The Journey Towards Confidence --

Bottom-Up PBK Modelling for Benzophenone 4

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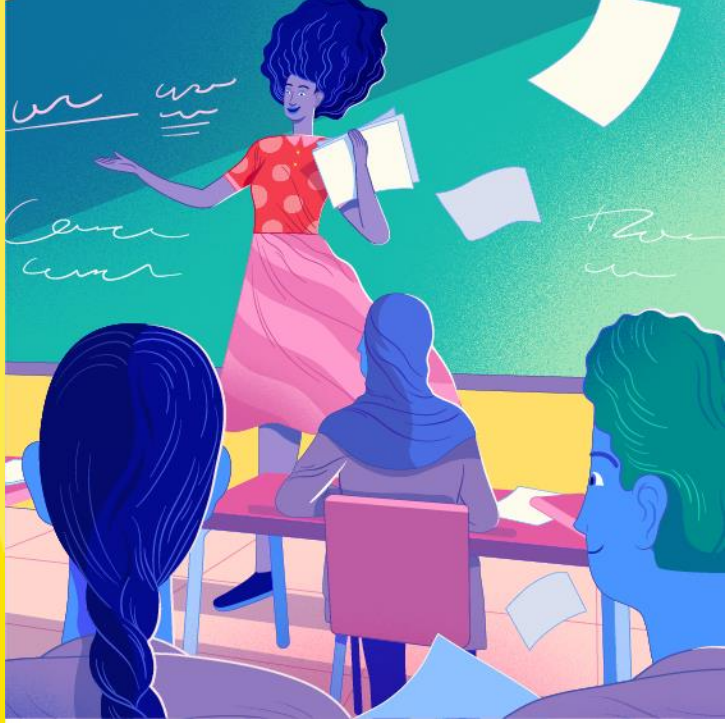


Unilever

At Unilever, our products must be safe

Can we make decisions on these people's safety?

The decisions we make about the safety of our products are for our consumers and workers all around the globe

Making safety decisions without generating data in animals

- Regulations ban animal testing of cosmetic products and their ingredients in over 40 countries
- Many of our consumers do not want to buy products associated with animal testing

From traditional risk assessment to next generation risk assessment

Traditional RA:

Hazard ID

Potency screening

NOAEL
(mg/kg/day)

Hazard confirmation

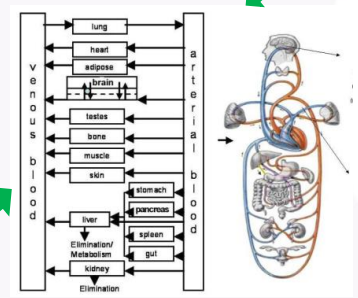
Potency assessment

consumer exposure

Animal testing

- may not be relevant to human
- slower and higher cost

internal exposure



QIVITE with PBK modelling
(Physiologically based kinetic modelling)

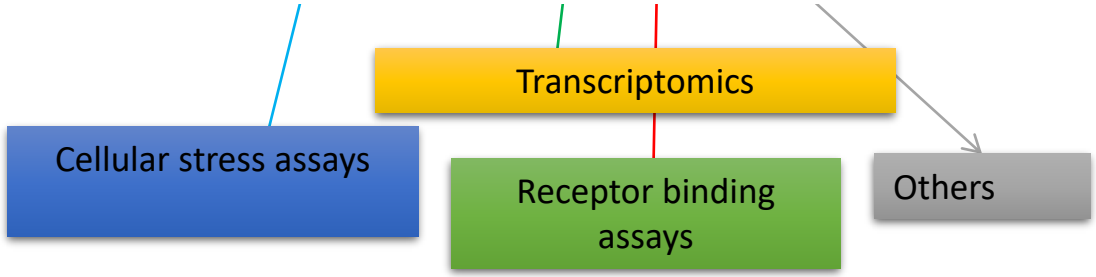
Next generation RA:

bioactivity characterization

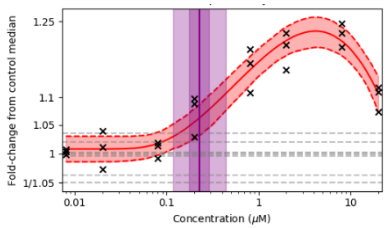
Next generation risk assessment (NGRA):
developing and applying new approach methodologies (NAMs) without generating new animal data

Approach to this Next Generation Risk Assessment

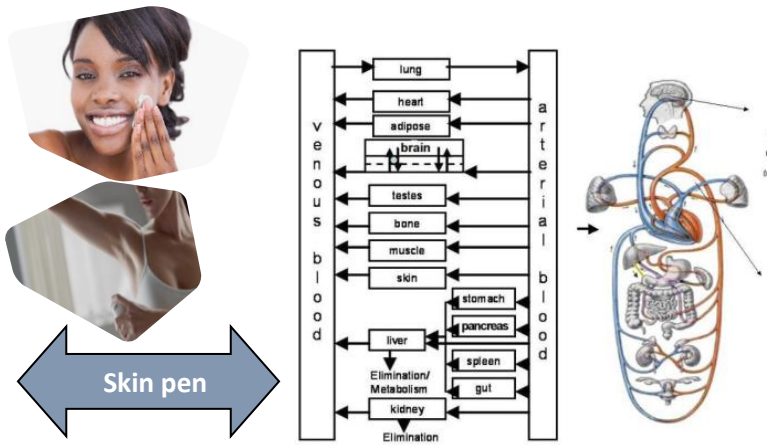
Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**



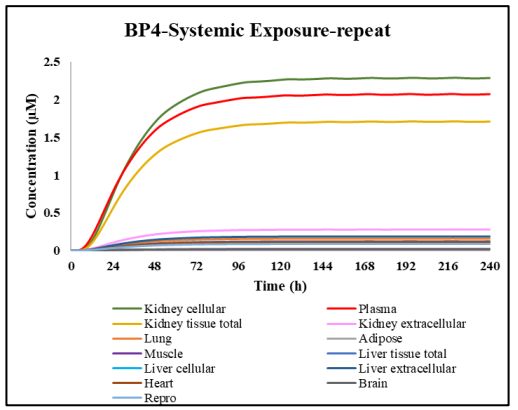
Point of departure (POD) derived from concentration-response data



Exposure models (PBK, free/total concentration)



Exposure estimation: Plasma C_{max}, organ distribution, AUC



Calculation of Bioactivity exposure ratio (BER) for safety decision making

The BER is defined as the ratio between the POD and the relevant exposure metric

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

- BP-4 is an UV-filter ingredient used in sunscreen cosmetics to prevent sunburns or photodegradation by inhibiting the infiltration of UV light.
- 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 on BP-4:
  - To assess whether a tiered NGRA approach is sufficiently protective for these types of ingredients following the framework and NAMs applied in previous case studies

Focus of this presentation

PBK model development of BP-4 based on NAMs to make estimates of systemic exposure levels so that a bioactivity-exposure ratio (BER) can be calculated in NGRA

Exposure assessment:

From topically applied dose to internal concentrations (e.g. C_{max}, AUC)

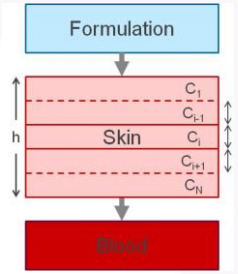
External dose

- Route of exposure
- Consumer use (Habits &Practices)
- Applied dose (external concentration)
- Duration and frequency

ADME parameters

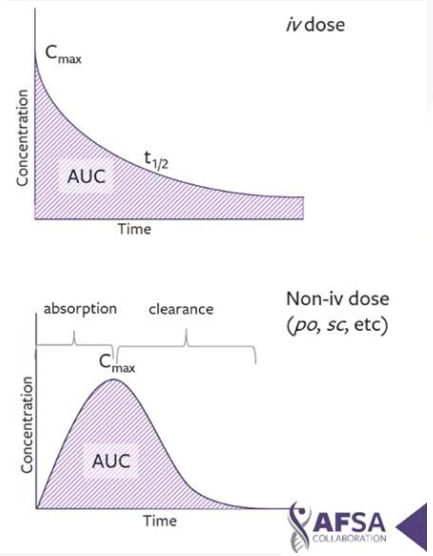
Absorption
Distribution
Metabolism
Elimination

- Skin penetration
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- Blood:plasma ratio



Kinetic profile of chemical

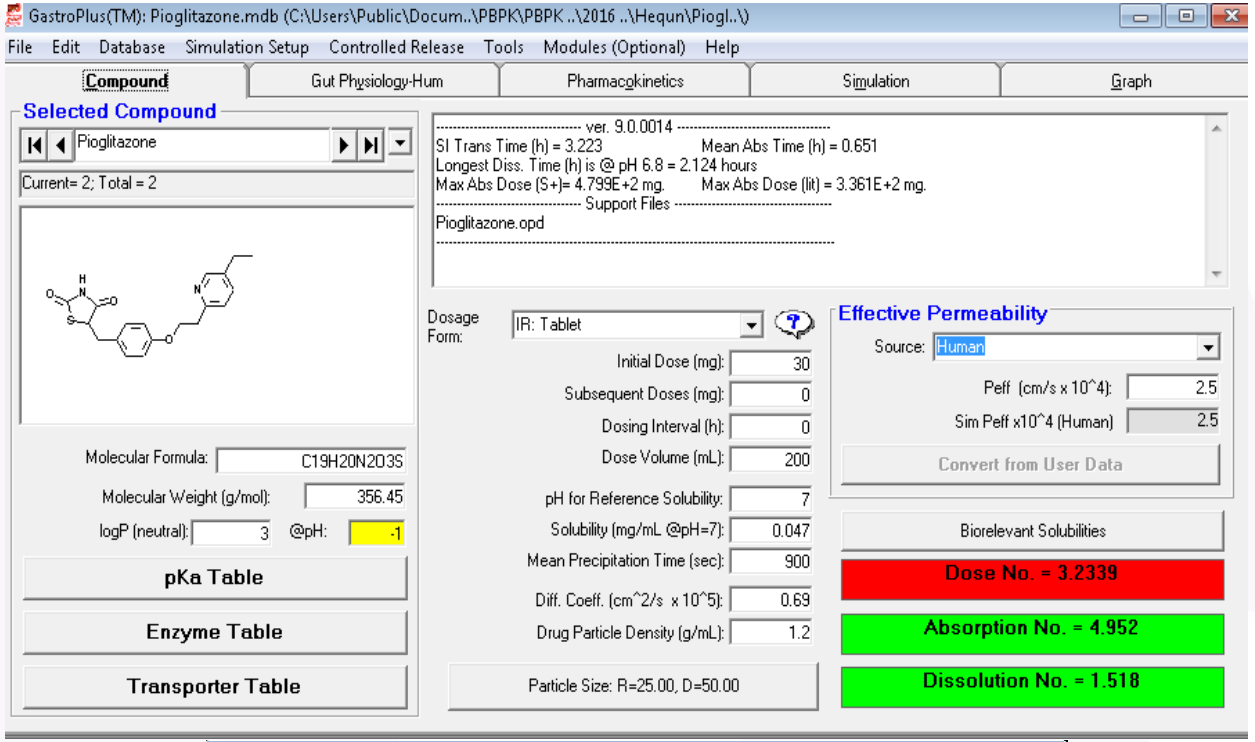
Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)

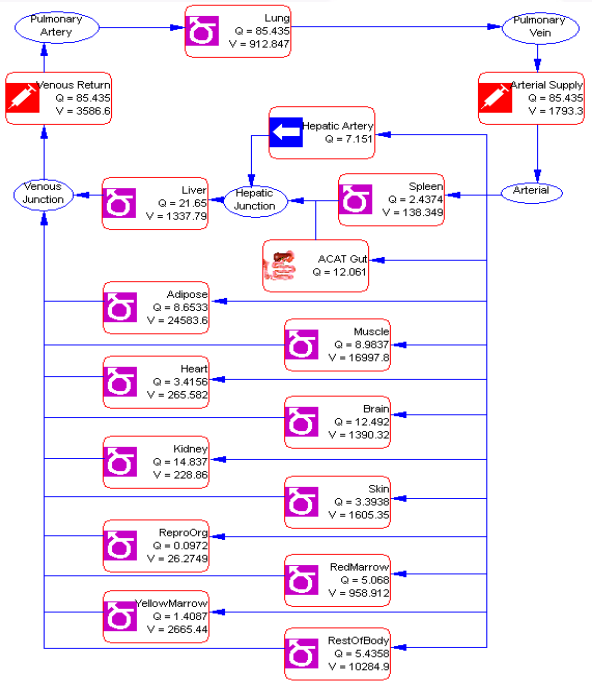


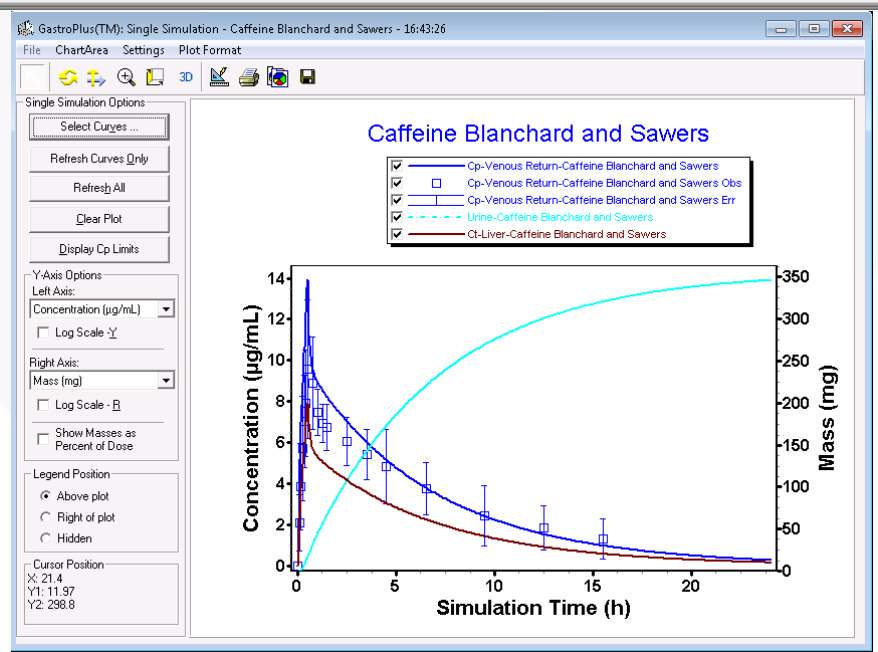
Images from: AFSA training module "Dosimetry (Internal Exposure)",2022

https://www.afsacollaboration.org/sciencex_event/dosimetry-internal-exposure-ivive/

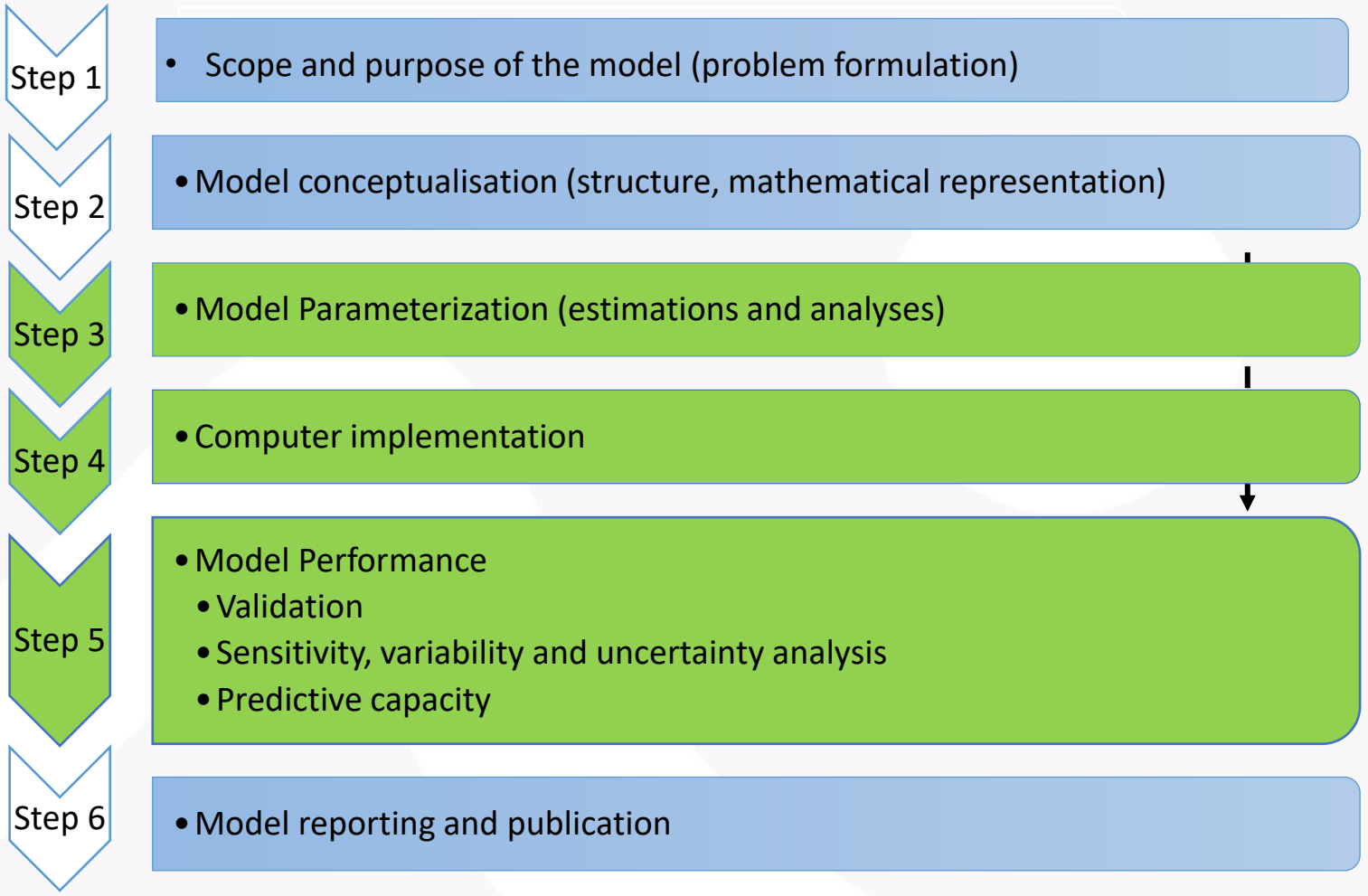
PBK modelling platform: GastroPlus







PBK Modelling Workflow and reporting template: compliant with OECD 2021 and WHO guidance



OECD Guidance, 2021

External applied dose

- 5% BP-4 in Sunscreen product
- 18g/day, two times, 9g/application, on body and face 17500cm^2 (Based on SCCS NoG)
- To closely simulate the real-life use scenarios, it was assumed that
  - the European individuals use this sunscreen body lotion in the daytime
  - each day apply the first dose (9g) at 9 am and the second dose (9g) at 2 pm
  - following a meal (fed condition) and this individual take a shower each morning at 7 am

	Dosage Form	Dose [mg]	TD Dose Vol [ml]	Start [h]	End [h]	Physiology or .cat file	PBPK Physiology or .pbk file
	TD: Liq Soln	450	9	0	22	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	5	22	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	24	46	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	29	46	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	48	70	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	53	70	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	72	94	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	77	94	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	96	118	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	101	118	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	120	142	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	125	142	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	144	166	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	149	166	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	168	190	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	173	190	Human - Physiological - Fed	european individual
	TD: Liq Soln	450	9	192	214	Human - Physiological - Fed	european individual

Mixed Multiple Doses (MMD) in GastroPlus to reflect multiple doses of specific amounts at varying intervals.

PhysChem and ADME data generation and parameterisation

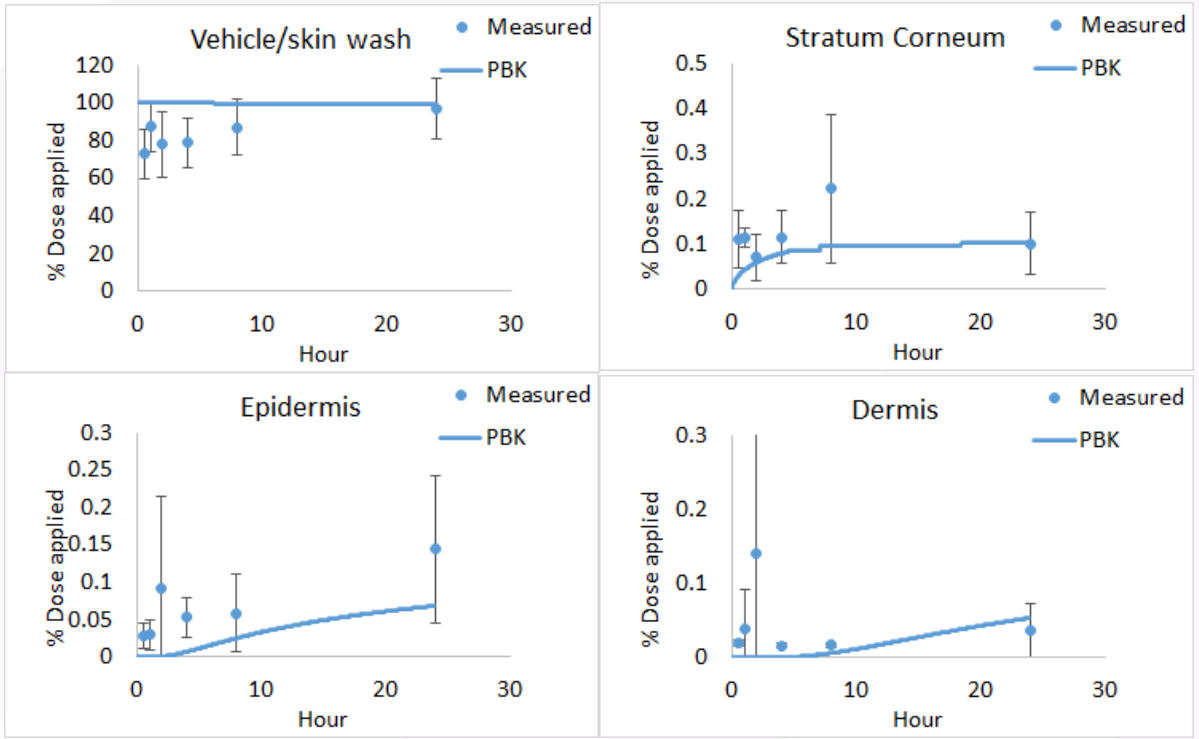
Strategy:

We took a stepwise approach to data generation and refinement, using relevant and robust approaches for parameter determination, support the reliability of input parameters and provide a sound biological basis for the model structure.

	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**
Blood: plasma ratio	0.6	**Measured**
Renal excretion	0.11L/h	GFR*Fup

Dermal absorption with *ex vivo* skin pen data

- *Ex vivo* skin penetration study designed according to *Davis et al. 2011* meeting OECD and SCCS guidance
- BP-4 in relevant formulation (oil in water emulsion)
- Full time course data in skin layers and kinetic in receptor fluid



Receptor fluid:
Below the Limit of Quantification

Results

- Very low skin penetration, therefore big variance of the data
- data used to fit important skin penetration parameters, i.e. diffusivity and partitioning parameters, in the TCAT module of GastroPlus

Michael Davies, Ruth U. Pendlington, Leanne Page, Clive S. Roper, David J. Sanders, Clare Bourner, Camilla K. Pease, Cameron MacKay, Determining Epidermal Disposition Kinetics for Use in an Integrated Nonanimal Approach to Skin Sensitization Risk Assessment, Toxicological Sciences, Volume 119, Issue 2, February 2011, Pages 308–318, https://doi.org/10.1093/toxsci/kfq326

Hepatic clearance

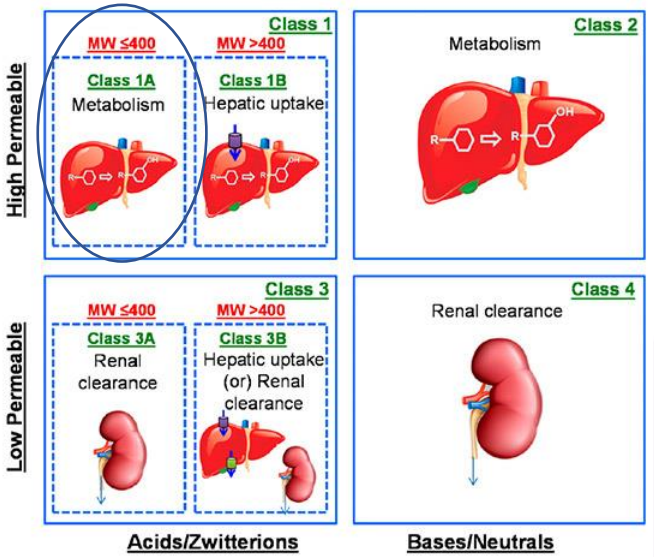
In silico:

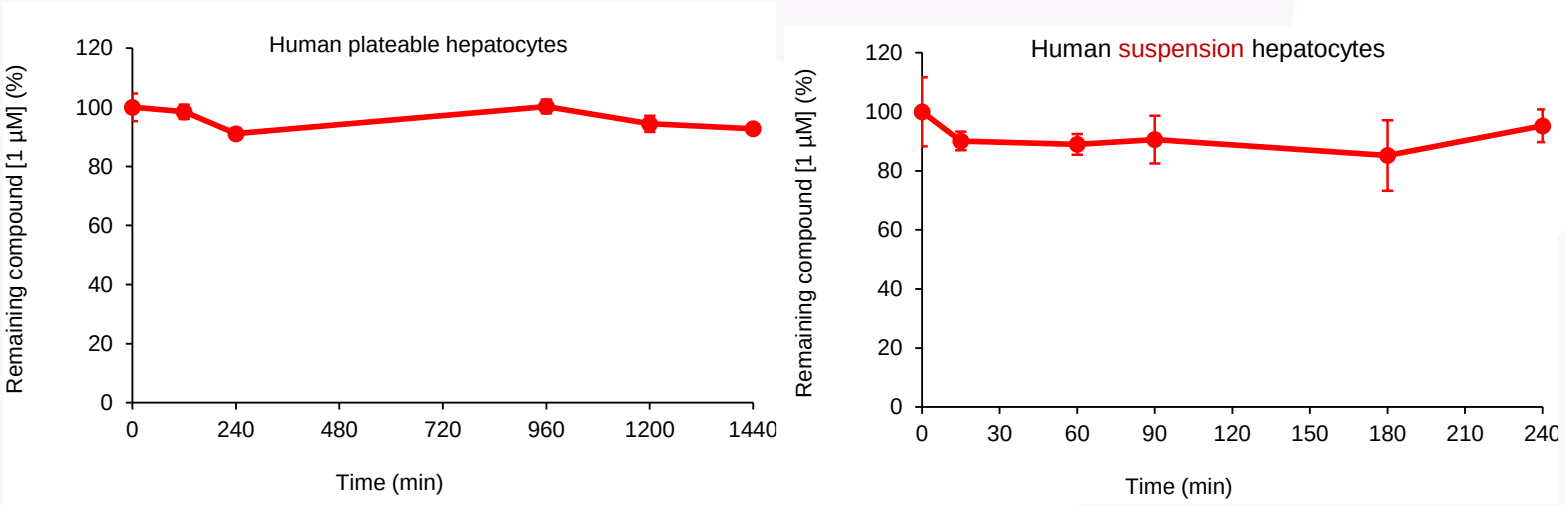
BP-4 was predicted to be mainly cleared via liver metabolism

In vitro data:

Primary human hepatocyte assay (using both suspension and plated cells):

Hepatic intrinsic clearance <2.5L/h (Below LOQ)





Initial ECCS (Extended Clearance Classification System):

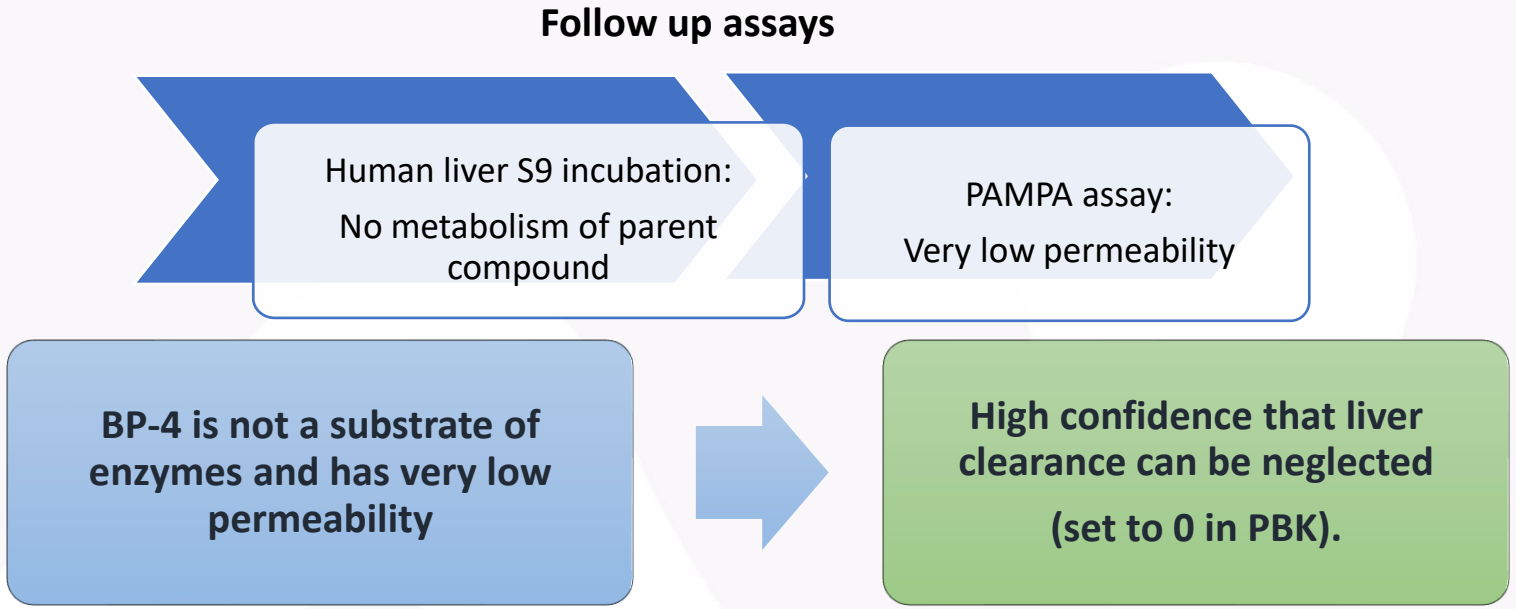
Class 1A

(*Varma et al., 2015)*

No metabolism of BP-4 seen in hepatocytes, conflicting with the ECCS Class 1A prediction.

Two hypotheses:

1) BP-4 is not a substrate of CYP enzymes – need to confirm with a second assay using S9 fraction
2) BP-4 has low membrane permeability– PAMPA assay



If BP-4 is not metabolised by the liver – what is the route of elimination?

How is BP-4 taken up by the cells?

Back to problem formulation…

Understanding chemical organ distribution and renal clearance

In silico predictions:

- BP-4 is an anion sulphonate
- Likely to be a substrate of Organic anion transporters (OATs)
- Renal clearance may be higher than GFR*Fup

In vitro:

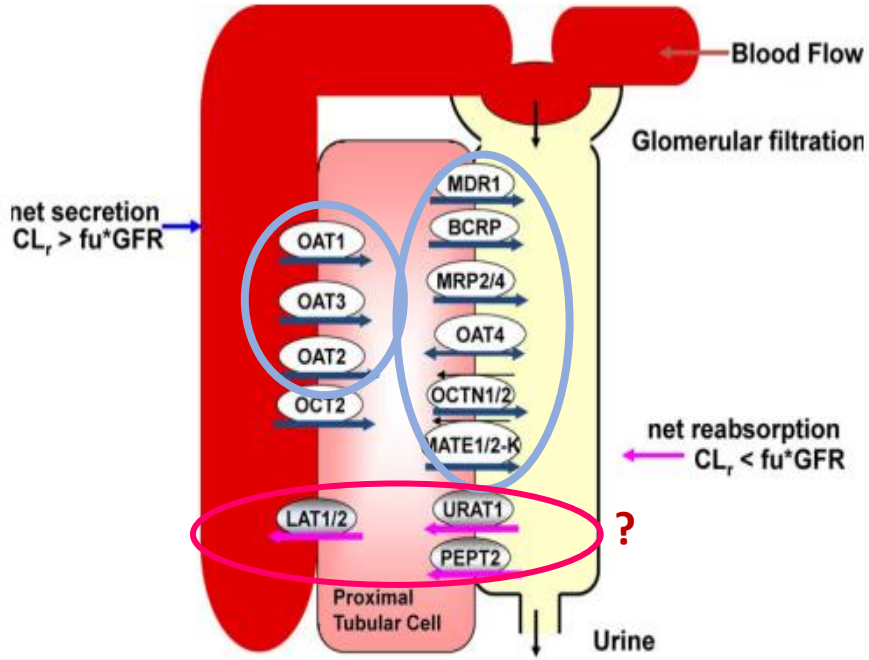
Transporter studies in transfected kidney cells in two different assays (uptake assay and vesicular assay)

Results:

- Substrate of the influx transporters, OAT1, OAT2, OAT3 and OCT2 and a substrate of the efflux transporters, BCRP and MRP4.
- All these transporters are expressed in the kidney, although OAT-2, BCRP and MRP4 are expressed both in kidney and liver

	Transporters	Uptake of efflux?	Substrate?
Uptake Transporter Substrate Assays	OAT1	Uptake	**Yes**
	OAT2	Uptake	**Yes**
	OAT3	Uptake	**Yes**
	OCT2	Uptake	No
	MATE1	Efflux	No
	MATE2-K	Efflux	No
Vesicular Transport Substrate Assays	MRP2	Efflux	No
	MRP4	Efflux	**Yes**
	MDR1/Pg-p	Efflux	No
	BCRP	Efflux	**Yes**

However…



Mechanism of drug elimination and major transporters in the kidney

https://doi.org/10.1002/jcph.702

Back to problem formulation…

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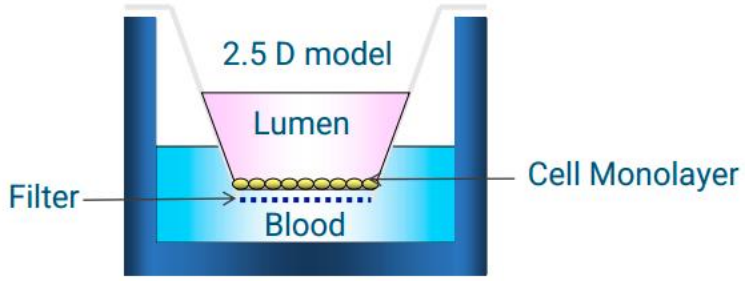
In vitro:

Transporter studies in transfected kidney cells in two different assays (uptake assay and vesicular assay)

In vitro:

Investigate the bi-directional transport profile in kidney where all the active transporters are present and functional (aProximate™).



B-A →blood to urine →active secretion
A-B → urine to blood →reabsorption

Human aProximate™ platform

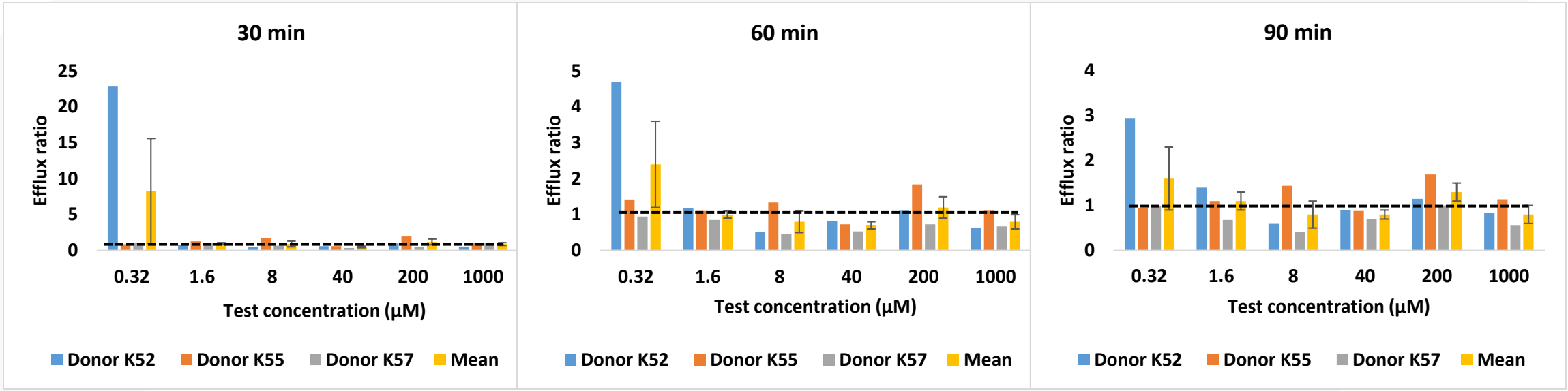
- Primary proximal tubule cells (PTCs) derived from fresh human kidneys
- Cultured on semi-permeable filters to form a tight monolayer
- Separating the two solute compartments, corresponding to the apical and basolateral sides of the proximal tubule, respectively
- Retains **a high degree of differentiation**
- Endogenously **express a variety of functional proteins and biomarkers**

Controls

- BP-4 was co-treated with Lucifer Yellow to account for paracellular leak, so that the contribution of transcellular transport of the compound could be derived.
- ^{14}C-P-aminohippurate (PAH) was tested in the absence and presence of the compound probenecid, an inhibitor of OAT proteins, to assess possible routes of transport across the monolayer

Efflux ratios

- Data is first presented as flux rate (pmol/cm²/h) in both directions (JA-B and JB-A)
- Efflux ratio= JB-A / JA-B
  - 1.5-2.5: secreted molecules
  - <1: reabsorbed molecules

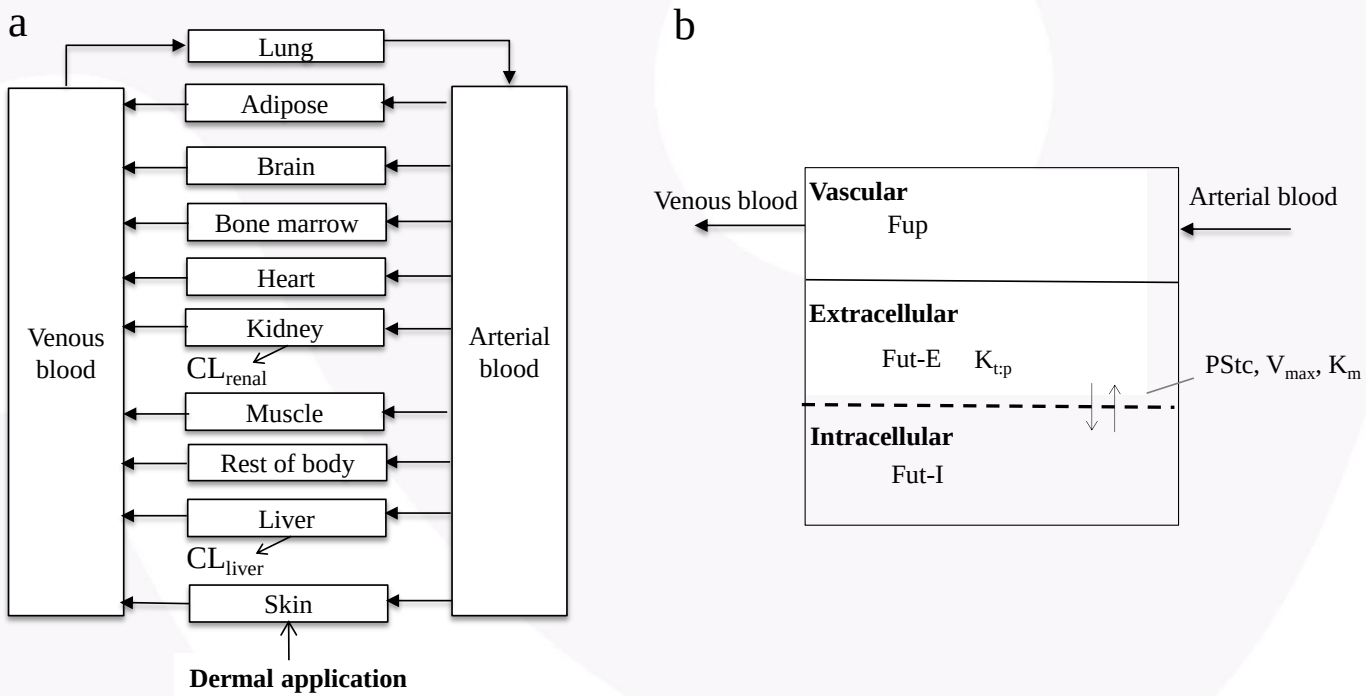


Results:

- Route of elimination in the kidney includes glomerular filtration, active tubular secretion and tubular reabsorption
- Transport in the proximal tubule cells is equally efficient in both directions
- However, donor variability has been observed that in 1 donor, active secretion was shown to be the main excretion route at biologically relevant concentrations

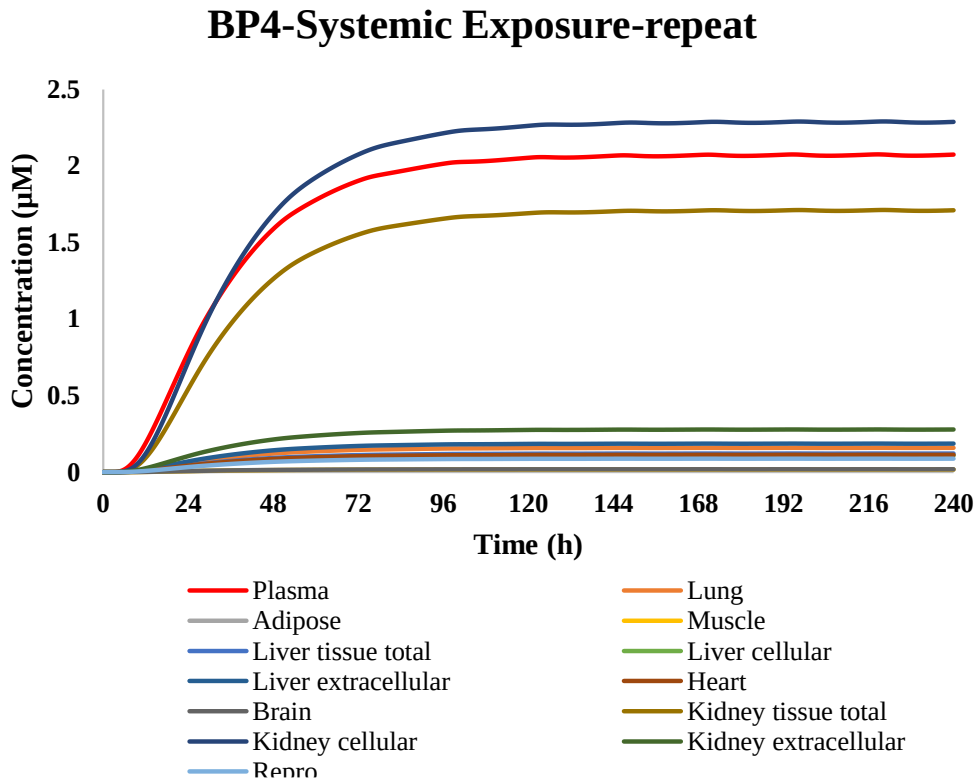
Updated PBK model in GastroPlus

- Set BP-4's distribution to each compartment to be modelled as permeability-limited
- Liver clearance set to 0
- Active transport in the liver was modelled by incorporating kinetic parameters (V_{max}, K_m, Protein expression) for the transporters (OAT-2, BCRP and MRP4).
- Biliary excretion not accounted for to be conservative
- GFR*Fup was used to calculate renal excretion of BP-4, accounting for filtration only to be conservative



Human PBK model structure for BP-4

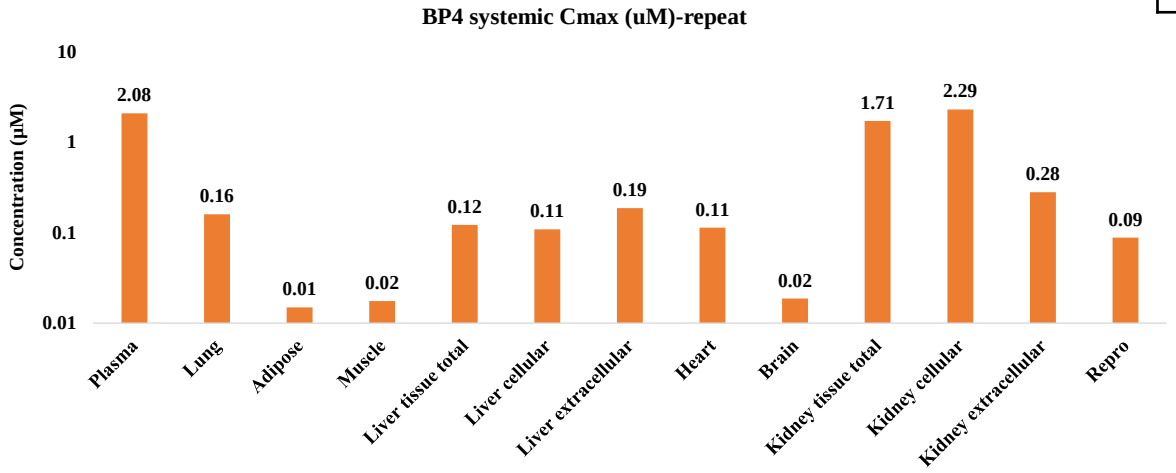
Deterministic PBK modelling



Kidney cellular
Plasma
Kidney total

for a female European
30 years-old 60 kg bodyweight

PK parameter	Value
Bioavailability (%)	0.4
CL_{renal} (L/h)	0.11
Plasma C_{max} (µM)	2.08
AUC_{24h} (ug-h/mL)	1.94
Volumes of distribution at steady state (L)	8.577
$t_{1/2}$ (h)	54.3



Human clinical PK data is not available for model verification

Strategies in addressing uncertainty in PBK estimation

Deterministic PBK modelling

Point estimate values for input parameters

Model

Individual modelled (30 year-old 60 kg female, European)

Probabilistic population PBK modelling

Parameter Uncertainty ('informed' distribution for the most sensitive parameters)

Model

Population Variability

Probabilistic population PBK+ CMED modelling

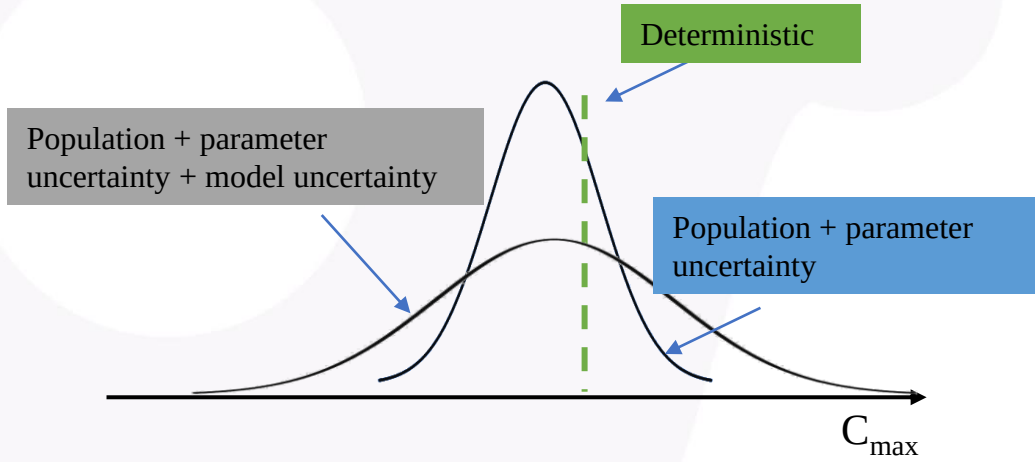
Parameter Uncertainty

Model Uncertainty

Model

Population Variability

Predicted C_{max} based on different approaches characterising uncertainty

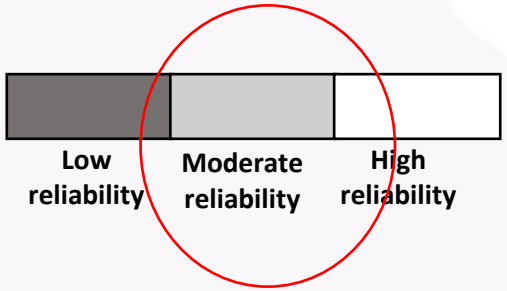


The output of the uncertainty and sensitivity analyses

A

Sensitivity	Uncertainty: High	Uncertainty: Medium	Uncertainty: Low
High		vehicle: water partition coefficient Stratum corneum water partition coefficient Stratum corneum diffusivity Fup	
Medium		K_m OAT2	
Low		V_{max} OAT2 Epidermis diffusivity Blood: plasma ratio	

Plasma Cmax



C

Sensitivity	Uncertainty: High	Uncertainty: Medium	Uncertainty: Low
High		vehicle: water partition coefficient Stratum corneum water partition coefficient Stratum corneum diffusivity	
Medium		K_m OAT2 V_{max} OAT2 Fup	
Low		Blood: plasma ratio	

Kidney intracellular Cmax

According to WHO/OECD guidance

Probabilistic PBK modelling to account for population variability and parameter uncertainty

Monte Carlo simulation

Population

Physiological characteristics

- 16-70 years old
- 40-85 kg
- 50% male and 50 % female
- European population

Parameter uncertainty analysis

- Set ranges (distributions) on values of influential parameters based on available information
- For uninfluential parameters, default distributions used

Note: a limitation of this approach is that parameter uncertainty and variability are considered together. Although separation of parameter uncertainty and variability is theoretically possible using hierarchical, population-based models, data are typically inadequate to achieve such a level or granularity

Distributions for parameters used in uncertainty analysis and probabilistic PBK simulations

Parameter	Mean	cv%		Distribution type	Lower Limit	Upper Limit
Fup	1.574	37.21	In vivo variability + In vitro standard deviation	lognormal	0.6095	4.0651
kidney volume	324.3	30	Table 2 from Clewell and Clewell III, 2008	normal	32.4348	616.261
Liver volume	1416.1	30		normal	141.612	2690.63
liver plasma partition coefficient	0.09	20		lognormal	0.05209	0.15555
kidney plasma partition coefficient	0.135	20		lognormal	0.07795	0.23277
OAT2 expression in liver	3.50E-03	56.63	Literature review	lognormal	0.00091	0.01345
Km MRP4	1.5	25	In vitro standard deviation	lognormal	0.768	2.92969
Vmax MRP4	2.60E-03	25		lognormal	0.00133	0.00508
Km OAT2	4.5	25		lognormal	2.304	8.78906
vehicle: water partition coefficient	120	25		lognormal	64.486	234.38
Stratum corneum water partition coefficient	1	70		lognormal	0.2035	4.913
Stratum corneum diffusivity	2.00E-11	70		lognormal	4.07E-12	9.83E-11
epidermis diffusivity	6.00E-10	130		lognormal	4.93E-11	7.30E-09

Table 2
Typical range of coefficients of variation for PBPK model input parameters

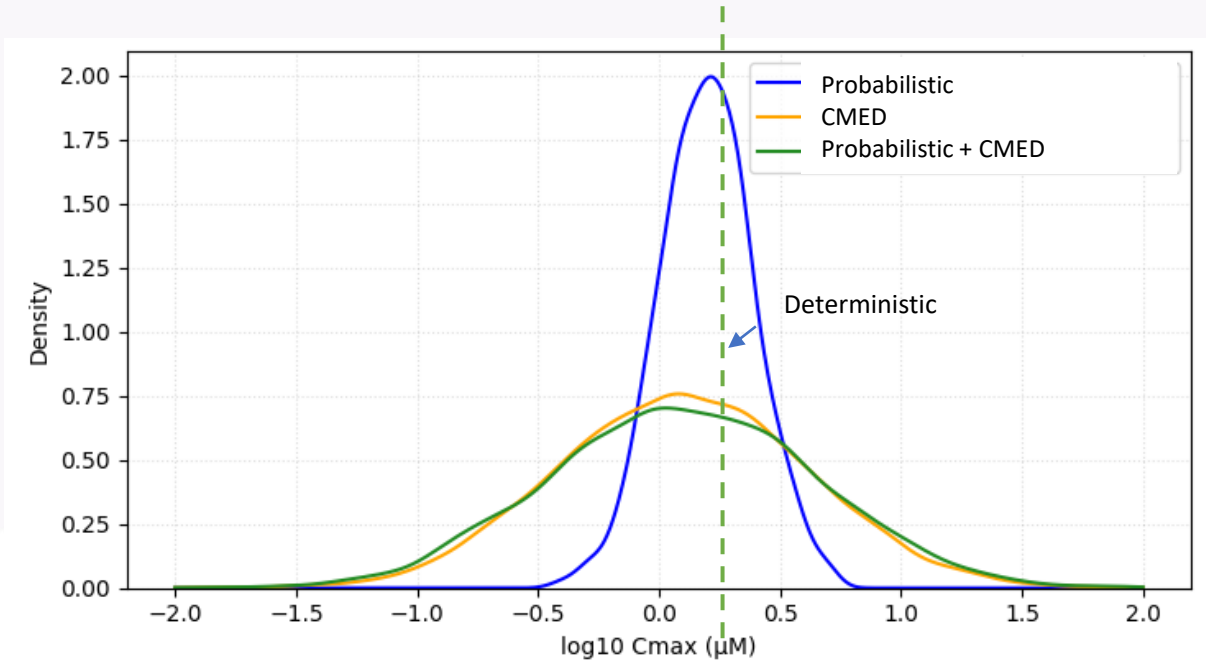
Parameters	CV (%)	Distribution
Tissue volumes	6–30	Truncated normal
Blood flows	8–30	Truncated normal
Ventilation	15–50	Truncated normal
Partitions	15–20	Truncated lognormal
Metabolism	30–70	Truncated lognormal

Clewell and Clewell III, 2008

Probabilistic PBK modelling + CMED model to account for population, parameter and model uncertainty

To account unknown-unknows e.g. model uncertainty

- C_{max} Error Distribution (CMED): A complementary approach to characterise PBK prediction uncertainty as published in *Li et al. 2022* and *Middleton et al.* 2022.
- This model seeks to quantify the error distribution of estimates of plasma C_{max} by looking at the difference between PBK predictions of C_{max} and existing measured values in human clinicals for several exposure scenarios.
- This model can be used to estimate the distribution of the possible prediction errors for future chemical and exposure scenario.



Deterministic PBK model for female adult 60 kg	Distribution of C_{max} (probabilistic simulation+CMED) (µM)	
Plasma C_{max} point estimate	**Median (95% interval)**	**95th percentile**
2.1	1.3 (0.11, 15)	9.8

Li H, Reynolds J, Sorrell I, Sheffield D, Pendlington R, Cubberley R, Nicol B. PBK modelling of topical application and characterisation of the uncertainty of C_{max} estimate: A case study approach. Toxicol Appl Pharmacol. 2022 May 1;442:115992. doi: 10.1016/j.taap.2022.115992. Epub 2022 Mar 25. PMID: 35346730.

Middleton, A.M., et al., Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicological Sciences, 2022. 189(1): p. 124-147.

To summarize BP-4's kinetic behavior in the human body:

- Overall, upon dermal absorption only a small amount of BP-4 enters systemic circulation, after which BP-4 remains unchanged due to negligible liver clearance.
- It has low tissue distribution due to low partitioning and limited passive diffusion of cell membranes (charged at physiological pH).
- It can be taken up into the kidney and then excreted to urine via active transport and can be reabsorbed back to into the bloodstream, however due to no preferred direction of movement glomerular filtration determines the overall renal excretion rate.
- BP-4 can also move into and then out of the liver cells.
- Successive doses result in accumulating concentrations of BP-4 in the body until a steady state is reached at around 100h when there is an equilibrium reached between the low absorption and low excretion into the urine.

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Confidence level

WHO questions for assessing the level of confidence in the BP-4 PBK modeling

Model evaluation aspect	level of confidence (towards the accuracy)	level of confidence (towards the conservatism)
Do the model **structure and parameters** have a reasonable **biological basis**?	High	High
How well does the PBK model **reproduce** the chemical-specific **PK data** under various experimental or exposure conditions?	Low	High
How **reliable** is the PBK model with regard to its predictions of dose metrics **relevant to risk assessment**?	High	High

Conclusions

- ✓ The stepwise way of data generation and refinement, using relevant and robust approaches for parameter determination, support the reliability of input parameters and provide a sound biological basis for the model structure.
- ✓ Although human clinical data are not available for validation, the sensitivity and uncertainty analyses and the probabilistic modelling performed provided assurance that the predictions are fit for purpose and provides conservative estimates of human systemic exposure.

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