Integration of Kinetics and Dynamics Data for Risk Assessment Purposes

Maria Baltazar
On behalf of the case study team
Cosmetics Europe

29th March 2022

We personally care

Cosmetics Europe
the personal care association

Outline

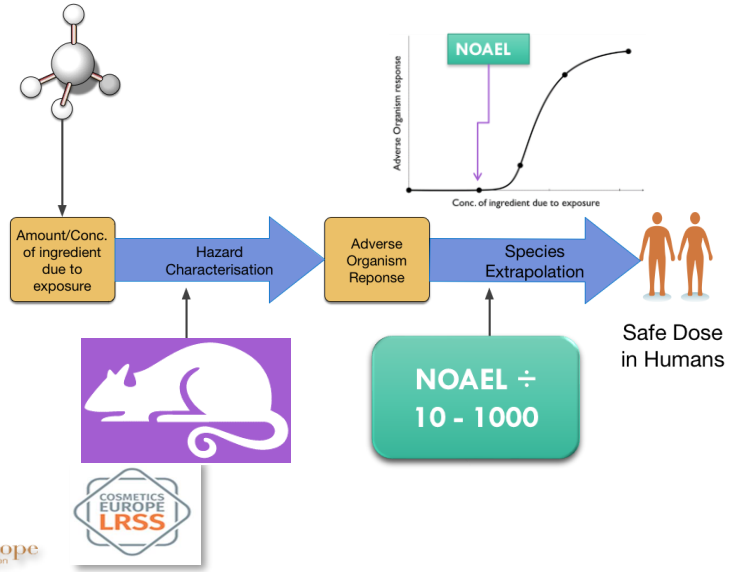
1. Case studies background & principles
2. Benzophenone-4 case study
3. Next steps & conclusions

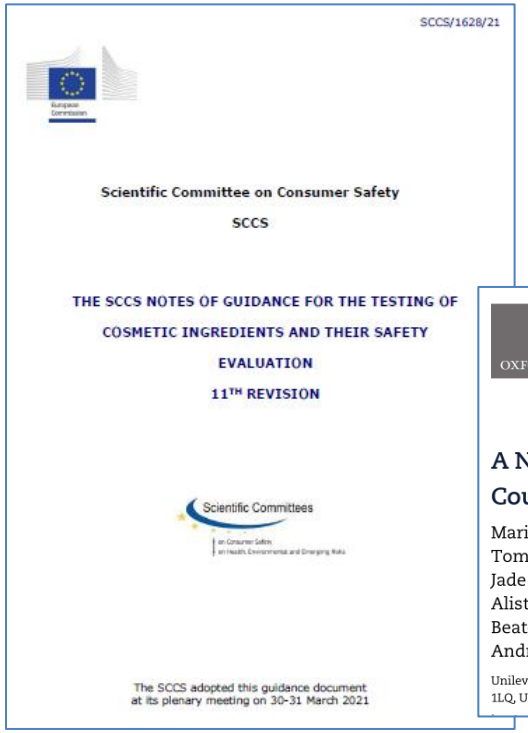
Context of the ab initio NGRA case studies

2007

2021

'Traditional' Risk Assessment





2021

'Next Generation' Risk Assessment

based on advances in human biology and in vitro/computational modelling

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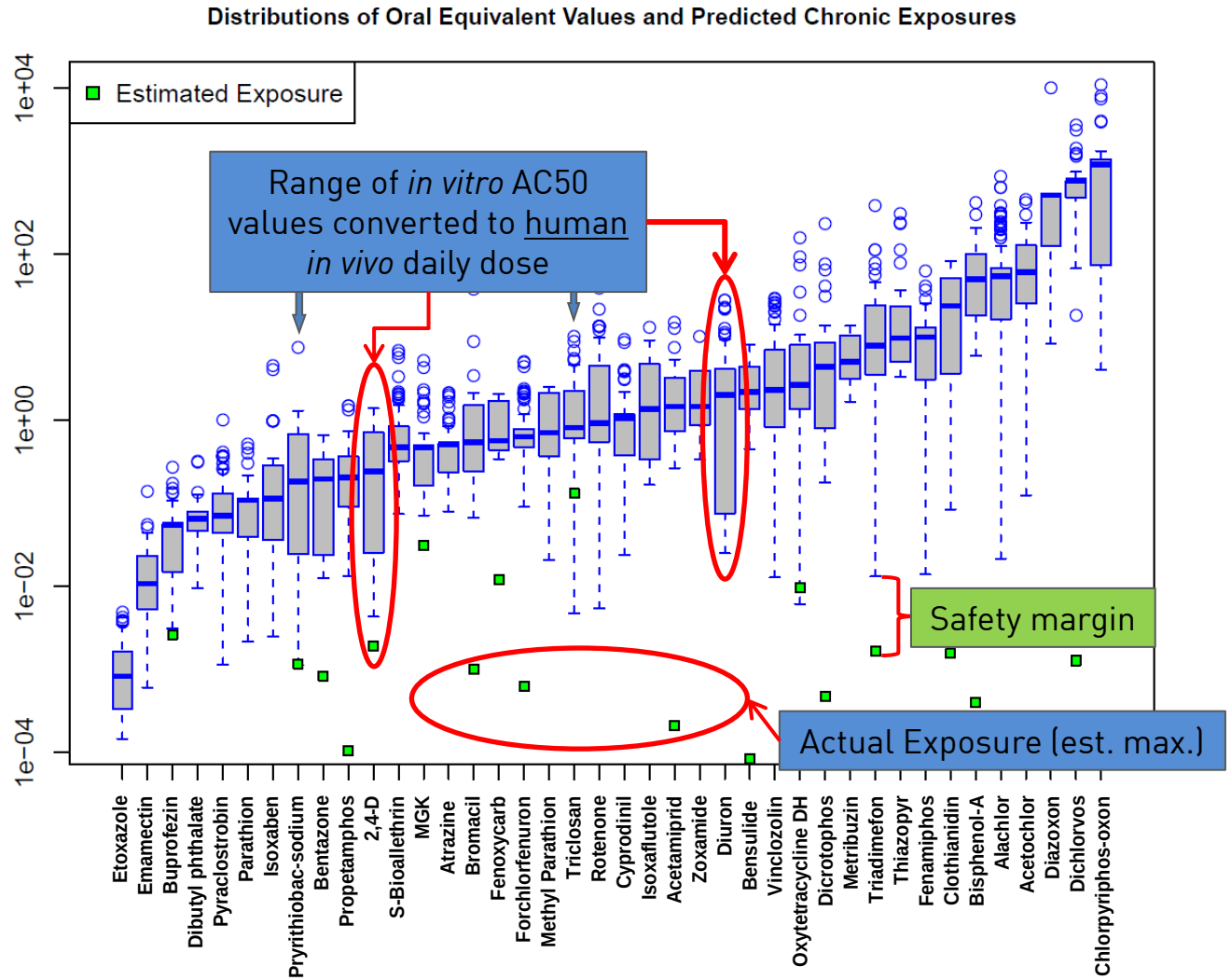
Paradigm shift for systemic safety - Protection not Prediction

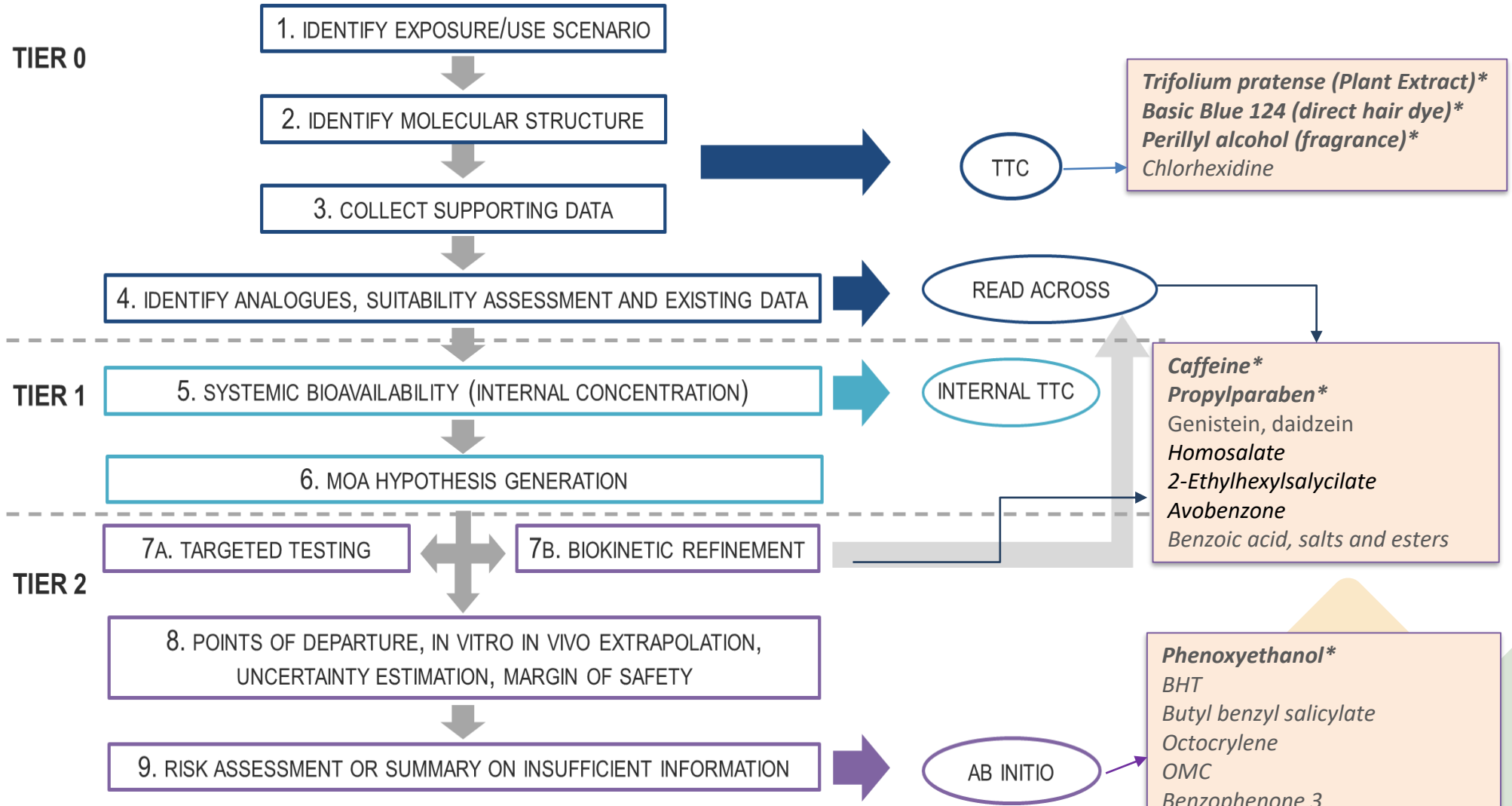


Slide from Dr Rusty Thomas, EPA, with thanks

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.**

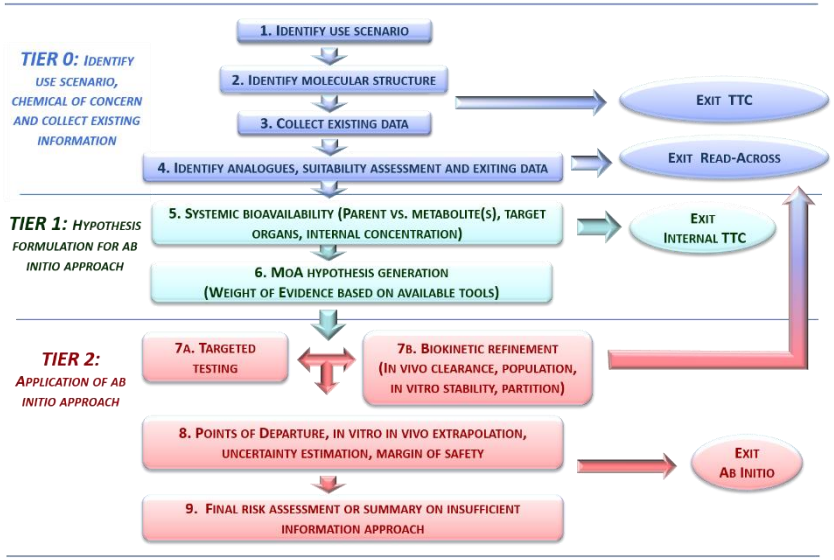
LRSS systemic Toxicity case studies



*Case studies finalized

https://ec.europa.eu/growth/sectors/cosmetics/cosmetic-products-specific-topics/endocrine-disruptors_en

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



SEURAT-1

Computational Toxicology 7 (2018) 20–26

Contents lists available at ScienceDirect

Computational Toxicology

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

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]

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

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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

ICCR International Cooperation on Cosmetics Regulation

Cosmetics Europe the personal care association

COSMETICS EUROPE LRSS

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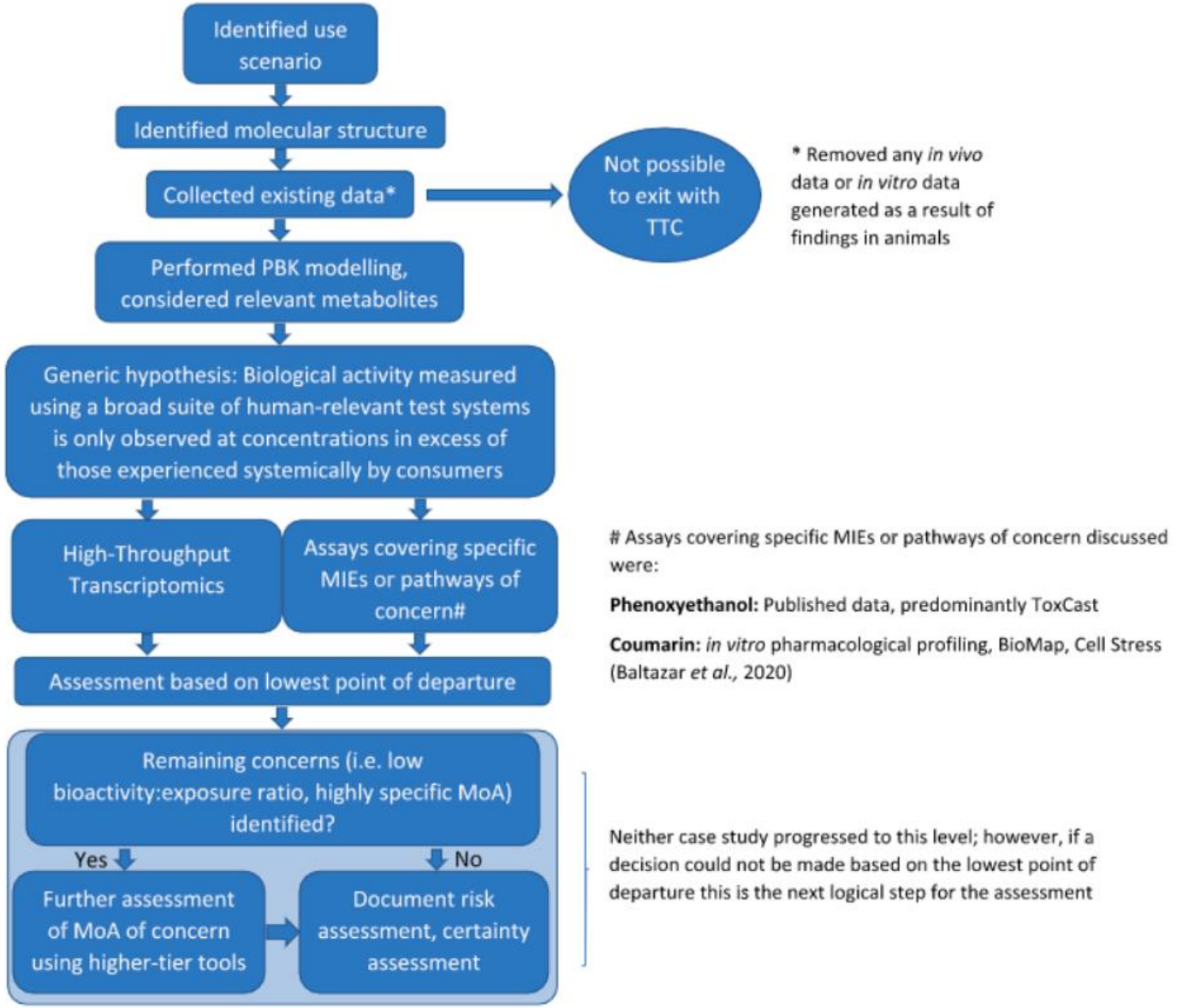
Benzophenone-4 (BP-4) case study: Objectives & Approach

- 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.

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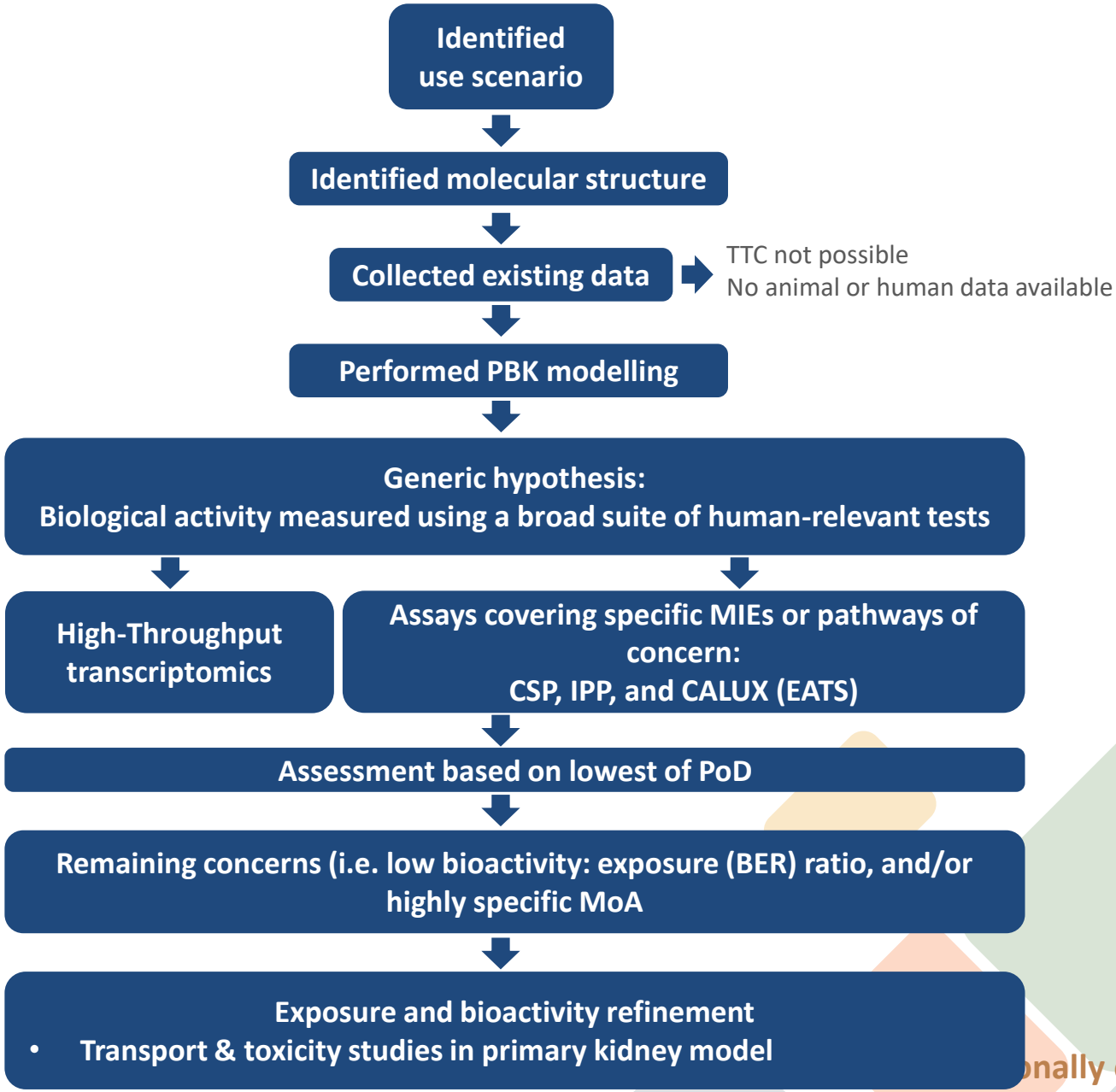


CSP= cell stress panel
IPP- in vitro pharmacological profiling

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

Tiered approach for Exposure estimation

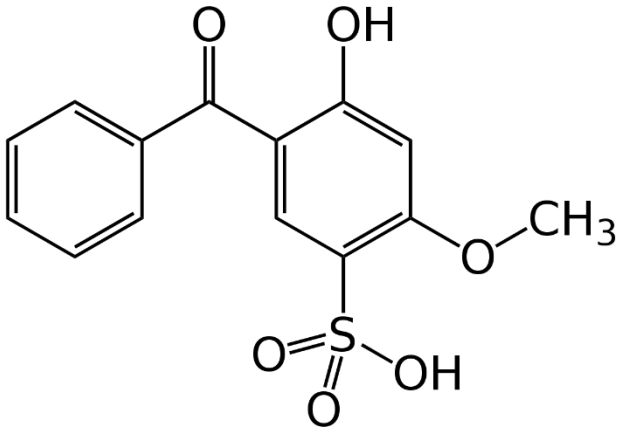
Level 0: Characterise exposure scenario

- 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



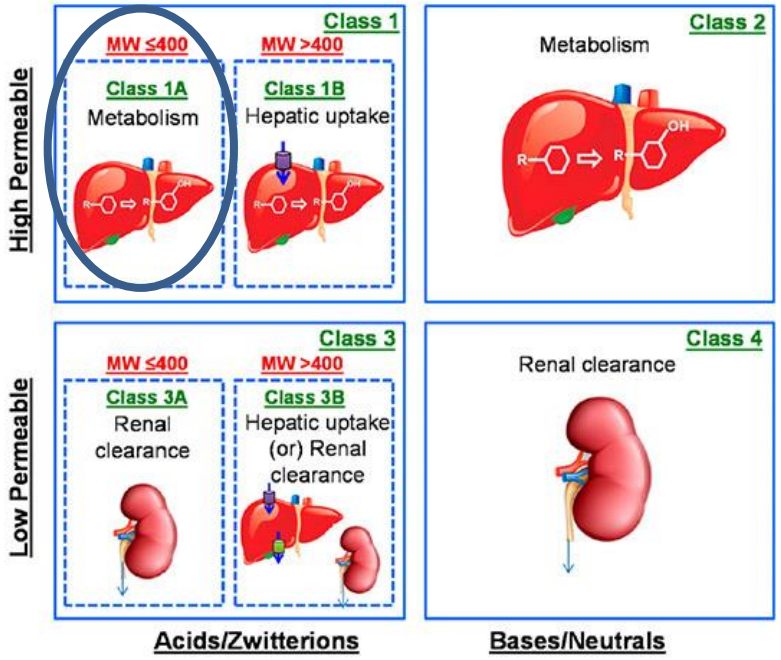
Moxon et al 2020. Toxicology in Vitro, Volume 63, 104746.

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 on 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?

Tiered approach for Exposure estimation: Further refinement on 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)

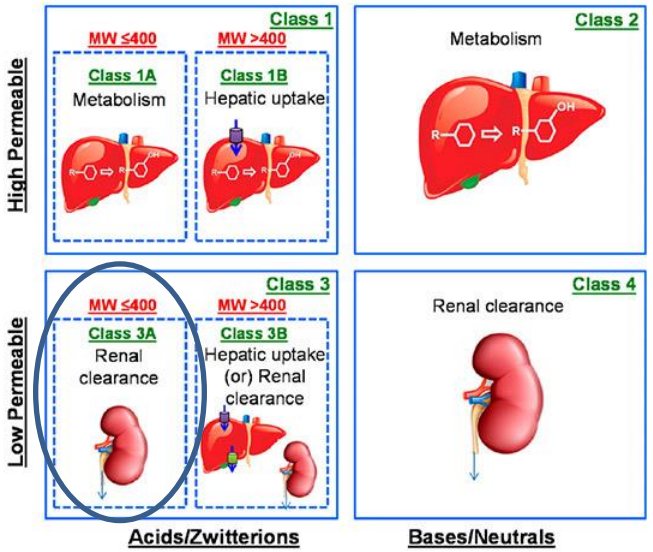
- Influx transporter substrate- OAT1, OAT2, OAT3
- Efflux transporter substrate- MRP4, BCRP
- Vmax and Km calculated for each transporter

Updated PBK model:

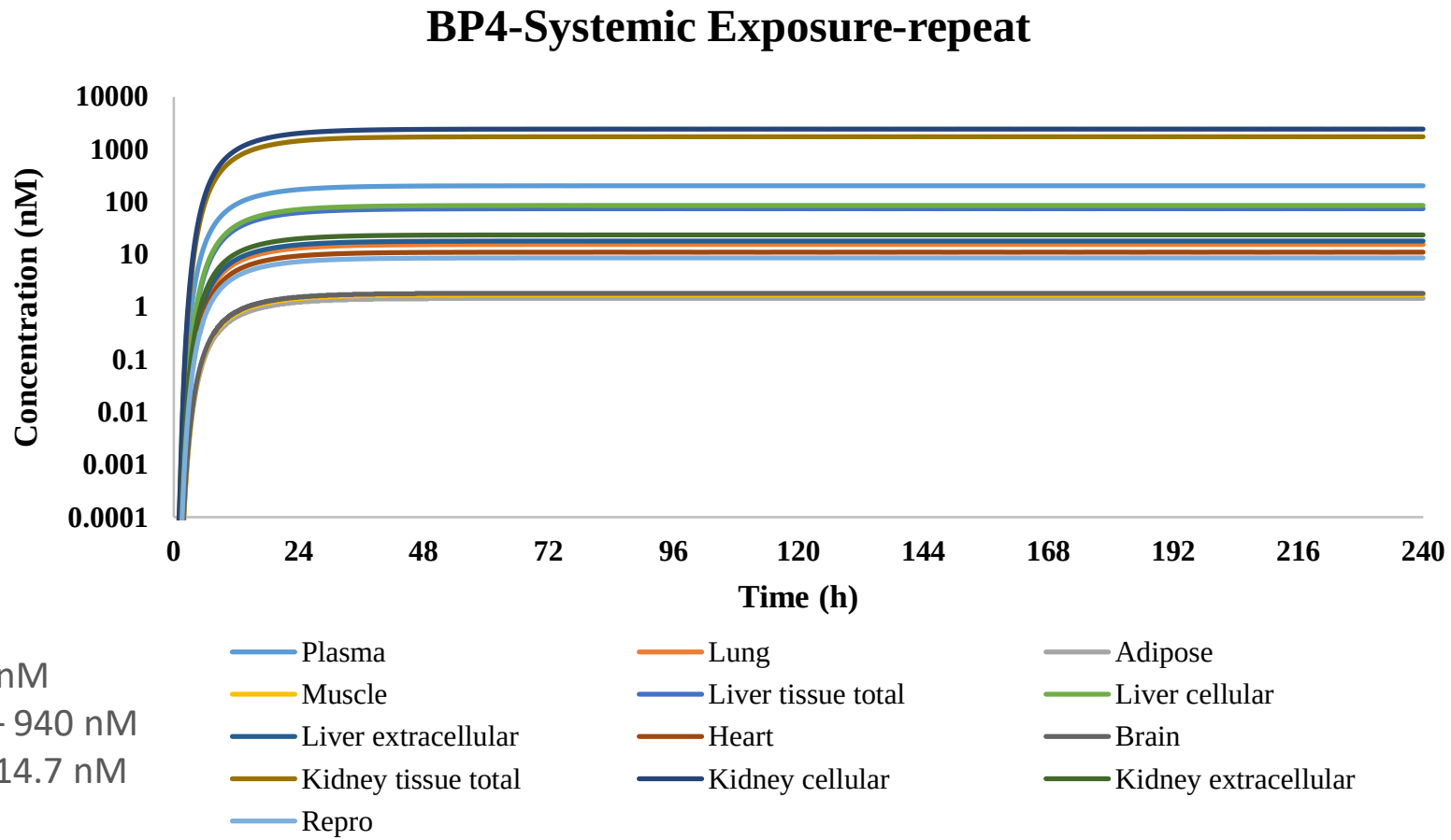
- Set BP-4's distribution to each compartment to be modelled as permeability-limited uptake; i.e. tissue permeability is set to 0.
- Active transport was modelled by incorporating kinetic and abundance parameters into the model

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

Revised ECCS: Class 3A



Deterministic PBK model simulation on Cmax



Plasma cmax – 112 nM
Kidney tissue total – 940 nM
Kidney cellular – 1314.7 nM

Characterisation of bioactivity- key NAMs

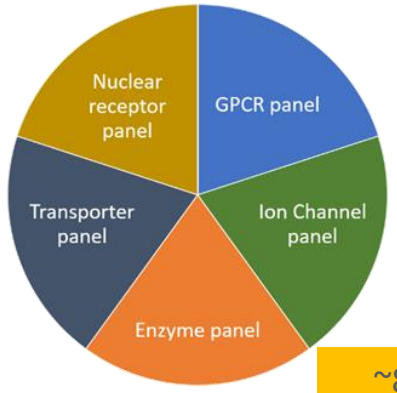
In vitro pharmacological profiling


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

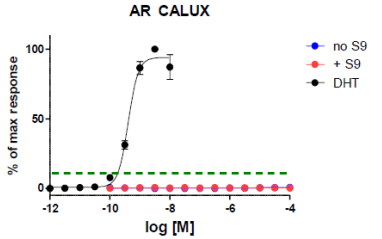


eurofins | Cerep

~83 targets

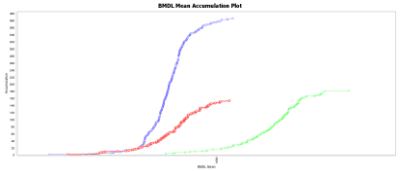
EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

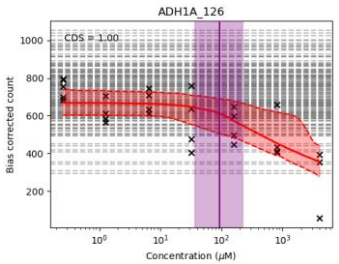
- **CALUX bioassays** and **binding assays: TTR-TRβ- and hTPO**
- **U2-OS** incorporating the firefly **luciferase reporter gene** coupled to Responsive Elements (REs)
- **12 concentrations**. Calculation of AC50, LOEC and NOEC



High-Throughput transcriptomics (HTTr)

- **TempO-se technology – full gene panel**
- **24hr exposure**
- **7 concentrations**
- **3 cell lines: HepG2, MCF7, and HepaRG**
- **Dose-response analysis using BMDExpress2 and BIFROST model**





Cell stress panel (CSP)

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

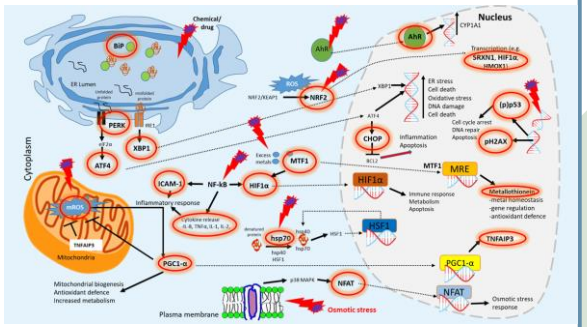


Image kindly provided by Paul Walker (Cyprotex)

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

Hatherell et al 2020. Tox Sci, 176, Issue 1, 11-33

Results from the 3 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**

EATS

•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

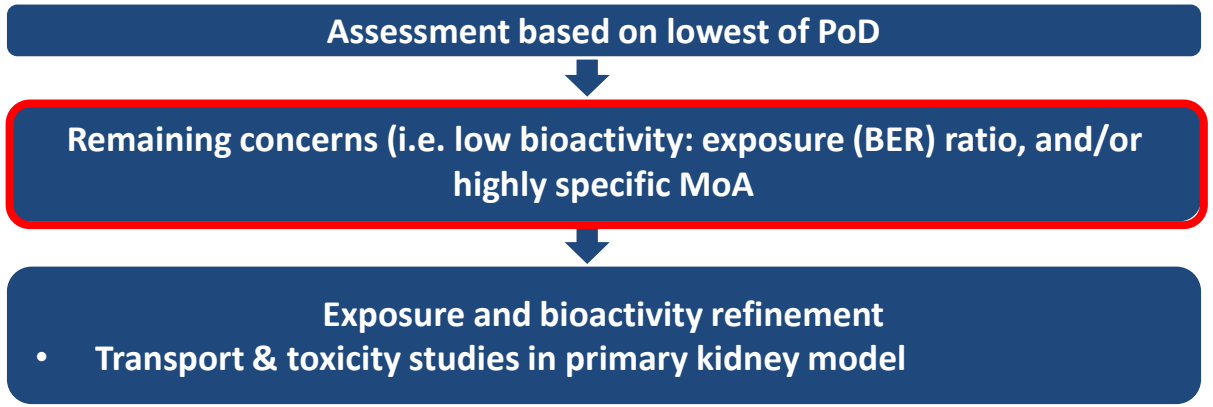
Reynolds et al 2020. Computational Toxicology, Volume 16, 100138
Hatherell et al 2020. Tox Sci, 176, Issue 1, 11-33
Baltazar et al, 2020. Tox Sci, 176, Issue 1, 236–252,

Bioactivity: exposure ratio calculation

Ratio between minimum PoD and predicted Cmax exposure

Minimum PoD: 4.2µM (HTTr, HepG2, BIFROST)
Plasma Cmax: 0.112 µM

BER = 4.2 / 0.112 ~ 37.5

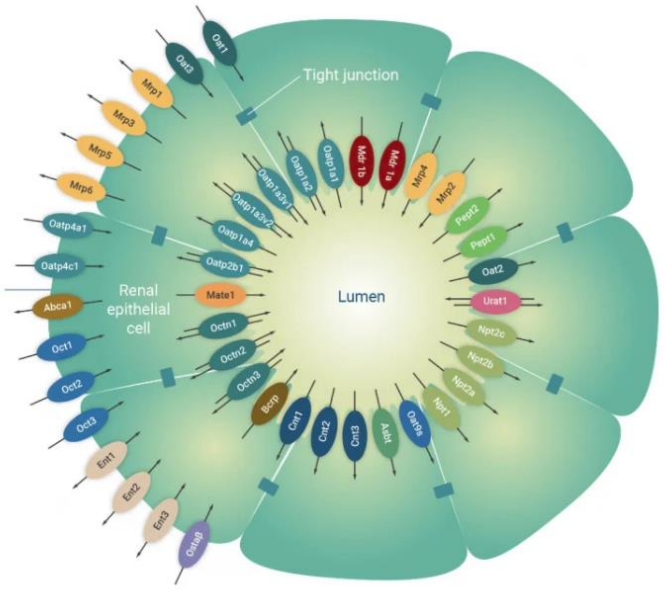


Next steps: Refinement of exposure and bioactivity – primary kidney model

Rationale:

- **BP-4 predicted exposure is higher in the kidney – are the PoDs derived in these cells models sufficiently protective?**
- **Limited evidence of presence of these transporters in HepG2, MCF7 and HepaRG**
- **Transporter studies were performed with transfected cell models overexpressing the transporters- ability to evaluate the full kinetics where a mixture of the transporters is present**

[Newcells aProximate™ platform]



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

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

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.

- Finalise the data generation & interpretation for BP-4 & rest of the 4 case studies (BHT, octocrylene, OMC, climbazole)

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Sandrine Spriggs
Ruth Pendlington
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Cyprotex
SOLVO
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NewCells