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Building Confidence in Bottom-Up PBK Model Development for NGRA Without Support of In Vivo Data

24 March 2026

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PBK modelling in next generation risk assessment (NGRA)

- PBK modelling critical in NGRA to predict **internal concentrations** for quantitative in vitro to in vivo extrapolations (QIVIVE)
- At present (human) in vivo data is still needed to validate a model, which are often not available
- Exploring means to establish scientific confidence in PBK **model prediction without in vivo data** is needed

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Bottom-up PBK model development

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External inhalation exposure

Fraction unbound plasma and B:P ratio

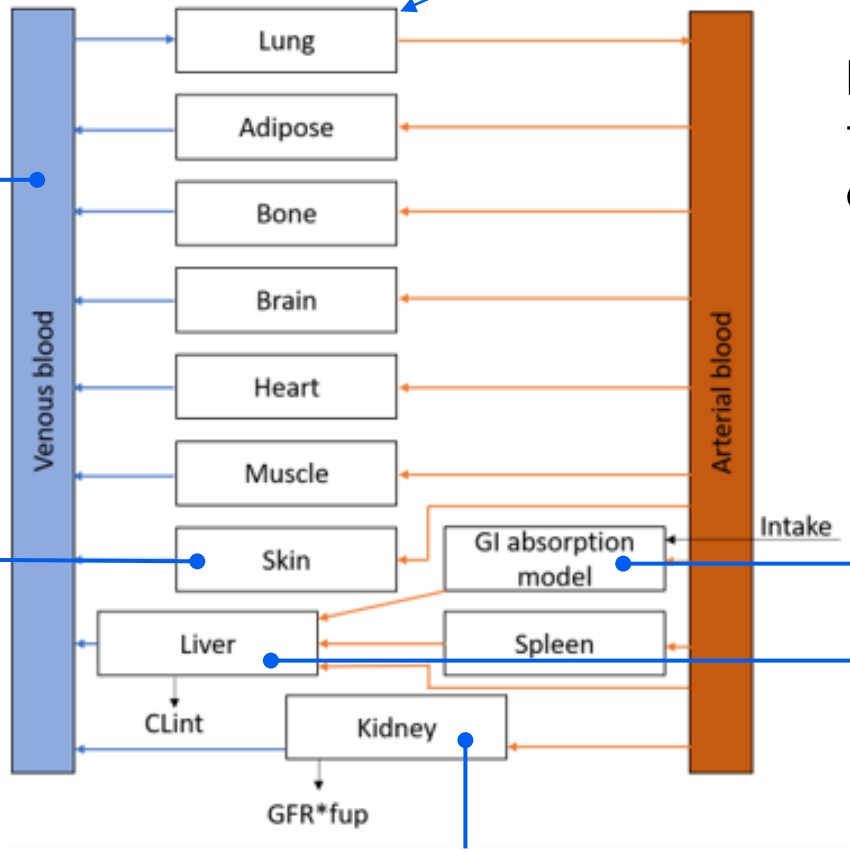
In silico calculations tissue:plasma partition coefficients (all organs)

Caco-2 transwell experiment

External oral exposure

External skin exposure

Ex vivo human skin absorption



In vitro Clint (hepatocytes/ microsomes/ S9)

Transporter kinetics when needed

Software: e.g. Gastroplus, PK-Sim

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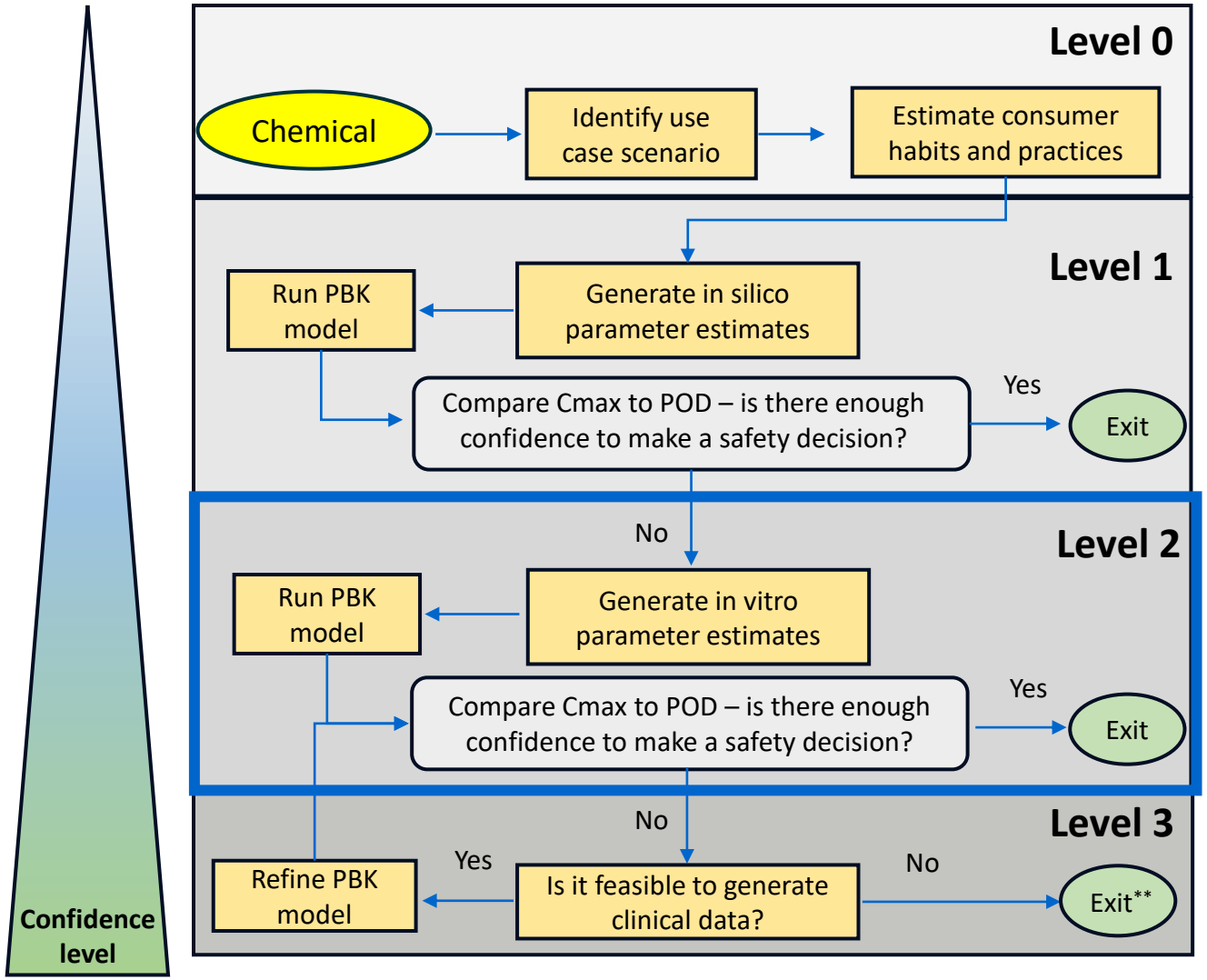
Parameterisation of PBK models within a tiered risk assessment framework



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

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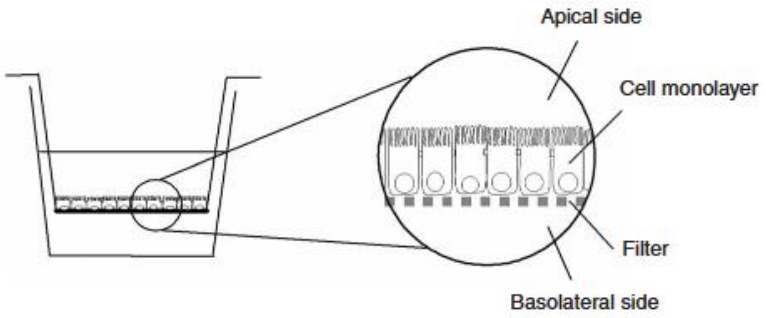
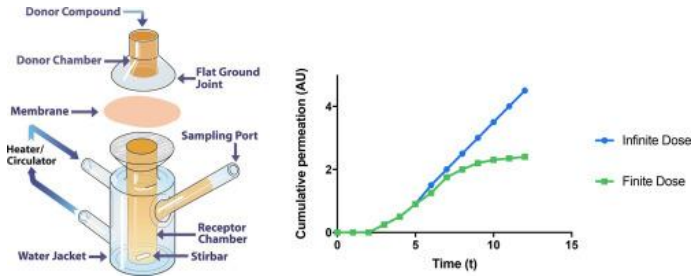
Caco-2 model for intestinal absorption



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)

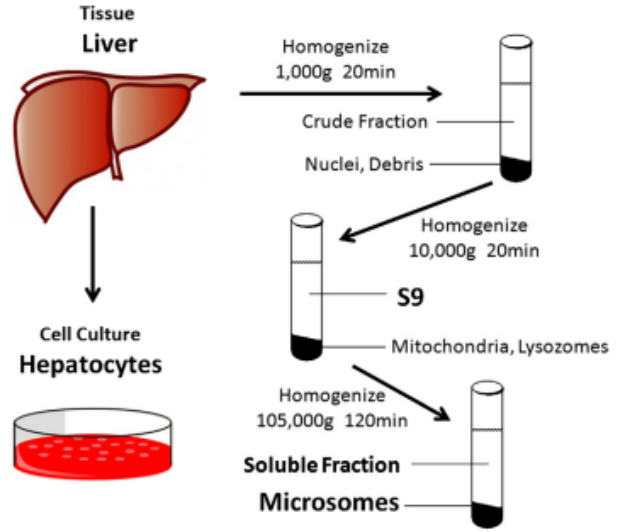
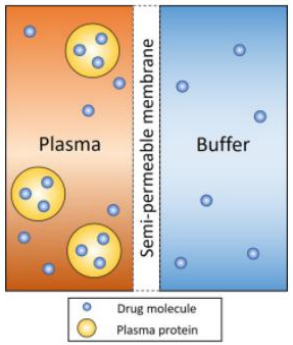


FIGURE 3.6 Preparation of microsomal, S9, and soluble fractions commonly used in drug metabolism studies.

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*

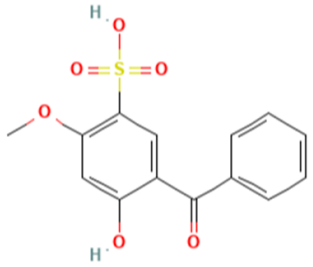
Partition coefficients (in silico based on logP and pKa)

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

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

Example case study: benzophenone-4

- **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, including BP-4.
- Case study work with Cosmetic Europe Long Range Science Strategy (LRSS) on developing new approaches for safety assessment without using animals.
- **PBK model development of BP-4 based on NAMs** to make estimates of systemic exposure levels in NGRA.



Chemical name: Benzophenone-4 (Sulisobenzone)

CAS: 4065-45-6

EINECS: 223-772-2

SMILES: COC1=C(C=C(C(=C1)O)C(=O)C2=CC=CC=C2)S(=O)(=O)O)

Microsoft PowerPoint Stock Image

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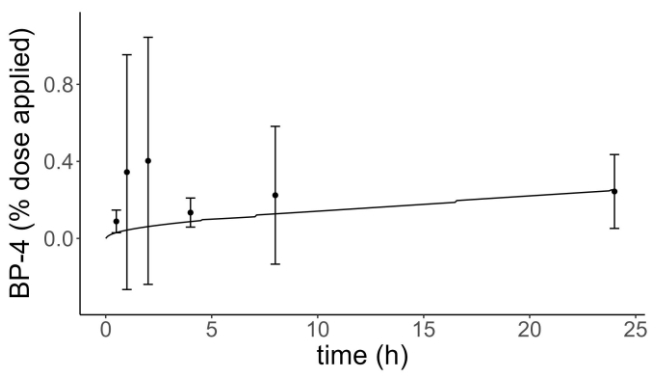
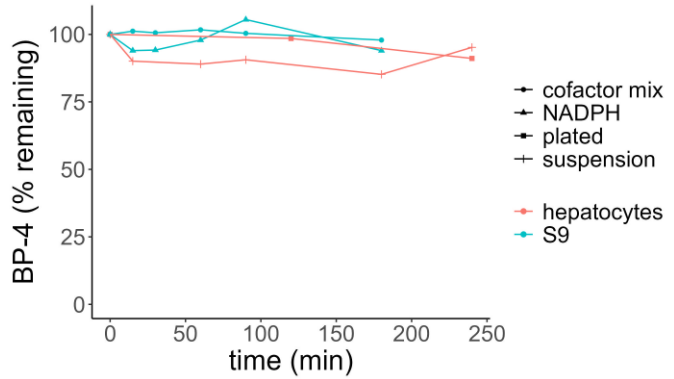
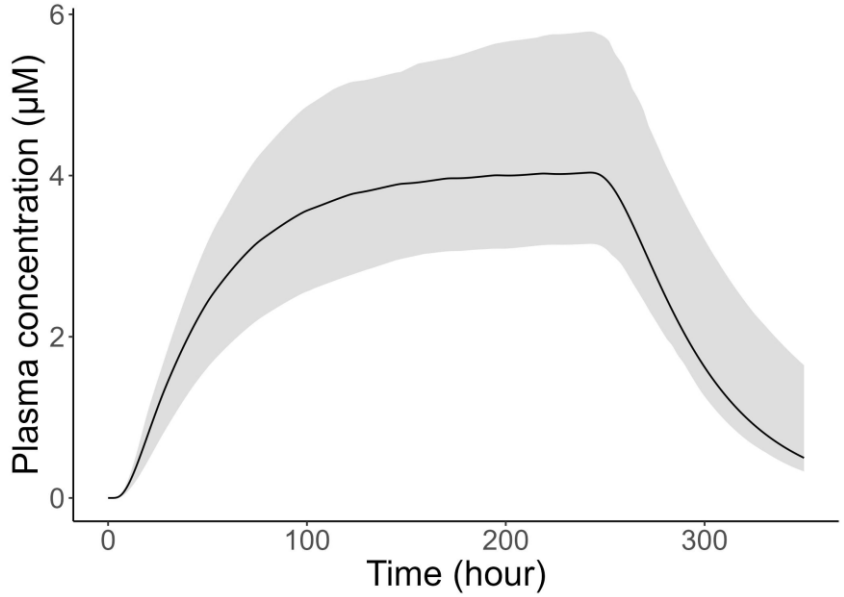
Core PBK model results BP-4

- Limited dermal absorption (±0.4% within 24h, based on an in vitro skin penetration study)
- No metabolic clearance (S9, hepatocytes)
- In silico partition coefficients, in vitro Fup (0.0157) and BP (0.6)
- Predicted plasma concentration 4 µM (3.2-5.8 µM) after 10 days of using 18 g (5%) product per day (2 applications, 1 shower/rinse off/day)
- Limited cell permeability and lack of metabolic clearance suggest a role of transporters in BP-4 kinetics







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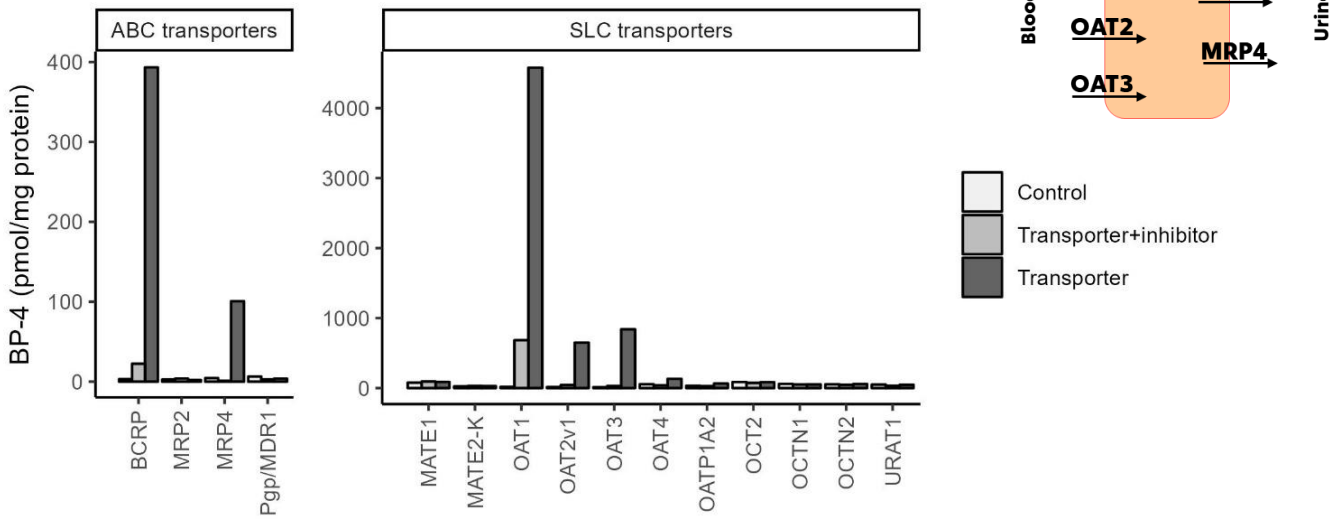
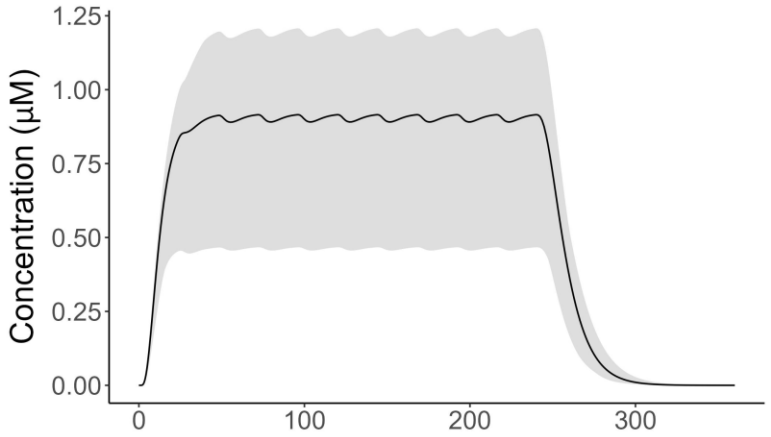
Punt *et al.* (2026), *Altex*, Volume 43, Issue 1

Advanced data collection BP-4

- BP-4 is a substrate of OAT1, OAT2, OAT3, BCRP, and MRP4.
- In vitro kinetic constants scaled in the PBK model to liver and kidney based on the relative expression.
- Net efflux is predicted
  - BP-4 moves from blood to cells via OAT1/2
  - BP-4 moves from cells to urine or bile via BCRP and MRP4.
- Predicted plasma concentration 0.9 µM (0.4-1.24 µM)
  - 10 days of using 18 mL product per day (2 applications, 1 shower/rinse off/day)
  - Sensitivity analysis: dermal absorption, OAT1 kinetics are the key input parameters





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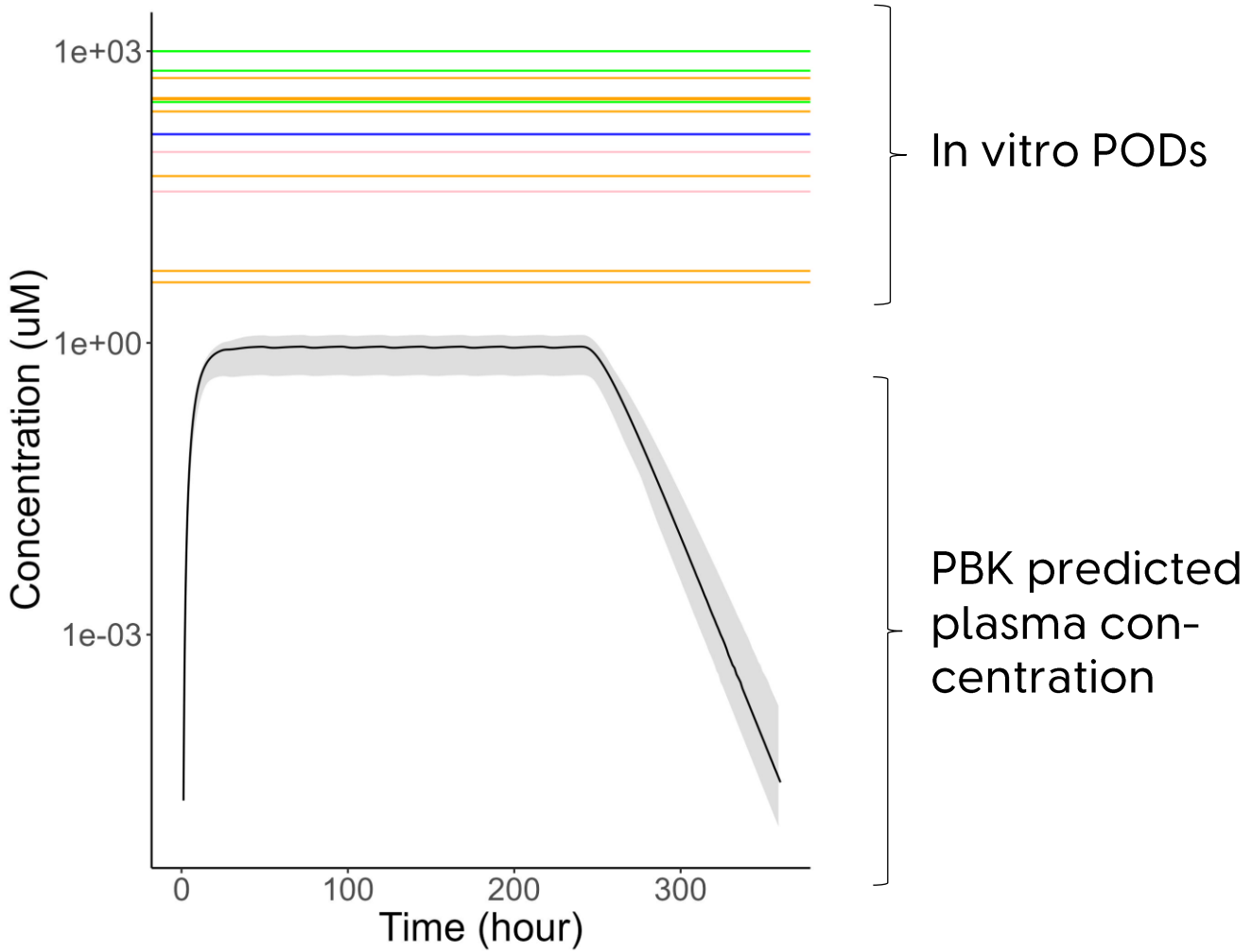
Punt *et al.* (2026), *Altex*, Volume 43, Issue 1

Use of the PBK model results in a NGRA

Conclusion: BP-4, used as a UV filter in body lotion at a concentration of 5%, does not exhibit significant biological activities at consumer-relevant exposures



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Baltazar *et al.* (2025), *Altex*, Volume 42, Issue 3

Establishing scientific confidence in PBK models without support of human in vivo data

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Model development

- Identify key kinetic processes from chemical properties.
  e.g. BP-4's low metabolic clearance + low tissue permeability → transporter involvement
- Making use of high-quality *in vitro* and *in silico* input data.
- Sensitivity analysis, uncertainty analysis, population variability.

Evaluation

- General predictive performance of bottom-up PBK models.
- Compare results across modelling platforms (e.g. PBK software vs differential-equation scripts.
- Incorporate read-across from structurally or mechanistically similar chemicals.

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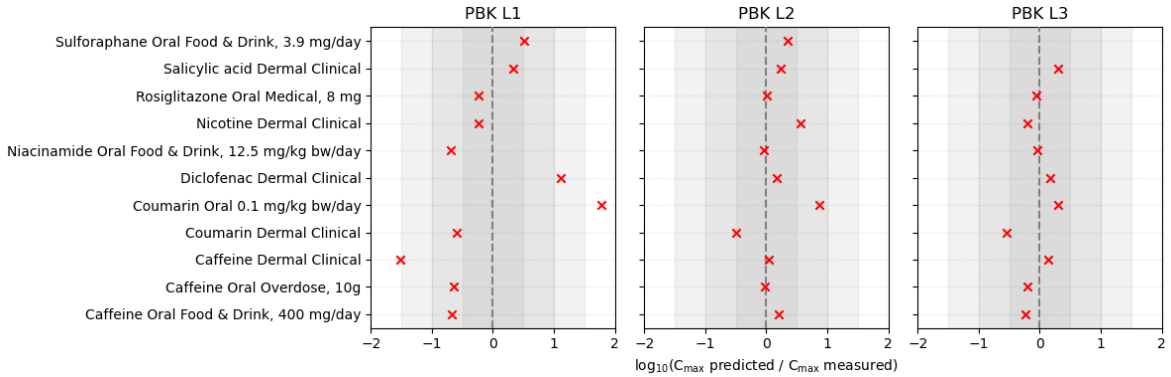
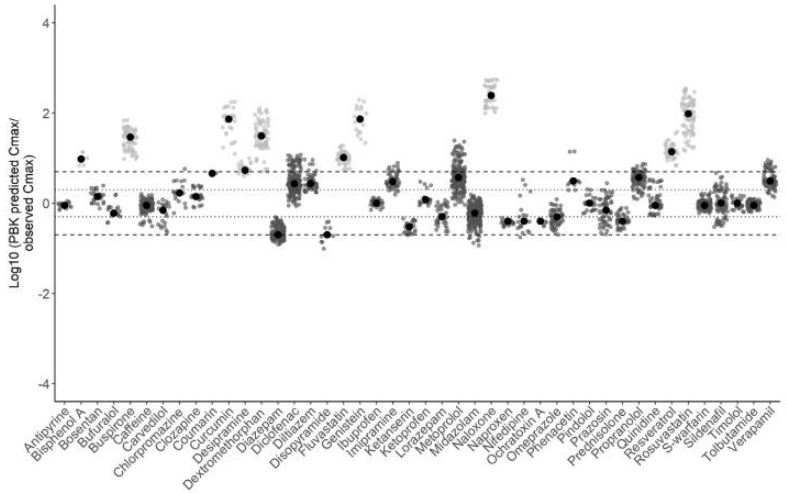
General predictive performance of bottom-up PBK models.

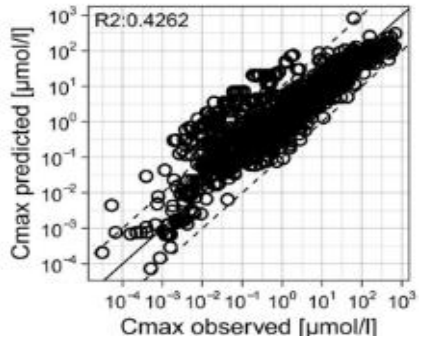
- Bottom-up PBK models predicted Cmax values within a 5- to 11-fold range of available human data.
- Human kinetic studies themselves also show variability across studies.



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



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)

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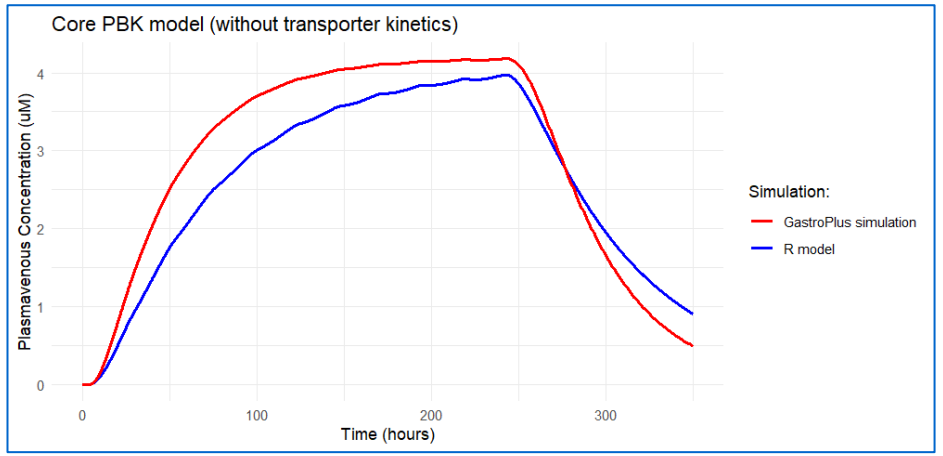
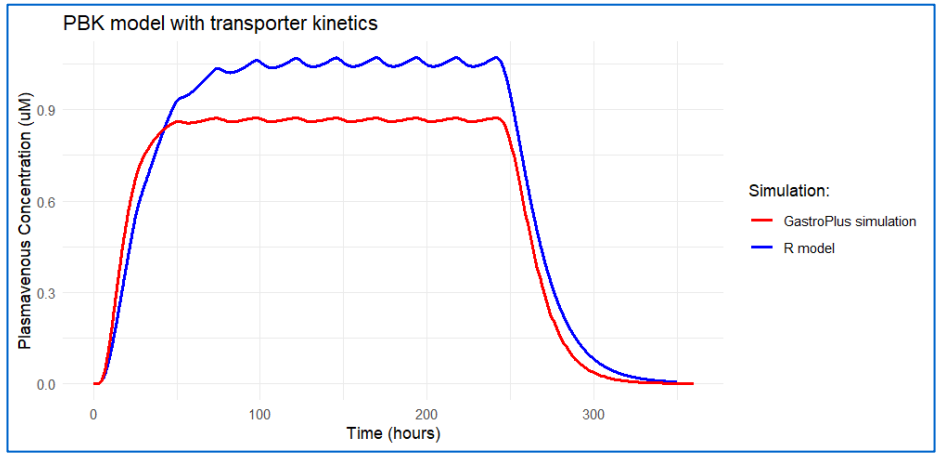
Comparison of results across modelling platforms

- PBK model comparison: **GastroPlus vs R** (differential-equation script)
- Both the core model (no transporters) and the model that includes transporters were evaluated
- **Similar model results** were obtained across platforms





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Unpublished data, do not cite.

Incorporate read-across from structurally or mechanistically similar chemicals.

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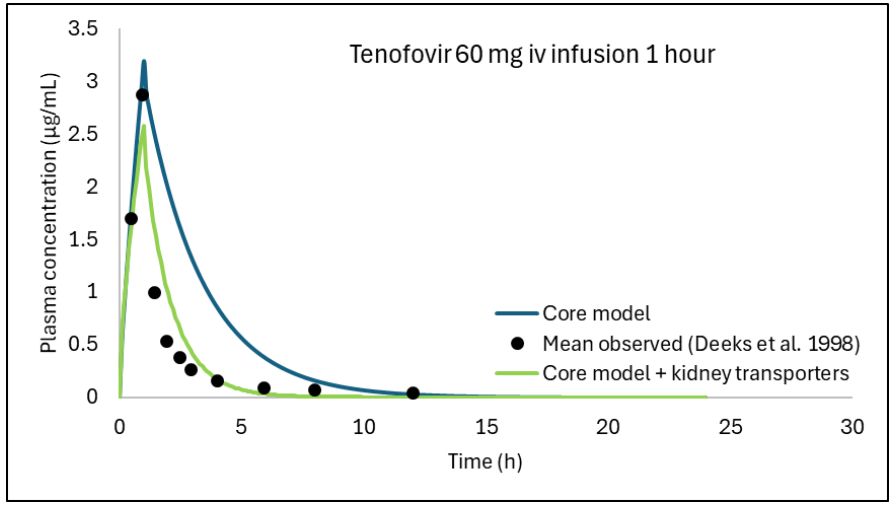
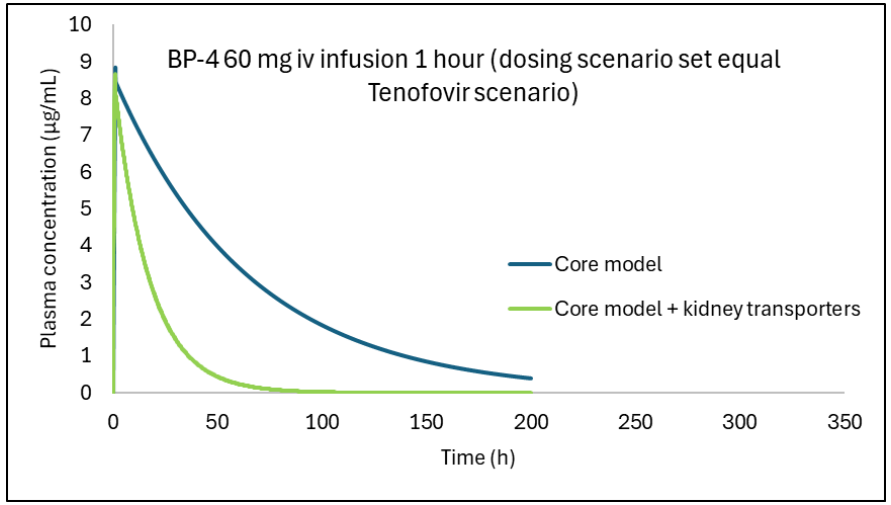
- No structural analogue available for BP-4 with existing human *in vivo* kinetic data
- Compare results with **mechanistically similar chemicals**
- Antiviral agent **Tenofovir** is not chemically related to BP4 but is subject to active renal excretion by OAT1 and MRP4 (like BP-4).
- Comparison allows to compare impact of transporter kinetics on plasma concentrations





Unpublished data, do not cite.

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Conclusions

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- PBK modelling has a crucial role in NGRA to translate human exposure scenarios to internal doses that can be compared with *in vitro* bioactivity data.
- Efforts are needed to obtain model-evaluation approaches without one-to-one validations against in vivo (human) data.
- Key kinetic processes in a model can be defined based on chemical properties.
- Model evaluation based on: general predictive performance of bottom-up PBK models, comparing results across modelling platforms and read-across from structurally or mechanistically similar chemicals.
- Confidence can be built in conservative model output without human in vivo data.

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Thank you

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