A Next Generation Risk Assessment – Coumarin case study

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Unilever

EPA NAM workplan

1. Primary focus for talk

The objective of a consumer product risk assessment is…

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

Framework Approach: The overall goal is a human safety risk assessment

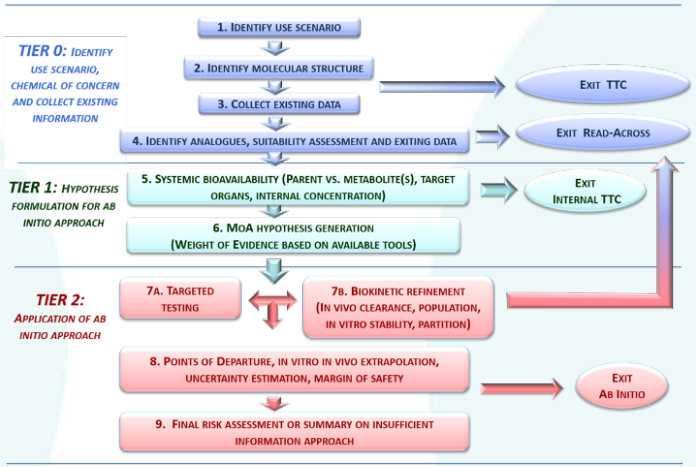
ICCR 9 principles of NGRA

4 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

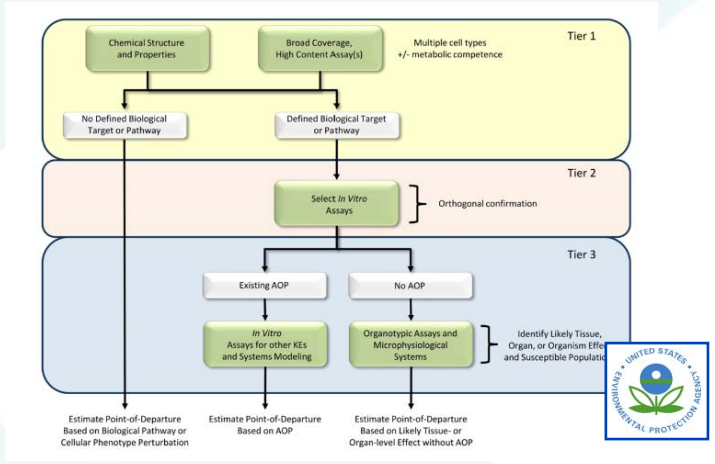
3 Principles describe how a NGRA should be conducted:
Following an appropriate appraisal of existing information
Using a tiered and iterative approach
Using robust and relevant methods and strategies

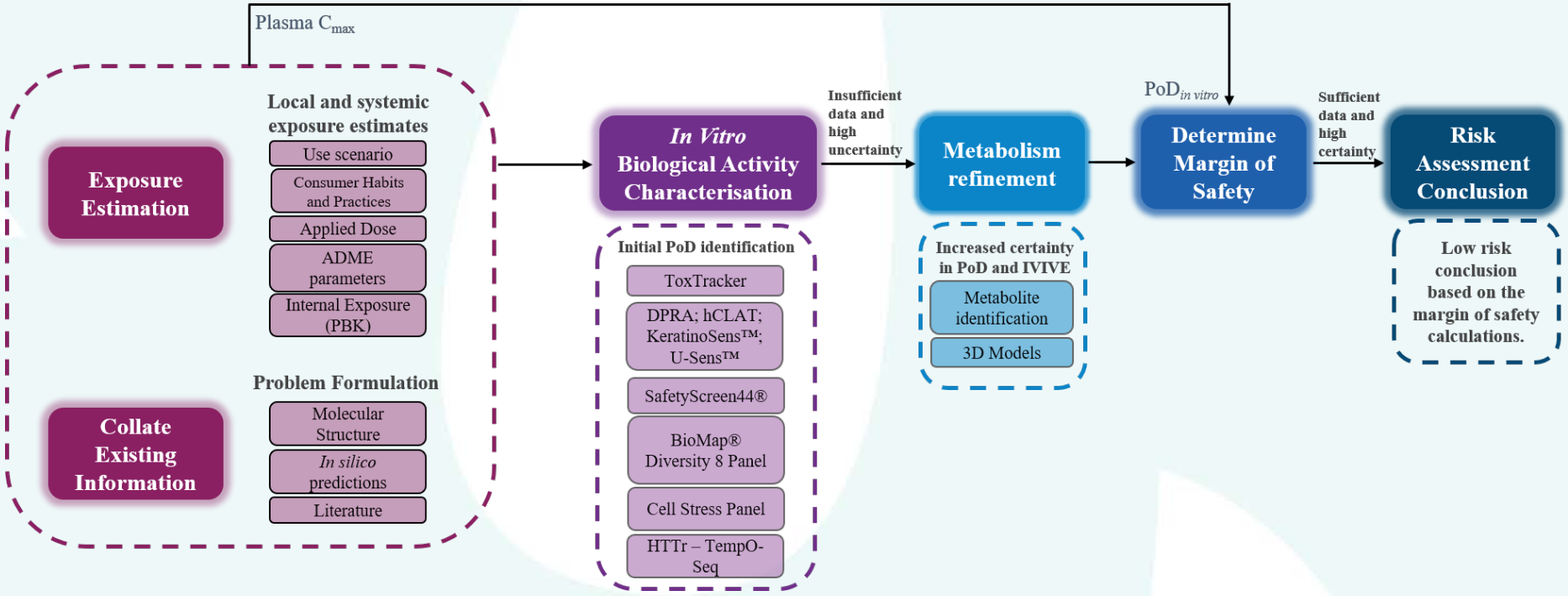
2 Principles for documenting NGRA:
Sources of uncertainty should be characterized and documented
The logic of the approach should be transparent and documented

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



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

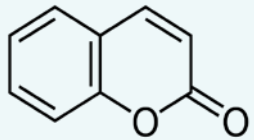




Problem Formulation

Case Study approach – Human Health Safety Assessment required for …

0.1% COUMARIN IN FACE CREAM



Assumed that:

- **Coumarin was 100% pure**
- **no *in vivo* data was available such as animal data, History of Safe Use (HoSU) info. 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**

Exposure Led

All safety assessments of cosmetic ingredients are exposure-driven:

Baltazar *et al.,* (2020) *Tox Sci* (vol 176: 236–252) https://doi.org/10.1093/toxsci/kfaa048

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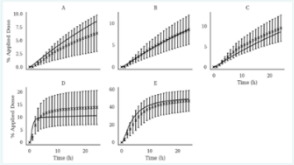
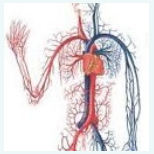
Application Scenario for determining PoD - still in development

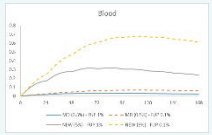
Exposure due to consumer use mg/kg/day

Skin pen





Free plasma concentration (uM) corresponding to consumer use, from PBPK modelling

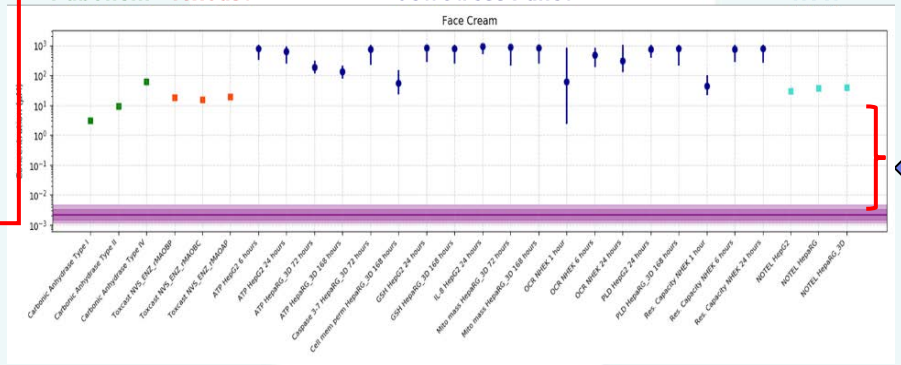


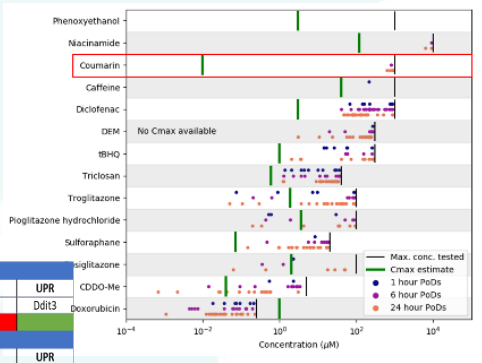
Consumer Exposure

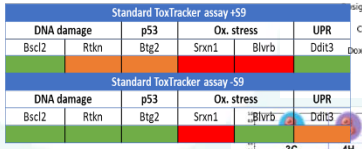
Potential hazards of the ingredients

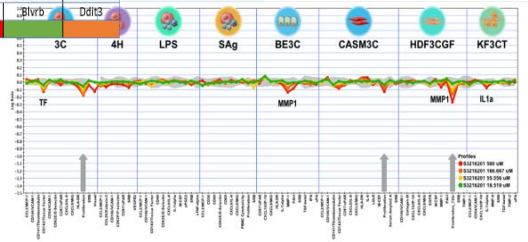
Risk Assessment

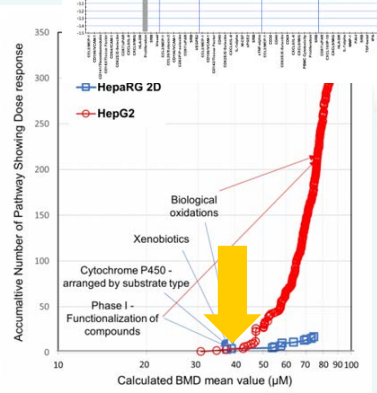
PubChem ToxCast Cell Stress Panel HTTr











MoS POD comparison

In vitro threshold concentration (uM) measured as free media concentration

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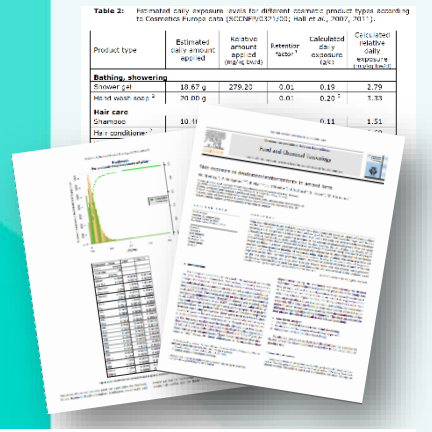
NGRA for 0.1% coumarin in face cream: exposure estimation

Exposure Estimation

Local and systemic exposure estimates

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

Creme GLOBAL

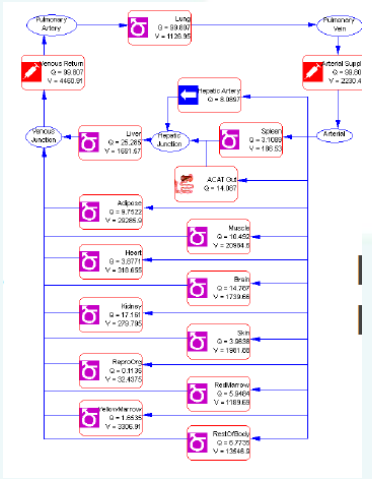


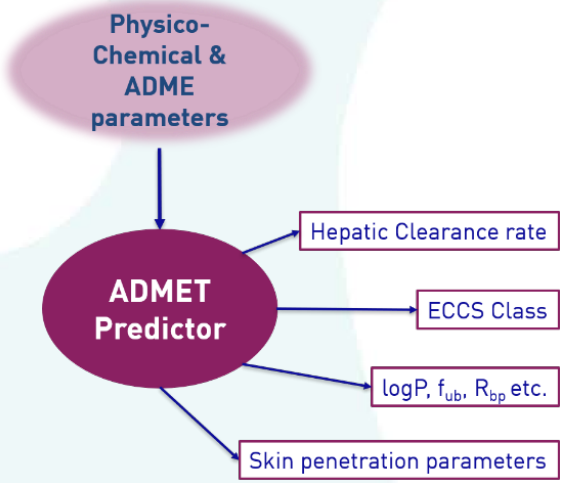
B. Hall et al./Food and Chemical Toxicology 49 (2011) 408–422

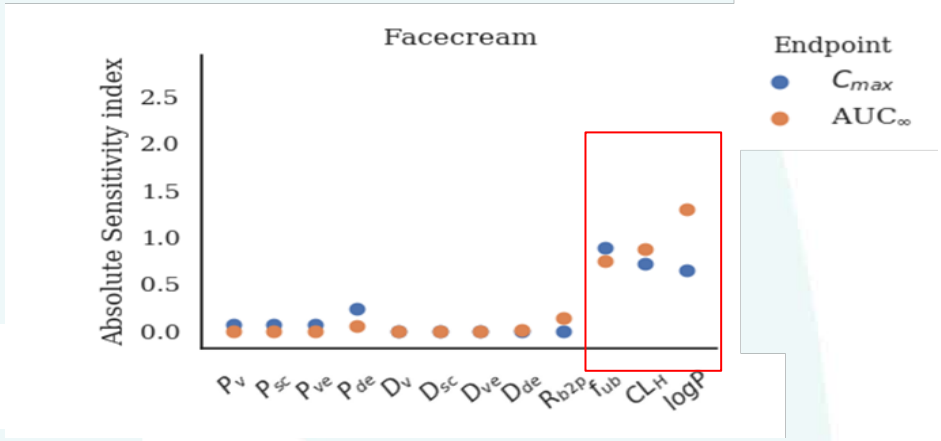
Assessment is exposure-led and uses available habits and practices data

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**

GastroPlus®
(Simulations Plus)







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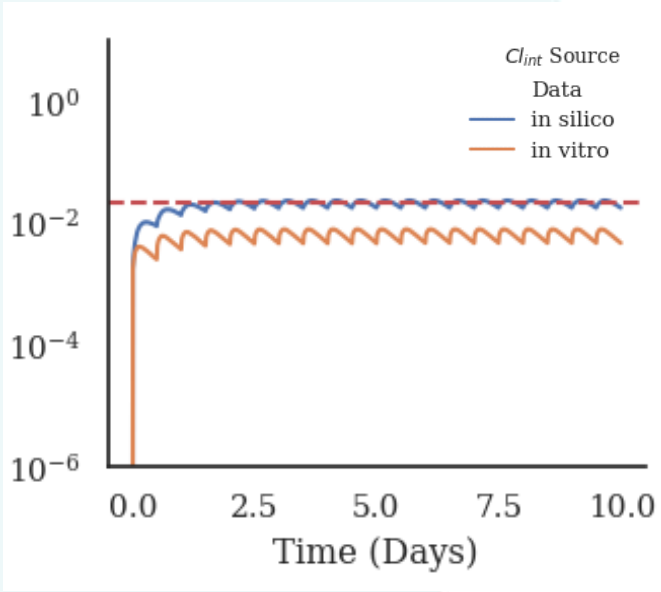
Exposure Estimation

Local and systemic exposure estimates

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

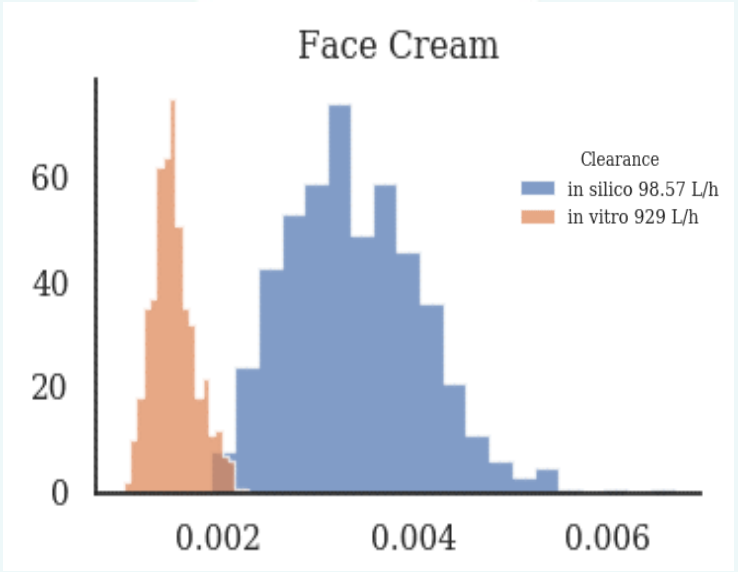
Exposure estimation- Internal concentration using PBK modelling- Model Outputs

Level 2- Simulated plasma concentration of coumarin after dermal exposure.



Level 2. Uncertainty and population variability

Distribution of Cmax values after performing Monte Carlo simulation.



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 63:104746

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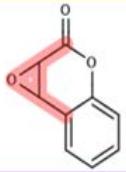
Summary of Existing Data

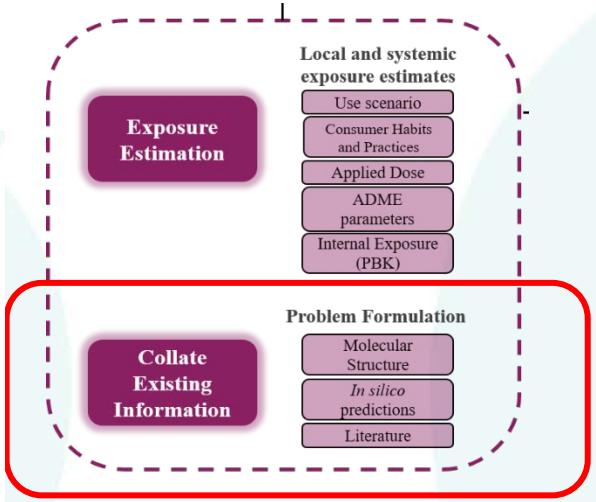
Generation of hypothesis for potential Molecular Initiating events – ToxTree, MIE ATLAS*, OECD toolbox

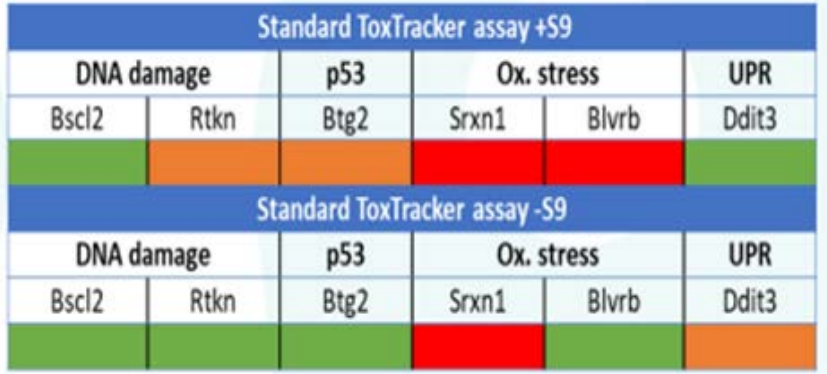


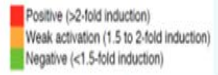
Initial Hypothesis

- 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





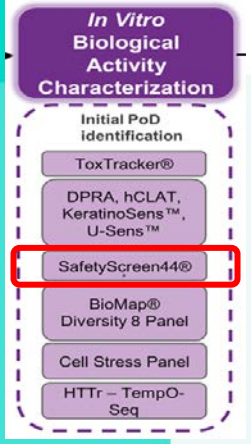


…ive assays among multiple assays (≈ 5000) …in inhibited both Monoamine oxidases and Carbonic anhydrases at concentrations between 3 µM- 40 µM

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

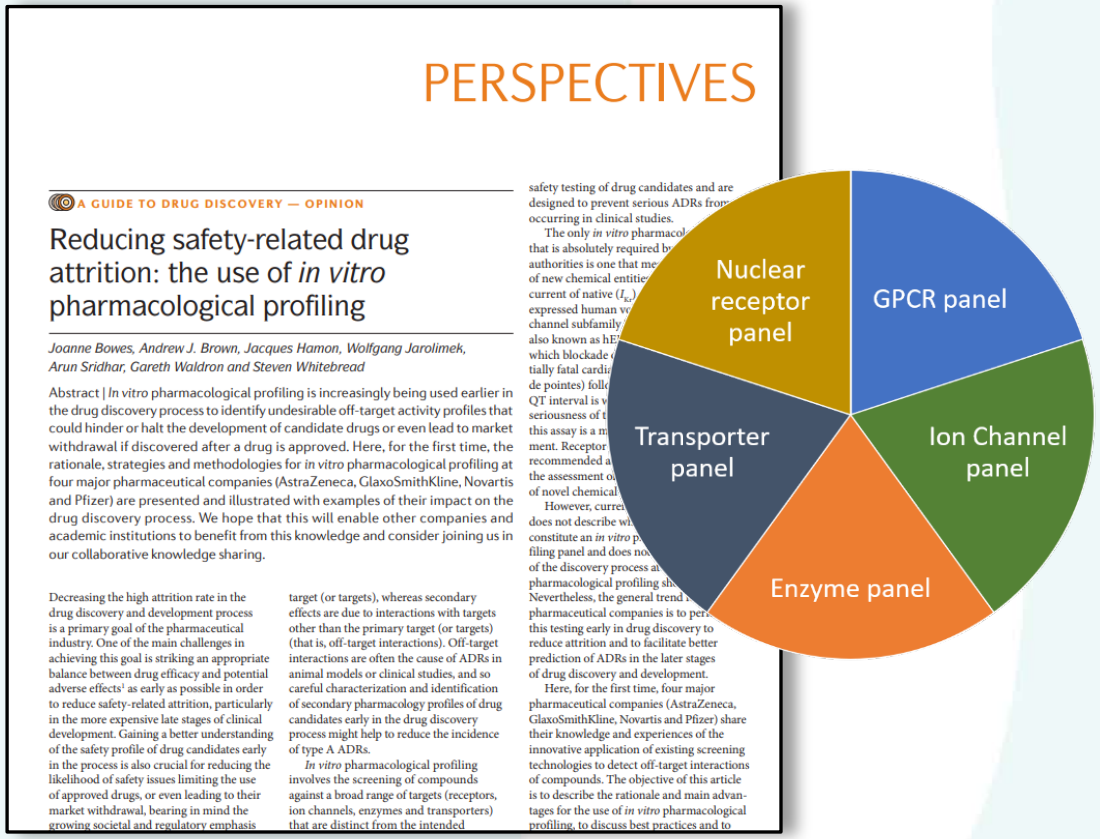
Results

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



NGRA for 0.1% coumarin in face cream: In vitro 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.

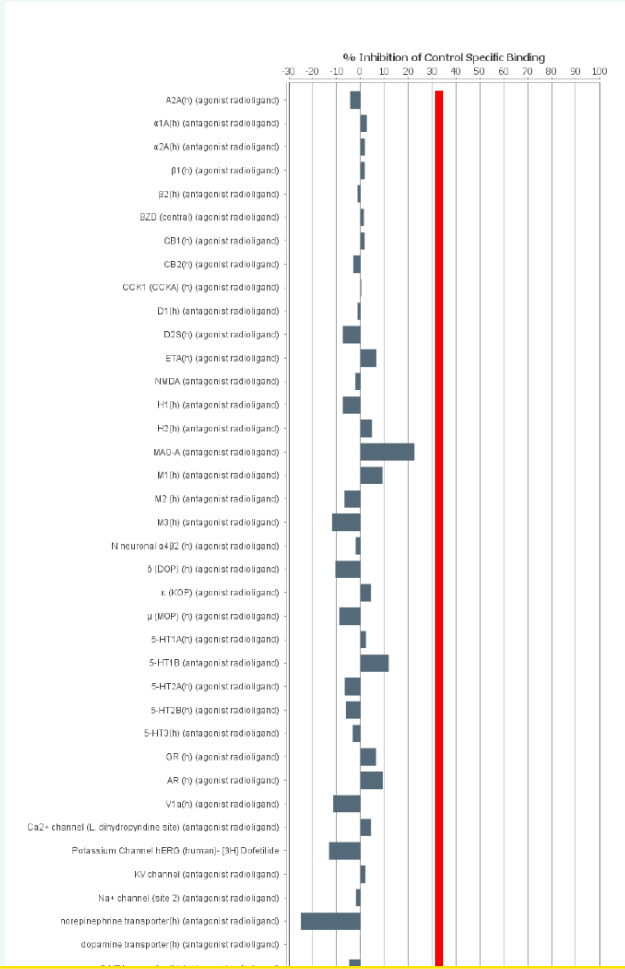
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

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

The only *in vitro* pharmacol... that is absolutely required b... authorities is one that me... of new chemical entities... current of native (I_{Kr})... expressed human v... channel subfamily... also known as hE... which blockade ... tially fatal cardi... de pointes) follo... QT interval is v... seriousness of t... this assay is a m... ment. Receptor ... recommended a... the assessment o... of novel chemical...

However, curre... does not describe wh... constitute an *in vitro* p... filing panel and does no... of the discovery process a... pharmacological profiling sh... Nevertheless, the general trend ... pharmaceutical companies is to per... 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



Results:

All binding and enzymatic assay results were negative at 10 µM

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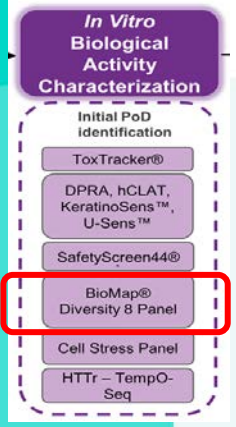
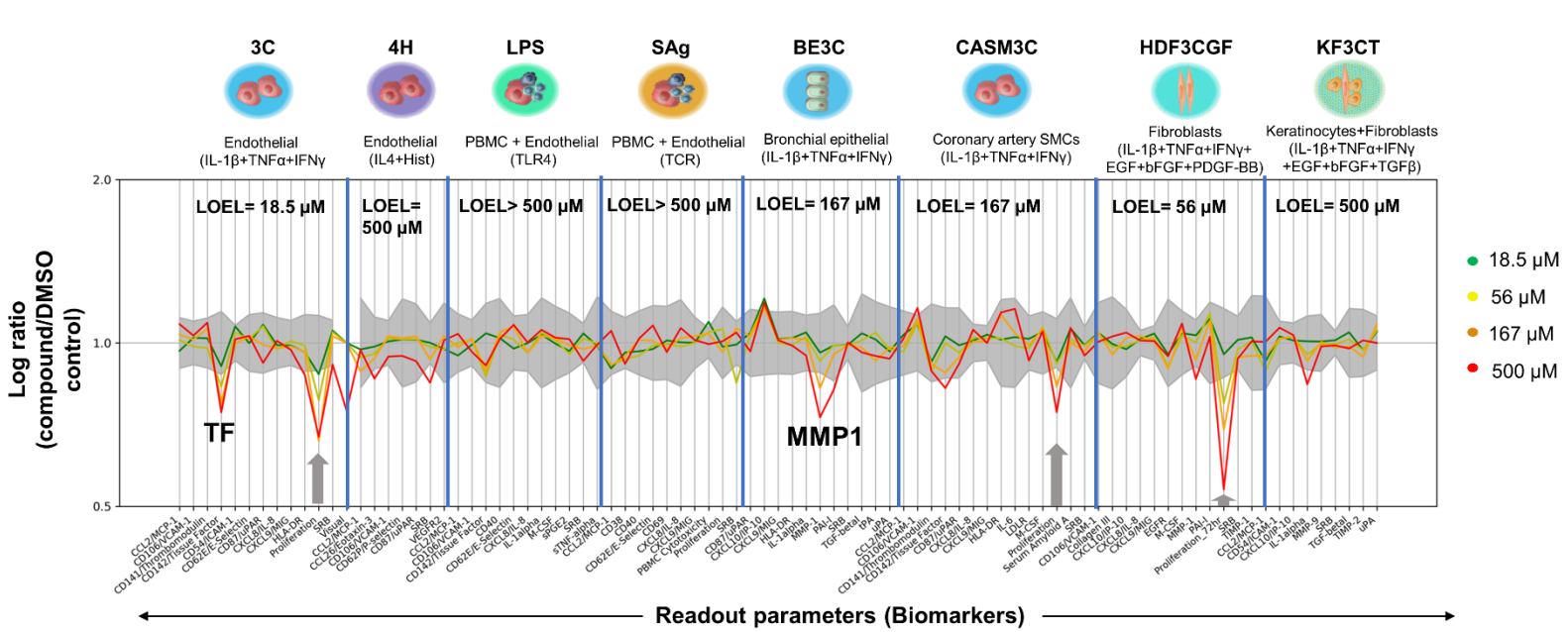


NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: Immunomodulatory screening assay: BioMap Diversity 8 Panel

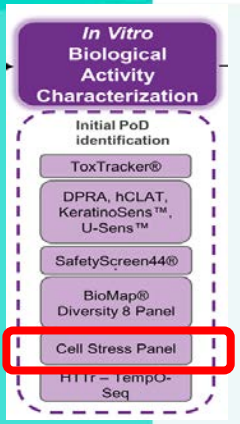
To investigate possible effects on vascular inflammation, immune activation and tissue remodelling



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

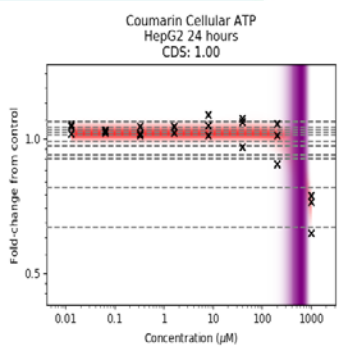
https://www.discoverx.com/services/drug-discovery-development-services/primary-cell-phenotypic-profiling/diversity-plus

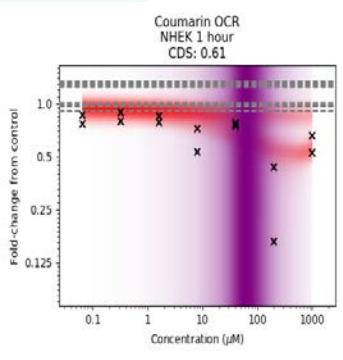
In vitro biological activity characterisation: In vitro cell stress panel



- Cellular stress response assays are useful to **characterize non-specific biological activity** which is not mediated via a specific protein/receptor interaction
- Measures a range of biomarkers covering **~10 cell stress pathways**
- Single exposure; 8 concentrations; 1h, 6h & 24hr timepoints; HepG2 & NHEK cells

- **Mitochondrial Toxicity:** MitoSOX, PGC1α, MMP, ATP, Glu/Gal
- **Oxidative Stress:** GSH, ROS, SRXN1, NRF2
- **DNA damage:** pH2AX, p53
- **Inflammation:** TNFAIP3, ICAM1, NFkB p65, IL-1β, IL-8, HMGB1
- **ER Stress:** PERK, ATF4, CHOP, XBP1, BiP, ER Tracker
- **Metal Stress:** MTF-1, Metallothionein
- **Osmotic Stress** (NFAT5); **Heat Shock** (HSP70); **Hypoxia** (HIF1α)
- **Cell Health**: LDH, Phospholipidosis, Steatosis, pHrodo indicator, apoptosis (caspase-3/7) & necrosis (ToPro-3)





Biomarkers	Cell type	Stress pathway	PoD (μM)	Effect	Concentration dependency score (CDS)
ATP (6h)	HepG2	cell health	794 (363-977)	down	0.98
ATP (24h)			617 (282-891)	down	1
Phospholipidosis (24h)	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 (1h)	NHEK	mitochondrial toxicity	62 (2.6-776)	down	0.6
OCR (6h)			468 (214-794)		1
OCR (24h)			309 (138-1000)		0.52
Reserve capacity (1h)	NHEK	mitochondrial toxicity	44 (23-96)	down	1
Reserve capacity (6h)			759 (302-1000)		0.9
Reserve capacity (24h)			794 (295-1000)		0.55

Hatherell *et al.,* 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. 176:11-33 https://doi.org/10.1093/toxsci/kfaa054

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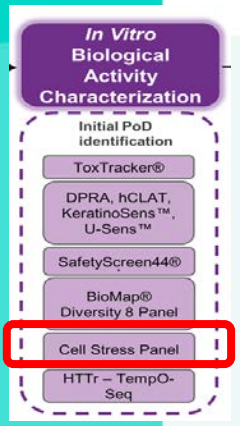
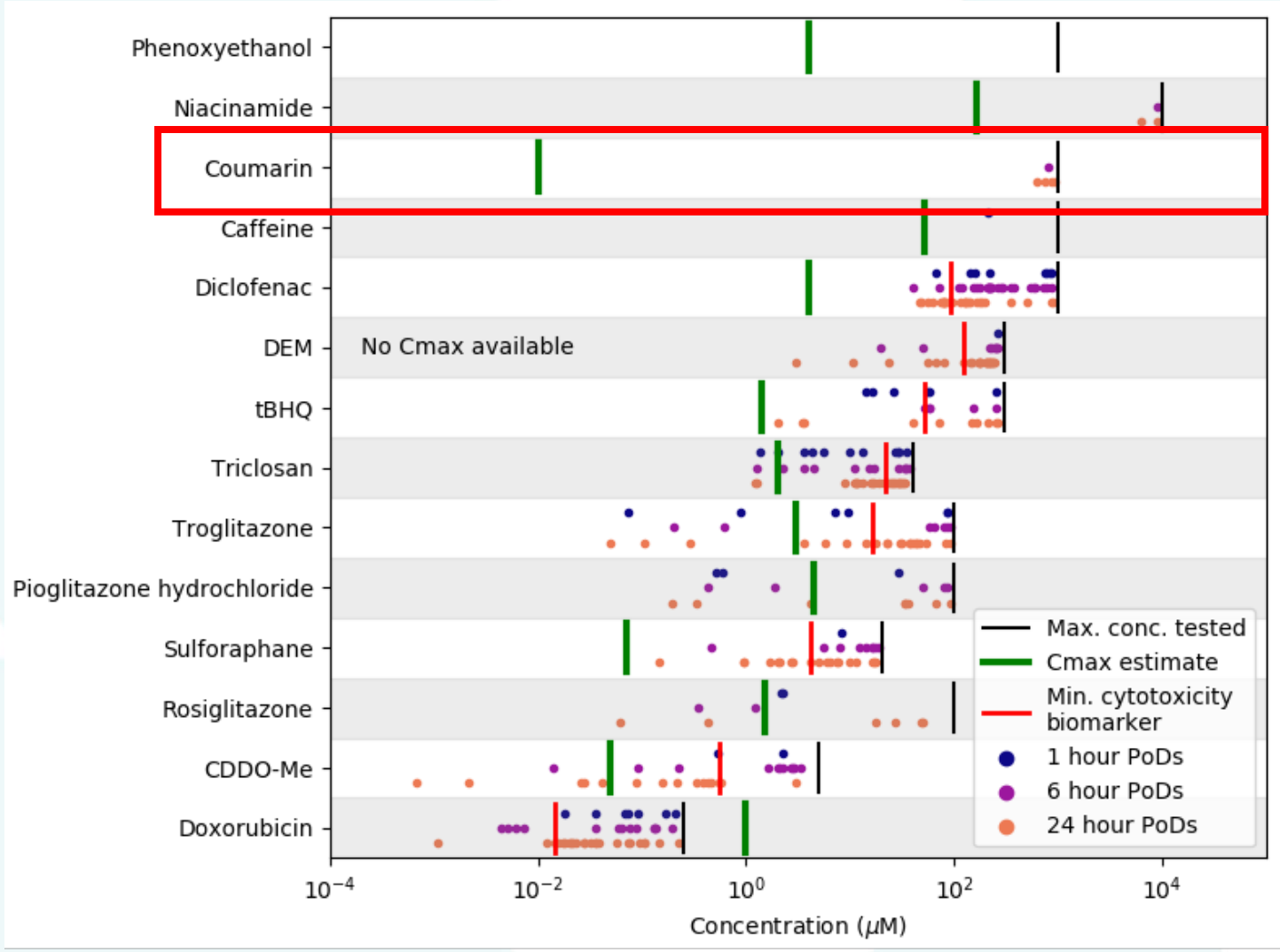


In vitro biological activity characterisation: In vitro cell stress panel



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. 176:11-33 https://doi.org/10.1093/toxsci/kfaa054

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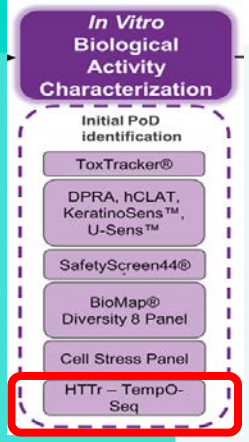


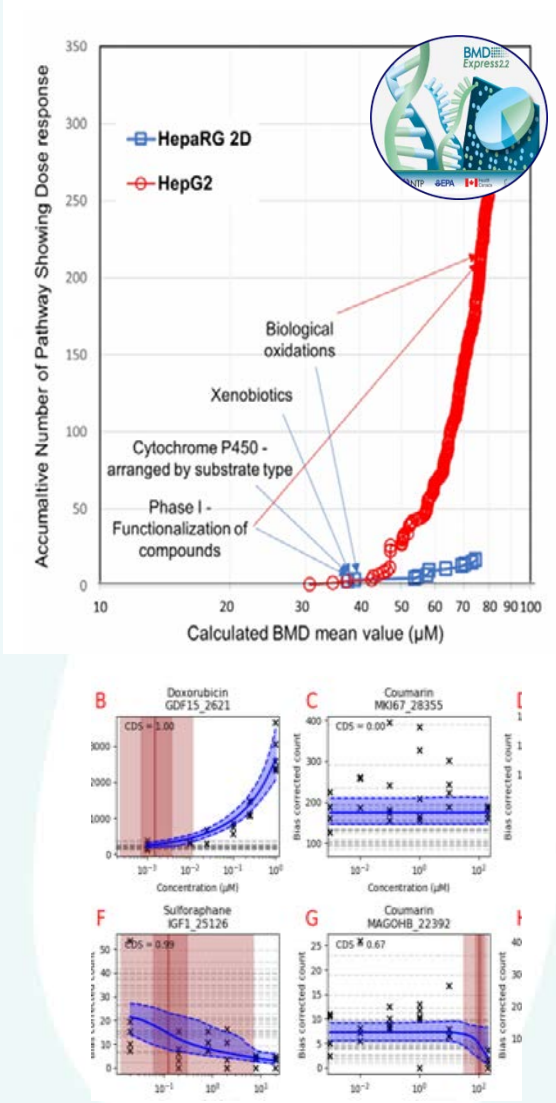
In vitro biological activity: High-Throughput Transcriptomics (HTTr)

Provide screen for biological activity across a broad biological coverage

- *Tempo-Seq*
- *Human gene panel ver1 ~ 21k*
- *3 cell lines*

Results:

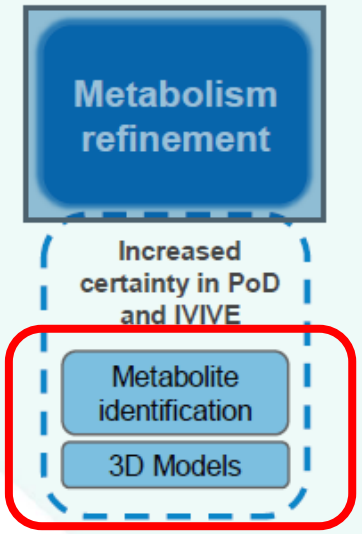
- The MCF7 PoD_T were not considered to be sufficiently robust to derive a MoS
- The lowest PoDT for each cell model was selected for the MoS calculation



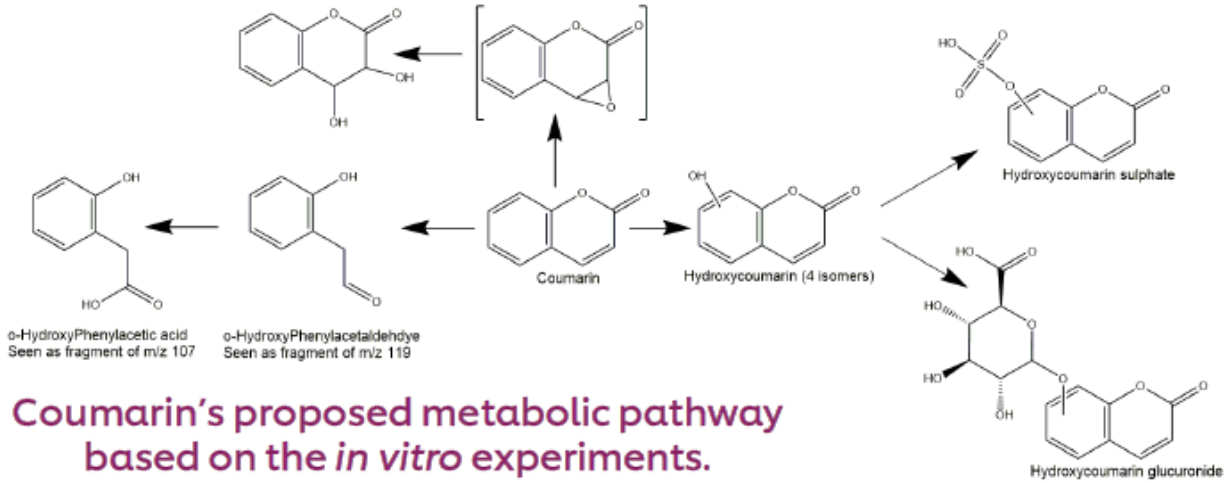
Cell model	HepG2	MCF7	HepaRG 2D
Pathway level tests PoD_T (µM)	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest p value Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38
Gene level tests PoD_T (µM)	(1570 genes)	(47 genes)	(87 genes)
Mean BMD of 20 genes with largest fold change	6	3	54
Mean BMD of genes between 25th and 75th percentile	17	1	59

Reynolds et al. A Bayesian approach for inferring global points of departure from transcriptomics data CompTox 2020 (accepted)

Tier 2 refinement: Metabolism prediction and activity



Human *In vitro* metabolism



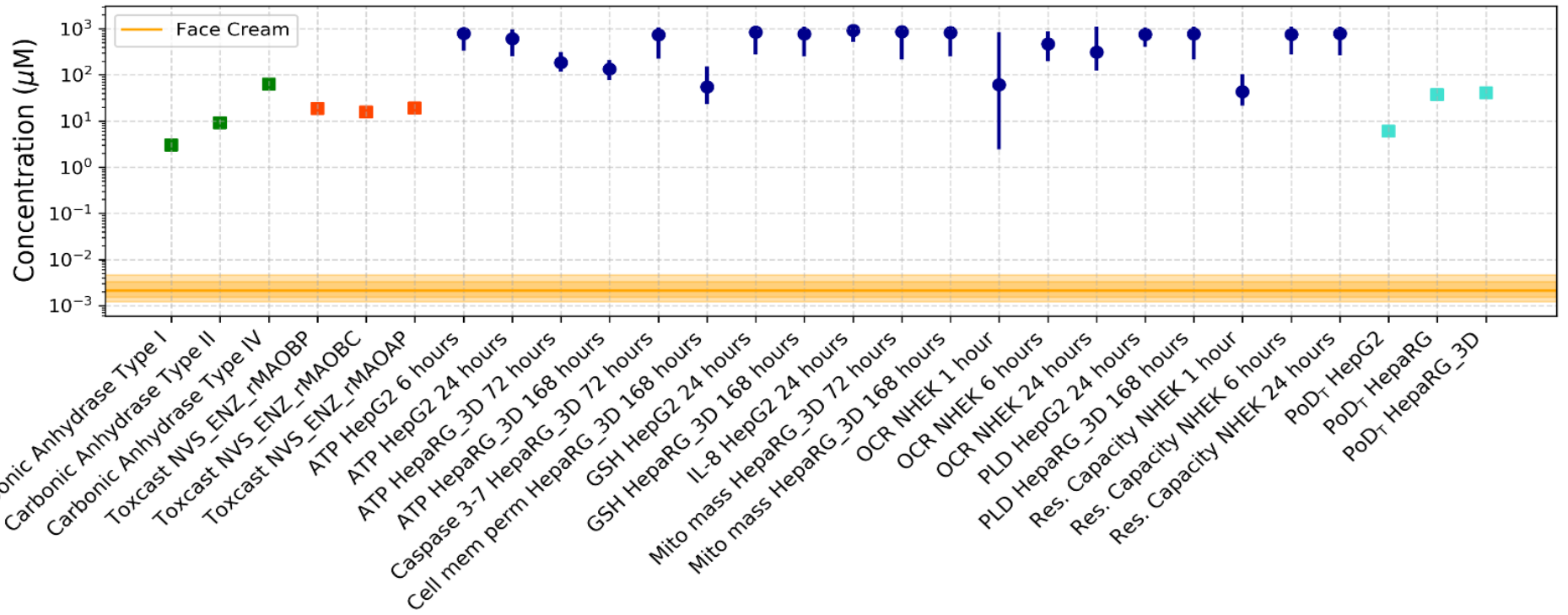
Coumarin's proposed metabolic pathway based on the *in vitro* experiments.

Cell stress & HTTr in 3D HepaRG models

- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871 µM – not very different from 2D cells

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

Determine Margin of Safety



- 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 use scenario**

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Summary

- Focus on weight of evidence to show tools can be integrated to make a safety decision - requires diverse expertise
- Exposure led approach to determine protection through a MoS
- Strength derived from a combination of targeted and broad unbiased tools – hypothesis led
- NAMs not standard - need to ensure robustness/quality of tools and include estimations of uncertainty to aid acceptance
- Utilise NAMs for further targeted follow where required to refine uncertainty e.g. metabolism
- Further evaluation, additional case studies internal/ in collaboration – ongoing EPA and EU-ToxRisk and assessment required to build out confidence for broader stakeholder community
- Additional research to progress on gaps

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