Relevance of desorption kinetics and permeability for *in vitro*-based predictions of hepatic clearance in fish

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Abstract

The impact of desorption kinetics and permeation kinetics on *in vitro*-based predictions of *in vivo* hepatic blood clearances is investigated in the present study. Most commonly, possible limitations due to slow desorption of chemicals from albumin or slow permeation of chemicals through cellular membranes are not considered when *in vivo* clearances are predicted from *in vitro* biotransformation rate constants. To evaluate whether the most commonly used extrapolation models might thus overlook important kinetic limitations, we compare predictions of *in vivo* clearance that explicitly consider desorption and permeation kinetics with predictions of *in vivo* clearance that neglect these aspects.

Our results show that strong limitations due to slow permeation kinetics are possible depending on the assumed permeability value. While permeability values estimated with a mechanistic approach are fast enough to avoid significant limitations, other experimentally derived permeability values lead to dramatically decreased *in vivo* clearance predictions. These latter values lead to unrealistically low *in vivo* biotransformation estimates. Furthermore, we also evaluated the implications of desorption kinetics using experimentally determined desorption rate constants. These evaluations show that slow desorption kinetics are unlikely to limit *in vivo* clearance.

Keywords:

desorption kinetics; permeability; *in vitro-in vivo* extrapolation; biotransformation; modeling

Introduction

In vitro experiments are powerful tools to study the biotransformation kinetics of chemicals. The purpose of these experiments is to predict the corresponding *in vivo* biotransformation from *in vitro* results, e.g. in the assessment of the bioaccumulation potential of a chemical (Krause and Goss, 2020; Nichols et al., 2013; Weisbrod et al., 2009). For doing so, *in vitro-in vivo* extrapolation (IVIVE) is performed. The origin of this approach is in the pharmacological field with a focus on mammalian applications (Brian Houston and Carlile, 1997; Houston, 1994; Obach, 1997). However, several years ago, the approach was also adapted in the environmental field for fish to use in bioaccumulation assessment of chemicals (Nichols et al., 2007; Nichols et al., 2006). Since then, two OECD guidelines (OECD, 2018b, c) and an accompanying guidance document (OECD, 2018a) have been published that focus on the methodology for estimating hepatic clearance from *in vitro* biotransformation data and demonstrate how the determined clearance can be used to predict fish bioconcentration factors (BCFs). The *in vitro-in vivo* extrapolation procedure has to correct for all differences that exist between the *in vitro* and the *in vivo* system. Crucial points in IVIVE are scaling, i.e. consideration of the different amounts of biotransforming components (like S9 or hepatocytes)

in vitro and *in vivo*, and binding correction, because the sorption effects differ *in vitro* and *in vivo* (Krause and Goss, 2018b).

Furthermore, kinetic limitations that might occur *in vivo* but are not represented in the *in vitro* system need to be accounted for. Neglecting such limitations would otherwise result in overestimating *in vivo* biotransformation and thus underestimating bioaccumulation (Han et al., 2008; Nichols et al., 2018; Saunders et al., 2019). A typical example for such a limitation is blood flow limitation (Rane et al., 1977), because *in vivo* chemical delivery to the metabolically active sites usually occurs via blood flow. Associated with that, slow permeation from blood into the eliminating tissue or slow desorption from binding sites within blood are other potential *in vivo* limitations (Kirichuk and Lutsevich, 1996; Weisiger et al., 1981). Currently, these two latter limitations are neglected in the extrapolation by using the well-stirred liver model (Rowland et al., 1973) for prediction of hepatic blood clearance.

Consideration of permeation and desorption kinetics could result in lower predicted *in vivo* clearances (Berezhkovskiy, 2012; Weisiger, 1985). One aspect that is often raised in the context of possible limitations occurring *in vivo* is the so-called facilitated transport (Bteich et al., 2019; Laue et al., 2020). Facilitated transport is the effect that the transport or uptake of chemicals across diffusional barriers is faster in the presence of certain mobile binding matrices (e.g. proteins) than the transport of only freely dissolved chemicals (Kramer et al., 2007; ter Laak et al., 2009). Mistakenly, facilitated transport is sometimes suggested as an explanation why *in vitro*-based estimates of *in vivo* clearance underestimate the actual clearance that is observed *in vivo*. However, this explanation would only be appropriate if the estimation of *in vivo* clearance had first considered any limitation due to slow uptake of the chemical into the biotransforming tissue. As mentioned above, however, this is usually not the case; *in vitro*-based estimates of *in vivo* clearance are usually calculated using the well-stirred liver model, which *a priori* assumes chemical uptake to be an instantaneous process. In contrast, in the model presented here, a kinetic limitation of chemical uptake is explicitly considered, so that the effect of facilitated transport may become relevant. Therefore, facilitated transport is also considered in this study.

In this manuscript, we investigate systematically in which scenarios limitations due to slow permeation or desorption kinetics might occur. For doing so, we use a steady state model that explicitly considers permeation and desorption kinetics in addition to blood flow and biotransformation kinetics (Krause and Goss, 2018a). For our analysis, we combine experimental data as well as estimated data on permeation kinetics, desorption kinetics and biotransformation kinetics. The used model is implemented into an excel calculation tool and made available so that users can evaluate the relevance of permeation and desorption limitation in their specific scenarios of interest. By this, the provided Microsoft Excel calculation

tool can be seen as an addition to the recently published B-compass fish tool for IVIVE and BCF prediction (Krause and Goss, 2020).

Methods

Model structure for evaluation of permeation and desorption limitation

The required model structure to consider permeation and desorption kinetics in combination with biotransformation kinetics in a steady-state scenario has been recently presented elsewhere and applied to estimate human hepatic clearance under consideration of desorption kinetics (Krause and Goss, 2018a). In short, the model distinguishes the freely dissolved chemical in blood, the bound chemical in blood and the total chemical in the hepatocytes (Figure 1). Individual mass balance for the distinguished compartments are formulated. Expressed in words the mass balances for the freely dissolved chemical in blood is:

$$freely\ dissolved\ chemical\ in\ blood\ over\ time = transport\ via\ blood\ flow - permeation\ into\ hepatocytes + desorption\ from\ bound\ state$$

For the bound chemical in blood the mass balance in words is:

$$bound\ chemical\ in\ blood\ over\ time = transport\ via\ blood\ flow - desorption\ from\ bound\ state$$

And for the chemical in the hepatocytes:

$$total\ chemical\ in\ the\ hepatocytes\ over\ time = permeation\ into\ hepatocytes - removal\ via\ biotransformation$$

The corresponding mathematic formulation of the mass balances can be found in SI section 1.

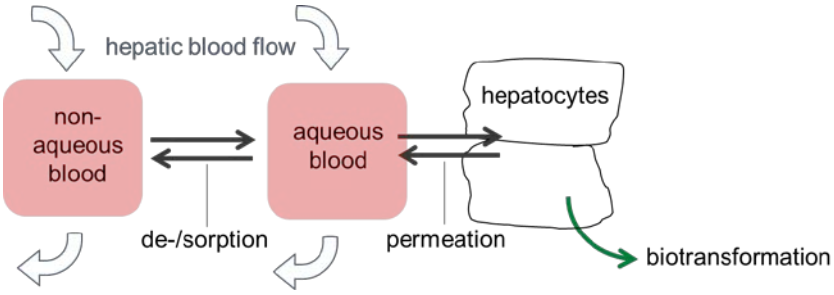

Figure 1: Conceptual overview of the model used to evaluate the impact of desorption and permeation kinetics on hepatic biotransformation.

Combination of the mass balances allows derivation of the hepatic extraction efficiency. The blood clearance CL_{blood} (in $mL_{blood}/h/kg_{fish}$) can be derived from the hepatic extraction efficiency E and the hepatic blood flow rate Q ($mL_{blood}/h/kg_{fish}$) as

$$CL_{blood} = Q * E \quad (1)$$

In the calculations, we increase complexity stepwise, meaning that we first evaluate the impact of permeation kinetics and, in a second step, consider desorption kinetics additionally. The purpose of this procedure is to be able to distinguish the impact of both parameters on the predicted clearances.

Input parameters

A table (Table **1**) of all input parameters can be found at the end of this section. In the following, details on the estimation of the input parameters are given.

Physiological data

Hepatic blood flow is calculated from the temperature adjusted cardiac output using the algorithm from Erickson and McKim (Erickson and McKim, 1990; Nichols et al., 2013). The bodyweight-normalized hepatocyte volume and exchange surface area between the liver sinusoids and hepatocytes are estimated using data from the literature: To calculate the hepatocyte volume, the hepatocellularity L_{hep} (510 * 10^6 hepatocytes/g_{liver}) (Nichols et al., 2013) is multiplied with the fractional liver weight (0.015 g_{liver}/g_{fish}) (Schultz and Hayton, 1999) and the volume of one single hepatocyte (calculated from diameter of 0.001 cm (Arnold et al., 1995), spherical shape assumed).The exchange surface area between hepatic sinusoids and hepatocytes can be calculated from the sinusoid diameter (0.001 cm, assumed) and the total sinusoid volume.

The total sinusoidal blood volume can either be estimated using the Krogh cylinder model (Krogh, 2010 (originally published in 1922)) or using volume fractions provided in the literature. Using the Krogh model yields a value that is roughly three times lower than the value calculated using volume fraction information. We thus use the Krogh model approach because a lower sinusoidal volume is worst case for the evaluation of desorption and permeation limitation due to the lower resulting exchange surface area and shorter residence time of blood in the sinusoids.

Partition data and biotransformation kinetics

Information on the partition properties of the compound is required in form of the blood-water, and hepatocyte-water partition coefficient of the compound. We derive these partition coefficients using a log K_{OW} based approach analogous to the one presented by Lee et al. (Lee et al., 2017) and Saunders et al. (Saunders et al., 2020). Note that for single chemicals, the required partition coefficients could also be calculated using poly-parameter free energy relationships (Endo et al., 2013) (ppLFERs), which in our opinion yields more accurate results. For the here presented general evaluation, however, this approach is not applicable.

To select a realistic range of hepatocyte biotransformation rate constants, we relied on *in vitro* biotransformation data for fish (Halder et al., 2018). These *in vitro* data can be extrapolated to the required hepatocyte biotransformation rate constants (Krause and Goss, 2018b). A recent

paper shows that the hepatocyte biotransformation rate constants for hydrophobic chemicals happen to be roughly equal to the *in vitro* measured rate constants without further scaling (Krause and Goss, 2020). The majority of the published *in vitro* rate constants are in the range of 0.1 /h to 10 /h, we thus use this range for the required hepatocyte biotransformation rate constants in the model.

Permeabilities and facilitated transport

To characterize the effect of permeation kinetics, we first estimate the actual value of the permeability: For hydrophobic compounds with octanol-water partition coefficients log K_{OW} > 1, the permeation kinetics can be assumed to be limited by the compound's diffusion through the aqueous boundary layer (ABL) adjacent to the cellular membranes and not by the membrane itself (Bittermann and Goss, 2017). The thickness of the ABL that needs to be crossed is thus an important parameter for the expected permeability. The thicknesses of *in vivo*-ABLs are difficult to determine and experimental data are thus scarce, but according to Avdeef (Avdeef, 2005) there is evidence that a value of 0.5 µm is realistic for small blood capillaries such as those found in the liver. Based on this value, the corresponding permeability can be estimated with the solubility-diffusion model (Avdeef, 2001; Bittermann and Goss, 2017). This approach assumes that the permeability of a chemical through a barrier is determined by the diffusion coefficient of the chemical in the barrier, the partition coefficient between barrier and water and the thickness of the barrier. The estimation yielded a value of 0.15 cm/s for all hydrophobic chemicals with log K_{OW} > 1. This value was thus used as default value for the calculations.

For scenarios in which permeation through an ABL is a limiting factor, facilitated transport can become crucial. Facilitated transport means that not only the freely dissolved chemical can overcome the aqueous diffusional barrier but also the chemical that is bound to certain 'carriers'. In blood, transport proteins like albumin are known to be typical carriers. According to the literature (Manera and Britti, 2006), rainbow trout blood also contains albumin or albumin-like proteins, we thus assume that transport facilitation via these proteins through an ABL is possible. To account for this facilitated transport, we use an approach analogous to the one from Kramer et al. (Kramer et al., 2007) (for details see SI): we calculate a so called 'facilitated transport ratio' (FTR) that reflects how much more chemical is transported through the ABL in presence of albumin per time unit. This FTR can then be multiplied with the given ABL permeability to yield an accelerated permeability that accounts for facilitated transport through the ABL by albumin. Other plasma proteins could also serve as potential carriers facilitating permeation through ABLs. By focusing on facilitation via albumin only, the estimated facilitation effect is a worst-case estimate. For calculation of the FTR the following input data are used: the required albumin concentration in rainbow trout blood in L/L can be calculated from the plasma concentration of 13.8 g/L (Manera and Britti, 2006), the albumin density of 1.4

189 g/cm³ (Endo and Goss, 2011) and the information that roughly 70 % of the total blood volume
190 is plasma volume (Gingerich and Pityer, 1989). The albumin-water partition coefficient for a
191 chemical is also derived from an empirical correlation with the K_{OW} that was presented by Endo
192 and Goss (Endo and Goss, 2011). Note that this correlation was originally developed to
193 describe partitioning into bovine serum albumin, but due to the lack of more suitable data we
194 still use this correlation to predict the partition data for fish albumin. The diffusion coefficient of
195 albumin in water ($3.6 * 10^{-7}$ cm²/s) is known from the literature (Wakeham et al., 1976).
196
197 For comparison, we also used experimental permeability values that were recently published
198 by Schug et al. (Schug et al., 2019) for intestinal cells in a study with fragrance molecules with
199 log K_{OW} values ranging from 2.17 to 6.25. The values from Schug et al., reported as intrinsic
200 membrane permeabilities, are notably smaller than the above predicted 0.15 cm/s. Schug et
201 al. interpret the significant differences such that in their experiments not the ABL is the
202 dominating resistance, but instead interactions with the cells constitute the main barrier (and
203 ABL transport resistance is implicitly contained in their values). The lowest value is found for
204 the most hydrophobic chemical in the dataset published by Schug et al. and amounts to 1.93
205 $* 10^{-4}$ cm/h (i.e. $5.4 * 10^{-8}$ cm/s). Here we select this lowest reported value from the Schug
206 dataset as an alternative permeability input for our simulations because this comparison is the
207 most informative.
208
209 Table 1: Required input parameters.

Parameter	value	equation/reference
liver blood flow Q [mL/h/kg$_{fish}$]	758.3	= 0.259 * (((0.23 * T) - 0.78) * (m_{body}/500)$^{-0.1}$) * 1000
hepatocyte volume in the liver $V_{hepliver}$ [mL$_{hep}$/kg$_{fish}$]	9.4	= L_{hep} * (L_{FBW} * 1000) * $V_{singlehep}$
sinusoid volume in the liver $V_{sinliver}$ [mL$_{sin}$/kg$_{fish}$]	0.3	estimated, see text
exchange surface sinusoids-hepatocytes A [cm²/kg$_{fish}$]	4369	= $V_{sinliver}$ / (d_{sin} / 4)
hepatocyte biotransformation rate constant k_{hep} [1/h]	0.1 - 10	generic range of values
permeability P_{hep} [cm/s]	0.15	estimated, see text
octanol-water partition coefficient log $K_{octanol/w}$ [L_w/$L_{octanol}$]	1 - 7	assumed
chemical diffusion coefficient in water [cm²/s]	10^{-6}	typical order of magnitude

albumin diffusion coefficient in water [cm²/s]	$3.6 * 10^{-7}$	(Wakeham et al., 1976)
The required partition coefficients are calculated according to:		
blood-water partition coefficient $K_{blood/w}$ [L_w/L_{blood}]	$= lipid_{blood} * K_{OW} + albumin_{blood} * K_{albumin/w} + protein_{blood} * 0.05 * K_{OW} + w_{blood}$	
hepatocyte-water partition coefficient $K_{hepatocyte/w}$ [$L_w/L_{hepatocyte}$]	$= lipid_{hepatocyte} * K_{OW} + protein_{hepatocyte} * 0.05 * K_{OW} + w_{hepatocyte}$	
albumin-water partition coefficient $K_{albumin/w}$ [$L_w/L_{albumin}$]	$= 10^{(0.71 * \log K_{OW} + 0.4)}$	

Results & discussion

Simplified scenario: Impact of permeability

To investigate the impact of permeability on hepatic biotransformation, we first combine the mechanistically derived permeability of 0.15 cm/s with fast biotransformation kinetics (k_{hep} = 10 1/h) and different partition coefficients at a typical blood flow rate (758 mL/h/kg_{fish} for a 10 g fish). The resulting blood clearances are represented by the dark blue line in Figure 1. For comparison, we also calculate the blood clearance under consideration of blood flow limitation only (orange line) and the blood clearance considering flow limitation and permeability but neglecting facilitated transport (light blue line).

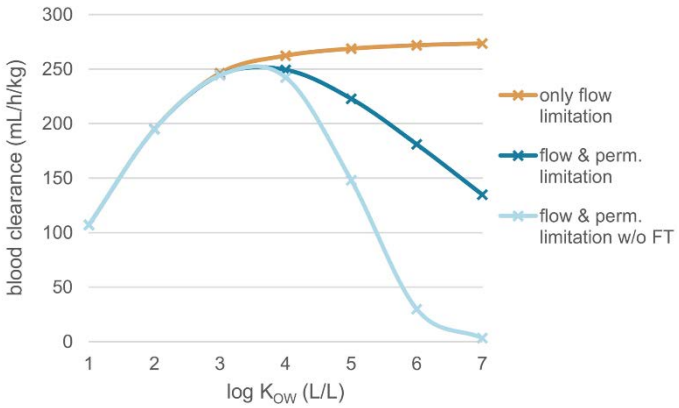

Figure 2: Blood clearances are calculated under consideration of blood flow limitation only (orange line) and under consideration of flow limitation and permeation limitation (with P_{hep} = 0.15 cm/s) including facilitated transport (dark blue line). For comparison, blood clearances resulting when flow limitation and permeation are considered but facilitated transport (FT) is neglected, are also shown (light blue line).

Figure 1 shows the blood clearances as a function of log K_{OW}. In the lower K_{OW} range (log K_{OW} 1 – 3), nearly identical blood clearances result for the three scenarios indicated by overlapping lines. This means that the permeability of 0.15 cm/s used in the calculations is fast enough to avoid any limiting effects on clearance of low K_{OW} chemicals. With increasing K_{OW}, however,

notably lower clearances are calculated for the scenario considering flow limitation and permeation limitation (including facilitation) than for the scenario that considers blood flow limitation only. Even lower clearances result when flow limitation and permeation limitation are considered but facilitation is neglected: The calculated blood clearances considering flow limitation and permeation limitation including facilitation are decreased by factor 2 to 3 maximum compared to those calculated considering blood flow limitation only, while the clearances calculated without consideration of facilitated transport are up to 80 times lower than those calculated considering blood flow limitation only. This illustrates the importance of facilitated transport for high log K_{OW} compounds that can occur when transport through an aqueous layer is dominating cross cellular transport.

The reason why the K_{OW} of a chemical is critical for the presented results is somewhat complex: In the calculations, a constant biotransformation rate constant of k_{hep} = 10 1/h was assumed over the whole range of K_{OW}. Assuming a constant k_{hep} for different K_{OW} actually reflects different intrinsic biotransformation capacities of freely dissolved chemical, because a much higher freely dissolved chemical clearance is required to yield a hepatocyte biotransformation rate constant of 10 1/h for a chemical with log K_{OW} = 6 compared to the freely dissolved chemical clearance that would be needed to yield the same hepatocyte biotransformation rate constant for a low K_{OW} chemical. As soon as this freely dissolved chemical clearance becomes faster than the permeation kinetics, limitations due to slow permeation result and the dark and light blue line diverge from the orange line that does not account for permeability.

We also performed these calculations for hepatocyte biotransformation rate constants of 0.1 1/h and 1 1/h (plots are shown in the SI). For these cases, the impact of permeability is less pronounced. The reason for this is that limitations due to permeation kinetics become less important when biotransformation itself is slower. Consequently, the data shown in Figure 1 already represent an extreme scenario with regard to the parameter combinations used.

In a second set of calculations we use a much lower membrane permeability of $5.4 * 10^{-8}$ cm/s from the literature as discussed above (Schug et al., 2019). For comparison, these results are also plotted together with the *in vivo* blood clearance that considers only blood flow limitation (Figure 2, note the logarithmic y-axis as compared to Figure 1). Note that facilitated transport is not considered here, because according to Schug et al. the ABL is not the limiting resistance for these permeabilities.

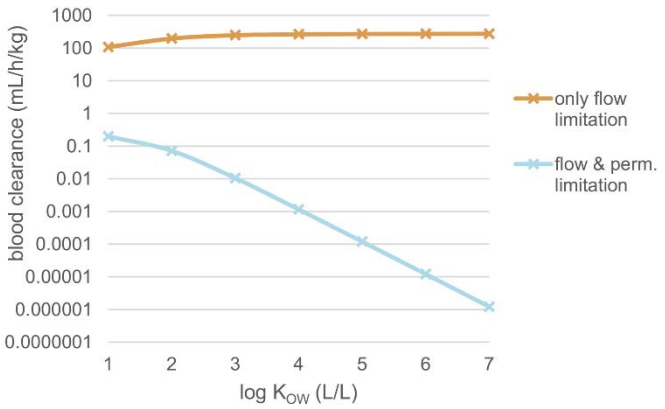

Figure 3: Blood clearances calculated under consideration of flow limitation only (orange line) and flow limitation combined with permeation limitation (with P_{hep} = 5.4 * 10^{-8} cm/s) (light blue line).

With this much lower permeability, the resulting *in vivo* blood clearances considering permeation limitation are orders of magnitude (500 to 10^8 times) lower than those presented in Figure 1. These huge differences between the calculations with a permeability of 0.15 cm/s and a permeability of 5.4 * 10^{-8} cm/s highlight the relevance of reliable permeability information. It seems possible that the permeability values published by Schug et al. suffer from an overparametrization in the multi-parameter model used for their data evaluation. In addition, the stirring rates applied in the experimental set-up by Schug et al. are low and according to the literature (Avdeef, 2012; Karlsson and Artursson, 1991; Korjamo et al., 2009; Korjamo et al., 2008) significant transport limitation due to a thick ABL (probably around 1000 um) should be expected different from what Schug et al. assumed. In fact, the *in vivo* blood clearances resulting from the calculation with a permeability of 5.4 * 10^{-8} cm/s are so low that biotransformation becomes negligible under virtually all circumstances for all chemicals with log K_{OW} > 1. If this was the case all existing IVIVE results would have been massively distorted towards an overestimated in vivo biotransformation. This is not what is seen when IVIVE results are compared with in vivo bioaccumulation studies.

The current weight of evidence thus indicates that the estimated permeability value of 0.15 cm/s is more realistic for hydrophobic chemicals. Hence, we suggest that consideration of permeation kinetics is not needed for prediction of *in vivo* clearance because even for the extreme combination with a fast biotransformation rate constant k_{hep} = 10 1/h in Figure 1, predicted clearances change only by factor 2 – 3 depending on permeation..

<u>Scenario with permeation and desorption</u>

For evaluation of the combined effect of permeation and desorption, we use a desorption rate constant of 0.1 1/s as the lowest desorption rate constant measured for fish plasma [Ref submitted manuscript]. For these calculations we use two biotransformation rate constants of

(10 1/h and 1/h), a fixed permeability of 0.15 cm/s, a typical blood flow rate and a range of partition coefficients.

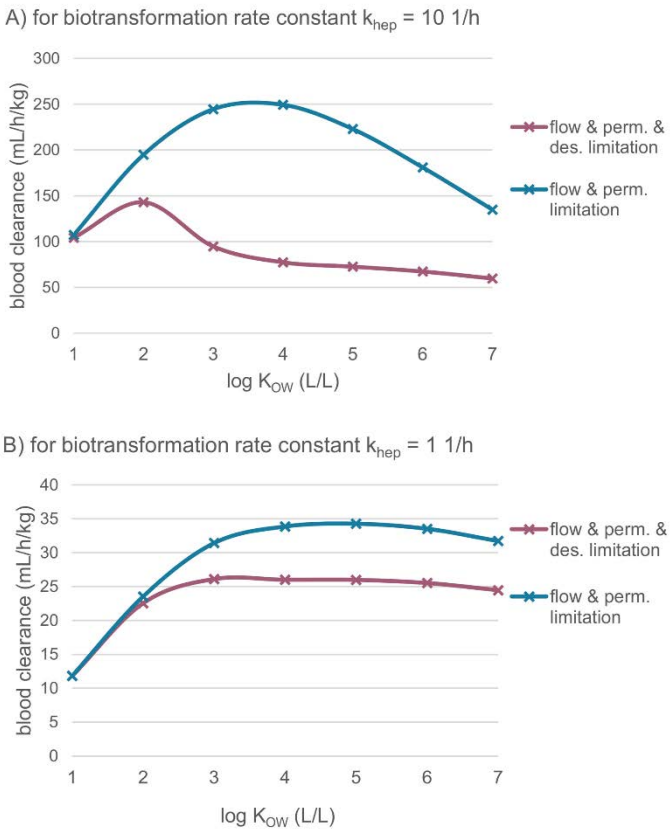

Figure 4: Blood clearances calculated under consideration of blood flow limitation and permeation limitation (with P_{hep} = 0.15 cm/s) including facilitated transport (dark blue lines) and blood clearances calculated under consideration of blood flow limitation, permeation limitation (including facilitated transport) and desorption limitation (purple lines). Panels A) and B) represent differences regarding the used hepatic biotransformation rate constant (k_{hep} = 10 1/h in panel A and k_{hep} = 1 1/h in panel B).

The differently colored lines in Figure 3 again represent scenarios differing in the considered limitations: The dark blue line represents a scenario considering blood flow limitation and permeation limitation including facilitation as reference (same as in Figure 1). The purple line shows the results with blood flow limitation, permeation limitation (including facilitation) and additionally desorption limitation. The upper panel of Figure 3 represents calculations with a fast biotransformation rate constant of k_{hep} = 10 1/h. In this case, the consideration of desorption kinetics leads to blood clearances decreased by up to a factor 3 for chemicals with log K_{OW} > 2 compared to the scenario neglecting desorption kinetics. The impact of desorption kinetics is related to the hydrophobicity of the chemical because a larger fraction of high K_{OW} chemicals is bound in blood and only this bound fraction is sensitive to slow desorption kinetics. In case of a lower biotransformation rate constant of k_{hep} = 1 1/h, the impact of desorption kinetics on the calculated blood clearance decreases (lower panel of Figure 3): the maximum difference in the calculated blood clearance is now less than factor 2. This illustrates that the

relevance of the desorption kinetics depends strongly on how fast the hepatic biotransformation is. In the above example with k_{hep} = 10 1/h (upper panel of Figure 3), a rapid biotransformation is combined with a very slow desorption, so that this scenario again represents an extreme parameter combination. For less extreme and, by that, more likely combinations, the impact of desorption kinetics on blood clearance will be smaller.

As mentioned above, we use a permeability of 0.15 cm/s for the calculations evaluating the combined effect of desorption and permeation. In case we use the notably smaller permeability (P_{hep} = 5.4 * 10^{-8} cm/s) for this evaluation, no additional effects due to desorption kinetics can be observed. The reason for this is that, in this case, the very slow permeation is the dominating factor of the two subsequent limitations.

Conclusion

The presented analysis shows that notable errors in the prediction of *in vivo* clearance from given *in vitro* biotransformation data due to the neglect of permeation and desorption kinetics are possible. Especially permeability could be a strong limitation depending on the used permeability value. Recently published permeabilities result in a tremendously decreased *in vivo* blood clearance while other permeability values (that we believe to be more reliable) have only little impact on the predicted *in vivo* blood clearance. Clarifying this permeability issue is thus of high priority. Furthermore, desorption kinetics appear to be of minor relevance.

Acknowledgements

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