

# A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria Baltazar



Unilever

We make many of the world's favourite brands



Many products means many ingredients=

**Need for robust safety assessment of ingredients in consumer products**

# RISK ASSESSMENT GOAL: Can we use a new ingredient safely?

## Can we safely use X% of ingredient Y in product Z?



All safety assessments of cosmetic ingredients are exposure-driven:





# Increasing numbers of global consumers want their consumer products not tested on animals+ transparency

Scientific, societal, regulatory and ethical reasons are demanding change; calls for non-animal, next generation risk assessments



# Introduction to Next generation risk assessment (NGRA)

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



*Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26*

## **Main overriding principles:**

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

## **Principles describe how a NGRA should be conducted:**

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

## **Principles for documenting NGRA:**

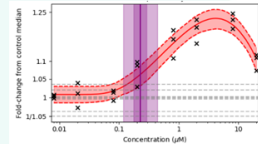
- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

# The overall goal is human safety assessment: exposure-led and human relevant

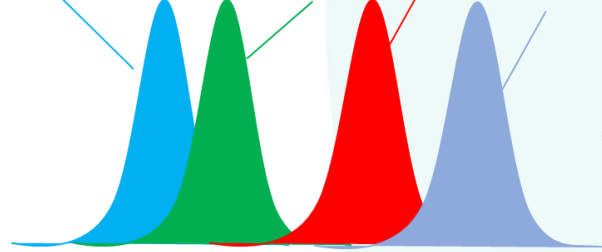
Potential hazards of the ingredients



Point of departure derived from concentration-response data



Cellular stress assays  
Transcriptomics  
Receptor binding  
Others



## Risk Assessment

Calculation of Margin of Safety (MoS) distribution



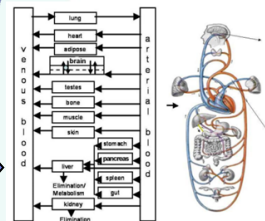
The MoS is defined as the ratio the PoD and the relevant plasma  $C_{max}$  estimate

Consumer Exposure

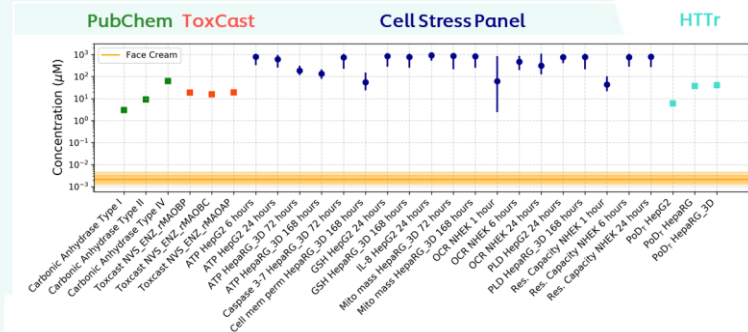
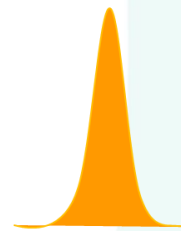


Skin pen

Exposure models (PBK, free/total concentration)



Exposure estimation: Plasma  $C_{max}$





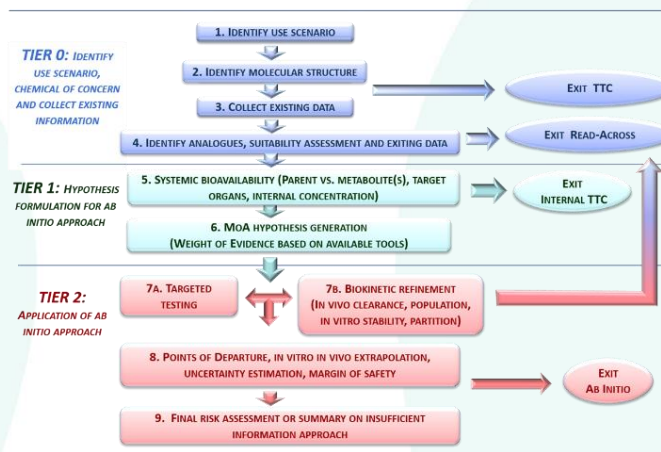
# NGRA should be conducted in a tiered and iterative approach



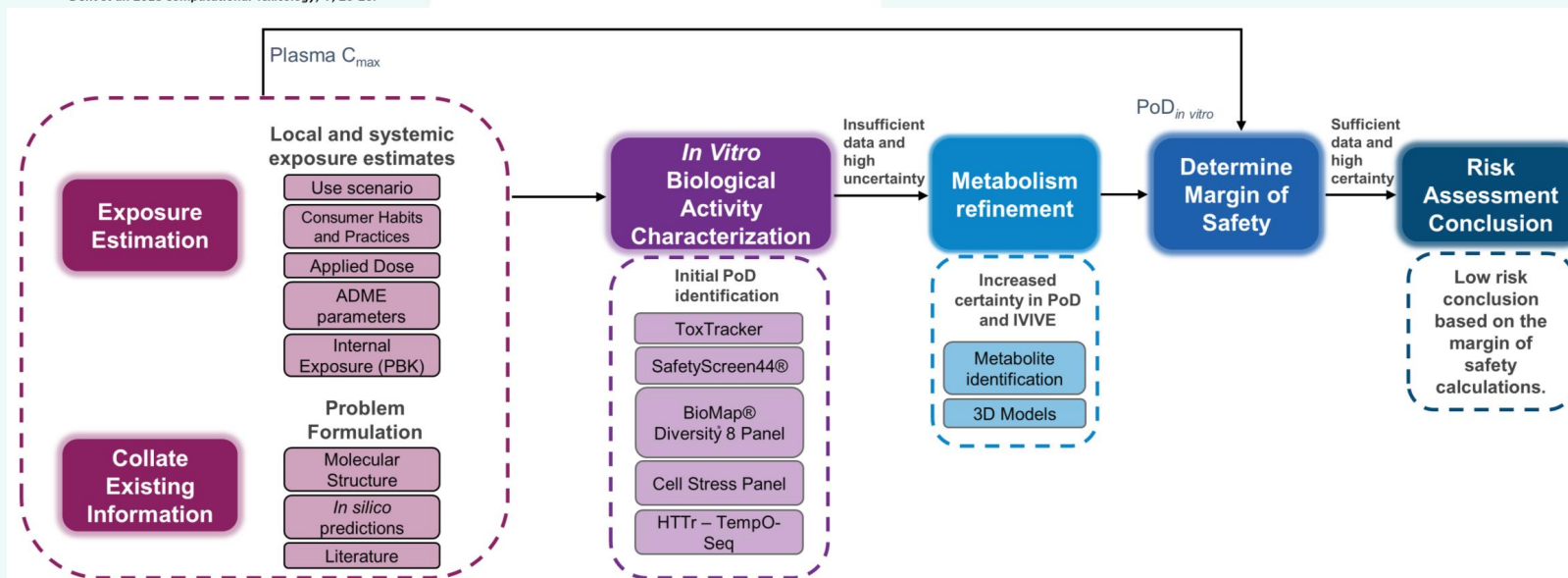
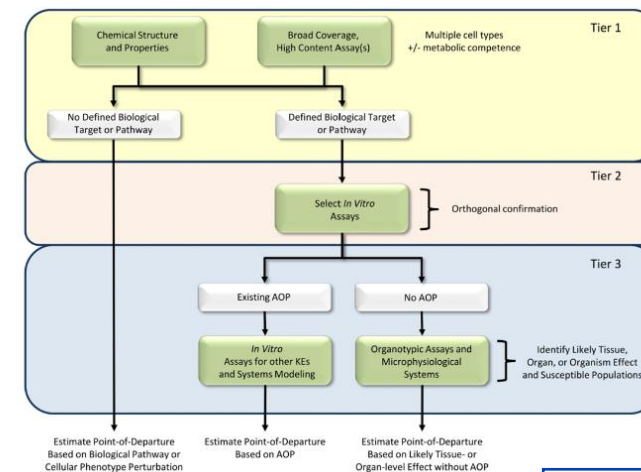
## ICCR NINE PRINCIPLES OF NGRA

- 4 **Main overriding principles:**
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- 2 **Principles for documenting NGRA:**
  - Sources of uncertainty should be characterized and documented
  - The logic of the approach should be transparent and documented

Dent et al. 2018 Computational Toxicology, 7, 20-26.

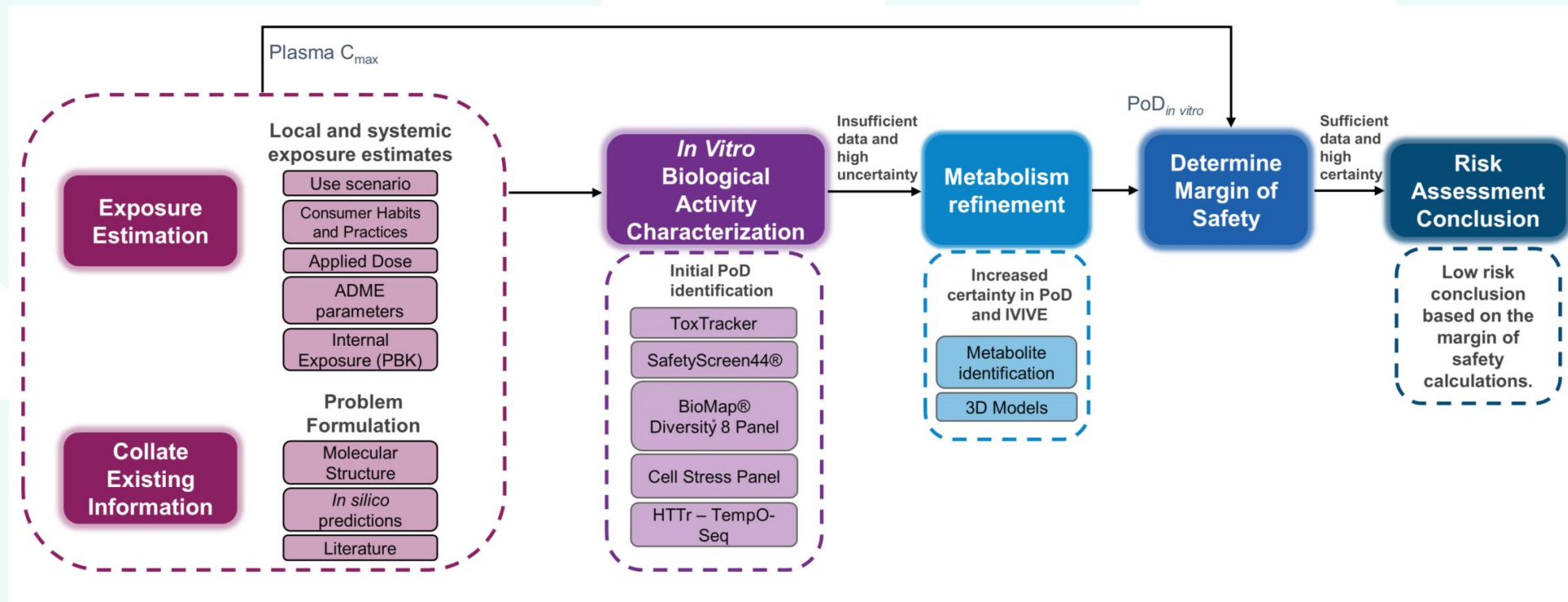
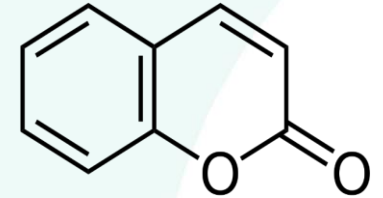


Berggren et al., (2017) Computational Toxicology 4: 31-44.



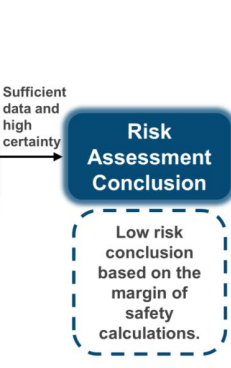
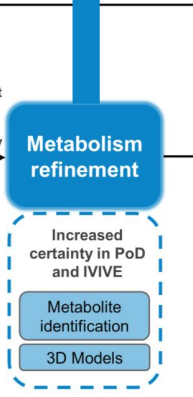
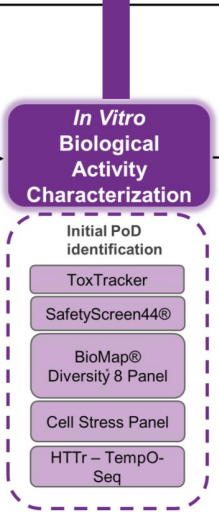
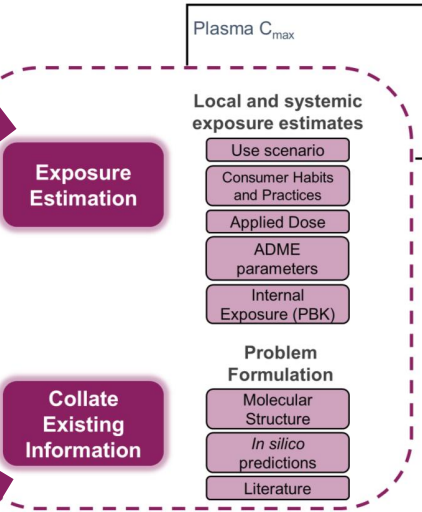
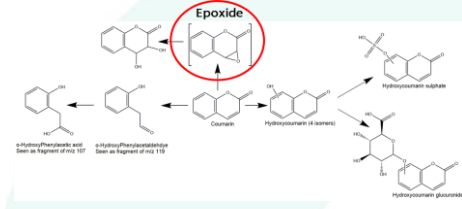
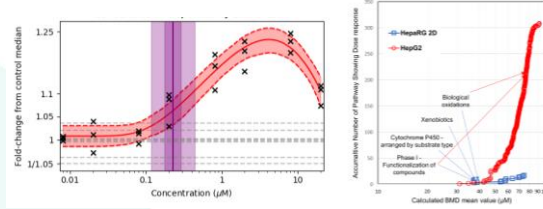
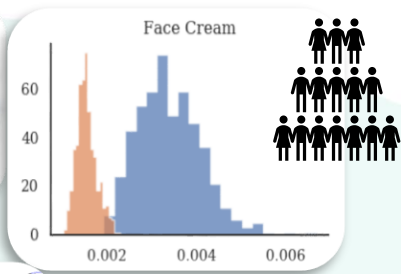
# A case study approach – human health safety assessment required for...

## 0.1% COUMARIN IN FACE CREAM AND BODY LOTION (NEW FRAGRANCE)





# Derivation of in vitro PoD across multiple cell models (HepG2, NHEK and MCF7) & refinement with HepaRG 2D and 3D & metabolism studies



**In this case study:**

- Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream is safe for the consumer

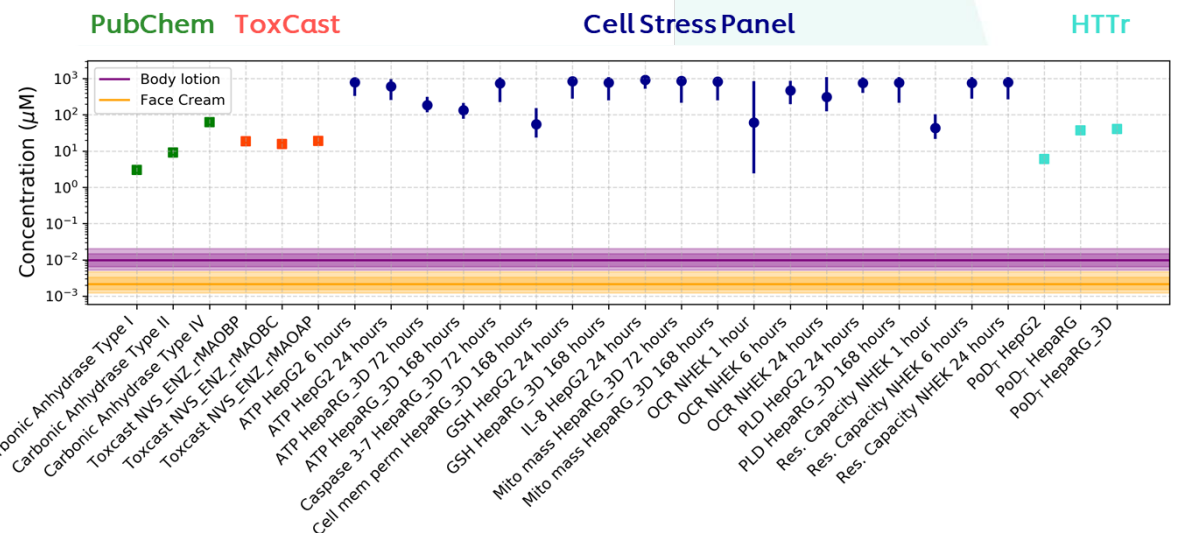


Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events  
 Timothy E. H. Allen,<sup>1</sup> Jonathan M. Goodman,<sup>1,2</sup> Steve Gutsell,<sup>1</sup> and Paul J. Russell<sup>1</sup>



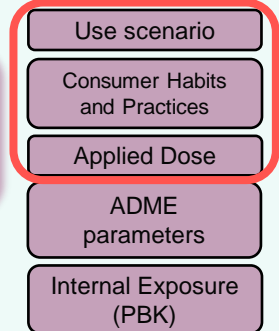
**Tox21/ToxCast**  
 ~700 HTS Biological Pathways Assays

**EPA iCSS ToxCast Dashboard**



# Exposure estimation: from applied dose to internal exposure

Local and systemic exposure estimates



Exposure Estimation

## 0.1% coumarin in face cream and body lotion

Table1. Summary of Habits and Practices Data and Applied Dose Estimates for Face Cream and Body Lotion for the European Consumer

Product Types	Face Cream	Body Lotion
Amount of product used per day (g/day) using 90th percentile <sup>a</sup>	1.54	7.82
Frequency of use <sup>b</sup>	2 times/day <sup>c</sup>	2 times/day <sup>d</sup>
Amount of product in contact with skin per occasion (mg)	770	3910
Ingredient inclusion level	0.1%	0.1%
Skin surface area (cm <sup>2</sup> ) <sup>b</sup>	565	15670 <sup>e</sup>
Leave on or rinse off	Leave on	Leave on
Exposure duration per occasion	12 h	12 h
Amount of ingredient in contact with skin per occasion (mg) <sup>f</sup>	0.77	3.91

<sup>a</sup>Hall et al. (2007).

<sup>b</sup>SCCS (2018).

<sup>c</sup>Rounded from 2.14 times/day.

<sup>d</sup>Rounded from 2.28 times/day.

<sup>e</sup>Specified as Leg region in GastroPlus.

<sup>f</sup>Based on 100% skin penetration and a body weight of 66.7 kg.

Source: Adapted from Moxon et al. (2020).

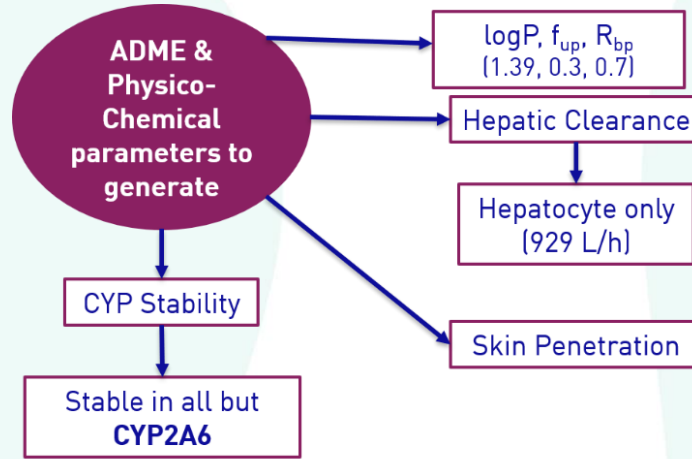


# Exposure estimation: from applied dose to internal exposure

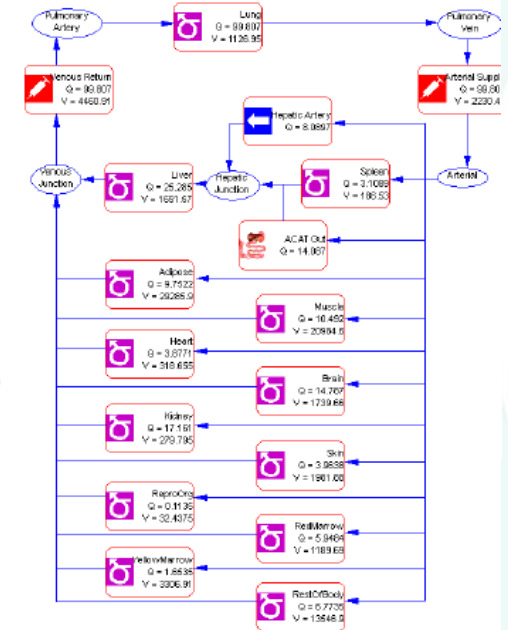
Local and systemic exposure estimates

- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters**
- Internal Exposure (PBK)**

Exposure Estimation



PBK modelling using GastroPlus® (Simulations Plus)



Simulation of plasma concentration of coumarin after dermal exposure.

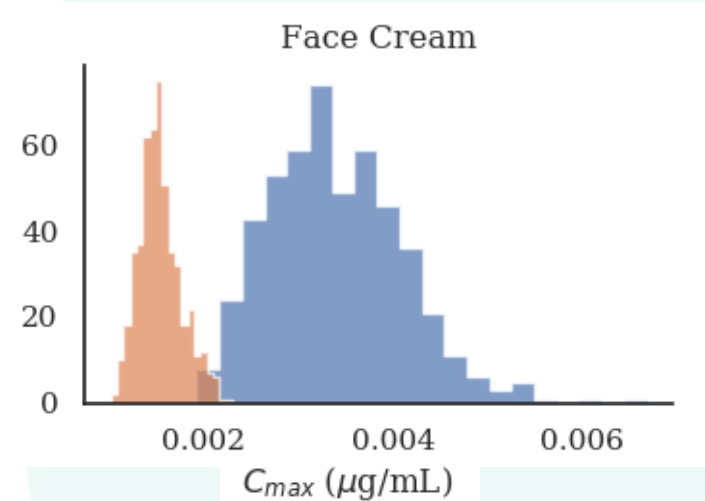
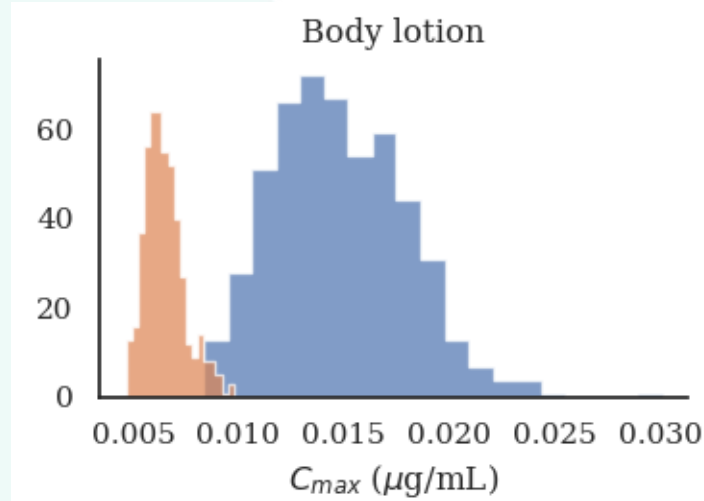


# Exposure estimation: from applied dose to internal exposure

Local and systemic exposure estimates

- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters**
- Internal Exposure (PBK)**

Exposure Estimation



Clearance  
■ in silico 98.57 L/h  
■ in vitro 929 L/h

Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

Total Plasma $C_{max}$ ( $\mu\text{M}$ )	Mean	Median	90th Percentile	95th Percentile	97.5th Percentile	99th Percentile
Body lotion	0.01	0.01	0.018	0.019	0.02	0.022
Face cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005



# Collation of existing information: in silico predictions

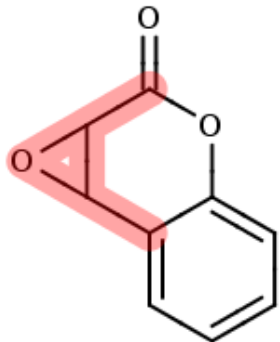
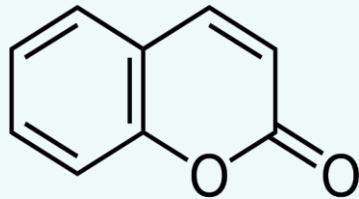
**Collate Existing Information**

**Problem Formulation**

Molecular Structure

*In silico* predictions

Literature



**In silico tools (ToxTree, MIE ATLAS\*, OECD toolbox, Meteor) predicted:**

- Protein binding- MIE for induction of skin sensitisation
- DNA binding alert - MIE for genotoxicity
- Reactive metabolites (e.g. epoxide formation)- alerts for both genotoxicity and skin sensitisation
- No binding alerts for the 39 targets in MIE atlas (e.g. nuclear receptors, enzymes, transporters)

# Collation of existing information: literature

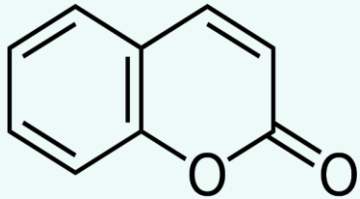
Collate  
Existing  
Information

Problem  
Formulation

Molecular  
Structure

*In silico*  
predictions

Literature

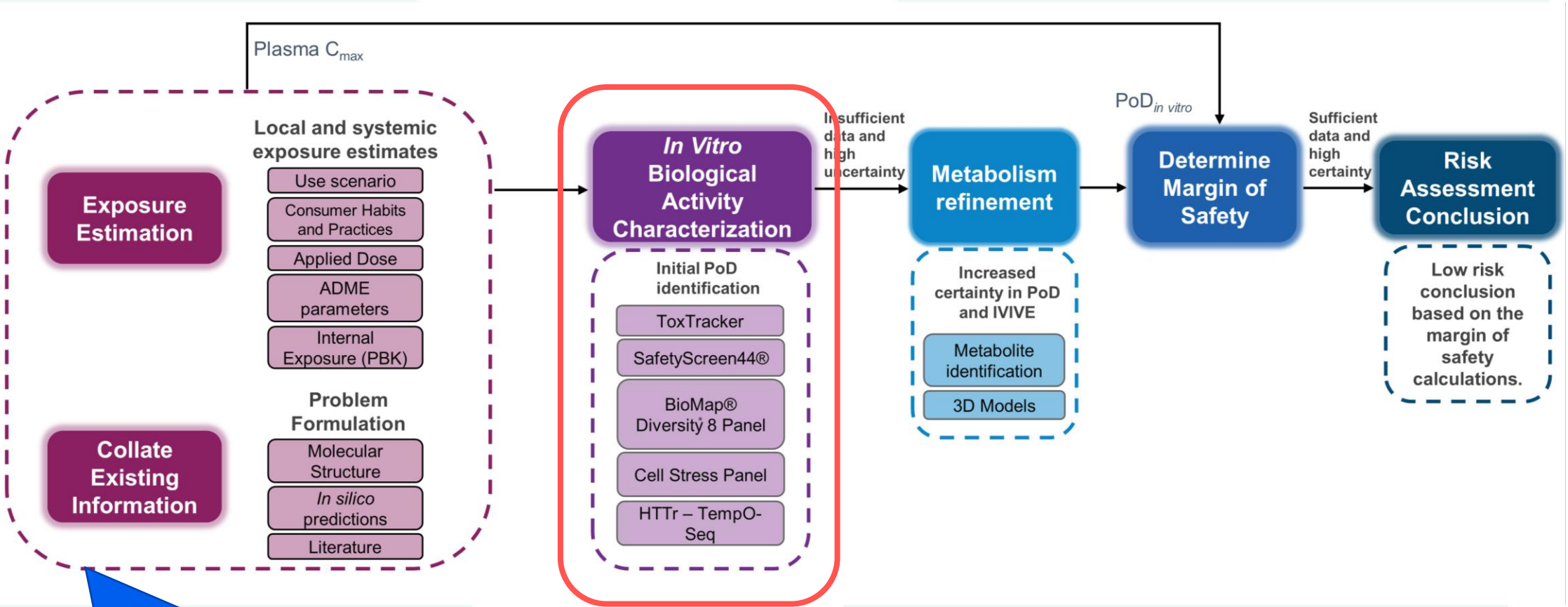


## PubChem and Toxcast databases results:

- Only few active assays among multiple assays ( $\approx 5000$ )
- Coumarin inhibited both Monoamine oxidases and Carbonic anhydrases at concentrations between  $3 \mu\text{M}$ -  $40 \mu\text{M}$
- The AC50\* from dose-response curves was used a PoD for MoS calculation



# Next-Generation Risk Assessment case study workflow



Q1: What other information would you like to see to increase your confidence in the conclusions?

Add your suggestions to Zoom Q&A

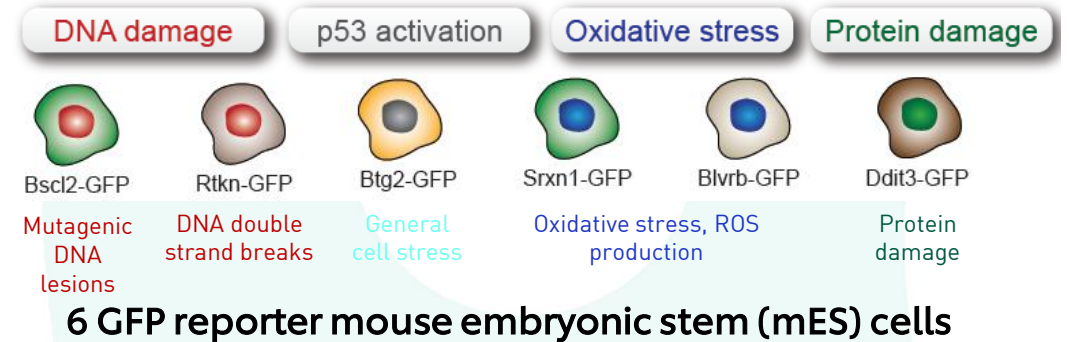
# In vitro biological activity characterisation – overview of the NAMs

## Genotoxicity assessment: ToxTracker

- Coumarin and its metabolites triggered genotoxicity alerts

## In vitro binding and enzymatic assays: Eurofins SafetyScreen44

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



**PERSPECTIVES**

**REDUCING SAFETY-RELATED DRUG ATTRITION: THE USE OF *IN VITRO* PHARMACOLOGICAL PROFILING**

*Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolim, Alan Smith, Gareth Holburn 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 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<sup>1</sup> 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 economic, societal and regulatory embargos.

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 characterisation 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

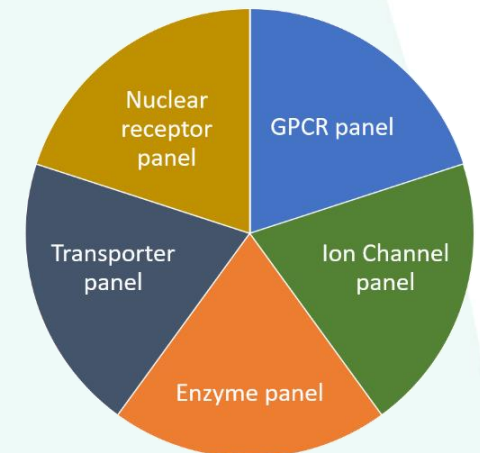
safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

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 time-current of native ( $I_h$ ) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (hKCNH2), also known as hERG. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (overlaid in the picture) following a prolongation of the QT interval is well characterised<sup>2</sup>, 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<sup>3</sup>.

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 need 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.

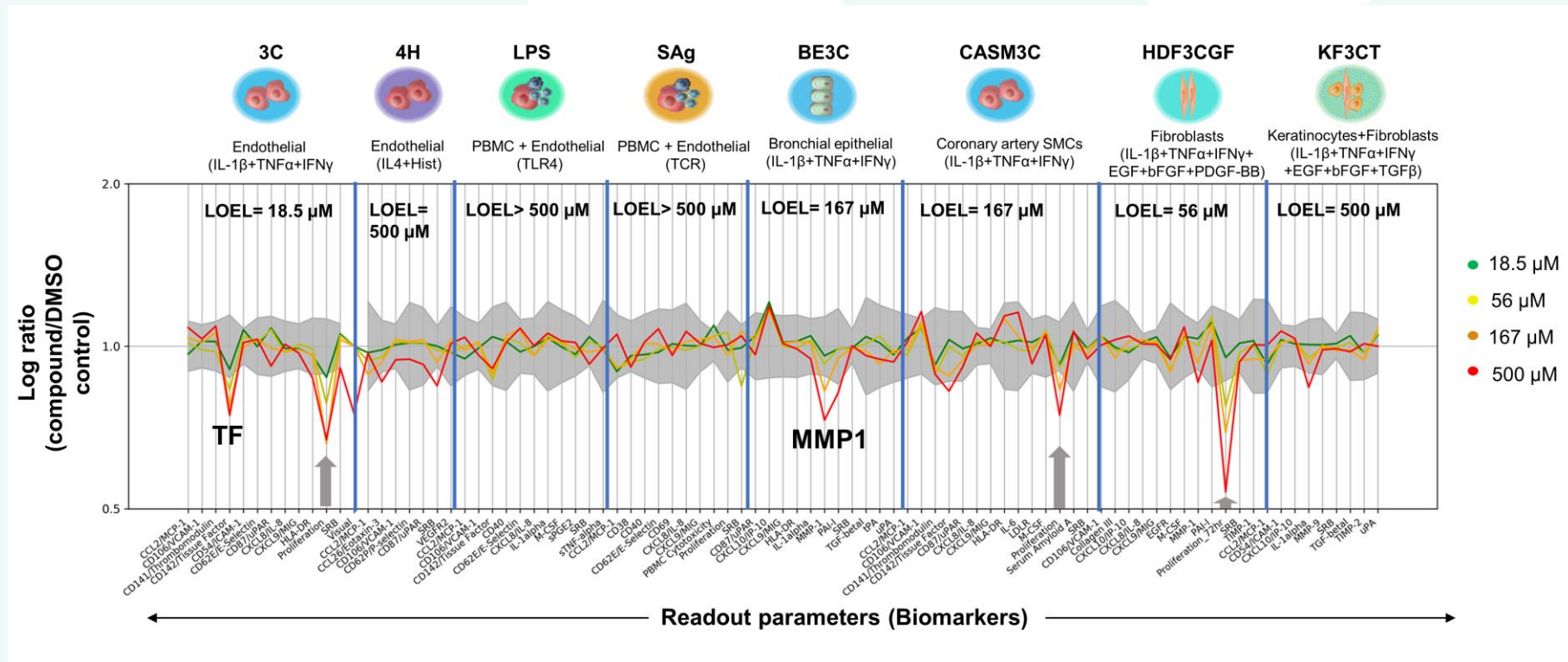
Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experience of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe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



# In vitro biological activity characterisation – overview of the NAMs

## Immunomodulatory screening assay: BioMap Diversity 8 panel

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





# In vitro biological activity characterisation – overview of the NAMs

## Cell stress panel

- To characterize non-specific biological activity which is not mediated via a specific protein/receptor interaction - covering ~10 cell stress pathways using high content imaging analysis

## High Throughput Transcriptomics (HTTr) – TempO-Seq

- Transcriptomics was applied as a broad nontargeted biological screen

- 36 Biomarkers;
- 3 Timepoints (1h,6h,24);
- 8 Concentrations;
- NHEK, HepG2, HepaRG
- Dose response analysis and derivation of PoD

- ~10 Stress Pathways: mitochondrial Toxicity, Oxidative Damage, DNA damage, Inflammation, ER stress, Metal stress, Heat Shock, Hypoxia, Cell Health

- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

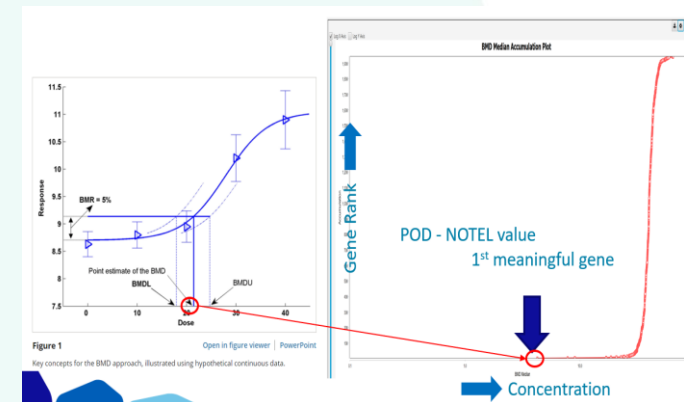


### FEATURED

#### Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

Sarah Hatherell,\* Maria T. Baltazar,\* Joe Reynolds,\* Paul L. Carmichael,\* Matthew Dent,\* Hequn Li,\* Stephanie Ryder,<sup>†</sup> Andrew White,\* Paul Walker ,<sup>†</sup> and Alistair M. Middleton\*<sup>1</sup>

\*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire

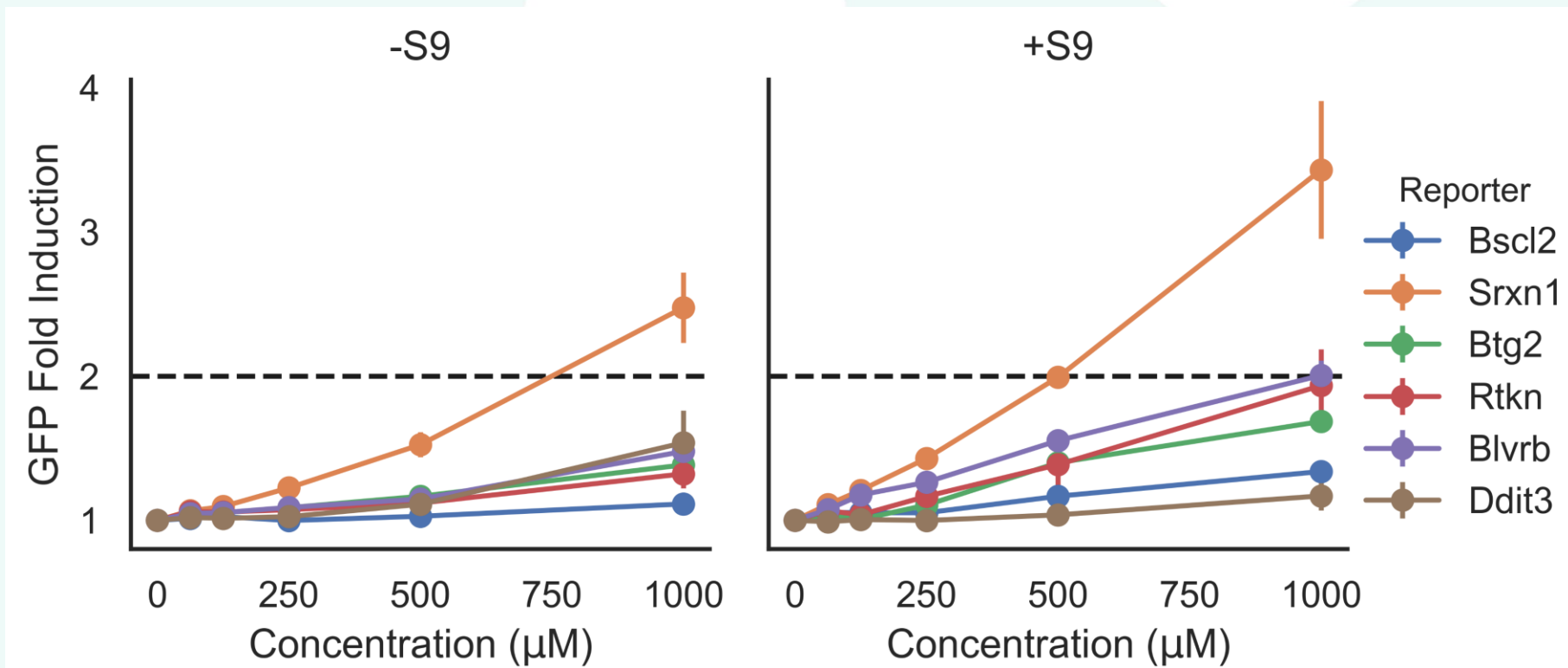


**BMDexpress 2**

# In vitro biological activity characterisation: Coumarin is not genotoxic in the Toxtracker assay

## Genotoxicity assessment: ToxTracker

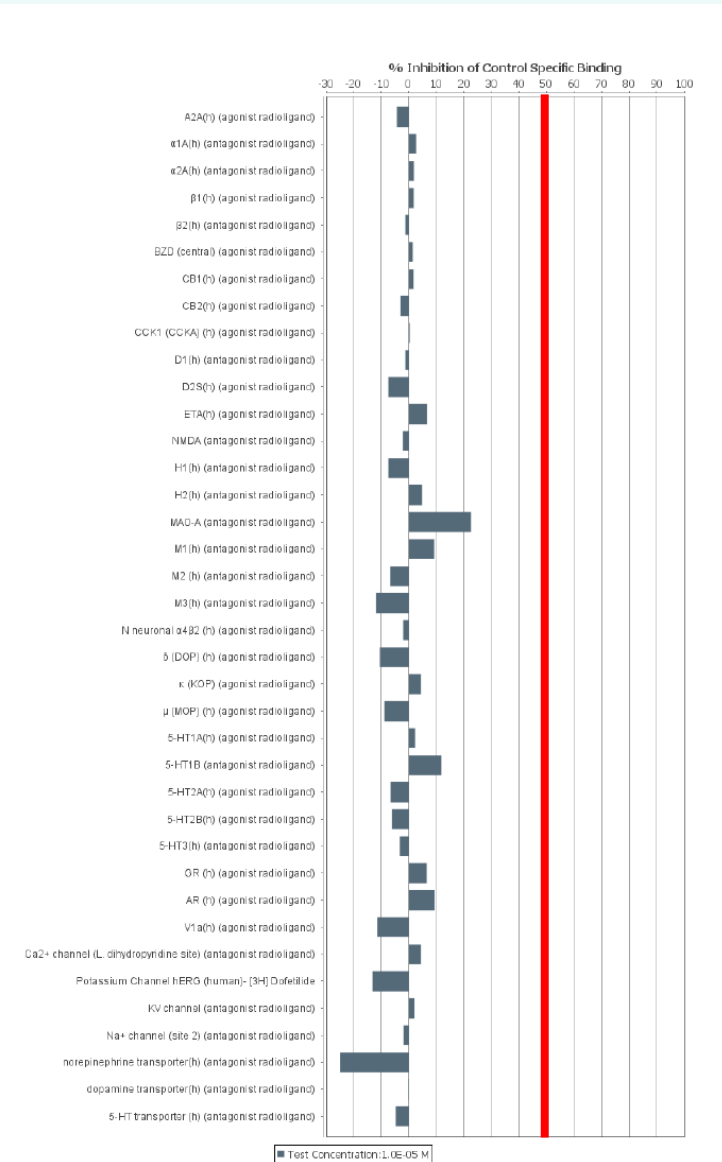
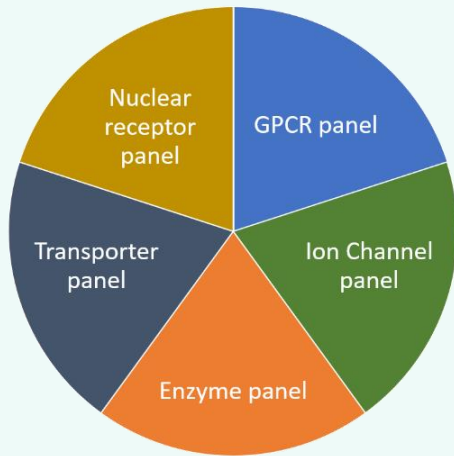
- ToxTracker negative
- Reactive coumarin metabolite(s) could induce DNA lesions secondary to oxidative stress



# In vitro biological activity characterisation: Coumarin does not bind to any of the 44 targets tested

## In vitro binding and enzymatic assays: Eurofins SafetyScreen44

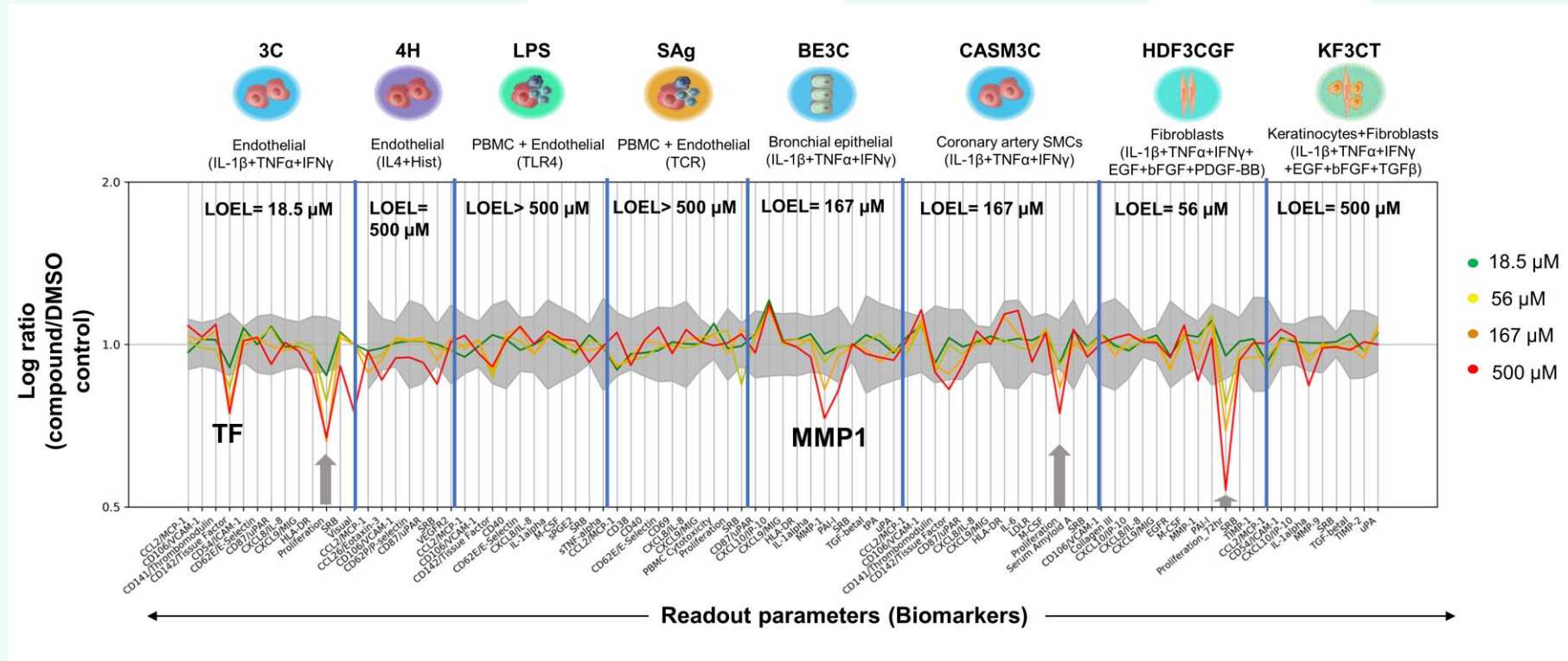
- All binding and enzymatic assay results were negative at 10  $\mu$ M



# In vitro biological activity characterisation: coumarin had no immunomodulatory effects

## Immunomodulatory screening assay: BioMap Diversity 8 panel

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





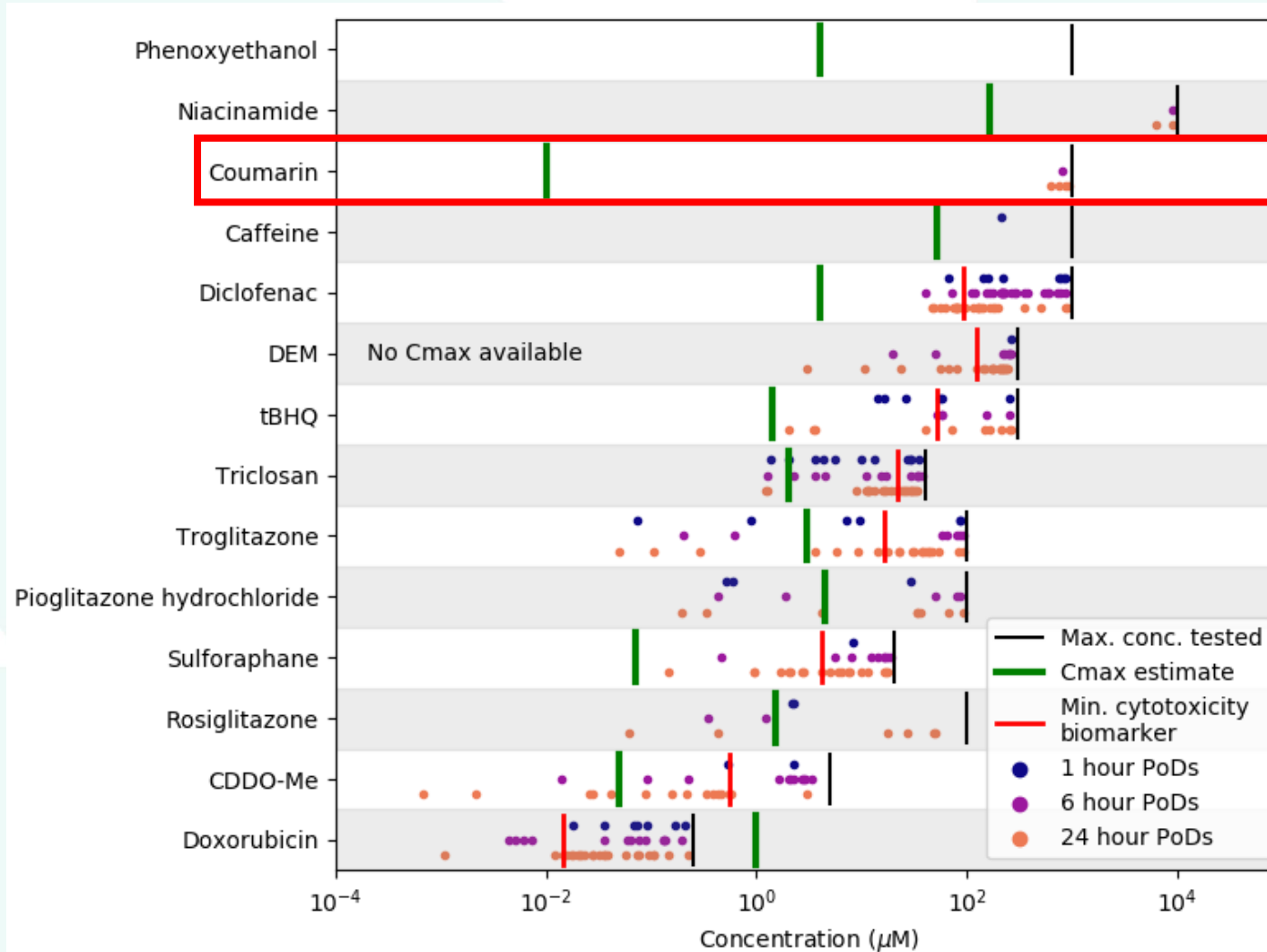
## In vitro biological activity characterisation: Coumarin showed low bioactivity in the cell stress panel

Table 4. PoDs From Cell Stress Panel After Acute Exposure (24 h) in HepG2 and NHEK and Long-term Exposure (168 h) in HepaRG 3D Spheroids

Biomarker	Cell Type	Stress Pathway	PoD ( $\mu\text{M}$ )	Effect	CDS
ATP (6 h)	HepG2	Cell health	794 (363–977)	Down	0.98
ATP (24 h)			617 (282–891)	Down	1
Phospholipidosis (24 h)	HepG2	Cell health	759 (437–977)	Down	0.93
GSH (24h)	HepG2	Oxidative stress	851 (301–1000)	Up	0.92
IL-8 (24h)	HepG2	Inflammation	912 (575–1000)	Down	0.61
OCR (1 h)	NHEK	Mitochondrial toxicity	62 (2.6–776)	Down	0.6
OCR (6 h)			468 (214–794)		1
OCR (24 h)			309 (138–1000)		0.52
Reserve capacity (1 h)	NHEK	Mitochondrial toxicity	44 (23–96)	Down	1
Reserve capacity (6 h)			759 (302–1000)		0.9
Reserve capacity (24 h)			794 (295–1000)		0.55
Caspase 3–7 (72 h)	HepaRG 3D	Cell health	741 (245–977)	Up	0.95
Cell membrane permeability (168 h)	HepaRG 3D	Cell health	55 (26–141)	Up	0.99
ATP (72h)	HepaRG 3D	Cell health	186 (129–288)	Down	1
ATP (168h)			135 (85–195)	Down	
Phospholipidosis (168h)	HepaRG 3D	Cell health	776 (234–1000)	Up	0.86
GSH (168 h)	HepaRG 3D	Oxidative stress	776 (275–1000)	Down	0.92
Mitochondrial mass (72 h)	HepaRG 3D	Mitochondrial toxicity	871 (234–1000)	Down	0.65
Mitochondrial mass (168 h)			831 (275–1000)	Down	0.73

Only PoDs from concentration-responses with CDS > 0.5 were considered as true representations of bioactivity. Reported values are the mode (most likely value in bold) and 95% highest-density-interval (in brackets) summarizing the distribution for the PoD as reported in Hatherell et al. (forthcoming).

## In vitro biological activity characterisation: Coumarin showed low bioactivity in the cell stress panel



### Results:

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

- PoDs shown for HepG2 only

## In vitro biological activity characterisation: Coumarin showed low bioactivity in the HTTr assay

Table 5. PoD<sub>T</sub> Values (μM) for Coumarin Treated Across 4 Cell Models for 24h Using a Subset of Proposed Approaches for Gene Selection Based on Those Proposed by Farmahin *et al.* (2017)

Cell Model	HepG2	MCF7	HepaRG 2D	HepaRG 3D
Pathway-level tests PoD <sub>T</sub> (μM)	(308 pathways)	(0 pathways)	(17 pathways)	(2 pathways)
20 pathways with the lowest <i>p</i> value Reactome	70	NA	58*	46*
20 pathways with the lowest BMD Reactome	44	NA	58*	46*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38	41
Gene-level tests PoD <sub>T</sub> (μM)	(1570 genes)	(47 genes)	(87 genes)	(9 genes)
Mean BMD of 20 genes with largest fold change	6	3	54	55
Mean BMD of genes between 25th and 75th percentile	17	1	59	46*

Highlighted (\*) are values where the number of pathways or genes was below the recommended number (ie, 20) for grouping. Abbreviation: NA, not applicable.

# All PoD are higher than the predicted plasma C<sub>max</sub> for both product types

Margin of Safety (5<sup>th</sup> percentile) for each product type and technology (lowest MoS per biomarkers)

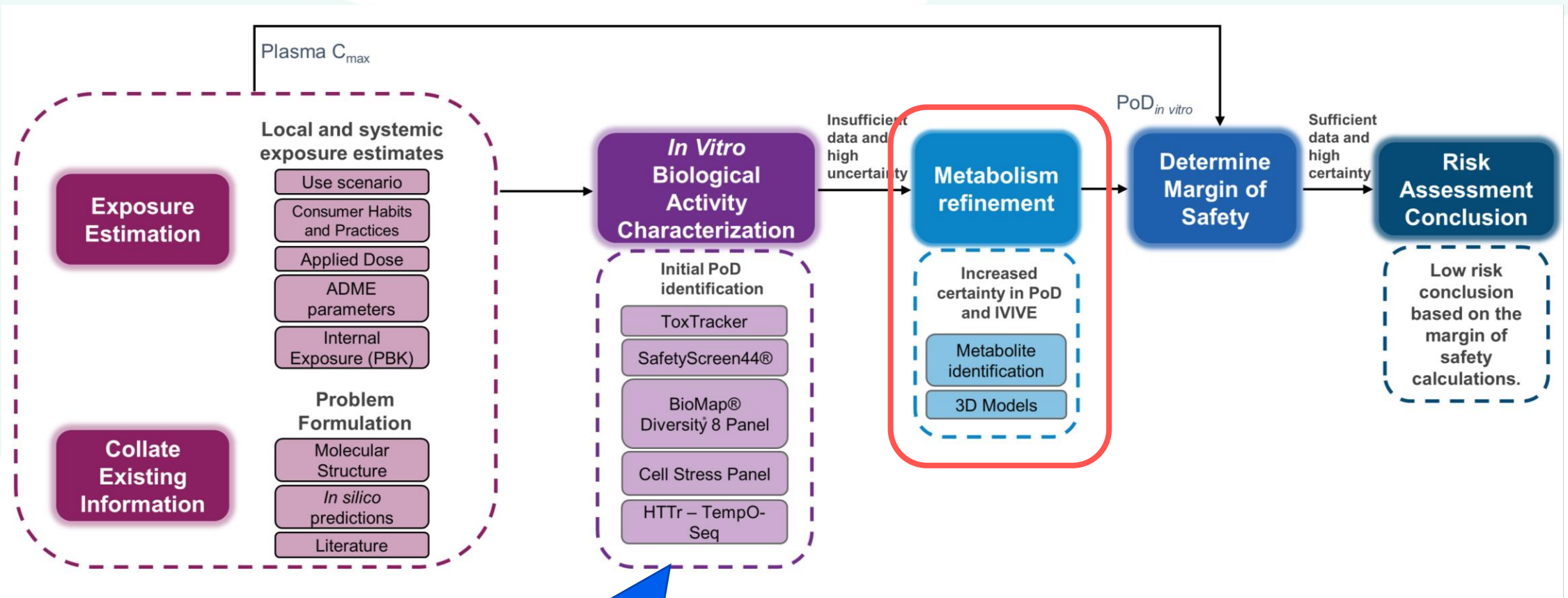
Source	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	Body Lotion Min. 5th percentile MoS	PoD provided as distribution?
PubChem	Carbonic Anhydrase Type I	706	158	No
PubChem	Carbonic Anhydrase Type II	2140	479	No
PubChem	Carbonic Anhydrase Type VI	14652	3282	No
Toxcast	MAO B (rat brain)	3711	831	No
Cell stress panel	HepG2 (ATP, 24 h)	96738	22048	Yes
Cell stress panel	NHEK (OCR 1 h) HepaRG_3D	1330	295	Yes
Cell stress panel	(cell membrane permeability 168 h)	9601	2197	Yes
HTTr	HepG2 (24 h)	1411	316	No
HTTr	HepaRG (24 h)	8864	1986	No
HTTr	HepaRG 3D (24 h)	9538	2137	No

Based on total concentrations for both C<sub>max</sub> and PoDs

- The lowest MoS across all assays was derived using the PoD (represented by Ki) for the inhibition of carbonic anhydrase I
- Potential metabolite-driven bioactivity not addressed

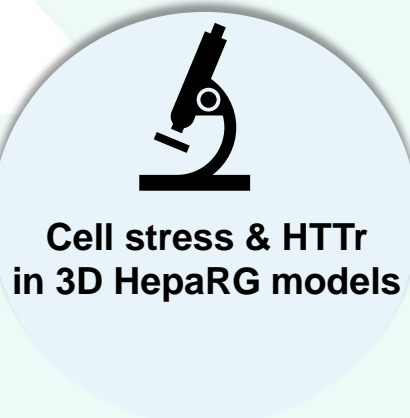
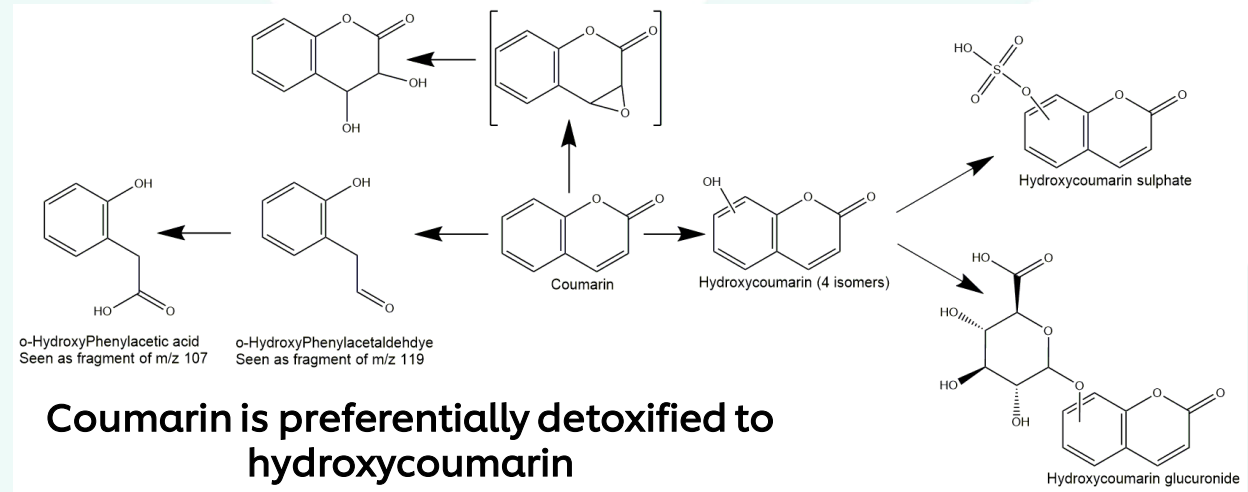


# Next-Generation Risk Assessment case study workflow



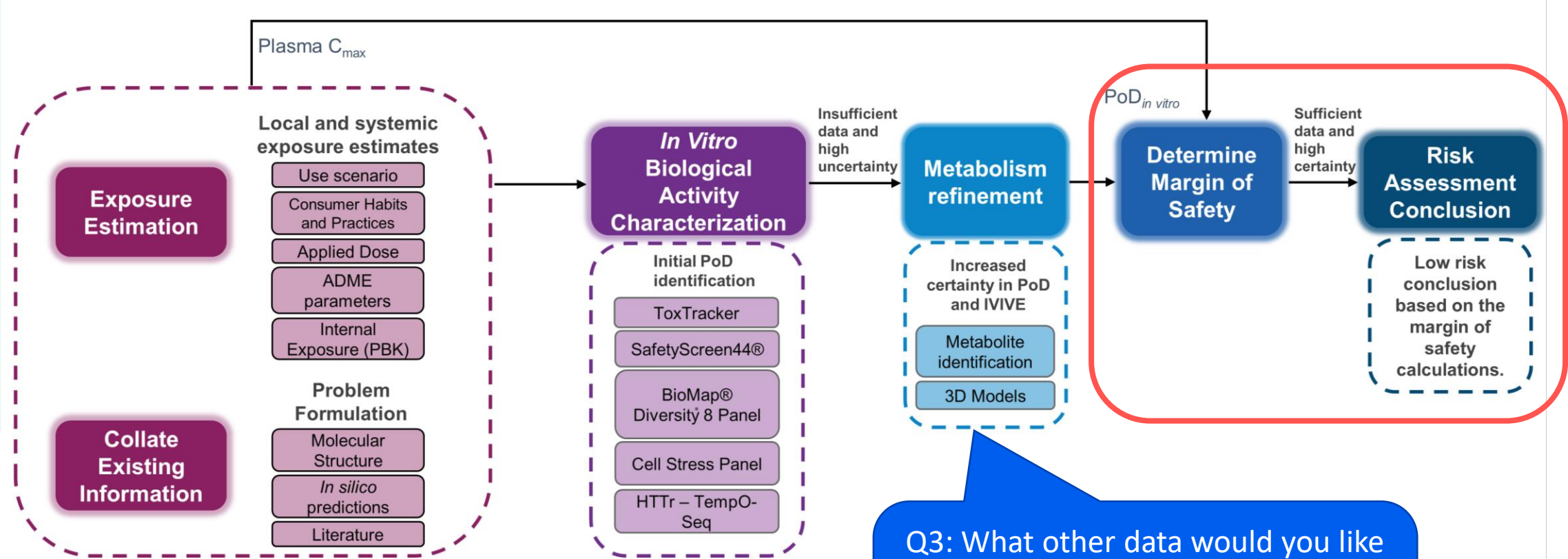
Q2: What other data would you like to generate to increase your confidence in the conclusions?  
 Add your suggestions to Zoom Q&A

# The metabolism refinement step increased our confidence that coumarin is preferentially detoxified



- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871  $\mu\text{M}$  – not very different from 2D cells

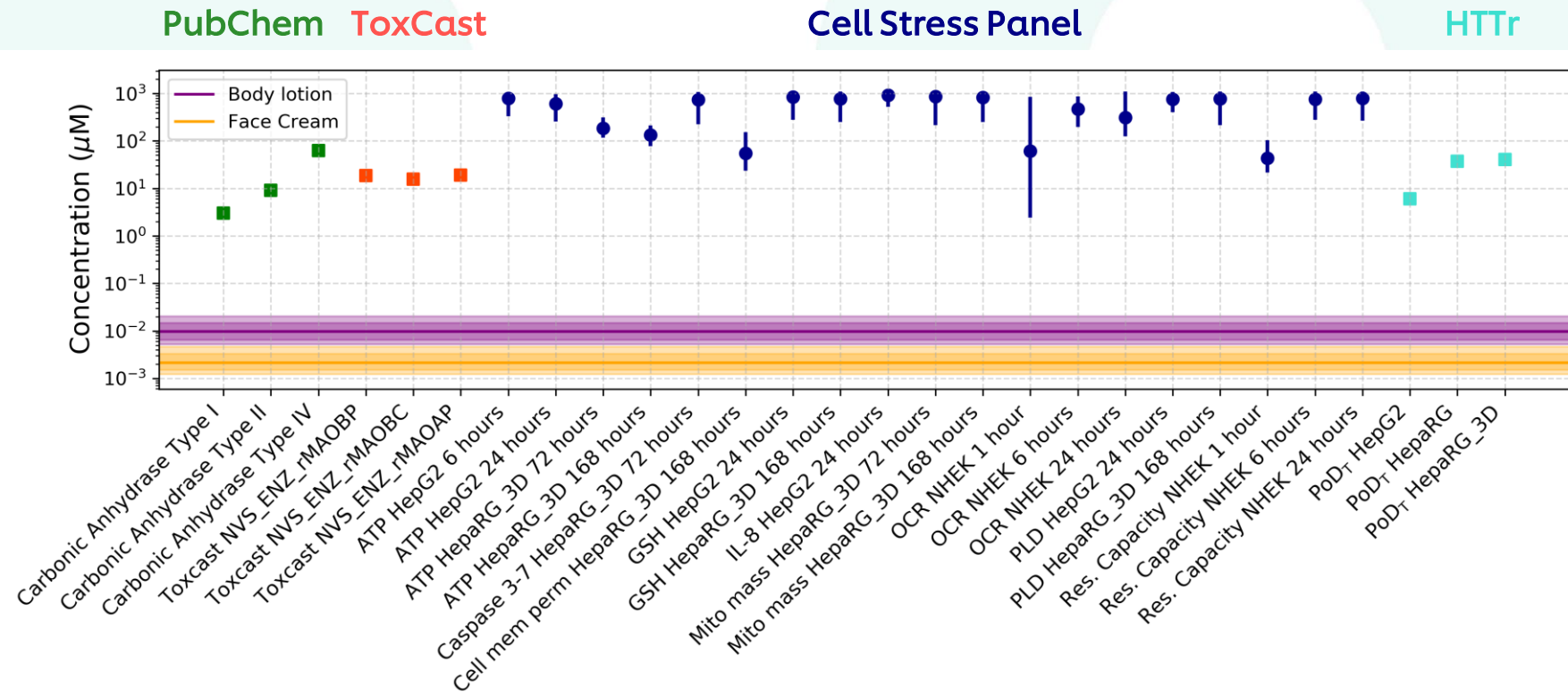
# Next-Generation Risk Assessment case study workflow



Q3: What other data would you like to generate to increase your confidence in the conclusions?

Add your suggestions to Zoom Q&A

# Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream and body lotion is safe for the consumer



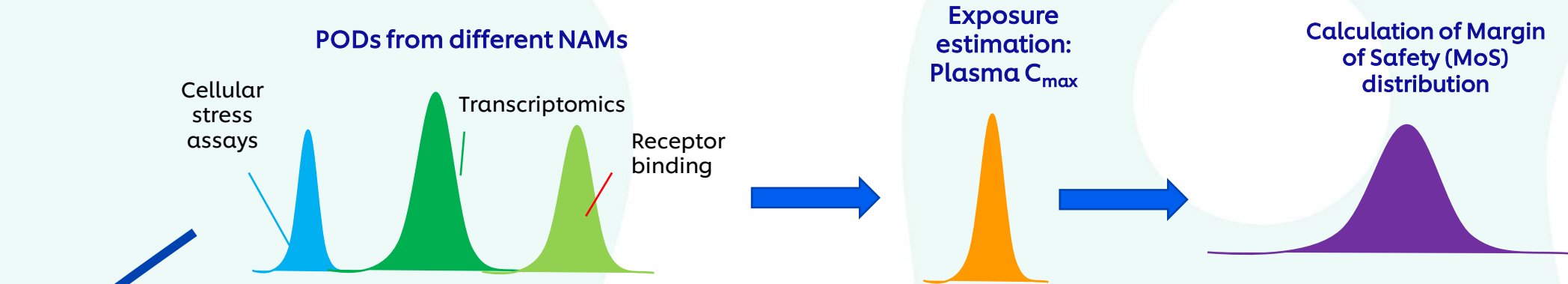
The 5<sup>th</sup> percentile of the MoS distribution ranged between 158 and 96738

### In this case study:

- Coumarin is not genotoxic, does not cause skin sensitisation, does not bind to any of the 44 targets and does not show any immunomodulatory effects at consumer relevant exposures

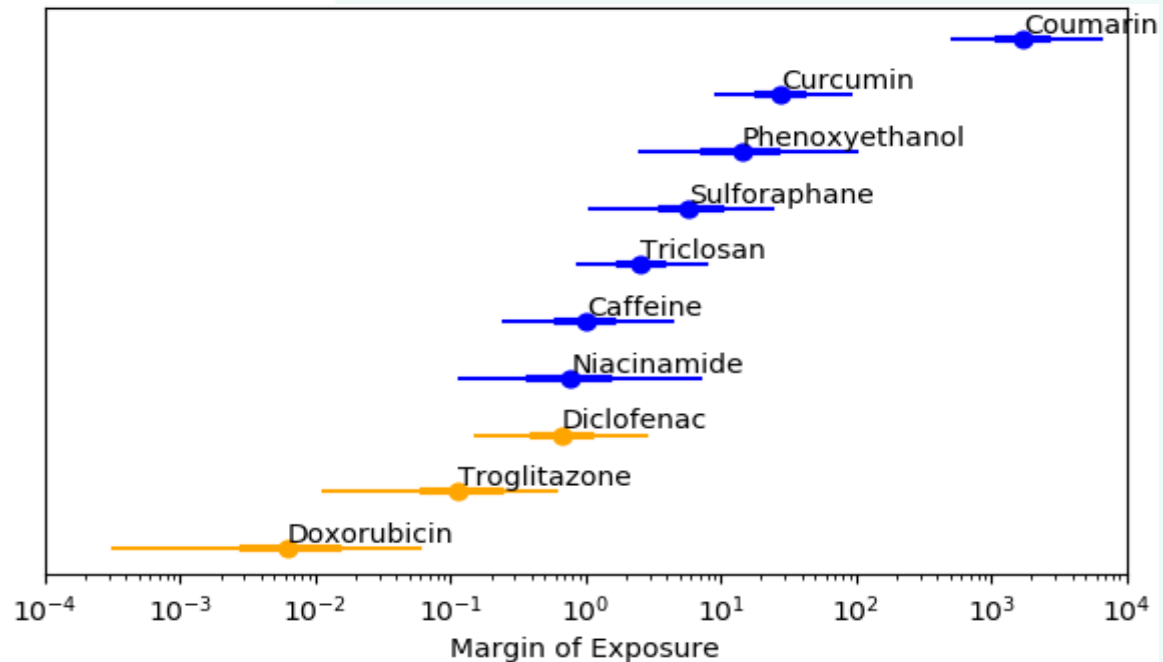


# Critical questions is: How can we conclude what MoS derived from NAMs is large enough to be protective of human health?



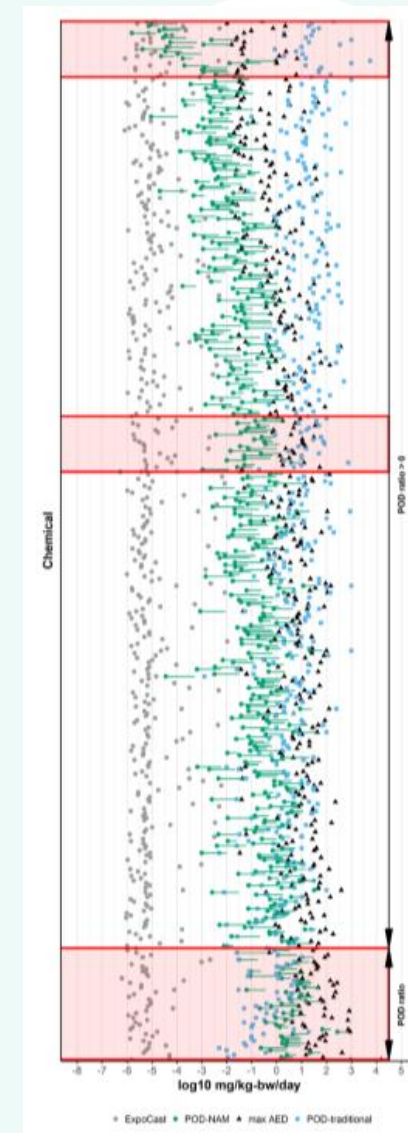
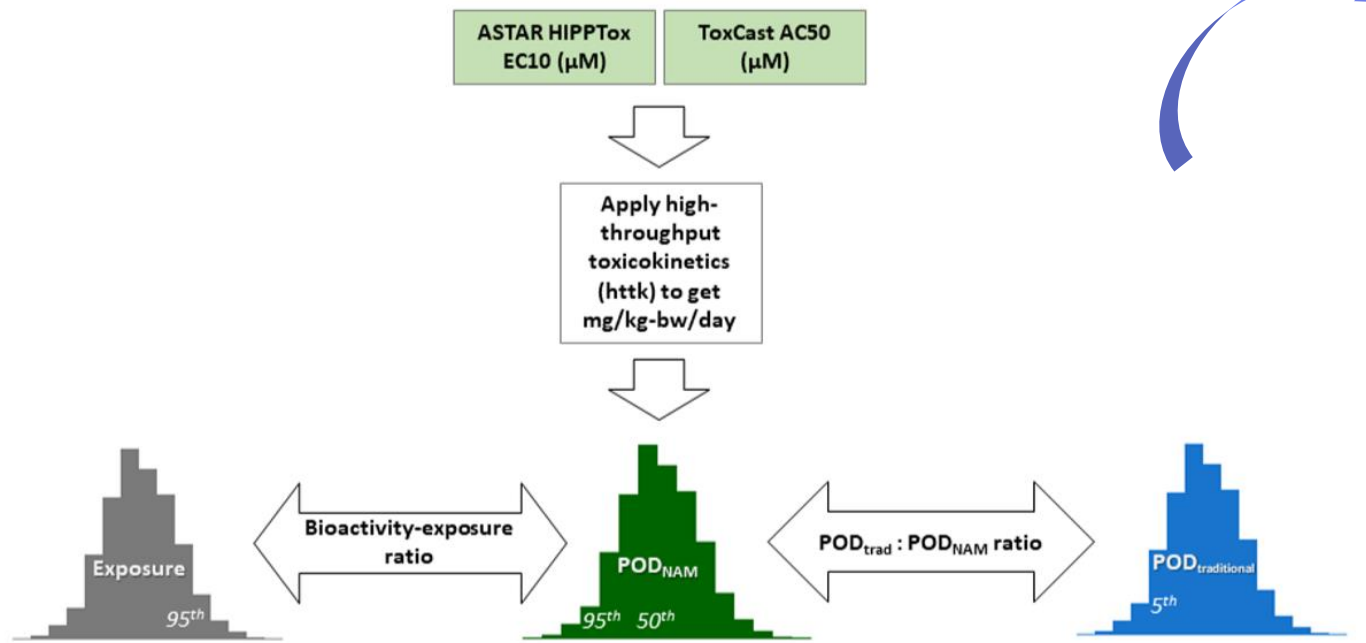
## Chemical exposures

- 'High' risk (from consumer goods perspective) – e.g. drugs
- 'Low' risk (from consumer goods perspective) – e.g. foods, cosmetics



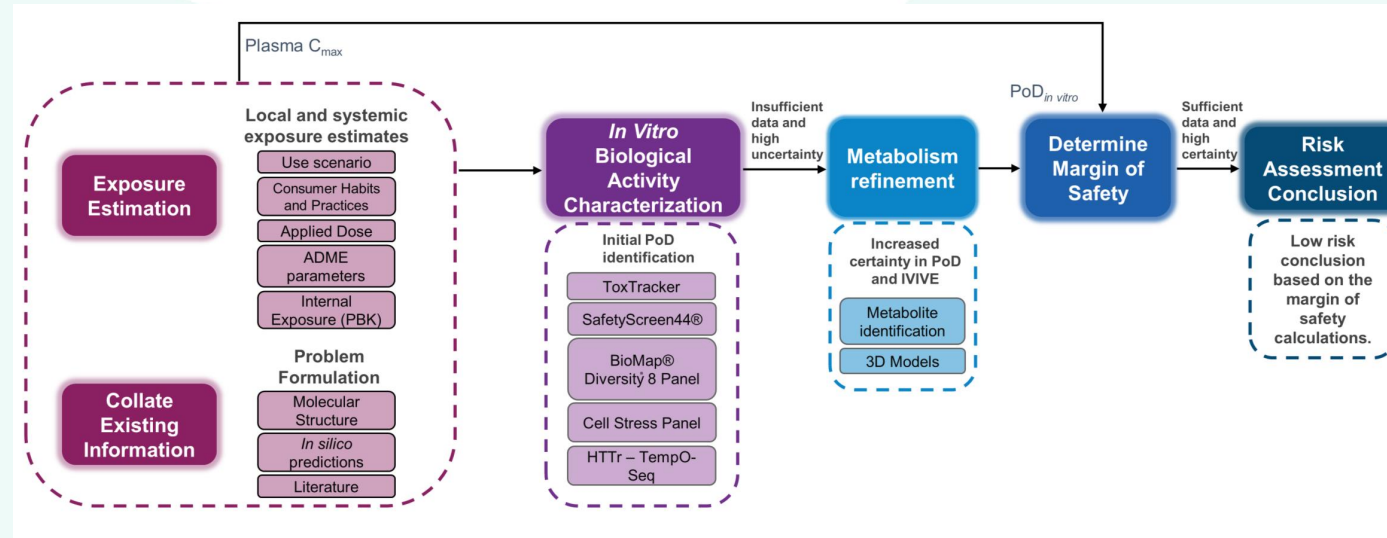
Recent research has shown that for 417 out of 448 chemicals tested the point of departure derived (PoD) from NAMS was more conservative than the in vivo PoD

EPA, NTP, HC, A\*STAR, ECHA, EFSA, JRC, RIVM...



Katie Paul-Friedman *et al.* 2019 *Tox Sci* 173(1): 202-225

# Concluding remarks



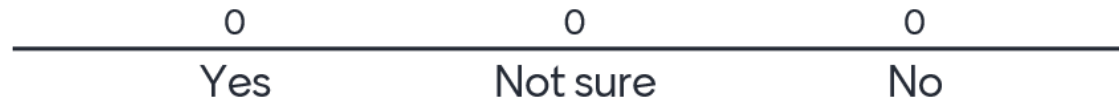
- NAMs can provide robust insights to support exposure estimation and mechanistic in vitro bioactivity data to inform non-animal safety assessment- **data generation is driven by the risk assessment questions**
- The approach focuses on **building a weight of evidence**- tools can be integrated to make a safety decision but **multidisciplinary team is needed!**
- Approach only possible with a change in mindset (**protection not prediction**)
- Uncertainty analysis incorporated across the framework allowed us to be **explicit about remaining uncertainties**
- **Rethinking MoS/MoE** – future evaluation of the approach to **infer a low risk space**
- Doing and sharing more **case studies will increase confidence in the applying of NAMs in decision-making**

Menti poll: please go to [menti.com](https://www.menti.com) and use code XX XX XX X

Go to [www.menti.com](https://www.menti.com) and use the code

Do you agree with the low risk decision?

 Mentimeter





# Acknowledgements



## Core Team:

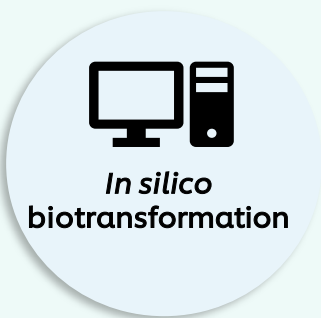
- Maria Baltazar, Alistair Middleton, Tom Cull, Joe Reynolds, Beate Nicol, Mi-Young Lee, Predrag Kukic, Alexis Nathanail, Sophie Cable, Georgia Reynolds, Mona Delagrange, Tom Moxon, Hequn Li, Mabel Cotter, Jade Houghton, Andy White, Matthew Dent, Paul Carmichael, Sarah Hatherell, Sophie Malcomber, Richard Cubberley, Ruth Pendlington

## Extended Team:

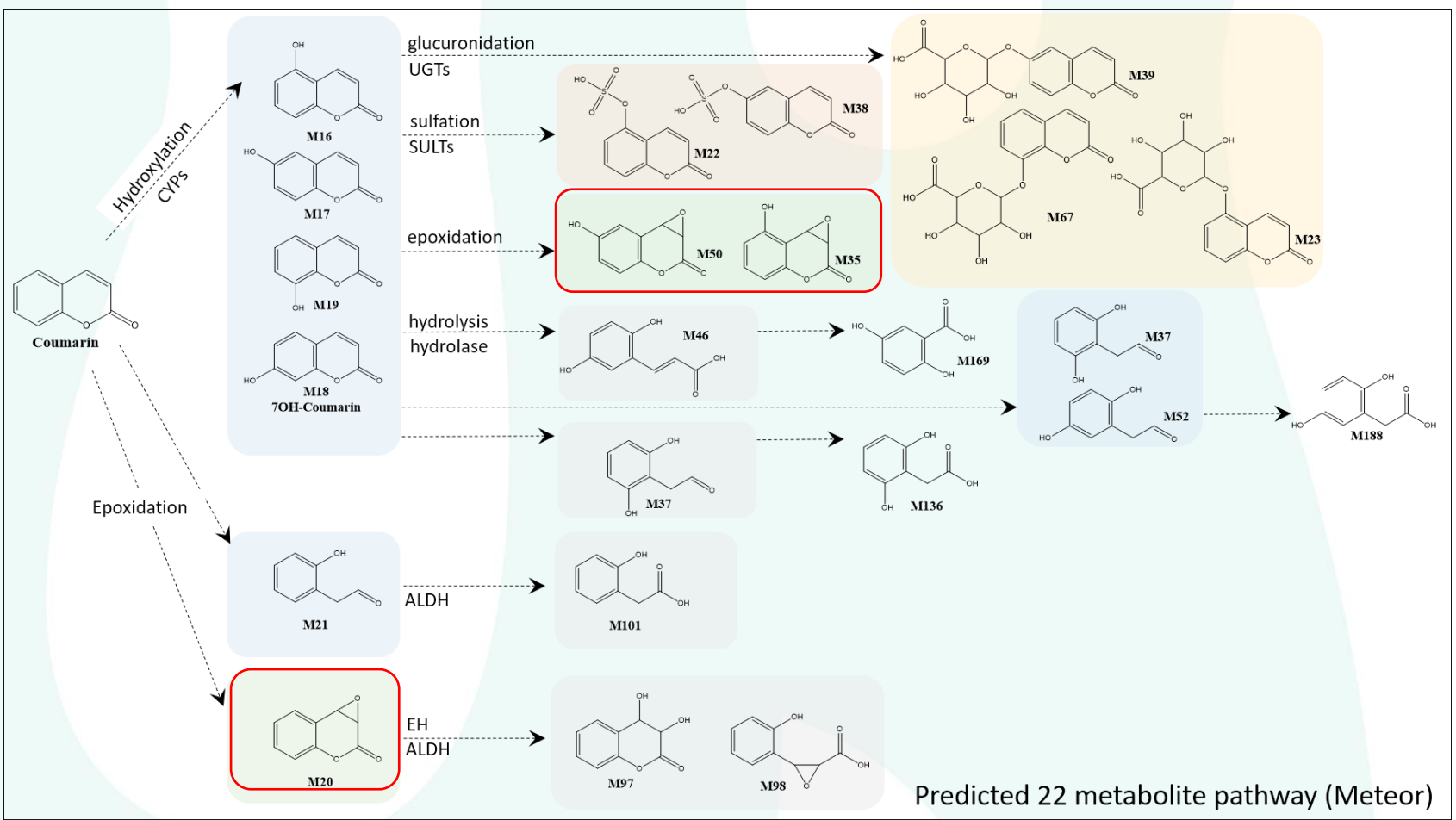
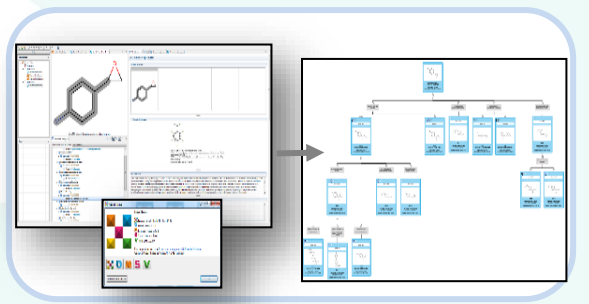
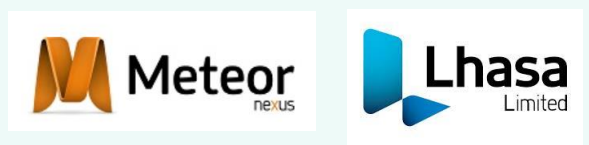
- Carl Westmoreland, Paul Russell, Gavin Maxwell, Ian Sorrell, Sam Piechota, Juliette Pickles, Karen Bonner, Sandrine Spriggs, Iris Muller, Katarzyna Przybylak, Paul Walker, Caroline Bauch, Rebecca Beaumont, Steve Clifton, Katie Paul-Friedman, Julia Fentem

# BACKUP SLIDES

# NGRA for 0.1% coumarin in face cream: in silico predictions - Metabolism



- Hydroxylation predicted as main route of biotransformation
- Reactive metabolites (e.g. epoxides) predicted.

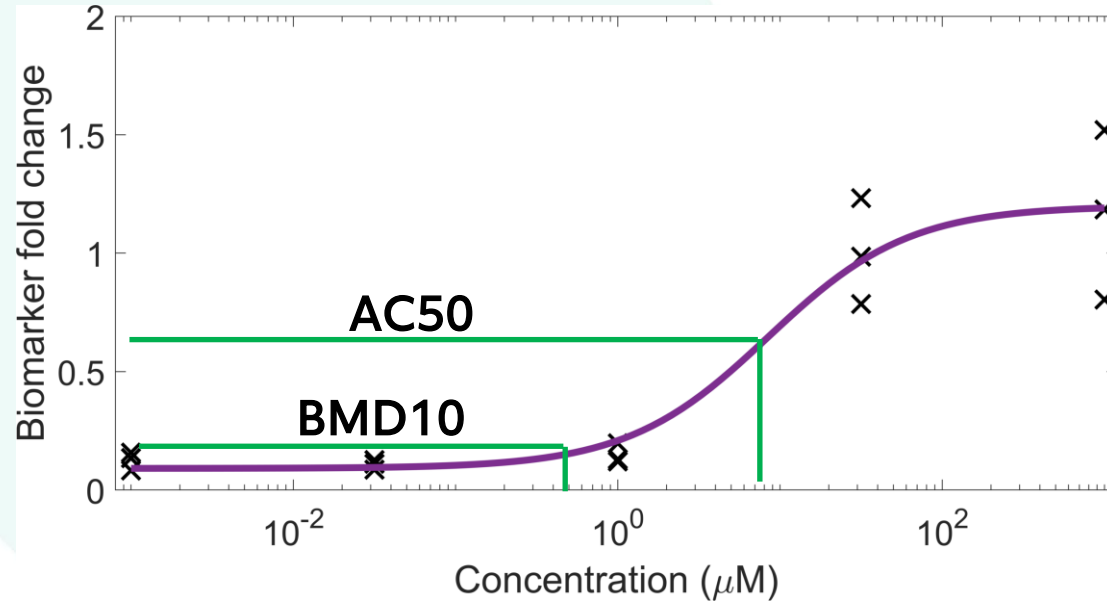


Predicted 22 metabolite pathway (Meteor)



# NGRA: dose-response analysis and PoD derivation

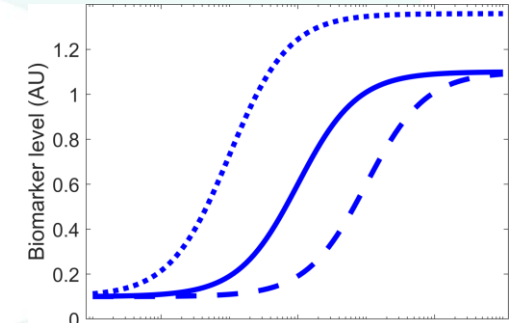
## Example dose response data



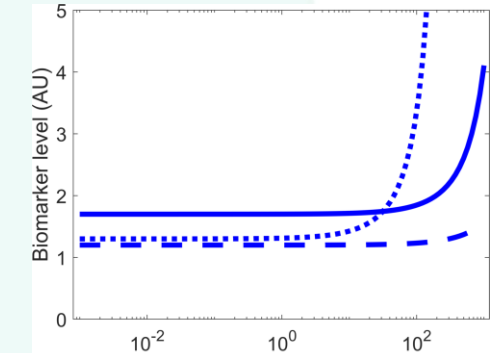
1. Fit different parametric models to the data
2. Identify the one with the 'best' fit
3. Use this to calculate the PoD...
4. Different PoDs exist, e.g:
  - AC50
  - BMD10

## Candidate dose-response models

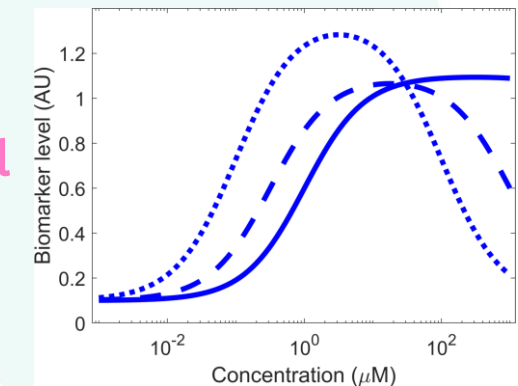
Hill function



Exponential



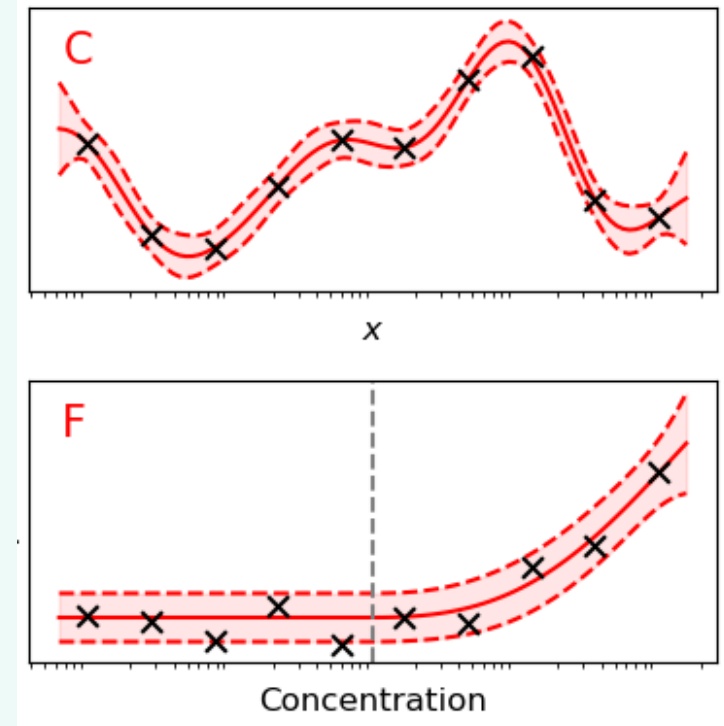
Gain-loss model





# NGRA: dose-response analysis and PoD derivation

1. Challenges with this can arise when e.g. none of the candidate models provide a good fit, or noise (e.g. outliers) in the data leads to spurious PoD estimates.
2. In NGRA it is important to quantify the uncertainty in a) whether there is a concentration-dependent response and b) the PoD estimate, if there is one.
3. Instead we used a non-parametric model (Gaussian processes) within a Bayesian statistical framework to model to data.



# NGRA: dose-response analysis and PoD derivation

