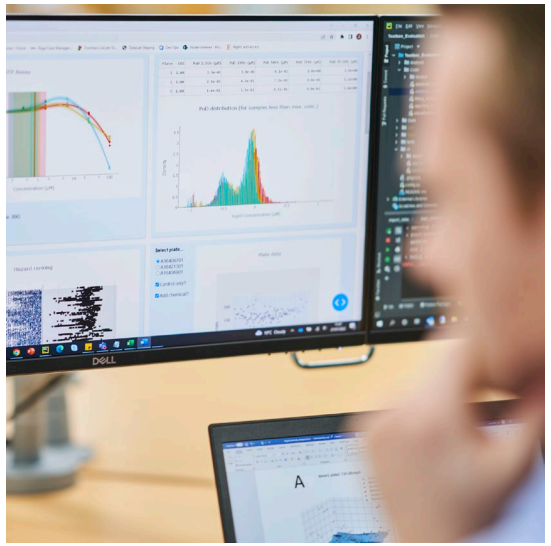
Next Generation Risk Assessment for Systemic Toxicity



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Science leader PBK
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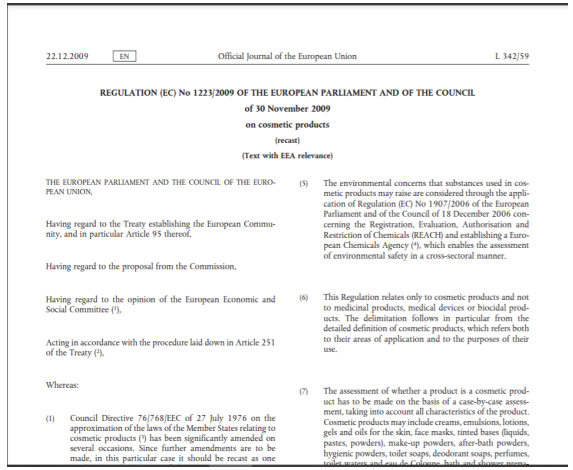
The need for non-animal safety assessments

Societal Attitudes/Consumer Preference

Human Relevance



22.12.2009 EN Official Journal of the European Union L 342/59

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 30 November 2009
on cosmetic products
(recast)
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee (1),

Acting in accordance with the procedure laid down in Article 251 of the Treaty (2),

Whereas:

(1) Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (3) has been significantly amended on several occasions. Since further amendments are to be made, in this particular case it should be recast as one

(5) The environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency (4), which enables the assessment of environmental safety in a cross-sectoral manner.

(6) This Regulation relates only to cosmetic products and not to medicinal products, medical devices or biocidal products. The delimitation follows in particular from the detailed definition of cosmetic products, which refers both to their areas of application and to the purposes of their use.

(7) The assessment of whether a product is a cosmetic product has to be made on the basis of a case-by-case assessment, taking into account all characteristics of the product. Cosmetic products may include creams, emulsions, lotions, gels and oils for the skin, face masks, tinted bases (liquids, pastes, powders), make-up powders, after-bath powders, hygienic powders, toilet soaps, deodorant soaps, perfumes, toilet waters and eau de Cologne bath and shower preps

Regulatory Changes (e.g. Cosmetics Regulation)

Archives of Toxicology (2023) 97:3075–3083
https://doi.org/10.1007/s00204-023-03601-5

REGULATORY TOXICOLOGY

Check for updates

Analysis of health concerns not addressed by REACH for low tonnage chemicals and opportunities for new approach methodology

Philip Botham[1] · Mark T. D. Cronin[2] · Richard Currie[1] · John Doe[2] · Dorothee Funk-Weyer[3] · Timothy W. Gant[4,5] · Marcel Leist[6] · Sue Marty[7] · Bennard van Ravenzwaay[8] · Carl Westmoreland[9]

Received: 20 July 2023 / Accepted: 30 August 2023 / Published online: 27 September 2023
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Resource/time constraints

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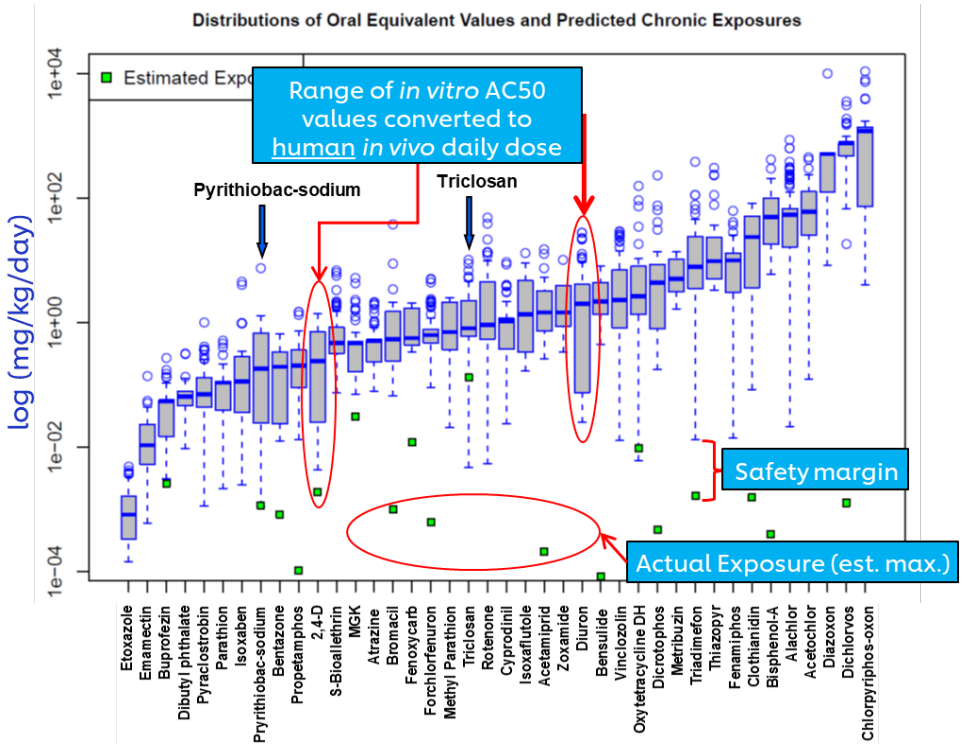
NGRA: an exposure-led and hypothesis-driven approach for protective decision making



Rotroff, et al. *Toxicological Sciences* 117.2 (2010): 348-358.

If there is **no** bioactivity observed at consumer-relevant concentrations, there is unlikely to be any adverse health effects.

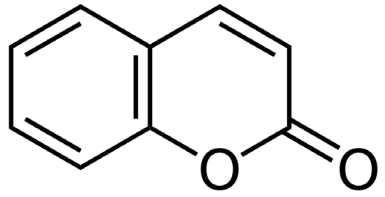
If there **is** bioactivity observed at consumer-relevant concentrations, follow up testing is required to determine whether that could result in an adverse effect

Unilever

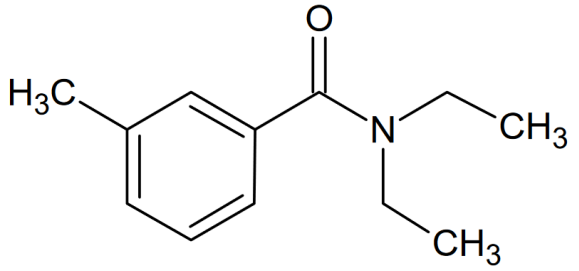
NGRA toolbox for systemic toxicity at Unilever

https://youtu.be/5Z2S8MnKp7g

Example exposure scenarios



Coumarin (flavouring and fragrance, naturally present in e.g. cinnamon)

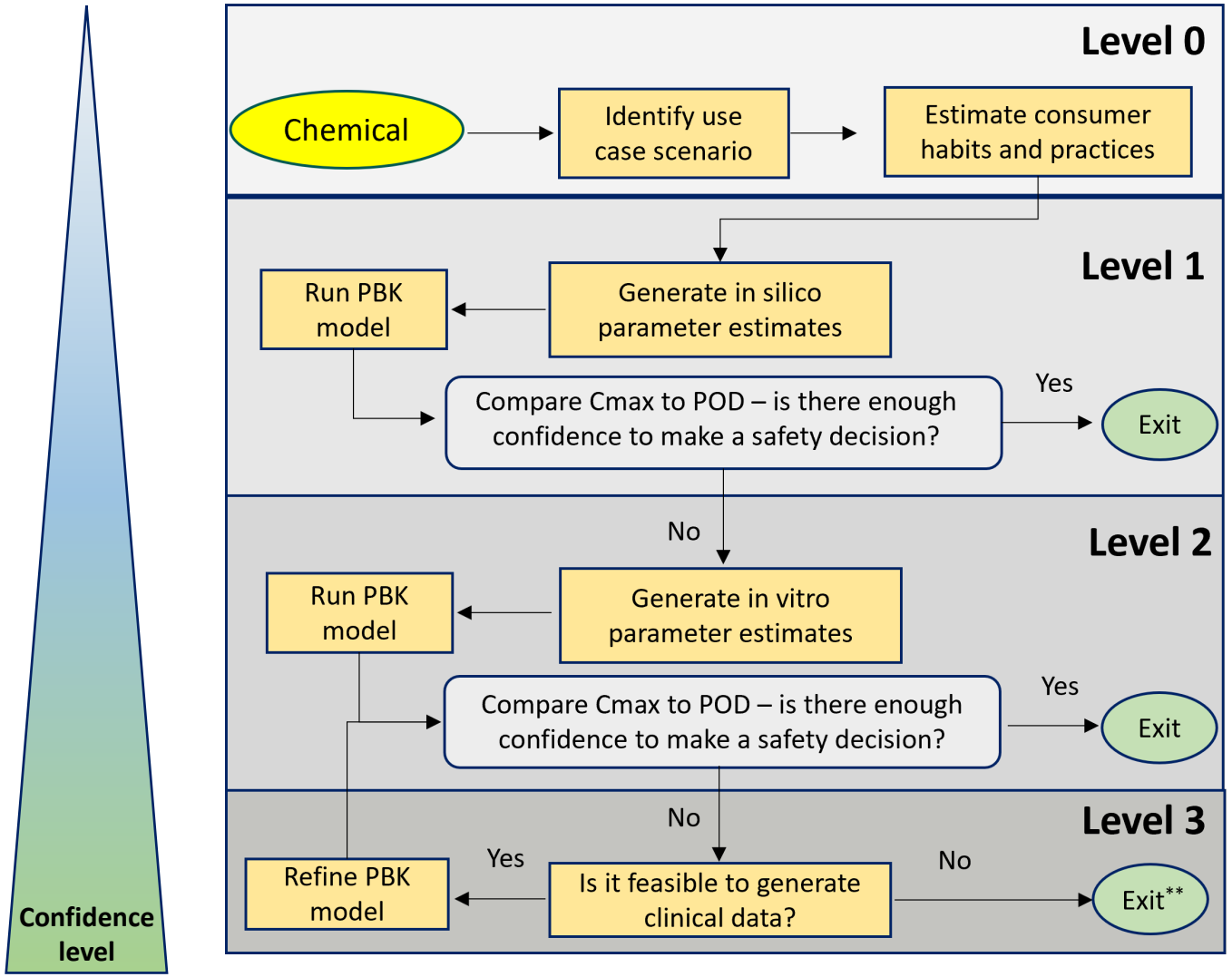


DEET (insect repellent, PT19)

Compound	Use Scenario	Exposure route	Risk classification
Coumarin	Dietary intake, 4 mg/day	Oral	Low risk
DEET	15% in a sun Lotion	Dermal	Acceptable risk based on risk-benefit

Unilever

Parameterisation of PBK models within a tiered risk assessment framework



PBK parameterisation levels

Level 1: Chemical-specific parameters informed using in silico predictions (e.g., using e.g., QSAR models)

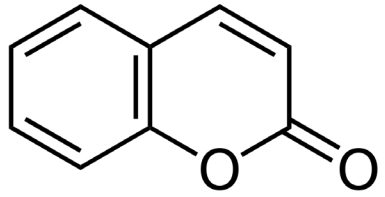
Level 2: Some chemical-specific parameters informed using *in vitro* data

Level 3: Some chemical-specific parameters are inferred by calibrating model against existing human PK data for the same chemical (by a different exposure scenario.

** While further refinement of the PBK model may not be possible, refinement of the bioactivity/POD estimates using higher tier tools (e.g., micro physiological systems) should be considered.

Figure adapted from **Moxon et al., 2020. Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. *Toxicology in Vitro*, *63*, p.104746.**

Example exposure scenarios



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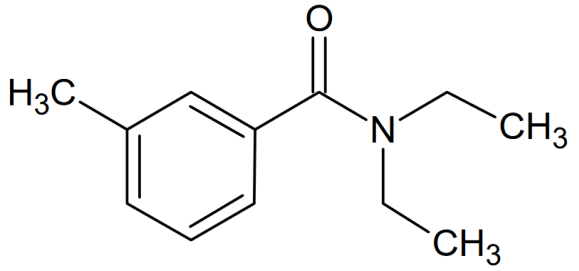

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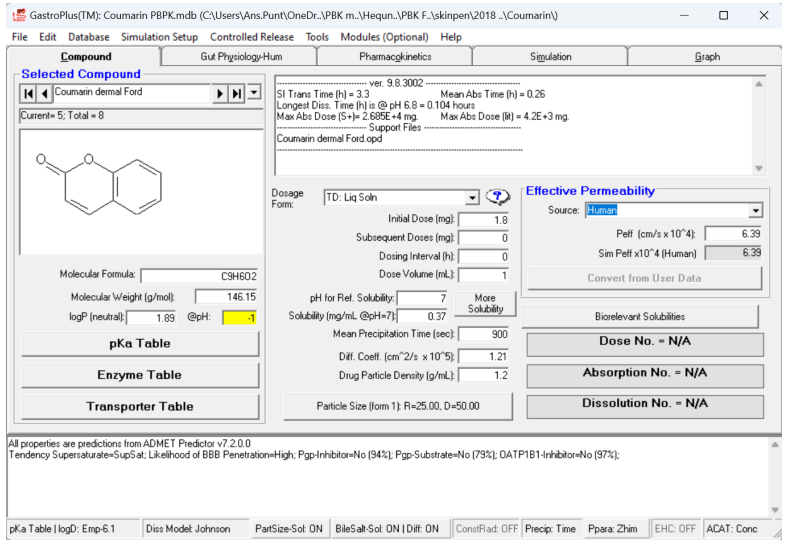
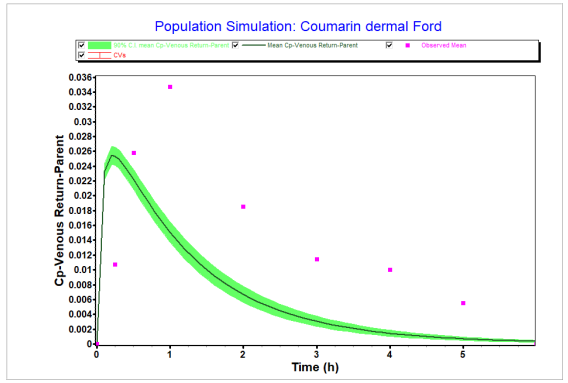
DEET	15% in a sun Lotion	Dermal	Acceptable risk based on risk-benefit

Unilever

Example 1: exposure to coumarin through oral dietary intake

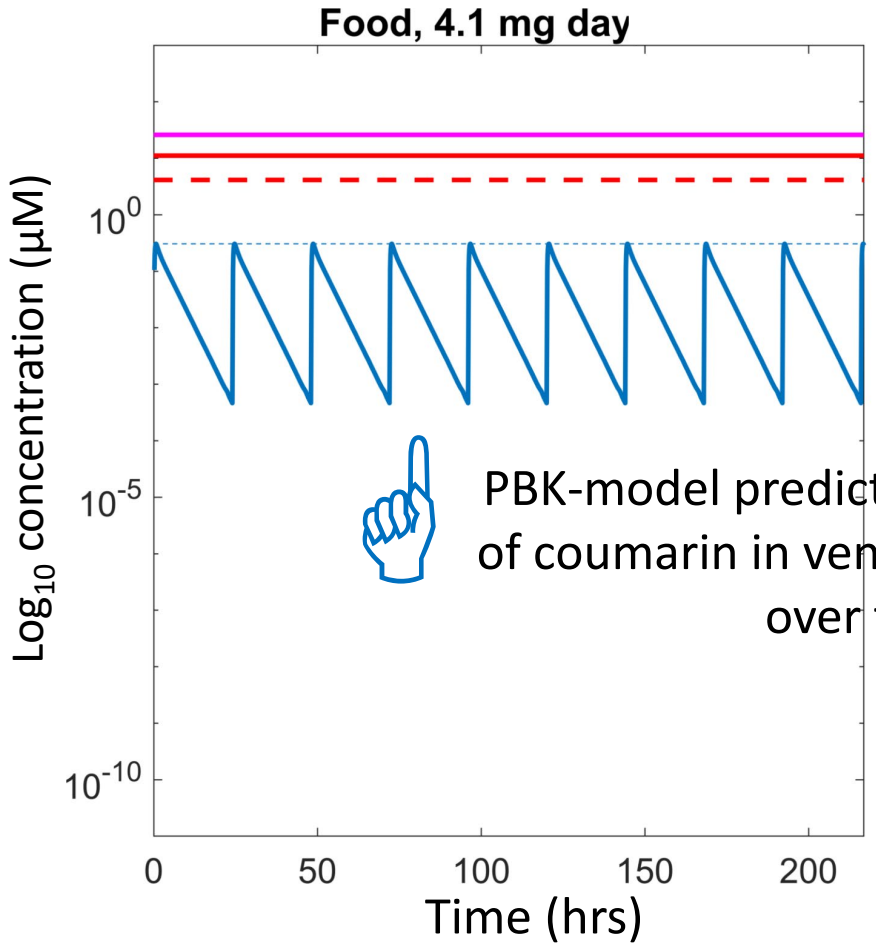
Parameter	Value	Source	Level
Molecular weight (g/mol)	147.1		
Log P	1.89	ADMET predictor	L1
	1.39	Measured[1]	L2
Hepatic intrinsic clearance (L/h)	105	ADMET predictor	L1
	929	Measured	L2
Unbound fraction in plasma (f_{up})	0.24	ADMET predictor	L1
	0.31	Measured[2]	L2
Blood: plasma ratio	1.08	ADMET predictor	L1
	0.7	Measured[2]	L2





1. Hansch, C., Leo, A., & Hoekman, D. (1995) *Exploring QSAR: Hydrophobic, electronic, and steric constants* (Vol. 2). American Chemical Society.
2. Moxon, T.E., et al (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicol In Vitro, 63, 104746

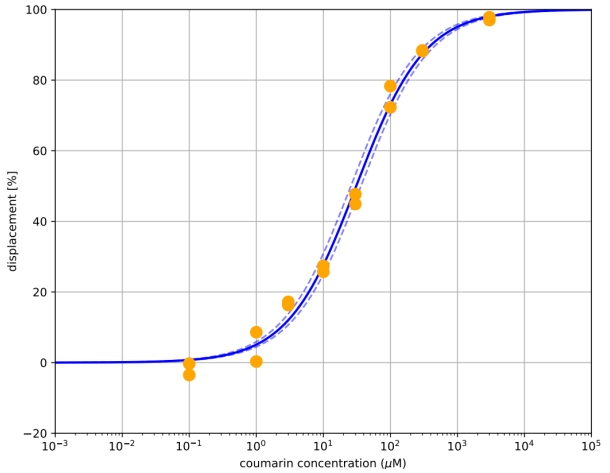
Example 1: exposure to coumarin through oral dietary intake (BER>1)



Points of Departure from in vitro cell assays, measured in µM

PBK-model predicted maximum concentration (C_{max})

PBK-model predicted concentration of coumarin in venous blood plasma over time



PODs:

- In vitro pharmacological profiling (MAO-A)
- High throughput transcriptomics (HepG2)
- High throughput transcriptomics (MCF-7)

Example 1: exposure to coumarin through oral dietary intake

Parameter	Value	Source	Level
Molecular weight (g/mol)	147.1		
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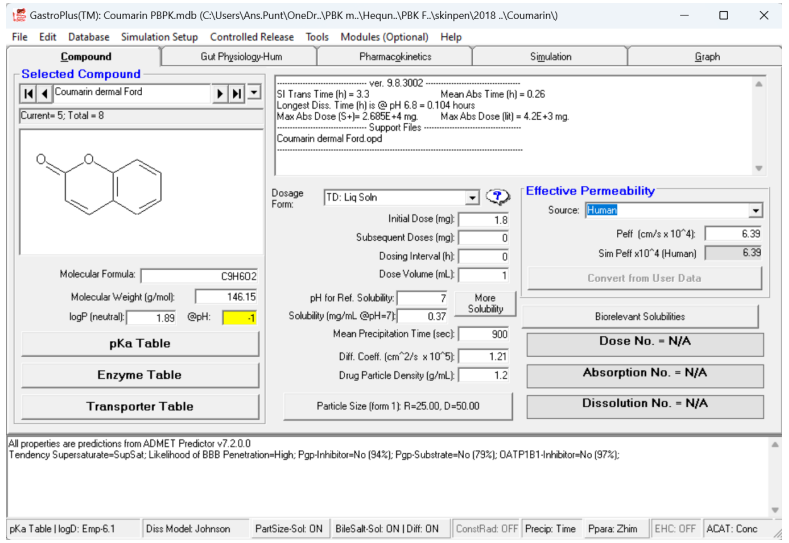

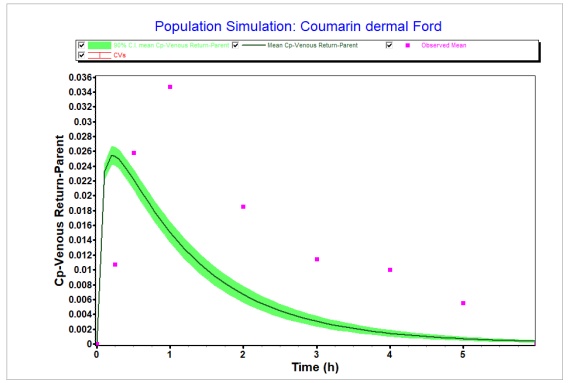

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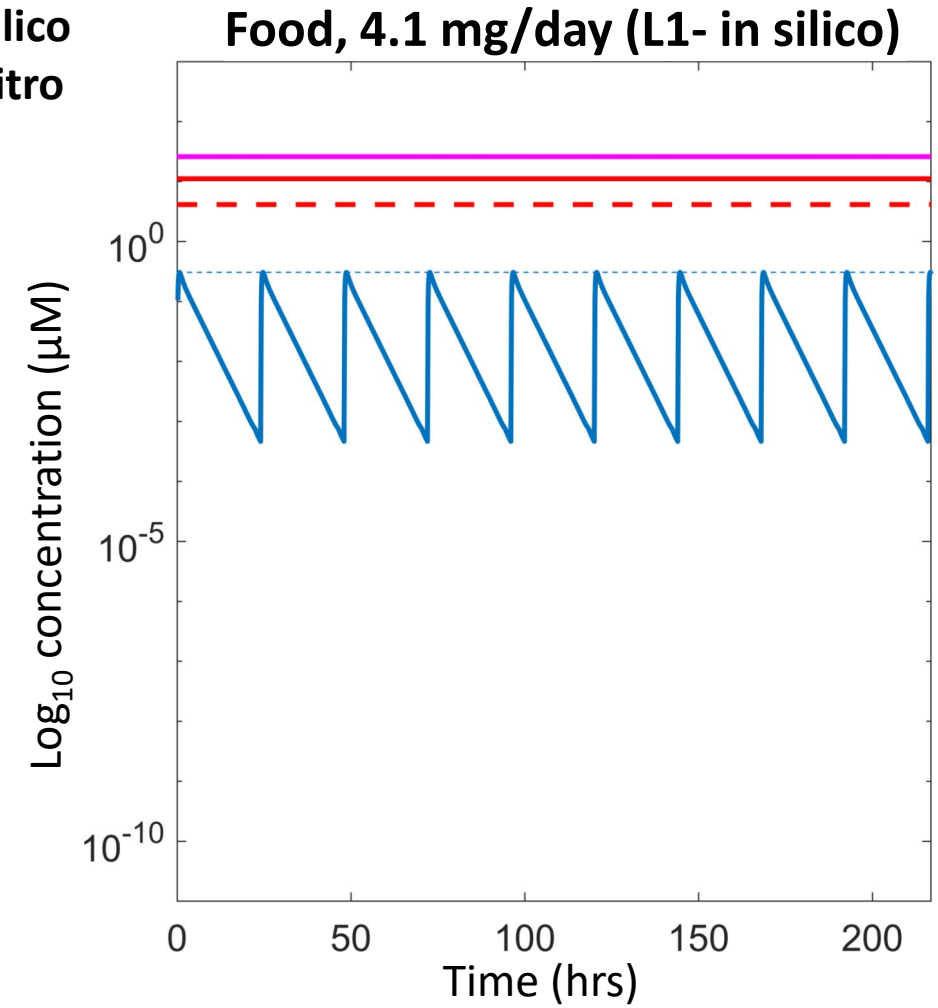
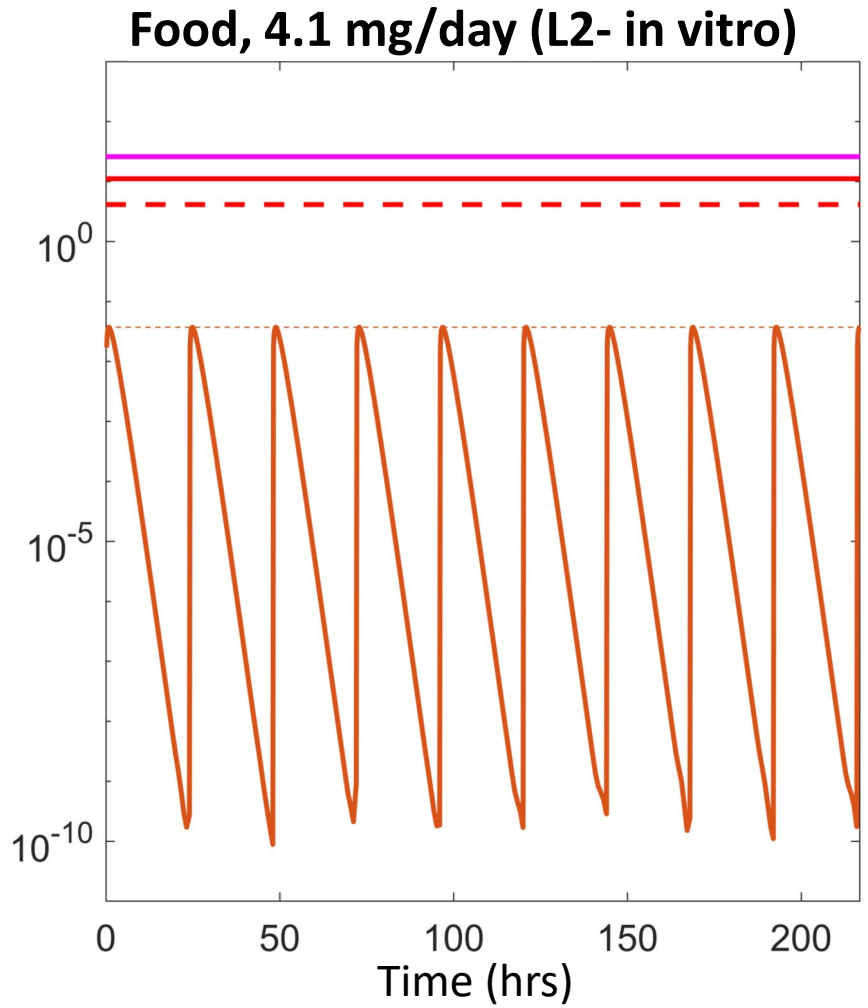
Example 1: exposure to coumarin through oral dietary intake

L1 – in silico
L2 – in vitro





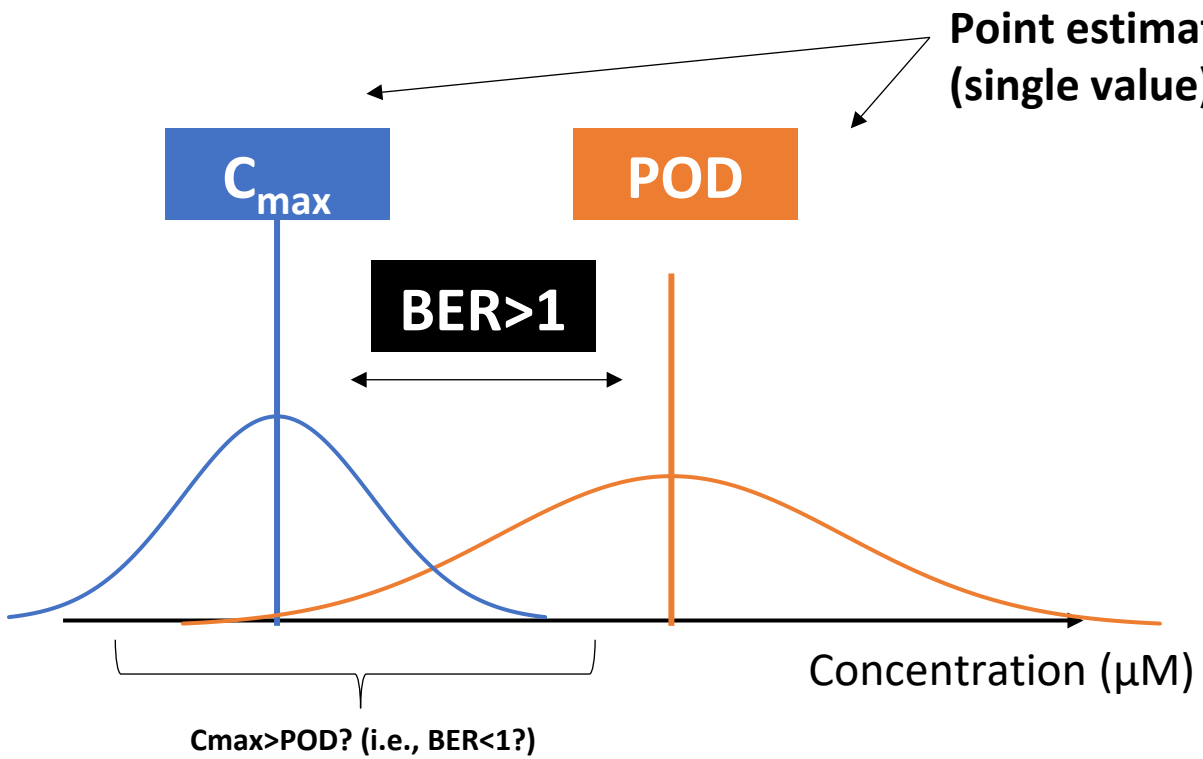
PODs:

- In vitro pharmacological profiling (MAO-A)
- High throughput transcriptomics (HepG2)
- High throughput transcriptomics (MCF-7)

Uncertainty quantification and decision making

Why do we care about quantifying uncertainty?

- In this example, using point estimates results in Cmax appearing below the POD (i.e., the BER>1).
- The true values of both metrics are subject to uncertainty.
- These uncertainties can be captured in terms of distributions.
- The distributions show the range of plausible values for the Cmax and POD.
- Quantifying uncertainty in quantities like Cmax and the POD can be helpful to determine when a safety decision can be made with confidence, or when more refinement is needed.

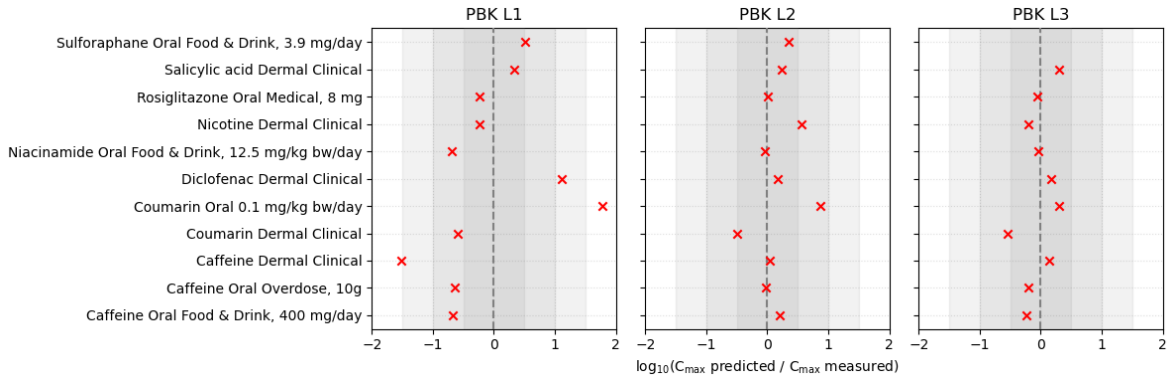


$$\text{Prob}(\text{BER} > 1)=?$$

Bayesian modelling of the PBK Cmax error

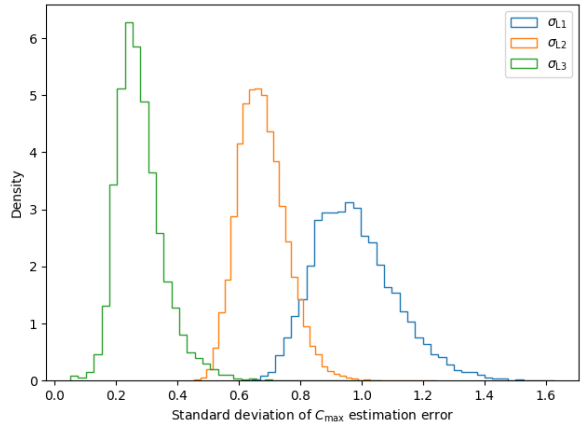
1. Model inference

Training data

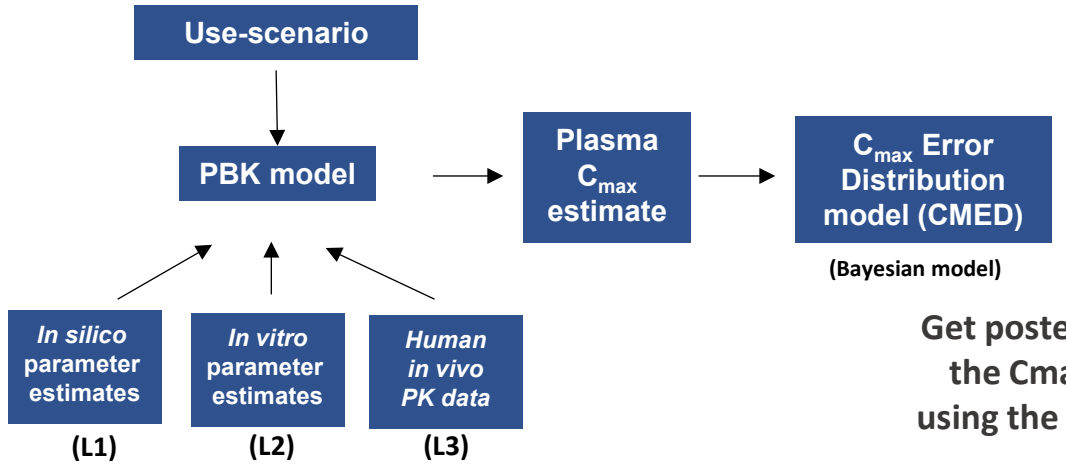


Use Bayesian inference to train model and learn the statistical model parameters

Posterior distributions of the Cmax at for different PBK levels

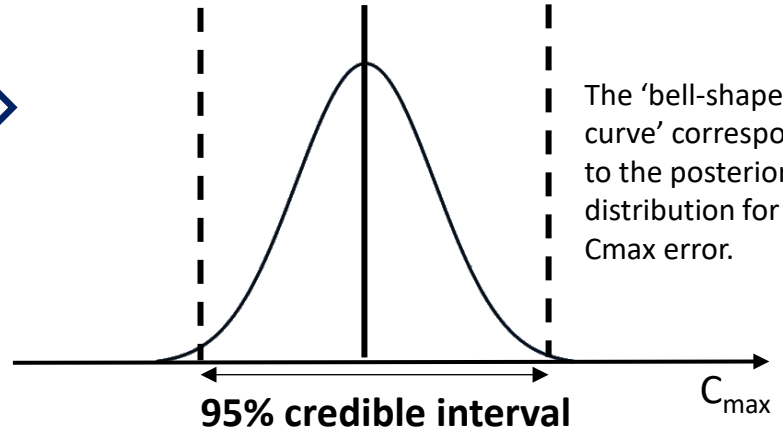


2. Application for novel exposure scenarios or chemicals



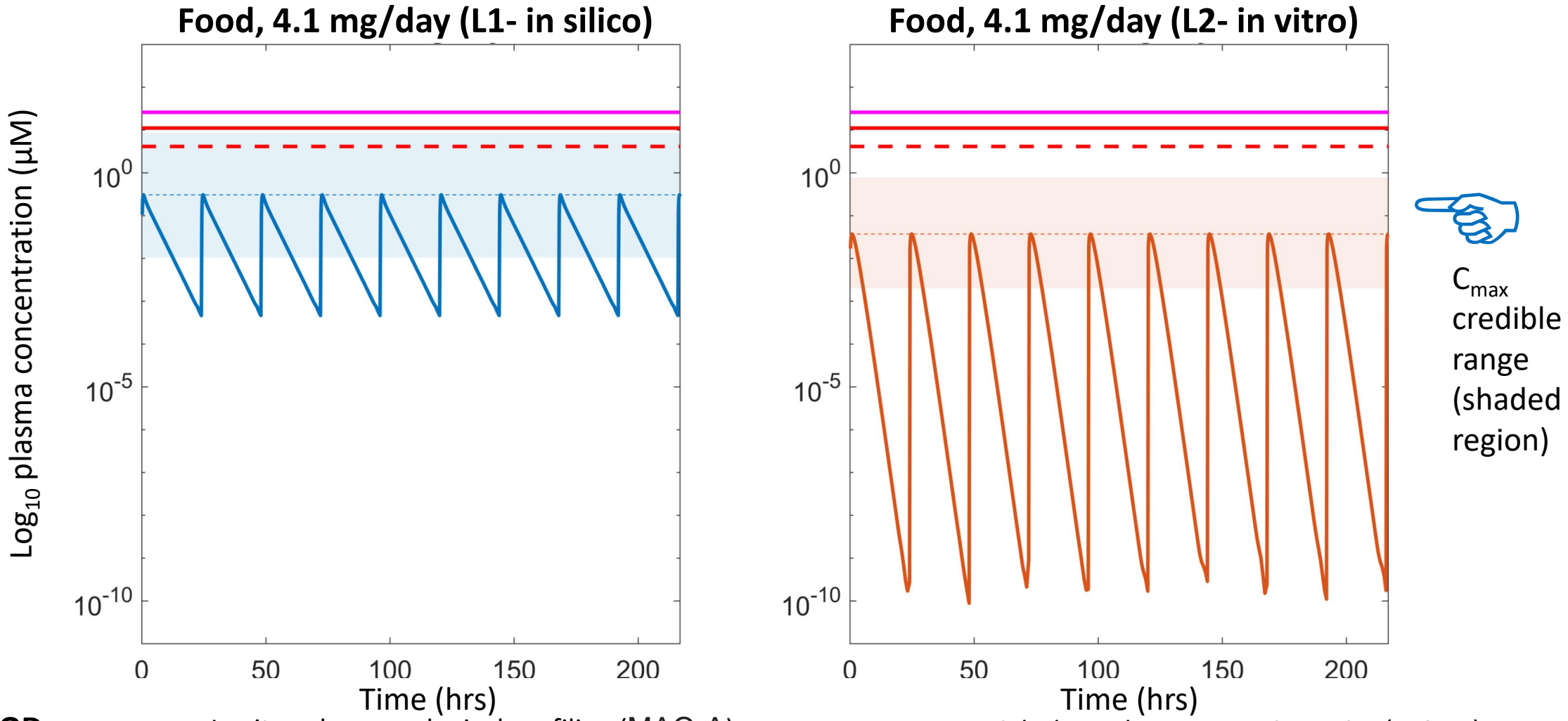
Get posterior distribution of the Cmax error obtained using the Bayesian statistical model

PBK model point estimate

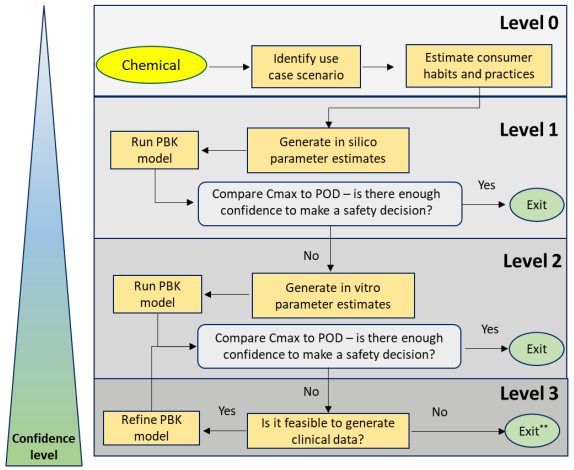


The 'bell-shaped curve' corresponds to the posterior distribution for the Cmax error.

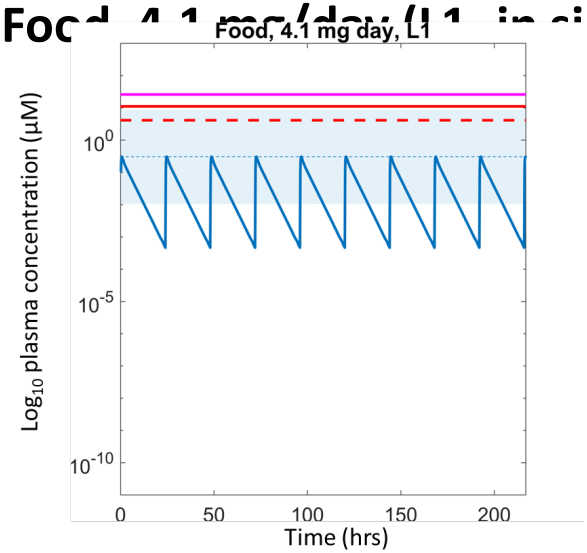
Adding credible range to Exposure estimates



Using different parameterisation levels with a tiered decision framework



Food, 4.1 mg/day (L1- in silico)



A small region of the C_{max} credible interval overlaps with the lowest POD. This can be quantified in terms of the probabilities:

$$\text{Prob}(\text{BER} > 1)=0.88$$

Can we confidently conclude low risk?

Food, 4.1 mg/day (L2- in vitro)

Go to the next level of refinement

Generate in vitro data on key parameter to obtain better estimates.

$$\cdot\ 1)=0.88$$

$$\text{Prob}(\text{BER} > 1) \approx 1$$

Use BER alongside other safety data to make decision

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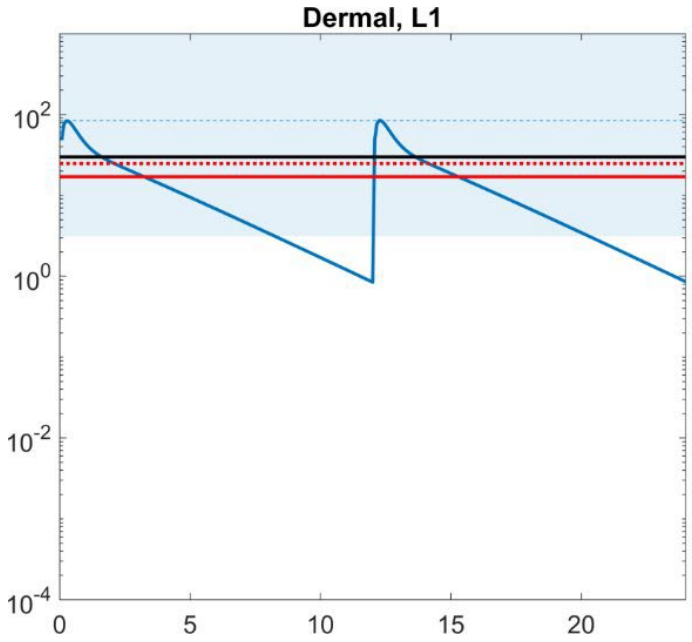
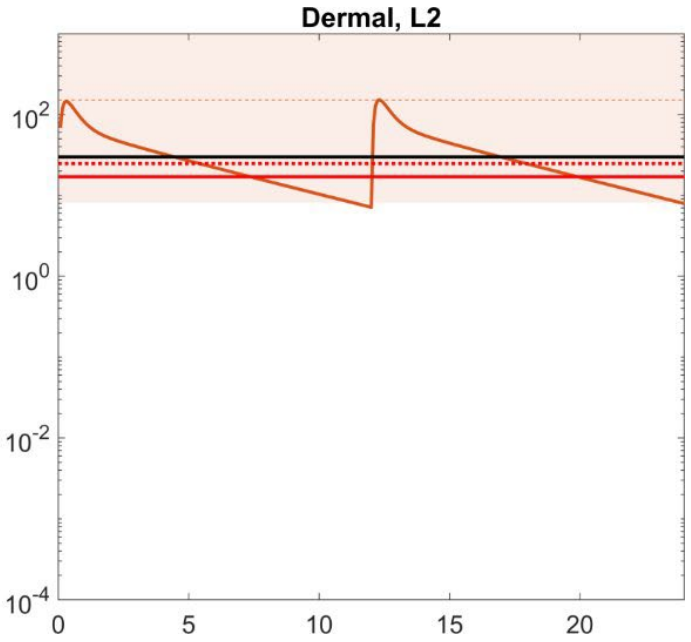
Examples 2: dermal exposure to DEET (BER ≤ 1)

- For the dermal scenario, we would assign this as uncertain risk.
- It may be possible to refine the DEET dermal exposure scenario further at higher tier tools.





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Evaluating the systemic safety toolbox across a wide range of chemicals and exposure scenarios

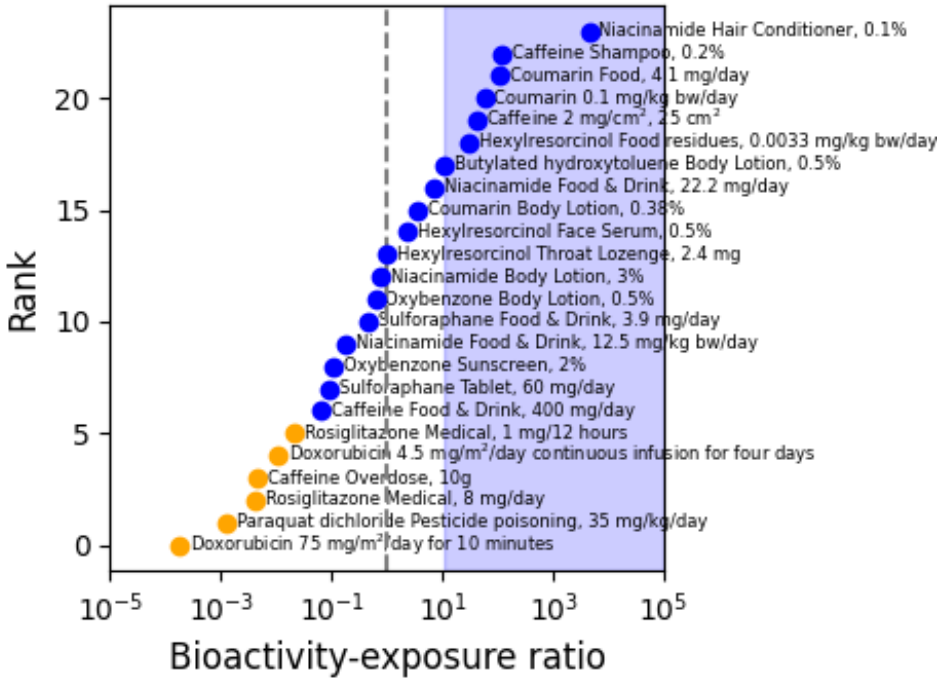
Selection of chemicals and exposure scenario

- Chemicals with well-defined human exposures
- Traditional safety assessment available

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
Paraquat	Accidental ingestion 35 mg/kg	High risk

10 chemicals – 25 exposure scenarios

PBK Level 2, Blue shaded region BER> 11



BER=lowest POD/Plasma Cmax

Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

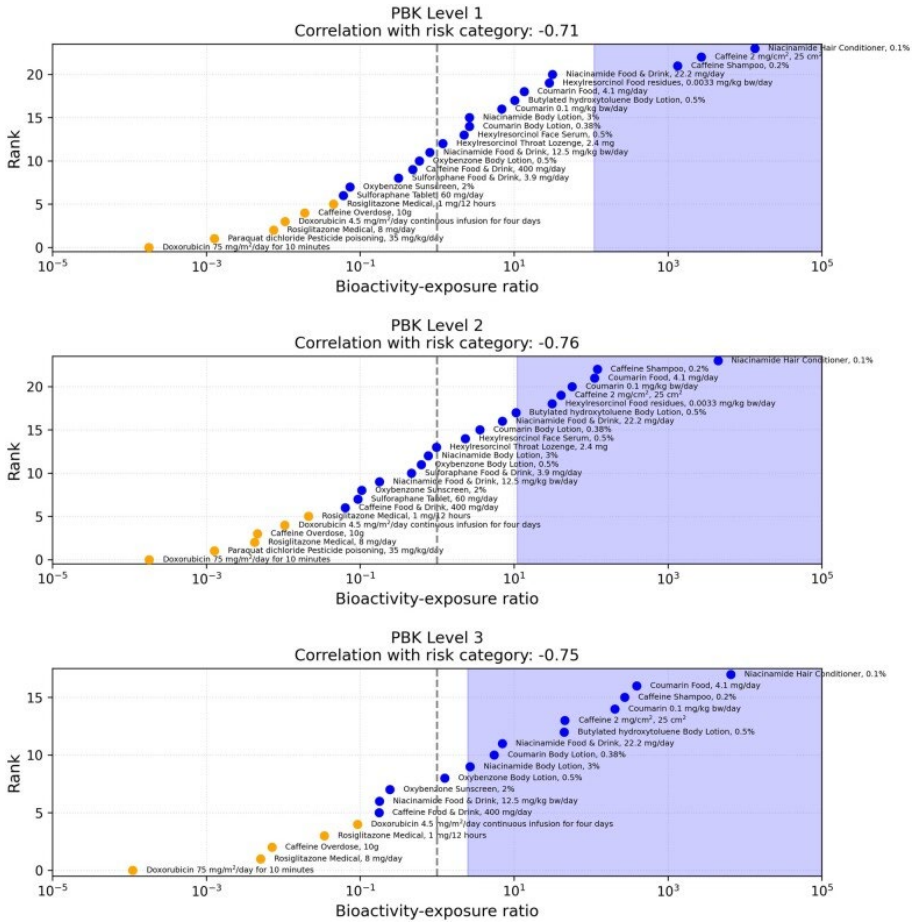
Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Evaluating the systemic safety toolbox across a wide range of chemicals and exposure scenarios

Low risk exposure scenarios are blue dots, high risk are yellow dots.

Chemical-exposure scenarios with a BER point estimate outside the blue-shaded region would be identified as “uncertain” risk under this decision model. The grey dashed line corresponds to BER = 1.

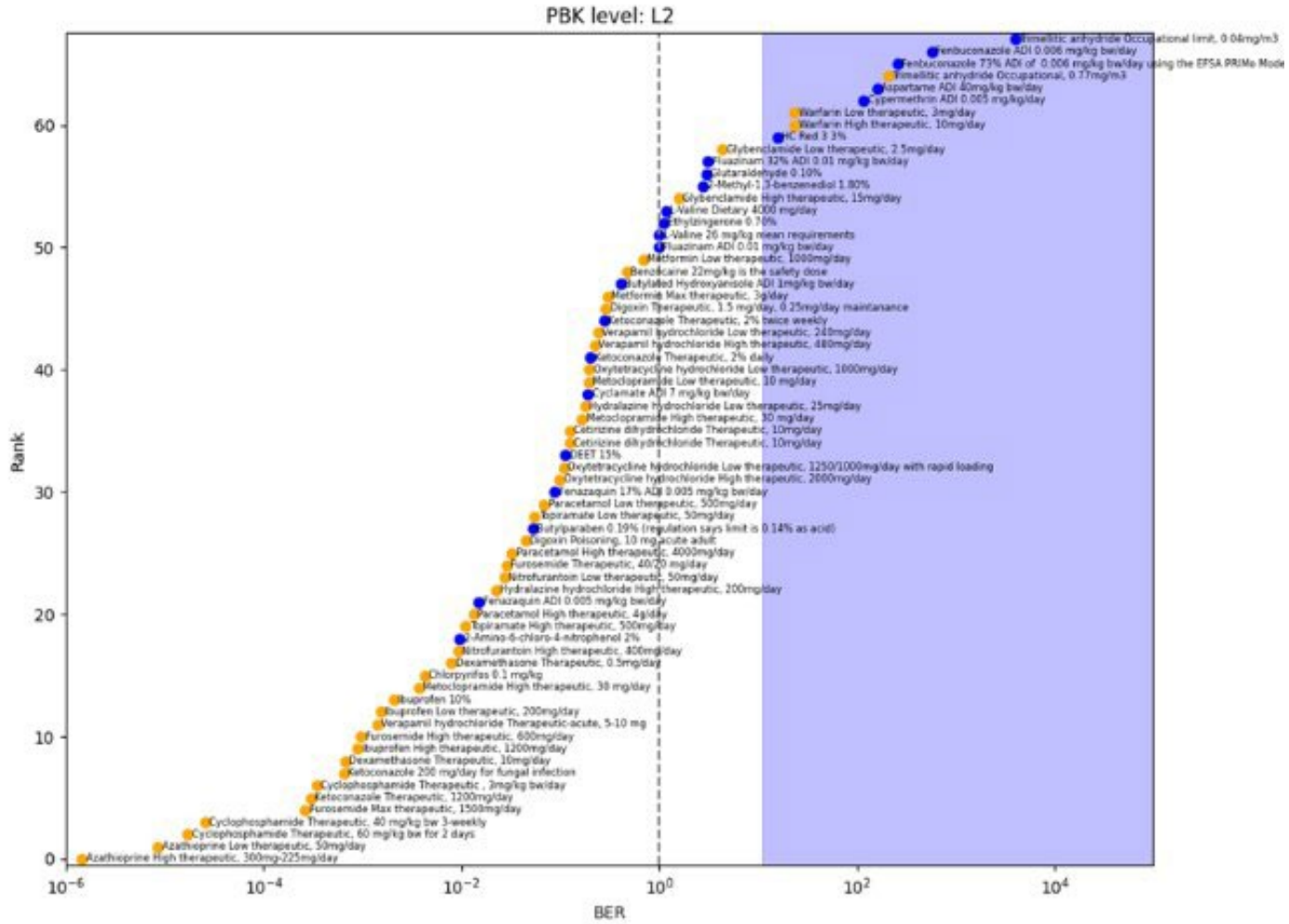
PBK Level	BER threshold	Empirical Protectiveness	Empirical Utility
1	110	6/6 (100%)	3/18 (17%)
2	11	6/6 (100%)	6/18 (33%)
3	2.5	5/5 (100%)	9/13 (69%)



Are these thresholds still protective if we increase the number and diversity of chemicals?

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

Extended evaluation (38 more chemicals)



PBK Level	BER threshold	Empirical Protectiveness	Empirical Utility
1	110	43/46 (93%)	2/24 (8%)
2	11	43/46 (93%)	6/22 (27%)
3	2.5	44/46 (96%)	0/3 (0%)
Highest	-	44/46 (96%)	7/24 (29%)

- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure

- **Further research is being performed to explore additional relevant in vitro assays to be added the toolbox.**

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Discussion and next steps

- The systemic safety toolbox and workflow has been evaluated for a range of chemicals covering different exposure scenarios.
- Here, a key metric is the bioactivity exposure ratio, which uses:
  - the minimum POD
  - the Cmax
- PBK models can be refined as required by the risk assessment.
- A Bayesian statistical model has been used to quantify uncertainty the Cmax estimates.
- Various areas of ongoing research include:
  - Further work to quantify the PBK model errors.
  - Comparing the results of different PBK models/software.
  - Evaluating different in vitro assays and POD estimation approaches.

Acknowledgements

Unilever: Alistair Middleton, Maria Baltazar, Sophie Cable , Joe Reynolds, Georgia Reynolds, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Katarzyna Przybylak, Predrag Kukic, Dawei Tang, Matthew Dent, Andrew White, Paul Carmichael, Sarah Hatherell, Richard Cubberley, Carl Westmoreland

US-EPA: Richard Judson, Josh Harrell, Logan Everett, Imran Shah, Joseph Bundy, Laura Taylor, Jacob Fredenburg, John Wambaugh