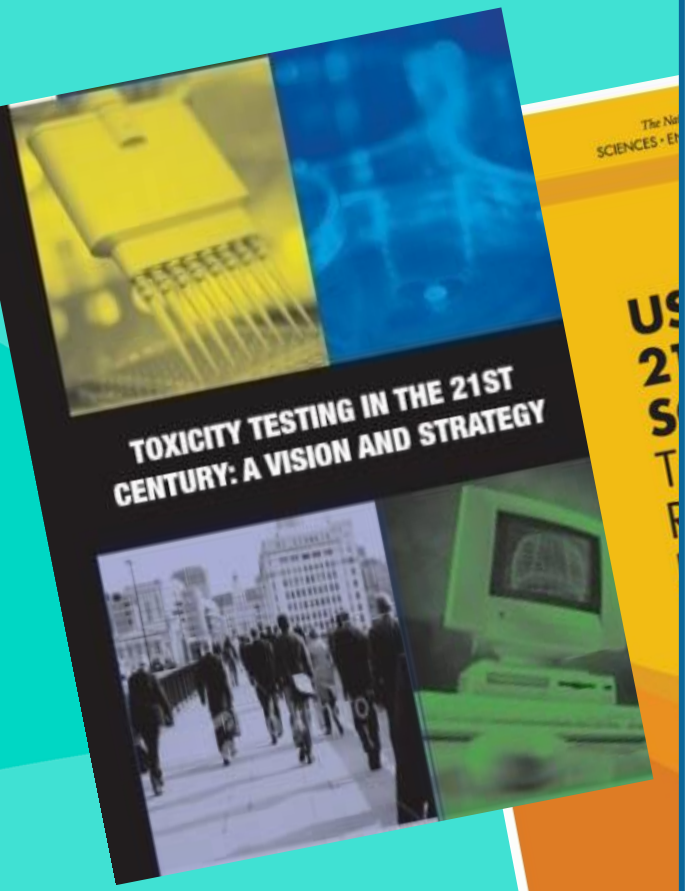
Hypothesis driven

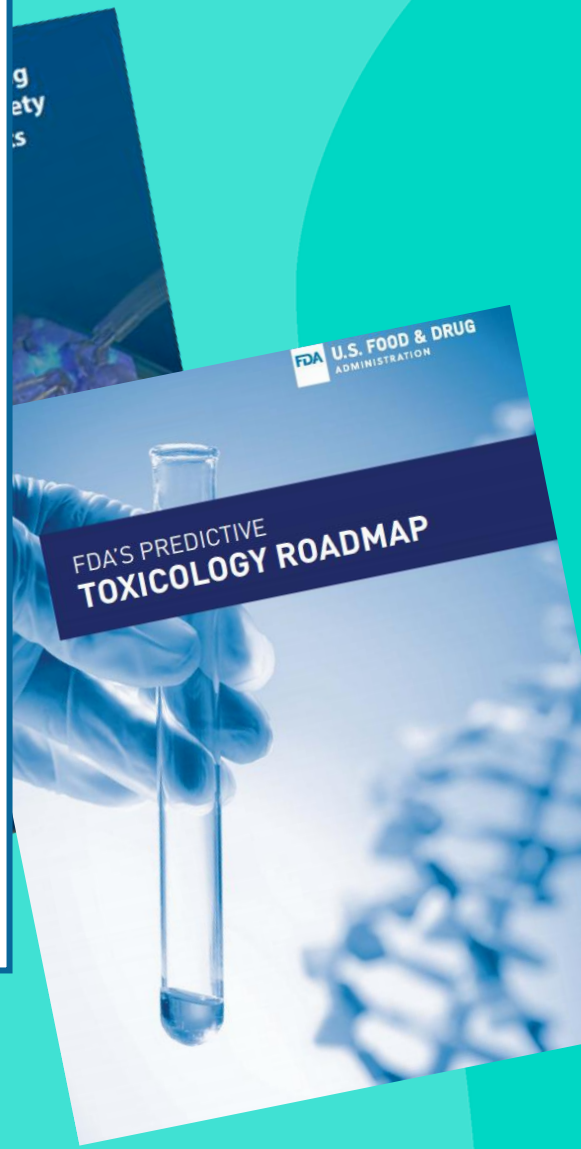
NGRA is an Exposure-led approach



2007 →

"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity **testing from a system based on whole-animal** testing **to one founded primarily on *in vitro* methods** that evaluate changes **in biologic processes using cells, cell lines, or cellular components, preferably of human origin**."





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ICCR Nine principles of NGRA

Main overriding principles:

4

- 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:

3

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

Principles for documenting NGRA:

2

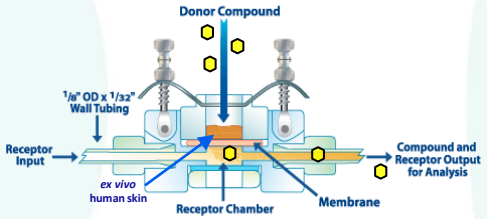
- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparent and documented

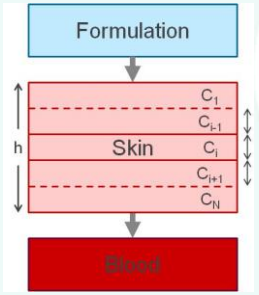
Dent et al, Computational Toxicology (2018) 7, 20-26

NGRA: The assessment is exposure-led

- **Route of exposure**
- **Consumer use (Habits & Practices)**
- **Applied dose (external concentration)**

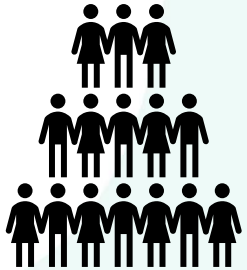
ADME parameters



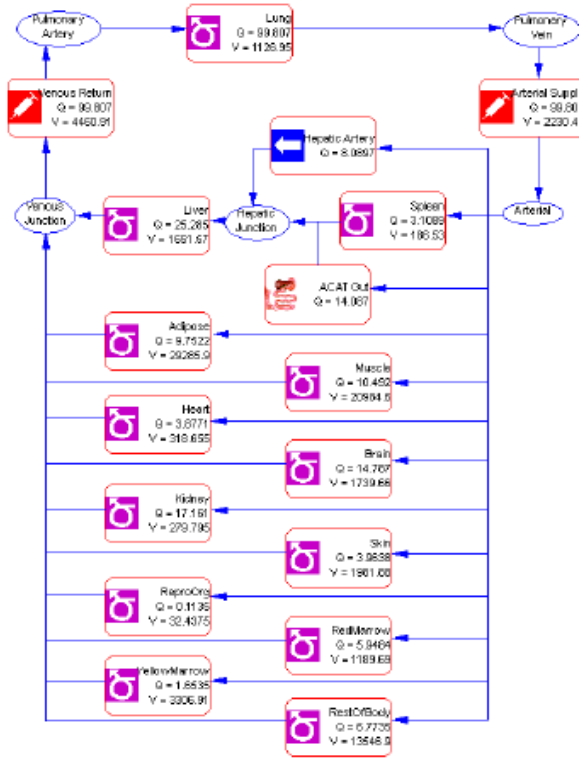


- **Skin penetration**
- **Phys-chem properties**
- **Hepatic clearance**
- **Fraction unbound**
- **blood:plasma ratio**

Uncertainty analysis- Population simulation



Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



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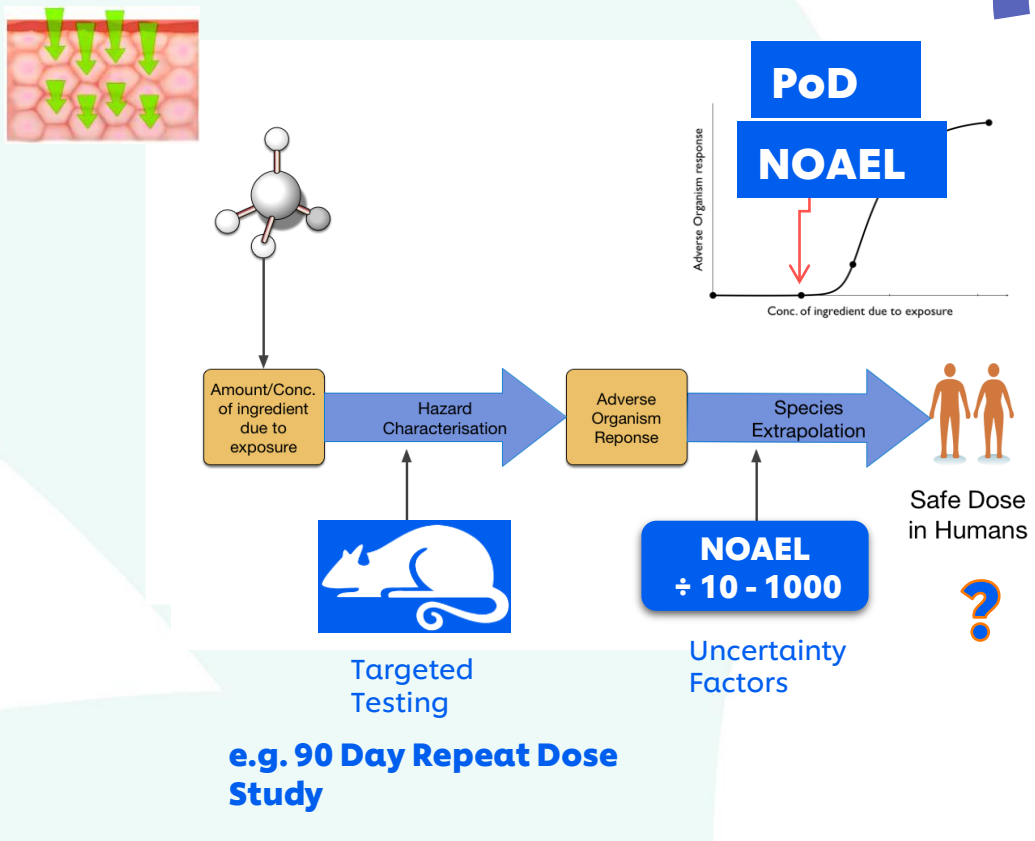
NGRA: the margin of safety (MoS) approach and decision making

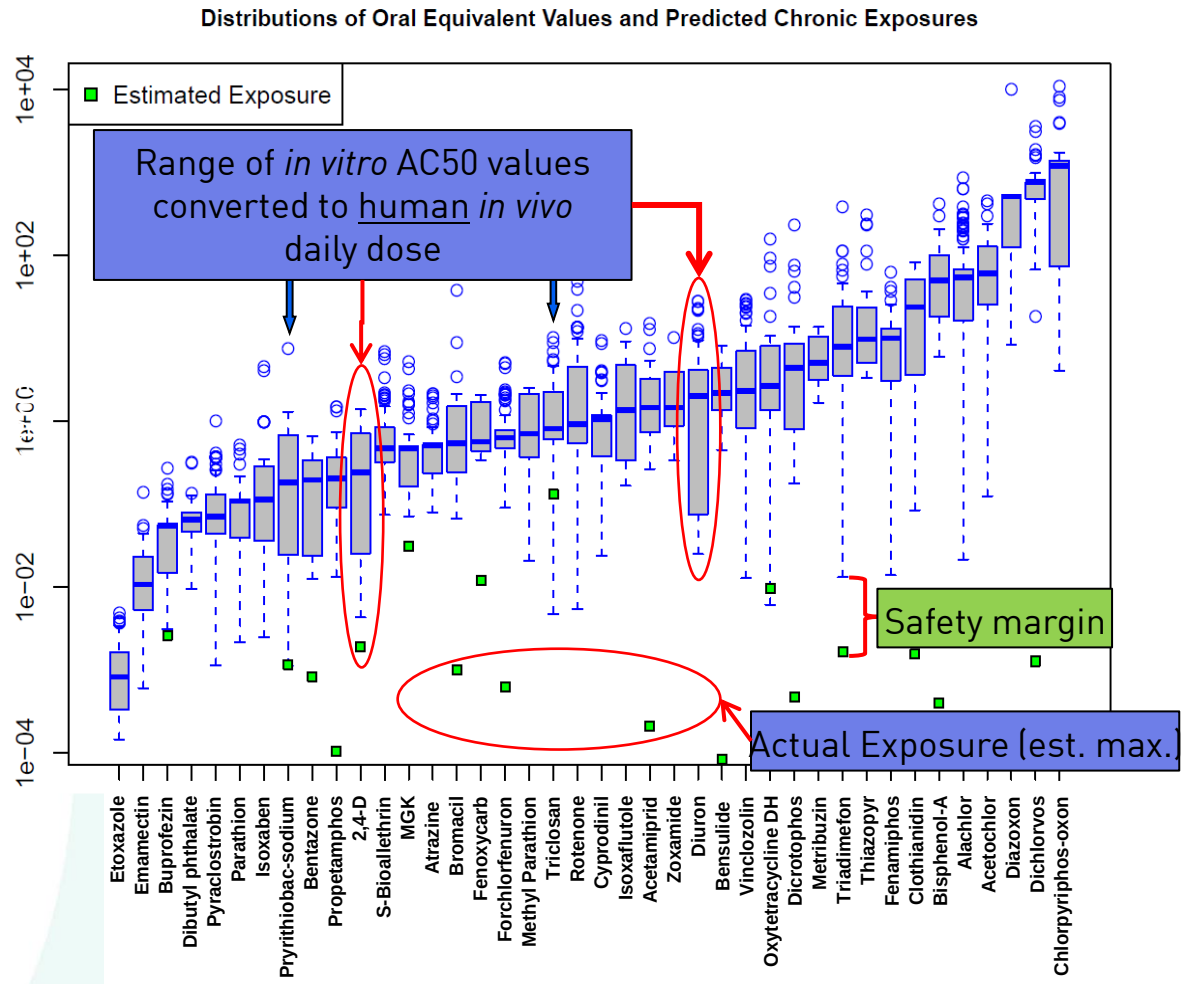
Is it safe?



e.g. 90 Day Repeat Dose Study

PoD – Point of Departure

NOAEL – No Observed Adverse Effect Level



Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

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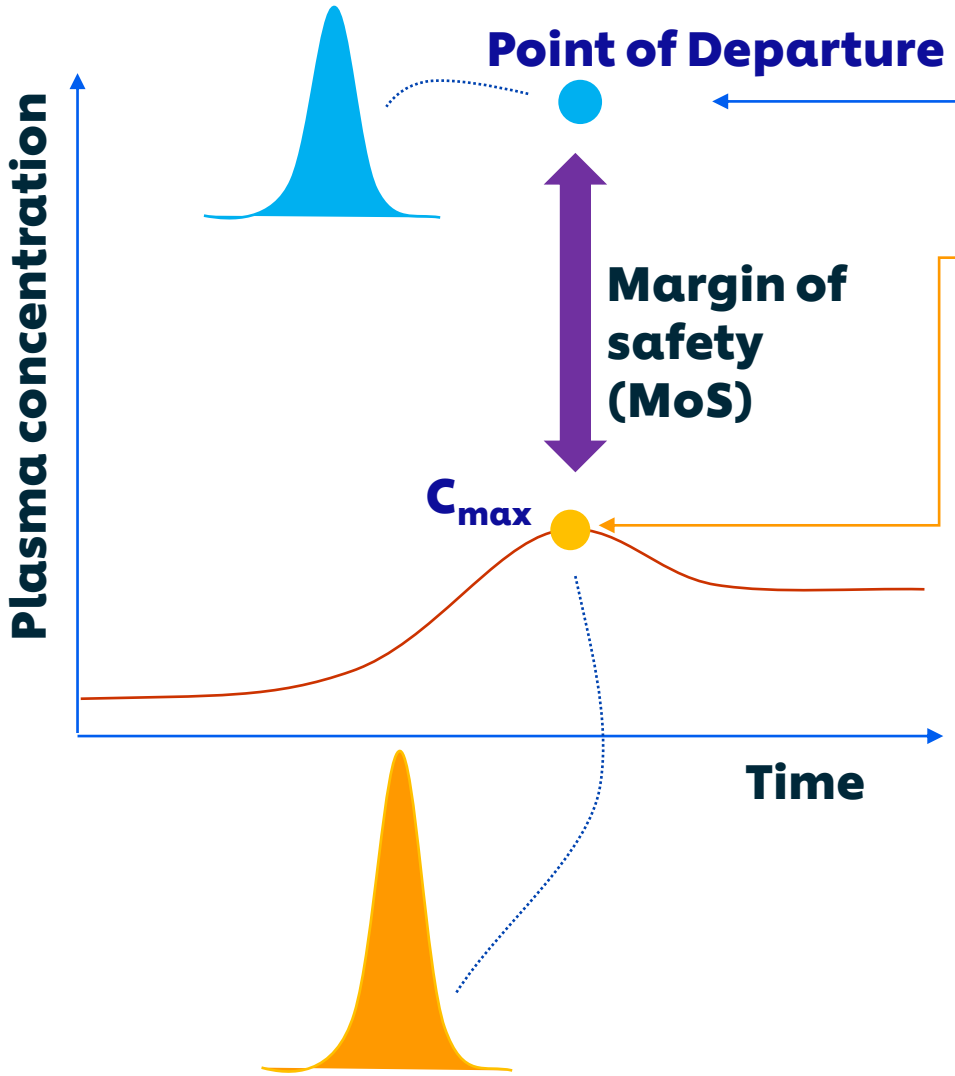
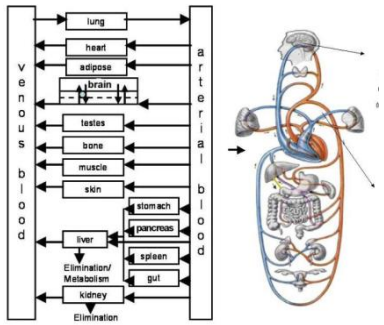
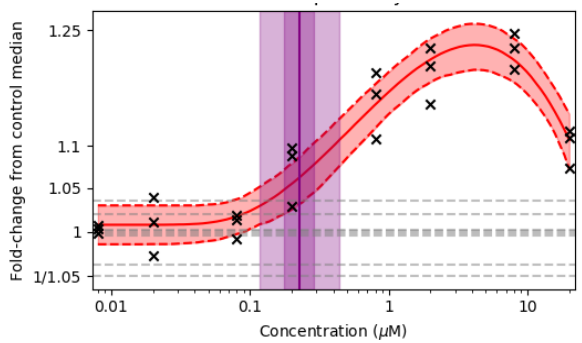
Margin of Safety



Exposure models (PBK, free/total concentration)

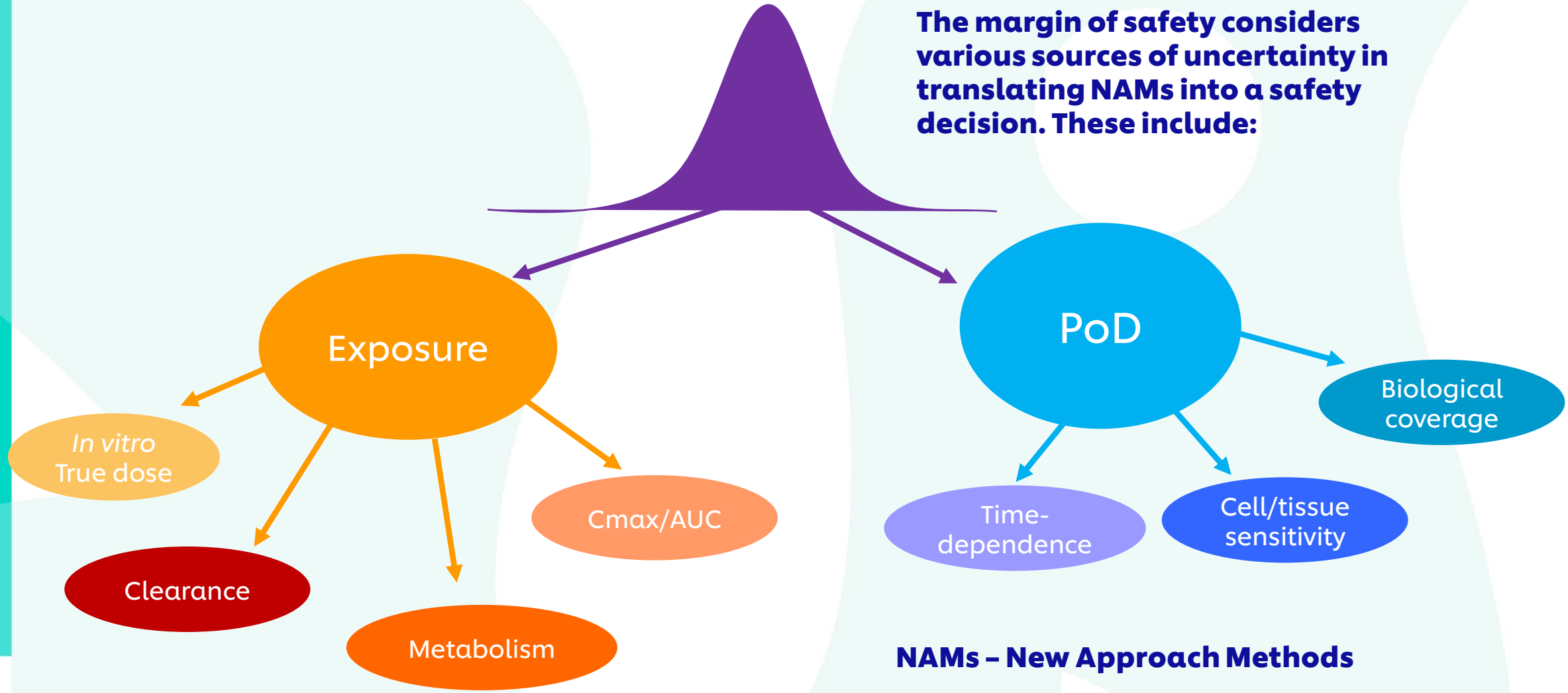


PoD derived from *in vitro* concentration-response



NGRA: Sources of uncertainty should be characterized and documented

The margin of safety considers various sources of uncertainty in translating NAMs into a safety decision. These include:



NAMs – New Approach Methods

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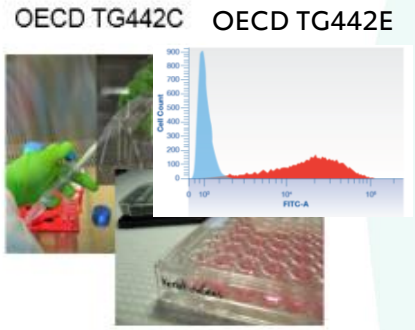
NGRA: Using relevant methods to test hypotheses

Established Methods

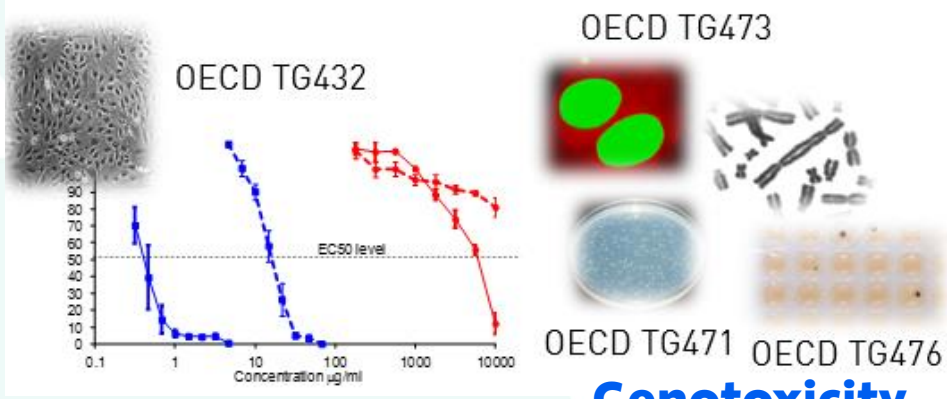
New Approach Methods (NAMs)

OECD test methods

Skin and eye irritation



Skin sensitisation

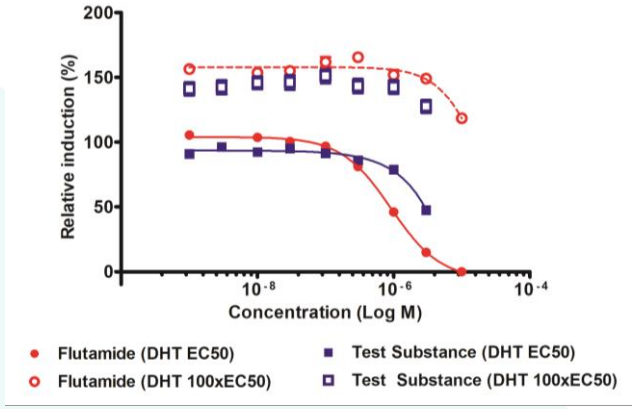


Phototoxicity

Genotoxicity

Receptor-binding assays

e.g. AR-CALUX® assay to measure androgen receptor activity

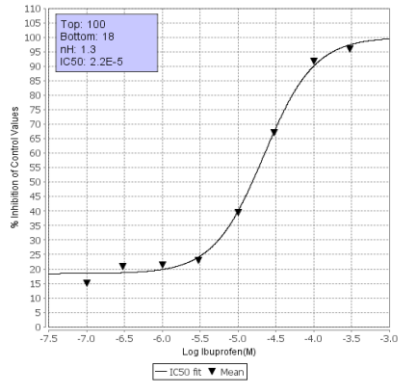


Dent et al (2019), Toxicological Science, 167, 375-384


PERSPECTIVES

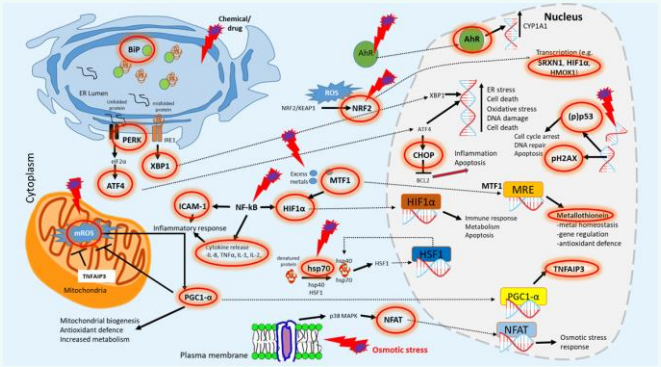
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Ibuprofen – Cox-1.



Biological activity characterisation using NAMs

Cellular stress



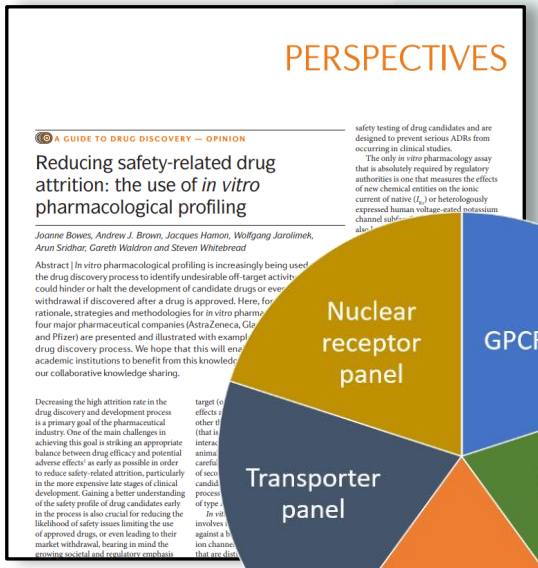
cyprotex AN EVOTEC COMPANY

Image kindly provided by Paul Walker (Cyprotex)

36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

Hatherell et al (2020), Toxicological Sciences, 176, 11-33

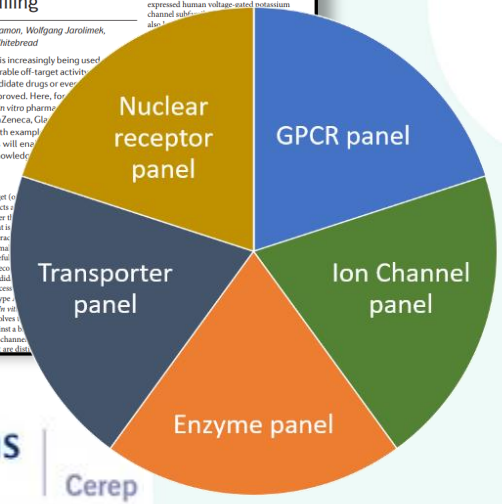
Receptor-binding assays


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

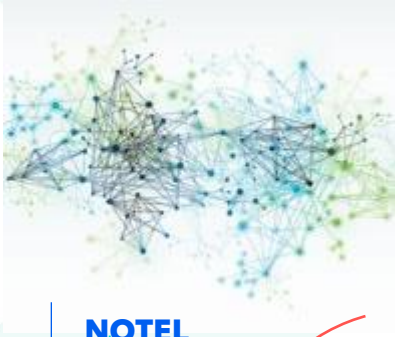
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

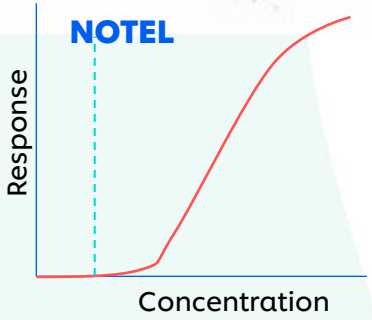
Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread



eurofins | Cerep

High throughput transcriptomics





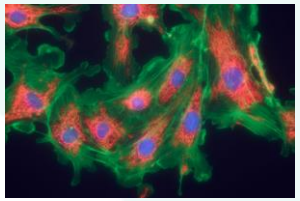
Mechanism based gentox assessment

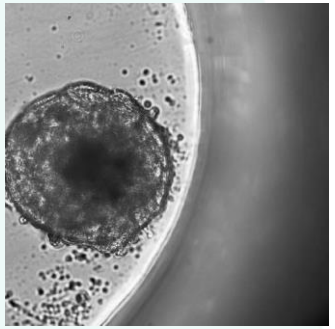
toxys®

ToxTracker®

DNA Damage
P53 Binding
Oxidative Stress
Protein Damage

Advanced cell systems and microtissues





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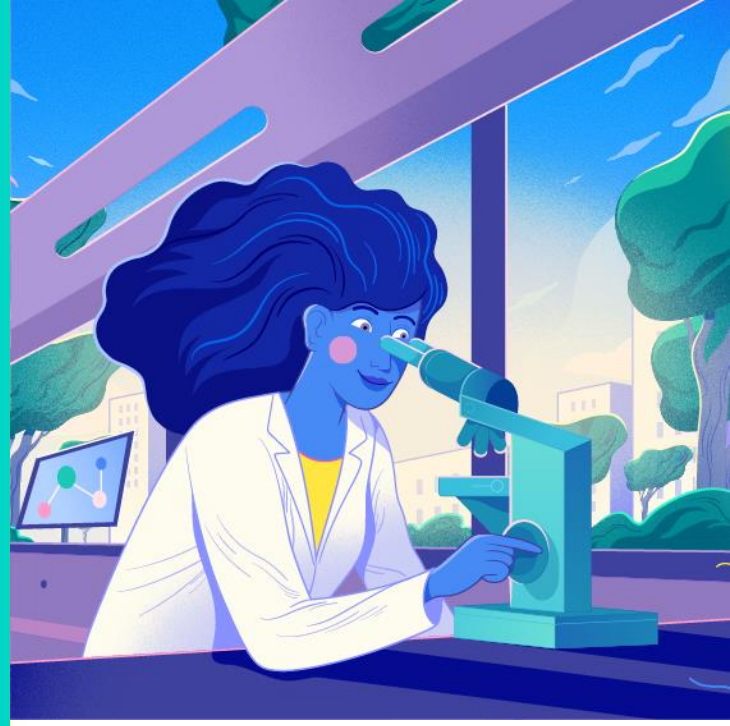
PART THREE

Case Study Examples

1) SYSTEMIC EFFECTS



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Case study example

Baltazar *et al*. (2020) A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Toxicological Sciences, 176, 236-252

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

TOXICOLOGICAL SCIENCES, 176(1), 2020, 236–252

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

Maria T. Baltazar,[1] Sophie Cable, Paul L. Carmichael, Richard Cubberley, Tom Cull, Mona Delagrange, Matthew P. Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Hequn Li, Mi-Young Lee, Sophie Malcomber, Alistair M. Middleton, Thomas E. Moxon, Alexis V. Nathanail, Beate Nicol, Ruth Pendlington, Georgia Reynolds, Joe Reynolds, Andrew White, and Carl Westmoreland

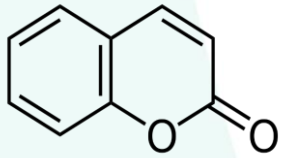
Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK

[1]To whom correspondence should be addressed. Fax: +44(0)1234 264 744. E-mail: maria.baltazar@unilever.com.

ABSTRACT

Next-Generation Risk Assessment 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. These principles were applied to a hypothetical safety assessment of 0.1% coumarin in face cream and body lotion. For the purpose of evaluating the use of NAMs, existing animal and human data on coumarin were excluded. Internal concentrations (plasma C_{max}) were estimated using a physiologically based kinetic model for dermally applied coumarin. Systemic toxicity was assessed using a battery of *in vitro* NAMs to identify points of departure (PoDs) for a variety of biological effects such as receptor-mediated and immunomodulatory effects (Eurofins SafetyScreen44 and BioMap Diversity 8 Panel, respectively), and general bioactivity (ToxCast data, an *in vitro* cell stress panel and high-throughput transcriptomics). In addition, *in silico* alerts for genotoxicity were followed up with the ToxTracker tool. The PoDs from the *in vitro* assays were plotted against the calculated *in vivo* exposure to calculate a margin of safety with associated uncertainty. The predicted C_{max} values for face cream and body lotion were lower than all PoDs with margin of safety higher than 100. Furthermore, coumarin was not genotoxic, did not bind to any of the 44 receptors tested and did not show any immunomodulatory effects at consumer-

0.1% COUMARIN IN FACE CREAM (NEW FRAGRANCE)



Assumptions:

- EU Market
- 100% purity
- no in vivo data was available such as animal data, History of Safe Use (HoSU) or Clinical data
- no use of animal data in Read Across
- In silico alerts known to be based on animal or in vivo data or on the structure of Coumarin itself were excluded

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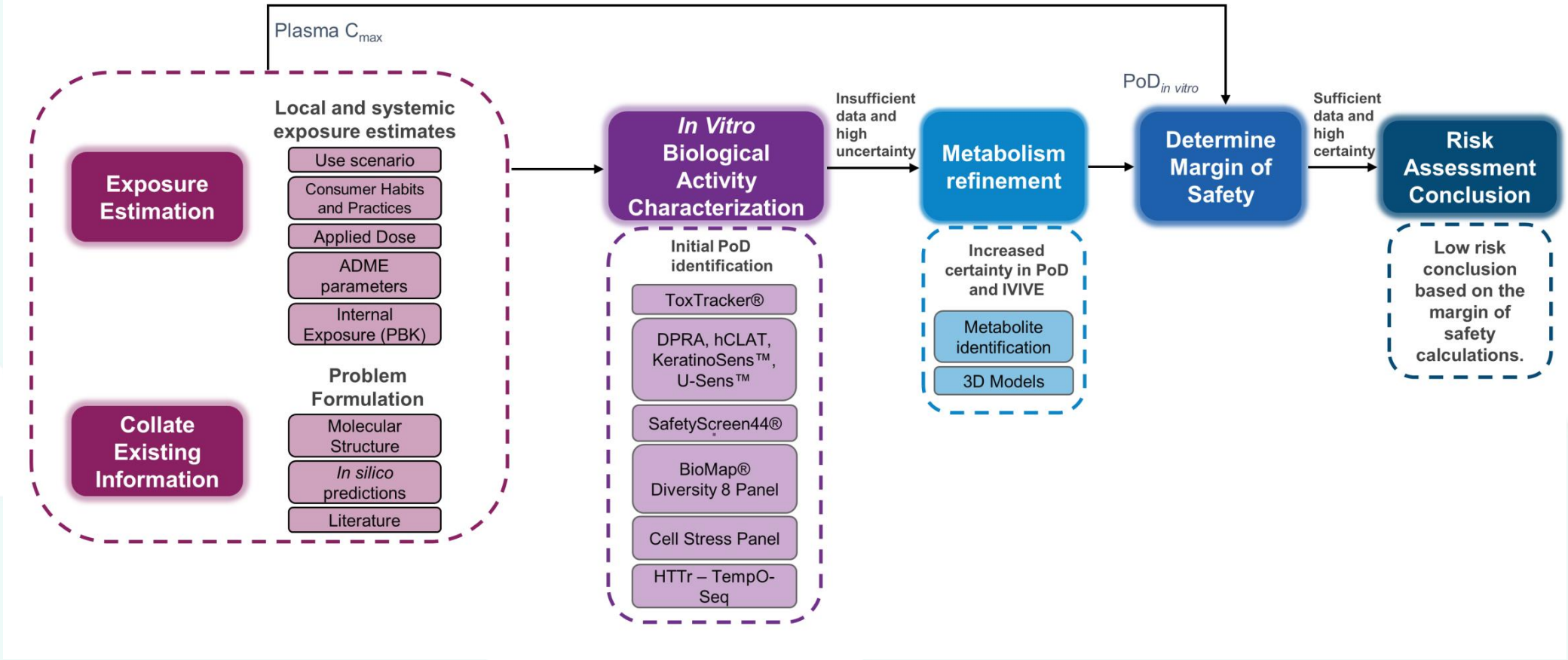
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



Baltazar *et al., Toxicological Sciences, Volume 176, Issue 1, July 2020, Pages 236–252*
https://doi.org/10.1093/toxsci/kfaa048

STEP ONE

Exposure information and collation of existing information

NGRA for 0.1% coumarin in face cream: exposure estimation

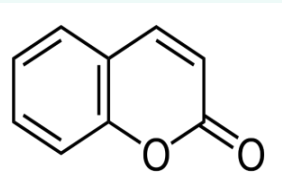
Creme GLOBAL

Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNFP/0321/00; Hall *et al.*, 2007, 2011).

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap [2]	20.00 g	-	0.01	0.20 [3]	3.33
Hair care					
Shampoo	10.46 g		0.01	0.11	1.51
Hair conditi...				0.04	0.60

B. Hall et al./Food and Chemical Toxicology 49 (2011) 408–422

Assessment is exposure-led and uses available habits and practices data



Parameter	Face cream
Amount of product used per day (g/day) using 90th percentile	1.54
Frequency of use	2 times/day
Amount of product in contact with skin per occasion (mg)	770
Ingredient inclusion level	0.1%
Skin surface area (cm2)	565
Exposure duration per occasion	12 hours
Amount of ingredient in contact with skin per occasion (mg)	0.77
Local dermal exposure per occasion (µg/cm2)	1.36
Systemic exposure per day (mg/kg)	**0.02**

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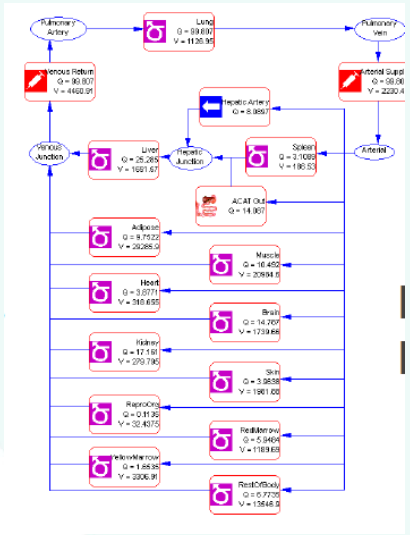
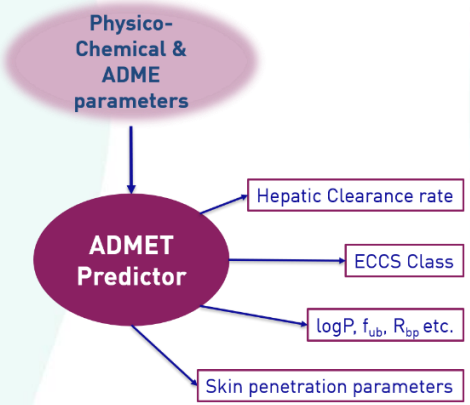
NGRA for 0.1% coumarin in face cream: exposure estimation- Internal concentration using PBK modelling - Model Inputs

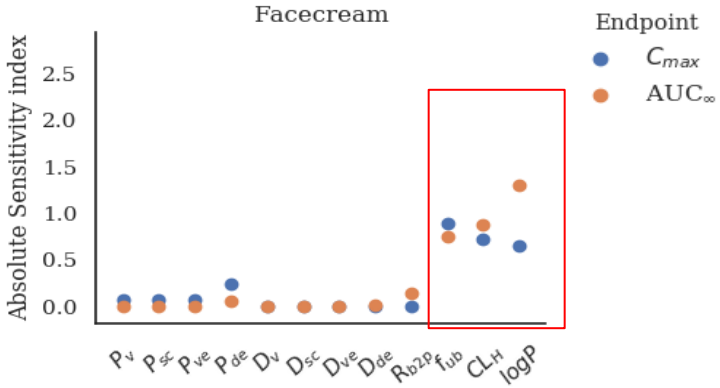
GastroPlus® (Simulations Plus)



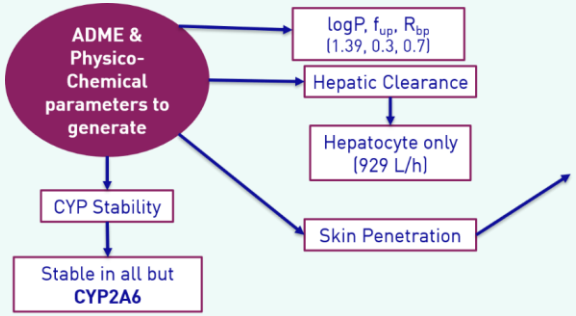
Use *in silico* parameters for modelling



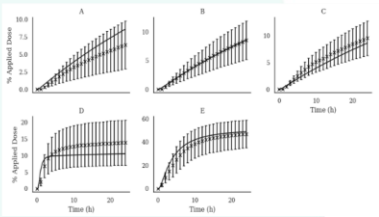
Sensitivity analysis



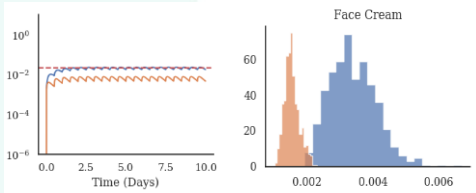
Experimental Refinement



Skin absorption study



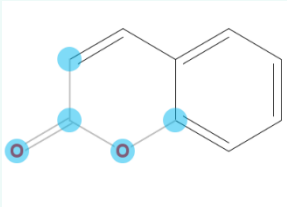
Exposure distribution



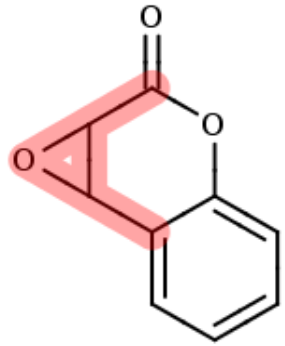
Total Plasma C_{max} (µM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

NGRA for 0.1% coumarin in face cream: in silico predictions



Generation of hypothesis for potential Molecular Initiating events –**ToxTree, MIE ATLAS*, OECD toolbox**



Initial Hypothesis

Next case study

- **Coumarin** might **bind to proteins- MIE for induction of skin sensitisation**
- **DNA binding alert + epoxide formation MIE for genotoxicity**
- **Reactive metabolites might be formed with alerts for both genotoxicity and skin sensitisation**
- **No binding alerts for the 39 targets in MIE atlas**

*Allen THE et al., 2018. Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events. Toxicol Sci. 2018 Sep 1;165(1):213-223

NGRA for 0.1% coumarin in face cream: *in vitro* existing information

Identification of potential biological targets – **PubChem and ToxCast**

Only few active assays among multiple assays (≈ 5000)

Coumarin inhibited both Monoamine oxidases and Carbonic anhydrases at concentrations between 3 µM- 40 µM

The AC50 from dose-response curves was used a PoD for MoS calculation

*AC50= activity concentration at 50% of maximal activity

NGRA for 0.1% coumarin in face cream: exposure estimation

Exposure Estimation

- Total plasma Cmax values obtained from PBK model: 0.002 µM (mean), 0.005 µM (99th percentile)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- *Genotoxicity and skin sensitisation alerts for parent compound*
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

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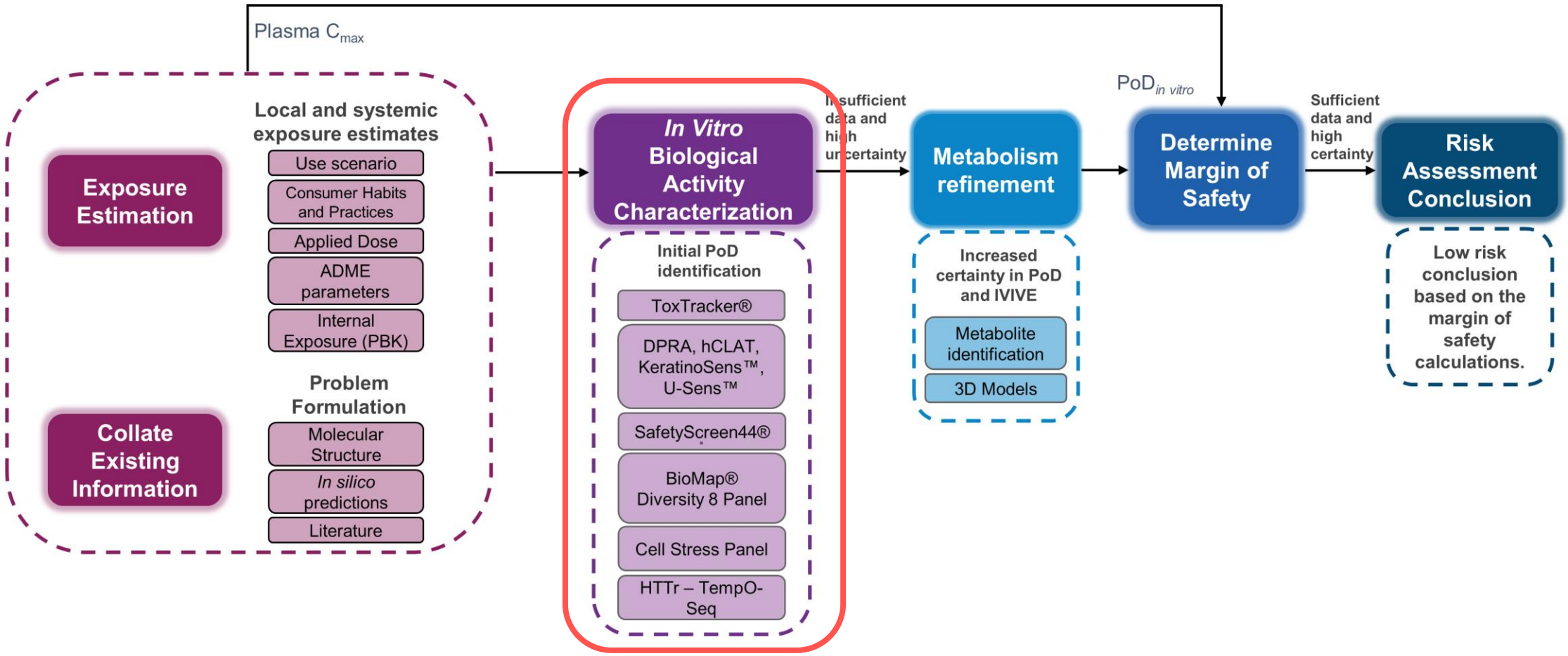
STEP TWO

In vitro biological activity characterisation

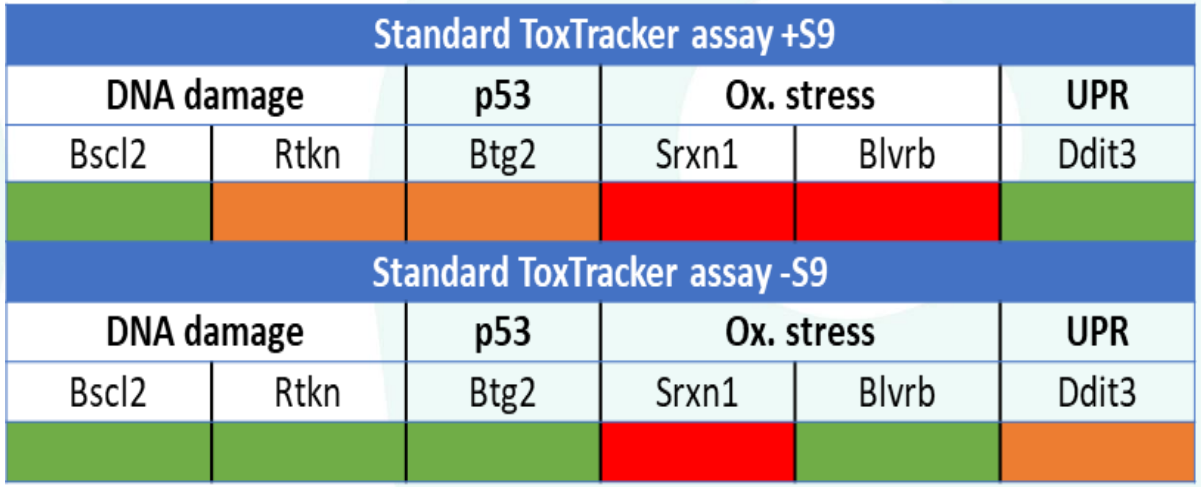
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: Genotoxicity assessment: ToxTracker

Initial hypothesis:

- **DNA binding alerts for coumarin and metabolites**



Results:

- **ToxTracker negative**
- **Reactive coumarin metabolite(s) could induce DNA lesions secondary to oxidative stress**

NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro binding and enzymatic assays – Eurofins SafetyScreen44

To investigate possible interactions between coumarin and the 44 key targets involved in drug attrition

PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

Abstract | *In vitro* pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in our collaborative knowledge sharing.

Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is striking an appropriate balance between drug efficacy and potential adverse effects[1] as early as possible in order to reduce safety-related attrition, particularly in the more expensive late stages of clinical development. Gaining a better understanding of the safety profile of drug candidates early in the process is also crucial for reducing the likelihood of safety issues limiting the use of approved drugs, or even leading to their market withdrawal, bearing in mind the growing societal and regulatory emphasis

target (or targets), whereas secondary effects are due to interactions with targets other than the primary target (or targets) (that is, off-target interactions). Off-target interactions are often the cause of ADRs in animal models or clinical studies, and so careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help to reduce the incidence of type A ADRs.

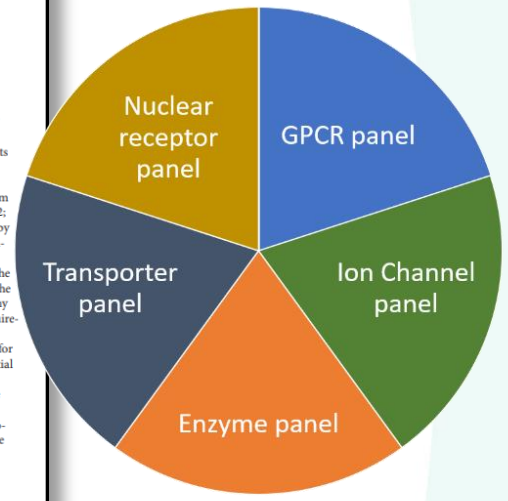
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

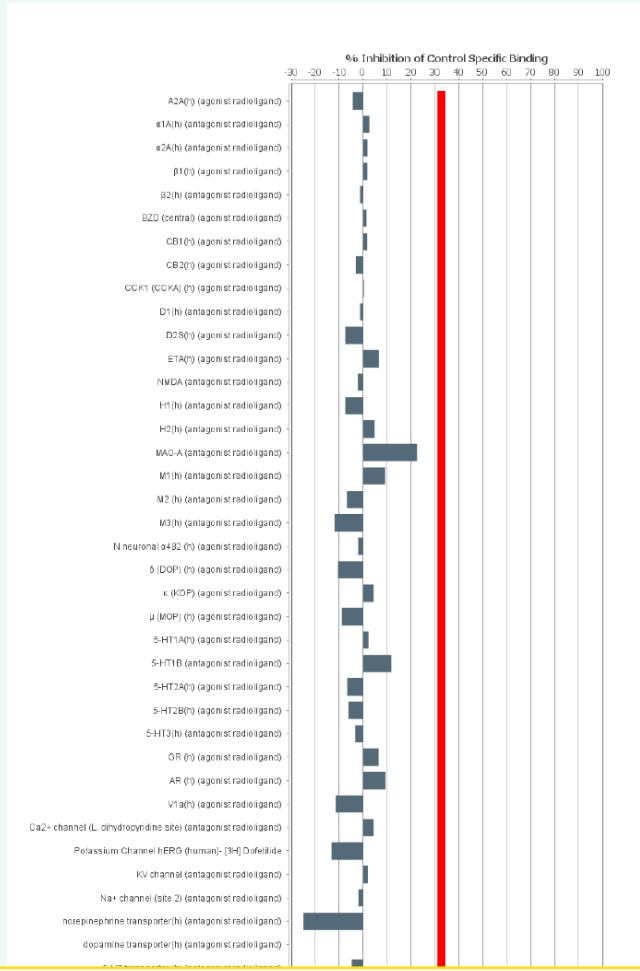
The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[2]. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized[2,3], and the seriousness of this ADR is one reason why this assay is a mandatory regulatory requirement. Receptor binding studies are also recommended as the first-tier approach for the assessment of the dependence potential of novel chemical entities[4].

However, current regulatory guidance does not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the stage of the discovery process at which *in vitro* pharmacological profiling should occur. Nevertheless, the general trend for most pharmaceutical companies is to perform this testing early in drug discovery to reduce attrition and to facilitate better prediction of ADRs in the later stages of drug discovery and development.

Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experiences of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe the rationale and main advantages for the use of *in vitro* pharmacological profiling, to discuss best practices and to



eurofins



Results:

All binding and enzymatic assay results were negative at 10 μM

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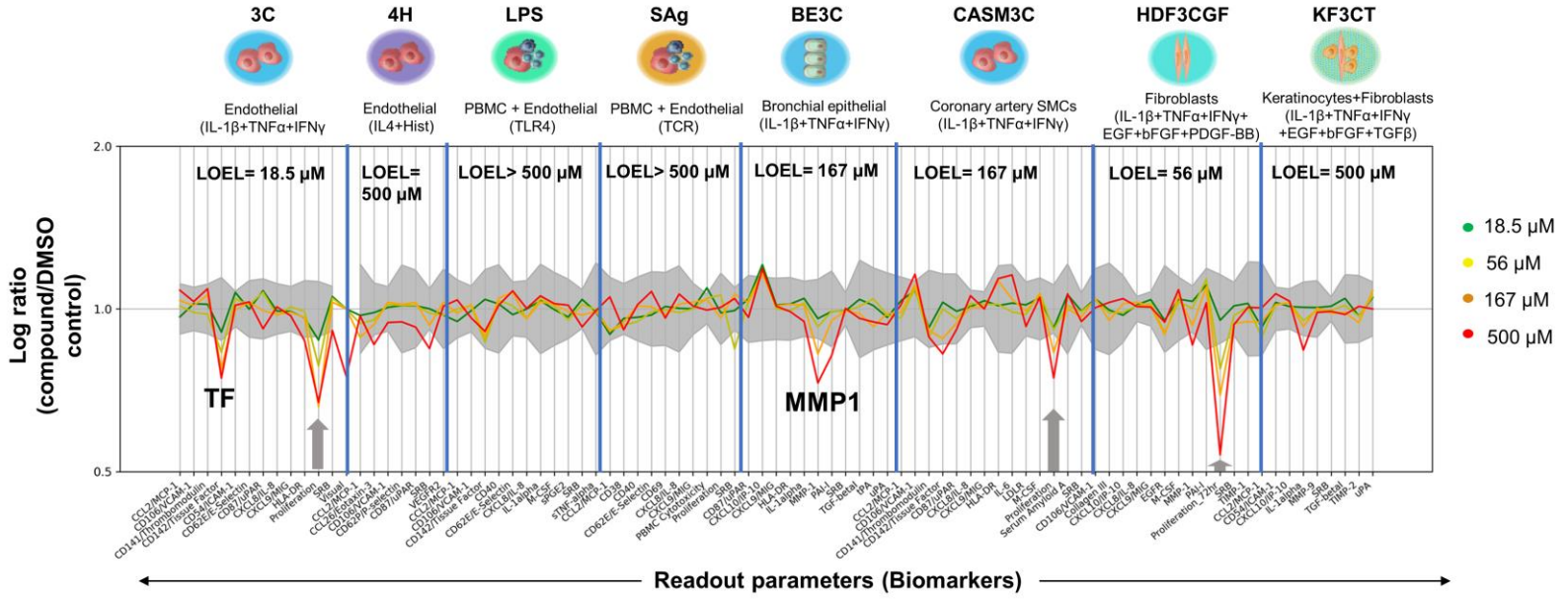
NGRA for 0.1% coumarin in face cream: biological activity characterisation: Immunomodulatory screening assay - BioMap Diversity 8 Panel

To investigate possible effects on vascular inflammation, immune activation and tissue remodelling

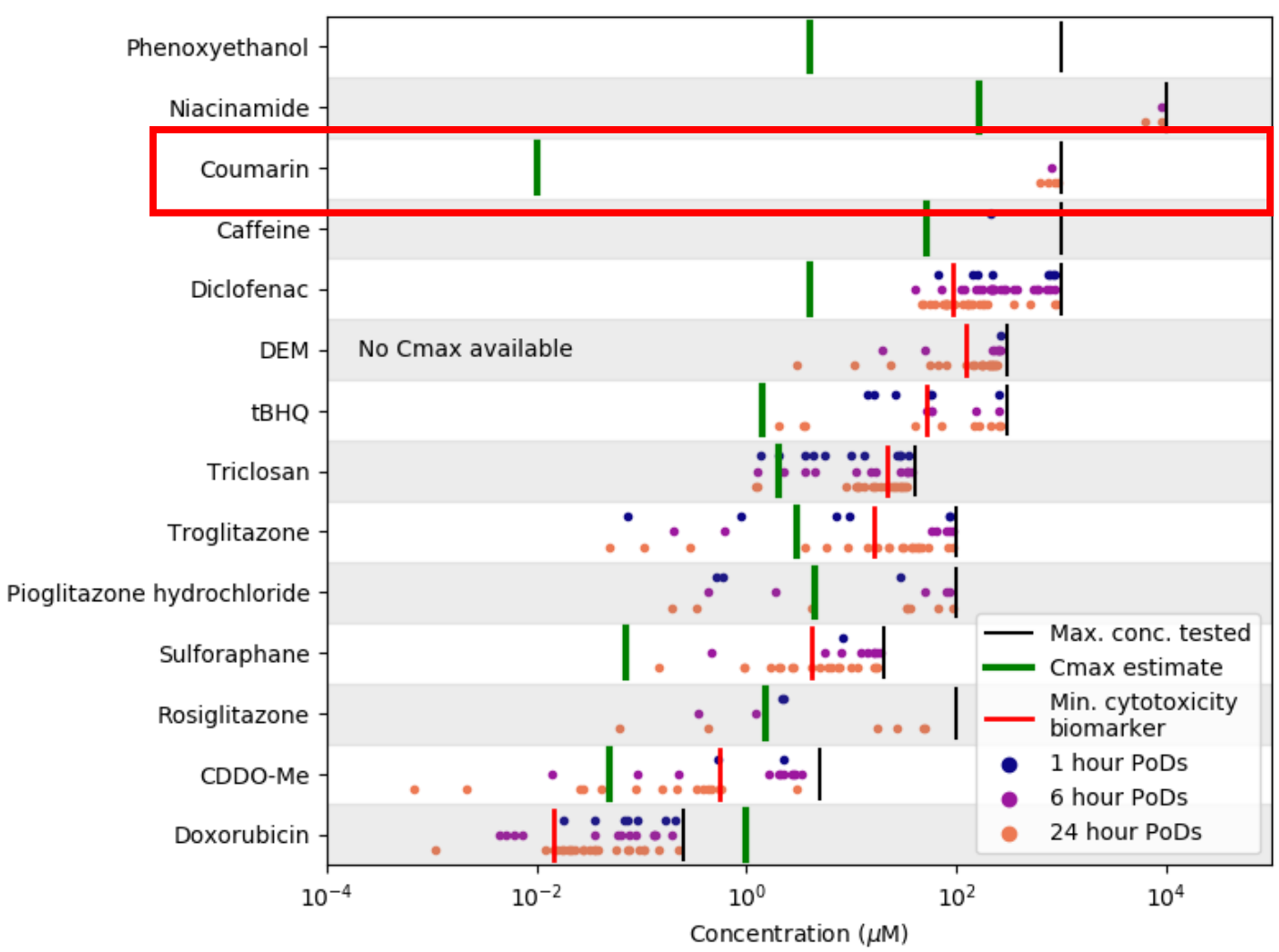


Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound

https://www.discoverx.com/services/drug-discovery-development-services/primary-cell-phenotypic-profiling/diversity-plus

NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro cell stress panel



Results:

Coumarin not very active in comparison to known "high risk compounds" like doxorubicin

- PoDs shown for HepG2 only

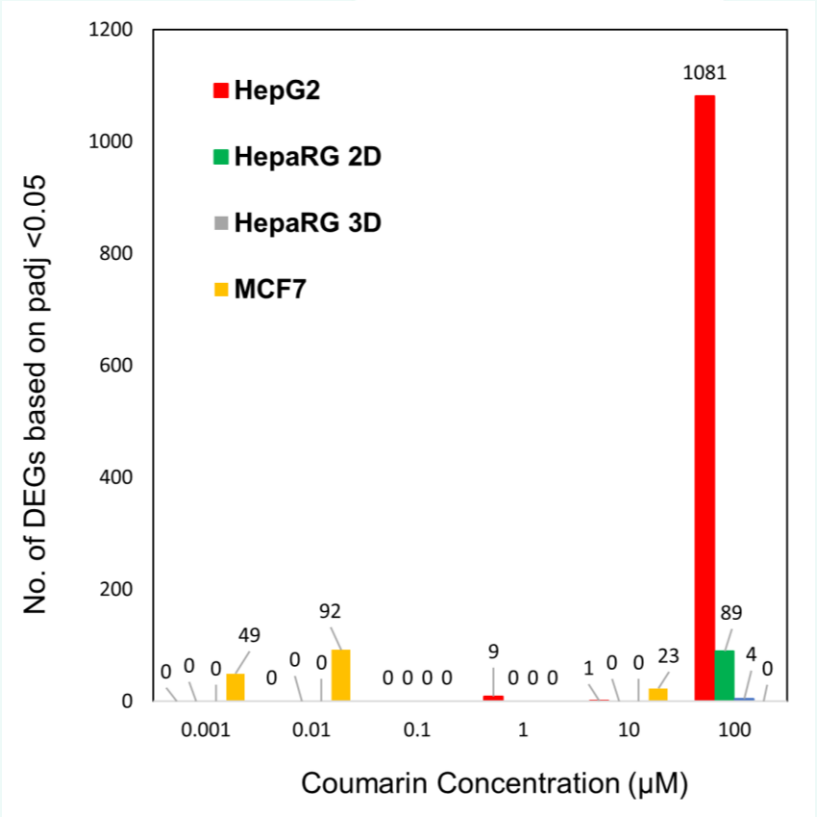
Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. in Press. https://doi.org/10.1093/toxsci/kfaa054

NGRA for 0.1% coumarin in face cream: *In vitro* biological activity characterisation:

High-Throughput Transcriptomics (HTTr) using TempO-SEQ technology

Transcriptomics was applied as a broad non-targeted biological screen

Differential expression analysis using DESeq2 analysis



Results:

Across the cell lines, treatment with coumarin resulted in limited gene-expression changes at concentrations below 100 µM, suggesting limited cellular effects at lower concentrations

NGRA for 0.1% coumarin in face cream: Key results

Exposure Estimation

- Total plasma Cmax values obtained from PBK model: 0.002 µM (mean), 0.005 µM (99th percentile)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- Genotoxicity and protein binding alerts for parent compound
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

In Vitro Biological Activity Characterisation

- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- No immunomodulation potential
- Low bioactivity confirmed by binding/enzymatic assays, HTTr and cell stress panel.
- PoD range: 6-912 µM

STEP THREE

Margin of Safety

NGRA for 0.1% coumarin in face cream: Preliminary Margin of Safety

Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	PoD provided as distribution?
Cell stress panel	HepG2 (ATP, 24h)	96738	Yes
Cell stress panel	NHEK (OCR 1h)	1330	Yes
HTTr	HepG2 (24h)	7223	No
HTTr	HepaRG (24h)	8864	No
Toxcast	MAO B (rat brain)	3711	No
PubChem	Carbonic Anhydrase Type I	**706**	No
PubChem	Carbonic Anhydrase Type II	2140	No
PubChem	Carbonic Anhydrase Type VI	14652	No

Based on total concentrations for both C_{max} and PoDs

- **The lowest MoS across all assays was derived using the PoD (represented by Ki) for the inhibition of carbonic anhydrase I**
- **All PoD are higher than predicted exposure**

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NGRA for 0.1% coumarin in face cream: Key results

Exposure Estimation

- Total plasma Cmax values obtained from PBK model: 0.002 µM (mean), 0.005 µM (99th percentile)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- Genotoxicity and protein binding alerts for parent compound
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- 90-100% coumarin predicted to be freely available *in vitro*
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

In Vitro Biological Activity Characterisation

- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- No immunomodulation potential
- Low bioactivity confirmed by binding/enzymatic assays, HTTr and cell stress panel.
- PoD range: 6-912 µM
- **Potential metabolite-driven bioactivity not addressed**

Determine Margin of Safety

Preliminary MoS

706 - 96738

NGRA for 0.1% coumarin in face cream: Next steps for refinement

1. **Coumarin metabolism in primary human hepatocytes - investigation of metabolites formed in human *in vitro* liver models**
2. **Short and long-term exposure in 3D tissues - longer exposure durations in a 3D HepaRG model with potentially higher metabolic capacity and in vivo-like physiology than HepG2 cells**

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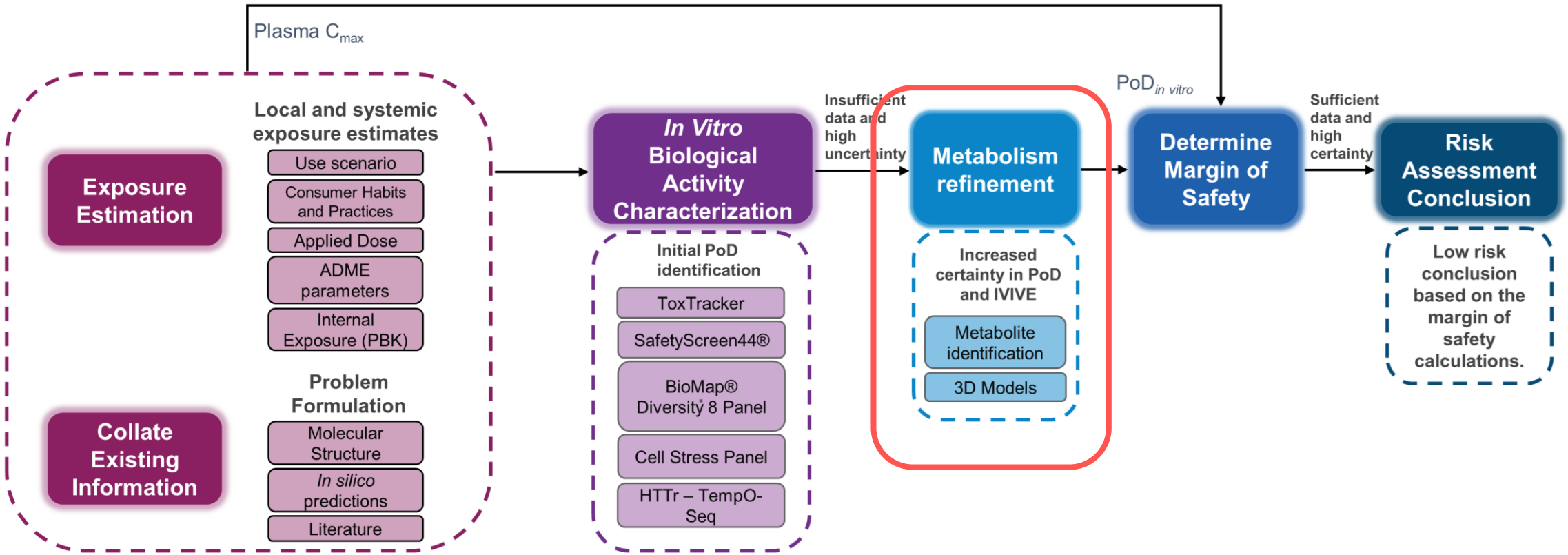
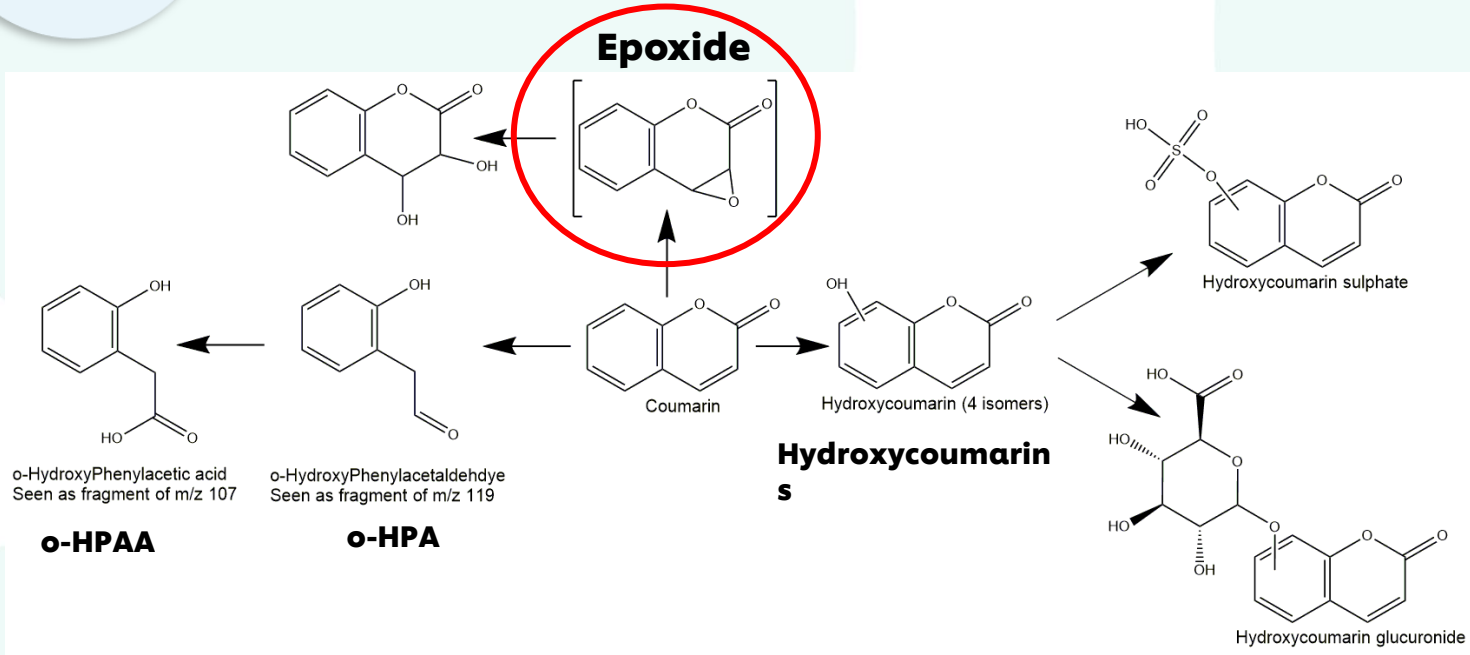
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



NGRA for 0.1% coumarin in face cream: Coumarin metabolism in primary human hepatocytes

Human *In vitro* metabolism

Metabolism study to investigate if reactive metabolites are likely to be formed at consumer relevant concentrations



Coumarin's proposed metabolic pathway based on the *in vitro* experiments.

Results:

- **Coumarin is preferentially detoxified** to hydroxycoumarins and respective glucuronides
- **Reactive metabolites** such as the epoxide, o-HPAA and o-HPA **were only detected at the highest concentration (1mM)**
- **Not expected to be formed in vivo for our consumer exposure scenario**

NGRA for 0.1% coumarin in face cream: Short and long-term exposure in 3D tissues

To increase our confidence in the initial PoDs from the 2D cell models

Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	PoD provided as distribution?
Cell stress panel	HepG2 (ATP, 24h)	96738	Yes
Cell stress panel	NHEK (OCR 1h)	1330	Yes
HTTr	HepG2 (24h)	7223	No
HTTr	HepaRG (24h)	8864	No
Toxcast	MAO B (rat brain)	3711	No
PubChem	Carbonic Anhydrase Type I	**706**	No
PubChem	Carbonic Anhydrase Type II	2140	No
PubChem	Carbonic Anhydrase Type VI	14652	No
Cell stress panel	HepaRG_3D (cell mem perm 168h)	9601	Yes
HTTr	HepaRG_3D_24h	9538	No

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NGRA for 0.1% coumarin in face cream: Key results

Exposure Estimation

- Plasma Cmax obtained (range 0.002- 0.02 µM) from PBK models (Table 2)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- Genotoxicity and protein binding alerts for parent compound
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- 90-100% coumarin predicted to be freely available *in vitro*
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

In Vitro Biological Activity Characterisation

- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- No immunomodulation potential
- Low bioactivity confirmed by binding/enzymatic assays, HTTr and cell stress panel.
- PoD range: 6-912 µM
- Potential metabolite-driven bioactivity not addressed

Metabolism refinement

- Hydroxylation confirmed as main route of biotransformation at 10 µM
- Reactive metabolites not formed at consumer relevant exposures
- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871 µM (Table 4 and 5).

Determine Margin of Safety

Updated MoS

9538- 9601

Preliminary MoS

706 - 96738

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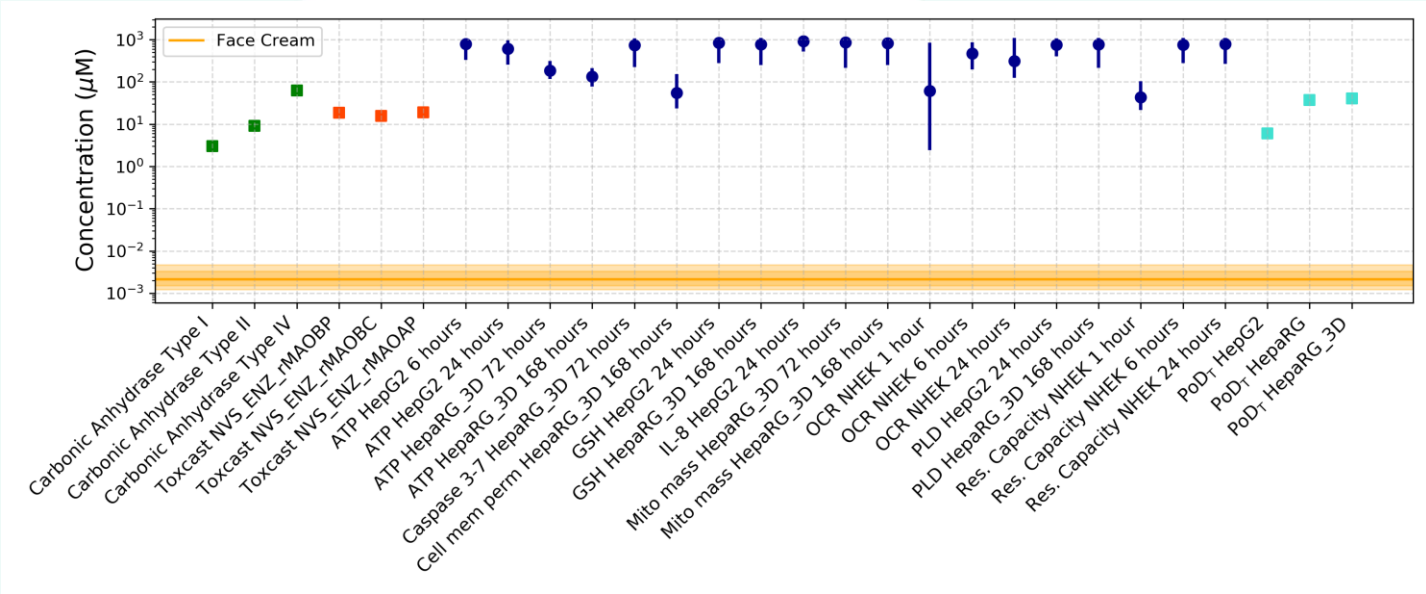
NGRA for 0.1% coumarin in face cream: Risk assessment conclusion



- The predicted C_{max} values for face cream were lower than all PoDs with a MoS (the 5th percentile) higher than 100
- Coumarin is not genotoxic, does not bind to any of the 44 targets and does not show any immunomodulatory effects at consumer relevant exposures
- **Weight of evidence suggests that the inclusion of 0.1% coumarin in face cream is safe for the consumer**

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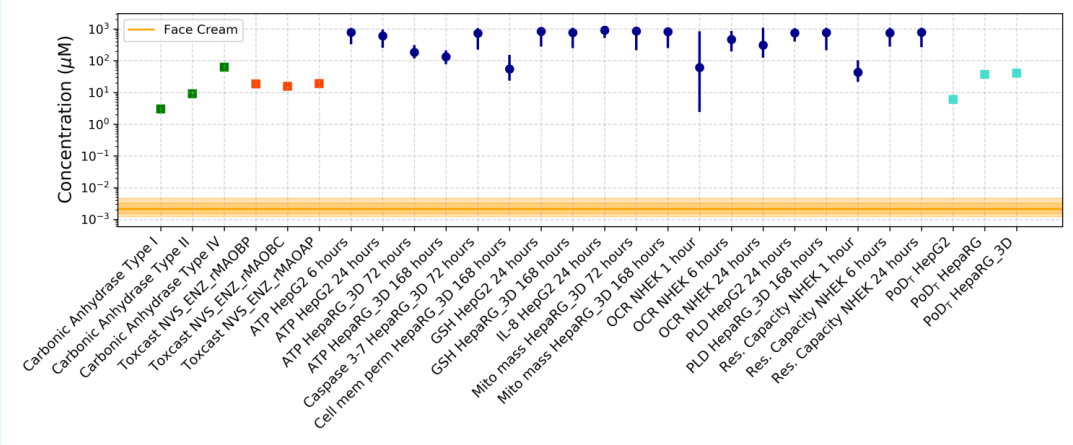
Concluding remarks

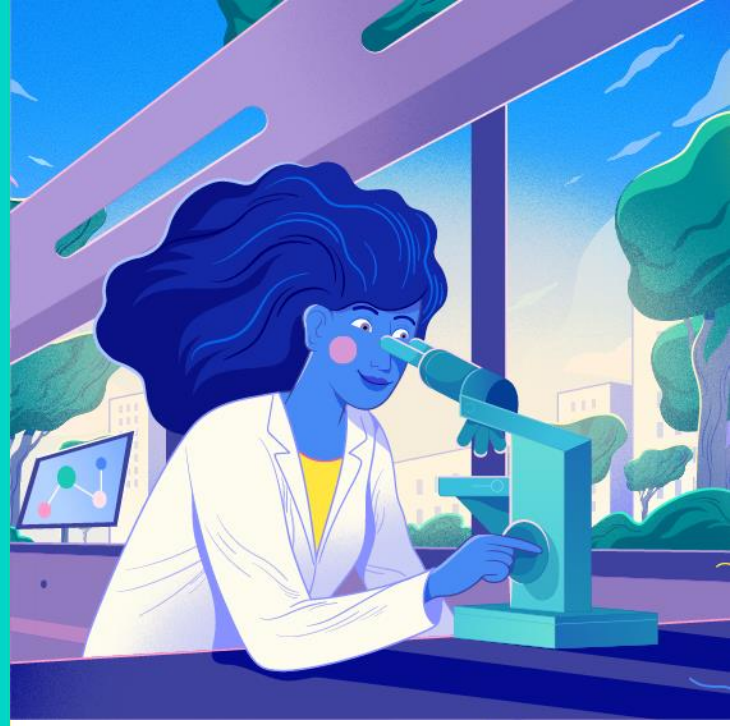
- **NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions**
  - **Exposure led**
  - **Human relevant**
  - ***in silico***
  - ***in vitro***
  - **weight of evidence**
- **Margin of safety is determined by the ratio of human exposure to the point of departure for the most sensitive assay**
- **NGRA tools are available now and research into more approaches continues**



PART THREE

Case Study Examples

2) SKIN SENSITISATION



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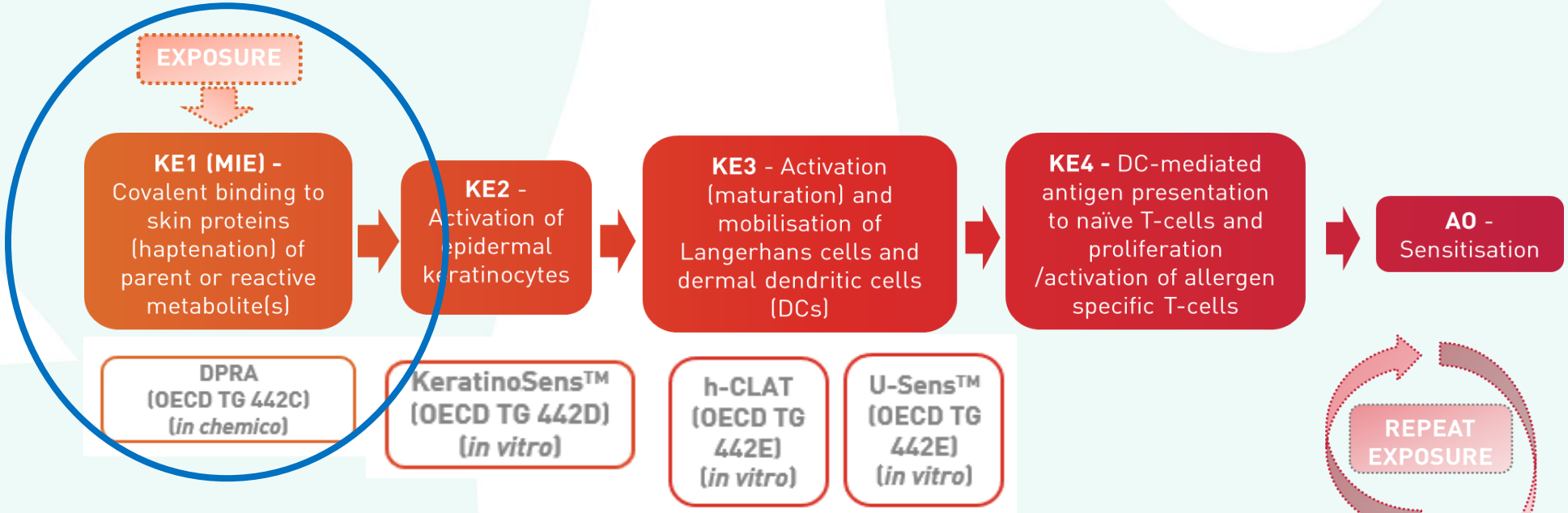
NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro skin sensitisation assessment

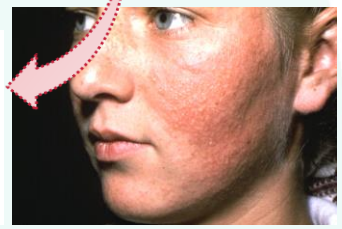
Initial hypothesis:

- **Protein binding alerts for coumarin and metabolites**



OECD (2014), *The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*, OECD Series on Testing and Assessment, No. 168, OECD Publishing, Paris, https://doi.org/10.1787/9789264221444-en.

Allergic contact dermatitis



NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro skin sensitisation assessment

Step 1: Generation of in vitro results for Coumarin

	DPRA (TG442C)		KeratinoSens (TG 442D)	h-CLAT (TG 442E)		U-SENS (TG 442E)
Call	**-ve**		**+ve**	**+ve**		**+ve**
Model Input	**%cys** depletion	**%lys** depletion	**EC1.5** (µM)	**CD54** (EC200 µg/mL)	**CD86** (EC150 µg/mL)	**CD86** (EC150 µg/mL)
RUNs	**1.0** **0.7** **2.2**	**0** **0** **0**	**200** **175** **NA**	**>637** **<178** **<178**	**>637** **>637** **>637**	**95** **96** **NA**

Initial results:

- **Coumarin is a skin sensitiser**
- **Likely to be due to metabolites (-ve DPRA)**

NGRA for 0.1% coumarin in face cream: biological activity characterisation
In vitro skin sensitisation assessment

Step 2. Generation of PoD for risk assessment- Skin allergy risk assessment (SARA) Defined approach (DA)

- The **SARA DA** is a Bayesian probabilistic model, which **estimates the human sensitiser potency via a prediction of an HRIPT 1% sensitising dose (ED_{01}) (i.e PoD) for a selected chemical**.

SARA Model Inputs

- Historical Local lymph node assay (LLNA)
- Historical Human repeated insult patch test (HRIPT)
- *In vitro* data: DPRA (TG442C), KeratinoSens (TG 442D), h-CLAT (TG 442E), U-SENS (TG 442E)
- First publication dataset of 30 chemicals – expanded to 53 core + 49 *in vitro* only

* Reynolds, J, MacKay C, Gilmour N, Miguel-Vilumbrales D and Maxwell G (Computational Toxicology, Volume 9, February 2019, Pages 36-49) Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment

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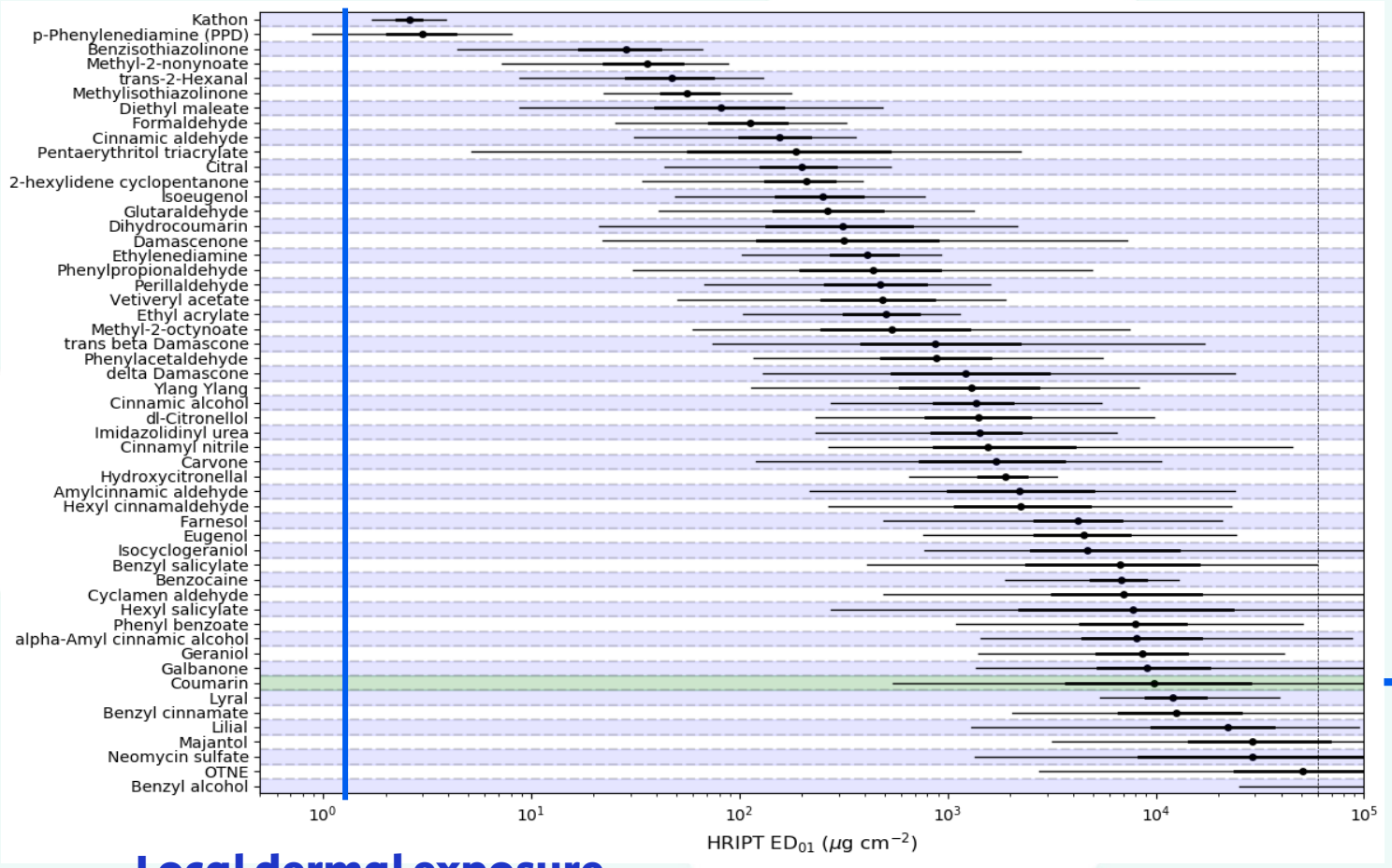
NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro skin sensitisation assessment

Step 2: PoD for risk assessment



Local dermal exposure (1.36 µg/cm²)

The PoD for coumarin has a central **95% credible interval** ranging from **546 – 217,603 µg/cm²**

Results:

- **Exposure is much lower than the predicted PoD**
- **MoS = 400 - 160 000**
- **Low risk conclusion**

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NGRA for 0.1% coumarin in face cream: Skin Sensitisation

Exposure Estimation

- Total plasma Cmax values obtained from PBK model: 0.002 µM (mean), 0.005 µM (99th percentile)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

Collate Existing Information

- Genotoxicity and protein binding alerts for parent compound
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

In Vitro Biological Activity Characterisation

- Predicted MoS (400-160,000) suggests that the risk of inducing skin allergy is low at the consumer exposure

AFSA
ANIMAL-FREE SAFETY ASSESSMENT COLLABORATION

www.afsacollaboration.org

Developing & disseminating a global training program in next-generation risk assessment (NGRA)

- Support regional capacity-building to achieve long-term acceptance & implementation of legislative measures
- Address the needs of regulatory & regulated communities, CROs & other stakeholders

www.afsacollaboration.org

Partner Organisations

HUMANE SOCIETY INTERNATIONAL

THE HUMANE SOCIETY OF THE UNITED STATES

AVON

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Delphic HSE SAFETY & REGULATORY SOLUTIONS

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symrise

L'ORÉAL

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ANIMAL-FREE SAFETY ASSESSMENT COLLABORATION

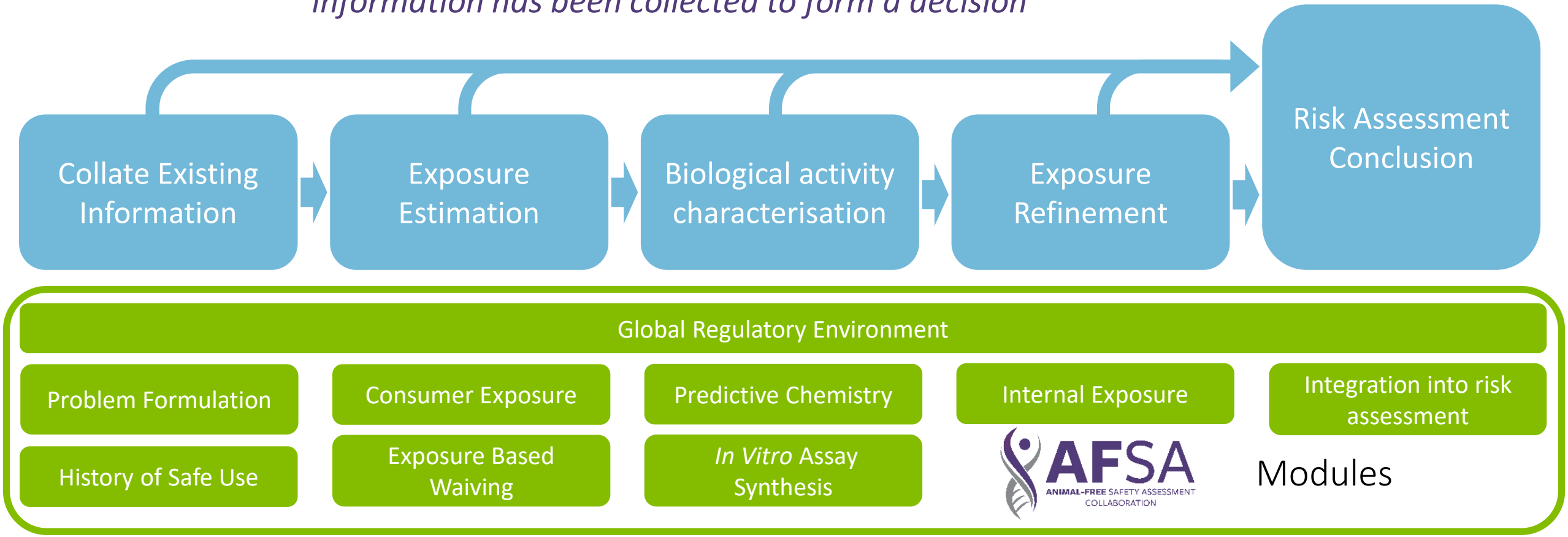
Scope of the course

Using information in decision making

- Safety assessment of cosmetics and cosmetic ingredients
- All aspects of the NGRA process for internal and regulatory safety decisions
- Covers the spectrum of available tools as well as some tools in development
- Focus on *understanding* the information generated from the tools and *how to use* this information vs. how to perform or build the individual methods
- Address the needs of regulatory & regulated communities, CROs and other stakeholders
- Support regional capacity-building to achieve long-term acceptance and implementation of non-animal approaches to chemical safety assessment

Risk assessment process

A tiered and iterative approach is needed until sufficient information has been collected to form a decision



Acknowledgements

paul.j.russell@unilever.com

renato.de-avila@unilever.com

Maria Baltazar
Sophie Cable
Paul Carmichael
Richard Cubberley
Tom Cull
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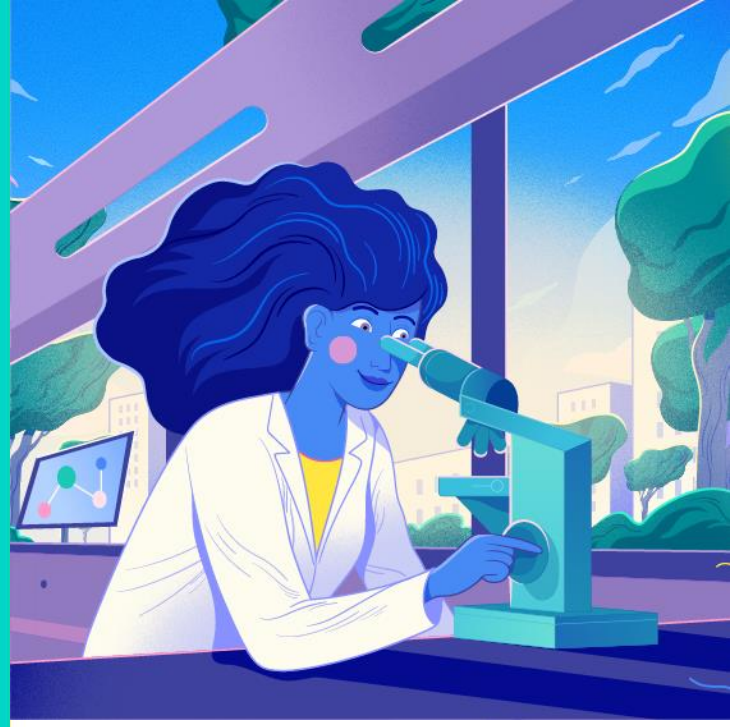
Sarah Hatherell
Jade Houghton
Predrag Kukic
Hequn Li
Sophie Malcomber
Alistair Middleton
Tom Moxon
Alexis Nathanail
Beate Nicol
Ruth Pendlington
Sam Piechota
Julia Fentem
Georgia Reynolds
Joe Reynolds
Nikol Simicek
Andy Scott
Carl Westmoreland
Andy White



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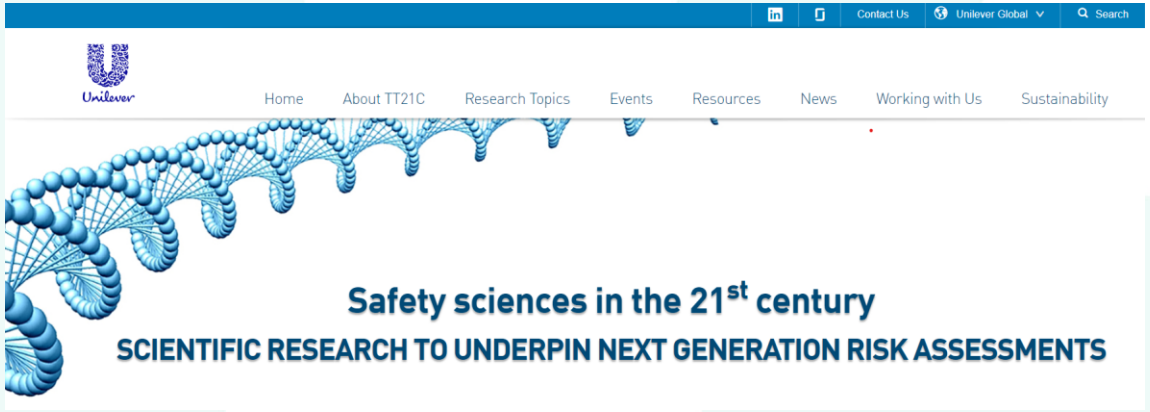
For more information on Unilever's ongoing research to develop non-animal approaches to safety assessment visit www.tt21c.org



Safety Risk Assessments in Unilever

Animal Testing Alternatives in Unilever