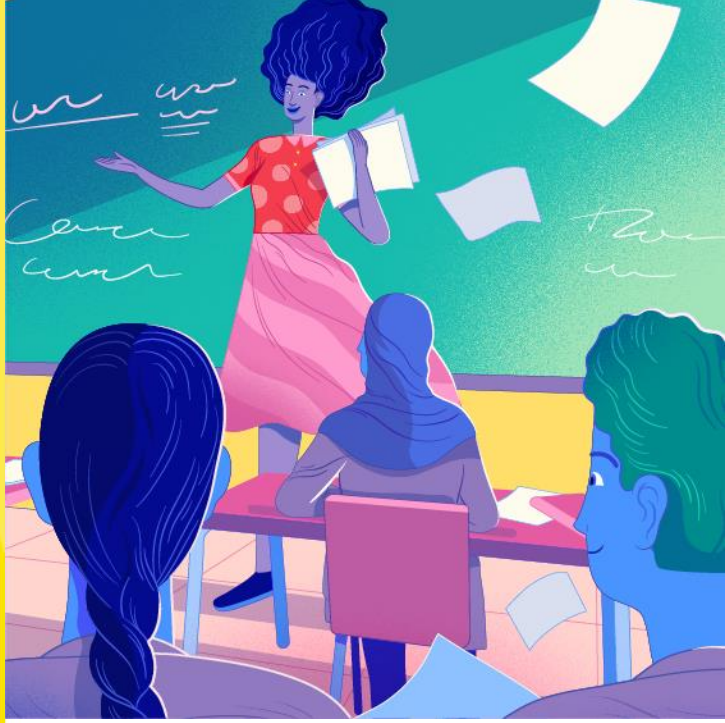
Next generation risk assessment (NGRA) case study: use of 0.1% coumarin in face cream



Maria Baltazar & Gavin Maxwell

Unilever

Outline

9h00 – 9h25 – Introduction to Next generation risk assessment (NGRA): concepts and tools (30 min)

9h25 – 9h35 – Exposure information and Collation of existing information (10 min)

9h35 – 10h – Breakout Discussion (25 min)

10h00 – 10h15 – Break (15 min)

10h15 – 10h55- In vitro biological activity characterisation (35 min)

10h55 – 11h20– Breakout Discussion (25 min)

11h20 – 11h30 – Metabolism refinement & Margin of Safety determination & Risk assessment conclusion (10 min)

11h30 – 11h55 – Poll questions & Discussion (25 min) (plenary)

11h55 – 12h00 – Concluding remarks (5 min)

Unilever

Introduction to Next generation risk assessment (NGRA): concepts and tools (30 min)

Unilever

The objective of a consumer product risk assessment is...

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

Unilever

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***

ICCR
9 principles of NGRA

Main overriding principles:
The overall goal is a human safety risk assessment
The assessment is exposure led
The assessment is hypothesis driven
The assessment is designed to prevent harm

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

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

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

NGRA: The overall goal is a human safety risk assessment

"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity **testing from a system based on whole-animal** testing **to one founded primarily on *in vitro* methods** that evaluate changes **in biologic processes using cells, cell lines, or cellular components, preferably of human origin**." 2007

Tox21/ToxCast
~700 HTS Biological Pathways Assays

National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP)

National Center for Advancing Translational Sciences (NCATS)

U.S. Food and Drug Administration (FDA)

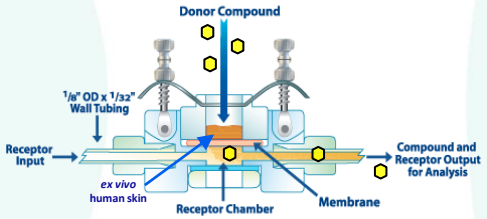
National Center for Computational Toxicology (EPA)

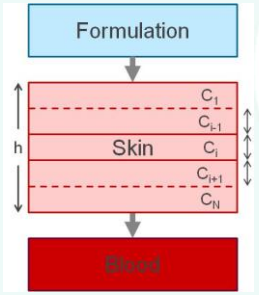
Unilever

NGRA: The assessment is exposure-led

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

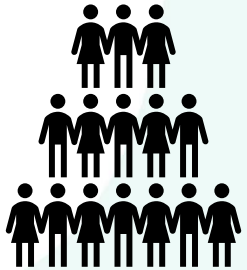
ADME parameters



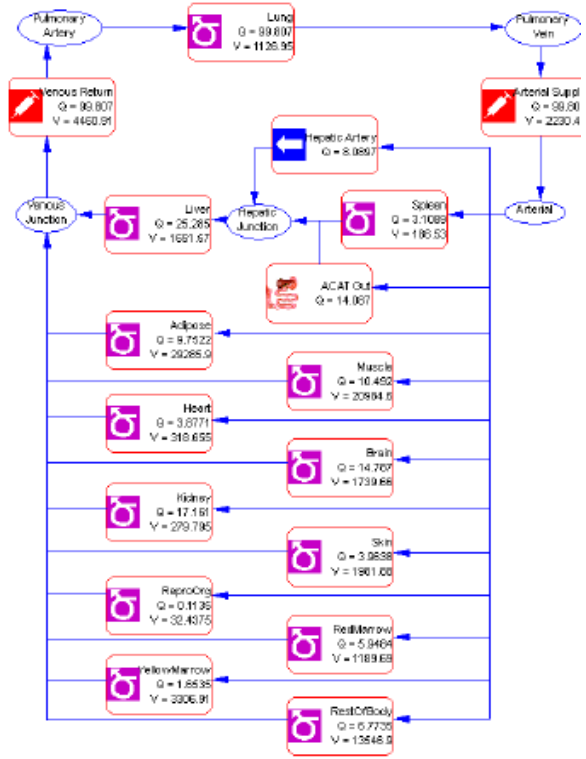


- Skin penetration
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- blood:plasma ratio

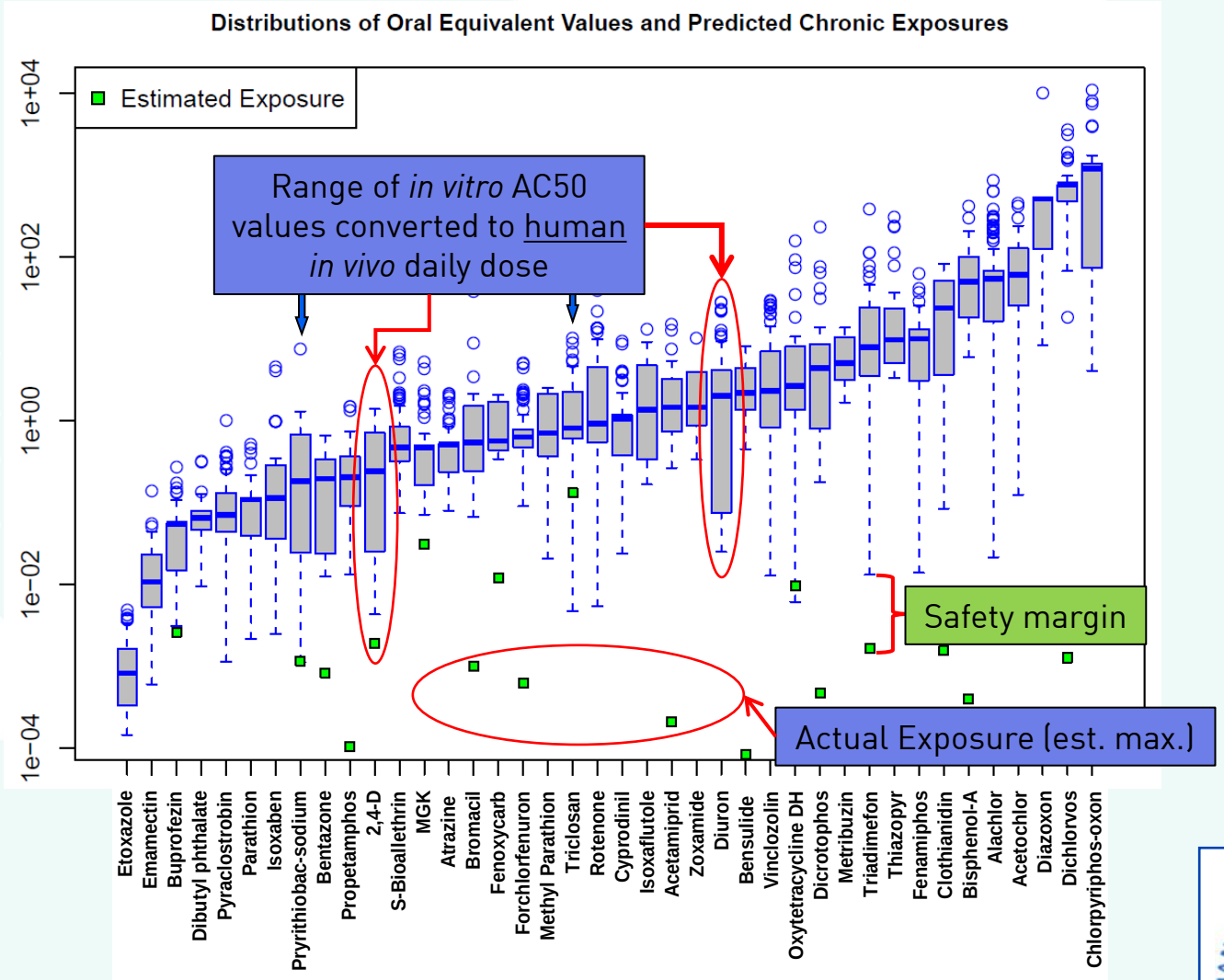
Uncertainty analysis- Population simulation



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



NGRA: The assessment is designed to prevent harm



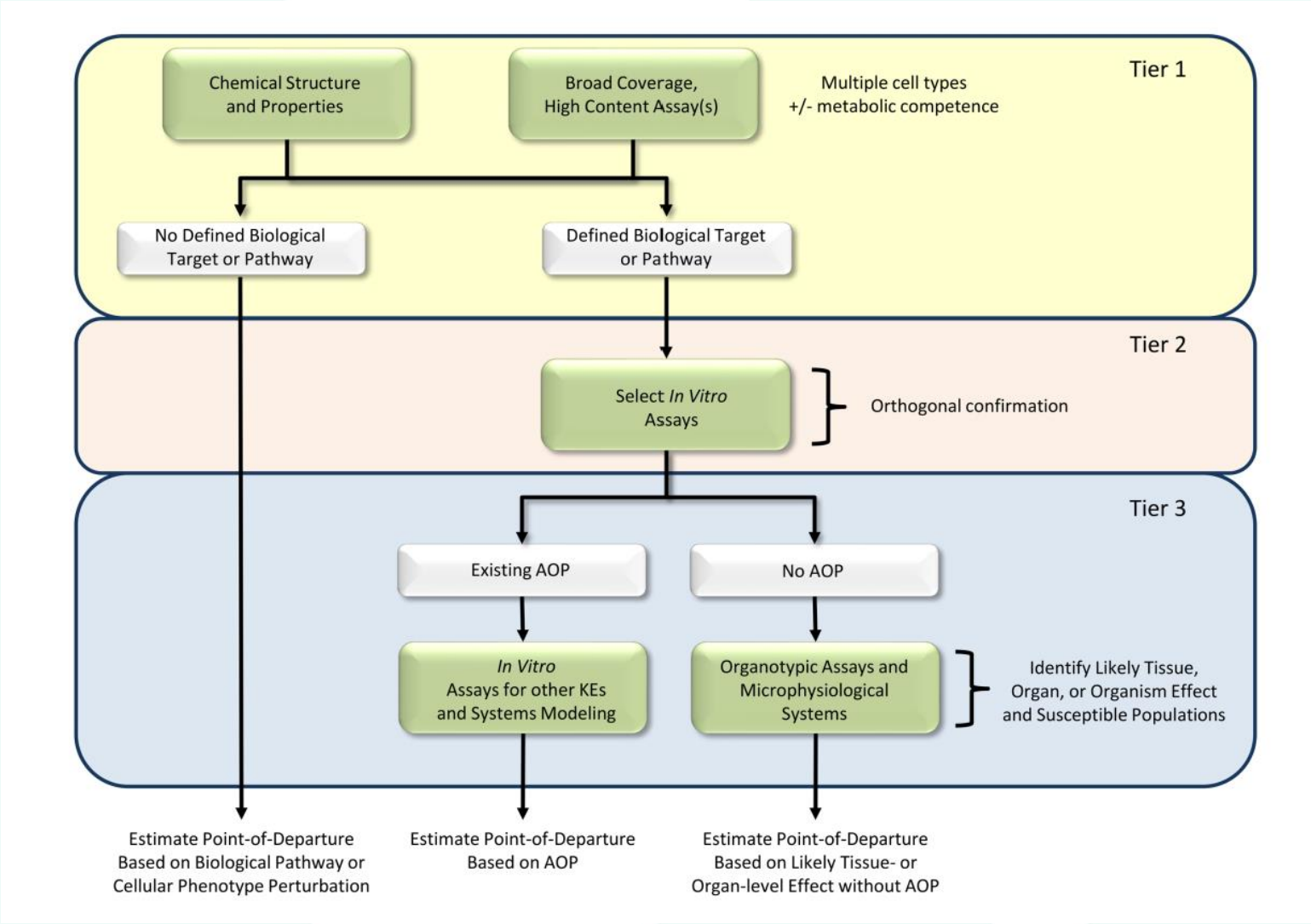
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

The philosophy behind this type of risk assessment aimed at preventing harm is **based on the premise of "Protection not Prediction".**

The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

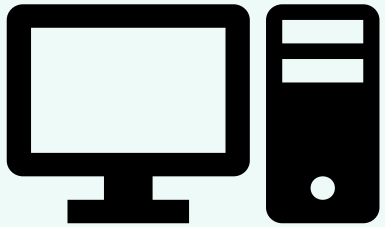
NGRA: The assessment is hypothesis driven & should be conducted Using a tiered and iterative approach



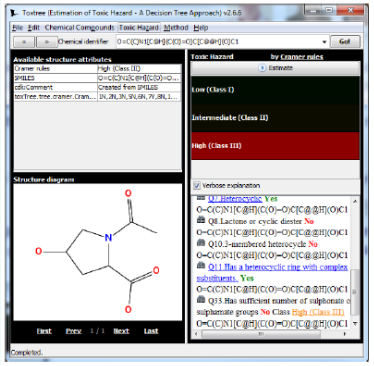
Russell S Thomas et al., 2019. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Tox Sci 169(2):317-332.

NGRA: Using robust and relevant methods and strategies to characterise bioactivity

In silico tools

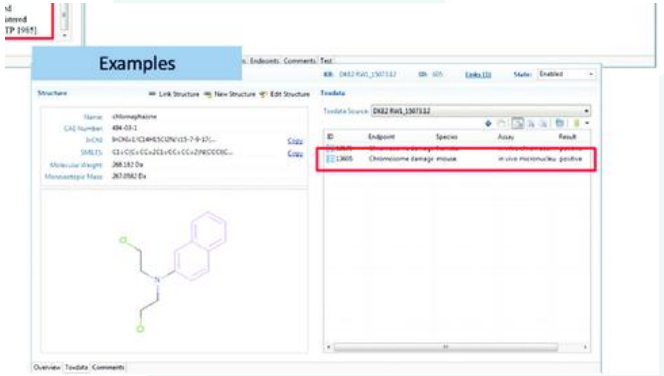


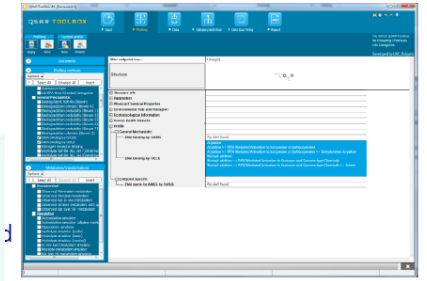
ToxTree



Derek nexus

OECD QSAR TOOLBOX





In silico models to predict Molecular initiating events (MIEs)

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

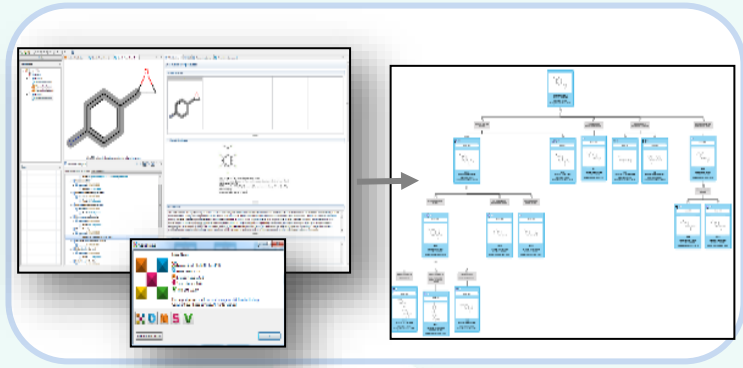
TOXICOLOGICAL SCIENCES, 165(1), 2018, 213–223

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

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

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

Meteor nexus



Metabolic fate predictions

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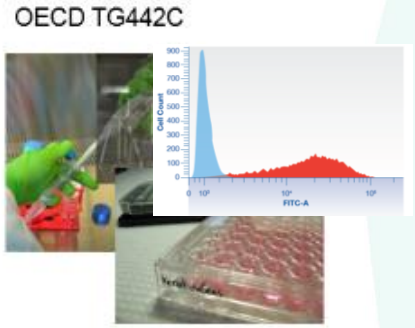
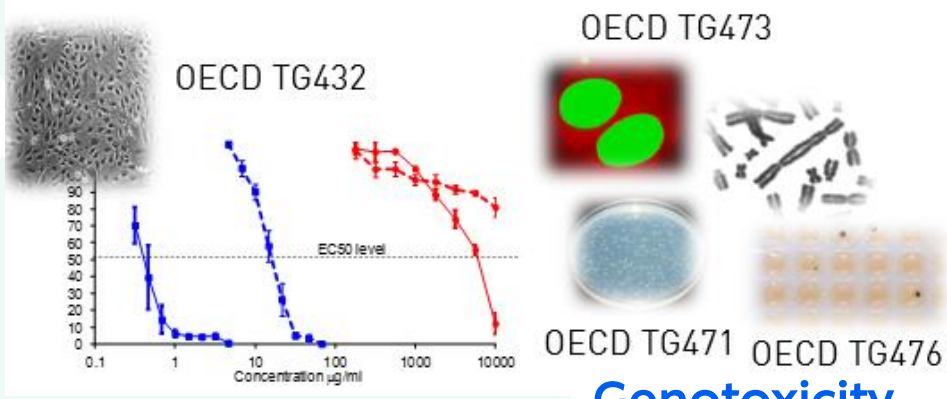
NGRA: Using robust and relevant methods and strategies to characterise bioactivity

OECD test methods

Skin and eye irritation



Skin sensitisation

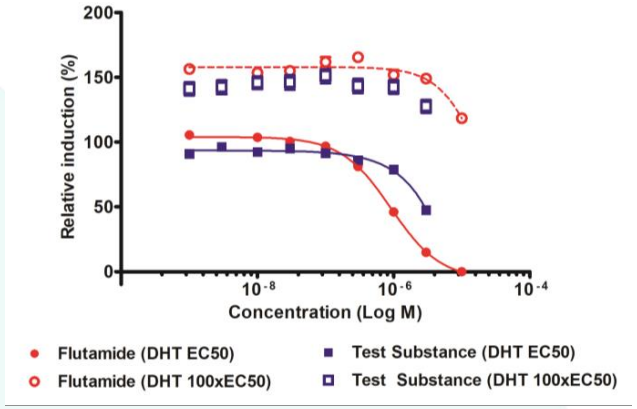


Phototoxicity

Genotoxicity

Receptor-binding assays

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

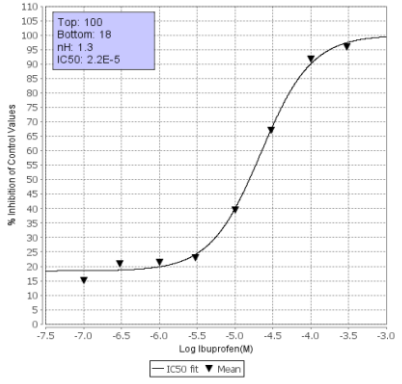


Dent et al (2019), Toxicological Science, 167, 375-384


PERSPECTIVES

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

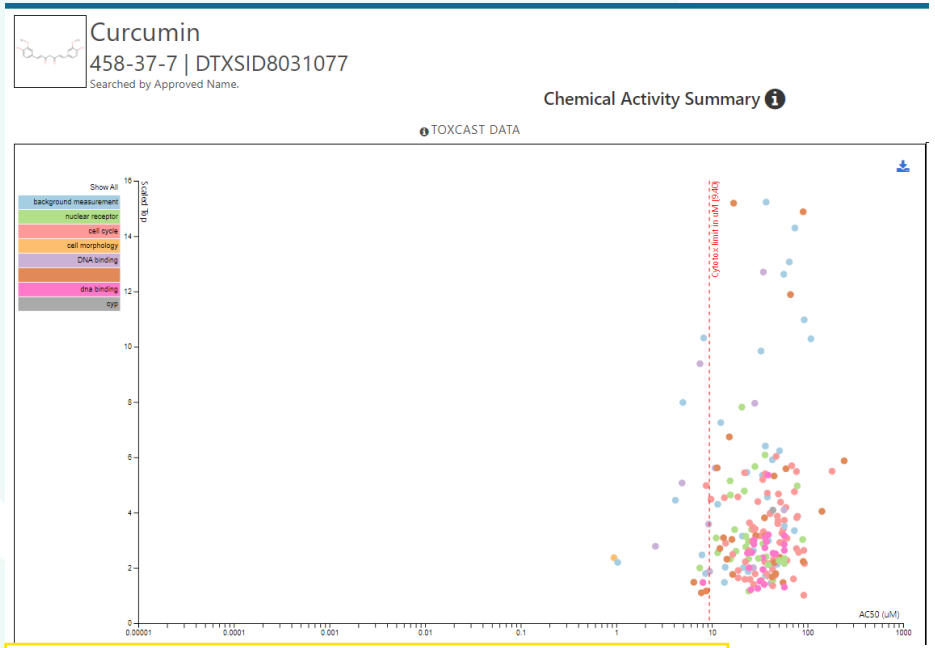
Ibuprofen – Cox-1.

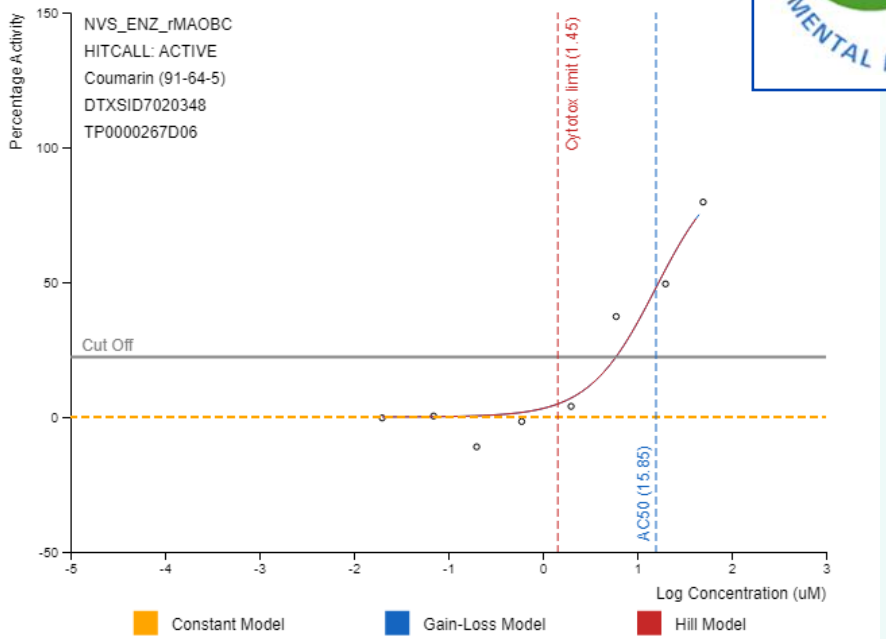


NGRA: Using robust and relevant methods and strategies to characterise bioactivity

Tox21/ToxCast
~700 HTS Biological Pathways Assays

EPA iCSS ToxCast Dashboard





Winning Model	Model	AIC	RMSE	Top	AC50	Slope
	Constant	81.4	35.91	-	-	-
	Gain-Loss	66.07	7.17	95.63	15.77	1.23
✓	Hill	62.07	7.17	95.63	15.78	1.23

- Nuclear receptors
- Transcription factors
- Cell stress/mitochondrial tox
- Enzymatic assays
- Receptor binding
- DNA damage/cell cycle

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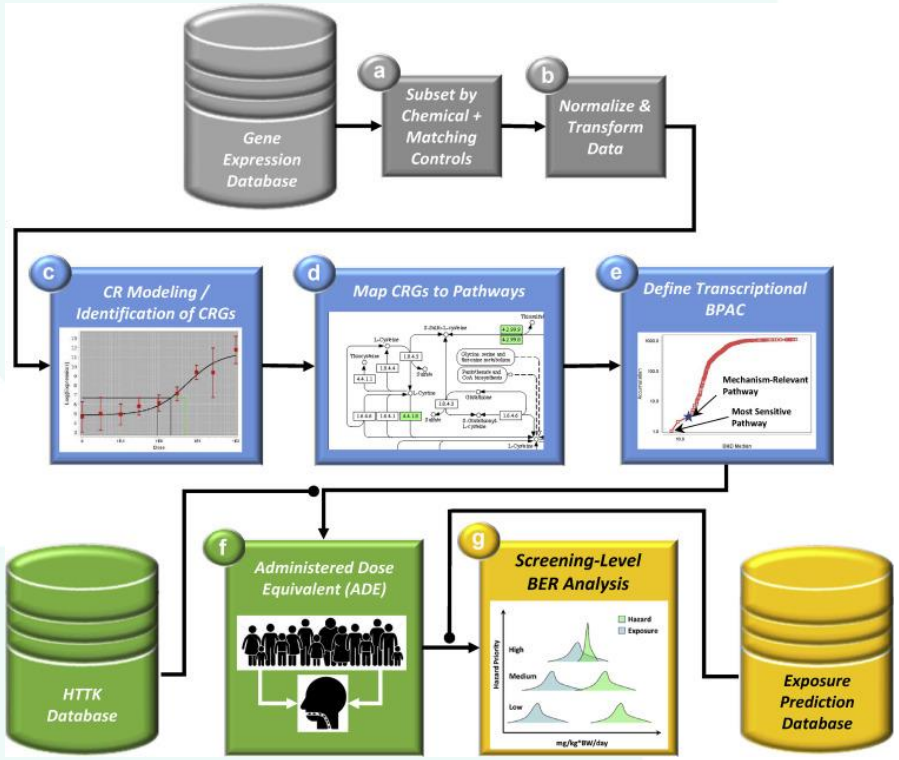
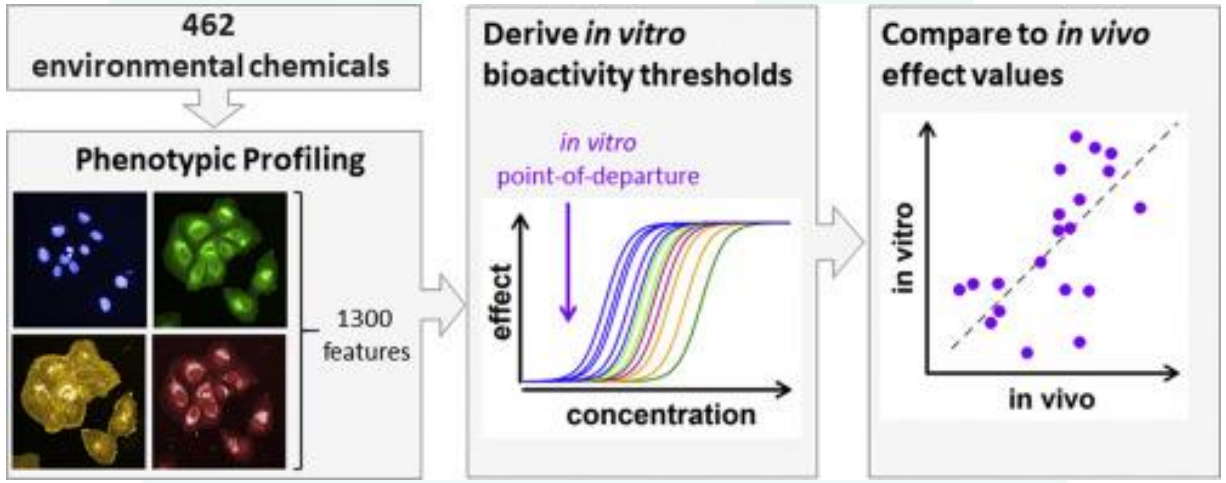
NGRA: Using robust and relevant methods and strategies to characterise bioactivity

High-throughput transcriptomics and High-throughput phenotypic profiling developed to increase biological coverage



Harrill J et al 2019. Considerations for strategic use of high-throughput transcriptomics chemical screening data in regulatory decisions. Current Opinion in Toxicology 15, 64-75



Nyffeler J et al 2019. Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling. *Toxicol Appl Pharmacol*. 2020;389:114876.

Thomas RS et al. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. *Toxicol Sci*. 2019;169(2):317-332.

NGRA: Using robust and relevant methods and strategies to characterise bioactivity

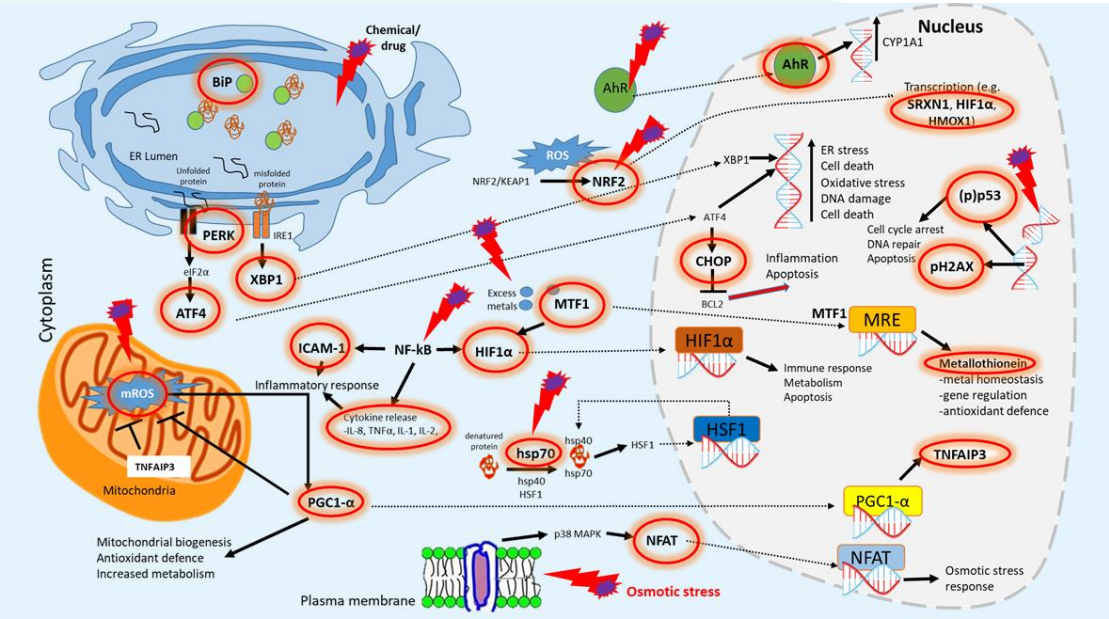


Image kindly provided by Paul Walker (Cyprotex)

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academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2020, 1–23

doi: 10.1093/toxsci/kfaa054
Advance Access Publication Date: May 6, 2020
Research article

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,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,1

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

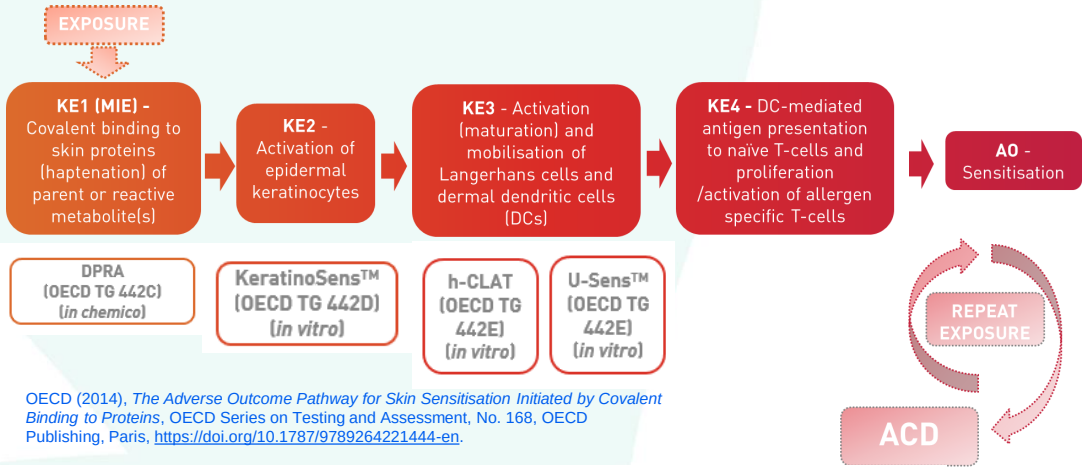
36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

Unilever

For some chemicals pathway-based risk assessment might be needed

Examples of Adverse Outcome Pathway (AOP) risk assessment

Induction of skin sensitisation that leads to allergic contact dermatitis



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.

Computational Toxicology 9 (2019) 36–49

Contents lists available at ScienceDirect

Computational Toxicology

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

Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment

Joe Reynolds*, Cameron MacKay, Nicola Gilmour, David Miguel-Vilumbrales, Gavin Maxwell

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

Anti-androgenic and estrogenic effects

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(2), 2019, 375–384

doi: 10.1093/toxsci/kfy245
Advance Access Publication Date: September 22, 2018
Research Article

Employing Dietary Comparators to Perform Risk Assessments for Anti-Androgens Without Using Animal Data

Matthew P. Dent,*,[1] Hequn Li,* Paul L. Carmichael,* and Francis L. Martin†

*Safety and Environmental Assurance Centre, Unilever, Colworth Science Park, Bedfordshire MK44 1LQ, UK; and †School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston, UK

Regulatory Toxicology and Pharmacology 71 (2015) 398–408

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

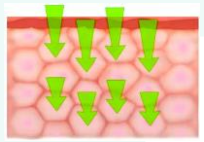
journal homepage: www.elsevier.com/locate/yrtph

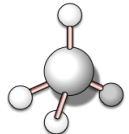
An exposure:activity profiling method for interpreting high-throughp screening data for estrogenic activity—Proof of concept

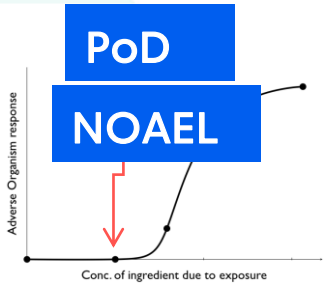
Richard A. Becker[a,*], Katie Paul Friedman[b], Ted W. Simon[c], M. Sue Marty[d], Grace Patlev J. Craig Rowlands[d]

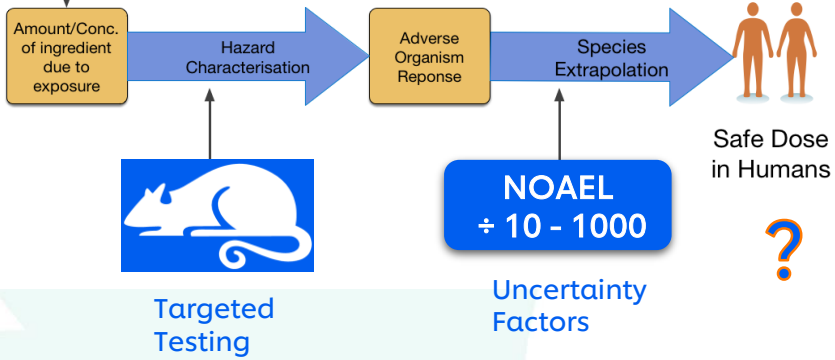
NGRA: the margin of safety (MoS) approach and decision making

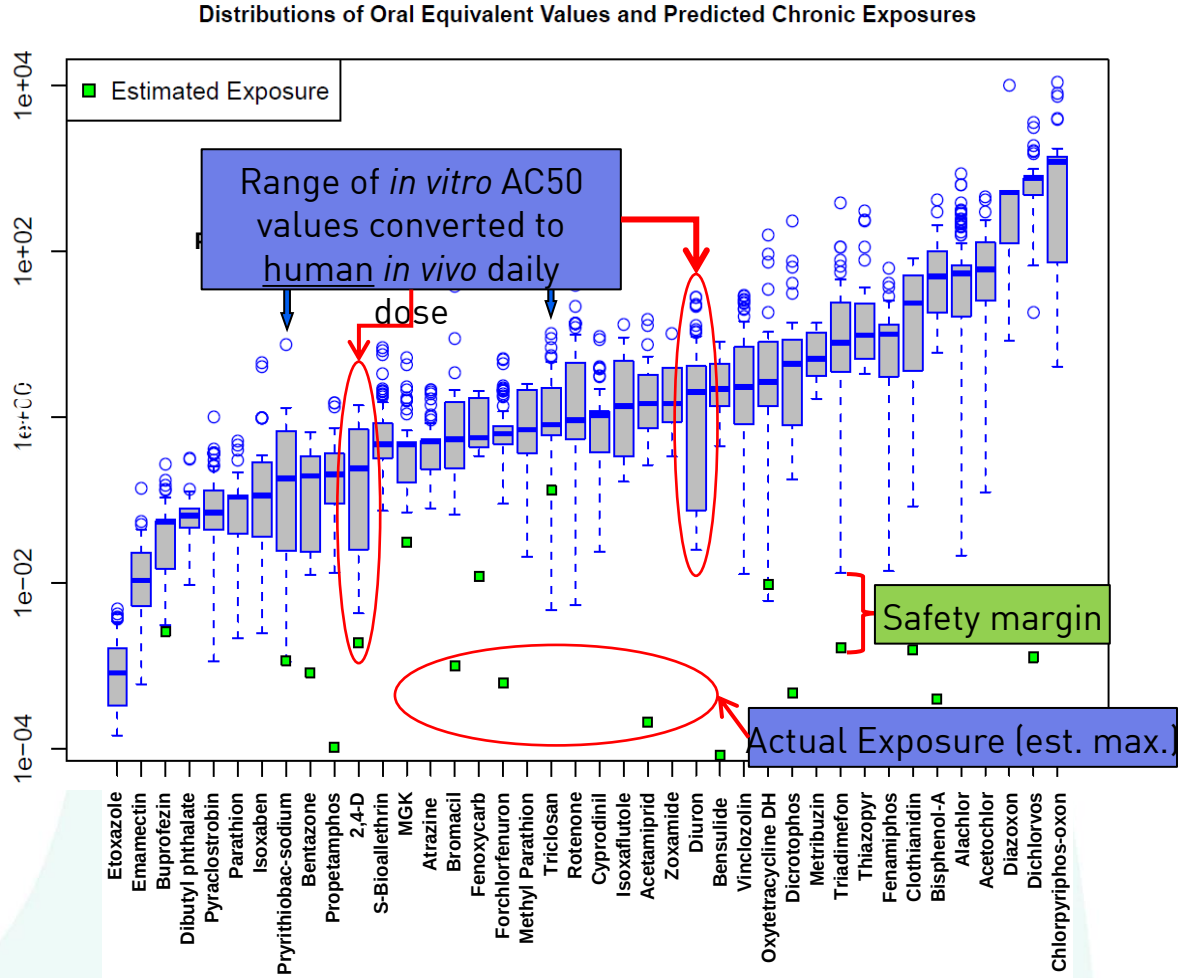
Is it safe?







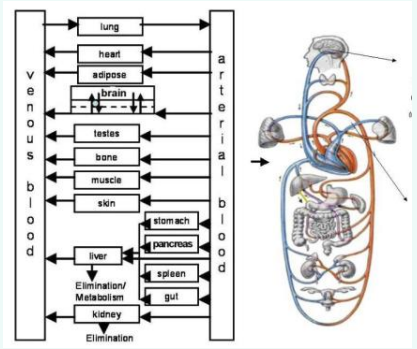


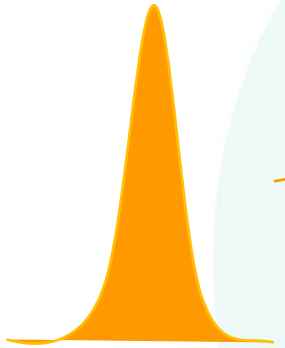


Unilever

NGRA: Sources of uncertainty should be characterized and documented

Exposure models (PBK, free/total concentration)

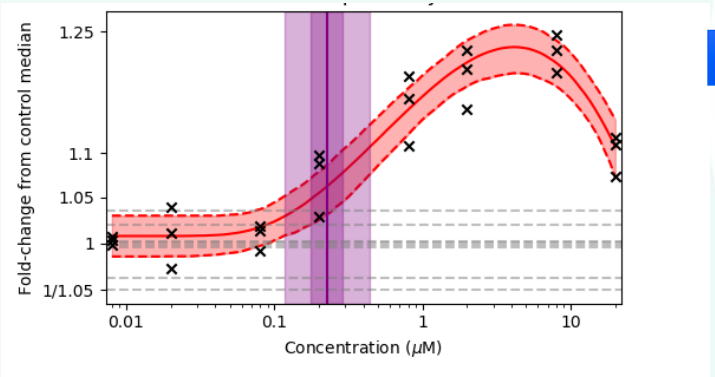


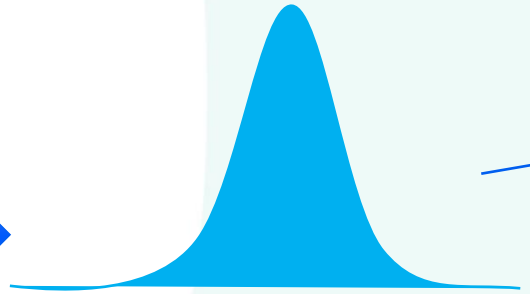


Plasma Cmax as a distribution

Uncertainty in the PBK inputs
Population variability

Point of departure derived from concentration-response data



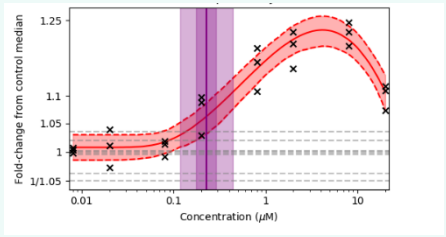


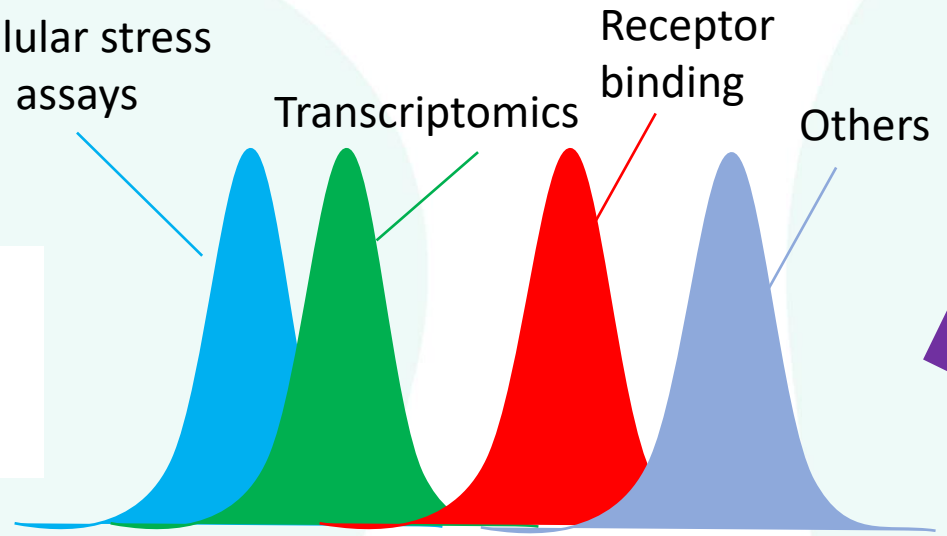
Point of Departure as a distribution

Variability in the data
Plate effects
Etc.

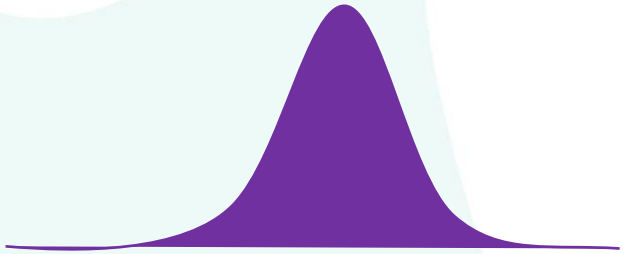
NGRA: the margin of safety (MoS) approach and decision making

Point of departure derived from concentration-response data

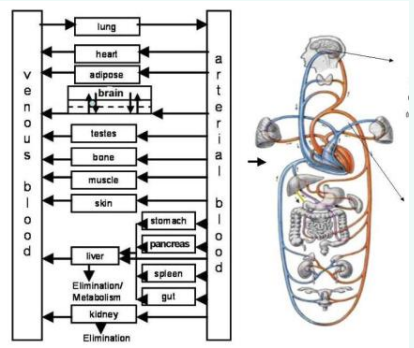




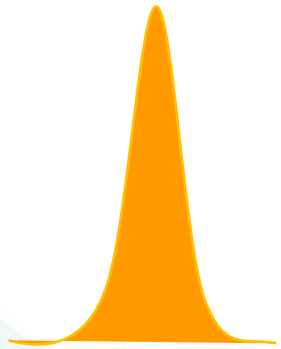
Calculation of Margin of Safety (MoS) distribution



Exposure models (PBK, free/total concentration)

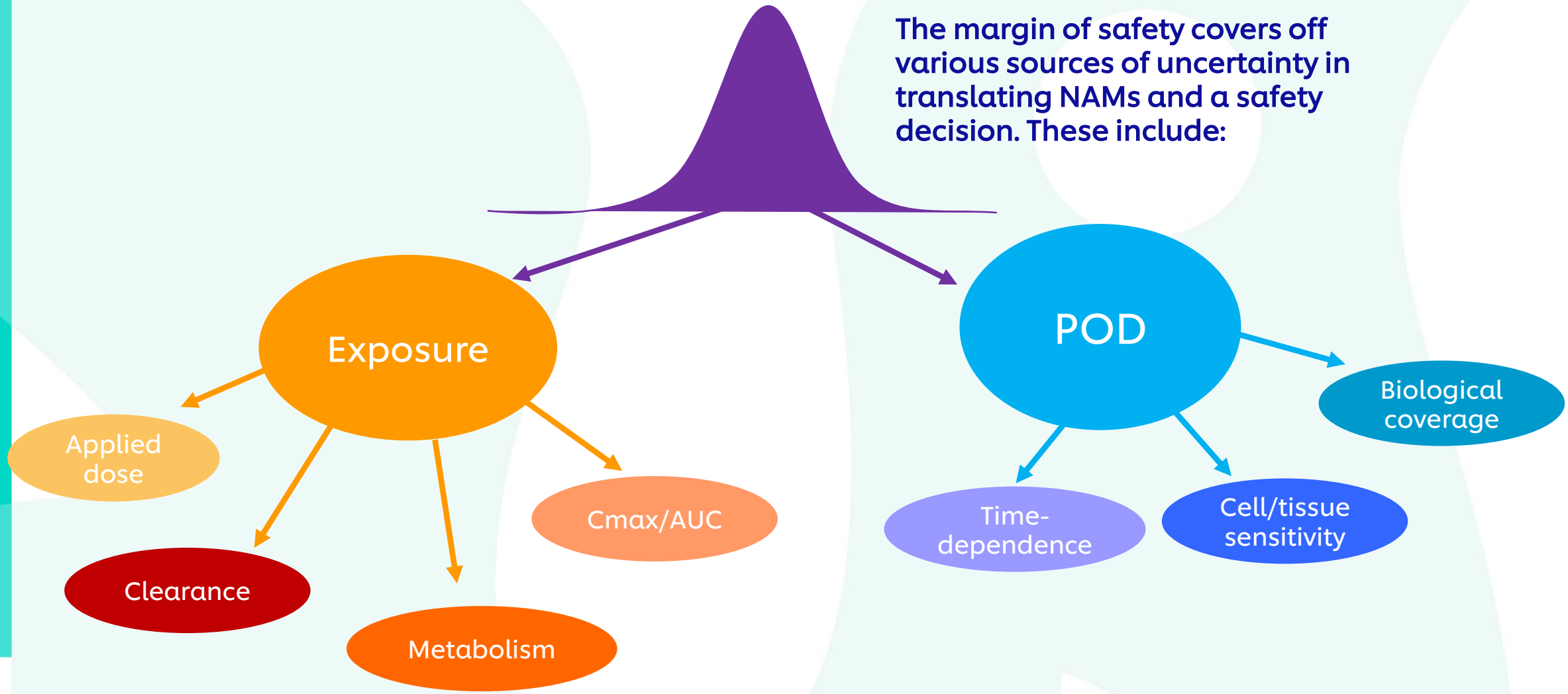


Exposure estimation: Plasma C_{max}



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

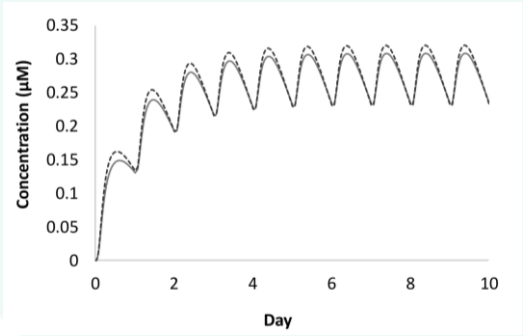
NGRA: Sources of uncertainty should be characterized and documented



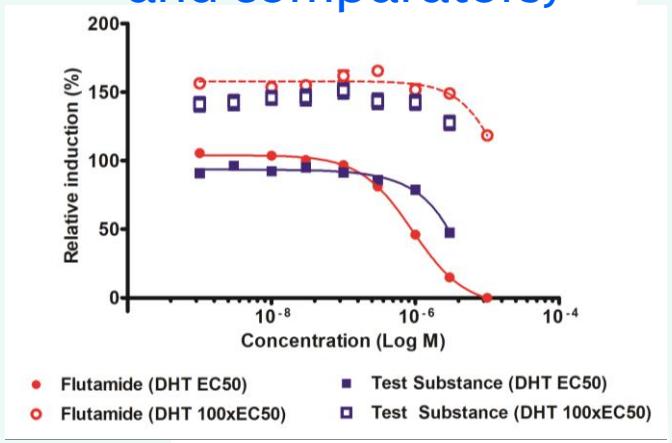
NGRA: Making sense of margins of safety by benchmarking

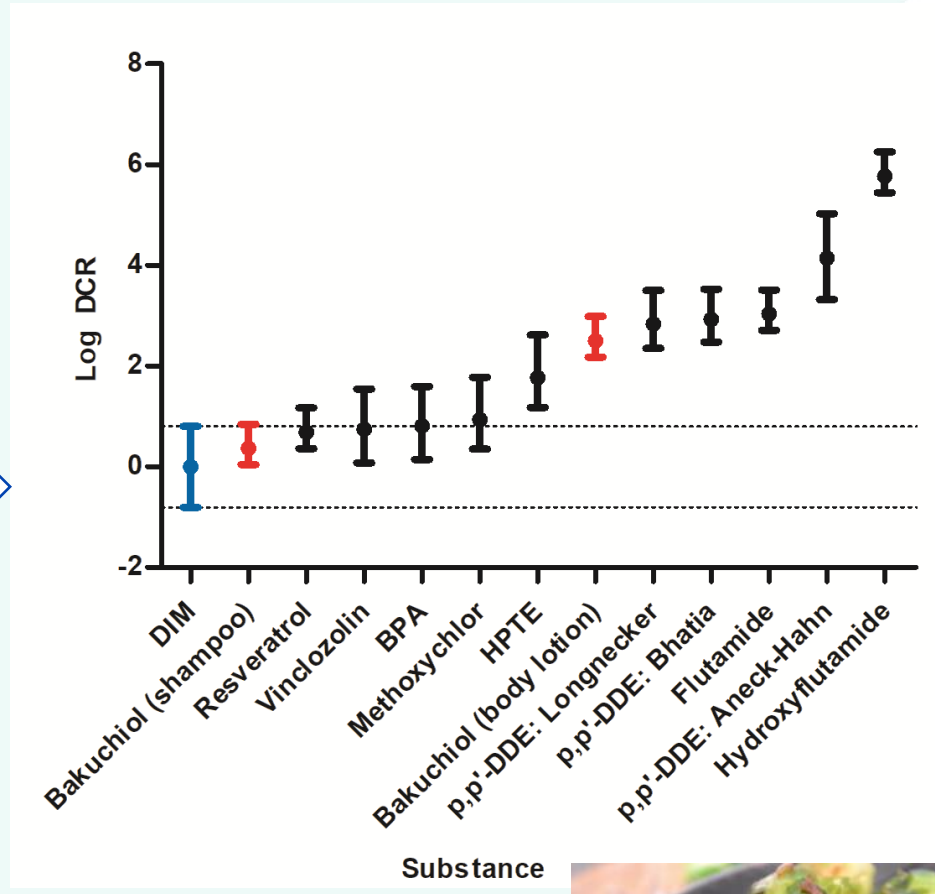
Dent *et al.*, (2019) *Tox Sci* 167(2): 375-384

Exposure



\+ Bioactivity data (substance and comparators)





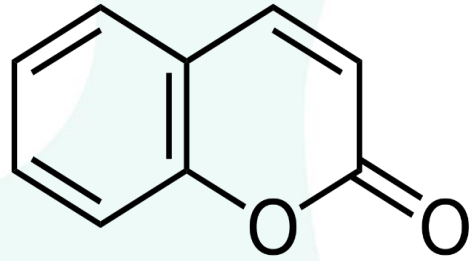
$$\text{Exposure: activity ratios} = \frac{\text{Exposure (plasma exposure in µM)}}{\text{Activity } (IC_{50}\ \mu M)}$$

$$\text{Dietary comparator ratio} = \frac{\text{EAR (test substance)}}{\text{EAR (dietary comparator)}}$$

(After Becker *et al.*, (2015) *Regul Toxicol Pharmacol* 71(3): 398–408)

A case study approach – human health safety assessment required for…

0.1% COUMARIN IN FACE CREAM FOR EU MARKET (NEW FRAGRANCE)

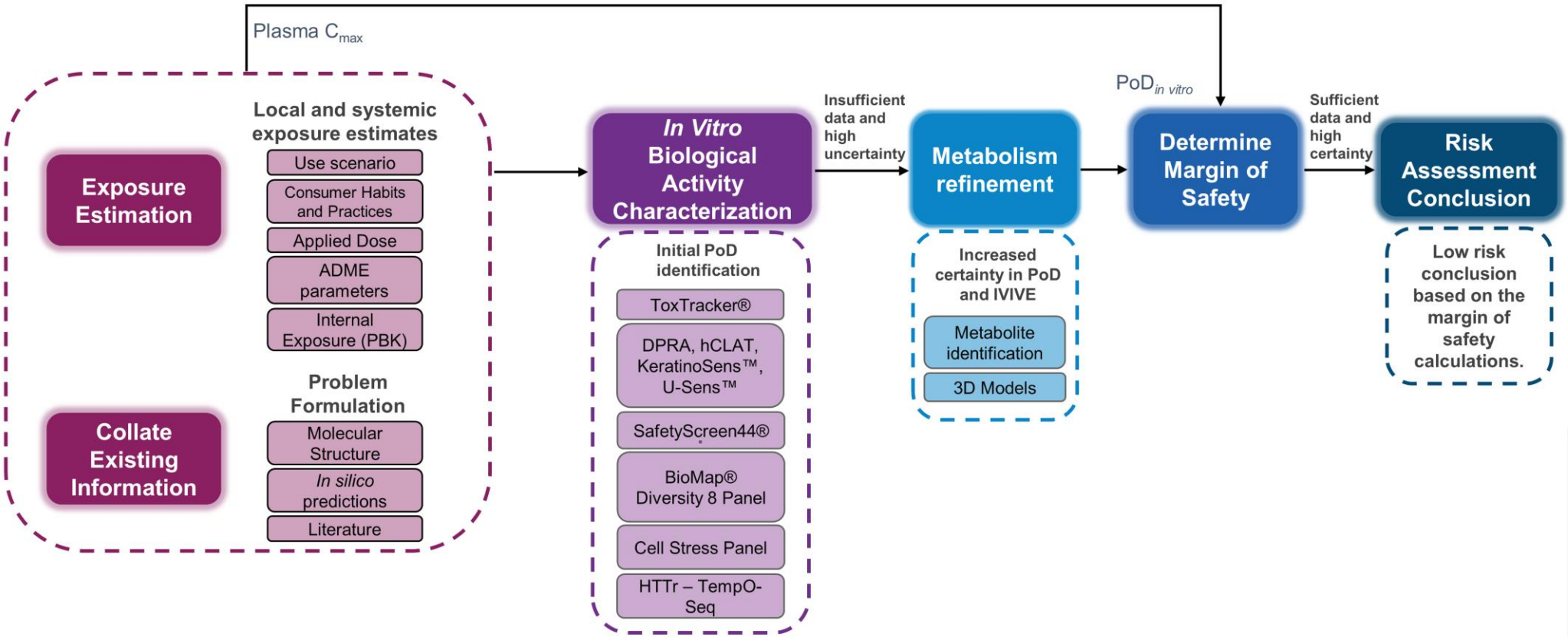


Assumed that:

- 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

Unilever

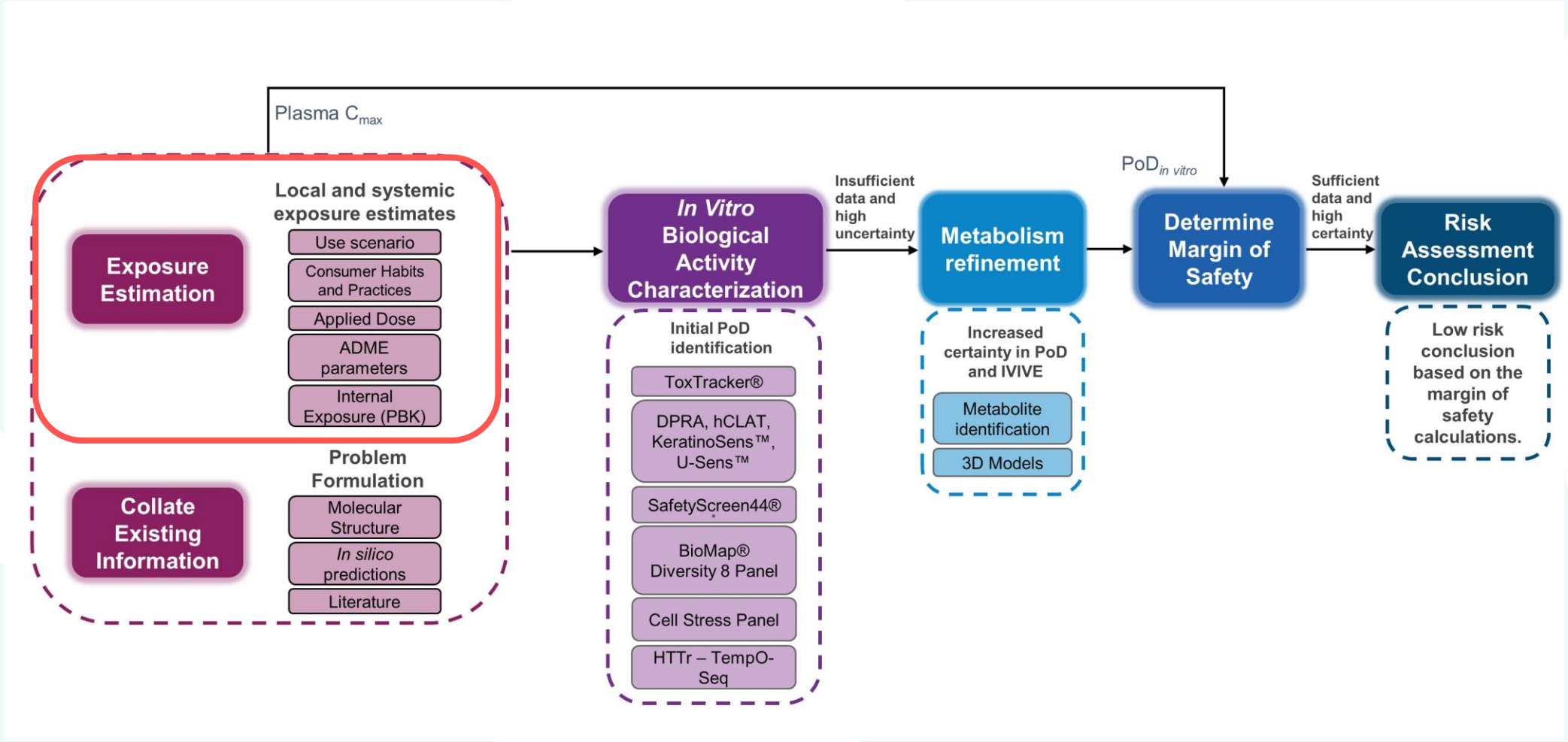
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



Baltazar *et al.*, (2020) *Tox Sci* (in press)
https://doi.org/10.1093/toxsci/kfaa048

Exposure information and Collation of existing information (10 min)

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



NGRA for 0.1% coumarin in face cream: exposure estimation

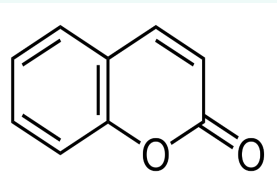
Creme GLOBAL

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				0.04	0.60

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

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

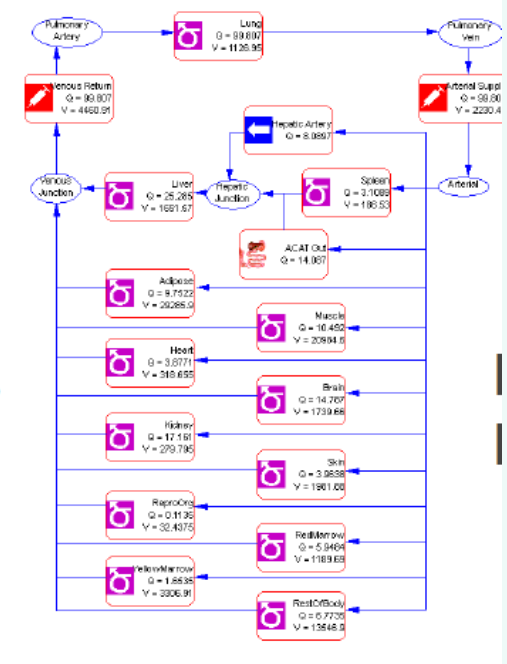


Parameter	Face cream
Amount of product used per day (g/day) using 90th percentile	1.54
Frequency of use	2 times/day
Amount of product in contact with skin per occasion (mg)	770
Ingredient inclusion level	0.1%
Skin surface area (cm2)	565
Exposure duration per occasion	12 hours
Amount of ingredient in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

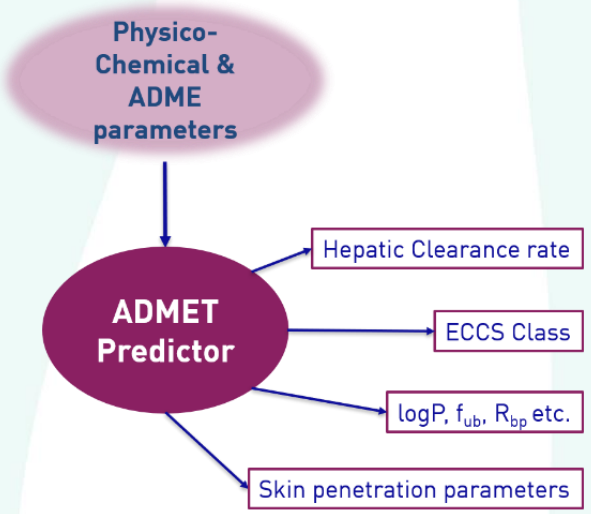
Unilever

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

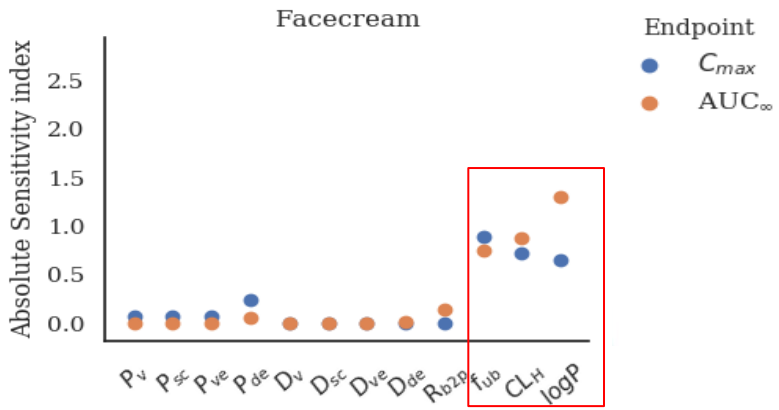
GastroPlus® (Simulations Plus)



Level 1. Use in silico parameters for modelling



Level 1. Sensitivity analysis

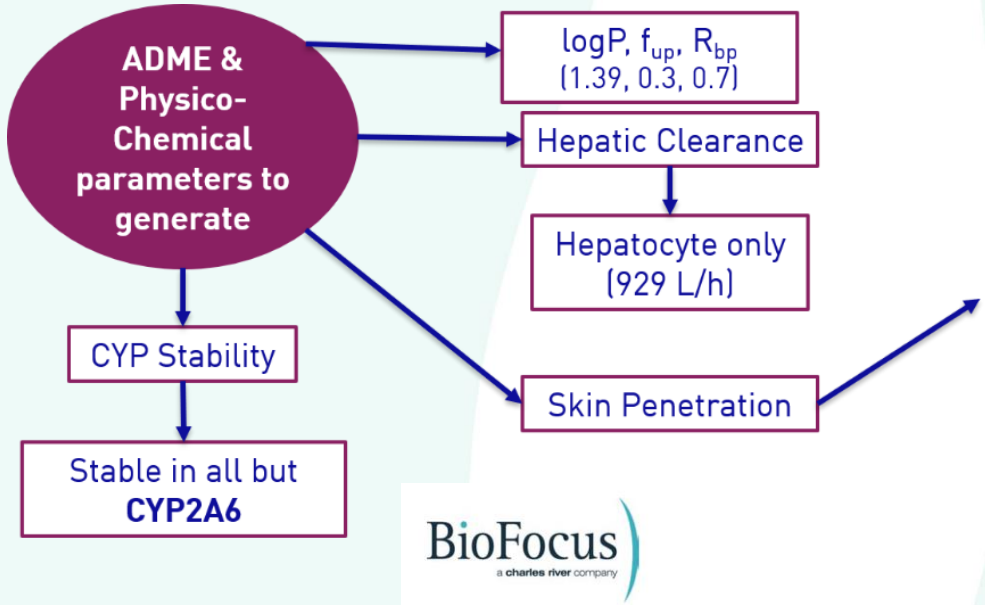


Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

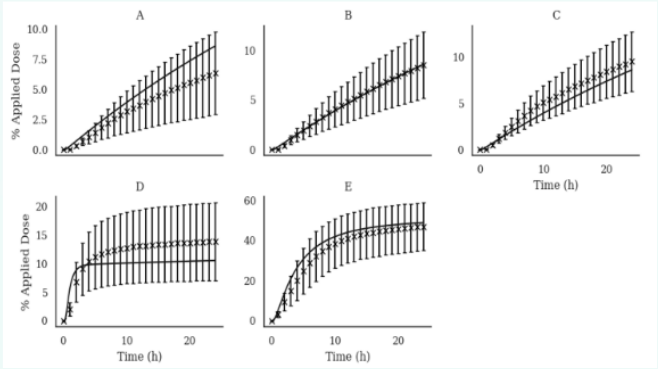
NGRA for 0.1% coumarin in face cream: exposure estimation- Internal concentration using PBK modelling-Model Inputs

Level 2.

- **In vitro data generation** for parameters with **high sensitivity &/or low confidence** in the predicted values require further refinement through
- **Update the model** with new parameters



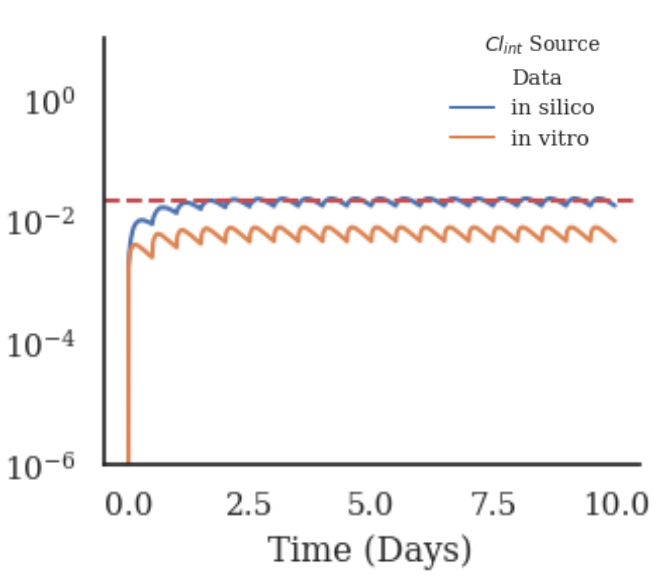
Skin absorption study



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

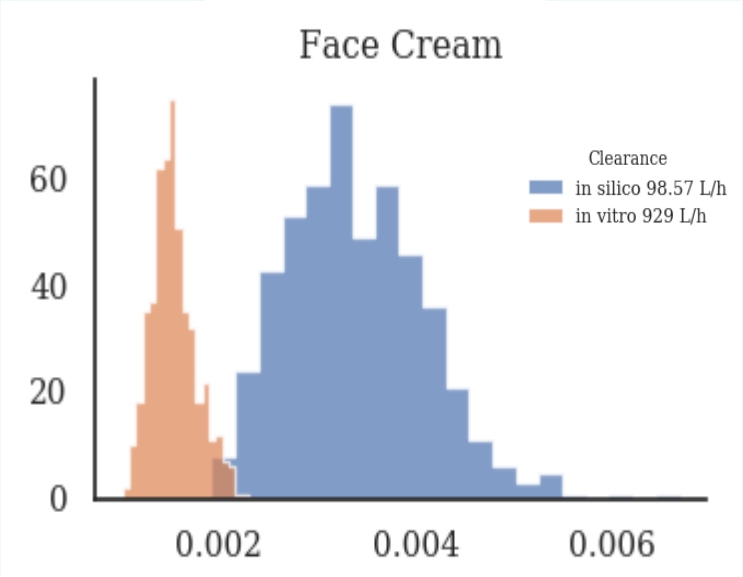
NGRA for 0.1% coumarin in face cream: exposure estimation- Internal concentration using PBK modelling- Model Outputs

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

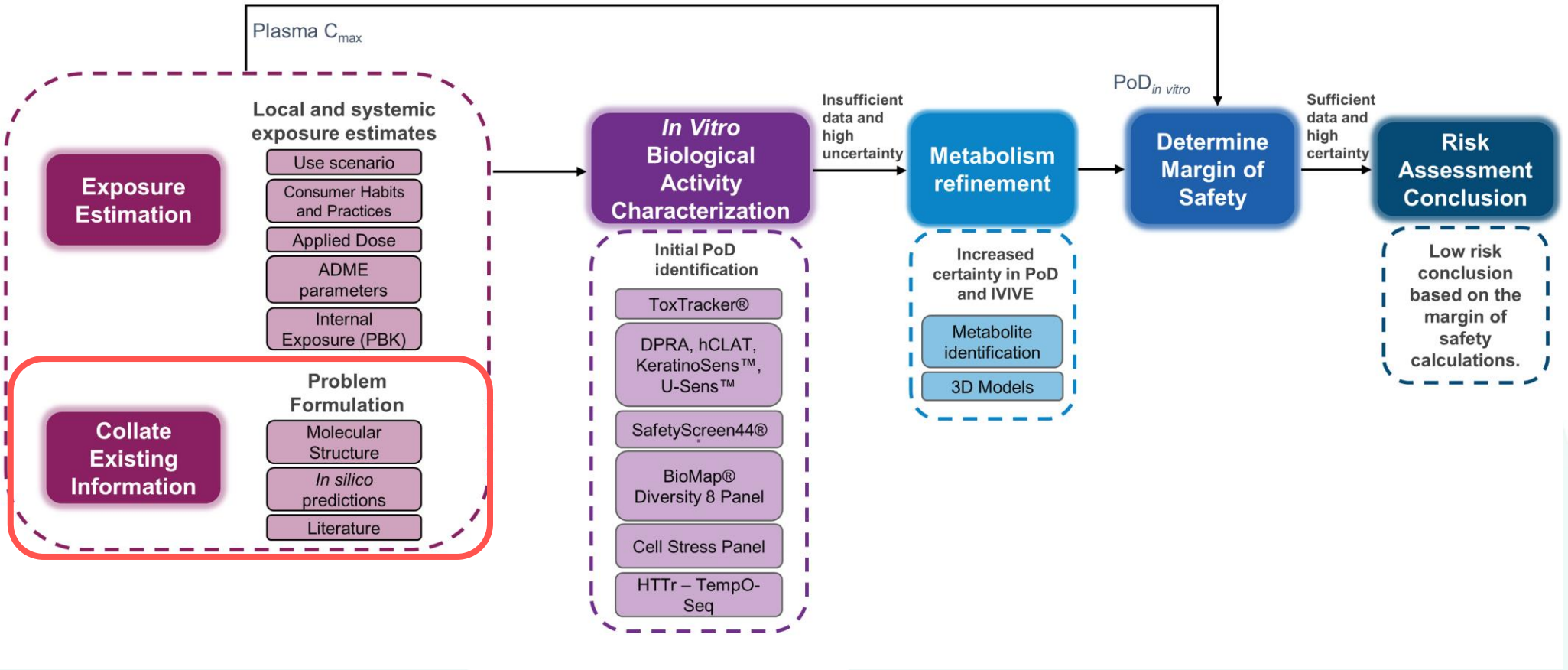
Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

NGRA for 0.1% coumarin in face cream: exposure estimation

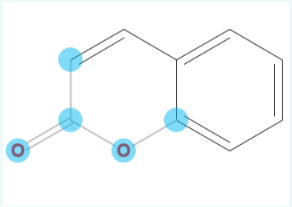
Exposure Estimation

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

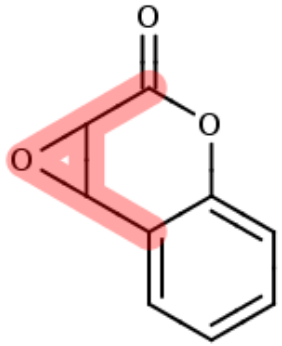
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



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

- 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

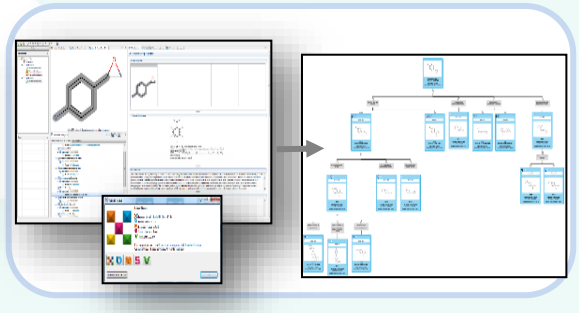
*Allen THE et al., 2018. Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events. Toxicol Sci. 2018 Sep 1;165(1):213-223

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

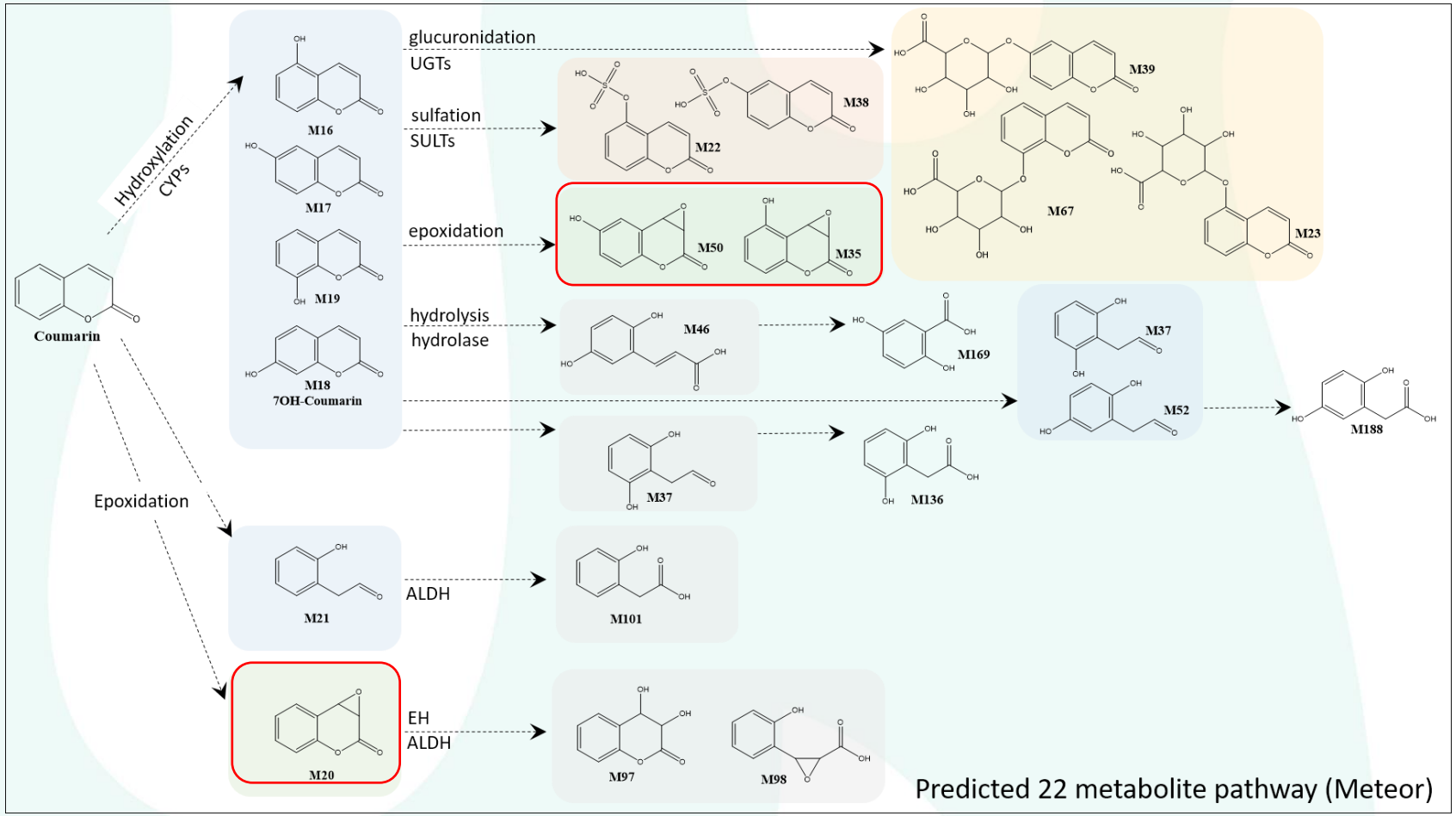
In silico biotransformation

Meteor nexus

Lhasa Limited



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



Predicted 22 metabolite pathway (Meteor)

Unilever

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

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

Only few active assays among multiple assays (≈ 5000)

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

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

Unilever

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)

Breakout Discussion (25 min)

Breakout group questions

1. Do you agree with the interpretation of the data/information? (Poll in menti, yes/no/not sure)
2. What other data/information would you like to generate/see? (please add your comment in Menti)
3. Any other questions? (please add your question in Menti)

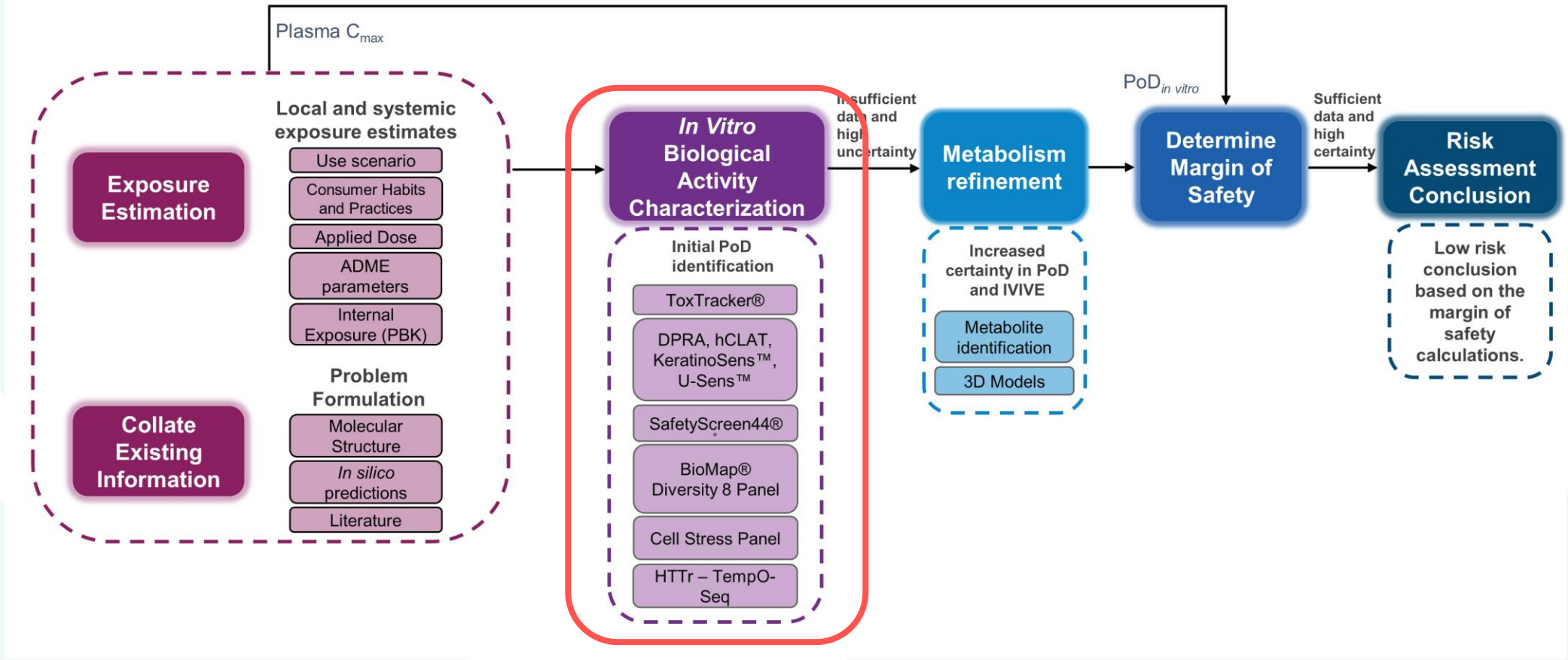
10 min breakout discussion

15 min plenary discussion

Break (15 min)

In vitro biological activity characterisation (35 min)

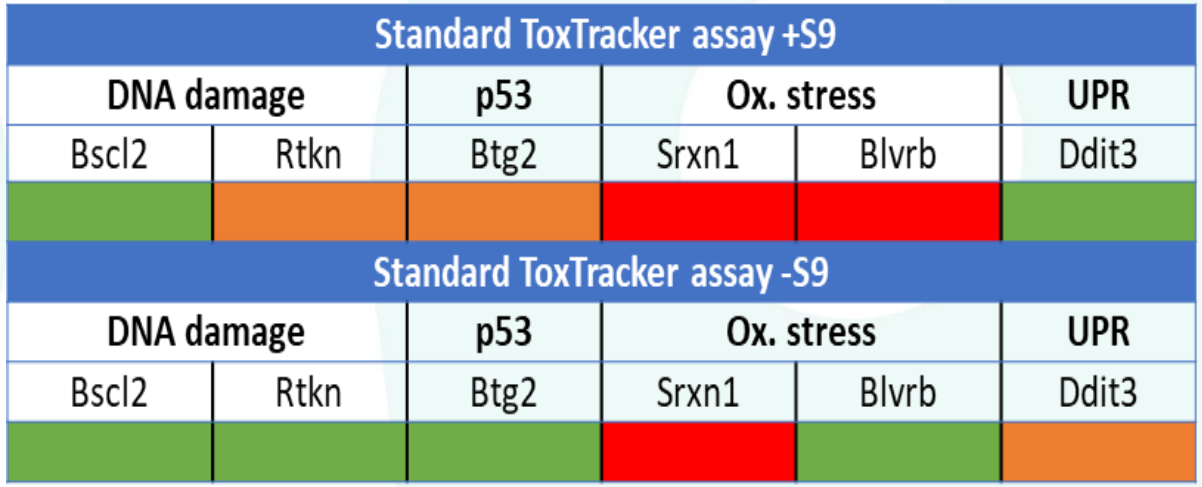
Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



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



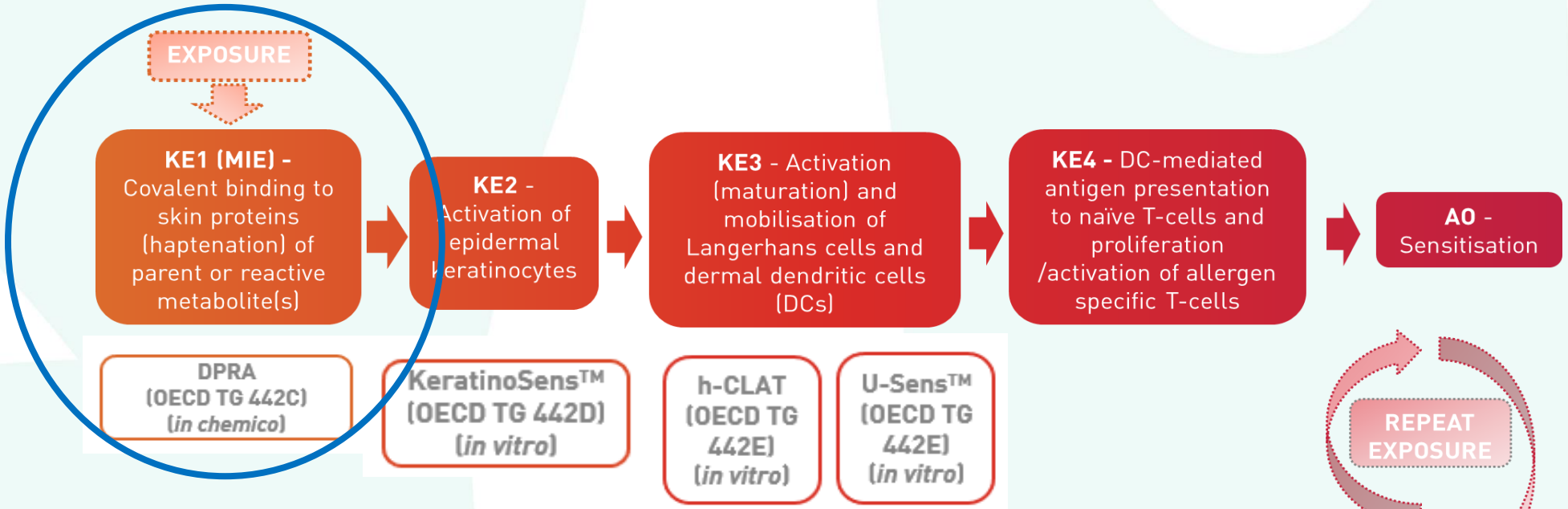
Results:

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

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

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: 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)	CD54 (EC150 µg/mL)	CD86 (EC150 µg/mL)
RUNs	1.0 0.7 2.2	0 0 0	200 175 NA	>637 <178 <178	>637 >637 >637	95 96 NA

Initial results:

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

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

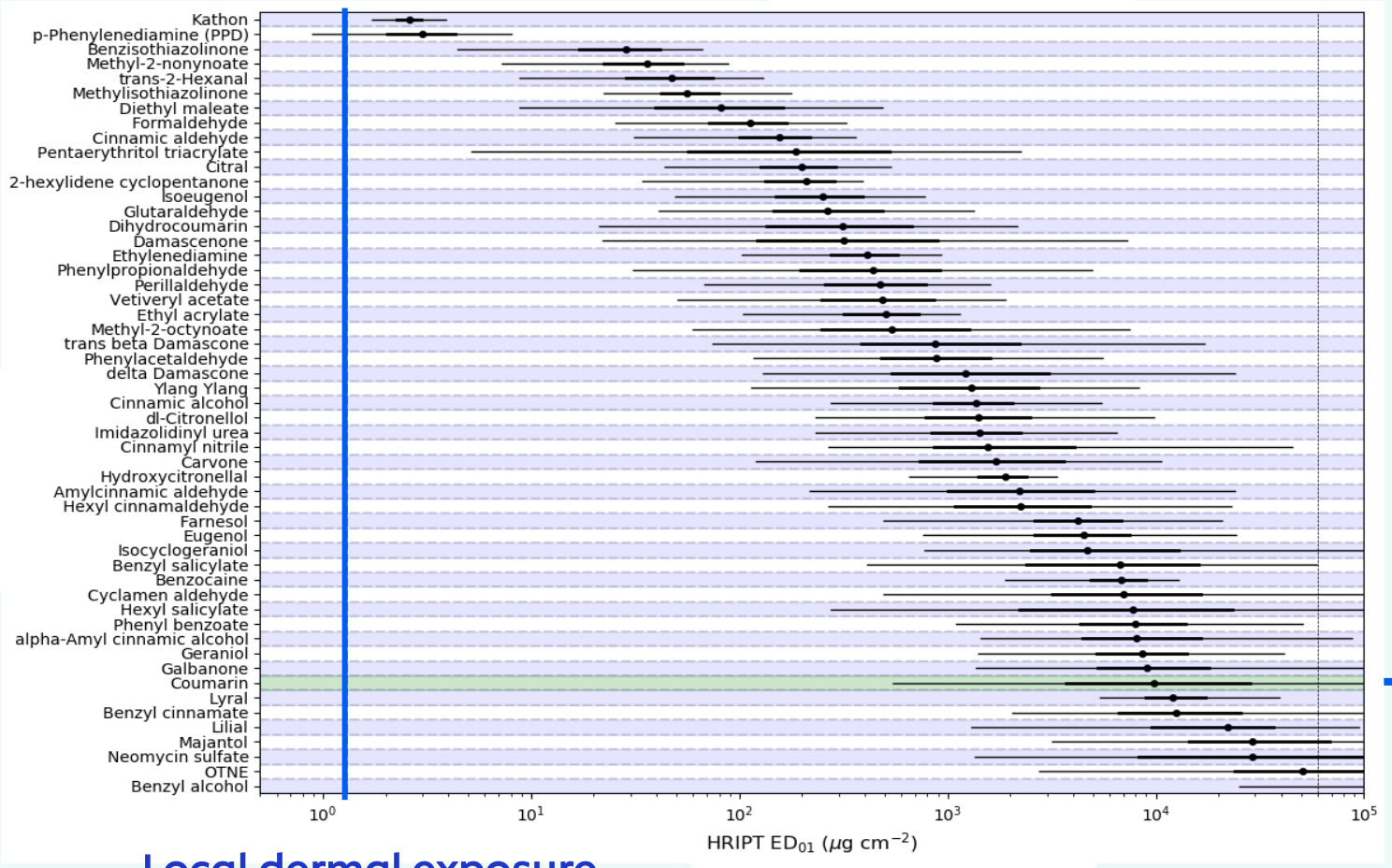
SARA Model Inputs

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

* Reynolds, J, MacKay C, Gilmour N, Miguel-Vilumbrales D and Maxwell G (Submitted for publication: Computational Toxicology) Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment

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

Step 2: PoD for risk assessment



Local dermal exposure (1.36 µg/cm²)

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

Results:

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

Unilever

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

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.

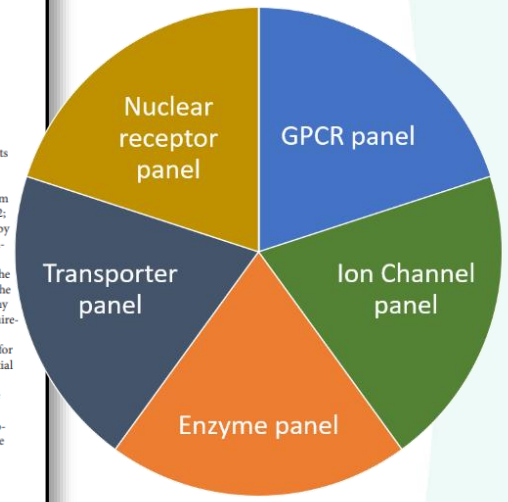
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

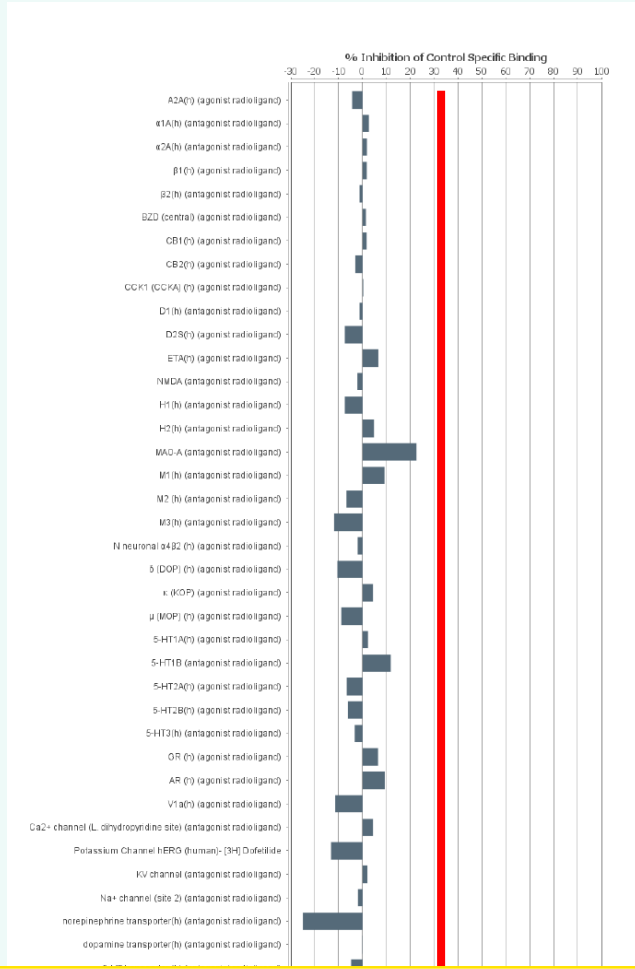
safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* 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



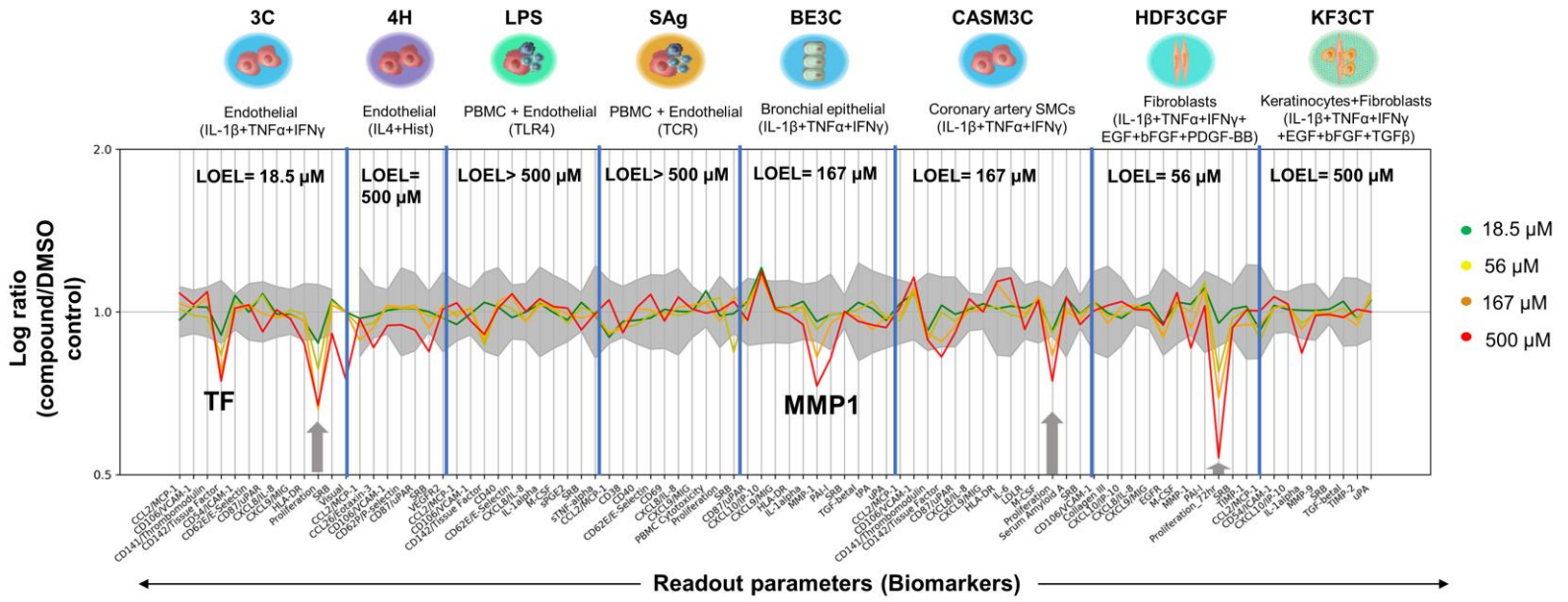


Results:

All binding and enzymatic assay results were negative at 10 µM

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

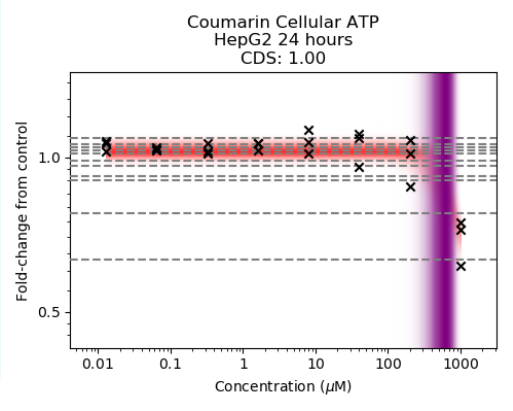


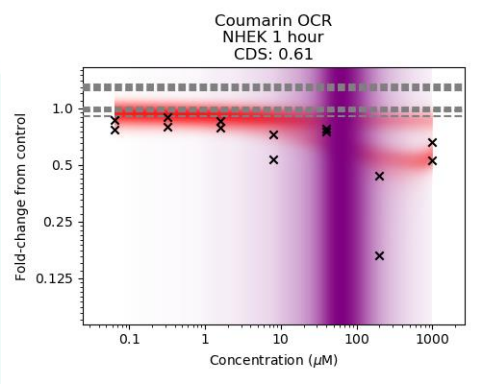
Data suggested that coumarin has no immunomodulatory effects at relevant concentrations and is not an anti-inflammatory compound

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

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

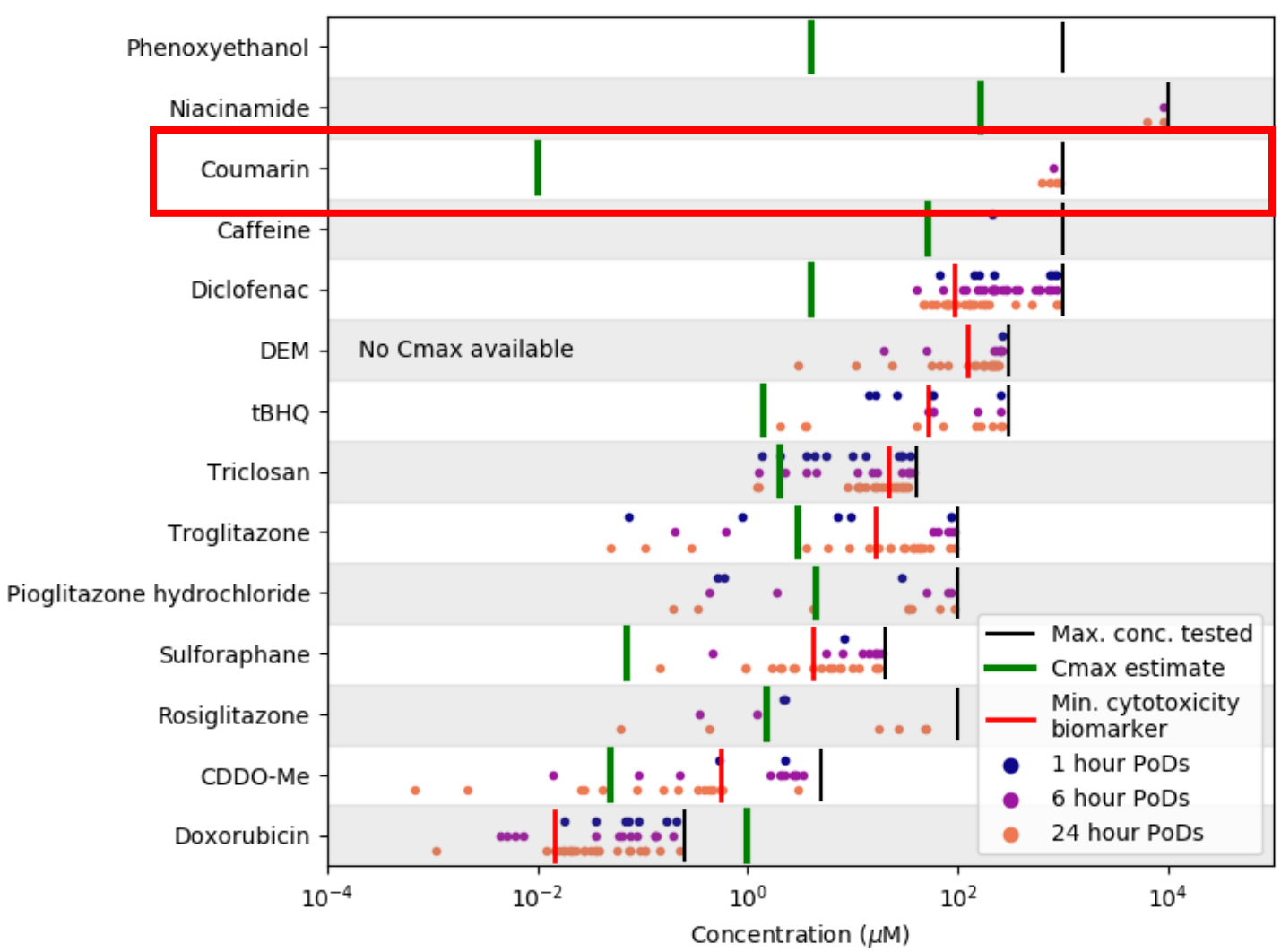




Biomarkers	Cell type	Stress pathway	PoD (μM)	Effect	Concentration dependency score (CDS)
ATP (6h)	HepG2	cell health	794 (363-977)	down	0.98
ATP (24h)			617 (282-891)	down	1
Phospholipidosis (24h)	HepG2	cell health	759 (437-977)	down	0.93
GSH (24h)	HepG2	oxidative stress	851 (301-1000)	up	0.92
IL-8 (24h)	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. in Press. https://doi.org/10.1093/toxsci/kfaa054

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel



Results:

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

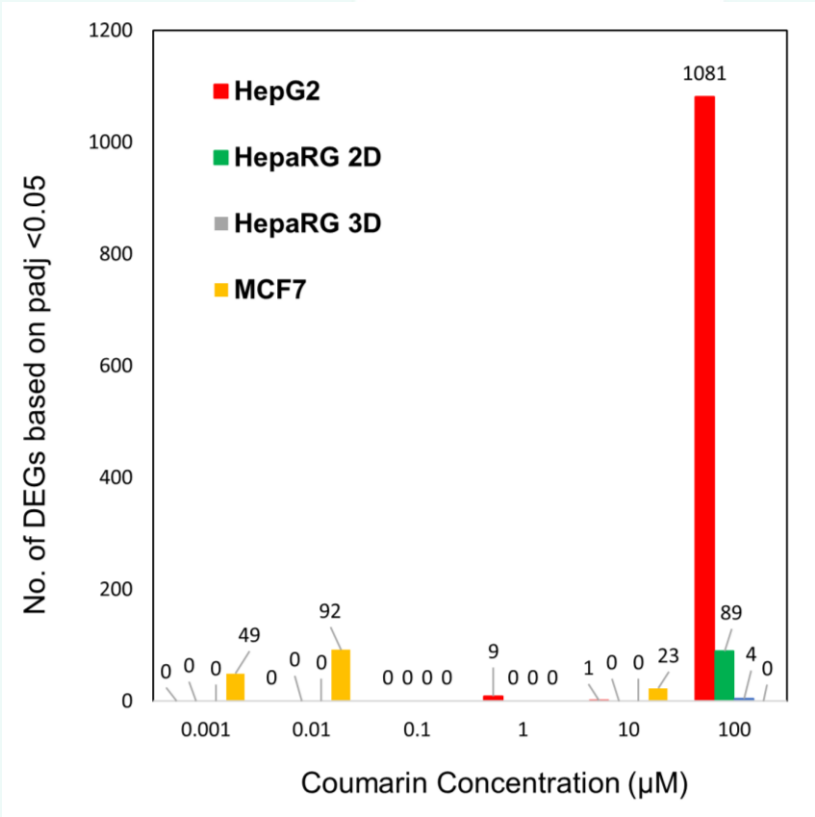
- PoDs shown for HepG2 only

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

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: High-Throughput Transcriptomics (HTTr) using TempO-SEQ technology

Transcriptomics was applied as a broad nontargeted 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: In vitro biological activity characterisation: High-Throughput Transcriptomics (HTTr), TempO-SEQ technology

Transcriptomics was applied as a broad nontargeted biological screen

PoD determination

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

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

Farmahin, R., Williams, A., Kuo, B. *et al.* Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment. *Arch Toxicol* **91,** 2045–2065 (2017). https://doi.org/10.1007/s00204-016-1886-5

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)
- The probability of coumarin inducing skin sensitisation at the consumer exposure is low
- No immunomodulation potential
- Low bioactivity confirmed by binding/enzymatic assays, HTTr and cell stress panel.
- PoD range: 6-912 µM

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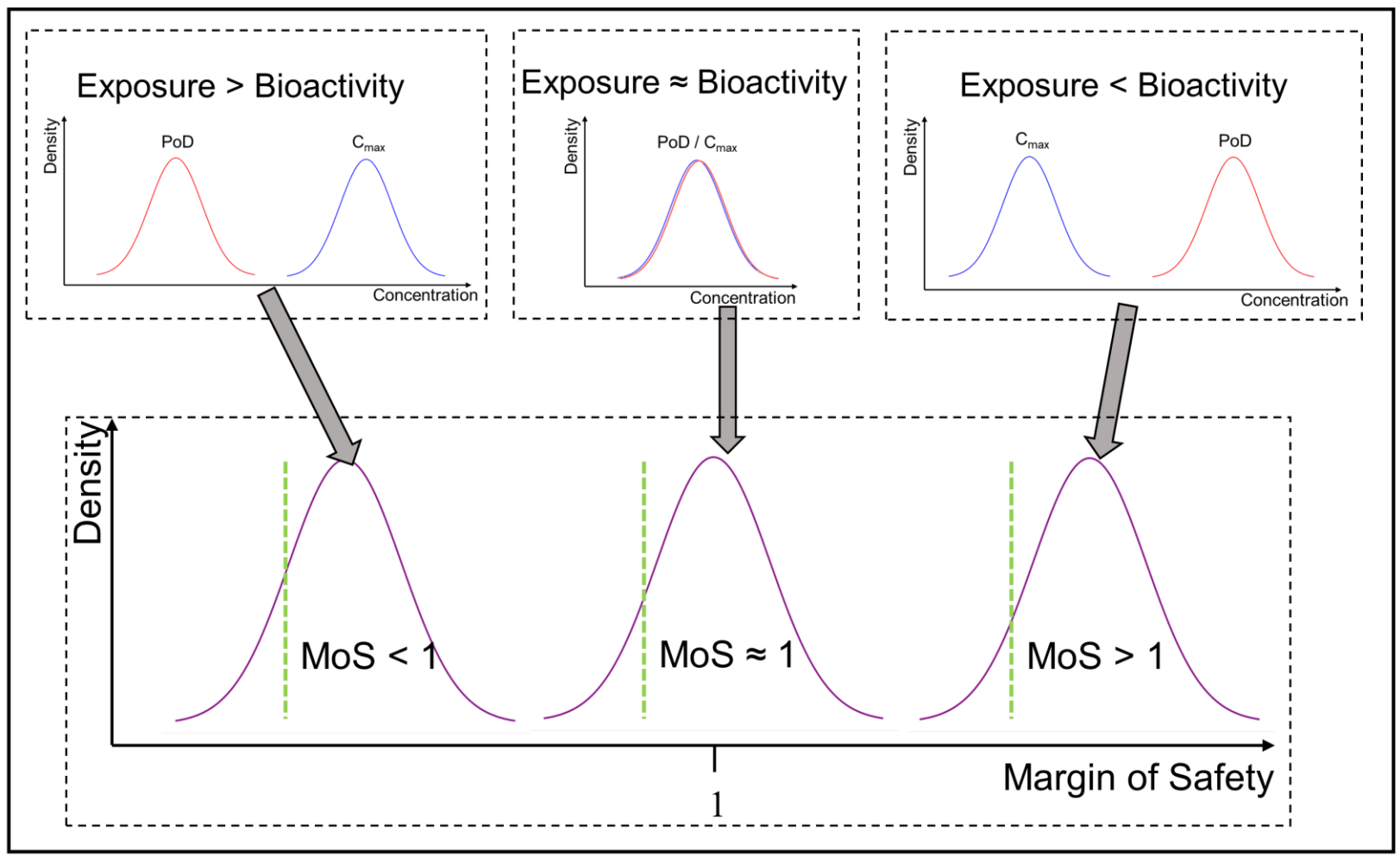
NGRA for 0.1% coumarin in face cream: Preliminary Margin of Safety – How MoS is calculated



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

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- 90-100% coumarin predicted to be freely available *in vitro*
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- Lowest PoD was 3 µM for carbonic anhydrase I (Figure 7)

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- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- The probability of coumarin inducing skin sensitisation at the consumer exposure is low
- 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

Breakout Discussion (25 min)

Breakout group questions

1. Do you agree with the interpretation of the data/information? (Poll in menti, yes/no/not sure)
2. What other data/information would you like to generate/see to increase your confidence in the conclusions? (please add your comment in Menti)
3. Any other questions? (please add to the chat)

10 min breakout discussion

15 min plenary discussion

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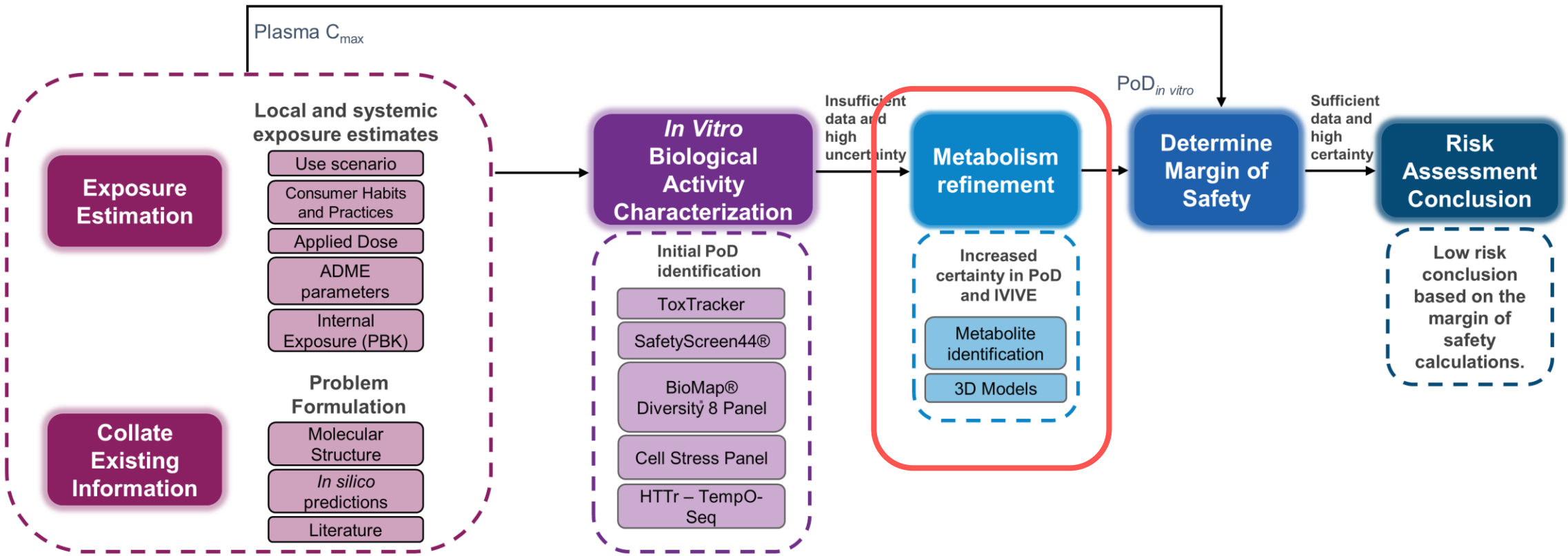
Metabolism refinement & Margin of Safety determination & Risk assessment conclusion (10 min)

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



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

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

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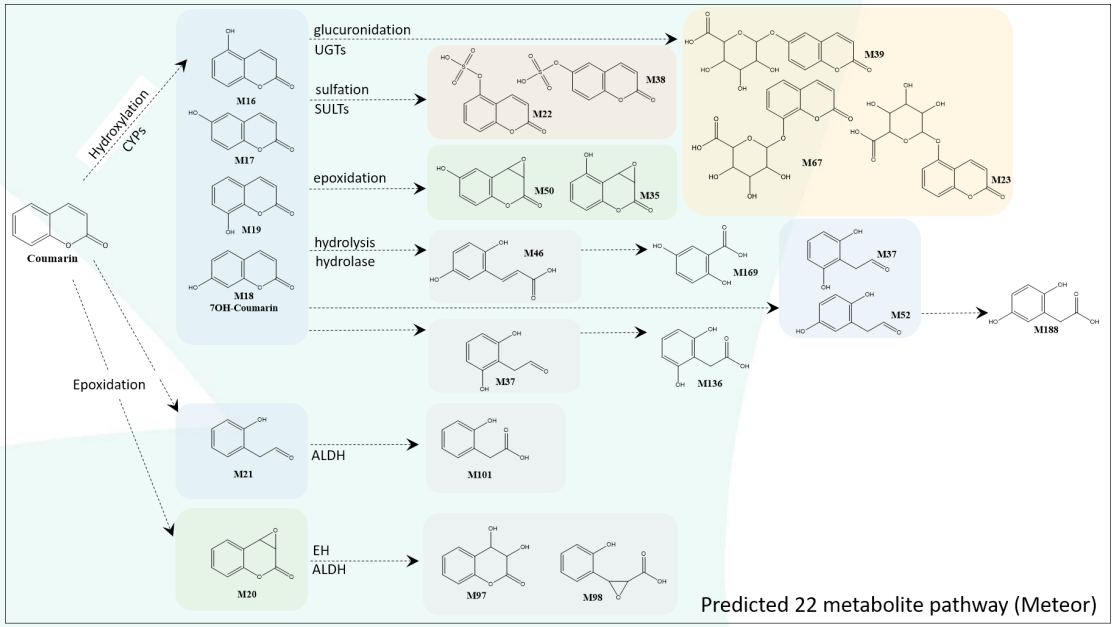
NGRA for 0.1% coumarin in face cream: Coumarin metabolism in primary human hepatocytes

Meteor nexus

In silico biotransformation

Metabolite profiling in **pooled human cryopreserved primary hepatocytes**

Human *In vitro* metabolism

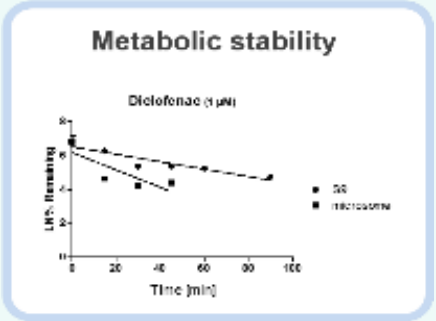


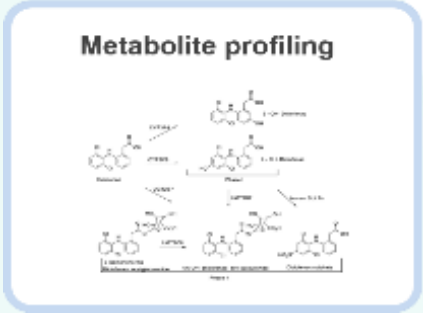
Predicted 22 metabolite pathway (Meteor)

Two approaches:

1. A high (1 mM) concentration of coumarin was used to saturate the CYP2A6 pathway.
2. A lower concentration of coumarin (10 µM) was used, both with and without inhibition of CYP2A6 (using either 0.5 or 2 µM tranylcypromine)

In vitro stability assays: CYP2A6 driven metabolism





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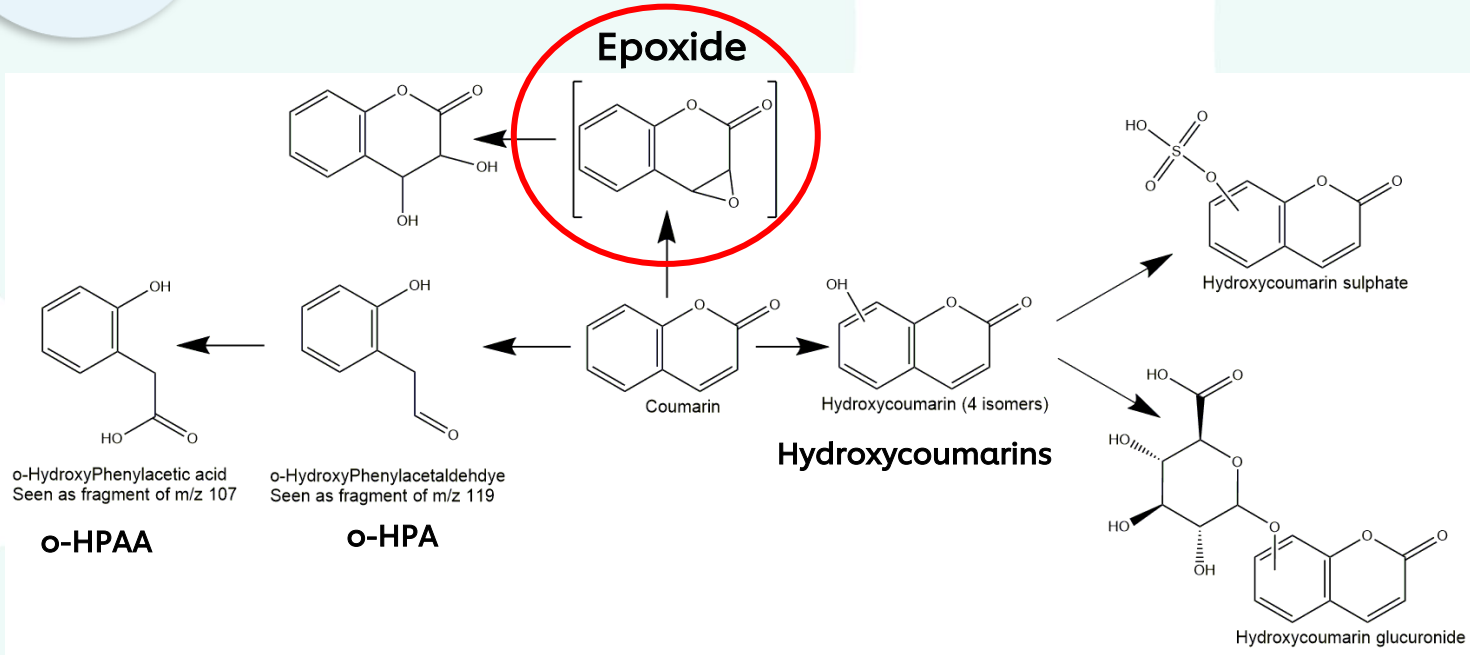
NGRA for 0.1% coumarin in face cream: Coumarin metabolism in primary human hepatocytes

Human *In vitro* metabolism

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



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

Results:

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

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

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

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

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NGRA for 0.1% coumarin in face cream: Key results

Exposure Estimation

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

Collate Existing Information

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

In Vitro Biological Activity Characterisation

- ToxTracker negative; weak activation of DNA damage reporters (only +S9)
- The probability of coumarin inducing skin sensitisation at the consumer exposure is low
- 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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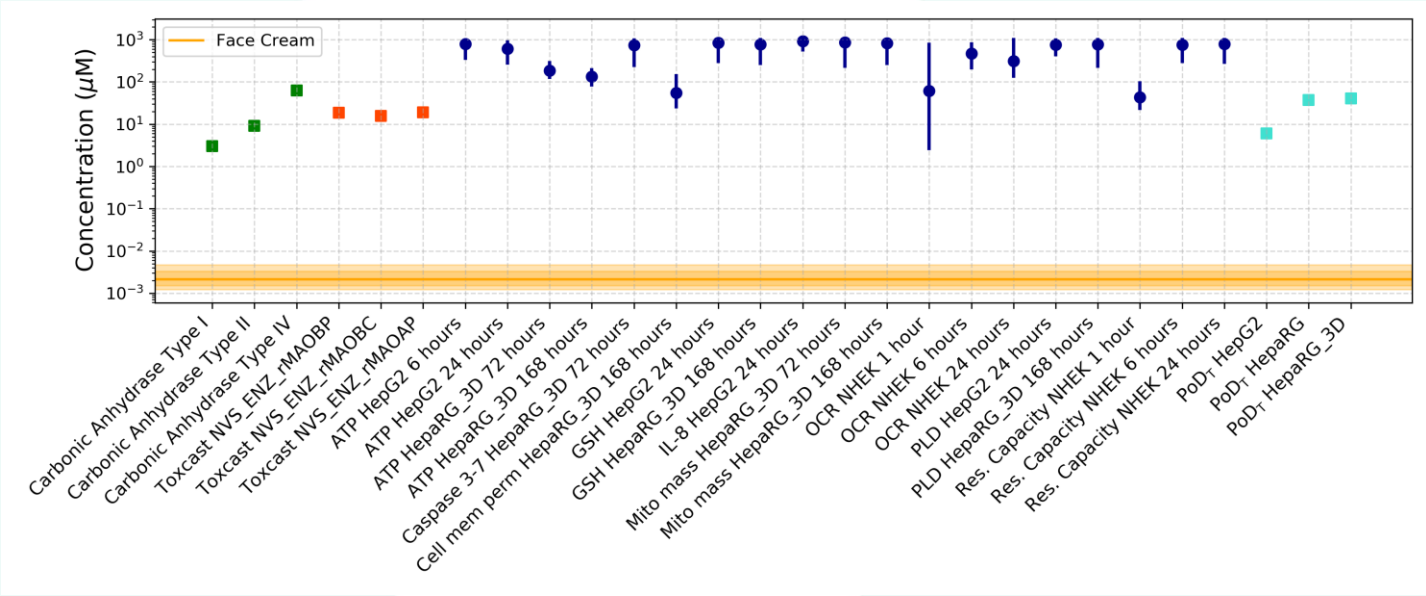
NGRA for 0.1% coumarin in face cream: Risk assessment conclusion



- 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 cause skin sensitisation, 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 these products is safe for the consumer**

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Poll questions & Discussion (25 min)

Discussion questions

1. Do you agree with the low risk decision? (Menti Poll: yes/no/not sure)
2. What additional data/information would you like to generate/see to increase your confidence in the decision? (Menti: post it note)
3. Has this case study increased your confidence in non animal approaches? (Menti Poll: yes/no/not sure)

10 min breakout

15 min discussion

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Concluding remarks

1. Available tools can be integrated to make a safety decision; multidisciplinary team needed!
2. NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
3. Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making
4. Rethinking MoS/MoE – future evaluation of the approach to infer a low risk space
5. Shortcomings will be addressed by current and future research
6. More research, creativity and examples needed to land this successfully across the community
7. Progress is only possible with a change in mindset (protection not prediction)

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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

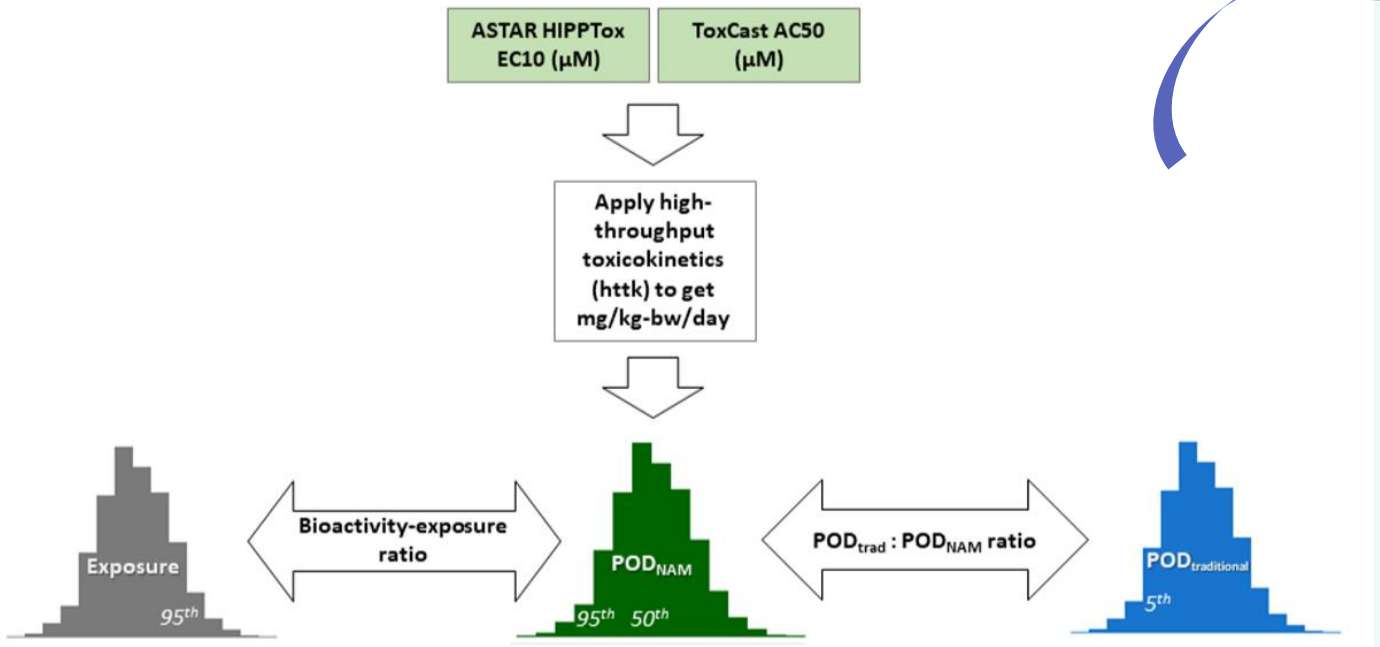
Extended Team:

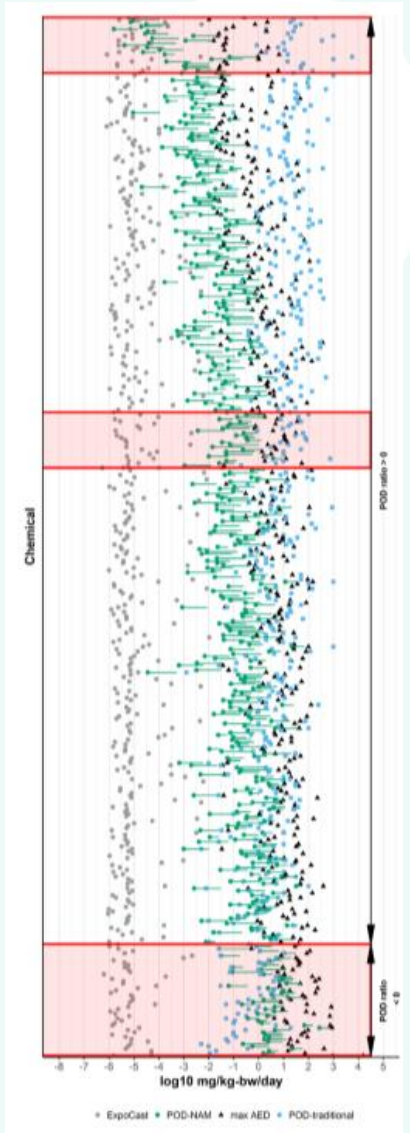
- Carl Westmoreland, Paul Russell, Gavin Maxwell, Ian Sorrell, Sam Piechota, Juliette Pickles, Karen Bonner, Sandrine Spriggs, Iris Muller, Katarzyna Przybylak, Paul Walker, Caroline Bauch, Rebecca Beaumont, Steve Clifton, Katie Paul-Friedman, Julia Fentem

BACKUP SLIDES

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

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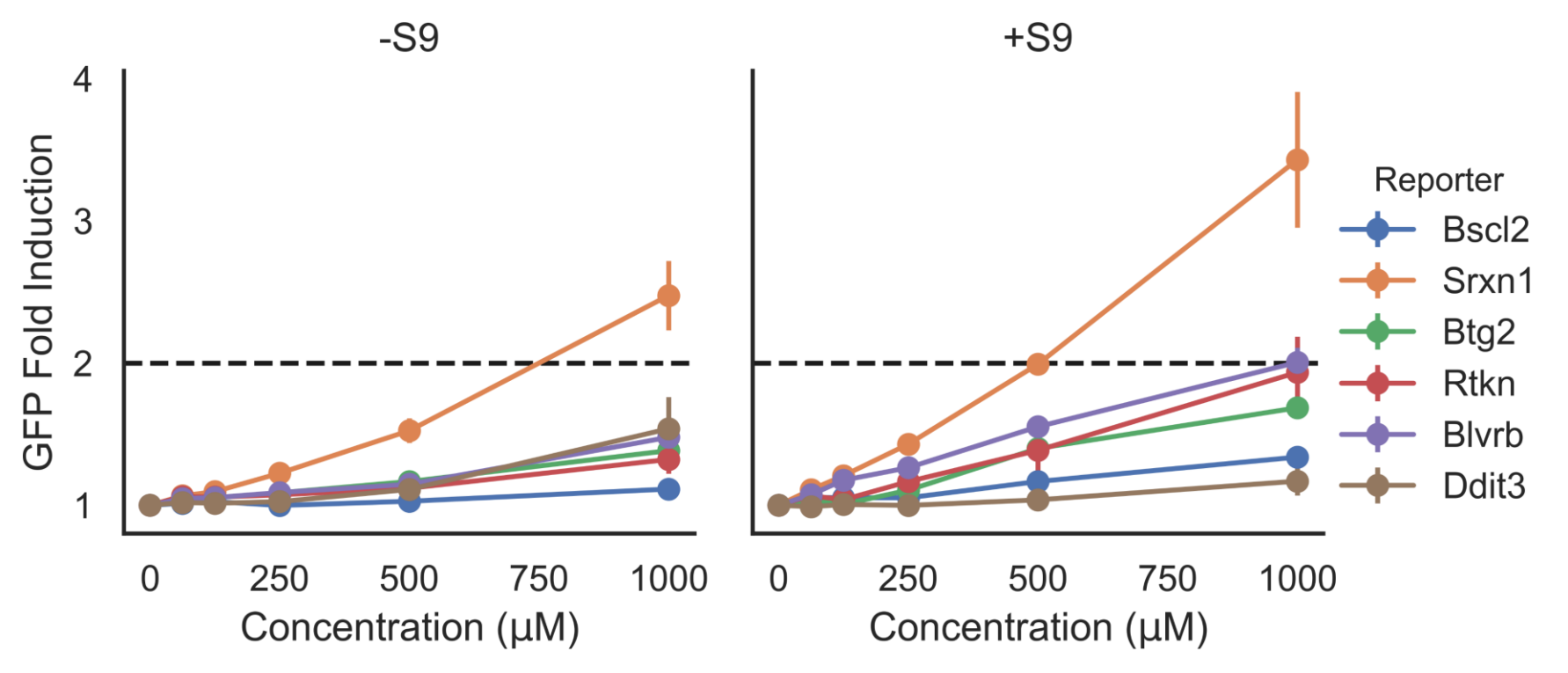
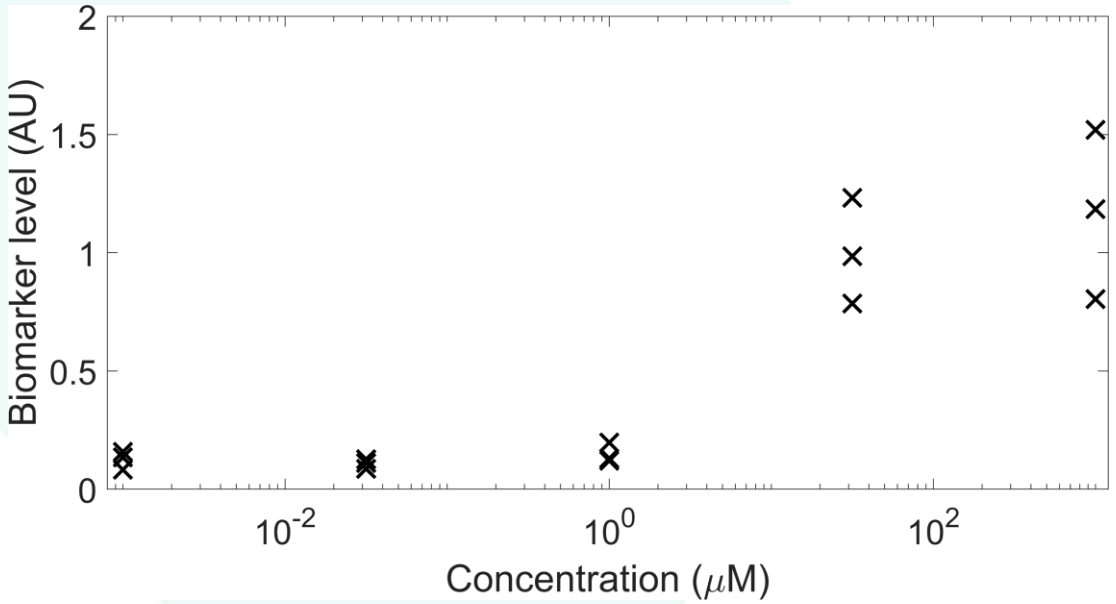
Backup slides- Toxtracker



NGRA: dose-response analysis and PoD derivation

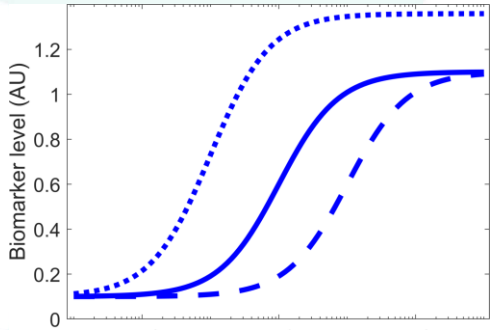
Example dose response data



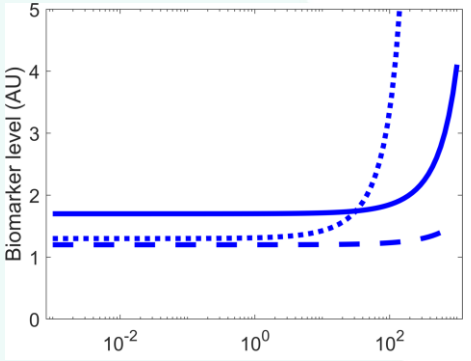
1. Fit different <u>parametric models</u> to the data
2. Identify the one with the 'best' fit
3. Use this to calculate the PoD…

Candidate dose-response models

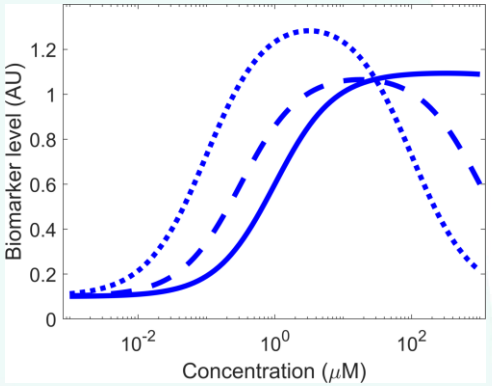
Hill function



Exponential

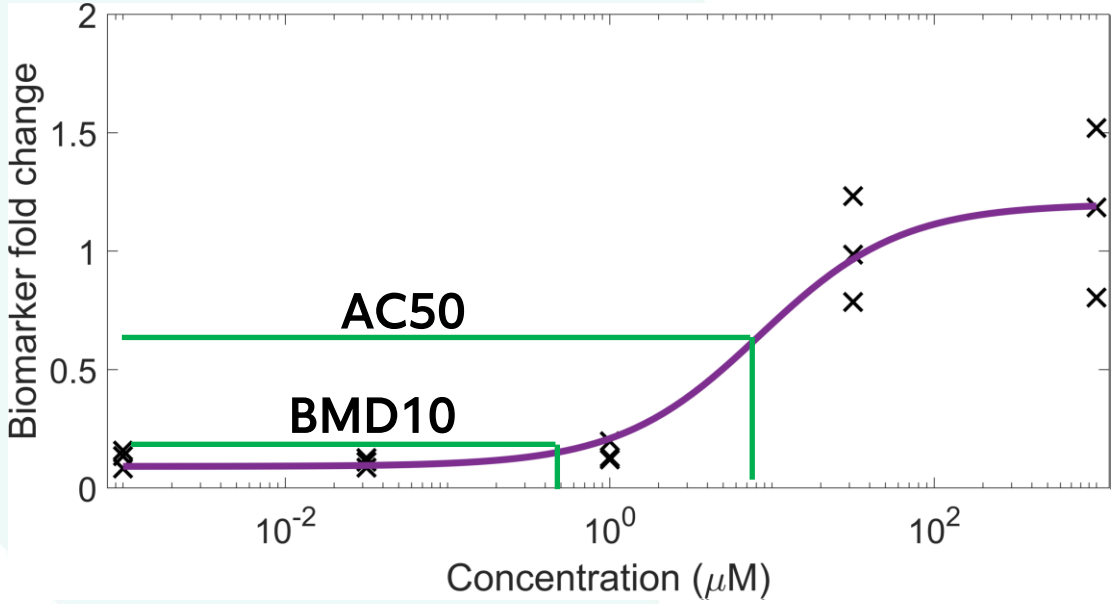


Gain-loss model



NGRA: dose-response analysis and PoD derivation

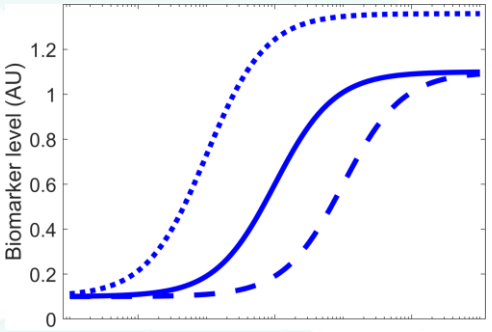
Example dose response data



1. Fit different <u>parametric models</u> 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



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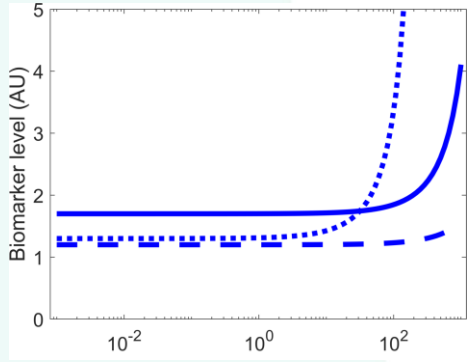
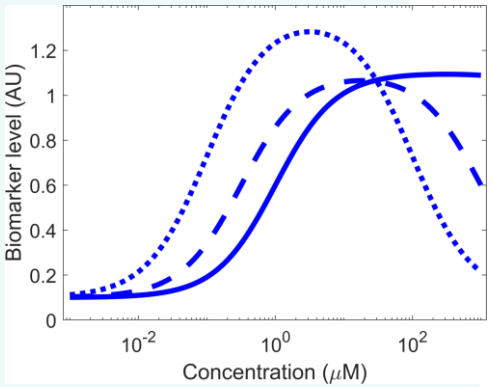


Gain-loss model



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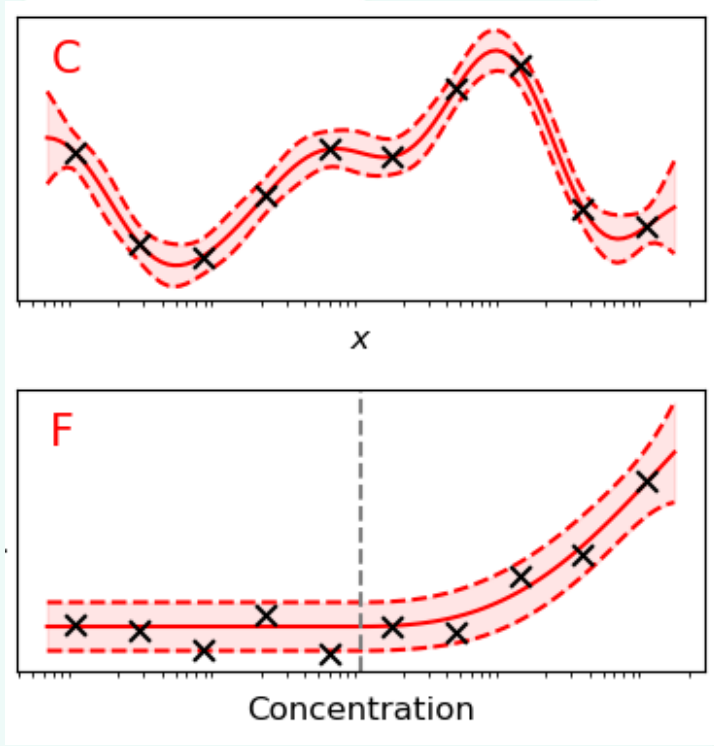
NGRA: dose-response analysis and PoD derivation

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.

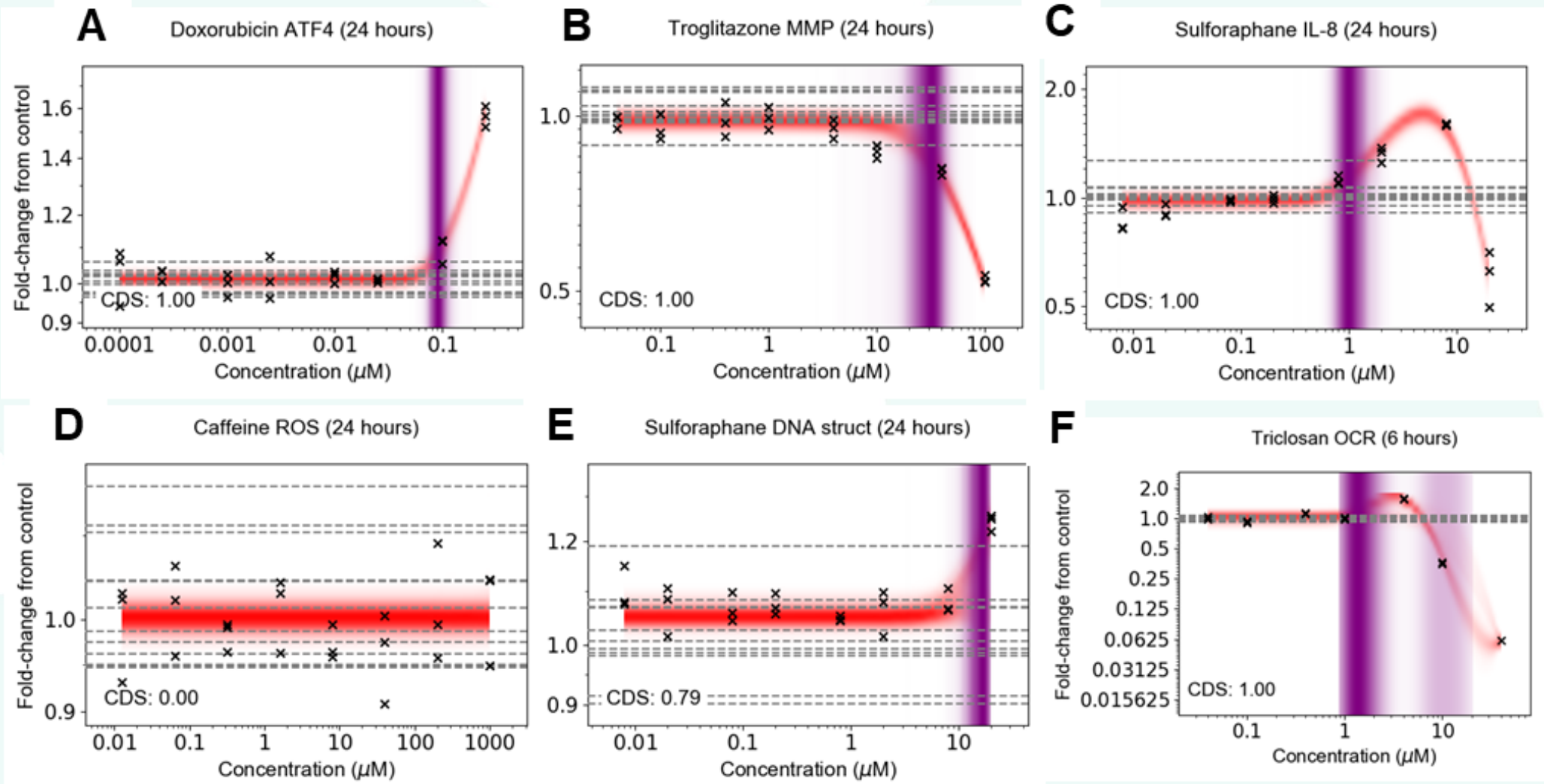
2. In NGRA it is important to quantify the uncertainty in a) whether there is a concentration-dependent response and b) the PoD estimate, if there is one.

3. Instead we used a non-parametric model (Gaussian processes) within a Bayesian statistical framework to model to data.



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

NGRA: dose-response analysis and PoD derivation



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

NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: High-Throughput Transcriptomics (HTTr)

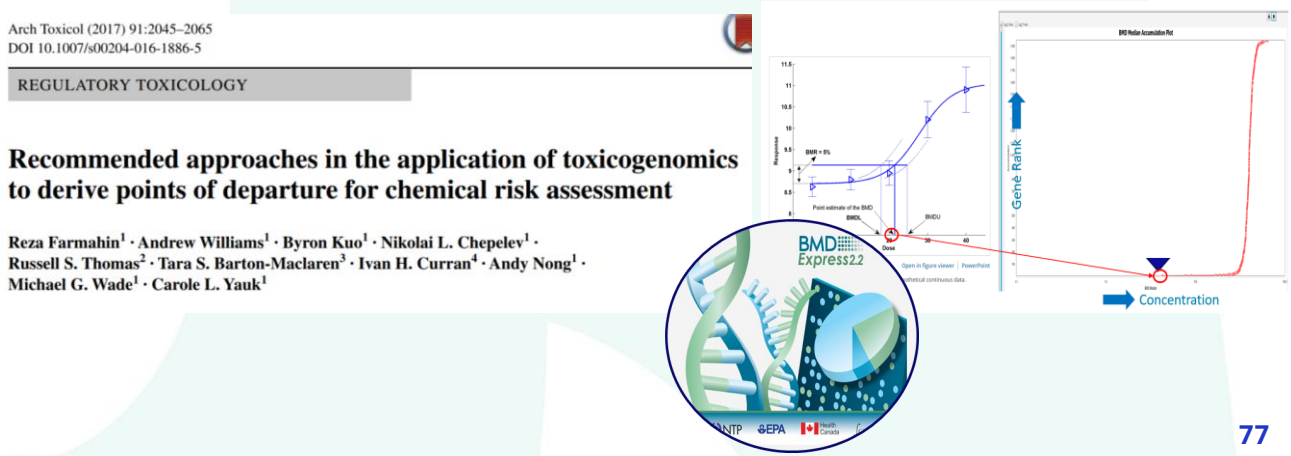
- Transcriptomics was applied as a broad nontargeted biological screen of *in vitro* cellular perturbation following coumarin treatment

Generation of HTTr using the TempO-SEQ technology

- TempO-SEQ technology advantages include simple sample preparation, high throughput, high accuracy and sensitivity, simplified bioinformatics analysis
- HepG2, MCF, and HepaRG 2D cell lines
- 24h exposure
- 7 concentrations

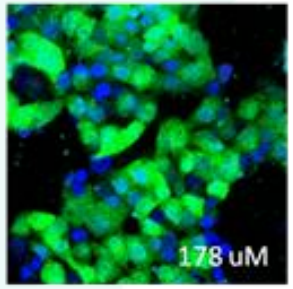
Data analysis: Differential expression analysis, pathway analysis and PoD determination

- Differential expression analysis was performed using DESeq2 analysis
- Concentration response analysis using BMDexpress2
- PoD was determined based on a subset of methods (1,3,4,5,9) outlined in (Farmahin et al. 2017

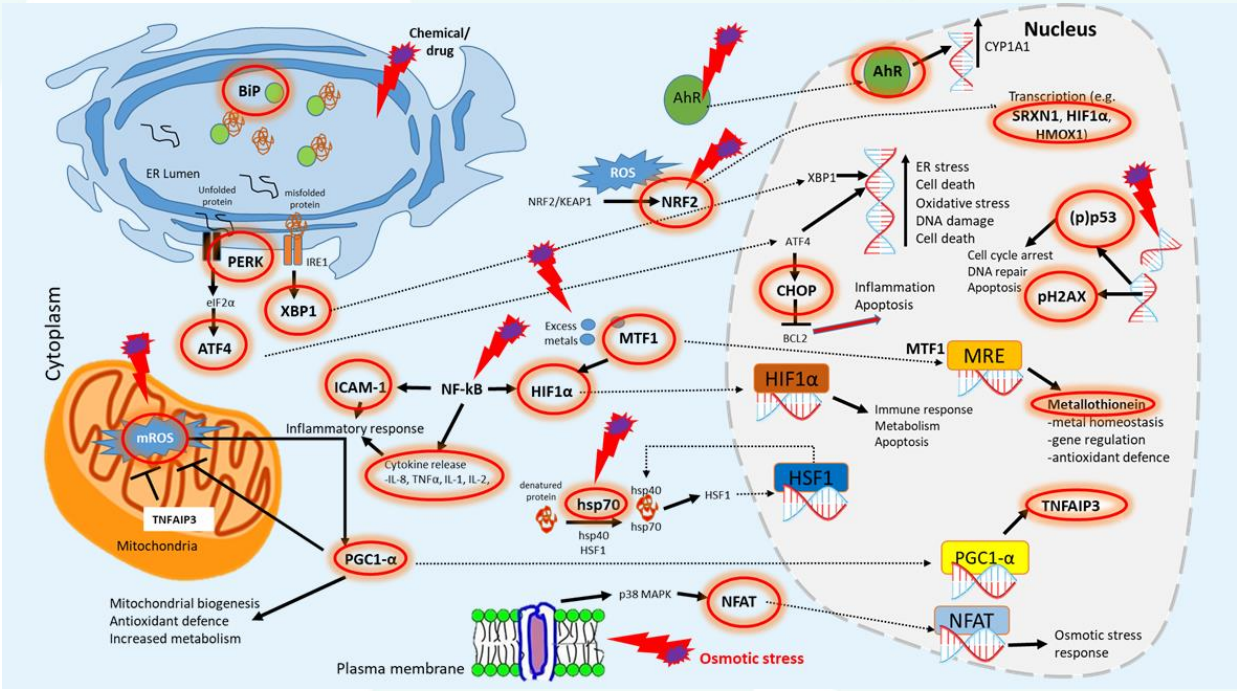


NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro cell stress panel

- 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)



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

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

PoD for cell stress biomarkers single dose up to 7 days in HepaRG 3D:

- Early signs of **cell damage** were observed at low concentrations (**PoD= 56 µM) after 168h incubation.**
- ATP decrease at 72 and 168h (PoD= 190 and 144 µM)
- At concentrations >700 µM) a mixture of biomarkers related to mitochondrial toxicity, oxidative stress and cell health were affected

HTTr in a HepaRG 3D model where cells were exposed to coumarin for 24h

- The response observed was very limited for DeSeq2 with only 4 genes meeting the padj value of 0.05, all seen at the top dose (200 µM)
- Lowest PoD across all methods was 41 µM

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