Making the transition to next generation risk assessment for systemic toxicity using two cosmetic ingredients as case studies

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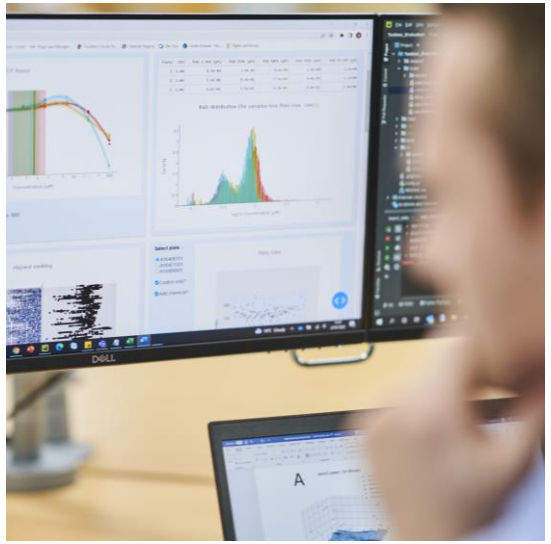

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Outline

- Principles of Next Generation Risk assessment (NGRA)
- Tiered approach for the case studies- what are the common tools?
- Coumarin case study – genotoxicity & metabolism considerations
- Benzophenone-4 – exposure, endocrine activity and bioactivity in relevant organ (kidney)
- Conclusions

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

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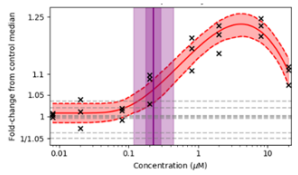
Approach to this Next Generation Risk Assessment – Protection of human health

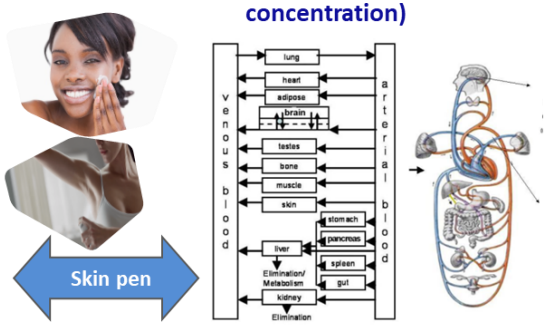
Point of departure (POD) derived from concentration-response data



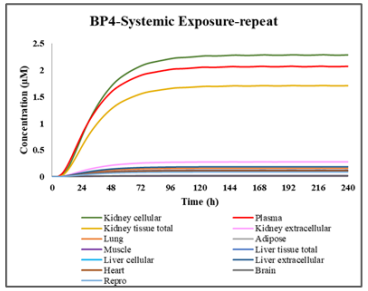
Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**

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

Exposure models (PBK, free/total concentration)



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



Calculation of Bioactivity exposure ratio (BER)

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

If there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.

If there is bioactivity observed at consumer-relevant concentrations -> is it adverse?

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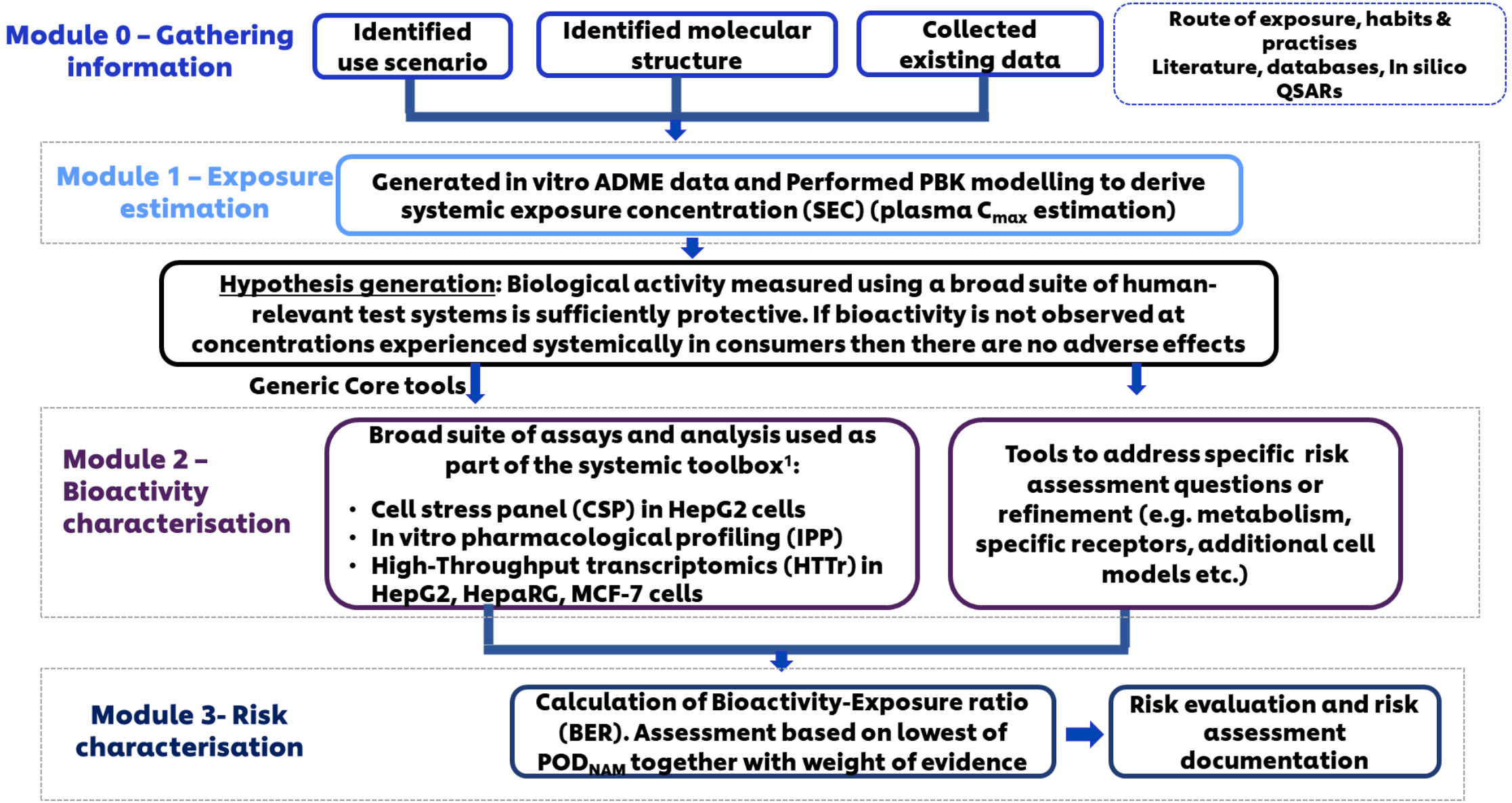
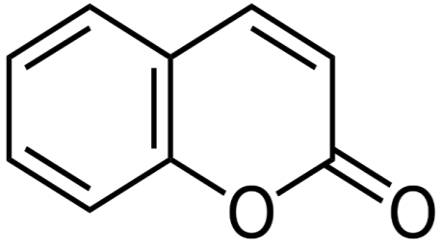
Tiered approach to risk assessment



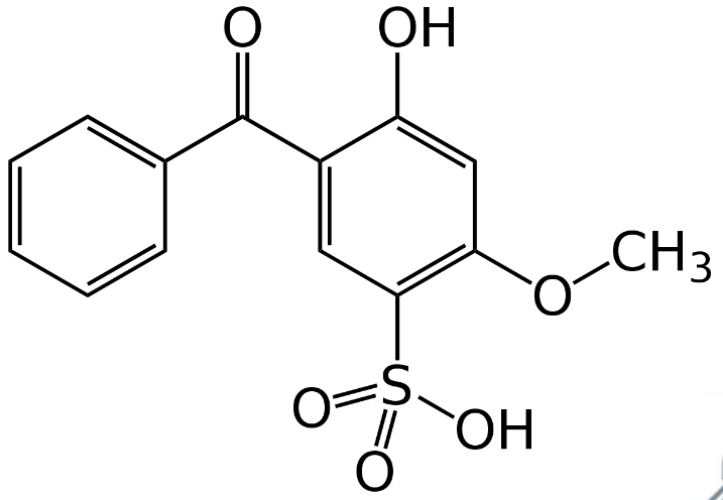
Introduction to the case studies

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



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

5% BENZOPHENONE-4 IN A SUNSCREEN BODY LOTION FOR EU MARKET



Assumptions/rules

- Focus on systemic toxicity
- 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 the chemicals themselves were excluded

Common tools used across the two case studies: Gathering existing information

- **Existing data: EPA ToxCast dashboard & PubChem**
- **QSARs tools for toxicity endpoints: OECD QSAR TOOLBOX, TOXTREE, DEREK NEXUS**
- **Metabolism prediction: DEREK METEOR**

Derek nexus

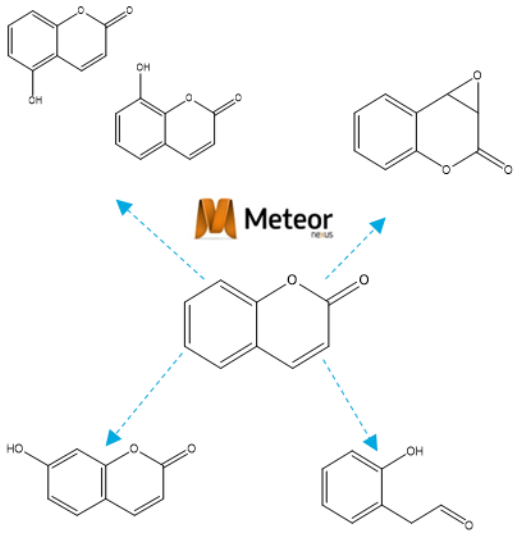
OECD

QSAR TOOLBOX

Toxtree

EPA iCSS ToxCast Dashboard

PubChem



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Common tools used across the two case studies: Exposure estimation

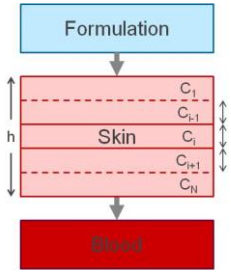
External dose

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

ADME parameters

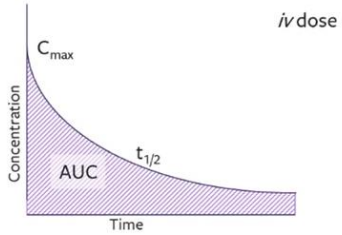
Absorption
Distribution
Metabolism
Elimination

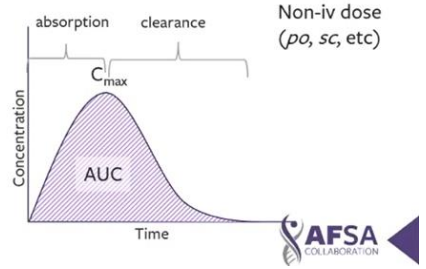
- Skin penetration
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- Blood:plasma ratio



Kinetic profile of chemical

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





Images from: AFSA training module "Dosimetry (Internal Exposure)",2022

https://www.afsacollaboration.org/sciencex_event/dosimetry-internal-exposure-ivive/

Common tools used across the two case studies: Tiered approach to exposure estimation

Level 0: Characterise exposure scenario

Sunscreen:

- 18g/day, two times/day, 9g/application,
- On body and face 17500 cm^2 (total body area)

Face cream:

- 1.54g/day , two times/day
- Face application, 565 cm^2

Level 1: PBK model built with in silico parameters only & sensitivity analysis

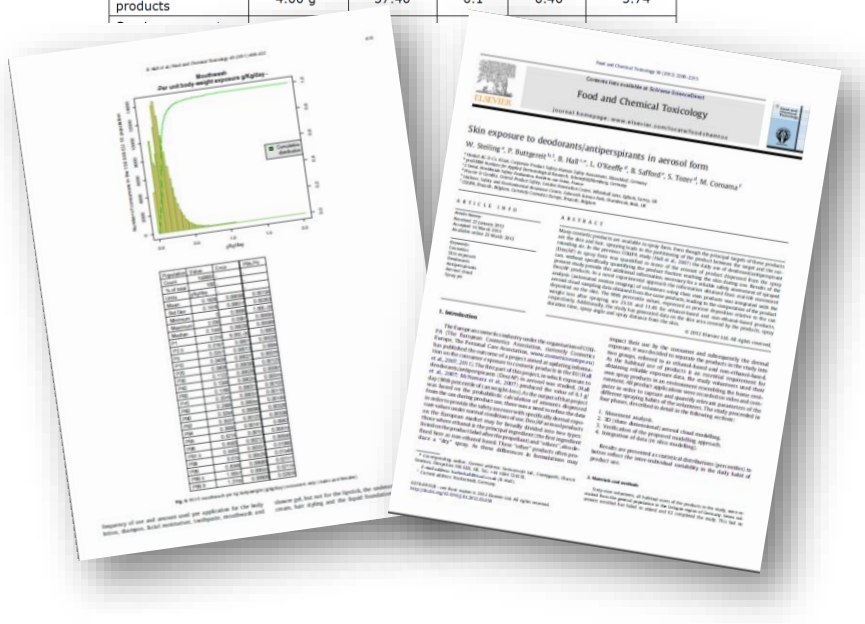
Level 2: PBK model built with in vitro-derived values for most important parameters:

- Dermal absorption
- Hepatic clearance
- Fraction unbound
- Blood to plasma ratio

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	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	0.1	0.40	5.74



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

Moxon et al. 2020. Toxicology in Vitro, Volume 63, 104746.

Li H,. Toxicol Appl Pharmacol. 2022 ;442:115992.

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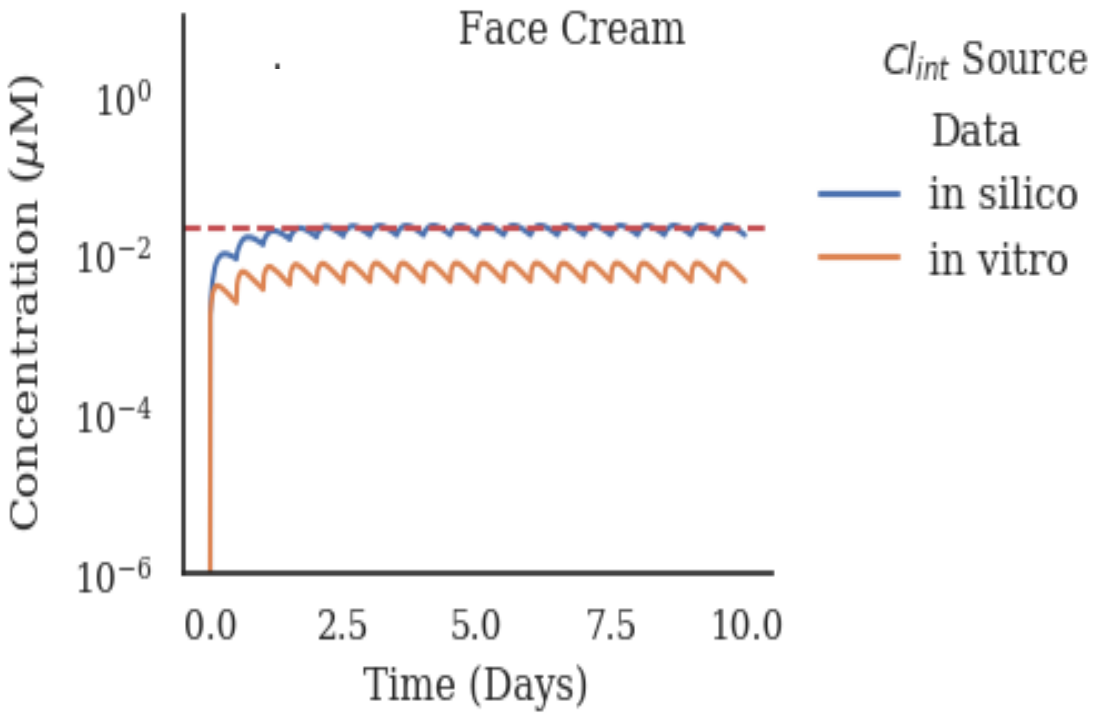
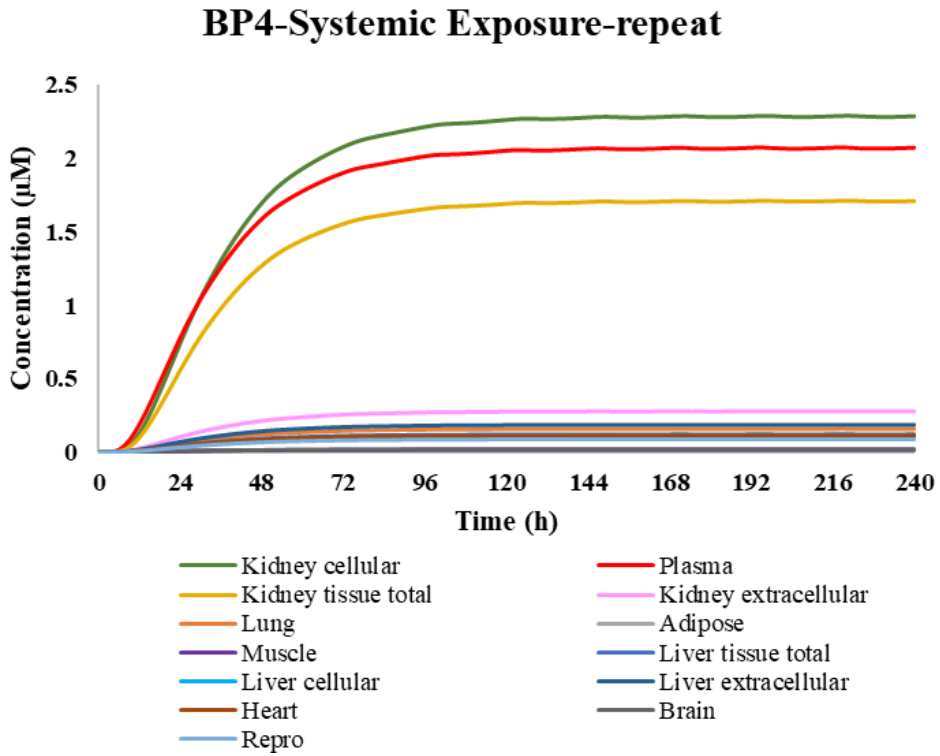
Common tools used across the two case studies: Tiered approach to exposure estimation- PBK modelling

Simulation of plasma concentration of coumarin after repeated dermal exposure.

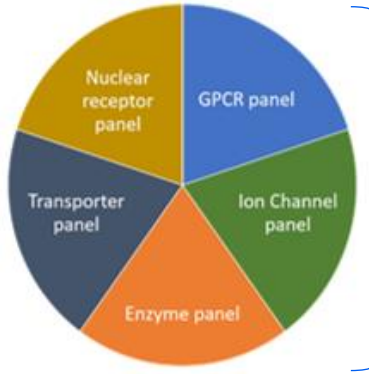


Simulation of plasma and organ concentration of benzophenone-4 after repeated dermal exposure.



Common tools used across the two case studies: biological activity characterisation- assays designed to cover a wider biological space

In vitro pharmacological profiling



eurofins | Cerep

~79 targets

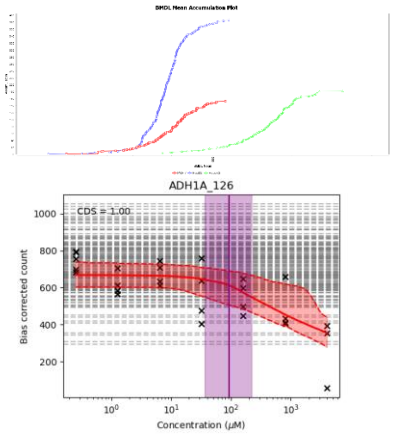

PERSPECTIVES

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

Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

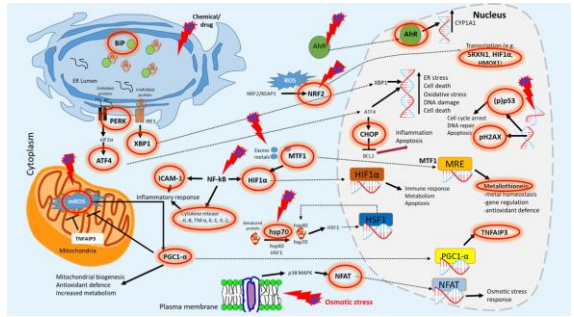


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

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Coumarin case study

Focus: genotoxicity & metabolism considerations

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Tiered approach to risk assessment

Module 0 – Gathering information

- Identified use scenario
- Identified molecular structure
- Collected existing data

Route of exposure, habits & practises
Literature, databases, In silico QSARs

Module 1 – Exposure estimation

Generated in vitro ADME data and Performed PBK modelling to derive systemic exposure concentration (SEC) (plasma C_{max} estimation)

Hypothesis generation: Biological activity measured using a broad suite of human-relevant test systems is sufficiently protective. If bioactivity is not observed at concentrations experienced systemically in consumers then there are no adverse effects

Generic Core tools

Module 2 – Bioactivity characterisation

Broad suite of assays and analysis used as part of the systemic toolbox[1]:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

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Module 0 – Gathering information

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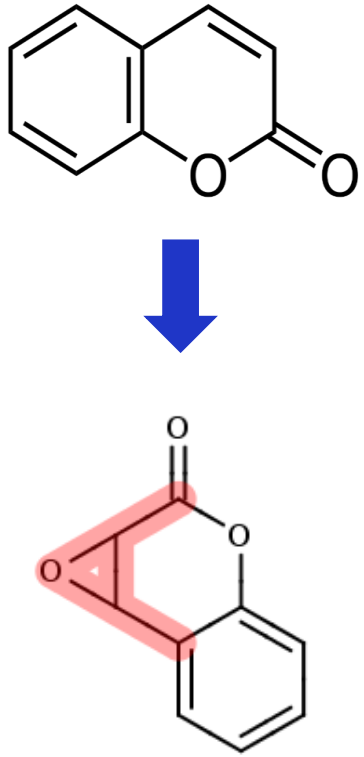
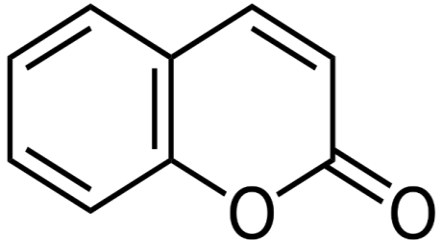


In silico tools (ToxTree, OECD toolbox, Meteor) predicted:

- Cramer class III
- 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

Coumarin case study – Problem formulation



- Exposure scenario – 0.1% in face cream for the European population
- Systemic exposure of 0.02 mg/kg above TTC for Cramer class III (2.3 µg/kg bw/day) and TTC not applicable for regulated chemicals
  - Need to estimate internal exposure using PBK models
- In silico tools identified the key areas of concern:
  - Potential anti-inflammatory activity via inhibition of COX-2
  - Potential formation of reactive metabolites
  - Genotoxicity of parent and reactive metabolites
- Absence of alerts ≠ no toxicity therefore a general bioactivity panel is required to exclude other potential toxicities

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**

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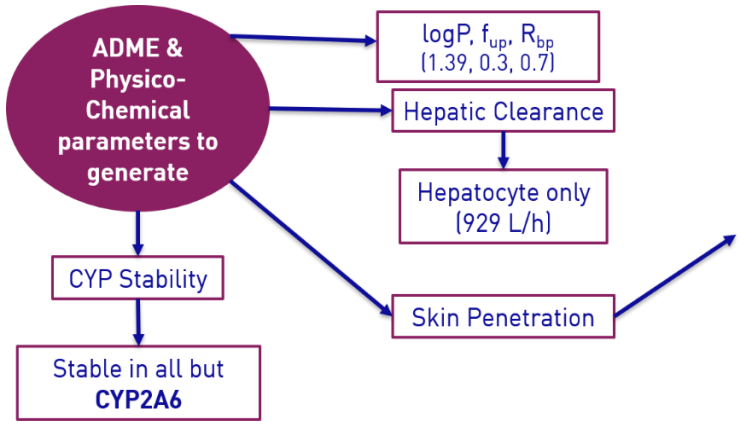
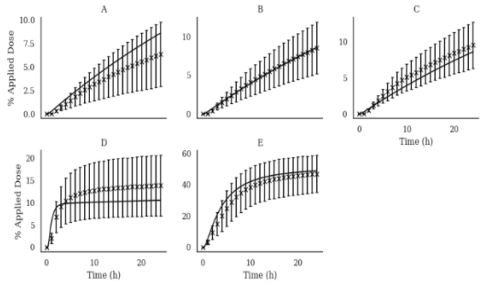
Module 1 – Exposure estimation

Generated in vitro ADME data and Performed PBK modelling to derive systemic exposure concentration (SEC) (plasma C_{max} estimation)





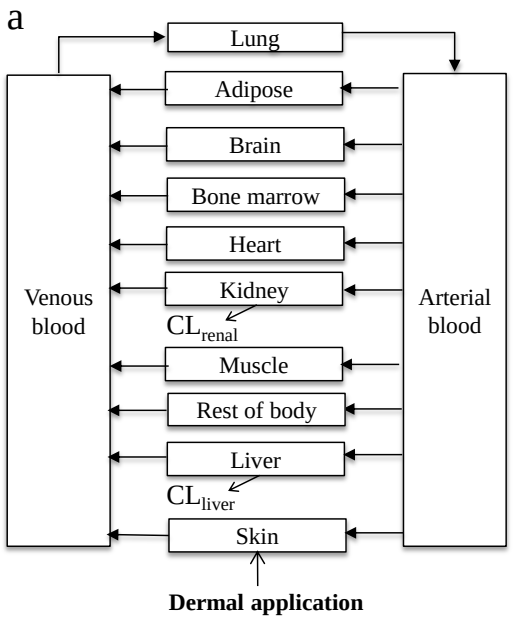


Table 2. Internal Exposures From Use of 0.1% Coumarin in Face Cream and Body Lotion Following the Exposure Scenario Outlined in Table 1

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

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Module 2 – Bioactivity characterisation

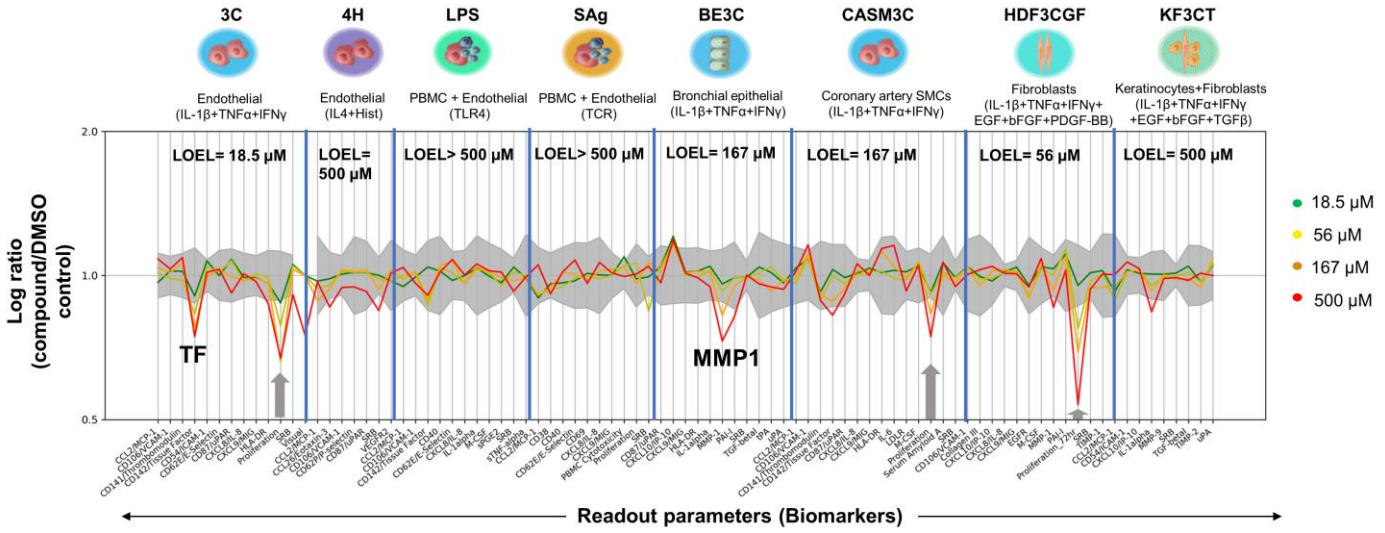
Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

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.

Module 2 – Bioactivity characterisation

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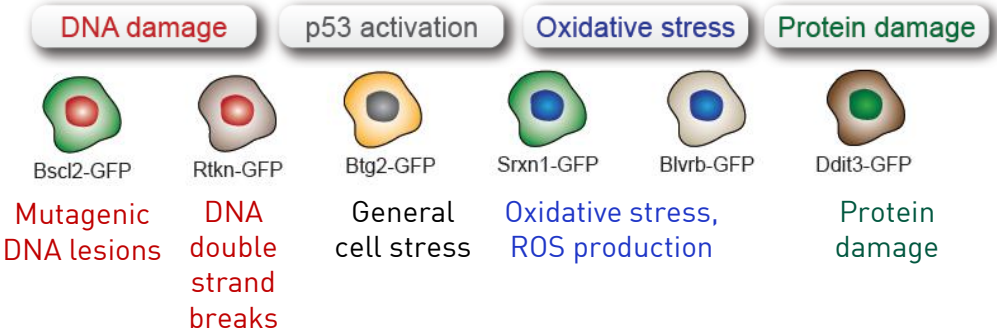
Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

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 <u>not genotoxic</u> (weak activation of DNA damage reporters likely due to metabolites)

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Module 2 – Bioactivity characterisation

Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
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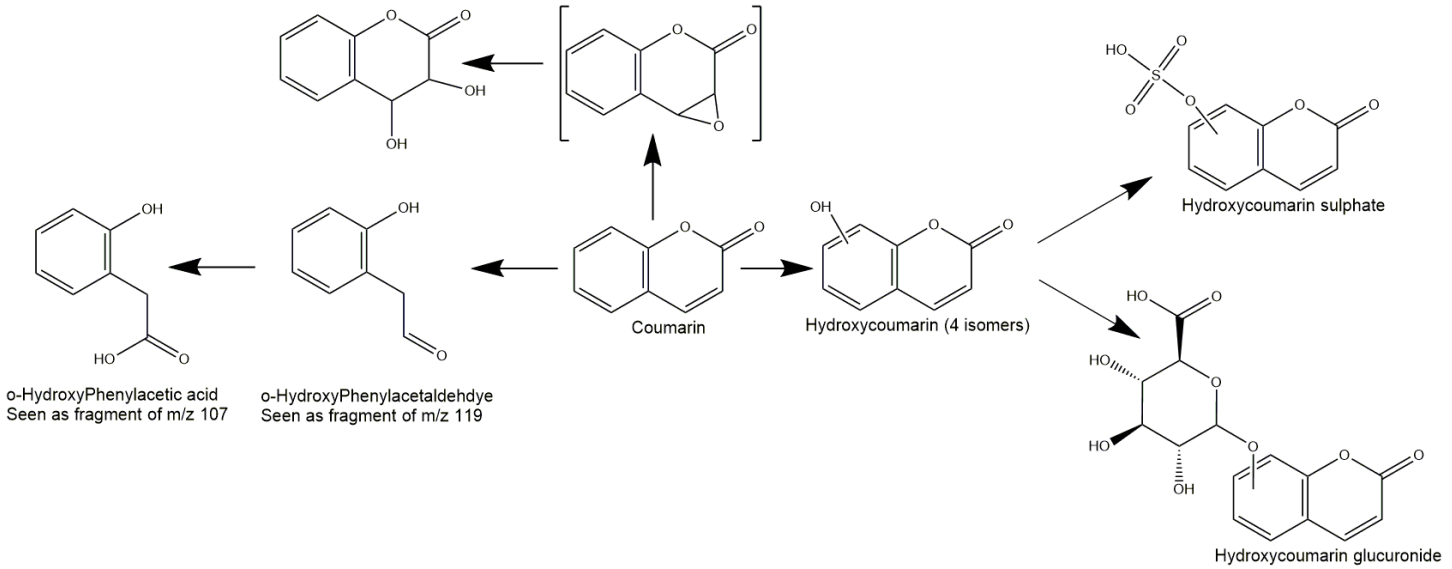
Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

Understanding the metabolic pathway of coumarin

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



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

Module 2 – Bioactivity characterisation

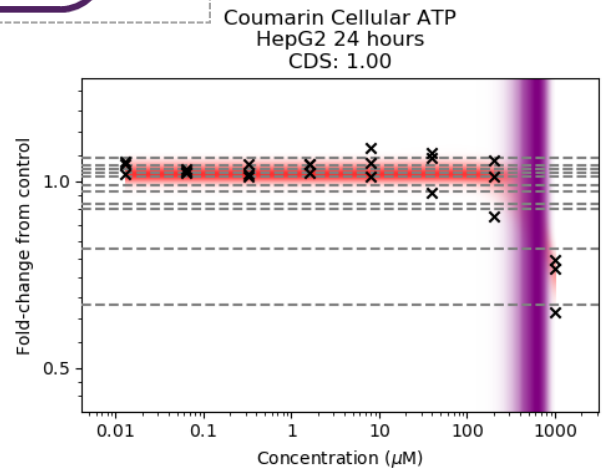
Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

In vitro Pharmacological profiling

- Tested up to 10 µM
- ~44 targets
- **No hits**

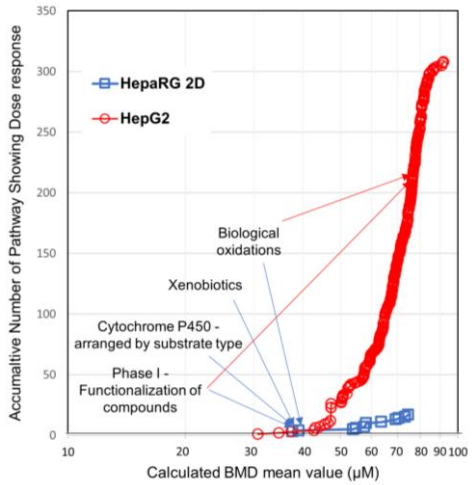


Cell Stress Panel

- 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

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Module 2 – Bioactivity characterisation

Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

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

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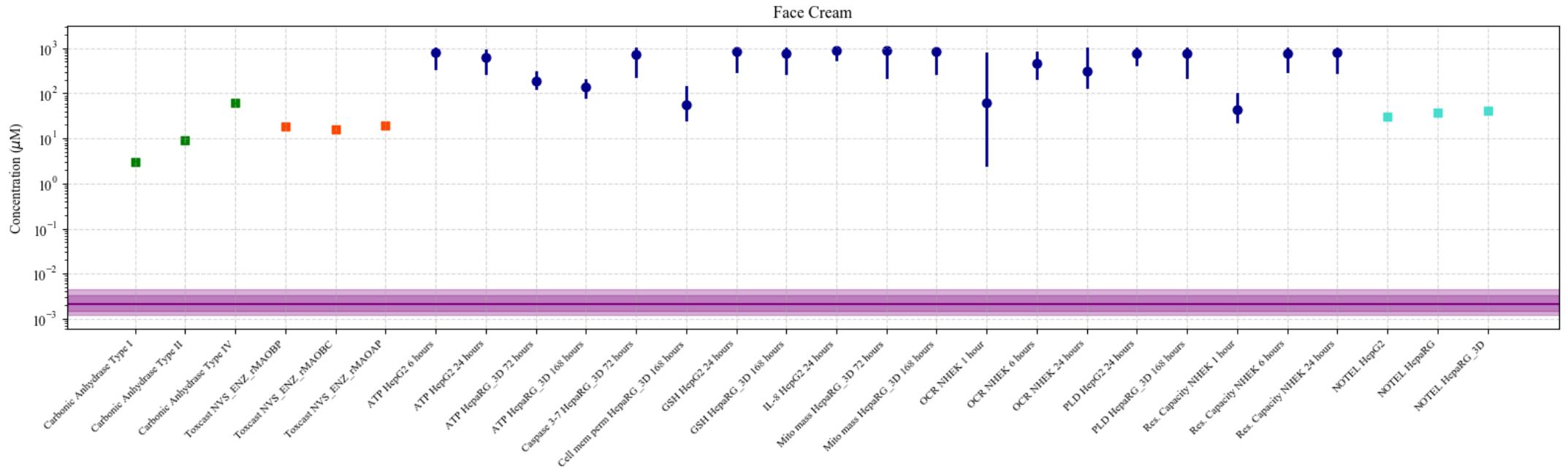
Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

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**

Benzophenone-4 case study

Focus: exposure, endocrine activity and bioactivity in relevant organ (kidney)

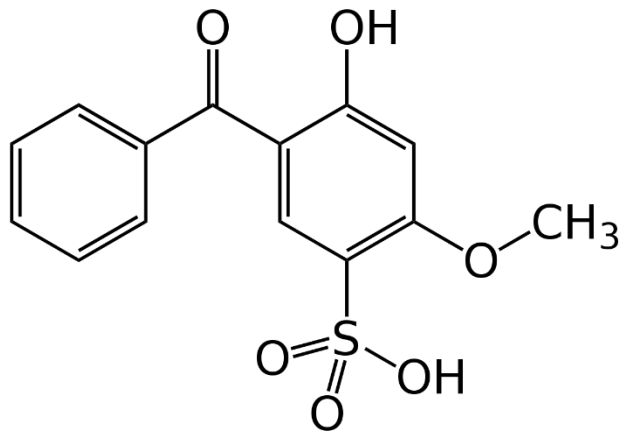
Module 0 – Gathering information

Identified use scenario

Identified molecular structure

Collected existing data

Route of exposure, habits & practises
Literature, databases, In silico QSARs



- **Benzophenone-4 did not trigger many alerts within the tools used**. The most common alert across the tools was for skin sensitisation, or protein binding as an indication of skin sensitisation, in the DEREK, TIMES and OECD Toolbox outputs.
- **Benzophenone-4 triggered one potential alert for estrogen receptor binding in the VEGA profiler**, however this was not consistent across other profilers that also assess estrogen receptor activity.

Module 1 – Exposure estimation

Generated in vitro ADME data and Performed PBK modelling to derive systemic exposure concentration (SEC) (plasma C_{max} estimation)

ADME data

	Value	Source
Molecular weight	308.3 g/mol	
Log P	1.28	ADMET predictor
pKa	acid 8.89, acid 0.5	ADMET predictor
Fraction unbound in plasma (f_{up})	0.0157	Measured
Blood: plasma ratio	0.6	Measured
Hepatic intrinsic clearance (L/h)	<2.5L/h Below LOQ	Measured, plated primary human hepatocyte assay, Pharmacelsus
ECCS classification	Class 1A metabolism	*Varma et al., 2015*
Renal excretion	0.11L/h	GFR*Fup
Dermal absorption parameters: Partition coefficient and diffusivity in skin layers	fitted against skin pen data	Measured, Eurofins, *Ex vivo* skin penetration study designed according to *Davis et al. 2011* meeting OECD and SCCS guidance

Main observations:

In silico

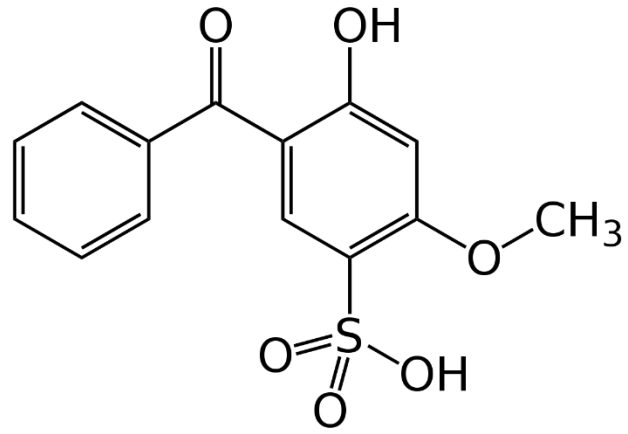
- BP-4 was predicted to be cleared via liver metabolism
- BP-4 is predicted to be substrate of several transporters by ADMET predictor

Experimental

- Very low skin penetration
- BP-4 stable in human hepatocytes. Hepatic intrinsic clearance <2.5L/h (Below LOQ)

Conclusion: Hepatic clearance needs more investigation

BP-4 case study – Problem formulation



- Exposure scenario – 5% in sunscreen for the European population
- Systemic exposure of 15 mg/kg/day. TTC not applicable for regulated chemicals
  - Need to estimate internal exposure using PBK models
- In silico tools and preliminary kinetics assessment key areas of concern:
  - Potential binding to estrogen receptor
  - Unclear route of elimination
  - Potential substrate of active transporters
- Absence of alerts ≠ no toxicity therefore a general bioactivity panel is required to exclude other potential toxicities

Parameter	Sunscreen
Amount of product used per day (g/day) using 90th percentile	18
Frequency of use	2 times/day
Amount of product in contact with skin per occasion (g)	9
Ingredient inclusion level	5%
Skin surface area (cm2)	17500
Exposure duration per occasion	5 hours
Systemic exposure per day (mg/kg)	**15**

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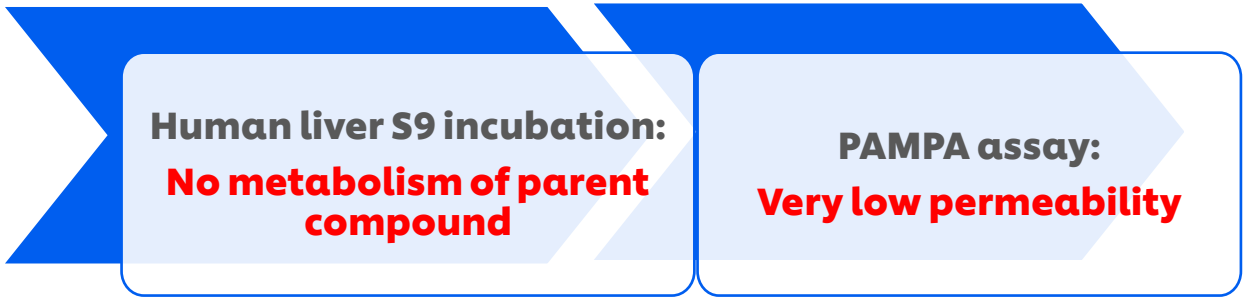
Back to problem formulation - Two hypotheses:

1) **Benzophenone-4 is not a substrate of CYP enzymes – need to confirm with a second assay using S9 fraction**
   - **Note,** BP-4 is an hydrophilic compound already
2) **Benzophenone-4 has low membrane permeability– Parallel artificial membrane permeability (PAMPA) assay**



BP-4 is not a substrate of enzymes and has very low permeability

High confidence that liver clearance can be neglected

(set to 0 in PBK).

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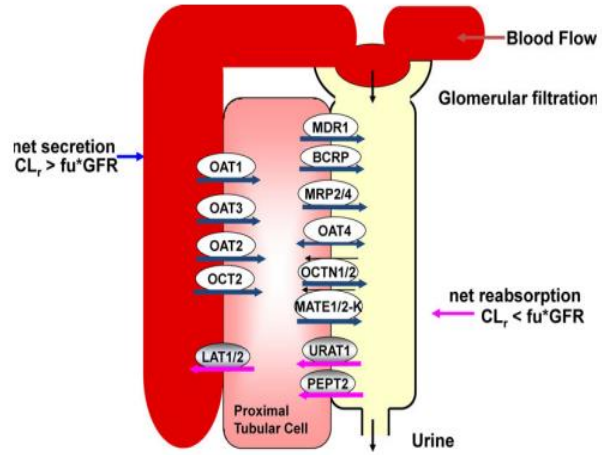
Understanding chemical organ distribution and renal clearance: Is BP-4 actively transported by active transporters in kidney?

Two experimental approaches:

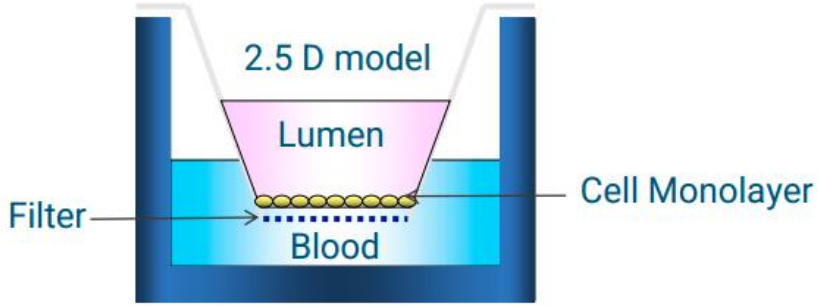
1. Transporter studies in transfected kidney cells in two different assays (uptake assay and vesicular assay)



https://doi.org/10.1002/jcph.702

Figure 1. Mechanism of drug elimination and major transporters in the kidney. Drug elimination in the kidney is through glomerular filtration, secretion, and reabsorption process. Major transporters localized in the proximal tubule cells are depicted. The blue arrows indicate secretion, and the pink arrows indicate reabsorption.

2. Investigate the transport profile in kidney where all the active transporters are present and functional (freshly isolated kidney proximal tubule cells monolayer (aProximate™).



B-A →blood to urine →active secretion
A-B → urine to blood →reabsorption

Newcells aProximate™ platform

BP-4 is a substrate of kidney and liver transporters and elimination in the kidney includes glomerular filtration, active tubular secretion and tubular reabsorption

Results:

- Substrate of the influx transporters, **OAT1, OAT2, OAT3** and substrate of the efflux transporters, **BCRP and MRP4.**
- All these transporters are expressed in the kidney, although OAT-2, BCRP and MRP4 are expressed both in **kidney and liver**
- Transport in the proximal tubule cells is equally efficient in both directions leading to no net movement

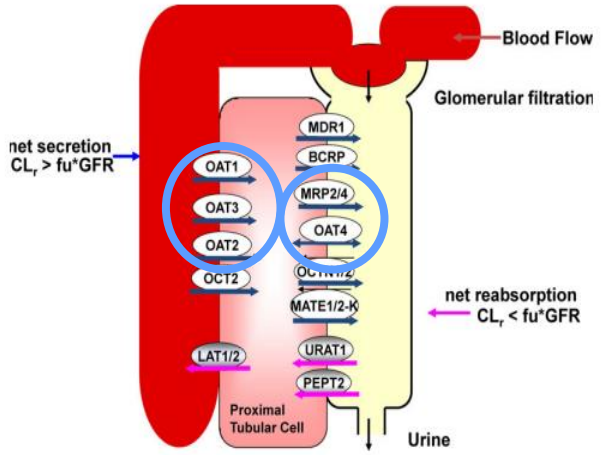
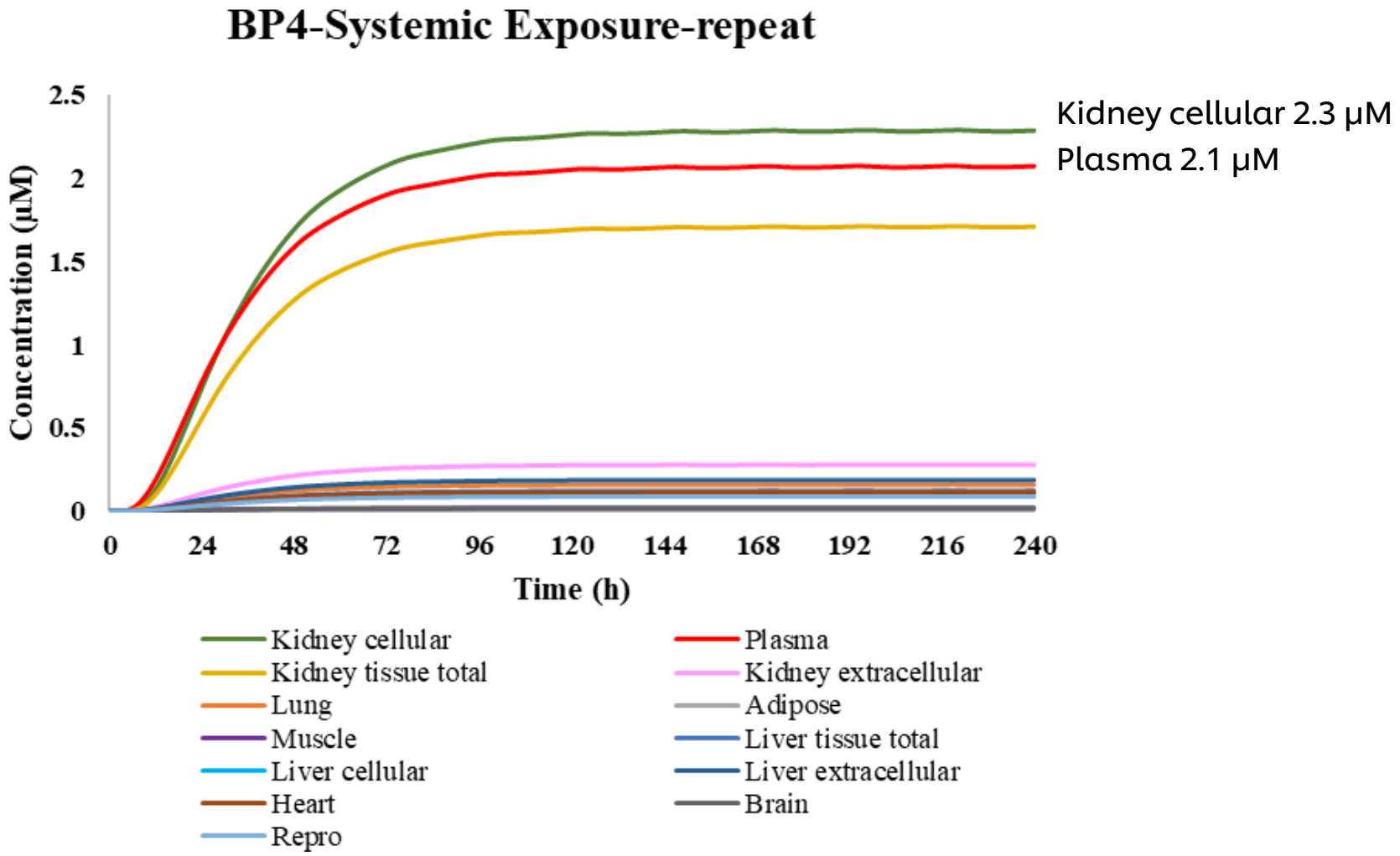


Figure 1. Mechanism of drug elimination and major transporters in the kidney. Drug elimination in the kidney is through glomerular filtration, secretion, and reabsorption process. Major transporters localized in the proximal tubule cells are depicted. The blue arrows indicate secretion, and the pink arrows indicate reabsorption.

Updated PBK model:

- Set BP-4's distribution to each compartment to be modelled as permeability-limited
- Active transport in the liver was modelled by incorporating kinetic parameters for the transporters (OAT-2, BCRP and MRP4).
- GFR*Fup was used to calculate renal excretion of benzophenone-4, accounting for filtration only to be conservative

PBK model simulation of plasma C_{max} for an American female with 60kg bodyweight



Kidney cellular 2.3 µM

Plasma 2.1 µM

Benzophenone-4 concentrations in plasma and different tissues after repeated exposure of body lotion 18g/day, i.e., 9g twice per day for a period of 10 days, with 5% benzophenone-4, on the whole body.

Module 2 – Bioactivity characterisation

Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

In vitro Pharmacological profiling

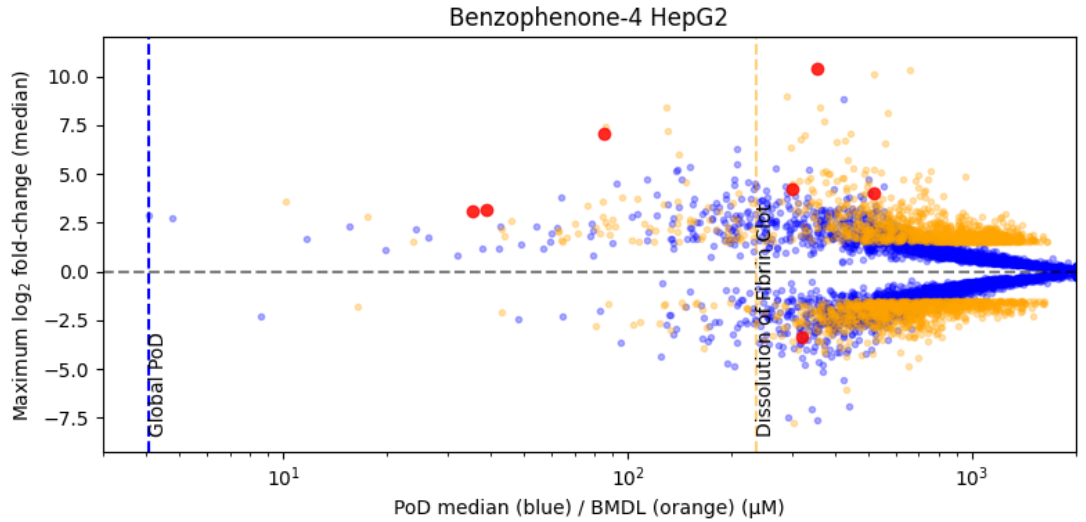
- Tested up to 10 uM
- ~83 targets compiled by Cosmetics Europe Safety pharmacology WG
- **No hits**

Cell Stress Panel

- Global POD_{NAM} = 140 µM (only 5 biomarkers out of 36 were affected)

HTTr (HepG2, HepaRG, MCF7, PTC)

- Two approaches to calculating POD – BIFROST (gene level) and BMDL (pathway level)
- POD range: 4.2 – 530 µM



Maximum fold-change in expression against BIFROST probe-level median POD (blue), and BMDExpress2 probe-level BMDLs (orange). Global POD calculated by BIFROST model (blue dotted line) and minimum pathway BMDL obtained from BMDExpress2 (orange dotted line). Red circles are the BMDexpress2 probe-level BMDLs contributing to the lowest pathway average. Global POD = CYP1A1 probe

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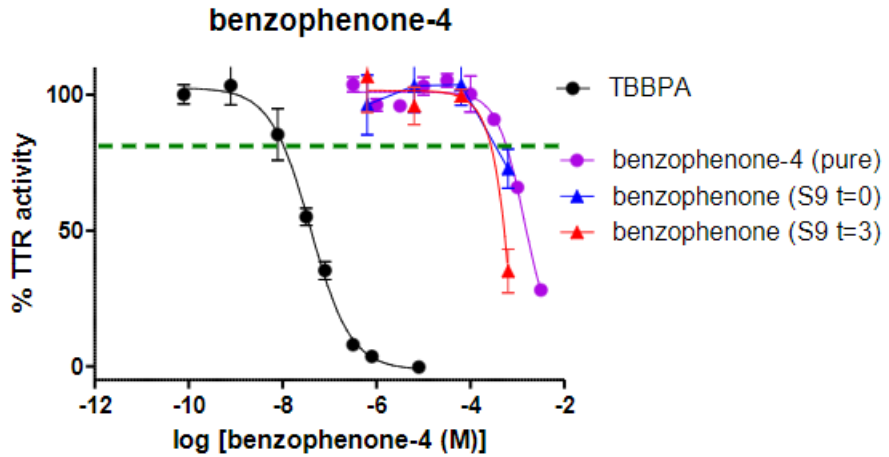
EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

- **CALUX bioassays** and **binding assays: TTR-TRβ- and hTPO**
- **U2-OS** incorporating the firefly **luciferase reporter gene** coupled to Responsive Elements (REs)
- **12 concentrations**. Calculation of AC50, LOEC and NOEC



- **No agonism or antagonism of ER, AR or TR** and no effect on production of oestrogens or androgens ±S9
- **Activity towards hTPO and TTR** was found at high concentrations (LOEC= 300-600 µM).
- Potency of benzophenone-4 is much lower than the positive control, genistein (dietary flavonoid) - 20-fold lower on the hTPO inhibition assay (LOEC genistein: 2.0E-5 M), and 3000-fold lower on the TTR-TRβ assay (LOEC genistein: 2.0E-7 M).

TTR: Transthyretin
hTPO: human thyroid peroxidase

Module 2 – Bioactivity characterisation

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- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

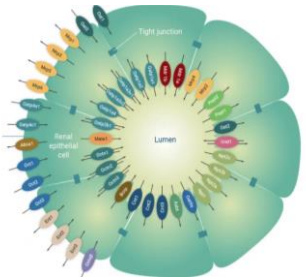
Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

- **Benzophenone-4 concentration was predicted to be higher in the kidney than any other organ**
- **Cell models in the toolbox have limited expression of the relevant transporters**

Renal Toxicity

Renal biomarkers (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints:

- KIM-1
- NGAL
- Clusterin
- TEER (Day 0 and Day 3)
- ATP
- LDH
- Toxicogenomics (3 donors, 2 duplicates per donor), 8 concentrations, 24h and 72h timepoints



- Omeprazole and cisplatin added as benchmarks/positive controls

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Piyush Bajaj et al. 2020. Toxicology. 442, 152535

- POD from renal biomarkers > 1000 µM for both timepoints
- POD from HTTR: 320 µM for both timepoints

- In conclusion, no additional markers of bioactivity were identified for benzophenone-4 in primary human kidney cells using additional biomarkers previously shown to be sensitive to nephrotoxins.

Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

NAM	Cell type	POD_{NAM} Type	POD_{NAM} Value (µM)	BER (using C_{max} of 2.1 µM)
Cell stress panel	HepG2	Global PoD	140	67
HTTr	HepG2	Global PoD	4.2	2
HTTr	HepaRG	Global PoD	52	25
HTTr	MCF7	Global PoD	5.5	2.6
HTTr	HepaRG	Lowest pathway BMDL	530	252
HTTr	HepG2	Lowest pathway BMDL	240	114
HTTr	MCF7	Lowest pathway BMDL	330	157

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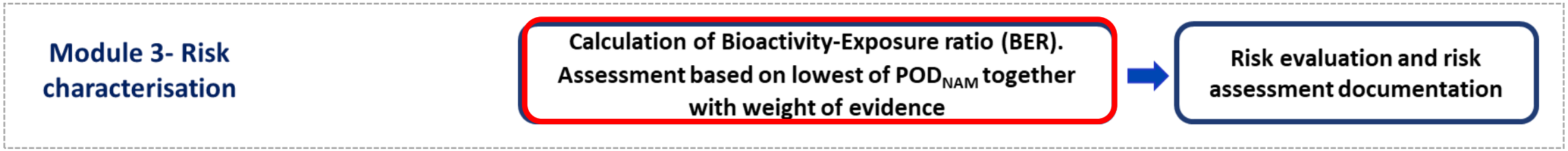
Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

NAM	Cell type	POD_{NAM} Type	POD_{NAM} Value (µM)	BER (using C_{max} of 2.1 µM)
Calux (hTPO-inhibition)	-	LOEC	300	143
Calux (T4 binding to TTR)	-	LOEC	630	300
Renal biomarkers (24 hr exposure)	PTC	Global PoD	>1000	NA
Renal biomarkers (72 hr exposure)	PTC	Global PoD	>1000	NA
HTTr (renal cells) (24 hr exposure)	PTC	Global PoD	320	152
HTTr (renal cells) (72 hr exposure)	PTC	Global PoD	320	152



Not yet consensus on best analysis method to provide HTTr POD

a) Most conservative in this assessment was 4.1 µM (BIFROST), giving a deterministic BER of 2
   a) *Single gene change of CYP 1A1 – is there toxicological significance?*

b) Also important to consider BMDL POD_{NAM} of 240 µM (HepG2), giving a deterministic BER of 114.

c) This provides some assurance that the gene changes seen at 4.1 µM may be of limited toxicological significance.

d) Consumer internal exposures would need to be greater than those predicted to lead to toxicologically significant systemic biological activity in consumers.

A Bayesian approach for inferring global points of departure from transcriptomics data - ScienceDirect

Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

How do we define an acceptable BER to conclude low risk?

Conceptually, with the following assumptions a BER>1 indicates a low risk of adverse effects in consumers following use of the product:

1. **The in vitro measures of bioactivity provide appropriate biological coverage**
2. **There is confidence that the test systems are at least as sensitive to perturbation as human cells in vivo**
3. **The exposure estimate is conservative for the exposed population**

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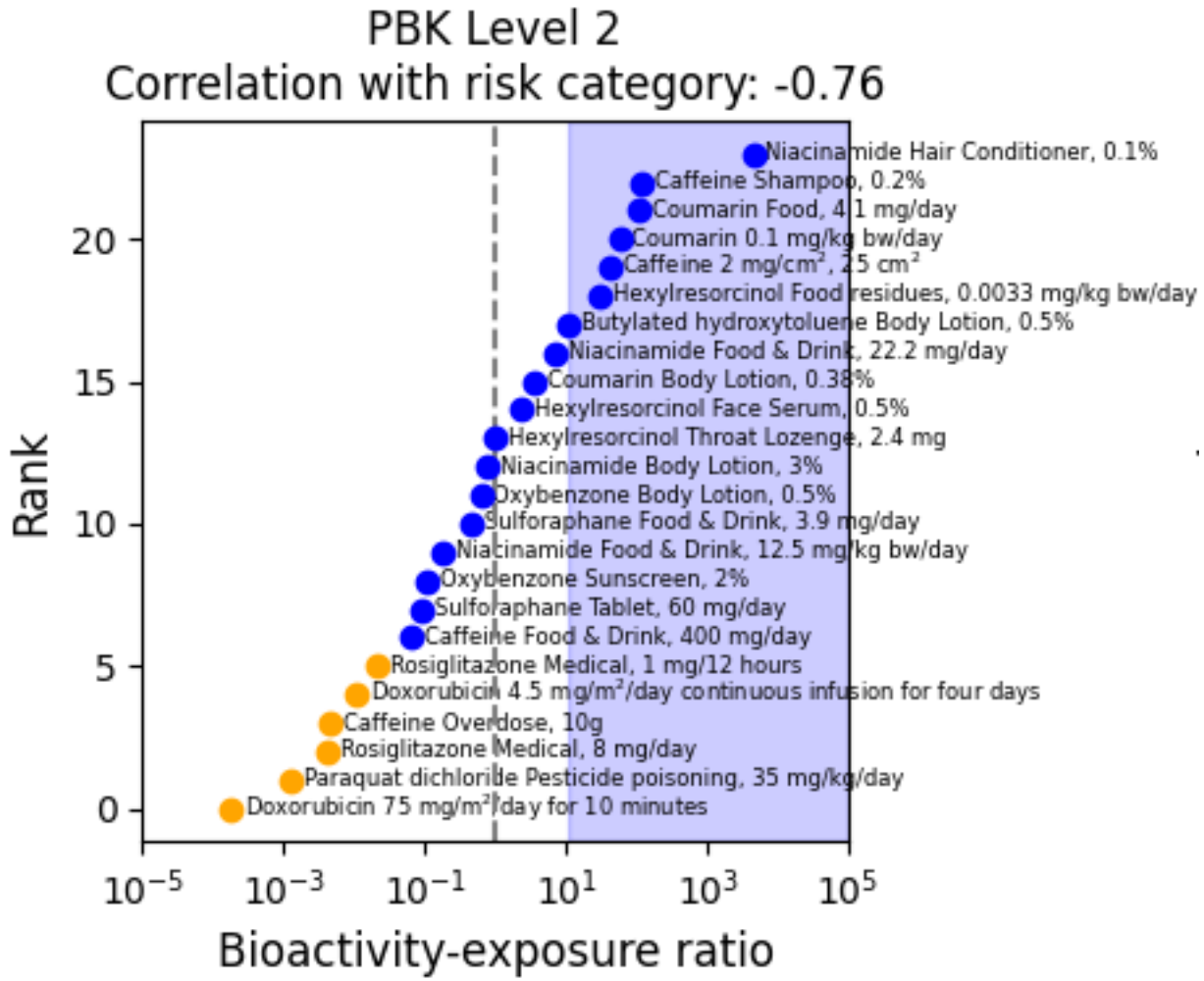
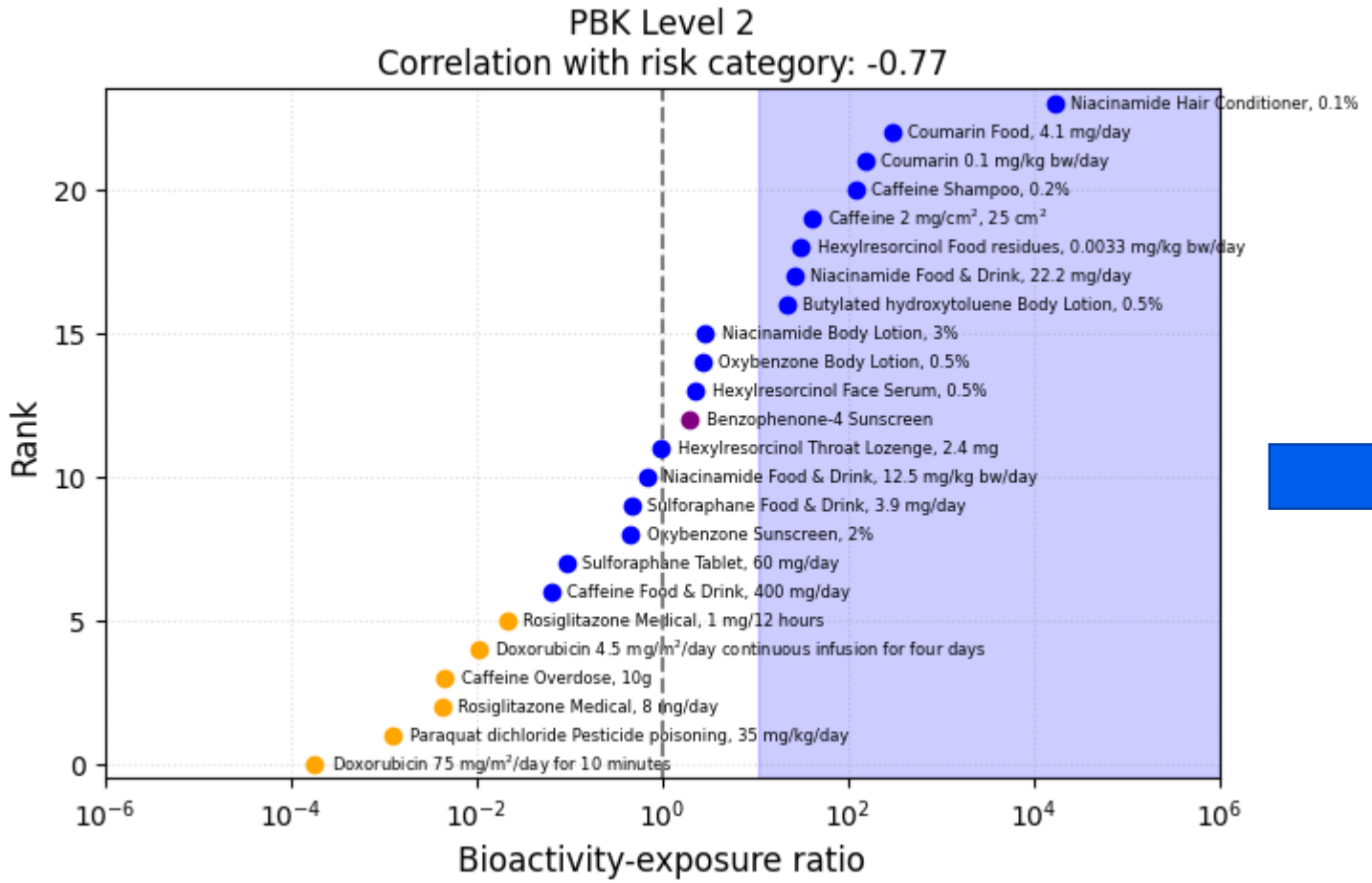
Is the assessment protective?



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)

Benzophenone-4 benchmarks with other low risk chemicals



BP4 is practically inert in a sub-chronic rat test → large traditional MoS

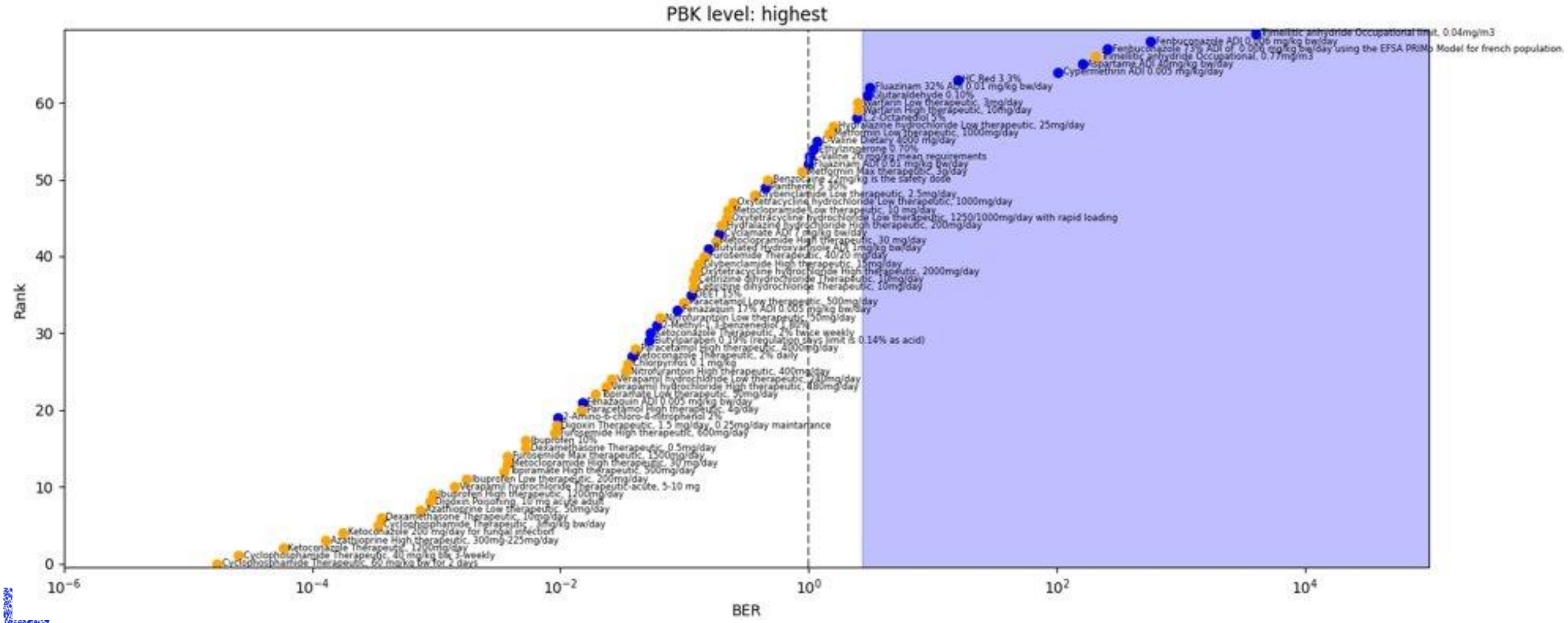
As of 17th of December 2023:

(…) opinion the SCCS is of the opinion that benzophenone-4 is safe when used as UV filter up to a maximum concentration of 5% in sunscreen, face and hand cream, lipstick, sunscreen propellant spray and pump spray, when used separately or in combination (based on deterministic aggregated exposure)

(https://health.ec.europa.eu/system/files/2023-12/sccs_o_283.pdf)

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

NAM Systemic toolbox remains protective (98%) when 38 additional chemicals and 70 exposure scenarios were tested (manuscript in preparation) using the previous BER thresholds



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!

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