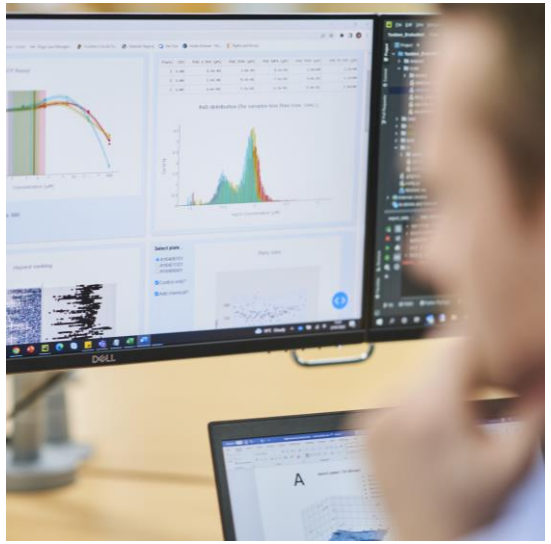
Non-animal methods for systemic toxicity

Matt Dent, Unilever Safety and Environmental Assurance Centre, UK



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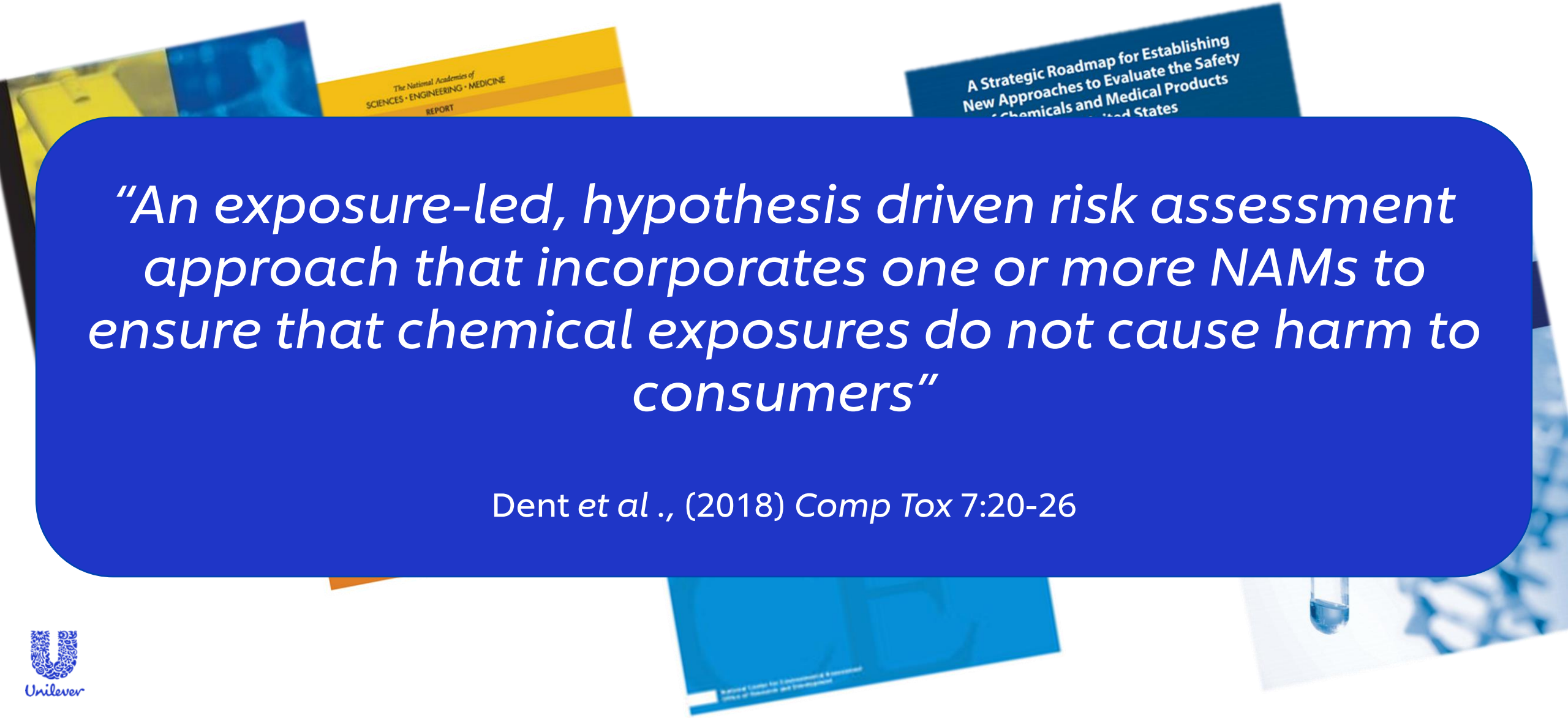
What is next generation risk assessment (NGRA)?



"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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Is NGRA even possible for systemic toxicity?

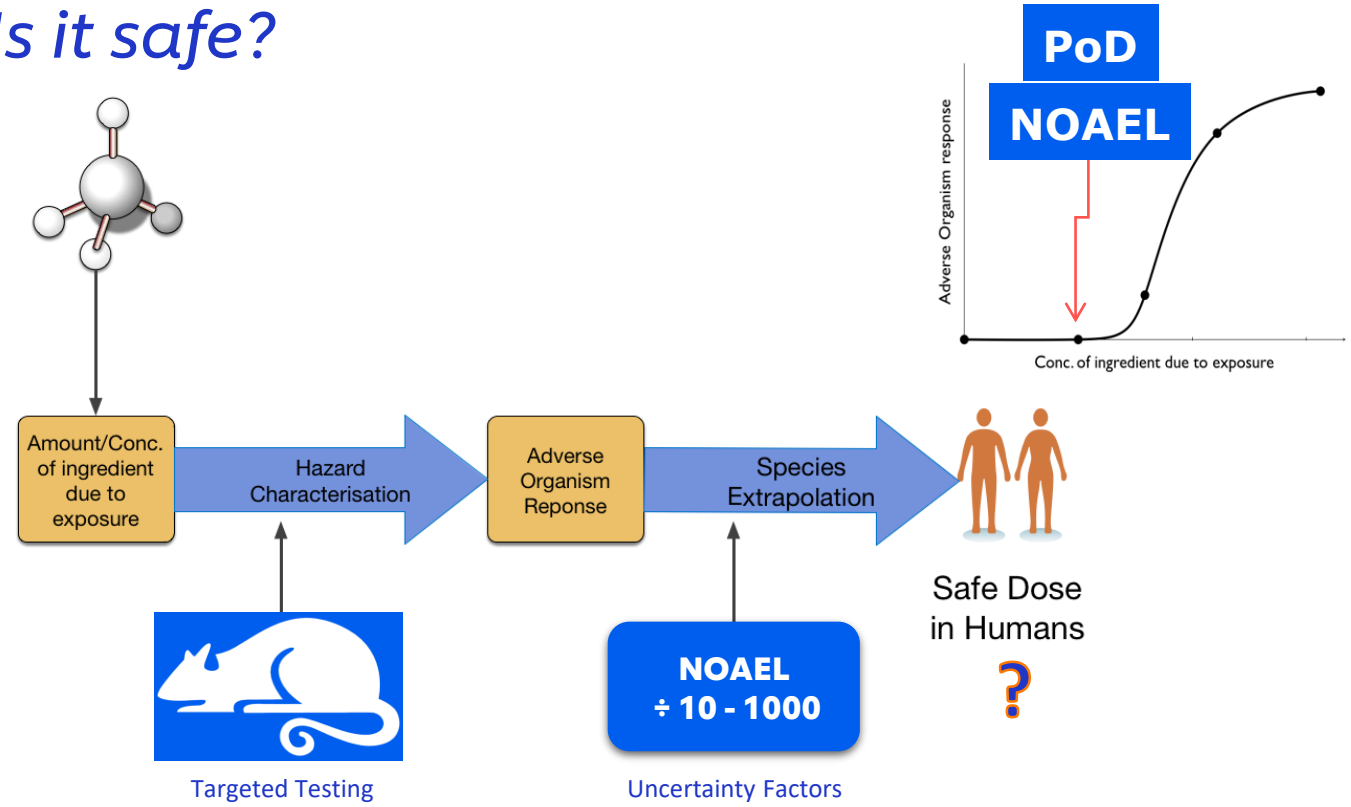
Systemic toxicity isn't like local toxicity

Many possible adversities

ADME considerations

Homeostasis

Is it safe?



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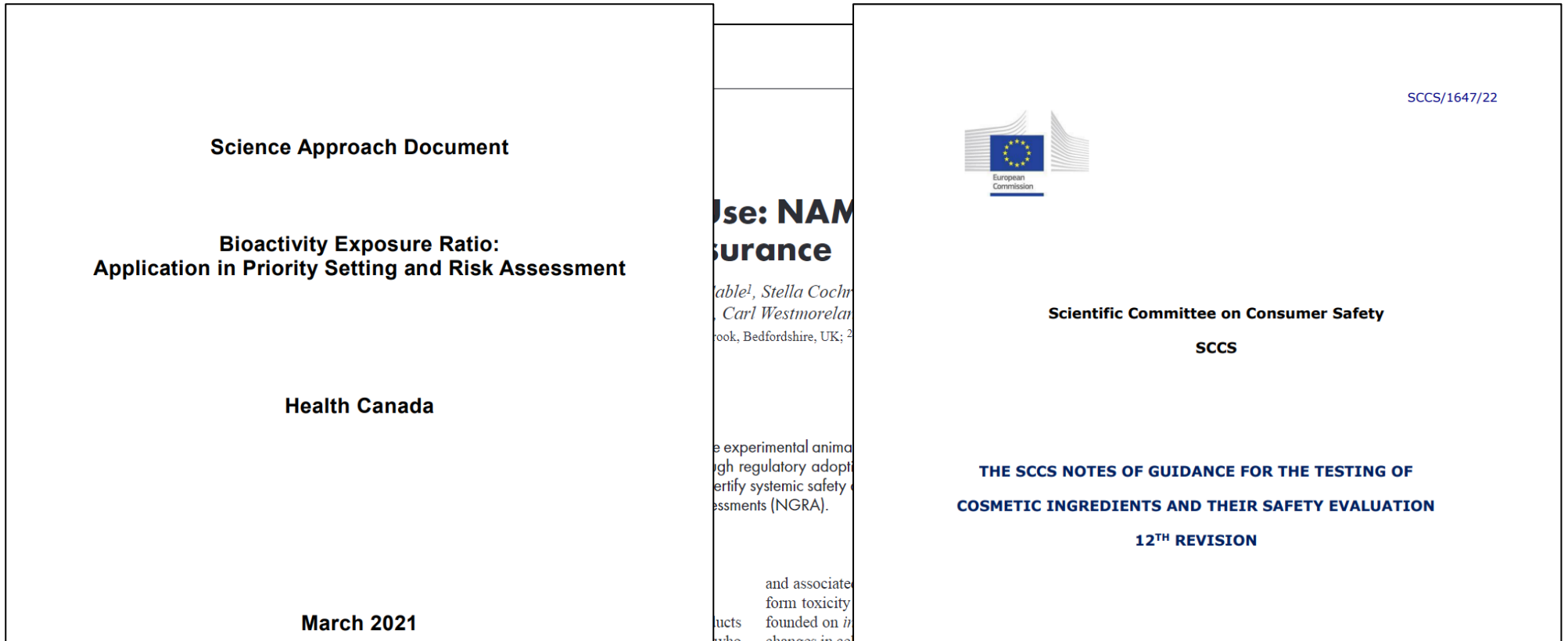
Tiered, exposure-led NGRA means we can make robust safety decisions *today*

- 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)
- Our knowledge will never be complete, but we know enough to start, and to ensure animal testing is only ever used as a last resort



Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

SCCS/1647/22

European Commission

Scientific Committee on Consumer Safety

SCCS

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

12TH REVISION

Principles of NGRA from ICCR

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparent and documented

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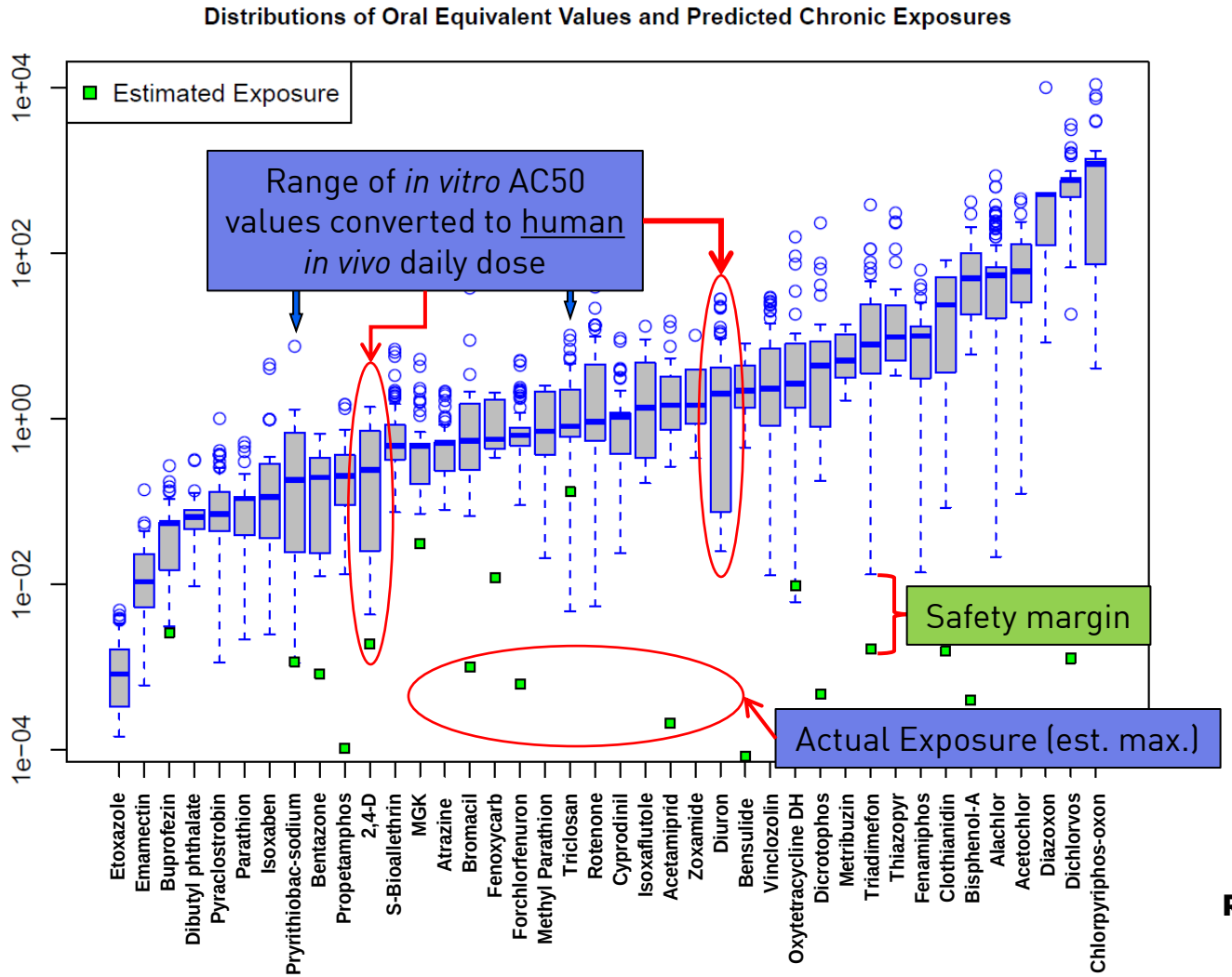
Dent *et al* ., (2018) *Comp Tox* 7:20-26

Paradigm shift for systemic safety - Protection not Prediction

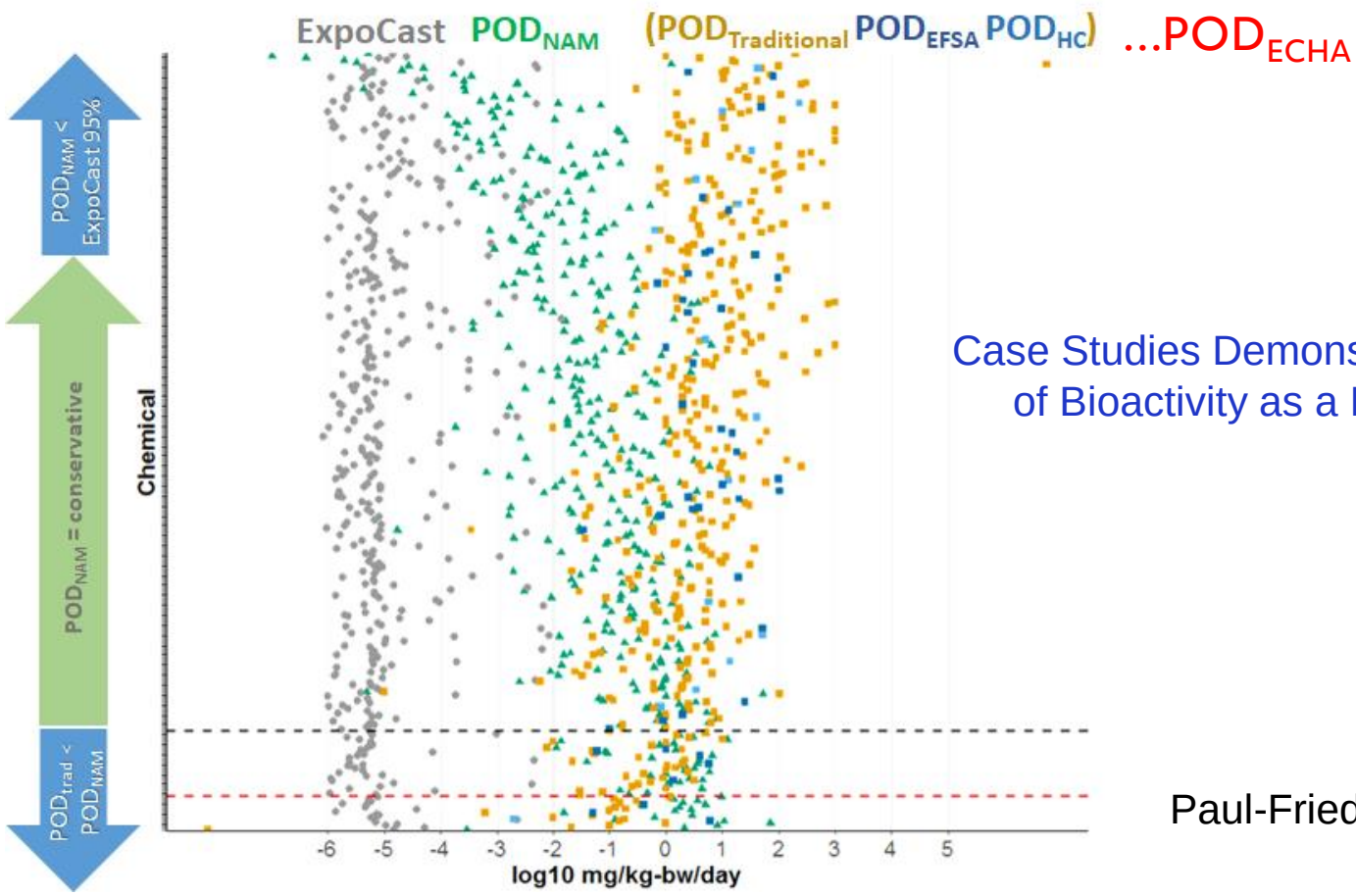


Graphic from Dr Rusty Thomas, EPA, with thanks

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

Points of Departure from NAMs can be protective



Case Studies Demonstrating Application of Bioactivity as a Protective POD

Paul-Friedman et al., 2020

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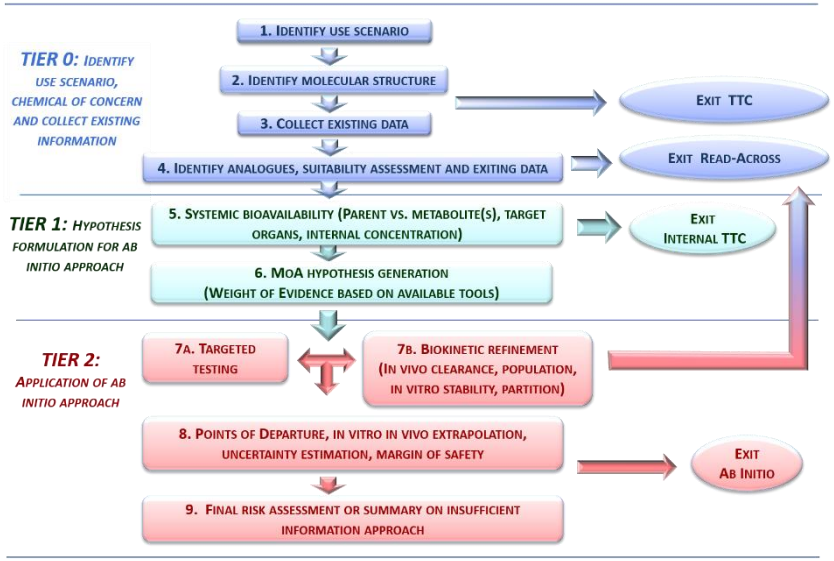
Guiding principles for the *ab initio* NGRA applied to the Benzophenone-4 case study



Computational Toxicology 7 (2018) 20–26


Contents lists available at ScienceDirect
Computational Toxicology
journal homepage: www.elsevier.com/locate/comtox
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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]

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
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

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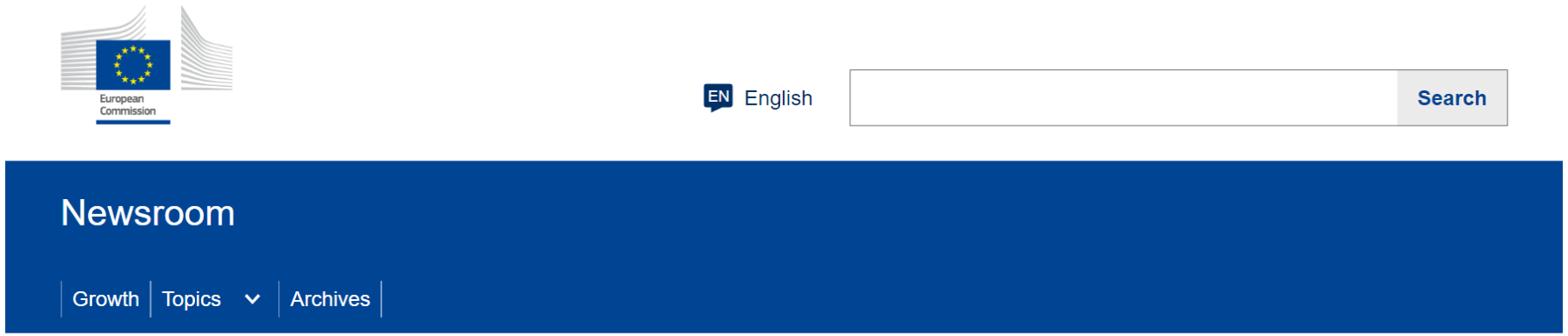
Benzophenone-4 (BP-4) case study



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

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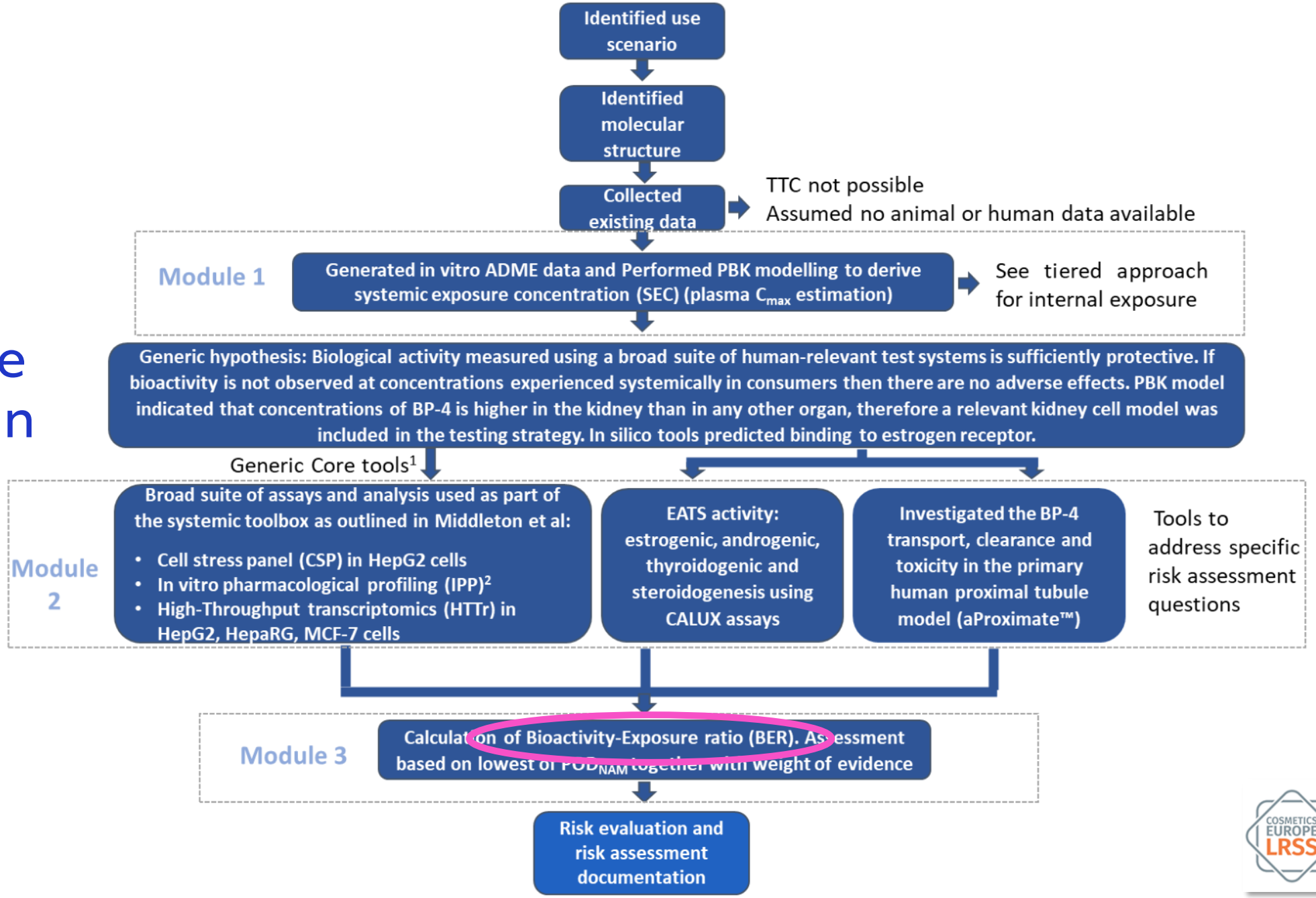
COSMETICS EUROPE LRSS

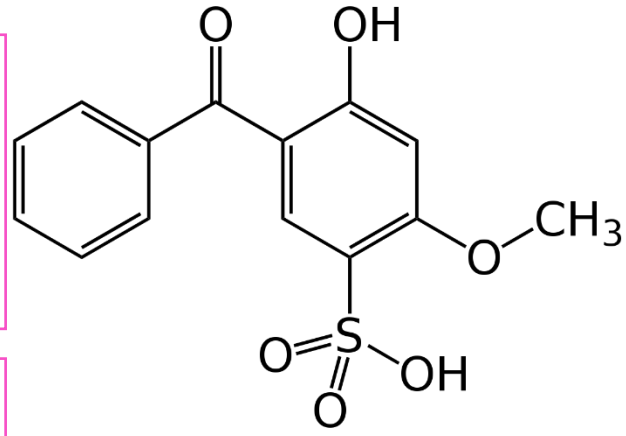
Approach based on previous case studies and in accordance with ICCR Principles



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 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

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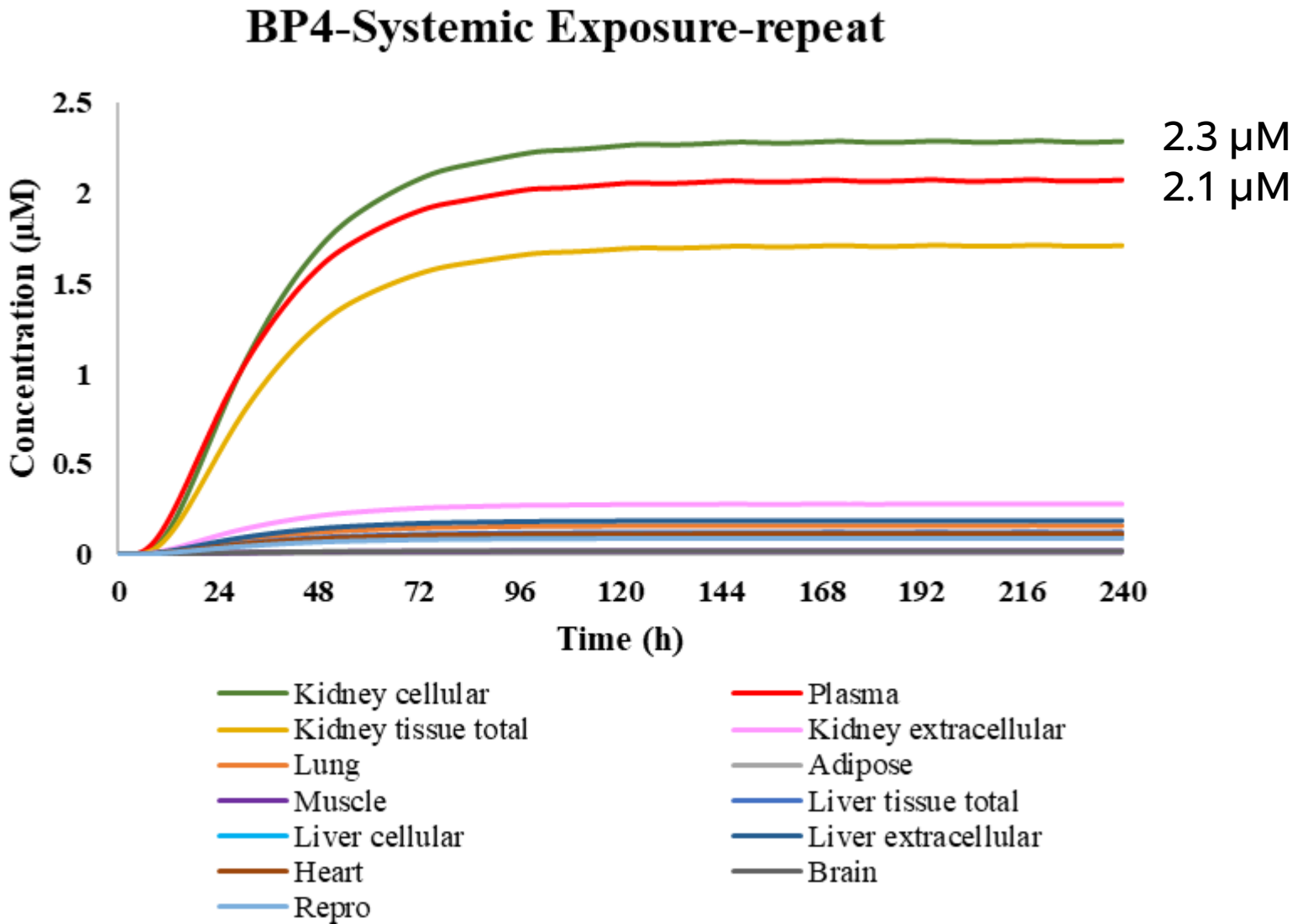
Moxon et al. 2020. Toxicology in Vitro, Volume 63, 104746.

PBK model simulation of C_{max}



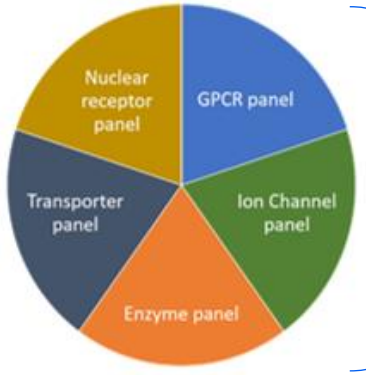
2.3 µM

2.1 µM

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

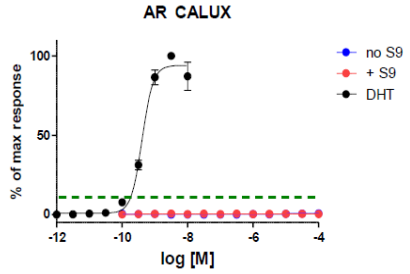
In vitro pharmacological profiling



~83 targets

eurofins | Cerep

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

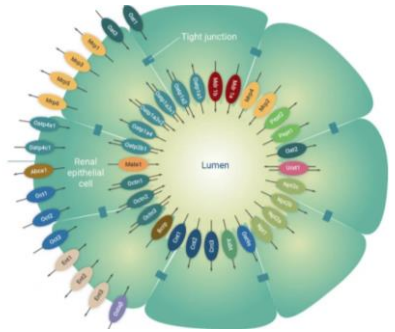


Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al. 2005. Toxciol Sci 83(1): 136-48

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

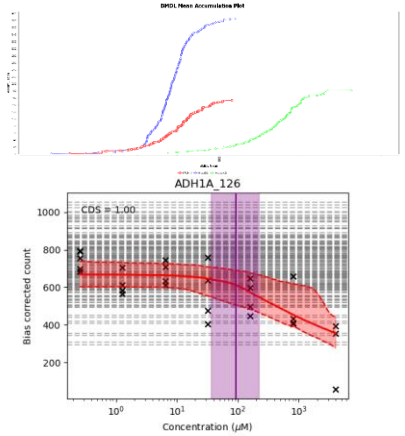


Newcells aProximate™ platform

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

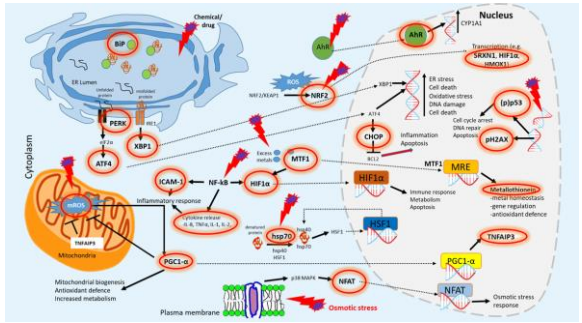


Image kindly provided by Paul Walker (Cyprotex)

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

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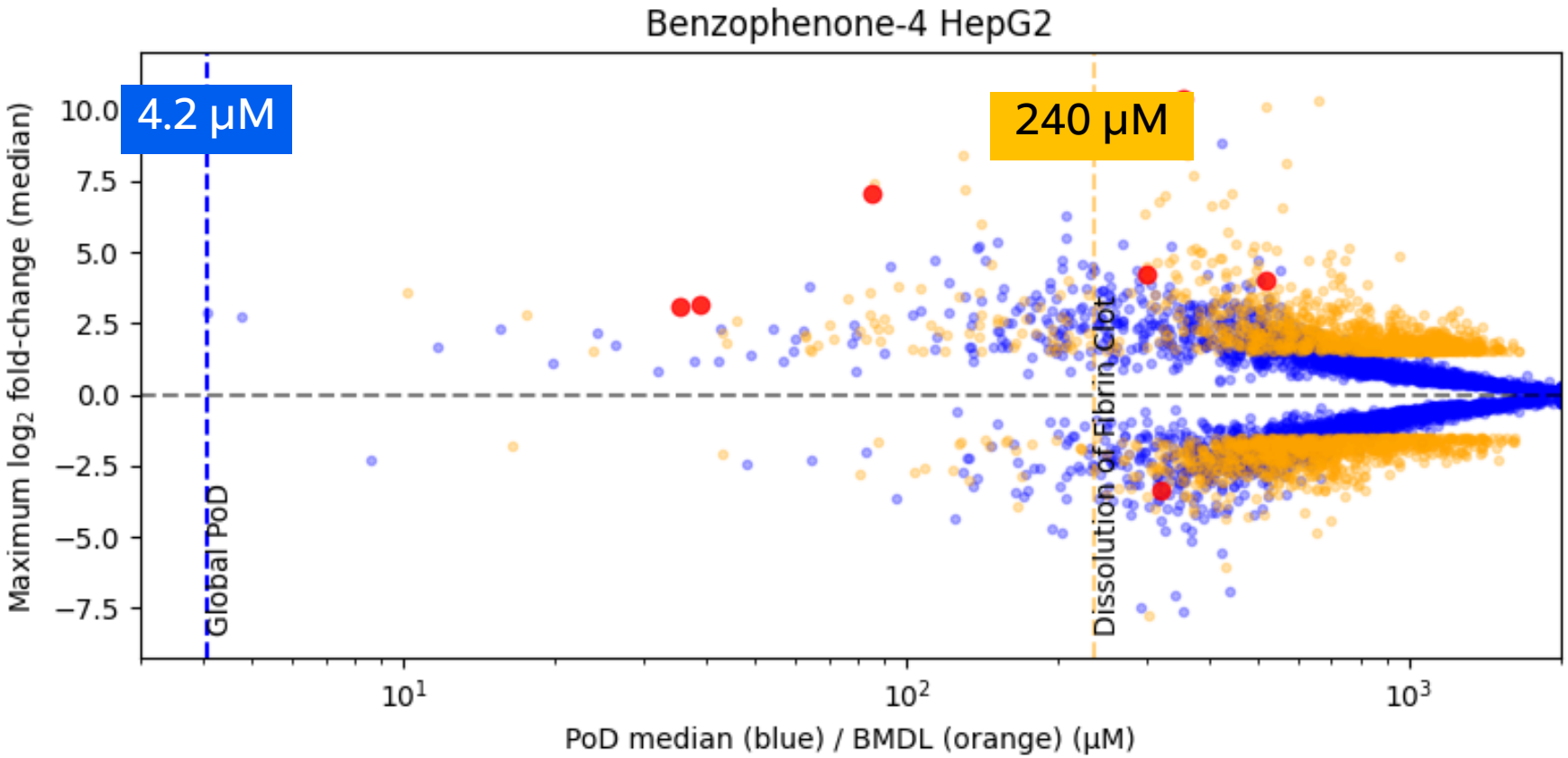
Results from the key NAMs- Deriving Points of Departure (PoDs)

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

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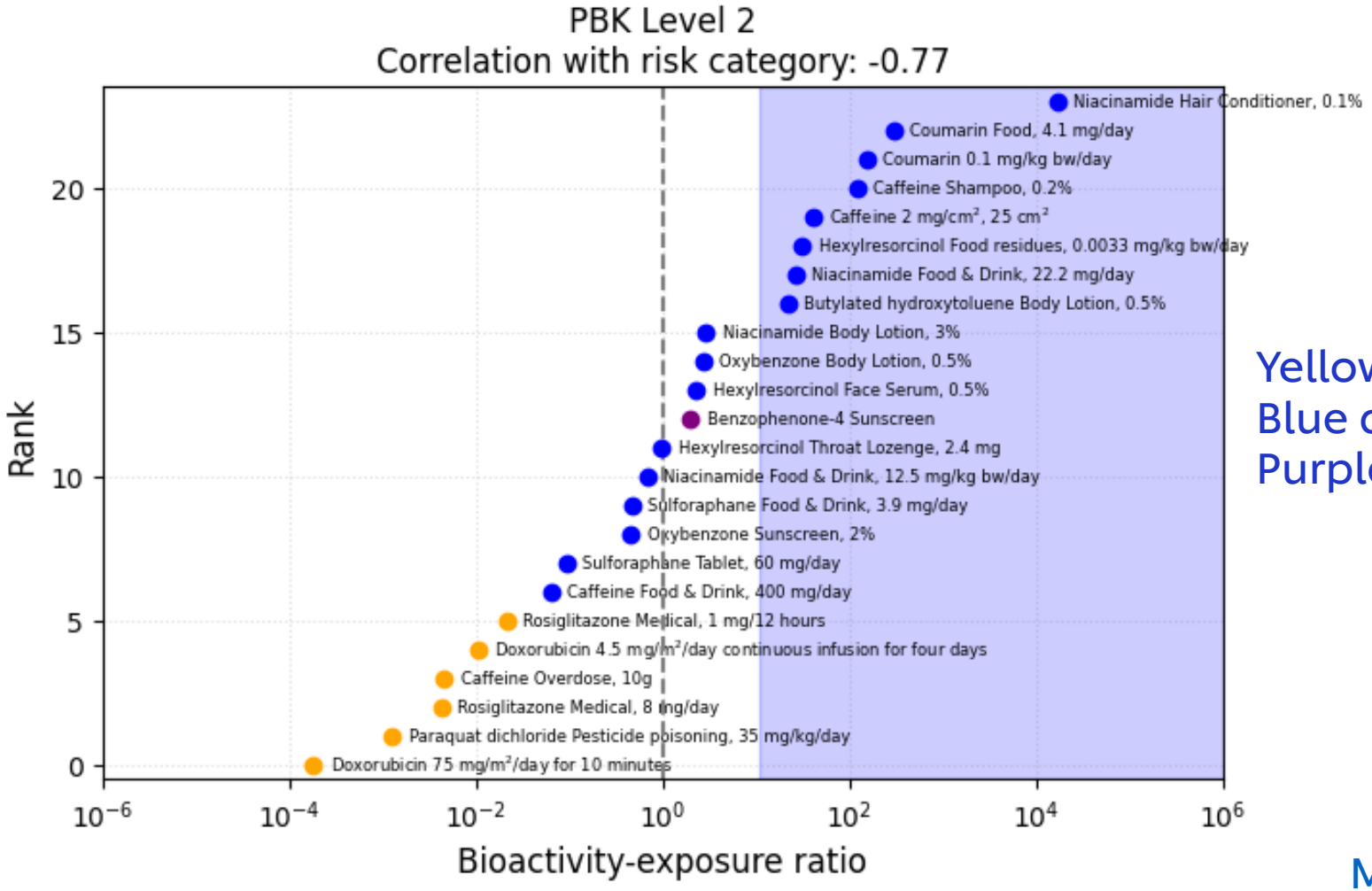
Benchmarking to determine a low-risk BER



Yellow dots: high risk benchmarks
Blue dots: low risk benchmarks
Purple dot: case study

[Middleton et al., 2022]

Conclusion

- Use of tiered, exposure-led approaches allows safety decisions to be made for systemic effects without animal test data
- The ICCR Principles help to formulate the problem and direct the assessment.
- ‘Early tier’ in vitro screening tools show promise for use in a protective rather than predictive capacity.

Acknowledgements

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Thank You

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