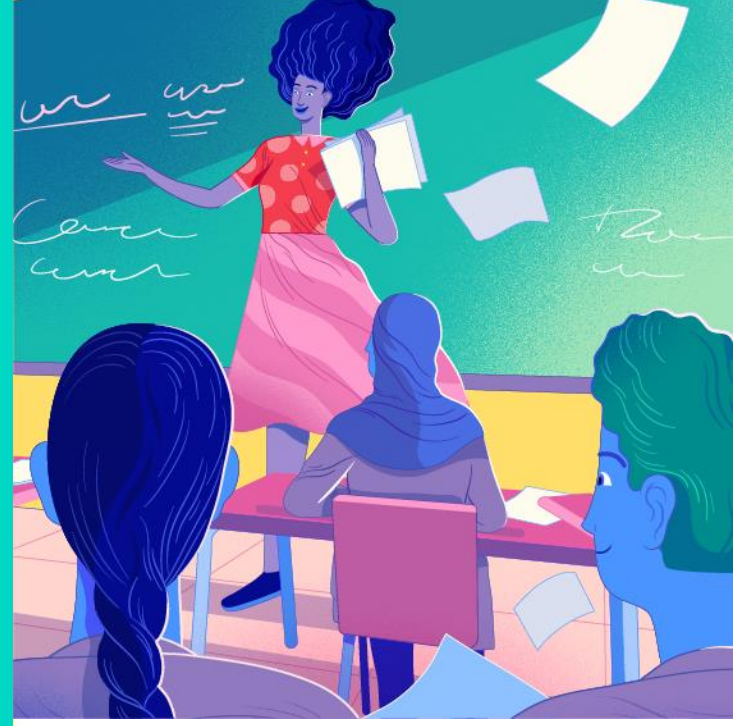
Making safety decisions using next generation risk assessment: case study



Matt Dent

29th March 2022

Outline

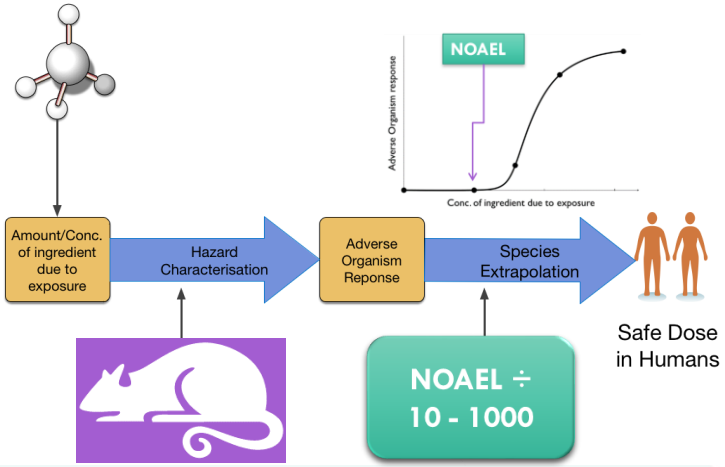
1. Case study background & principles
2. Case study approach
3. Next steps & conclusions

Context of case study

2007

2021

‘Traditional’ Risk Assessment

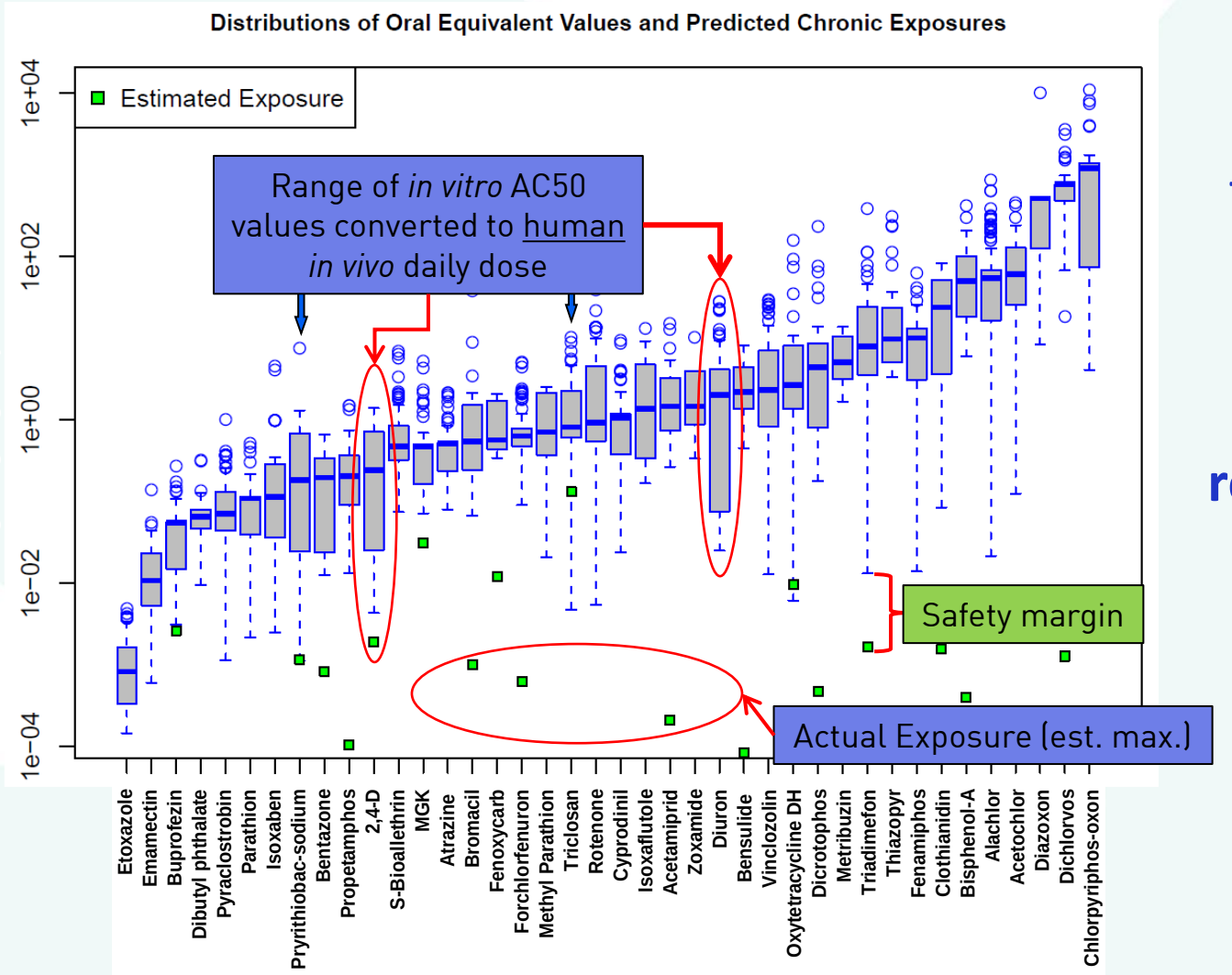


2021

‘Next Generation’ Risk Assessment

based on advances in <u>human</u> biology and in vitro/computational modelling

Paradigm shift for systemic safety - Protection not Prediction

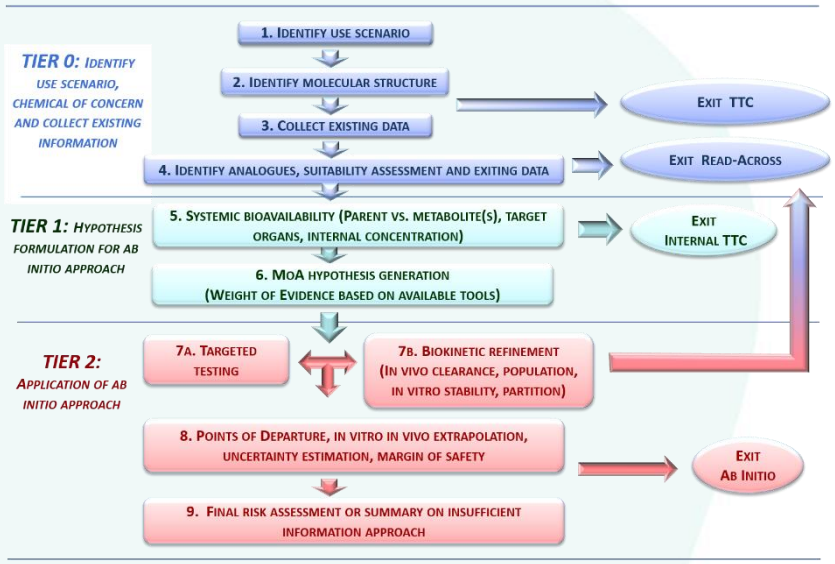


Slide from Dr Rusty Thomas, EPA, with thanks

Thomas RS et al., 2019. Tox Sci. 1;169(2):317-332.
Rotroff, *et al.* Tox.Sci 2010

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.**

Guiding principles for the *ab initio* NGRA applied to the Benzophenone-4 case study



SEURAT-1

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

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Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o], Rajeshwar Verma[j], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

ICCR International Cooperation on Cosmetics Regulation

OECD Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified — English - Or. English

27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testing and Assessment, No. 349

Unilever

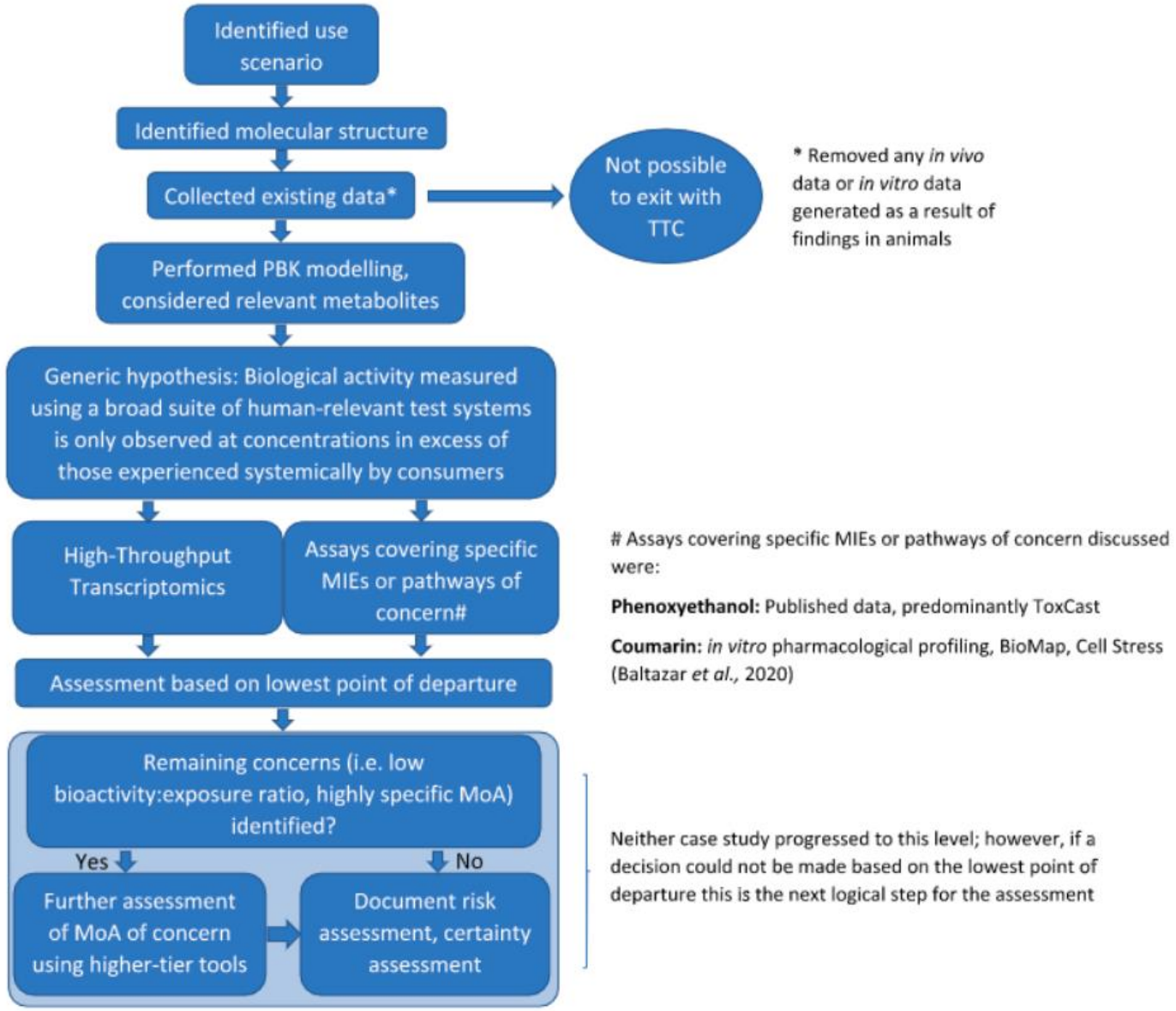
Benzophenone-4 (BP-4) case study: Objectives & Approach

In 2019, the European Commission defined a list of 28 cosmetic ingredients with potential endocrine activity

BP-4 is one of the 28 chemicals for which the call for data took place.

Objective of the case studies & BP-4:

- To assess whether a tiered NGRA approach is sufficiently protective for these types of ingredients following the framework and NAMs applied in previous case studies

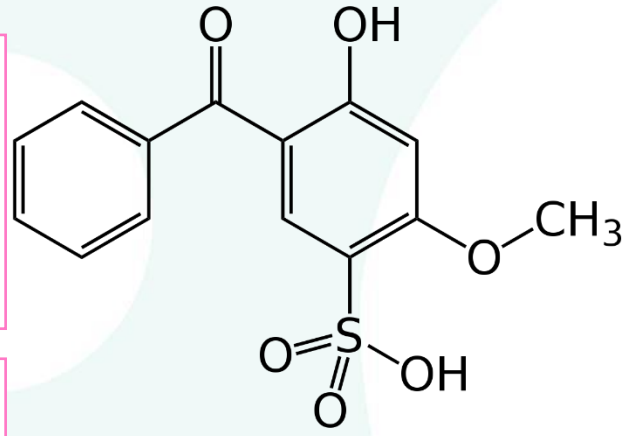


Dent et al 2021. Reg. Tox. Pharm. Volume 125, 105026.

Tiered approach for Exposure estimation

Level 0: Characterise exposure scenario

- 5% in Sunscreen product,
- 18g/day, two times, 9g/application,
- On body and face 17500cm2 (total body area)



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

- Predicted plasma C_{max} at steady state = 33µM
- Predicted sensitive parameters
  - Fup (Fraction unbound in plasma)
  - Liver CL_{int} (intrinsic clearance)
  - Dermis water partition coefficient
  - Dermis diffusivity

Level 2: PBK model built with vitro parameters

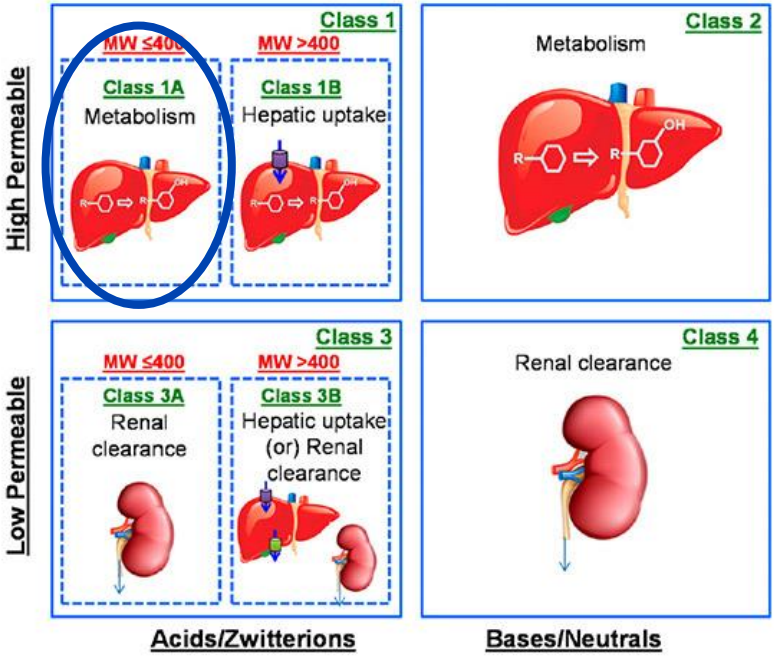
Moxon et al 2020. Toxicology in Vitro, Volume 63, 104746.

Tiered approach for Exposure estimation: LEVEL 2 PBK Model

	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, Pharmacelsus
Blood: plasma ratio	0.6	Measured, Pharmacelsus
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

ECCS classification

(Extended Clearance Classification System)



Davies et al.,2011. Toxicological Sciences, Volume 119, Issue 2, Pages 308–318.

Tiered approach for Exposure estimation: Further refinement of hepatic clearance

Primary human hepatocyte assay :
Hepatic intrinsic clearance <2.5L/h (Below LOQ)

PAMPA assay:
Very low permeability

Human liver S9 incubation:
No metabolism of parent compound

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

→

Low clearance chemical

→

High confidence that liver clearance can be neglected

(set to 0 in PBK).

If ECCS classification is not Class 1A, what's the route of elimination?

How is BP-4 taken up by the cells?

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Tiered approach for Exposure estimation: Further refinement of renal clearance

In silico predictions:

- BP-4 is an anion sulphonate
- BP-4 is predicted to be substrate of several transporters in kidney and liver
- Likely to be a substrate of Organic anion transporters (OATs)
- Renal clearance is likely to be higher than GFR*Fup

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

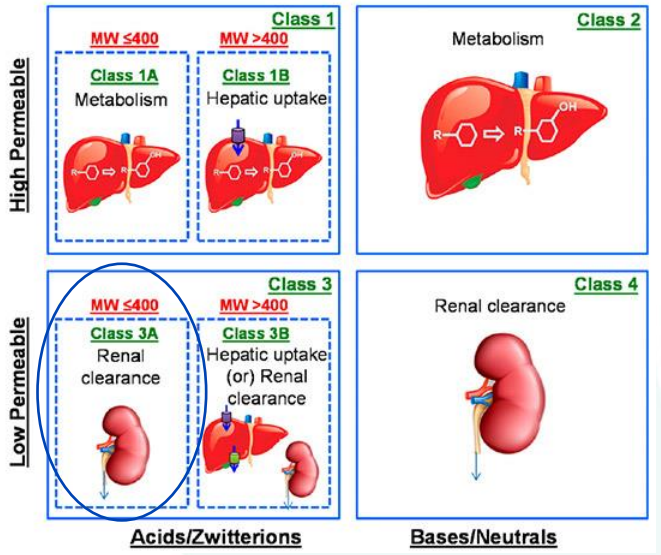
- Influx transporter substrate- OAT1, OAT2, OAT3
- Efflux transporter substrate- MRP4, BCRP
- V_{max} and Km calculated for each transporter

Updated PBK model:

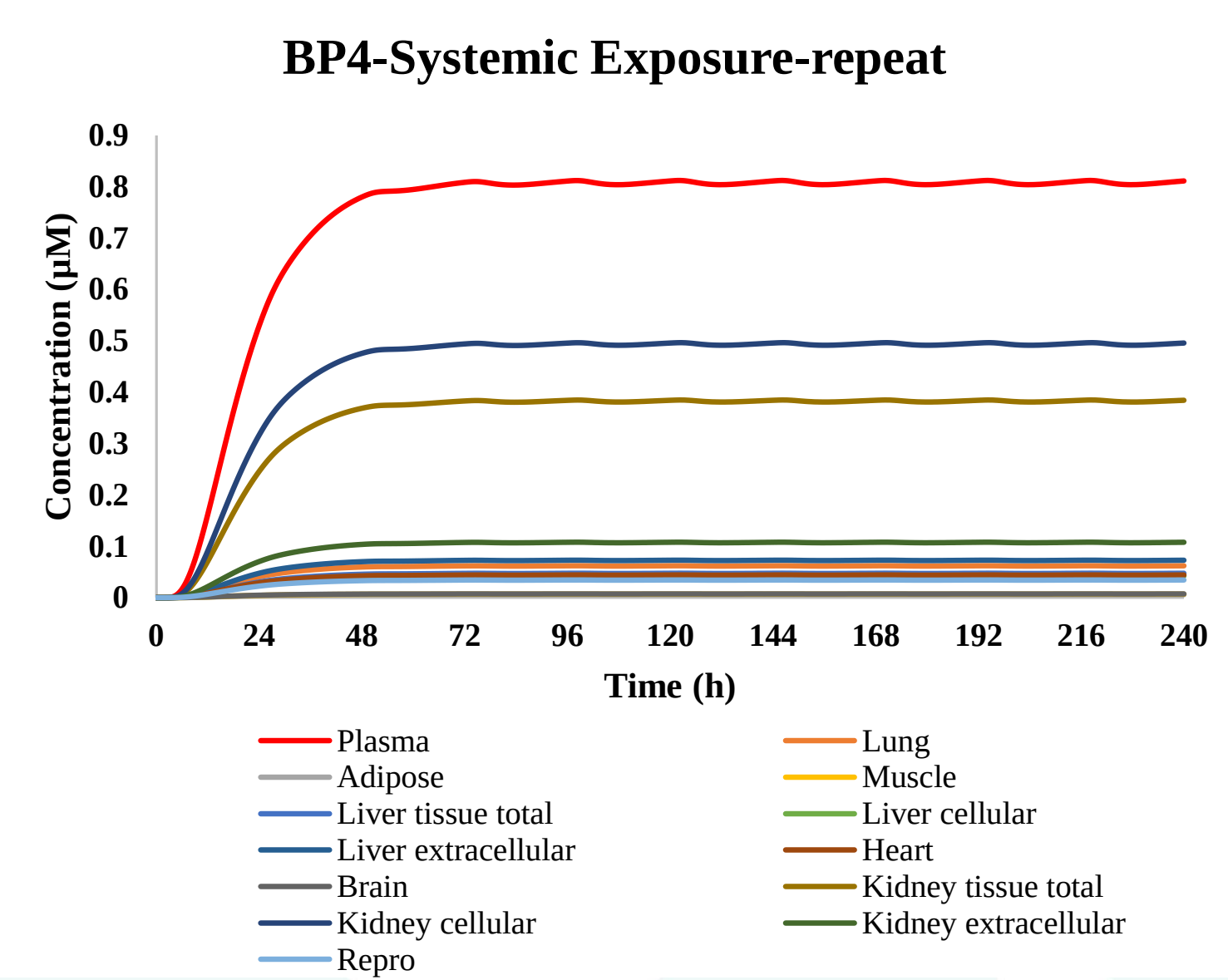
- Set BP-4's distribution to each compartment to be modelled as permeability-limited uptake; i.e. tissue permeability is set to 0.
- Active transport was modelled by incorporating kinetic and abundance parameters into the model

High confidence that BP-4 is substrate of transporters and actively transported into the liver and kidney

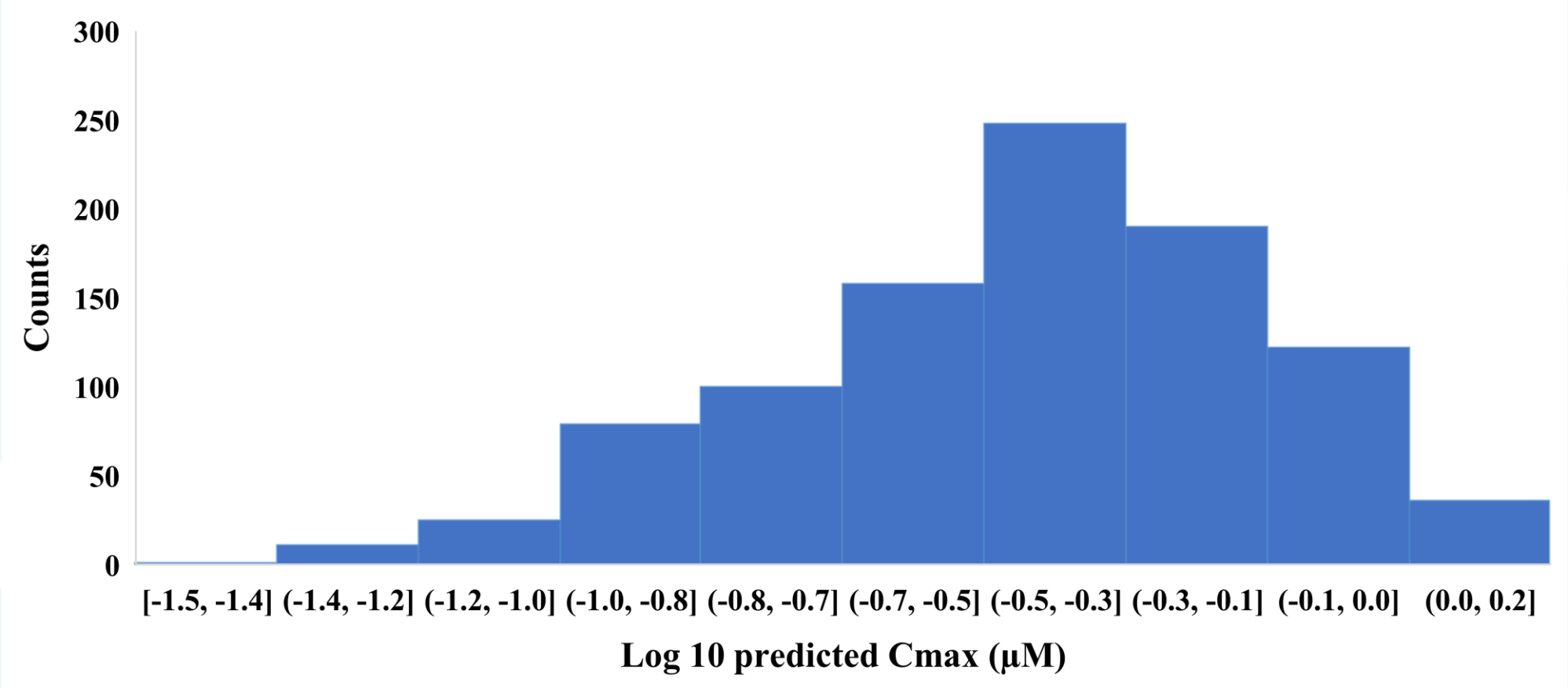
Revised ECCS: Class 3A



Deterministic PBK model simulation of C_{max}



Probabilistic PBK model simulation of C_{max}



	5th percentile (µM)	Median (µM)	95th percentile (µM)
Plasma C_{max}	0.1	0.4	1.0

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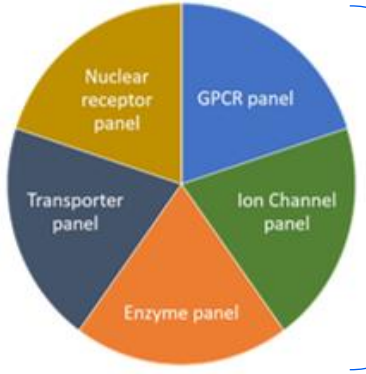
Characterisation of bioactivity- key NAMs

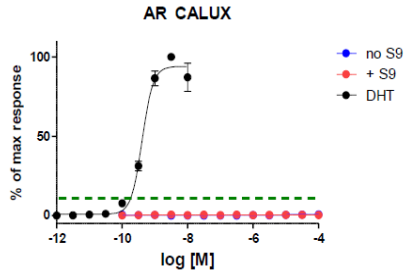
In vitro pharmacological profiling



~83 targets

eurofins | Cerep

CALUX bioassays and binding assays: TTR-TRβ- and hTPO

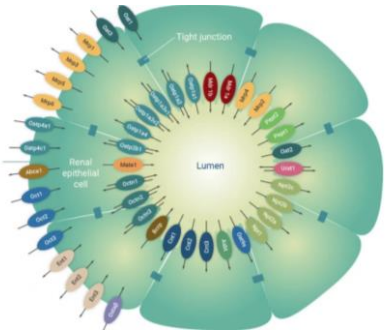


Bowes et al 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al 2005. Toxicol Sci 83(1): 136-48

Renal Toxicity

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

- KIM-1
- NGAL
- Clusterin
- TEER (Day 0 and Day 3)
- ATP
- LDH

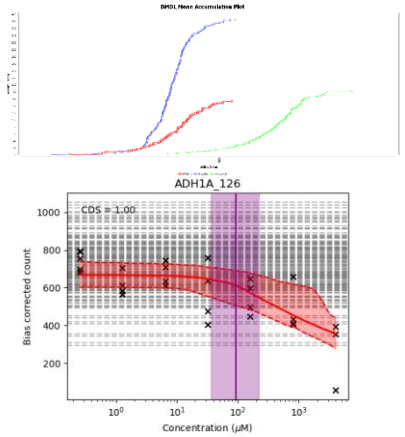


Newcells aProximate™ platform

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

High-Throughput transcriptomics (HTTr)

- TempO-se technology – full gene panel
- 24hr exposure
- 7 concentrations
- 4 cell models: HepG2, MCF7, HepaRG and aProximate cells
- 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

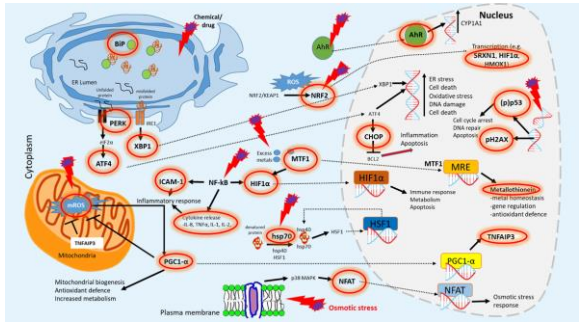


Image kindly provided by Paul Walker (Cyprotex)

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

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Results from the key NAMs- Deriving Points of Departure (PoDs)

In vitro Pharmacological profiling

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

Calux assays

•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).

Platform/NAM	Cell type	Analysis method	PoD (µM)
Cell stress panel	HepG2	BIFROST	140
HTTr	HepG2	BIFROST	4.2
HTTr	HepaRG	BIFROST	52
HTTr	MCF7	BIFROST	5.5
HTTr	HepaRG	Lowest pathway BMDL	650
HTTr	HepG2	Lowest pathway BMDL	240
HTTr	MCF7	Lowest pathway BMDL	280

Concentrations (µM) 0.128, 0.64, 3.2, 16, 80, 400, 2000
Dose response modelling using various methods- BMDExpress2 & BIFROST

Bioactivity:exposure ratio (BER) = Ratio between minimum PoD and predicted C_{max} exposure

Reynolds et al 2020. Computational Toxicology, Volume 16, 100138; Hatherell et al 2020. Tox Sci, 176, Issue 1, 11-33; Baltazar et al, 2020. Tox Sci, 176, Issue 1, 236–252

Bioactivity: exposure ratio calculation (awaiting aProximate™ data)

NAM	Cell type	Analysis method	PoD (µM)	BER from individual C_{max} (µM)
				Median (95% interval)
Cell stress panel	HepG2	BIFROST	140	280 (19, 4400)
HTTr	HepG2	BIFROST	4.2	8.5 (0.58, 130)
HTTr	HepaRG	BIFROST	52	110 (7.2, 1600)
HTTr	MCF7	BIFROST	5.5	11 (0.77, 170)
HTTr	HepaRG	Lowest pathway BMDL	530	1100 (74, 17000)
HTTr	HepG2	Lowest pathway BMDL	240	480 (33, 7500)
HTTr	MCF7	Lowest pathway BMDL	330	670 (46, 10000)
Calux (hTPO-inhibition)	Nthy-ori 3-1	LOEC	300	610 (42, 9500)
Calux (T4 binding to TTR)	U2OS	LOEC	630	1300 (88, 20000)

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Bioactivity: exposure ratio calculation (awaiting aProximate™ data)

NAM	Cell type	Analysis method	PoD (µM)	BER from individual C_{max} (µM)	Prob. BER>1
				Median (95% interval)	
Cell stress panel	HepG2	BIFROST	140	280 (19, 4400)	1.0
HTTr	HepG2	BIFROST	4.2	8.5 (0.58, 130)	0.95
HTTr	HepaRG	BIFROST	52	110 (7.2, 1600)	1.0
HTTr	MCF7	BIFROST	5.5	11 (0.77, 170)	0.96
HTTr	HepaRG	Lowest pathway BMDL	530	1100 (74, 17000)	1.0
HTTr	HepG2	Lowest pathway BMDL	240	480 (33, 7500)	1.0
HTTr	MCF7	Lowest pathway BMDL	330	670 (46, 10000)	1.0
Calux (hTPO-inhibition)	Nthy-ori 3-1	LOEC	300	610 (42, 9500)	1.0
Calux (T4 binding to TTR)	U2OS	LOEC	630	1300 (88, 20000)	1.0

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What does this bioactivity: exposure ratio mean?

Ratio between minimum PoD and predicted C_{max} exposure

Margins of safety (MoS) using animal data have been around for many years and we generally accept that a MoS >100 is protective

More data needed to understand how large a BER needs to be to assure safety

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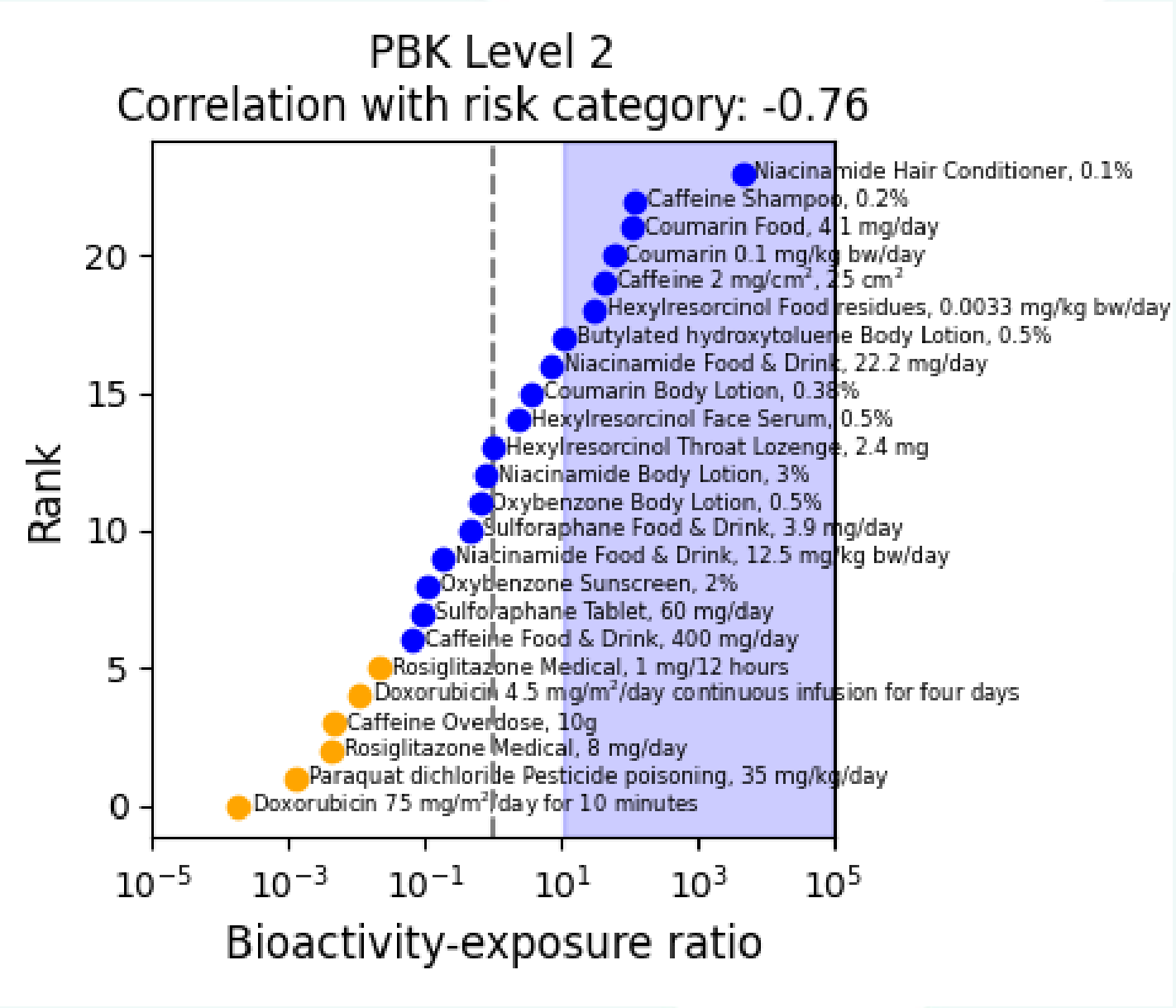
Is the assessment protective?



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* in press

Conclusion & Next steps

- Case studies have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure to make a safety decision.
- This case study 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.

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BioClavis
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SOLVO
BioDetection Systems
NewCells

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