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

Maria Baltazar, SEAC Safety Science Leader

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

Unilever

Principles of Next Generation Risk assessment (NGRA)

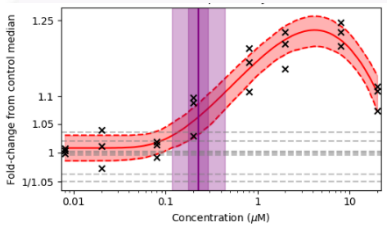
*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

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

- **Using new tools and approaches** to build a risk assessment to enable decisions to be made (without animal tests)
- **An exposure-led risk assessment** solution to biological pathway-indicated hazard concerns in human cells
- **Move away from high-dose apical endpoint pathology in rodents**; adverse effect levels; uncertainty factors
- **Move to NAMs in human cells that cover broad biological perturbations** (cell stress, pharmacological effects and gene expression changes)
  - Bioactivity not pathology
  - Protection not prediction

Principles of Next Generation Risk assessment (NGRA)

Point of departure (POD) derived from concentration-response data

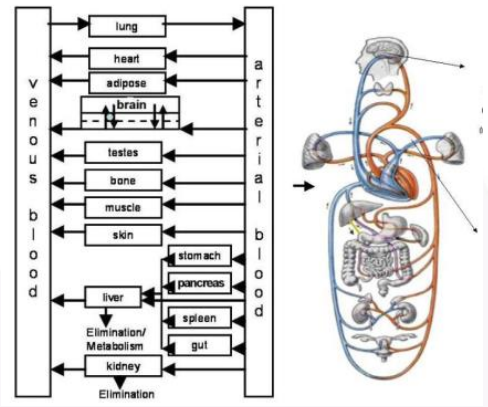


Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**

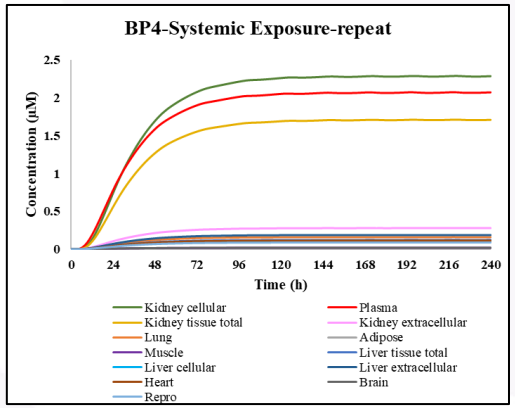
- Cellular stress assays
- Transcriptomics
- Receptor binding assays
- Others

Calculation of Bioactivity exposure ratio (BER)

Exposure models (PBK, free/total concentration)



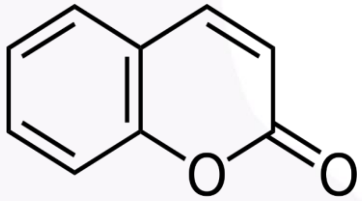
Exposure estimation: Plasma C_{max}, organ distribution, AUC



The BER is defined as the ratio between the POD and the relevant exposure metric

A case study approach using only non-animal data to assess the safety of…

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



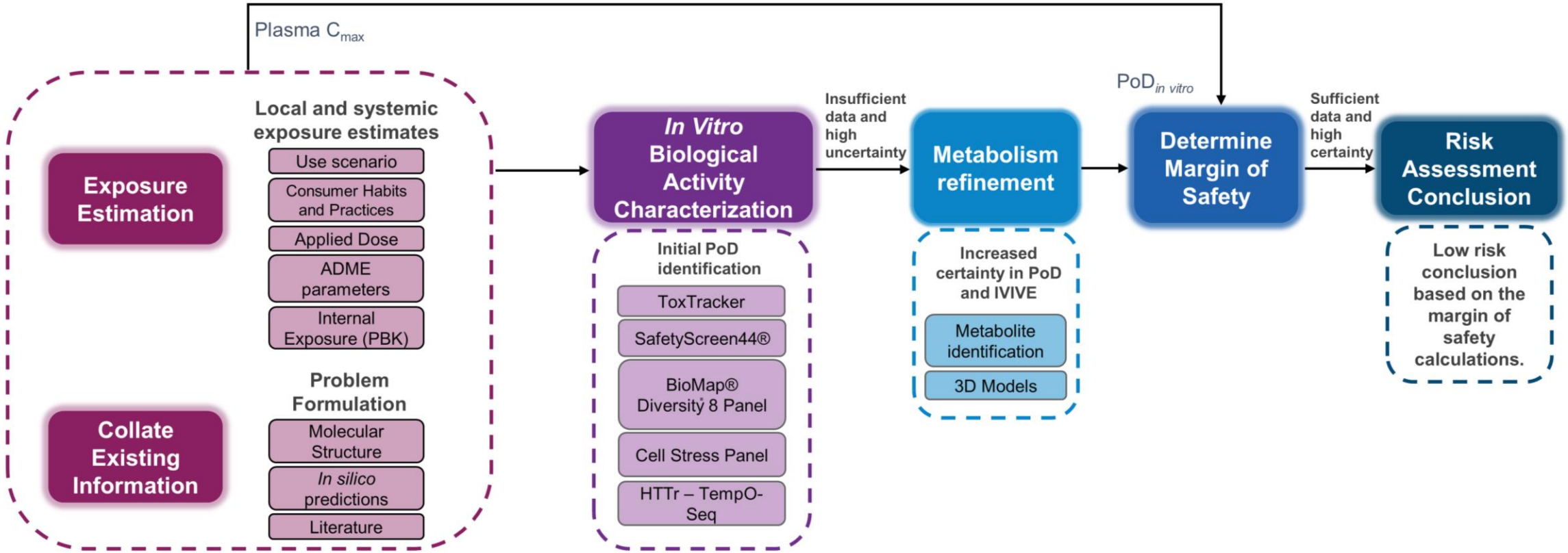
Assumed that:

- **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**

Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

Unilever

Framework for the NGRA of 0.1% Coumarin in a face cream.



Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

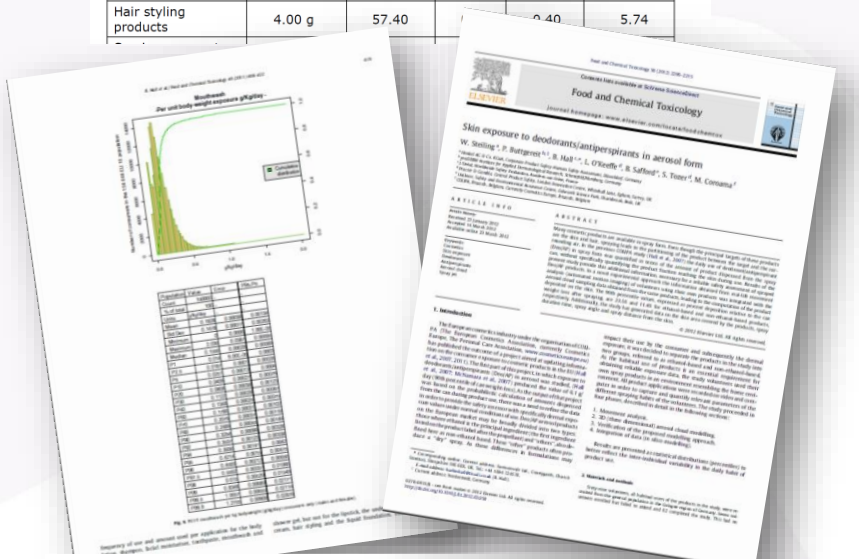
Applied Dose Exposure Estimation for 0.1% Coumarin in a face cream

Exposure Route:

- Oral exposure is unlikely due to use pattern
- Inhalation exposure is unlikely due to product type
- Dermal exposure will happen

Tab :osmetic product types accordir
hall *et al.*, 2007, 2011).

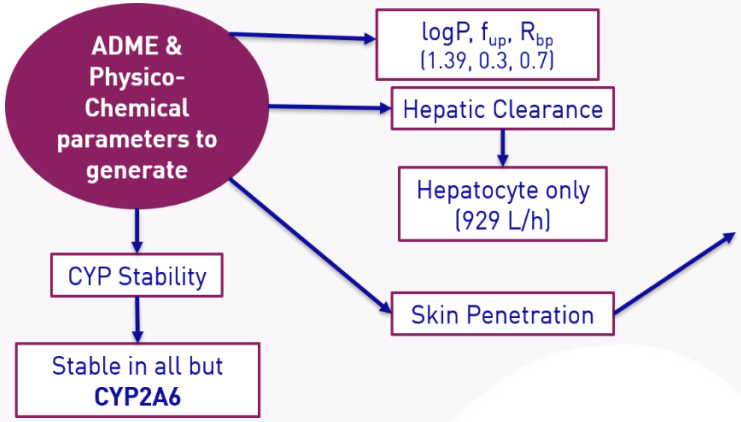
Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap [2]	20.00 g	-	0.01	0.20 [3]	3.33
Hair care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

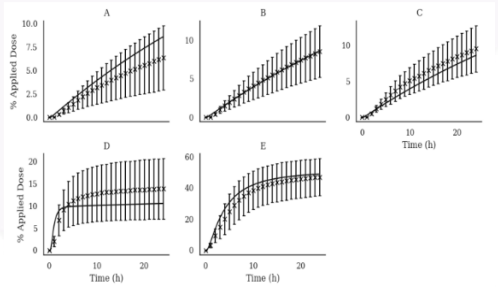


B. Hall et al./Food and Chemical Toxicology 49 (2011) 408–422

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
Bodyweight (kg)	70
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**

Modelling internal exposure using Physiologically Based Kinetic Modelling (PBK)





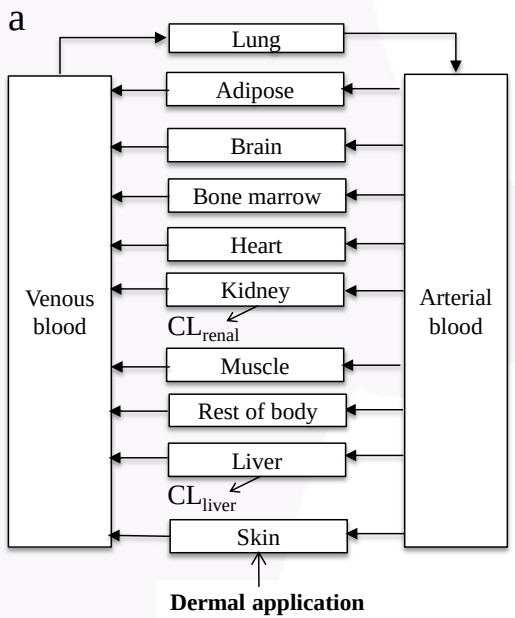
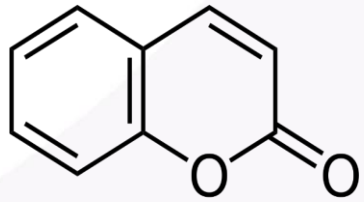
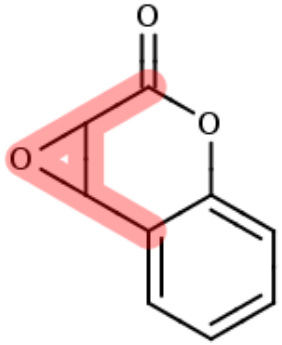


Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

Total Plasma C_{max} (μM)	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 existent information – in silico tools



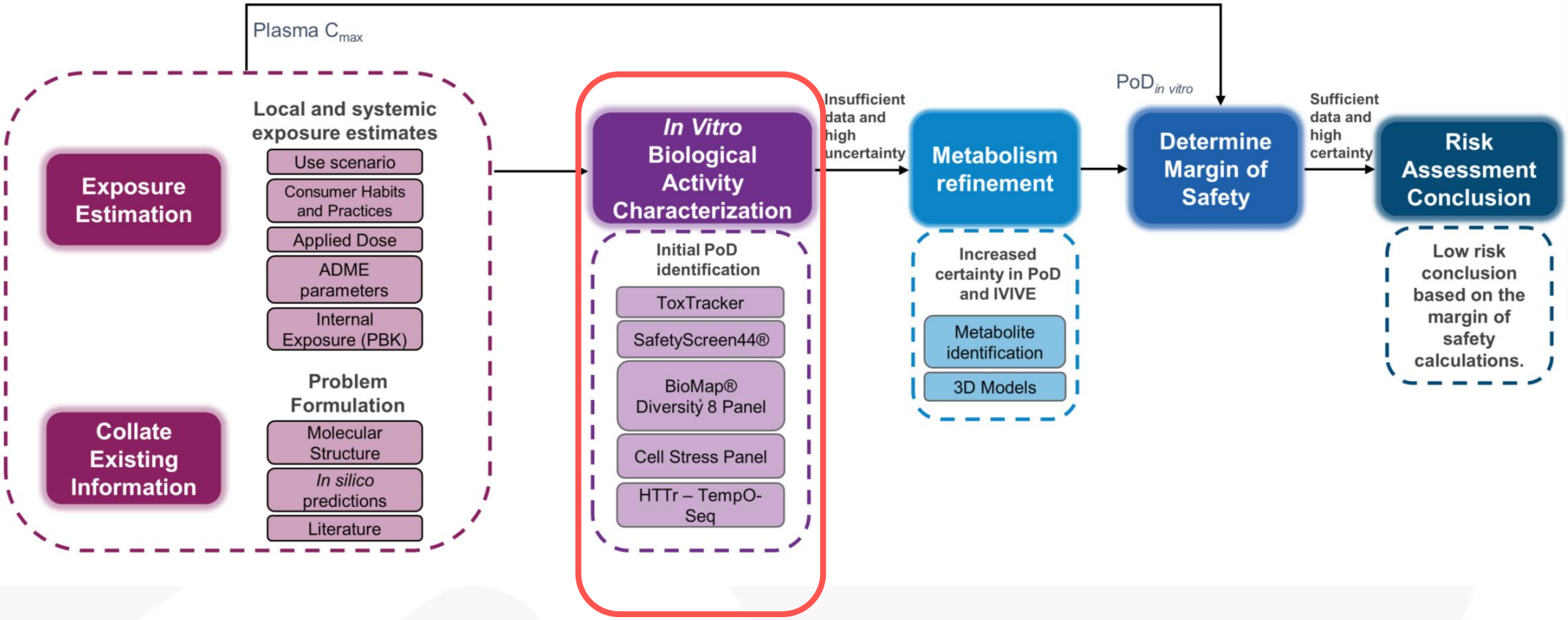


In silico tools (ToxTree, OECD toolbox, Meteor) predicted:

- Protein binding- MIE for induction of skin sensitisation*
- Prediction of COX-2 inhibition – anti-inflammatory effects
- DNA binding alert - MIE for genotoxicity
- Reactive metabolites (e.g. epoxide formation)- alerts for both genotoxicity and skin sensitisation

*not covered today

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

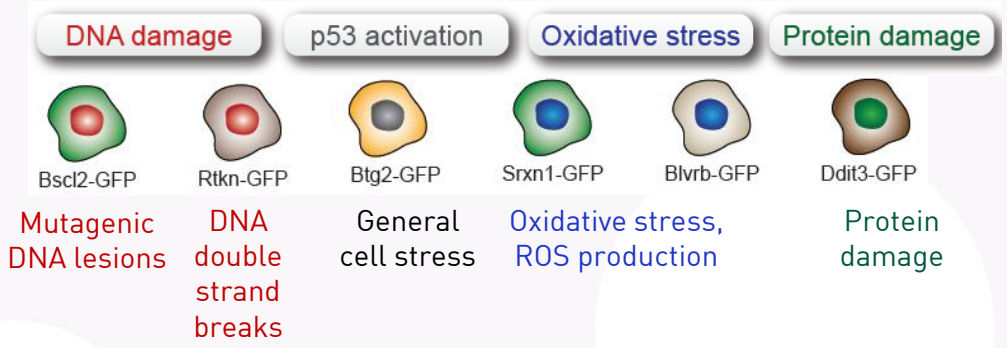


In vitro biological activity characterisation: assays targeting initial in silico flags

Genotoxicity assessment: ToxTracker®

Coumarin and its metabolites triggered genotoxicity alerts

6 GFP reporter mouse embryonic stem (mES) cells



Standard ToxTracker assay +S9					
DNA damage		p53	Ox. stress		UPR
Bscl2	Rtkn	Btg2	Srxn1	Blvrb	Ddit3

Standard ToxTracker assay -S9					
DNA damage		p53	Ox. stress		UPR
Bscl2	Rtkn	Btg2	Srxn1	Blvrb	Ddit3

Positive (>2-fold induction)
Weak activation (1.5 to 2-fold induction)
Negative (<1.5-fold induction)

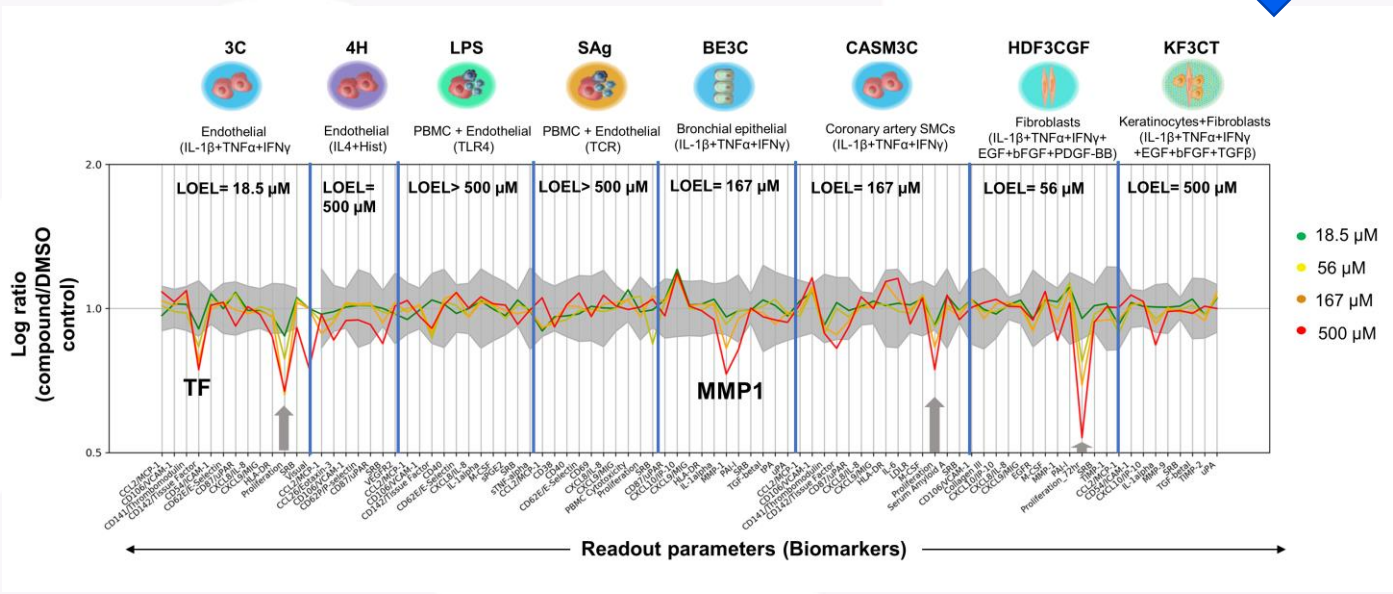
Conclusions: Coumarin is not genotoxic (weak activation of DNA damage reporters likely due to metabolites)

Unilever

In vitro biological activity characterisation: assays targeting initial in silico flags

Immunomodulatory screening assay: BioMap® Diversity 8 Panel

- **Coumarin predicted to have anti-inflammatory properties**
- **To investigate possible effects on vascular inflammation, immune activation and tissue remodelling**
- **8 individual BioMAP human primary cell-based co-culture systems which predictively model drug effects on multiple tissues and disease states**



Conclusions: Coumarin does not cause immunomodulatory effects.

In vitro biological activity characterisation: assays designed to cover a wider biological space

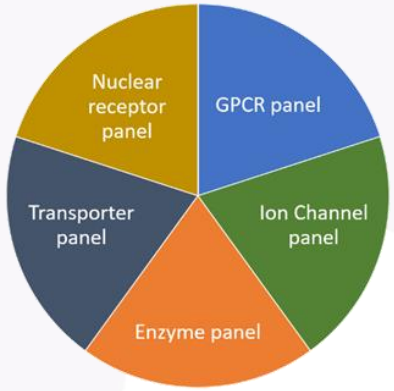
In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

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



eurofins | Cerep

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

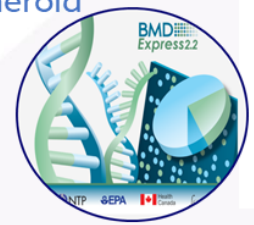
Transcriptomics was applied as a broad nontargeted biological screen

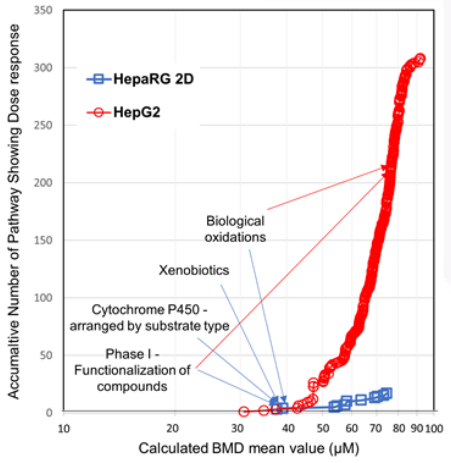
To characterize non-specific biological activity which is not mediated via a specific protein/receptor interaction

Transcriptomics

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

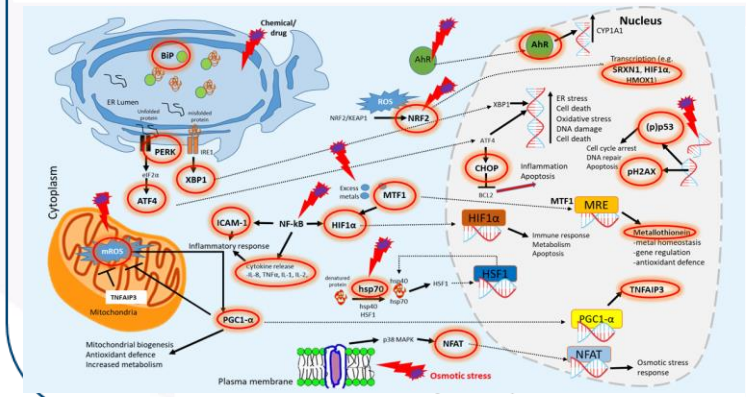
BMDExpress 2



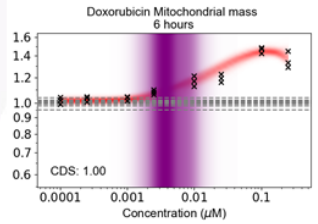


Cellular Stress Pathways

13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways



cyprotex AN EVOTEC COMPANY



Toxicol Sci (2020), **176**, 11-33

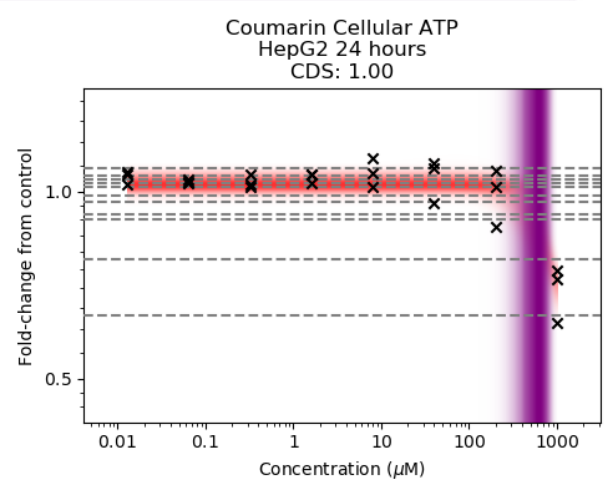
Unilever

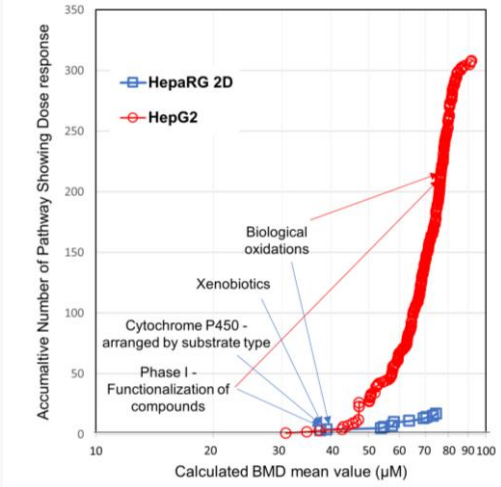
In vitro biological activity characterisation: assays designed to cover a wider biological space

In vitro Pharmacological profiling
- Tested up to 10 µM
- ~44 targets
- **No hits**

Cell Stress Panel
- 6 out of the 36 biomarkers significantly affected
- PoDs 44-912 µM

HTTr (HepG2, HepaRG 2D, MCF7)
- Two approaches to calculating POD – BIFROST (gene level) and BMDL (pathway level)
- PoD range 6-70 µM





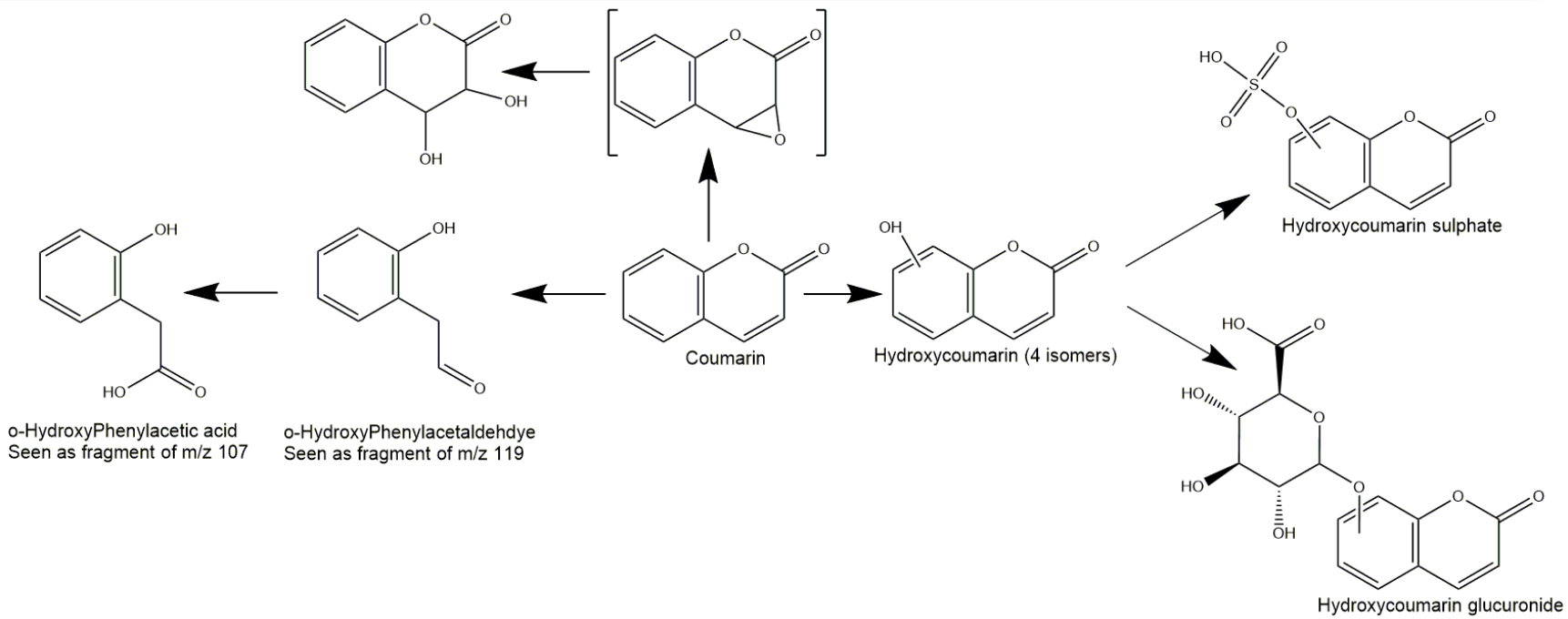
Cell models in the toolbox have limited metabolic competency

Unilever

Increasing the confidence in the risk assessment: metabolite identification

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

Understanding the metabolic pathway of coumarin



Conclusions: Coumarin is mainly detoxified to 7-OH coumarin and respective glucuronide. Saturation of CYP2A6 (at high concentration) leads to the formation of reactive metabolites

Increasing the confidence in the risk assessment: metabolite identification

Addressing the limitation of the toolbox cell models with a metabolic competent cell model - HepaRG 3D model

Cell stress & HTTr
3D HepaRG models

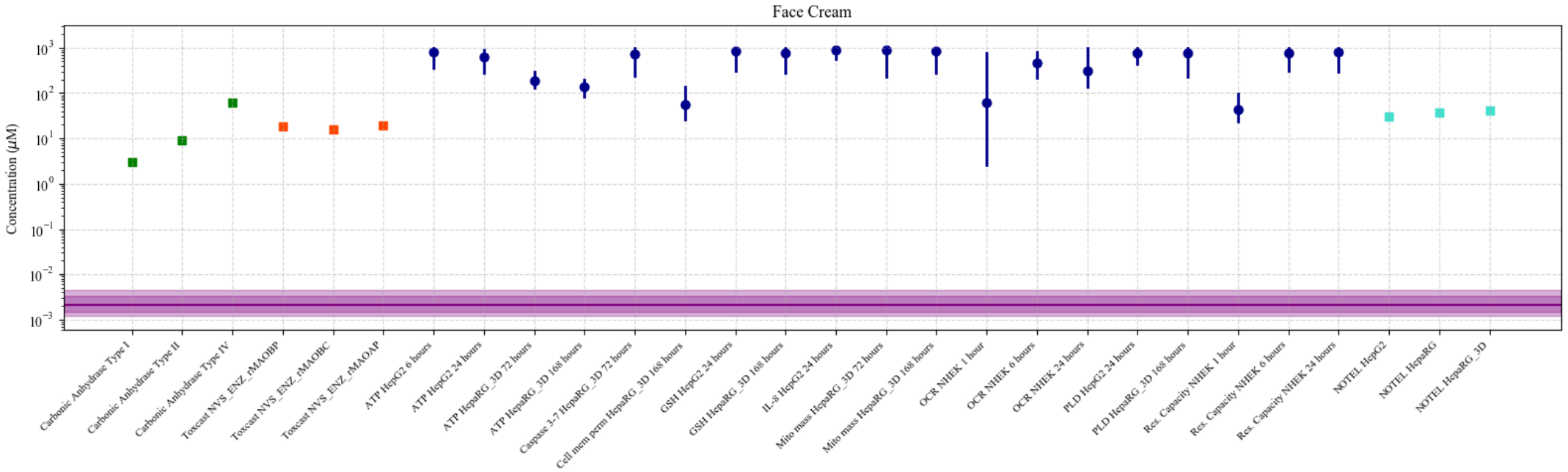
- **Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)**
- **PoDs range: 41-871 µM – not very different from 2D cells**

Conclusions: The metabolism refinement step increased our confidence in the PoDs and allowed for a safety decision to be made

Unilever

Safety assessment: calculation of bioactivity: exposure ratio and weight of evidence

PubChem ToxCast Cell Stress Panel HTTr

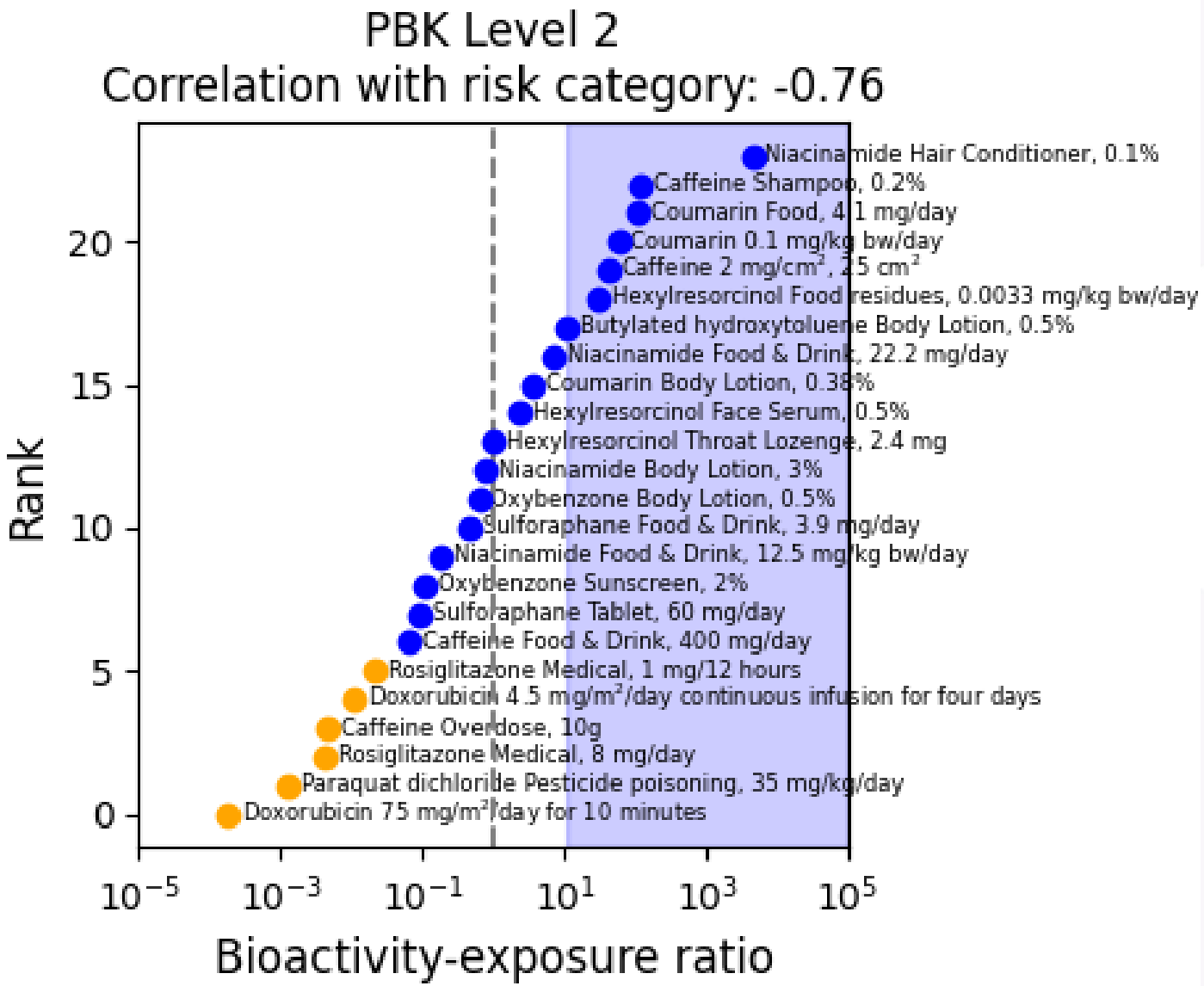


Conclusions:

- **The 5th percentile of the BER distribution ranged between 158 and 96738**
- **Coumarin is not genotoxic**
- **Coumarin does not bind to any of the 44 targets**
- **Coumarin does not show any immunomodulatory effects**

Unilever

Is the assessment protective?



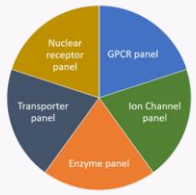
Evaluation of ~40 substances to assess toolbox and workflow: Are NAM-based assessments protective? What BER is needed to assure safety?

Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

Application of NGRA concepts to occupational exposure

NAM toolbox

In vitro pharmacological profiling



eurofins | Cerep

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

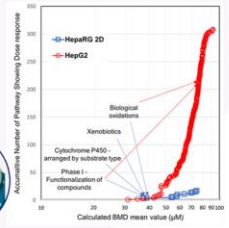
Transcriptomics was applied as a broad nontargeted biological screen

To characterize non-specific biological activity which is not mediated via a specific protein/receptor interaction

Transcriptomics

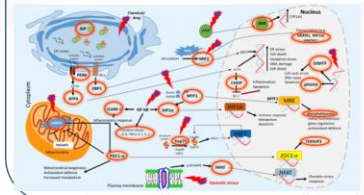
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

BMDExpress 2

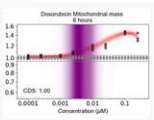


Cellular Stress Pathways

13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

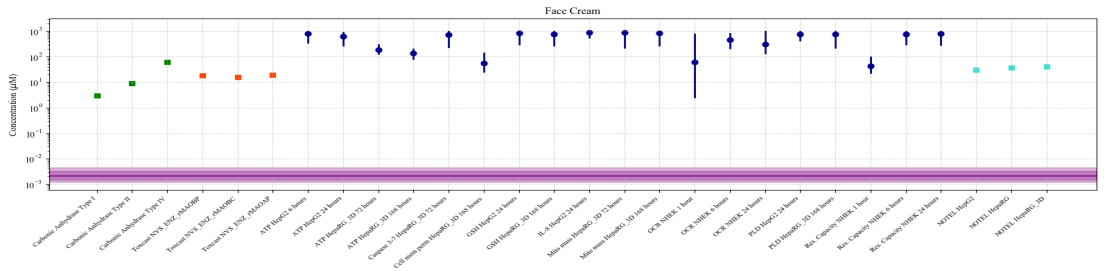


cyprotex

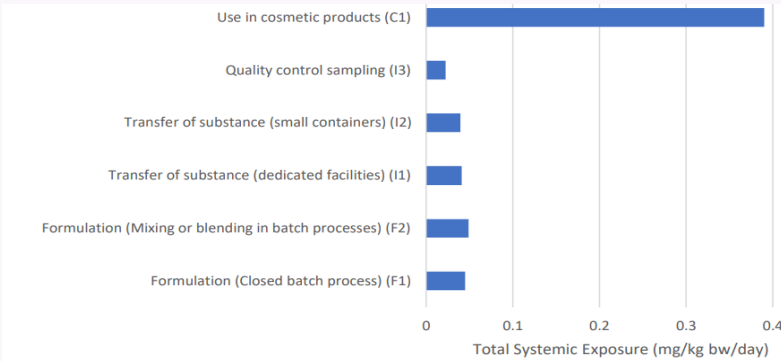


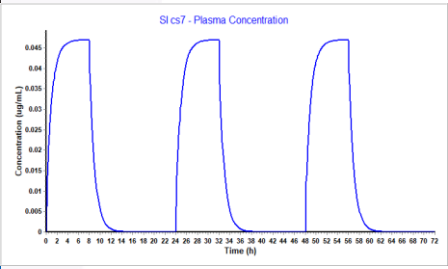
Toxicol Sci (2020), 176, 11-33

BER calculation



Exposure





- Understanding worker exposure
  - Routes
  - Levels of exposure
  - Personal protection equipment, engineering controls, ventilation etc.
  - PBK for worker exposure

Conclusions & reflections

- Case studies have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure to make a safety decision.
- These case studies showed that the approach is exposure-led and follows a tiered approach for both exposure and bioactivity
    - Bespoke NAMs can be added to the NGRA to fill gaps identified along the process
- ‘Early tier’ in vitro screening tools show promise for use in a protective rather than predictive capacity.
- NGRA requires a mindset shift and a multidisciplinary team!

Unilever

Acknowledgements

Matt Dent
Sophie Cable
Hequn Li
Beate Nicol
Joe Reynolds
Sophie Malcomber
Sharon Scott
Jade Houghton
Predrag Kukic
Andrew White
Richard Cubberley
Sandrine Spriggs
Ruth Pendlington
Katie Przybylak
Alistair Middleton
Sarah Hatherell
Carl Westmoreland
Paul Carmichael

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

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

doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

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

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

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

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

[2022 Publications « Safety Science in the 21st Century (tt21c.org)]