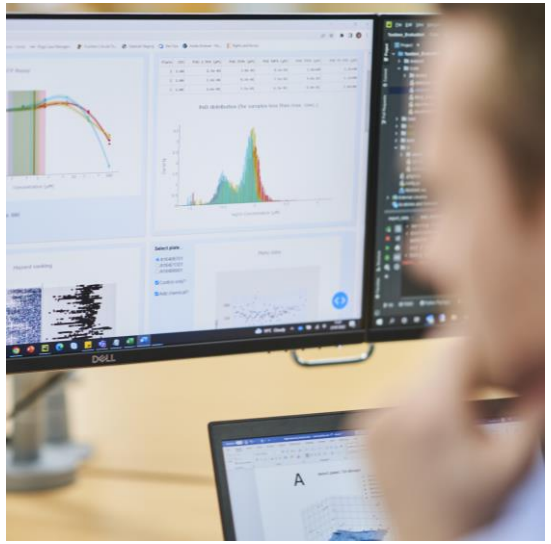
Making the transition to next generation risk assessment for systemic toxicity.

Maria Baltazar,
Unilever Safety and Environmental Assurance Centre, UK



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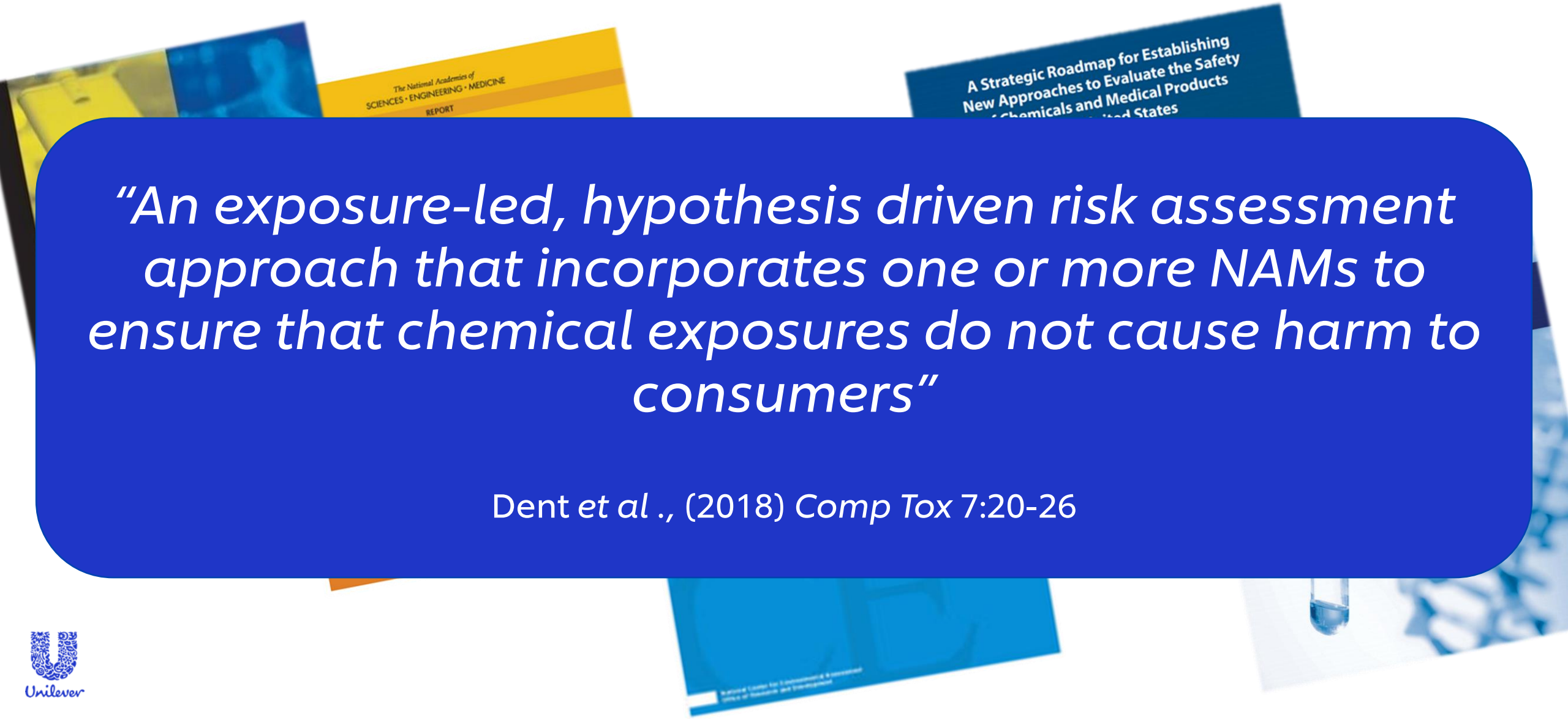
What is next generation risk assessment (NGRA)?



"An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm to consumers"

Dent *et al* ., (2018) *Comp Tox* 7:20-26

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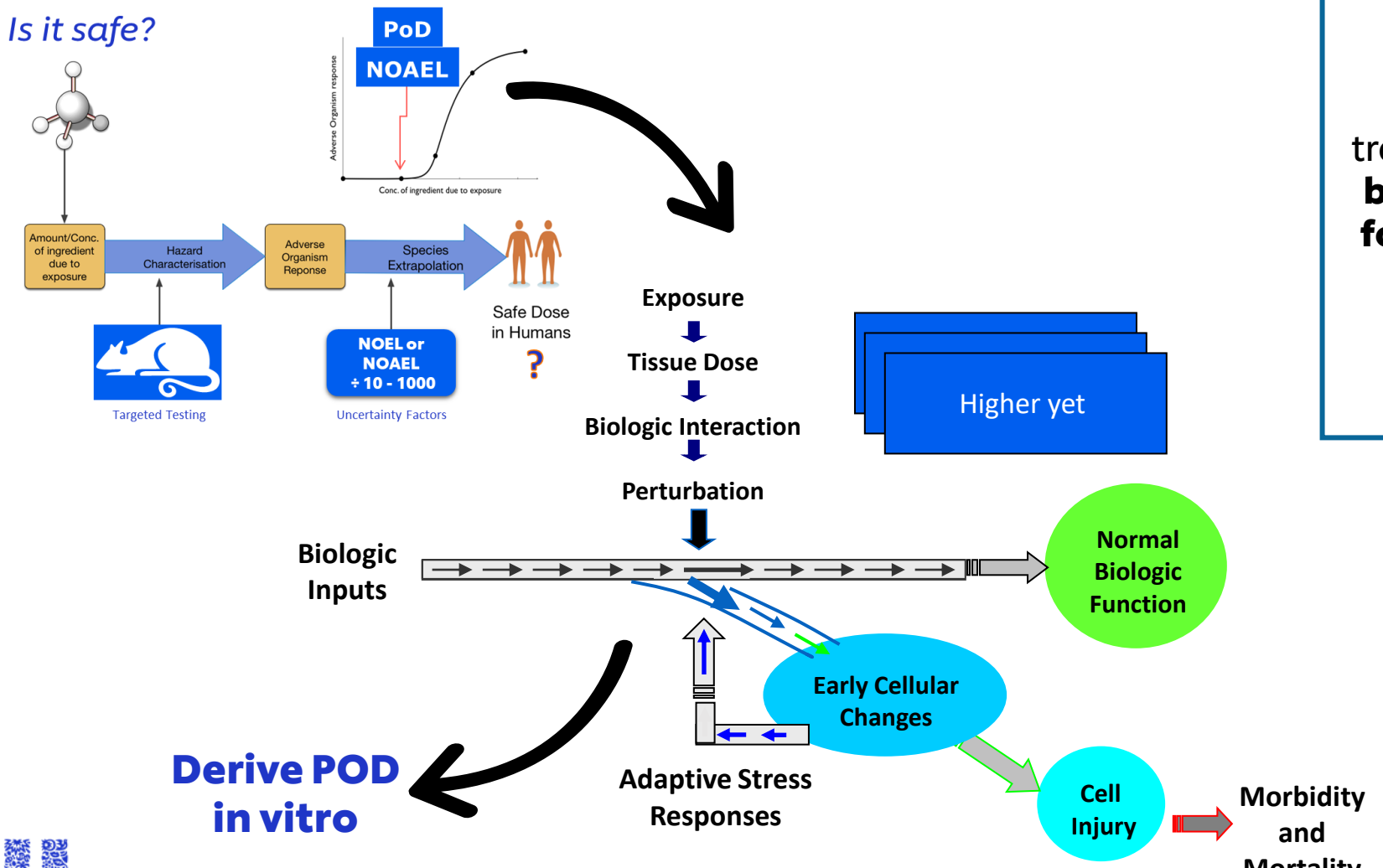
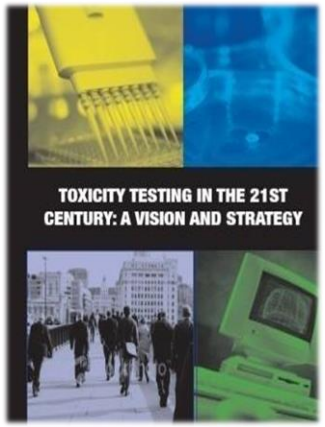
Transition from apical endpoints in animal to cellular perturbations using human relevant in vitro models

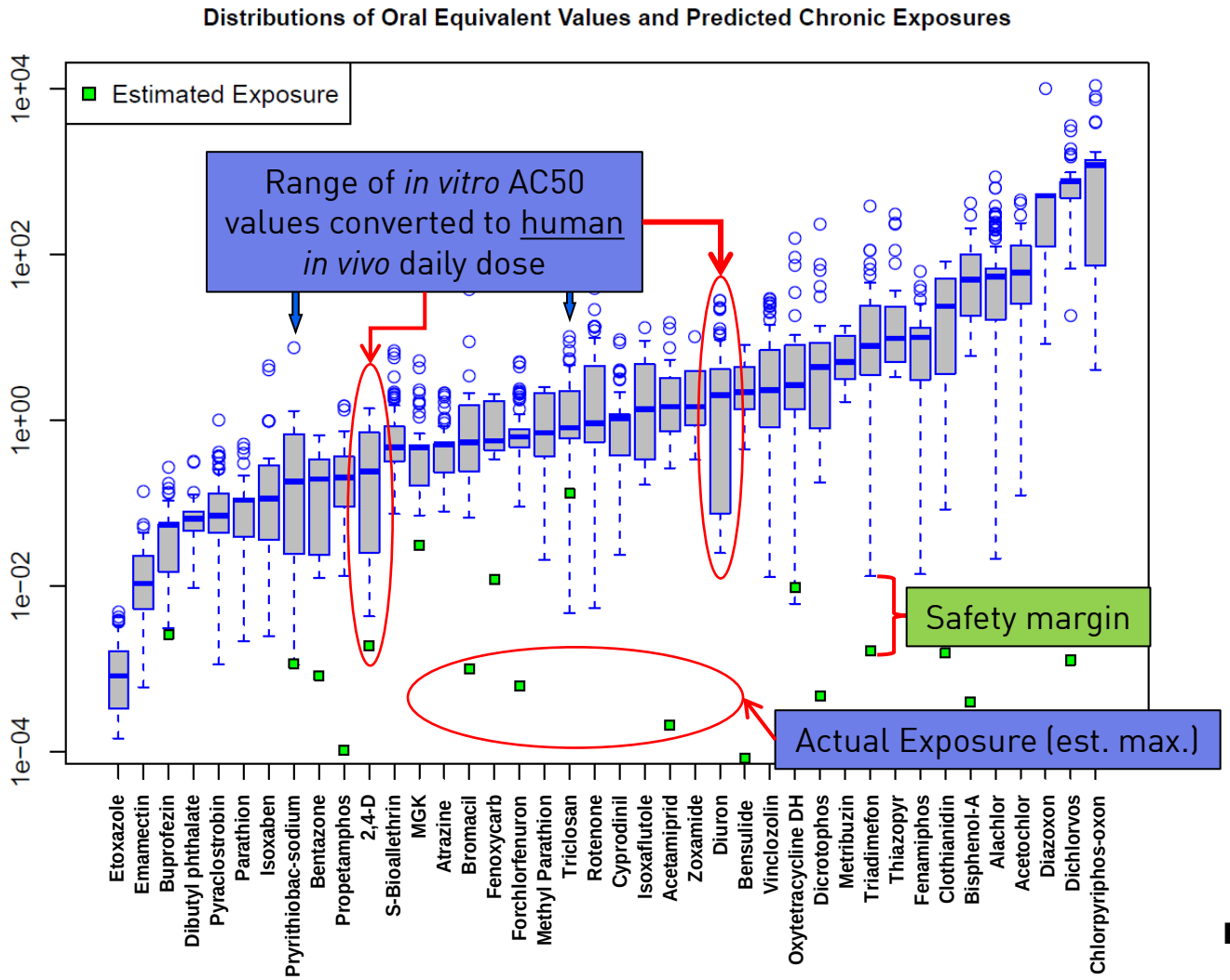


"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity **testing from a system based on whole-animal** testing **to one founded primarily on *in vitro* methods** that evaluate changes **in biologic processes using cells, cell lines, or cellular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



Krewski. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):51-138

Paradigm shift for systemic safety - Protection not Prediction



Graphic from Dr Rusty Thomas, EPA, with thanks

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.**

Rotroff, *et al.* Tox.Sci 2010

Tiered, exposure-led NGRA means we can make robust safety decisions

- Many tools available (exposure-based waiving, read across, history of safe use)
- Increasing recognition that *in vitro* bioactivity is a part of this tiered approach (e.g. Health Canada, SCCS)

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

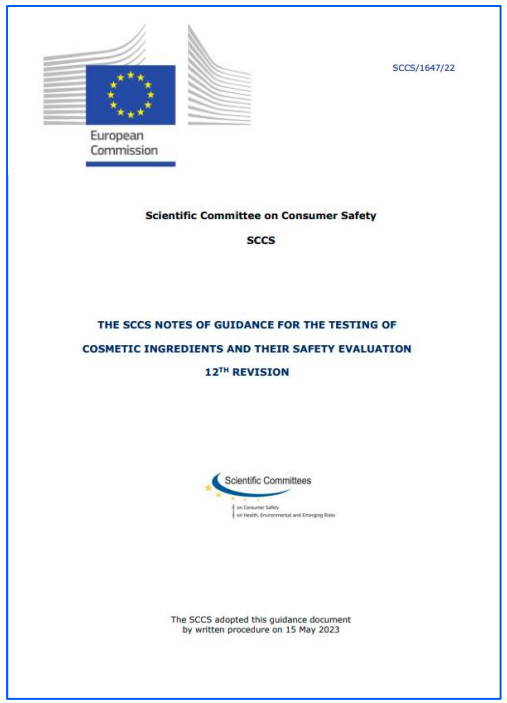
Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o],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

ARTICLE INFO

ABSTRACT

Consumer safety is a prerequisite for any cosmetic product. Worldwide, there is an ever-increasing desire to bring safe products to market without animal testing, which requires a new approach to consumer safety. 'Next Generation Risk Assessment' (NGRA), defined as an exposure-led, hypothesis driven risk assessment approach that integrates *in silico*, *in chemico* and *in vitro* approaches, provides such an opportunity. The customized nature of each NGRA means that the development of a prescriptive list of tests to assure safety is not possible, or appropriate. The International Cooperation on Cosmetics Regulation (ICCR) therefore tasked a group of scientists from regulatory authorities and the Cosmetic Industry to agree on and outline the principles for incorporating these new approaches into risk assessments for cosmetic ingredients. This ICCR group determined the overall goals of NGRA (to be human-relevant, exposure-led, hypothesis-driven and designed to prevent harm); how an NGRA should be conducted (using a tiered and iterative approach, following an appropriate literature search and evaluation of the available data, and using robust and relevant methods and strategies); and how the assessment should be documented (transparent and explicit about the logic of the approach and sources of uncertainty). Those working on the risk assessment of cosmetics have a unique opportunity to lead progress in the application of novel approaches, and cosmetic risk assessors are encouraged to consider these key principles

ICCR International Cooperation on Cosmetics Regulation



European Commission

SCCS/1647/22

Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 12TH REVISION

Scientific Committees

The SCCS adopted this guidance document by written procedure on 15 May 2023

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

OECD

JT03483903

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

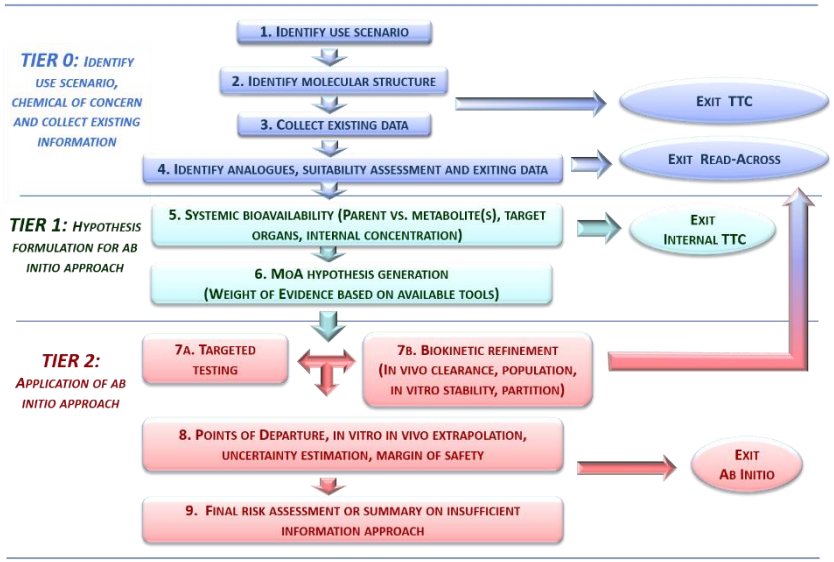
International Cooperation on Cosmetics Regulation (2018)

European Commission: Scientific Committee on Consumer Safety (2021, 2023)

OECD (2021)

Unilever

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



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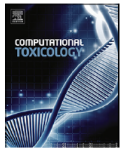

SEURAT-1

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

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

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified English - Or. English
27 October 2021

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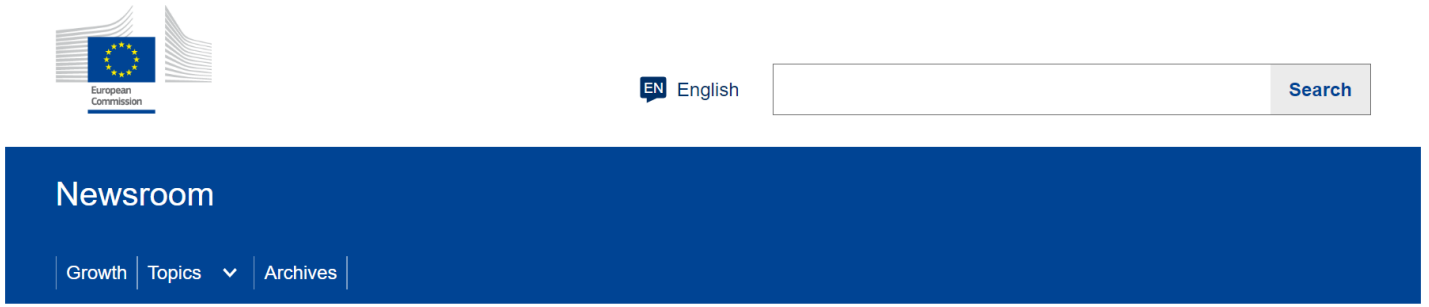
Benzophenone-4 (BP-4) case study



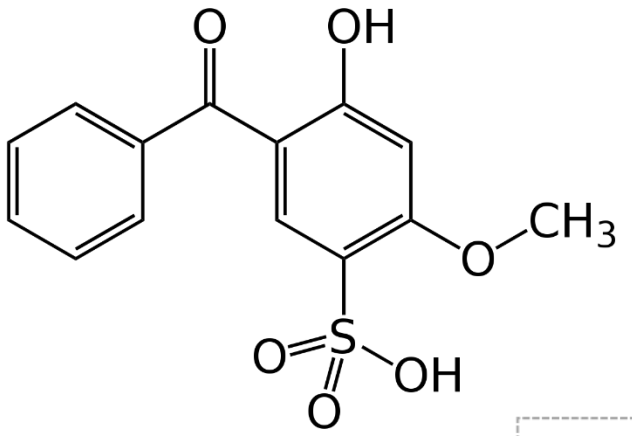
Is a tiered NGRA approach is sufficiently protective and useful to answer a real-life question?

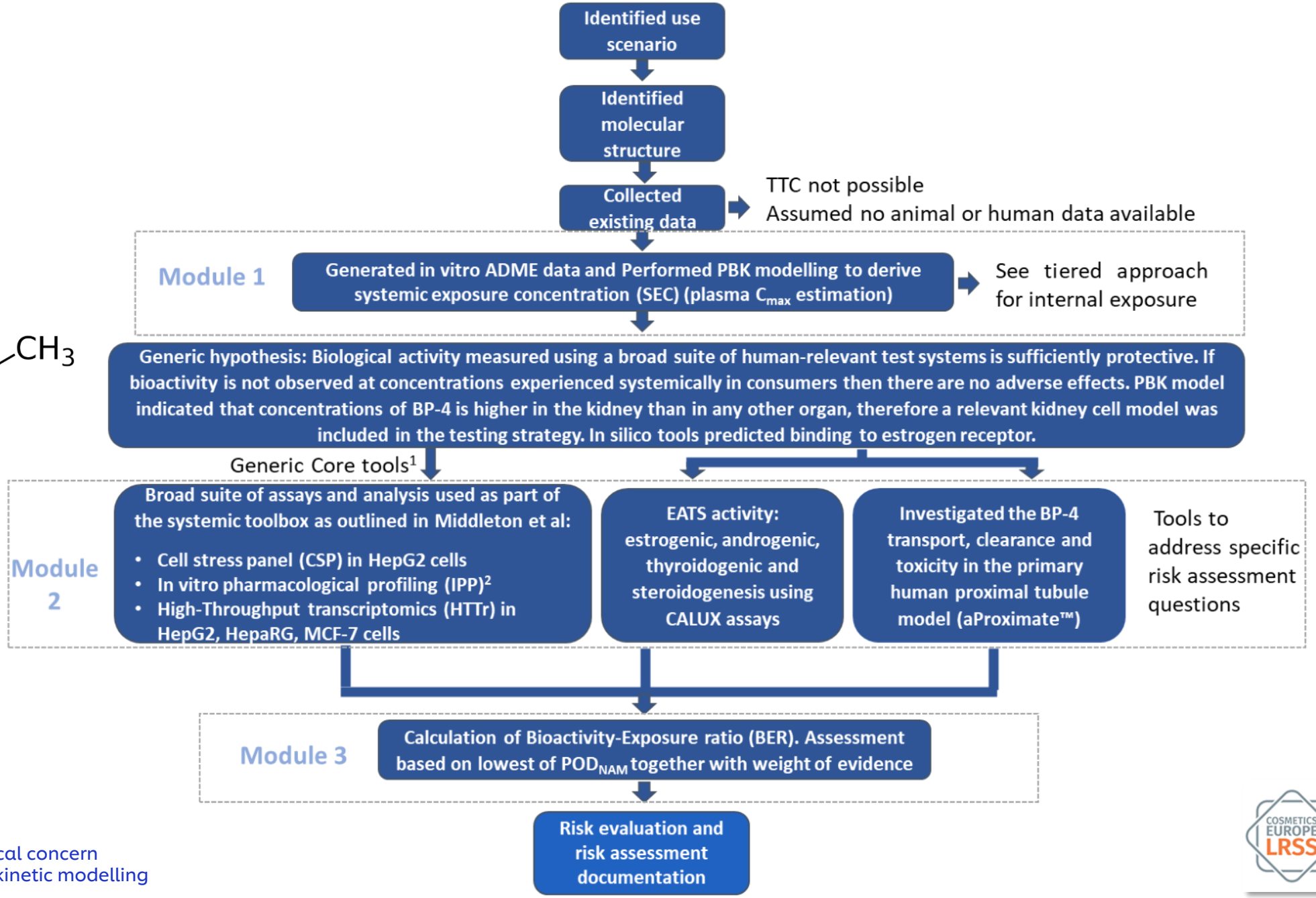
Is Benzophenone-4 safe in a sunscreen product at the maximum approved level of 5%?

Unilever

COSMETICS EUROPE LRSS

Framework used for BP-4





TTC: threshold of toxicological concern
PBK: physiologically based kinetic modelling

Gathering information: In silico results

- **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.

Follow up with in vitro assays to confirm whether or not BP-4 binds to estrogen receptor and other endocrine related endpoints – CALUX EATS (estrogenic, androgenic, thyroidogenic and steroidogenesis

Tiered approach for Exposure estimation

Level 0: Characterise exposure scenario[1]

- 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[1,2]

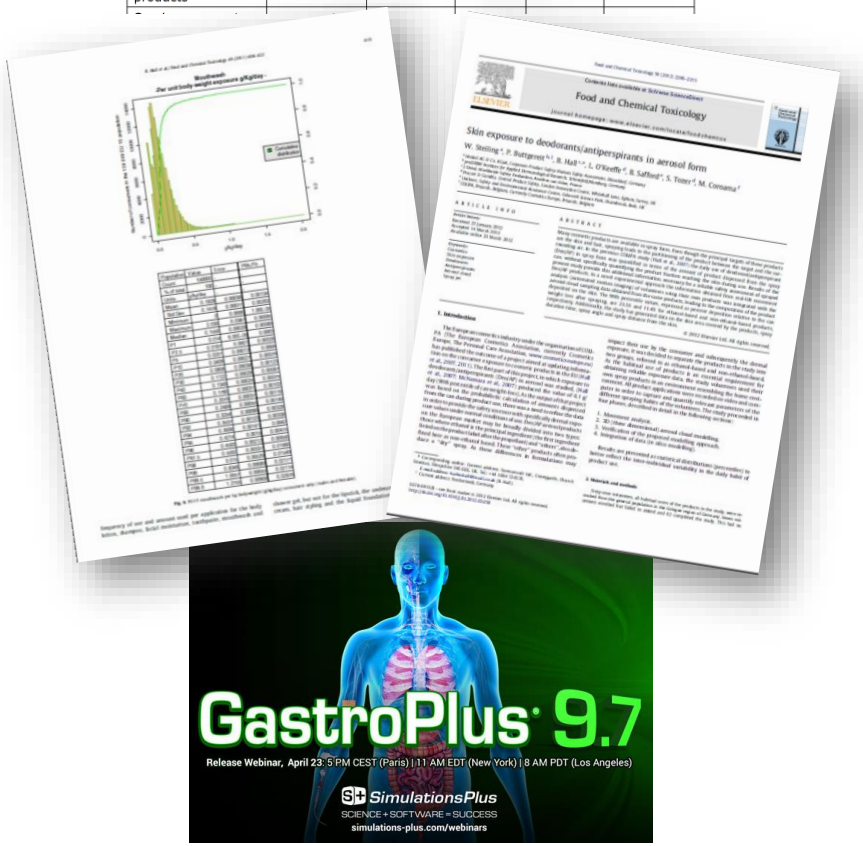
- Predicted sensitive parameters
  - Fup (Fraction unbound in plasma)
  - Liver CL_{int} (intrinsic clearance)
  - Dermis water partition coefficient
  - Dermis diffusivity

Level 2: PBK model built with vitro parameters[1,2]

Creme GLOBAL

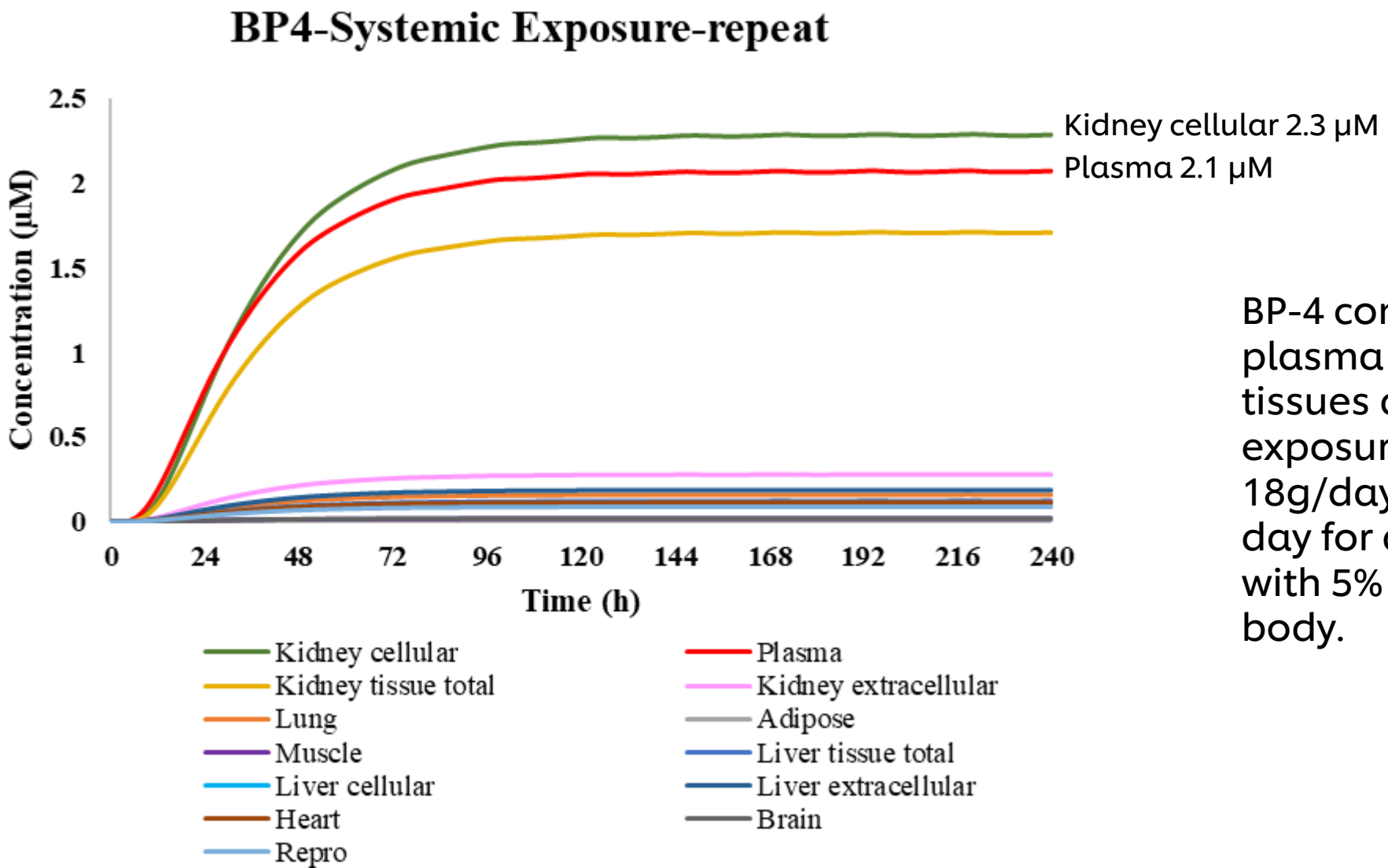
Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNFP/0321/00; Hall *et al.*, 2007, 2011).

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



[1]Hall et al., Food and Chemical Toxicology 49 (2011) 408-422.
[2]Moxon et al. 2020. Toxicology in Vitro, Volume 63, 104746.
[3]Li H,. Toxicol Appl Pharmacol. 2022 ;442:115992.

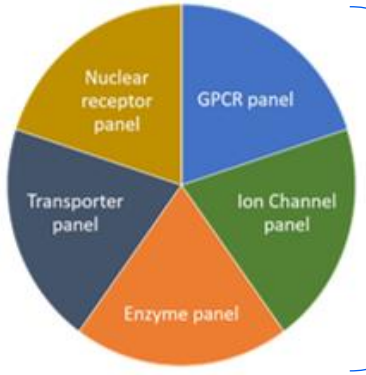
PBK model simulation of C_{max} for an American female with 60kg bodyweight



BP-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% BP-4, on the whole body.

Key bioactivity NAMs

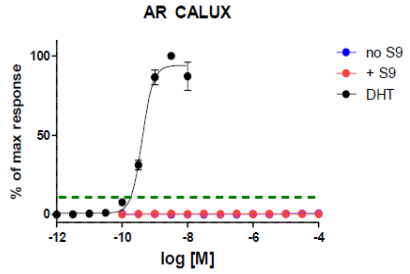
In vitro pharmacological profiling



~79 targets

eurofins | Cerep

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

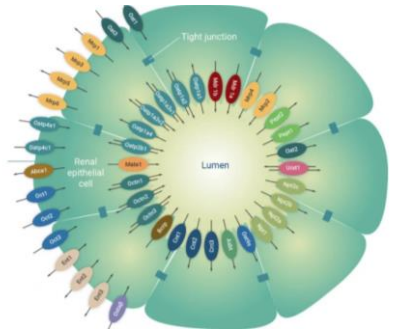


Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al. 2005. Toxciol Sci 83(1): 136-48

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

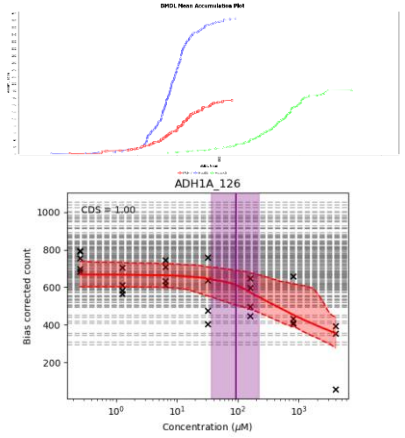


Newcells aProximate™ platform

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

High-Throughput transcriptomics

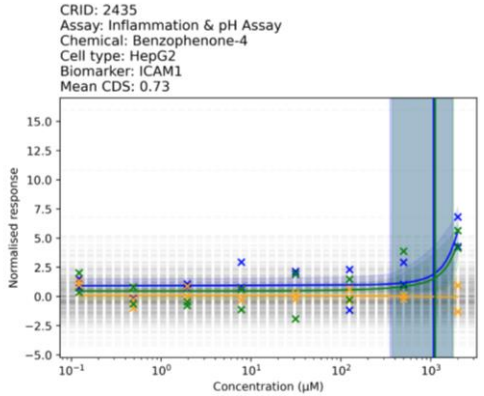
- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- 4 cell models: HepG2, MCF7, HepaRG and aProximate cells
- Dose-response analysis using BMDExpress2 and BIFROST model



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



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

Unilever

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

BERs calculated for all individual NAMs tested

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

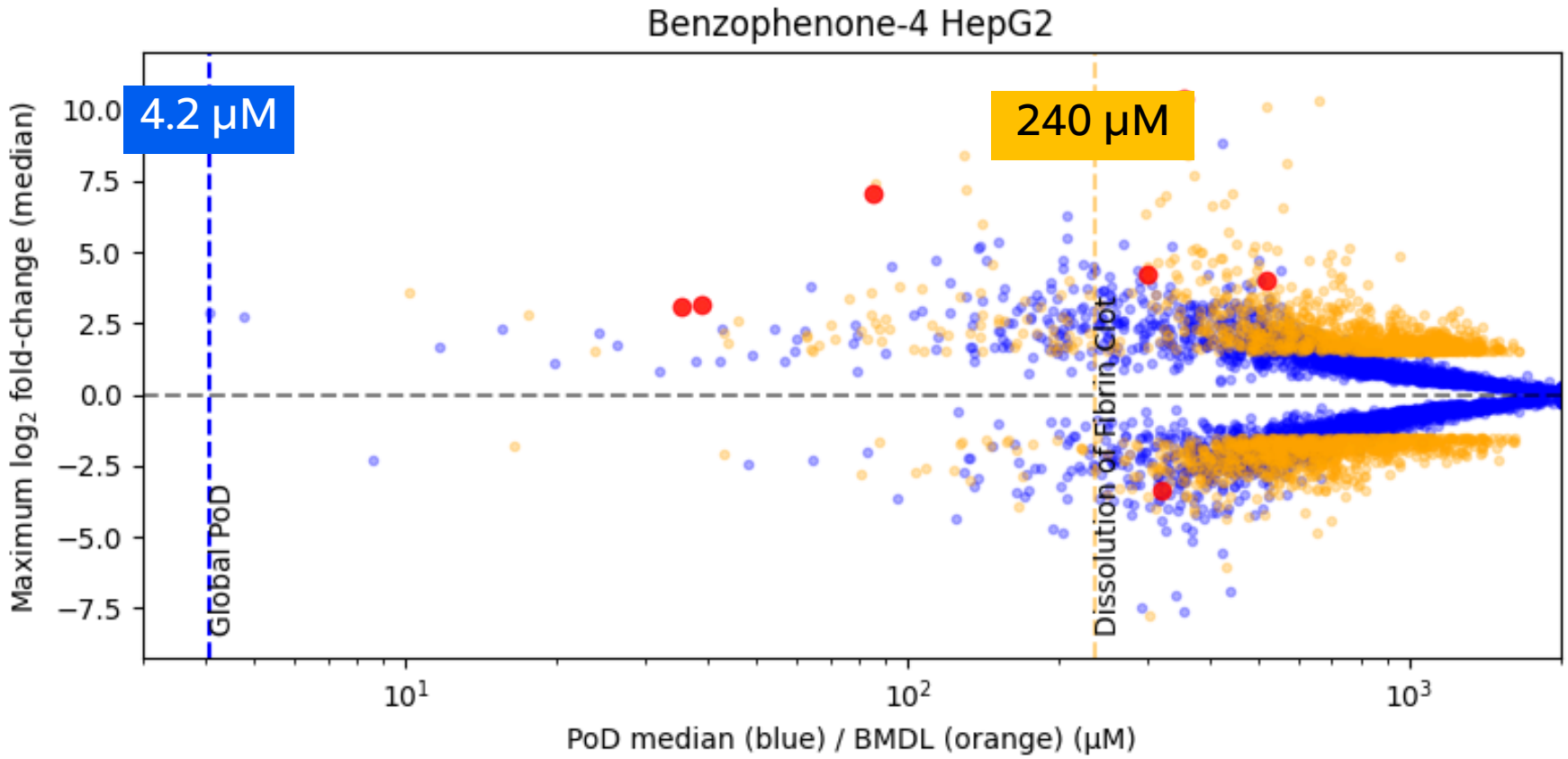
NAM	Cell type	POD_{NAM} Type	POD_{NAM} Value (µM)	BER (using C_{max} of 2.1 µM)
Calux (hTPO-inhibition)	-	LOEC	300	143
Calux (T4 binding to TTR)	-	LOEC	630	300
Renal biomarkers (24 hr exposure)	PTC	Global PoD	>1000	NA
Renal biomarkers (72 hr exposure)	PTC	Global PoD	>1000	NA
HTTr (renal cells) (24 hr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

BER= PoD/Plasma C_{max}

Unilever

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

Very little bioactivity: high throughput transcriptomics in HepG2 cells gave the lowest point of departure



Bioactivity:Exposure Ratios

Gene level = $\frac{4.2}{2.3}$ = 2

Pathway = $\frac{240}{2.3}$ = 114

Acceptable BER?

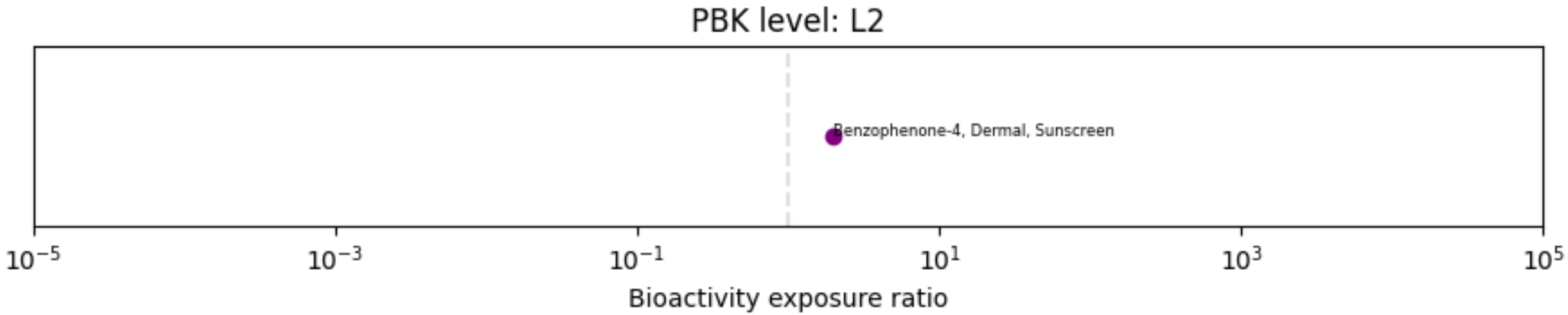
Conceptually, with the following assumptions a BER>1 indicates a low risk of adverse effects in consumers following use of the product:

a) **The in vitro measures of bioactivity provide appropriate biological coverage**

b) **There is confidence that the test systems are at least as sensitive to perturbation as human cells in vivo**

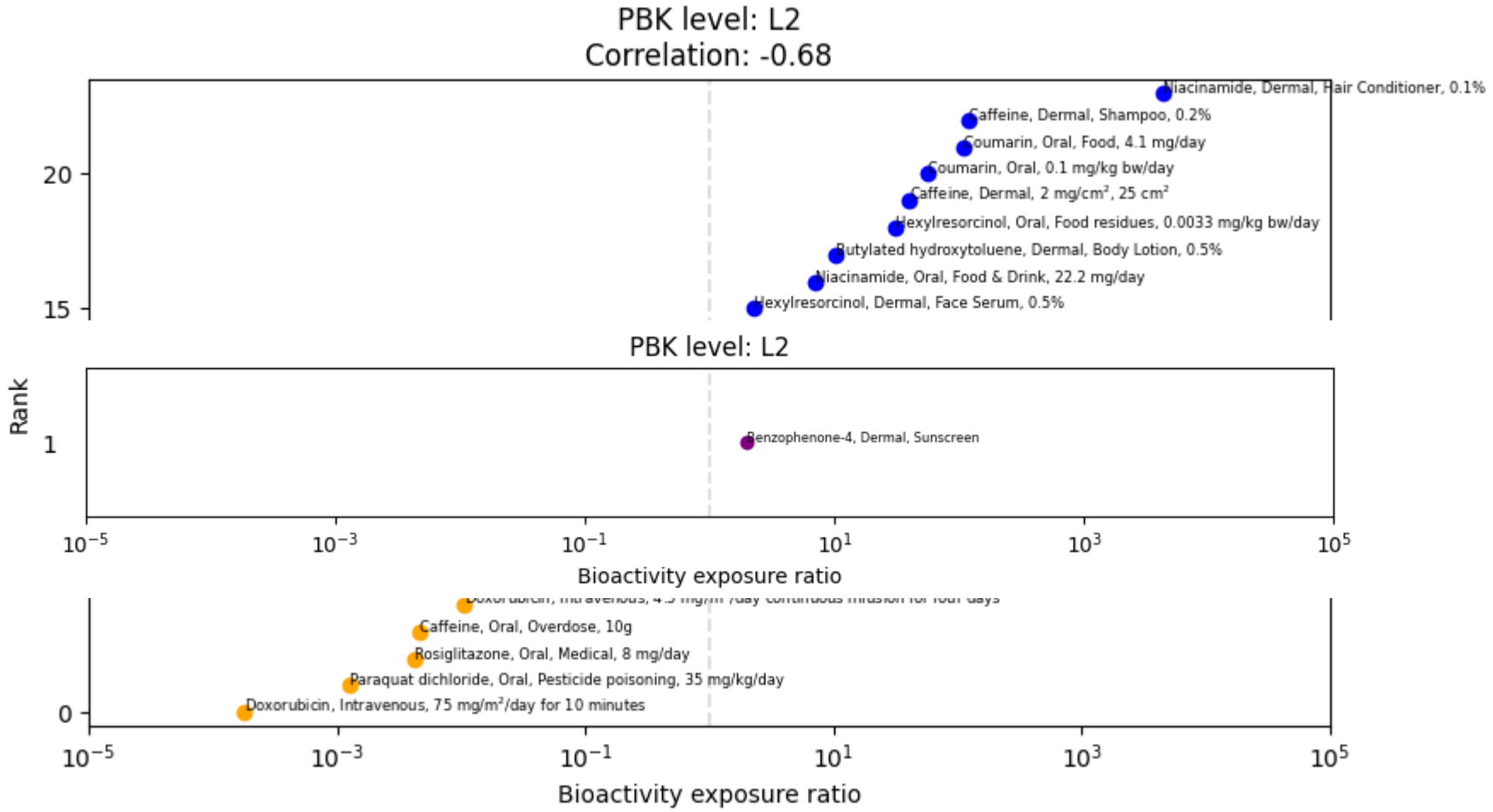
c) **The exposure estimate is conservative for the exposed population**

Considering the determinist BER using lowest PoD (BER=2)



Given all the information before, how confident would you be to conclude low risk?

What if the same approach was applied to 10 other chemicals with varying risk classifications



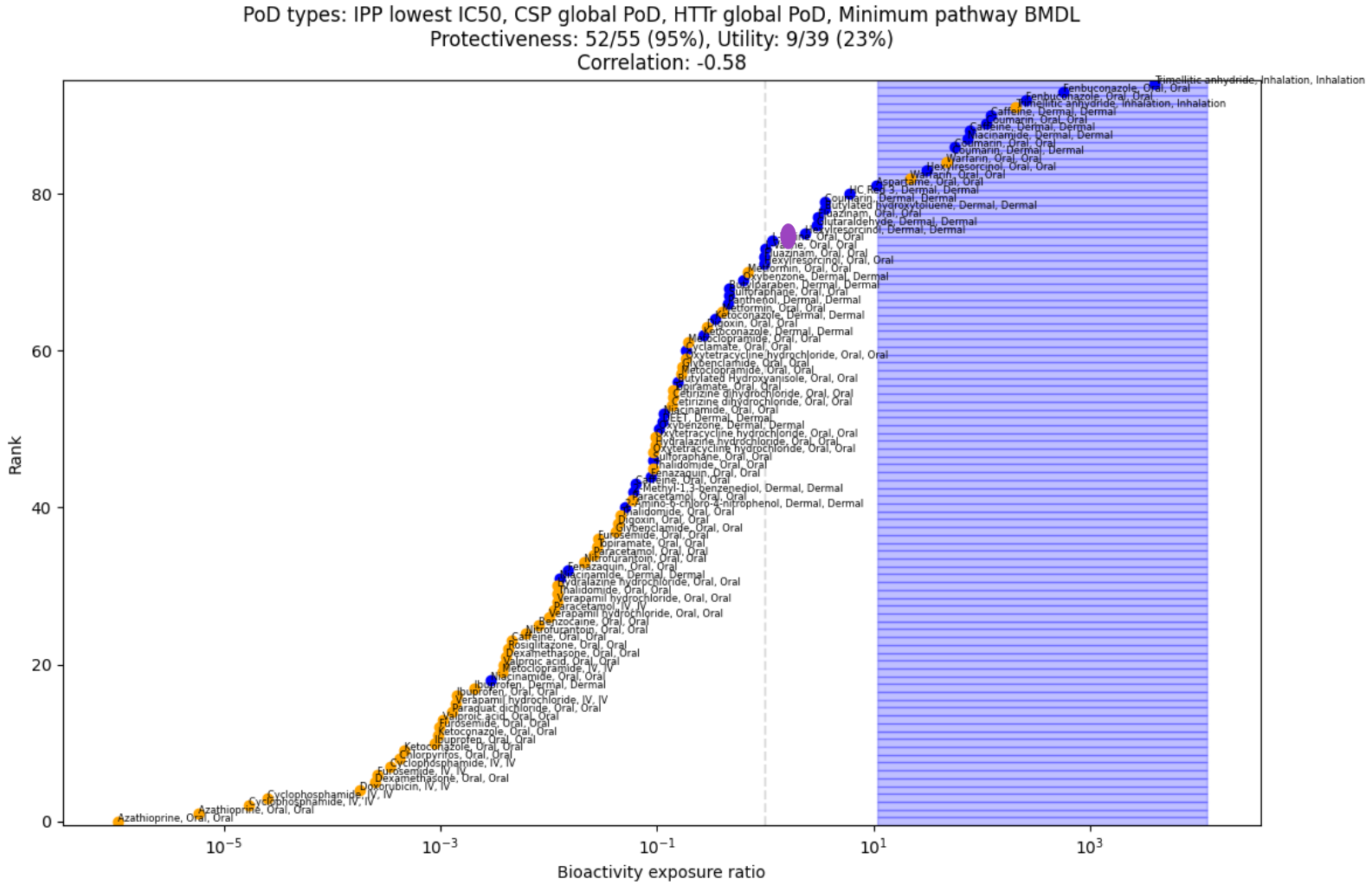
Chemical exposures scenarios

- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs

Note: Low risk is different than low toxicity; it is all about integrating exposure.

Middleton et al., 2022

NAM Systemic toolbox remains protective (95%) when 38 additional chemicals and 70 exposure scenarios were tested



Manuscript in preparation

- **Toolbox not protective for 3/55 of the high-risk exposure scenarios**
- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure
- **Using BER >11, only 23% of the low-risk chemical-scenarios would be correctly identified as such**
  - For the other 77%, refinement by using approaches to distinguish bioactivity from adversity would be needed.

*BER > 11 from Middleton et al., 2022

Conclusion & reflections

- Case studies have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure to make a safety decision without generating animal testing.
- 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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SOLVO
BioDetection Systems
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Thank You

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