Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

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Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Ensuring Safe Ingredients for Foods, Drinks, Homecare and Cosmetic Products

Risk Based Approach:
Considers both the hazard and the exposure to evaluate the risk

Can we safely use % of ingredient in product?

For **consumers**; **workers**;
the **environment**

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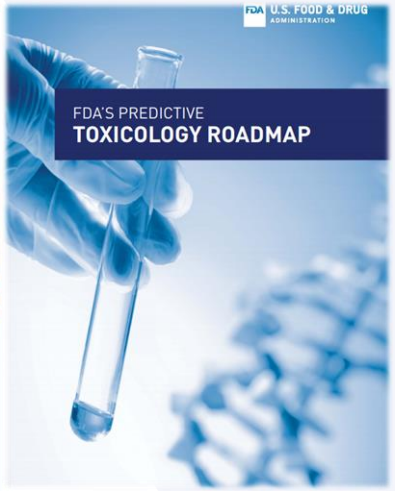
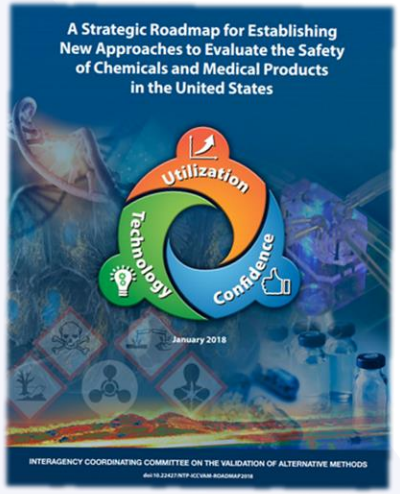
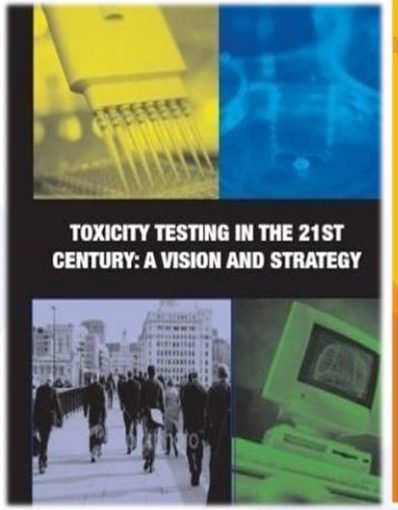
Next Generation Risk Assessment (NGRA)

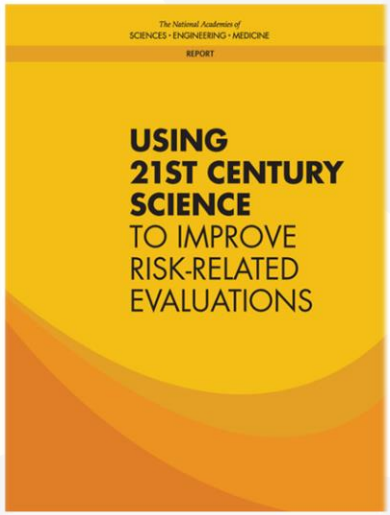
*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Safety without animal testing









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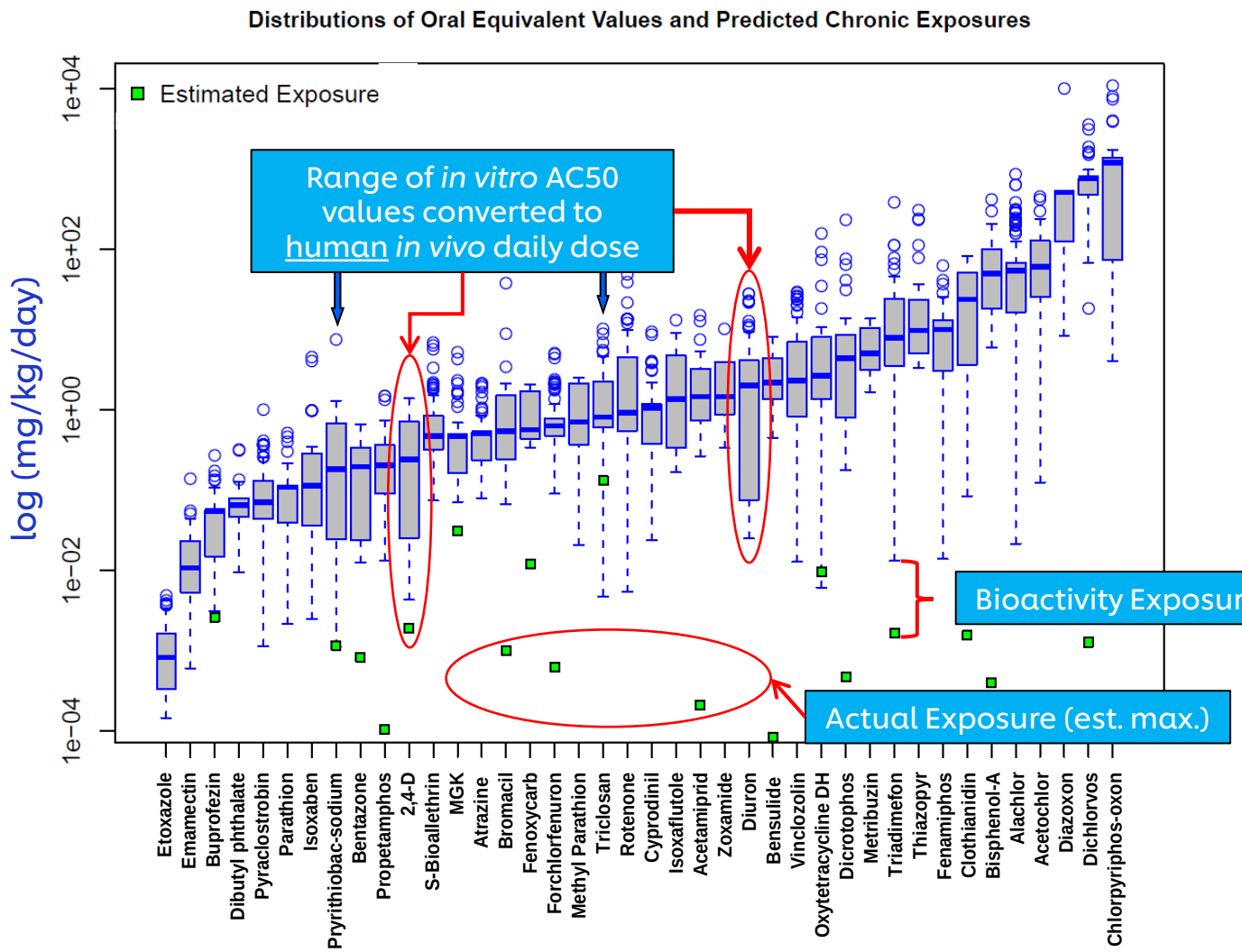
NGRA: aim is protection, not prediction of animal data



The hypothesis underpinning NGRA is that **if no bioactivity is observed at consumer-relevant concentrations, there can be no adverse health effects.**

At no point does NGRA attempt to predict the results of high dose toxicology studies in animals.

NGRA **uses new exposure science and understanding of human biology.**

Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

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Integration of exposure and bioactivity for decision-making – Case studies

NAMs to support hypothetical read-across NGRA case studies (e.g. caffeine and parabens)

Regulatory Toxicology and Pharmacology 123 (2021) 104931

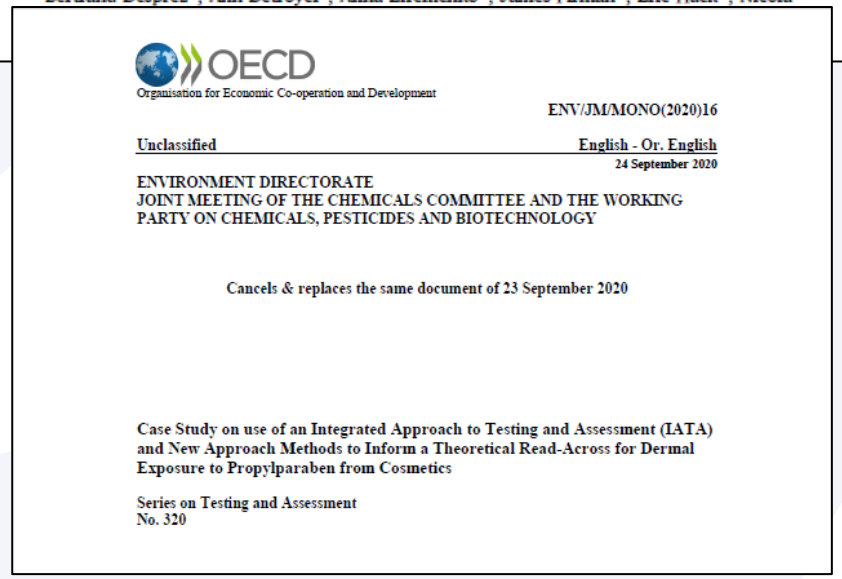
Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

New framework for a non-animal approach adequately assures the safety of cosmetic ingredients – A case study on caffeine

Dagmar Bury [a], Camilla Alexander-White [b], Harvey J. Clewell III [c], Mark Cronin [d], Bertrand Desprez [e], Ann Detroyer [f], Alina Efremenko [g], James Firman [d], Eric Hack [g], Nicola



OECD

Organisation for Economic Co-operation and Development

ENV/JM/MONO(2020)16

Unclassified — English - Or. English

24 September 2020

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 23 September 2020

Case Study on use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics

Series on Testing and Assessment
No. 320

NAMs applied in an *ab initio* hypothetical/NGRA case study

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

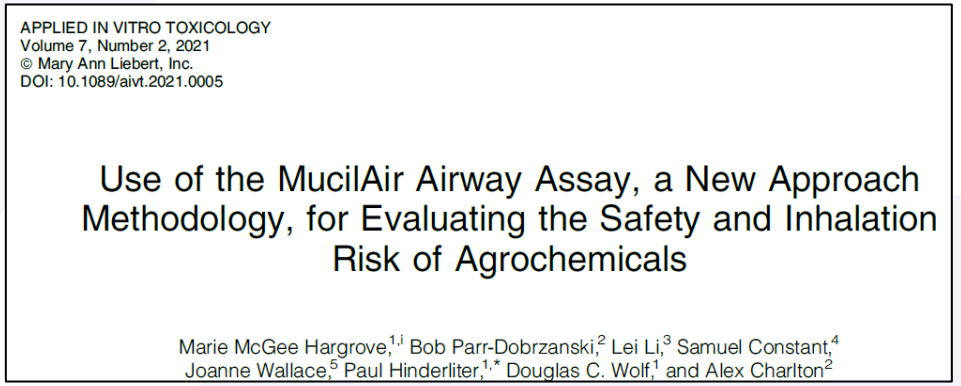
A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

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

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK

[1]To whom correspondence should be addressed. Fax: +44(0)1234 264 744. E-mail: maria.baltazar@unilever.com.

NAMs applied in real-life chemical safety assessments



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DOI: 10.1089/aivt.2021.0005

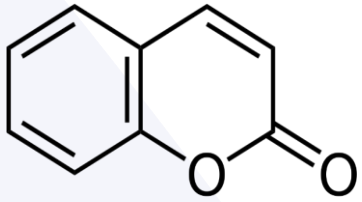
Use of the MucilAir Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals

Marie McGee Hargrove,[1,i] Bob Parr-Dobrzanski,[2] Lei Li,[3] Samuel Constant,[4] Joanne Wallace,[5] Paul Hinderliter,[1,*] Douglas C. Wolf,[1] and Alex Charlton[2]

https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080

Example how to integrate NAMs for a NGRA: coumarin case study

0.1% COUMARIN IN FACE CREAM AND BODY LOTION (NEW FRAGRANCE)



Plasma C_{max}

Exposure Estimation

Local and systemic exposure estimates
- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters
- Internal Exposure (PBK)

Collate Existing Information

Problem Formulation
- Molecular Structure
- *In silico* predictions
- Literature

***In Vitro* Biological Activity Characterization**

Initial PoD identification
- ToxTracker
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Insufficient data and high uncertainty

Metabolism refinement

Increased certainty in PoD and IVIVE
- Metabolite identification
- 3D Models

$PoD_{in\ vitro}$

*** Determine Margin of Exposure**

*or Bioactivity exposure ratio (BER)

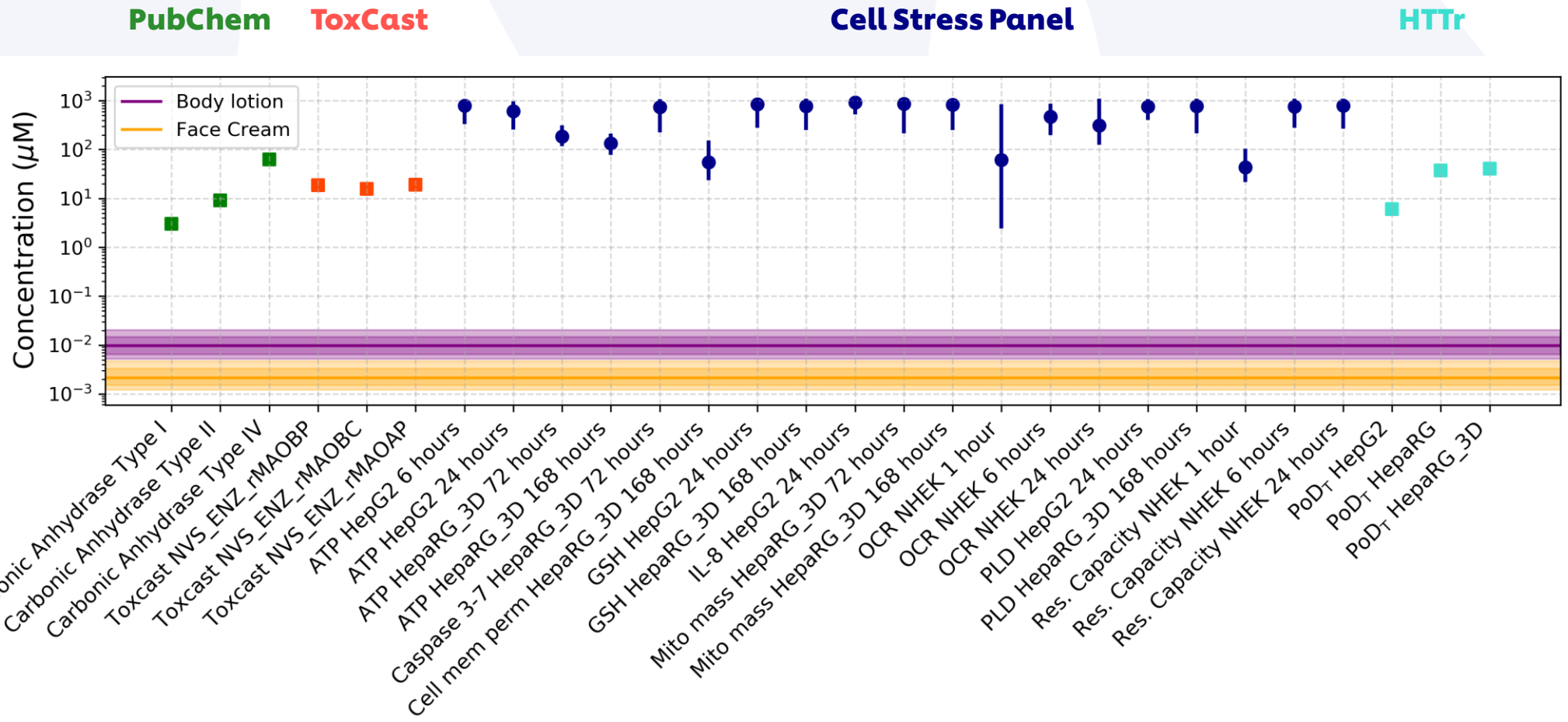
Sufficient data and high certainty

Risk Assessment Conclusion

Low risk conclusion based on the margin of safety calculations.

Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

Exposure and PoD are plotted and used to derive a Bioactivity-Exposure Ratio (MoE/BER)



The 5th percentile of the BER distribution ranged between 158 and 96738

In this case study: Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream or a body lotion is safe for the consumer

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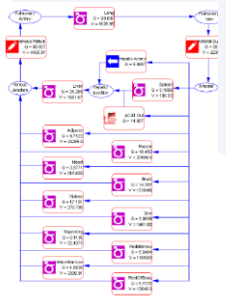
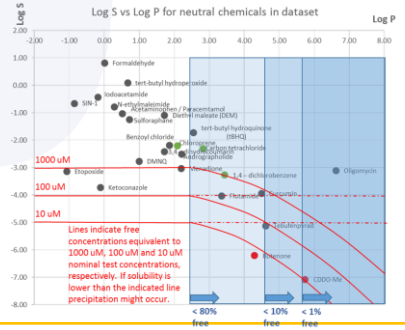
Can we develop a systemic safety toolbox for estimating BERs?

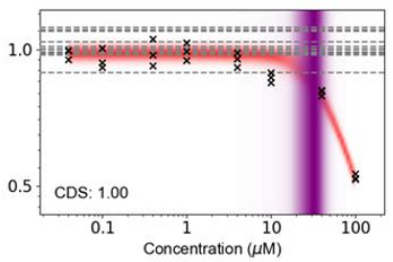
PBK models



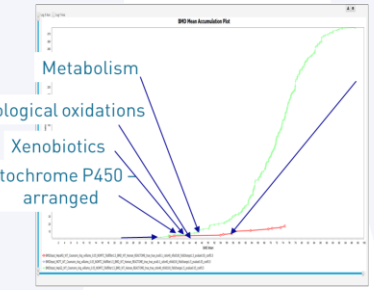
Free concentration



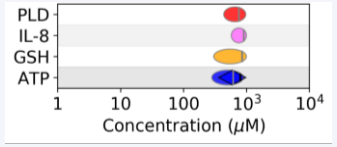
Conc. Resp. models



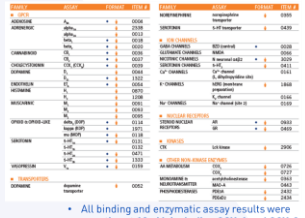
HTTr



CSP

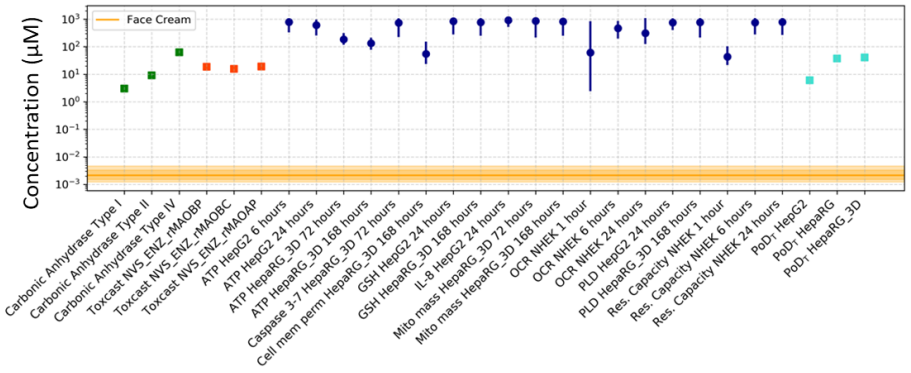


IPP



- All binding and enzymatic assay results were **negative at 10 μM, including COX-1 and COX-2.**
- Highest inhibition [22%] was for MAO-A

Bioactivity exposure ratio



Inform safety decision

HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

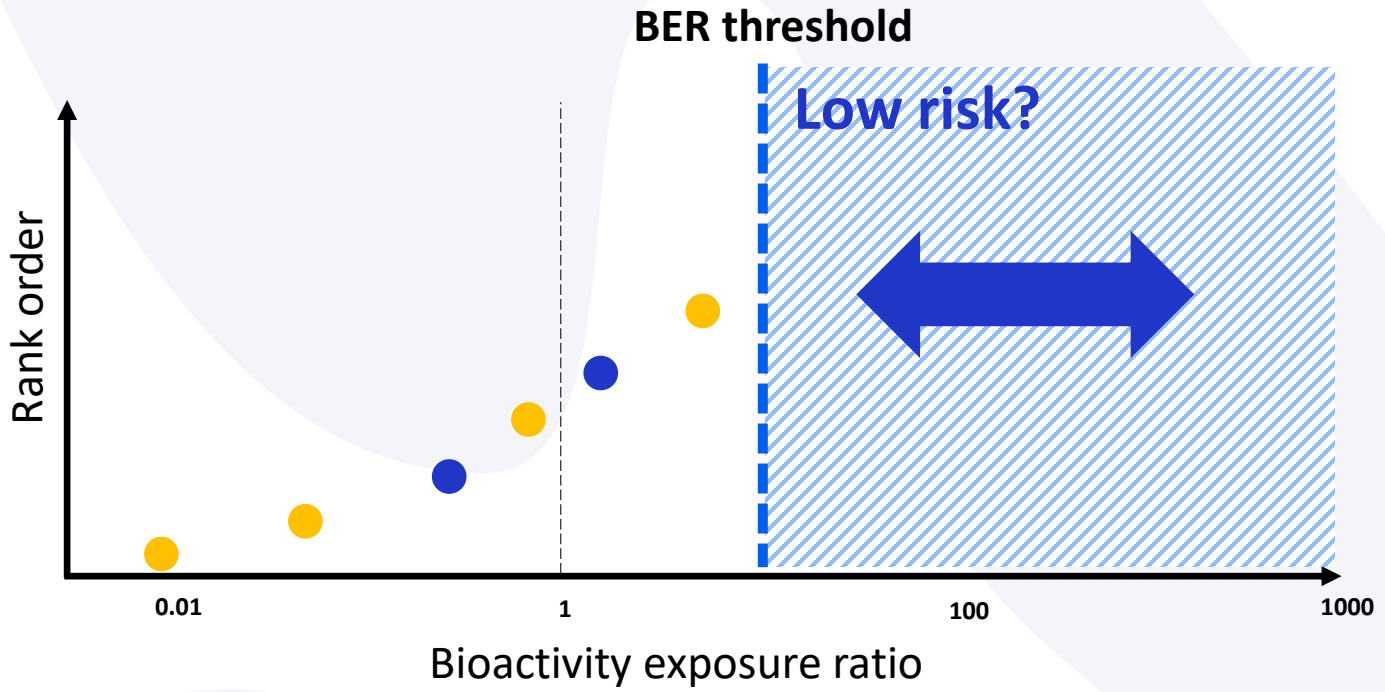
Some challenges to developing and evaluating a systemic safety toolbox

- Biological coverage:
  - Are we using the right in vitro models?
  - Are we measuring the right biomarkers?
- Accuracy of internal exposure estimates (PBK models)
- How large should be bioactivity exposure ratio to identify an exposure as **low risk**?

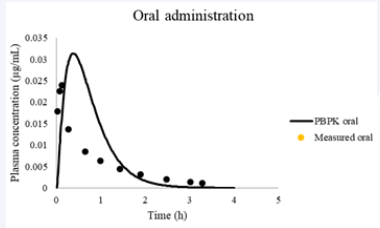
An approach for evaluating the toolbox

Chemical exposures scenarios

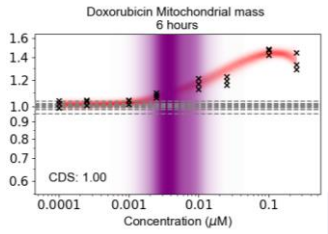
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



Define typical use-case scenarios benchmark chemical-exposures; Mixture of High and low risk



PBK models of systemic exposure



In-vitro cell assays, estimate PoDs

Calculate the bioactivity exposure ratio

Overall evaluation strategy

Step 1 (pilot study)*

- Define what the toolbox contains (which NAMs) and the workflow through which they should be used.
- Define process of how the toolbox will be evaluated, and the metrics that will be used to determine it's 'performance'
- Explore using a small set of chemicals and exposure scenarios (11 chemicals, 25 exposure scenarios)
- Define **prototype decision model** for determining the BER threshold.

Step 2 (full evaluation)

- Evaluate the toolbox using ~40 chemicals with ~100 exposure scenarios based on the toolbox established in the pilot study.
- Use learnings from the toolbox evaluation to refine the toolbox in terms of **NAM composition** and the **decision model**.

*Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Stage 1: defining the benchmark chemical exposure scenarios

Chemical	Exposure scenario	Risk classification
Oxybenzone	**2 scenarios:** 0.5%; 2% sunscreen	Low risk
Caffeine	**2 scenarios:** 0.2% shampoo & coffee oral consumption 50 mg	Low risk
Caffeine	10g – fatal case reports	High risk
Coumarin	**3 scenarios:** 4 mg/d oral consumption; 1.6% body lotion (dermal); TDI 0.1 mg/kg oral	Low risk
Hexylresorcinol	**3 scenarios:** Food residues (3.3 ug/kg); 0.4% face cream; throat lozenge 2.4 mg	Low risk
BHT	Body lotion 0.5%	Low risk
Sulforaphane	**2 scenarios:** Tablet 60 mg/day; food 4.1-9.2 mg/day	Low risk
Niacinamide	**4 scenarios:** oral 12.5-22 mg/kg; dermal 3% body lotion and 0.1 % hair condition	Low risk
Doxorubicin	75 mg/m2 IV bolus 10 min; 21 days cycles; 8 cycles	High risk
Rosiglitazone	8 mg oral tablet	High risk
Valproic Acid (VPA)	**2 scenarios:** oral tablet 1000 mg & > 60 mg/kg	High risk
Paraquat	Accidental ingestion 35 mg/kg	High risk

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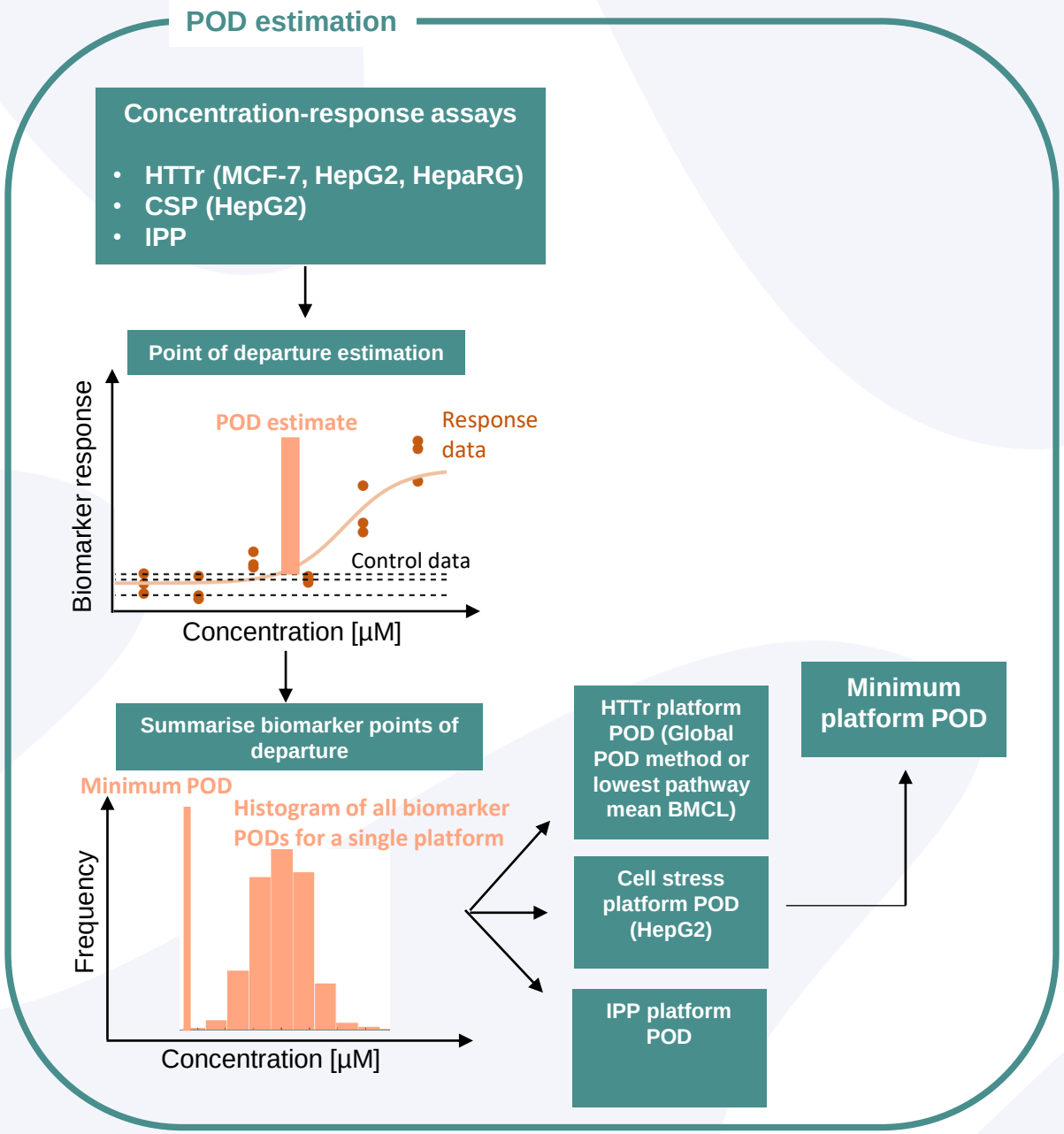
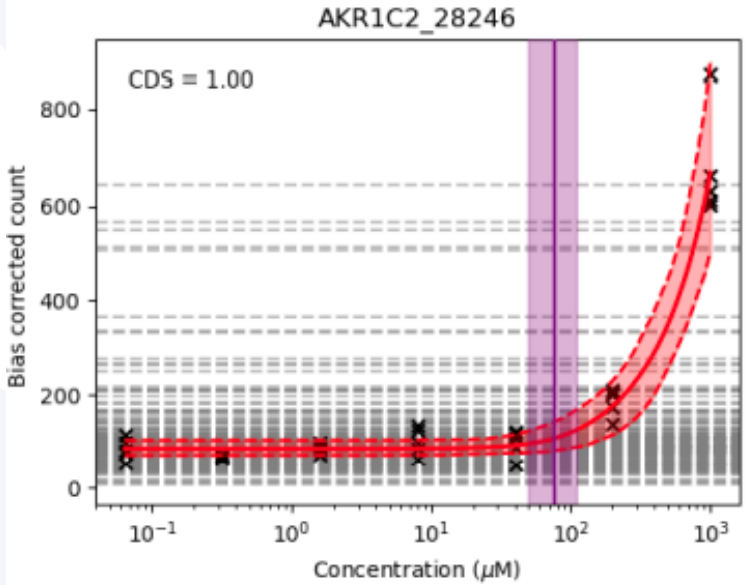
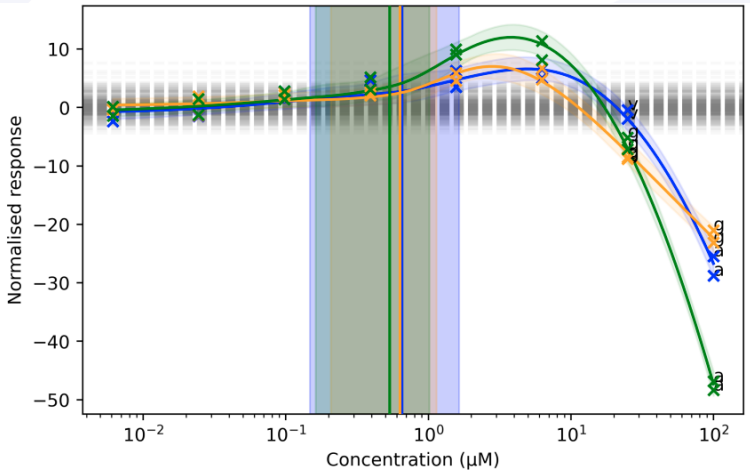
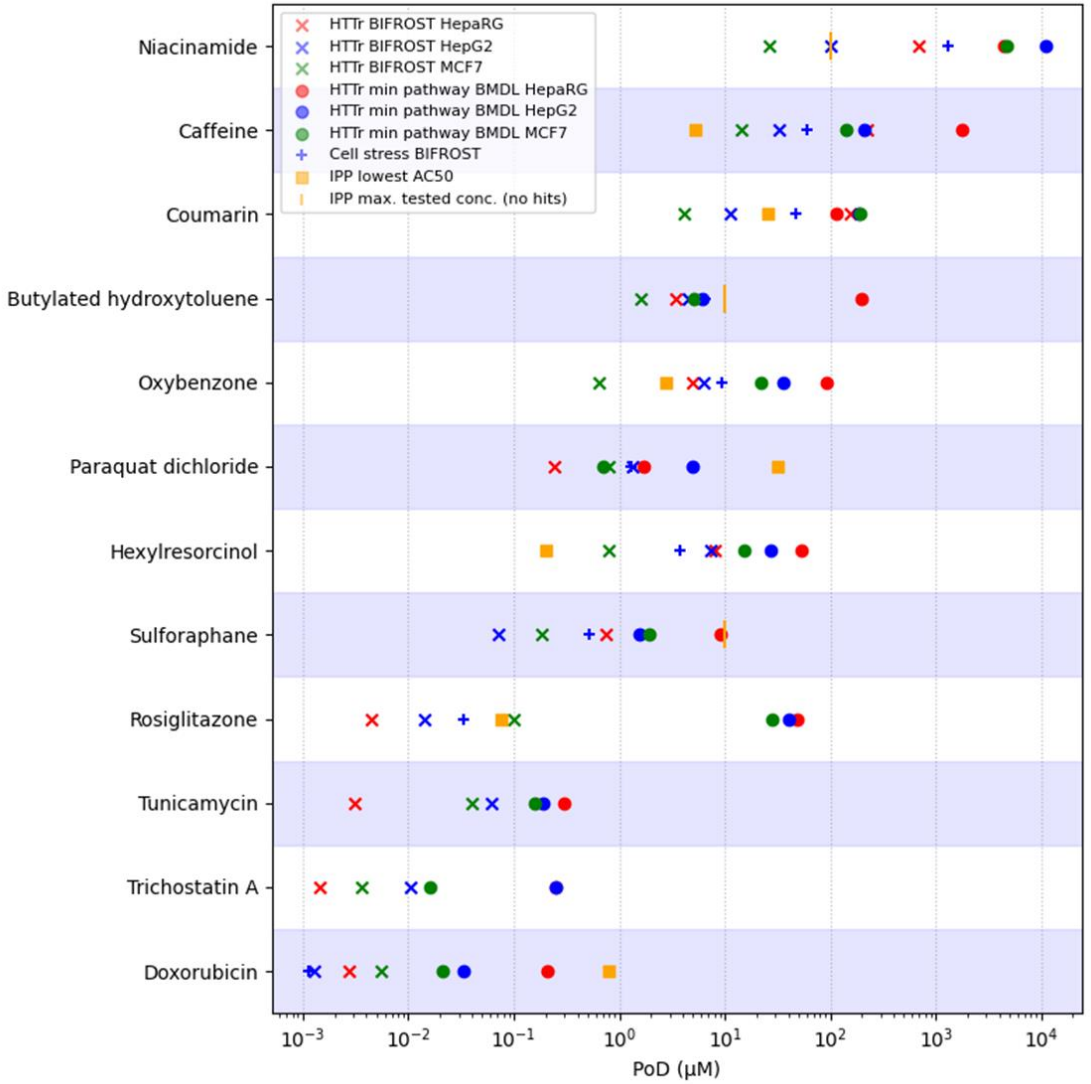
Stage 2: Estimating PODs from bioactivity platforms



Stage 2: Estimating PODs from bioactivity platforms







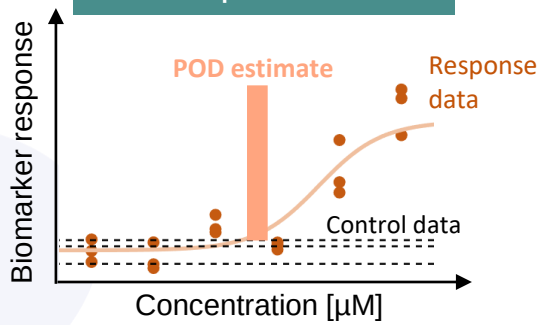
Stage 2: Estimating Cmax using PBK models

POD estimation

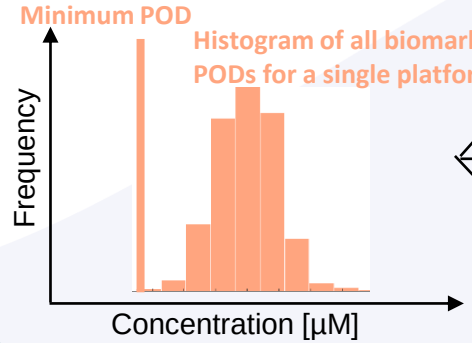
Concentration-response assays

- HTTr (MCF-7, HepG2, HepaRG)
- CSP (HepG2)
- IPP

Point of departure estimation



Summarise biomarker points of departure



HTTr platform POD (Global POD method or lowest pathway mean BMCL)

Cell stress platform POD (HepG2)

IPP platform POD

Minimum platform POD

Exposure estimation

Use-scenario

PBK model
(Gastroplus)

Plasma C_{max} estimate

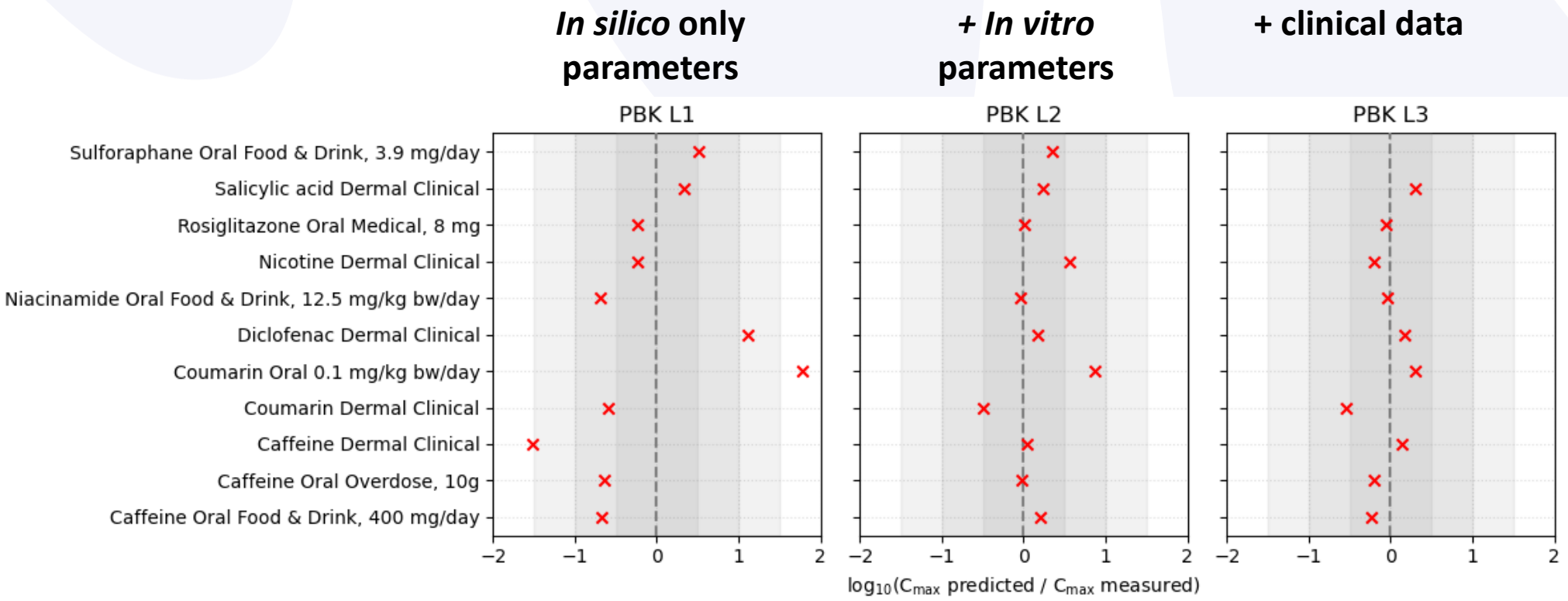
C_{max} Error Distribution model (CMED)
(Bayesian model)

In silico **parameter estimates** **(L1)**

In vitro **parameter estimates** **(L2)**

Human in vivo PK data **(L3)**

Considering the error in PBK models based on parameterisation level



- The PBK prediction error decreases as we go ‘up’ parameterisation levels
- Developed a Bayesian statistical model to quantify the error for a novel chemical

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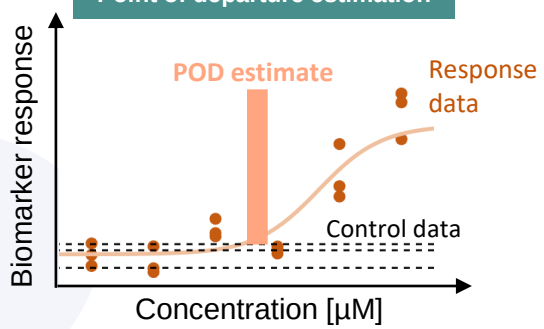
Stage 3: Estimating the BER from the toolbox

POD estimation

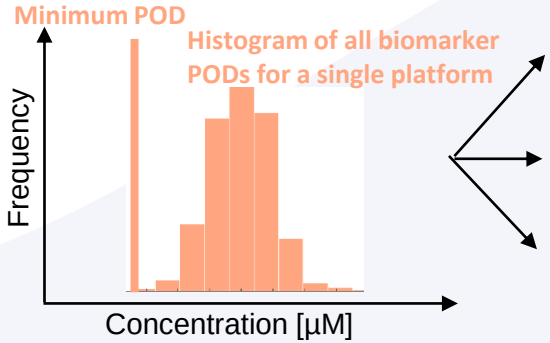
Concentration-response assays

- HTTr (MCF-7, HepG2, HepaRG)
- CSP (HepG2)
- IPP

Point of departure estimation



Summarise biomarker points of departure



- HTTr platform POD (Global POD method or lowest pathway mean BMCL)
- Cell stress platform POD (HepG2)
- IPP platform POD

Minimum platform POD

Exposure estimation

Use-scenario

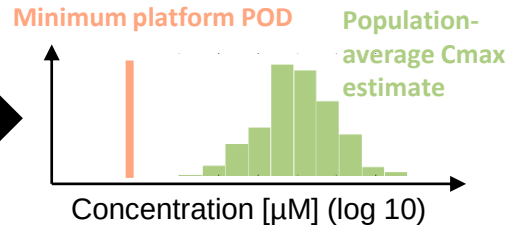
PBK model (Gastroplus)

- *In silico* parameter estimates (L1)
- *In vitro* parameter estimates (L2)
- *Human in vivo PK data* (L3)

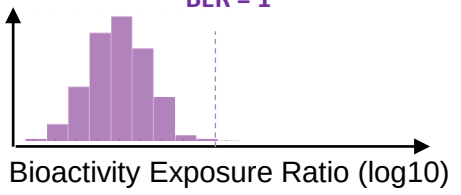
Plasma C_{max} estimate

C_{max} Error Distribution model (CMED) (Bayesian model)

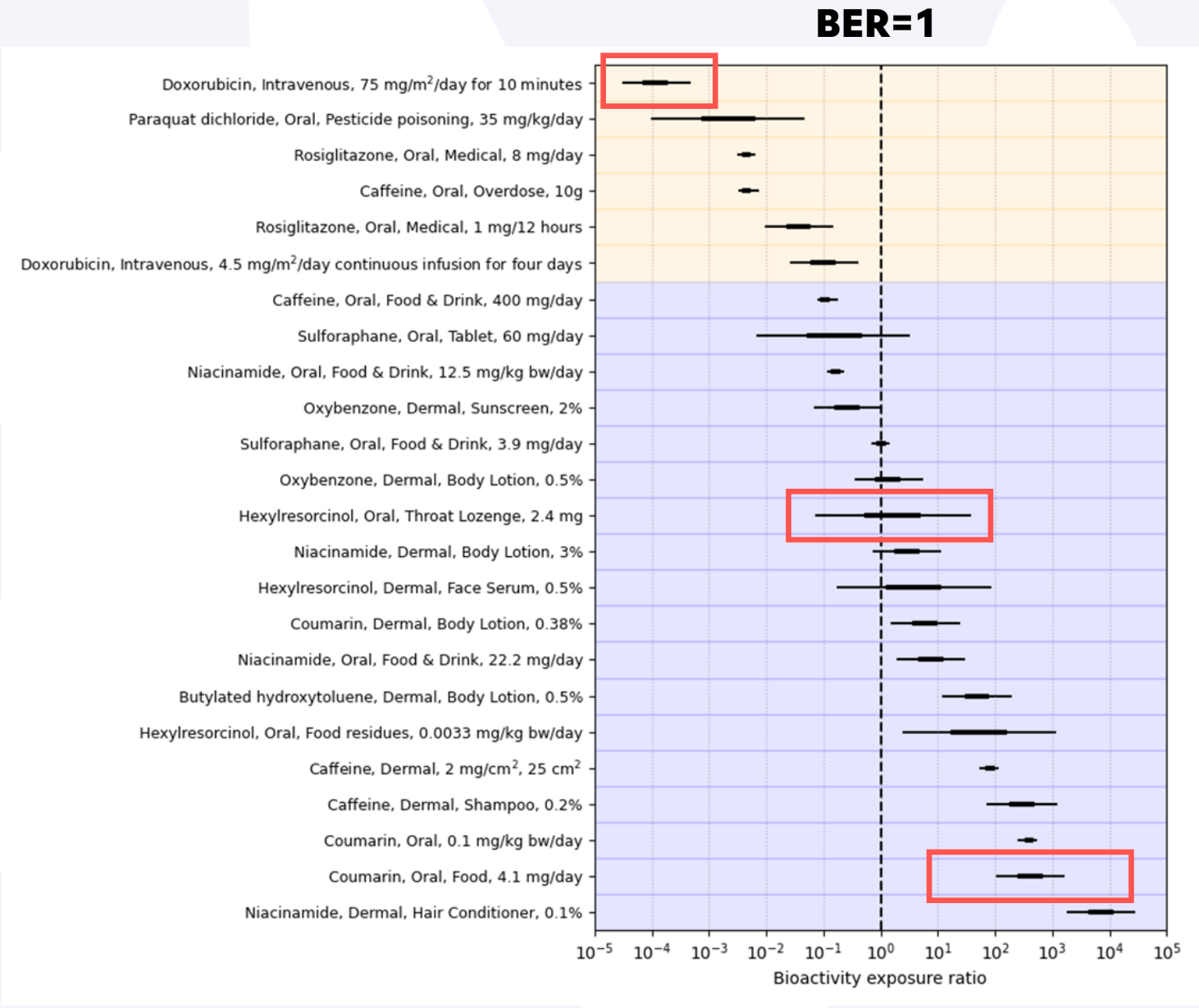
Estimate Bioactivity Exposure Ratio and Decision model



Bioactivity Exposure Ratio Distribution



Stage 3: Estimating the BER from the toolbox



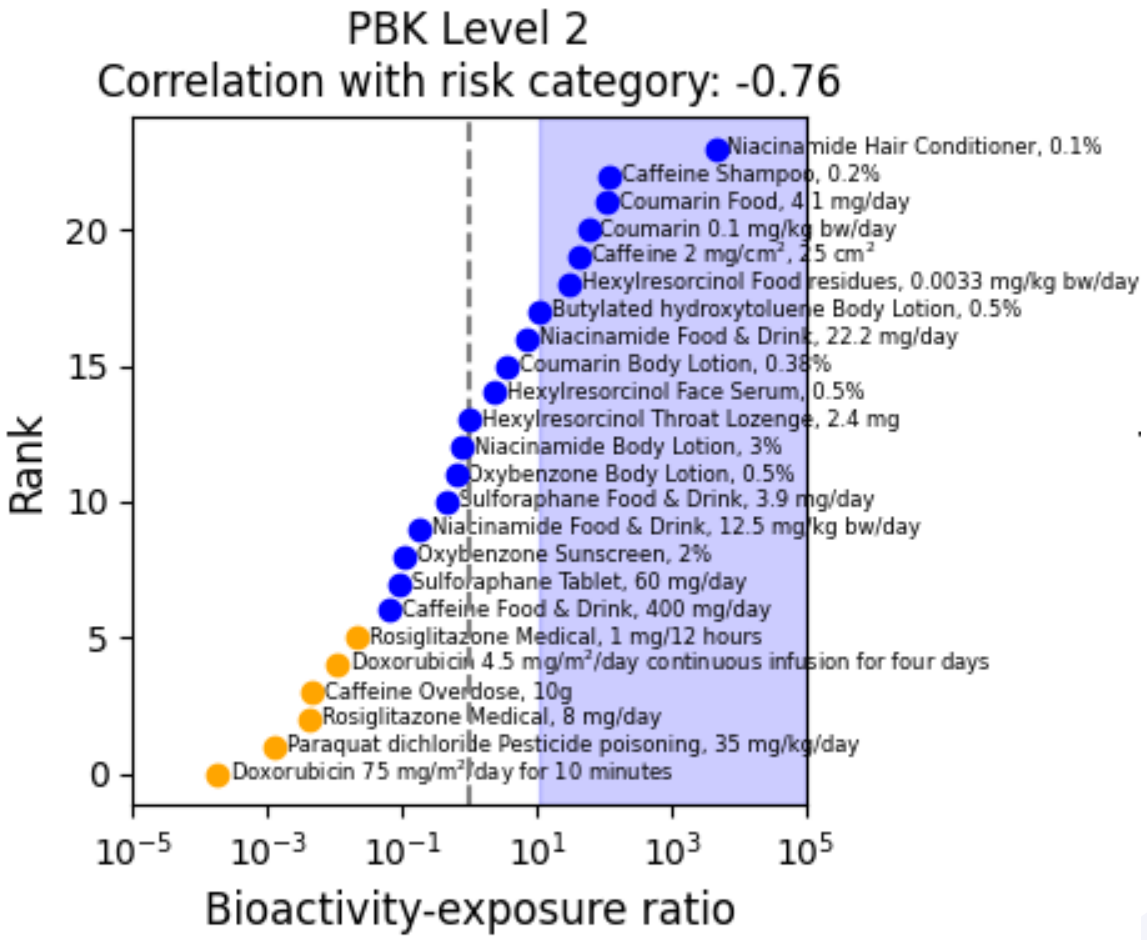
Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

BER=1:
Cmax estimates coincide with the minimum POD

What threshold value of the BER is needed to assure safety?

Visualising how the toolbox performs against the pilot study data



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

Exposure scenarios within the **blue shaded region** are identified as **low risk**

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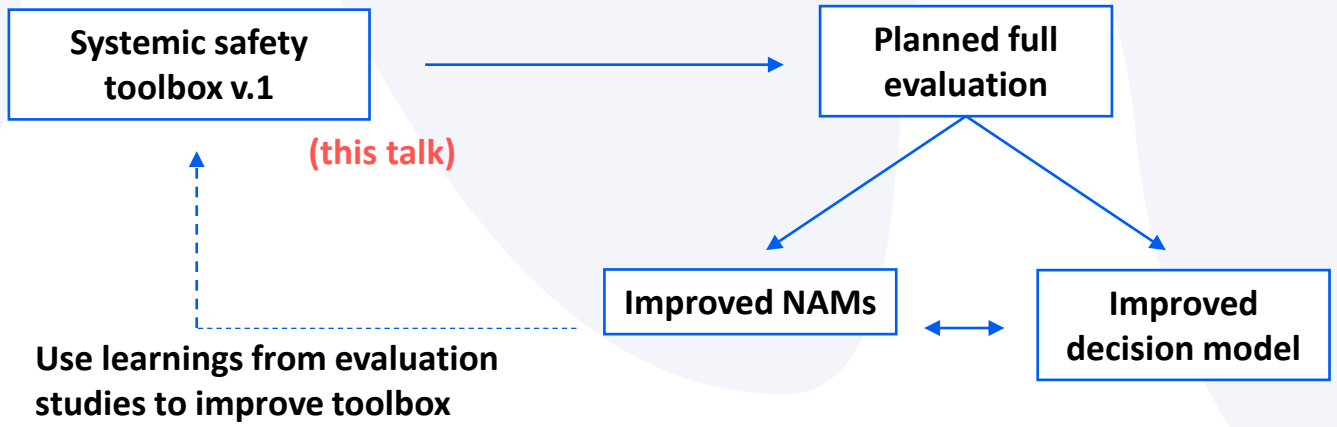
An iterative approach to evaluating the toolbox



- Have now extended the evaluation to ~40 chemicals with ~100 associated **high risk** and **low risk** exposure scenarios.
- Adopt **iterative approach** to evaluating and then identifying potential improvements to the toolbox.
- Use of concepts from used model evaluation and development should help build confidence in the approach.
- **Unilever-EPA CRADA**: Generating data for 10 cell lines, using high-throughput transcriptomics and phenotypic profiling.

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Acknowledgements

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US-EPA: Richard Judson, Josh Harrell, Logan Everett, Imran Shah

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