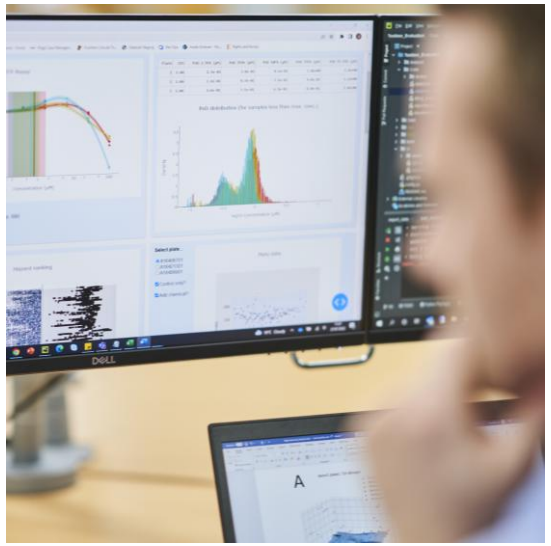
Building confidence in PBK modelling in Next Generation Risk Assessment for systemic toxicity



Dawei Tang and Ans Punt

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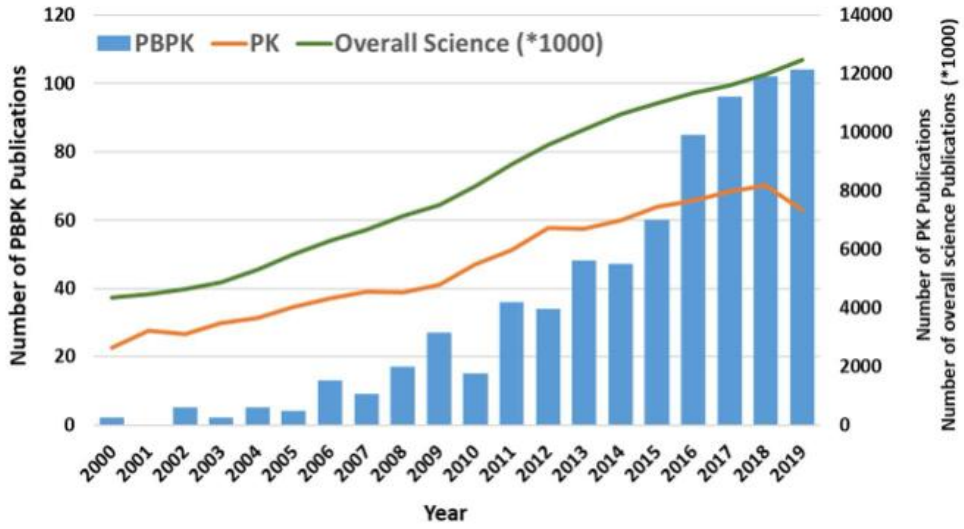
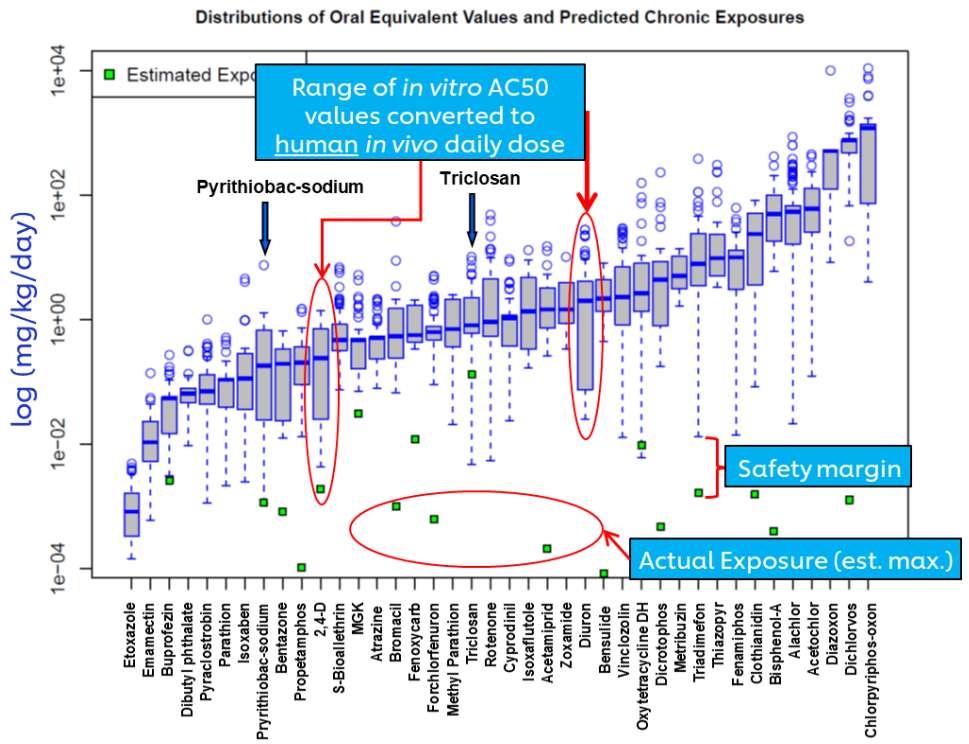
Applications of PBK modelling

- Species differences in kinetics
- Simulating special populations and interindividual human variation
- Chemical interactions like drug-drug or drug-food interactions
- High-to low-dose extrapolations
- Route-to-route extrapolations (e.g. oral to inhalation)
- Interpretation of human biomonitoring studies
- **Quantitative in vitro-in vivo extrapolations (QIVIVE) in non-animal testing strategies**



Number of PBPK publications (El-Khateeb et al, Biopharmaceutics & Drug Disposition 42.4 (2021): 107-117,)

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

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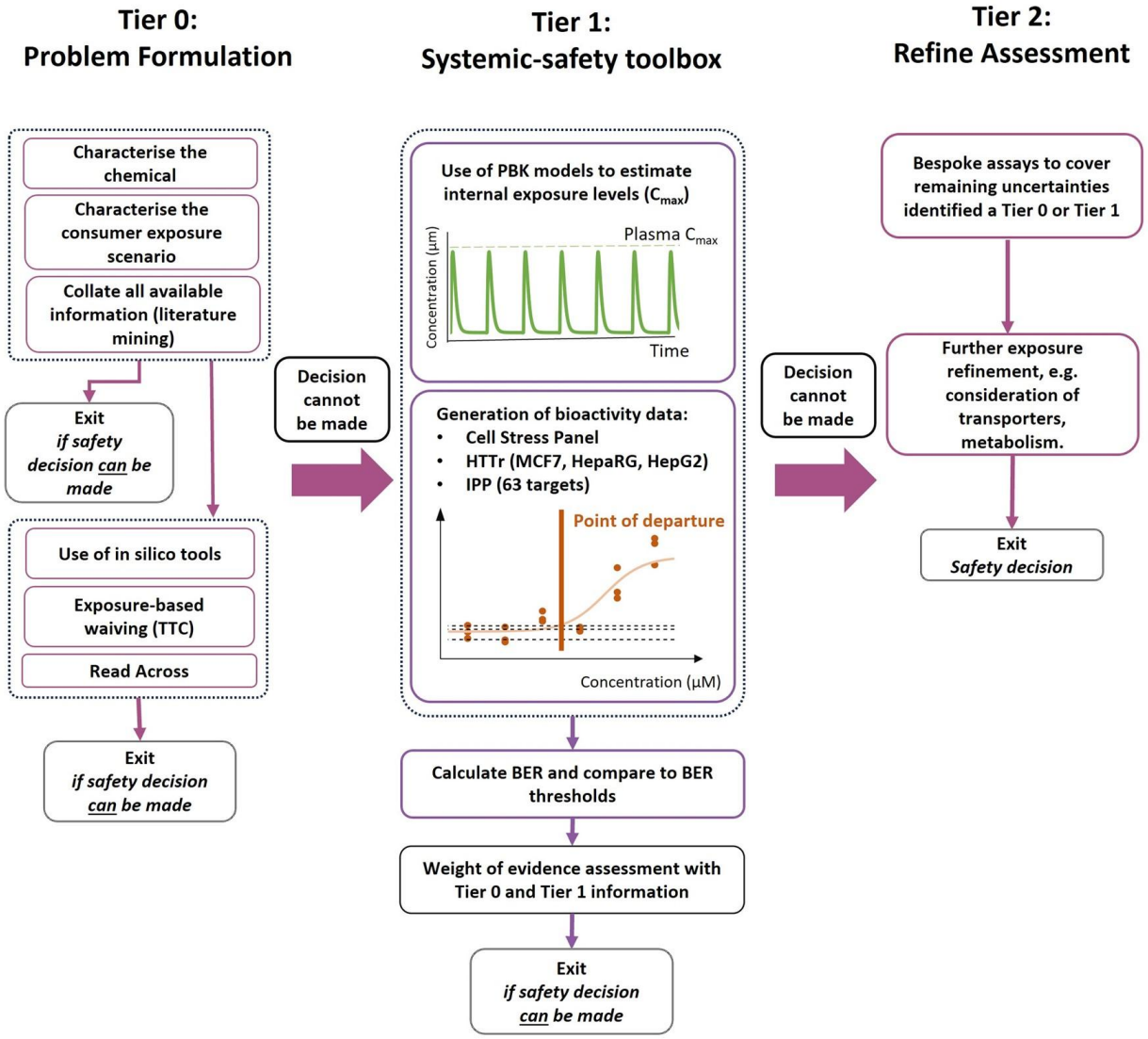
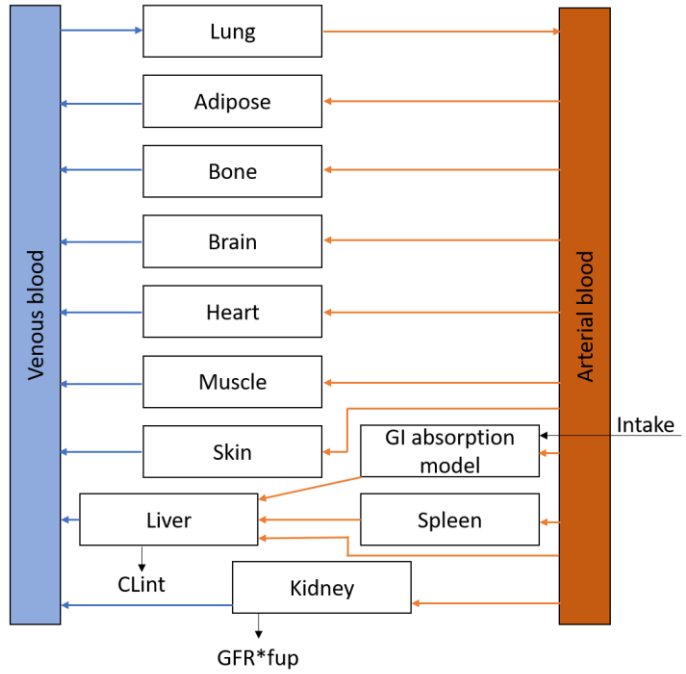
NGRA toolbox for systemic toxicity at Unilever



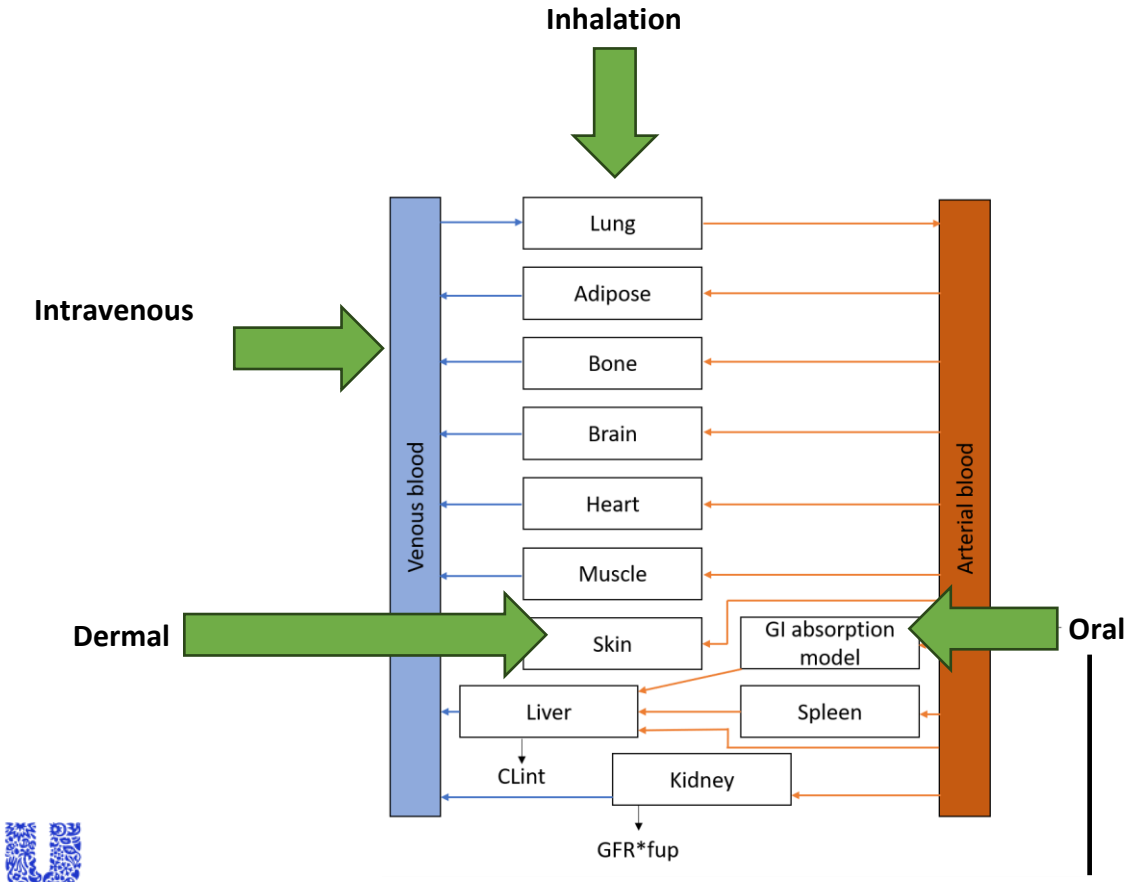
PBK modelling key in Tier 1 to understand internal exposure in relation to in vitro bioactivity data



Cable et al (2025), *Toxicological Sciences 204 (1), 79-95*

PBK modelling in NGRA

Physiologically based kinetic (PBK) models are used to simulate the behaviour of a chemical in the body for a given exposure scenario.

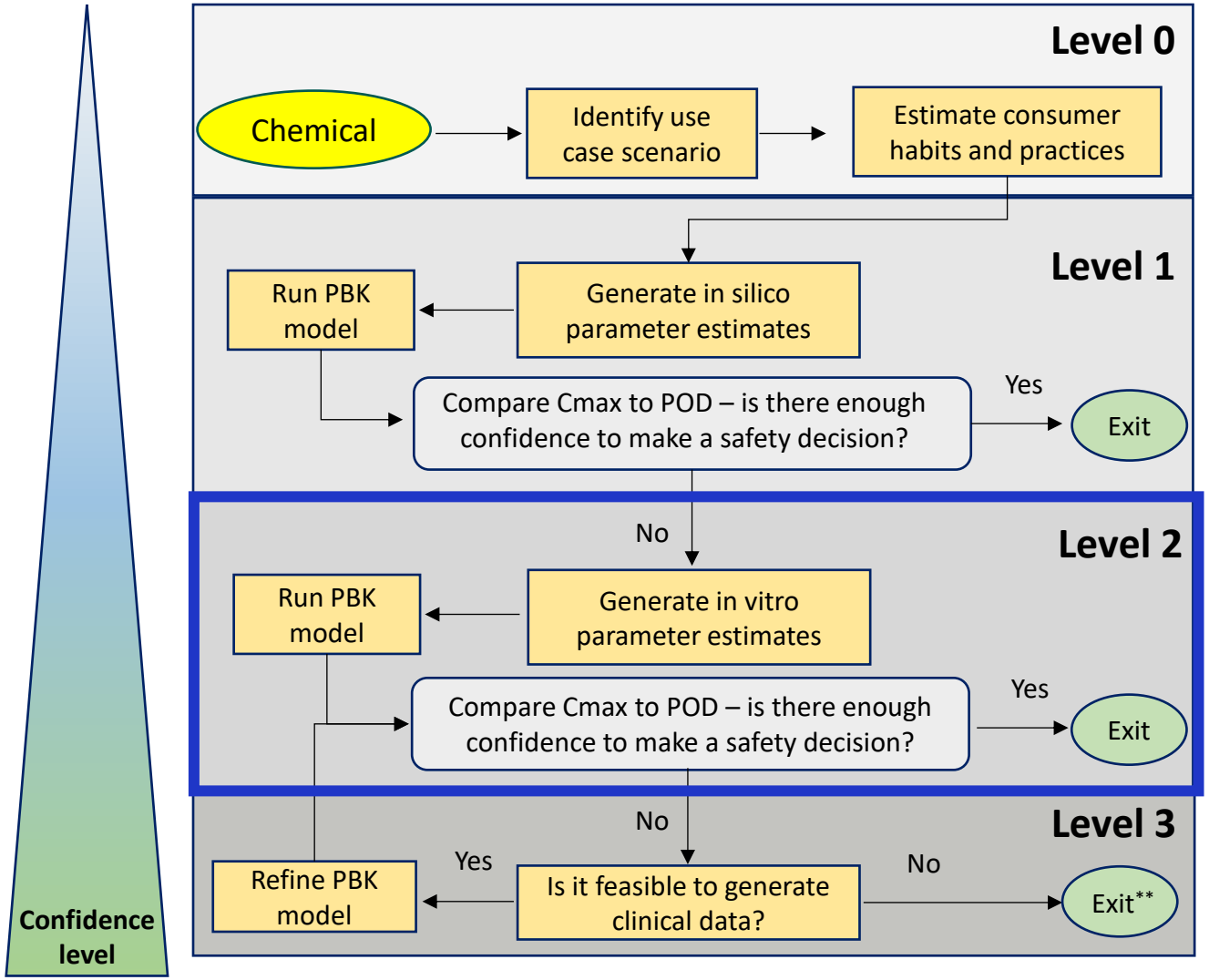


- PBK models are composed of multiple coupled ordinary differential equations.
- The model have various parameters that need to be determined.
- Example equation for determining the concentration of chemical in the liver:

$$V_{\text{Liver}}\frac{dC_{\text{Liver}}}{dt} = Q_{\text{Liver}}\left(C_{\text{Arterial}} - \frac{C_{\text{Liver}}}{P_{\text{Liver}}}\right) - CLint\left(\frac{C_{\text{Liver}}}{P_{\text{Liver}}} * Fup\right)$$

- $V_{\text{Liver}}\frac{dC_{\text{Liver}}}{dt}$: [Rate of change of concentration in the liver]
- Q_{Liver}: [Blood flow rate]
- C_{Arterial}: [Concentration of chemical in the arterial blood]
- $\frac{C_{\text{Liver}}}{P_{\text{Liver}}}$: [Concentration of chemical in the liver plasma]
- $CLint\left(\frac{C_{\text{Liver}}}{P_{\text{Liver}}} * Fup\right)$: [Clearance rate via metabolism in the liver]

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

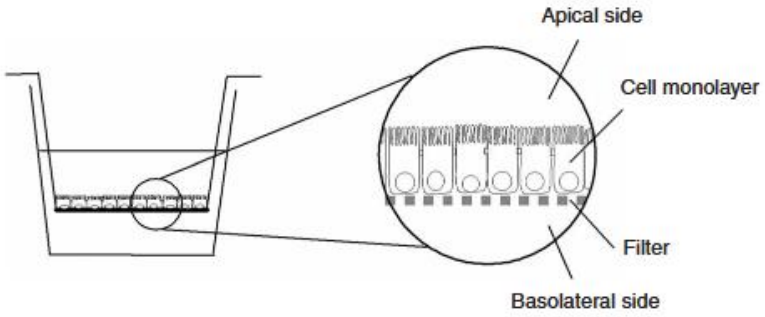
Level 3: Some chemical-specific parameters are inferred by calibrating model against existing human PK data for the same chemical (by a different exposure scenario.

** 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.**

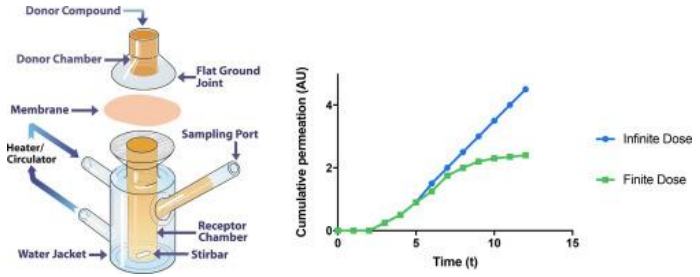
In vitro and in silico methods to parameterize PBK models

Caco-2 model for intestinal absorption



e.g. Hubatsch et al (2007), *Nature Protocols 2, 2111-2119*

Skin absorption (Franz cell)



e.g. Lane (2024), *European Journal of Pharmaceutical Sciences 201, 106873*

Metabolic clearance (hepatocytes, S9 or microsomes)

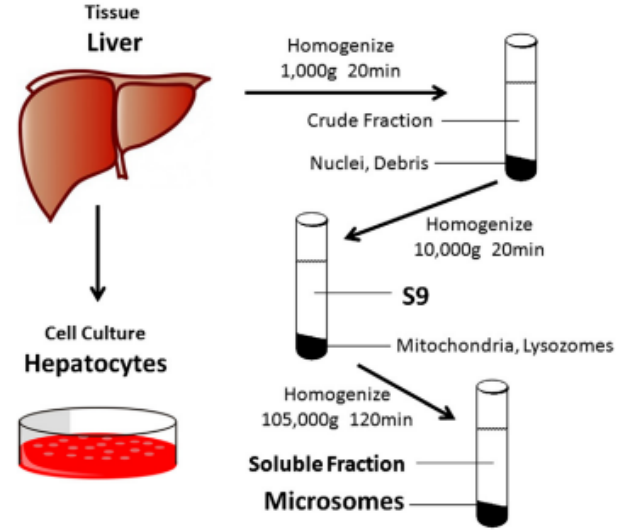
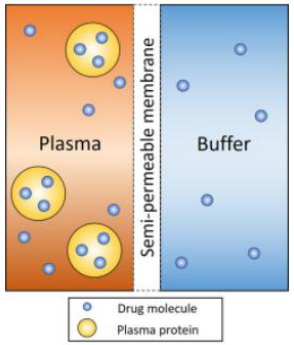


FIGURE 3.6 Preparation of microsomal, S9, and soluble fractions commonly used in drug metabolism studies.

e.g. Vrbanac and Slauter (2016), *ADME in Drug Discovery. Book: A Comprehensive Guide to Toxicology in Nonclinical Drug Development, pp. 39-67*

Plasma protein binding



e.g. Hann et al. (2022), Chapter Three - The importance of plasma protein and tissue binding in a drug discovery program to successfully deliver a preclinical candidate. *Book: Progress in Medicinal Chemistry, pp. 163 - 214*

Partition coefficients (in silico based on logP and pKa)

$$Kpu = \left[\begin{array}{l} f_{EW} + \left(\dfrac{1+X}{1+Y} \cdot f_{IW} \right) \\ \quad + \left(\dfrac{(P \cdot f_{NL} + ((0.3P + 0.7) \cdot f_{NP}))}{1+Y} \right) \\ \quad + \left(\dfrac{Ka \cdot [AP^-]_T \cdot X}{1+Y} \right) \end{array} \right]$$

e.g. Rodgers and Rowland (2006), *Journal of Pharmaceutical Sciences 95, 1238-1257*

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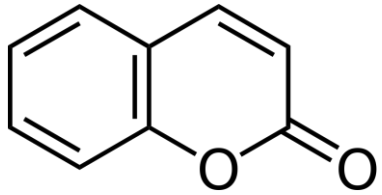
Example exposure scenarios

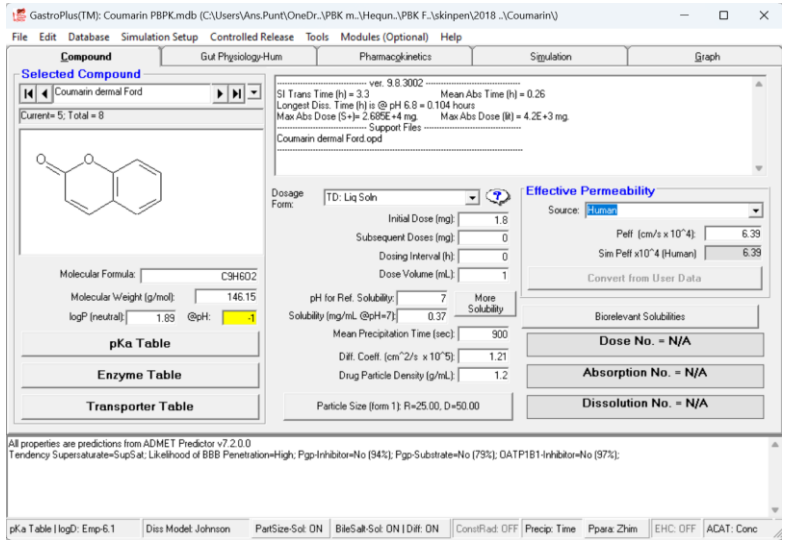


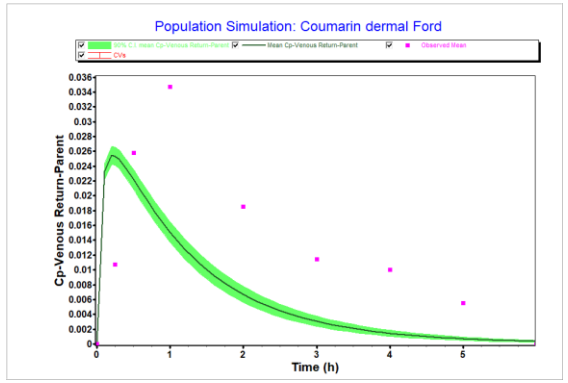
Coumarin (flavouring and fragrance, naturally present in e.g. cinnamon)

Compound	**Use Scenario**	**Exposure route**	**Risk classification**
Coumarin	Dietary intake, 4.1 mg/day	Oral	Low risk

Example: exposure to coumarin through oral dietary intake

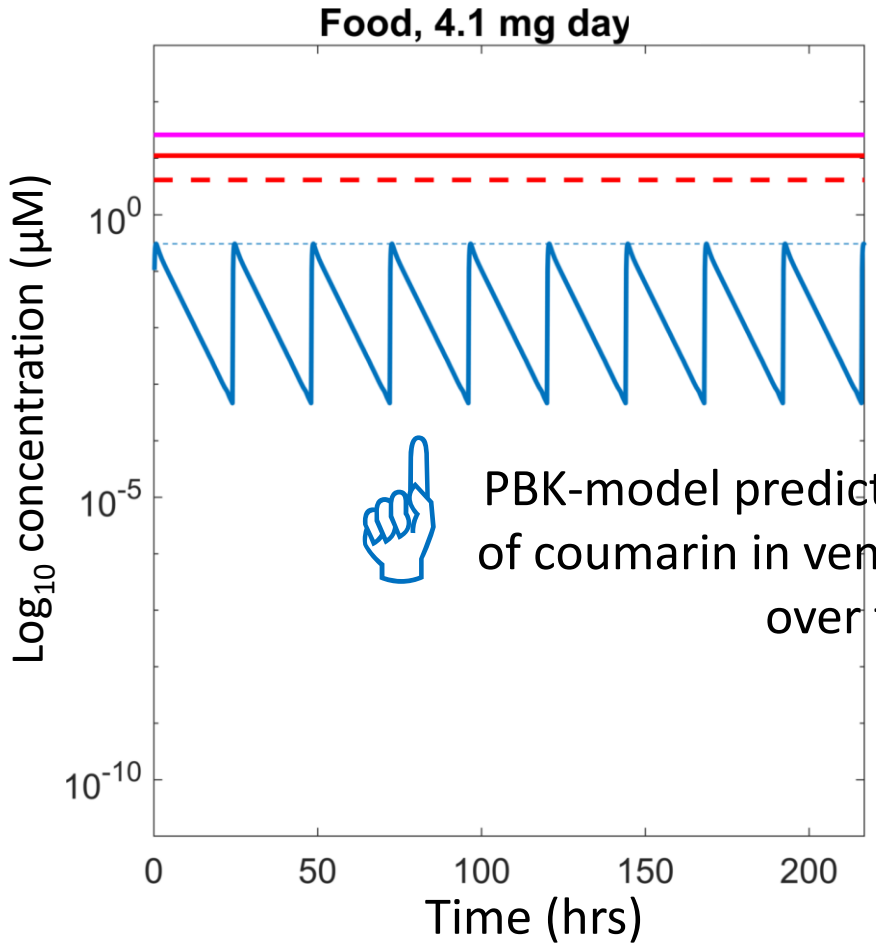
Parameter	Value	Source	Level
Molecular weight (g/mol)	147.1		
Log P	1.89	ADMET predictor	L1
	1.39	Measured[1]	L2
Hepatic intrinsic clearance (L/h)	105	ADMET predictor	L1
	929	Measured	L2
Unbound fraction in plasma (f_{up})	0.24	ADMET predictor	L1
	0.31	Measured[2]	L2
Blood: plasma ratio	1.08	ADMET predictor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

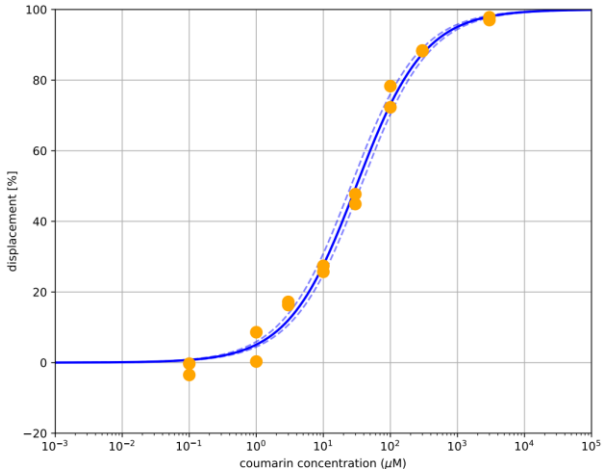
Example: exposure to coumarin through oral dietary intake (BER>1)



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: exposure to coumarin through oral dietary intake

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Molecular weight (g/mol)	147.1		
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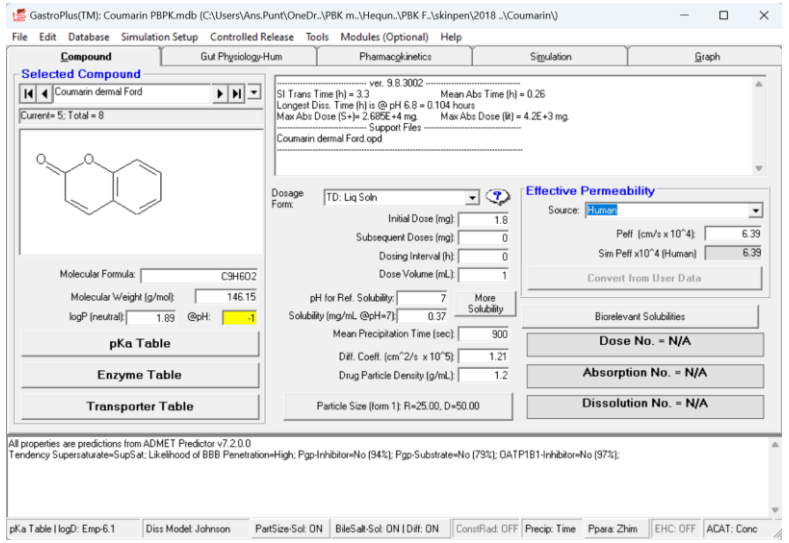

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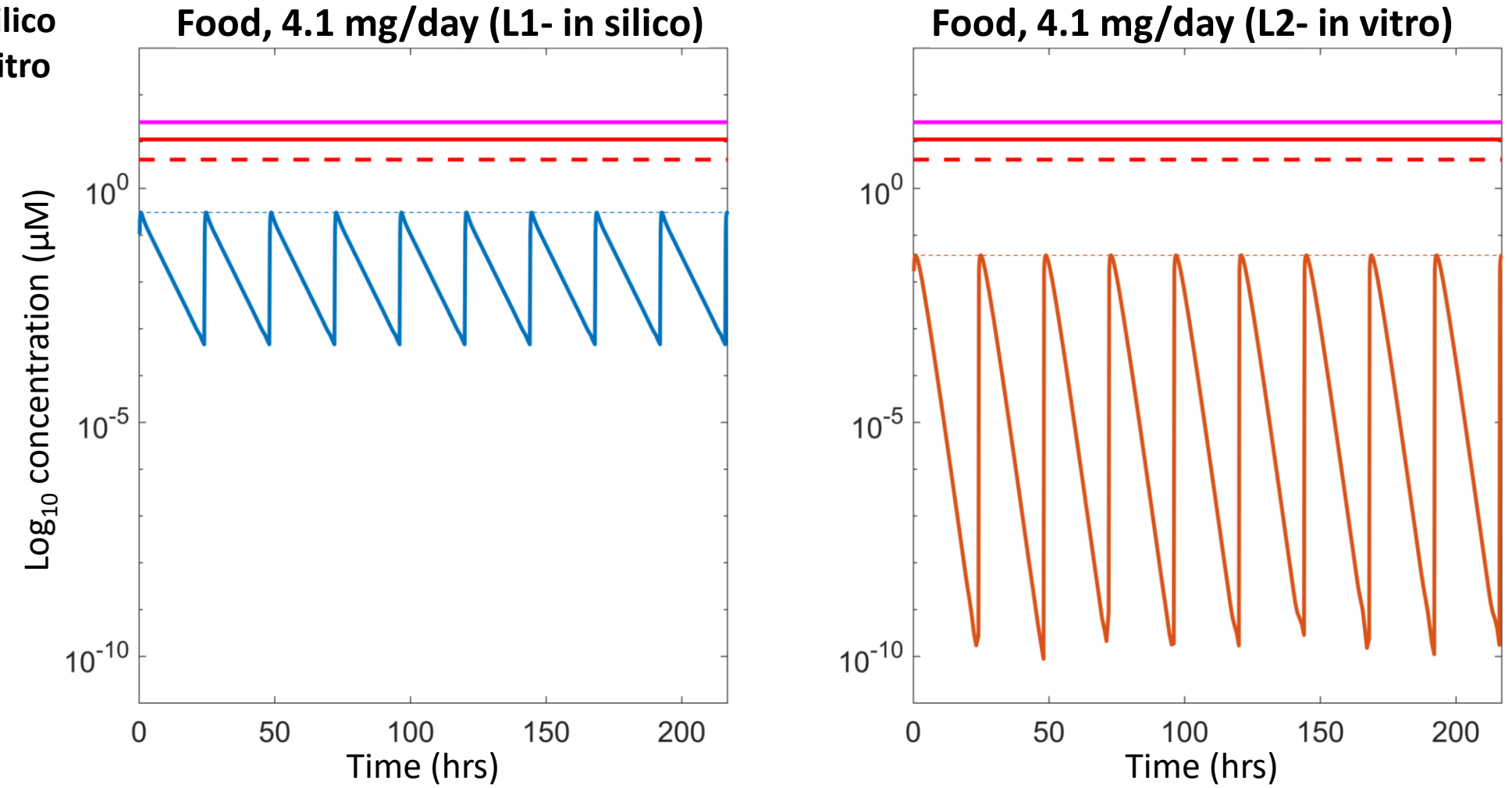
Example: exposure to coumarin through oral dietary intake

L1 – in silico
L2 – in vitro



PODs:
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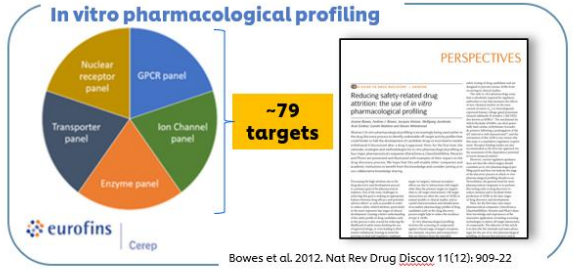
Risk Assessment Outcome

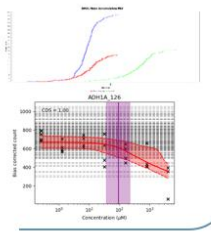
BIOACTIVITY



Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- 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

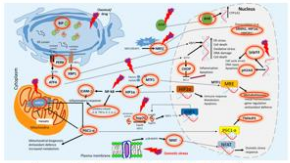
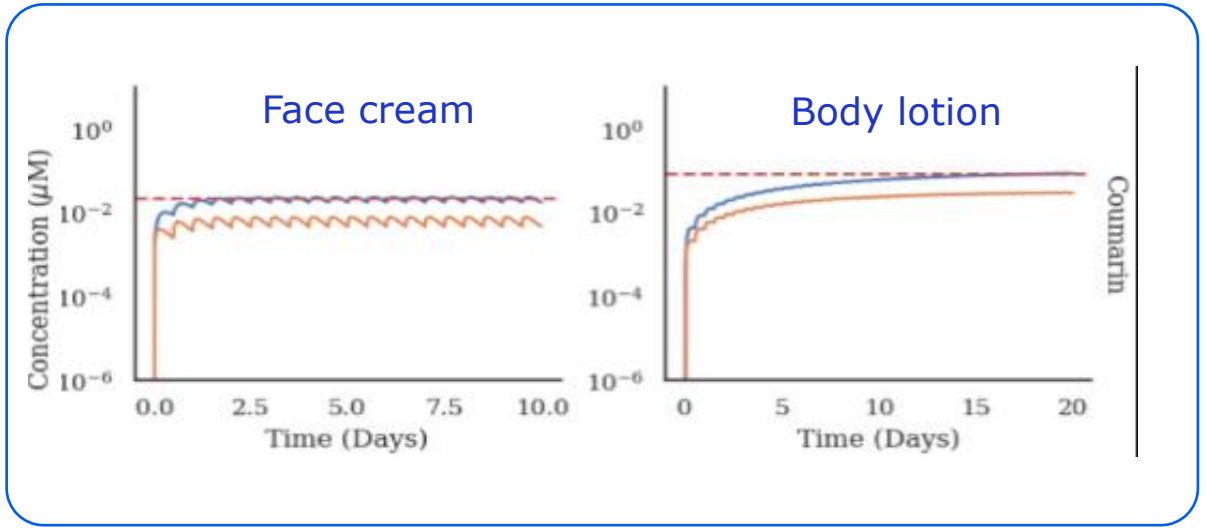


Image kindly provided by Paul Walker (Cyprotex)

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

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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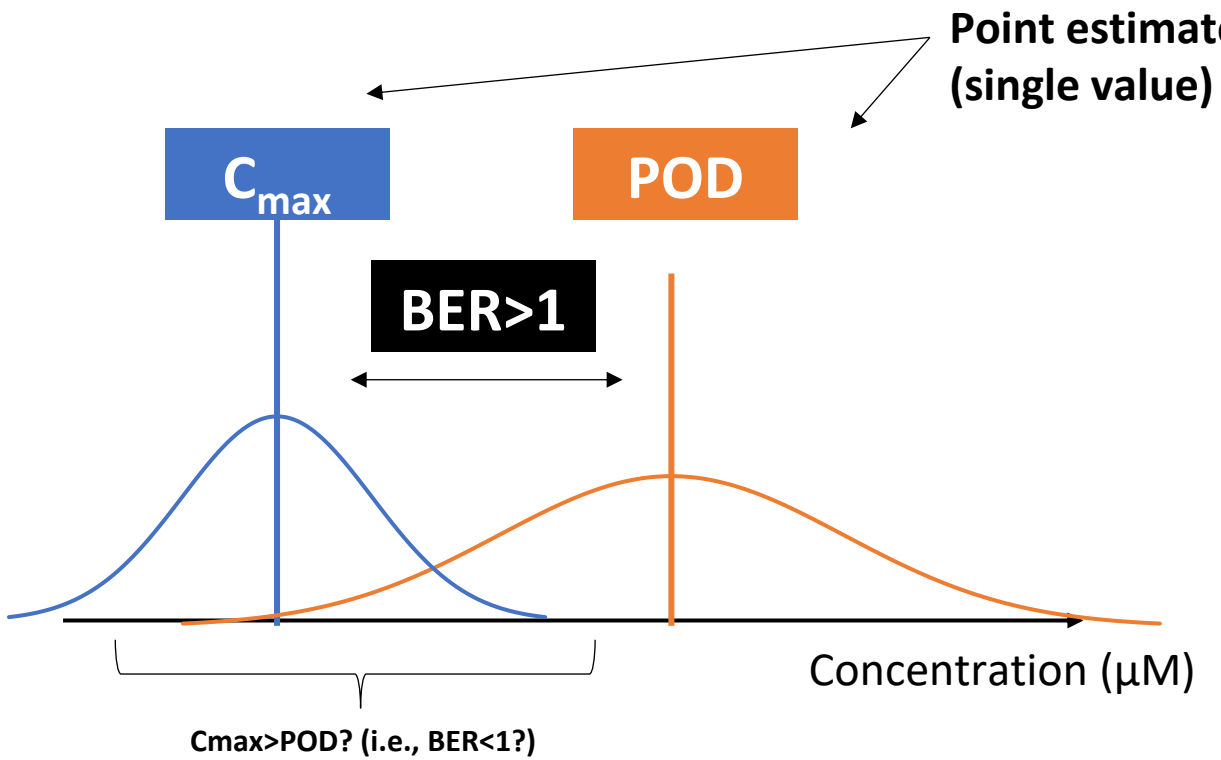
Uncertainty quantification and decision making

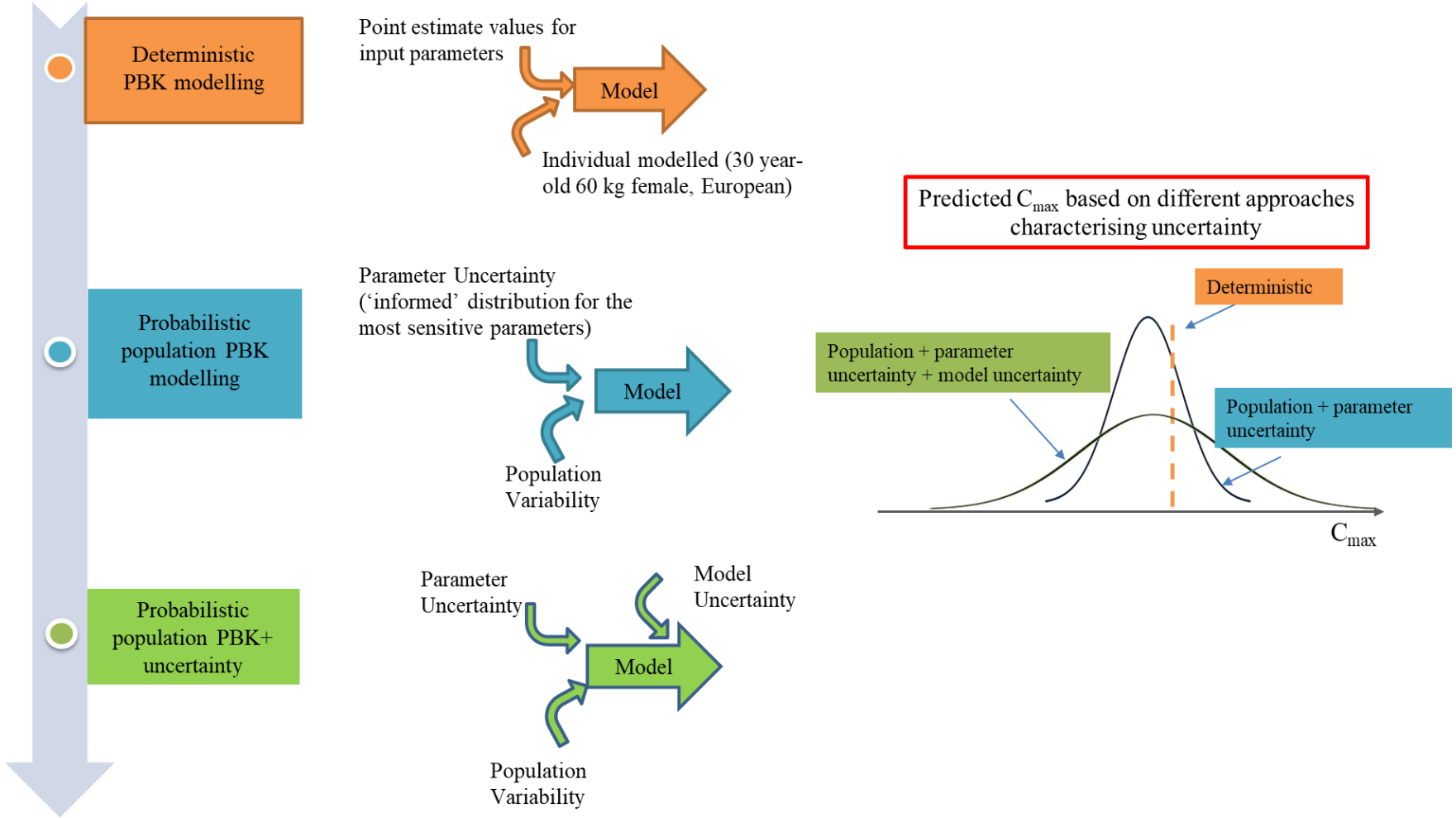
Why do we care about quantifying uncertainty?

- 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.



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

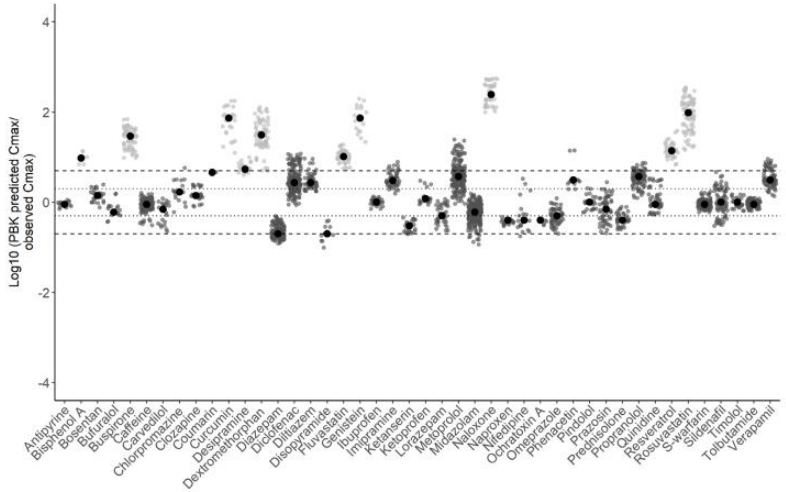
Strategies in addressing uncertainty and variability in PBK model predictions



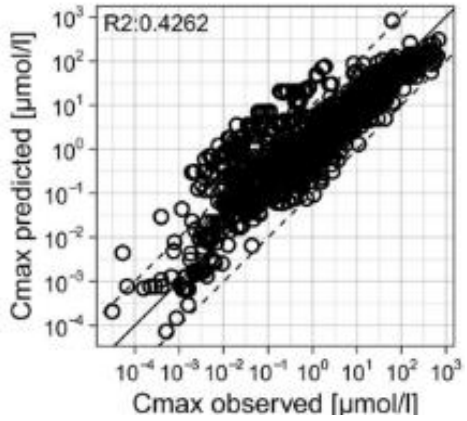
Unilever

Uncertainty in PBK model predictions, comparing predicted and observed Cmax

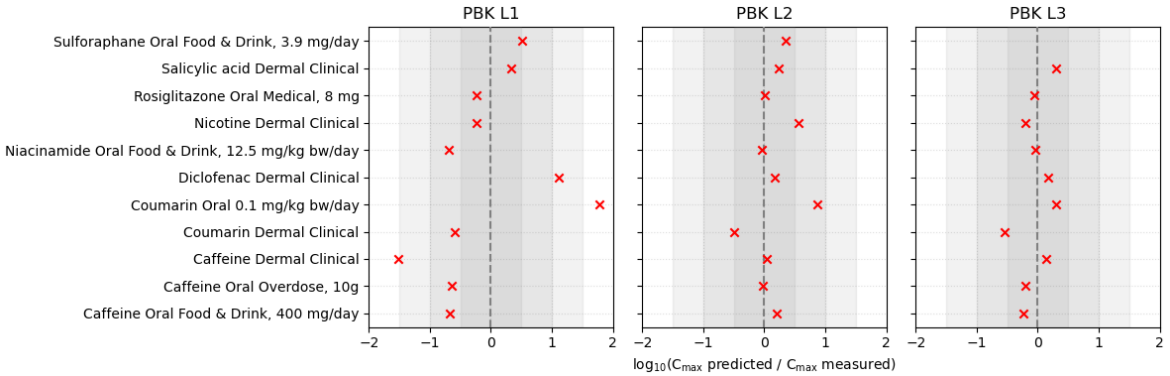
- L2: Cmax predicted within 5- to 11-fold of observed Cmax values.
- L1: uncertainty is higher.
- Note: human kinetic studies also vary from each other.



Punt et al (2022), *Altex,* Volume 39, Issue 2
(In vitro, Level 2)



Geci et al (2022), *Arch Tox,* Volume 99.
(In silico, Level 1)

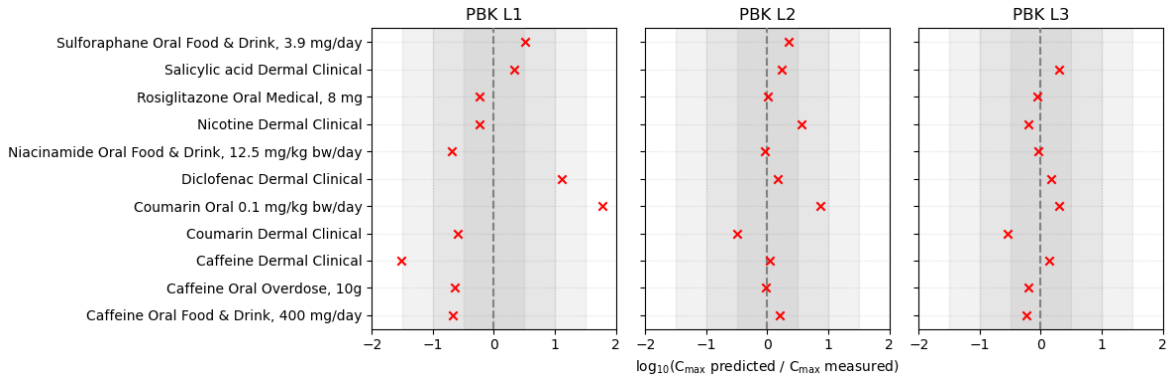


Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1
(Level 1, 2 and 3)

Bayesian modelling of the PBK Cmax error

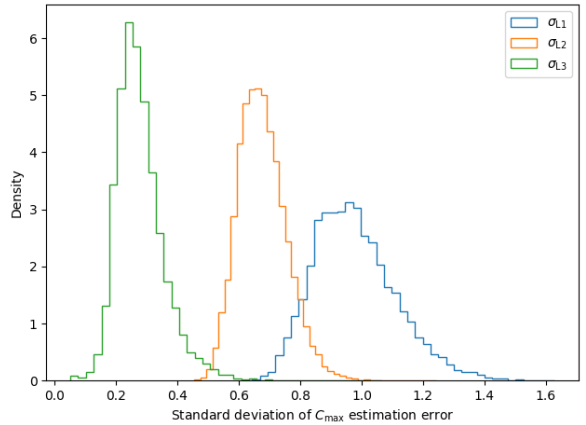
1. Model inference

Training data

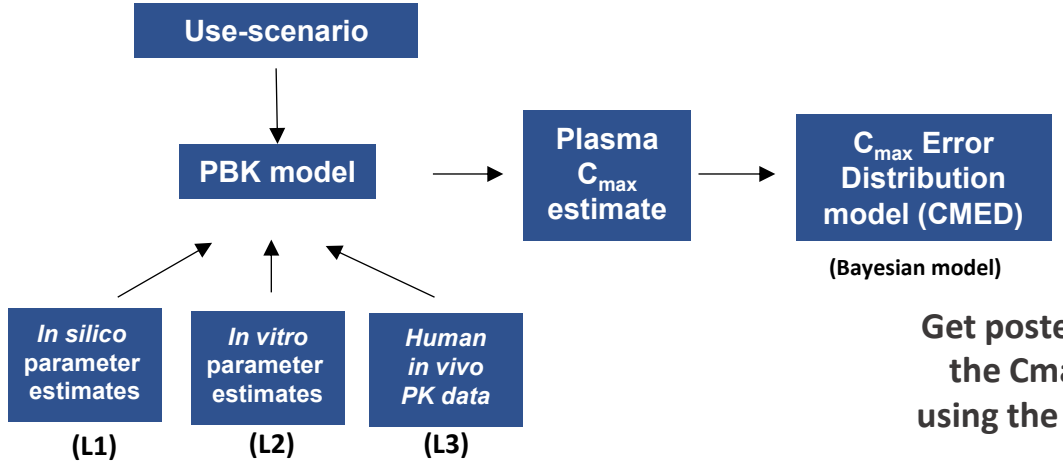


Use Bayesian inference to train model and learn the statistical model parameters

Posterior distributions of the Cmax at for different PBK levels

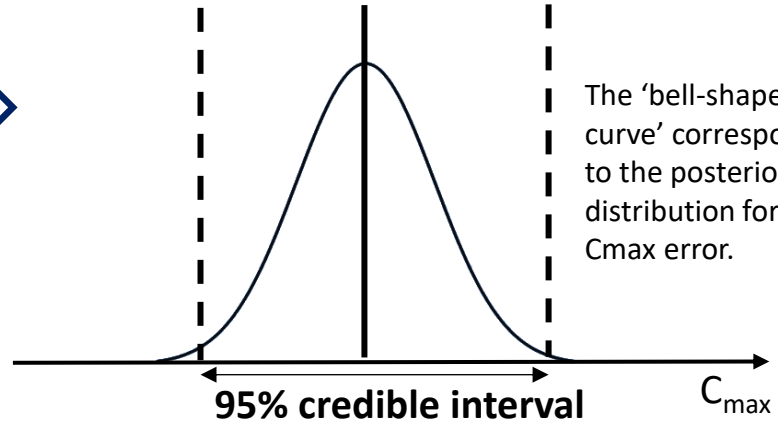


2. Application for novel exposure scenarios or chemicals



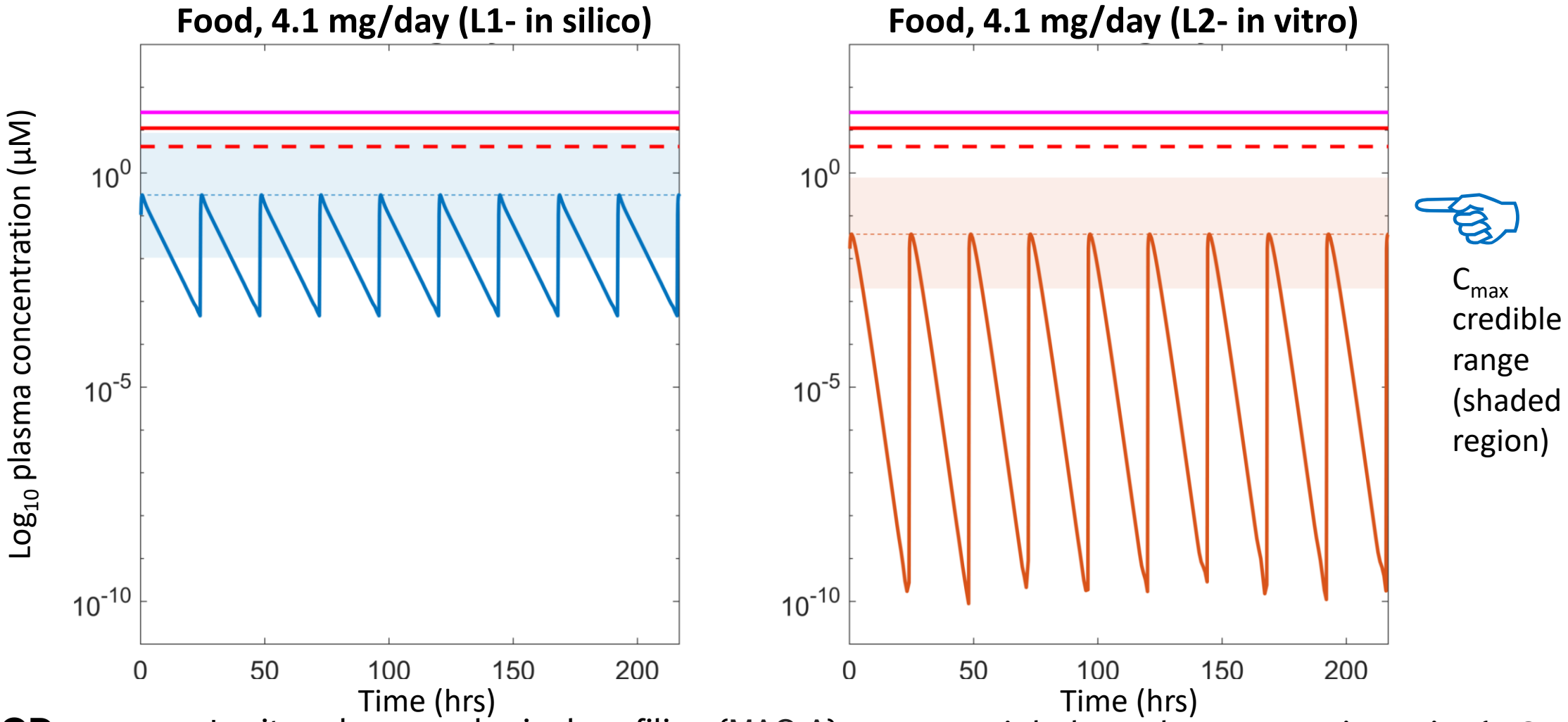
Get posterior distribution of the Cmax error obtained using the Bayesian statistical model

PBK model point estimate



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

Adding credible range to exposure estimates



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

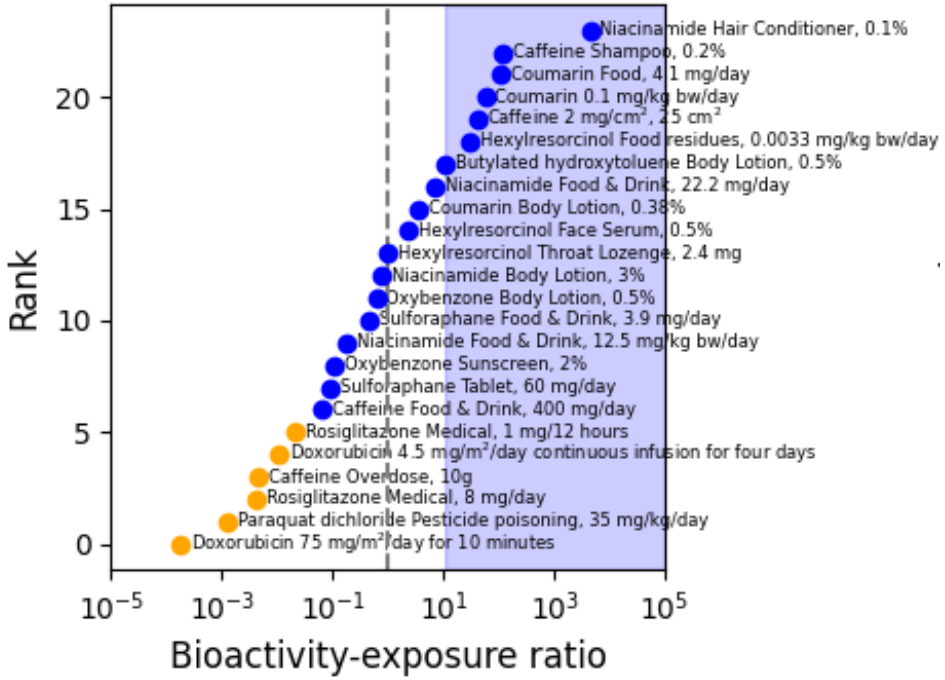
Selection of chemicals and exposure scenario

- 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

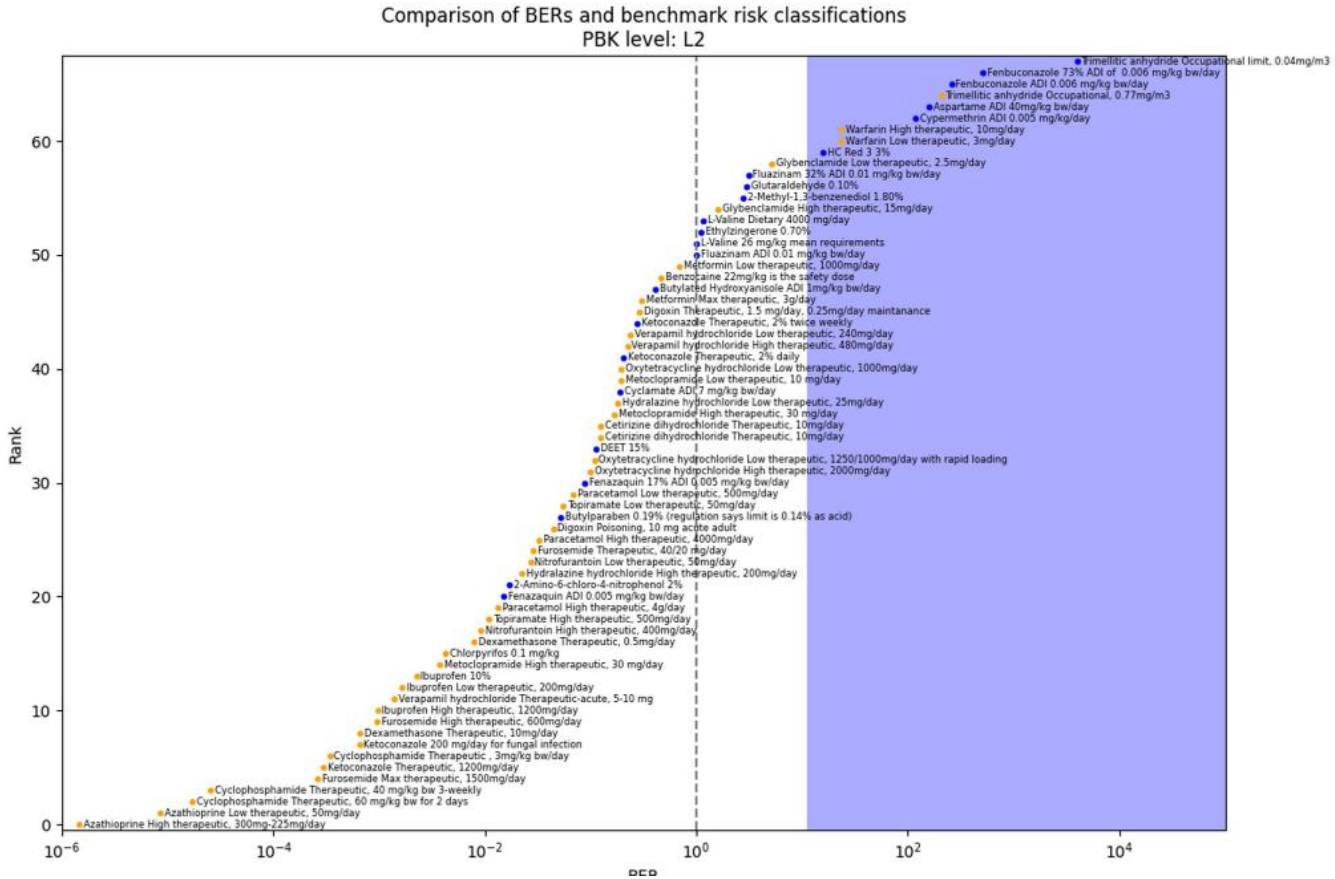
PBK Level 2, Blue shaded region BER> 11



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

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	40/41 (98%)	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 (tier 2) assays to be combined with the toolbox.**

Cable et al (2025), *Toxicological Sciences 204 (1), 79-95*

The NEW Gold Standard

Was:

- Rodents
- Pathology
- High-dose apical endpoints
- No adverse effect level
- Uncertainty factors

Is Now:

- Human focused
- Broad-based NAMs
- Bespoke new NAMs
- **Exposure led (PBK)**
- Bioactivity not pathology
- Protection not prediction
- Underpinned by Computational modelling

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