



HAL
open science

PBPK modeling: What is the role of CYP3A4 expression in the gastrointestinal tract to accurately predict first-pass metabolism?

Justine Henriot, André Dallmann, François Dupuis, Jérémy Perrier, Sebastian Frechen

► To cite this version:

Justine Henriot, André Dallmann, François Dupuis, Jérémy Perrier, Sebastian Frechen. PBPK modeling: What is the role of CYP3A4 expression in the gastrointestinal tract to accurately predict first-pass metabolism?. CPT: Pharmacometrics and Systems Pharmacology, In press, 10.1002/psp4.13249 . hal-04722025

HAL Id: hal-04722025

<https://hal.univ-lorraine.fr/hal-04722025v1>

Submitted on 4 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



ARTICLE

PBPK modeling: What is the role of CYP3A4 expression in the gastrointestinal tract to accurately predict first-pass metabolism?

Justine Henriot^{1,2} | André Dallmann³ | François Dupuis⁴ | Jérémy Perrier⁵ | Sebastian Frechen²

¹Université de Lorraine, Faculty of Pharmacy, Nancy, France

²Bayer AG, Pharmacometrics/Modeling and Simulation, Systems Pharmacology & Medicine – PBPK, Leverkusen, Germany

³Bayer HealthCare SAS (on behalf of Bayer AG, Model-Informed Drug Development (MIDD), Research & Development Pharmaceuticals, Leverkusen, Germany), Lille, France

⁴Université de Lorraine, CITHEFOR, Nancy, France

⁵PhinC Development, Massy, France

Correspondence

Justine Henriot, SCK CEN, Boeretang 200, 2400 Mol, Belgium.

Email: justine.henriot@sckcen.be

Present address

Justine Henriot, Research in Dosimetry Applications, Belgian Nuclear Research Centre (SCK CEN), Mol, Belgium; and Nuclear Medicine and Molecular Imaging, Department of Imaging and Pathology, Katholieke Universiteit Leuven (KUL), Leuven, Belgium

Jérémy Perrier, esqLABS GmbH, Saterland, Germany

Abstract

Gastrointestinal first-pass metabolism plays an important role in bioavailability and in drug–drug interactions. Physiologically-based pharmacokinetic (PBPK) modeling is a powerful tool to integrate these processes mechanistically. However, a correct bottom-up prediction of GI first-pass metabolism is challenging and depends on various model parameters like the level of enzyme expression and the basolateral intestinal mucosa permeability (P_{mucosa}). This work aimed to investigate if cytochrome P450 (CYP) 3A4 expression could help predict the first-pass effect using PBPK modeling or whether additional factors like P_{mucosa} do play additional roles using PBPK modeling. To this end, a systematic review of the absolute CYP3A expression in the human gastrointestinal tract and liver was conducted. The resulting CYP3A4 expression profile and two previously published profiles were applied to PBPK models of seven CYP3A4 substrates (alfentanil, alprazolam, felodipine, midazolam, sildenafil, triazolam, and verapamil) built in PK-Sim[®]. For each compound, it was assessed whether first-pass metabolism could be adequately predicted based on the integrated CYP3A4 expression profile alone or whether an optimization of P_{mucosa} was required. Evaluation criteria were the precision of the predicted interstudy bioavailabilities and area under the concentration–time curves. It was found that none of the expression profiles provided upfront an adequate description of the extent of GI metabolism and that optimization of P_{mucosa} as a compound-specific parameter improved the prediction of most models. Our findings indicate that a pure bottom-up prediction of gastrointestinal first-pass metabolism is currently not possible and that compound-specific features like P_{mucosa} must be considered as well.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2024 The Author(s). *CPT: Pharmacometrics & Systems Pharmacology* published by Wiley Periodicals LLC on behalf of American Society for Clinical Pharmacology and Therapeutics.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The current CYP3A4 expression profile implemented in PK-Sim[®], an open-source PBPK modeling software, can result in an underestimation of the simulated gastrointestinal first-pass metabolism, which in turn, can be corrected by the optimization of the basolateral intestinal mucosa permeability.

WHAT QUESTION DID THIS STUDY ADDRESS?

Does a refined CYP3A4 expression in the gastrointestinal tract based on a systematic literature review lead to more accurate bottom-up predictions of the first-pass metabolism in PK-Sim[®]?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Despite an accurate integration of CYP3A4 expression profiles in PK-Sim[®], bottom-up predictions of the intestinal first-pass metabolism remain challenging, and the basolateral intestinal mucosa permeability needs to be considered on a case-by-case basis.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

This study emphasizes once more the importance of quantifying the extent of intestinal metabolism as early as possible in the clinical development phase, with ideally an IV dose in conjunction with PBPK modeling to better characterize the pharmacokinetic profile of a given drug candidate.

INTRODUCTION

After oral administration, a drug is absorbed, distributed, and then eliminated. Metabolism is a crucial step in the drug's elimination. First-pass metabolism occurs before the drug enters the systemic circulation and is mainly carried out by the intestines and the liver.¹ Cytochromes P450 (CYP) are drug-metabolizing enzymes, and the 3A4 isoform (CYP3A4) in particular is responsible for the metabolism of the majority of marketed drugs.²

When developing a new drug, applicants seeking approval of the drug must investigate the drug–drug interaction (DDI) potential of their product to ensure its safety. Regulatory agencies recommend *in vitro*, *in silico* and clinical investigation of these processes.³ Physiologically-based pharmacokinetic (PBPK) modeling is a compartmental modeling technique that is now widely used for *in silico* prediction of drug pharmacokinetics. In the context of *in silico* DDI assessments, the use of PBPK modeling involves combining a substrate (or perpetrator) model validated for DDI applications with the model of the new chemical drug under development, for which clinical DDI data might not (yet) be available. To achieve an adequate predictive performance of the PBPK model, first-pass metabolism must be well described in each of the drug models before they can be combined to simulate the DDI. Numerous studies have focused on hepatic first-pass metabolism, which is now well described and

predicted by PBPK modeling in most cases. In contrast, intestinal first-pass metabolism remains poorly understood. When the enzyme is expressed in both gut and liver it is also important to capture the right proportion between the two tissues. A better understanding of the intestinal metabolism may allow PBPK modeling to predict more accurately bioavailability, concentration–time profiles, and thus DDI. For this purpose, three unknowns can be identified as playing a major role in determining the extent of intestinal first-pass:

- The expression of drug-metabolizing enzymes in the gastrointestinal (GI) tract.
- The fraction unbound of the drug in enterocytes ($f_{u,g}$), and
- The intestinal mucosa permeability (P_{mucosa}) of the compound.

Typically, a recurrent under-prediction of intestinal metabolism is observed when building PBPK models for CYP3A4 substrates, which is corrected by optimizing, e.g., $f_{u,g}$ ⁴ or, alternatively, P_{mucosa} .⁵ When P_{mucosa} is decreased, the molecule's residence time in the enterocytes is increased, thereby enhancing its GI first-pass metabolism.

In this study, we investigated the impact of intestinal CYP3A4 expression on the prediction performance of intestinal first-pass metabolism using the Open Systems Pharmacology (OSP) software suite. OSP is an open-source

software dedicated to system pharmacology modeling. It includes PK-Sim[®] which is used for PBPK modeling.^{6,7} More specifically, we aimed to determine whether a more precise CYP3A4 expression at the intestinal level would, on its own, enable more accurate prediction of GI metabolism, and thus overcome the need to optimize P_{mucosa} . The influence of $f_{u,g}$ has not been studied here, as informing this parameter is challenging, whereas CYP expression has been extensively reported in the literature. Thus, the impact of three different CYP3A4 expression profiles was compared on PBPK models of seven CYP3A4 substrates.

The first intestinal CYP expression profile we used is based on the RT-PCR database described by Meyer et al.⁸ and is currently implemented in PK-Sim[®]. It compiles intestinal CYP expressions obtained by Nishimura et al.,^{9–11} expressed relatively to the liver.^{12,13} The second one is based on the work of Cordes and Rapp,¹⁴ who recently proposed a new gene expression database interoperable with PK-Sim[®] using publicly available data.¹⁵ In both databases, CYP expressions were obtained from mRNA data. Although mRNA expression levels have been widely described, various studies suggested that the relationship between gene and protein levels is rather inconsistent, since mRNA may or may not be translated into protein.^{14,16–18} Thus, we aimed to perform a systematic review of CYP expression derived from protein-level data rather than mRNA to create our third intestinal CYP expression profile. The work focused on CYP3A4 expression since it is the most abundant CYP in both gut and liver.¹⁸

Following the above-mentioned considerations, the objectives of this study were:

- (i) To create a new CYP3A4 expression profile and compare it to existing ones.
- (ii) To apply these three CYP3A4 expression profiles to PBPK models of the seven selected CYP3A4 substrates.
- (iii) To evaluate the role of these expression profiles in the prediction of GI metabolism.

METHODS

Software

The database structure for the systematic review was created using Microsoft 365 Excel (Version 2108 – Microsoft Corporation – Redmond, Washington, USA). The reported plasma concentration–time data were digitized using WebPlotDigitizer version 4.5 (Ankit Rohatgi, Austin, United States). Plot creation and statistical analysis were conducted with R (Version 4.2.1 – R foundation for Statistical Computing – Vienna, Austria). PBPK modeling and parameter identification were performed using the

free and open-source software PK-Sim[®] as part of the Open Systems Pharmacology Suite (OSP version 11.1, <https://www.open-systems-pharmacology.org/>).⁶ Phoenix WinNonlin[®] (Version 8.3.5.340 – Certara – Princeton – USA) was used for non-compartmental analyses.

Systematic review

A systematic review of the absolute expression of four cytochrome isoforms belonging to the CYP3A subfamily was conducted. To this end, two systematic literature searches occurred on April 20, 2022 using PubMed (<https://pubmed.ncbi.nlm.nih.gov/>). The first search used the following keywords simultaneously: “human,” “CYP3A*,” “liquid chromatography-mass spectrometry” or “western blot,” and “quantification” or “protein expression.” The second literature search used the keyword “human” in combination with both “protein quantification” and “cytochrome.” Only English articles were examined, with no limitations concerning the publication date. The inclusion and exclusion criteria for selecting relevant publications are defined in Table S1. Selected articles were scrutinized for possible additional references that were not covered by the literature search and further examined for eligibility. In the end, 1165 articles were sought for retrieval, among which 59 were included with a total of 788 measurements of CYP3A expression (Figure 1). When individual data were available, means were not collected to avoid redundancy and include interindividual variability.

Measurements were directly available and extracted from the articles or digitized from figures. The database gathering all collected measurements is available as supplementary material to this article (*abundance database*) and on OSP GitHub (<https://github.com/Open-Systems-Pharmacology/CYP3A-abundance-database-and-expression-profile/>). The collected measurements came from different experimental systems. Results were then homogenized and scaled to obtain a standardized expression in μmol of CYP per liter of organ. Human scaling factors for lysates, human stomach microsomes, and intestinal homogenates were not available in the literature. Thus, the corresponding measurements were later excluded. The scaling factors used for the selected systems – human liver and intestinal microsomes, human hepatocytes, liver homogenates, intestinal mucosa – are displayed in Table S2. Liver and small intestine densities were both rounded to 1 g/mL.^{19,20}

Analysis of collected expression data

One-way analyses of variances were performed using simple linear models to identify potential differences between

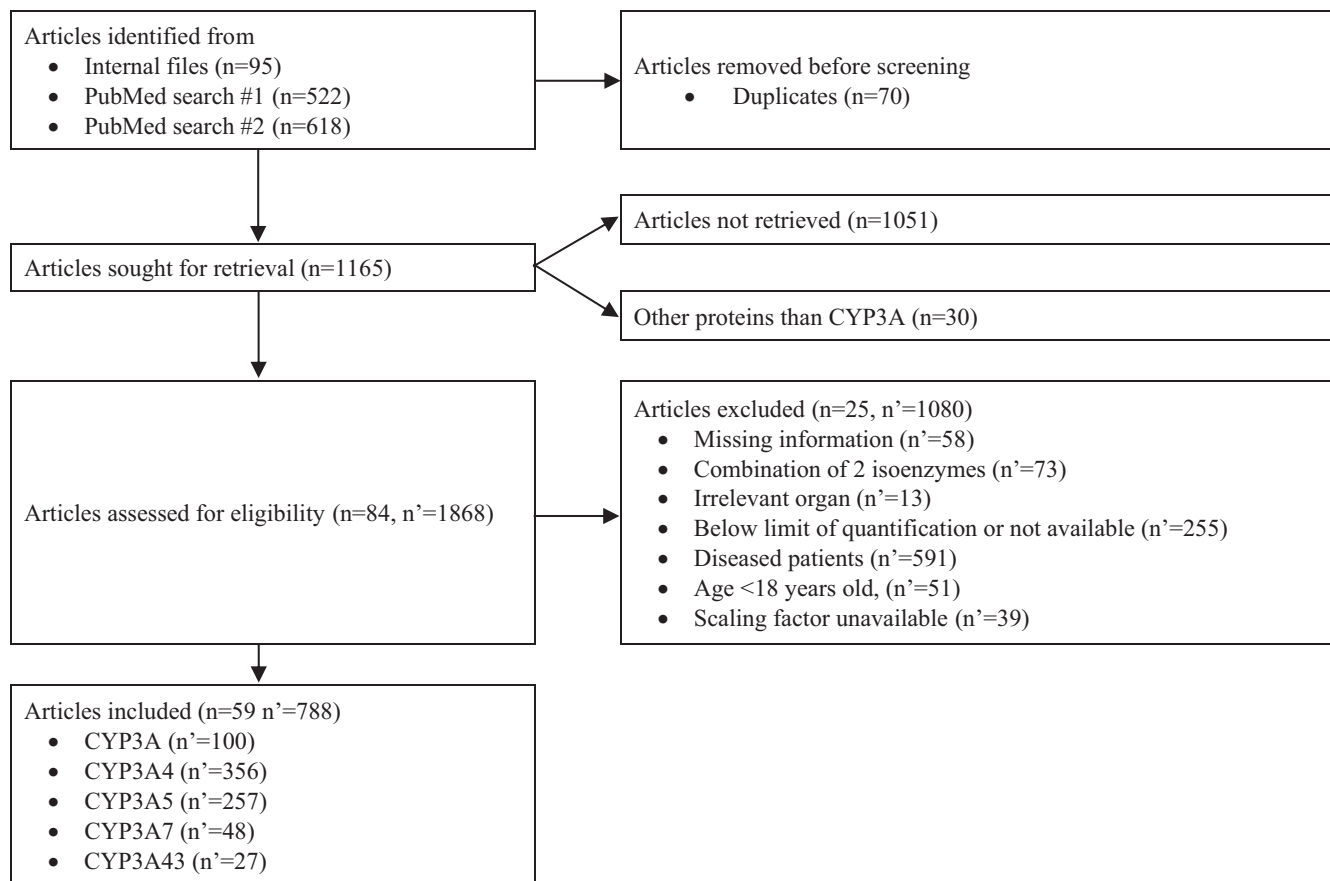


FIGURE 1 Flow diagram. CYP, cytochrome P450; *N*, number of articles; *n'*, Number of measurements.

groups, with the significance level set at 0.05. The resulting expressions were then described as the weighted geometric mean, geometric standard deviation, and 95% confidence interval.

As cytochromes are expressed in the intracellular space of each organ, the expressions resulting from the analyses needed to be scaled once more. To this end, the weighted geometric means were divided by the intracellular fraction provided by PK-Sim[®] (Table S3). A CYP3A4 expression profile was built where only liver and intestinal mucosa expressions were informed.

BPBK modeling

Seven CYP3A4 substrates were selected for this study: alfentanil, alprazolam, felodipine, midazolam, sildenafil, triazolam, and verapamil. For each of these substrates, a PBPK model was previously developed and published on OSP GitHub.²¹ Further information on model building and evaluation can be found in the evaluation report of the respective substrate published on OSP GitHub.

The same workflow was applied to each of the seven CYP3A4 substrates: each published PBPK model, featuring

either the CYP3A4 expression profile from Meyer et al.⁸ currently implemented in PK-Sim[®], from Cordes and Rapp¹⁴ or from the present systematic review, was used to simulate the pharmacokinetics of the respective drug. Of note, the structure of the model was kept the same and only the expression profiles were exchanged. Overall, three different models were built and compared for each compound, for a total of 21 models. Additionally, for each of these models, it was evaluated whether an optimization of P_{mucosa} —while keeping all other parameters fixed—improved the simulation results. Parameter optimization was conducted in PK-Sim[®] using the built-in module for parameter identification. The Levenberg Marquardt algorithm was selected for all compounds but felodipine, for which the Monte Carlo algorithm was used due to the long optimization time.

Model evaluation was started by determining the interest of optimizing P_{mucosa} for each compound and expression profile. Then, model performance between expression profiles was compared. Two evaluation criteria helped the evaluation of the GI first-pass metabolism prediction. First, since the GI first-pass metabolism impacts the bioavailability of a drug, we looked at the bioavailability prediction. Precisely, for each substrate, one intravenous

(IV) and one oral scenario, thought to be representative of the pharmacokinetic behavior of the drug, were selected (Table S4). Then, the interstudy bioavailabilities (BA_{η}) were computed according to Equation 1, enabling the calculation of simulated vs. observed interstudy bioavailability ratios (BAR, Equation 2). The closer BAR was to 1, the better the model predicted the bioavailability.

$$BA_{\eta} = \frac{AUC_{po} \cdot D_{iv}}{AUC_{iv} \cdot D_{po}} \quad (1)$$

$$BAR = \frac{BA_{\eta, simulated}}{BA_{\eta, observed}} \quad (2)$$

Second, observed and simulated areas under the concentration–time curve (AUC) were compared. To this end, observed AUCs were retrieved from publications. The simulated AUC was computed by using a non-compartmental analysis. The geometric mean fold error (GMFE) was calculated gathering all scenarios with available observed data (Equation 3).

$$GMFE = 10 \sum \left| \frac{\log\left(\frac{AUC_{simulated}}{AUC_{observed}}\right)}{n} \right| \quad (3)$$

where GMFE and n respectively stand for the GMFE of all AUC ratios and the number of scenarios (i.e., pairs of observed and simulated AUC values). The lower the GMFE was the better the model performed regarding the AUC prediction. The percentage of scenarios whose ratios were falling in the following ranges were calculated as well: model performance was very good when AUC ratios were falling between 0.80 and 1.25 (derived from bioequivalence requirements²²), and good in the 0.50–2.00 (twofold) criterion. When two models yielded the same results, the one with fewer optimized parameters was designated as better, following the parsimony principle.

RESULTS

Systematic review

For each organ and sub-organ, expressions from human liver and intestinal microsomes were statistically different from the other experimental systems (p -values $< 2 \times 10^{-16}$). Thus, only microsomal data were kept and the expressions for the liver, duodenum, jejunum, and ileum were differentiated. Expressions in the small intestine without specification of the segment were therefore excluded. In the end, from the 356, 257, 48, and 27 measurements initially selected for CYP3A4, CYP3A5, CYP3A7, and

CYP3A43, respectively (Figure 1), only 327, 240, 46, and 27 measurements were included for the analysis, respectively (Table S5).

Concentrations resulting from the systematic review are provided in Table 1 and illustrated in Figure 2. CYP3A expressions were statistically different between the liver and small intestine, but also between the three segments duodenum, jejunum, and ileum (p -values $< 2 \times 10^{-16}$). Expressions of CYP3A4 and CYP3A5 were available in both liver and small intestine. It was observed that CYP levels were the highest in the liver and decreased along the GI tract. No small intestine data were available for CYP3A7 and CYP3A43. CYP3A4 was the most abundant CYP3A isoform in the liver (2.288 $\mu\text{mol/L}$), followed by 3A7 (0.162 $\mu\text{mol/L}$), 3A5 (0.134 $\mu\text{mol/L}$) and 3A43 (0.035 $\mu\text{mol/L}$).

For the subsequent model evaluation, only CYP3A4 was implemented in PK-Sim[®]. The software provided the relative expressions, and the corresponding expression profile interoperable with PK-Sim[®] is available as supplementary material (*Systematic review – CYP3A4 expression profile*) and on OSP GitHub (<https://github.com/Open-Systems-Pharmacology/CYP3A-abundance-database-and-expression-profile/>). The three expression profiles are summarized in Table S6. Relatively to the liver, the present systematic review features a slightly higher expression of CYP3A4 in the duodenum (10%) than the one from Meyer et al.⁸ (7%). The expression profile from Cordes and Rapp¹⁴ largely exceeds both others (70%).

PBPK modeling

The interstudy bioavailabilities of each model are presented in Table 2 and illustrated in Figure 3. In most cases (16 out of 21), the interstudy bioavailability was better predicted when P_{mucosa} was optimized. Indeed, looking at alfentanil, felodipine, and triazolam models, the model with optimized P_{mucosa} – compared with calculated P_{mucosa} – resulted in a BAR closer to one regardless of the expression profile. For five models, the optimization of P_{mucosa} gave an equal or weaker prediction of the interstudy bioavailability: alprazolam (1.309 for both BAR) and midazolam (BAR of 1.708 vs. and 1.729) with the expression profile from the systematic review, and midazolam (BAR of 1.145 vs. 1.182), sildenafil (BAR of 1.504 vs. 1.532) and verapamil (BAR of 0.918 vs. 0.880) with the expression profile from Cordes and Rapp.¹⁴ The improvement of the predicted interstudy bioavailability following the P_{mucosa} optimization was particularly notable using the expression profile from Meyer et al.,⁸ and the systematic review with larger differences in BAR between models. Indeed, in the triazolam model, BAR was decreased from 1.982 to 1.103 and

TABLE 1 CYP3A absolute expressions in the liver and small intestine from the systematic review.

Organ	Weighted geo. Mean	Weighted geo. SD	95% confidence interval	Number of measurements	Total sample size
CYP3A4					
Liver	2.288	2.244	[2.088; 2.506]	254	1256
Duodenum	0.232	1.285	[0.157; 0.344]	8	68
Jejunum	0.147	2.528	[0.083; 0.263]	31	31
Ileum	0.076	2.240	[0.044; 0.133]	34	34
CYP3A5					
Liver	0.134	3.330	[0.114; 0.157]	178	1022
Duodenum	0.036	4.479	[0.014; 0.092]	8	28
Jejunum	0.002	1.496	[0.001; 0.006]	27	27
Ileum	0.002	1.917	[0.001; 0.005]	27	27
CYP3A7					
Liver	0.162	3.004	[0.117; 0.223]	46	129
CYP3A43					
Liver	0.035	3.347	[0.022; 0.053]	27	58

Note: All concentrations are given in $\mu\text{mol/L}$.

Abbreviations: Geo.mean, geometric mean; geo. SD, geometric standard deviation.

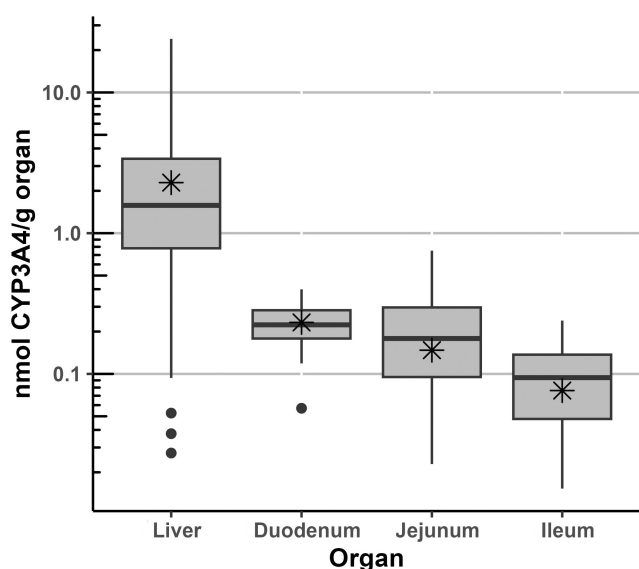


FIGURE 2 Comparison of CYP3A4 expression between organs from the systematic review. Boxes represent the interquartile range (IQR); the thick lines represent the median; stars represent the geometric means; whiskers show the minimum and maximum values falling within a distance of 1.5 times the IQR, measured out from above the upper quartile and below the lower quartile, and the black dots represent outliers beyond that range.

2.140–1.059 with the expression profile from Meyer et al.⁸ and the systematic review, respectively, when optimizing P_{mucosa} . Similarly, BAR of 3.951 and 3.831 with the expression from Meyer et al.⁸ were lowered to 1.776 and 1.671 with the optimization of P_{mucosa} in the sildenafil model.

The descriptive statistics for the AUC of each model are presented in Table 3. All GMFE were between 1.04 and 1.65. For 16 out of the 21 models, lower GMFEs and higher percentages of studies falling in both the twofold and the bioequivalence limits were achieved when P_{mucosa} was optimized. Conversely, the AUC considering all six alprazolam models were comparable (GMFE from 1.11 to 1.14), and optimizing P_{mucosa} did not improve the prediction of AUC. Additionally, the calculated value of P_{mucosa} allowed for a better or equivalent AUC prediction in the midazolam (GMFE of 1.04 vs. 1.05) and verapamil (both GMFEs of 1.08) models with the expression profiles from Cordes and Rapp¹⁴ and the systematic review, respectively.

DISCUSSION

The expression of drug-metabolizing enzymes in the GI tract, $f_{\text{u,g}}$, and P_{mucosa} of the compound were previously identified as playing a major role in determining the extent of intestinal first-pass metabolism.^{5,23} This work aimed to investigate if CYP3A4 expression alone can explain the extent of GI metabolism or whether compound-specific factors (like P_{mucosa}) do play an important role as well, while the impact of $f_{\text{u,g}}$ was not studied here. To this end, two existing expression profiles for CYP3A4^{8,14} were used in addition to a new one built from a systematic review. These three expression profiles were successfully applied to investigate their respective impact on simulated concentrations and GI first-pass metabolism using PBPK models of seven CYP3A4 substrates.

TABLE 2 Comparison of interstudy bioavailabilities between the three CYP3A4 expression profiles.

Compound	Cordes and Rapp ⁴⁹						Meyer et al. ⁸						Systematic review ^a					
	P _{mucosa} not optimized			P _{mucosa} optimized			P _{mucosa} not optimized			P _{mucosa} optimized			P _{mucosa} not optimized			P _{mucosa} optimized		
	BA _{0,obs}	BA _{0,sim}	BAR	BA _{0,sim}	BAR	BA _{0,sim}	BA _{0,sim}	BAR	BA _{0,sim}	BAR	BA _{0,sim}	BA _{0,sim}	BAR	BA _{0,sim}	BAR	BA _{0,sim}	BA _{0,sim}	BAR
Alfentanil	0.231	0.460	1.990	0.420	1.817	0.669	0.669	2.893	0.362	1.567	0.669	0.669	2.893	0.383	1.658	0.669	0.669	2.893
Alprazolam	0.736	0.970	1.317	0.969	1.316	0.965	0.965	1.310	0.921	1.251	0.965	0.965	1.309	0.964	1.309	0.965	0.965	1.309
Felodipine	0.123	0.233	1.895	0.230	1.875	0.255	0.255	2.076	0.253	2.060	0.255	0.255	2.060	0.220	1.792	0.255	0.255	2.060
Midazolam	0.313	0.358	1.145	0.370	1.182	0.521	0.521	1.664	0.442	1.413	0.521	0.521	1.708	0.541	1.729	0.521	0.521	1.708
Sildenafil	0.319	0.480	1.504	0.489	1.532	1.261	1.261	3.951	0.567	1.776	1.261	1.261	3.831	0.533	1.671	1.261	1.261	3.831
Triazolam	0.358	0.515	1.438	0.376	1.049	0.710	0.710	1.982	0.395	1.103	0.710	0.710	2.140	0.379	1.059	0.710	0.710	2.140
Verapamil	0.216	0.198	0.918	0.190	0.880	0.247	0.247	1.146	0.213	0.988	0.247	0.247	0.958	0.223	1.034	0.247	0.247	0.958

Note: Cells filled in blue show which model performed better between the one with P_{mucosa} not optimized or optimized, considering the prediction of the interstudy bioavailability.

Abbreviations: BAR, interstudy bioavailability ratio; BA_{0,obs}, interstudy bioavailability; obs, observed; P_{mucosa}, basolateral intestinal mucosa permeability; sim, simulated.

^aThe systematic review CYP3A4 expression profile resulted from the analysis of microsomal CYP3A4 measurements available in the literature. This profile included CYP3A4 expressions in the liver, duodenum, jejunum and ileum, where the respective weighted geometric means divided by the fraction intracellular were implemented in PK-Sim (see Section 2).

The presented results of the systematic literature review show that CYP3A4 was the most abundant CYP3A isoform in the liver (2.288 μmol/L), followed by CYP3A7 (0.162 μmol/L), CYP3A5 (0.134 μmol/L) and CYP3A43 (0.035 μmol/L). Although CYP3A7 is considered to be a fetal-specific enzyme, with very low and negligible concentrations in adults,^{24,25} CYP3A7 concentrations were found to be higher than those for CYP3A5 in adults (over 18 years old; Table 1). Geometric standard deviations ranged from 1.3 to 4.5, showing a large spread of the values around the geometric means, and therefore important interindividual and interstudy variabilities, which were already emphasized in the literature.^{25,26} CYP3A4 and CYP3A5 concentrations were higher in the liver (2.288 and 0.134 μmol/L, respectively) than in the small intestine and decreased along the GI tract, as described previously.^{27–29} Expressions in the large intestine could further inform this trend, but no scaling factors were available to scale the reported values.

The experience in building PBPK models with the CYP3A4 expression profile reported by Meyer et al. expression⁸ implemented in PK-Sim[®] showed that in some cases, the GI metabolism can be underrepresented which is typically corrected by the optimization of P_{mucosa}. In this study, the new CYP3A4 expression profile based on the results from the systematic review and the one published by Cordes and Rapp¹⁴ were utilized to investigate their impact on simulated concentrations and parameters using PBPK models of seven CYP3A4 substrates and compared with the models featuring the expression from Meyer et al.⁸ The results from the two evaluation criteria – the AUC and the interstudy bioavailability—were consistent with each other. AUC GMFE was below 2 for all models, showing good predictions of the AUC. Looking at the two evaluation criteria and considering all expression profiles, model predictions were more accurate when P_{mucosa} was optimized. This was particularly pronounced for the models using the expression profiles from Meyer et al.⁸ or from the presented systematic review. However, in models featuring the CYP3A4 expression from Cordes and Rapp¹⁴—which had a relatively high intestinal CYP3A4 expression—optimizing P_{mucosa} improved model performance only slightly.

We initially expected that the CYP3A4 expression profiles from our systematic review and Cordes and Rapp,¹⁴ which had a higher relative CYP3A4 intestinal expression compared with the liver than the one from Meyer et al.⁸ (Table S6), would enhance overall model performance and supersede the need to optimize P_{mucosa}. However, for most of the tested compound models, optimizing P_{mucosa} improved predictions of bioavailabilities and AUC, even when models were built with these two expression profiles.

Nevertheless, this observation remained compound-specific. It was noted that the interest in optimizing P_{mucosa}

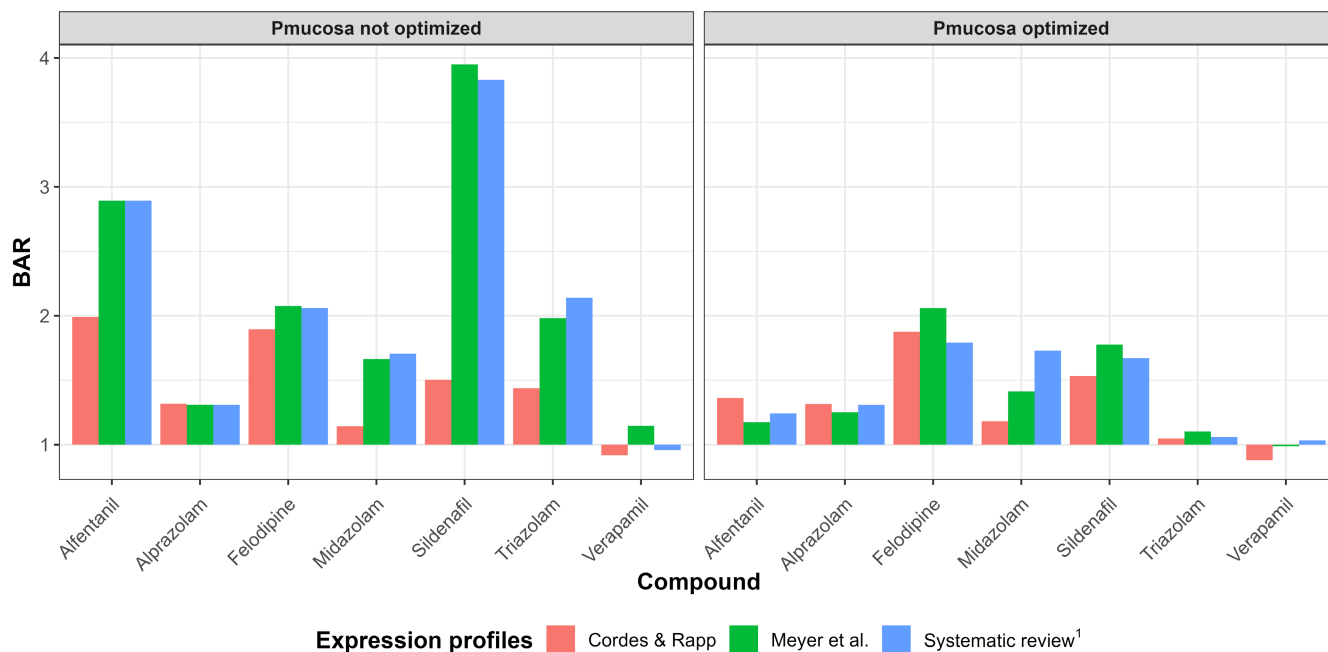


FIGURE 3 Comparison of the interstudy bioavailabilities between the three CYP3A4 expression profiles. BAR, ratio of simulated vs. observed interstudy bioavailabilities; P_{mucosa} , Basolateral intestinal mucosa permeability. ¹The systematic review CYP3A4 expression profile resulted from the analysis of microsomal CYP3A4 measurements available in the literature. This profile included CYP3A4 expressions in the liver, duodenum, jejunum, and ileum, where the respective weighted geometric means divided by the fraction intracellular were implemented in PK-Sim (see Section 2).

was limited for alprazolam, as the model with the calculated value often performed better. Conversely, triazolam was a good example where P_{mucosa} optimization enabled a much better match of observed data. Therefore, CYP3A4 expression certainly plays a role in predicting GI metabolism, but other compound-specific factors also come into play. Similarly, when largely increasing CYP3A4 gut expression (Cordes and Rapp¹⁴), the interest in optimizing P_{mucosa} was reduced, but still needed for some compounds, although this expression profile was less physiologically plausible. Indeed, CYP3A4 expressions of around 70% and 80% in the small and large intestine compared with the liver, respectively, differ from reported data³⁰ (Table 1) and from the two other expression profiles. Overall, among the three expression profiles that were used in this study, none allowed us to find a compromise between a sufficiently high CYP3A4 expression in the gut to prevent from optimizing P_{mucosa} , while keeping this expression consistent with physiological values. Optimization of P_{mucosa} as a compound-specific parameter improved the prediction in most models. Even the CYP expression profile from the systematic review, which seemed to best describe the human physiology, was not sufficient on its own to accurately predict GI metabolism and required optimizing P_{mucosa} using clinical pharmacokinetic data after oral and intravenous administration.

Across various PBPK software platforms, intestinal metabolism is structurally handled in different ways. For example, GastroPlusTM and Simcyp[®] introduce a parameter for the fraction unbound in the gut ($f_{u,g}$), which can have a big impact on intestinal metabolism, and consequently, on the predicted magnitude of DDIs. Owing to the challenges in accurately measuring $f_{u,g}$, it has been recommended to test different values for this parameter and to conduct a sensitivity analysis to quantify the impact of the uncertainty in this parameter on the simulation results.⁴ This parameter influencing the intestinal first-pass metabolism is hard to inform and is often set to one by default.^{23,31} Another research group worked on the impact of $f_{u,g}$ on DDI predictions, and concluded that this parameter influences AUC and C_{max} and could also be compound-specific.³² For example, while it might not have an impact on midazolam first-pass effect,²⁸ it was found to be highly relevant for the predicted first-pass effect of alfentanil and terfenadine.²³ In PK-Sim[®], this parameter is not explicitly present. Instead, the free fraction in the enterocyte is determined by the partition coefficient between intracellular space and water (i.e., the ratio of the fraction unbound in plasma and the partition coefficient between intracellular space and plasma). Hence, the intracellular free fraction is handled consistently across different tissues, including GI tissues, depending solely on the model used for estimating the tissue-to-plasma partition coefficients.

TABLE 3 Comparison of areas under the concentration–time curve between the three CYP3A4 expression profiles.

Compound	<i>n</i>	<i>P</i> _{mucosa} not optimized			<i>P</i> _{mucosa} optimized		
		GMFE	% in two-fold	% in Bioeq	GMFE	% in two-fold	% in Bioeq
Cordes and Rapp ⁴⁹							
Alfentanil	11/12	1.10	100	91	1.09	100	100
Alprazolam	20/32	1.11	100	85	1.12	100	85
Felodipine	13/15	1.45	85	38	1.45	92	38
Midazolam	4/4	1.04	100	100	1.05	100	100
Sildenafil	16/17	1.40	87	44	1.30	94	37
Triazolam	16/28	1.25	100	50	1.21	100	56
Verapamil	6/6	1.11	100	83	1.08	100	100
Meyer et al. ⁸							
Alfentanil	11/12	1.24	100	55	1.10	100	100
Alprazolam	20/32	1.12	100	85	1.14	100	85
Felodipine	13/15	1.50	85	31	1.46	85	46
Midazolam	4/4	1.24	100	25	1.13	100	100
Sildenafil	16/17	1.70	81	19	1.23	100	56
Triazolam	16/28	1.35	100	44	1.21	100	56
Verapamil	6/6	1.20	100	67	1.10	100	100
Systematic review ^a							
Alfentanil	11/12	1.24	100	55	1.10	100	100
Alprazolam	20/32	1.12	100	85	1.12	100	85
Felodipine	13/15	1.45	85	46	1.43	85	46
Midazolam	4/4	1.25	100	25	1.24	100	75
Sildenafil	16/17	1.65	81	12	1.22	100	62
Triazolam	16/28	1.33	100	56	1.21	100	56
Verapamil	6/6	1.08	100	100	1.08	100	100

Note: Cells filled in blue show which model performed better between the one with *P*_{mucosa} not optimized or optimized, considering the prediction of areas under the curves.

Abbreviations: % in bioeq, percentage of AUC falling in the bioequivalence limits ([0.80; 1.25]); % in two-fold, percentage of AUC ratios falling in the two-fold limits ([0.50; 2.00]); AUC, area under the curve; GMFE, geometric mean fold error; *n*, number of studies with available observed AUC / total number of studies; *P*_{mucosa}, basolateral intestinal mucosa permeability.

^aThe systematic review CYP3A4 expression profile resulted from the analysis of microsomal CYP3A4 measurements available in the literature. This profile included CYP3A4 expressions in the liver, duodenum, jejunum, and ileum, where the respective weighted geometric means divided by the fraction intracellular were implemented in PK-Sim (see Section 2).

This study has some limitations that need to be addressed. The systematic review included data from various quantification methods, mainly western blotting and liquid chromatography with tandem mass spectrometry. Data were analyzed regardless of the measurement technique. However, liquid chromatography with tandem mass spectrometry is known to be more accurate and able to better differentiate CYP isoforms.^{33,34} Looking at the data of the systematic review, liver concentrations obtained by LC–MS/MS and western blot were indeed statistically different (p -value = 4.13×10^{-6}). Similarly, differences in gender, age, weight, ethnicity, genotype, and smoking status were not investigated, although these factors have been

proved to influence CYPs expression.^{24,35–39} For example, hepatic CYP3A4 mRNA levels are significantly higher in females versus males,⁴⁰ and while smoking is known to induce CYP1A2,⁴¹ it might possibly also induce CYP3A4.⁴² The relatively large variability in the presented CYP3A concentrations (with geometric standard deviations spanning from 1.3 to 4.5; Table 1) might largely stem from these covariates that were not consistently accounted for across the different studies included in this systematic review. Finally, we worked with seven drugs, which remains a limited dataset.

Many drugs are either CYP substrates, inhibitors, or inducers, and their co-administration may lead to DDI. As

detailed by Galetin et al.,^{43–46} GI metabolism is an important factor to account for when looking at DDI since CYPs are expressed in both the intestine and the liver. Besides, for a given metabolized fraction, the higher the intestines contribute to the metabolism, the higher is the potential for DDI.⁴³ It is thus of the utmost importance to understand and accurately predict GI first-pass metabolism in PBPK modeling prior to simulating DDI. PBPK modeling is a powerful tool to explore and quantitatively predict the DDI liability of drugs. To this end, the extent of interaction is determined in vitro, and PBPK modeling enables the translation to humans through in vitro and in vivo extrapolations. The FDA and the EMA are becoming increasingly inclined to use this approach and to accept model-based DDI results as it may offer a valuable alternative to dedicated clinical trials.^{47,48} Simulating DDI could be the next step in the evaluation of the GI metabolism using the three expression profiles. Such work has already been published for CYP3A4-mediated DDI⁴⁹ using alfentanil, alprazolam, midazolam, triazolam, and verapamil PBPK models. The models all featured the expression profile from Meyer et al.⁸ and the optimization of P_{mucosa} . It resulted in an overall GMFE of ~1.4 for both AUC and C_{max} ratios, showing a good prediction precision.

DDI simulations can be highly informative and time and cost-efficient during drug development, from both the clinical and industrial perspectives. The seven drugs included in this study are well-established with a lot of published data. The experience acquired on these drugs will be a great asset for new chemical entities under development with few or poor available data. Indeed, this work could enhance and support the precision of GI metabolism and therefore DDI predictions for these potential medications.

CONCLUSION

In summary, while the CYP3A4 expression, in particular the expression in the GI tract relative to the hepatic expression, obviously plays a role in the prediction of GI metabolism, compound-specific features like P_{mucosa} on top influence its extent and must be considered. We would therefore recommend keeping on using the expression profile from Meyer et al.,⁸ which is currently implemented in PK-Sim[®] and provided acceptable predictions of the GI metabolism while being physiologically relevant. The need for P_{mucosa} optimization should be checked individually for each PBPK model: we recommend an iterative modeling approach, performing an initial parameter identification without optimizing P_{mucosa} . If intestinal metabolism seems under-predicted, optimization of P_{mucosa} should

be considered along with measures to avoid non-identifiability issues. In both cases, model calibration using the totality of available data (e.g., in vitro permeability data and in vivo pharmacokinetic data after IV dosing) is recommended to better characterize the pharmacokinetic profile. The prediction of P_{mucosa} in the absence of clinical data seems challenging. Thus, an accurate quantification of the extent of GI metabolism in early clinical drug development seems to require an IV dosing already during phase I. Microdosing could be an option to reduce overdose and adverse effect issues due to the potential lack of knowledge at this step. As more data on compounds with known first-pass metabolism profiles become available, our refined CYP3A4 expression profile could be used to conduct comparative analyses and to develop a framework for scenario-based recommendations, identifying specific compound classes where the optimization of P_{mucosa} significantly improves prediction accuracy.

AUTHOR CONTRIBUTIONS

J.H., A.D., F.D., J.P., and S.F. wrote the manuscript; J.H., A.D. and S.F. designed the research; J.H., A.D., and S.F. performed the research; J.H., A.D., and S.F. analyzed the data.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Henrik Cordes (Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) for providing the expression profile, Juri Solodenko (Bayer AG, Leverkusen, Germany) for his guidance in implementing the new expression profile in PK-Sim[®], and Luc Ferrari (Université de Lorraine, F-54000 Nancy, France) for his advice and feedback throughout the course of this research project.

FUNDING INFORMATION

No funding was received for this work.

CONFLICT OF INTEREST STATEMENT

A.D. and S.F. use Open Systems Pharmacology software, tools, and models in their professional roles. S.F. is a member of the Open Systems Pharmacology Sounding Board. All other authors declared no competing interests for this work.


ORCID

Justine Henriot  <https://orcid.org/0000-0001-7793-1637>

André Dallmann  <https://orcid.org/0000-0003-1108-5719>

François Dupuis  <https://orcid.org/0000-0003-1947-3839>

Jérémy Perrier  <https://orcid.org/0009-0001-9372-6315>

Sebastian Frechen  <https://orcid.org/0000-0003-1170-9392>

Jérémy Perrier  <https://orcid.org/0009-0001-9372-6315>

Sebastian Frechen  <https://orcid.org/0000-0003-1170-9392>

Sebastian Frechen  <https://orcid.org/0000-0003-1170-9392>

REFERENCES

- Lin JH, Lu AY. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu Rev Pharmacol Toxicol.* 2001;41:535-567. doi:10.1146/annurev.pharmtox.41.1.535
- Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther.* 2013;138(1):103-141. doi:10.1016/j.pharmthera.2012.12.007
- United States Food and Drug Administration. Guidance for Industry: In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions. 2020. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions>. Accessed October 9, 2023.
- Ezuruike U, Zhang M, Pansari A, et al. Guide to development of compound files for PBPK modeling in the Simcyp population-based simulator. *CPT Pharmacomet Syst Pharmacol.* 2022;11(7):805-821. doi:10.1002/psp4.12791
- Wendl T, Frechen S, Gerisch M, Heinig R, Eissing T. Physiologically-based pharmacokinetic modeling to predict CYP3A4-mediated drug-drug interactions of finerenone. *CPT Pharmacometrics Syst Pharmacol.* 2022;11(2):199-211. doi:10.1002/psp4.12746
- Open Systems Pharmacology. <https://www.open-systems-pharmacology.org/>. Accessed October 7, 2023.
- Lippert J, Burghaus R, Edginton A, et al. Open systems pharmacology community – an open access, open source, open science approach to modeling and simulation in pharmaceutical sciences. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(12):878-882. doi:10.1002/psp4.12473
- Meyer M, Schneckener S, Ludewig B, Kuepfer L, Lippert J. Using expression data for quantification of active processes in physiologically based pharmacokinetic modeling. *Drug Metab Dispos.* 2012;40(5):892-901. doi:10.1124/dmd.111.043174
- Nishimura M, Yaguti H, Yoshitsugu H, Naito S, Satoh T. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi.* 2003;123(5):369-375. doi:10.1248/yakushi.123.369
- Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab Pharmacokinet.* 2005;20(6):452-477. doi:10.2133/dmpk.20.452
- Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human phase I metabolizing enzymes except for cytochrome P450 and phase II metabolizing enzymes. *Drug Metab Pharmacokinet.* 2006;21(5):357-374. doi:10.2133/dmpk.21.357
- Rodrigues AD. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem Pharmacol.* 1999;57(5):465-480. doi:10.1016/s0006-2952(98)00268-8
- Barter ZE, Bayliss MK, Beaune PH, et al. Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values of human micro-somal protein and hepatocellularity per gram of liver. *Curr Drug Metab.* 2007;8(1):33-45. doi:10.2174/13892000779315053
- Cordes H, Rapp H. Gene expression databases for physiologically based pharmacokinetic modeling of humans and animal species. *CPT Pharmacometrics Syst Pharmacol.* 2023;12(3):311-319. doi:10.1002/psp4.12904
- Bastian FB, Roux J, Niknejad A, et al. The Bgee suite: integrated curated expression atlas and comparative transcriptomics in animals. *Nucleic Acids Res.* 2021;49(D1):D831-D847. doi:10.1093/nar/gkaa793
- Ohtsuki S, Schaefer O, Kawakami H, et al. Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: comparison with mRNA levels and activities. *Drug Metab Dispos.* 2012;40(1):83-92. doi:10.1124/dmd.111.042259
- Berggren S, Gall C, Wollnitz N, et al. Gene and protein expression of P-glycoprotein, MRP1, MRP2, and CYP3A4 in the small and large human intestine. *Mol Pharm.* 2007;4(2):252-257. doi:10.1021/mp0600687
- Zhou SF. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Curr Drug Metab.* 2008;9(4):310-322. doi:10.2174/138920008784220664
- Niehues SM, Unger JK, Malinowski M, Neymeyer J, Hamm B, Stockmann M. Liver volume measurement: reason of the difference between in vivo CT-volumetry and intraoperative ex vivo determination and how to cope it. *Eur J Med Res.* 2010;15(8):345-350. doi:10.1186/2047-783x-15-8-345
- Mcintosh RL, Anderson V. A comprehensive tissue properties database provided for the thermal assessment of a human at rest. *Biophys Rev Lett.* 2010;5(03):129-151. doi:10.1142/S1793048010001184
- Open Systems Pharmacology. Library of released PBPK substance models and evaluation reports. <https://github.com/Open-Systems-Pharmacology/OSP-PBPK-Model-Library>. Accessed May 15, 2023.
- United States Food and Drug Administration. Guidance for Industry: Statistical Approaches to Establishing Bioequivalence. 2001. <https://www.fda.gov/media/70958/download>. Accessed July 4, 2023.
- Heikkinen AT, Baneyx G, Caruso A, Parrott N. Application of PBPK modeling to predict human intestinal metabolism of CYP3A substrates - an evaluation and case study using GastroPlus. *Eur J Pharm Sci.* 2012;47(2):375-386. doi:10.1016/j.ejps.2012.06.013
- Sim SC, Edwards RJ, Boobis AR, Ingelman-Sundberg M. CYP3A7 protein expression is high in a fraction of adult human livers and partially associated with the CYP3A7*1C allele. *Pharmacogenet Genomics.* 2005;15(9):625-631. doi:10.1097/01.fpc.0000171516.84139.89
- Lacroix D, Sonnier M, Moncion A, Cheron G, Cresteil T. Expression of CYP3A in the human liver – evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem.* 1997;247(2):625-634. doi:10.1111/j.1432-1033.1997.00625.x
- Achour B, Al-Majdoub ZM, Grybos-Gajniak A, et al. Liquid biopsy enables quantification of the abundance and interindividual variability of hepatic enzymes and transporters. *Clin Pharmacol Ther.* 2021;109(1):222-232. doi:10.1002/cpt.2102
- Grangeon A, Clermont V, Barama A, Gaudette F, Turgeon J, Michaud V. Determination of CYP450 expression levels in the human small intestine by mass spectrometry-based targeted

- proteomics. *Int J Mol Sci.* 2021;22(23):12791. doi:[10.3390/ijms222312791](https://doi.org/10.3390/ijms222312791)
28. Paine MF, Khalighi M, Fisher JM, et al. Characterization of interintestinal and intrainestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther.* 1997;283(3):1552-1562.
 29. von Richter O, Burk O, Fromm MF, Thon KP, Eichelbaum M, Kivistö KT. Cytochrome P450 3A4 and P-glycoprotein expression in human small intestinal enterocytes and hepatocytes: a comparative analysis in paired tissue specimens. *Clin Pharmacol Ther.* 2004;75(3):172-183. doi:[10.1016/j.cpt.2003.10.008](https://doi.org/10.1016/j.cpt.2003.10.008)
 30. Wenzel C, Drozdziak M, Oswald S. Mass spectrometry-based targeted proteomics method for the quantification of clinically relevant drug metabolizing enzymes in human specimens. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2021;1180:122891. doi:[10.1016/j.jchromb.2021.122891](https://doi.org/10.1016/j.jchromb.2021.122891)
 31. Yang J, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A. Prediction of intestinal first-pass drug metabolism. *Curr Drug Metab.* 2007;8(7):676-684. doi:[10.2174/138920007782109733](https://doi.org/10.2174/138920007782109733)
 32. Sinha VK, Snoeys J, Osselaer NV, Peer AV, Mackie C, Heald D. From preclinical to human—prediction of oral absorption and drug-drug interaction potential using physiologically based pharmacokinetic (PBPK) modeling approach in an industrial setting: a workflow by using case example. *Biopharm Drug Dispos.* 2012;33(2):111-121. doi:[10.1002/bdd.1782](https://doi.org/10.1002/bdd.1782)
 33. Achour B, Russell MR, Barber J, Rostami-Hodjegan A. Simultaneous quantification of the abundance of several cytochrome P450 and uridine 5'-diphospho-glucuronosyltransferase enzymes in human liver microsomes using multiplexed targeted proteomics. *Drug Metab Dispos.* 2014;42(4):500-510. doi:[10.1124/dmd.113.055632](https://doi.org/10.1124/dmd.113.055632)
 34. Langenfeld E, Zanger UM, Jung K, Meyer HE, Marcus K. Mass spectrometry-based absolute quantification of microsomal cytochrome P450 2D6 in human liver. *Proteomics.* 2009;9(9):2313-2323. doi:[10.1002/pmic.200800680](https://doi.org/10.1002/pmic.200800680)
 35. Dennison JB, Jones DR, Renbarger JL, Hall SD. Effect of CYP3A5 expression on vincristine metabolism with human liver microsomes. *J Pharmacol Exp Ther.* 2007;321(2):553-563. doi:[10.1124/jpet.106.118471](https://doi.org/10.1124/jpet.106.118471)
 36. Westlind-Johnsson A, Malmebo S, Johansson A, et al. Comparative analysis of CYP3A expression in human liver suggests only a minor role for CYP3A5 in drug metabolism. *Drug Metab Dispos.* 2003;31(6):755-761. doi:[10.1124/dmd.31.6.755](https://doi.org/10.1124/dmd.31.6.755)
 37. Zane NR, Chen Y, Wang MZ, Thakker DR. Cytochrome P450 and flavin-containing monooxygenase families: age-dependent differences in expression and functional activity. *Pediatr Res.* 2018;83(2):527-535. doi:[10.1038/pr.2017.226](https://doi.org/10.1038/pr.2017.226)
 38. Miyauchi E, Tachikawa M, Declèves X, et al. Quantitative atlas of cytochrome P450, UDP-glucuronosyltransferase, and transporter proteins in jejunum of morbidly obese subjects. *Mol Pharm.* 2016;13(8):2631-2640. doi:[10.1021/acs.molpharmaceut.6b00085](https://doi.org/10.1021/acs.molpharmaceut.6b00085)
 39. Wegler C, Wisniewski JR, Robertsen I, et al. Drug disposition protein quantification in matched human jejunum and liver from donors with obesity. *Clin Pharmacol Ther.* 2022;111(5):1142-1154. doi:[10.1002/cpt.2558](https://doi.org/10.1002/cpt.2558)
 40. Wolbold R, Klein K, Burk O, et al. Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology.* 2003;38(4):978-988. doi:[10.1053/jhep.2003.50393](https://doi.org/10.1053/jhep.2003.50393)
 41. Tantcheva-Poór I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. *Pharmacogenetics.* 1999;9(2):131-144.
 42. Scherf-Clavel M, Deckert J, Menke A, Unterecker S. Smoking is associated with lower dose-corrected serum concentrations of escitalopram. *J Clin Psychopharmacol.* 2019;39(5):485-488. doi:[10.1097/JCP.0000000000001080](https://doi.org/10.1097/JCP.0000000000001080)
 43. Galetin A, Gertz M, Houston JB. Potential role of intestinal first-pass metabolism in the prediction of drug-drug interactions. *Expert Opin Drug Metab Toxicol.* 2008;4(7):909-922. doi:[10.1517/17425255.4.7.909](https://doi.org/10.1517/17425255.4.7.909)
 44. Galetin A, Gertz M, Houston JB. Contribution of intestinal cytochrome p450-mediated metabolism to drug-drug inhibition and induction interactions. *Drug Metab Pharmacokinet.* 2010;25(1):28-47. doi:[10.2133/dmpk.25.28](https://doi.org/10.2133/dmpk.25.28)
 45. Galetin A, Hinton LK, Burt H, Obach RS, Houston JB. Maximal inhibition of intestinal first-pass metabolism as a pragmatic indicator of intestinal contribution to the drug-drug interactions for CYP3A4 cleared drugs. *Curr Drug Metab.* 2007;8(7):685-693. doi:[10.2174/138920007782109805](https://doi.org/10.2174/138920007782109805)
 46. Galetin A, Houston JB. Intestinal and hepatic metabolic activity of five cytochrome P450 enzymes: impact on prediction of first-pass metabolism. *J Pharmacol Exp Ther.* 2006;318(3):1220-1229. doi:[10.1124/jpet.106.106013](https://doi.org/10.1124/jpet.106.106013)
 47. Zhang X, Yang Y, Grimstein M, et al. Application of PBPK modeling and simulation for regulatory decision making and its impact on US prescribing information: an update on the 2018–2019 submissions to the US FDA's Office of Clinical Pharmacology. *J Clin Pharmacol.* 2020;60(Suppl 1):S160-S178. doi:[10.1002/jcph.1767](https://doi.org/10.1002/jcph.1767)
 48. Edginton AN, Theil FP, Schmitt W, Willmann S. Whole body physiologically-based pharmacokinetic models: their use in clinical drug development. *Expert Opin Drug Metab Toxicol.* 2008;4(9):1143-1152. doi:[10.1517/17425255.4.9.1143](https://doi.org/10.1517/17425255.4.9.1143)
 49. Frechen S, Solodenko J, Wendl T, et al. A generic framework for the physiologically-based pharmacokinetic platform qualification of PK-Sim and its application to predicting cytochrome P450 3A4-mediated drug-drug interactions. *CPT Pharmacometrics Syst Pharmacol.* 2021;10(6):633-644. doi:[10.1002/psp4.12636](https://doi.org/10.1002/psp4.12636)

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Henriot J, Dallmann A, Dupuis F, Perrier J, Frechen S. PBPK modeling: What is the role of CYP3A4 expression in the gastrointestinal tract to accurately predict first-pass metabolism? *CPT Pharmacometrics Syst Pharmacol.* 2024;00:1-12. doi:[10.1002/psp4.13249](https://doi.org/10.1002/psp4.13249)