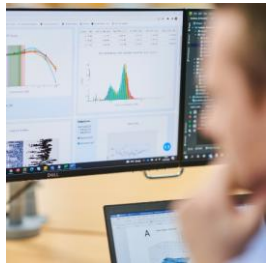
Next generation risk assessment case study for a cosmetic ingredient

Matt Dent, Unilever

SERS
Safety, Environmental & Regulatory Science



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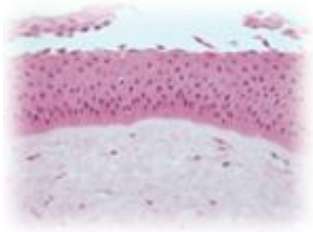
Cosmetic safety assessment: key safety considerations

Exposure data (external/applied dose and internal exposure)

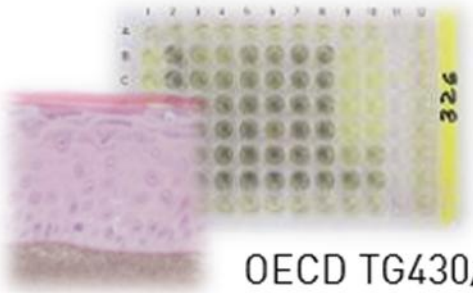
Corrosion/irritation (skin/eye)
Phototoxicity
Mutagenicity/genotoxicity
Skin sensitisation
Systemic toxicity (focus on repeat dose)
Reproductive toxicity
Carcinogenicity

SCCS Notes of Guidance, 12th Revision

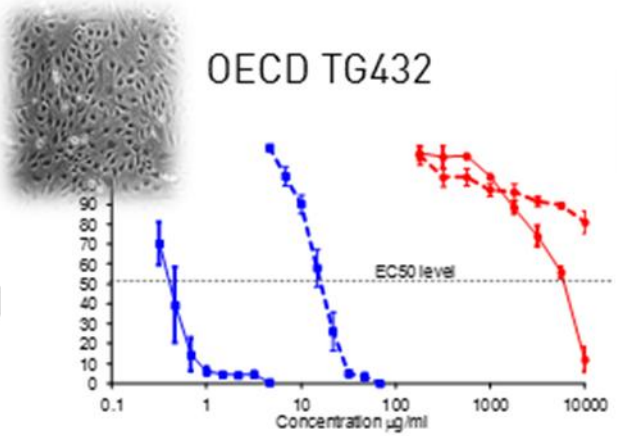
Use of Existing OECD *In Vitro* Approaches



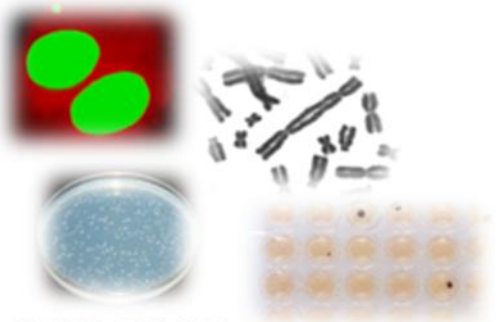
OECD TG437



OECD TG430/431
OECD TG439

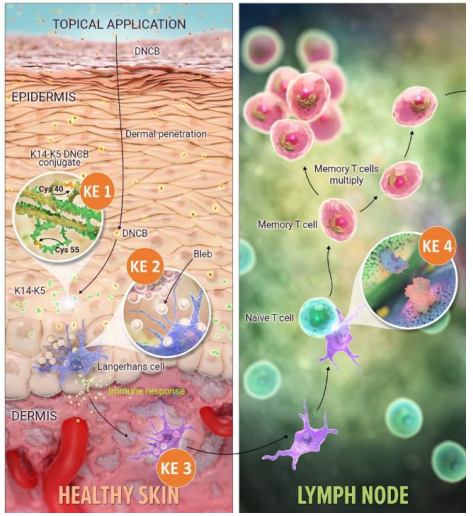


OECD TG432



OECD TG473
OECD TG471
OECD TG476

OECD TG442A-E



https://doi.org/10.1080/10408444.2024.2308816

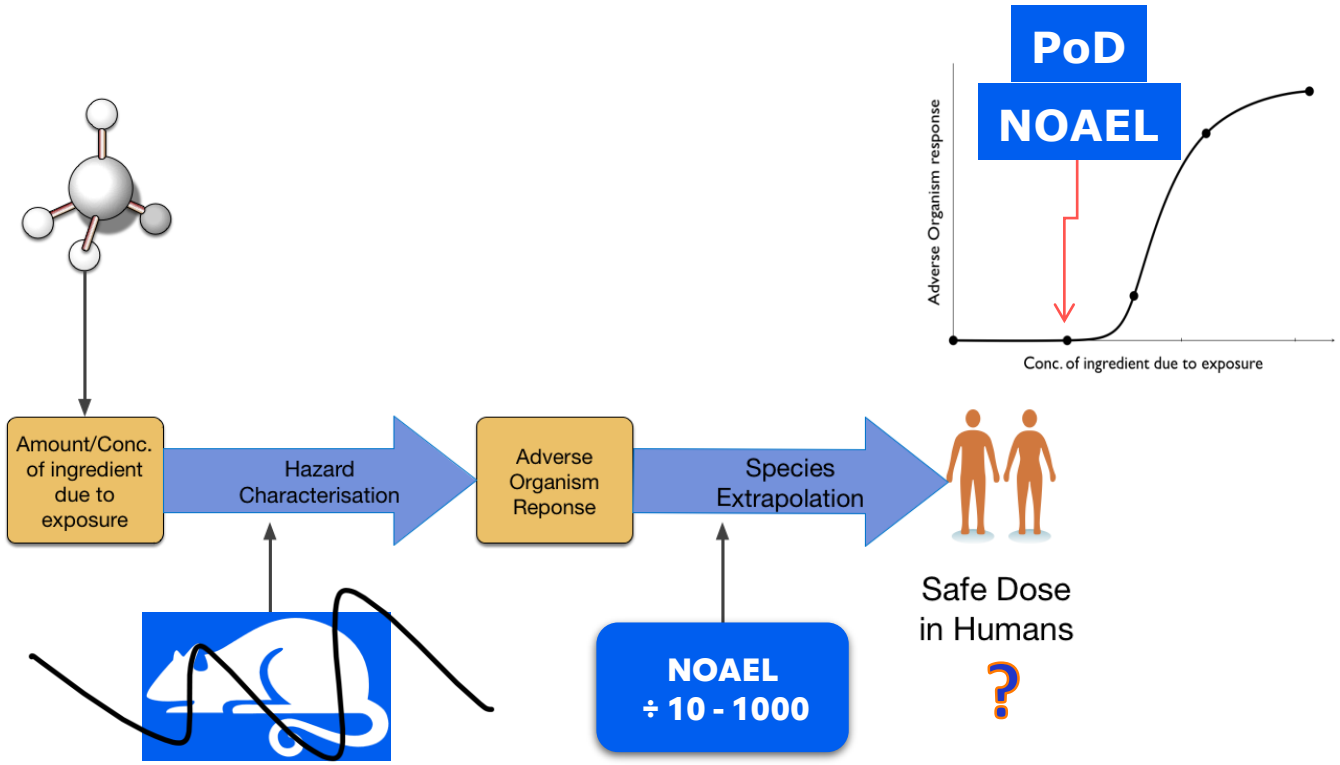
+OECD G 497

Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity…

…what about systemic effects?

Are non-animal safety assessments even possible for systemic toxicity?

Systemic toxicity isn't like local toxicity



Many possible adversities...ADME considerations...Homeostasis

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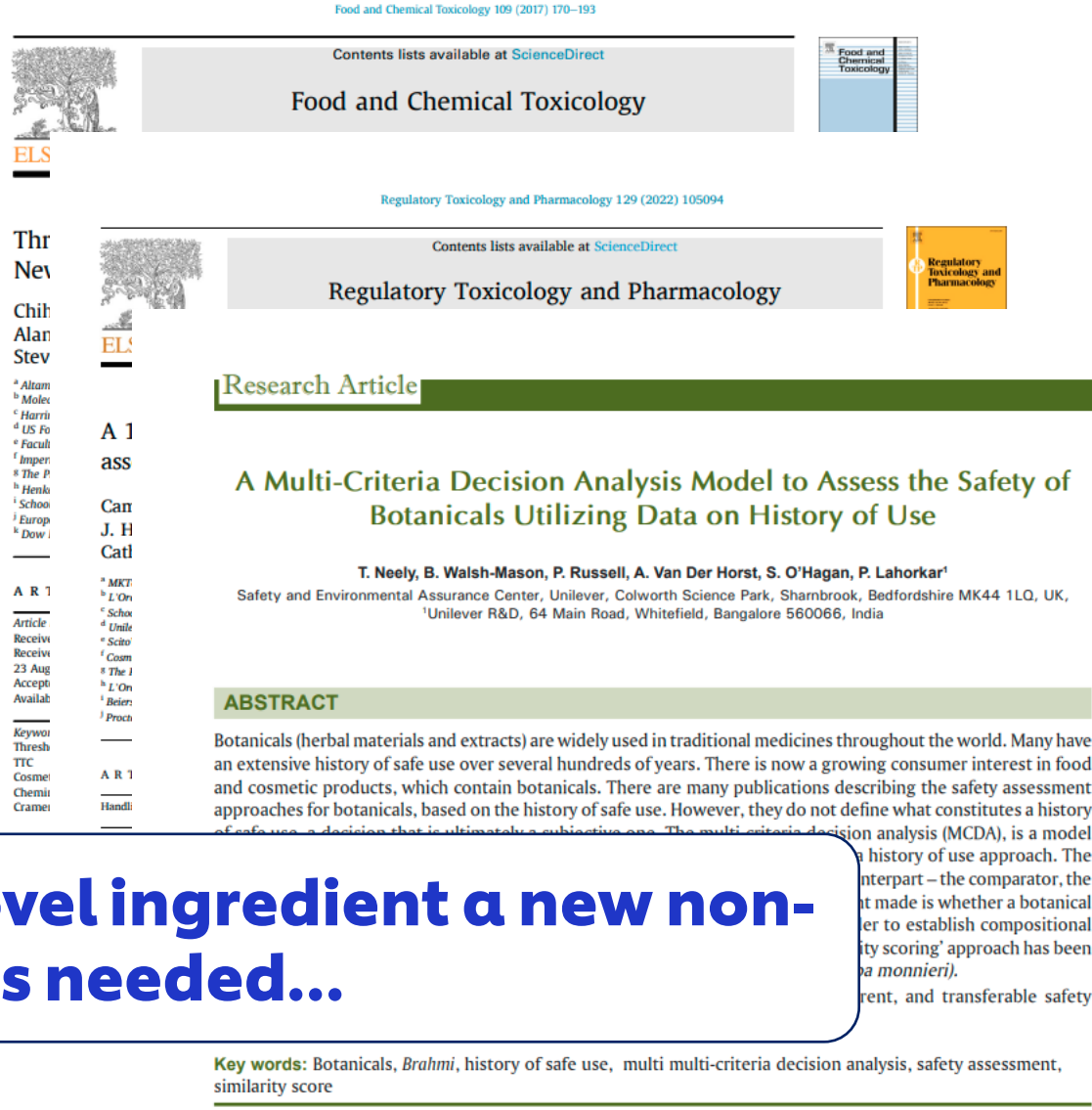
Well-established approaches for systemic toxicity

Threshold of Toxicological Concern
(Yang et al 2017) https://doi.org/10.1016/j.fct.2017.08.043

Read across
(Alexander-White et al 2022)
https://doi.org/10.1016/j.yrtph.2021.105094

History of Safe Use
(Neely et al 2011) PMID: 22025816



Food and Chemical Toxicology 109 (2017) 170–193

Contents lists available at ScienceDirect

Food and Chemical Toxicology

Regulatory Toxicology and Pharmacology 129 (2022) 105094

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

Research Article

A Multi-Criteria Decision Analysis Model to Assess the Safety of Botanicals Utilizing Data on History of Use

T. Neely, B. Walsh-Mason, P. Russell, A. Van Der Horst, S. O'Hagan, P. Lahorkar[1]
Safety and Environmental Assurance Center, Unilever, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK,
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ABSTRACT

Botanicals (herbal materials and extracts) are widely used in traditional medicines throughout the world. Many have an extensive history of safe use over several hundreds of years. There is now a growing consumer interest in food and cosmetic products, which contain botanicals. There are many publications describing the safety assessment approaches for botanicals, based on the history of safe use. However, they do not define what constitutes a history of safe use, a decision that is ultimately a subjective one. The multi-criteria decision analysis (MCDA), is a model history of use approach. The nterpart – the comparator, the t made is whether a botanical er to establish compositional ity scoring' approach has been *a monnieri).* rent, and transferable safety

Key words: Botanicals, *Brahmi*, history of safe use, multi multi-criteria decision analysis, safety assessment, similarity score

For 'significant' exposures to a novel ingredient a new non-animal paradigm is needed...

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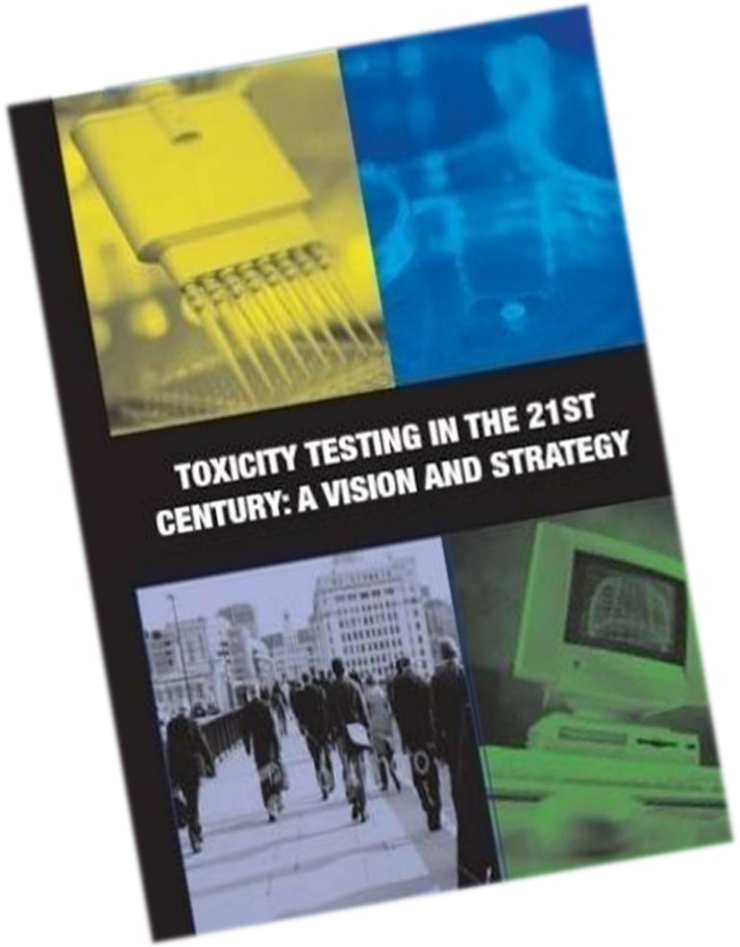
2007 Toxicity Testing in the 21st Century (TT21C)



"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."

Perturbation of 'toxicity pathways' and stress responses

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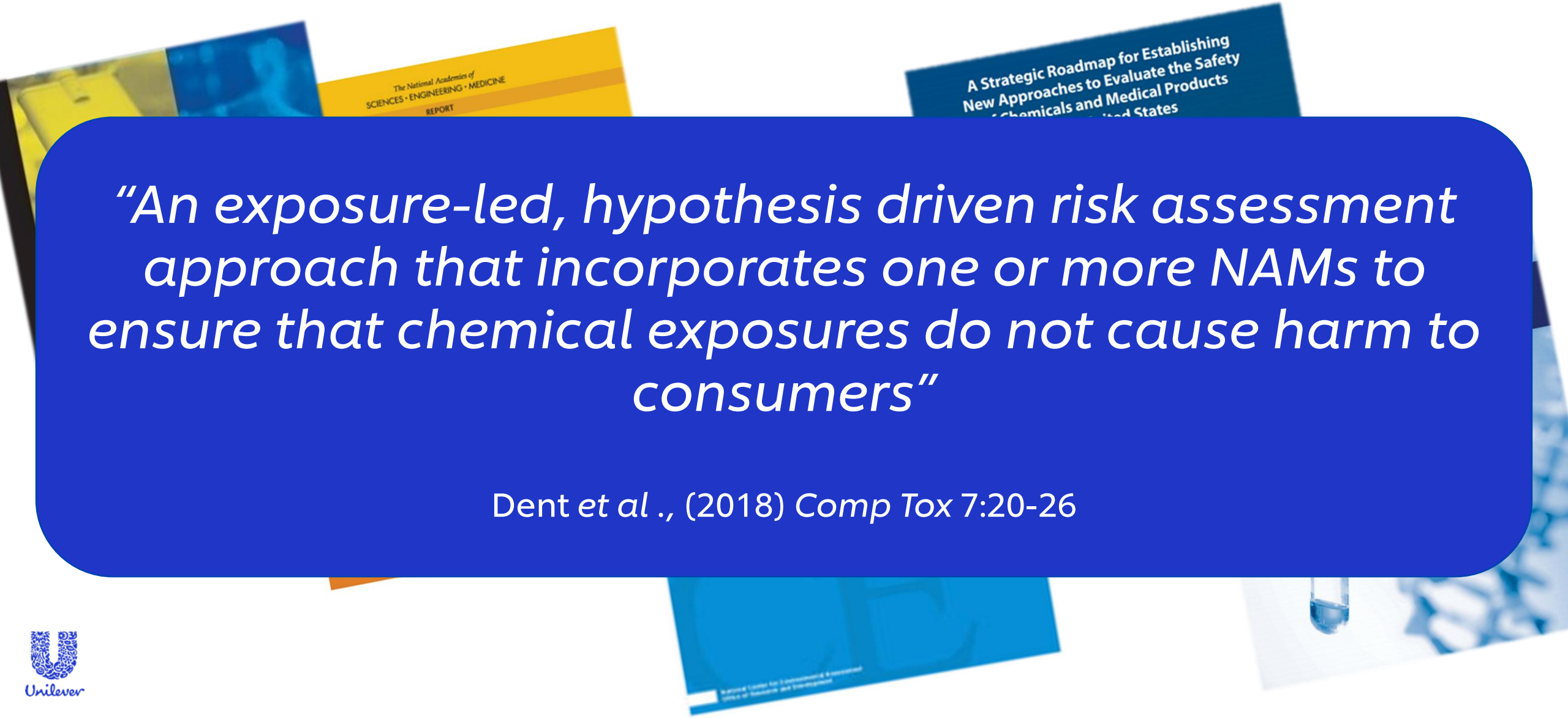
What is next generation risk assessment (NGRA)?



"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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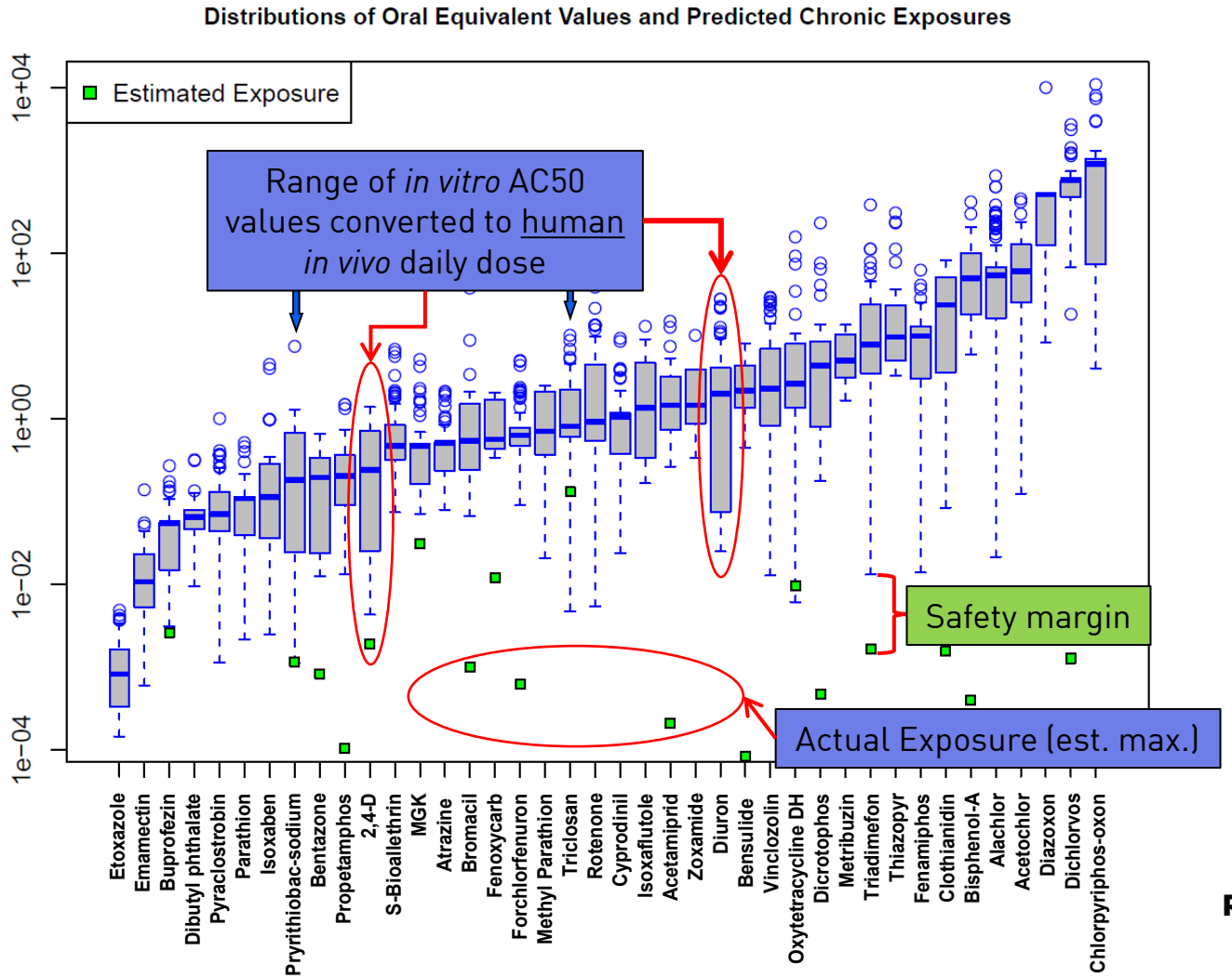
Paradigm shift for systemic safety - Protection not Prediction

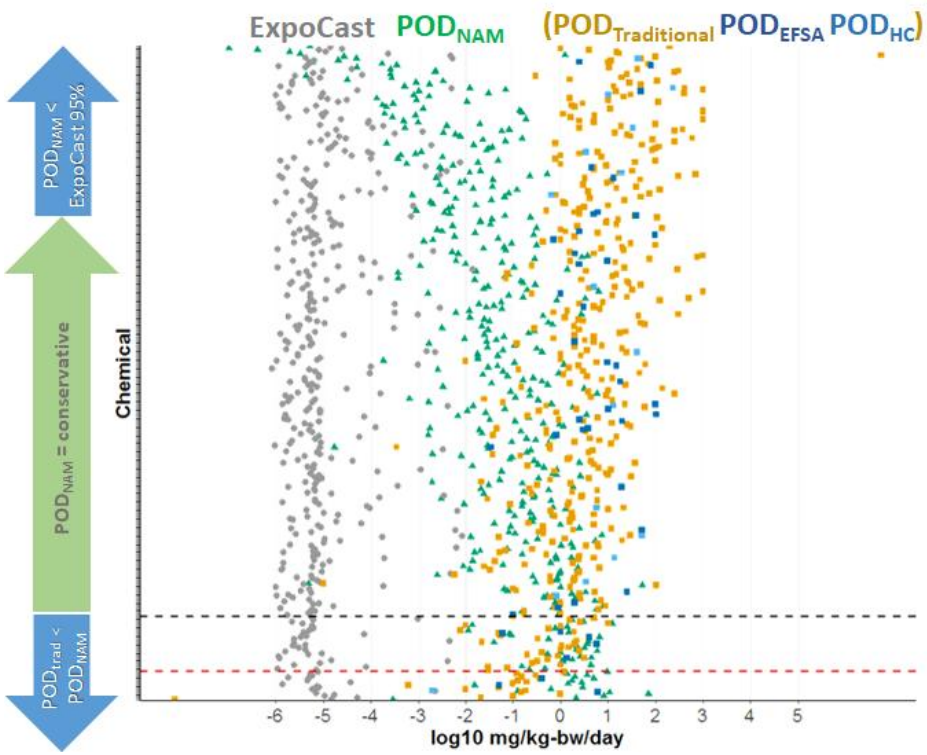


Graphic from Dr Rusty Thomas, EPA, with thanks

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.**

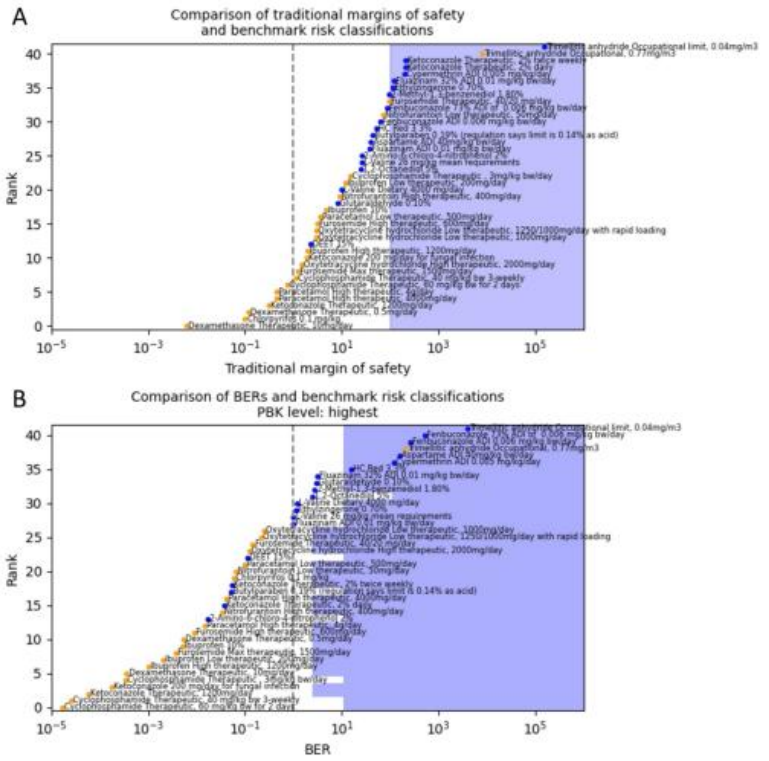
Rotroff, *et al.* Tox.Sci 2010

Points of Departure from NAMs can be protective



Case Studies Demonstrating Application of Bioactivity as a Protective POD

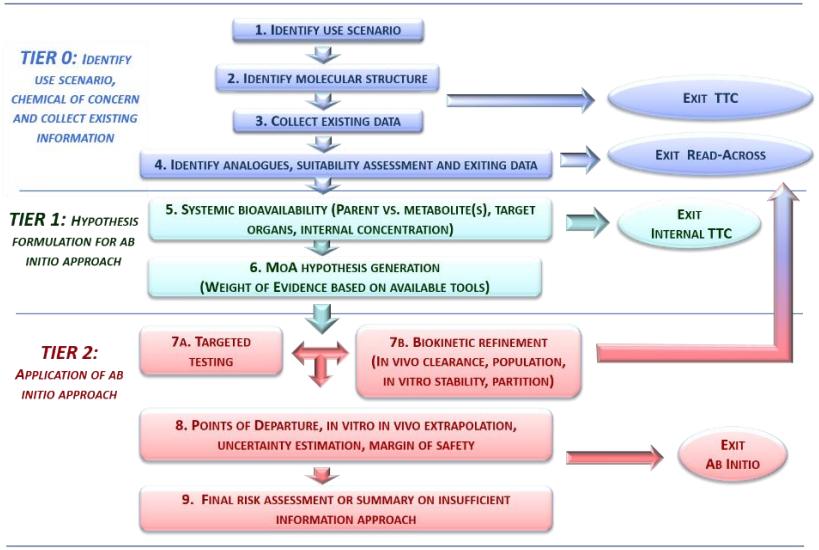
Paul-Friedman et al., 2020



NAM-based assessments can be at least as protective as animal-based assessments

Cable et al., 2025 (Toxicological Sciences)

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



https://doi.org/10.1016/j.comtox.2017.10.001

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

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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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

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]

ICCR International Cooperation on Cosmetics Regulation

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

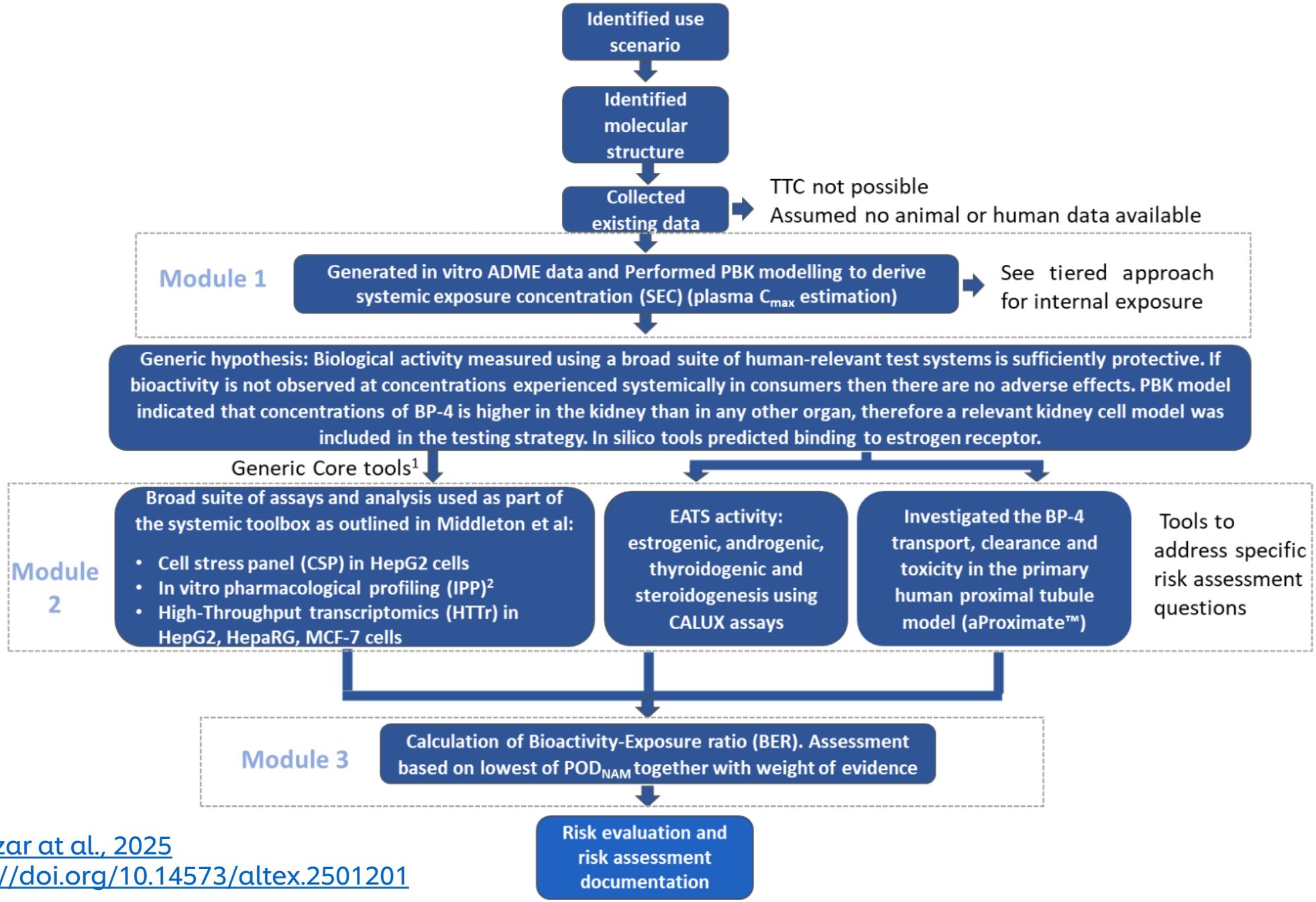
In 2019, the European Commission defined a list of 28 cosmetic ingredients with potential endocrine activity

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

Objective of the case study:

- To assess whether a tiered NGRA approach is sufficiently protective and useful to answer a real-life question

Baltazar at al., 2025
https://doi.org/10.14573/altex.2501201

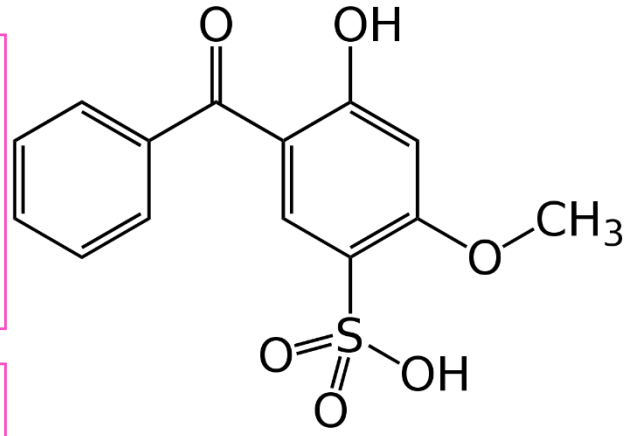


Baltazar at al., 2025
https://doi.org/10.14573/altex.2501201

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

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

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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

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 is negligible (set to 0 in PBK).**

Can BP-4 be taken up by the cells?

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 may 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
- Overall it appeared that rate of efflux similar to influx – net 0

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.
- Renal clearance by GF

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

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PBK model simulation of C_{max}

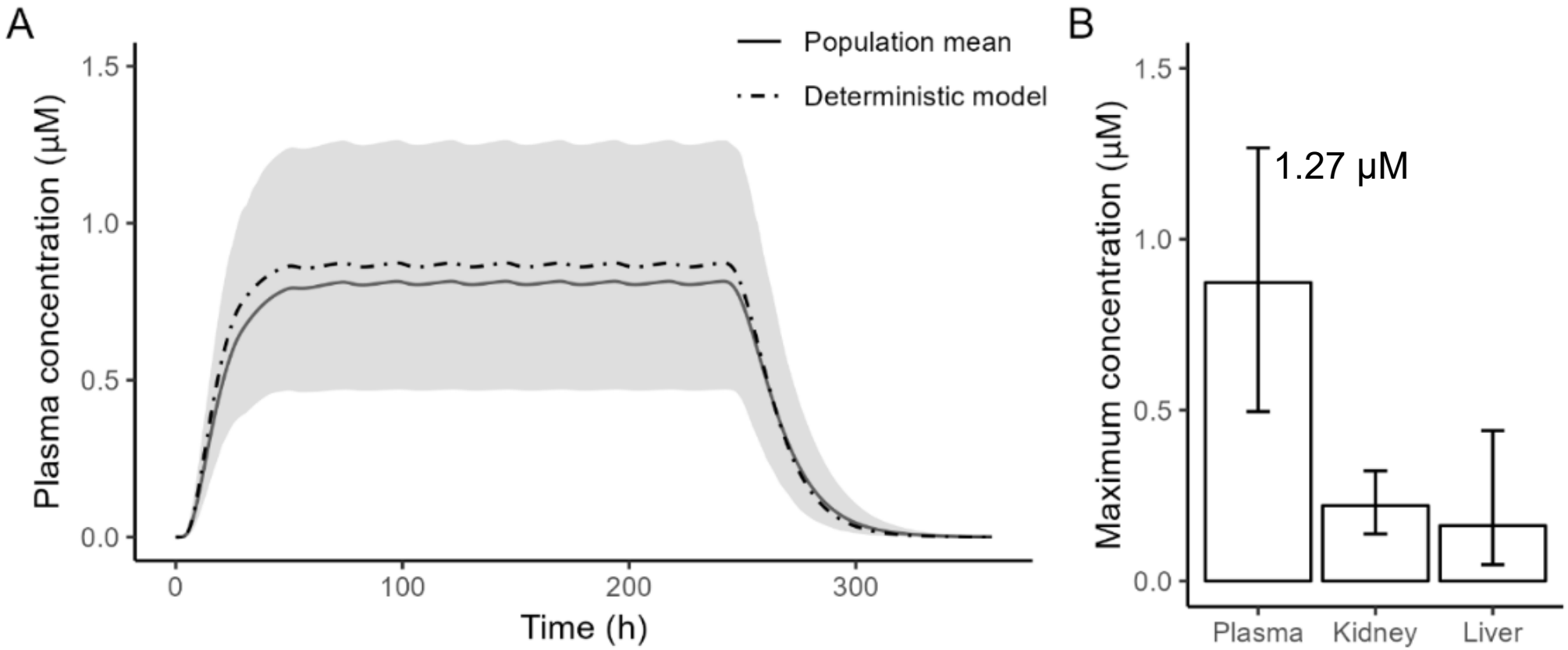
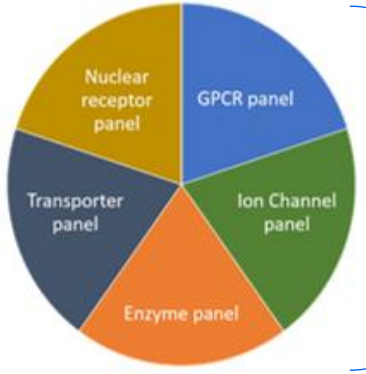


Fig. 2: Kinetic profile of BP-4 after dermal application of a body lotion
A) Population PBK simulation results (time course data and C_{max}) on benzophenone-4 concentrations in plasma after repeated exposure of body lotion 18g/day, i.e., 9g two times per day for a period of 10 days, with 5% benzophenone-4, on the whole body. Solid line represents the population mean and grey band represents the 90% credible interval. Dashed line corresponds to the deterministic plasma C_{max} value for a 30-year-old Caucasian 60 kg female. B) Peak plasma and organ concentrations for population.

Baltazar at al., 2025 https://doi.org/10.14573/altex.2501201

Characterisation of bioactivity- key NAMs

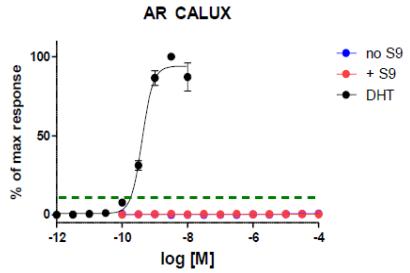
In vitro pharmacological profiling



79 targets

eurofins | Cerep

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

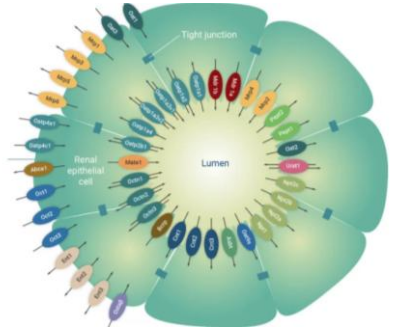


Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al. 2005. Toxciol Sci 83(1): 136-48

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

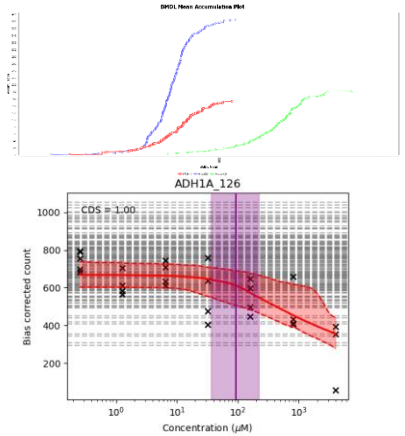


Newcells aProximate™ platform

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

High-Throughput transcriptomics (HTTr)

- 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

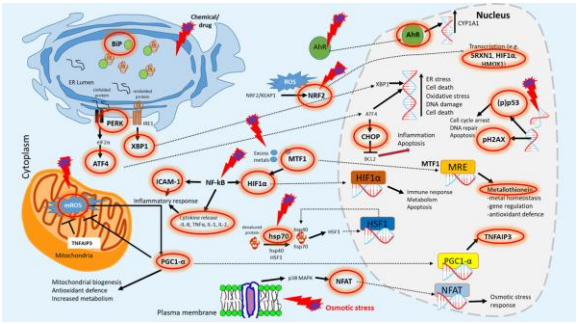


Image kindly provided by Paul Walker (Cyprotex)

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

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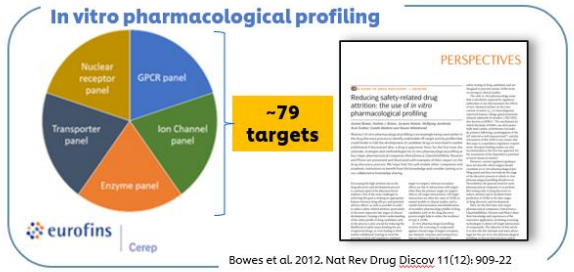
Risk Assessment Outcome

BIOACTIVITY



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High-Throughput transcriptomics (HTTr)

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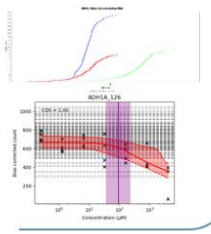

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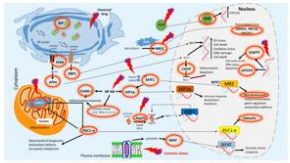
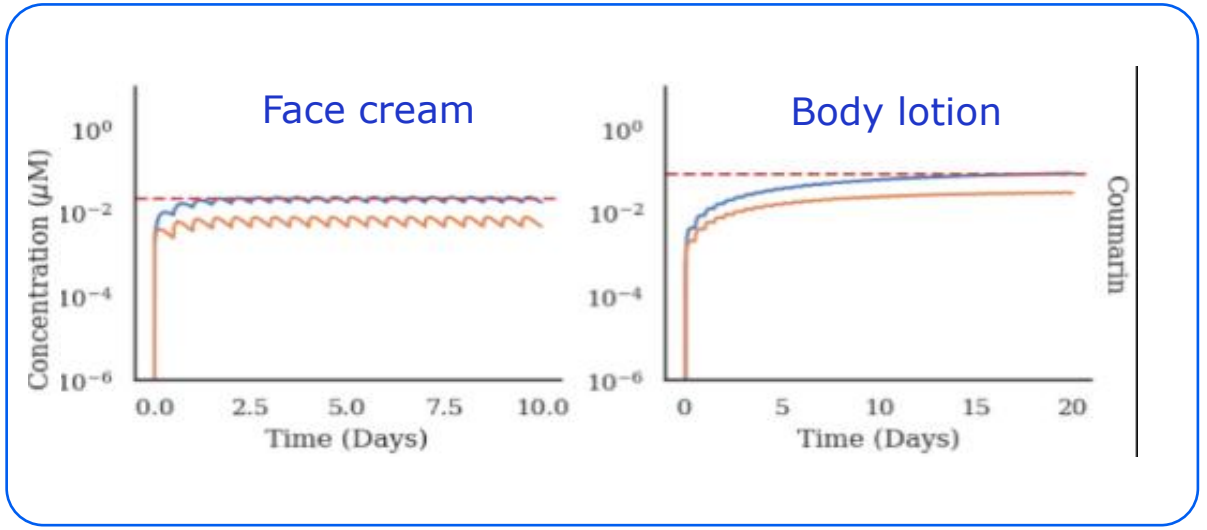


Image kindly provided by Paul Walker (Cyprotex)

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

EXPOSURE



Identify lowest (most sensitive) point of departure, expressed in µM

Identify realistic worst-case plasma exposure (C_{max}) expressed as µM

$$\text{BIOACTIVITY EXPOSURE RATIO} = \frac{\text{BIOACTIVITY}}{\text{EXPOSURE}}$$

The bigger the BER, the greater the confidence that bioactivity will not occur in exposed consumers

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

In vitro Pharmacological profiling

- Tested up to 10 µM
- **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).

Cell Stress Panel

- Global POD_{NAM} = 140 µM

HTTr (HepG2, HepaRG, MCF7, PTC)

- Two approaches to calculating POD – BIFROST (gene level) and BMDL (pathway level)
- Gene level PoD = 4.2 µM(HepG2 cells)
- Pathway level PoD = 240 µM (HepG2 cells)

Renal biomarkers (PTC)

- No significant response for BP-4 (Cisplatin and Omeprazole gave expected dose-response 72-h)

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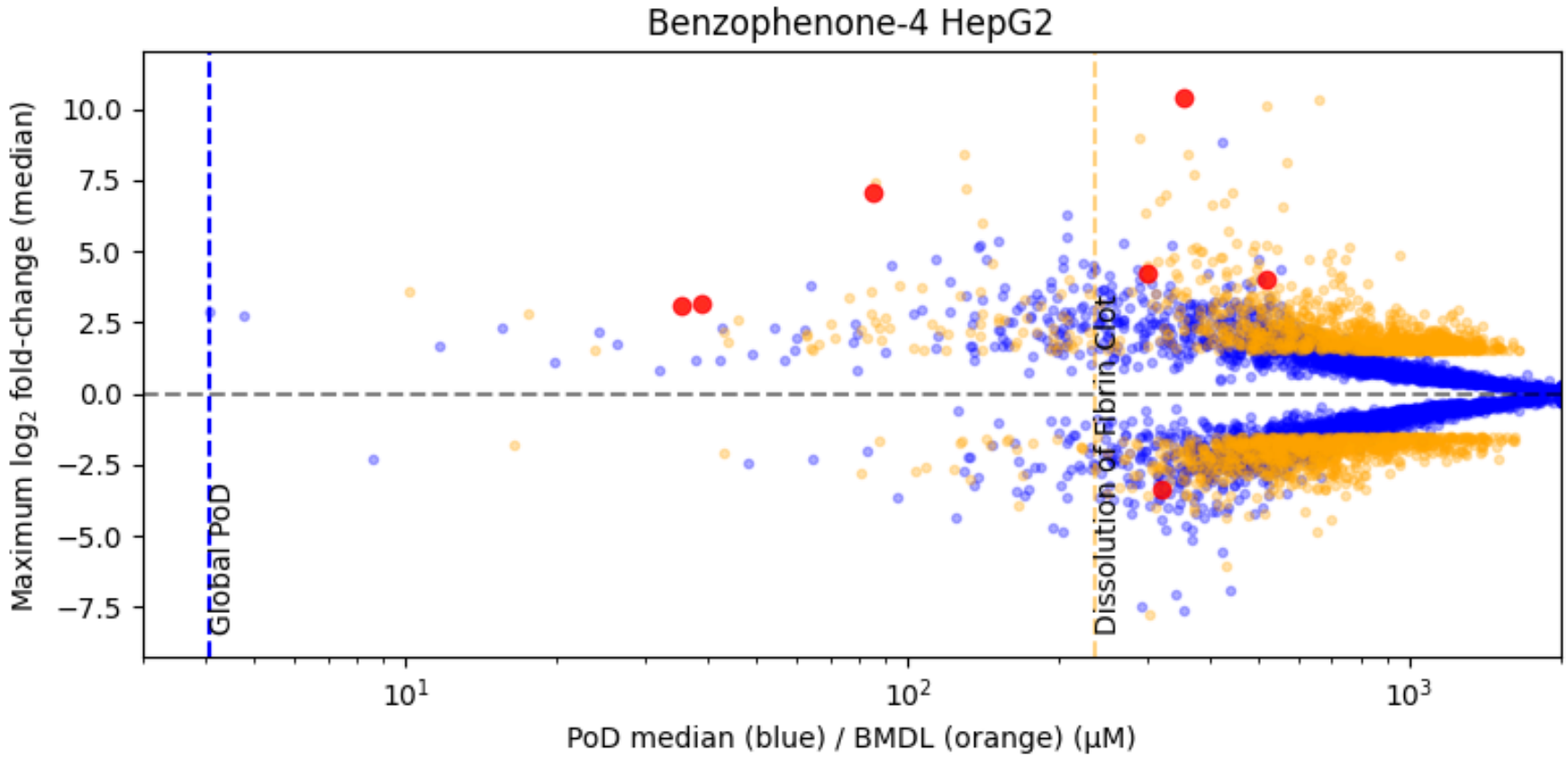
HTTr PODs in HepG2 Cells



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

Baltazar at al., 2025 https://doi.org/10.14573/altex.2501201

Bioactivity: exposure ratios

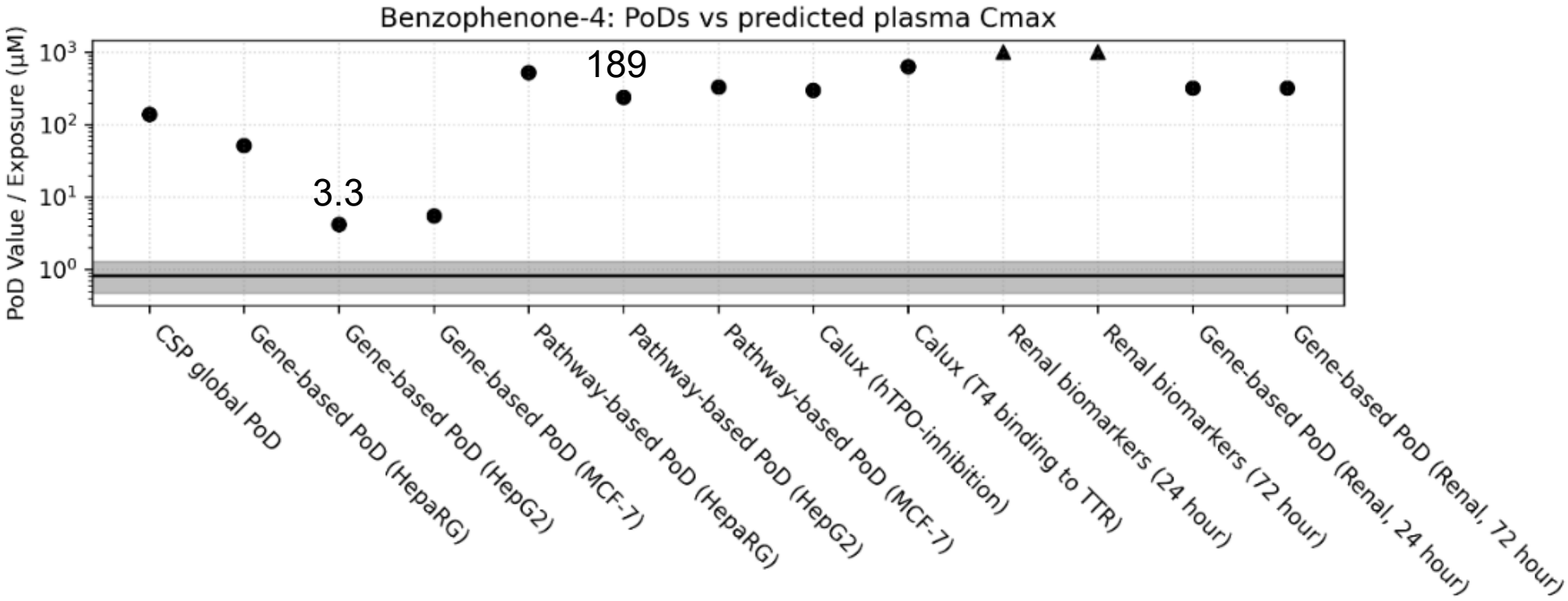


Fig. 6: Bioactivity exposure ratio comparing the PoD_{NAM} for the various NAM assays to the predicted plasma C_{max} (total, µM) expressed as distribution of the population (90^{th} credible interval in grey, mean of the population as solid line)
Points of departure (PoDs) are expressed as nominal concentration (µM) and represented as a black circle. For the renal biomarkers (24h and 72h), it was not possible to calculate a PoDNAM, and therefore maximum tested concentration is represented as a triangle.

Baltazar at al., 2025 https://doi.org/10.14573/altex.2501201

HTTr BER summary

- Not yet consensus on best analysis method to provide HTTr POD
- Most conservative in this assessment was 4.2 µM (BIFROST), giving a deterministic BER of 3.3

  ? toxicological significance – 1A1 a very common lowest affected probe)

3. Also important to consider BMDL POD_{NAM} of 240 µM (HepG2), giving a deterministic BER of 189.

4. This provides assurance that the gene changes seen at 4.2 µM are likely to be of limited toxicological significance.

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

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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

Different NAMs and analysis techniques will provide different PoDs and therefore different BERs, so it's important to benchmark the toolbox used and to understand its strengths and limitations

Cable et al., 2025 (Toxicological Sciences)

Conclusion

- 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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