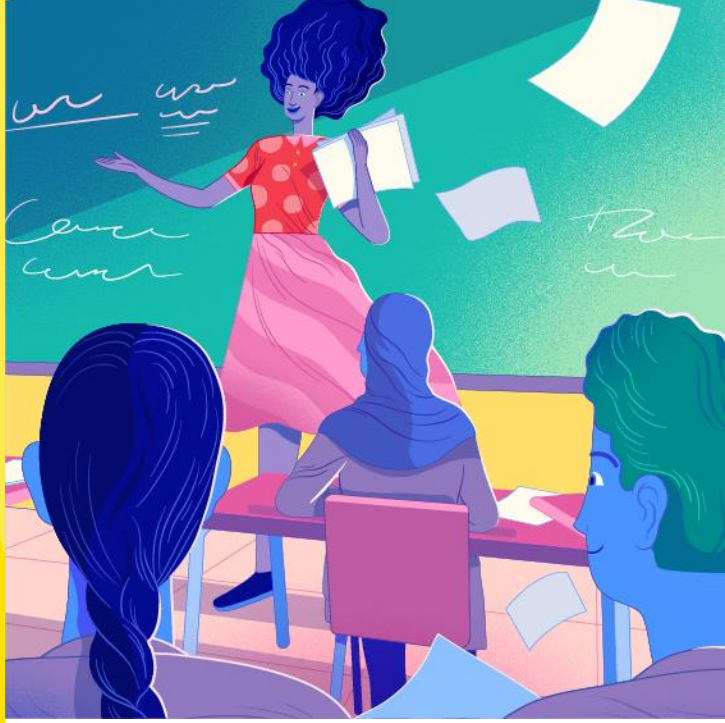
Case study application using non-animal approaches for local and systemic safety assessment of cosmetic ingredients



Renato Ivan de Ávila, PhD

Safety Scientist - Toxicology

Unilever Safety & Environmental Assurance Centre (SEAC)

Renato.de-Avila@unilever.com

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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

https://www.omo.com/br/sem-testes-em-animais.html

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Assuring consumer safety without animal testing:
maximising use of existing information and animal-free approaches

- 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 (e.g. toxicological threshold of concern)
- History of safe use
- *In silico* predictions
- Read across
- Use of existing *in vitro* data and approaches
- **Next Generation Risk Assessment (NGRA)**

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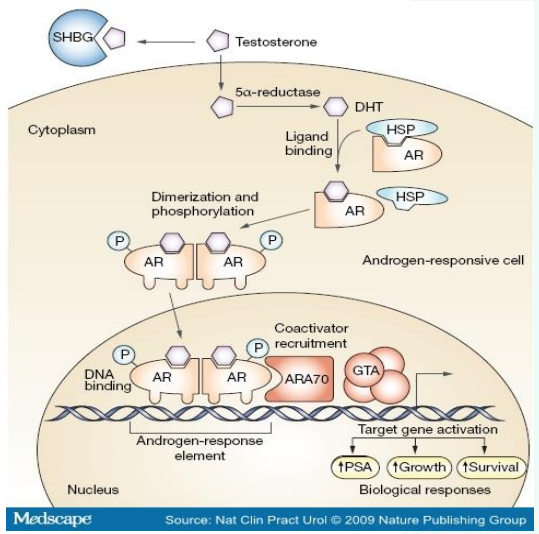
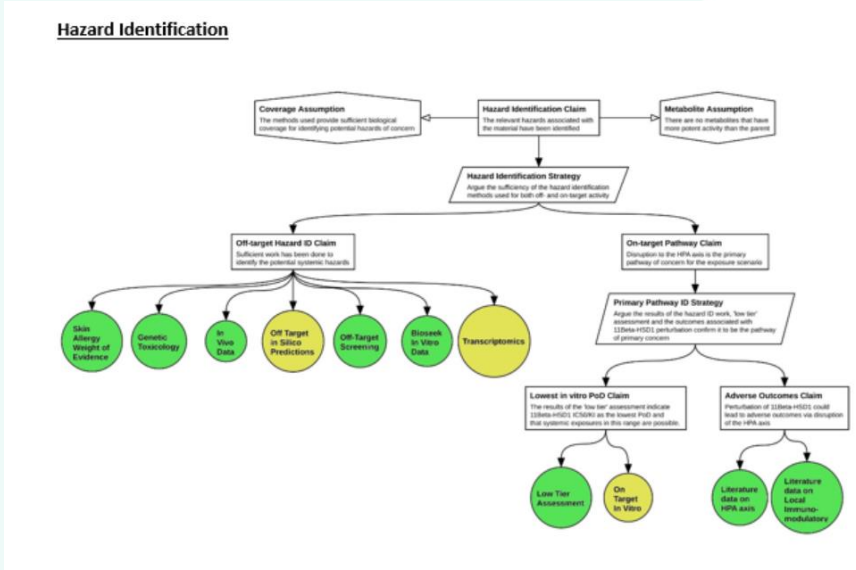
Next Generation Risk assessment (NGRA)

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





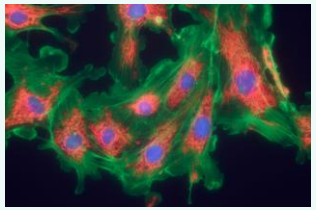
Exposure led **Mechanistic** **Hypothesis driven**

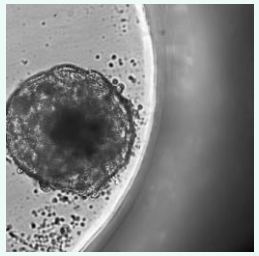
Dent *et al* . Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. *Comp Tox* 7:20-26, 2018.

NGRA: Using relevant methods to test hypotheses

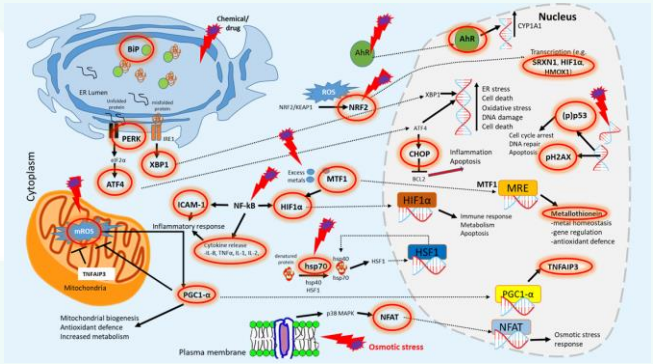
New Approach Methods (NAMs)

Advanced cell systems and microtissues





Cellular stress

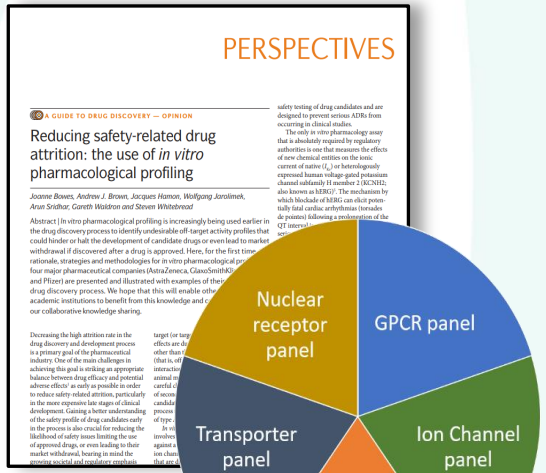


cyprotex AN EVOTEC COMPANY Image kindly provided by Paul Walker (Cyprotex)

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

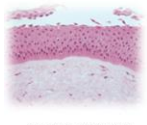
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

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

eurofins | Cerep

Established Methods

OECD test methods

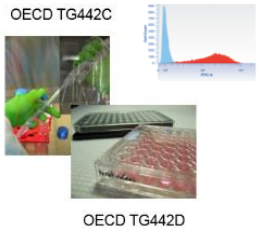


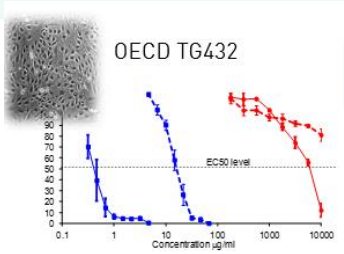
OECD TG437

OECD TG430/431
OECD TG439

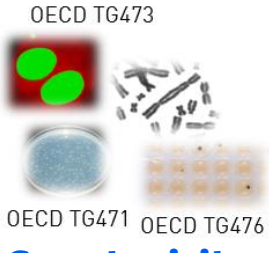
Skin and eye irritation

Skin sensitisation





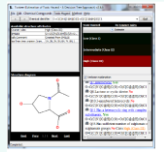
Phototoxicity



Genotoxicity

In silico tools

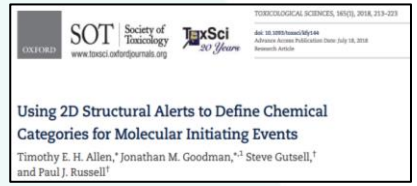
ToxTree



Derek nexus

Meteor nexus

OECD QSAR TOOLBOX



High throughput transcriptomics

Mechanism based gentox assessment

toxys ToxTracker®

DNA Damage
P53 Binding
Oxidative Stress
Protein Damage

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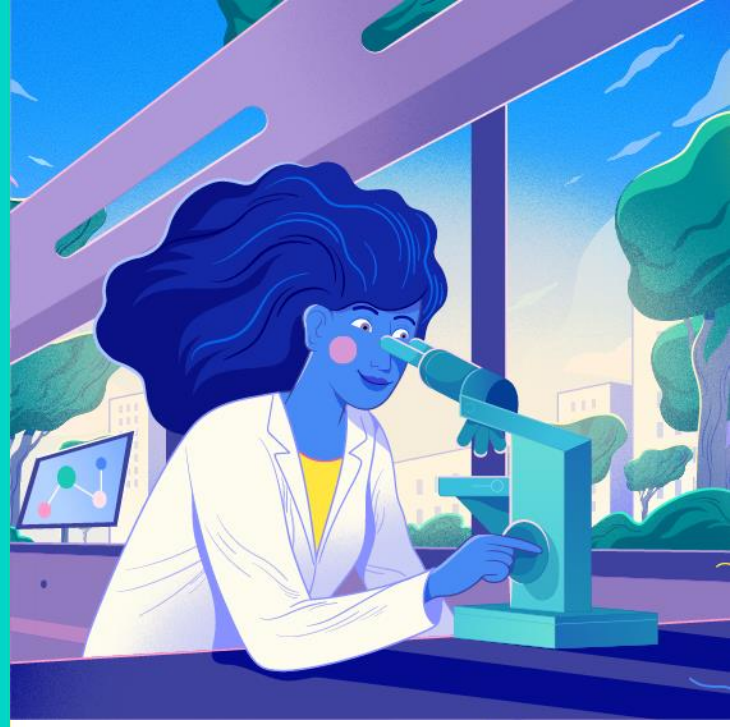
Case study examples

1) Systemic effects

2) Local effect - skin sensitisation



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Case study: hypothetical example for 0.1% coumarin in face cream

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

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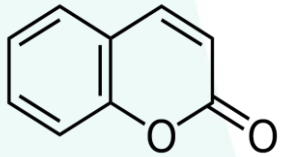
ABSTRACT

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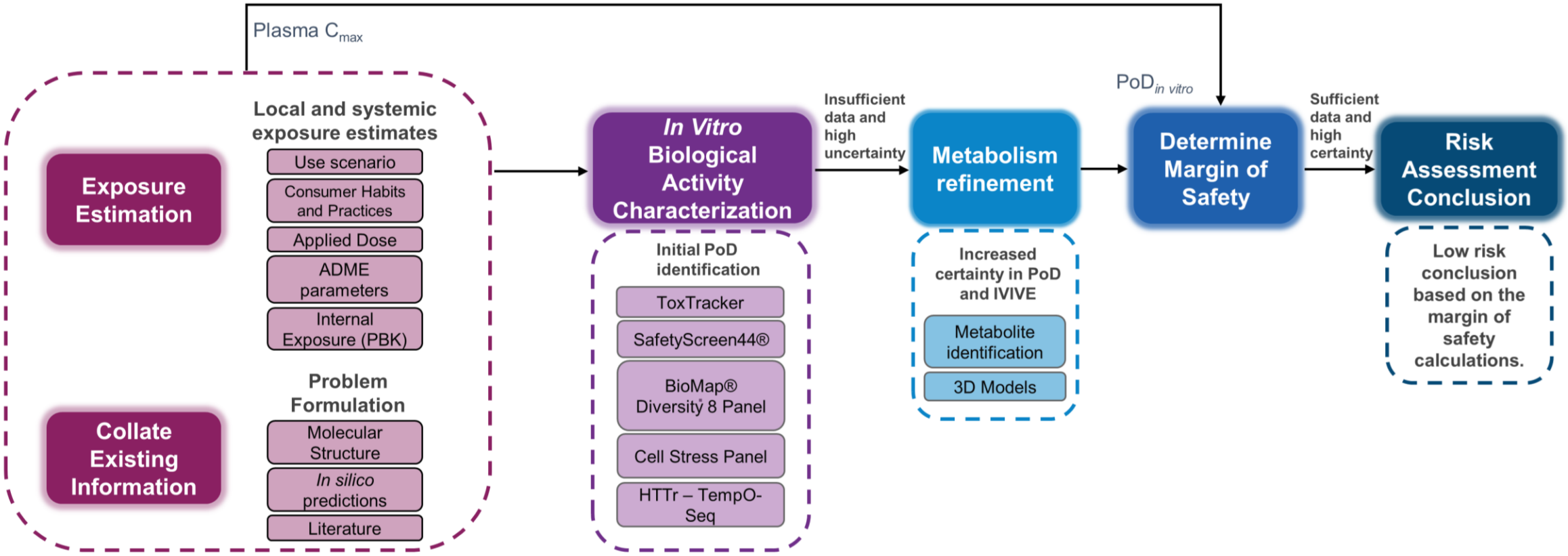
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



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

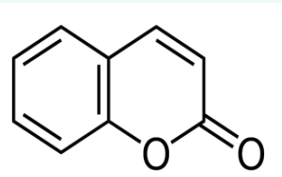
Creme GLOBAL

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 conditioner [2]				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)	**2.73**
Systemic exposure per day (mg/kg)	**0.02**

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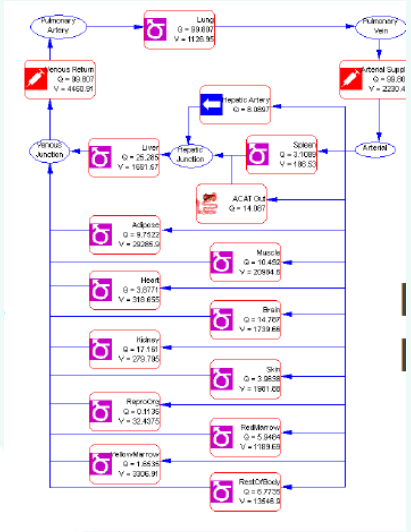
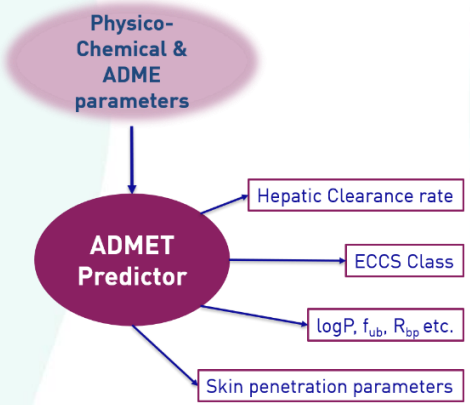
NGRA for 0.1% coumarin in face cream: exposure estimation- Internal concentration using PBK modelling - Model Inputs

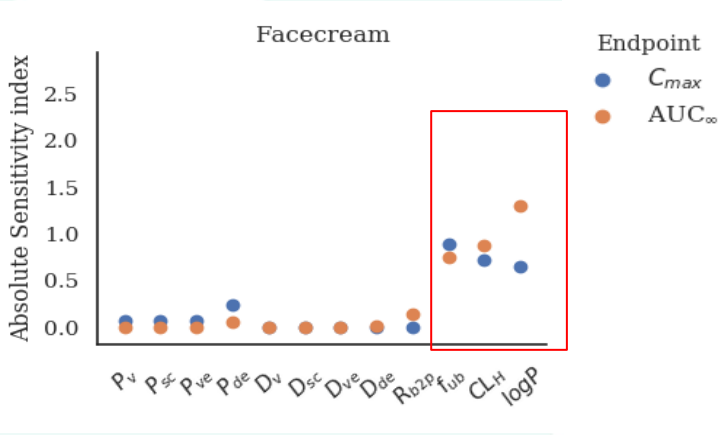
GastroPlus® (Simulations Plus)



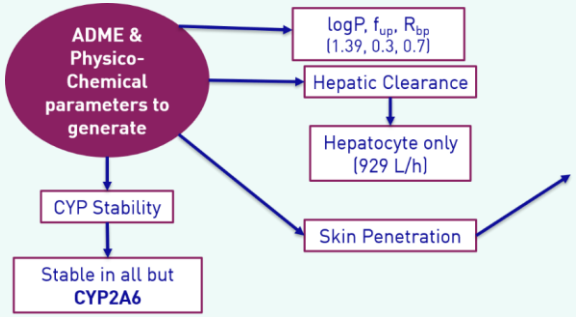
Use *in silico* parameters for modelling



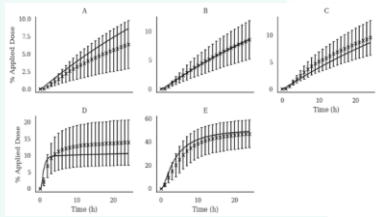
Sensitivity analysis



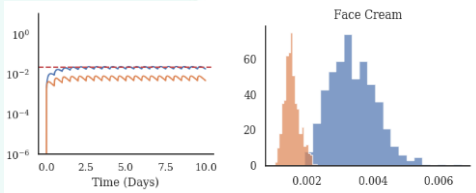
Experimental Refinement



Skin absorption study



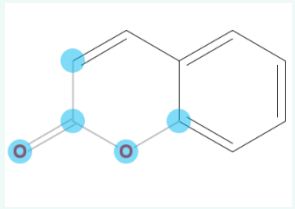
Exposure distribution



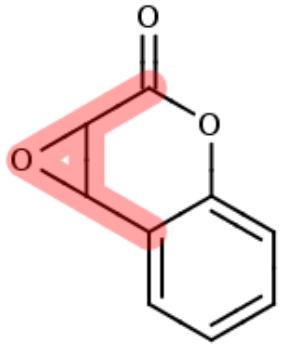
Total Plasma C_{max} (µM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

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 - 40 µM

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

*AC50= activity concentration at 50% of maximal activity

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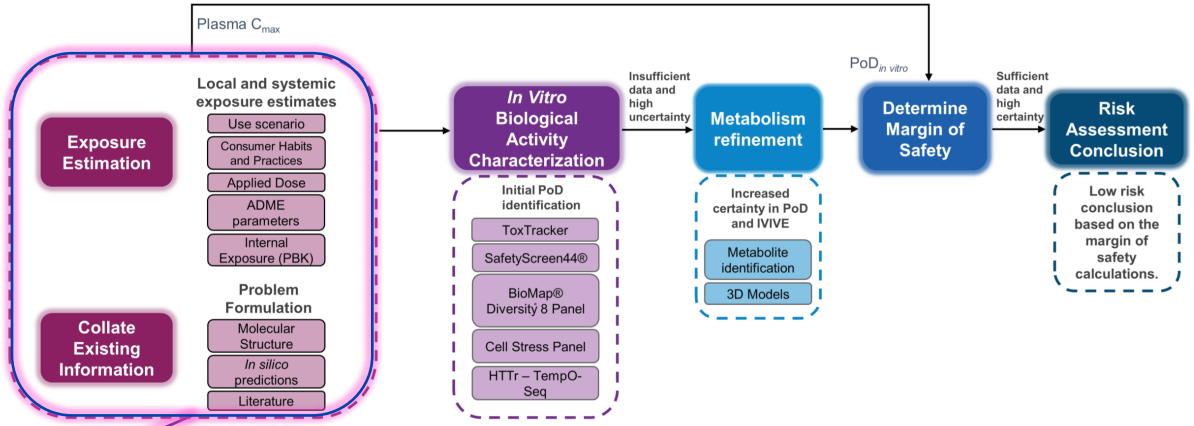
NGRA for 0.1% coumarin in face cream: exposure estimation

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

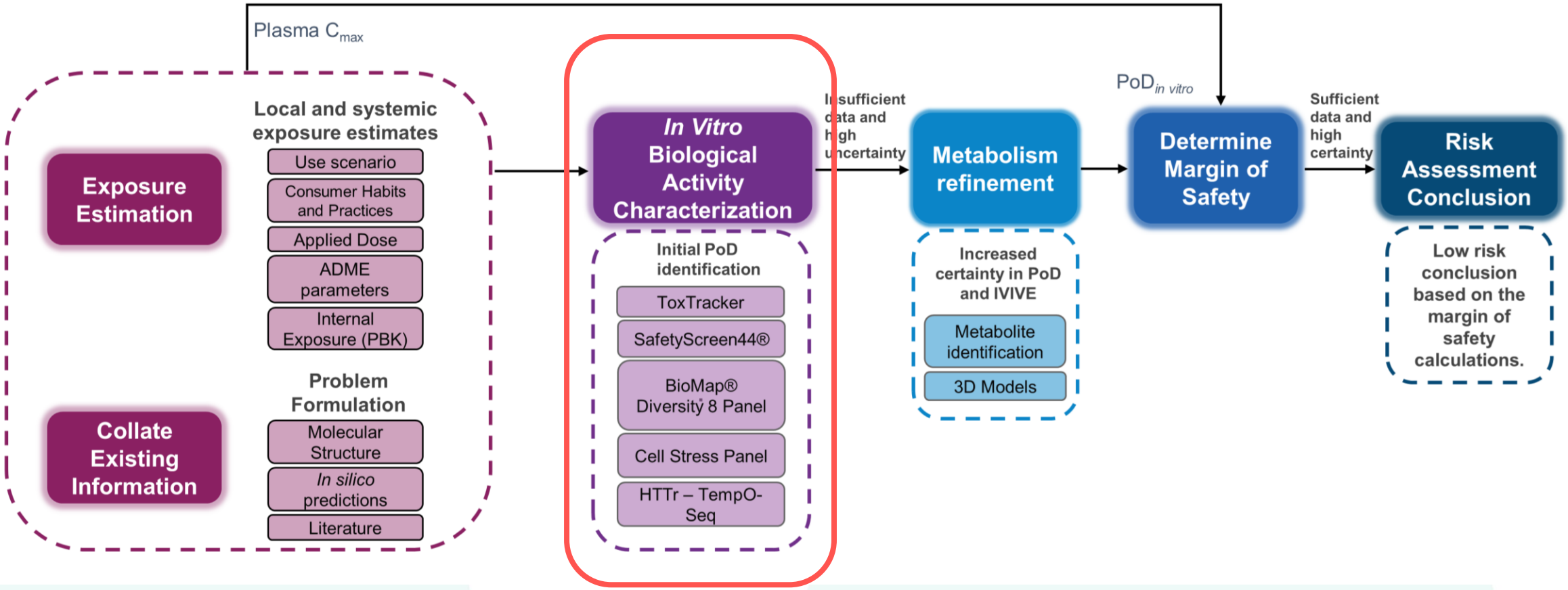
- *Genotoxicity and skin sensitisation alerts for parent compound*
- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.
- Low bioactivity in ToxCast and Pubchem: binding to Carbonic Anhydrases and MAO-A/B reported
- Lowest PoD was 3 µM for carbonic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



STEP TWO

In vitro biological activity characterisation

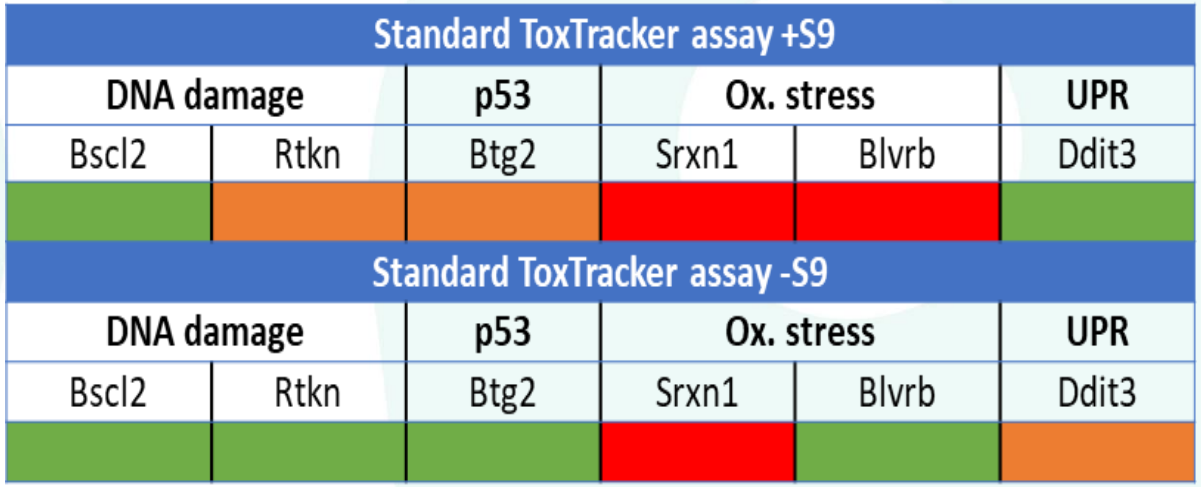
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



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.

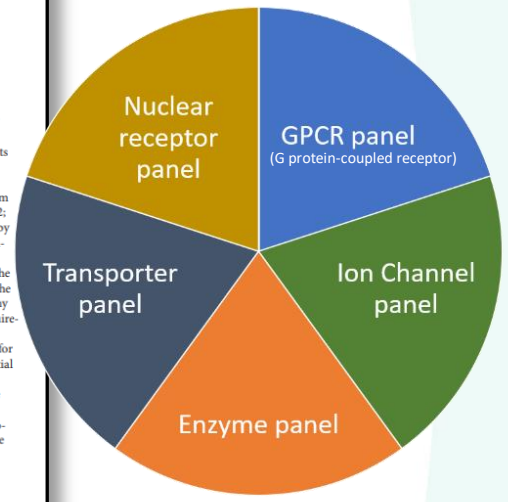
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

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

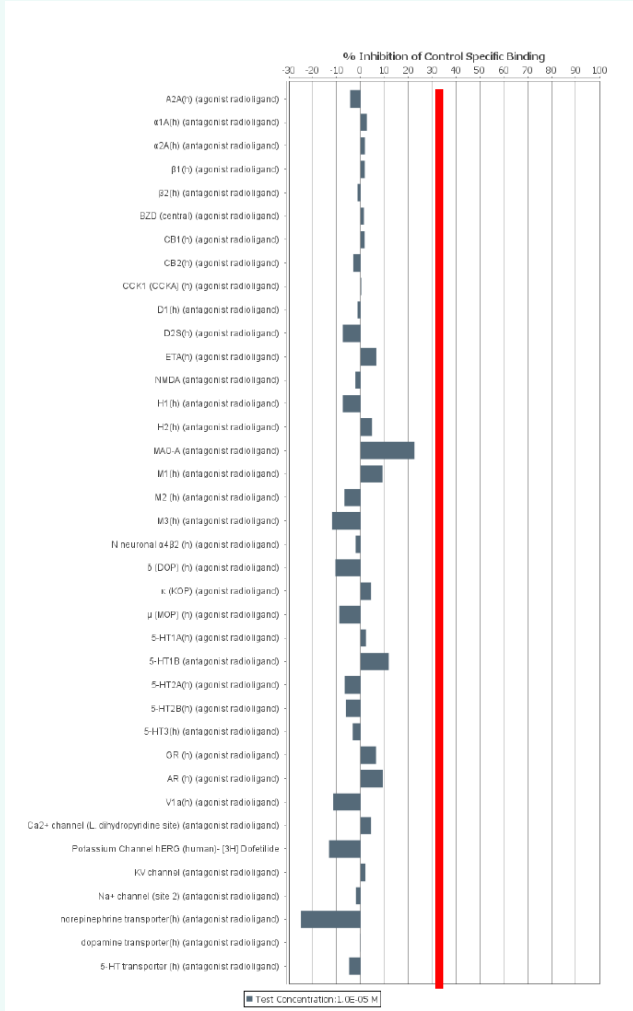
The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[2]. 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:

No significant activity was observed for all binding and enzymatic assay results

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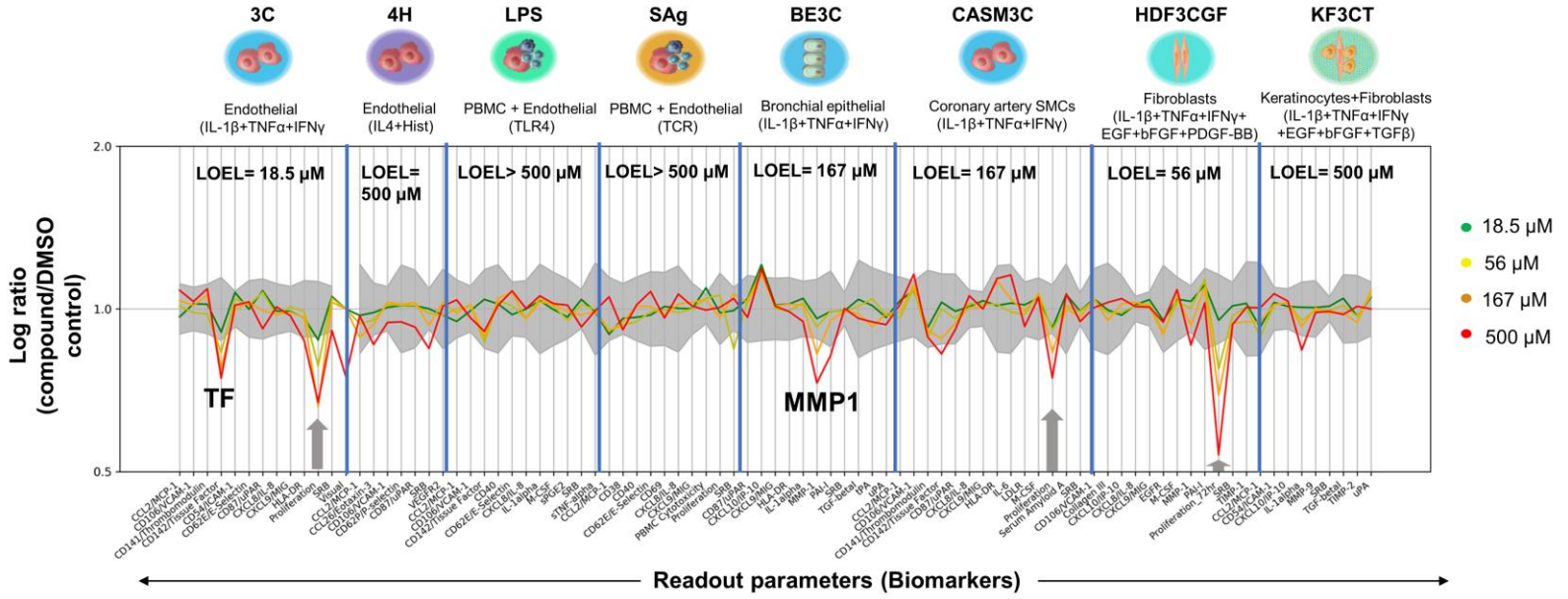
NGRA for 0.1% coumarin in face cream: biological activity characterisation: Immunomodulatory screening assay - BioMap Diversity 8 Panel

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

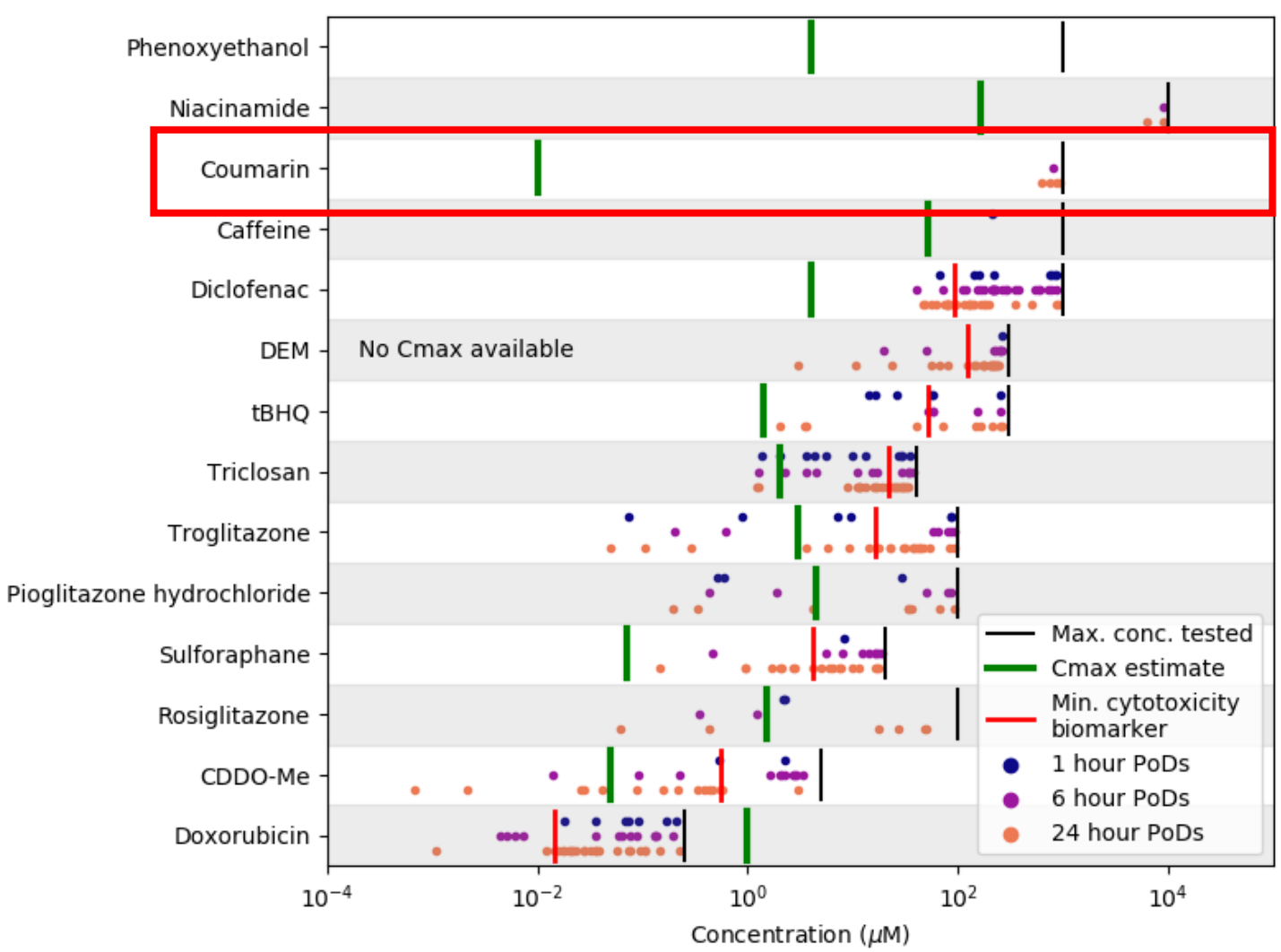


Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound

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

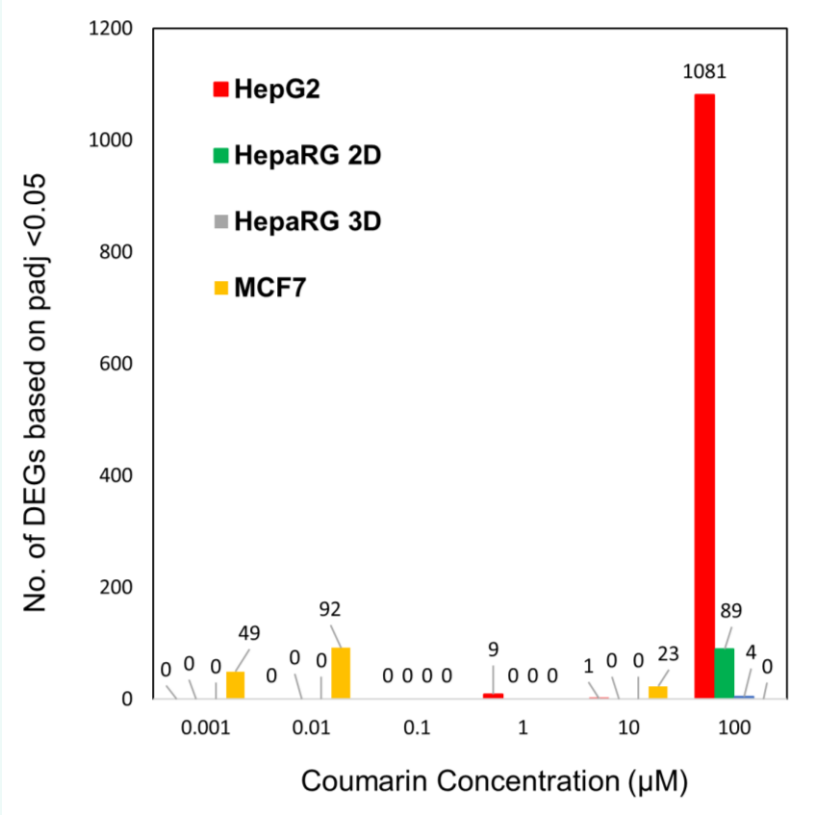
Hatherell et al. Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment. Tox. Sci., v. 176, p. 11-33, 2020.

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

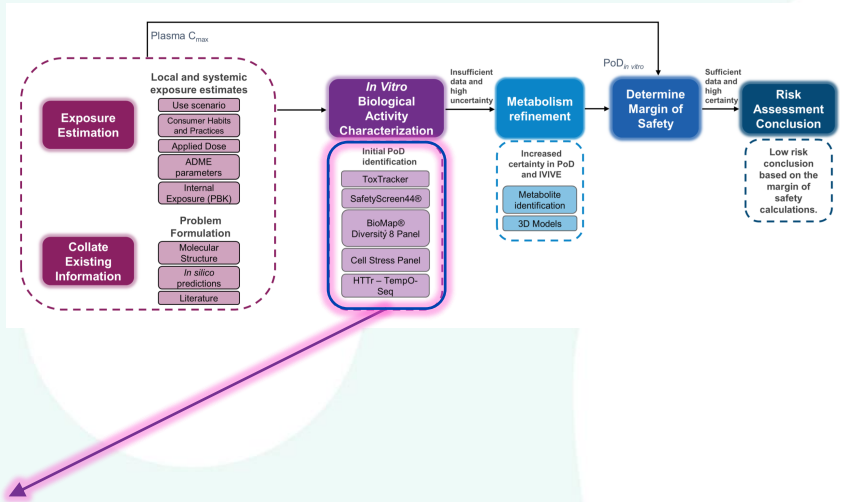
- Total plasma Cmax values obtained from PBK model: 0.002 µM (mean), 0.005 µM (99th percentile)
- Stability assays indicated coumarin rapidly metabolized mainly via CYP2A6

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

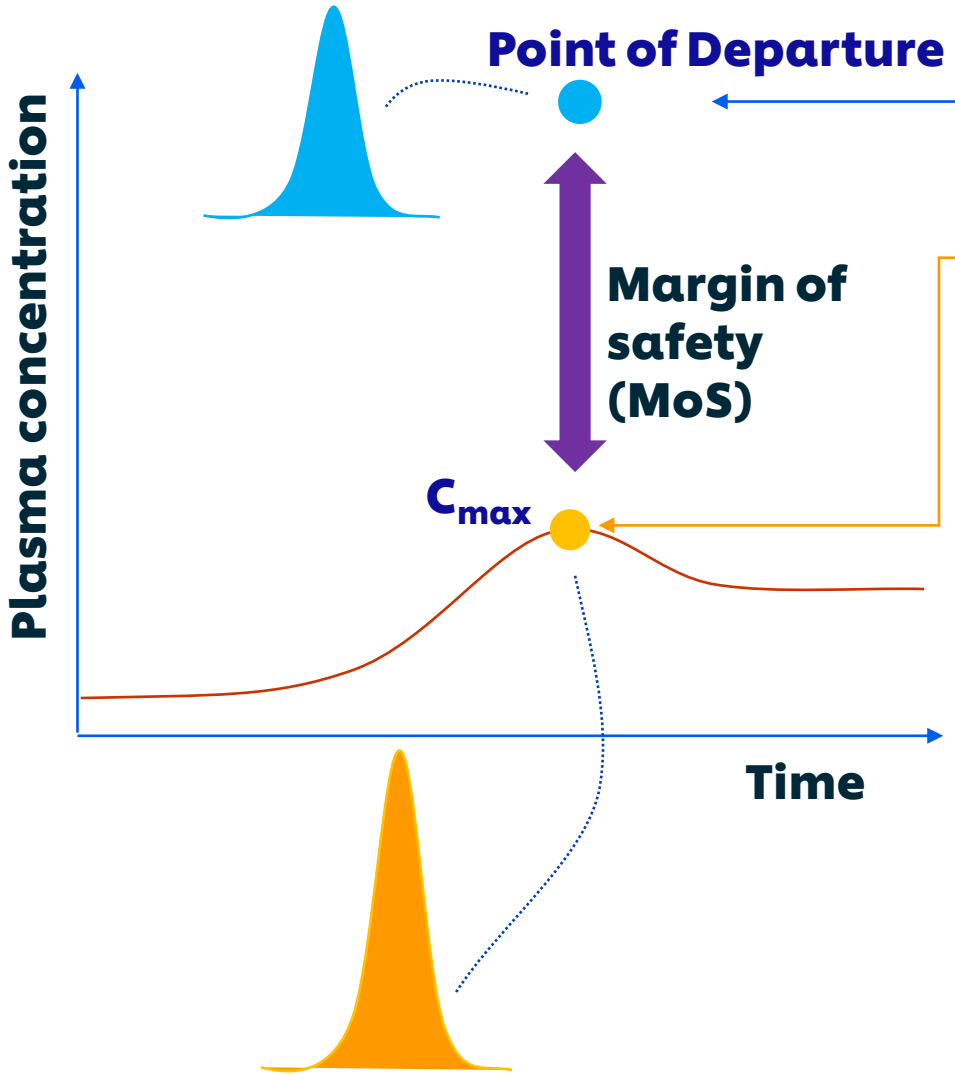
- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- No immunomodulation potential
- Low bioactivity confirmed by binding/enzymatic assays, HTTr and cell stress panel.
- PoD range: 6-912 µM



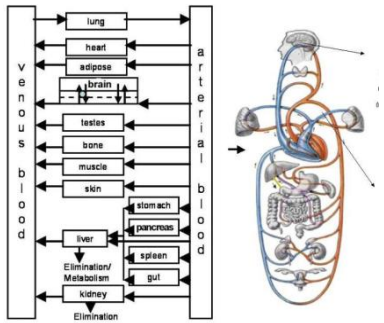
STEP THREE

Margin of Safety

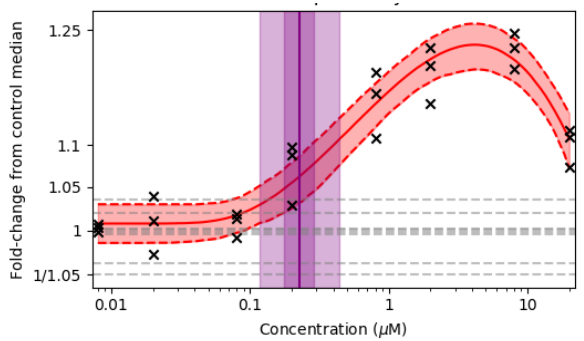
Margin of Safety



Exposure models (PBK, free/total concentration)



PoD derived from *in vitro* concentration-response



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

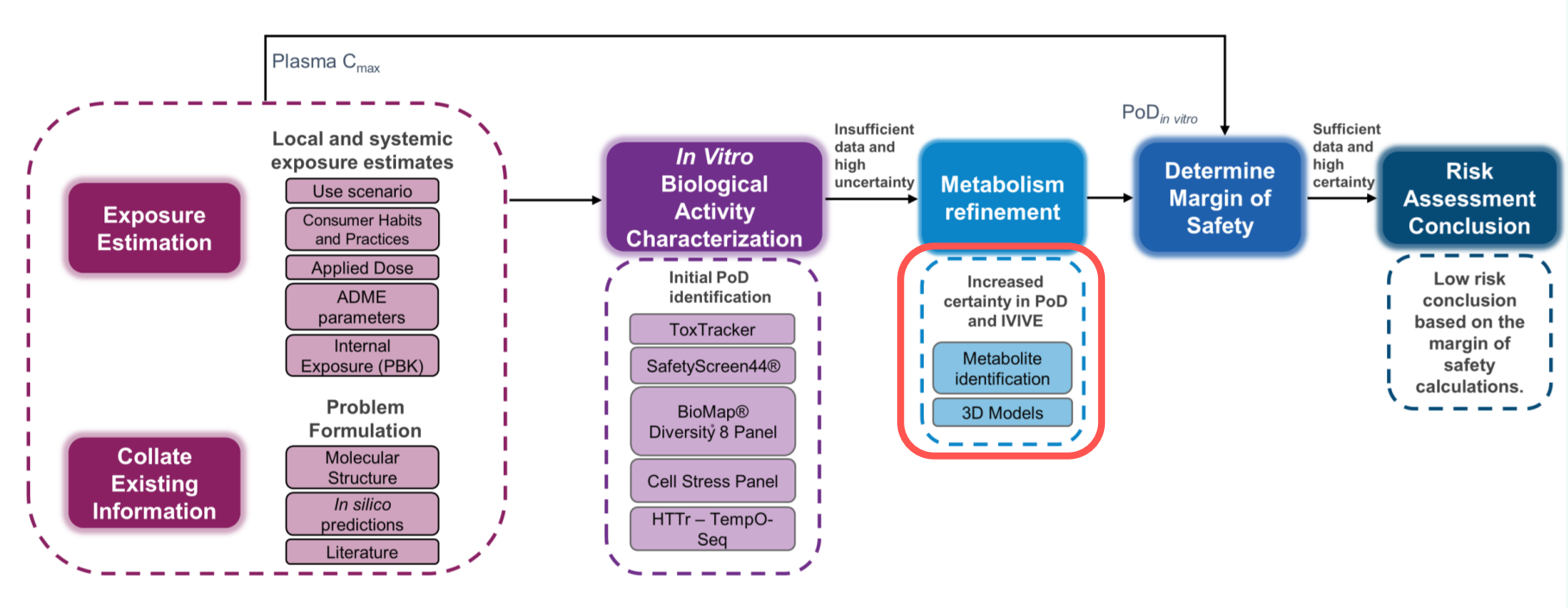
Preliminary MoS

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**

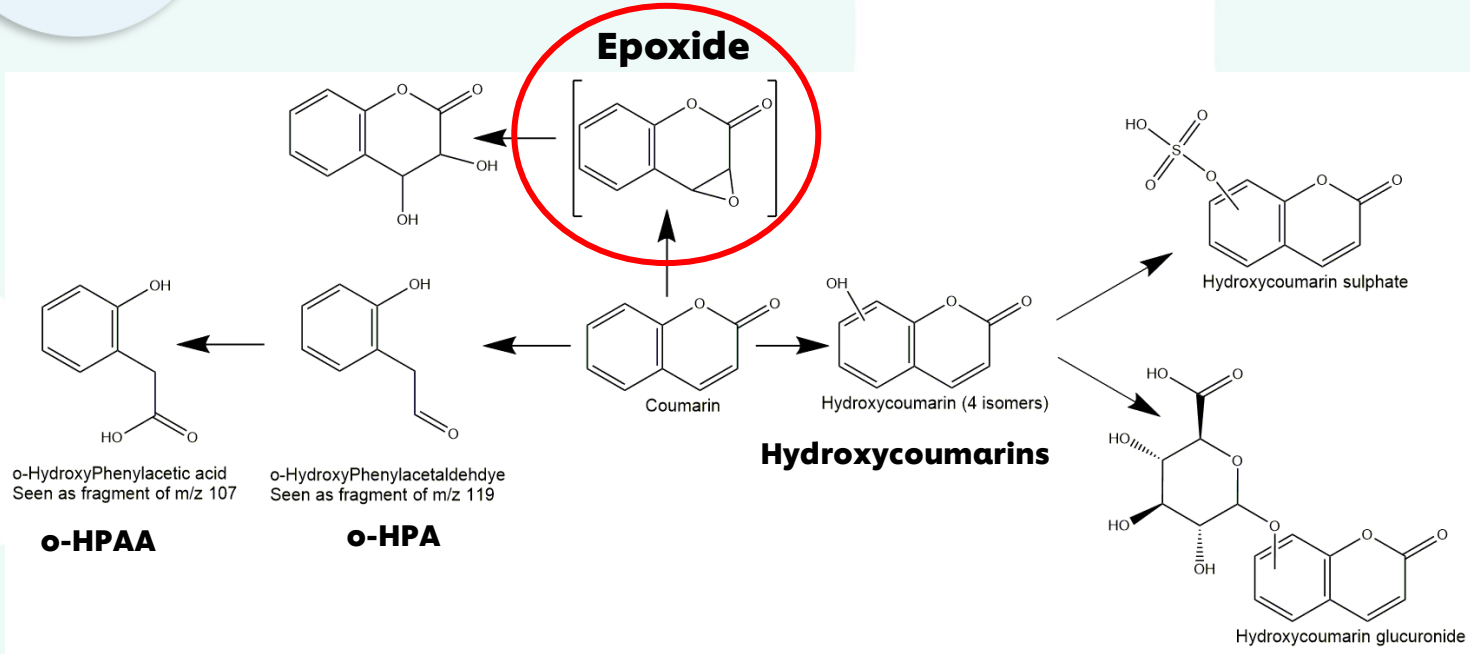
2. **Short and long-term exposure in 3D tissues - longer exposure durations in a 3D HepaRG model with potentially higher metabolic capacity and in vivo-like physiology than HepG2 cells**



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**

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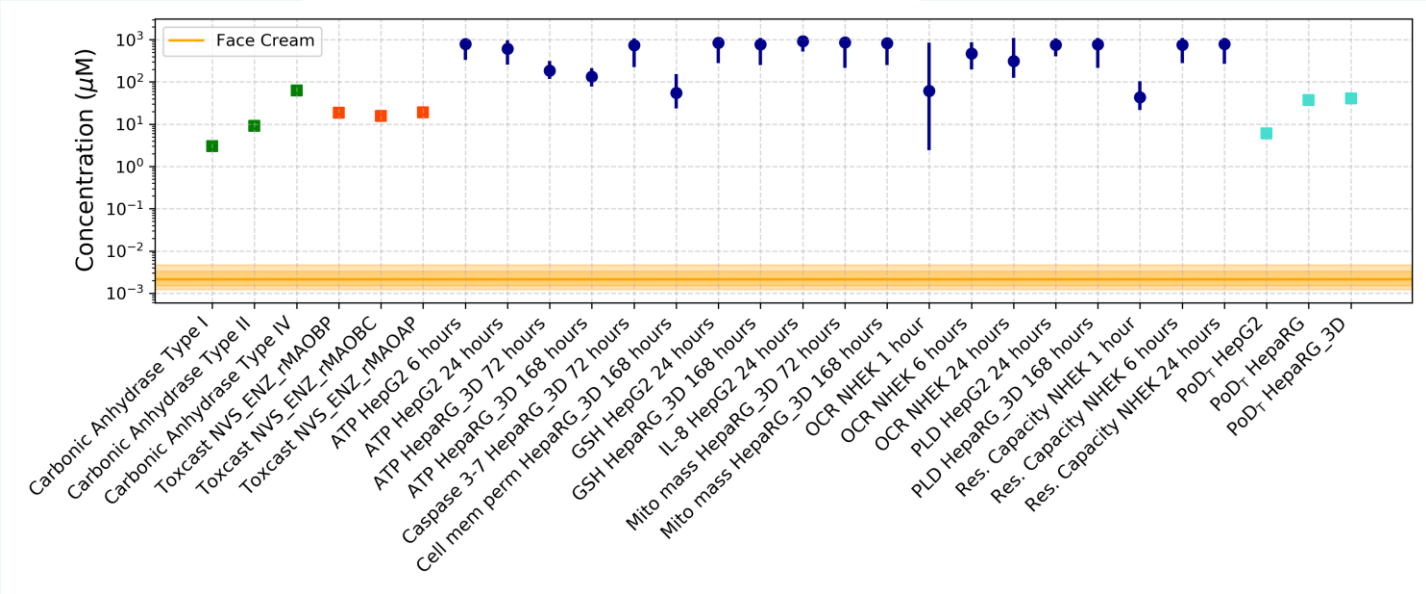
NGRA for 0.1% coumarin in face cream: Risk assessment conclusion



- 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 low risk for the consumer**

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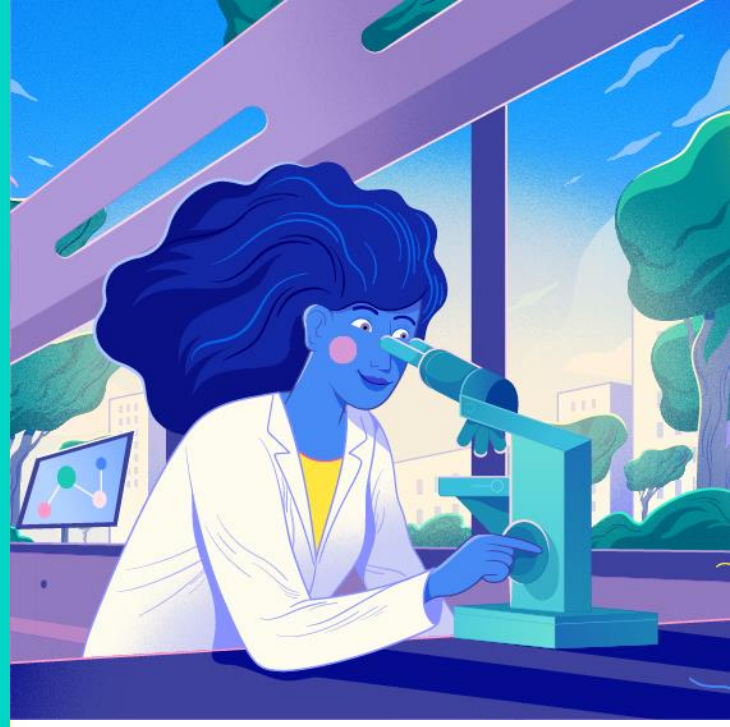
Case study examples

1) Systemic effects

2) Local effect - skin sensitisation



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Case study: hypothetical example for 0.1% coumarin in face cream

Reynolds et al. (2021). A hypothetical skin sensitisation next generation risk assessment for coumarin in cosmetic products. Reg. Tox. Pharm., 127, 2021.

Regulatory Toxicology and Pharmacology 127 (2021) 105075

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A hypothetical skin sensitisation next generation risk assessment for coumarin in cosmetic products

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New approach methodologies
Consumer exposure
Uncertainty analysis
Decision making
Metabolism

ABSTRACT

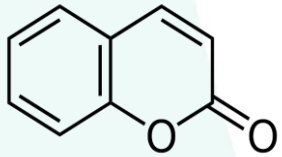
Next generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach which integrates new approach methodologies (NAMs) to assure safety without generating animal data. This hypothetical skin allergy risk assessment of two consumer products – face cream containing 0.1% coumarin and deodorant containing 1% coumarin – demonstrates the application of our skin allergy NGRA framework which incorporates our Skin Allergy Risk Assessment (SARA) Model. SARA uses Bayesian statistics to provide a human relevant point of departure and risk metric for a given chemical exposure based upon input data that can include both NAMs and historical *in vivo* studies. Regardless of whether NAM or *in vivo* inputs were used, the model predicted that the face cream and deodorant exposures were low and high risk respectively. Using only NAM data resulted in a minor underestimation of risk relative to *in vivo*. Coumarin is a predicted pro-hapten and consequently, when applying this mechanistic understanding to the selection of NAMs the discordance in relative risk could be minimized. This case study demonstrates how integrating a computational model and generating bespoke NAM data in a weight of evidence framework can build confidence in safety decision making.

1. Introduction

Skin sensitisation, which can ultimately result in development of allergic contact dermatitis (ACD), is a common human health problem (Alinaghi et al., 2019). To ensure consumer safety, particularly for proliferation of antigen-specific T-cells (OECD 2014). The developed NAMs adopted by the OECD align to the first three key events and can be used in various combinations incorporated into defined approaches (DA) within a framework for a weight of evidence (WoE) chemical safety assessment (Gilmour et al., 2020; Kleinstreuer et al., 2018; OECD 2017,

0.1% COUMARIN IN FACE CREAM (NEW FRAGRANCE)

Assumptions:



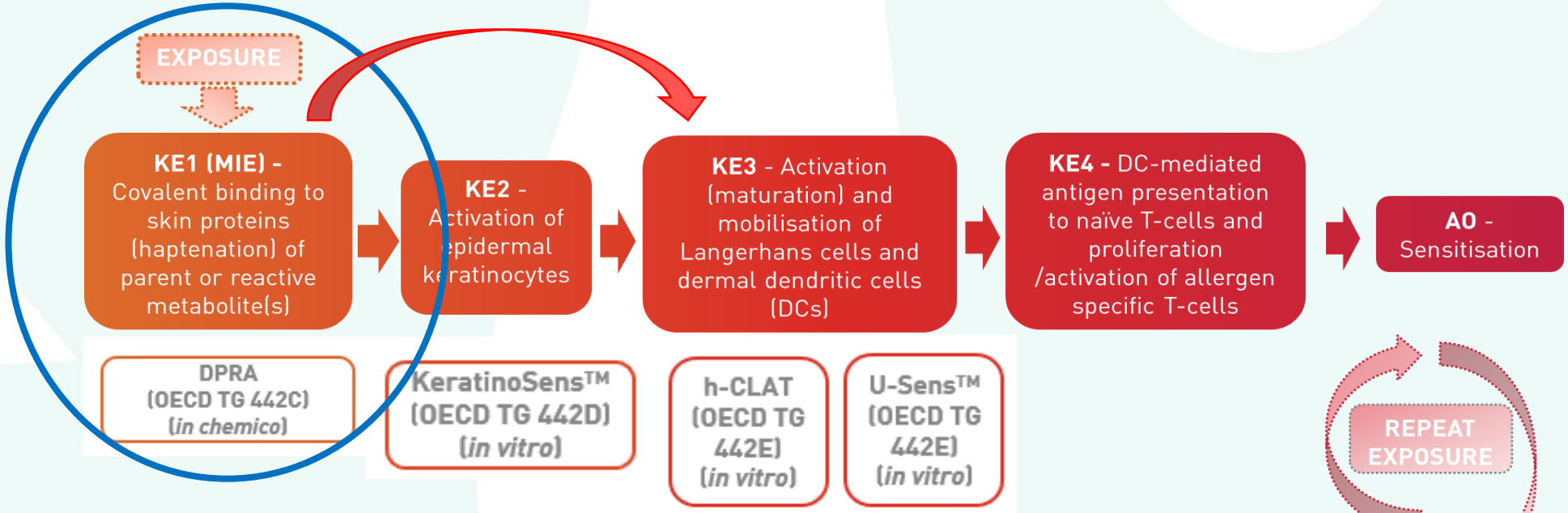
- EU Market
- 100% purity
- no in vivo data was available such as animal data, History of Safe Use (HoSU) or Clinical data
- no use of animal data in Read Across
- In silico alerts known to be based on animal or in vivo data or on the structure of Coumarin itself were excluded

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

In vitro skin sensitisation assessment

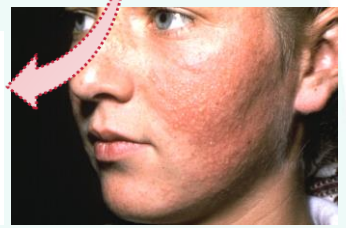
Initial hypothesis:

- **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)
Results	1.3	0	187.5	<178	>637	95.5

Initial results:

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

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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 a HRIPT 1% sensitising dose (ED_{01}) (i.e PoD) for a selected chemical**.

- The SARA model uses Bayesian statistics to infer a probability that a consumer exposure to some chemical can be considered low risk, to inform risk assessment decisions.

- The SARA Model uses a database of public NAM data covering AOP KEs 1-3, and historic LLNA and HRIPT data for the AOP AO.

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 et al. Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment. Computational Toxicology, v. 9, p. 36-49, 2019.

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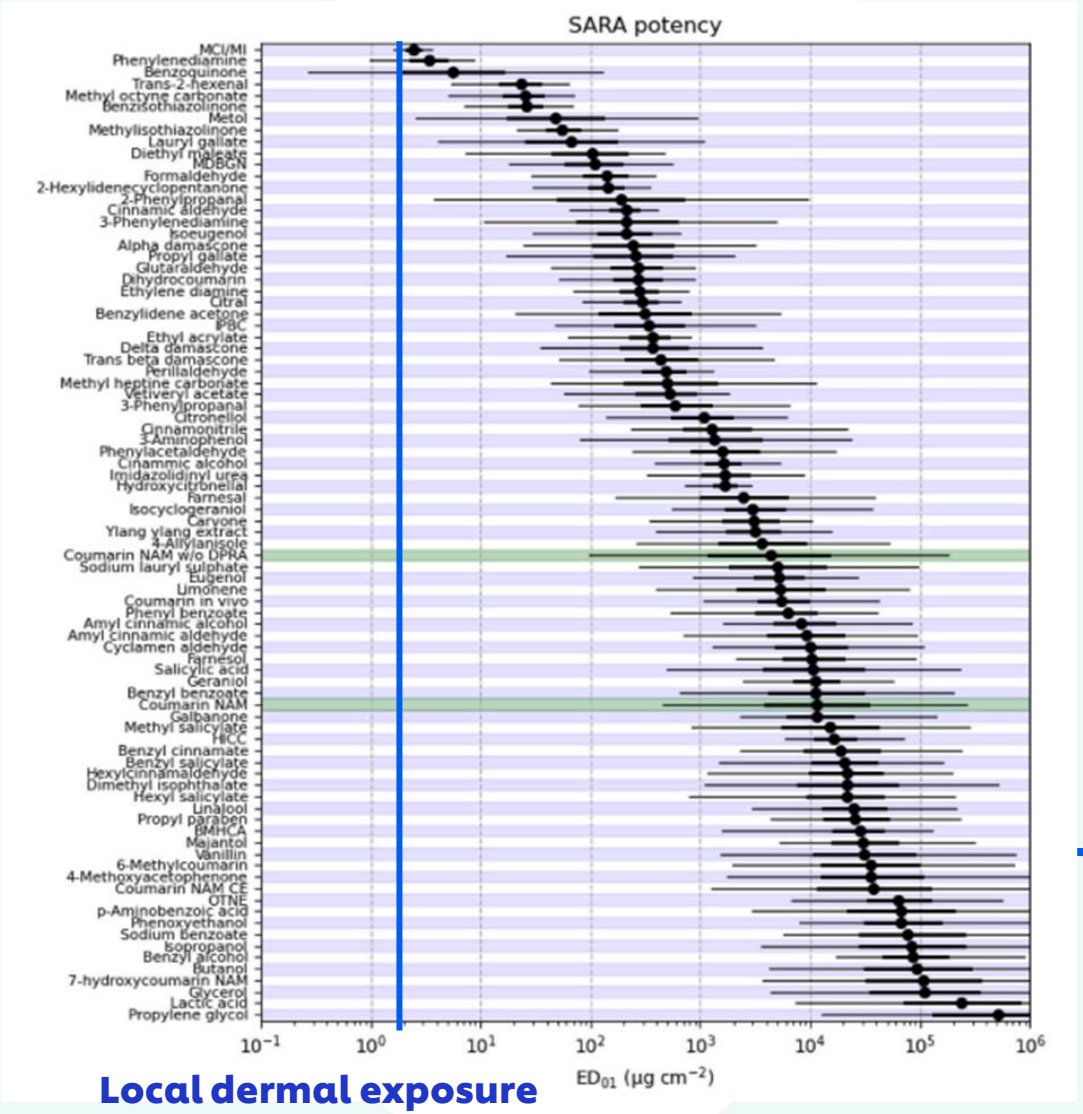
NGRA for 0.1% coumarin in face cream: biological activity characterisation

In vitro skin sensitisation assessment

Step 2: PoD for risk assessment



Local dermal exposure (2.7 µg/cm²)

The ED01 for coumarin has a ranging from **420 – 260,000 µg/cm²**

Results:

- **Exposure is much lower than the predicted ED01**
- **Low risk conclusion**

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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

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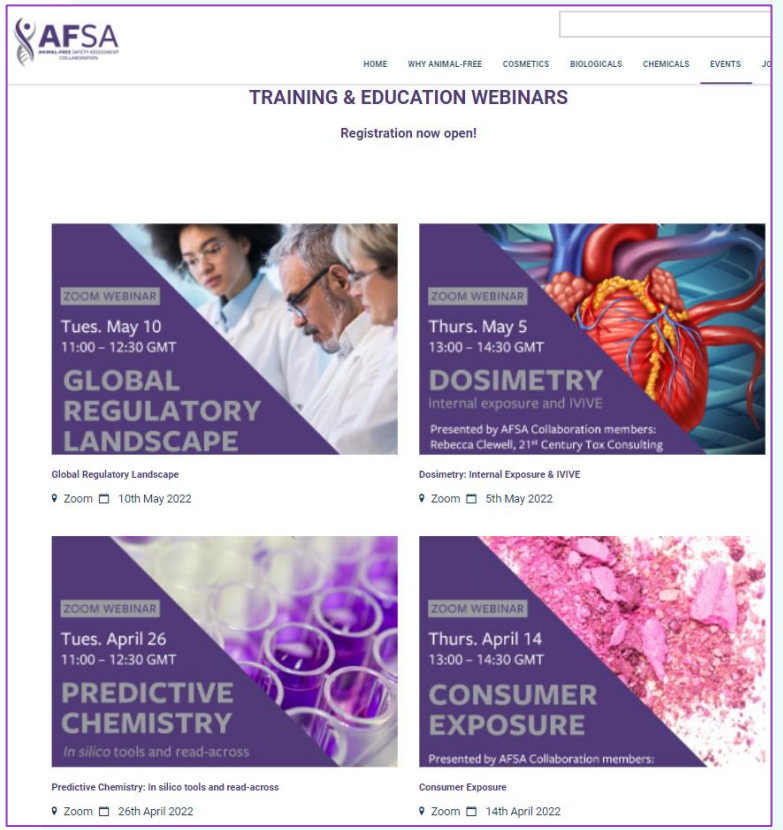
Recordings of past webinars are available:

https://www.afsacollaboration.org/event/



Invitation:

Bianca Marigliani, an AFSA partner, will present the AFSA E&T Platform,

27th May, 14:40 (sala D):

"Animal-Free Safety Assessment of Cosmetics: a global education and training program"

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Maja Aleksic
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Gavin Maxwell
Joe Reynolds
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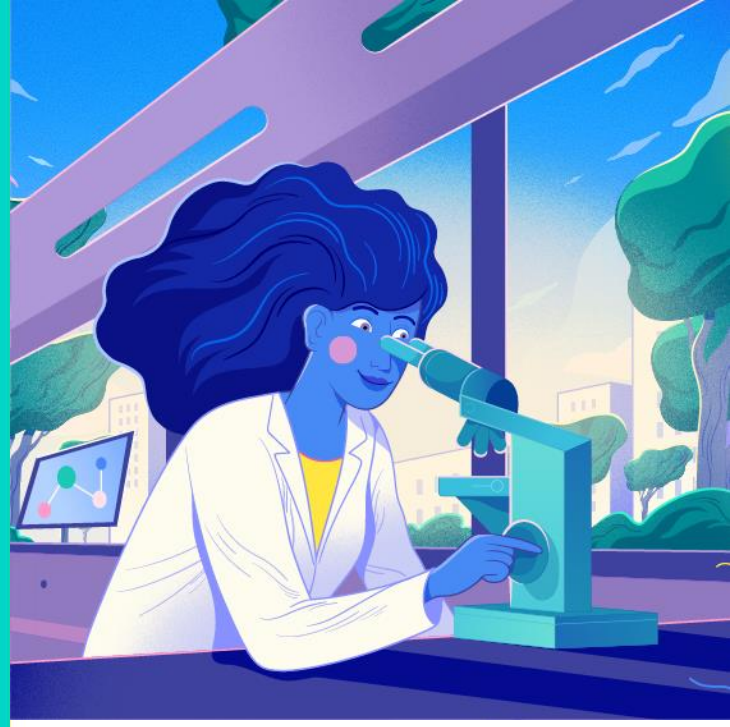
Andy Scott
Carl Westmoreland
Andy White



RENAMA

Congratulations to RENAMA on your 10th anniversary!

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