# How to scale clearance from adults to children for drugs undergoing hepatic metabolism? Insights from advanced PBPK modelling and simulation

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# Chapter 6

## Scaling drug clearance from adults to the young children for drugs undergoing hepatic metabolism: Striving for a simple solution

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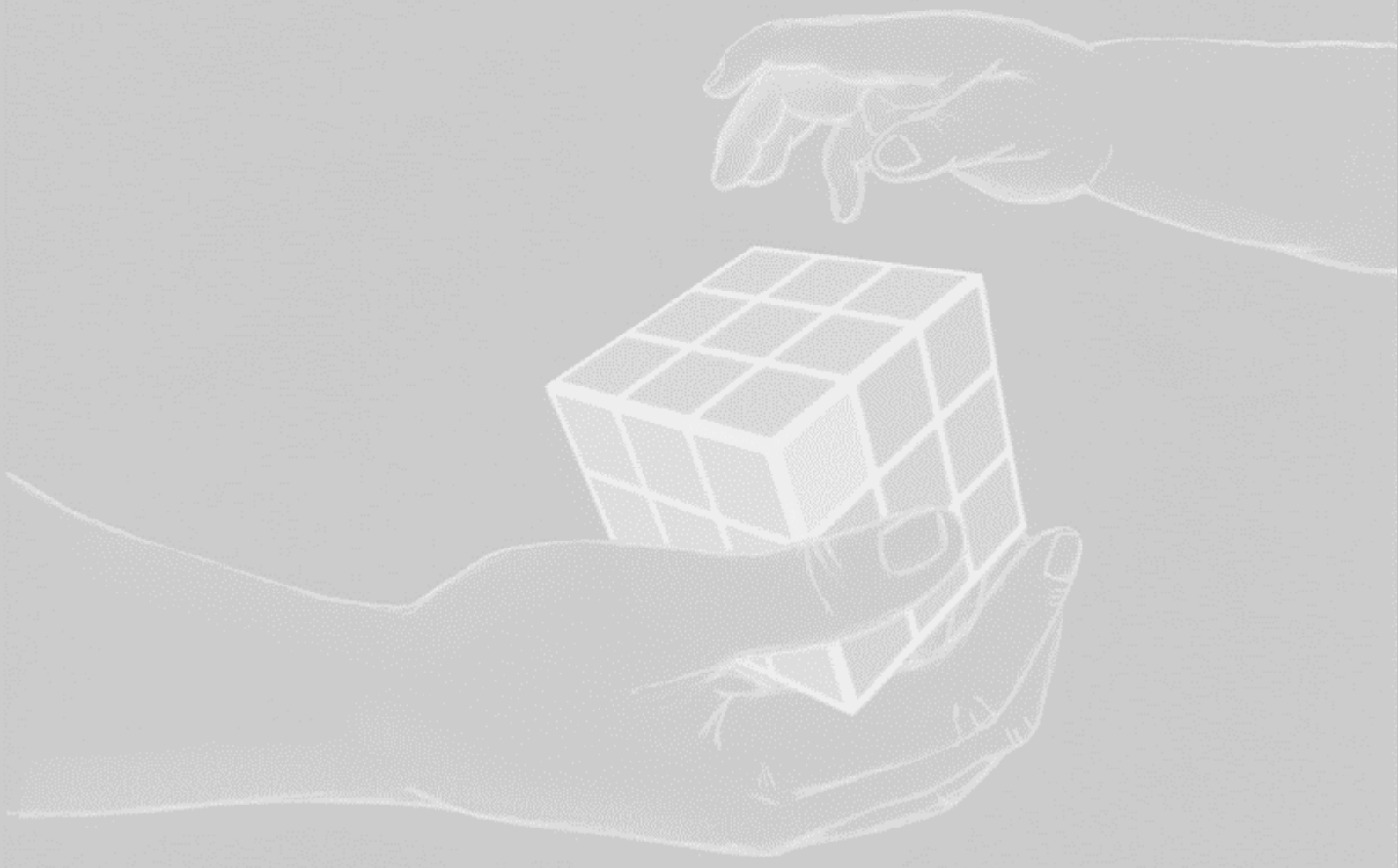

Submitted

**Abstract**

In drug development, accurate scaling of drug clearance from adults to young children is important. Previous research showed that, for very young children, scaling based on bodyweight alone is not accurate for all hepatically cleared drugs. The aim of this study was to systematically assess, for drugs undergoing hepatic metabolism in children younger than five years, the accuracy of two scaling methods that in addition to bodyweight also take age-based variables into account. These two methods comprise scaling with: (1) a bodyweight-based function using an age-dependent exponent (ADE), and (2) a body weight-based function with fixed exponent of 0.75 (AS0.75) combined with isoenzyme maturation functions ($MF_{PBPK}$) similar to those implemented in physiologically-based pharmacokinetic (PBPK) models (AS0.75+$MF_{PBPK}$). A PBPK-based simulation workflow was used that included hypothetical drugs with a wide range of properties and metabolized by different isoenzymes for seven typical children between one day and four years of age. We found that isoenzyme maturation is an important driver of developmental changes in clearance in all children younger than five years, which ultimately prevents the systematic accuracy of ADE scaling. AS0.75+$MF_{PBPK}$, when accounting for isoenzyme maturation and maturation in microsomal protein per gram of liver (MPPGL), was found to accurately scale clearance for all low and intermediate extraction ratio drugs except for drugs binding to alpha-1-acid glycoprotein in neonates. No other simple and generalizable scaling method was found to accurately predict paediatric clearance for other drugs, due to the wide variations of the impact of drug properties on clearance ontogeny.

## 6.1 Introduction

Accurate scaling of drug plasma clearance (CLp) from adults to children is important for the definition of first-in-child doses and hence robust study design involving younger children. To date, physiologically-based pharmacokinetic (PBPK) models represent the most mechanistic method to scale CLp across the paediatric age range due to considerations given to biological changes (maturation) of drug metabolising enzymes. PBPK models quantify the interactions between drug-specific and system-specific parameters and predict paediatric CLp by accounting for developmental changes in the system-specific parameters and how they impact drugs with specific properties. Application of these models is considered best practice in pharmaceutical industry, but obtaining PBPK ontogeny functions for a given drug, is time-consuming and complex due to the requirement of a wide range of drug-specific and system-specific information. Moreover, all this information may not always be available for each drug or each population. This leads to a need for simplified scaling functions which are more convenient for defining paediatric CLp in pharmacometrics. As multiple system-specific parameters may change in the paediatric population and as the impact of each of these changes on paediatric CLp may be different for each given drug with different characteristics, the challenge in developing simplified scaling functions is to aggregate all relevant information in functions with a limited number of scaling variables. Various simplified clearance scaling methods for the paediatric population have been proposed. Allometric scaling using a fixed exponent of 0.75 (AS0.75) is one of the simplest scaling methods, as it only uses bodyweight as scaling variable. However, it has been shown to lead to large over-predictions of CLp in children younger than 5 years, especially when isoenzymes are immature [1,2].

As scaling based on bodyweight alone was found not to lead to systematic accurate scaling, other proposed scaling functions that rely on the use of additional age-based variables are of interest. Mahmood *et al*. have proposed the age-dependent exponent method (ADE) that was found to outperform AS0.75 in young children [1,3]. ADE relies on the use of an allometric equation with exponents of 1.1, 1.0, and 0.9 for ages 0 (term neonates)–3 months, > 3 months–2 years, and > 2–5years respectively for all drugs as most recently reported [3]. While this method is claimed to be applicable to any drug irrespective of their elimination route, inaccurate scaling can be anticipated for some drugs undergoing hepatic metabolism, since this method does not account for the differences in isoenzyme maturation, which are known to vary greatly [4].

Another proposed scaling method uses AS0.75 together with isoenzyme maturation functions that are similar to those implemented in PBPK models (AS0.75+$MF_{PBPK}$) [4]. In addition to bodyweight, this method also requires information on the fraction of the drug metabolized by each isoenzyme in adults, as well as on isoenzymes maturation. This method does not explicitly account for maturation in haematocrit and plasma proteins abundance. Based on data of five CYP3A substrates, AS0.75+$MF_{PBPK}$ was found to be accurate in children older than 3 months, but could lead to inaccurate predictions in younger children for some drugs [4].

While ADE and AS0.75+$MF_{PBPK}$ represent potentially viable options to accurately scale clearance in children under five years of age [1,3,4], no systematic investigation of their accuracy has been undertaken. The aim of this study was therefore to systematically assess the accuracy of paediatric CLp scaling with ADE and AS0.75+$MF_{PBPK}$ in children younger than five years for drugs undergoing hepatic metabolism that are not substrates for transporters, to identify drug properties that are predictive for accurate scaling with these methods. This was performed using a previously developed PBPK-based simulation workflow [5]. In this workflow, hypothetical drugs that are substrates for common hepatic isoenzymes are generated, covering the entire potential drug parameter space. PBPK modelling principles are used to obtain 'true' CLp values for all hypothetical drugs in adults and children of various ages. Subsequently CLp values scaled from 'true' adult values to paediatric values with ADE and AS0.75+$MF_{PBPK}$ are compared to 'true' CLp values in children, and drug properties that lead to systematically accurate scaling in various ages are identified.

## 6.2 Methodology

A PBPK-based simulation workflow was used [5] that was running in R (a software environment for statistical computing and graphics) version 3.3.1 with R studio interface version 0.99.902 [6]. In this workflow, 'true' adult and paediatric CLp values for hypothetical drugs with a wide range of properties that are substrates for known hepatic metabolic enzymes were generated using PBPK-based simulations, based on the dispersion model for hepatic metabolic CLp [7,8]. This model was selected as it has been reported to better predict CLp than the well-stirred model for drugs with a high extraction ratio, while both models lead to equivalent CLp prediction for other drugs [7,9]. Subsequently, the accuracy of

scaling the 'true' adult CLp values to paediatric CLp values with the two scaling methods was assessed, by comparing CLp values scaled by ADE and AS0.75+$MF_{PBPK}$ to 'true' paediatric CLp values.

### 6.2.1 PBPK simulation workflow

#### *6.2.1.1 Hypothetical drugs*

A total of 84,000 hypothetical drugs were generated, with all possible combinations of values for the following three drug-specific variables.

Plasma protein binding: The hypothetical drugs were assumed to exclusively bind to either human serum albumin (HSA) or alpha-1 acid glycoprotein (AAG). The unbound drug fraction in plasma (fu) in adults ranged from 1% to 100%, with 8 equidistant intermediate values. Equations by Rodgers and Rowland [10] were used to derive the affinity to plasma proteins from the fu and the concentration of the binding proteins in plasma in adults [11]. The affinity to plasma proteins in adults was assumed to remain constant with age.

Blood-to-plasma partition coefficient (Kp): Kp values of 0.35, 0.8, and values from 1 to 40 with 38 intermediate equidistant values were selected, reflecting different extents of drug diffusion into the red blood cells [12,13]. Kp was assumed to not change with age.

Total unbound intrinsic clearance value of one microgram of liver microsomes ($CL_{int,mic}$): Total $CL_{int,mic}$ ranged between $0.56 \cdot 10^{-6}$ and $0.209 \cdot 10^{-3}$ $mL.min^{-1}.\mu g^{-1}$ microsomal protein in adults [14], with 98 equidistant intermediate values. These different values reflect difference in both affinities for and abundances of isoenzymes.

#### *6.2.1.2 System-specific variables*

The assessment of the accuracy of the two scaling methods was performed in seven typical paediatric individuals including term neonates of one or fifteen days, infants of one month, six months or one year and children of two or four years. CLps were scaled from adult values in a typical twenty-five-year-old. The demographic and system-specific parameters of the PBPK model for these typical individuals can be found in Appendix 1.

For each investigated paediatric age, isoenzyme maturation ($CL_{int,mic}$ maturation) was implemented as a near continuous variable. To do so, first a realistic range of isoenzyme

maturation was defined for each age by taking the maximum and minimum isoenzyme maturation value reported for 14 isoenzymes from the Simcyp® library. For SULT1A1, maturity was taken to have been reached at birth [15]. A minimum limit of 5% isoenzyme maturation was set. Then intermediate values across these ranges were taken with 1% increments, to allow for the investigation of clearance maturation of drug metabolized to different extents by all possible combinations of multiple isoenzymes, an important feature since most drugs are metabolized by several isoenzymes.

### 6.2.2 Computations

#### *6.2.2.1 Step 1: 'True' CLp*

For each hypothetical drug, 'true' CLp values for the typical adult and children were generated. In children, 'true' CLp values were generated for each hypothetical drug and each isoenzyme maturation value defined for that age. Details can be found in Appendix 1.

For each paediatric age, 'true' relative paediatric CLps were computed as in equation 1, reflecting 'true' paediatric CLp as a percentage of 'true' adult CLp:

$$\text{'true' relative paediatric CLp} = \frac{\text{'true' paediatric CLp}}{\text{'true' adult CLp}} \times 100 \quad (1)$$

#### *6.2.2.2 Step 2: CLp scaling*

First, for each hypothetical drug and for each of the different percentages of isoenzyme maturation defined for each age, the 'true' adult CLp values from step 1 were scaled to each typical paediatric individual using ADE and AS0.75+$MF_{PBPK}$ scaling functions according to equation 2 and 3 respectively.

$$\text{ADE} - \text{based paediatric CLp} = \text{'true' adult CLp x } \left(\frac{\text{BWpaediatric)}}{\text{BWadult}}\right)^{\text{ADE}} \quad (2)$$

$$\text{AS0.75} + \text{MF}_{\text{PBPK}} - \text{based paediatric CLp} = \text{'true' adult CLp x } \left(\frac{\text{BWpaediatric)}}{\text{BWadult}}\right)^{0.75} \times \text{MF}_{\text{PBPK}} \quad (3)$$

In these equations, BW stands for bodyweight, ADE equals 1.1, 1.0, and 0.9, for ages 0 (term neonate)–3 months, >3 months–2 years, and >2–5 years respectively [3], and $MF_{PBPK}$ corresponds to the different percentages of isoenzyme maturation defined for each age, as also used in the PBPK model for the generation of 'true' relative paediatric CLps (see Appendix 1).

In literature there are two different interpretations of $MF_{PBPK}$ in use and both were investigated in this work. $MF_{PBPK}$ was either expressed as percentage of adult unbound intrinsic clearance per gram of liver ($MF_{PBPK\text{-}liver}$), which accounts for maturation in both isoenzyme activity and microsomal protein per gram of liver (MPPGL) or $MF_{PBPK}$ was expressed as percentage of adult unbound intrinsic clearance per microgram of microsomes ($MF_{PBPK\text{-}microsomes}$), which only accounts for maturation of isoenzyme activity. Therefore, for $MF_{PBPK\text{-}liver}$, maturation in MPPGL as implemented in the PBPK model for the generation of 'true' relative paediatric CLps was also used.

For comparative purposes, equation 4 was used to calculate the exponent that, in the allometric equation of the ADE method, would yield perfect scaling of 'true' adult hepatic metabolic CLp to 'true' paediatric hepatic metabolic CLp.

$$\text{'true' EXP} = \frac{\ln(\text{'true' relative paediatric CLp})}{\ln(\frac{\text{BWpaediatric}}{\text{BWadult}})} \quad (4)$$

#### *6.2.2.3 Step 3: Assessment of CLp scaling accuracy*

For each drug and percentage of isoenzyme maturation in each paediatric age, the accuracy for both ADE and AS0.75+$MF_{PBPK}$-based CLp scaling was numerically assessed using the prediction error (PE). PE was computed for each 'true' paediatric CLp generated in step 1 and its corresponding scaled value in step 2 using equation 5.

$$\text{PE (\%)} = \frac{\text{scaled CLp} - \text{'true' paediatric CLp}}{\text{'true' paediatric CLp}} \times 100 \quad (5)$$

For each paediatric age and investigated percentage of isoenzyme maturation, the scaling performance of both methods was visually assessed in plots of 'true' and scaled relative paediatric CLp values. The scaling accuracies were also compared to scaling accuracy of AS0.75. Analogue to previous systematic assessments of simplified scaling methods, accurate CLp scaling was defined as scaled values having a PE within ±30% [2,5,16].

#### *6.2.2.4 Step 4: Drug properties predictive for accurate scaling*

To define scenarios in which each scaling method systematically yields accurate paediatric CLp values, the combined impact of plasma protein binding to HSA or AAG and diffusion in red blood cells was assessed using the following drug categorization:

- drugs not influenced by plasma protein maturation (fu =1) or haematocrit maturation (Kp=1)

- all hypothetical drugs binding to HSA, including drugs with fu=1

- all hypothetical drugs binding to AAG, including drugs with fu=1

These categories were then further subcategorized based on the extraction ratio (ER) as having either a low (ER $\leq$ 0.3), intermediate (0.3 < ER $\leq$ 0.7) or high (ER > 0.7) ER in adults.

## 6.3 Results

### 6.3.1 CLp scaling accuracy

Table 1 provides for each age the range of 'true' relative paediatric CLp values as well as the range of PE obtained when scaling hepatic metabolic CLp with ADE, AS0.75+$MF_{PBPK\text{-}liver}$ and AS0.75+$MF_{PBPK\text{-}microsomes}$. The table reflects that 'true' relative paediatric CLp values in children of the same age may vary with both isoenzyme maturation and drug properties, while relative paediatric CLp values in children of the same age scaled using ADE or AS0.75+$MF_{PBPK}$ do not vary with drug properties nor, for ADE only, with isoenzyme maturation. For comparative purposes, PE values upon AS0.75 scaling are provided as well (Table 1).

ADE, AS0.75+$MF_{PBPK\_liver}$ and AS0.75+$MF_{PBPK\_microsomes}$ capture changes in 'true' CLp for part of the hypothetical drugs, as can be seen from the PE ranges which all include ±30% in each age for each of these scaling methods. However, each of these methods also leads to inaccurate paediatric CLp predictions for some other hypothetical drugs in each age, due to drug properties or isoenzyme maturation or a combination of both, resulting in PE ranges including values outside the range of ±30%. More specifically, scaling with ADE, AS0.75+$MF_{PBPK\_liver}$ and AS0.75+$MF_{PBPK\_microsomes}$ yields extreme PE values that on an absolute scale are at least 437%, 80%, or 77%, respectively, with higher values for lower age ranges (Table 1). While ADE was found to be less accurate than AS0.75+$MF_{PBPK}$, as shown by the increased PE range, this method yields a range of PEs that is greatly reduced as compared to the use of AS0.75.

***Table 1*** *Assessment of paediatric CLp scaling accuracy, expressed as prediction error, for different ages and 'true' relative paediatric CLp*

| Age | 'True' relative paediatric CLp [a] [range] | Prediction error [range] | | | |
|---|---|---|---|---|---|
| | | ADE [b] | AS0.75 + $MF_{PBPK\_liver}$ [c] | AS0.75 + $MF_{PBPK\_microsomes}$ [d] | AS0.75 [e] |
| One day | [0.26% - 13.3%] | [-74% - 1224%] | [-87% - 23%] | [-79% - 92%] | [-24% - 3745%] |
| Fifteen days | [0.29% - 8.14%] | [-54% - 1220%] | [-87% - 20%] | [-81% - 87%] | [31% - 3645%] |
| One month | [0.32% - 9.15%] | [-51% - 1305%] | [-87% - 22%] | [-80% - 89%] | [31% - 3679%] |
| Six months | [1.09% - 15.6%] | [-33% - 853%] | [-85% - 19%] | [-77% - 82%] | [18% - 1578%] |
| One year | [1.62% - 21.3%] | [-36% - 739%] | [-84% - 22%] | [-76% - 83%] | [5% - 1281%] |
| Two years | [2.67% - 29.3%] | [-42% - 536%] | [-83% - 22%] | [-75% - 77%] | [-10% - 890%] |
| Four years | [5.07% - 43.6%] | [-38% - 437%] | [-80% - 33%] | [-72% - 82%] | [-22% - 567%] |

[a] *Paediatric CLp expressed as percentage of adult value*
[b] *Age-dependent exponent*
[c] *Scaling using AS0.75 in combination with a maturation function expressed in percentage of adult unbound intrinsic clearance per gram of liver*
[d] *Scaling using AS0.75 in combination with a maturation function expressed in percentage of adult unbound intrinsic clearance per microgram of microsomes*
[e] *Allometric scaling using a fixed exponent of 0.75*

### 6.3.2 Impact of isoenzyme maturation on CLp scaling accuracy

Figure 1 and 2 compare for each investigated paediatric age and across their respective isoenzyme maturation range, the scaled relative paediatric CLp with a ±30% PE using respectively ADE or AS0.75+$MF_{PBPK}$ (solid and dotted black lines) versus the 'true' relative paediatric CLp (coloured areas) for all hypothetical drugs with different properties. The x-axis in Figures 1 and 2A displays isoenzyme maturation per gram of liver ($MF_{PBPK\_liver}$) which reflects both $MF_{PBPK\_microsomes}$ and maturation in MPPGL, while the x-axis of Figure 2B displays isoenzyme maturation per microgram microsomes ($MF_{PBPK\_microsomes}$).

Figure 1 shows that while ADE (solid and dotted black lines) can accurately scale hepatic metabolic CLp for some of the hypothetical drugs and for some percentages of isoenzyme maturation in each age, this scaling method can lead to a wide range of PEs due to the large variation in 'true' relative paediatric CLp values (coloured areas). Figure 1 also shows that for each typical paediatric individual, 'true' CLp values lower and higher than those predicted with ADE and a ±30% PE range are found, with over-predictions for the lowest isoenzyme maturation values and under-predictions for highest isoenzyme maturation values.

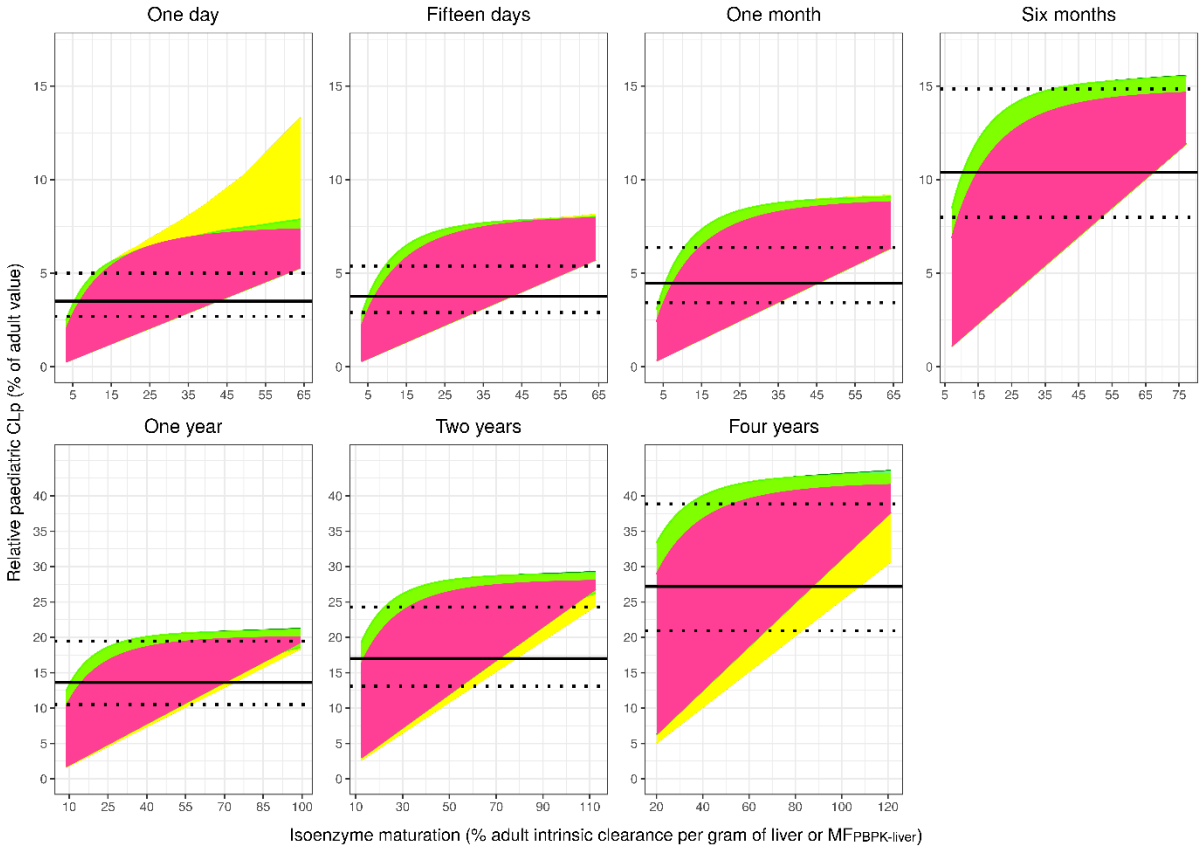


***Figure 1*** *Relative paediatric CLp (% of adult value) obtained with ADE scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green or yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Different colours represent hypothetical drugs with different properties, with pink representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). Green and yellow are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG, respectively, to different extents (including fu=1). Under the pink area, the pink, yellow, and green areas overlap completely, therefore the combination of pink and green areas shows the results for all drugs binding to HSA and the combination of pink, green and yellow areas shows the results for drugs binding to AAG. Note that the scales on the x- and y-axes may be different for different ages.*

Figure 2A shows that AS0.75+$MF_{PBPK\text{-}liver}$ does generally not lead to over-prediction of hepatic metabolic CLp in the studied age-range, but under-predictions may occur, especially when isoenzyme maturation is low. When enzyme maturation in this approach is expressed relative to adult intrinsic activity per microgram microsomes (AS0.75+$MF_{PBPK\text{-}microsomes}$), both over and under-prediction of paediatric CLp for different drugs are observed in all ages (Figure 2B).

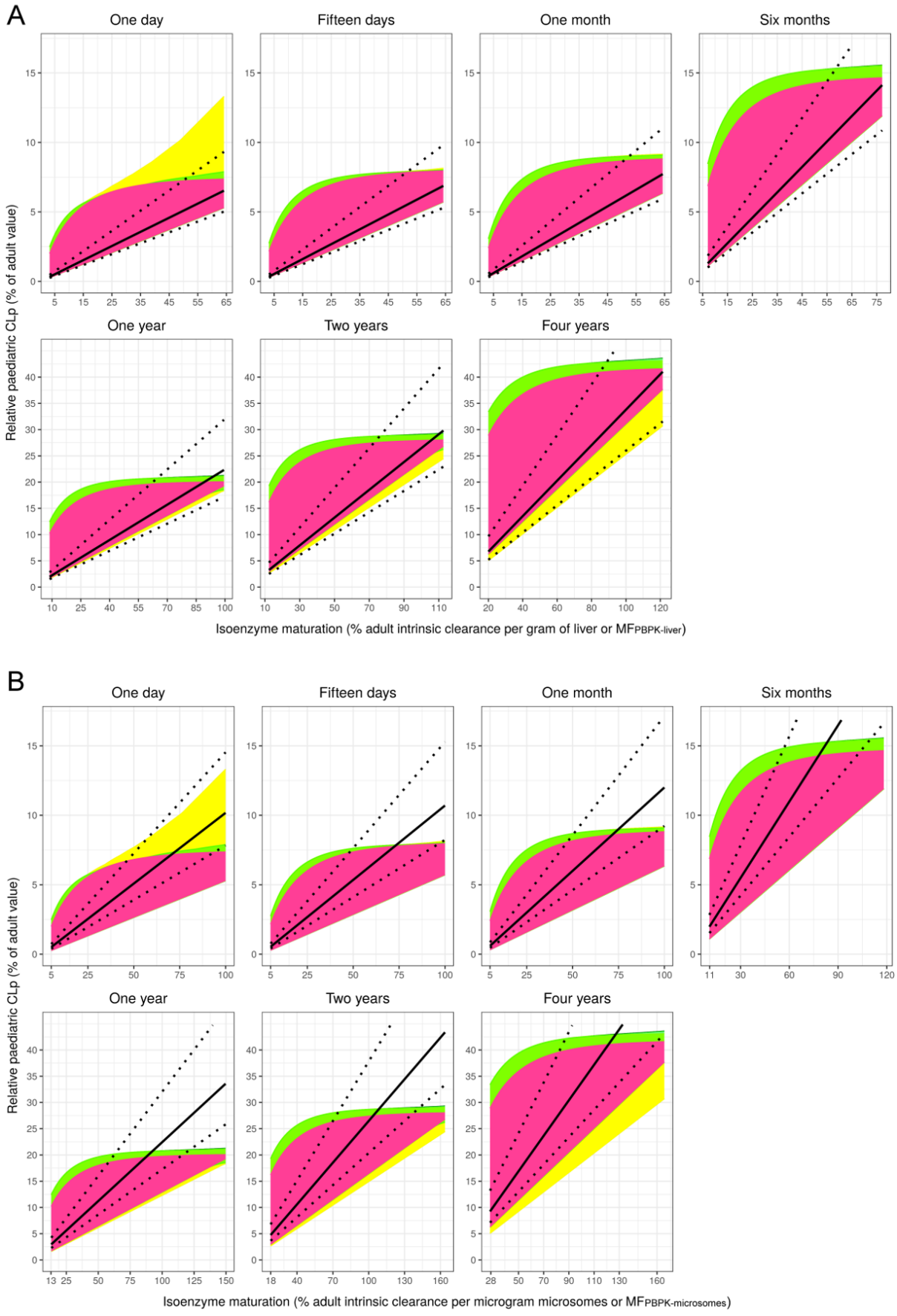


***Figure 2*** *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}liver}$ scaling (A) and AS0.75+MF$_{PBPK\text{-}microsomes}$ scaling (B) (solid black line with ± 30% PE as dotted*

*black lines) and 'true' relative paediatric CLp (pink, green and yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Different colours represent hypothetical drugs with different properties, with pink representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). Green and yellow are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG respectively to different extents (including fu=1). Under the pink area, the pink, yellow, and green areas overlap completely, therefore the combination of pink and green areas shows the results for all drugs binding to HSA and the combination of pink, green and yellow areas shows the results for drugs binding to AAG. Note that the scales on the x- and y-axes may be different for different ages.*

For all hypothetical drugs, it was determined what the 'true' allometric exponent would be if it was estimated in the typical paediatric patients within the respective isoenzyme maturation range. Figure 3 illustrates how the range of 'true' allometric exponent compares to the allometric exponent used in ADE scaling. High values of 'true' relative paediatric CLp will yield low values for the 'true' allometric exponent and therefore the reverse trends with isoenzyme maturation and drug properties can be observed in Figure 3 as compared to Figure 1. The 'true' allometric exponent varies considerably within each paediatric age ranging from 0.57 to 2.07 across all ages. Table 1 and Figure 3 show that changing the allometric exponent in the scaling function with age, as proposed with ADE scaling, will lead to an overall improved scaling for more hypothetical drugs, but it also illustrates that for each age it is unlikely that a single exponent will accurately scale hepatic metabolic CLp for all drugs.

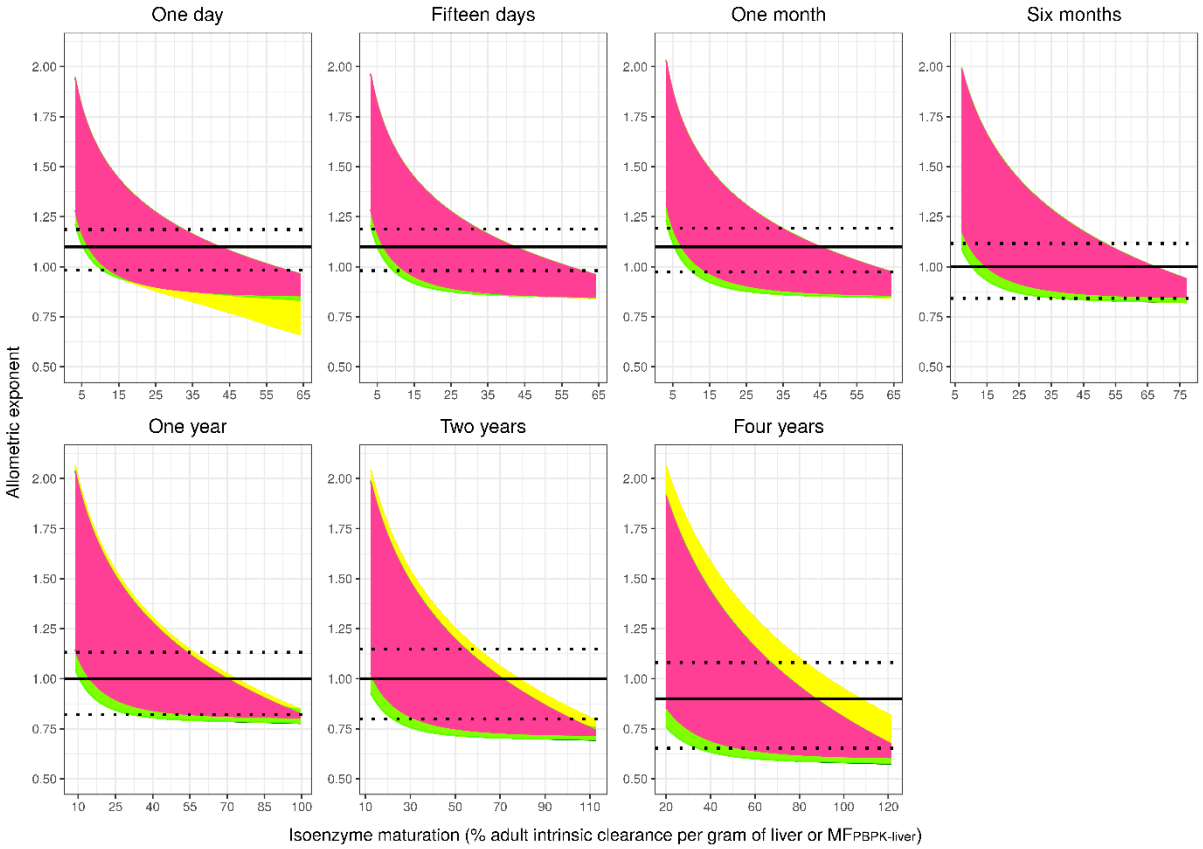


***Figure 3*** *'True' allometric exponent (pink, green and yellow areas) and ADE exponent used to scale CLp (solid black line with ± 30% PE in CLp as dotted black lines) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Different colours represent hypothetical drugs with different properties, with pink representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). Green and yellow are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG respectively to different extents (including fu=1). Under the pink area, the pink, yellow, and green areas overlap completely, therefore the combination of pink and green areas shows the results for all drugs binding to HSA and the combination of pink, green and yellow areas shows the results for drugs binding to AAG. Note that the scales on the x-axis may be different for different ages.*

### 6.3.3 Identification of drug properties predictive for accurate CLp scaling

As explained above, in both Figure 1 and 2, results were grouped in 3 categories to assess the combined impact of plasma protein binding to HSA or AAG and diffusion in red blood cells, on 'true' relative paediatric CLp. This categorization does not explain the observed variability in 'true' relative paediatric CLp values, which can be seen by the spread of each colour outside the ±30% PE range of the scaling methods defined by the dotted lines. As such, plasma protein binding to HSA or AAG and diffusion in red blood cells do not allow for the

definition of drug variables for which ADE or AS0.75+$MF_{PBPK}$ systematically lead to accurate scaling.

Further categorization of these results based on ER ratio of drugs in adults was not found to allow for the definition of drug variables for which ADE systematically leads to accurate hepatic metabolic CLp scaling either (Supplementary Figure 1 and Supplementary Table 1). For ADE, Supplementary Figure 1, which is the same as Figure 1 but stratified on the adult ER (i.e., low, intermediate and high ER drug), shows the wide variability in 'true' relative paediatric CLp with isoenzyme maturation in each age, leading to both over and under-predictions of 'true' relative paediatric CLp for each ER category. Supplementary Table 1 also shows that although PE ranges decrease with increasing ER, every category still includes PE values above 100%.

For hepatic metabolic CLp scaling using AS0.75+$MF_{PBPK}$, further categorization based on ER of drugs in adults did reveal scenarios for which CLp scaling is systematically accurate (Supplementary Figure 2 and 3 and Table 2). Table 2 shows the PE ranges in each age for each drug category when scaling CLp using AS0.75+$MF_{PBPK\text{-}liver}$ (Table 2A) or $MF_{PBPK\text{-}microsomes}$ (Table 2B). On one hand, Table 2A shows that after further categorization of the results based on ER, PEs for scaled CLp values of drugs with low and intermediate ER lie within a ±30% and ±50% range respectively when $MF_{PBPK\text{-}liver}$ was used for the predictions, except for AAG bound drugs in term neonates of one day. Similarly, Supplementary Figure 2 reveals a close agreement between CLp values scaled using AS0.75+$MF_{PBPK\text{-}liver}$ and the 'true' relative paediatric CLp for low and intermediate ER drugs which leads to the acceptable accuracy of CLp scaling in all studied ages, except for drugs binding to AAG in neonates of one day. On the other hand, for high ER drugs, there are no scenario based on age and drug properties that systematically leads to accurate CLp scaling with AS0.75+$MF_{PBPK\text{-}liver}$.

Regarding hepatic metabolic CLp scaling using $MF_{PBPK\text{-}microsomes}$, Table 2B shows that after additional categorization of the results based on ER, all PE ranges included values above 30% and most of them included PE values above 50% regardless of the drug category. Supplementary Figure 3 shows a shift in which scaling with this approach moves from predominantly over-estimation of relative paediatric CLp for drugs with a low ER in all ages towards under-prediction of relative paediatric CLp in all ages with increasing ER of the hypothetical drugs. For this method, no scenario can however be defined based on age and drug properties that leads to systematically accurate scaling.

***Table 2*** *Range of prediction errors in each investigated paediatric age categorized per drug property for CLp values obtained when scaling the CLp of the hypothetical drugs using either AS0.75+MF$_{PBPK\text{-}liver}$ (A) or AS0.75+MF$_{PBPK\text{-}microsomes}$ (B)*

A

| Drug category | | Age | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | One day | Fifteen days | One month | Six months | One year | Two years | Four years |
| Low ER | HSA bound | [-17% - 23%] | [-18% - 20%] | [-10% - 22%] | [-7% - 19%] | [-8% - 18%] | [-9% - 12%] | [-14% - 9%] |
| | AAG Bound | [-55% - 23%] | [-22% - 20%] | [-19% - 22%] | [-4% - 19%] | [-5% - 21%] | [-9% - 22%] | [-9% - 33%] |
| | fu=1 & Kp=1 | [4% - 23%] | [1% - 20%] | [2% - 21%] | [0% - 18%] | [-2% - 16%] | [-5% - 11%] | [-7% - 9%] |
| Inter. ER | HSA bound | [-46% - 18%] | [-47% - 13%] | [-42% - 16%] | [-40% - 17%] | [-40% - 20%] | [-40% - 14%] | [-42% - 9%] |
| | AAG Bound | [-70% - 18%] | [-50% - 13%] | [-48% - 16%] | [-38% - 17%] | [-38% - 22%] | [-40% - 20%] | [-38% - 28%] |
| | fu=1 & Kp=1 | [-37% - 16%] | [-38% - 13%] | [-38% - 14%] | [-37% - 14%] | [-38% - 15%] | [-40% - 10%] | [-38% - 7%] |
| High ER | HSA bound | [-87% - 9%] | [-87% - 2%] | [-87% - 5%] | [-85% - 13%] | [-84% - 20%] | [-83% - 14%] | [-80% - 8%] |
| | AAG Bound | [-87% - 9%] | [-87% - 2%] | [-87% - 5%] | [-85% - 13%] | [-84% - 21%] | [-83% - 18%] | [-80% - 19%] |
| | fu=1 & Kp=1 | [-84% - 3%] | [-84% - 1%] | [-84% - 2%] | [-81% - 6%] | [-81% - 13%] | [-80% - 8%] | [-77% - 3%] |

B

| Drug category | | Age | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | One day | Fifteen days | One month | Six months | One year | Two years | Four years |
| Low ER | HSA bound | [30% - 92%] | [28% - 87%] | [41% - 89%] | [43% - 82%] | [39% - 77%] | [32% - 63%] | [17% - 49%] |
| | AAG Bound | [-30% - 92%] | [22% - 87%] | [26% - 89%] | [47% - 82%] | [42% - 82%] | [33% - 77%] | [24% - 82%] |
| | fu=1 & Kp=1 | [62% - 92%] | [57% - 87%] | [59% - 88%] | [54% - 81%] | [47% - 74%] | [38% - 62%] | [28% - 49%] |
| Inter. ER | HSA bound | [-16% - 83%] | [-18% - 76%] | [-11% - 80%] | [-7% - 79%] | [-9% - 81%] | [-13% - 65%] | [-20% - 49%] |
| | AAG Bound | [-53% - 83%] | [-22% - 76%] | [-19% - 80%] | [-5% - 79%] | [-7% - 83%] | [-12% - 75%] | [-16% - 75%] |
| | fu=1 & Kp=1 | [-1% - 81%] | [-4% - 76%] | [-3% - 78%] | [-4% - 74%] | [-7% - 72%] | [-12% - 60%] | [-16% - 46%] |
| High ER | HSA bound | [-79% - 70%] | [-81% - 59%] | [-80% - 64%] | [-77% - 73%] | [-76% - 81%] | [-75% - 65%] | [-72% - 48%] |
| | AAG Bound | [-79% - 70%] | [-81% - 59%] | [-80% - 64%] | [-77% - 73%] | [-76% - 82%] | [-75% - 71%] | [-72% - 63%] |
| | fu=1 & Kp=1 | [-75% - 61%] | [-75% - 57%] | [-75% - 59%] | [-71% - 62%] | [-71% - 70%] | [-71% - 58%] | [-68% - 41%] |

*Low, intermediate and high extraction ratios are defined as ER ≤ 0.3, 0.3 < ER ≤ 0.7, and ER > 0.7. fu=1 & Kp=1 corresponds to drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). HSA bound corresponds to drugs that diffuse into red blood cells to different extents and that bind to HSA to different extents (including fu=1). AAG bound corresponds to drugs that diffuse into red blood cells to different extents and that bind to AAG to different extents (including fu=1). HSA, human serum albumin; AAG, alpha-1 acid glycoprotein; AS0.75+MF$_{PBPK_liver}$, AS0.75 in combination with a maturation function expressed in percentage of adult unbound intrinsic clearance per gram of liver; AS0.75+MF$_{PBPK_microsomes}$, AS0.75 in combination with a maturation function expressed in percentage of adult unbound intrinsic clearance per microgram microsomes. Colours indicate the PE category, with PE range for all hypothetical drugs lying within ±30% in green, within ±50% in orange, and including absolute values higher than 50% in red.*

## 6.4 Discussion

As previous analyses have shown that hepatic metabolic CLp scaling based on bodyweight alone is not systematically accurate in patients younger than 5 years [5,16], the aim of this study was to systematically assess the hepatic metabolic CLp scaling accuracy of ADE and AS0.75+$MF_{PBPK}$ in children younger than five years. Since this systematic assessment was performed using a PBPK-based simulation workflow analogue to previous analyses of other scaling methods [2,5,16], the reported accuracy of the different methods can be directly compared.

Whereas ADE scaling was found to perform better than standard AS0.75 scaling in all ages, ADE does not systematically lead to accurate scaling of hepatic metabolic CLp from adult to children younger than 5 years (Table 1). This is due to the significant impact of isoenzyme maturation and drug properties on the 'true' relative paediatric CLp, which is not properly accounted for in all cases by ADE (Figure 1, Supplementary Table 1 and Supplementary Figure 1). This explains the lack of accuracy of scaling methods solely accounting for age and bodyweight that has been reported for some drugs in young children [1,3,17]. Therefore, although ADE scaling leads to accurate hepatic metabolic CLp scaling for some drugs and isoenzyme maturations in each age, it has not been possible to develop guidelines to *a priori* predict whether this will be the case for a specific individual drug.

The wide variations in 'true' CLp values within the typical individuals of each age, translate into a wider range of 'true' allometric exponents of 0.57 to 2.07 across all ages (Figure 3), compared to the range of 0.8 to 1.2 that we reported earlier for children younger than 5 years [2]. The previously reported range of allometric exponent values were derived from scenarios in which size-related changes were accounted for in the absence of maturation in system-specific parameters. The range reported here corresponds to allometric exponents needed to scale 'true' adult CLp values to 'true' paediatric CLp values which are impacted by size-related changes as well as by maturational changes in isoenzyme activity, plasma protein concentration and haematocrit. Results in Figure 1 show that a single exponent cannot scale CLp with an accuracy of ±30% for all drugs at young ages, even when the exponent changes with age.

AS0.75+$MF_{PBPK}$ is a simplified scaling method that, in addition to scaling based on bodyweight, includes an age-based PBPK function for enzyme maturation. This scaling method does not take maturational changes in haematocrit and plasma proteins abundance into

account, but accounting for isoenzyme maturation is sufficient for accurate hepatic metabolic CLp scaling of drugs with a low or intermediate ER in adults. When isoenzyme maturation is expressed as percentage of adult intrinsic clearance per gram of liver ($MF_{PBPK\text{-}liver}$), this method leads to PEs lying within a ±30% and a ±50% range for all hypothetical drugs with a low and intermediate ER respectively, except for drugs binding to AAG in term neonates of 1 day. This is due to the decreasing variability in relative paediatric CLp with decreasing ER values, because isoenzyme maturation is the main driver of relative paediatric CLp for drugs that have a low or intermediate ER in adults. The lack of accuracy in one day term neonates for AAG bound drugs (Figure 2A, Supplementary Figure 2 and Table 2) is due to the steep increase in AAG concentration in the first days of life, leading to a wide variation in relative paediatric CLp for different hypothetical drugs binding to this plasma protein to varying extents [18]. For drugs that have a high ER in adults, the ER decreases in children with decreasing enzyme maturation and as a result the impact of hepatic blood flow on CLp will decrease as well. This shift in the contribution of hepatic blood flow is not accounted for in the scaling method. As such, for AAG bound drugs and for drugs with a high ER in adults, PBPK models are required for accurate CLp scaling from adults to neonates of one day and to children younger than 5 years respectively.

In scaling CLp with the $AS0.75+MF_{PBPK}$ method, the choice of the PBPK function ($MF_{PBPK}$) to use is of high importance. While both $MF_{PBPK\text{-}liver}$ and $MF_{PBPK\text{-}microsomes}$ account for isoenzyme maturation, only $MF_{PBPK\text{-}liver}$ also accounts for age-related changes in MPPGL (microsomal protein per gram of liver). Indeed, expressing isoenzyme maturation as percentage of adult intrinsic clearance per microgram microsomes ($MF_{PBPK\text{-}microsomes}$), leads to inaccurate CLp predictions regardless of drug properties in almost all ages (Figure 2B, Supplementary Figure 3 and Table 2). Until 2008, MPPGL maturation with age had not been characterized and therefore isoenzyme maturation was expressed as percentage of adult intrinsic clearance per gram of liver ($MF_{PBPK\text{-}liver}$) [19]. Afterwards, MPPGL maturation was implemented in commercial PBPK software packages and isoenzyme maturation functions were adapted accordingly to be expressed in percentage of adult intrinsic clearance per microgram microsomes. As the units of isoenzyme maturation functions are not always reported in literature [20] and because selecting the appropriate $MF_{PBPK}$ is of utmost importance when using $AS075+MF_{PBPK}$, reporting these units for enzyme maturation functions should be encouraged.

In those cases where after scaling plasma clearance *a priori* with AS0.75, a maturation function is estimated from clinical PK data instead of using enzyme maturation functions as implemented in PBPK models, it is often assumed that the estimated maturation function reflects isoenzyme maturation for drugs undergoing hepatic metabolism. From our results as depicted in Figure 2, it can be deduced that this is not always the case, as there is only limited overlap between the true relative paediatric CLp (coloured areas) and the AS0.75+$MF_{PBPK}$ scaled predictions (black lines with 30% PE) (Figure 2). The explanation may be that these estimated maturation functions also aggregate the impact of drug properties on clearance maturation that are not properly accounted for. This is in line with previous finding from Strougo *et al.* [4,21].

The application of the PBPK-based framework was an essential part of the current investigation as a clean and systematic evaluation on the impact of individual drug-specific and system-specific parameters is not possible with real data. In a clinical situation elimination pathways and the impact of changes in individual drug-specific and system-specific parameters cannot be studied in isolation. Indeed, the total number of drugs prescribed in the paediatric population is far too limited to be able to perform a systematic assessment that can support generalizable conclusions for all current and future small molecule drugs. Moreover, values of 'true' CLp are at best approximated by deriving them from observed concentration values that are inevitable obtained with experimental error. Thanks to this PBPK-based analysis workflow, we could identify the theoretical boundaries in PE and 'true' allometric exponents for hepatic metabolic CLp between which all current and future small molecular drugs can be predicted to lie *a priori*.

Because isoenzyme maturation was studied as a near continuous variable within the range of reported enzyme maturation values for each age, this analysis covers all possible combinations of hepatic metabolism by multiple isoenzymes contributing to hepatic metabolic CLp to various extents. However, the analysed scenarios do assume the maturation profile of the isoenzymes to be known. For drugs with low or intermediate ER that are metabolized by multiple isoenzymes, scaling CLp therefore requires knowledge on the fraction metabolized by each isoenzyme in adults and the $MF_{PBPK\text{-}liver}$ of each isoenzyme involved in the drug clearance.

CYP3A7 is an example of an isoenzyme often found to be involved in drug metabolism in the paediatric population when other isoenzymes are highly immature. As this isoenzyme is not functionally present in adults, CLp values could not be scaled from adult values based

on the maturation profile of this isoenzyme. Although clinically observed total CLp values cannot be directly compared to the hepatic metabolic CLp studied in isolation in the current work, we accounted for the observation that in clinical situations when elimination routes are highly immature other elimination routes take over, by setting a lower limit of 5% isoenzyme maturation. The scaling accuracy of ADE and AS0.75+$MF_{PBPK}$ for other elimination routes, including renal excretion, and for scenarios involving multiple elimination mechanisms, remains subject of further investigation.

Finally, information on maturation of most system-specific parameters in preterm neonates is currently still lacking. Similarly, there is a lack of information on transporters ontogeny in the entire paediatric population. Therefore, further investigation on the systematic accuracy of CLp scaling for all drugs in preterm neonates and for substrates of transporters on hepatocytes in all paediatric ages, remains to be performed once the required information for these assessments becomes available.

In conclusion, when scaling CLp from adults to children younger than five years, solely accounting for age and bodyweight without taking drug properties and enzyme maturation into consideration, will likely not yield systematically accurate CLp scaling. All paediatric CLp values for low and intermediate ER drugs can be scaled using AS0.75+$MF_{PBPK}$ except for drugs binding to AAG in neonates of one day, provided the $MF_{PBPK\text{-}liver}$ is used thereby accounting for both isoenzyme and MPPGL maturation. For other drugs, no simple scaling method is systematically accurate and their CLp should be scaled using PBPK models.

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quality products for the life-long treatment of chronic endocrine conditions. The author has completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declares no support from any organization for the submitted work. All other authors have no conflicts of interest to declare.

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**Appendix 1** Methodology

System-specific parameters

PBPK simulations were performed for typical paediatric individuals of various ages and for a typical 25 years old adult. Demographic values (average for males and females) were taken from the CDC growth charts [1] for the typical paediatric individuals and from the Simcyp® (Simcyp Ltd, Sheffield, UK) V15.R1 library for a typical adult (see Appendix Table 1). Body surface area (BSA) was estimated using the equations of Dubois and Dubois [2] for children weighing >15kg, and of Haycock *et al.* [3] for those weighing ≤15kg, as implemented in Simcyp® V15.R1.

PBPK system-specific parameters were taken from the Simcyp® V15.R1 library, including maturation patterns in the hepatic blood flow (Qh), plasma protein concentrations for human serum albumin (HSA) and alpha-1 acid glycoprotein (AAG), haematocrit, liver size, and microsomal protein per gram of liver (MPPGL).

For $CL_{int,mic}$ maturation ($MF_{PBPK\text{-}microsomes}$), a realistic range was defined for each paediatric age using the Simcyp® library for all isoenzymes, except for SULT1A1 for which maturity was taken to have been reached at birth [4]. These $MF_{PBPK\text{-}microsomes}$ ranges correspond to the minimum and maximum $MF_{PBPK\text{-}microsomes}$ for each investigated paediatric age (see Appendix Table 1), with a minimum limit of 5% being set when minimum reported values were below this limit.

***Appendix Table 1*** *Demographic characteristics of the 8 typical individuals implemented in the PBPK-based simulation workflow and their corresponding system-specific parameters values*

| **Demographic values** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 1 day | 15 days | 1 months | 6 months | 1 year | 2 years | 4 years | 25 |
| Bodyweight(kg) | 3.45 | 3.69 | 4.30 | 7.55 | 9.90 | 12.35 | 17.10 | 72.65 |
| Height (cm) | 49.75 | 53.40 | 54.25 | 66.00 | 74.75 | 86.00 | 101.98 | 172.30 |
| BSA ($m^2$) | 0.22 | 0.24 | 0.26 | 0.38 | 0.46 | 0.55 | 0.69 | 1.86 |
| **System-specific parameters** | | | | | | | | |
| Qh[a] (L/h) | 6.55 | 7.07 | 7.83 | 12.95 | 17.65 | 24.65 | 36.64 | 87.92 |
| HSA[b] (g/L) | 35.78 | 36.25 | 39.94 | 42.07 | 42.90 | 43.73 | 41.26 | 43.94 |
| AAG[c] (g/L) | 0.2678 | 0.5340 | 0.5497 | 0.6774 | 0.7172 | 0.7512 | 0.8406 | 0.6847 |
| Hematocrit (%) | 51.93 | 41.66 | 38.14 | 35.11 | 35.78 | 36.79 | 36.75 | 40.74 |
| Liver size (g) | 133 | 143 | 159 | 249 | 313 | 385 | 501 | 1614 |
| MPPGL[d] | 25.53 | 25.57 | 25.60 | 25.99 | 26.45 | 27.36 | 29.12 | 39.79 |
| $MF_{PBPK\text{-}microsomes}$[e] | | | | | | | | |
| CYP1A2[f] (%) | 24 | 28 | 35 | 118 | 150 | 164 | 166 | 100 |
| CYP2A6[f] (%) | $2.10^{-9}$* | $1.10^{-2}$* | 0.48 | 99 | 100 | 100 | 100 | 100 |
| CYP2B6[f] (%) | 15 | 17 | 19 | 34 | 47 | 62 | 78 | 100 |
| CYP2C8[f] (%) | 38 | 77 | 86 | 97 | 99 | 99 | 100 | 100 |
| CYP2C9[f] (%) | 40 | 68 | 74 | 87 | 90 | 92 | 100 | 100 |
| CYP2C18-19[f] (%) | 30 | 31 | 33 | 84 | 95 | 97 | 98 | 100 |
| CYP2D6[f] (%) | 6 | 32 | 47 | 84 | 91 | 95 | 98 | 100 |
| CYP2E1[f] (%) | 10 | 29 | 37 | 59 | 67 | 74 | 80 | 100 |
| CYP3A4[f] (%) | 11 | 11 | 13 | 48 | 78 | 96 | 103 | 100 |
| UGT1A1[f] (%) | 0.2* | 7 | 23 | 98 | 104 | 100 | 100 | 100 |
| UGT1A4[f] (%) | 74 | 74 | 74 | 74 | 75 | 77 | 80 | 100 |
| UGT1A6[f] (%) | 15 | 23 | 30 | 63 | 76 | 87 | 94 | 100 |
| UGT1A9[f] (%) | 9 | 10 | 12 | 34 | 52 | 71 | 86 | 100 |
| UGT2B7[f] (%) | 8 | 9 | 9 | 11 | 13 | 18 | 27 | 100 |
| SULT1A1[g] (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Studied range (%) | [5 – 100] | [5 – 100] | [5 – 100] | [11 – 118] | [13 – 150] | [18 – 164] | [27 – 166] | NA |

[a] *Hepatic blood flow*
[b] *Plasma protein concentrations for human serum albumin*
[c] *Plasma protein concentrations for alpha-1 acid glycoprotein*
[d] *Milligram microsomal protein per gram of liver*
[e] *Isoenzyme maturation expressed as percentage of adult microsomal unbound intrinsic clearance*
[f] *values obtained from the Simcyp ® V15.R1 library*
[g] *values obtained from Hines et al.* [4]
** A lowest value of 5% was used in the simulations*

System and drug specific parameters

To generate PBPK-based CLp values, Qh was taken from Appendix Table 1. Unbound drug fraction in plasma (fu), blood to plasma ratio (B:P) and whole liver unbound intrinsic clearance (CLint) were derived from system-specific parameters in Appendix Table 1 and from the drug-specific parameters defined under hypothetical drugs in the Methodology section of the manuscript.

Fu in adults was taken as a drug property, with different fu values (ranging from 1% to 100%, with 8 equidistant intermediate values) reflecting different affinities for plasma proteins. Adult fu values were scaled to paediatric patients using the relevant plasma concentration of HSA or AAG in adults ([P]adult) and in paediatric patients ([P]paediatric) (see values in Appendix Table 1) according to equation 1. The hypothetical drugs were assumed to exclusively bind to either HSA or AAG.

$$\mathrm{fu_{paediatric}} = \frac{1}{1+\frac{(1-\mathrm{fu_{adult}})\times[\mathrm{P}]_{\mathrm{paediatric}}}{[\mathrm{P}]_{\mathrm{adult}}\times \mathrm{fu_{adult}}}} \quad (1)$$

B:P was computed based on the defined Kp, and on haematocrit and fu values in the corresponding age, according to equation 2 [5].

$$\mathrm{B{:}P} = 1 + [\mathrm{Hematocrit} \times (\mathrm{fu} \times \mathrm{Kp} - 1)] \quad (2)$$

Kp values of 0.35, 0.8, and values from 1 to 40 with 38 intermediate equidistant values were selected, reflecting different extents of drug diffusion into the red blood cells [6,7]. Kp was assumed to not change with age.

CLint values were computed according to equation 3.

$$\mathrm{CLint} = \mathrm{Liver\ size} \times \mathrm{MPPGL} \times \mathrm{MF_{PBPK-microsomes}} \times \mathrm{CL_{int,mic}} \quad (3)$$

In this equation, Liver size and MPPGL are system-specific parameters taken from Appendix Table 1, $MF_{PBPK\text{-}microsomes}$ is the isoenzyme maturation expressed as percentage of adult microsomal intrinsic clearance for which the range at each age is also defined in Appendix Table 1, and $CL_{int,mic}$ was taken as a drug-specific parameter. As defined under hypothetical drugs in the Methodology section of the manuscript, $CL_{int,mic}$ is the adult unbound intrinsic clearance value of one microgram of liver microsomes, ranging between $0.56 \cdot 10^{-6}$ and $0.209 \cdot 10^{-3}$ $mL.min^{-1}.\mu g^{-1}$ microsomal protein [8], with 98 equidistant intermediate values. These different values reflect difference in both affinities for and abundances of isoenzymes between different drugs.

Dispersion model

'True' total hepatic plasma clearance (CLp) values were computed using the dispersion model (Equations 4 to 9). The dispersion model was selected as it has been reported to more accurately predict hepatic CLp than the well-stirred model for highly cleared drugs, while both models lead to equivalent clearance predictions for other drugs [9].

$$\mathrm{CLp} = \mathrm{CL_B} \times \mathrm{B{:}\,P} \quad (4)$$

$$\mathrm{CL_B} = \mathrm{Qh} \times \mathrm{ER} \quad (5)$$

$$\mathrm{ER} = 1 - \mathrm{F_H} \quad (6)$$

$$\mathrm{F_H} = \frac{4a}{(1+a)^2 \exp\{(a-1)/2D_N\} - (1-a)^2 \exp\{-(a+1)/2D_N\}} \quad (7)$$

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (8)$$

$$R_N = (fu/B{:}\,P) \times CLint/Qh \quad (9)$$

In these equations, CLp is the overall total (i.e., bound and unbound) hepatic plasma clearance, $CL_B$ is the total whole blood clearance, B:P is the blood to plasma ratio, Qh is the hepatic blood flow, ER is the hepatic extraction ratio, fu is the unbound drug fraction in plasma, CLint is the whole liver unbound intrinsic clearance, $R_N$ is the efficiency number and $D_N$ is the axial dispersion number. For the axial dispersion number ($D_N$) a value of 0.17 was used [10].

$MF_{PBPK}$ computation.

For the scaling of clearance using AS0.75+ $MF_{PBPK,}$ two different $MF_{PBPK}$ were used:

- $MF_{PBPK\text{-}liver}$ which is expressed as percentage of adult unbound intrinsic clearance per gram of liver and accounts for maturation in both isoenzyme activity and MPPGL. $MF_{PBPK\text{-}liver}$ was computed as the product of MPPGL maturation (i.e., the paediatric to adult MPPGL ratio) and the isoenzyme maturation expressed as percentage of adult microsomal intrinsic clearance (i.e., $MF_{PBPK-microsomes}$) according to equation 10.

$$MF_{PBPK-liver} = \frac{MPPGL_{paediatric}}{MPPGL_{adult}} \times MF_{PBPK-microsomes} \quad (10)$$

- $MF_{PBPK\text{-}microsomes}$ is expressed as percentage of adult unbound intrinsic clearance per microgram of microsomes and only accounts for maturation of isoenzyme activity.

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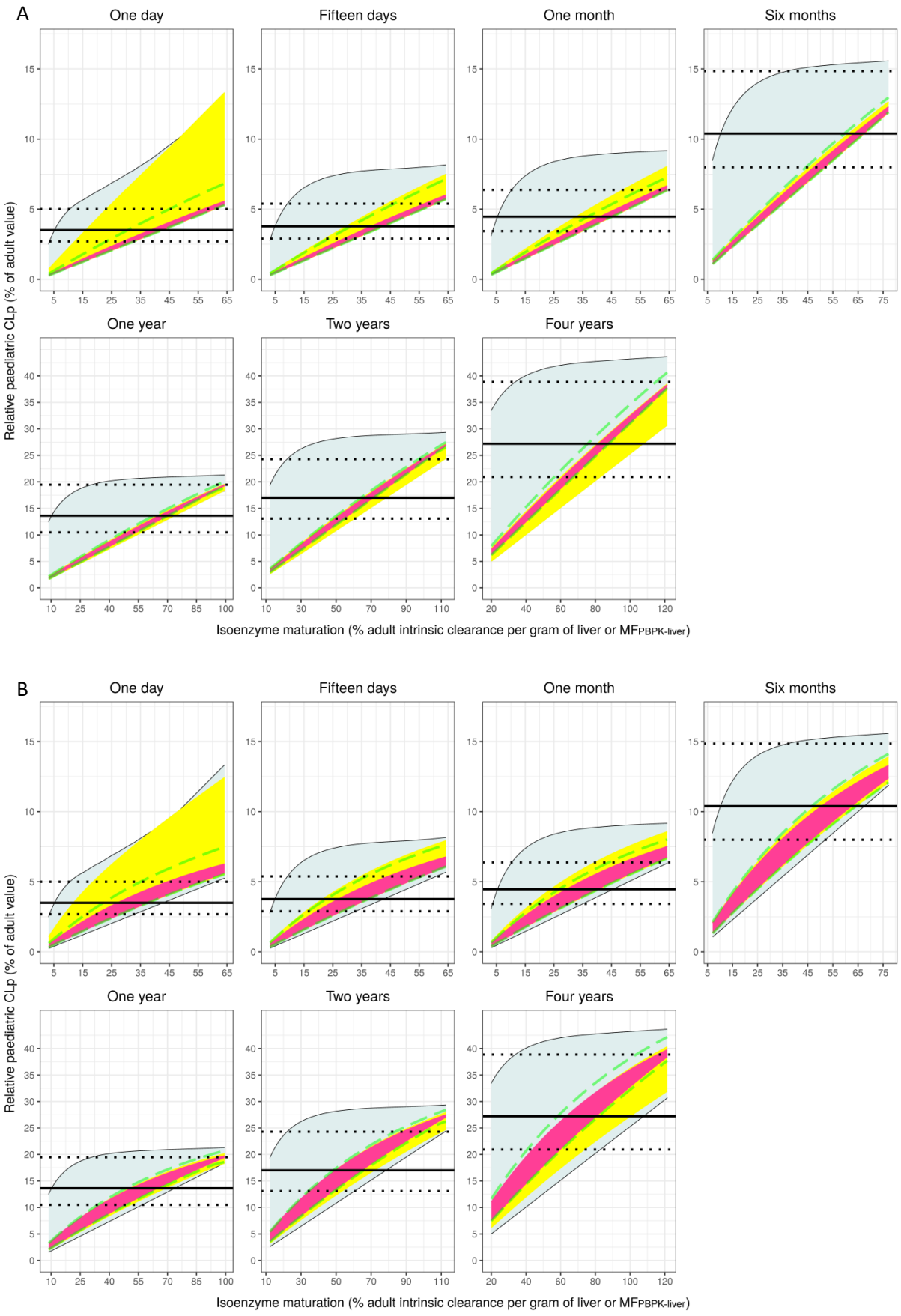
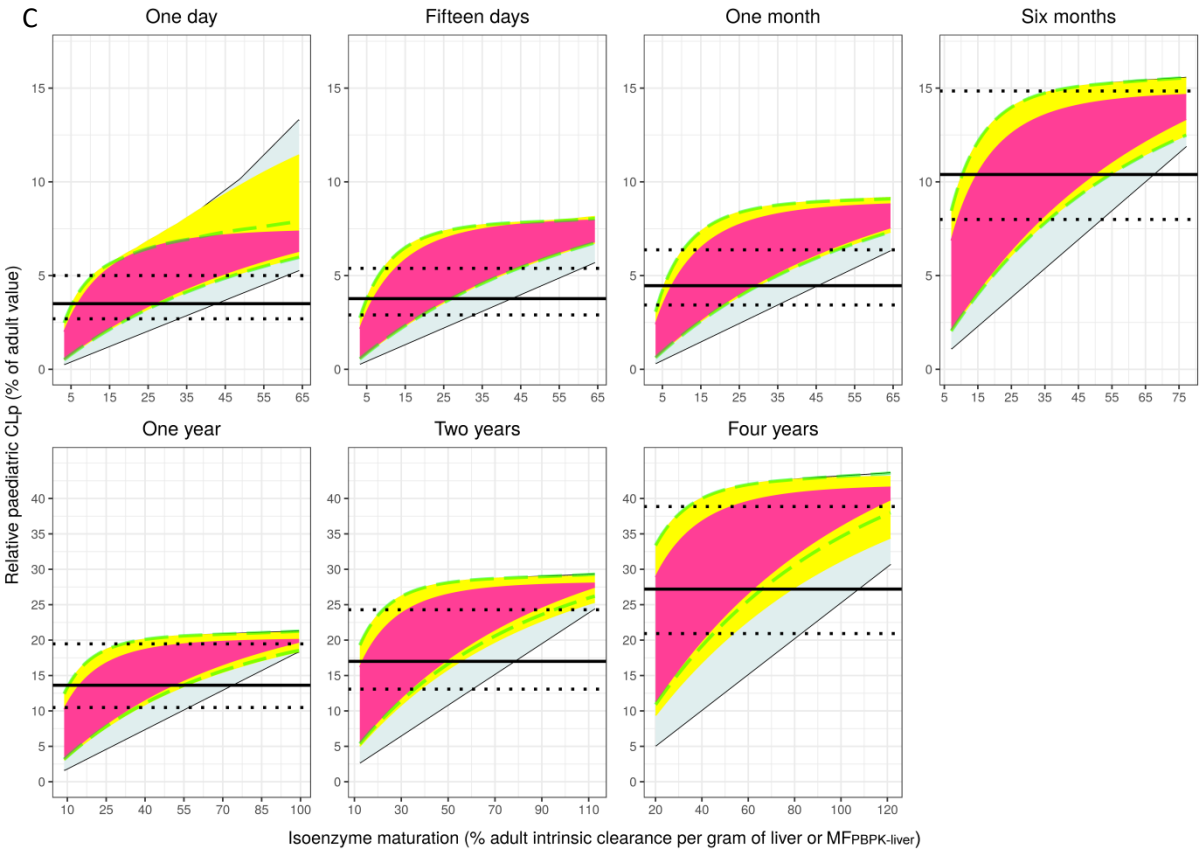
***Table S1*** *Range of prediction errors of CLp values obtained when scaling the CLp of the hypothetical drugs using ADE for the investigated paediatric ages categorized per drug property*

| Drug category | | Age | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | One day | Fifteen days | One month | Six months | One year | Two years | Four years |
| Low ER | HSA bound | [-49% - 1224%] | [-47% - 1220%] | [-39% - 1305%] | [-20% - 853%] | [-32% - 701%] | [-38% - 479%] | [-33% - 338%] |
| | AAG Bound | [-74% - 1224%] | [-50% - 1220%] | [-45% - 1305%] | [-18% - 853%] | [-30% - 739%] | [-37% - 536%] | [-29% - 437%] |
| | fu=1 & Kp=1 | [-37% - 1205%] | [-37% - 1201%] | [-33% - 1285%] | [-16% - 840%] | [-30% - 690%] | [-37% - 472%] | [-29% - 332%] |
| Inter. ER | HSA bound | [-53% - 968%] | [-51% - 966%] | [-44% - 1036%] | [-26% - 679%] | [-34% - 556%] | [-40% - 377%] | [-35% - 264%] |
| | AAG Bound | [-72% - 968%] | [-52% - 966%] | [-48% - 1036%] | [-25% - 679%] | [-32% - 583%] | [-39% - 417%] | [-33% - 336%] |
| | fu=1 & Kp=1 | [-44% - 959%] | [-44% - 956%] | [-41% - 1024%] | [-22% - 670%] | [-31% - 549%] | [-38% - 372%] | [-32% - 260%] |
| High ER | HSA bound | [-56% - 580%] | [-53% - 576%] | [-51% - 621%] | [-33% - 409%] | [-36% - 332%] | [-42% - 217%] | [-38% - 150%] |
| | AAG Bound | [-69% - 580%] | [-54% - 576%] | [-51% - 621%] | [-33% - 409%] | [-36% - 345%] | [-42% - 239%] | [-37% - 191%] |
| | fu=1 & Kp=1 | [-52% - 557%] | [-53% - 555%] | [-49% - 597%] | [-29% - 390%] | [-32% - 316%] | [-39% - 206%] | [-35% - 141%] |

*Low, intermediate and high extraction ratios are defined as ER $\leq$ 0.3, 0.3 < ER $\leq$ 0.7, and ER > 0.7. fu=1 & Kp=1 corresponds to drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). HSA bound corresponds to drugs that diffuse into red blood cells to different extents and that bind to HSA to different extents (including fu=1). AAG bound corresponds to drugs that diffuse into red blood cells to different extents and that bind to AAG to different extents (including fu=1). HSA, human serum albumin; AAG, alpha-1 acid glycoprotein*

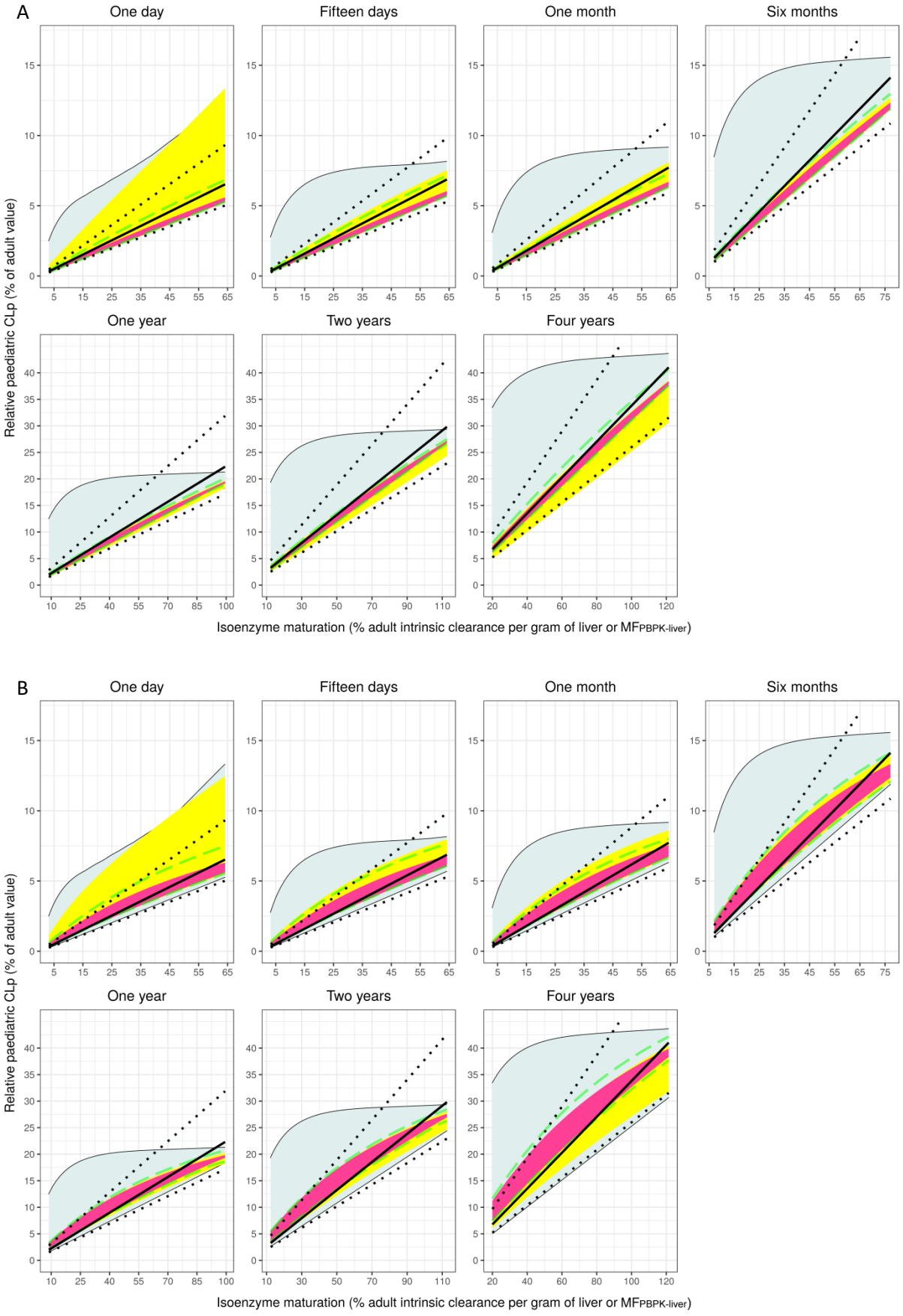
## Supplementary Figure 1

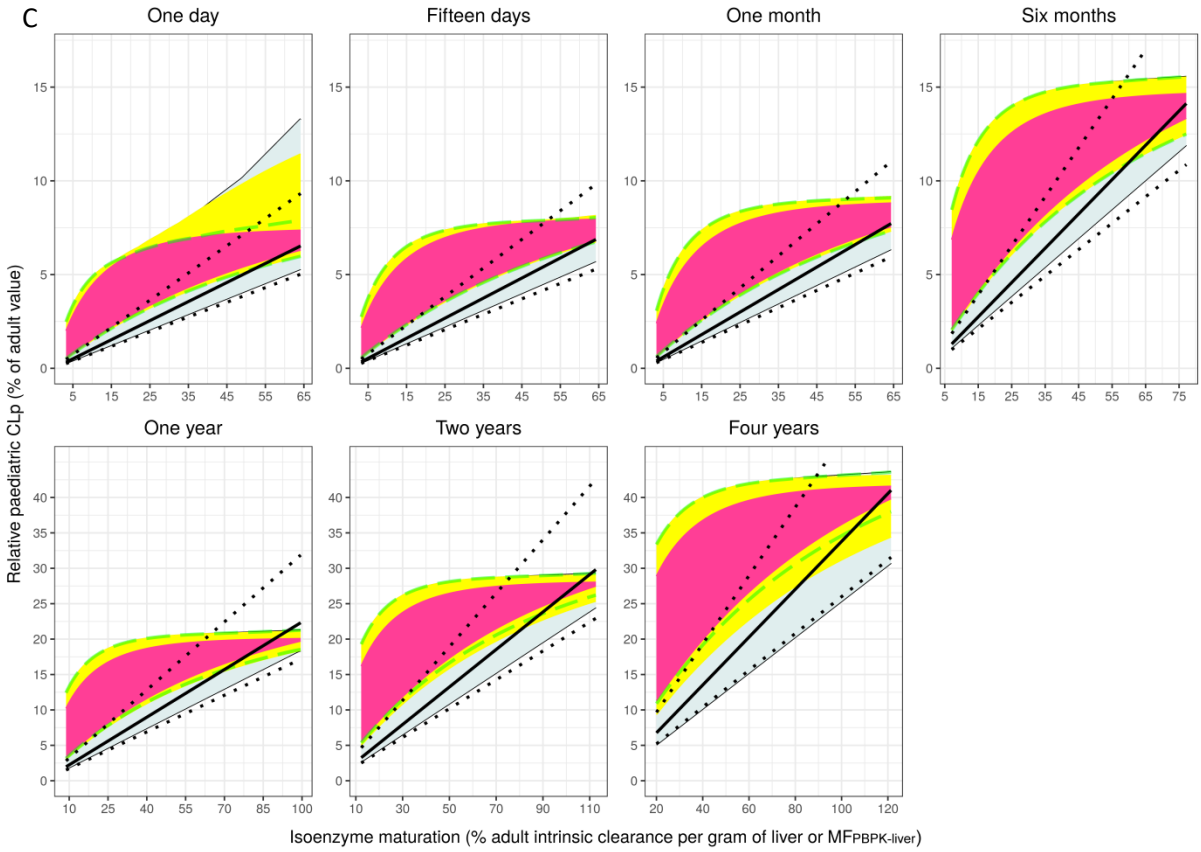





***Supplementary Figure 1*** *Relative paediatric CLp (% of adult value) obtained with ADE scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green or yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Drugs are categorized by extraction ratio (ER) in adults with low ER (≤ 0.3) in panel A, intermediate ER (0.3-0.7) in panel B, and high ER (> 0.7) in panel C. Different colours represent hypothetical drugs with different properties, with the pink shaded area representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). The area delimited by the two green dashed lines and the yellow shaded area are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG, respectively, to different extents (including fu=1). Under the pink area, the pink and yellow areas overlap completely, therefore the combination of pink and yellow areas shows the results for drugs binding to AAG. The blue shaded area depicts all drugs. Note that the scales on the x- and y-axes may be different for different ages.*

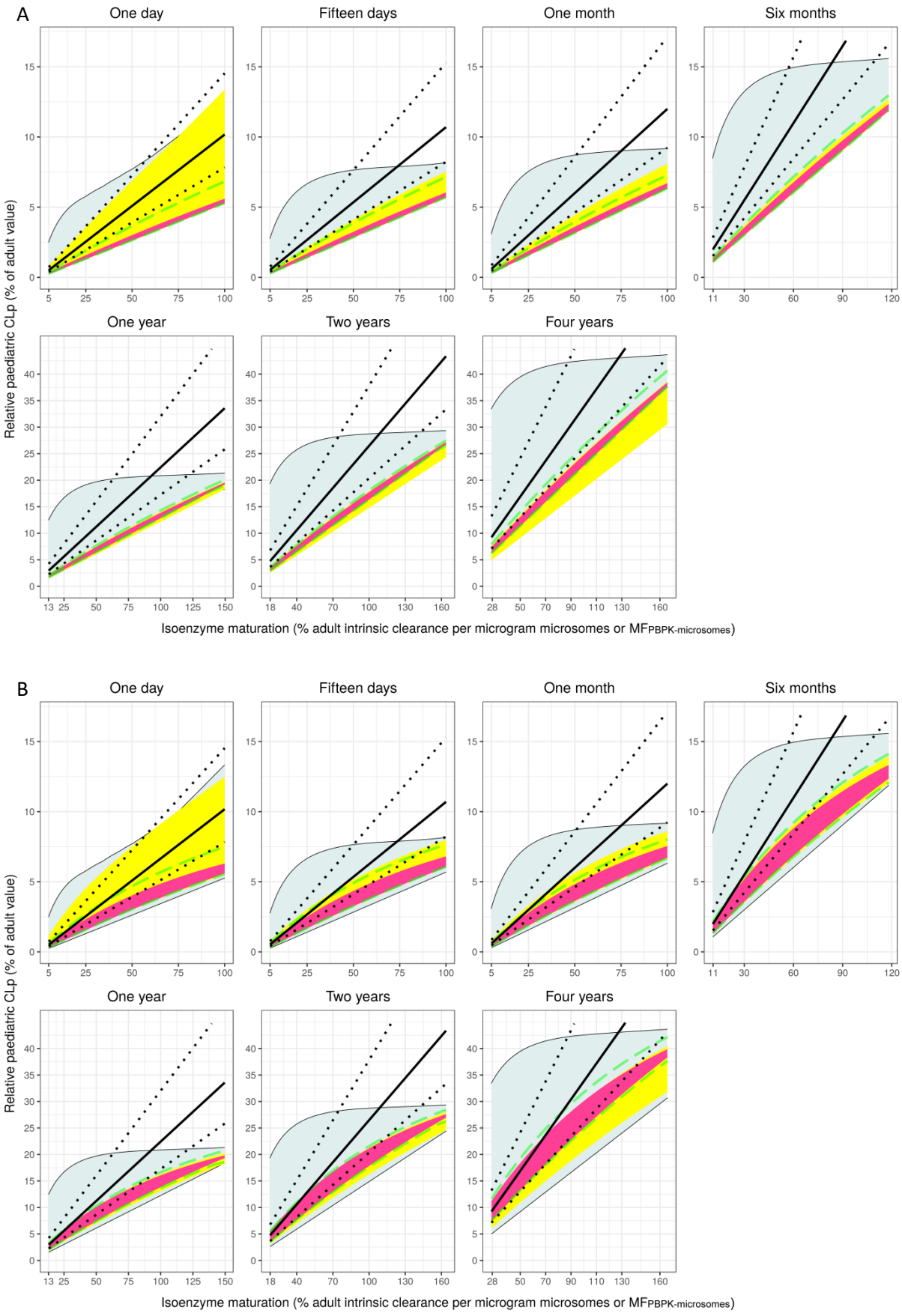
## Supplementary Figure 2

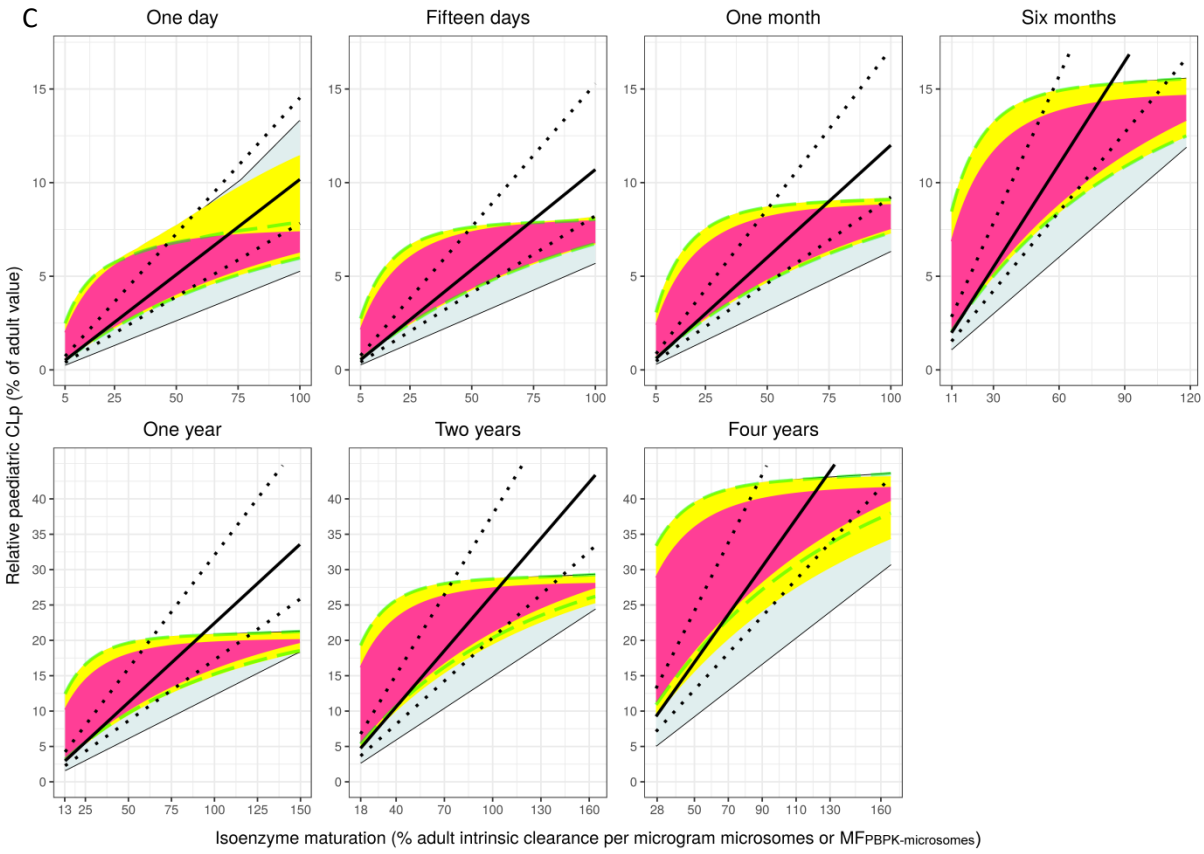





***Supplementary Figure 2*** *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}liver}$ scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green and yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Drugs are categorized by extraction ratio (ER) in adults with low ER (≤ 0.3) in panel A, intermediate ER (0.3-0.7) in panel B, and high ER (> 0.7) in panel C. Different colours represent hypothetical drugs with different properties, with the pink shaded area representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). The area delimited by the two green dashed lines and the yellow shaded area are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG, respectively, to different extents (including fu=1). Under the pink area, the pink and yellow areas overlap completely, therefore the combination of pink and yellow areas shows the results for drugs binding to AAG. The blue shaded area depicts all drugs. Note that the scales on the x- and y-axes may be different for different ages.*

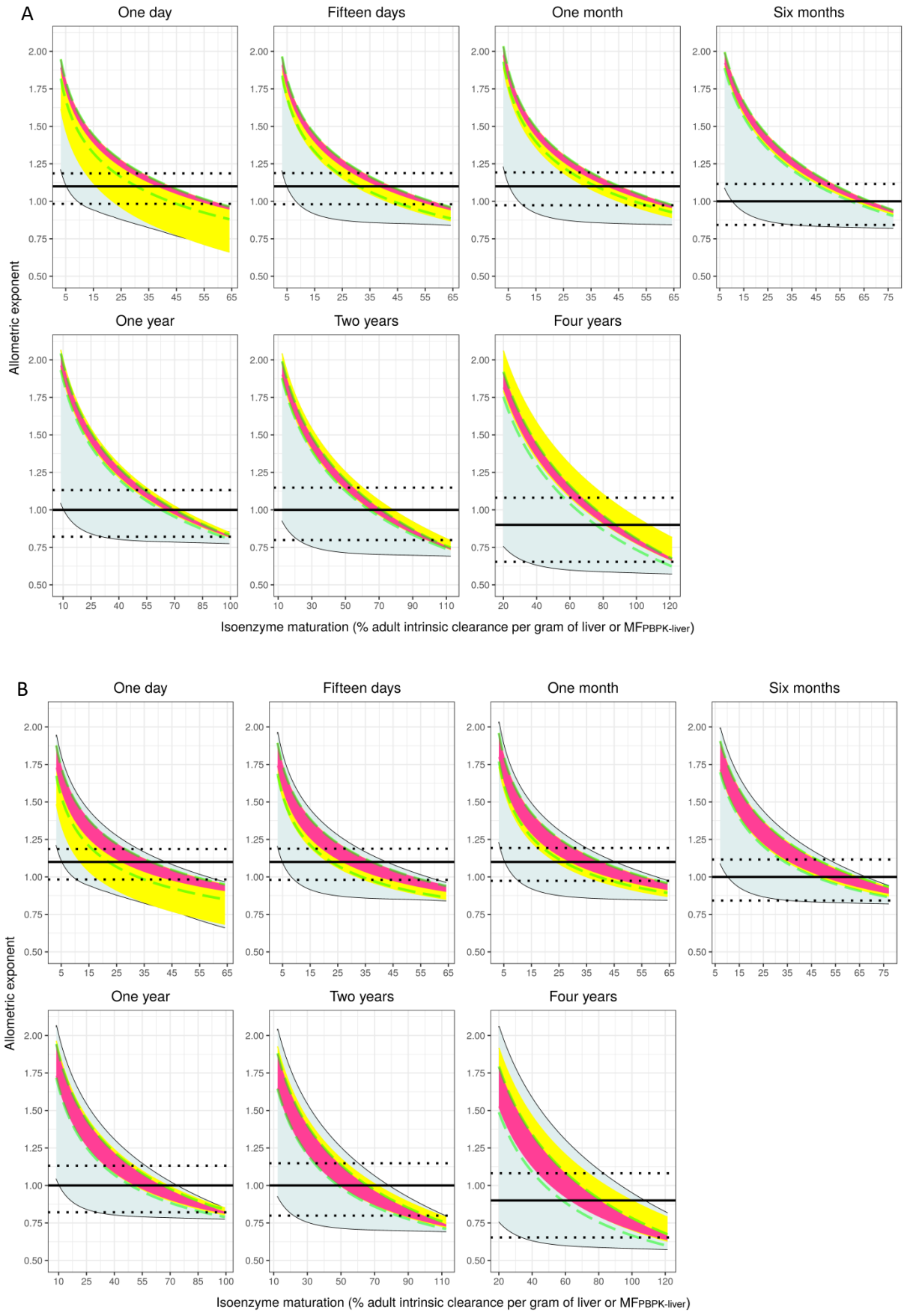
## Supplementary Figure 3

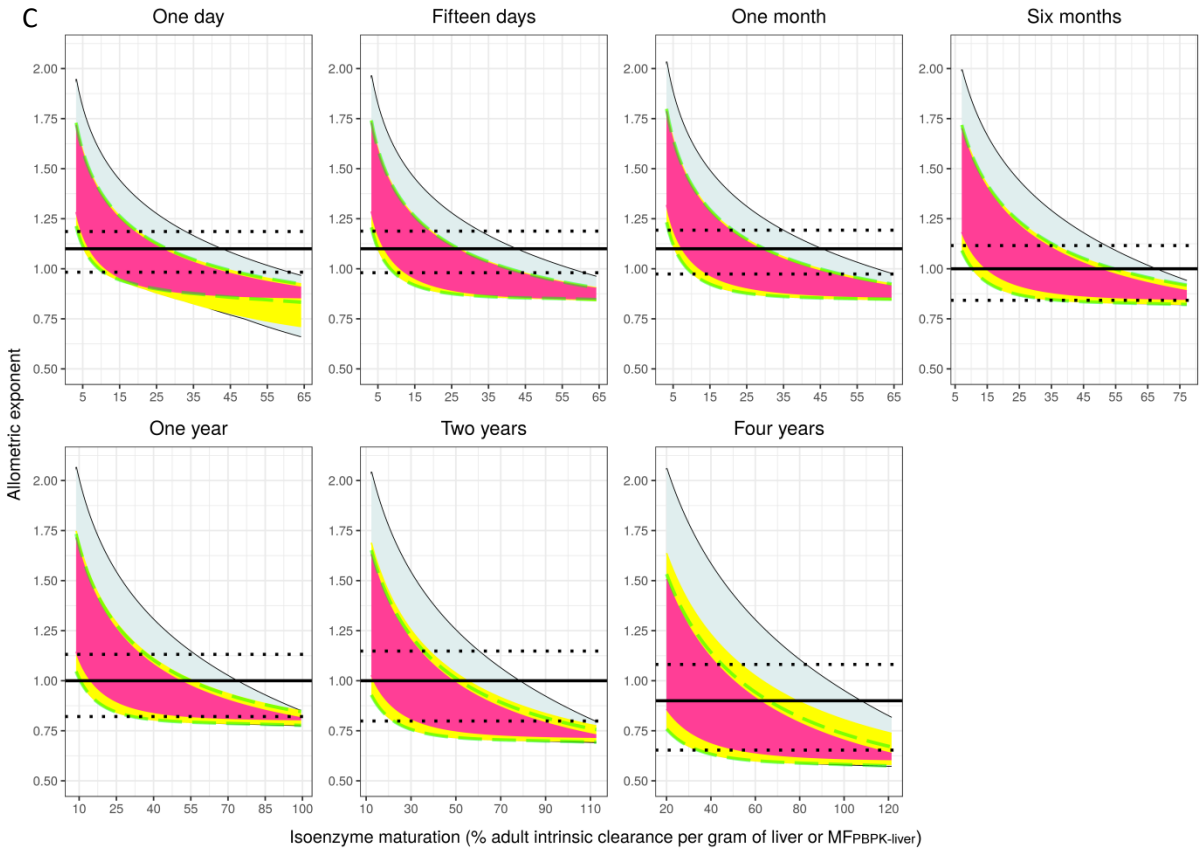





***Supplementary Figure 3*** *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}microsomes}$ scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green and yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Drugs are categorized by extraction ratio (ER) in adults with low ER (≤ 0.3) in panel A, intermediate ER (0.3-0.7) in panel B, and high ER (> 0.7) in panel C. Different colours represent hypothetical drugs with different properties, with the pink shaded area representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). The area delimited by the two green dashed lines and the yellow shaded area are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG, respectively, to different extents (including fu=1). Under the pink area, the pink and yellow areas overlap completely, therefore the combination of pink and yellow areas shows the results for drugs binding to AAG. The blue shaded area depicts all drugs. Note that the scales on the x- and y-axes may be different for different ages.*

## Supplementary Figure 4






***Supplementary Figure 4*** *'True' allometric exponent (pink, green and yellow areas) and ADE exponent used to scale CLp (solid black line with ± 30% PE in CLp as dotted black lines) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Drugs are categorized by extraction ratio (ER) in adults with low ER (≤ 0.3) in panel A, intermediate ER (0.3-0.7) in panel B, and high ER (> 0.7) in panel C. Different colours represent hypothetical drugs with different properties, with the pink shaded area representing drugs not binding to plasma proteins (fu=1) that are also in equilibrium between plasma and red blood cells (Kp=1). The area delimited by the two green dashed lines and the yellow shaded area are used to depict drugs that diffuse into red blood cells to different extents and that bind to HSA or AAG, respectively, to different extents (including fu=1). Under the pink area, the pink and yellow areas overlap completely, therefore the combination of pink and yellow areas shows the results for drugs binding to AAG. The blue shaded area depicts all drugs. Note that the scales on the x-axis may be different for different ages.*

# Section IV

## Towards improved paediatric clearance predictions with the combined use of population PK and PBPK modelling approaches