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

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How to scale clearance from adults to children for drugs undergoing hepatic metabolism?

Insights from advanced PBPK modelling and simulation

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How to scale clearance from adults to children for drugs undergoing hepatic metabolism?

Insights from advanced PBPK modelling and simulation

Proefschrift

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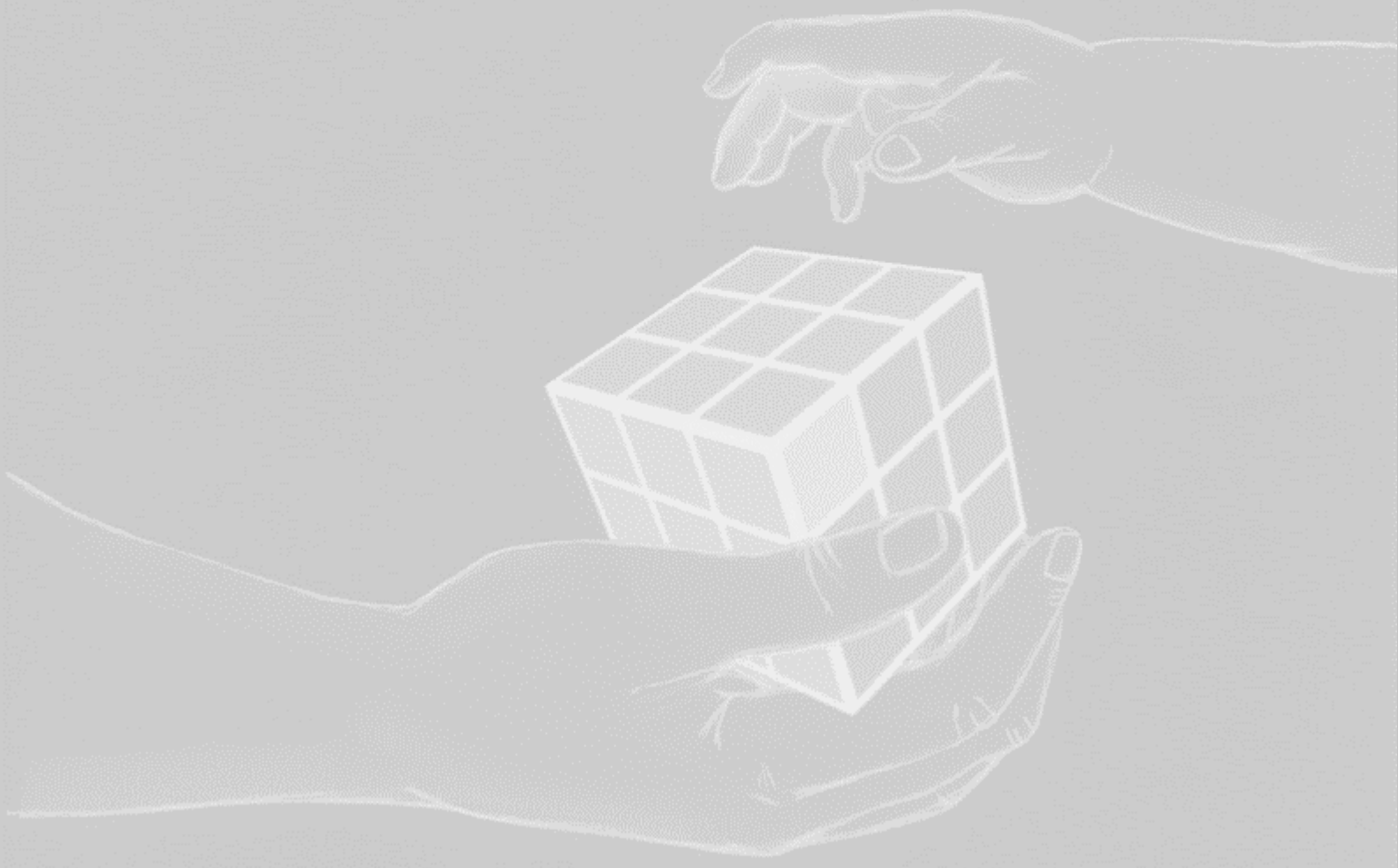
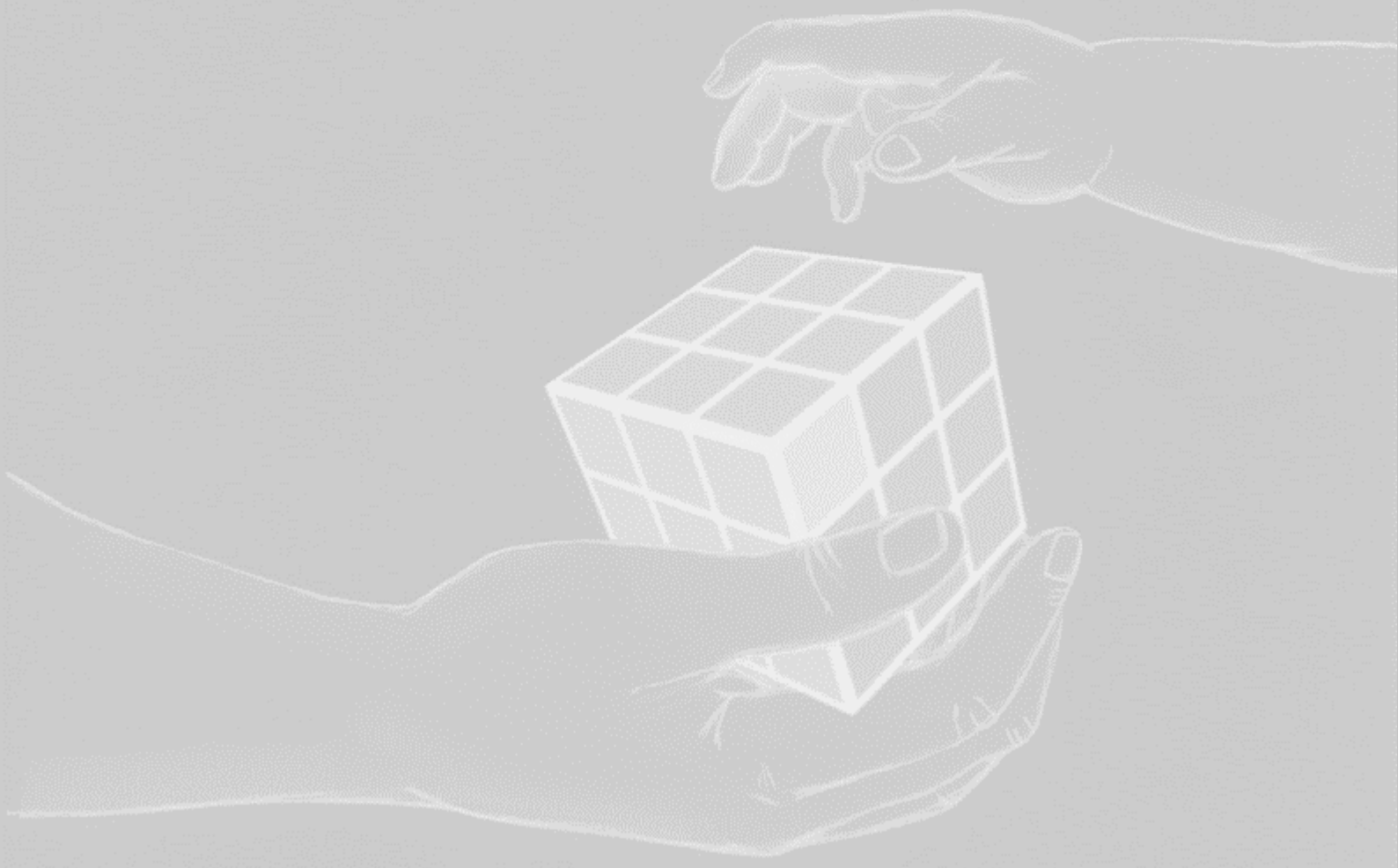
Section I

Background and introduction



Chapter 1

General introduction



While in adult patients clinical trials have long been considered mandatory in order to ensure safe and efficacious drug treatment, they have long been thought unethical in paediatric patients [1]. Additionally, paediatric drug evaluation has often been neglected due to the diversity of challenges in performing pharmacological studies in paediatrics. These challenges include practical, ethical and economical concerns as well as challenges stemming from the major physiological changes taking place over the life spam of children [2]. This has led to the observed high paediatric unlicensed and off-label prescription rate, the latter being largely due to a lack of information on the recommended dosage [3–6]. Unlicensed drug prescription is the prescription of a drug that does not have a license, as for instance modified formulations, extemporaneous preparations or imported medicines. Off-label prescription is a prescription made outside the terms indicated in the product license, as for instance off-label use with regard to dose, age group, route of administration, or different indication [3].

To date there is agreement that off-label and unlicensed use of drugs is itself a suboptimal and at times potentially unethical situation that must be addressed [2]. In face of the urgent need to ensure safe and efficacious drug dosing in children for new drugs, but also currently available drugs, efforts have focussed on the advancement of dedicated paediatric clinical trials for each of these drugs across the paediatric age range.

Paediatric clinical trials enable the estimation of changes in clearance across the paediatric population, a key parameter for safe and efficacious paediatric dose tailoring. Characterizing clearance in paediatrics is challenging because this parameter varies across the paediatric population due to ontogenic processes and because few samples per patients and few patients per age group are available. Model-based approaches such as population modelling are necessary to overcome these challenges and enable the characterization of clearance ontogeny [7,8]. In addition, tremendous efforts have been made to accurately predict clearance ontogeny in children in order to derive individualized paediatric dosing regimen based on adult data and to integrate and maximize information throughout paediatric drug development, for which physiologically-based pharmacokinetic modelling (PBPK) approaches have been instrumental [9–12]. Such modelling and scaling methods are essential to overcome the problems related to the data analysis of underpowered studies and/or the lack of clinical data. Gaining a mechanistic insight into clearance ontogeny enables a rational choice of appropriate clearance scaling methods for their use throughout paediatric drug development and in clinical practice and could steer efforts of future research in this field.

1.1 Clearance: a key parameter for efficacious drug dosing

Pharmacokinetics is the study of the fate of substances in an organism, which involves four processes, namely absorption, distribution, metabolism and excretion (ADME) [13]. Pharmacokinetic (PK) studies are an important part of drug development, ultimately aiming at ensuring safe and efficacious drug treatment. Pharmacokinetic processes are described by pharmacokinetic parameters, such as clearance. Clearance describes the elimination of a substance, which may be due to both metabolism and excretion. This pharmacokinetic parameter is expressed as the volume of plasma or blood from which a compound is completely removed per unit of time and is a key parameter for efficacious and safe dose tailoring. Indeed, clearance, together with the administered dose and bioavailability for non-intravenous administration, drives the drug exposure which in turn, drives the drug effect and toxicity [14–16].

According to the paediatric study decision tree of the Food and Drug administration which has also been proposed in Europe (Figure 1) [17,18], PK studies should be systematically conducted in order to establish paediatric dosing so as to achieve a target concentration. This target concentration is the required drug concentration to achieve the desired effect and is derived based on exposure-response relationship. When disease progression and exposure-response relationship are expected to be the same as in adults, no pharmacodynamics (PD) studies are undertaken and the target concentration is taken to be the same as in adults (option C in the paediatric decision tree). When differences in disease progression and/or exposure-response relationship are expected compared to adults, these differences are assessed through the conduct of PK/PD or efficacy trials in order to define the target concentration in paediatric patients [19]. While classically randomized placebo controlled trials are performed for PK/PD and efficacy studies, the use of a placebo arm is sometimes unethical in paediatric patients and therefore in these cases, comparative effectiveness or non-inferiority designs are the preferred approach [20].

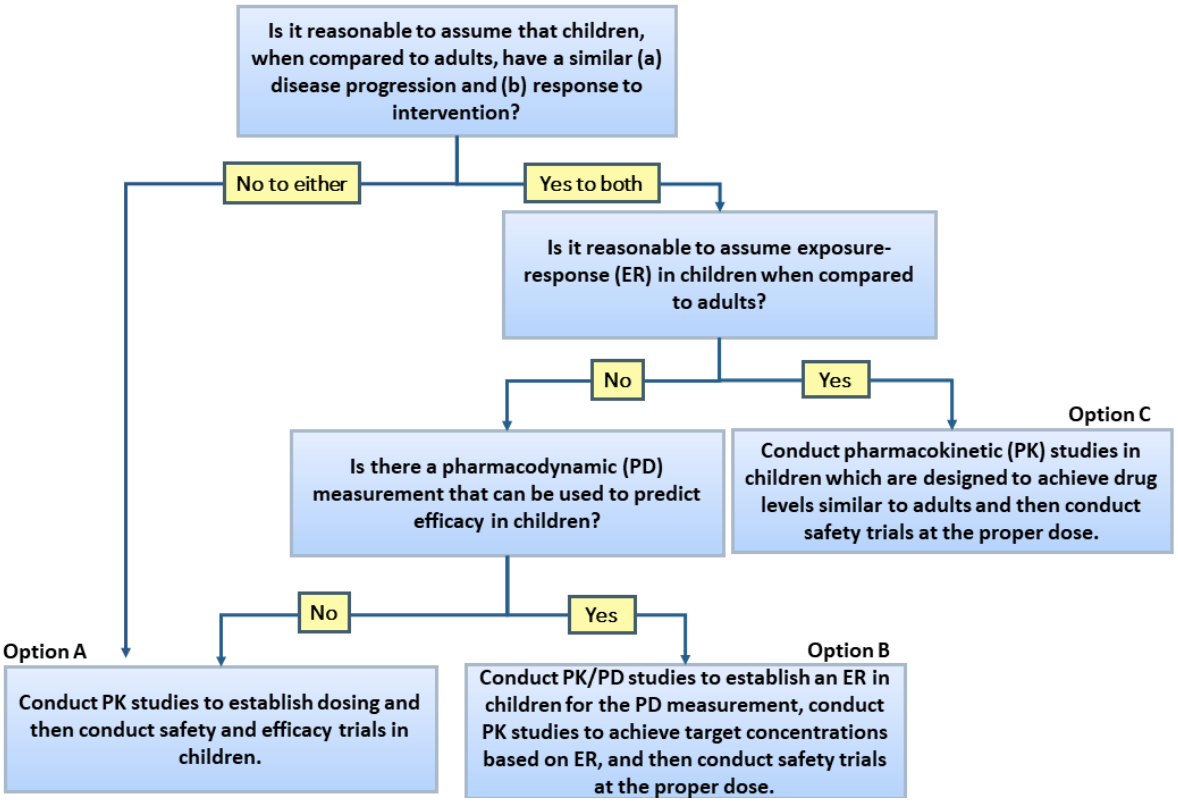


Figure 1 *FDA paediatric study decision tree* [18]

1.2 Clearance across the paediatric population: a moving target

Clearance of a drug, as other PK parameters, is driven by complex interactions between system-specific parameters, which are represented by anatomical and physiological measures, and drug-specific parameters. This is for instance reflected in different PK profiles of drugs with different drug properties. An illustration of the relationship between system- and drug-specific parameters on paediatric clearance of drugs undergoing hepatic metabolism is given in Figure 2. Relevant parameters to mechanistically describe clearance of such drugs are hepatic blood flow (Qh), the unbound drug fraction in plasma (fu), the blood to plasma ratio (B:P), and the intrinsic metabolic clearance in the liver based on unbound drug concentration (CLint). Qh and for instance isoenzyme abundance are purely system-specific parameters, whereas fu, B:P, and CLint are derived from both physiological and drug-specific parameters (Figure 2).

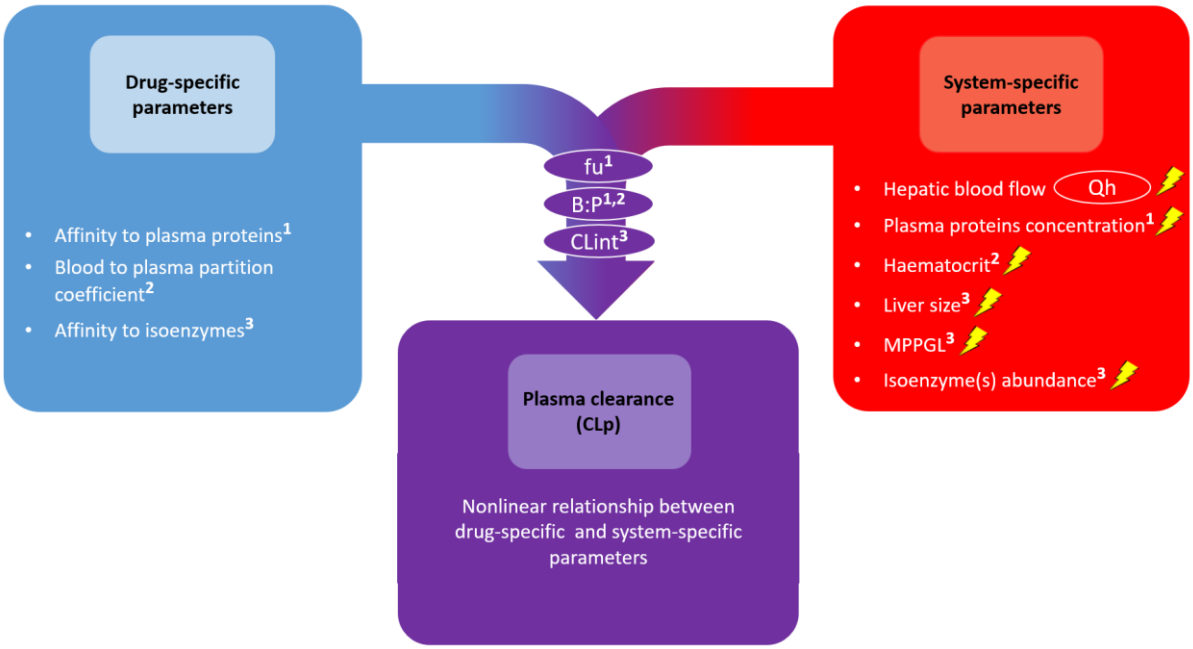


Figure 2 *Schematic representation of the relationship between drug-specific and system-specific parameters driving hepatic plasma clearance (CLp). Parameters within circles are directly used in the physiologically based pharmacokinetic hepatic clearance model (e.g., dispersion model). Parameters in the purple circles represent composite parameters that are derived from the system-specific parameters and the drug-specific parameters indicated by the numbers in the superscripts. In children, each of the system-specific parameters change with age, each represented by a lightning bolt. B:P, blood to plasma ratio; CLint, intrinsic metabolic clearance in the liver based on unbound drug concentrations; fu, unbound drug fraction in plasma; MPPGL, microsomal protein per gram of liver. Figure taken from Calvier et al.* [21].

From birth to adulthood, children undergo physiological changes due to ontogenic (or maturational) processes in system-specific parameters, which are highlighted by lightning bolts in Figure 2. The ontogeny of each of these system-specific parameters has their own profile. Figure 3 for instance shows ontogeny profiles of some of these system-specific parameters as relative values to the corresponding adult level. Ontogeny in system-specific parameters drives clearance ontogeny in a drug-dependent manner due to the interaction between system-specific and drug-specific parameters. Indeed, clearance ontogeny depends on the drug elimination pathway and drug properties, as illustrated in Figure 3. Panel A of this figure shows the ontogeny in system-specific parameters impacting clearance of all drugs undergoing hepatic metabolism, while panel B shows the ontogeny of system-specific parameters solely impacting drugs with specific properties and eliminated by specific isoenzymes. For instance, on one hand, ontogeny in liver volume impacts clearance ontogeny

of all drugs undergoing hepatic metabolism. On the other hand, albumin ontogeny only impacts clearance ontogeny of drugs binding to albumin, and ontogeny in CYP1A2 only impacts clearance ontogeny of drugs eliminated (completely or partially) by this isoenzyme. This impact of ontogenic processes on the pharmacokinetics of drugs has been extensively reviewed in the literature [22–27], as it is responsible for the high pharmacokinetic variability between drugs and different paediatric ages that is observed in paediatric patients.

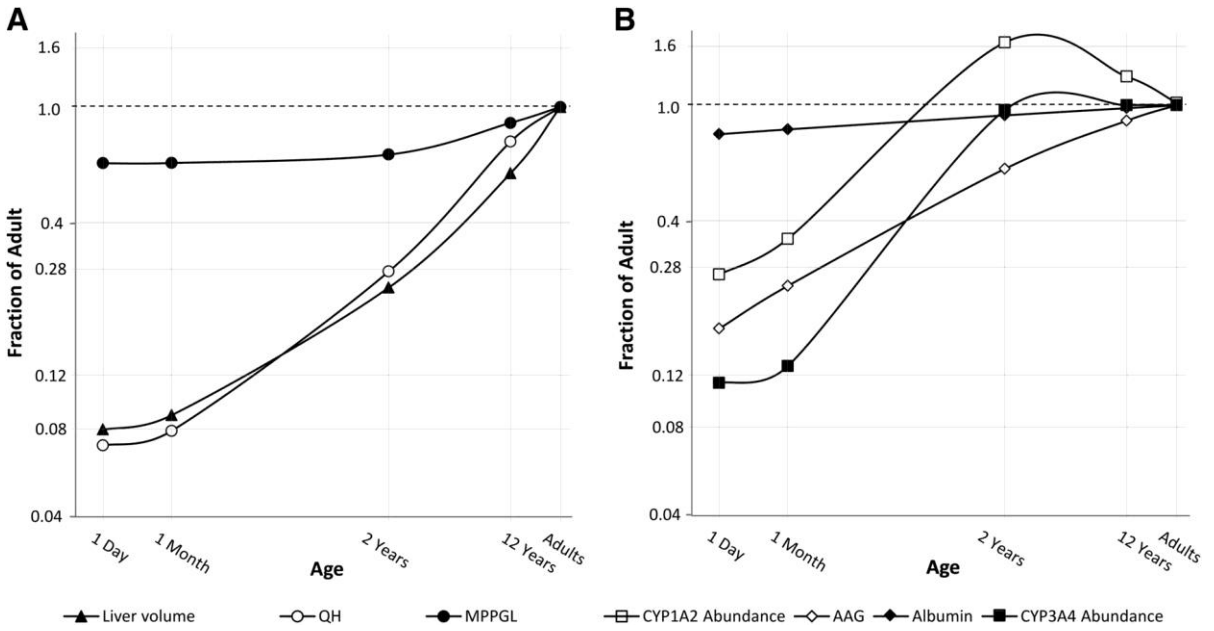


Figure 3 *Age-related variations in parameters defining clearance are shown as relative values to the corresponding adult level of each parameter, taken from Salem et al.* [28]*. (A) Changes in liver volume* [29]*, hepatic blood flow (Qh)* [30]*, and amount of microsomal protein per gram of liver (MPPGL)* [31] *that impact clearance of all drugs. (B) Relative values of serum albumin* [22,32,33]*, CYP3A4 abundance* [34] *alongside age variation in serum alpha-acid glycoprotein (AAG)* [32,33]*, and abundance of CYP1A2* [34]*, that impact clearance in a drug-dependent manner. The impact of the parameters shown in B will depend on the relative importance of the protein binding to each protein and the role of the specific enzyme to overall elimination.*

Additionally, transporters can also have a significant impact on clearance ontogeny but this impact is currently poorly characterized and hence often not considered [35–37]. Because clearance is a moving target across different drugs and different paediatric ages, quantifying its ontogenetic changes for each drug is of utmost importance for paediatric dose tailoring so as to achieve the required exposure in all paediatric patients.

1.3 Quantification of clearance ontogeny across the paediatric age range on the basis of paediatric clinical data

Clearance ontogeny should be accounted for in paediatric dose individualization in order to prevent variability in drug exposure across the paediatric age range. This requires the precise and accurate quantification of clearance ontogeny, which can be achieved through population pharmacokinetic modelling of paediatric clinical data. This model-based approach is recommended in paediatrics by the EMA [38,39] and FDA [40,41] because it can handle opportunistic, and limited blood samples sometimes available in children and, through covariate model building, allows for the quantification and explanation of the large inter-individual variability in pharmacokinetic parameters which is characteristic of the paediatric population [7,8].

Population pharmacokinetic models enable the estimation of individual pharmacokinetic parameters, as described in equation 1 for clearance. CL_i is the clearance value in an individual i, CLpop is the clearance value in the population, $f(cov_i)$ is the covariate model which is a function describing the changes in individual clearance values with the individual covariate value (cov_i), ε_i is the unexplained random variability in clearance for an individual i.

$$CL_i = CLpop \times f(cov_i) + \varepsilon_i \quad (1)$$

A covariate model $f(cov_i)$ that describes inter-individual variability in a pharmacokinetic parameter captures the covariation of this parameter and a variable that can be measured in individual patients, so-called covariate [42], thereby reducing the random unexplained variability in a pharmacokinetic parameter.

During covariate model building, the correlation of different variables with individual pharmacokinetic parameters are tested in order to identify statistically significant and clinically relevant covariates [43]. Most common covariates for clearance in paediatrics are bodyweight alone or together with age (post-natal age, post-conceptional or post-menstrual age, and gestational age) [44]. In case these covariates are identified as predictors of clearance ontogeny across the paediatric age range, they can serve as the basis of paediatric dose adjustment (individualized therapy) [45]. In this respect, since the shape of clearance ontogeny can greatly vary with the covariate distribution in the observed data (e.g., age range studied) and with the studied drug and population**,** the evaluation of different covariate relationships is also of importance [43,46,47].

While in this thesis we focus on developmental changes in clearance, other covariates may be of importance in the pharmacotherapy of paediatric patients, similarly as in other populations, such as abnormal body composition, pharmacogenetics, critical illness and inflammatory status, and the impact of these covariates should also be investigated during covariate model building in order to optimize individualized therapy [48].

1.4 Scaling clearance from adults to paediatric patients in absence of paediatric clinical data

While paediatric dose tailoring should ideally be performed based on covariate models built on paediatric clinical data, such information is lacking for first–in-child clinical trials and for drugs administered in paediatric patients but for which no pharmacological study has been undertaken in this population. To cope with this lack of data, information on clearance ontogeny across the paediatric age range is obtained using scaling functions which rely on covariates such as weight and/or age. Often, paediatric clearance is scaled from available adult values, since adult data is normally available before paediatric clinical trials are performed. To date, a diversity of scaling functions has been proposed to scale clearance across the paediatric population (Table 1). These models differ in their covariate, covariate relationship and hypothesis on which they are based.

From all scaling methods available, PBPK is the most accurate method to scale clearance from adults to young children [33], with its accuracy mainly limited in preterm neonates, as the changes in system-specific parameters have not been well characterized in this population. PBPK models quantify how drug properties, characterized by drug-specific parameters, interact with system-specific parameters that reflect anatomical and physiological measures, and account for the impact of diverse ontogenetic changes in system-specific parameters on paediatric pharmacokinetics. However, PBPK scaling is complex and requires accurate measurements of drug-specific parameters. These parameters can be obtained either from *in vitro* experiments, by extrapolating experimental *in vivo* values from animals to humans, by estimation/prediction using specific algorithms [9], or by estimation based on adult clinical data. These measurements are often not available, particularly for existing drugs, and can be biased, which is for instance the case for historical *in vitro* measurements of drug fraction unbound (fu) in plasma [49] and *in vitro* measurements of unbound microsomal intrinsic clearance (CLint) [49,50]. To ensure accurate PBPK scaling from adults to children, these models

require first validation on adult data, in order to overcome problems arising from biased or missing measurements, during which parameters are refined (estimated) such as unbound intrinsic clearance which is otherwise under predicted based on *in vitro* data [10,51]. However, all parameters cannot be estimated due to structural identifiability problems [52], and accurate *in vitro* measurements of some of the parameters might have to be carried out.

Table 1 *Approaches to scale clearance across the paediatric population*

Method	Covariate model	Covariate
AS0.75 [53]	$\left(\frac{BW}{BW_adult}\right)^{0.75}$	BW
Clark's rule or linear scaling [54,55]	$\left(\frac{BW}{BW_adult}\right)^{1}$	BW
ADE [56]	$\left(\frac{BW}{BW_adult}\right)^{k}$ With k equalling 1.1, 1.0, 0.9 and 0.75, for 0 (term neonates)–3 months, >3 months–2 years, >2–5 years and >5 years respectively	BW and PNA
AS0.75+MF_{PBPK} [57]	$\left(\frac{BW}{BW_adult}\right)^{0.75} \times MF_{PBPK}(AGE)$	BW and AGE
Between drug extrapolation of covariate models [58,59]	Any type of covariate relationship for a drug eliminated by a specific pathway	Any covariate
PBPK [33]	Complex covariate model scaling each physiological parameter, and linking physiological parameters to drug parameters for clearance predictions	PNA and diverse drug properties

AS0.75, allometric scaling using a fix exponent of 0.75; ADE, age-dependent allometric exponent; MF_{PBPK}, isoenzyme maturation function as implemented in PBPK models; PBPK, physiologically-based pharmacokinetics; BW, bodyweight; PNA, post-natal age.

While PBPK is the best scaling method to accurately scale clearance across the paediatric age range, this approach is time consuming, requires specific expertise and sometimes necessitates the conduct of *in vitro* or *in vivo* experiments. Simpler scaling

functions represent a valuable alternative to expedite clearance scaling from adults to children, since they require less information and can be used without extended training. The simplest of these scaling functions solely rely on bodyweight, such as allometric scaling using a fixed exponent of 0.75 (AS0.75) and linear scaling (Table 1). Allometric scaling methods for clearance correlate clearance with size (e.g., bodyweight). Different allometric scaling methods only differ in their allometric exponent which drives the shape of the correlation between clearance and the size descriptor such as bodyweight or body surface area. Because these functions have a poor predictive value in young children, other functions have been proposed.

Inaccuracy of allometric scaling methods often being attributed to the maturational process occurring simultaneously with growth at young ages [47,60,61], Mahmood *et al.* [56,62] have proposed the use of an age-dependent allometric exponent (ADE) in order to account for maturational processes. This method, like AS0.75 or linear scaling, disregards the route of elimination of drugs and can be used to scale clearance of any drug, but relies on the use of different allometric exponents for different age groups. More specifically, with this method, an allometric exponent of 1.1, 1.0, 0.9 and 0.75 is applied for ages 0 (term neonates)–3 months, >3 months–2 years, >2–5 years and >5 years respectively as most recently proposed [56]. More flexible functions than ADE have been proposed to account for the difference in clearance ontogeny of drugs eliminated through different pathways, such as AS0.75+MF_{PBPK} and extrapolation of covariate models between drugs sharing the same elimination pathway (Table 1). AS0.75+MF_{PBPK} has been proposed to scale clearance of drugs undergoing hepatic metabolism. This method scales bodyweight-dependent changes in clearance based on AS0.75 and age-dependent changes in clearance based on isoenzyme ontogeny functions as implemented in PBPK models. Extrapolation of covariate models between drugs sharing the same elimination pathway have been proposed for drugs mainly eliminated by one isoenzyme, but also for drugs undergoing glomerular filtration [63]. For instance, a covariate model describing clearance ontogeny of a drug eliminated through glomerular filtration has been used to predict clearance ontogeny of other drugs eliminated via the same pathway [64]. These functions are semi-physiological covariate models reflecting important mechanisms underlying drug clearance ontogeny [58,59]. Because population PK covariate models are the basis of dose recommendations, this approach would suggest that paediatric dosing recommendations from a drug of which the changes in clearance have been quantified can be

extrapolated to other drugs for which no paediatric PK studies have been undertaken, provided these drugs share the same elimination pathway [65].

1.5 Accurate clearance scaling from adults to paediatric patients: lost in translation

Accurate clearance scaling is important in paediatrics since paediatric dosing regimen of many drugs is derived based on adult clearance scaling due to a lack of paediatric pharmacological data and children may not be subject to dose escalation studies similar to those carried out in adults [66]. While there is a diversity of simple scaling methods available, a clear overview of the applicability of these methods is lacking, leaving clinical pharmacologists without guidance on how to best extrapolate clearance from adult to paediatric patients. Indeed, the accuracy of these different methods has been assessed only on a few case examples, and their comparison to each other is limited due to the use of different types of data, drugs and criterion to assess their accuracy.

AS0.75, which solely relies on the covariate bodyweight, has been recognized as a useful tool to extrapolate clearance from adult values to adolescents [67], but was found to lead to overpredictions in children younger than 5 years [62], reaching up to more than 200 and 3000% in infants and premature neonates, respectively [68]. The latter is often attributed to the maturational process occurring simultaneously with growth at young ages [47,60,61]. ADE, which accounts for maturational processes in young children through the use of different allometric exponents in different paediatric age groups [56,62], was found to lead to prediction error ≤ 63% in children as young as 1 day term neonates based on case examples (n drugs =6) [62].

AS0.75+MF_{PBPK} [57] and extrapolation of covariate models between drugs sharing the same elimination pathway [58,59,64,69,70] both lead to more flexible scaling functions as compared to ADE since differences in maturation (or ontogeny) between different elimination pathways are accounted for. These two scaling methods were found to be accurate in children older than 3 months, and across the entire paediatric age range respectively [57,58,70]. However, both methods have only been investigated for drugs eliminated by one main pathway, while most drug undergo metabolism via several isoenzymes. Moreover, their investigation was limited to CYP3A substrates, and drug eliminated through glomerular filtration or glucuronidation by UGT2B7 respectively [58,59,64,69,70].

In face of this jumble of accuracy assessments, a clear overview of the systematic accuracy of these scaling methods is needed in order to guide their application, which would

ultimately facilitate and accelerate paediatric dose tailoring. Accuracy of these scaling methods depends on their ability to aggregate the influence of ontogeny in diverse system-specific parameters impacting paediatric clearance for diverse drugs. This can be investigated using PBPK simulations of clearance ontogeny across the paediatric age range of diverse drugs [59], since paediatric PBPK models link ontogeny in system-specific parameters and drug properties with clearance ontogeny (Figure 2). Developing a decision tree for a rational choice of appropriate clearance scaling methods would ensure accurate scaling of clearance based on the minimum amount of necessary information, making sure that neither important ontogeny processes impacting clearance nor clinicians get lost in translation.

While PBPK models represent a valuable tool to understand important ontogenic changes that should be accounted for to accurately predict clearance ontogeny of diverse drugs, further development of these models for different understudied paediatric populations is needed. For instance, it is known that premature birth can lead to different clearance ontogeny patterns than birth at term. Information on system-specific parameters in these populations is important and currently attempts in developing such models have been published [71]. However, development of such models currently requires an extensive amount of data which is difficult and sometimes impossible to collect in young children, such as hepatic blood flow measurements or intrinsic clearance values for hepatic metabolic routes, and therefore these models need to go through many learn and confirm circles before one can become confident in their predictive performance. Hence, accurate scaling of clearance for different paediatric subpopulations will require development of innovative approaches that can tackle the lack of data on their system-specific parameters.

1.6 Conclusion

There is an urgent need to accurately scale drug clearance in children. Gaining a mechanistic insight on the most important drivers of clearance ontogeny for diverse drugs would enable for the definition of guidance for the application of clearance scaling functions and the development of new ones in order to accurately extrapolate clearance from adults to children. This would ultimately facilitate and accelerate dose tailoring in paediatrics.

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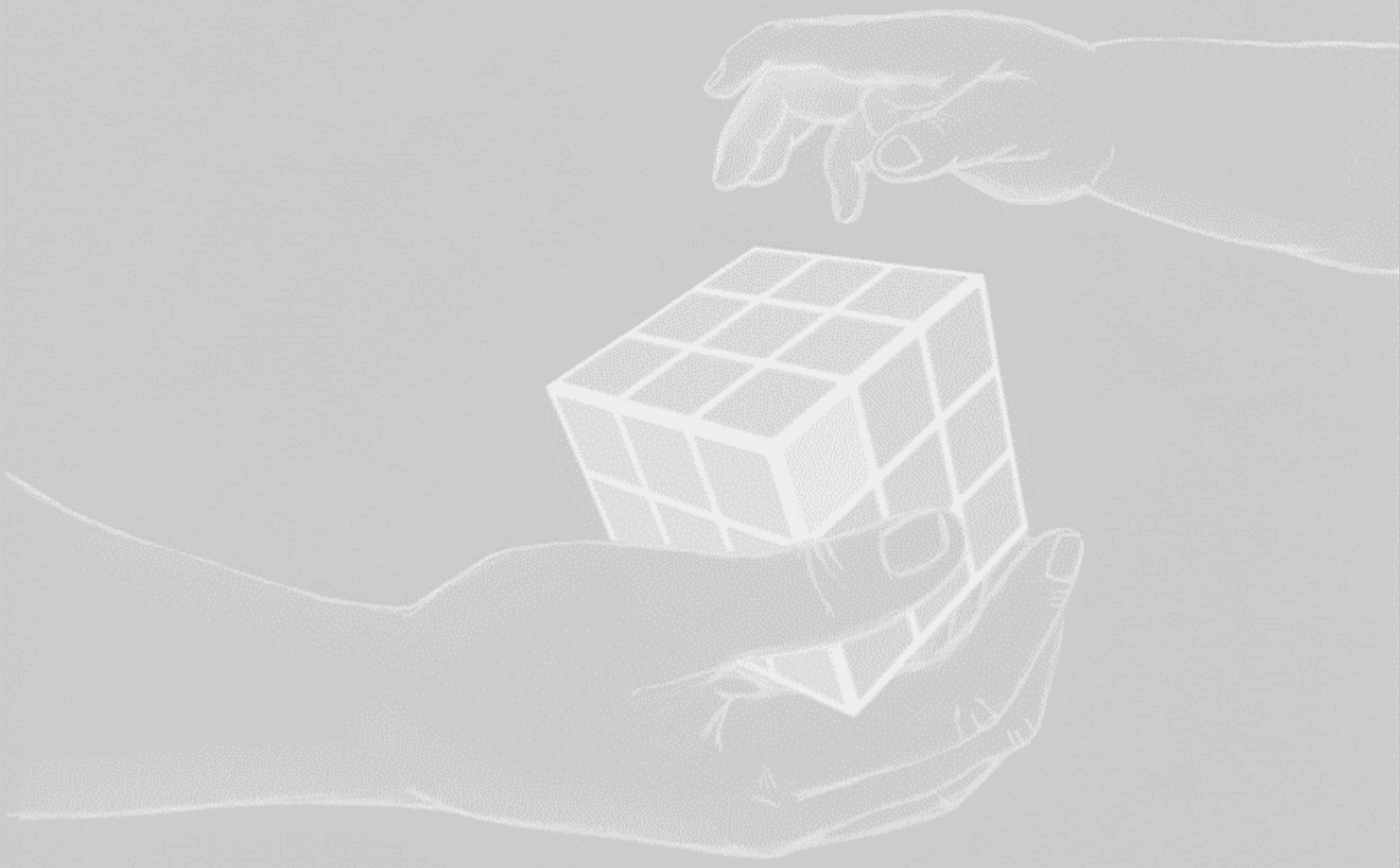
Chapter 2

Scope and intent of the investigations



Clearance is a key parameter for efficacious dose tailoring in paediatric patients. This pharmacokinetic parameter undergoes ontogenic changes, which can be quantified across the paediatric age range using model-based approaches. Models describing ontogenic changes in clearance are the basis of individualized therapy, ultimately preventing variability in drug exposure across the paediatric age range. Such models should be developed and validated on paediatric clinical data in order to ensure safe and efficacious drug treatment. However, such data is lacking for many marketed drugs and for first-in-child clinical trials, leaving many children potentially over- or under-dosed. To ensure safe and efficacious dosing while coping with this lack of data, information on clearance ontogeny across the paediatric age range is obtained using scaling functions which rely on covariates such as weight and/or age. Often, paediatric clearance is scaled from previously measured adult values, since adult data is generally available before undertaking paediatric clinical trials. To date, a diversity of scaling functions has been proposed to scale clearance across the paediatric population. These models require different extents of information for their application.

While Physiologically-Based PharmacoKinetic (PBPK) is the best scaling method to accurately scale clearance across the paediatric age range in absence of paediatric clinical pharmacokinetic data, this approach is time consuming, requires specific expertise and sometimes necessitates the conduct of *in vitro* or *in vivo* experiments. Simpler scaling functions represent a valuable alternative to expedite clearance scaling from adults to children, since they require less information and can be used without extended training. While there are many simple scaling methods available (Chapter 1, Table 1), a clear overview of their applicability is lacking, leaving clinical pharmacologists without guidance on how to best extrapolate clearance from adult to paediatric patients.

Accuracy of these scaling methods depends on their ability to aggregate the influence of ontogeny in diverse system-specific parameters impacting paediatric clearance for diverse drugs (Chapter 1, Figure 2). This can be investigated using PBPK simulations of clearance ontogeny of diverse drugs across the paediatric age range [1], since paediatric PBPK models link ontogeny in system-specific parameters and drug properties with clearance ontogeny. Developing a decision tree for a rational choice of appropriate clearance scaling methods would ensure accurate clearance scaling while using the minimum amount of prior data, which would ultimately facilitate and accelerate paediatric dose tailoring and would steer efforts of future research in this field. While PBPK models represent a valuable tool to understand important ontogenic changes that should be accounted for to accurately predict clearance

ontogeny of diverse drugs, further development of these models for different understudied paediatric populations is needed. Therefore, the aim of this thesis is to expedite and ensure the systematic accuracy of clearance scaling from adults to paediatric patients, with a special focus on drugs undergoing hepatic metabolism.

In **Section II**, using a PBPK-based simulation workflow, we systematically investigate two methods that scale clearance by solely accounting for bodyweight differences across the paediatric age range. The accuracy of clearance scaling from adults to different paediatric ages, from term neonates of one day to adolescent of 15 years, is investigated for both drugs undergoing hepatic metabolism and drugs undergoing glomerular filtration presenting a wide range of drug properties. Scenarios for which these scaling methods systematically lead to accurate paediatric clearance predictions are defined in order to guide their use. **Chapter 3** specifically investigates allometric scaling using a fixed exponent of 0.75 (AS0.75). Additionally, in this chapter, drugs for which clearance ontogeny is only influenced by size-related changes are selected, and the value of the allometric exponent scaling the clearance of these drugs from adults to the different investigated paediatric ages is estimated. This is performed in order to investigate the strong belief that size-related changes scale with a unique allometric exponent of 0.75 across the paediatric age range. **Chapter 4** specifically investigates allometric scaling using a fixed exponent of 1, also known as linear scaling. This simple scaling method is often used to adapt paediatric doses from adult dosing in the clinic when no dosing guidelines are available. Additionally, in this chapter, scaling accuracy of linear scaling is compared to scaling accuracy of AS0.75.

In **Section III**, using the same PBPK-based simulation workflow, we systematically investigate the accuracy of scaling methods accounting for age-related changes in general or ontogeny in hepatic isoenzyme responsible for drug clearance. Scenarios for which these scaling methods systematically lead to accurate paediatric clearance predictions are defined in order to guide their use. **Chapter 5** identifies conditions for which extrapolation of covariate models between drugs sharing the same elimination pathway consistently leads to accurate clearance scaling from adults to different paediatric ages, from term neonates of one day to adolescent of 15 years. Because population PK covariate models are the basis of dose recommendations, this investigated scaling approach could also allow for extrapolation of paediatric dosing recommendations from a drug of which the changes in clearance have been quantified to other drugs for which no paediatric PK studies have been undertaken, provided these drugs share the same elimination pathway. Since most drugs are metabolized by several

isoenzymes, the impact of elimination through multiple elimination pathways on drug clearance is assessed.

Chapter 6 systematically assesses and compares the scaling accuracy of age-dependent exponent (ADE) [2] and AS0.75+MF_{PBPK} [3] in children younger than five years for drugs undergoing hepatic metabolism. These two scaling methods have been proposed to circumvent the reported inaccuracy of AS0.75 in young children, which is believed to be due to maturational processes occurring simultaneously with growth at young ages [4–6]. ADE relies on the use of different allometric exponents for different age groups, and is proposed to scale clearance of any drugs, disregarding their route of elimination. AS0.75+MF_{PBPK} scales bodyweight-dependent changes in clearance based on AS0.75 and age-dependent changes in clearance based on isoenzyme ontogeny functions as implemented in PBPK models. AS0.75+MF_{PBPK} is a scaling method for drugs undergoing hepatic metabolism and is a more flexible function than ADE since it accounts for the different ontogeny patterns of isoenzymes responsible for drug clearance. Comparison of these two methods allows for the assessment of whether specific ontogeny patterns of different isoenzyme pathways are needed for accurate clearance predictions in young children.

In **Section IV**, **Chapter 7**, we propose the estimation of key PBPK parameters based on clinical data using population modelling as a solution to circumvent the lack of data on system-specific parameters of PBPK models which imped their development for specific paediatric subpopulations. We develop a methodology to investigate the feasibility and requirements for precise and accurate estimation of PBPK parameters using population modelling of clinical data and illustrate this for two key PBPK parameters for hepatic metabolic clearance, namely whole liver unbound intrinsic clearance and hepatic blood flow in children.

In **Section V, Chapter 8**, results and conclusions of this thesis are summarized and discussed and a decision tree for the applicability of the scaling methods investigated is provided based on the results of this thesis. Moreover, future perspectives are presented. Perspectives are given concerning the use of the decision tree to select an appropriate clearance scaling method and ideas are provided to enable further improvement of paediatric PBPK models to extend their use to understudied drugs and patient populations. Finally, we provide good modelling practice for the estimation of the allometric exponent to scale both size- and age-related changes in clearance.

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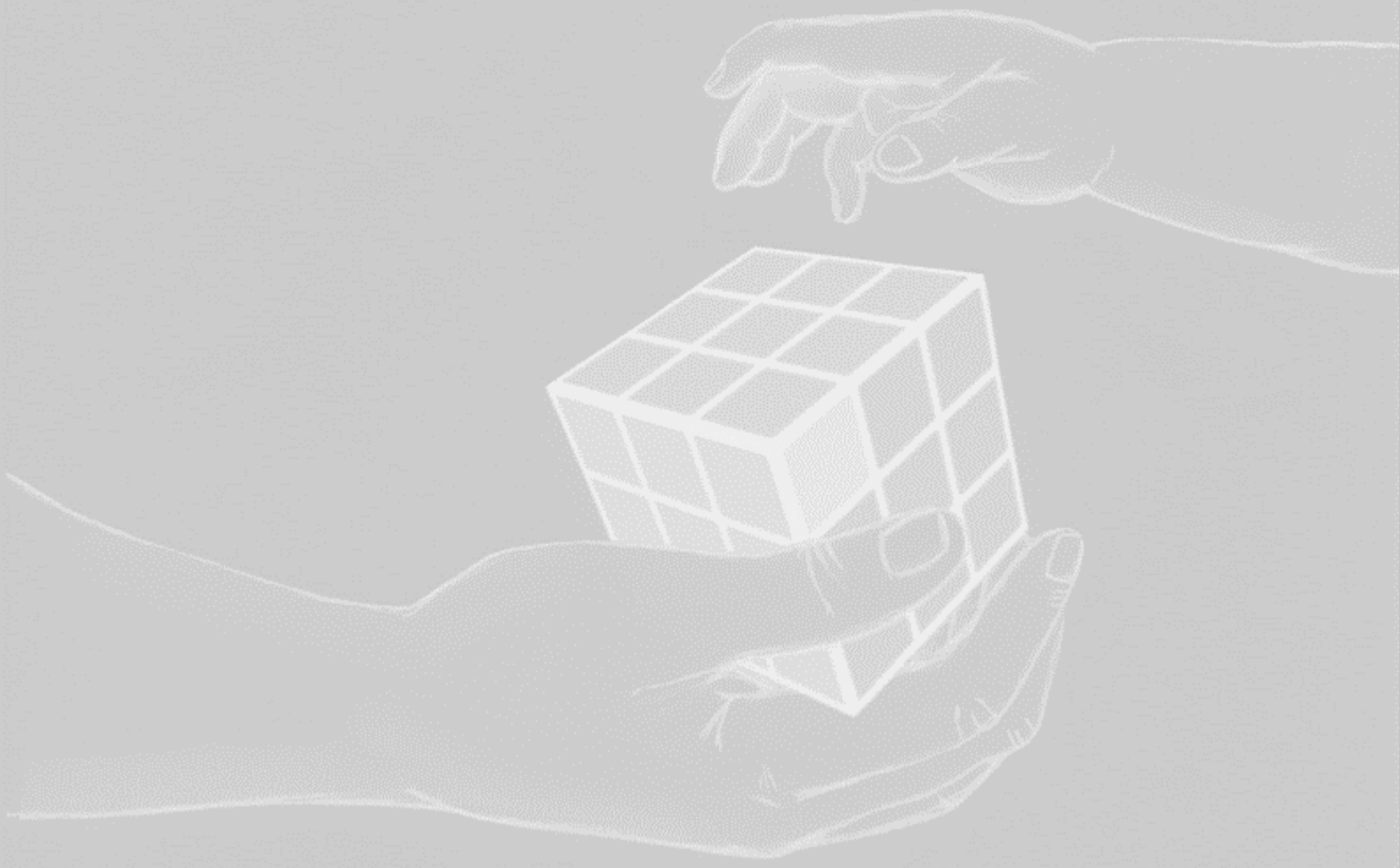
Section II

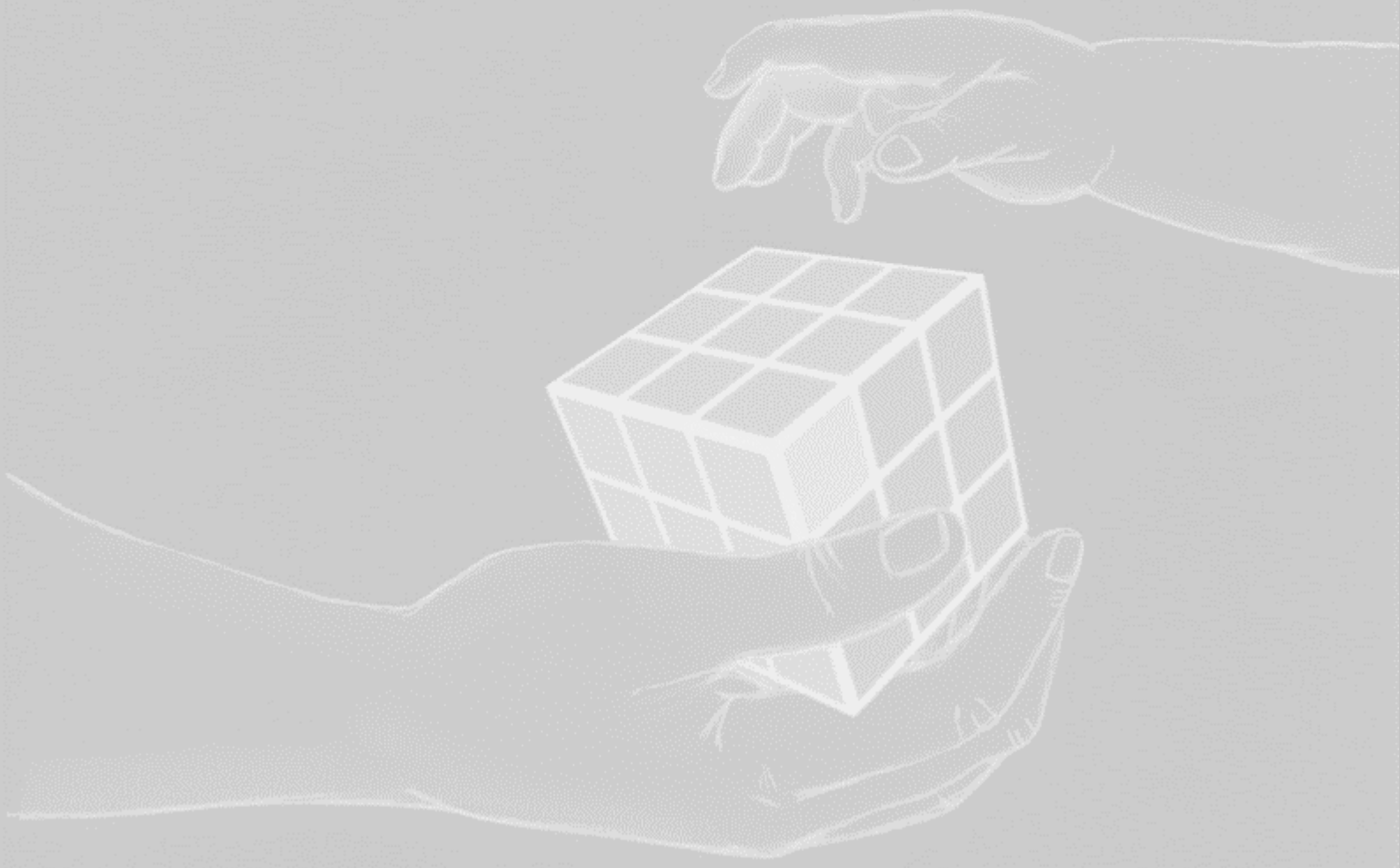
Scaling methods based on bodyweight to extrapolate adult clearance to paediatric patients



Chapter 3

Allometric scaling of clearance in paediatric patients: when does the magic of 0.75 fade?

Elisa A. M. Calvier, Elke H. J. Krekels, Pyry A. J. Välitalo, Amin Rostami-Hodjegan, Dick Tibboel, Meindert Danhof, Catherijne A. J. Knibbe



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Abstract

Allometric scaling on the basis of bodyweight raised to the power of 0.75 (AS0.75) is frequently used to scale size-related changes in plasma clearance (CLp) from adults to children. A systematic assessment of its applicability is undertaken for scenarios considering size-related changes with and without maturation processes. A physiologically-based pharmacokinetic (PBPK) simulation workflow was developed in R for 12,620 hypothetical drugs. In scenario one, only size-related changes in liver weight, hepatic blood flow, and glomerular filtration were included in simulations of 'true' paediatric CLp. In a second scenario, maturation in unbound microsomal intrinsic clearance ($CL_{int,mic}$), plasma protein concentration, and haematocrit were also included in these simulated 'true' paediatric CLp values. For both scenarios, the prediction error (PE) of AS0.75-based paediatric CLp predictions was assessed, while, for the first scenario, an allometric exponent was also estimated based on 'true' CLp. In the first scenario, the PE of AS0.75-based paediatric CLp predictions reached up to 278% in neonates, and the allometric exponent was estimated to range from 0.50 to 1.20 depending on age and drug properties. In the second scenario, the PE sensitivity to drug properties and maturation was higher in the youngest children, with AS0.75 resulting in accurate CLp predictions above 5 years of age. Using PBPK principles, there is no evidence for one unique allometric exponent in paediatric patients, even in scenarios that only consider size-related changes. As PE is most sensitive to the allometric exponent, drug properties and maturation in younger children, AS0.75 leads to increasingly worse predictions with decreasing age.

Key points

- There is no evidence for a universal allometric exponent for scaling drug plasma clearance (CLp) from adults to children.
- When scaling CLp to children, the prediction error (PE) is not sensitive to the allometric exponent in children above the age of 5 years, resulting in accurate CLp predictions with allometric scaling in this age range for drugs eliminated by glomerular filtration or for drugs undergoing hepatic metabolism when enzyme activity is close to adult values.
- In children below the age of 5 years, the PE in scaled CLp becomes increasingly more sensitive to the allometric exponent, drug properties and maturation, leading to biased CLp predictions with allometric scaling.

3.1 Introduction

Allometric scaling's first main domain of application was scaling of the basal metabolic rate between species, after which a series of investigations (principally in mammals) suggested that interspecies differences in the basal metabolic rate were best described by a bodyweight-based exponential relationship with an exponent close to 0.75 [1–7]. Several theories have been developed to support the existence of a universal allometric scaling exponent [8–11]; however, these theories have been criticized, and discrepancies between the theory and observations have been reported [12–18].

The use of the allometric equation was extended to the scaling of size-related changes in clearance between species [19] and thereafter to the scaling of clearance from adults to children [20]. While different universal values [1,21] for the allometric exponent have been proposed, a value of 0.75 is commonly applied in order to scale clearance from adults to children. While allometric scaling is applicable to both plasma and whole blood clearance, in this work we focus on allometric scaling of plasma clearance (CLp) using an exponent of 0.75, as this is the most commonly used for clearance parameters. This scaling approach will be referred to as AS0.75 in this work. This approach is appealing because it is a simple and fast method to scale paediatric CLp. However, this use of AS0.75 relies on two important implicit assumptions, i.e., the assumption of equivalence between basal metabolic rate and clearance, and the assumption of equivalence between inter- and intraspecies scaling. As illustrated in Figure 1, for each of these assumptions separately, AS0.75 was found not to be supported by experimental data, challenging the belief of the strong theoretical and empirical basis of AS when applied in scaling paediatric clearance [22–31].

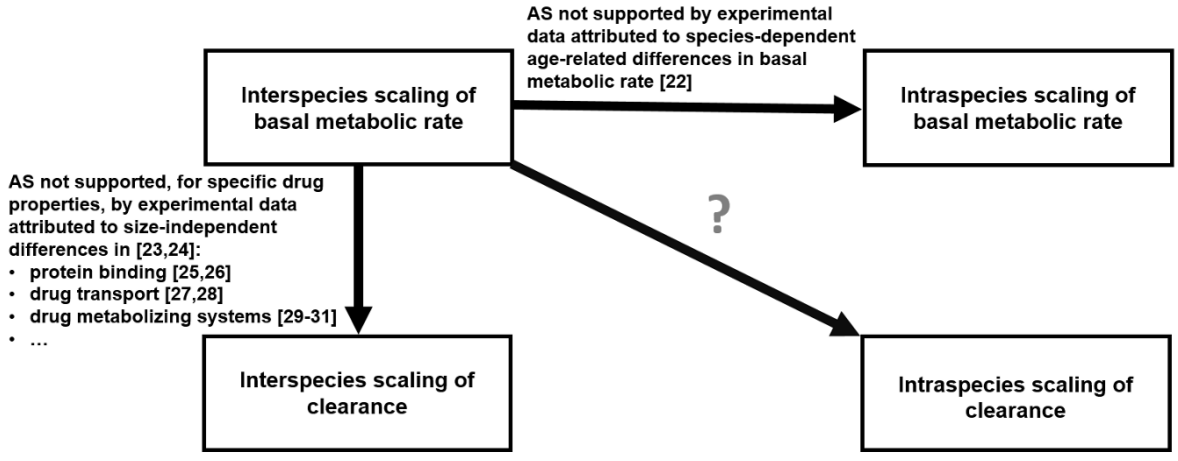

Figure 1 *Implicit assumptions underlying the use of AS to scale CLp from adults to children. AS, allometric scaling; CLp, plasma clearance.*

For the scaling of CLp in the paediatric population, AS0.75 has been recognized as a useful tool to extrapolate CLp from adult values to adolescents [32], while overpredictions in children younger than 5 years [33], reaching up to more than 200 and 3000% in infants and premature neonates, respectively, have been reported [34]. The latter is often attributed to the maturational process occurring simultaneously with growth at young ages [35], similar to what was concluded for the intraspecies scaling of the basal metabolic rate [22]. To cope with this, the estimation of a maturation function (MF) in addition to AS0.75 has been proposed [35]. Because of the high correlation between age and size, and consequently the difficulty in disentangling the influence of size-related changes from the influence of age-related changes, their distinction has hitherto been mainly driven by the AS0.75 theory, according to which all changes in CLp that are not accounted for by AS0.75 are due to maturation. Moreover, the accuracy of AS0.75 of CLp from adults to children has been evaluated for a limited number of drugs and it is unknown how the accuracy is impacted by various drug properties.

The aim of this study was to unravel in which situations AS0.75 consistently leads to accurate paediatric CLp predictions. To perform a systematic investigation, we developed a physiologically-based pharmacokinetic (PBPK) workflow for a wide range of hypothetical drugs cleared through hepatic metabolism or glomerular filtration (GF), and explored two scenarios—a scenario including size-related changes only, and a scenario including size-related changes as well as maturational changes. This methodology allowed for the disentanglement of the impact of drug properties, size-related changes, and maturation processes on the performance of AS0.75 in predicting paediatric CLp from adult values.

3.2 Methods

A PBPK simulation workflow was developed in R version 3.0.2 (Figure 2). This workflow was used to investigate the accuracy of AS0.75 in predicting paediatric CLp, simulated based on PBPK principles, for a total of 12,620 hypothetical drugs (X).

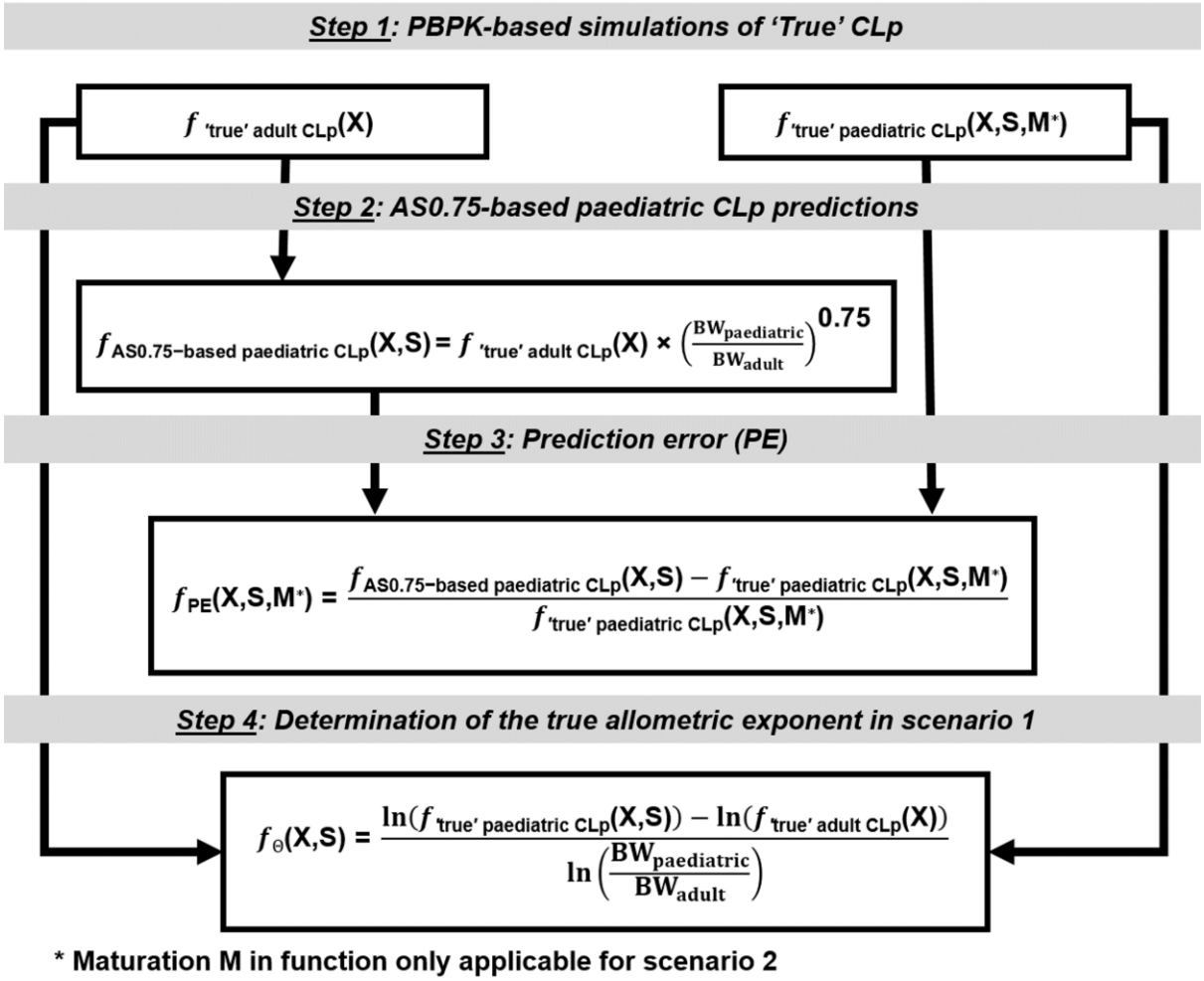

Figure 2 *PBPK simulation workflow investigating the predictive performance of AS0.75 on the basis of bodyweight to the power of 0.75, when scaling paediatric CLp from adult CLp values. The simulations include two scenarios: scenario 1 includes size-related changes only, while scenario 2 includes both size-related changes and maturation in the PBPK-based predictions of paediatric CLp. Simulated CLp values are functions of X representing drug-specific properties (i.e., fu, type of binding plasma protein, $CL_{int,mic}$, and Kp). Paediatric CLp predictions also depend on S representing size-related changes (changes in liver weight, hepatic blood flow, and glomerular filtration) and, in scenario 2, also on M, which represents maturational changes (changes in plasma protein concentration, haematocrit and $CL_{int,mic}$). PBPK, physiologically-based pharmacokinetic; CLp, plasma clearance; fu, unbound drug fraction in plasma; $CL_{int,mic}$, unbound microsomal intrinsic clearance; Kp, blood to plasma partition coefficient.*

3.2.1 Hypothetical drugs (X)

In the simulations, numerous different hypothetical drugs were investigated to explore the dependence of AS0.75 predictive performance on drug properties, as drug properties have been shown to influence the predictive performance of AS0.75 in interspecies scaling of clearance (see Figure 1). Five drug-specific properties were used to generate a wide range of

hypothetical drugs (X); namely, route of elimination, type of binding plasma protein, affinity to the plasma protein, blood to plasma partition coefficient (Kp), and the unbound intrinsic clearance value of one microgram of liver microsomes ($CL_{int,mic}$). Thus, the design space was a five-dimensional hypercube, with each drug property corresponding to one dimension.

The two investigated routes of elimination were hepatic metabolism and GF. The hypothetical drugs were exclusively bound either to human serum albumin (HSA) or α1-acid glycoprotein (AGP). The affinity to plasma proteins were derived from the unbound drug fraction in plasma (fu) in adults and the plasma concentration of the binding plasma protein in adults [36], using equations derived from Rodgers and Rowland [37]. The fu in adults ranged from 1 to 100%, with eight equidistant intermediate values. Because the fu is more frequently reported than drug affinity to plasma protein, the fu in adults, together with the type of binding plasma protein, was retained for drug categorization. Kp values of 0, 1, 2, 3 and 4 were selected, reflecting different extents of drug diffusion in the red blood cells. $CL_{int,mic}$ ranged between 0.56×10^{-3} and 0.209 mL min^{-1} mg^{-1} microsomal protein [38], with 124 equidistant intermediate values. The different $CL_{int,mic}$ values reflect different affinities for and abundances of enzymes.

Hypothetical drugs undergoing hepatic metabolism were generated based on all possible combinations of the aforementioned drug properties, while hypothetical drugs undergoing GF were generated by all possible combinations of affinity to plasma protein and type of binding plasma protein only, as $CL_{int,mic}$ and Kp do not impact their clearance.

3.2.2 Simulation scenarios

Two different scenarios regarding maturation (scenario 1 and scenario 2) were included in the PBPK-based simulation workflow, and an overview of these scenarios is displayed in Table 1.

The first scenario (scenario 1) was designed in order to answer the question as to whether scaling using an allometric exponent of 0.75 accounts for all size-related changes in the processes underlying CLp in the paediatric population. Therefore, for this scenario, only size-related changes were included in the simulations of PBPK-based 'true' paediatric CLp values. Size-related changes (S) included in the PBPK simulations are changes in liver weight, hepatic blood flow, and GF rate (GFR) [see step 1 of the PBPK simulation workflow for details on size-related changes]. In this scenario, for drugs undergoing hepatic metabolism, enzymes

were considered to have reached maturity ($CL_{int,mic}$ was set to be 100% of the adult value). Furthermore, for this scenario, only drugs that do not bind to plasma proteins and which are in equilibrium between plasma and red blood cells (Kp of 1) were included in order to exclude the potential influence of maturational changes on CLp (see Eqs. 15 and 22–24 in the Supplementary Material). This drug selection is equivalent to a selection of probe drugs for which only size-related changes would impact the clearance.

A second scenario (scenario 2) was designed to investigate how maturation processes influence the applicability of AS0.75 in the paediatric population, in order to unravel the conditions in which AS0.75 consistently leads to accurate paediatric CLp predictions. Therefore, for this scenario, size-related changes (S) were included in the simulations of PBPK-based 'true' paediatric CLp values, as well as maturation processes (M), which are believed not to be corrected for by AS0.75. Maturation processes included maturation in plasma protein concentration and haematocrit, as well as hepatic enzyme maturation (see step 1 of the PBPK simulation workflow for details on maturation). For this scenario, all hypothetical drugs were included.

Table 1 *Overview of the two scenarios investigated in the PBPK-based simulation workflow*
PBPK, physiologically-based pharmacokinetic.

	Scenario 1	Scenario 2
Aim and research question	Investigation of AS0.75 theory	Investigation of general applicability of AS0.75 in paediatrics
	Does an allometric exponent of 0.75 account for size-related changes in paediatric CLp?	Under which conditions AS0.75 consistently leads to accurate paediatric CLp predictions?
PBPK-based 'true' CLp	Only size-related changes (S) were included in the PBPK simulations of true CLp	Both size-related changes (S) and maturation (M) were included in the PBPK simulations of true CLp
	S includes changes in liver weight, hepatic blood flow, and glomerular filtration rate	M includes maturation in plasma protein concentration and in haematocrit as well as hepatic enzyme maturation
Selection of hypothetical drugs	Selection of hypothetical drugs equivalent to a selection of probe drugs for which only size-related changes would impact the clearance, by retaining drugs with: fu = 1 Kp = 1	All hypothetical drugs
	The number of selected drugs:	The number of drugs:
	undergoing GF was 1	undergoing GF was 20
	undergoing hepatic metabolism was 126	undergoing hepatic metabolism was 12600

3.2.3 Physiologically-Based Pharmacokinetic (PBPK) simulation workflow

3.2.3.1 Step 1: PBPK-based simulations of 'true' plasma clearance (CLp)

For both scenarios, PBPK principles were used to simulate 'true' CLp values for the hypothetical drugs with different properties (X; see the 3.2.1 section) in term neonates aged 1 day, infants aged 1 and 6 months, children aged 1, 2, and 5 years, adolescents aged 15 years, and adults (Figure 2). For drugs undergoing hepatic metabolism, CLp was computed using the dispersion model (Eqs. 1–6), with an axial dispersion number (D_N) of 0.17 [39]. The dispersion model was selected as it has been reported to better predict clearance than the well-stirred model for high-clearance drugs, which are also included in the hypothetical drugs, while both models lead to equivalent clearance predictions for the rest of the drugs [40]:

$$\mathrm{CLp} = \mathrm{CL_B} \times \mathrm{B{:}P} \quad (1)$$

$$\mathrm{CL_B} = \mathrm{Q_H} \times \mathrm{E_H} \quad (2)$$

$$\mathrm{E_H} = 1 - \mathrm{F_H} \quad (3)$$

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

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (5)$$

$$\mathrm{R_N} = (\mathrm{fu/B{:}P}) \times \mathrm{CLint/Q_H} \quad (6)$$

In these equations, CLp is the plasma clearance, B:P is the blood to plasma ratio, CL_B is the whole blood clearance, Q_H is the hepatic blood flow, E_H is the hepatic extraction ratio, fu is the unbound drug fraction in plasma, CLint is the hepatic intrinsic clearance, and D_N is the dispersion number. For drugs undergoing GF, CLp was computed as the product of GFR [36] and fu.

Adult demographic values (height and weight) and adult values for cardiac output were taken from the ICRP annals [41]. CLint in adults was computed as the product of $CL_{int,mic}$, amount of microsomal protein per gram of liver, and liver weight [36]. B:P in adults was derived from the Kp value, adult haematocrit [42], and fu in adults. $CL_{int,mic}$, Kp and fu in adults were taken from the values defined to generate the hypothetical drugs (see the 3.2.1 section).

Paediatric demographic values (height and weight) were taken from the CDC growth charts [43], and paediatric cardiac output values were compiled from Johnson *et al.* [36]. Size-related changes in CLint were accounted for by changes in liver weight, and size-related

changes in GF were accounted for by changes in GFR [36]. Maturation of CLint was accounted for by selected extents of enzyme maturation [36]. For all hypothetical drugs undergoing hepatic metabolism, different extents of enzyme maturation, ranging between 10 and 200% of the adult $CL_{int,mic}$ value, were investigated for each paediatric age. Additionally, scenarios were simulated with published enzyme maturation values for different hepatic isoenzymes [36,44–47]. For these scenarios, values for enzyme maturation in neonates aged 1 day are based on values for term neonates. Fu was scaled from adults to children by accounting for maturation in plasma protein concentration (HSA and AGP) [36]. B:P was scaled from adults to children by accounting for maturation in haematocrit [42] and plasma protein concentrations [36].

The 'true' CLps herein simulated are a function of the properties of the hypothetical drug (X), size-related changes (S), and, in scenario 2 (*), maturation (M): $f_{\text{'true' CLp}}(X,S,M^*)$. In order to allow for computation across a wide parameter space and improve interpretability of the results, single-point estimates of $f_{\text{'true' CLp}}(X,S,M^*)$ were studied by transforming X, S, and M in categorical variables using typical values for the model parameters. For the same reasons, variability or uncertainty in demographic parameters or model parameters was not taken into account. Details on all equations used in the PBPK model and parameter values used in the simulations can be found in the Supplementary Material.

3.2.3.2 Step 2: AS0.75-based paediatric CLp predictions

In the AS0.75 equation (Figure 2), bodyweight (BW) is used as a descriptor of size. AS0.75-based CLp predictions for children [$f_{\text{AS0.75-based paediatric CLp}}(X,S)$] were performed in both scenarios for all included combinations of hypothetical drugs (X) and size-related changes (S), using the AS0.75 relationship. For scenario 1, only drugs that do not bind to plasma proteins (fu = 1) and which are in equilibrium between plasma and red blood cells (Kp of 1) were included in order to exclude the potential influence of maturational changes on CLp (see the 3.2.2 section, as well as Eqs. 15 and 22–24 in the Supplementary Material).

In this AS0.75 relationship, the typical bodyweight for the different investigated paediatric ages (BWpaediatrics) was normalized to the bodyweight of a typical adult (BWadult). The typical bodyweight for the different paediatric ages and adults were kept the same as for the PBPK-based CLp simulations (see Supplementary Material).

3.2.3.3 Step 3: Prediction error (PE)

For both scenarios, the prediction error (PE) of AS0.75 using the fixed allometric exponent of 0.75 in predicting 'true' PBPK-based paediatric CLp was determined for all included combinations of hypothetical drugs (X) and size-related changes (S) [scenarios 1 and 2] and, where relevant, maturation (M) [scenario 2]. For scenario 1, only drugs that do not bind to plasma proteins (fu = 1) and which are in equilibrium between plasma and red blood cells (Kp of 1) were included in order to exclude the potential influence of maturational changes on CLp (see the 3.2.2 section, as well as Eqs. 15 and 22–24 in the Supplementary Material). Because this workflow relies on 'true' CLp predictions from simulations that do not take parameter uncertainty into account, predictions leading to PEs within an interval of ±30% were considered accurate.

For both scenarios, results were categorized by age and route of elimination (hepatic metabolism or GF). For scenario 2, in which both size-related changes and maturation were included, results were also categorized by type of binding plasma protein and extent of enzyme maturation. The fu, Kp, B:P, and extraction ratio were screened for potential additional categorization.

3.2.3.4 Step 4: Determination of the true allometric axponent in scenario 1

To investigate patterns in potential misspecification by AS0.75, which could be useful in deriving general rules regarding the applicability of AS0.75, the true allometric exponent (Ɵ) was derived for the scenario including only size-related changes (scenario 1); the true allometric exponent Ɵ was determined by rearranging the AS0.75 equation (Figure 2). For each of the hypothetical drugs of this scenario, the PE obtained with AS0.75 was plotted against the true allometric exponent in order to evaluate the impact of the fixed 0.75 exponent instead of the true allometric exponent on the predictions accuracy.

3.3 Results

3.3.1 Scenario 1

Figure 3 shows the PE of AS0.75 in predicting 'true' paediatric CLp versus the true allometric exponent for each paediatric age for the scenario including size-related changes

only (scenario 1) in the simulations of PBPK-based 'true' paediatric CLp values (see the 3.2.2 section). Figure 3a shows the results for 126 drugs undergoing hepatic metabolism only differing in their $CL_{int,mic}$ values, and Figure 3b shows the results for one drug undergoing GF, as, for this scenario, only drugs that do not bind to plasma proteins (fu = 1) and which are in equilibrium between plasma and red blood cells (Kp of 1) were included in order to exclude the potential influence of maturational changes in these parameters on CLp (see the 3.2.1 and 3.2.2 sections, as well as Eqs. 15 and 22–24 in the Supplementary Material). In this scenario, the true allometric exponent ranged from 0.52 to 0.88 for drugs undergoing hepatic metabolism, and from 0.50 to 1.20 for drugs undergoing GF. For both elimination routes, the true allometric exponent was higher in the youngest age groups. For drugs undergoing hepatic metabolism, the true allometric exponent also slightly increased with decreasing $CL_{int,mic}$ in children aged 5 years and older, with opposite results in children younger than 5 years of age.

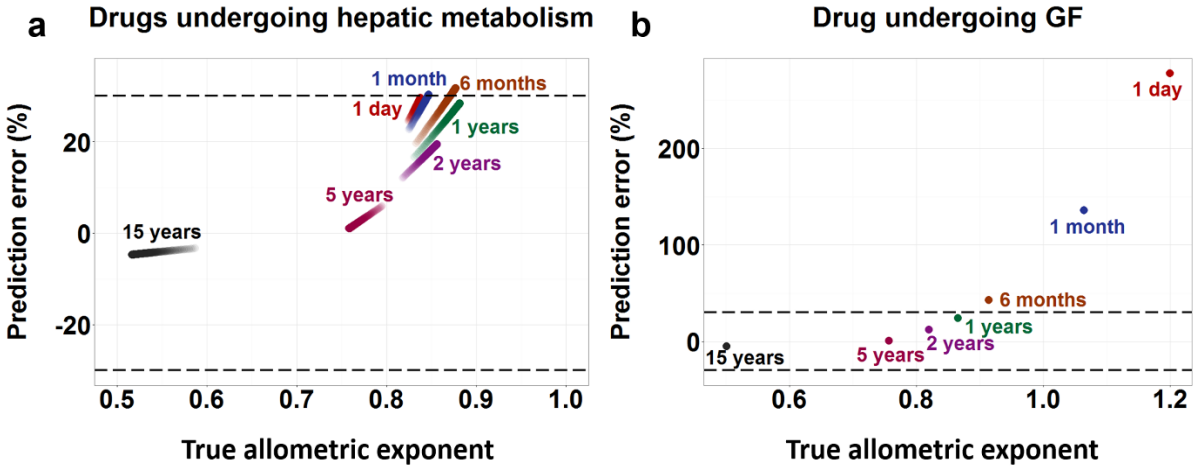

Figure 3 *PE of AS0.75 using the fixed allometric exponent of 0.75 in predicting 'true' paediatric CLp versus the true allometric exponent for each paediatric age, for drugs undergoing (a) hepatic metabolism or (b) glomerular filtration in scenario 1, where only size-related changes were included in the PBPK-based simulations of 'true' paediatric CLp. The values for the different ages are represented with different colours. Colour intensity increases with $CL_{int,mic}$ values of the hypothetical drugs (a). Dotted black lines indicate a PE of ±30%. PE, prediction error; CLp, plasma clearance; PBPK, physiologically-based pharmacokinetic; $CL_{int,mic}$, unbound microsomal intrinsic clearance.*

The PE resulting from the use of the fixed allometric exponent of 0.75 instead of the true allometric exponent was higher in the youngest children. For drugs undergoing hepatic metabolism, PEs mostly remained within the ±30% limits, with highest values reaching up to

32% in children aged 6 months. However, for drugs undergoing GF, PE was higher than 30% in children aged 6 months, and reached up to 278% in neonates aged 1 day. Moreover, the absolute PE increased with decreasing $CL_{int,mic}$ in children aged 5 years and older, while it decreased with decreasing $CL_{int,mic}$ in children younger than 5 years of age. For children aged 2 years and younger, drugs undergoing hepatic metabolism with similar true allometric exponents were found to lead to increased PE with decreasing age, showing a higher sensitivity of the PE to the use of a fixed allometric exponent of 0.75 in younger children.

3.3.2 Scenario 2

3.3.2.1 PE for hypothetical drugs undergoing hepatic metabolism

Figure 4 shows the PE for the AS0.75-based paediatric CLp predictions in scenario 2 for drugs undergoing hepatic metabolism. Results are categorized by age, type of binding plasma protein, and extent of enzyme maturation. The extraction ratio in adults was found to be most predictive of the PE compared with fu, Kp or B:P. For this reason, results were also categorized by adult extraction ratio in boxplots, displaying the maximum, third quartile, median, first quartile and minimum PE for each category.

The PE range increased with decreasing age and was wider for drugs binding to AGP than for drugs binding to HSA. The more the enzyme maturation differed from 100% of the adult $CL_{int,mic}$ value, the more the PE range increased, leading to the smallest PE range for enzyme maturation of 100%. Enzyme maturation had less effect on the PE for high extraction ratio drugs compared with low extraction ratio drugs.

For drugs undergoing hepatic metabolism, AS0.75 led to accurate predictions for all hypothetical drugs in adolescents aged 15 years and children aged 5 years when $CL_{int,mic}$ was between 75% and 100%, and at 100% of the adult value, respectively. In a situation where $CL_{int,mic}$ is at 100% of the adult value, 'true' CLp with a PE within ±30% for all investigated ages is predicted only in cases of drugs with a low extraction ratio and binding to HSA. For the remaining drugs, the absolute PE ranged up to 50% in children as young as 6 months of age, and even higher in neonates. Regardless of age, AS0.75 performed poorly in predicting 'true' paediatric CLp of drugs metabolized by enzymes for which maturation is at or below 50% of the adult $CL_{int,mic}$ value, leading to overprediction of more than 50% for a large part of the investigated hypothetical drugs.

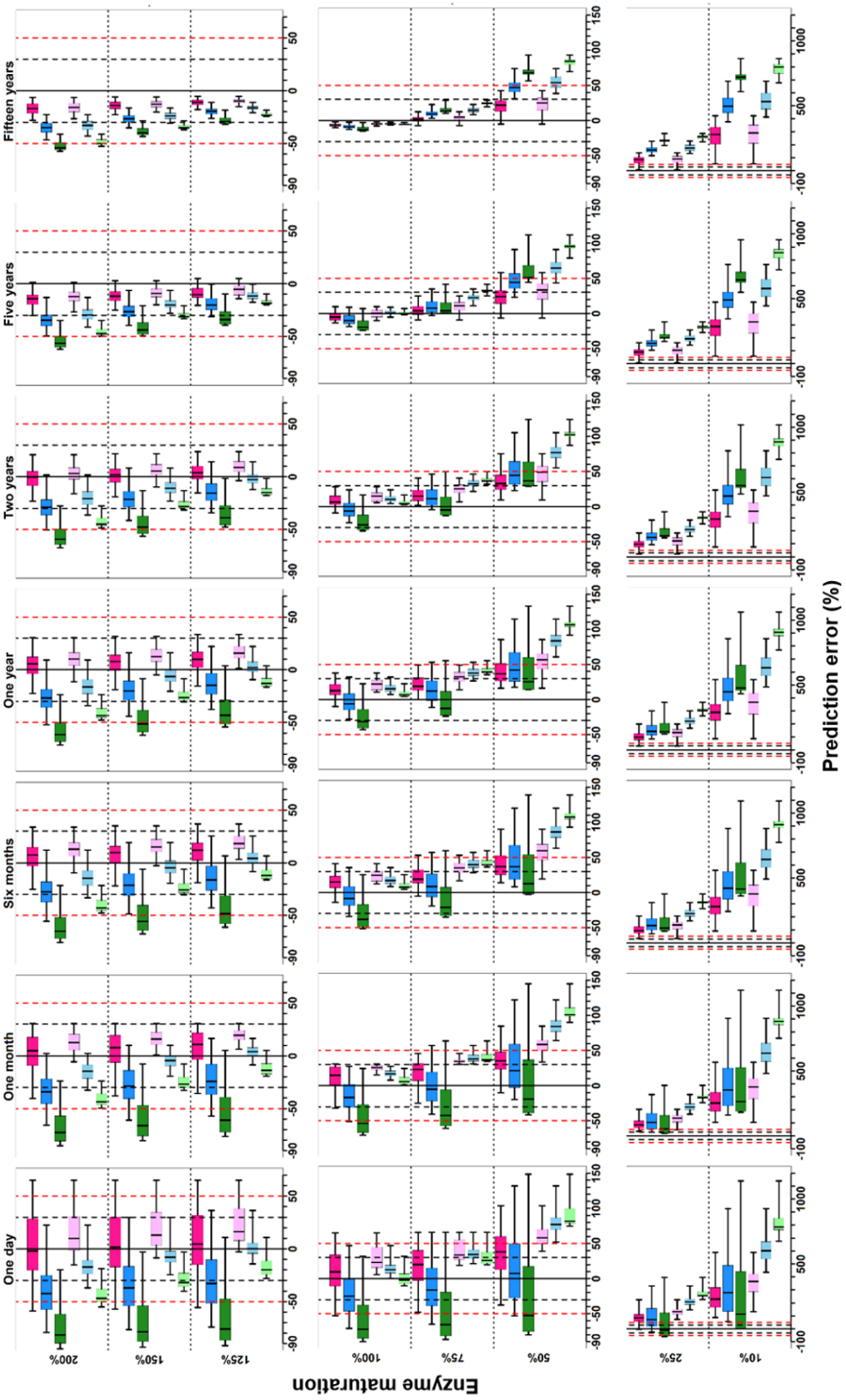

Figure 4 *PE for AS0.75-based CLp predictions of drugs undergoing hepatic metabolism in scenario 2, where both size-related changes and maturation were included in the simulations of 'true' paediatric CLp, with enzyme maturation in $CL_{int,mic}$ ranging from 10 to 200% of the adult value. PE, prediction error; CLp, plasma clearance; $CL_{int,mic}$, unbound microsomal intrinsic clearance.*

Table 2 displays published $CL_{int,mic}$ maturation values for hepatic isoenzymes for each paediatric age, together with their corresponding category of PE for AS0.75-based CLp predictions for all hypothetical drugs. Three PE categories were defined, with the PE range for all hypothetical drugs lying within ±30% (green), within ±50% (orange), and including absolute PE values higher than 50% (red), with PE values rounded to the tenth (i.e., 32% was rounded to 30%). The PE of AS0.75-based CLp predictions for all hypothetical drugs was within ±30% in children aged 5 years and older, except for one of the three cytochrome P450 (CYP)1A2 enzyme maturation patterns and one of the two CYP2E1 enzyme maturation patterns published in children aged 5 years. For neonates aged 1 day and infants aged 1 month, the PE range systematically included absolute values >50% for hypothetical substrates of all isoenzymes. Intermediate results were found for children between 6 months and 2 years of age.

Table 2 *Published enzyme maturation values and their corresponding PE category of AS0.75-based CLp predictions for drugs undergoing hepatic metabolism for scenario 2*

Metabolic route	One day	One month	Six months	One year	Two years	Five years	Fifteen years
CYP1A2 [36]	0.022	3	25	47	70	90	98
CYP1A2 [44]	5	20	29	35	NA	NA	100
CYP1A2 [45]	27	41	122	153	159	152	123
CYP2B6 [36]	0.22	6	30	46	65	85	98
CYP2C8 [36]	39	88	99	100	101	101	102
CYP2C9 [36]	39	94	101	102	103	103	103
CYP2C18_19 [36]	23	30	52	66	80	95	103
CYP2D6 [36]	6	49	88	95	100	103	104
CYP2E1 [36]	11	26	41	49	57	71	90
CYP2E1 [44]	21	40	46	100	100*	100*	100
CYP3A4 [44]	20	50	110	130	130	NA	100
CYP3A4 [46]	10	NA	43	54	72	101	100
CYP3A4_5 [36]	2	29	64	76	85	92	97
UGT2B7 [44]	5	10	70	100	100*	100*	100
UGT1A6 [44]	10	16	36	50	NA	NA	100
SULT1A1 [47]	100	100	100	100	100	100	100

Age category

*Numbers within cells correspond to the value of enzyme maturation, as a percentage of adult enzyme activity, for each age and isoenzyme. NA was used when no data on enzyme maturation for the corresponding age and isoenzyme were reported. * Indicates replacement of missing enzyme maturation values (NA) by 100% when reported values in younger and older children were equal to 100%. 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. PE values were rounded to the tenth. PE, prediction error; CLp, plasma clearance.*

3.3.2.2 PE for hypothetical drugs undergoing glomerular filtration

Table 3 displays the PE of AS0.75-based CLp predictions for each paediatric age and each hypothetical drug undergoing GF. Drugs undergoing GF only differed by their fu and type of binding plasma protein. AS0.75 led to a PE within ±30% for all these drugs in children as young as 1 year of age, with the exception of those drugs highly bound to AGP (adult fu ≤ 0.12%) in children aged 1 and 2 years. AS0.75 predictions in neonates aged 1 day and infants aged 1 month led to a PE of up to more than 100%.

Table 3 *PE for AS0.75-based CLp predictions for each drug binding to AGP or HSA and undergoing glomerular filtration in scenario 2, where both size-related changes and maturation were included in the simulations of 'true' paediatric CLp*

Adult fu	Binding protein	One day	One month	Six months	One year	Two years	Five years	Fifteen years
0.01	AGP	-70	-44	-42	-39	-35	-28	-17
	HSA	172	94	25	11	3	-5	-7
0.12	AGP	-31	-24	-33	-32	-29	-25	-15
	HSA	184	99	27	13	4	-4	-7
0.23	AGP	7	-4	-23	-25	-24	-22	-14
	HSA	196	103	29	14	5	-4	-7
0.34	AGP	46	16	-14	-18	-19	-18	-13
	HSA	207	108	31	16	6	-3	-7
0.45	AGP	85	36	-4	-11	-14	-15	-11
	HSA	219	113	33	17	7	-2	-6
0.56	AGP	123	56	5	-4	-8	-12	-10
	HSA	231	117	35	19	8	-2	-6
0.67	AGP	162	76	15	3	-3	-9	-9
	HSA	243	122	37	20	9	-1	-6
0.78	AGP	201	96	24	10	2	-6	-8
	HSA	255	127	39	22	10	0	-5
0.89	AGP	239	116	33	18	7	-2	-6
	HSA	266	131	41	23	11	0	-5
1	AGP	278	136	43	25	13	1	-5
	HSA	278	136	43	25	13	1	-5

Colours indicate the prediction error categories for each drug. Three prediction error categories were defined, with PE lying within ±30% in green, within ±50% in orange, and including absolute values higher than 50% in red. PE, prediction error; CLp, plasma clearance; AGP, a1-acid glycoprotein; HSA, human serum albumin.

Figure 5 displays a summary of the results regarding the applicability of AS0.75 to scale CLp from adults to children. Scenarios for which AS0.75 led to accurate CLp predictions were defined, including ages, enzyme maturation and drug properties.

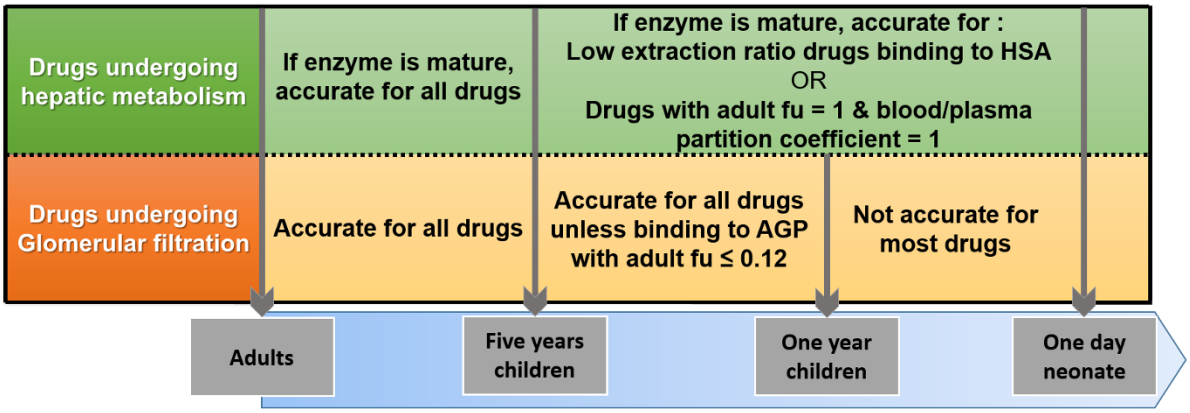

Figure 5 *Scenarios for which AS0.75-based paediatric CLp predictions are accurate, based on a PBPK simulation workflow. CLp, plasma clearance; PBPK, physiologically-based pharmacokinetic.*

3.4 Discussion

In this study, a PBPK simulation workflow was developed in order to systematically investigate, for the first time, the accuracy of AS0.75-based paediatric CLp predictions for a wide range of hypothetical drugs, undergoing hepatic metabolism or GF. The use of a systematic approach increases our current knowledge on the applicability of AS0.75 to predict paediatric CLp based on adult values, which has, until now, been based on studies of a limited number of existing drugs and a limited number of observed concentrations. The use of PBPK principles allowed for the screening of the impact of size-related changes, maturation, and drug properties on the accuracy of AS0.75-based paediatric CLp predictions, which is not possible with other methods. This allowed us to define scenarios, including ages, drug properties (including elimination pathways), and maturation, for which AS0.75 is likely to lead to accurate paediatric CLp predictions (Figure 5).

In contrast with previously proposed applications of AS0.75 theory in the paediatric population [20], but in line with more recent work [18], the current study shows that there is no universal allometric exponent that can be used to accurately scale size-related changes in CLp

from adults to children of various ages. Even when estimated in scenarios in which only size-related changes are included (scenario 1), the allometric exponent was found to vary from 0.50 to 1.20, changing with age and drug properties (Figure 3). The corresponding PE was found to be more sensitive to the use of a fixed exponent of 0.75 among the youngest children, leading to increased bias in very young children, with values around 30% for drugs undergoing hepatic metabolism and up to 278% for drugs undergoing GF (Figure 3).

The inaccurate paediatric CLp predictions resulting from AS0.75 in the very young are often attributed to the influence of the many maturation processes that are known to occur in these children. Therefore, in model building, AS0.75 is usually used in combination with an MF that should account for these maturational changes, to describe CLp in this population for a specific drug. However, in scenario 1, which did not include maturation in the PBPK-based simulations of 'true' paediatric CLp, the PE of the AS0.75-based CLp predictions was still large in children aged 6 months and younger (ranging from 43 to 278%) for drugs undergoing GF. From this it can be concluded that in these scenarios, when modelling CLp in very young children using AS0.75 in combination with MF, the MF likely also corrects for bias introduced by the use of a fixed allometric exponent of 0.75. Therefore, while the use of AS0.75 + MF may prove to be a viable method in some cases, there is no sufficient evidence to support its scientific basis, either for predicting drug clearance between individuals of different ages or for covariate relationships in model-building procedures. Moreover, there are no grounds to reject the well-established data-driven (i.e., stepwise covariate model building) strategies for the inclusion of bodyweight in a paediatric pharmacokinetic model, which have recently been used to develop more flexible functions to accurately scale CLp across the entire paediatric age range using only bodyweight in model development [48–51].

As similarly reported for interspecies AS0.75 of CLp [23–31] (Figure 1), drug properties were found to impact the accuracy of the predictions (Figures 3 and 4). While drug properties not amenable to AS0.75 could be defined in the case of interspecies AS0.75 of CLp [23,24], no unique trends (in terms of direction and extent) in the impact of drug properties on the PE were observed for AS0.75 of CLp in paediatric patients as this impact varied with maturation and size (Table 3 and Figure 4).

We found that enzyme activity close to adult values is a requirement for accurate AS0.75-based CLp predictions at all paediatric ages, which is in agreement with previous reports [44–52]. However, mature enzyme activity alone is not sufficient to lead to accurate AS0.75-based CLp predictions, as, in children younger than 5 years, AS0.75 can lead to a

PE >30%, depending on the drug properties, even when enzyme activity is close to adult values (Figure 4). With the exception of certain publications on CYP2E1 and CYP1A2, it is commonly believed that hepatic enzymes are mature in children aged 5 years and older (Table 2), which is in line with previous reports on the use of AS0.75 to predict CLp in children older than 5 years of age [34,53].

Drugs undergoing GF were found to be amenable to AS0.75 of CLp until a younger age, than drugs undergoing hepatic metabolism. Our results show that clearance through GF can be accurately predicted in children as young as 1 year of age for all drugs, with the exception of drugs highly bound to AGP ($fu \leq 0.12$) in children aged between 1 and 5 years. While this result seems in line with previous findings that GFR reaches adult values at 8–12 months of age [54], we emphasize that secretion and reabsorption processes are not yet included in the simulations.

Sethi *et al.* [55] recently suggested that the fu for drugs binding to albumin was smaller than what can be predicted using MFs for albumin currently in use, in children younger than 4 years of age. The underestimated fu in children would impact the results but would not impact our conclusion. Indeed, for children aged 3 months and younger, we found that AS0.75 led to inaccurate CLp prediction for drugs undergoing both hepatic metabolism and GF.

The use of a PBPK approach allowed for the disentanglement of size-related changes, drug properties, and maturation processes due to its unique feature to incorporate multiple levels of information to predict CLp for different paediatric ages. Over the past decade, the scaling of an adult PBPK model to paediatric patients has been proven to lead to accurate CLp predictions in children [36,46,56,57]. The investigation of hypothetical drugs in the current study allowed the avoidance of bias in PBPK-based CLp predictions due to the inaccurate measurements of drug-specific parameters, such as fu, which can occur when studying existing drugs [58]. The parameters defining drug properties should be interpreted as apparent parameters. For instance, a drug with a Kp of 0 could translate into a drug that can enter membranes and red blood cells, but for which distribution in red blood cells is not a limiting factor for its metabolism in the hepatocytes (i.e., fast drug repartitioning compared with the residence time of blood in the capillaries [59]). This simulation workflow does not account for illness, and different results might be expected in patients for whom physiological changes, for instance in AGP concentration or organ function, can impact the drug clearance [60]. However, this allows for the investigation of AS0.75 predictive performance without confounding factors such as

illness, sparse paediatric clinical data, or bias in the estimation of adult CLp used in the AS0.75-based paediatric CLp predictions.

The herein applied workflow is intended to give an overview of the impact of drug properties (including elimination pathways), size-related changes, and maturation on the accuracy of AS0.75-based CLp predictions. To do so, unrealistic enzyme maturation scenarios for part of the paediatric ages, as well as uniform distribution of drug properties (equidistant values), were included in the simulations using a global sensitivity analysis with a fractional design. Indeed, keeping enzyme maturation scenarios the same for different ages allows to differentiate between the impact of age and enzyme maturation, thereby unravelling an increased sensitivity of the accuracy of AS0.75-based CLp predictions to enzyme maturation with decreasing age (see Figure 4). Therefore, the poor predictive performance of AS0.75 in young children is not solely due to enzyme immaturity in itself, but also due to an increased sensitivity of drug clearance to enzyme maturation at that age.

Because this study was intended to be systematic and not probabilistic, results were not weighted on the likelihood of the investigated drug properties. This approach can be considered a risk assessment, where rare drug properties are considered as important as frequent drug properties.

3.5 Conclusions

Based on current PBPK knowledge, we found no evidence for a universal allometric exponent for scaling CLp in children. This finding holds even when only size-related changes are considered and maturation of processes underlying drug clearance are not taken into account. This work reveals that AS0.75 accurately predicts CLp in children over 5 years of age for drugs eliminated by GF and/or undergoing hepatic metabolism, when enzyme activity is close to adult values, due to the lack of sensitivity of PE to the allometric exponent and to the drug properties in this scenario.

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Conflict of interest

Amin Rostami-Hodjegan is an employee of the University of Manchester, seconded part-time to Simcyp Limited (a Certara company) whose research is funded by a consortium of pharmaceutical companies.

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Supplementary material

Type of parameters	Parameters	Values for the different typical patients investigated								[Ref] (Eq)
Age		1 day	1 month	6 months	1 year	2 years	5 years	15 years	35 years	
Demographic values	Bodyweight [1] (kg)	3.45[a]	4.3 [a]	7.55 [a]	9.9 [a]	12.35 [a]	18.25 [a]	54.25 [a]	66.5 [b]	[1] [a], [2] [b]
	Height[1] (cm)	49.75 [a]	54.25[a]	66 [a]	74.75 [a]	86 [a]	108.25 [a]	166 [a]	169.5 [b]	[1] [a], [2] [b]
	Body surface area (m^2)	0.22 [c]	0.26 [c]	0.38 [c]	0.46 [c]	0.55 [c]	0.74 [d]	1.60 [d]	1.77 [d]	[3](1) [c], [4](2) [d]
Drug properties	CLint,mic ($L.min^{-1}.mg^{-1}$ microsomal protein)	between $0.56 \cdot 10^{-6}$ and $0.209 \cdot 10^{-3}$ with 124 intermediate equidistant values								[5]
	Kp	0, 1, 2, 3 or 4								
	fu in adults	From 0.01 to 1 with 8 intermediate equidistant values								
Size and age independent system specific parameters	Liver density ($g.L^{-1}$)	1080								[6]
	MPPGL ($mg.g^{-1}$)	33								[6]
	D_N	0.17								
System specific parameters influenced by size-related changes	Liver weight (g)	133	159	249	313	385	544	1351	1523	[6](3-5)
	Cardiac output ($L.min^{-1}$)	0.52[e]	0.61[e]	0.92[e]	1.15[e]	1.46[e]	2.33 [e]	5.59 [e]	6.2 [f,2]	[6](6) [e], [2] [f]
	Hepatic blood flow ($L.min^{-1}$)	0.14	0.17	0.25	0.32	0.40	0.64	1.54	1.70	[6](7)
	Glomerular filtration rate ($mL.min^{-1}$)	3.97	7.49	18.90	26.56	34.71	51.90	85.84	138.05	[6](8)
System specific influenced by maturational changes	Albumin concentration	27	31	33	34	35	36	37	38	[6](9)
	Alpha 1 glycoprotein concentration	0.04	0.13	0.22	0.27	0.32	0.40	0.49	0.56	[6](10)
	Hematocrit	56	44	36	36	36.5[3]	37	42 [2]	44 [2]	[7]

Table of the parameters implemented in the PBPK-workflow. Demographic values are typical size parameters correlated to age. Drug properties are parameters chosen for the generation of the different hypothetical drugs. System specific parameters are parameters reflecting the physiology and are categorized based on their dependence on age (maturation) and size (size-dependent changes). $CL_{int,mic}$ stands for unbound microsomal intrinsic clearance, Kp stands for blood to plasma partition coefficient, fu stands for unbound drug fraction in plasma, MPPGL stands for amount of microsomal protein per gram of liver and D_N stands for dispersion number. Superscript letters show the different source of the data between different ages; [1], mean of the 50th percentile in males and females; [2], values based on the mean between males and females; [3], average between two age groups when age corresponds to cut off age for the reported hematocrit value.

Formulas used to obtain the demographic and system specific parameters

Formulas for demographic values

- *Body surface area, as implemented in the PBPK model from Johnson et al. [6]:*
    - Body surface area (BSA) based on Haycock *et al.* formula [3] for children with a bodyweight smaller than 15kg

$$\text{BSA } (\text{m}^2) = 0.024265 \times \text{Bodyweight (kg)}^{0.5378} \times \text{Height (cm)}^{0.3964} \quad (1)$$

    - Body surface area (BSA) based on Dubois and Dubois formula [4] for children with a bodyweight greater than 15 kg and for adults

$$\text{BSA } (\text{m}^2) = 0.007184 \times \text{Bodyweight (kg)}^{0.425} \times \text{Height (cm)}^{0.725} \quad (2)$$

Formulas for size-related changes

- *Liver weight based on Johnson et al. [6]:*

$$\text{Liver weight (g)} = \text{Liver density (g.L}^{-1}) \times \text{Liver volume (L)} \quad (3)$$

$$\text{Liver density (g.L}^{-1}) = 1080 \quad (4)$$

$$\text{Liver volume (L)} = 0.722 \times \text{BSA}^{1.176} \quad (5)$$

- *Cardiac output based on Johnson et al. [6]:*

$$\text{Cardiac output} = \left[\text{u} + \frac{(\text{Age} - \text{v}) \times (\text{w} - \text{x})}{\text{y} - \text{z}}\right] \times \text{BSA} \quad (6)$$

where u = 2.5, v = 1, w = 4, x = 2.5, y = 10 and z = 1 for ages ≤ 10 years, and u = 4, v = 10, w = 3, x = 4, y = 20 and z = 10 for ages > 10 years, but ≤ 20 years.

- *Hepatic blood flow (Q_H) based on Johnson et al. [6]:*

$$\text{Q}_\text{H} = 0.275 \times \text{Cardiac output} \quad (7)$$

- *Glomerular filtration rate (GFR) based on Johnson et al. [6]:*

$$\text{GFR (L)} = (-6.1604 \times \text{BSA}^2) + (99.054 \times \text{BSA}) - 17.74 \quad (8)$$

Formulas for maturational changes of system specific parameters (independent of drug properties)

- *Plasma protein concentration based on Johnson et al. [6] :*
    - Albumin:

$$\text{Plasma albumin concentration}(\text{g.L}^{-1}) = 1.1287 \times \ln(\text{Age}) + 33.746 \quad (9)$$

With Age in years

    - α1-acid glycoprotein (AGP):

$$\text{Plasma AGP concentration}(\text{g.L}^{-1}) = \frac{0.887 \times \text{Age}^{0.38}}{8.89^{0.38} + \text{Age}^{0.38}} \quad (10)$$

With Age in years

Formulas for the drug and system specific parameters

Clearance is dependent on drug and system specific parameters, that is to say parameters dependent on both drug properties and system specific parameters.

Formulas for maturational changes of system and drug specific parameters (dependent on drug properties)

- *Fraction unbound based on Johnson et al. [6] :*

$$\text{fu}_{\text{paediatric}} = \frac{1}{1 + \frac{(1 - \text{fu}_{\text{adult}}) \times [\text{P}]_{\text{paediatric}}}{[\text{P}]_{\text{adult}} \times \text{fu}_{\text{adult}}}} \quad (11)$$

With maturation in [P] (concentration in plasma protein for either albumin or AGP) driving the maturation of fu

- *Blood to plasma ratio (B:P) based on Maharaj et al. [8] :*

$$\text{B:P} = 1 + [\text{Hematocrit} \times (\text{fu} \times \text{Kp} - 1)] \quad (12)$$

With maturation in hematocrit and in plasma protein driving the maturation in B:P.

- *Intrinsic clearance (CLint) based on Johnson et al.* [6] *:*

$$\mathrm{CLint} = \mathrm{MPPGL} \times \mathrm{Liver\ weight} \times \mathrm{CL_{int,mic}} \times \mathrm{enzyme\ maturation} \quad (13)$$

With enzyme maturation (expressed as a percentage of adult $CL_{int,mic}$) driving the maturation of CLint. In the PBPK-workflow, different enzyme maturations were investigated.

Plasma clearance (CLp) for drugs undergoing glomerular filtration for adults and children, based on Johnson *et al.* [6]

$$\mathrm{CLp} = \mathrm{GFR} \times \mathrm{fu} \quad (14)$$

For scenario 1, selection of the drug property fu=1 allows for the simplification equation 14 into an equation only reflecting size-related changes:

$$\mathrm{CLp} = \mathrm{GFR} \quad (15)$$

Clearance for drugs undergoing hepatic metabolism based on Naritomi *et al.* [9]

$$\mathrm{CLp} = \mathrm{CL_B} \times \mathrm{B{:}\,P} \quad (16)$$

$$\mathrm{CL_B} = \mathrm{Q_H} \times \mathrm{E_H} \quad (17)$$

$$\mathrm{E_H} = 1 - \mathrm{F_H} \quad (18)$$

$$\mathrm{F_H} = \frac{4a}{(1+a)^2 \exp\{(a-1)/2D_N\} - (1-a)^2 \exp\{-(a+1)/2D_N\}} \quad (19)$$

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (20)$$

$$\mathrm{R_N} = (\mathrm{fu}/\mathrm{B{:}\,P}) \times \mathrm{CLint}/\mathrm{Q_H} \quad (21)$$

For scenario 1, selection of fu = 1 and Kp =1 (drugs for which B:P=1 for at ages) and enzyme maturation = 100% allows for the simplification equations 16, 23 and 13 into the equations 22,23 and 24 respectively leading to CLp equation only reflecting size-related changes:

$$\mathrm{CLp} = \mathrm{CL_B} \quad (22)$$

$$\mathrm{R_N} = \mathrm{CLint}/\mathrm{Q_H} \quad (23)$$

$$\mathrm{CLint} = \mathrm{MPPGL} \times \mathrm{Liver\ weight} \times \mathrm{CL_{int,mic}} \quad (24)$$

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Chapter 4

Children are not small adults, but can we treat them as such?

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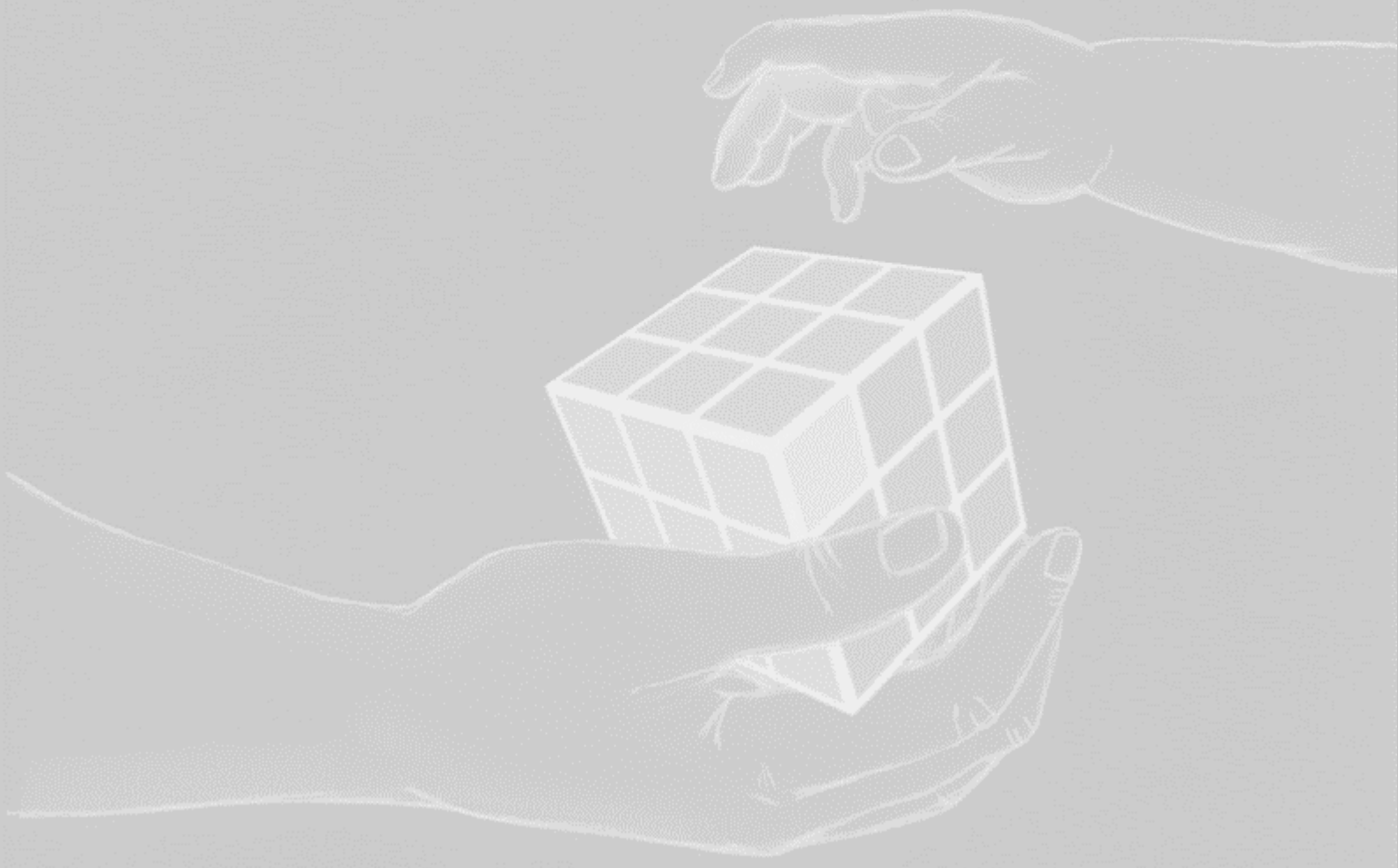

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Abstract

While children cannot be considered small adults due to nonlinear processes underlying the pharmacokinetics of drugs, pediatric doses are typically still expressed per kg. We use a physiologically-based pharmacokinetic workflow to assess the accuracy of linear scaling of plasma clearance (CLp) for hypothetical drugs with range of realistic parameter values in pediatric patients of different ages. The results are compared with 0.75 fixed allometric scaling (AS0.75). Linear CLp scaling is accurate down to the age of 1 month for drugs undergoing glomerular filtration, except when these drugs are highly bound to AGP. For hepatically cleared drugs, linear scaling is reasonably accurate down the age of 2 years, except for AGP bound drugs with a low ER and mature isoenzymes. In neonates, linear scaling outperforms AS0.75 for HSA and AGP bound drugs excreted through glomerular filtration. These results suggest that pediatric patients can in many cases be treated as small adults.

Study highlights

What is the current knowledge on the topic?

Clearance is the most important determinant for drug dosing. In children, nonlinear processes underlying the pharmacokinetics of drugs result in complex maturational patterns in drug clearance.

What question did this study address?

Using physiologically-based pharmacokinetic modeling principles, this study investigates how accurate linear (bodyweight-based) clearance scaling, and therefore linear dose scaling, is in the pediatric population and compares this accuracy to the accuracy of allometric (bodyweight-based) scaling with a fixed exponent of 0.75.

What does this study add to our knowledge?

This study defines that linear scaling is accurate down to the age of 1 month for most drugs undergoing glomerular filtration and reasonably accurate down the age of 2 years for most hepatically cleared drugs. In neonates, linear scaling outperforms fixed allometric scaling, however both scaling methods can be highly inaccurate and systematic scaling accuracy cannot be achieved without taking drug properties and enzyme maturation into account.

How might this change drug discovery, development and/or therapeutics?

Accurate simple scaling methods allow for easy establishment of pediatric study doses or therapeutic doses.

4.1 Introduction

In pharmacokinetics, the adagio "children are not small adults" reflects the non-linear impact of changes in body size and composition, and in the maturational status of physiological systems, on the plasma clearance (CLp) of drugs in children. Given that CLp is one of the most important determinants for drug dosing, it is therefore unlikely that optimal dosing can be achieved by linearly scaling drug doses from adults to children according to equation 1:

$$\mathrm{Dose_P} = \mathrm{Dose_A} \cdot \frac{\mathrm{BW_P}}{\mathrm{BW_A}} \quad (1)$$

in which BW stands for bodyweight and subscripts P and A stand for pediatric and adult respectively, in which the bodyweight of adults is often taken to be 70 kg.

The ease of linear scaling is preferred by many, as reflected in the fact that both in pediatric clinical practice and in pediatric clinical trials, drug doses are still typically expressed per kg bodyweight, albeit with dose adjustments for different age-groups. To scale pediatric doses during the design of first-in-child studies during drug development, 0.75 fixed allometric scaling (AS0.75) is probably the most commonly used empiric scaling method. However, Fisher and Shafer have recently pointed out that absolute scaled CLp values in the pediatric bodyweight-range are practically indistinguishable when using linear scaling or AS0.75 [1]. Physiologically-based pharmacokinetics (PBPK) modeling prospectively generates pediatric CLp values by collating all known information on physiological processes underlying CLp and maturation in these physiological processes and should therefore be considered the gold standard for accurate predictions of pediatric CLp values and dose selection. As PBPK modeling is time consuming, needs to be performed by trained experts and relies on knowledge on drug-specific and system-specific properties that may not always be available, there remains a need to explore the performance of empiric scaling methods.

Surprisingly, there are hardly any scientific reports investigating the basis for linear scaling of pediatric CLp. We recently developed a PBPK-based workflow to systematically investigate the accuracy of scaling methods for pediatric CLp for more than ten thousand hypothetical drugs, with combinations of realistic drug properties covering the entire theoretical parameter space [2], that can be used for this purpose. Based on this work, we concluded that the commonly applied AS0.75 method, that uses a bodyweight-based function with an exponent of 0.75 to scale CLp from adults to children, yields accurate pediatric CLp predictions for all hypothetical drugs down to the age of five years in case isoenzymes are

mature. For certain drugs, the predictions are even accurate down to the age of 1 year and younger, depending on their properties. Interestingly, this study showed that this was not because the value of 0.75 for the exponent was accurate. On the contrary, the results showed the 'true exponents' to be highly variable and dependent both on the age of the children and the properties of the drugs. Rather, the observed accuracy of the CLp predictions was the result of the CLp predictions being insensitive to deviations between the 'true' exponent and an exponent of 0.75, thereby providing a basis to explore the performance of other exponents.

In this study we evaluated to what extent linear scaling (i.e., using a bodyweight-based exponential equation with an exponent of 1) would lead to accurate CLp scaling in children of various ages for drugs undergoing elimination through glomerular filtration or hepatic metabolism. For this we use our previously developed PBPK-based workflow that was developed to systematically assess the accuracy of CLp scaling methods in children of various ages and for a large number of hypothetical drugs with a wide yet realistic range of parameter values. The results of linear scaling are subsequently compared to those obtained with AS0.75, particularly for the situations where linear scaling does not lead to accurate scaling.

4.2 Methods

Details of the PBPK-based workflow, have been presented in detail elsewhere [2] and this methodology is only briefly repeated here. Model equations and parameter values can also be found in the Supplementary Methods.

To cover the entire hypothetical parameter space, a total of 12,620 hypothetical drugs were generated based on all possible combinations of the following realistic ranges in drug properties:

1) drugs were either fully eliminated through glomerular filtration or through hepatic metabolism.

2) drugs were either exclusively bound to alpha-1-acid glycoprotein (AGP) or to human serum albumin (HSA), yielding two different groups. Published maturation profiles were used to scale the abundance of these proteins in children.

3) affinities for the plasma proteins were selected to be such that the fraction unbound (fu) in adults ranged between 1 and 100%, with eight equidistant intermediate values, yielding 10 different values in total.

4) for drugs eliminated through hepatic metabolism, the dispersion model was used and unbound intrinsic microsomal clearance ($CL_{int,mic}$) values in adults were selected to range between $0.56 \cdot 10^{-6}$ - $0.209 \cdot 10^{-3}$ L. $min^{-1}.mg^{-1}$ microsomal protein, with 124 equidistant intermediate values, yielding 126 values in total for drugs eliminated through hepatic metabolism.

5) the blood-to-plasma partition coefficient (Kp) was selected to be 0, 1, 2, 3, or 4, and published maturation profiles were used to scale hematocrit in children, these 5 values are only relevant in the calculation of CLp for drug eliminated through hepatic metabolism.

This yields 20 hypothetical drugs eliminated through glomerular filtration and 12,600 eliminated through hepatic metabolism. For each of these hypothetical drugs, PBPK principles were used in combination with reported age-appropriate demographic values to calculate a point estimate of the 'true' CLp values in a typical adult and in typical children of 1 day, 1 month, 6 months, and 1, 2, 5, and 15 years of age. CLp predictions were made for scenarios in which enzyme maturation (parameterized as percentage of $CL_{int,mic}$ relative to adult values) was 100% or at the lowest or highest reported percentage for each age. For the latter, reported literature values for the maturation of various isoenzymes were collected. As for some isoenzymes in the very young extremely low or absent enzyme activity is reported, a lower cut-off of 10% was used. The lowest and highest values of enzyme maturation used at each age were: 10% and 100% at 1 day and 1 month, 25% and 122% at 6 months, 35% and 153% at 1 year, 57% and 159% at 2 years, 71% and 152% at 5 years, and 90% and 125% at 15 years. To obtain scaled CLp values for each typical child by either linear extrapolation or AS0.75 scaling from adult CLp values, the 'true' CLp value of each hypothetical drug in a typical adult of 66.5 kg was used in combination with the age-appropriate bodyweights obtained from CDC growth charts for each pediatric age [3].

The prediction error (PE) between the scaled pediatric CLp value and the 'true' PBPK-based pediatric CLp value was calculated. PE cutoff value of between ±30% were considered to indicate accurate scaling, scaled values outside the range of ±50% were considered to be inaccurate, values in between were considered to be reasonably accurate.

4.3 Results

Table 1 illustrates the results upon linear CLp scaling for drugs eliminated through glomerular filtration by showing the obtained PE values for different combinations of fu and binding plasma protein (i.e., AGP or HSA) at various ages. The table illustrates that for these drugs, the PE is dependent on the fu and the type of plasma protein the drug is binding to. For drugs binding to AGP, the range in absolute PE values is generally higher compared to those for drugs binding to HSA, but linear scaling still leads to reasonably accurate CLp values (i.e., PE within ± 50%) for children as young as 1 month, except for drugs with an fu < 0.34 for children below 2 years. For drugs binding to HSA, CLp can be accurately scaled (i.e., PE within ± 30%) using linear scaling down to the age of 1 month. For all ages included in this analysis, the absolute PE upon linear scaling for drugs excreted through glomerular filtration never exceeds 90% for any drug (Table 1).

Table 1 *Prediction error for linearly scaled CLp values for hypothetical drugs undergoing glomerular filtration binding to various extends to alpha-1-acid glycoprotein (AGP) or human serum albumin (HSA)*

fu in adults	*Binding plasma protein*	1 day	1 month	6 months	1 year	2 years	5 years	15 years
0.01	*AGP*	-86	-72	-66	-62	-57	-48	-21
0.01	*HSA*	30	-2	-27	-31	-32	-31	-12
0.12	*AGP*	-67	-62	-61	-58	-54	-46	-20
0.12	*HSA*	36	0	-26	-30	-32	-31	-12
0.23	*AGP*	-49	-52	-55	-53	-50	-43	-18
0.23	*HSA*	41	3	-25	-29	-31	-30	-11
0.34	*AGP*	-30	-41	-50	-49	-47	-41	-17
0.34	*HSA*	47	5	-24	-28	-30	-30	-11
0.45	*AGP*	-12	-31	-44	-45	-43	-39	-16
0.45	*HSA*	52	7	-23	-27	-30	-29	-11
0.56	*AGP*	7	-21	-39	-40	-40	-36	-15
0.56	*HSA*	58	10	-22	-26	-29	-29	-11
0.67	*AGP*	25	-11	-33	-36	-36	-34	-13
0.67	*HSA*	64	12	-21	-25	-28	-28	-10
0.78	*AGP*	44	-1	-28	-31	-33	-32	-12
0.78	*HSA*	69	14	-19	-24	-28	-28	-10
0.89	*AGP*	62	9	-23	-27	-30	-29	-11
0.89	*HSA*	75	17	-18	-24	-27	-27	-10
1	*AGP*	80	19	-17	-23	-26	-27	-10
1	*HSA*	80	19	-17	-23	-26	-27	-10

Green indicates PE values within ±30%, red indicates values outside the range of ±50%, and orange indicating absolute PE values between 30% and 50%. Fu, adult unbound drug fraction in plasma.

Figure 1 shows the results of linear CLp scaling for the hypothetical drugs eliminated by hepatic metabolism. The figure illustrates that the PE depends on the extraction ratio (categorized as low, intermediate, or high) and the type of plasma protein the drug is binding to (AGP or HSA) as depicted with different colors. Additionally, the maturation of the metabolizing isoenzyme is of importance for the accuracy of pediatric CLp scaling (results depicted in different rows). For the various ages included in this analysis, results are shown for 100% isoenzyme maturation (upper row), meaning that $CL_{int,mic}$ values in the children were 100% of the adult values, as well as for the highest (middle row) and lowest reported percentage for isoenzyme maturation at each age (lowest row). Supplemental Figure S1, shows the same results for children of 1 day, 1 month and 6 months, but with an enlarged scale to allow for the assessment of PE values in the younger individuals with low enzyme maturation.

Figure 1 shows that for drugs that are cleared through hepatic metabolism by mature isoenzymes (upper row), linear scaling can be used down to the age of 2 years for AGP bound drugs metabolized by mature isoenzymes, except for AGP bound drugs with a low ER and isoenzyme activity similar to or higher than adult values. For AGP bound drugs with a high extraction ratio, linear scaling can be used down to the age of 6 months. For HSA bound drugs linearly scaled CLp values are accurate or reasonably accurate down to the age of 1 day when isoenzymes are mature, although in some cases PE can reach up to -60%. For drugs cleared by isoenzymes with $CL_{int,mic}$ values higher than adult values (middle row), accuracy seems limited down to adolescents for AGP bound drugs and down to 5 years of age for HSA bound drugs, as the PE can go up to around -70%. When isoenzymes have the lowest reported enzyme maturation value for each age (lowest row), PE values upon linear scaling are accurate in children down to the age of 2 years, but then gradually increase towards considerable over-prediction with decreasing age, with the highest PE values reaching up to values of around 500%.

For the situations when linear scaling does not lead to accurate predictions, Supplemental Table S1 and Supplemental Figure S2 and S3 show the results obtained upon AS0.75 scaling, which were taken from or adapted from Calvier *et al*. [2]. These figures show that for newborns of 1 day and 1 month, linear scaling is more accurate, especially for renally cleared drugs and for hepatically metabolized drugs which are substrates for enzymes that are not mature, although both methods yield extreme and unacceptable values for some drugs.

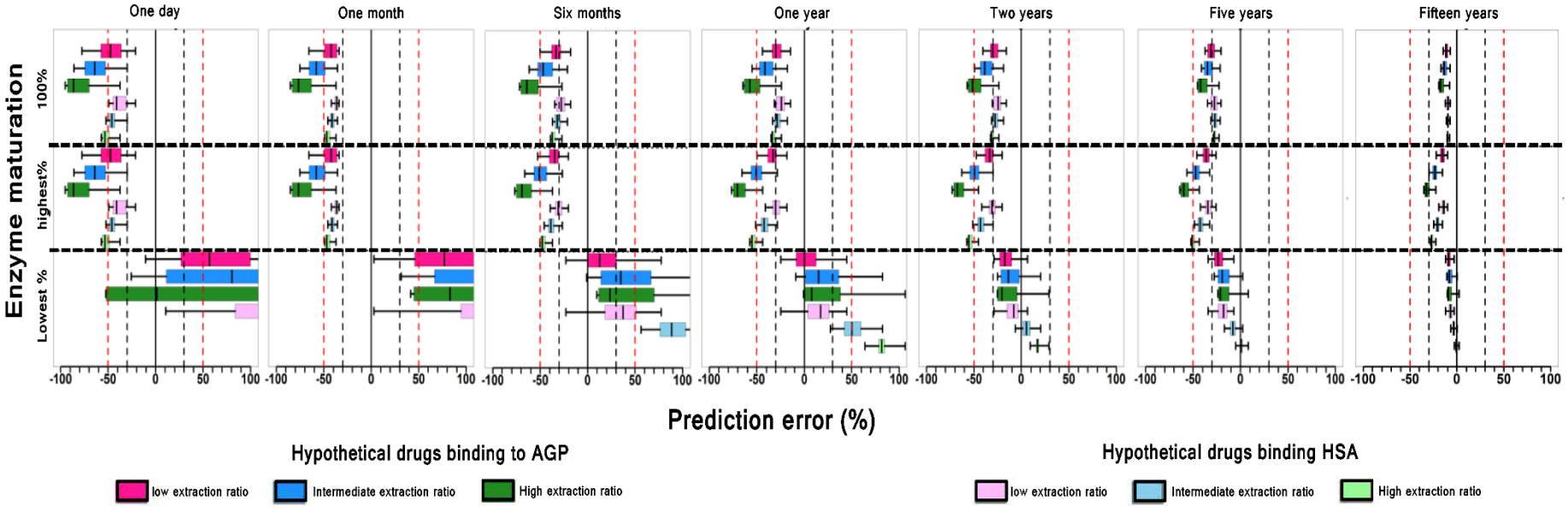

Figure 1 *Prediction error for linearly scaled CLp values for hypothetical drugs undergoing hepatic metabolism, binding to various extends to alpha-1-acid glycoprotein (AGP, dark colors) or human serum albumin (HSA, light colors) and having low (green), intermediate (blue), or high (pink) extraction ratio. At the top row, results are shown for scenarios in which enzyme maturation is 100% of adult values, the bottom and middle row show results for the lowest and highest reported enzyme maturation value for each age, respectively, which were 10% and 100% at 1 day and 1 month, 25% and 122% at 6 months, 35% and 153% at 1 year, 57% and 159% at 2 years, 71% and 152% at 5 years, and 90% and 125% at 15 years. Black and red vertical dotted lines correspond to a prediction error of 30 and 50% respectively.*

4.4 Discussion

Here we report on the accuracy of linear scaling of pediatric CLp values from adult CLp values, using a previously developed PBPK-based simulation platform [2]. This platform allows for the systematic assessment of pediatric CLp scaling methods by comparing scaled CLp values to 'true' pediatric CLp values obtained with PBPK-based simulations, for a large number of hypothetical drugs with drug-specific parameter values that cover a wide range of realistic values.

As was observed for other empiric scaling methods [2,4], the accuracy of linear scaling of pediatric CLp is dependent on drug properties (elimination pathway, extraction ratio and binding plasma proteins and unbound drug fraction) and on the age of children and the maturity of isoenzymes. This may lead to unacceptably biased CLp values for at least a subset of drugs in all studied ages, except in 15-year-olds. And although Fisher and Shafer have shown that absolute values of pediatric CLp may not differ greatly between linear scaling and AS0.75 [1], when expressed on a relative scale distinct differences do become apparent.

Our results show that linear CLp scaling is systematically accurate for all drugs in adolescents of 15 years (Table 1 and Figure 1) and for HSA bound drugs that are cleared through glomerular filtration in newborns down to the age of 1 month (Table 1). For hepatically cleared drugs there is some under-prediction when enzyme maturation is close to maturity, leading to PEs that are acceptable for some drugs, but that may with decreasing age lead down to almost -100% for others (Figure 1). This is in line with observations by Lack *et al.*, who also reported trends in under-prediction of drug dosing with linear scaling [5]. In drug development systematic under-prediction may however be favorable, as drug doses derived from under-predicted CLp values yield more conservative pediatric starting doses. On the other hand, under-dosing in early pediatric clinical trials may lead to a lack of therapeutic effect in the children participating in the trial which raises questions about the trial-related burden that would be ethically acceptable. In case hepatic enzymes are not mature, the systematic under-prediction of CLp upon linear scaling is reversed (Figure 1). This leads to reasonably accurate CLp values in children of 2 years and older when enzyme maturity is low, but also leads to extreme over-prediction of CL in newborns (Figure 1).

We have previously reported that AS0.75 scaling of CLp is accurate in children of 5 years and older for all drugs that are cleared through glomerular filtration and for drugs undergoing hepatic metabolism, when enzyme activity is close to adult values [2]. Comparison

of these results with the results obtained with linear scaling, suggest that in the aforementioned situations, AS0.75 scaling is on average less biased than linear scaling. In newborns of 1 month and younger for drugs cleared through glomerular filtration and for drugs that are hepatically cleared by isoenzymes that are not close to maturity, linear scaling will on average lead to less biased scaling, although in these young children both methods can lead to unacceptable bias for some drugs depending on the drug properties.

Given the wide range in PE values in all scenarios included in our current investigation, even within a specific age, it seems that simple scaling methods that do not take drug properties and isoenzyme maturation into account are not likely to systematically yield accurate pediatric CLp values. This suggests that especially in the very young only physiologically-based scaling approaches have a rational basis for CLp scaling, because linear scaling or AS0.75 scaling may lead to both accurate predictions or to unacceptable over- or under-prediction and without taking drug properties or maturational status into account, it cannot be predicted what situation will apply.

Our results are predicated on the validity of pediatric PBPK modeling principles. In our opinion PBPK models are most suitable to integrate and leverage all existing knowledge on drug clearance in children, which is supported by a number of publications assessing the accuracy of predictions by PBPK models in the pediatric population [6–9]. In their most recent addendum on "*Clinical Investigation of Medicinal Products in the Pediatric Population – Guidance for Industry*", the FDA recommends that all existing knowledge should be used to design the pediatric drug development program and that modeling and simulation is a useful approach to integrate and leverage existing knowledge [10]. Regarding the drug-specific properties of the hypothetical drugs used in this analysis, it has to be noted that although the range in each of drug-specific parameters separately is realistic, it cannot be excluded that hypothetical drugs with an unrealistic combination of parameters have been generated.

To enhance the interpretability of our results, it has to be noted that the PBPK-based 'true' CLp values in this work were generated without taking variability or uncertainty in parameter values into account. To compensate for this, a very stringent PE value of ±30% was arbitrarily chosen to indicate accurate scaling and a value of 50% for reasonably accurate scaling. Moreover, as very little is known about maturation of transporters in the kidney and liver, these were not taken into account in the generation of PBPK-based 'true' CLp values. Finally, for enzyme maturation a lower limit of 10% was chosen, because in case an isoenzyme is inactive in the very young, generally other elimination routes take over, at least partially. As

a result, in Supplemental Figure 2, the scaled values for children of 1 day and 1 month, do not reflect scaling for the least mature metabolic route, but they may reflect the CLp that is observed for a drug in real life for which other elimination routes take over.

In summary, even though children are not small adults due to the known complex physiological changes across the pediatric age range, linear CLp scaling is (reasonably) accurate down to the age of 1 month for drugs undergoing glomerular filtration, except for drugs highly bound to AGP. For hepatically cleared drugs, linear scaling is reasonably accurate down the age of 2 years, except for AGP bound drugs with a low ER and mature isoenzymes. In neonates of 1 day and 1 month, linear scaling outperforms AS0.75 for drugs excreted through glomerular filtration and for hepatically metabolized drugs which are substrates for enzymes that are not mature, however at very young ages simple scaling approaches that do not take drug properties and enzyme maturation into account can generally not reach systematic accuracy. Altogether these results suggest however that in many cases pediatric patients can be treated as small adults.

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Supplementary Methods

PBPK-based simulations of 'true' CLp values

System-specific parameters

PBPK simulations were performed for a typical adult of 35 years and in typical children of 1 day (term neonate), 1 month, 6 months, and 1, 2, 5, and 15 years of age. Demographic values (average for males and females) were taken from the CDC growth charts [1] for the typical pediatric individuals. For a typical adult, these values as well as cardiac output value were taken from the ICRP annals [2]. Body surface area (BSA) was estimated using the equations of Dubois and Dubois [3] for children weighing > 15kg, and of Haycock *et al.* [4] for those weighing ≤ 15kg.

Physiological parameters and ontogeny patterns in the hepatic blood flow (Qh), plasma protein concentrations for human serum albumin (HSA) and alpha-1 acid glycoprotein (AGP), liver size, milligram protein per gram of liver (MPPGL) were taken from Johnson *et al.* [5] and ontogeny pattern in hematocrit was taken from Hinderling *et al.* [5]. Isoenzymes maturations were taken from published values [6–10]. The table below provides an overview of the system-specific parameters used in the PBPK-based simulations for each typical individual.

Type of parameter	Parameter	Parameter values for the typical individuals							
	Age	***1 day***	***1 month***	***6 months***	***1 year***	***2 years***	***5 years***	***15 years***	***35 years***
Demographic parameters	Bodyweight (kg)	3.45	4.30	7.55	9.90	12.35	18.25	54.25	66.50
	Height (cm)	49.75	54.25	66.00	74.75	86.00	108.25	166.00	169.5
System-specific parameters	Liver density ($g.L^{-1}$)	1080							
	MPPGL ($mg.g^{-1}$)	33							
	Liver weight (g)	133	159	249	313	385	544	1351	1523
	Cardiac output ($L.min^{-1}$)	0.52	0.61	0.92	1.15	1.46	2.33	5.59	6.2
	Hepatic blood flow ($L.min^{-1}$)	0.14	0.17	0.25	0.32	0.40	0.64	1.54	1.70
	Glomerular filtration rate ($mL.min^{-1}$)	3.97	7.49	18.9	26.6	34.7	51.9	85.8	138
	Albumin concentration ($g.L^{-1}$)	27	31	33	34	35	36	37	38
	alpha-1 acid glycoprotein ($g.L^{-1}$)	0.04	0.13	0.22	0.27	0.32	0.40	0.49	0.56
	Hematocrit (%)	56	44	36	36	36.5	37	42	44

MPPGL, milligram microsomal protein per gram of liver.

Drug-specific parameters of hypothetical drugs

Hypothetical drugs were generated to serve as model and test drugs in the PBPK-based simulation workflow. For each individual drug-specific property, a realistic range of values was selected.

The model parameters to calculate hepatic plasma clearance (CLp) in this workflow which are based on a combination of drug-specific and system-specific parameters are: the unbound drug fraction in plasma (fu), the blood to plasma ratio (B:P), and the whole liver unbound intrinsic clearance (CLint). These parameters therefore reflect drug properties in a population of a specific age. fu values in adults were used as surrogates of their contributing drug-specific parameters (i.e., affinity to plasma proteins), since fu is most often reported.

To derive the affinity to plasma proteins from the unbound drug fraction in plasma (fu) and the concentration of the binding plasma protein in adults, equations by Rodgers and

Rowland [6,11] were used. The hypothetical drugs were assumed to be exclusively bound to either HSA or AGP. The fu in adults ranged from 1% to 100%, with 8 equidistant intermediate values. Values for the blood to plasma partitioning coefficient (Kp) of 0, 1, 2, 3, and 4 were selected, reflecting different extents of drug diffusion into the red blood cells. $CL_{int,mic}$ in adults ranged between $0.56 \cdot 10^{-6}$ and $0.209.10^{-3}$ $L.min^{-1}.mg^{-1}$ microsomal protein [12], with 124 equidistant intermediate values. These different values reflect difference in both affinities for and abundances of isoenzymes in adults.

Prediction of 'true' CLp values for drug eliminated through glomerular filtration

Analogous to Johnson *et al.* [6], equation 1 was used to compute 'true' CLp for drugs eliminated through glomerular filtration, in which GFR represents an age-specific value for the glomerular filtration rate.

$$CLp = GFR \times fu \quad (1)$$

Prediction of 'true' CLp values for drug eliminated through hepatic metabolism

'True' total hepatic plasma clearance (CLp) values were computed using the dispersion model (Equations 2 to 7). 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 [13].

$$CLp = CL_B \times B{:}P \quad (2)$$

$$CL_B = Qh \times ER \quad (3)$$

$$ER = 1 - F_H \quad (4)$$

$$F_H = \frac{4a}{(1+a)^2 \exp\{(a-1)/2D_N\} - (1-a)^2 \exp\{-(a+1)/2D_N\}} \quad (5)$$

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (6)$$

$$R_N = (fu/B{:}P) \times CLint/Qh \quad (7)$$

In these equations, CLp is the total 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 [14].

For each hypothetical drugs, CLint in adults was computed as the product of $CL_{int,mic}$, the MPPGL, and the liver weight. B:P in adults was derived from the adult haematocrit, from the Kp value, and the value of fu in adults [15]. Values of adult $CL_{int,mic}$, Kp, and adult fu were taken from the values defined to generate the hypothetical drugs. Qh, CLint, fu, and B:P in pediatric patients were scaled using the ontogeny functions of the relevant system-specific parameters.

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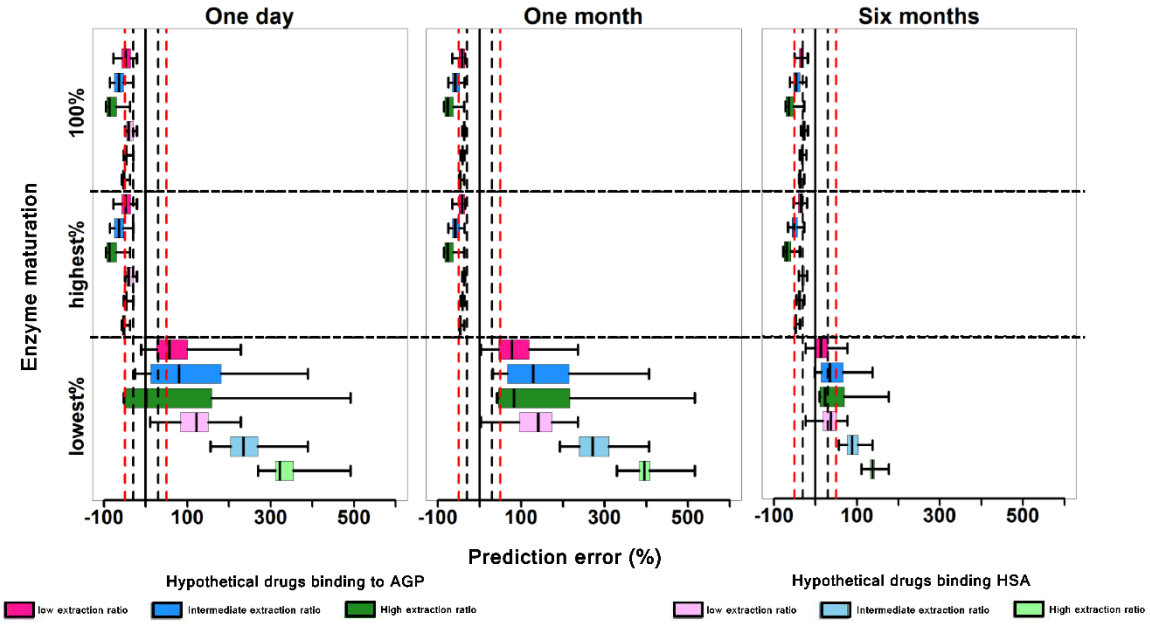
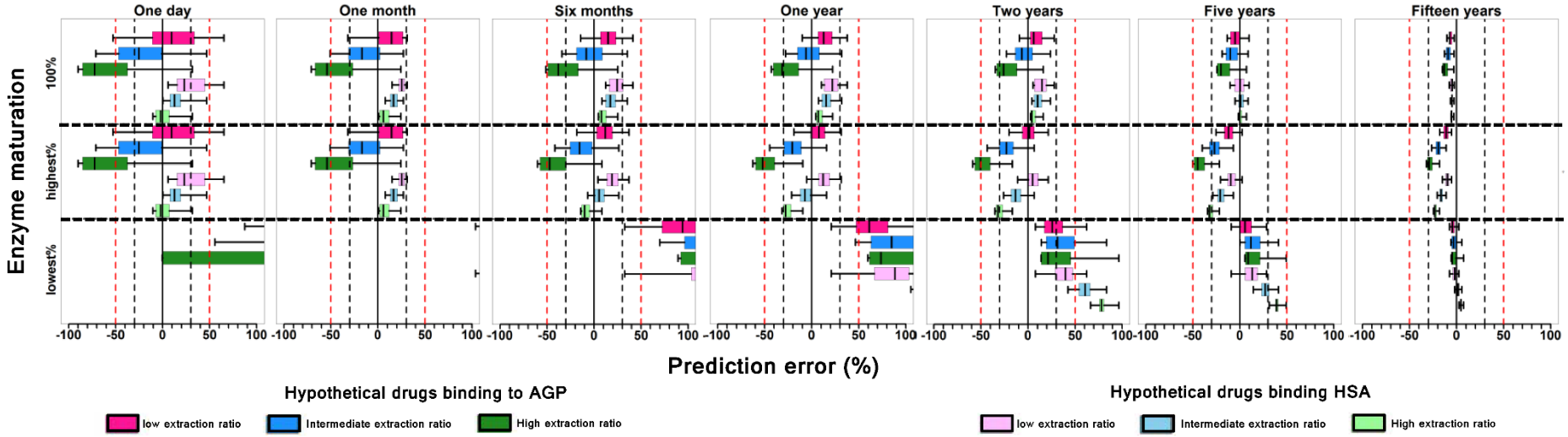
Supplemental Table S1 *Prediction error for AS0.75-scaled CLp values for hypothetical drugs undergoing glomerular filtration binding to various extends to alpha-1-acid glycoprotein (AGP) or human serum albumin (HSA)*

fu in adults	*Binding plasma protein*	1 day	1 month	6 months	1 year	2 years	5 years	15 years
0.01	*AGP*	-70	-44	-42	-39	-35	-28	-17
0.01	*HSA*	172	94	25	11	3	-5	-7
0.12	*AGP*	-31	-24	-33	-32	-29	-25	-15
0.12	*HSA*	184	99	27	13	4	-4	-7
0.23	*AGP*	7	-4	-23	-25	-24	-22	-14
0.23	*HSA*	196	103	29	14	5	-4	-7
0.34	*AGP*	46	16	-14	-18	-19	-18	-13
0.34	*HSA*	207	108	31	16	6	-3	-7
0.45	*AGP*	85	36	-4	-11	-14	-15	-11
0.45	*HSA*	219	113	33	17	7	-2	-6
0.56	*AGP*	123	56	5	-4	-8	-12	-10
0.56	*HSA*	231	117	35	19	8	-2	-6
0.67	*AGP*	162	76	15	3	-3	-9	-9
0.67	*HSA*	243	122	37	20	9	-1	-6
0.78	*AGP*	201	96	24	10	2	-6	-8
0.78	*HSA*	255	127	39	22	10	0	-5
0.89	*AGP*	239	116	33	18	7	-2	-6
0.89	*HSA*	266	131	41	23	11	0	-5
1	*AGP*	278	136	43	25	13	1	-5
1	*HSA*	278	136	43	25	13	1	-5

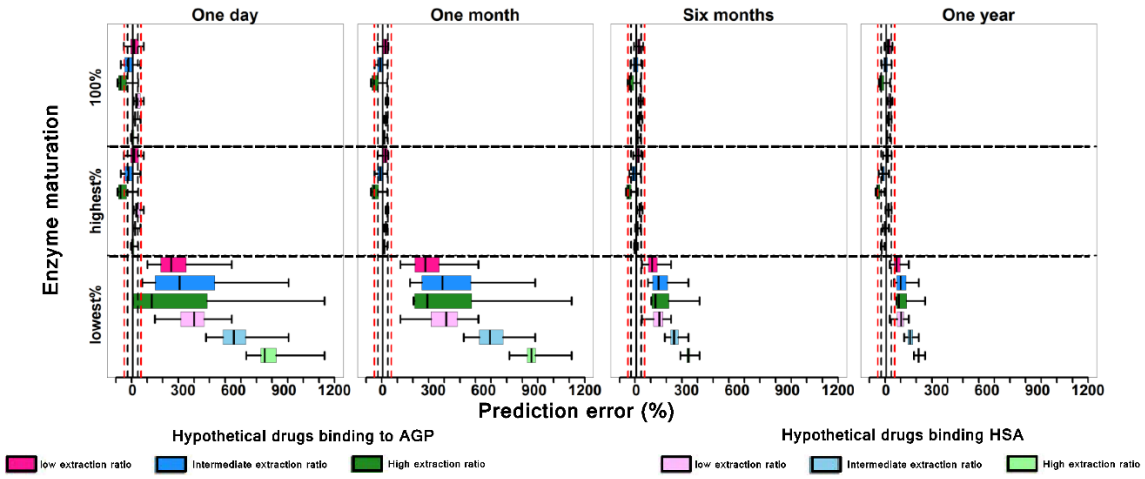
Green indicates PE values within ±30%, red indicates values outside the range of ±50%, and orange indicates absolute PE values between 30% and 50%. Fu, adult unbound drug fraction in plasma.



Supplemental Figure S1 *Enlarged scale depiction of prediction error for linearly scaled CLp values for hypothetical drugs undergoing hepatic metabolism, binding to various extends to alpha-1-acid glycoprotein (AGP, dark colors) or human serum albumin (HSA, light colors) and having low (green), intermediate (blue), or high (pink) extraction ratio. At the top row, results are shown for scenarios in which enzyme maturation is 100% of adult values, the bottom and middle row show results for the lowest and highest reported enzyme maturation value for each age, respectively, which were 10% and 100% at 1 day and 1 month, 25% and 122% at 6 months. Black and red vertical dotted lines correspond to a prediction error of 30 and 50% respectively.*



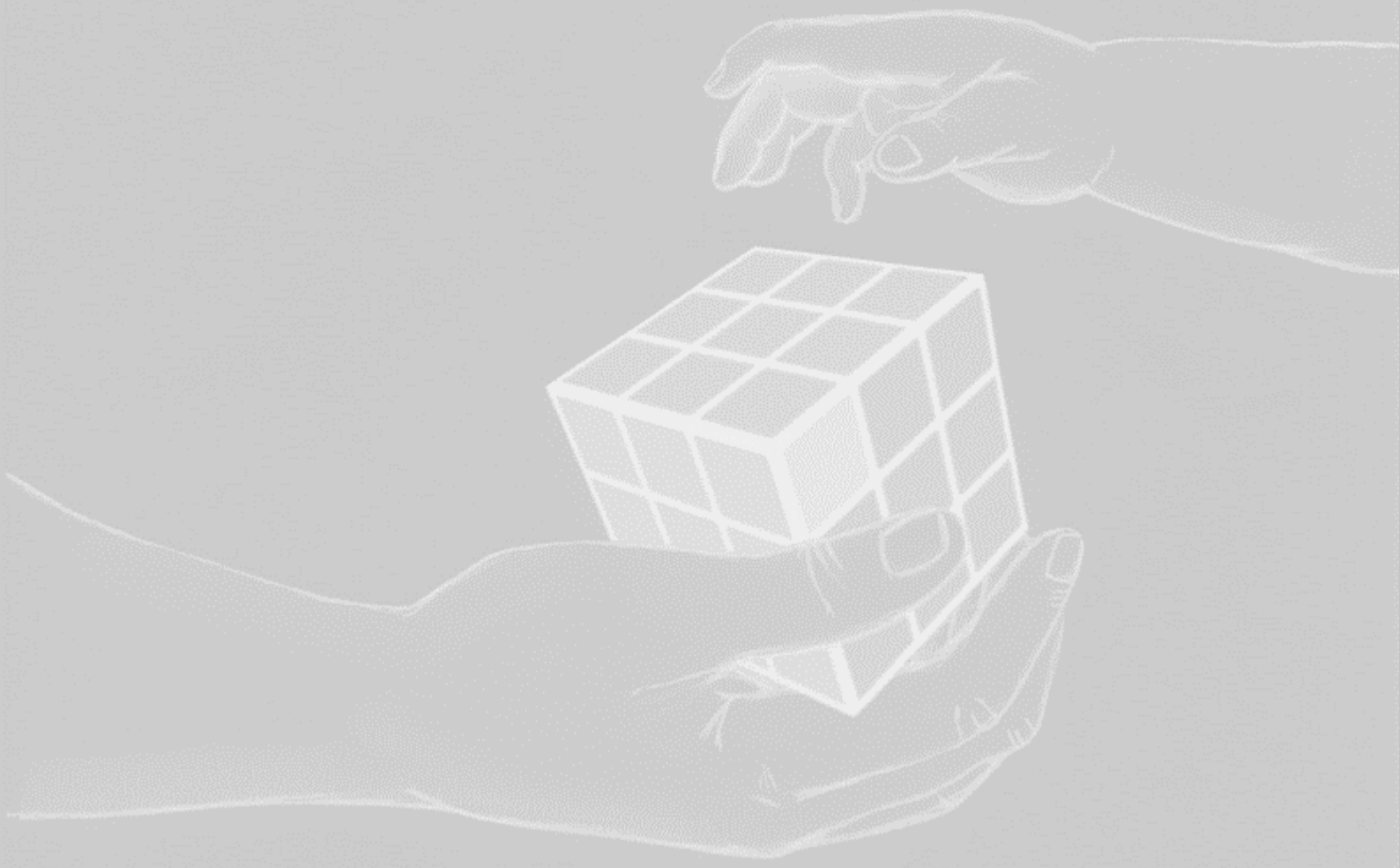
Supplemental Figure S2 *Prediction error for CLp values scaled with AS0.75 for hypothetical drugs undergoing hepatic metabolism, binding to various extends to alpha-1-acid glycoprotein (AGP, dark colors) or human serum albumin (HSA, light colors) and having low (green), intermediate (blue), or high (pink) extraction ratios. Results are shown for scenarios in which enzyme maturation is 100% of adult values (top), the bottom and middle row show results for the lowest and highest reported enzyme maturation value for each age, respectively, which were 10% and 100% at 1 day and 1 month, 25% and 122% at 6 months, 35% and 153% at 1 year, 57% and 159% at 2 years, 71% and 152% at 5 years, and 90% and 125% at 15 years. Black and red vertical dotted lines correspond to a prediction error of 30 and 50% respectively.*



Supplemental Figure S3 *Enlarged scale depiction of prediction error for CLp values scaled with AS0.75 for hypothetical drugs undergoing hepatic metabolism, binding to various extends to alpha-1-acid glycoprotein (AGP, dark colors) or human serum albumin (HSA, light colors) and having low (green), intermediate (blue), or high (pink) extraction ratios. Results are shown for scenarios in which enzyme maturation is 100% of adult values (top), the bottom and middle row show results for the lowest and highest reported enzyme maturation value for each age, respectively, which were 10% and 100% at 1 day and 1 month, 25% and 122% at 6 months, 35% and 153% at 1 year. Black and red vertical dotted lines correspond to a prediction error of 30 and 50% respectively.*

Section III

Scaling methods accounting for isoenzyme maturation for prediction of clearance in children



Chapter 5

Drugs being eliminated via the same pathway will not always require similar pediatric dose adjustments

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Abstract

For scaling drug plasma clearance (CLp) from adults to children, extrapolations of population pharmacokinetic covariate models between drugs sharing an elimination pathway have enabled accelerated development of pediatric models and dosing recommendations. This study aims at identifying conditions for which this approach consistently leads to accurate pathway-specific CLp scaling from adults to children for drugs undergoing hepatic metabolism. A physiologically-based pharmacokinetic simulation workflow utilizing mechanistic equations defining hepatic metabolism was developed. We found that drugs eliminated via the same pathway require similar pediatric dose adjustments only in specific cases, depending on drugs extraction ratio (ER), fraction unbound, type of binding plasma protein and the fraction metabolized by the isoenzyme pathway for which CLp is scaled. Overall, between-drug extrapolation of pediatric covariate functions for CLp is mostly applicable to low and intermediate ER drugs eliminated by one isoenzyme and binding to human serum albumin, in children older than 1 month.

Study Highlights

What is the current knowledge on the topic?

Proofs of concept for extrapolation of pediatric covariate functions for plasma clearance (CLp) between drugs sharing the same elimination pathway have been published for a limited number of drugs eliminated through glucuronidation and renal excretion.

What question did the study address?

The study identifies the conditions upon which between-drug extrapolation of isoenzyme specific pediatric covariate functions consistently leads to accurate CLp scaling from adults to pediatric ages, for drugs metabolized by one or multiple hepatic isoenzymes.

What this study adds to our knowledge?

Between-drug extrapolation of pediatric covariate functions for CLp is mostly applicable to low and intermediate extraction ratio drugs eliminated by one isoenzyme and binding to human serum albumin in children older than 1 month.

How might this change drug discovery?

We now have a tool available that can establish *a priori* whether two drugs metabolized by the same isoenzyme will require the same or different dose adjustments in pediatrics.

5.1 Introduction

Accurate scaling of plasma clearance (CLp) of drugs from adults to the pediatric population is crucial for the definition of first-in-child doses and for the development of pediatric dose recommendations [1–4]. As illustrated in Figure 1, for drugs undergoing hepatic metabolism, CLp values are driven by the complex interplay between drug-specific and system-specific properties. Relevant parameters to describe hepatic clearance are hepatic blood flow (Qh), the unbound drug fraction in plasma (fu), the blood to plasma ratio (B:P), and the intrinsic metabolic clearance in the liver based on unbound drug concentration (CLint) [5]. Qh is a purely system-specific parameter, whereas unbound fraction, B:P and CLint are derived from both system-specific and drug-specific parameters. Moreover, in children, system-specific parameters vary with age due to ontogenic processes, which in turn drive CLp changes across the pediatric age range (Figure 1) [6]. Because physiologically-based pharmacokinetic (PBPK) modeling integrates all the above-mentioned information [6,7], it can provide a yard-stick for scaling drug CLp from adults to various pediatric ages in the absence of pediatric clinical data [6,8–11], which is relevant for first-in-child dose definition. In addition to PBPK modeling, there is a need for model-based scaling methods that aggregate the influence of ontogeny of the system-specific parameters in a smaller set of equations, thereby facilitating scaling of pediatric CLp in drug development and clinical practice.

In pediatric population pharmacokinetic (popPK) models, the net influence of ontogeny of system-specific parameters on CLp of a drug is described using empirical covariate models derived from clinical data (i.e., concentration-time profiles). Although these covariate models can be directly used as the basis for pediatric dose adjustments [12–14], this approach requires clinical data obtained upon the administration of every single drug of interest in every pediatric sub-population.

Semimechanistic or semiphysiological popPK pediatric scaling approaches have been proposed to bridge the gap between PBPK and popPK methodologies, allowing for accelerated development of pediatric popPK models and subsequent dosing recommendations. One of these approaches relies on extrapolations of popPK covariate models between drugs that share an elimination pathway [15–19]. Because popPK covariate models are the basis of dose recommendations, this approach would also allow for extrapolation of pediatric dosing recommendation from a drug of which the changes in clearance have been quantified to other drugs for which no pediatric pharmacokinetic (PK) studies have been undertaken, provided

these drugs share the same elimination pathway. To date, proofs of concept for this method have been published for a limited number of drugs eliminated through glucuronidation or renal excretion [15–19]. However, as illustrated in Figure 2, with this method, between drug differences in CLp of drugs sharing the same elimination pathway are solely accounted for by the absolute value of the scaled CLp (e.g., adult CLp values), whereas CLp ontogeny is assumed to be purely system-specific and, therefore, drug-independent. This assumption is challenged by the fact that CLp of drugs with different properties might be impacted differently by the various ontogenic changes in system-specific parameters (Figure 1).

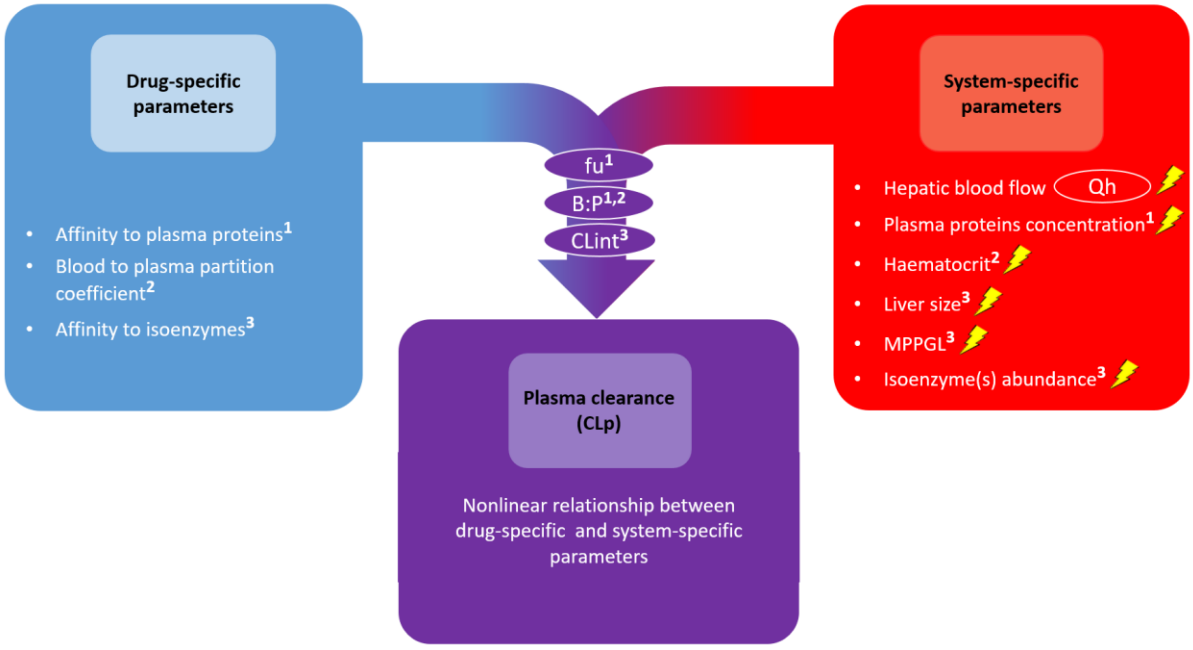


Figure 1 *Schematic representation of the complex interplay between drug-specific and system-specific parameters driving hepatic CLp values. Parameters within circles are directly used in the PBPK hepatic clearance model (e.g., dispersion model). Parameters in the purple circles represent composite parameters that are derived from the system-specific parameters and the drug-specific parameters indicated by the numbers in the superscripts. In children each of the system-specific parameters change with age, each represented by a lightning bolt. MPPGL stands for microsomal protein per gram of liver, fu stands for the unbound drug fraction in plasma, CLint stands for the intrinsic metabolic clearance in the liver based on unbound drug concentrations and B:P stands for the blood to plasma ratio.*

Therefore, the aim of the current study is to identify the conditions for which between-drug extrapolation of pediatric covariate functions for hepatic metabolic CLp consistently leads to accurate pathway-specific CLp scaling from adults to children of various ages (absolute prediction error ≤ 30%). We developed a PBPK-based simulation workflow utilizing mechanistic equations defining hepatic metabolism to systematically screen a wide parameter

space for both system-specific and drug-specific variables impacting hepatic CLp by one specific isoenzyme. Additionally, we investigated the impact of multiple elimination pathways on the between-drug extrapolation potential of pediatric covariate functions for hepatic metabolic CLp. This allowed us to define a decision tree to identify the conditions leading to consistently accurate pediatric CLp scaling using between-drug extrapolations of pathway-specific pediatric covariate models.

5.2 Methods

5.2.1 Model drug and test drug

Total (i.e., bound and unbound) hepatic metabolic plasma clearance will be referred to as CLp in this paper. We investigate the extrapolation potential of pediatric covariate functions scaling CLp from adults to pediatric patients between a model drug and a test drug both exclusively eliminated by the same hepatic isoenzyme. This method is illustrated in Figure 2. The impact of elimination by multiple isoenzymes is investigated as described under the section "Multiple elimination pathways".

- Model drug and determination of the pediatric covariate function: A pediatric covariate function is developed to describe the ontogeny of CLp of what will be referred to as the model drug (M).
- Test drug and between-drug extrapolation of the pediatric covariate function: The pediatric covariate function developed based on the model drug (M) is used to scale CLp for what will be referred to as the test drug (T) from adult to various pediatric ages.

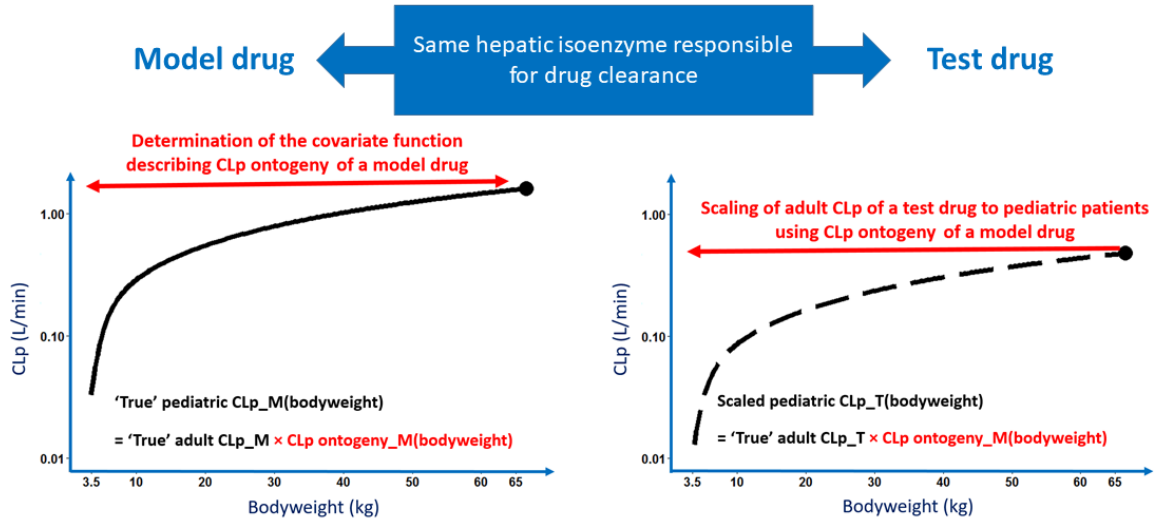


Figure 2 *Illustration of between-drug extrapolation of pediatric covariate functions to scale hepatic plasma clearance (CLp) from adults to pediatric patients for drugs eliminated by the same isoenzyme. The black dot in both graphs shows the adult hepatic CLp value for the model drug ('True' adult CLp_M) and the test drug ('True' adult CLp_T). The solid black line represents the change in CLp of the model drug throughout the pediatric age range, which is described by a pediatric covariate function based, in this example, on bodyweight (CLp ontogeny_M(bodyweight)). The dashed black line represents the scaled pediatric CLp predicted for the test drug by between-drug extrapolation of the pediatric covariate function obtained for the model drug (CLp ontogeny_M(bodyweight)).*

5.2.2 PBPK-based simulation workflow

A four steps PBPK-based simulation workflow (Figure 3) was developed in R software [20] version 3.3.1 with R studio interface version 0.99.902 following a similar approach, as previously published [21]. This workflow investigates the impact of two main variables on the accuracy of pediatric CLp predictions based on extrapolation of pediatric covariate functions from a model drug to a test drug. These variables are the drug-specific parameters of both the model drug and test drug and the ontogeny of the system-specific parameters (Figure 1). This investigation was undertaken for 15 different elimination pathways. These elimination pathways correspond to elimination by the following isoenzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18_19, CYP2D6, CYP2E1, CYP3A4, UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and SULT1A1. Ultimately, conditions for which extrapolations consistently led to accurate CLp predictions were identified.

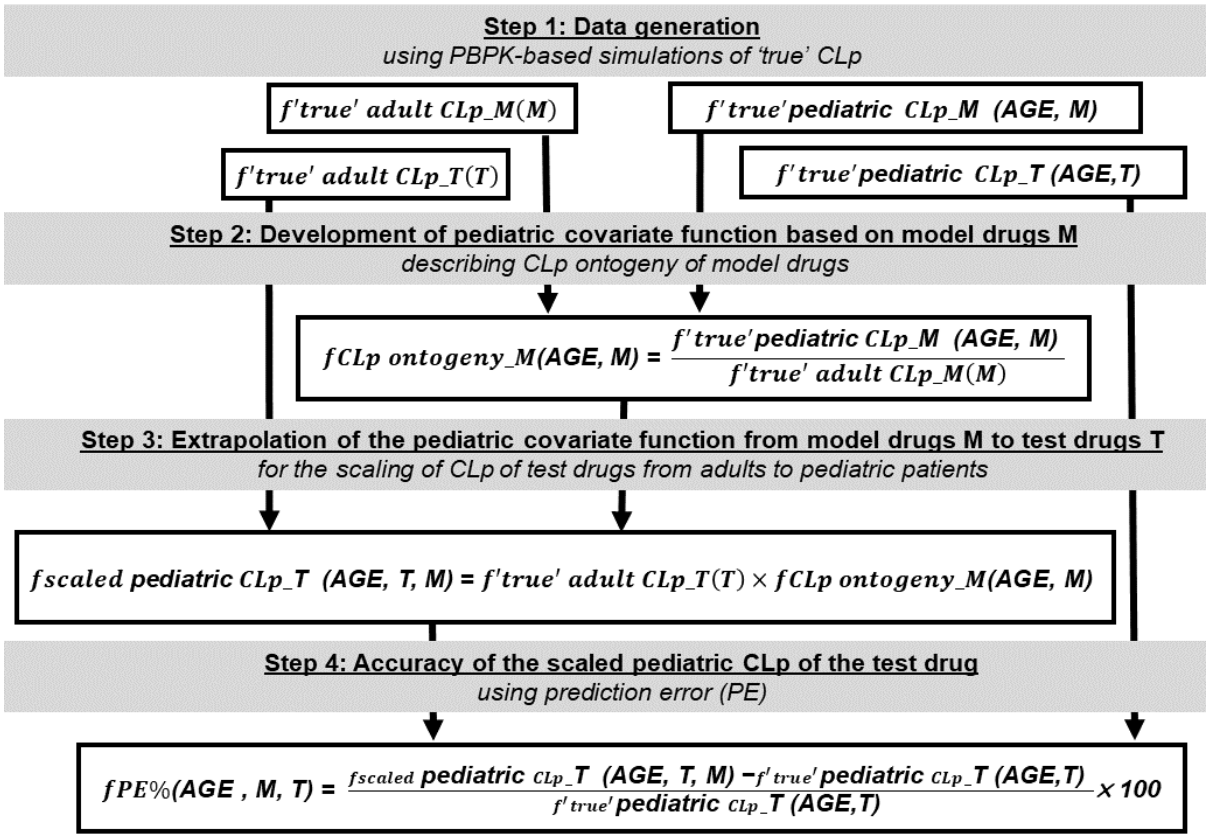


Figure 3 *PBPK-based simulation workflow used to investigate the between-drug extrapolation potential of pediatric covariate models when scaling total plasma clearance (CLp) from adults to pediatric patients. The model drug is denoted with M and the test drug with T. AGE stands for pediatric postnatal age. All steps are performed for model drugs and test drugs binding to the same plasma proteins and eliminated by the same isoenzyme and repeated for each of the 15 isoenzymes and each of the 2 binding plasma proteins investigated.*

5.2.3 Variables in the PBPK-based simulation workflow

For a systematic investigation of the impact of each variable, we chose a factorial design (i.e., a global sensitivity analysis approach) with a wide yet realistic parameter space for each individual variable. For this purpose, continuous variables were transformed into categorical variables using a number of intermediate values within the defined parameter space allowing for the generation of single point estimates of the different functions of the PBPK-based simulation workflow. To enable the computation across a wide parameter space and interpretability of the results, variability and uncertainty in both demographics and model parameters were not accounted for.

5.2.3.1 Ontogeny of system-specific parameters

To unravel the impact of ontogeny of the system-specific parameters (see Figure 1) on the extrapolation potential of pediatric covariate functions between drugs sharing the same elimination pathway, simulations were performed for 8 typical individuals for whom demographic characteristics and system-specific parameters are specified in Table 1. The PBPK model parameters for typical individuals were computed as average values of men and women. Ontogeny functions for each system-specific model parameter were taken from the Simcyp® software (Simcyp Ltd, Sheffield, UK) V15.R1 library, except for the isoenzyme ontogeny of SULT1A1. For SULT1A1 maturity was taken to have been reached at birth [22], and, therefore, isoenzyme ontogeny of SULT1A1, defined as the pediatric to adult ratio of microsomal intrinsic clearance, was set to 1. More information can be found in Supplementary Material 1.

5.2.3.2 Drug-specific properties of the model drugs and test drugs

To unravel the impact of drug-specific properties on the between-drug extrapolation potential of pediatric covariate functions, a total of 7,560 hypothetical drugs with unique combinations of drug-specific properties were generated and used as model drugs, as well as test drugs. This set of hypothetical drugs was generated using all possible combinations of different values of the following three drug-specific properties: adult unbound fraction in plasma (range: 0.01-1, n=10), blood-to-plasma partition coefficient or Kp (range: 0.35-40, n=9), and adult total unbound intrinsic clearance value of one microgram of liver microsomes or adult $CL_{int,mic,total}$ (range: $0.56 \cdot 10^{-6}$ - $0.209 \cdot 10^{-3}$ L. $min^{-1}.mg^{-1}$ microsomal protein, n=42). The hypothetical drugs were assumed to be exclusively bound to either human serum albumin (HSA) or alpha-1 acid glycoprotein (AAG). More details can be found in Supplementary Material 1.

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	1 month	6 months	1 year	2 years	5 years	15 years	25 years
Bodyweight, kg	3.45	4.30	7.55	9.90	12.35	18.25	54.25	72.65
Height, cm	49.75	54.25	66.00	74.75	86.00	108.25	166.00	172.30
BSA, m^2	0.22	0.26	0.38	0.46	0.55	0.74	1.60	1.86
System-specific parameters								
Qh, L/h	6.55	7.83	12.95	17.65	24.65	41.14	89.75	87.92
HSA, g/L	35.78	39.94	42.07	42.90	43.73	44.82	44.68	43.94
AAG, g/L	0.2678	0.5497	0.6774	0.7172	0.7512	0.7877	0.6711	0.6847
Hematocrit, %	51.93	38.14	35.11	35.78	36.79	38.29	40.01	40.74
Liver size, g	133	159	249	313	385	544	1351	1614
MPPGL	25.53	25.60	25.99	26.45	27.36	29.97	36.80	39.79
IO CYP1A2, %	24	35	118	150	164	161	126	100
IO CYP2A6, %	2.10^{-9}	0.48	99	100	100	100	100	100
IO CYP2B6, %	15	19	34	47	62	83	99	100
IO CYP2C8, %	38	86	97	99	99	100	100	100
IO CYP2C9, %	40	74	87	90	92	100	100	100
IO CYP2C18-19, %	30	33	84	95	97	98	100	100
IO CYP2D6, %	6	47	84	91	95	98	100	100
IO CYP2E1, %	10	37	59	67	74	82	88	100
IO CYP3A4, %	11	13	48	78	96	104	106	100
IO UGT1A1, %	0.2	23	98	104	100	100	100	100
IO UGT1A4, %	74	74	74	75	77	81	97	100
IO UGT1A6, %	15	30	63	76	87	95	100	100
IO UGT1A9, %	9	12	34	52	71	90	100	100
IO UGT2B7, %	8	9	11	13	18	32	79	100
IO SULT1A1, %	100	100	100	100	100	100	100	100

AAG, alpha-1 acid glycoprotein; BSA, body surface area; HSA, human serum albumin; MPPGL, milligram microsomal protein per gram of liver; IO, isoenzyme ontogeny expressed as percentage of adult microsomal intrinsic clearance; Qh, hepatic blood flow.

5.2.4 Steps of the PBPK-based simulation workflow

Between-drug extrapolation of covariate models was studied for model and test drugs sharing the same elimination pathway and binding to the same plasma protein. For each of the 15 elimination pathways investigated and each type of binding plasma protein, the following steps were undertaken as also illustrated in Figure 3.

5.2.4.1 Step 1: Data generation

Using the dispersion model (Equations 1 to 6), PBPK-based simulations of 'true' CLp were performed for all hypothetical model drugs M and test drugs T in adults ('true' adult CLp_M and 'true' adult CLp_T respectively) as well as in each investigated pediatric age ('true' pediatric CLp_M and 'true' pediatric CLp_T respectively).

$$\mathrm{CLp} = \mathrm{CL_B} \times \mathrm{B{:}P} \quad (1)$$

$$\mathrm{CL_B} = \mathrm{Qh} \times \mathrm{ER} \quad (2)$$

$$\mathrm{ER} = 1 - \mathrm{F_H} \quad (3)$$

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

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (5)$$

$$R_N = (\mathrm{fu/B{:}P}) \times \mathrm{CLint/Qh} \quad (6)$$

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 total hepatic 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 [23]. See Supplementary Material 1 for more details.

5.2.4.2 Step 2: Development of pediatric covariate function based on model drugs M

The pediatric covariate function (*f* CLp ontogeny_M) describes the ontogenic changes in 'true' CLp from adults to pediatric patients and is derived from the model drug M. For each model drug M and investigated pediatric postnatal age (AGE), single point estimates of *f* CLp ontogeny_M were computed (see equation in Figure 3).

5.2.4.3 Step 3: Extrapolation of the pediatric covariate function from model drugs M to test drugs T

Analogous to Figure 2, scaled pediatric CLp of the test drugs (*f* scaled pediatric CLp_T) was computed by scaling the 'true' adult CLp of each test drug T ('true' adult CLp_T) to each pediatric age. This scaling was performed by extrapolating the pediatric covariate function (*f* CLp ontogeny_M) from all model drugs to each test drug, following the equation in Figure 3. This led, for each pediatric age and test drug, to as many scaled pediatric CLps as the number of model drugs.

5.2.4.4 Step 4: Accuracy of the scaled pediatric CLp of the test drug

The prediction errors (PEs) of scaled pediatric CLps obtained in step 3 was calculated by comparing, for each test drug and each age, the scaled pediatric CLps with the 'true' CLp value obtained in step 1. This led, for each pediatric age and test drug, to as many PEs as the number of model drugs. Predictions were considered to be accurate when the absolute prediction error was 30% or lower.

5.2.5 Conditions leading to accurate CLp predictions

First, for the diversity of investigated ages and isoenzymes, trends in prediction errors (PEs) with all drug properties of the model and the test drug were separately assessed. These drug properties were the type of binding plasma protein, Kp, and fraction unbound in plasma, B:P, CLint, and ER in adults, as well as the difference in the latter parameters between the model drug and test drug.

In order to define scenarios consistently leading to accurate CLp scaling from adults to the different pediatric ages using between-drug extrapolation of pediatric covariate functions, drug properties allowing to best discriminate between accurate and inaccurate scaled pediatric CLp were identified by a hierarchical tree analysis (see Supplementary Material 1) [24].

All model-test drug combinations were grouped into scenarios based on the most discriminative drug properties. Within each of these scenarios, multiple model-test drug combinations are included and for all combinations, both model and test drug are eliminated

by the same isoenzyme and binding to the same plasma protein. Overall accuracy of the test drug CLp predictions for each model-test drug scenario was summarized per pediatric age group as follow:

- Model-test drug scenario systematically leading to accurate CLp scaling of the test drug: all CLp predictions within the defined scenario are accurate (absolute PE ≤ 30%).
- Model-test drug scenario not systematically leading to accurate CLp scaling of the test drug: at least one of the CLp predictions within the defined scenario is inaccurate (absolute PE > 30%).

5.2.6 Multiple elimination pathways

Because many drugs are metabolized by multiple isoenzymes, we also evaluated the situation in which the model drug and/or the test drug are metabolized by two isoenzymes, namely I_A and I_{NA}. I_A is the isoenzyme representing the elimination pathway accounted for by the covariate function, whereas I_{NA} is the isoenzyme representing the elimination pathway not accounted for by the covariate function. In order to include model drugs and/or test drugs undergoing metabolism through multiple hepatic isoenzymes, adaptations of the PBPK-based simulation workflow earlier described were performed. Details on these alterations can be found in Supplementary Material 1.

5.3 Results

5.3.1 Results for scenarios in which model and test drugs are metabolized by one isoenzyme

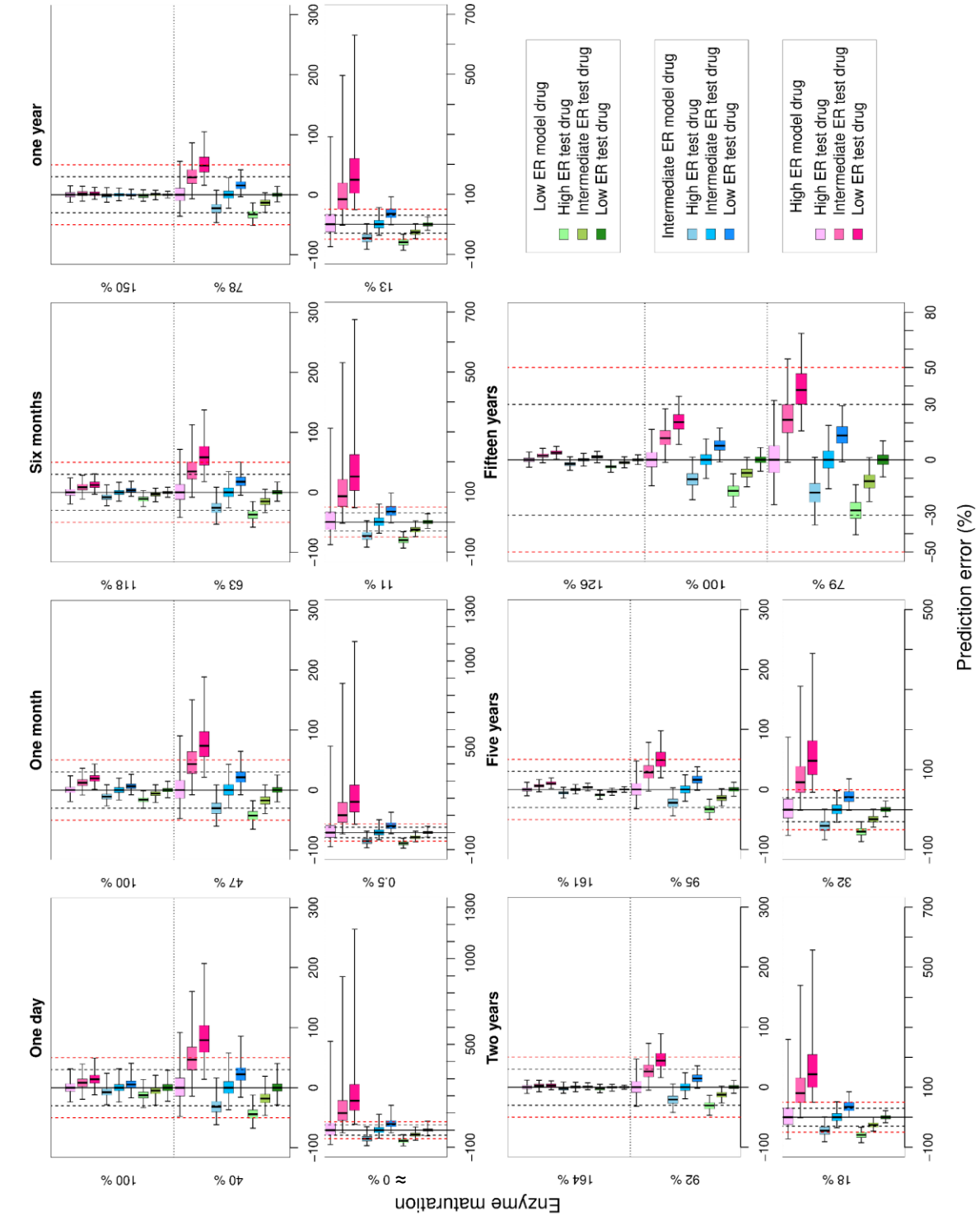
Visual inspection of the PEs revealed trends with age, type of metabolizing isoenzyme, the type of binding plasma protein and extraction ratio in adults of the model drug and test drug. Figure 4 displays these trends in PEs for all hypothetical drugs that are exclusively metabolized by one isoenzyme and that are either exclusively binding to HSA (Figure 4A) or to AAG (Figure 4B). Results are categorized by age and by adult extraction ratio category of the model and test drug, with increasing absolute PE values indicating increasing between-drug differences in CLp ontogeny, with CLp standing for total (i.e., bound and unbound) hepatic metabolic plasma clearance. For each age, results are reported for the lowest,

intermediate and highest isoenzyme ontogeny values of the 15 isoenzymes included in the analysis.

Figure 4 shows similar trends in PEs between drugs binding to HSA and drugs binding to AAG, except in neonates of 1 day of age. However, for AAG bound drugs, the ranges in PE were either similar or higher than PE ranges for HSA bound drugs. For both HSA and AAG bound drugs, the range of absolute PE values tend to decrease with increasing age. Decreased isoenzyme ontogeny value tends to increase the range of the absolute PEs, with the highest absolute PEs being > 1000% in neonates of one day (for isoenzyme ontogeny ≈ 0%) and the highest absolute PE value in adolescents being only about 70% (for isoenzyme ontogeny = 79%).

Generally, within each age category and isoenzyme ontogeny category, the median PE can be seen to be closest to 0 when extrapolating pediatric covariate models between drugs of the same extraction ratio category, although even for these drugs the range in PE values shows a number of scenarios in which absolute PE values are higher than 30%. The absolute median PE was the highest when model and test drugs belonged to extreme extraction ratio categories. Except for drugs binding to AAG in neonates of one day, extrapolating pediatric covariate functions to a test drug of a lower extraction ratio category than the model drug systematically yields a positive median PE, indicating a bias towards overprediction of CLp of the test drug. The reverse trend is observed when extrapolating pediatric covariate functions to a test drug of a higher extraction ratio category than the model drug. These trends increase as the isoenzyme ontogeny decreases to values below 100%.

Overall, the hierarchical tree analysis revealed that the most discriminating drug properties for identifying systematically accurate CLp predictions across the pediatric age range were the extraction ratio of the test drug and/or the model drug, and the difference in extraction ratio and in unbound fraction in plasma between model drug and test drug, (extraction ratio and fu defined in adults, see Supplementary Material 1). The additional influence of the difference in fraction unbound between the model drug and test drug on the PEs was not observed upon visual inspection of the trends in PEs, likely because trends in PE with fu are smaller than trends with extraction ratio and they depend on the extraction ratio of the model drug and test drug. As the extrapolations to the test drug require the use of a pediatric covariate model that is defined for the model drug, scenarios leading to accurate between-drug extrapolations were defined using the extraction ratio of the model drug.


4A
Enzyme maturation
Prediction error (%)
One day
One month
Six months
one year
Two years
Five years
Fifteen years
≈ 0 %
0.5 %
11 %
13 %
18 %
32 %
79 %
40 %
47 %
63 %
78 %
92 %
95 %
100 %
100 %
118 %
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164 %
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Low ER model drug
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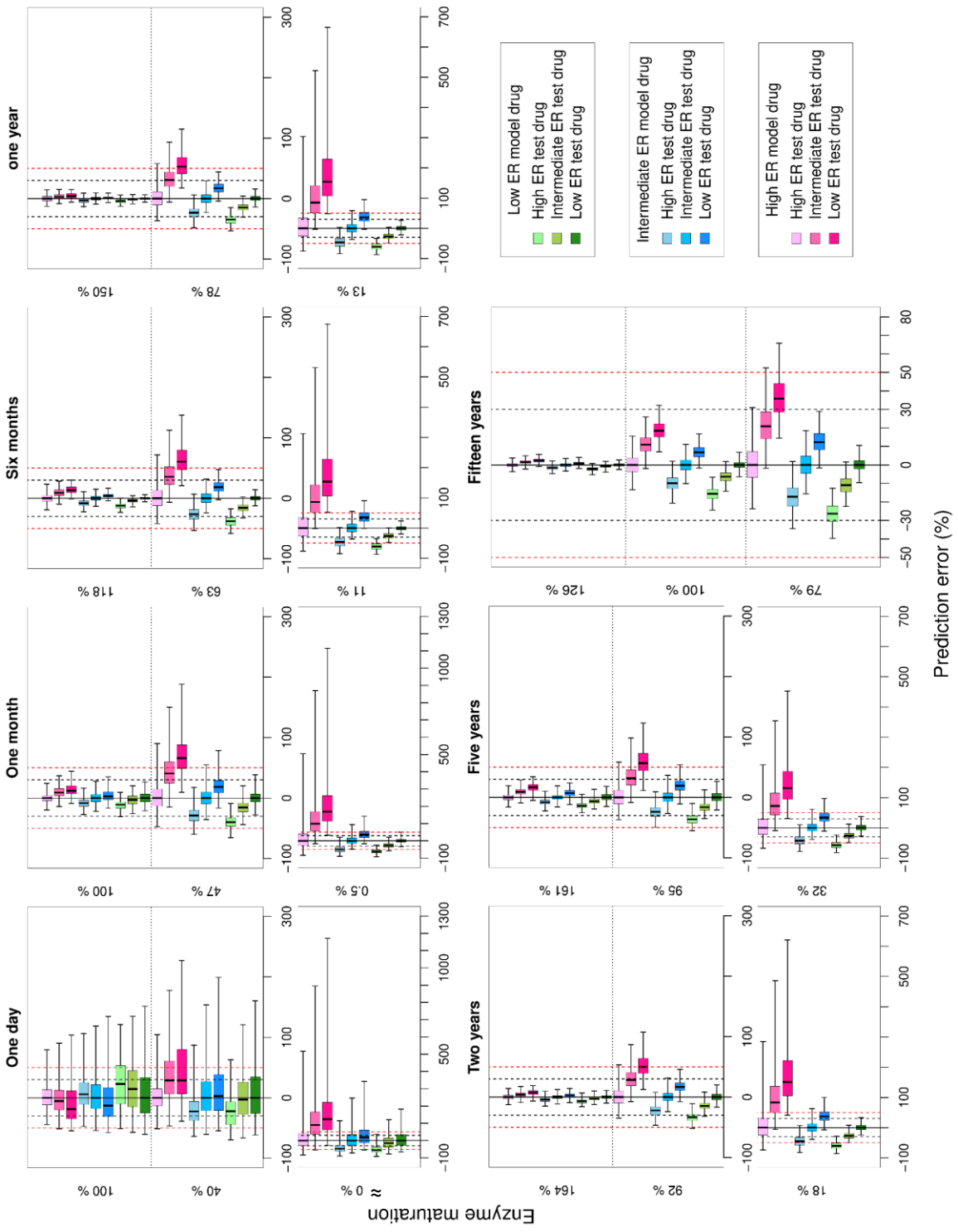
4B
Enzyme maturation
≈ 0 %
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One day
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Five years
Fifteen years
Low ER model drug
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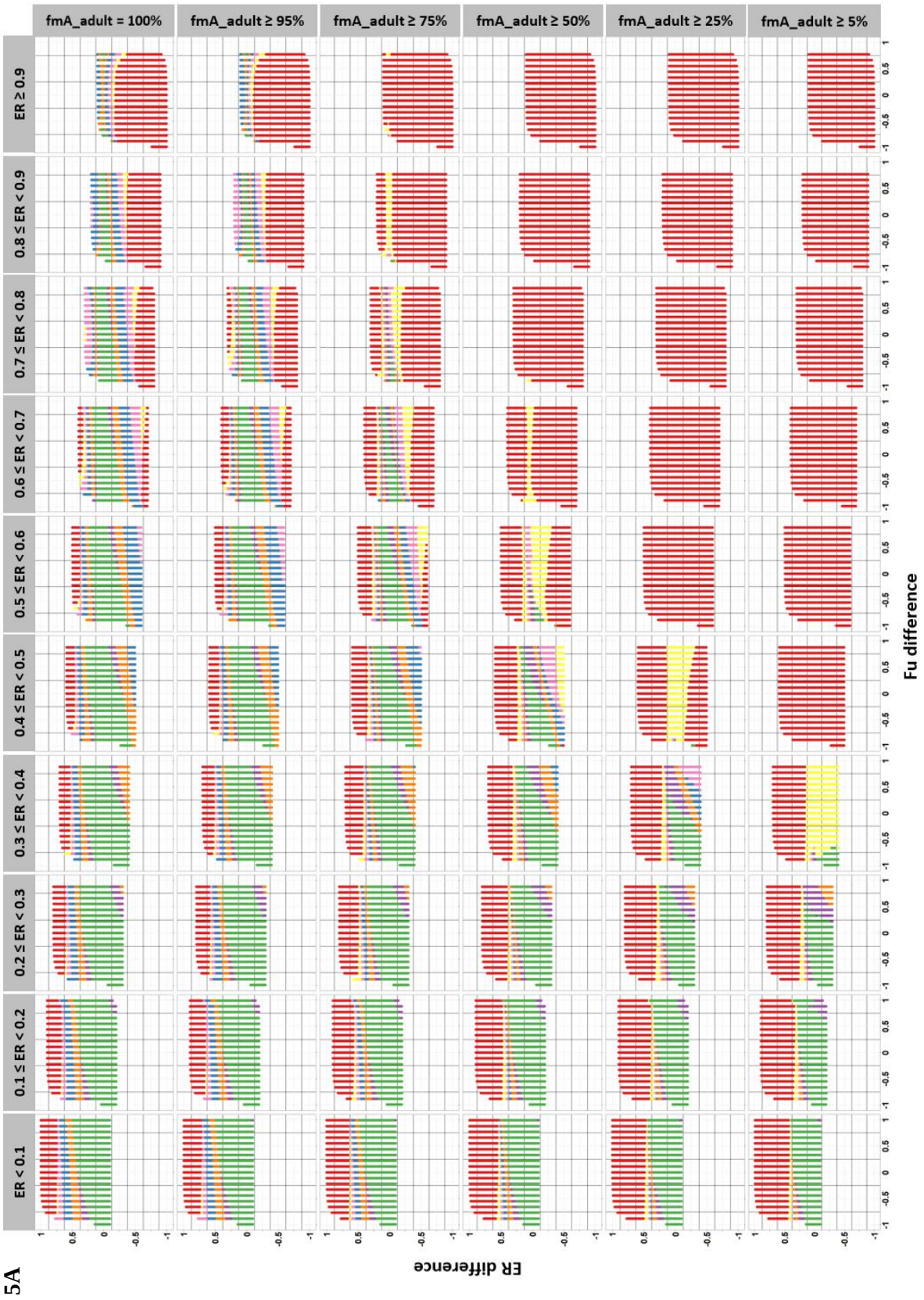
Intermediate ER test drug
Low ER test drug
Intermediate ER model drug
High ER test drug
Intermediate ER test drug
Low ER test drug
High ER model drug
High ER test drug
Intermediate ER test drug
Low ER test drug
Prediction error (%)

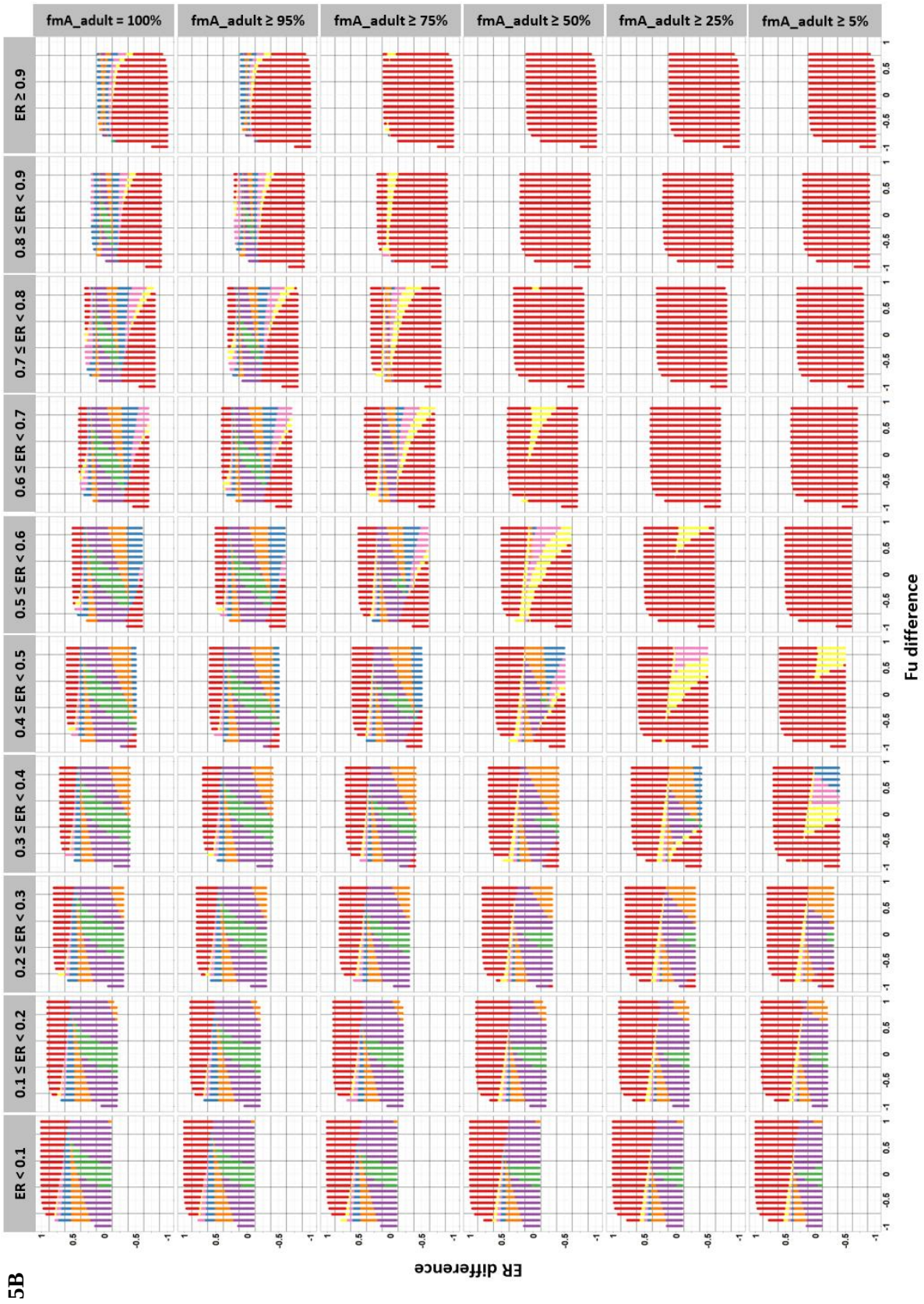
Figure 4 *The prediction error (PE) of the total (bound and unbound) drug plasma clearance (CLp) predictions for scenarios in which model and test drugs are exclusively metabolized by one isoenzyme (fmA_adult = 100%) and exclusively binding to human serum albumin (Figure 4A) or to alpha-1 acid glycoprotein (Figure 4B). The boxplots represent the minimum, 1st quartile, median, 3rd quartile, and maximum PE and are categorized by low (green), intermediate (blue), and high (pink) adult extraction ratio (ER) of the model drug and low (light color), intermediate (intermediate color), and high (dark color) adult ER of the test drug. For each age, the lowest, intermediate, and highest isoenzyme ontogeny values (percentage of adult $CL_{int,mic}$) reported for the 15 isoenzymes are shown. The intermediate isoenzyme ontogeny was defined as the isoenzyme ontogeny value the closest to the mean of the lowest and highest isoenzyme ontogeny values for a specific age. Low, intermediate, and high ER correspond to $ER \leq 30\%$, $30\% < ER \leq 70\%$ and $ER > 70\%$ respectively. The vertical solid black line indicates a PE of 0. The dotted black and dotted red lines indicate PE intervals of ±30% and ±50% respectively. Note that the x-axes are different for different ages.*

These most discriminative drug properties (i.e., extraction ratio of the model drug, and the difference in extraction ratio and in fraction unbound between model drug and test drug) were used to define model-test drug scenarios systematically leading to accurate CLp predictions in different age ranges, as displayed in the first row of Figure 5A, 5B and of the figures in Supplemental Material 2 and 3. The first row in Figure 5 presents the results for drugs that are exclusively metabolized by CYP3A4 and that bind to HSA (Figure 5A) or AAG (Figure 5B), while Supplementary Material 2 and 3 show the results for substrates of all investigated isoenzymes binding to HSA and AAG respectively. Although the results differ between the different isoenzymes and plasma proteins, they do reveal similar trends.

The between-drug extrapolation potential of pathway-specific pediatric covariate functions generally decreases with decreasing age, with patterns in model-test drug scenarios systematically leading to accurately scaled pediatric CLp being highly dependent on both ontogeny of the system-specific parameters and drug properties. For all ages, the between-drug extrapolation potential increases with decreasing ER values of the model drug, with this effect being most pronounced in younger children (see Supplementary Material 2 and 3). Moreover, the extrapolation potential increases when the difference in ER between model and test drugs decreases. Regarding plasma protein binding, it can be seen that between-drug extrapolation of pathway-specific pediatric covariate functions generally yields more accurately scaled pediatric CLp for drugs binding to HSA compared to drugs binding to AAG. Additionally, the difference in plasma protein binding between model and test drugs was found to mostly impact the method applicability in infants of one month or younger.


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference

5A


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference

5B

Figure 5 *Illustration of model-test drug scenarios that lead to accurate pathway-specific CLp predictions for a test drug after between-drug extrapolation of a pathway-specific pediatric covariate function. Results are presented for drugs that are metabolized by CYP3A4 and that bind to human serum albumin (Figure 5A) or alpha-1 acid glycoprotein (Figure 5B). Each column corresponds to a range of extraction ratio (ER) values for the model drug in adults and each row to a specific range of fraction of drug (model and test drug) that is metabolized by CYP3A4 in adults. For each graph, the y-axis represents the difference in ER between the test drug and the model drug (ER test drug – ER model drug) in adults, and the x-axis represents the difference in fraction unbound (fu) between the test drugs and the model drug in adults (fu test drug – fu model drug). Each dot represents a model-test drug scenario, including multiple model-test drug combinations. A color code is used to indicate systematically accurate CLp predictions for all model-test drug combinations within a model-test drug scenario, for children ≥ 5 years (yellow), ≥ 2 years (pink), ≥ 1 year (blue), ≥ 6 months (orange), ≥ 1 month (purple), and ≥ 1 day term neonates (green). Red dots indicate model-test drug scenarios leading to inaccurate predictions in children older than 5 years for at least one model-test drug combination within the model-test drug scenario. As an example, systematically accurate CLp scaling in children of 6 months and older is represented by the combination of the green, purple and orange dots.*

5.3.2 Results for model drugs and/or test drugs metabolized by several isoenzymes

When the model drug and/or the test drug are metabolized by two isoenzymes, PE increases or decreases, as compared to drugs eliminated by one isoenzyme, depending on the ontogeny of the isoenzyme representing the elimination pathway not accounted for by the covariate model (I_{NA}), and the fmA_adult values of the test drug and model drug. For the specific case in which the isoenzyme representing the elimination pathway accounted for by the covariate model (I_A) and I_{NA} have similar ontogeny, the PEs are similar to the PE for drugs exclusively metabolized by one isoenzyme I_A.

For model drugs and/or test drugs metabolized by several isoenzymes, the most discriminating drug properties to identify systematically accurate CLp predictions were the same as those found when both model and test drugs are metabolized by one unique isoenzyme. However, the larger the contribution of alternative metabolic pathways to the overall CLp in adults, the lower the extrapolation potential of the pathway-specific covariate function will be, as can be seen in the bottom rows of Figure 5 and Supplementary Material 2 and 3.

5.4 Discussion

In this work, for the first time we systematically investigated the applicability of between-drug extrapolation of pathway-specific pediatric covariate functions for CLp of drugs undergoing hepatic metabolism in which CLp stands for total (i.e., bound and unbound) hepatic metabolic plasma clearance. Our results show that pediatric changes in CLp of drugs that are eliminated by the same hepatic elimination pathway will not always follow similar patterns and therefore, in specific cases, these drugs will require a different pediatric PK-based dose adjustment.

CLp ontogeny of a specific elimination pathway was found to mainly depend on the following drug properties: the type of binding plasma protein, the adult fu and ER of the drugs and – in case of multiple elimination pathways - the number and type of isoenzymes responsible for the drug metabolism. This finding disproves the often implicitly made assumption that the ontogeny of CLp is drug-independent [25,26]. Additionally, it highlights the importance of ontogeny processes other than isoenzyme ontogeny alone on CLp scaling from adults to children for drugs undergoing hepatic metabolism (Figure 1). Therefore, the identified drug properties should be taken into account when extrapolating pathway-specific pediatric covariate functions between drugs. Figure 5 and Supplementary Material 2 and 3 were developed to guide the selection of scenarios that will systematically lead to accurate between-drug extrapolation of pediatric covariate functions. These guides can also be interpreted as defining scenarios for which between-drug extrapolation of pediatric covariate functions will approximate PBPK-based predictions with an accepted error of ± 30%. In these scenarios the use of this semi-mechanistic scaling method can be encouraged to expedite pediatric CLp scaling and the development of dosing recommendations.

To illustrate the use of these guides, we can take the example of midazolam (ER = 0.44 [27], fu = 0.022 [28] with binding to HSA [9], fm_A ≈ 93% [28,29]), sildenafil (ER = 0.45 [30], fu ≈ 0.04 with binding to HSA [31], fm_A = 79% [31]), and simvastatin (ER = 0.97 [32], fu = 0.02 [33] with binding to HSA [34], fm_A = 92% [29]), all mainly metabolized by CYP3A4. Based on the results for CYP3A4, we can anticipate that the popPK covariate model describing CYP3A4-mediated clearance ontogeny from adults to neonates of 1 day of midazolam, could be extrapolated to sildenafil, because ER model = 0.44, fu difference = 0.018, ER difference = 0.01, fm_A ≥ 75% corresponds to a green area in Figure 5A. The same plot also shows that this extrapolation from midazolam to simvastatin cannot be performed,

because ER model = 0.44, fu difference = -0.002, ER difference = 0.53, fm_A ≥ 75% corresponds to a red area. Importantly, this scaling method is isoenzyme specific, and one should be careful not to overlook minor pathways in adults which can become major pathways in children when aiming at scaling total clearance.

It should be noted that model-test drug scenarios leading to systematically accurate CLp scaling were defined according to very strict criteria. Each dot in Figure 5 and the accompanying Supplementary Material represents a scenario that summarizes the PEs for many different model-test drug combinations. CLp predictions were defined as not systematically accurate for a scenario if only one model-test drug combination within this scenario and, for drugs metabolized by several isoenzymes, if at least one specific I_{NA} (isoenzyme not accounted for by the covariate model) led to scaled CLp deviating > 30% from its 'true' value. However, it is likely for CLp scaling to be accurate for a (large) number of model-test drug combinations within such scenarios, but in these scenarios the accuracy of the scaling method cannot be easily predicted *a priori* without PBPK modeling.

Overall, our results show that the between-drug extrapolation potential of pathway-specific pediatric covariate functions for CLp increases when the ER of the model drug in adults decreases. The applicability of this method decreases with age and with a decreased adult fraction of the test drug and/or model drug being metabolized by the isoenzyme pathway accounted for by the covariate model (fmA_adult). Moreover, these trends increase with increased ER of the model drug. Plasma protein binding to AAG also limits the between-drug extrapolation potential of pediatric covariate functions for CLp, especially in young children, which can be explained by the more pronounced ontogeny pattern of AAG compared to HSA for these ages [35].

In this work, we discovered that, for drugs metabolized by several isoenzymes, the ontogeny of CLp due to one specific isoenzyme (I_A) is influenced by the ontogeny of the other isoenzymes responsible for the drug clearance (I_{NA}). We also found that this impact increases with increased adult ER and decreased fmA_adult, with fmA_adult standing for adult fraction of drug CLp due to I_A. This is due to changes in ER with age contributed by the ontogeny of all isoenzymes involved in the drug CLp [36], changes which in turn modify each isoenzyme specific CLp ontogenies. This was shown by the reduced applicability of the between-drug extrapolation potential of pathway-specific covariate models for CLp with decreased fmA_adult. This is further supported by the increase of these trends with increased ER of the model drug (see Figure 5 and Supplementary Material 2 and 3).

Although this workflow only investigated drugs undergoing hepatic metabolic CLp, the results on multiple elimination routes also apply to drugs undergoing hepatic metabolism in combination with renal clearance, as renal clearance does not impact the hepatic ER or the ontogeny of hepatic CLp. For these drugs, fmA_adult should be interpreted as the fraction of the total hepatic metabolic CLp in adults due to the isoenzyme pathway accounted for by the covariate model (I_A). Additionally, this workflow can be used to develop guidance on the need for PK-based dose adjustments in clinical situations of reduced plasma protein binding, like for instance uremia and hypoalbuminemia, or drug-drug interactions. In this situation, the model drug properties correspond to the drug properties in normal clinical situations and the test drug properties are changed to fu and ER values adapted to the calculated values for plasma protein binding in adults. If such a model-test drug combination yields an accurate CLp prediction for the test drug, the pediatric dose can be derived by applying the same dose adjustment factor used for maintenance doses in adults with a similar clinical condition.

An important limitation of the PBPK-based simulation workflow is that active influx or efflux of drugs into or out of hepatocytes by transporters is not included, thereby implicitly assuming this process to be passive. The impact of active drug transport in the membranes of hepatocytes on the between-drug extrapolation of pathway-specific pediatric covariate functions requires further investigation.

In conclusion, the developed PBPK-based simulation workflow utilizing mechanistic equations defining hepatic metabolism allowed, for the first time, to unravel the variables most impacting CLp ontogeny and to define scenarios for which extrapolation of pediatric covariate functions from one drug to another systematically leads to accurate isoenzyme specific CLp scaling from adults to pediatric patients for 15 hepatic isoenzymes.

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Conflict of interest

Trevor Johnson is a paid employee of Simcyp Limited (a Certara company). Amin Rostami-Hodjegan holds shares in Certara, a company focusing on model-informed drug development and also has shares in Diurnal, which focuses on developing high quality products for the life-long treatment of chronic endocrine conditions. As Editor-in-Chief for CPT: Pharmacometrics & Systems Pharmacology, Piet H. van der Graaf was not involved in the review or decision process for this article.

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Supplementary material 1 Methodology

PBPK-based simulation workflow

Variables in the PBPK-based simulation workflow

System-specific parameters

PBPK simulations were performed for typical pediatric 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 pediatric individuals and from the Simcyp® (Simcyp Ltd, Sheffield, UK) V15.R1 library for a typical adult (see table below). 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.

	1 day	1 month	6 months	1 year	2 years	5 years	15 years	25 years
Bodyweight (kg)	3.45	4.30	7.55	9.90	12.35	18.25	54.25	72.65
Height (cm)	49.75	54.25	66.00	74.75	86.00	108.25	166.00	172.30

PBPK parameters were taken from the Simcyp® V15.R1 library as were ontogeny patterns in the hepatic blood flow (Qh), plasma protein concentrations for human serum albumin (HSA) and alpha-1 acid glycoprotein (AAG), haematocrit, liver size, milligram protein per gram of liver (MPPGL) and isoenzyme abundance, excepted for the isoenzyme ontogeny of SULT1A1 for which maturity was taken to have been reached at birth [4].

Drug-specific properties of the model drugs and test drugs

Hypothetical drugs were generated to serve as model and test drugs in the PBPK-based simulation workflow. For each individual drug-specific property, a realistic range of values was selected.

The model parameters to calculate hepatic plasma clearance (CLp) in this workflow which are based on a combination of drug-specific and system-specific parameters are: the unbound drug fraction in plasma (fu), the blood to plasma ratio (B:P), and the intrinsic metabolic clearance in the liver based on unbound drug concentration (CLint). These parameters therefore reflect drug properties in a population of a specific age. In this analysis, fu and CLint in adults were used as surrogates of their contributing drug-specific parameters (i.e., affinity to plasma proteins and affinity to isoenzymes respectively), since they are most often reported.

To derive the affinity to plasma proteins from the unbound drug fraction in plasma (fu) and the concentration of the binding plasma protein in adults, equations by Rodgers and Rowland [5,6] were used. The hypothetical drugs were assumed to be exclusively bound to either HSA or AAG. The fu in adults ranged from 1% to 100%, with 8 equidistant intermediate values. Values for the blood to plasma partitioning coefficient (Kp) of 0.35, 1, 2, 3, 4, 5, 10, 20, and 40 were selected, reflecting different extents of drug diffusion into the red blood cells. $CL_{int,mic,total}$ in adults ranged between $0.56.10^{-6}$ and $0.209.10^{-3}$ L. $min^{-1}.mg^{-1}$ microsomal protein [7], with 40 equidistant intermediate values. These different values reflect difference in both affinities for and abundances of isoenzymes in adults. Equations 3 to 6 below were used to calculate the extraction ratio (ER) of each hypothetical drug in adults.

Step 1 of the PBPK-based simulation workflow

'True' total hepatic plasma clearance (CLp) values were computed using the dispersion model (Equations 1 to 6). 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 [8].

$$\mathrm{CLp} = \mathrm{CL_B} \times \mathrm{B{:}\,P} \quad (1)$$

$$\mathrm{CL_B} = \mathrm{Qh} \times \mathrm{ER} \quad (2)$$

$$\mathrm{ER} = 1 - \mathrm{F_H} \quad (3)$$

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

$$a = (1 + 4R_N \times D_N)^{1/2} \quad (5)$$

$$R_N = (fu/B{:}\,P) \times CLint/Qh \quad (6)$$

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 total hepatic 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 [9].

For each hypothetical drugs, CLint in adults was computed as the product of $CL_{int,mic,total}$, the MPPGL, and the liver weight. B:P in adults was derived from the adult haematocrit, from the Kp value, and the value of fu in adults [10]. Values of adult $CL_{int,mic,total}$, Kp, and adult fu were taken from the values defined to generate the hypothetical drugs (see under Hypothetical drugs). Qh, CLint, fu, and B:P in pediatric patients were scaled using the ontogeny functions of the relevant system-specific parameters. The R model code for the CLp simulations is provided in the Model Code Supplementary Material.

Conditions leading to accurate CLp predictions

In order to identify the drug-specific properties allowing to best discriminate between accurate and inaccurate scaled pediatric CLp, the prediction errors (PEs) obtained in step 4 of the PBPK-based simulation workflow were first split into different groups. A group was defined as containing PEs for all ages for one combination of model and test drugs eliminated by the same isoenzyme and binding to the same plasma protein (both including drugs not binding to plasma proteins). Then, the PEs within each group were transformed into a binary outcome according to the following definitions:

- Positive outcome: all CLp predictions within the defined group are accurate (absolute PE $\leq$ 30%).
- Negative outcome: at least one CLp prediction within the defined group is inaccurate (absolute PE >30%).

Then, for each isoenzyme and type of binding plasma protein, drug-specific parameters were tested for their discriminative power between positive and negative outcome using a hierarchical tree method with a penalty function. The hierarchical tree method used was the recursive partitioning and regression tree from the rpart R package [11]. The penalty function was implemented in order to obtain variables best discriminating positive outcomes, while accepting a decreased discriminative power for negative outcomes. The drug specific parameters tested were Kp, and fu, CLint, and B:P in adults of the model drug and of the test

drug, as well as the difference in these parameters between the model drug and test drug. The most discriminatory variables were defined as the variables allowing to discriminate the maximum of positive outcomes from negative outcomes. A maximum of 3 most discriminatory variables was set in order to allow for readability of the results.

Multiple elimination pathways

For model drugs metabolized by several isoenzymes, the CLp_A corresponds to the part of the overall drug CLp due to isoenzyme A (I_A), this is the isoenzyme for which ontogeny is accounted for by the covariate function. Likewise, for test drugs metabolized by several isoenzymes, CLp_A corresponds to the part of the overall CLp due to I_A and the adult CLp_A is scaled to pediatric patients using the covariate function describing the changes in CLp_A from adults to pediatric patients of a model drug. This is equivalent to scaling part of the drug clearance responsible for the formation of a specific metabolite due to a specific isoenzyme. An isoenzyme that was not accounted for (I_{NA}) in the pediatric covariate function was included in the simulations since it impacts the extraction ratio of the drug which in turn might impact the ontogeny of CLp_A.

In this part of our investigation, the same steps of the PBPK-based simulation workflow were performed as for the case described for only one metabolizing isoenzyme, with thereinafter adaptations.

All 15 isoenzymes that were included in the simulation workflow were included as I_A and I_{NA} in all possible combinations. For the fraction metabolized by I_A in adults (fmA_adult), values of 5, 25, 50, 75, and 95% were selected and the remaining fraction was assumed to be metabolized by I_{NA}. fmA_adult was included in the PBPK-based simulation workflow as a drug property, leading to a greater set of hypothetical model drug and test drugs to be investigated compared to the investigation of drugs only eliminated by one isoenzyme. Equations 7 to 9 were used to determine intrinsic microsomal clearance for each isoenzyme.

$$CL_{int,mic,A} = CL_{int,mic,total} \times fmA \quad (7)$$

$$CL_{int,mic,NA} = CL_{int,mic,total} \times fmNA \quad (8)$$

$$\text{With } CL_{int,mic,A} + CL_{int,mic,NA} = CL_{int,mic,total} \quad (9)$$

In step 1 of the workflow, 'true' CLp_A were derived from the $'true'\ total\ CLp$ using equation 10.

$$\text{'true' } CLp_A = \text{'true' total } CLp \times fmA \quad (10)$$

Adult 'true' total CLp_A was derived as described for drugs eliminated by one isoenzymes, using the adult $CL_{int,mic,total}$ (to obtain the adult 'true' total CLp),and adult fmA (fmA _adults), both defined as a drug property.

Pediatric 'True' total CLp from Eq. 10 was computed as described earlier, by scaling $CL_{int,mic,total}$ from adult to pediatric values. Since for drugs metabolized by several isoenzymes, $CL_{int,mic,total}$ corresponds to the sum of $CL_{int,mic,A}$ and $CL_{int,mic,NA}$ (Eq. 9), adult values of $CL_{int,mic,A}$, and $CL_{int,mic,NA}$ were scaled to pediatric values by accounting for the isoenzyme ontogeny of I_A (IO_A) and I_{NA} (IO_{NA}) respectively and then summed up to derive pediatric $CL_{int,mic,total}$. The fmA in pediatric patients (fmA_pediatrics) was derived according to Eq.11.

$$\text{fmA_pediatrics} = \frac{\text{fmA_adult} \times IO_A}{\text{fmA_adult} \times IO_A + \text{fmNA_adult} \times IO_{NA}} \quad (11)$$

In this equation, IO_A and IO_{NA} are the isoenzyme ontogeny defined as the ratio of pediatric $CL_{int,mic}$ and adults $CL_{int,mic}$ of I_A and I_{NA} respectively, and fmA_adult and fmNA_adult are the fraction metabolized in adults by I_A and I_{NA} respectively. The R model code for the CLp_A simulations is provided in the Model Code Supplementary Material.

In step 2 and 3 of the workflow the pediatric covariate function is developed for the elimination of model drug M via I_A only and is extrapolated to predict the elimination of the test drug T via I_A only. The isoenzyme that is not accounted for (I_{NA}) in the pediatric covariate function is responsible for CLp_{NA}. While I_{NA} is contributing to the overall CLp of the model and/or test drugs and thereby influencing the extraction ratio of these drugs, CLp_{NA} is not part of the pediatric covariate function that is extrapolated between the model and the test drug.

In step 4, the PE is only calculated for CLp_A, the plasma clearance by I_A.

The identification of drug properties allowing to best discriminate between accurate and inaccurate scaled pediatric CLp_A was done by defining PEs groups similarly as for drugs eliminated by one isoenzyme, but drugs contained in each group take all possible values of fmA_adult, and are co-metabolized by all possible I_{NA}.

Finally, to define model-test drug scenarios systematically leading to accurate scaled pediatric CLp, PE not only were grouped based on the most discriminative drug properties (similarly as for drug eliminated by one isoenzymes), but also were grouped per fmA-adult category, disregarding the I_{NA} involved in drug clearance. FmA_adult categories were: fmA_adult = 100%, fmA_adult ≥ 95%, ≥ 75%, ≥ 50%, ≥ 25% and ≥ 5%.

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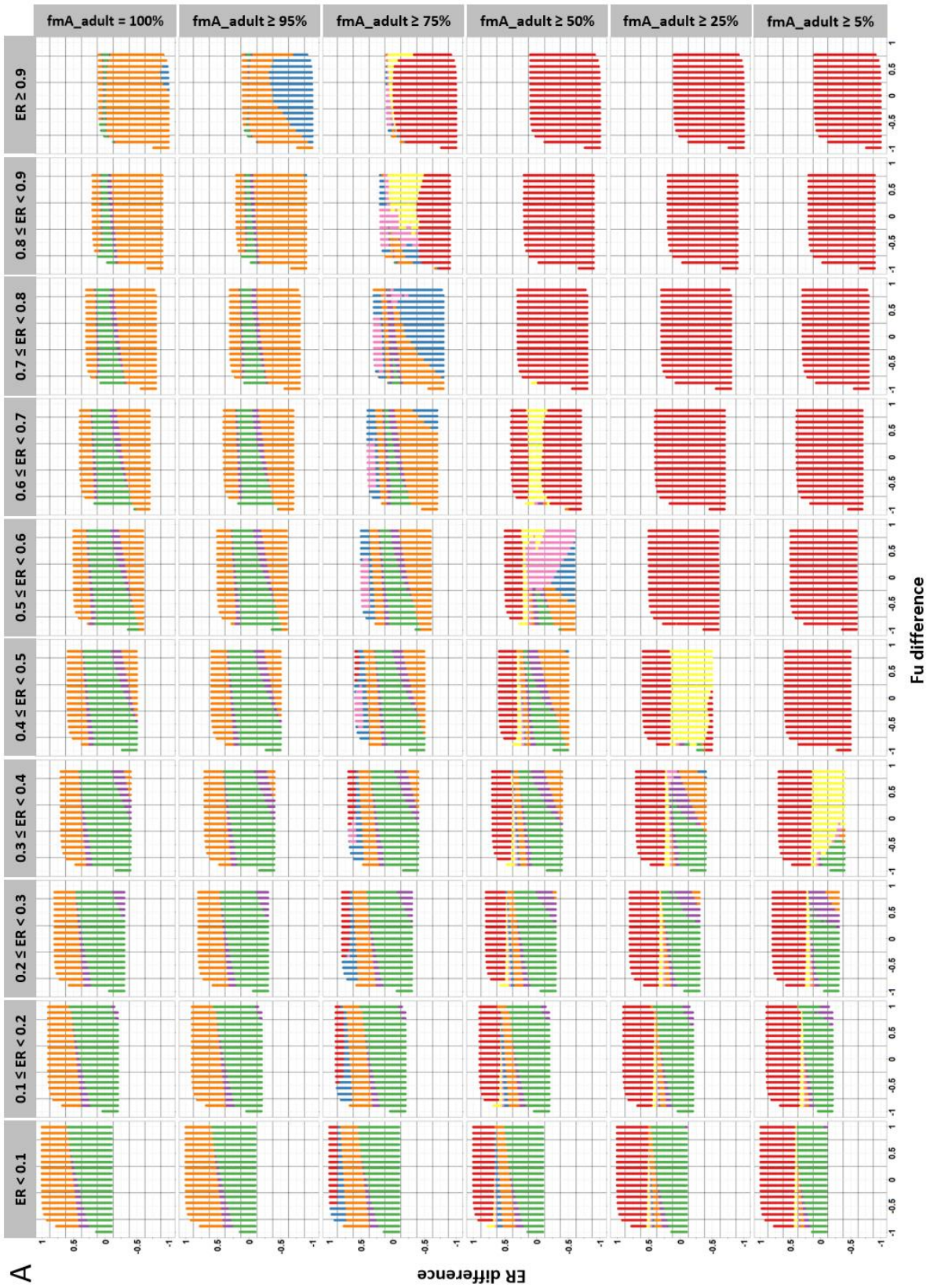
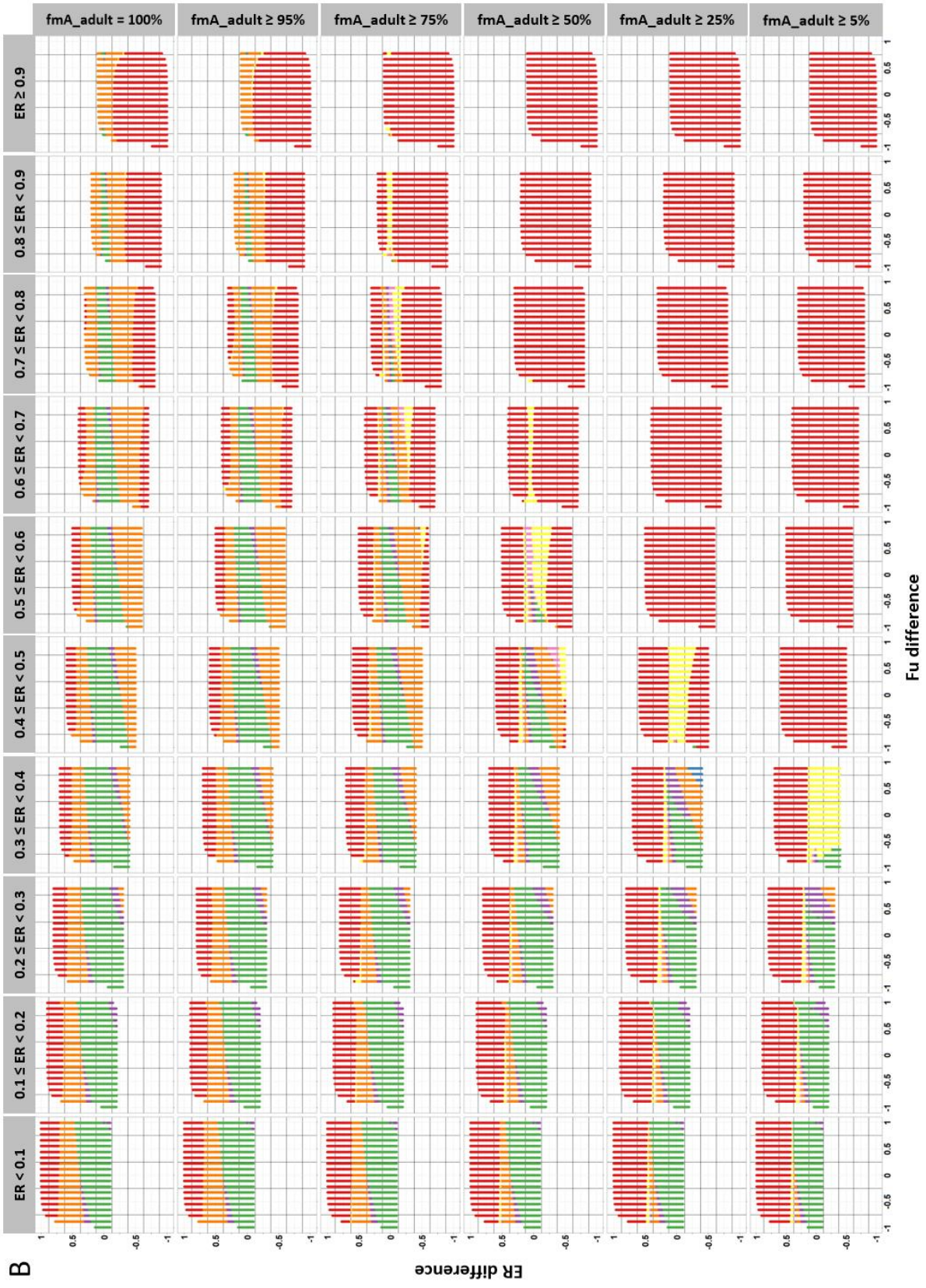
Supplementary material 2 Model-test drug scenarios, for drugs binding to human serum albumin, that lead to accurate pathway specific CLp predictions for a test drug after between-drug extrapolation of a pathway specific pediatric covariate function.

Figures A to O display model-test drug scenarios that lead to accurate pathway specific CLp predictions for a test drug after between-drug extrapolation of a pathway specific pediatric covariate function. Results are displayed for drugs binding to human serum albumin (HSA) and eliminated through CYP1A2 (A), CYP2A6 (B), CYP2B6 (C), CYP2C8 (D), CYP2C9 (E), CYP2C18_19 (F), CYP2D6 (G), CYP2E1 (H), CYP3A4 (I), SULT1A1 (J), UGT1A1 (K), UGT1A4 (L), UGT1A6 (M), UGT1A9 (N) and UGT2B7 (O) hepatic metabolism. Each column corresponds to a range of extraction ratio (ER) values for the model drug in adults and each row to a specific range of fraction of drug (model and test drug) that is metabolized by CYP1A2 in adults (fmA_adult). For each graph, the y-axis represents the difference in ER between the test drug and the model drug (ER test drug – ER model drug) in adults, and the x-axis represents the difference in fraction unbound (fu) between the test drugs and the model drug in adults (fu test drug – fu model drug). Each dot represents a model-test drug scenario, including multiple model-test drug combinations. A color code is used to indicate systematically accurate CLp predictions for all model-test drug combinations within a model-test drug scenario, for children ≥ 5 years (yellow), ≥ 2 years (pink), ≥ 1 year (blue), ≥ 6 months (orange), ≥ 1 month (purple), and ≥ 1 day term neonates (green). Red dots indicate model-test drug scenarios leading to inaccurate predictions in children older than 5 years for at least one model-test drug combination within the model-test drug scenario. As an example, systematically accurate CLp scaling in children of 6 months and older is represented by the combination of the green, purple and orange dots. The table below summarizes the color code used to indicate systematically accurate scaled pediatric CLp (+) and inaccurate scaled pediatric CLp (-) in the different ages studied.

1 day	1 month	6 months	1 year	2 years	≥ 5 years	
+	+	+	+	+	+	**green**
-	+	+	+	+	+	**purple**
-	-	+	+	+	+	**orange**
-	-	-	+	+	+	**blue**
-	-	-	-	+	+	**pink**
-	-	-	-	-	+	**Yellow**
-	-	-	-	-	-	**Red**


A
fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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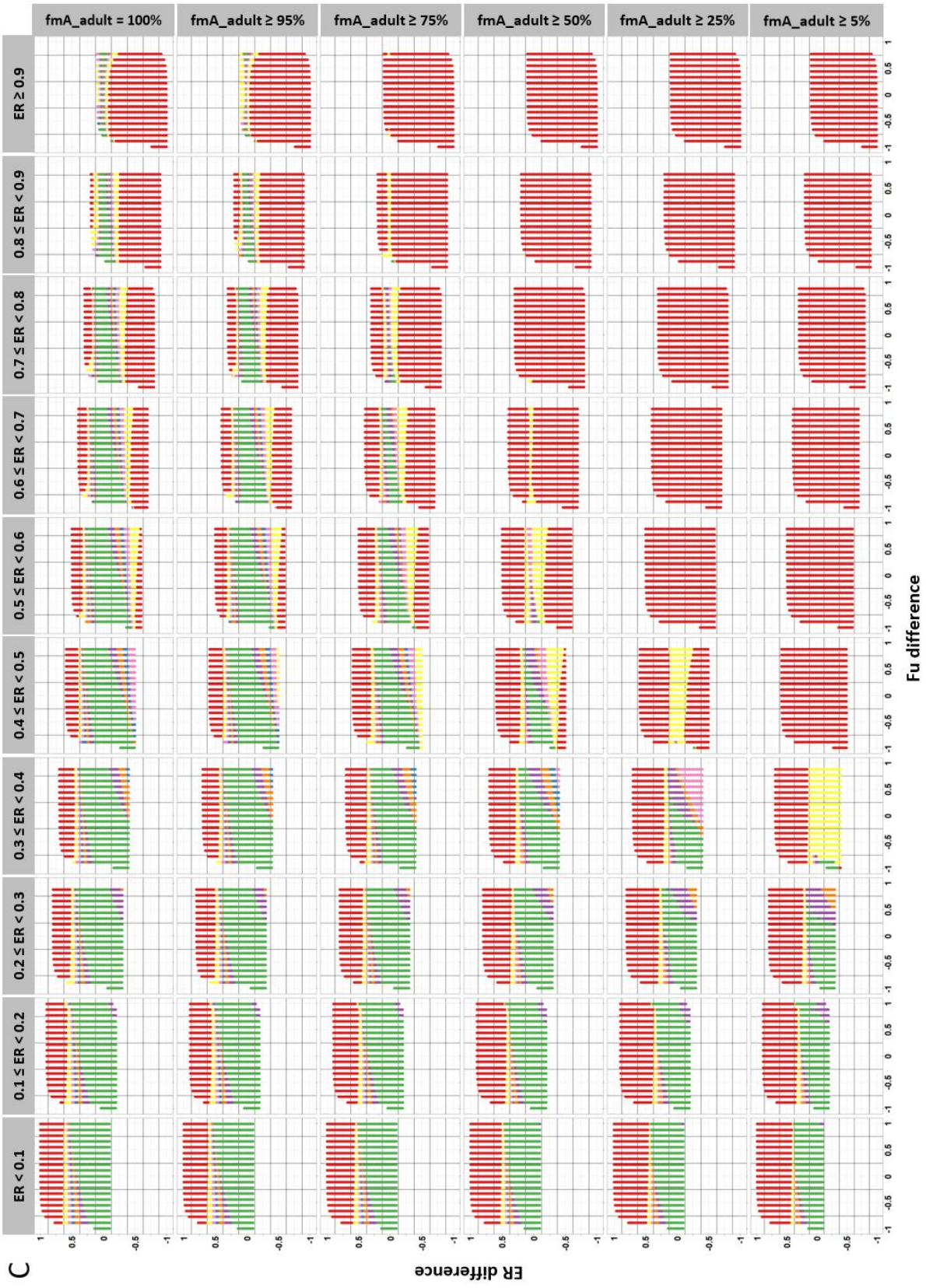
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference


C
fmA_adult = 100%
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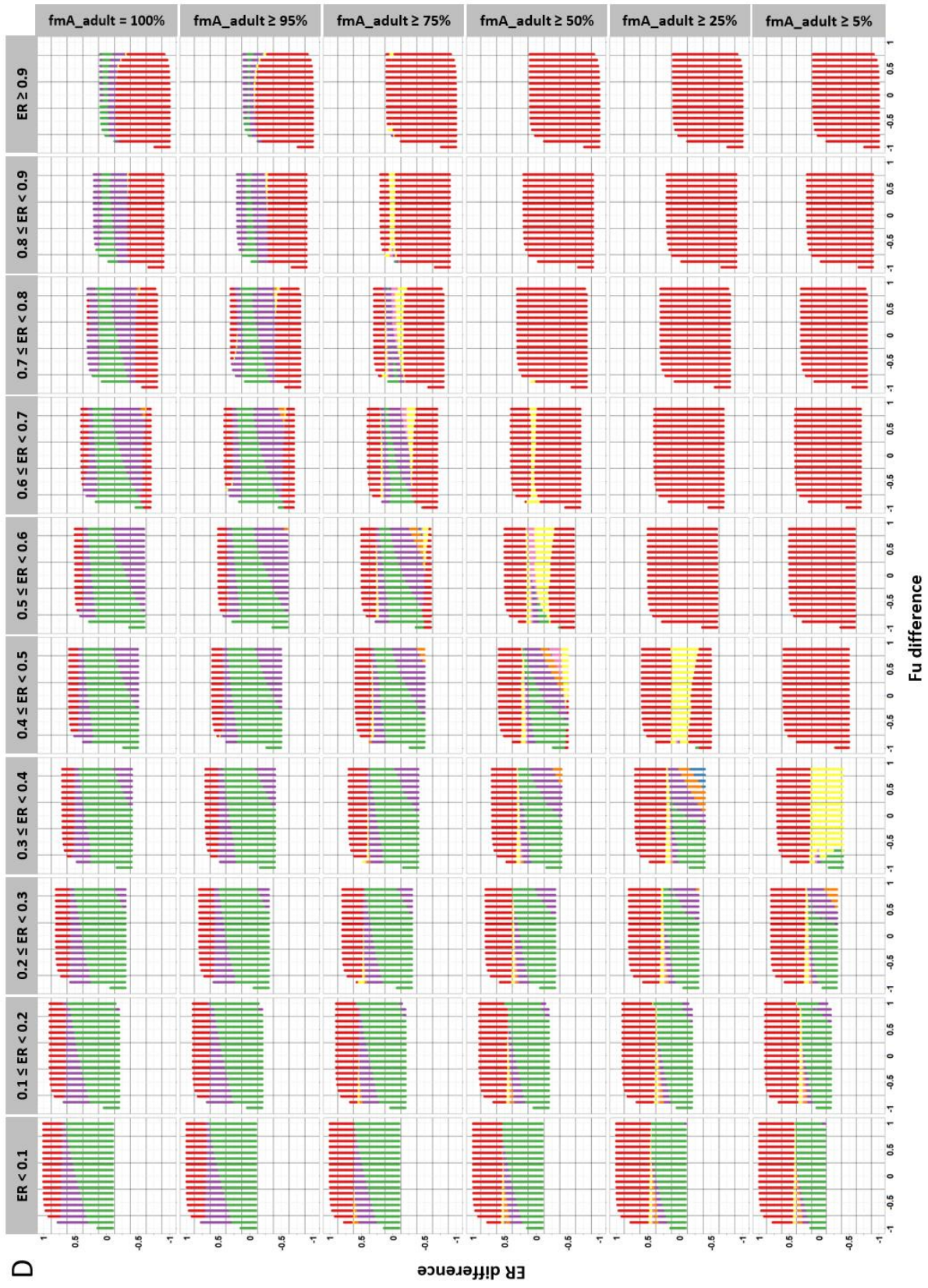
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
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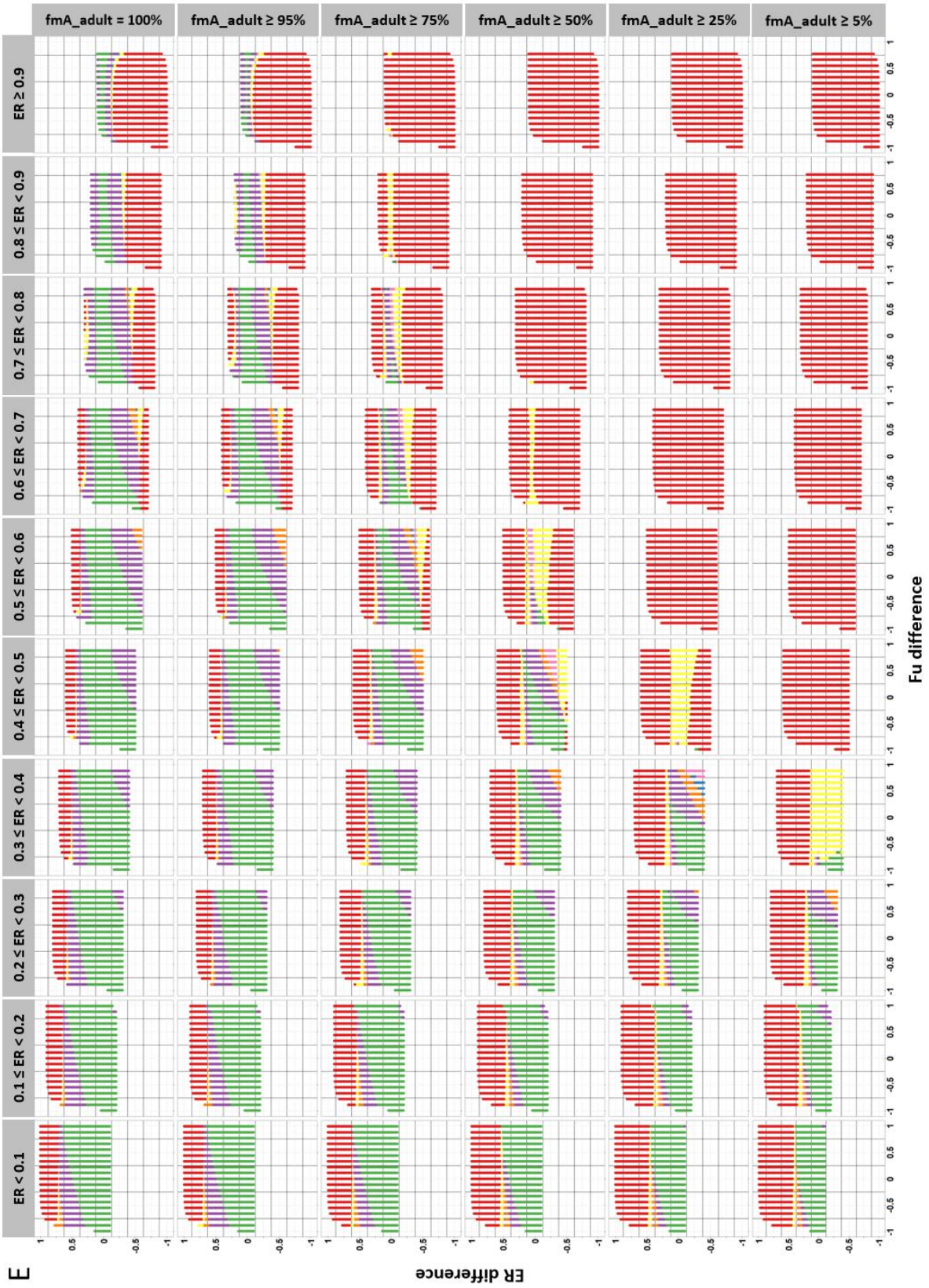
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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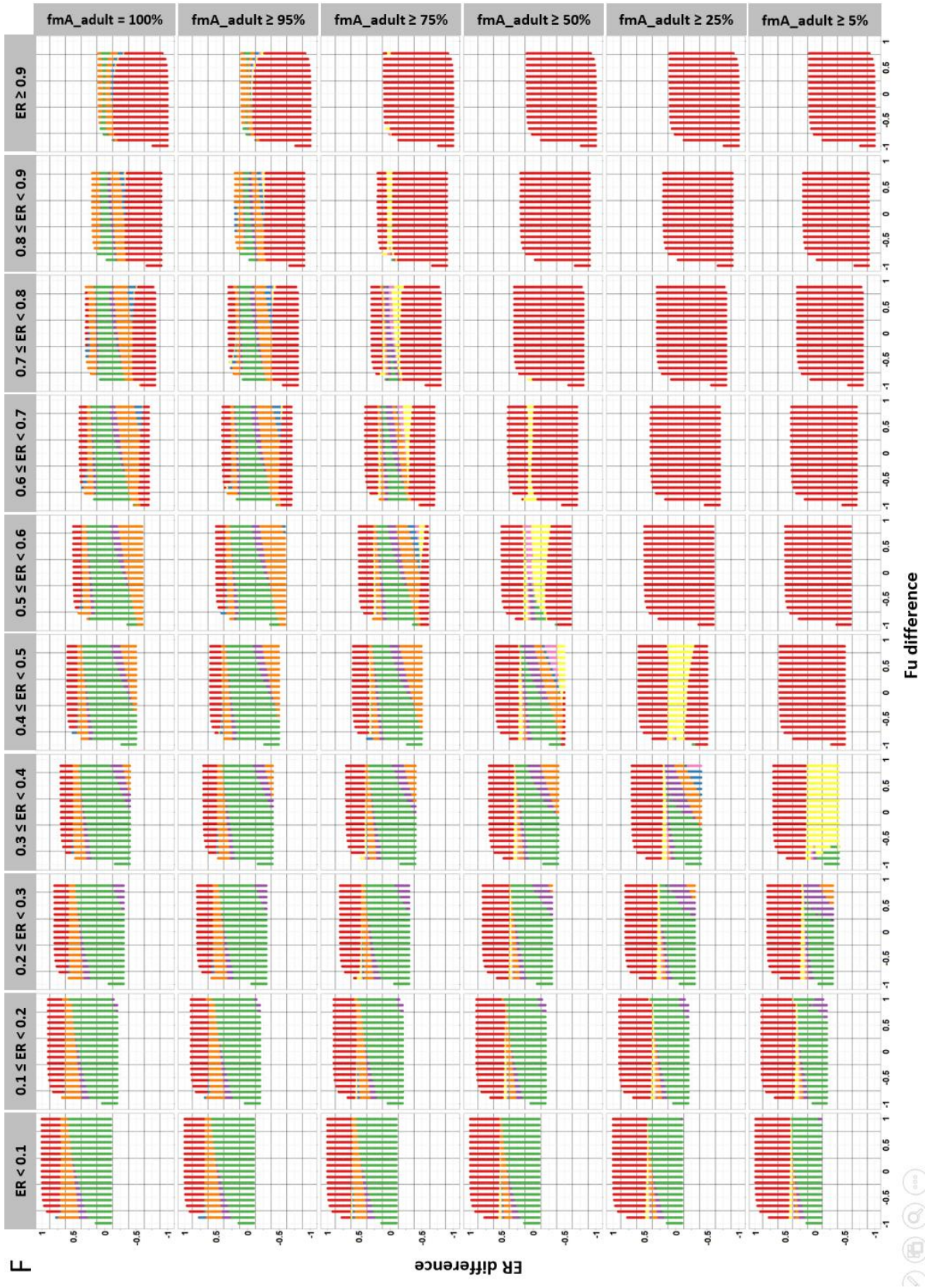
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
E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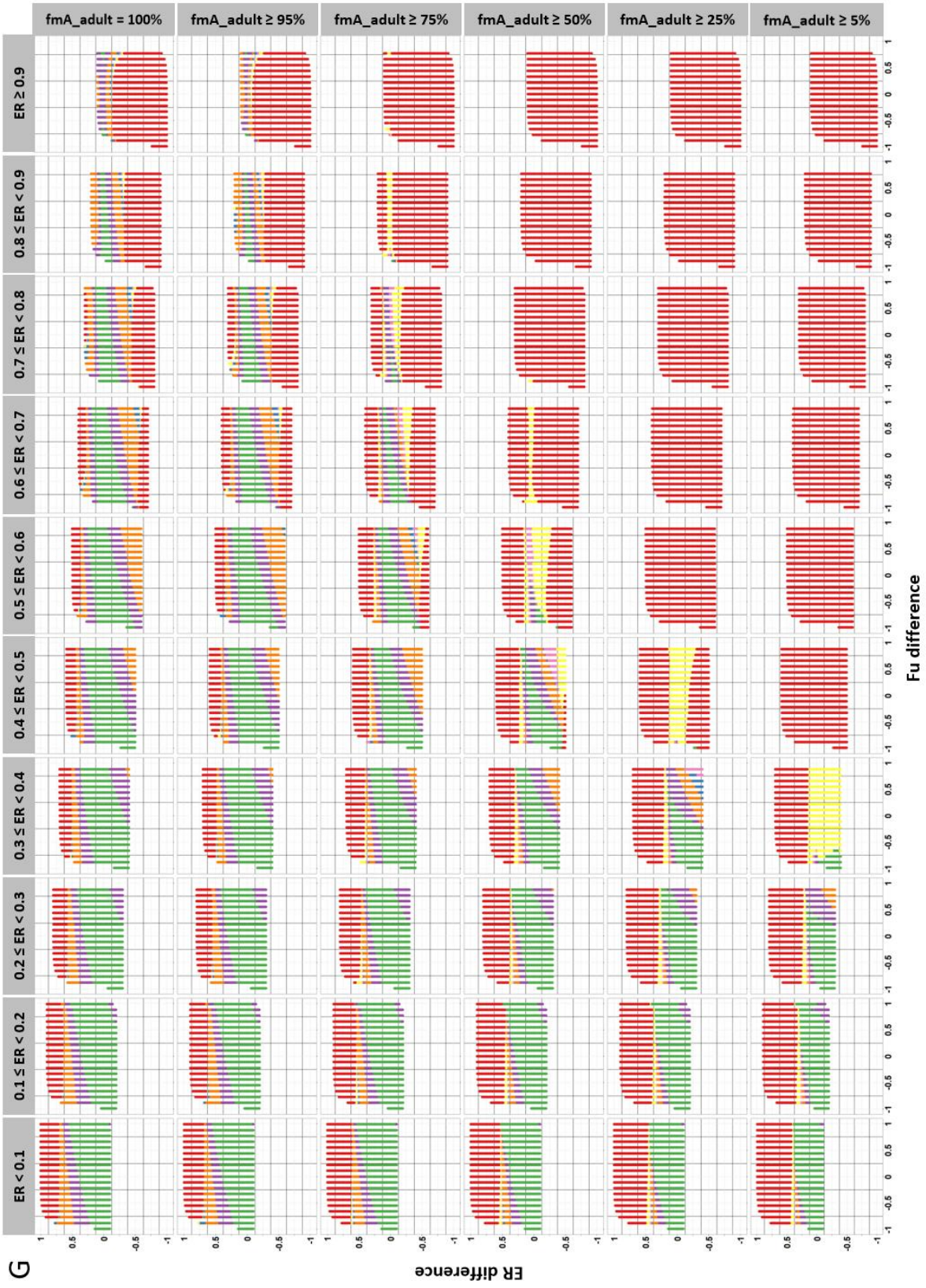
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
F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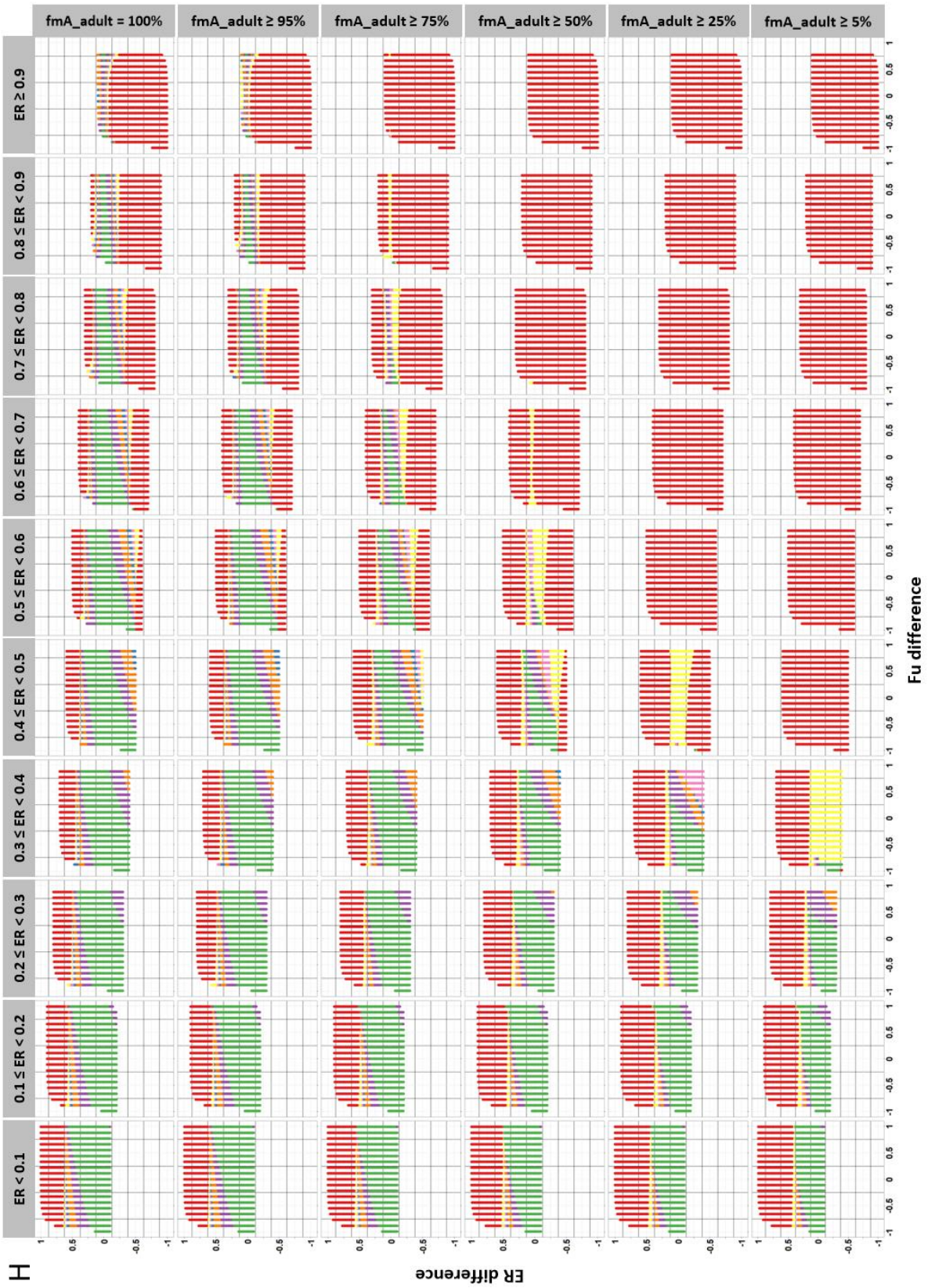
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
G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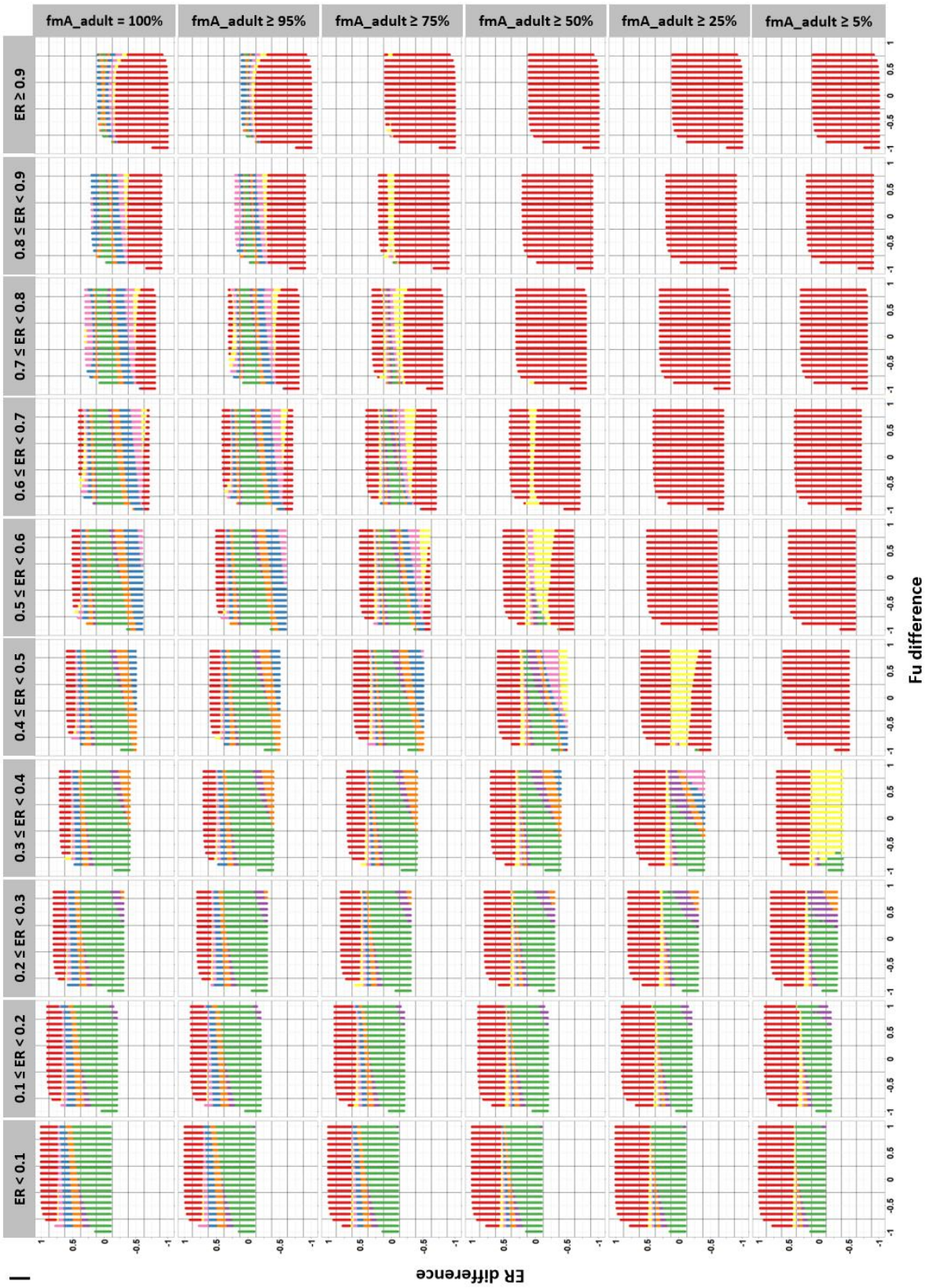
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
H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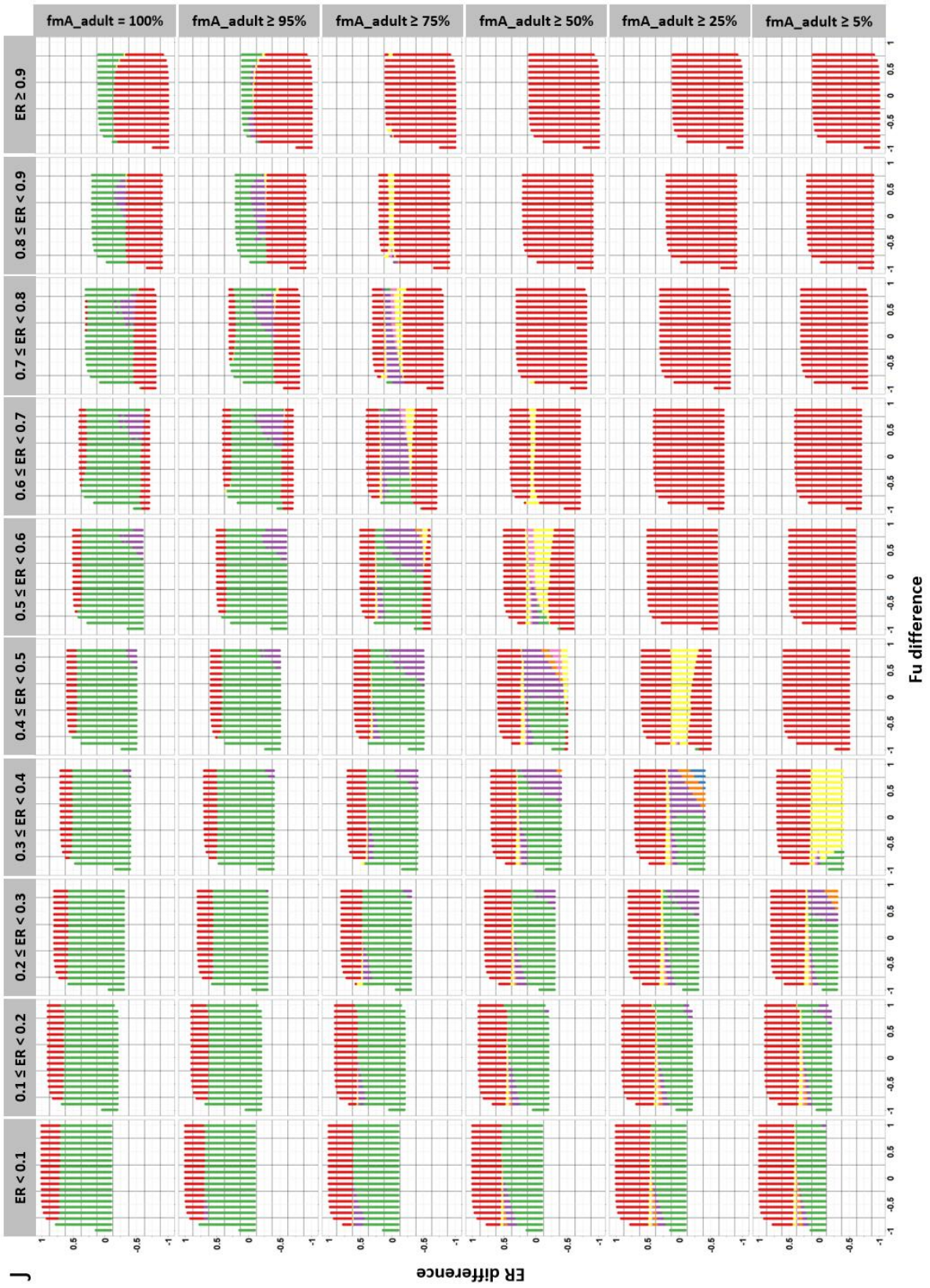
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
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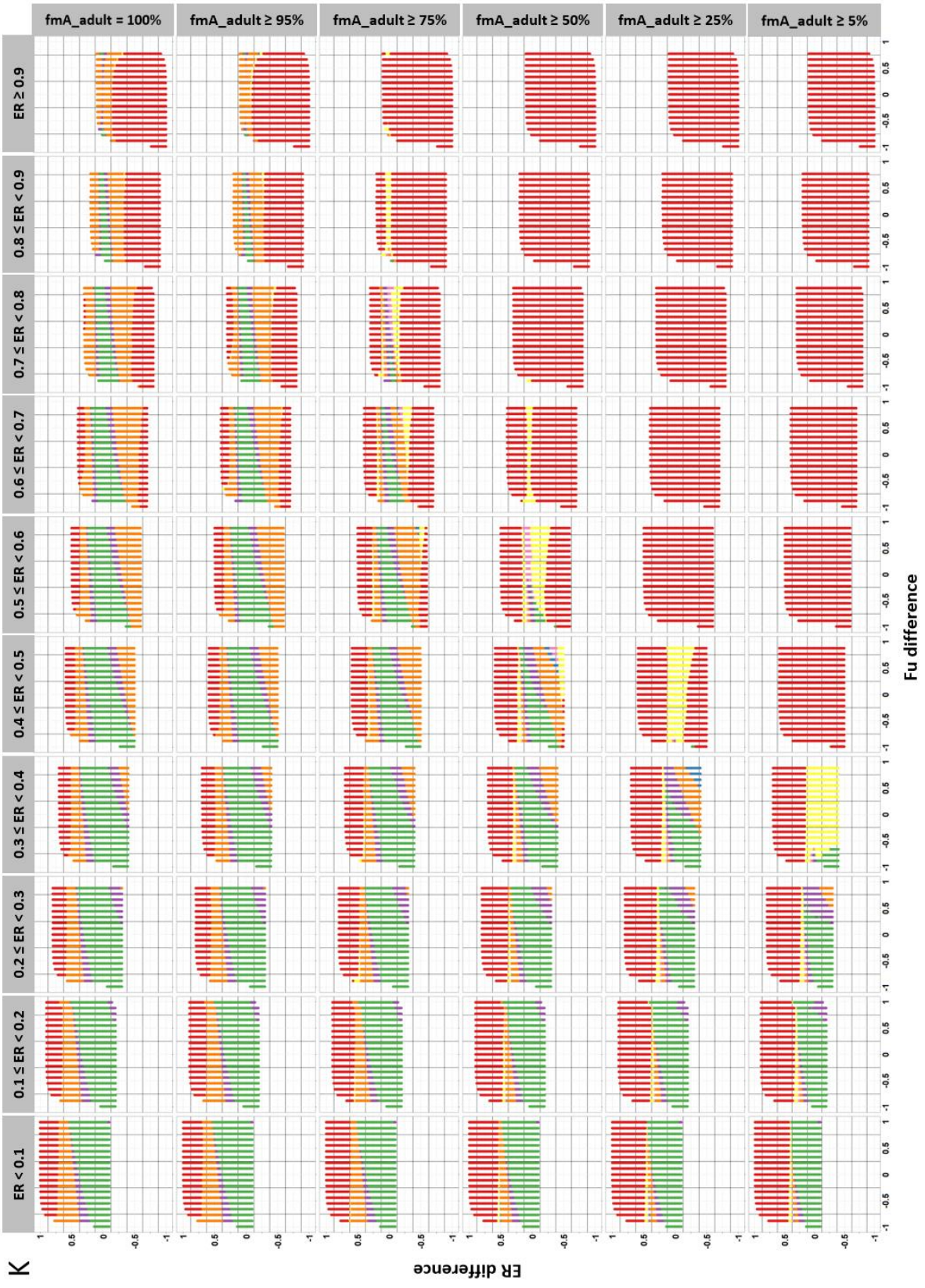
0.1 ≤ ER < 0.2
ER < 0.1
J
ER difference
Fu difference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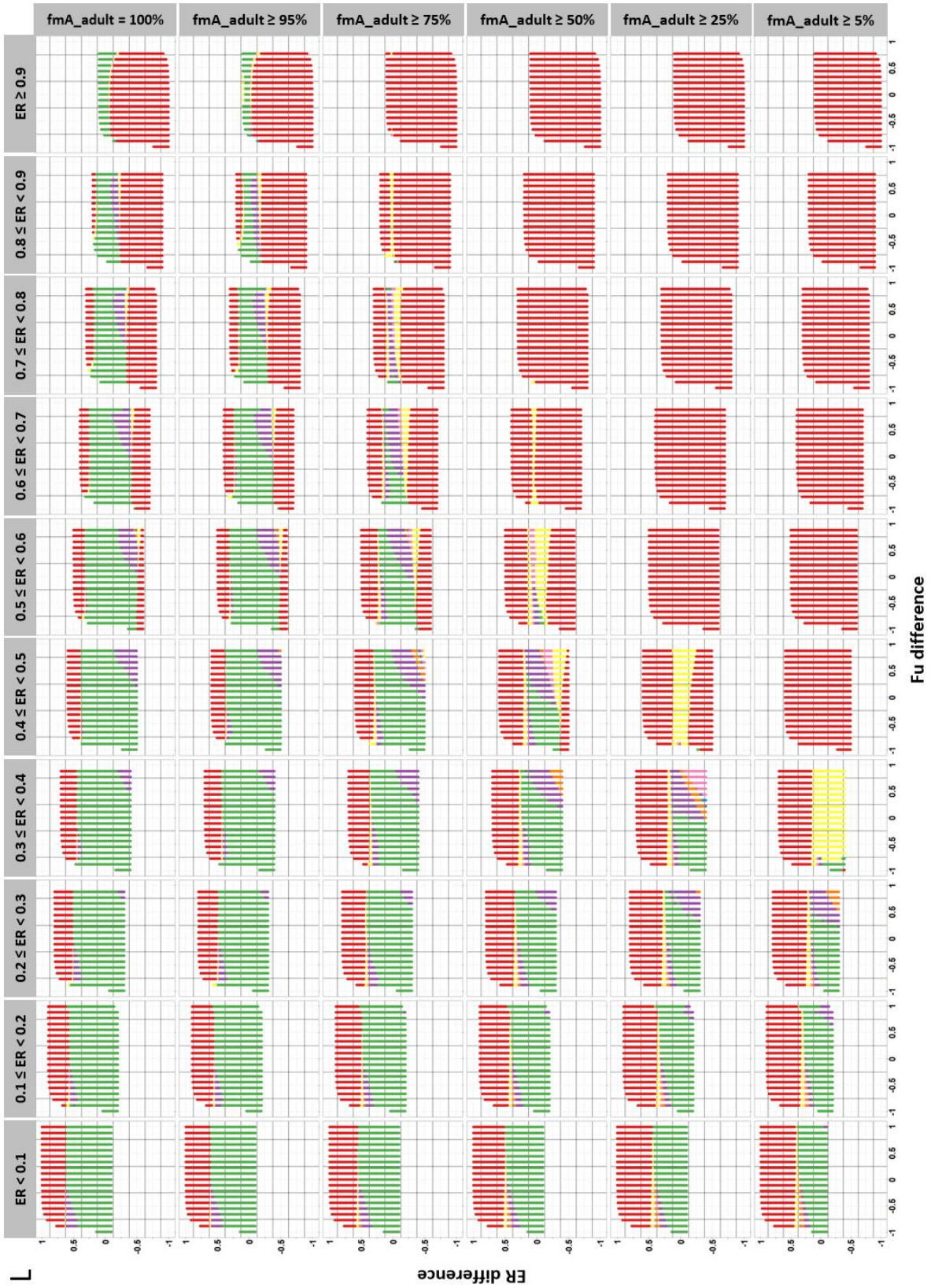
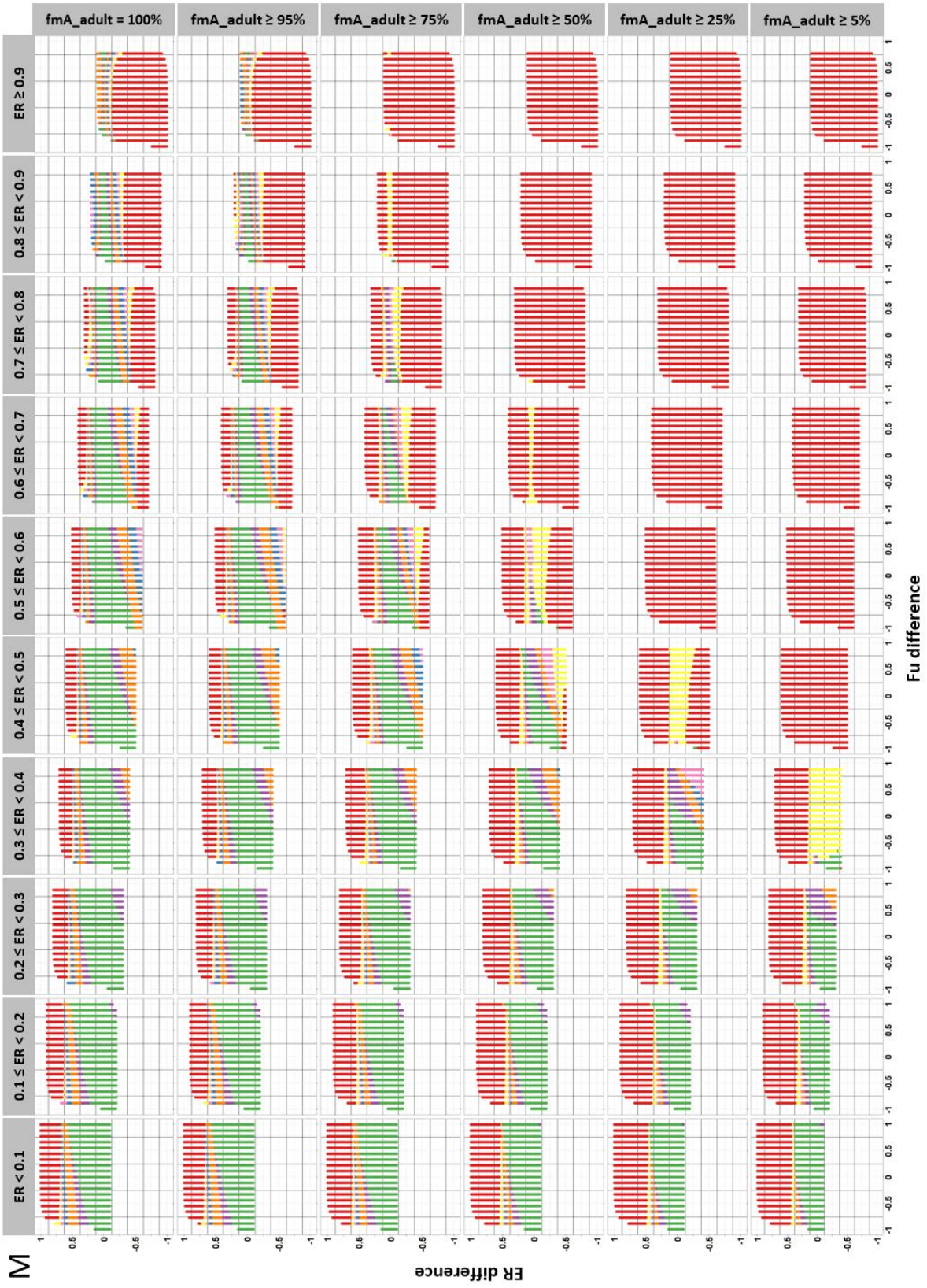
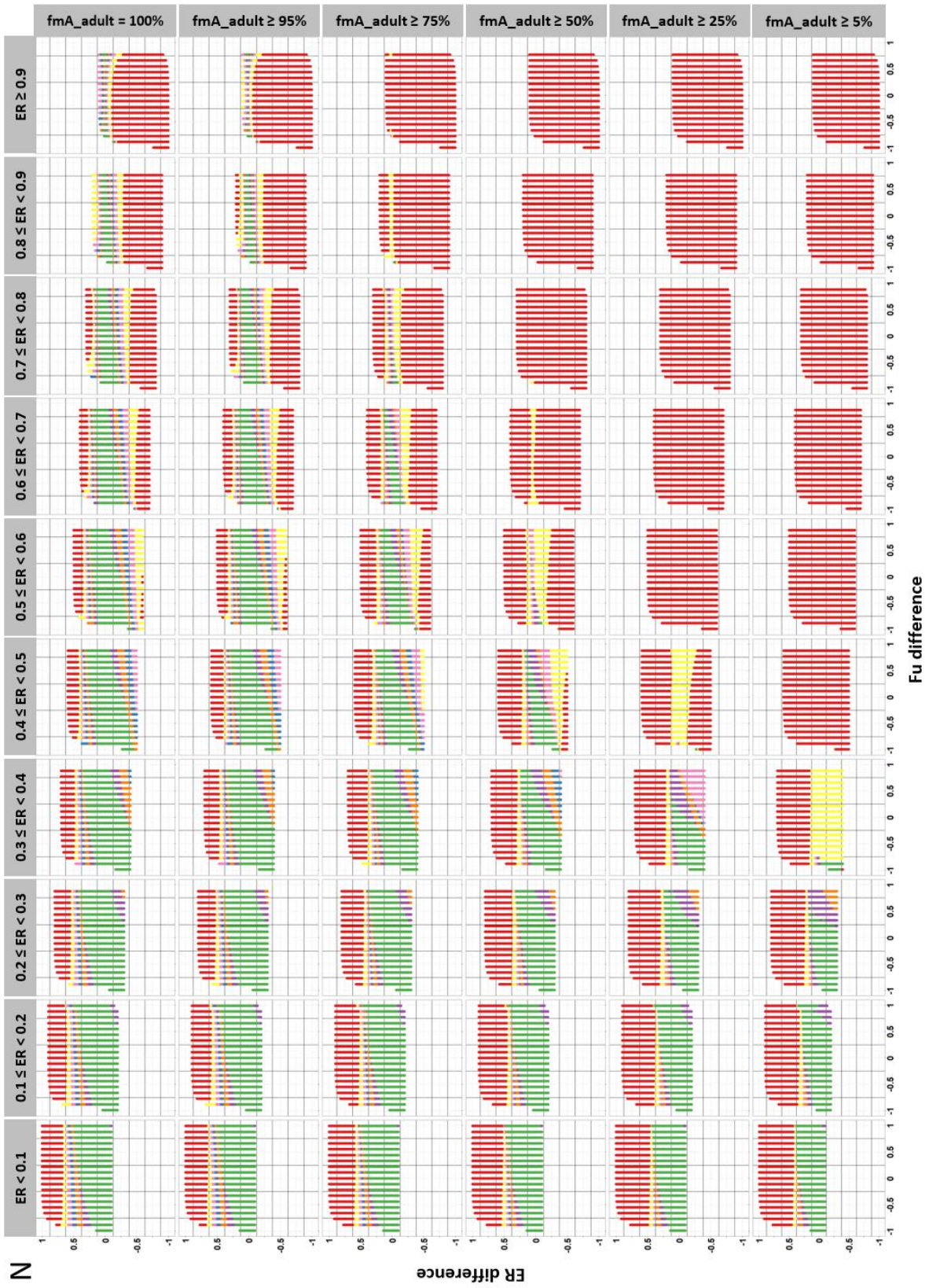
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
K
ER difference
Fu difference


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
1
0.5
0
-0.5
-1


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
M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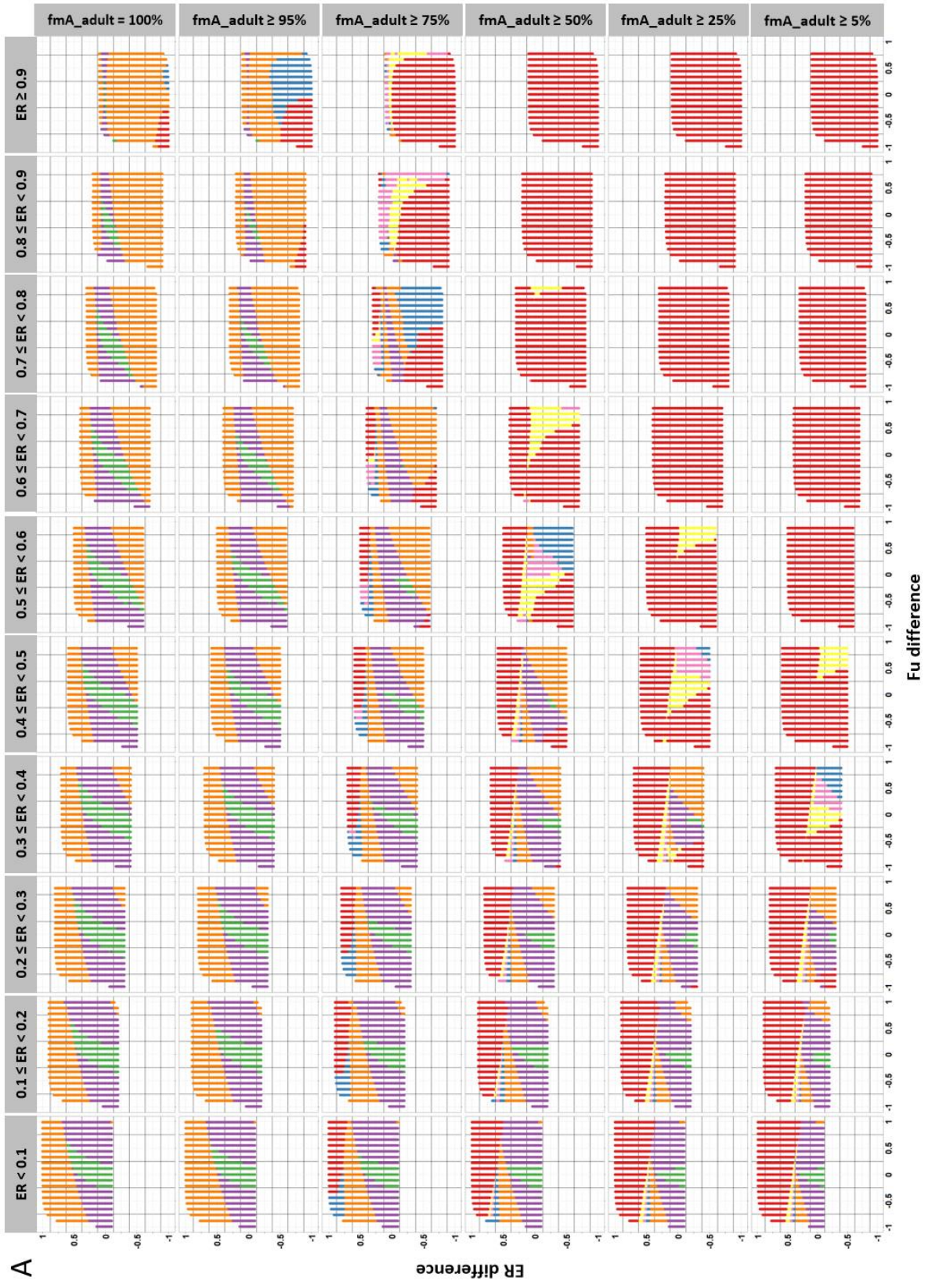
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
N

fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference

Supplementary material 3 Model-test drug scenarios, for drugs binding to alpha-1 acid glycoprotein, that lead to accurate pathway specific CLp predictions for a test drug after between-drug extrapolation of a pathway specific pediatric covariate function.

Figures A to O display model-test drug scenarios that lead to accurate pathway specific CLp predictions for a test drug after between-drug extrapolation of a pathway specific pediatric covariate function. Results are displayed for drugs binding to alpha-1 acid glycoprotein (AAG) and eliminated through CYP1A2 (A), CYP2A6 (B), CYP2B6 (C), CYP2C8 (D), CYP2C9 (E), CYP2C18_19 (F), CYP2D6 (G), CYP2E1 (H), CYP3A4 (I), SULT1A1 (J), UGT1A1 (K), UGT1A4 (L), UGT1A6 (M), UGT1A9 (N) and UGT2B7 (O) hepatic metabolism. Each column corresponds to a range of extraction ratio (ER) values for the model drug in adults and each row to a specific range of fraction of drug (model and test drug) that is metabolized by CYP1A2 in adults (fmA_adult). For each graph, the y-axis represents the difference in ER between the test drug and the model drug (ER test drug – ER model drug) in adults, and the x-axis represents the difference in fraction unbound (fu) between the test drugs and the model drug in adults (fu test drug – fu model drug). Each dot represents a model-test drug scenario, including multiple model-test drug combinations. A color code is used to indicate systematically accurate CLp predictions for all model-test drug combinations within a model-test drug scenario, for children ≥ 5 years (yellow), ≥ 2 years (pink), ≥ 1 year (blue), ≥ 6 months (orange), ≥ 1 month (purple), and ≥ 1 day term neonates (green). Red dots indicate model-test drug scenarios leading to inaccurate predictions in children older than 5 years for at least one model-test drug combination within the model-test drug scenario. As an example, systematically accurate CLp scaling in children of 6 months and older is represented by the combination of the green, purple and orange dots. The table below summarizes the color code used to indicate systematically accurate scaled pediatric CLp (+) and inaccurate scaled pediatric CLp (-) in the different ages studied.

1 day	1 month	6 months	1 year	2 years	≥ 5 years	
+	+	+	+	+	+	**green**
-	+	+	+	+	+	**purple**
-	-	+	+	+	+	**orange**
-	-	-	+	+	+	**blue**
-	-	-	-	+	+	**pink**
-	-	-	-	-	+	**Yellow**
-	-	-	-	-	-	**Red**


A
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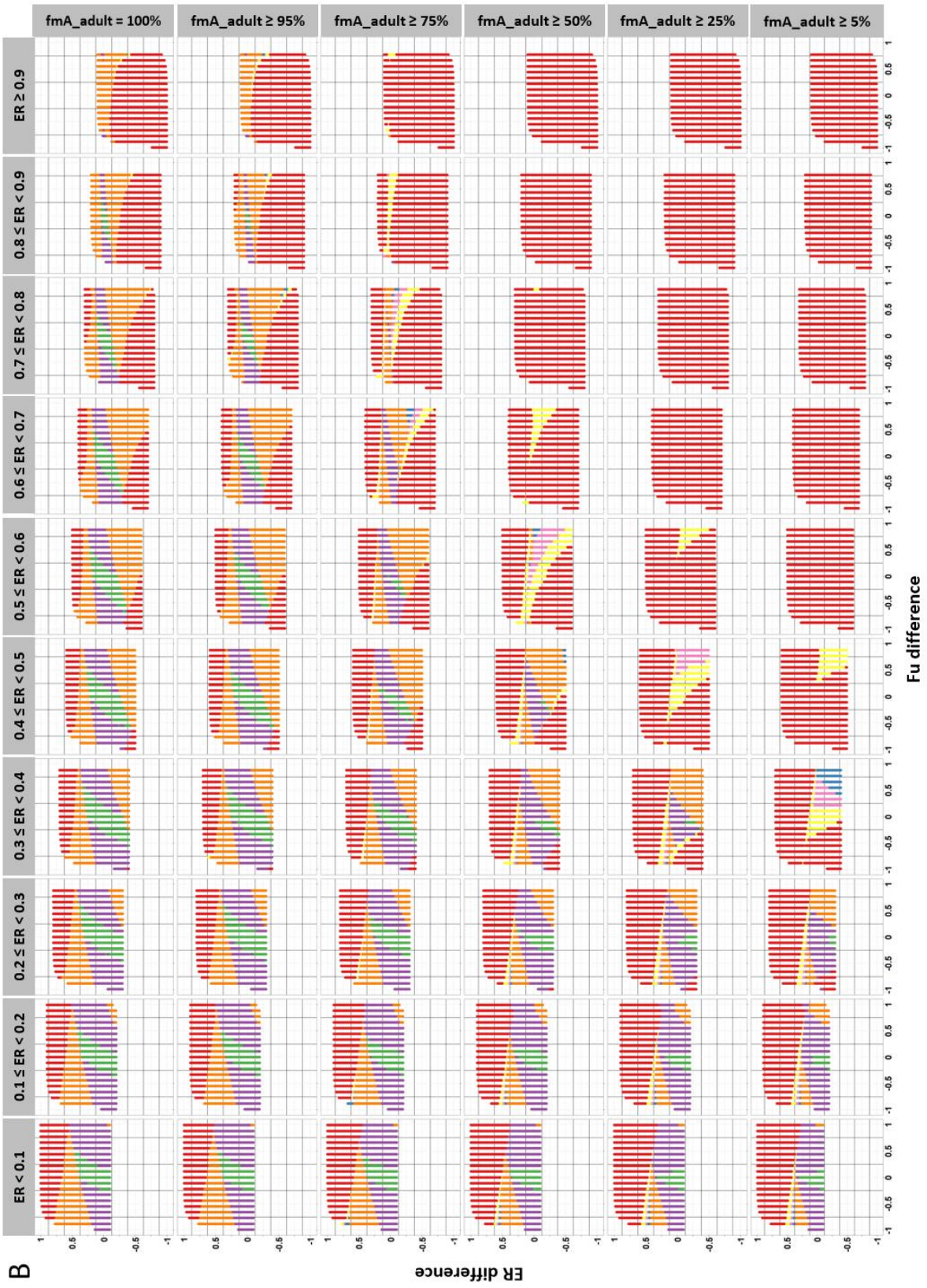
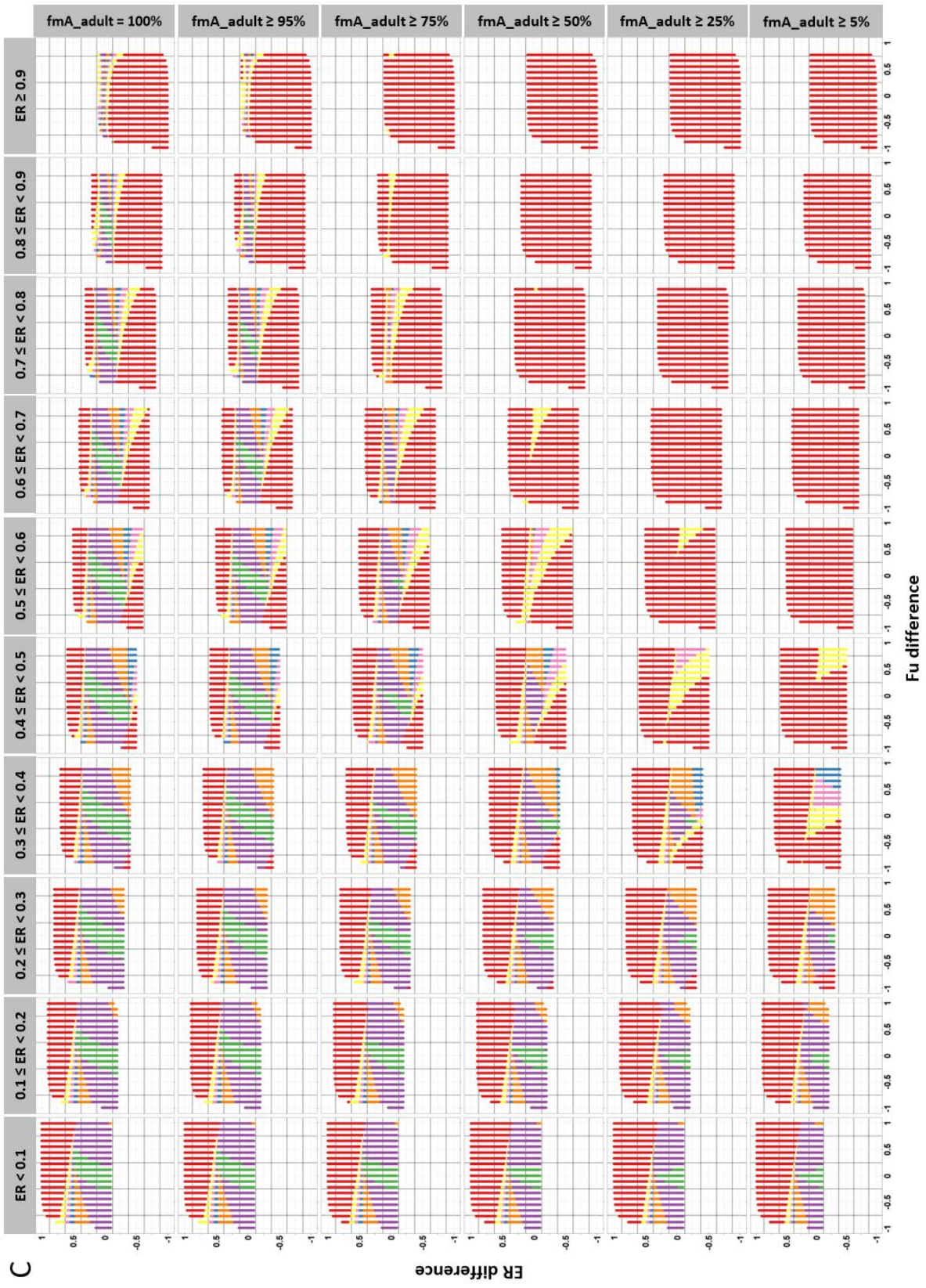
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference


B
fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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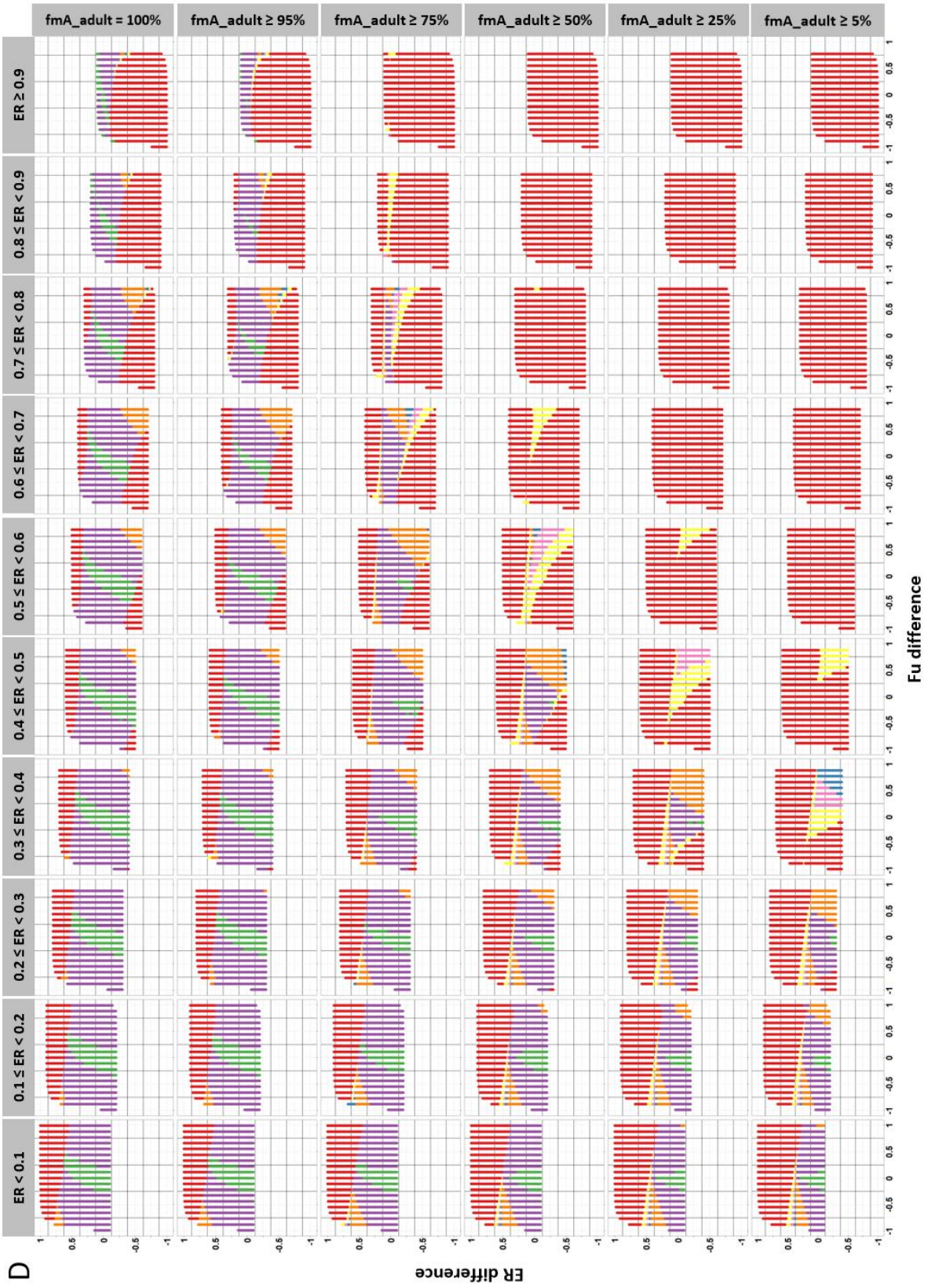
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference
C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fmA_adult ≥ 75%
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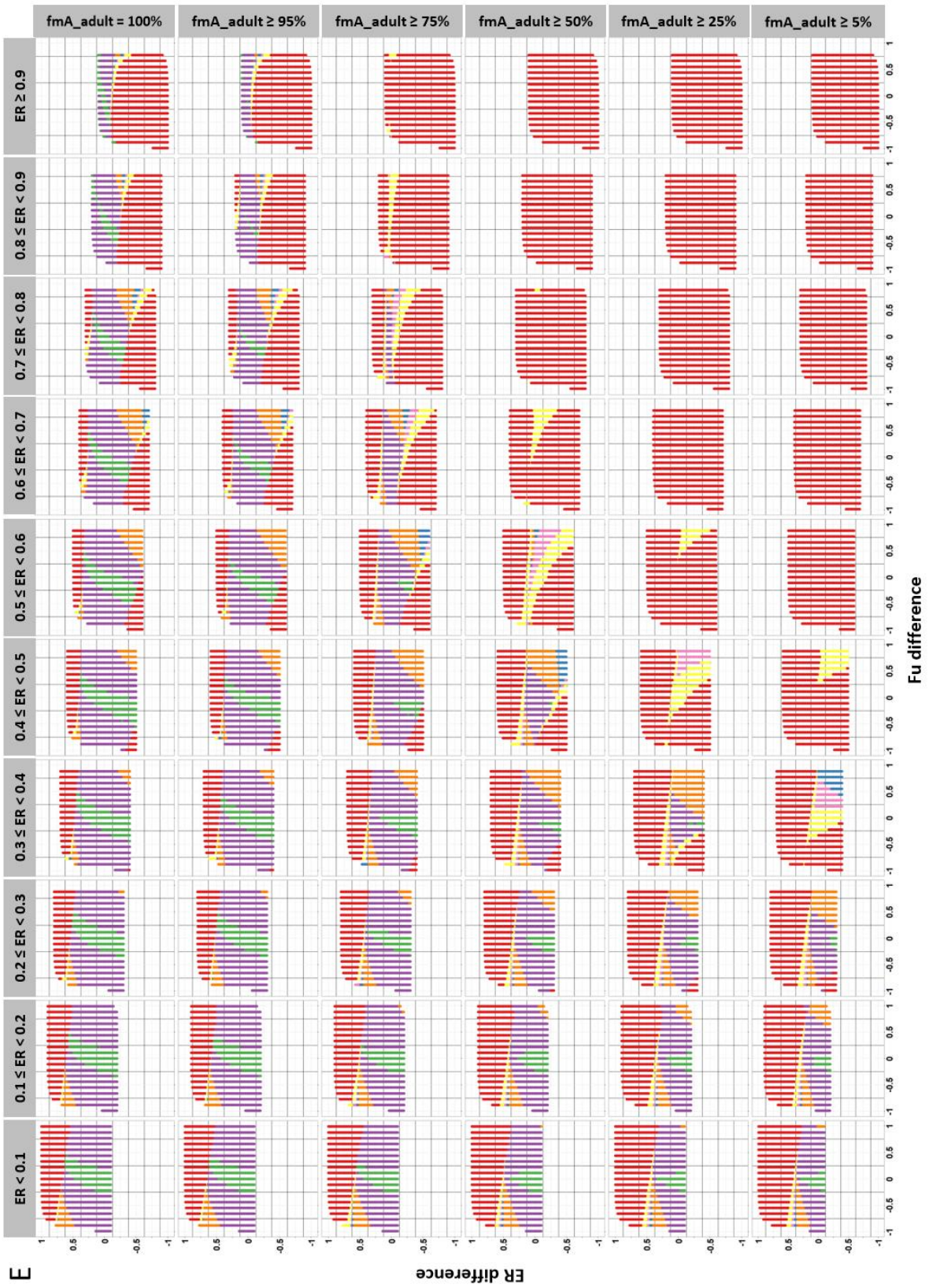
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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0.7 ≤ ER < 0.8
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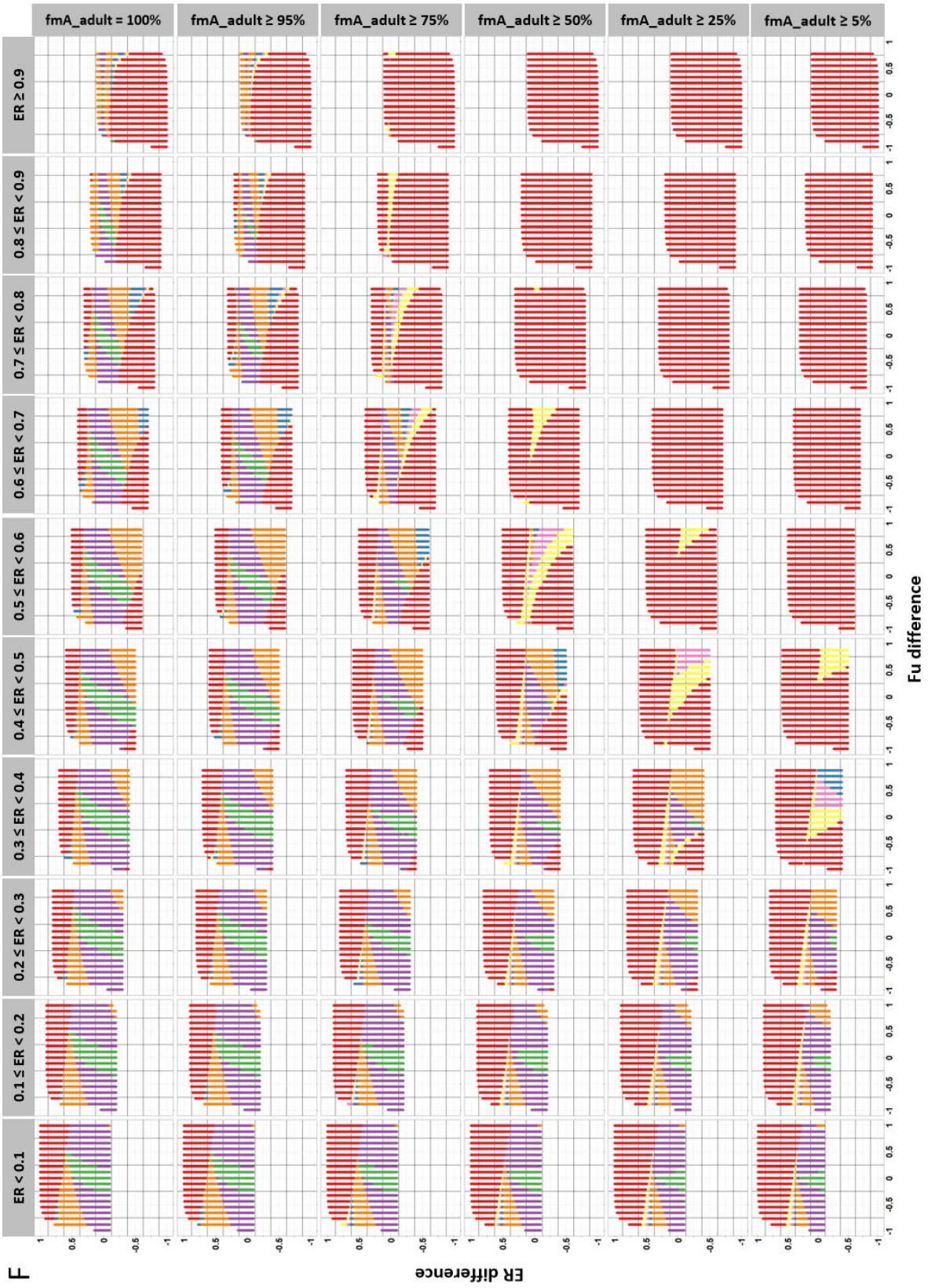
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference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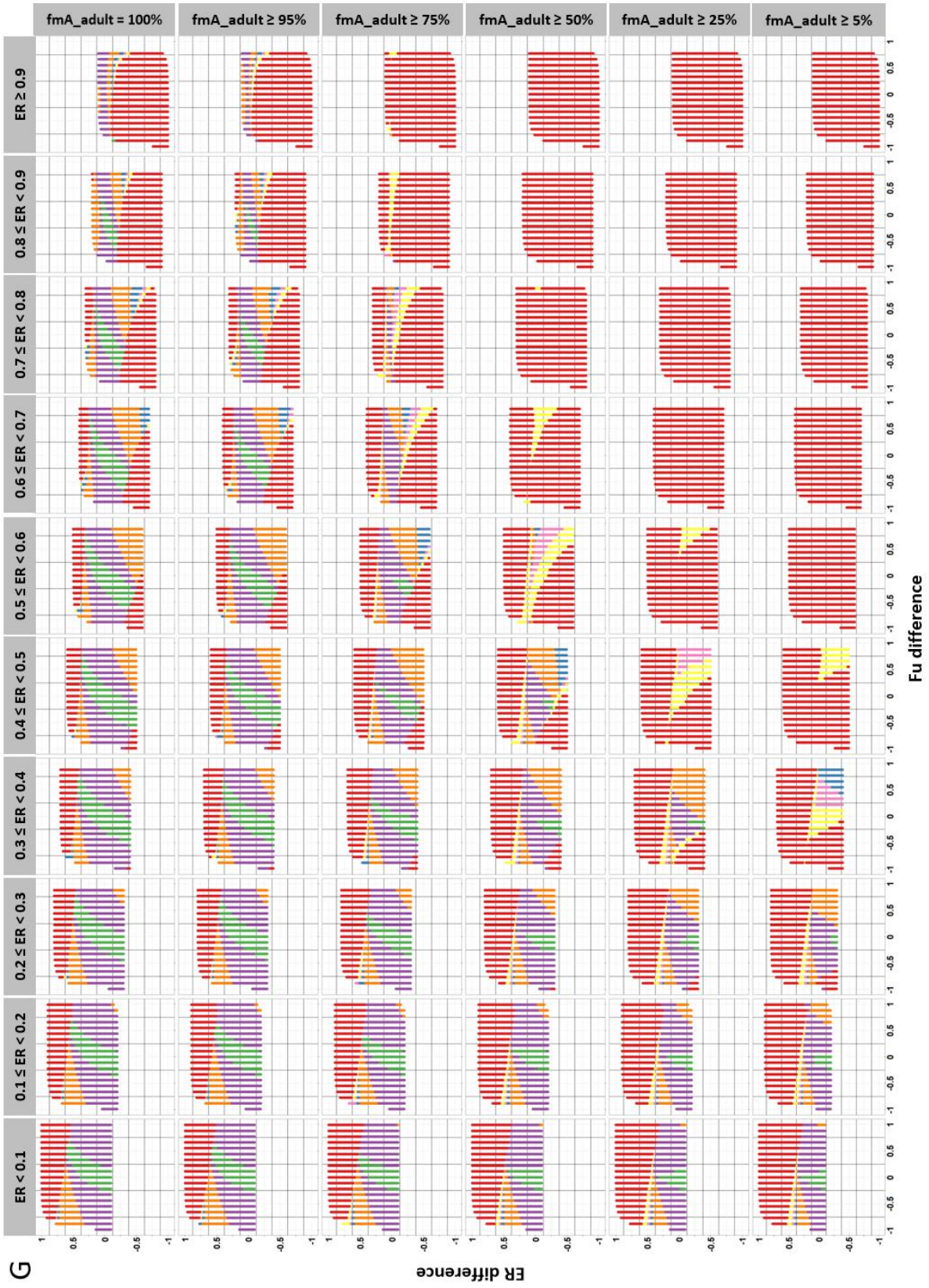
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
1
0.5
0
-0.5
-1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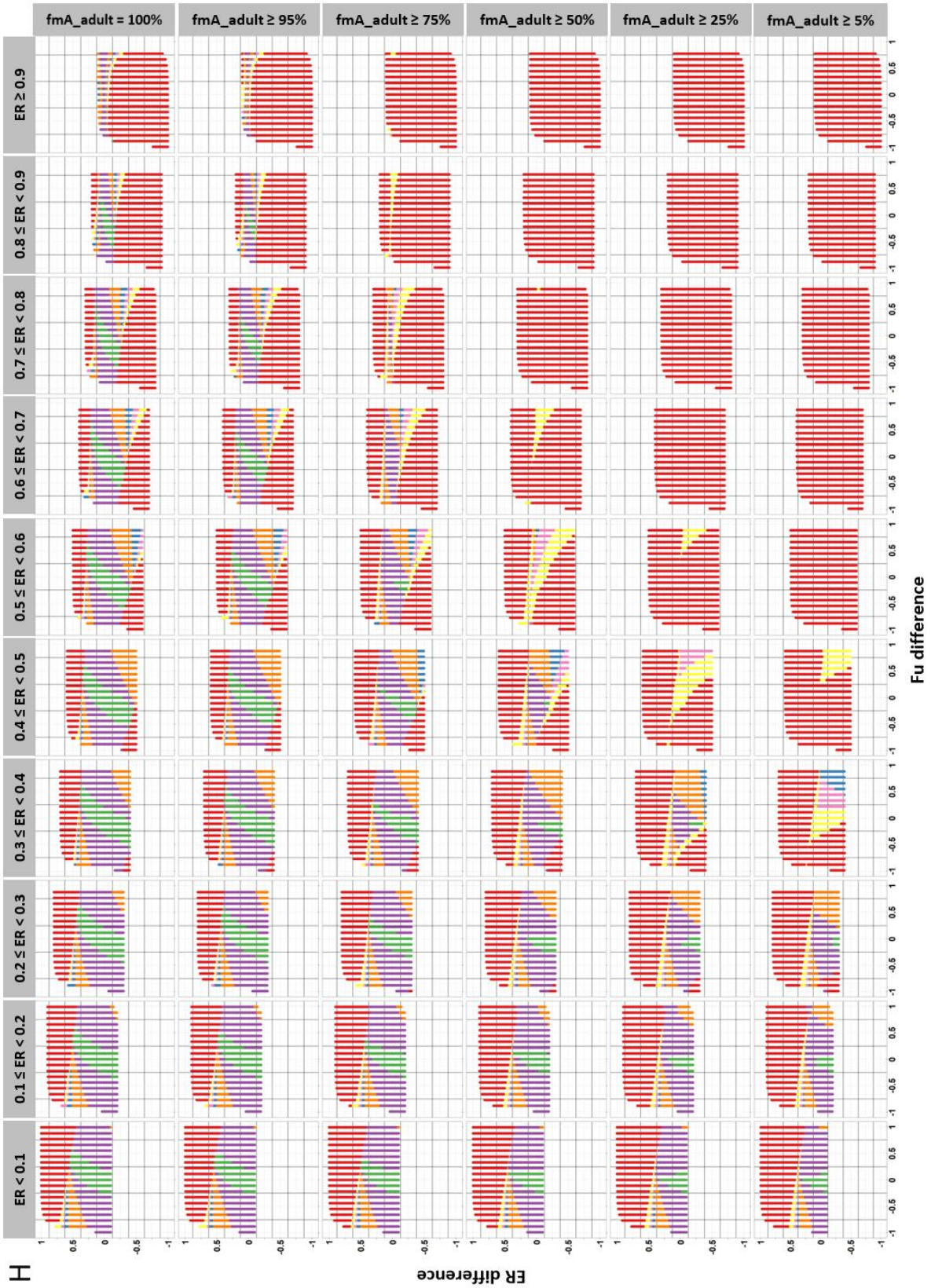
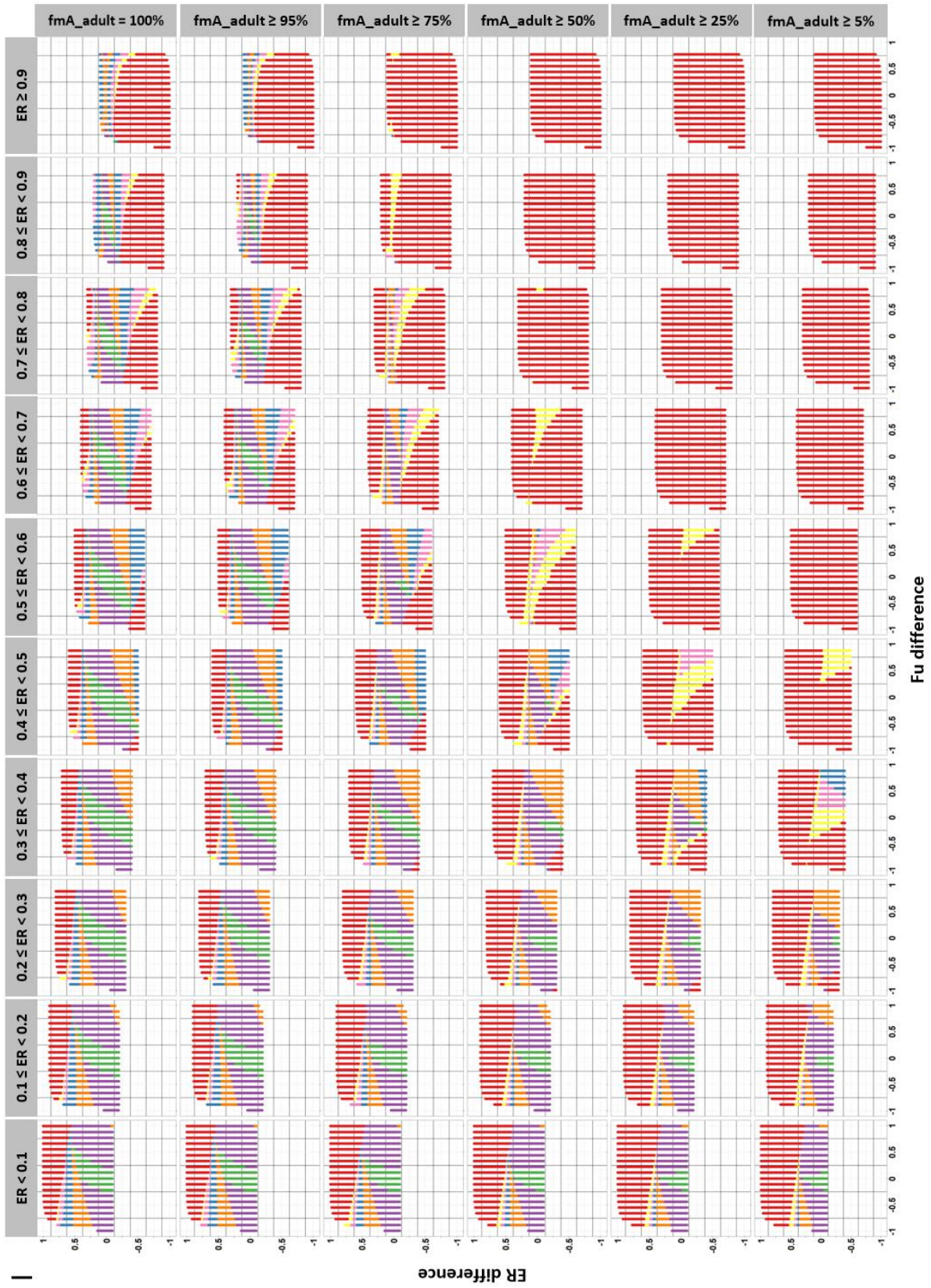
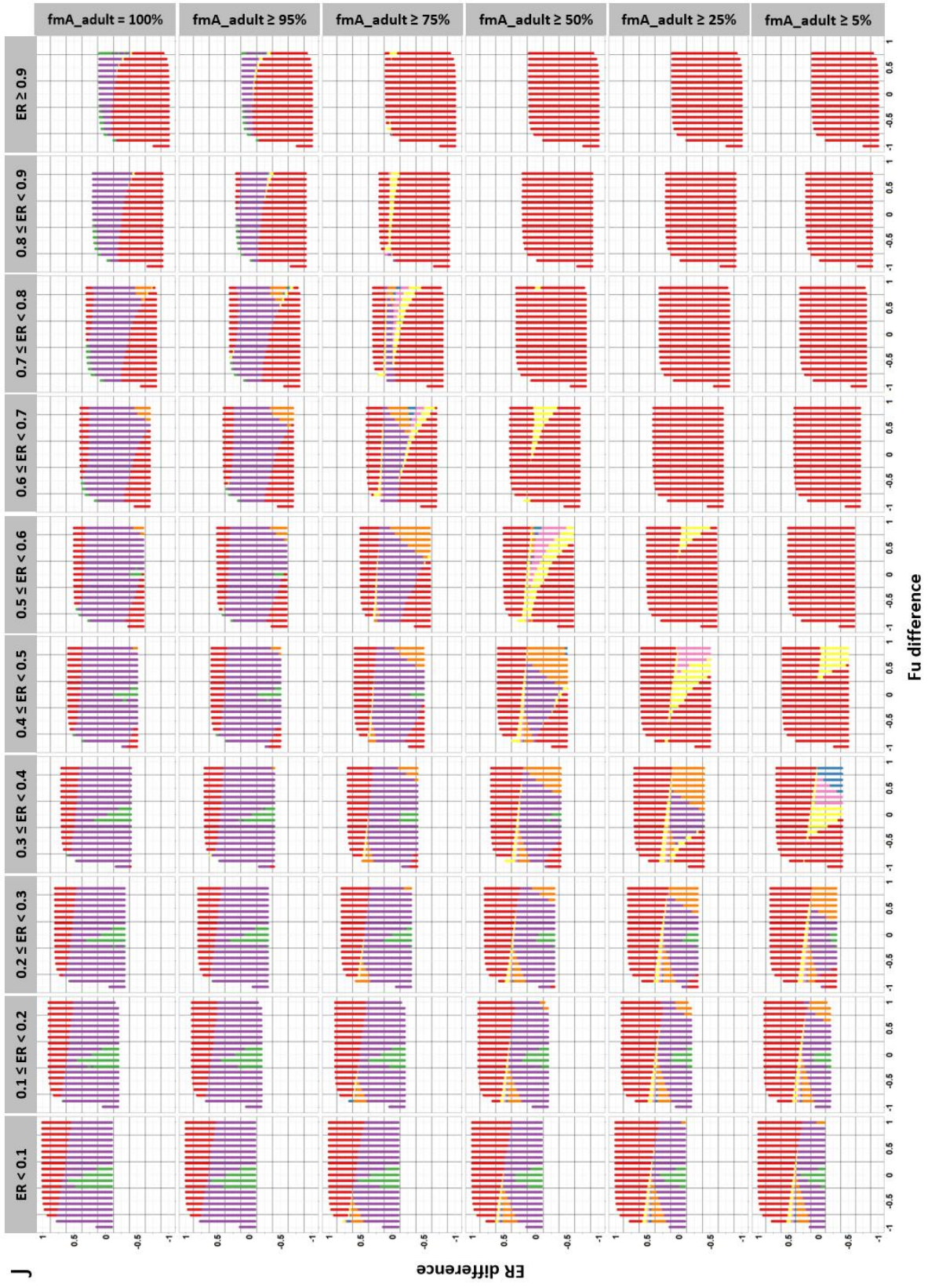
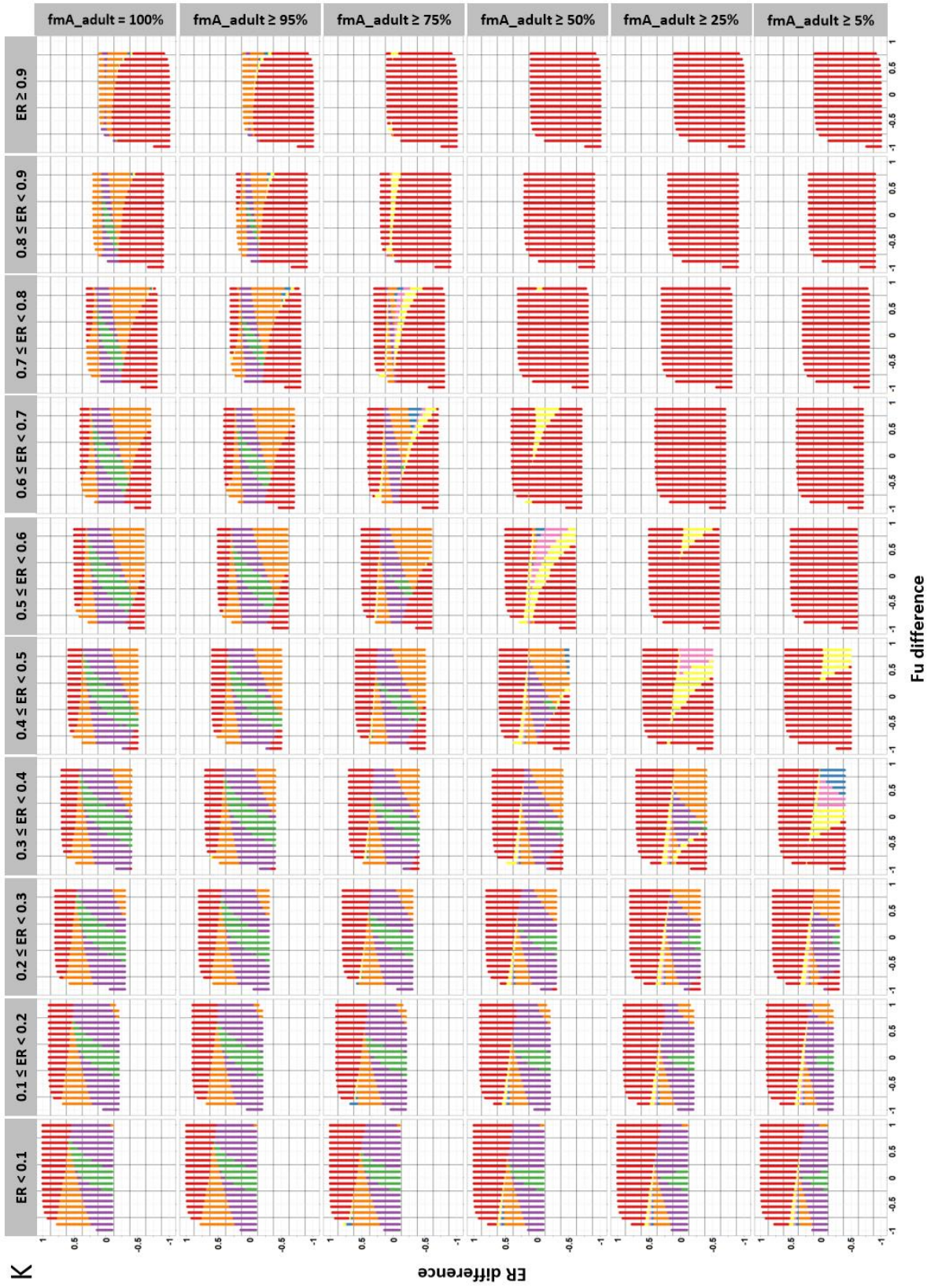
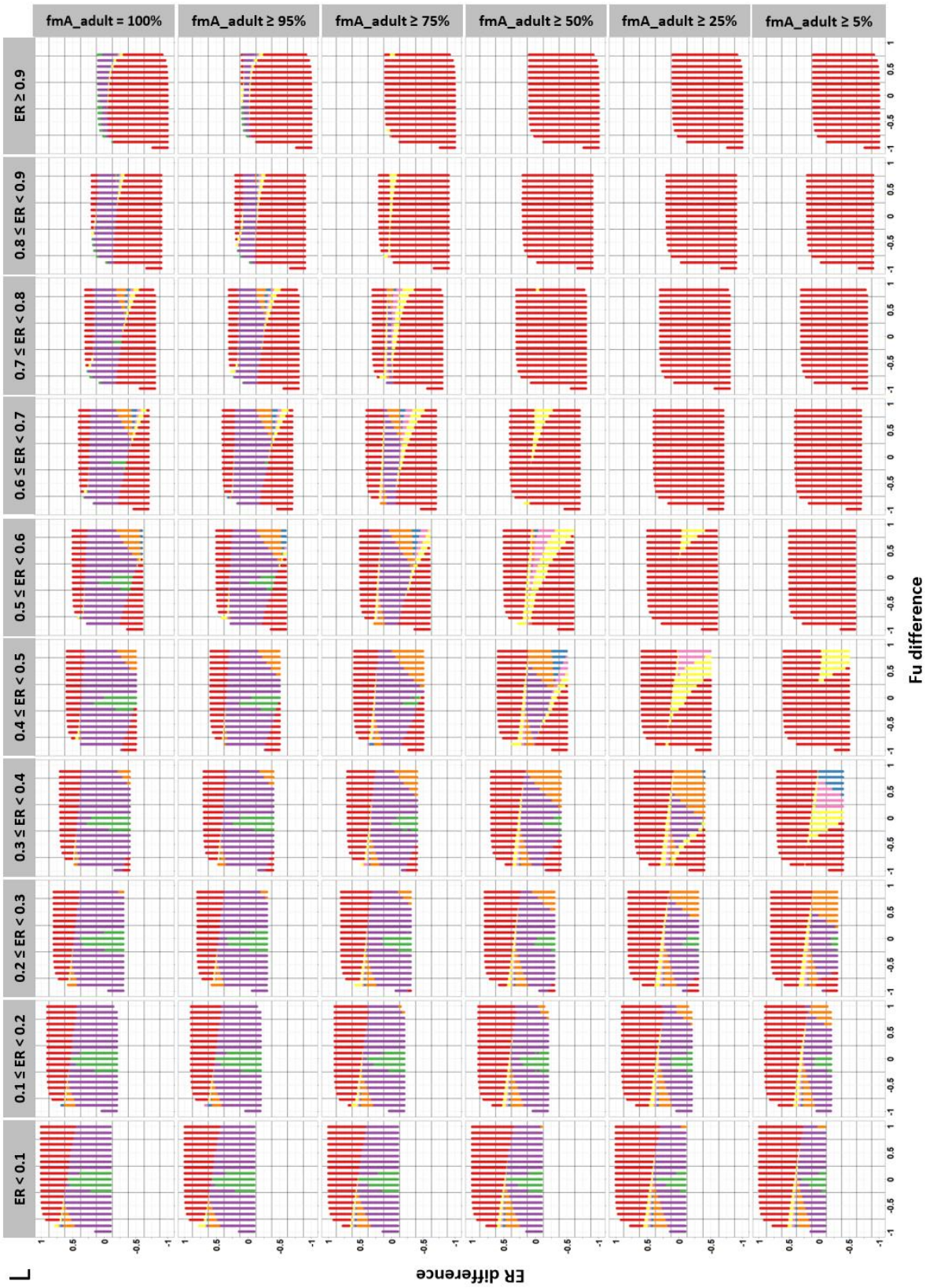
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
G

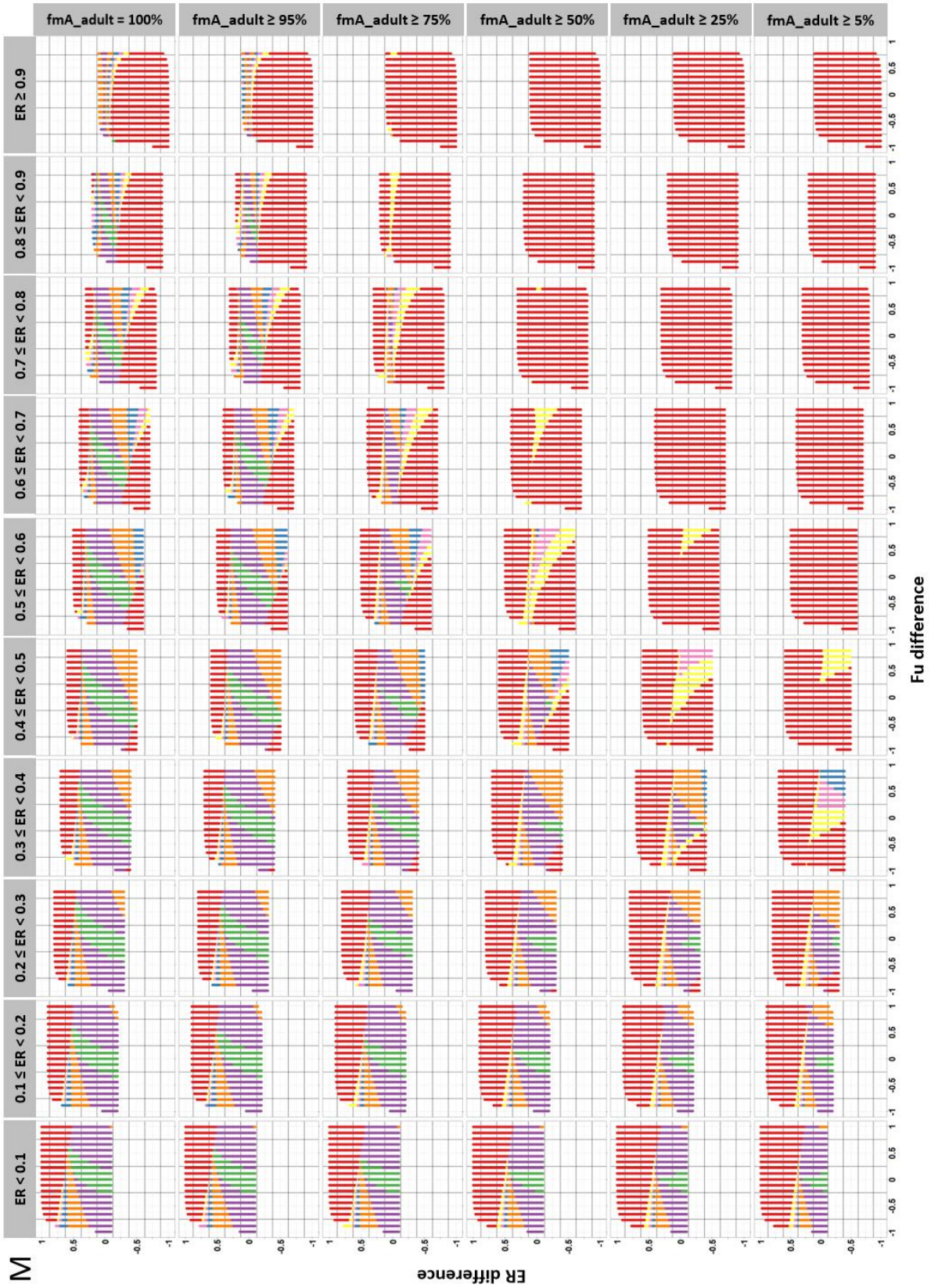

H
ER difference
Fu difference
fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1

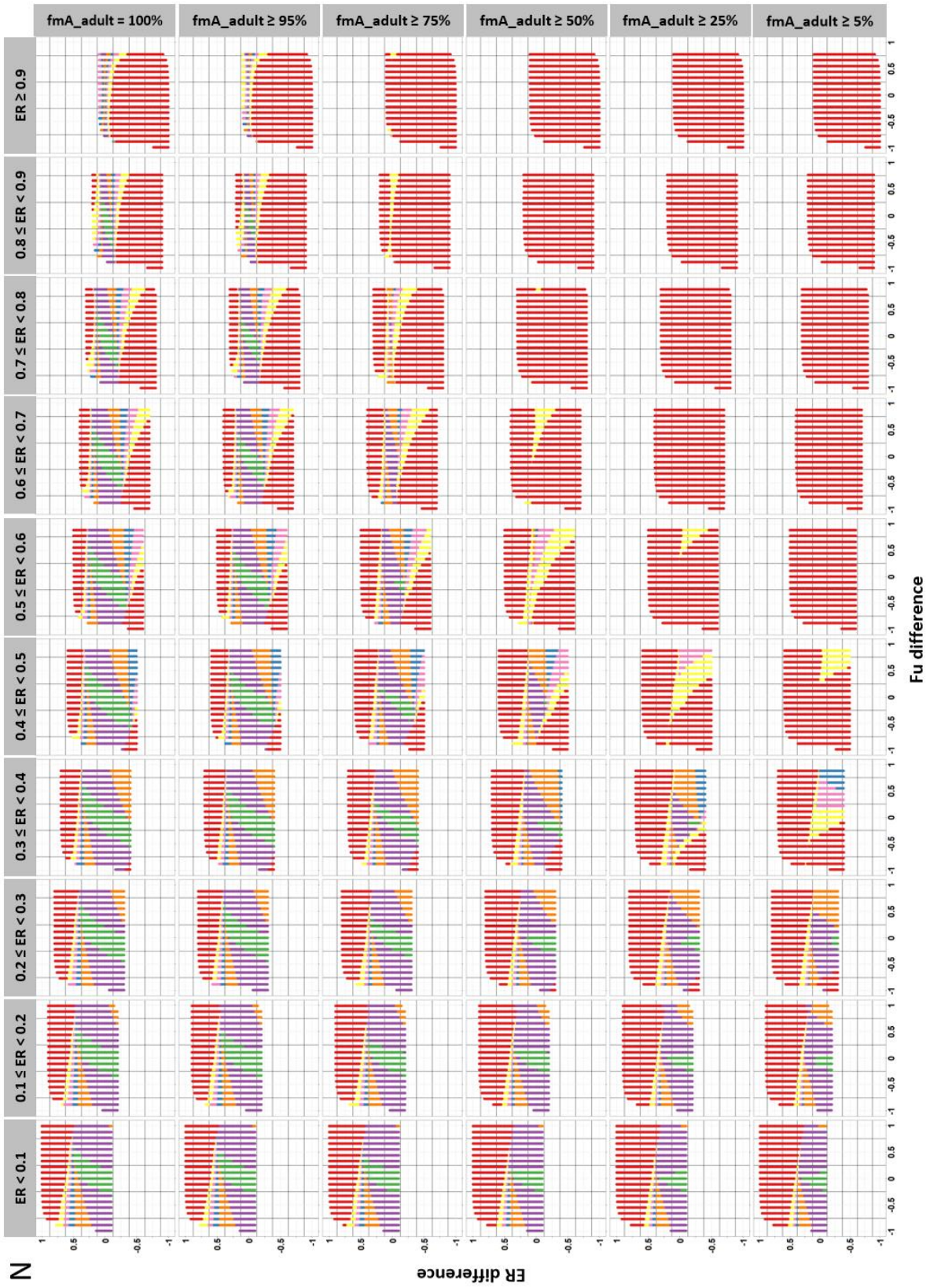

fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference

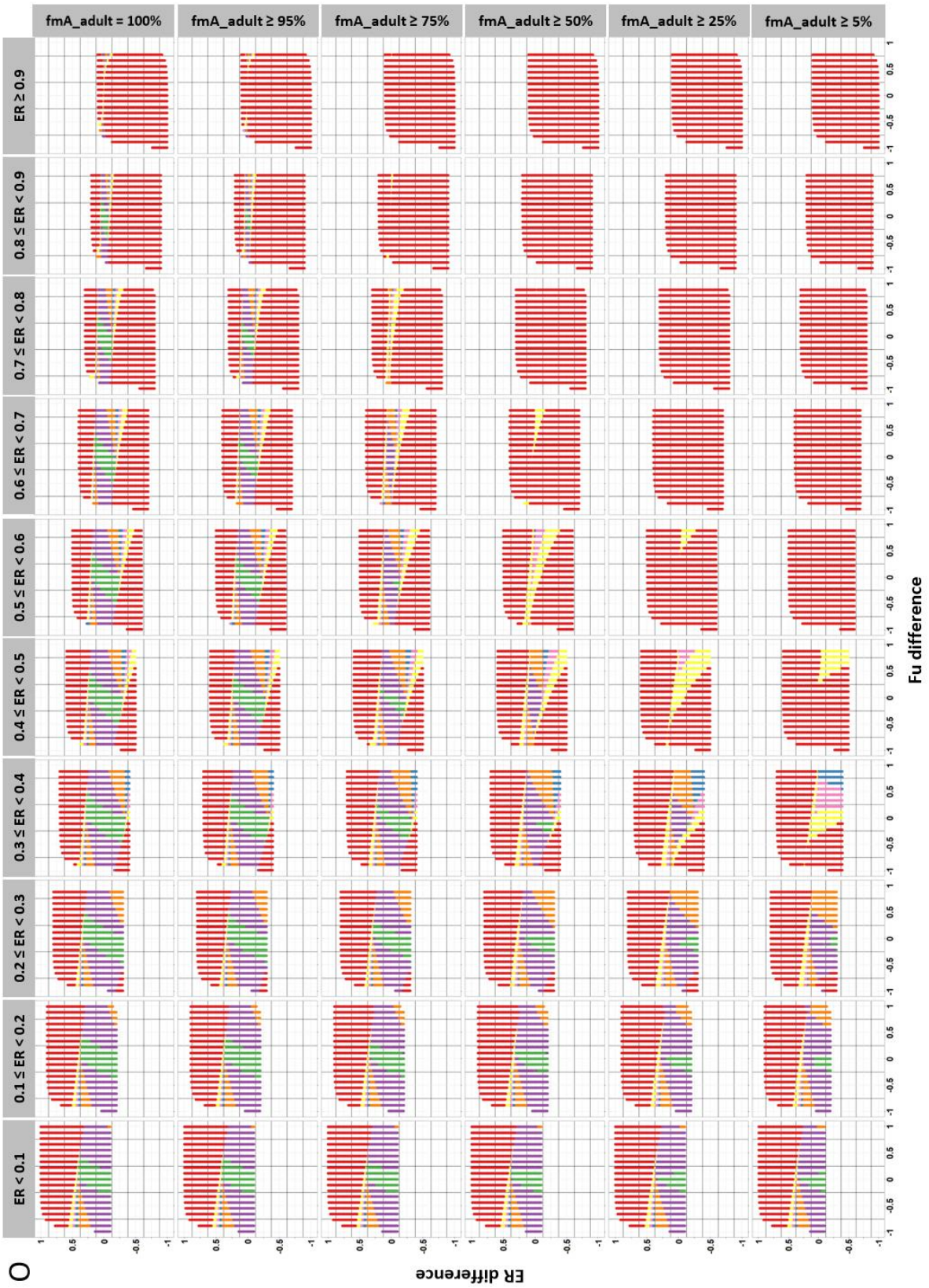

fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
J
ER difference
Fu difference


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
K
ER difference
Fu difference


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
1
0.5
0
-0.5
-1


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
M
ER difference
Fu difference


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
Fu difference
ER difference
N


fmA_adult = 100%
fmA_adult ≥ 95%
fmA_adult ≥ 75%
fmA_adult ≥ 50%
fmA_adult ≥ 25%
fmA_adult ≥ 5%
ER ≥ 0.9
0.8 ≤ ER < 0.9
0.7 ≤ ER < 0.8
0.6 ≤ ER < 0.7
0.5 ≤ ER < 0.6
0.4 ≤ ER < 0.5
0.3 ≤ ER < 0.4
0.2 ≤ ER < 0.3
0.1 ≤ ER < 0.2
ER < 0.1
ER difference
Fu difference

Model Code Supplementary Material

R model code for CLp, ER and CLpA simulations

```
################################################################################
############################## Demographic values ##############################
################################################################################

# Typical individuals are characterized by a specific post-natal age, bodyweight and height
# AGE: post-natal age (years)
# BW: bodyweight (kg)
# HT: height (cm)
# BSA: body surface area (square meters), derived based on HT and BW

# Vector of investigated post-natal ages (years)
AGE_vector<- c(1/365,30/365,0.5,1,2,5,15,25)

# Vector of investigated bodyweights (kg)
BW_vector <- c(3.45,4.3,7.55,9.9,12.35,18.25,54.25,72.65)

# Vector of investigated heights (cm)
HT_vector<- c(49.75,54.25,66,74.75,86,108.25,166,172.30)

# Selection of the age of the typical pediatric patient for which simulations are performed (1 for
neonates of 1 day, 2 and 3 for infants of 1 and 6 months respectively, 4, 5 and 6 for for children
of 1, 2 and 5 years respectively, 7 for adolescent of 15 years and 8 for adults of 25 years)
Age_index <- 1

# Post-natal age (years) for the selected typical individual
AGE<- AGE_vector[Age_index]

# Bodyweight (kg) for the selected typical individual
BW<- BW_vector[Age_index]

# Height (cm) for the selected typical individual
HT<- HT_vector[Age_index]


# Body surface area for children of 15kg or less, formula from Haycock et al.
BSA_Haycock <- function(HT,BW){
BSA <- 0.024265 * HT**0.3964 * BW**0.5378
BSA
}

# Body surface area for children >15kg and adults, formula from Dubois and Dubois
BSA_Dubois <- function(HT,BW){
BSA <- 0.007184 * HT**0.725 * BW**0.425
BSA
}

# Selection of the BSA formula according to the bodyweight of the selected typical individual
(BW) for which simulations are performed
```

```
BSA <- ifelse(BW>15,BSA_Dubois(HT,BW),BSA_Haycock(HT,BW))

################################################################################
########################### Drug-specific parameters ###########################
################################################################################

### Selection of drug specific parameters using the index of the selected values within each
vector (value between square brackets)

# Adult total unbound intrinsic microsomal clearance (L/min/mg microsome)
CLintmictotal_adult <- seq(from=0.56/1000000, to=0.209/1000, length.out= 42) [4]

# Adult unbound drug fraction in plasma (similar for HSA bound drugs and AAG bound drugs).
fu_adult <- seq(from=0.01,to=1, by=0.11)[10]

# Blood to plasma partition coefficient.
Kp <- c(0.35,1,2,3,4,5,10,20,40)[2]

# Fraction of the drug metabolized by IA in adults. For drugs metabolized by one
isoennzyme,fmA_adult = 1
fmA_adult <- c(1,0.95,0.75,0.5,0.25,0.05) [2]


################################################################################
########################### System-specific parameters #########################
################################################################################

### Hepatic blood flow (QH)

# Cardiac output (L/min)
CO <- (BSA*(110 + 184.974*(exp(-0.0378*AGE)-exp(-0.24477*AGE)))/60)

# QH: hepatic blood flow (L/min), expressed as a fraction of the cardiac output (CO). 26.75% is
the average between males (25.5%) and females (28%).
QH <- CO * 0.2675


### Human serum albumin (HSA) concentration (g/L). HSA concentration changes with age (age
dependent)

# HSA concentration (g/L) for the different investigated post-natal ages (AGE)
HSA_vector <- c(35.78,39.94,42.07,42.90,43.73,44.82,44.68,43.94)
# HSA concentration (g/L) in adults
HSA_adult <- HSA_vector[8]

# HSA concentration (g/L) for a selected post-natal age
HSA <- HSA_vector[Age_index]
### Alpha-1-acid glycoprotein concentration (g/L). AAG concentration changes with age (age
dependent)

# AAG concentration (g/L) for the different investigated post-natal ages (AGE)
AAG_vector <- c(0.2678,0.5497,0.6774,0.7172,0.7512,0.7877,0.6711,0.6847)
```

```
# AAG concentration (g/L) in adults
AAG_adult <- AAG_vector[8]

# AAG concentration (g/L) for a selected post-natal age
AAG <- AAG_vector[Age_index]


### Hematocrit

# Vector of investigated hematocrit values for the different investigated post-natal ages
Hematocrit_vector <- c(51.93,38.14,35.11,35.78,36.79,38.29,40.01,40.74)/100

# Hematocrit value for a selected post natal age
Hematocrit <- Hematocrit_vector[Age_index]


### Microsomal Protein Per Gram of Liver (mg/g)
# Microsomal Protein Per Gram of Liver (mg/g) for a selected postnatal age
MPPGL <- 10**(1.407+0.01579*AGE-0.0003824*AGE**2+0.00000237*AGE**3)

### Isoenzyme ontogeny values
CYP1A2 <- c(24.03,35.14,118.15,149.64,163.82,161.05,125.83,100)/100
CYP2A6 <- ifelse(AGE_vector<=1,1*AGE_vector**5.68/(0.21**5.68+AGE_vector**5.68),1)
CYP2B6 <- ifelse(AGE_vector<=16,(1.1-
0.15)*AGE_vector**1/(2**1+AGE_vector**1)+0.15,1)
CYP2C8 <- ifelse(AGE_vector<=2,(1-0.3)*AGE_vector**1/(0.02**1+AGE_vector**1)+0.3,1)
CYP2C9 <- ifelse(AGE_vector<=3,(0.98-
0.17)*AGE_vector**0.53/(0.0157**0.53+AGE_vector**0.53)+0.17,1)
CYP2C18_19 <- ifelse(AGE_vector<=5,(0.98-
0.3)*AGE_vector**2.44/(0.29**2.44+AGE_vector**2.44)+0.3,1)
CYP2D6 <- ifelse(AGE_vector<=10,(1-
0.036)*AGE_vector**1/(0.1**1+AGE_vector**1)+0.036,1)
CYP2E1 <- ifelse(AGE_vector<=18,0.99*AGE_vector**0.5/(0.23**0.5+AGE_vector**0.5),1)
CYP3A4_5 <- ifelse(AGE_vector<=25,(1.06-
0.11)*AGE_vector**1.91/(0.64**1.91+AGE_vector**1.91)+0.11,1)
UGT1A1 <- ifelse(AGE_vector<=1,(1.064-
0.0015)*AGE_vector**2.07/(0.154**2.07+AGE_vector**2.07)+0.0015,1)
UGT1A4 <- 0.7354 + AGE_vector*0.0157
UGT1A6 <- ifelse(AGE_vector<=15,(1.025-
0.142)*AGE_vector**0.97/(0.411**0.97+AGE_vector**0.97)+0.142,1)
UGT1A9 <- ifelse(AGE_vector<=15,(1.041-
0.086)*AGE_vector**1.2/(1.16**1.2+AGE_vector**1.2)+0.086,1)
UGT2B7 <- 0.0846 + AGE_vector*0.0472
SULT1A1 <- c(100,100,100,100,100,100,100,100)/100
Isoenzyme_Ontogeny <-
data.frame(rbind(CYP1A2,CYP2A6,CYP2B6,CYP2C8,CYP2C9,CYP2C18_19,CYP2D6,
CYP2E1,CYP3A4_5,UGT1A1,UGT1A4,UGT1A6,UGT1A9,UGT2B7,SULT1A1))
names(Isoenzyme_Ontogeny) <- AGE_vector

# All isoenzymes are mature in adults
Isoenzyme_Ontogeny[,8] <- 1
```

```
# Selection of a specific iosenzyme A (IA), the value corresponds to the row number of the relevant isoenzyme in the table Isoenzyme_Ontogeny. 1 for instance selects CYP1A2
IsoenzymeA_index <- 1

# Selection of a specific iosenzyme B (IB), the value corresponds to the row number of the relevant isoenzyme in the table Isoenzyme_Ontogeny. 2 for instance selects CYP2A6
IsoenzymeB_index <- 2

# IOA: isoenzyme ontogeny value for a specific isoenzyme A (IA) and age, expressed as the pediatric to adult CLintmicA (CLintmic due to IA) ratio. In adults, IOA=1 (IA mature in adults)
IOA <- Isoenzyme_Ontogeny[IsoenzymeA_index,Age_index]

# IOB: isoenzyme ontogeny value for a specific isoenzyme B (IB) and age, expressed as the pediatric to adult CLintmicB (CLintmic due to IB) ratio. In adults, IOB=1 (IB mature in adults)
IOB <- Isoenzyme_Ontogeny[IsoenzymeB_index,Age_index]


################################################################################
################### Drug and system-specific parameters ####################
################################################################################

####################### Unbound drug fraction in plasma (fu) ###################
nKa_HSA <- (1/fu_adult-1)/HSA_adult
fu_HSA <- 1/(1+nKa_HSA*HSA)
# nKa_HSA: drug affinity to HSA dervied from the adult unbound drug fraction in plasma (fu_adult) and HSA concentration in adults (HSA_adult) using the equations by Rodgers and Rowland
# fu_adult: unbound plasmatic drug fraction in adults (identical between HSA bound drugs and AAG bound drugs in the simulations, considered as a drug_specific parameter)
# HSA_adult and HSA: human serum albumin (HSA) concentration in adult and for a specific post-natal age respectively (g/L). HSA changes with age.
# fu_HSA: unbound drug fraction for drugs binding to HSA for a specific typical individual
nKa_AAG <- (1/fu_adult-1)/AAG_adult
fu_AAG<- 1/(1+nKa_AAG*AAG)
# nKa_AAG: drug affinity to AAG dervied from the adult unbound drug fraction in plasma (fu_adult) and AAG concentration in adults (AAG_adult) using the equations by Rodgers and Rowland
# fu_adult: unbound plasmatic drug fraction in adults (identical between HSA bound drugs and AAG bound drugs in the simulations, considered as a drug_specific parameter)
# AAG_adult and AAG_pediatric: alpha-1 acid glycoprotein (AAG) concentration in adult and and for a specific post-natal age respectively (g/L). AAG changes with age.
# fu_AAG: unbound drug fraction for drugs binding to AAG for a specific typical individual


############################ Blood to plasma ratio (BP) ########################
# B:P for drugs binding to HSA
BP_HSA <- 1+(Hematocrit*(fu_HSA*Kp-1))

# B:P for drugs binding to AAG
BP_AAG <- 1+(Hematocrit*(fu_AAG*Kp-1))
# Hematocrit is the hematocrit for a specific age (age dependent)
```

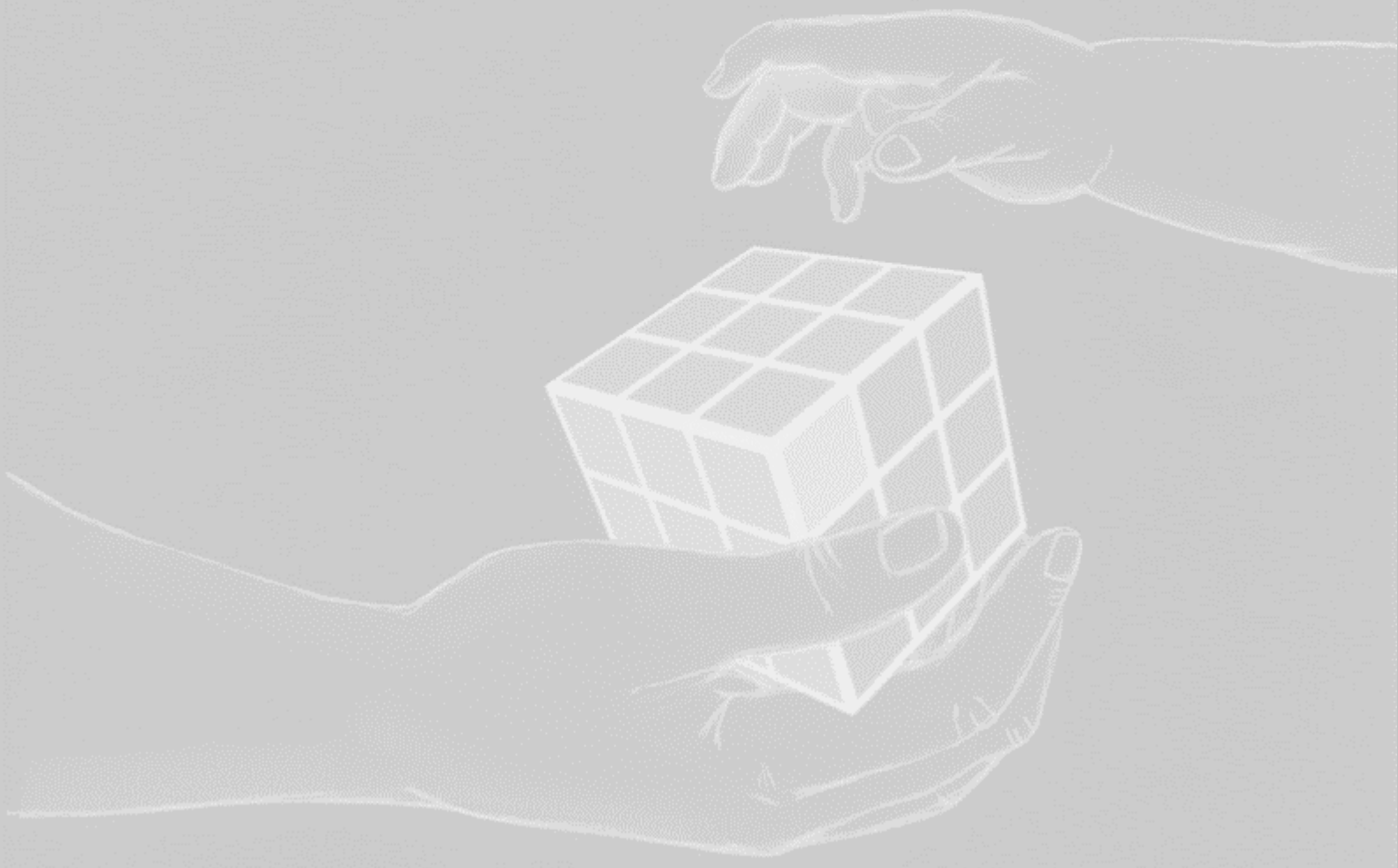
```
# Kp is the blood to plasma partition coefficient (drug-specific property)


################### Total intrinsic metabolic clearance in the liver ##################
################### based on unbound drug concentration (CLint)###################

### CLint (L/min)

CLint_function <- function(CLintmictotal_adult,BSA,AGE,IOA,IOB,fmA_adult){

# LIVER_VOLUME: liver volume (L), dependent on BSA
LIVER_VOLUME <- 0.722 * BSA**1.176

# LIVER_DENSITY: Liver density (g/L), identical for all ages
LIVER_DENSITY <- 1080

# LIVERWT: liver weight  (g)
LIVERWT <- LIVER_VOLUME * LIVER_DENSITY
# CLint: total intrinsic metabolic clearance in the liver based on unbound drug concentration
(L/min)

CLint <- (CLintmictotal_adult*fmA_adult*IOA+CLintmictotal_adult*(1-fmA_adult)*IOB) *
LIVERWT*MPPGL

CLint
}

# CLint: total intrinsic metabolic clearance in the liver based on unbound drug concentration
(L/min). CLint is a function of CLintumictotal_adult, BSA, AGE, IOA, IOB and fmA_adult

CLint <- CLint_function(CLintmictotal_adult,BSA,AGE,IOA,IOB,fmA_adult)

# CLintumictotal_adult: adult total unbound intrinsic microsomal clearance (L/min/mg
microsome)
# BSA: bodysurface area (square meters)
# AGE: post natale age (years)
# IOA: isoenzyme ontogeny value for a specific isoenzyme A (IA) and age, expressed as the
pediatric to adult CLintmicA (CLintmic due to IA) ratio. In adults, IOA=1 (IA mature in adults)
# IOB: isoenzyme ontogeny value for a specific isoenzyme B (IB) and age, expressed as the
pediatric to adult CLintmicB (CLintmic due to IB) ratio. In adults, IOB=1 (IB mature in adults)
# fmA_adult: fraction of the drug metabolized by IA. For drugs metabolized by one
isoennzyme,fmA_adult = 100%
# (1-fmA_adult): fraction of the drug metabolized by IB (fmB_adult). For drugs metabolized by
one isoennzyme,fmB_adult = 0%


##########################################################################
#################### Plasma clearance and extraction ratio ###################
##########################################################################

### Overall total (bound and unbound) hepatic metabolic plasma clearance (CLp) based on the
dispersion model, with a dispersion number (Dn) of 0.17
```

```
dispersionmodelCLp <- function(CLint,fu,BP,QH){
Rn <- fu*CLint/BP/QH
Dn <- 0.17
a <- sqrt(1+4*Rn*Dn)
CLp <- (QH*(1-(4*a/((1+a)**2*exp((a-1)/(2*Dn))-(1-a)**2*exp(-(a+1)/(2*Dn))))))*BP
CLp
}


#### Extraction ratio (ER) based on the dispersion model, with a dispersion number (Dn) of 0.17

dispersionmodelER <- function(CLint,fu,BP,QH){
Rn <- fu*CLint/BP/QH
Dn <- 0.17
a <- sqrt(1+4*Rn*Dn)
ER <- 1-(4*a/((1+a)**2*exp((a-1)/(2*Dn))-(1-a)**2*exp(-(a+1)/(2*Dn))))
ER
}

# CLp for HSA bound drugs
CLp_HSA <- dispersionmodelCLp(CLint,fu_HSA,BP_HSA,QH)

# CLp for AAG bound drugs
CLp_AAG <- dispersionmodelCLp(CLint,fu_AAG,BP_HSA,QH)

# ER for HSA bound drugs
ER_HSA <- dispersionmodelER(CLint,fu_HSA,BP_HSA,QH)

# CLp for AAG bound drugs
ER_AAG <- dispersionmodelER(CLint,fu_AAG,BP_HSA,QH)


### Total (bound and unbound) hepatic metabolic plasma clearance due to isoenzyme A (CLpA)

# CLpA for HSA bound drugs
CLpA_HSA <- CLp_HSA*fmA_adult*IOA/(fmA_adult*IOA+((1-fmA_adult)*IOB))

# CLpA for AAG bound drugs
CLpA_AAG <- CLp_AAG*fmA_adult*IOA/(fmA_adult*IOA+((1-fmA_adult)*IOB))

# fmA_adult*IOA/(fmA_adult*IOA+((1-fmA_adult)*IOB)) corresponds to the fmA of the
selected typical individual
```

Chapter 6

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

Elisa A. M. Calvier, Elke H. J. Krekels, Trevor N. Johnson, Amin Rostami-Hodjegan, Dick Tibboel, Catherijne A. J. Knibbe



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.

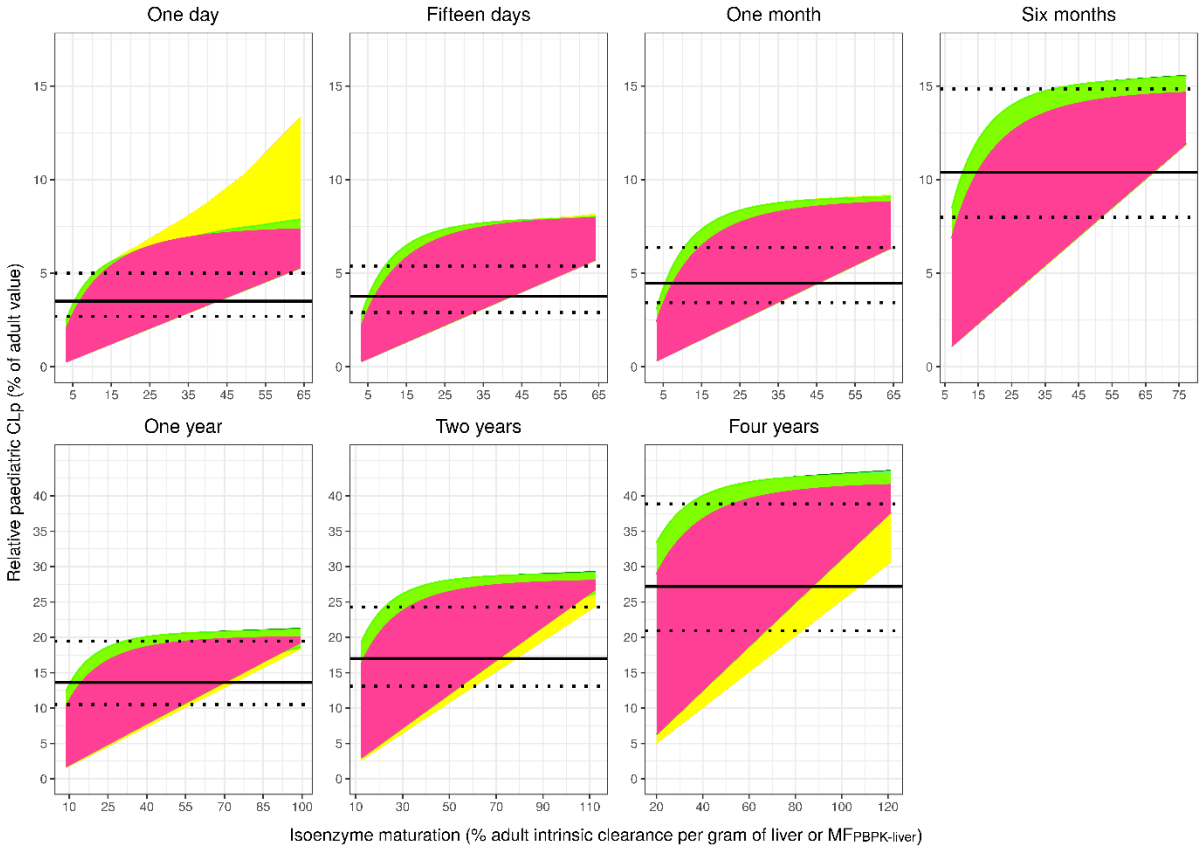


Figure 1 *Relative paediatric CLp (% of adult value) obtained with ADE scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green or yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric 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).

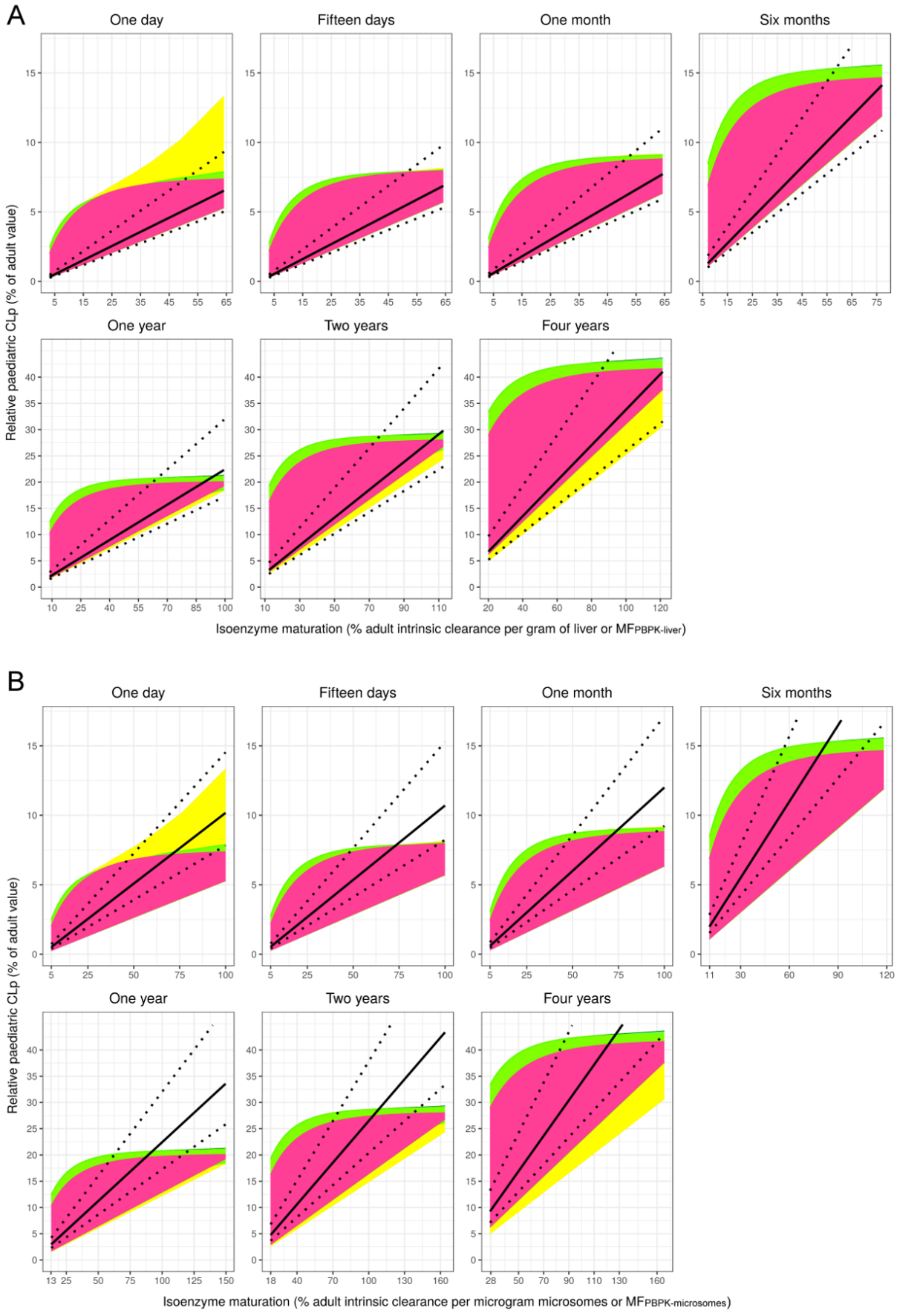


Figure 2 *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}liver}$ scaling (A) and AS0.75+MF$_{PBPK\text{-}microsomes}$ scaling (B) (solid black line with ± 30% PE as dotted*

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.

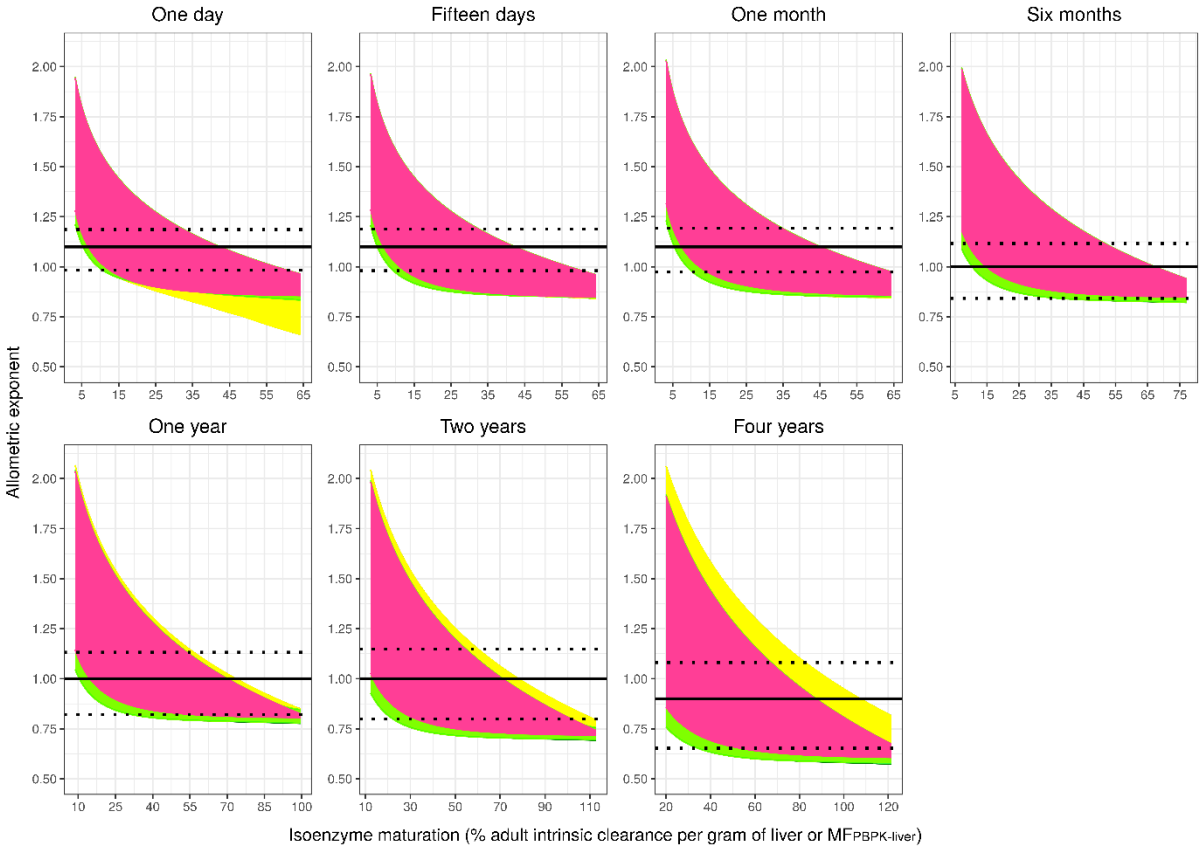


Figure 3 *'True' allometric exponent (pink, green and yellow areas) and ADE exponent used to scale CLp (solid black line with ± 30% PE in CLp as dotted black lines) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. Different colours 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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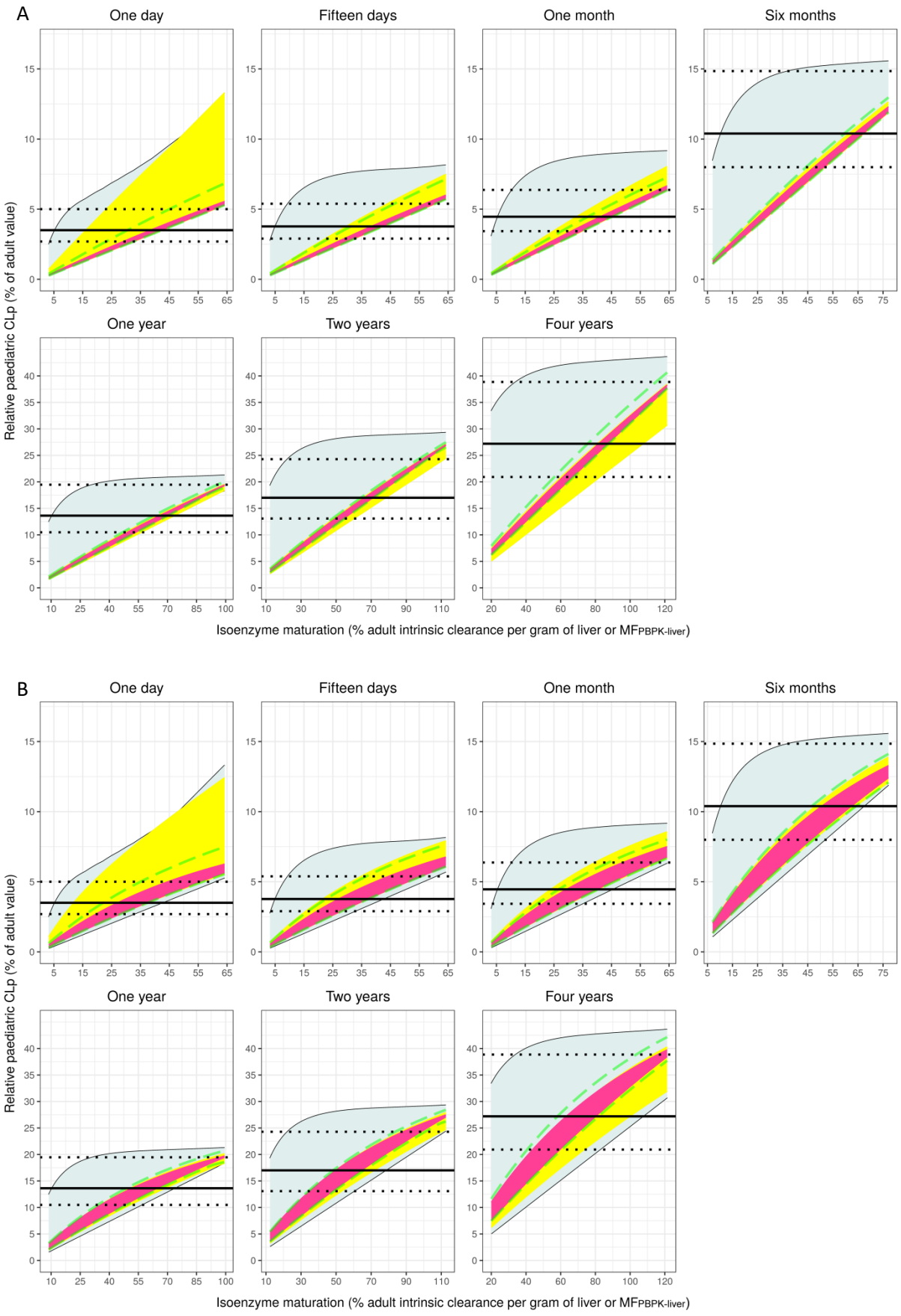
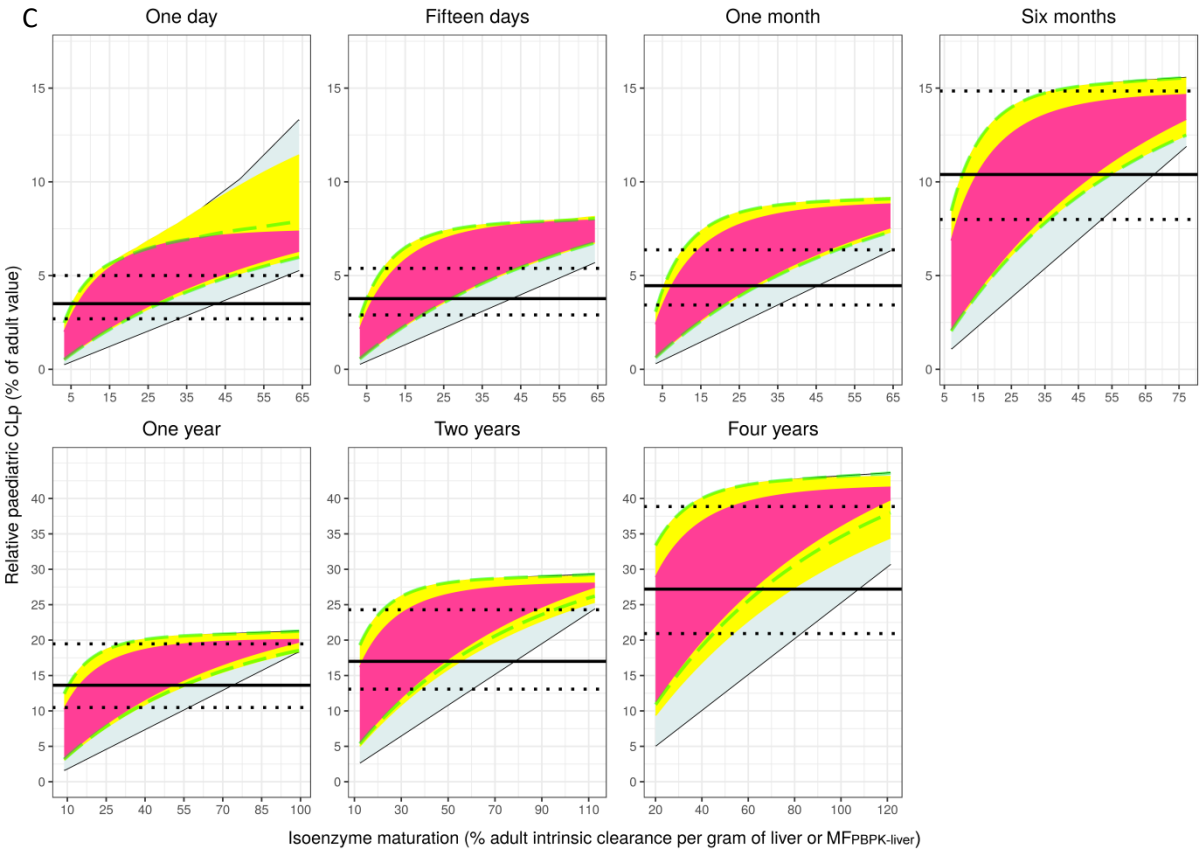
Table S1 *Range of prediction errors of CLp values obtained when scaling the CLp of the hypothetical drugs using ADE for the investigated paediatric ages categorized per drug property*

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

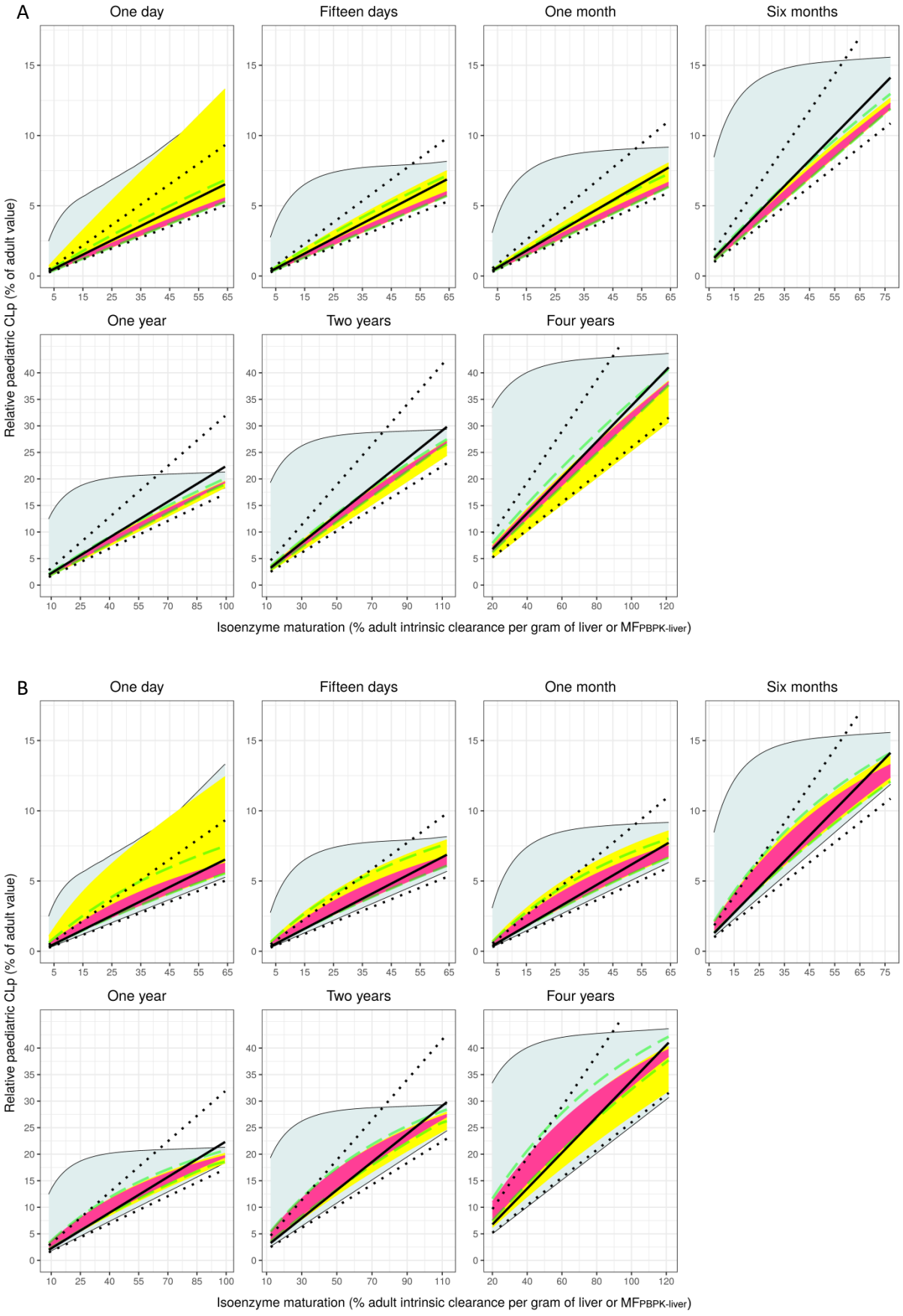
Supplementary Figure 1

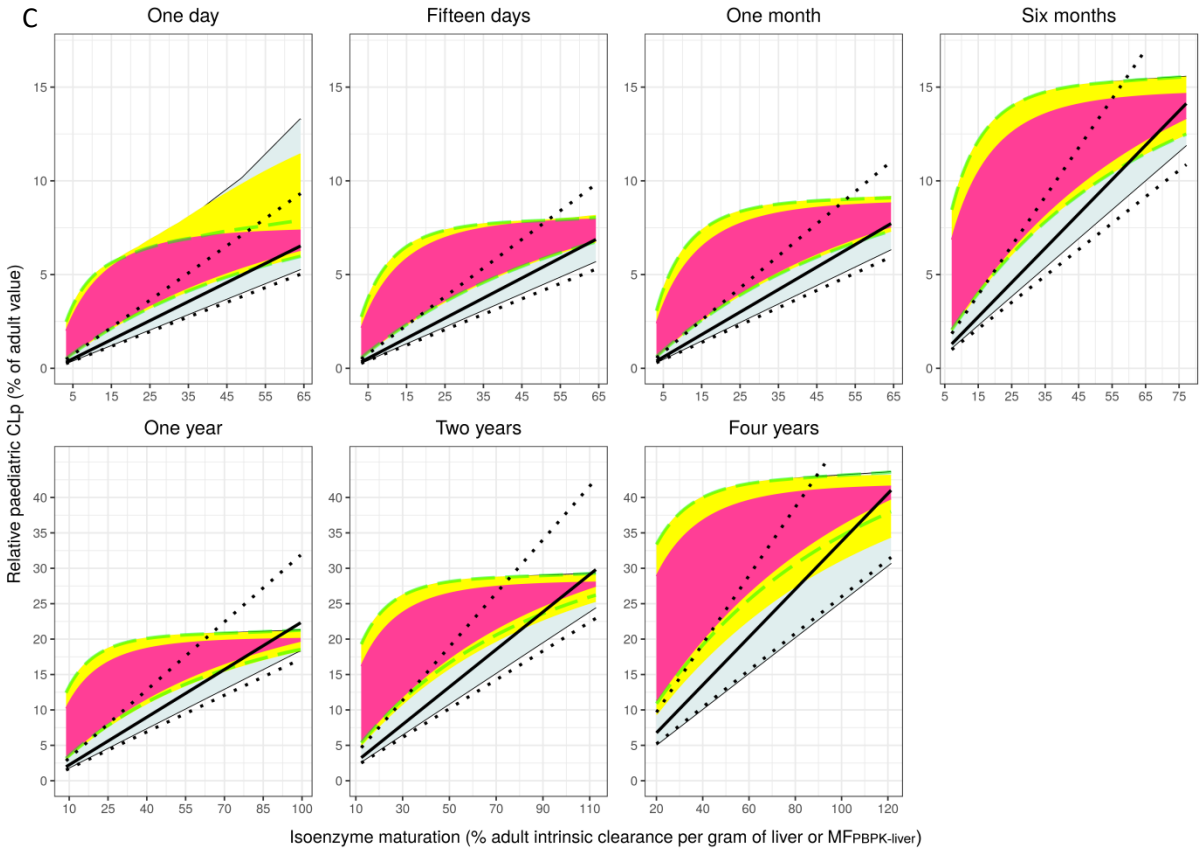




Supplementary Figure 1 *Relative paediatric CLp (% of adult value) obtained with ADE scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green or yellow areas) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied 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.*

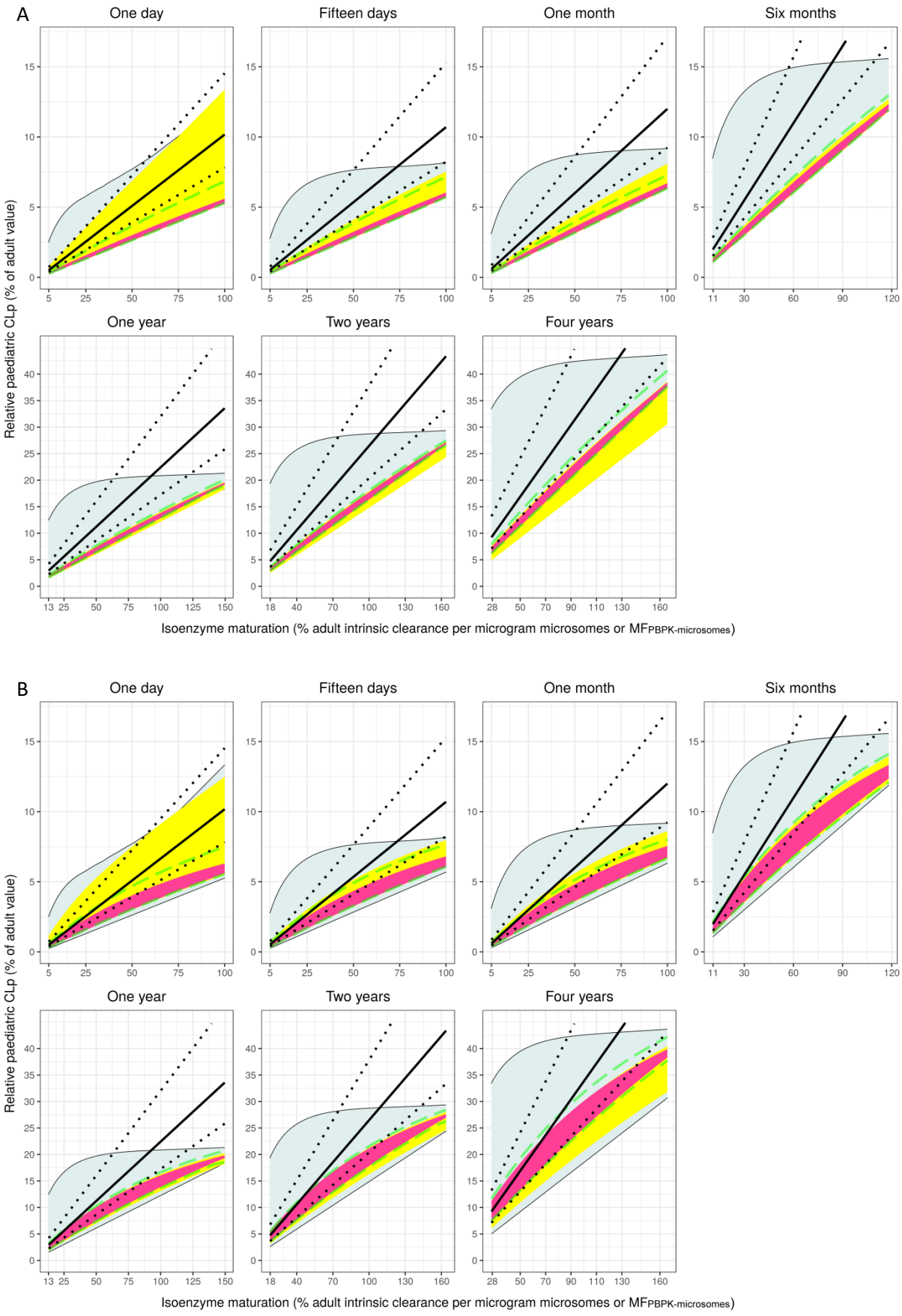
Supplementary Figure 2

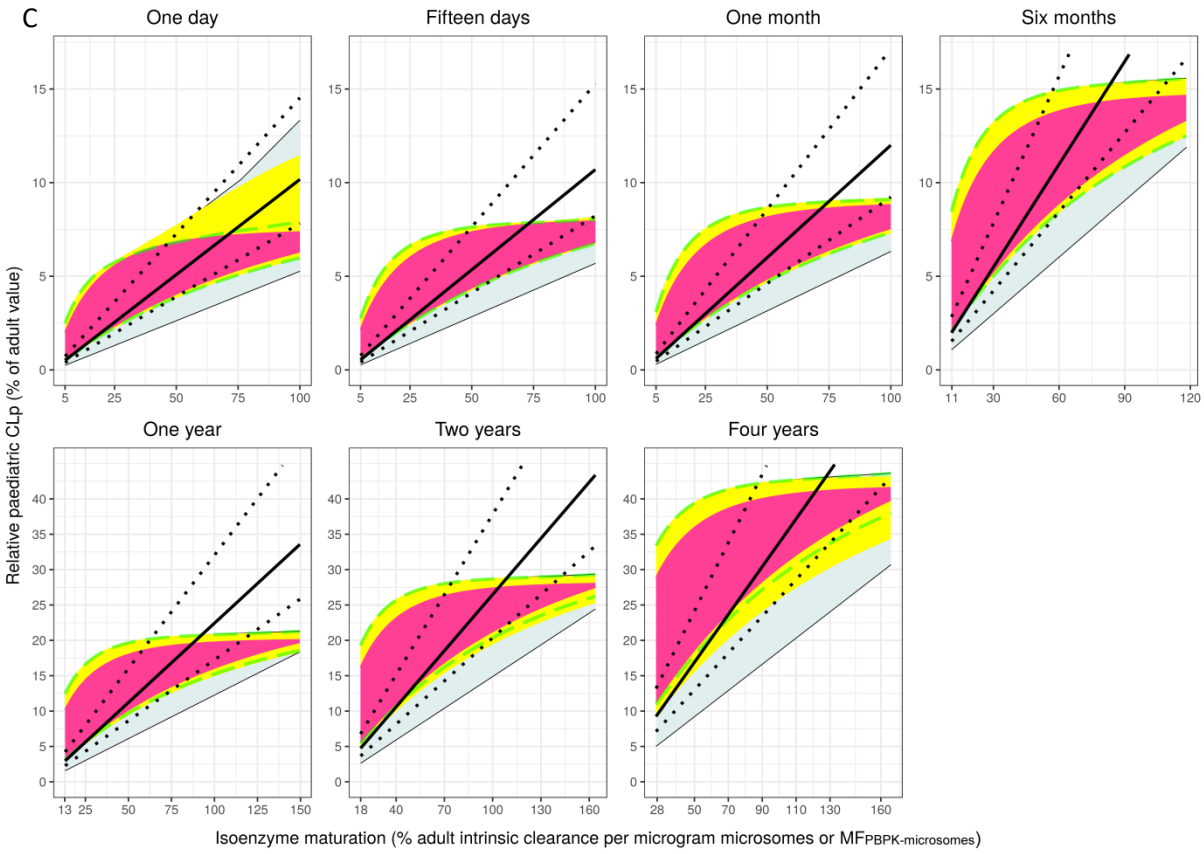




Supplementary Figure 2 *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}liver}$ scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green and yellow areas) for all hypothetical drugs versus the respective isoenzyme 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.*

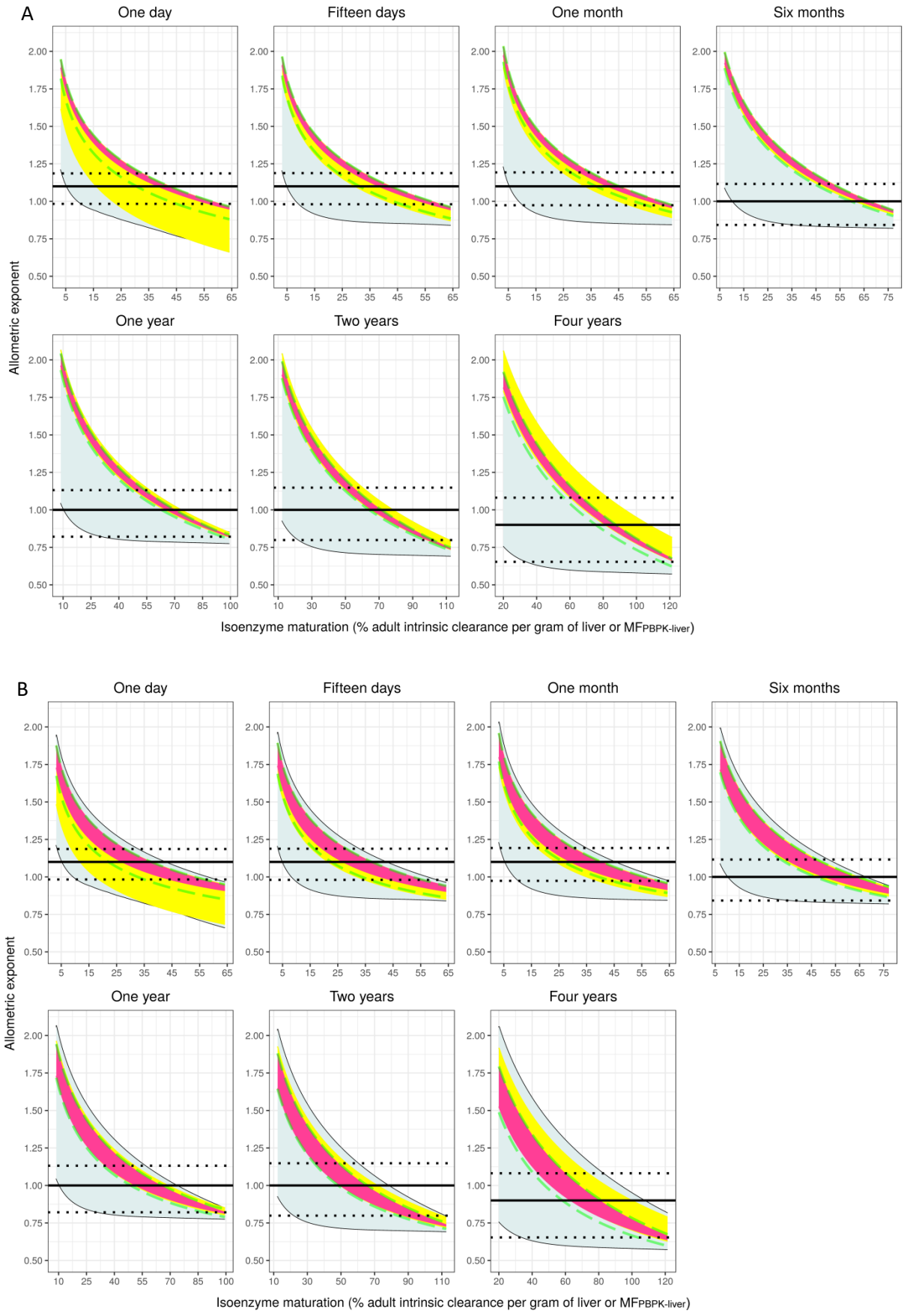
Supplementary Figure 3

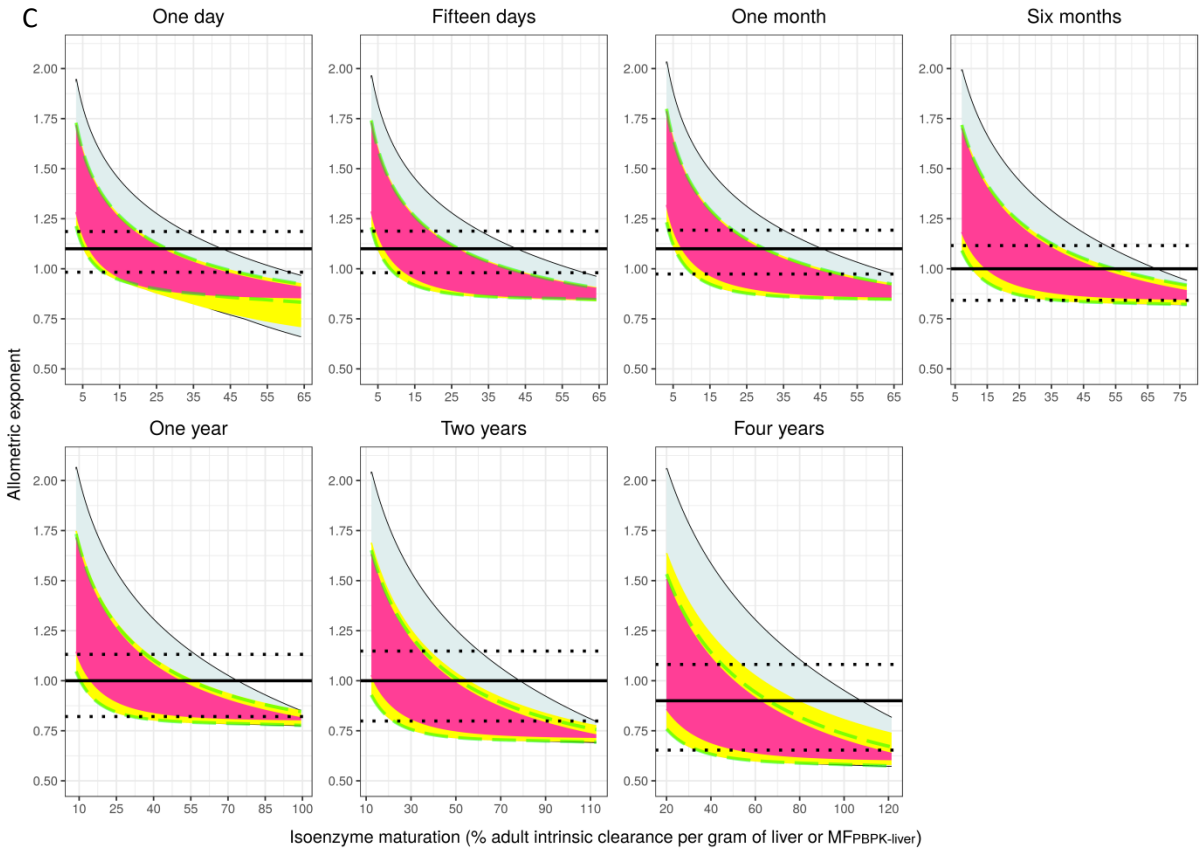




Supplementary Figure 3 *Relative paediatric CLp (% of adult value) obtained with AS0.75+MF$_{PBPK\text{-}microsomes}$ scaling (solid black line with ± 30% PE as dotted black lines) and 'true' relative paediatric CLp (pink, green and yellow areas) for all hypothetical drugs versus the respective isoenzyme 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

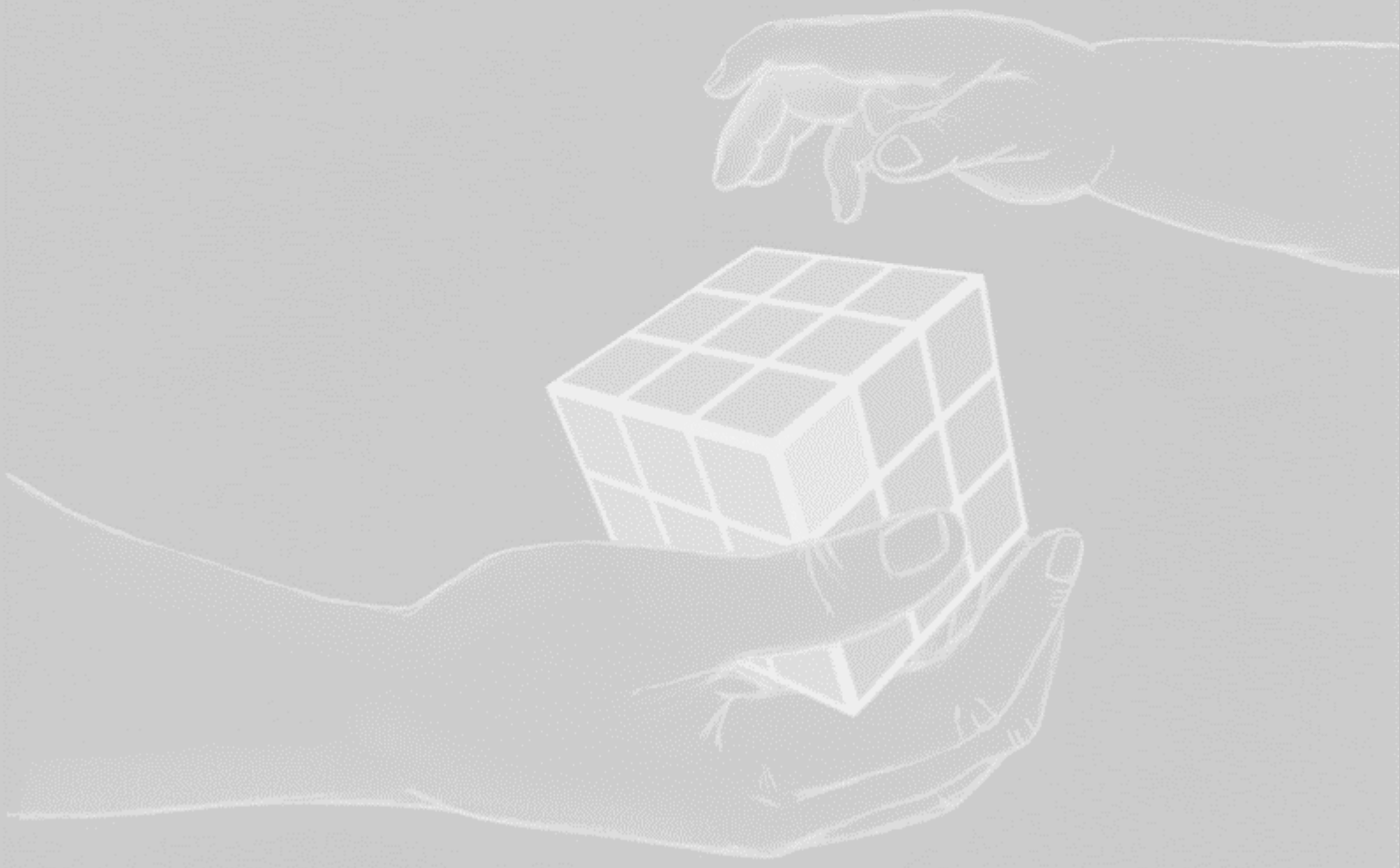




Supplementary Figure 4 *'True' allometric exponent (pink, green and yellow areas) and ADE exponent used to scale CLp (solid black line with ± 30% PE in CLp as dotted black lines) for all hypothetical drugs versus the respective isoenzyme maturation range in the studied typical paediatric individuals. 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

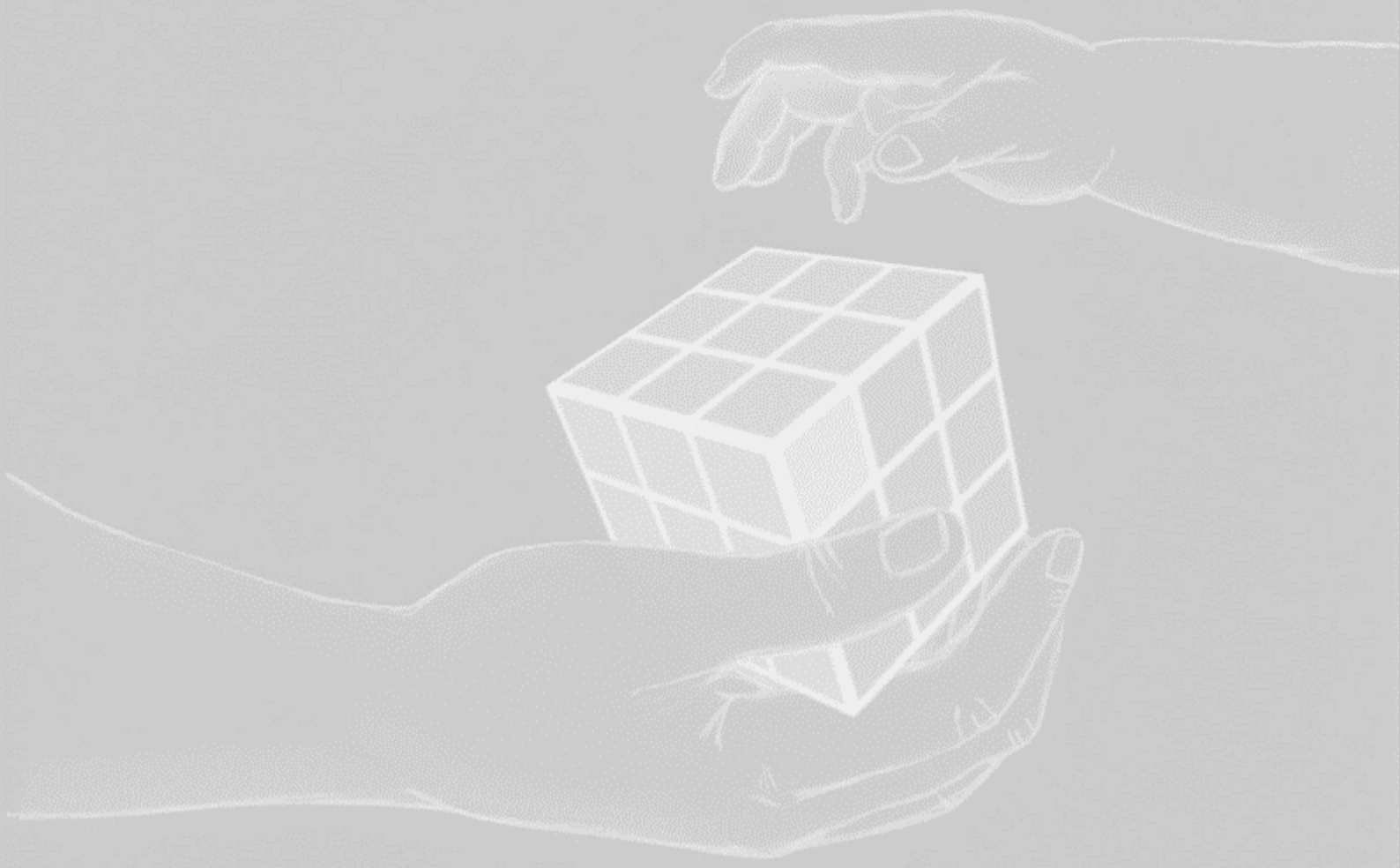
Towards improved paediatric clearance predictions with the combined use of population PK and PBPK modelling approaches



Chapter 7

Can population modelling principles be used to identify key PBPK parameters for paediatric clearance predictions? An innovative application of optimal design theory

Elisa A. M. Calvier, Thu Thuy Nguyen, Trevor N. Johnson, Amin Rostami-Hodjegan, Dick Tibboel, Elke H. J. Krekels, Catherijne A. J. Knibbe



Pharm Res (2018) 35: 209

Abstract

Purpose: Physiologically-based pharmacokinetic (PBPK) models are essential in drug development but require parameters that are not always obtainable. We developed a methodology to investigate the feasibility and requirements for precise and accurate estimation of PBPK parameters using population modelling of clinical data and illustrate this for two key PBPK parameters for hepatic metabolic clearance, namely whole liver unbound intrinsic clearance ($CLint_{,u,WL}$) and hepatic blood flow (Qh) in children.

Methods: First, structural identifiability was enabled through re-parametrization and the definition of essential trial design components. Subsequently, requirements for the trial components to yield precise estimation of the PBPK parameters and their inter-individual variability were established using a novel application of population optimal design theory. Finally, the performance of the proposed trial design was assessed using stochastic simulation and estimation.

Results: Precise estimation of $CLint_{,u,WL}$ and Qh and their inter-individual variability was found to require a trial with two drugs, of which one has an extraction ratio (ER) ≤ 0.27 and the other has an ER ≥ 0.93. The proposed clinical trial design was found to lead to precise and accurate parameter estimates and was robust to parameter uncertainty.

Conclusion: The proposed framework can be applied to other PBPK parameters and facilitate the development of PBPK models.

7.1 Introduction

Physiologically-based pharmacokinetic (PBPK) models are an essential tool to predict the pharmacokinetics (PK) of new compounds in various human populations. PBPK models quantify how drug molecules, characterized by drug-specific parameters reflecting their properties, interact with organisms which are defined by system-specific parameters reflecting anatomical and physiological measures. Predictions are made by feeding drug-specific parameters into a PBPK model with the system-specific parameter values of the population of interest. This has been proven useful for instance to define first-in-child doses [1–3] or support paediatric clinical trial design [2,4].

System-specific parameter values for PBPK models can be either obtained experimentally by direct measurements or they can be derived from clinical PK data through model parameter estimation [5–7]. The latter not only allows for coping with a lack of experimental data, which may be particularly relevant in special patient populations, but is also the most robust approach which has been well described by Tsamandoura *et al.* [8]. For instance, it has been found that ontogeny functions estimated from clinical PK data performed markedly better than those developed from *in vitro* measurements [7]. However, estimating parameter values is not always trivial due to structural identifiability issues. Structural identifiability refers to the possibility to uniquely estimate model parameters given a model and ideal, error-free data [9,10]. Without a guarantee of structural identifiability, parameter estimates will be either non obtainable or random and unreliable [11]. In such cases global structural identifiability, meaning that only one unique solution exists for each parameter, can be obtained by fixing or using priors for some model parameters, while estimating the others.

Once global structural identifiability of model parameters is achieved, population modelling of longitudinal data can be used for estimation of the PBPK parameters and their inter-individual variability. However, structural identifiability of a model does not guarantee precise and unbiased parameter estimates, as this depends on the information content of the data. Therefore, model parameters should also be numerically identifiable, meaning that accurate and precise estimates can be obtained given real, observed data. Numerical identifiability can in general terms be achieved by designing studies that yield sufficiently rich data [12]. Evaluation and optimization of trial designs for population PK analyses can be achieved without time-consuming clinical trial simulations using optimal design software [13]. Optimal design software packages approximate the Fisher information matrix (FIM) of

population models and rely on the Rao and Cramer bound that states that the inverse of the FIM is the lower bound of the variance-covariance matrix of any unbiased estimator of the parameters. While to date population optimal design is routinely used in optimizing trials for the precise estimation of population PK parameters [14], it has never been applied to the estimation of population PBPK parameters.

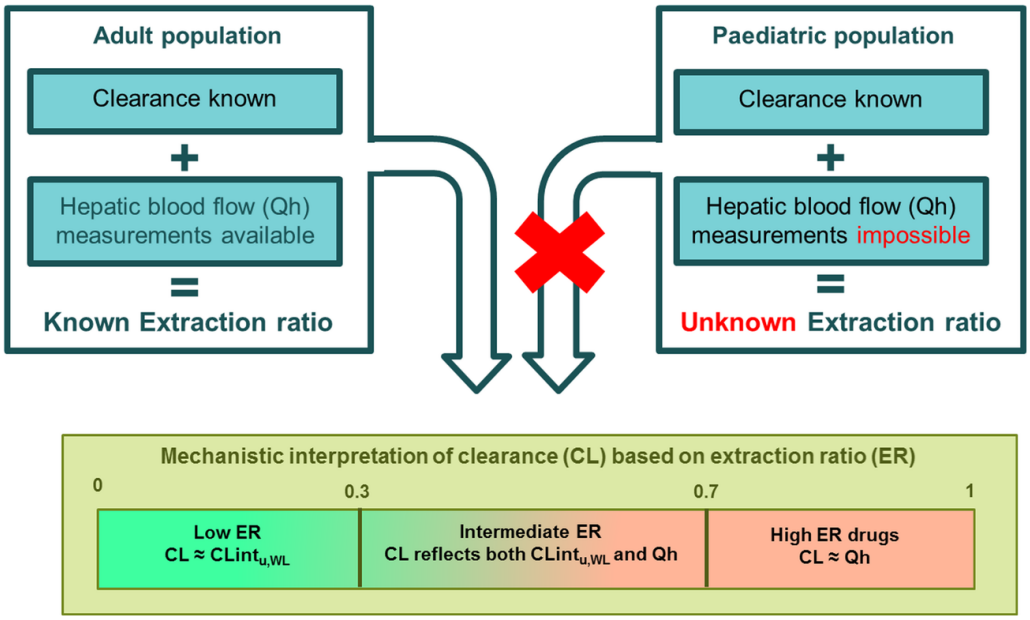


Figure 1 *The relationships between extraction ratio (ER), whole liver unbound intrinsic clearance ($CLint_{,u,WL}$) and hepatic blood flow (Qh) for the adult and paediatric population. Without knowing Qh, the ER of a drug is unknown, making it mathematically impossible to disentangle Qh and $CLint_{,u,WL}$ from clinical concentration-time data of one drug. CL is the blood clearance of the unbound drug, see equations 1-6.*

For drugs undergoing hepatic metabolism, the part of a paediatric PBPK model describing this clearance contains two key parameters that cannot be directly measured and that cannot be simultaneously estimated from the PK data of one drug, due to identifiability issues, namely whole liver unbound intrinsic clearance ($CLint_{,u,WL}$) and hepatic blood flow (Qh). As illustrated in Figure 1, both contribute to the overall hepatic metabolic clearance. The extraction ratio (ER) of a drug reflects the relative contribution of these two parameters to hepatic metabolic clearance. On one hand, clearance of high ER drugs reflects Qh, while clearance of low ER drugs reflects $CLint_{,u,WL}$. To achieve global structural identifiability, Qh is often fixed while $CLint_{,u,WL}$ is estimated based on PK data [5,15]. As Qh cannot be directly measured in very young children its value is often fixed to an assumed percentage of cardiac output [5,15]. When this assumed percentage is however not correct, the estimation of $CLint_{,u,WL}$

values and enzyme ontogeny functions may be biased. Therefore, there is a need for other methodologies that allow us to cope with the lack of experimental data on system-specific parameters. Even though paediatric PBPK models have been proven to predict clearance of many drugs with reasonable accuracy [15–17] , such methods would ultimately improve and accelerate the development and validation of these models in various paediatric populations.

The aim of this paper was to develop an analysis framework to investigate whether population modelling approach can be used to estimate PBPK model parameters from clinical PK data and establish the required criteria for such estimations. The developed analysis framework depends on firstly establishing the data requirements for structural identifiability of PBPK model parameters. Then on a subsequent application of innovative population optimal design theory to define a clinical trial design that yields precise estimates of the relevant model parameters. And lastly on the evaluation of the performance of the proposed trial design in terms of bias and imprecision of parameter estimates using stochastic simulation and estimation. The approach is illustrated using the simultaneous estimation of Qh and $CLint_{,u,WL}$ in the paediatric population as an example.

7.2 Materials and Methods

An analytical workflow was developed, which is composed of the three following steps: structural identifiability, optimal design and evaluation of the optimized design performance. R version 3.3.1 was used for calculations, data management and visualizations [18]. For optimal design procedures of step 2 of the analytical workflow, the PFIM program 4.0 running in R was used [19]. PBPK simulations were performed with Simcyp software (Simcyp, Sheffield, UK) V15.R1 to derive PBPK model parameters value for optimal design procedures in step 2 and the stochastic simulations and estimations in step 3. Stochastic simulations and estimations were performed in step 3 using NONMEM version 7.3 [20] and Perl-speaks-NONMEM software package version 4.6.0 [21].

7.2.1 Step 1: Structural identifiability

The dispersion model (equation 1 – 6) was used to describe hepatic plasma clearance based on PBPK-principles. This model was selected as it has been reported to better predict clearance than the well-stirred model for high clearance drugs, while both models lead to equivalent clearance predictions for other drugs [22,23].

$$\mathrm{CLp} = \mathrm{CLb} \times \mathrm{B{:}P} \quad (1)$$

$$\mathrm{CLb} = \mathrm{Qh} \times \mathrm{ER} \quad (2)$$

$$\mathrm{ER} = 1 - \mathrm{F_H} \quad (3)$$

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

$$a = (1 + 4\mathrm{R_N} \times \mathrm{D_N})^{1/2} \quad (5)$$

$$\mathrm{R_N} = (\mathrm{fu}/\mathrm{B{:}P}) \times CLint_{u,WL}/\mathrm{Qh} \quad (6)$$

In these equations CLp is the total (bound and unbound) plasma clearance, B:P is the blood to plasma ratio, CLb is the whole blood clearance, Qh is the hepatic blood flow, ER is the hepatic extraction ratio, FH is the hepatic availability, fu is the unbound drug fraction in plasma, $CLint_{u,WL}$ is the whole liver unbound intrinsic clearance, R_N is the efficiency number and D_N is the dispersion number, which was taken to be 0.17 [23].

In the dispersion model, CLp values can be directly derived from PK data of intravenously administered drugs. In the paediatric population, the unbound drug fraction in plasma (fu) and the blood to plasma ratio (B:P) of a drug can be obtained experimentally and, assuming these values were obtained precisely and accurately, they were fixed in the model (equation 1-6). Qh and $CLint_{u,WL}$ are thereafter the only two parameters that remain to be estimated. As in this case the dispersion model can be written as a single equation with two unknowns, clinical data on the PK of one drug will not yield structural identifiability. Therefore, clinical trial scenarios were explored based on obtaining PK profiles of two different drugs, in which case there would be two equations for clearance, each with two unknowns. Global structural identifiability can then be obtained if the unknowns are the same in both equations.

Using this approach, Qh is a system-specific parameter that will be the same for two drugs administered to individuals from the same population, assuming the drugs do not alter Qh. $CLint_{,u,WL}$ is however a parameter that combines system-specific properties (i.e., isoenzyme abundance) and drug-specific properties (i.e., isoenzyme activity measured in *in vitro* systems as intrinsic clearance (µl / min) per functional unit of system). This means that even when two drugs are metabolized by the same isoenzyme, their $CLint_{,u,WL}$ value is likely to be different. Therefore a re-parameterization of $CLint_{,u,WL}$ was performed to separate system-specific parameters from drug-specific parameters.

The ratio in $CLint_{,u,WL}$ ($CLint_{ratio}$) of drug A and drug B that are metabolized by the same isoenzyme is equivalent to the ratio in the activity for the metabolizing isoenzyme of the two drugs according to equation 7. Therefore, this parameter is a drug-specific parameter.

$$CLint_{ratio} = \frac{CLint_{,u,WL_B}}{CLint_{,u,WL_A}} = \frac{\text{isoenzyme activity B} \times \text{isoenzyme abundance}}{\text{isoenzyme activity A} \times \text{isoenzyme abundance}} = \frac{\text{isoenzyme activity B}}{\text{isoenzyme activity A}} \quad (7)$$

This ratio does not vary with age, as the isoenzyme activity towards a drug is believed to be unaffected by maturational processes. When fixing $CLint_{ratio}$ obtained for the two drugs, one unique $CLint_{,u,WL}$ value can be estimated in a patient population for the two drugs according to the re-parametrization in equations 8 and 9:

$$CLint_{,u,WL} = CLint_{,u,WL_A} \quad (8)$$

$$CLint_{,u,WL_B} = CLint_{,u,WL} \times CLint_{ratio} \quad (9)$$

7.2.2 Step 2: Optimal design for precise population parameter estimates

Because population PK modelling disentangles inter-individual variability in PK parameters from residual unexplained variability, this method allows for the estimation of population PK parameter values Θ and their inter-individual variability ω^2 which is characterized as described in equation 10. In order to obtain precise estimates of Θ and ω^2 for $CLint_{,u,WL}$ and Qh, a global sensitivity analysis of the uncertainty of these parameters with respect to the drugs ERs was undertaken using PFIM 4.0 (see under uncertainty section in methods). Defining the relationship between parameter uncertainty and the drugs ERs enables optimization of the trial design, by selecting drugs based on their ER. ER was chosen as the trial design parameter to optimize because it reflects the relative information content of drug clearance values regarding $CLint_{,u,WL}$ and Qh (see Figure 1).

7.2.2.1 Clinical trial design

The clinical trial design is illustrated in Figure 2. To allow for structural identifiability, the clinical trial design was composed of two arms. The patients in each arm belonged to the same paediatric population, for which 1-year-olds were chosen, and the drugs administered in each arm were eliminated by the same isoenzyme and had different extraction ratios. In order to ensure that the sampling design was informative enough, while only focusing on the optimization of drug's ER, the number of patients and blood samples were chosen to be high, with 45 patients included in each arm and 7 blood samples drawn in each patient. This choice allowed us to assess the impact of the drug's ER on the performance of the trial design without a confounding impact of patient or sample number. Finally, in order to assess the impact of the drug's ER on the performance of the design without any confounding impact of sampling times, drug dosing, and number of patients and sampling times, these parameters were adapted to the drugs' properties. To do so, drugs were administered as a constant rate infusion with infusion rate and sampling times adapted to the drugs clearance and half-life respectively, and each arm of the study included the same number of patients and sampling times. The infusion rate was set to reach the same arbitrary steady state concentration of 70 mg/L. Sampling times were drawn every half-life, from the first half life after the start of the infusion up to seven half-lives.

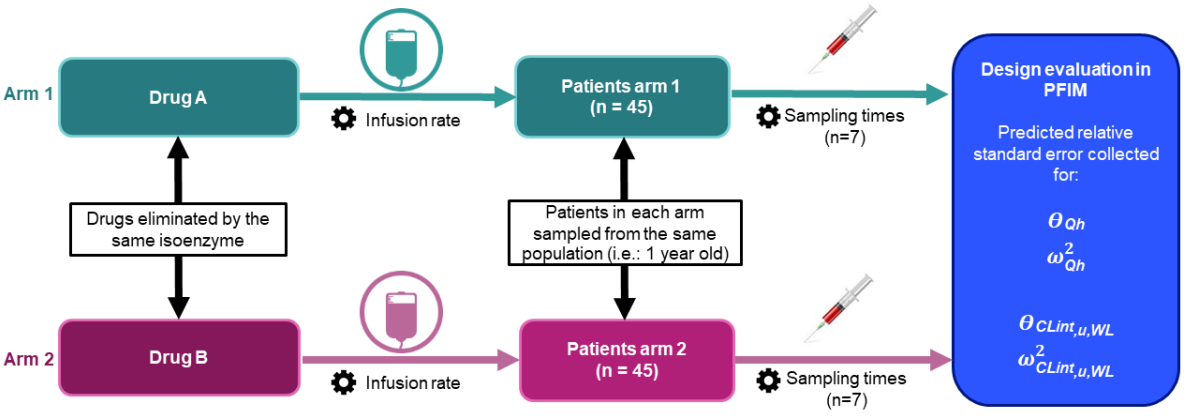


Figure 2 *Overview of the clinical trial design. Qh is the hepatic blood flow, $CLint_{u,WL}$ is the whole liver unbound intrinsic clearance as define in equations 8 and 9. Θ represents the fixed effect and ω^2 represents the inter-individual variance. Cogs indicate trial design variables which are adapted to the drug properties in order to prevent them from being confounding factors when assessing the impact of the drug's ER on the performance of the design.*

7.2.2.2 Hypothetical drug combinations

A total of 99 hypothetical drugs were generated and each unique combination was tested in the design. In order to reduce computational costs and increase interpretability of the results, fu and B:P were taken to be 1. Using the dispersion model, population values of $CLint_{,u,WL}$ ($\Theta_{CLint_{,u,WL}}$) were calculated to be such that ER values ranged from 0.01 to 0.99. This required population values of Qh (Θ_{Qh}) which was obtained by simulating 500 male and 500 female individuals of 1 year in Simcyp and estimating the population parameter value using the "fitdist" function from the fitdistrplus R package [24] in R.

7.2.2.3 Studied models and parameters

For illustration, the proposed workflow is applied to identifying Qh and $CLint_{,u,WL}$ in the paediatric population. As none of the parameters in the dispersion model are impacted by absorption or distribution processes, it is possible to study this PBPK sub-model in full while simplifying the remaining aspects of the PK model for the purpose of this work. Therefore, for each drug in the design (drug A and B), the structural model was a one compartment PK model with a volume of distribution VA and VB respectively and with constant intravenous infusion (see PFIM model code in Supplementary Material 2 and corresponding model equations in Supplementary Material 1). Clearance was parameterized as defined by the dispersion model (equation 1–6). $CLint_{,u,WL}$ for drug A and B was parameterized according to equations 8 and 9 respectively. The differential equations of the models, as implemented in PFIM can be found in Supplementary Material 2. Inter-individual variability was implemented on all fixed effects except on fu, B:P, DN and $CLint_{ratio}$. Inter-individual variability on fu and B:P can be measured and are therefore attainable parameters. D_N is a model specific parameter with a fixed value taken from literature. And as a drug-specific parameter, $CLint_{ratio}$ is not subject to inter-individual variability. For the other parameters, inter-individual variability was assumed to follow a log normal distribution and implemented using equation 10, in which η is normally distributed with a mean of 0 and a variance of ω^2 (see eq.10).

$$Pi = \theta \times e^{\eta} \quad (10)$$

A proportional residual error model for each arm in the design with a standard deviation σ of 0.1 was assumed, to yield the same impact of the model error on the parameter uncertainty between study arms and study designs. Information on the values and estimation

of the fixed effects, inter-individual variability and residual errors implemented in the models can be found in Table 1.

Table 1 *Estimated and fixed model parameters*

Parameters	Values	Estimated and fixed
	Fixed effects	
$\Theta_{\mathrm{CLint_{u,WL}}}$	[0.10 – 156] L/h	Estimated
Θ_{Qh}	20.3 L/h	Estimated
Θ_{fu}	1	Fixed
$\Theta_{B:P}$	1	Fixed
$\Theta_{\mathrm{V_A}}$	4 L	Estimated
$\Theta_{\mathrm{V_B}}$	4 L	Estimated
Θ_{D_N}	0.17	Fixed
$\Theta_{\mathrm{CLint_{ratio}}}$	[0.00065 – 0.97000]	Fixed
	Inter-individual variability	
$\omega^2_{\mathrm{CLint_{u,WL}}}$	[0.1 – 0.8]	Estimated
ω^2_{Qh}	[0.1 – 0.6]	Estimated
$\omega^2_{\mathrm{V_A}}$	0.25	Estimated
$\omega^2_{\mathrm{V_B}}$	0.25	Estimated
	Residual error	
$\boldsymbol{\sigma}_A$	0.1	Estimated
$\boldsymbol{\sigma}_B$	0.1	Estimated

Θ fixed effect; ω^2 inter-individual variance. Θ_{V_A}, Θ_{V_B}, correspond to fixed effects for the volume of distribution of drug A and B respectively.

7.2.2.4 Design evaluation with PFIM 4.0

The models, parameters and designs described above were implemented in PFIM 4.0 [19]. Concentration-time profiles obtained for both drug A and B were simultaneously analysed to enable for the estimation of Θ and ω^2 for $CLint_{u,WL}$ and Qh. The clinical design parameter to optimize for precise estimation of these parameters was the ER of each of the

two drugs in the design. To do so, the population Fisher information matrix (FIM) was evaluated for each drug combination in PFIM 4.0. The expected standard errors for each population parameter were calculated as the square root of the diagonal of the inverse of the FIM and reported in PFIM outputs with corresponding relative standard errors (rse). Parameter uncertainties were assessed using their rse with precise estimates being defined as having an rse $\leq$ 30%.

7.2.2.5 Uncertainty

ER, which is the design variable that was optimized, is dependent on the relative contribution of $CLint_{,u,WL}$ and Qh to overall hepatic metabolic clearance, but not on their absolute values since there is an infinity of combinations of $CLint_{,u,WL}$ and Qh values leading to a specific value of ER. Hence, combinations of $\Theta_{CLint_{,u,WL}}$ and Θ_{Qh} that lead to similar ER values are estimated with the same rse%. Therefore, no uncertainty on the $\Theta_{CLint_{,u,WL}}$ and Θ_{Qh} was implemented in the design evaluation procedure to avoid unnecessary computations, meaning that only one value for $\Theta_{CLint_{,u,WL}}$ and one value for Θ_{Qh} as displayed in Table 1 was used in the PFIM runs.

For each combination of hypothetical drugs, the analysis was repeated for different values of $\omega^2_{CLint_{,u,WL}}$ and ω^2_{Qh} in order to account for uncertainty in these parameters. The range in $\omega^2_{CLint_{,u,WL}}$ was set from 0.1 to 0.8, while the range in ω^2_{Qh} was set from 0.1 to 0.6, both reflecting realistic ranges (see Supplementary Material 1). Then, design evaluations were repeated using each combination of the upper and lower uncertainty value of the defined range for $\omega^2_{CLint_{,u,WL}}$ and ω^2_{Qh}, running 4 possible inter-individual variance scenarios in total, yielding a global sensitivity analysis of the uncertainty of $CLint_{,u,WL}$ and Qh. Only the lowest and highest values for $\omega^2_{CLint_{,u,WL}}$ and ω^2_{Qh} were retained in the uncertainty analysis since this yields the extreme values for the rse values. Rse results of all variance scenarios were collapsed for each hypothetical drug combination into one single precision category reflecting the worst-case scenario of all variance scenarios. The PFIM model file, input file, and the R command to launch the PFIM evaluation runs for all combinations of hypothetical drugs for one uncertainty scenario are provided in Supplementary Material 2 to 4. The table containing the drug properties used for the PFIM runs are provided in Supplementary Table 1.

7.2.3 Step 3: Investigation of performance of the proposed design

In step 2, the requirements for ER of the two drugs yielding numerical identifiability of fixed effect Θ and inter individual variability ω^2 of $CLint_{u,WL}$ and Qh were identified. In the last step of the developed approach, the performance of the proposed design was investigated in terms of bias and imprecision using stochastic simulation and estimation (sse).

First, two drugs meeting the ER requirements defined in step 2 were selected and their ER was converted to expected plasma clearance (CLp) values in a one year old using the expected age-appropriate Θ_{Qh} value derived from Simcyp in step 2. The volume of distribution of each drug was used together with their expected CLp to derive their expected half-life. Since the clinical trial design depends on the half-life and CLp of the two drugs for establishing sampling times and infusion rate respectively these parameters were used for its implementation.

Because the true value of Θ_{Qh} might differ from the value derived from Simcyp in step 2, the selected drugs might have an ER differing from their expected ER. Therefore, uncertainty in both Θ and ω^2 values of $CLint_{u,WL}$ and Qh, were accounted for in the sse. This was performed by sampling each of these parameters 1000 times from a uniform distribution. For the population parameters the range of the distribution entailed the typical values from step 2 ±50%. For the variance parameters the range of the distribution entailed the range defined for the parameter uncertainty in step 2. Sampling of population parameter values Θ_{Qh} and $\Theta_{CLint_{u,WL}}$ was restricted, such that their corresponding CLp values yielded a variation of ±30% of the drugs' CLps value derived from the ERs of the selected drug A and drug B. This mimics the accepted 30% uncertainty of the reported CLp value in literature. The performance of the design settings were assessed using relative estimation error re (equation 11), mean relative estimation error mre (equation 12), and and relative root mean square error rrmse (equation 13) for Θ and ω^2 $CLint_{u,WL}$ and Qh, using only the runs for which minimization was successful.

$$\text{Relative estimation error (\%)} = \frac{est_i - true_i}{true_i} \times 100 \quad (11)$$

$$\text{Mean relative estimation error (\%)} = \frac{1}{N}\sum_{i=1}^{N} \frac{est_i - true_i}{true_i} \times 100 \quad (12)$$

$$\text{Relative root mean square error (\%)} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} \frac{(est_i - true_i)^2}{true_i{}^2} \times 100} \quad (13)$$

In these equations, *true* is the true parameter value used in the simulation step, *est* is the estimated parameter value from the estimation step, and *i* is the run index, ranging from 1 to N, the total number of runs for which minimization was successful.

7.3 Results

7.3.1 Step 1: Structural identifiability

Qh and $CLint_{,u,WL}$ were found to be structurally identifiable when the concentration-time profiles from a clinical trial with 2 arms were simultaneously analysed. Each arm of the trial required patients from the same population and administration of different drugs metabolized by the same isoenzyme for which the ratio in intrinsic clearance ($CLint_{ratio}$) is known.

7.3.2 Step 2: Optimal design for precise population parameter and variance estimates

Precision of fixed effects and variance values for $CLint_{,u,WL}$ and Qh obtained with combinations of two drugs in the described clinical trial design are summarized in Figure 3. Except for drug combinations with similar extraction ratio ER (i.e., drug combinations near the line of unity), most drug combinations will lead to a precise $\Theta_{CLint_{,u,WL}}$ estimate. However, for the estimate of $\omega^2_{CLint_{,u,WL}}$ to be precise, at least one of the drugs should have a low extraction ratio. For Θ_{Qh}, most drug combinations leading to precise estimation include one drug with a high ER, while for precise estimation of ω^2_{Qh}, at least one drug with an ER $\geq$ 0.93 is required. Finally, precise estimation of all parameter estimates (rse $\leq$ 30%) requires one drug with an ER $\geq$ 0.93 and one drug with an ER $\leq$ 0.27. Parameter estimation with rse $\leq$ 50% (corresponding to the blue and green areas in Figure 3) for all parameters, requires one drug with an ER $\geq$ 0.85 and one drug with an ER $\leq$ 0.41.

Supplemental Figure 1 shows the results presented in Figure 3, but separated for the four variance scenarios. As shown by both Figure 3 and Supplementary Figure 1, $\omega^2_{CLint_{,u,WL}}$ and ω^2_{Qh} drive the threshold for the highest and lowest ER value required in the drug combination for precise estimation of all parameters respectively. Supplementary Figure 1 also shows that the value of each of these thresholds are driven by both $\omega^2_{CLint_{u,WL}}$ and

ω^2_{Qh}. For precise parameter estimates, an increase in ω^2_{Qh} from 0.1 to 0.6 leads on one hand to a decrease in the threshold for the highest ER value required in the drug combination from 0.79 to 0.56 when $\omega^2_{CLint,u,WL}$ is equal to 0.1 and from 0.93 to 0.80 when $\omega^2_{CLint,u,WL}$ is equal to 0.8. On the other hand, such an increase in ω^2_{Qh} also leads to a decrease in the threshold for the lowest ER value required in the drug combination from 0.48 to 0.27 when $\omega^2_{CLint,u,WL}$ is equal to 0.1 and from 0.76 to 0.55 when $\omega^2_{CLint,u,WL}$ is equal to 0.8. The reverse is observed regarding $\omega^2_{CLint,u,WL}$, with an increase in $\omega^2_{CLint,u,WL}$ leading to an increasing threshold for the highest ER value required in the drug combination for precise estimates of ω^2_{Qh} and Θ_{Qh}, and an increasing threshold for the lowest ER value required in the drug combination for precise $\omega^2_{CLint,u,WL}$ estimates.

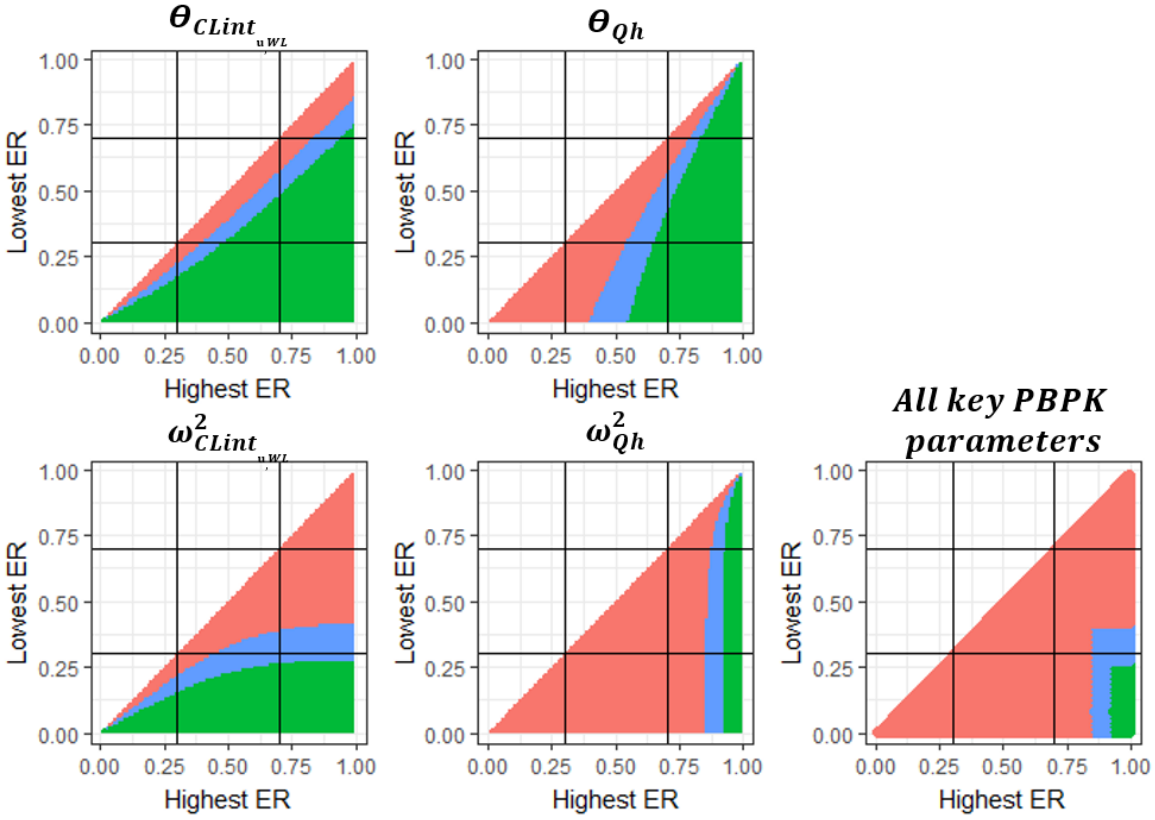


Figure 3 *Parameter precision as a function of the extraction ratios (ER) of the two drugs studied in the clinical trial. Θ represents the fixed effect, ω² the inter-individual variance in the parameter. $CLint_{u,WL}$ is the whole liver unbound intrinsic clearance as defined in equations 8 and 9, Qh is the hepatic blood flow. For each tested drug combination, the precision of the parameter estimates are summarized, with each pixel representing the results for all four variance scenarios (i.e., $\omega^2_{CLint_{u,WL}}$ and ω^2_{Qh} of 0.1 and 0.1, 0.1 and 0.6, 0.8 and 0.1 or 0.8 and 0.6 respectively) according to the following colour scheme: green indicates relative standard errors (rse%) ≤ 30% for all scenarios, blue indicates rse% is between 30 and 50% for at least one variance scenario, and red indicates rse% > 50% for at least one variance scenario, and red indicates rse% > 50% for at least one variance scenario.*

The results on ER requirements for drug A and B are independent of the age of children as the ER does not depend on absolute values of Θ_{Qh} and $\Theta_{CLint_{,u,WL}}$. However, the ERs of drugs are often unknown, especially in special patient populations, because as presented in Figure 1, ER in children changes due to maturation of both $CLint_{,u,WL}$ and Qh and the impact of these changes are isoenzyme-dependent due to different maturation patterns.

To facilitate the identification of model drugs for which clinical data could be obtained to precisely estimate population values and variance of $CLint_{,u,WL}$ and Qh at different ages, maturation patterns of different isoenzymes were used to identify isoenzymes and ages for which drugs with the lowest and highest ER required for such estimation are likely to exist. To do so, the dispersion model in combination with maturation patterns for $CLint_{,u,WL}$ of various isoenzymes as implemented in Simcyp V15 (see Supplementary Material 1) were used to calculate expected ER values for the hypothetical drugs that are substrates of these isoenzymes in children of various ages. We found that drugs with an $ER \leq 0.27$ are likely to exist for all investigated isoenzymes at all ages (results not shown). However, drugs with an $ER \geq 0.93$ are likely to exist only for specific isoenzyme pathways and ages, as shown in Table 2 by the increased blue and red boxes with younger ages for most isoenzyme pathways. This is, because low enzyme maturation will reduce the overall ER of drugs. As a result, drugs metabolized by slowly maturing isoenzymes such as CYP2E1 and UGT2B7, are unlikely to have a high ER at young ages. On the other hand, drugs metabolized by fast maturing isoenzymes, such as CYP1A2 or UGT1A4, with a very high ER over a wide range of paediatric ages are likely to exist and could be used as model drugs.

For convenience, to identify drugs with desired ER values, the ER values have also been translated into the required total plasma clearance values (CLp) for various paediatric ages. This is presented in Table 3, which shows for instance that in a one-year old child, $ER \leq 0.27$ and $ER \geq 0.93$ translate into a $CLp \leq 5.5$L/h and a $CLp \geq 18.9$ L/h, respectively.

Table 2 *Identification of isoenzyme pathways and ages for which combinations of two drugs leading to precise Qh and $CLint_{,u,WL}$ fixed effects and inter-individual variability estimates is theoretically possible*

Isoenzyme	Uncertainty	1 day	1 month	6 months	1 year	2 years	5 years	15 years	25 years
CYP1A2	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
CYP2C18_19	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
CYP2D6	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
CYP2E1	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
CYP3A4_5	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
UGT1A1	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
UGT1A4	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
UGT1A6	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								
UGT2B7	Qh - 50%								
	$CLint_{,u,WL}$ +50%								
	none								
	Qh + 50%								
	$CLint_{,u,WL}$ - 50%								

The cells indicate whether within the set of hypothetical drugs (see Supplementary Material 1) a combination of two drugs exist with which Qh and $CLint_{,u,WL}$ fixed effects and inter-individual variability estimates can be obtained with rse ≤ 30% (green), 30% < rse ≤ 50% (blue), or rse >50% (red). Calculations are performed for a situation in which Θ_{Qh} and isoenzyme maturation are set to values as implemented in Simcyp V15 or to a value that is 50% higher or lower reflecting extreme scenarios of uncertainty on Θ_{Qh} and $\Theta_{CLint_{u,WL}}$.

Table 3 *Conversion table to convert the extraction ratio (ER) values of 0.27 and 0.93 to total plasma clearance (CLp, equation 1) in various postnatal ages using expected hepatic blood flow (Qh) values and fu and B:P values taken to be 1*

	Expected Θ_{Qh} (L/h)	CLp (L/h) corresponding to ER = 0.27	CLp (L/h) corresponding to ER = 0.93
25 years	87.0	23.5	80.9
15 years	89.2	24.1	83.0
5 years	41.5	11.2	38.6
2 years	30.0	8.1	27.9
1 year	20.3	5.5	18.9
6 months	13.0	3.5	12.1
1 month	7.5	2.0	7.0
1 day	6.4	1.7	6.0

Expected Θ_{Qh} is the fixed effect for the hepatic blood flow derived from Simcyp simulations.

7.3.3 Step 3: Investigation of bias

The performance of a clinical trial design resulting from step 1 was evaluated using sse with one drug with an ER of 0.94 and one drug with an ER of 0.2 in one-year old children. The uncertainty on $\Theta_{\mathrm{CLint_{u,WL}}}$ and Θ_{Qh} included in the sse, lead to the simulation of 1000 combinations of $\Theta_{\mathrm{CLint_{u,WL}}}$ and $\Theta_{Qh}$with a range of ER from 0.09 to 0.38 and from 0.76 to 0.99 for the drug with the lowest and the highest ER respectively. Minimization was successful in 78.3% of the sse runs. The mre was ≤ 7.5% for all parameters (Table 4), and the rrmse was below 31%. The confidence interval (5^{th} and 95^{th} percentile) of the re was below or around 20% for $\Theta_{\mathrm{CLint_{u,WL}}}$ and Θ_{Qh}. Higher values were found for $\omega^2_{\mathrm{CLint_{u,WL}}}$and ω^2_{Qh} , with values below or around 47%.

Table 4 *Assessment of the clinical trial performance based on the precision and accuracy of the parameter estimates of the sse with uncertainty*

	$\Theta_{\mathrm{CLint_{u,WL}}}$	Θ_{Qh}	$\omega^2_{\mathrm{CLint_{u,WL}}}$	ω^2_{Qh}
re 5th percentile	-16.2935	-18.356	-44.4268	-47.0063
re 50th percentile	0.715404	-1.6823	-9.7909	-10.7792
re 95th percentile	23.68881	16.63842	41.03665	46.80308
mre	1.548568	-1.19669	-7.38877	-7.24315
rrmse	12.21483	10.9555	26.24813	30.54991

Re, relative estimation error; mre, mean relative estimation error; rrmse, relative root mean square error; Θ, fixed effect, ω^2, inter-individual variance. $CLint_{,u,WL}$ is the whole liver unbound intrinsic clearance as define in equations 8 and 9 and Qh is the hepatic blood flow.

7.4 Discussion

The aim of this paper was to develop an analysis framework to investigate the feasibility and clinical trial requirements for the estimation on clinical data of PBPK parameters with a population PK approach. This work represents, to our knowledge, the first application of population optimal design principles for the estimation of PBPK parameters. Being able to *a priori* define trial requirements that yield sufficiently informative data (i.e., numerical identifiability), is essential for a decision-making process when costs and benefits of performing a study need to be weighed. The complex design requirements derived in our example for instance, would not be easy to define and would likely not have been met using conventional study design approaches. The execution of clinical trials that yield uninformative data that do yield numerical identifiability is both unethical, especially in vulnerable populations, and cost-inefficient.

While we focused on $CLint_{,u,WL}$ and Qh in a paediatric population, the analysis workflow herein developed can be applied to other PBPK parameters, a different number of parameters, and for other populations. In these cases, the workflow would contain the same steps as outlined here: structural identifiability, optimal design and evaluation of the optimized design performance.

In our example, in the first step of the workflow, structural identifiability for the estimation of both $CLint_{,u,WL}$ and Qh was found possible when PK data of a minimum of two drugs were simultaneously analysed. In addition, both drugs must be administered in patients groups from the same population, metabolized by the same isoenzyme, and their $CLint_{ratio}$

should be known. $CLint_{ratio}$ is the ratio of the *in vitro* measured intrinsic clearance of the drugs and is a drug-specific parameter (equation 7).

In the second step, the use of optimal design not only allowed to optimize the characteristics of drugs to include in the clinical trial design in order to solve numerical identifiability issues, but also acts as a safeguard ensuring global identifiability of the model parameters. Indeed, optimal design identifies models that are either globally or locally structurally non identifiable since their Fisher information matrix is non invertible [25]. We found that given the described trial design, the two drugs included in the trial should have an $ER \geq 0.93$ and an $ER \leq 0.27$, in order to precisely ($rse \leq 30\%$) estimate Θ and ω^2 for $CLint_{,u,WL}$ and Qh. These requirements might not be easy to meet since drugs with $ER \geq 0.93$ are rare, especially in very young children whose isoenzyme maturation can lead to a decrease in ER with decreasing age [26]. This is shown in Table 2, displaying isoenzymes and ages for which drugs with ER values that meet these criteria exist within the set of hypothetical drugs tested. These results show that characterization of isoenzyme specific ontogeny is challenging in very young children not only due to the sparsity and lack of available data in this population, but also due to intrinsic characteristics of the system which leads to a decrease in ER with decreasing age. Drugs metabolized by fast maturing isoenzymes were found to be the best model drugs to estimate Qh and $CLint_{,u,WL}$ simultaneously. For instance, CYP1A2 substrates are likely to have the required ERs in children as young as 6 months even in situations where $\Theta_{\mathrm{CLint_{u,WL}}}$ and Θ_{Qh} deviate up to ±50% of their expected values. UGT1A4 substrates are likely to have the required ERs in children as young as term neonates of one day, but only in situations where $\Theta_{\mathrm{CLint_{u,WL}}}$ and Θ_{Qh} values correspond to their expected value or are up to +50% and -50% of their expected value respectively. To support the selection of such model drugs from existing drugs, the requirements in ER were translated into CLp values in the conversion table (see Table 3) using reported hepatic blood flow values. Overall, these results do highlight the importance of investigating the clinical trial requirements *a priori*, as otherwise the chances of successfully estimating PBPK model parameters from clinical PK data using population approach will be very low.

In the last step of our analysis workflow, stochastic simulations and estimations were used to assess the performance of the optimized study design, since optimal design only addresses parameter precision. While step 2 defined the ER of the two drugs required in the clinical trial, in practice the selected drugs might have an ER deviating from their expected values, which was accounted for in the sse. Uncertainty in fixed effects and inter-individual

variability for $CLint_{u,WL}$ and Qh was also accounted for in the sse. This step allowed for the assessment of whether further investigation to optimize the clinical trial design would be needed to ensure unbiased parameter estimates. Absolute mean ree was below 7.5% for all parameters and the rrmsee was below 31%, meaning that on average the estimates both are accurate and precise. The confidence interval (5^{th} and 95^{th} percentile) of the relative estimation error was below or around 20% for $\Theta_{CLint_{u,WL}}$ and Θ_{Qh}. Higher values were found for $\omega^2_{CLint_{u,WL}}$ and ω^2_{Qh} which was expected due to parameter uncertainty and the general difficulty in estimating inter-individual variability. Overall, the sse showed that the proposed clinical trial design is robust to parameter uncertainty.

Once existing model drugs with the required ER are identified, clinical data to estimate Θ and ω^2 for $CLint_{u,WL}$ and Qh can be sought. The source of these data could either be new clinical trials or historical data from studies meeting the defined design requirements.

An advantage of PBPK models is that quantification of system-specific model parameters, like Qh and the maturation profile of $CLint_{u,WL}$, for each isoenzyme, needs to be performed only once. Once Qh has been quantified for instance using substrates for the CYP1A2 or UGT1A4 isoenzymes, this can be implemented in the PBPK model either as fixed value or as prior in order to estimate Θ and ω^2 for $CLint_{u,WL}$, for any other drug or other isoenzyme pathway in the same population. This is important because both $CLint_{u,WL}$ and Qh cannot be directly measured and because identifiability is important for the estimation of PBPK model parameters [27].

While in this work we focus on popPBPK parameter estimation for a specific age, the scalability of the rse results with regard to ER allows for the applicability of design requirements across the entire age range. Since most paediatric PK studies include patients of a range of ages, estimation of a maturation function would be preferable over the estimation of parameter values for each specific paediatric age. In the future this work could be extended to the design of studies for the estimation of such maturation functions. This will require the *a priori* definition of the most suitable covariate for maturation function and optimization of study designs for the estimation of parameters in continuous covariate functions, the latter of which is not yet available in the PFIM software.

In the developed analytical workflow, optimal design principles were applied in an innovative manner. The clinical design parameter optimized was the ER of the drugs included in the clinical trial. In a classical optimal design setting, optimized clinical design parameters

include the number of patients and samples, sampling times, and drug dose. In this work, in order to assess the impact of the drug's ER on the performance of the design without any confounding impact of these design parameters, these parameters were set such as to have the same impact for each hypothetical drug in the study. To do so, drugs were administered as a constant rate infusion with infusion rate and sampling times adapted to the drugs clearance and half-life respectively, and each arm of the study included the same number of patients and number of blood samples. Practically, such design adaptation on half-life and clearance means that when normalizing sampling times over the drug's half-life, sampling times and measured concentrations are identical between different drugs (between each arm of the design and between different drug combinations tested), which in turn allows for the same impact of sampling times and dosing regimen on the design performance. Another innovative feature of the workflow is that the optimal design is not given as a unique solution as classically performed, but as a range of solutions. This was done by investigating the parameter space of the design variable to optimize (ER) and categorization of the results by level of precision.

The number of patients and samples in the assessed trial designs in step 2 of the workflow were selected to be relatively high, so that these variables would not be limiting to the optimization of trial requirements regarding the definition of ER values. Once the important design features relevant for the research question of interest have been defined in the proposed workflow, traditional optimal design procedures could be applied to further optimize the trial design regarding these variables.

In the developed workflow, computational cost was tremendously reduced through the optimization of a scalable variable and the use of extreme variance scenarios to account for parameter uncertainty. The ER of a drug is a scalable variable, as it can be converted to clearance values in any paediatric age by using the expected hepatic blood flow in the corresponding age which represents the scaling factor. Indeed, ER reflects the relative contributions of Qh and $CLint_{u,WL}$ to the hepatic metabolic clearance, but is independent of the absolute value of these two parameters. Therefore, the results of the optimal design analysis obtained for one age, one-year-olds in our example, can be extended to any other ages, allowing to reduce the computational cost and to facilitate results interpretation. Moreover, the ER summarizes the influence of all drug-specific parameters (e.g., drug binding to plasma proteins, drug distribution in red blood cells, etc.), and therefore, accounting for uncertainty in $\Theta_{CLint_{u,WL}}$ and Θ_{Qh} becomes unnecessary in the optimal design phase and the result obtained in our example for drugs with an unbound drug fraction in plasma or fu of 1 and blood to

plasma ratio or B:P of 1 can be translated to drugs for which these parameters take different values. Testing all combinations of extreme ω^2_{Qh} and $\omega^2_{CLint_{u,WL}}$ values lead to results reflecting the best- and worst-case scenarios in terms of parameter precision. Because we defined precise parameter estimates as rse < 30% in all tested variance scenarios, the final results reflect the worst-case scenarios and account for all untested intermediate variance scenarios.

In conclusion, this work presents an analysis framework that allows for the *a priori* identification of clinical trial requirements that would allow for the estimation of PBPK model parameters from clinical data using population modelling. The example on $CLint_{,u,WL}$ and Qh shows that it may be unlikely to design an adequate clinical trial, without the knowledge obtained by the application of this analysis framework. Being able to identify PBPK parameters that cannot be obtained by direct experimental measurements in a time and cost-efficient manner would greatly improve the development of PBPK models and their predictive performance.

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Supplementary Material 1

Realistic extraction ratio ranges for various isoenzymes and ages

To facilitate the identification of existing compounds that would be suitable model drugs, the maximum ranges in extraction ratio that are expected for hypothetical drugs that are substrates for various isoenzymes in children of various ages were calculated. This was done using the dispersion model in combination with maturation patterns of various isoenzymes as implemented in Simcyp V15. Θ_{Qh} values were used as obtained for each age as displayed in table III and $\theta_{CLint,u,WL}$ values were computed as described in equation 1.

$$CLint_{,u,WL} = CL_{int,mic} \times MPPGL \times \text{liver weight} \times IO \quad (1)$$

$CL_{int,mic}$ stands for intrinsic microsomal clearance for which values between $0.56 \cdot 10^{-6}$ and $0.209.10^{-3}$ $L.min^{-1}.mg^{-1}$ microsomal protein were used according to literature [1]. The population mean of the product of MPPGL (microsomal protein per gram of liver) and liver weight was derived from the Simcyp simulation similarly as for the computation of Θ_{Qh}. Isoenzyme ontogeny (IO) values for the different ages and isoenzymes were taken from Simcyp V15 and are displayed in the table below. IO is expressed as percentage of adult isoenzyme activity.

	1 day	1 month	6 months	1 year	2 years	5 years	15 years	25 years
IO CYP1A2 (%)	24	35	118	150	164	161	126	100
IO CYP2C18-19	30	33	84	95	97	98	100	100
IO CYP2D6 (%)	6	47	84	91	95	98	100	100
IO CYP2E1 (%)	10	37	59	67	74	82	88	100
IO CYP3A4_5 (%)	11	13	48	78	96	104	106	100
IO UGT1A1 (%)	0.2	23	98	104	100	100	100	100
IO UGT1A4 (%)	74	74	74	75	77	81	97	100
IO UGT1A6 (%)	15	30	63	76	87	95	100	100
IO UGT2B7 (%)	8	9	11	13	18	32	79	100

In order to account for uncertainty on Qh and $Clint_{,u,WL}$, extraction ratio ranges were also computed with Θ_{Qh} values of +/50% and with $\theta_{CLint,u,WL}$ values of ±50% of the values described above. For each isoenzyme, the age range was identified in which drugs with realistic extraction ratio values exist that are required for the precise estimation of Qh and $CLint_{,u,WL}$.

Definition of realistic ranges for $\omega^2_{CLint,u,WL}$ and ω^2_{Qh} for the implementation of parameter uncertainty in PFIM

In step 2 and 3 of the analytical workflow, uncertainty on $\omega^2_{CLint,u,WL}$ and ω^2_{Qh} were implemented using realistic ranges for these parameters.

For $\omega^2_{CLint,u,WL}$, the range of variance was set to 0.1-0.8, including the minimum and maximum hepatic $\omega^2_{CLint,u,WL}$ estimates derived using Simcyp (0.46 and 0.77 respectively) and reported clearance variance of low extraction ratio drugs [2]. For populations of 1 day, 1 month, 6 months, 1, 2, 5, 15 and 25 year-olds, 1000 Simcyp simulations of hepatic $CLint_{,u,WL}$ for each population were performed, with 50% females and 50% males, assuming drug metabolism by different isoenzymes. These isoenzymes were isoenzymes for which model drugs have been reported (drugs mainly metabolized by the isoenzyme), namely CYP1A2, CYP2C19, CYP2D6, CYP2E1, CYP3A4-5, UGT1A1, UGT1A4, UGT1A6 and UGT2B7 [34]. For each population and isoenzyme, $\omega^2_{CLint,u,WL}$ was estimated using the fitdist function from the fitdistrplus R package assuming a log normal distribution.

For Qh a range of variance of 0.1-0.6 was selected, which includes recently reported portal vein flow variance values in healthy adults (ω^2_{Qh} = 0.13) and in cirrhotic adults (omega =0.38) using phase contrast MRI sequence with compressed sensing acceleration and high spatial resolution [5]. The chosen range also included higher values (0.6 against the highest reported value of 0.38 in adults) in order to account for potentially higher hepatic blood flow variability in paediatric (sub)populations.

Model equations

The equations of the pharmacokinetic model (structural model) for drug A and drug B implemented in PFIM in the step 2 of the analytical workflow are given below:

Plasma clearance of drug A

$$\mathrm{RnA} = (\mathrm{fuA}/\mathrm{BPA}) \times \mathrm{CLint}_{,u,WL}\mathrm{A}/\mathrm{Qh}$$

$$\mathrm{aA} = \sqrt{(1 + 4\mathrm{RnA} \times \mathrm{Dn})}$$

$$\mathrm{CLA} = \mathrm{Qh} \times \mathrm{BPA} \times \left(1 - \frac{4\mathrm{aA}}{(1+\mathrm{aA})^2 \exp\{(\mathrm{aA}-1)/2\mathrm{D_N}\} - (1-\mathrm{aA})^2 \exp\{-(\mathrm{aA}+1)/2\mathrm{D_N}\}}\right)$$

Plasma clearance of drug B

$$\mathrm{RnB} = (\mathrm{fuB}/\mathrm{BPB}) \times \mathrm{CLint}_{,u,WL}\mathrm{A} \times \mathrm{CLint}_{\mathrm{ratio}}/\mathrm{Qh}$$

$$\mathrm{aB} = \sqrt{(1 + 4\mathrm{RnB} \times \mathrm{Dn})}$$

$$\mathrm{CLB} = \mathrm{Qh} \times \mathrm{BPB} \times \left(1 - \frac{4\mathrm{aB}}{(1+\mathrm{aB})^2 \times \exp\{(\mathrm{aB}-1)/2\mathrm{D_N}\} - (1-\mathrm{aB})^2 \times \exp\{-(\mathrm{aB}+1)/2\mathrm{D_N}\}}\right)$$

Plasma concentration time profile of drug A

$$\frac{d\mathrm{AA}}{dt} = \mathrm{K0A} - \frac{\mathrm{CLA}}{\mathrm{VA}} \times \mathrm{AA}$$

$$\mathrm{CA(t)} = \frac{\mathrm{AA(t)}}{\mathrm{VA}}$$

$$\frac{d\mathrm{AB}}{dt} = \mathrm{K0B} - \frac{\mathrm{CLB}}{\mathrm{VB}} \times \mathrm{AB}$$

$$\mathrm{CB(t)} = \frac{\mathrm{AB(t)}}{\mathrm{VB}}$$

With estimated parameters VA (volume of distribution for drug A), $\mathrm{CLint}_{,u,WL}$(whole liver intrinsic unbound clearance of drug A), Qh (hepatic blood flow) and VB (volume of distribution for drug A) and fixed parameters K0A (infusion rate of drug A), K0B (infusion rate of drug B), $\mathrm{CLint}_{\mathrm{ratio}}$ (ratio $\mathrm{CLint}_{,u,WL}\mathrm{B}$ /$\mathrm{CLint}_{,u,WL}\mathrm{A}$), equivalent to the ratio of microsomal intrinsic clearance as could be measured in vitro, fuA (fraction unbound of drug A), fuB (fraction unbound of drug B), BPA (blood to plasma ratio of drug A), BPB (blood to plasma ratio of drug B) and Dn (Dispersion number).

References

1. Nikolic, K. & Agababa, D. Prediction of hepatic microsomal intrinsic clearance and human clearance values for drugs. *J. Mol. Graph. Model.* **28**, 245–52 (2009).

2. Wang, C. *et al.* Population pharmacokinetics of paracetamol across the human age-range from (pre)term neonates, infants, children to adults. *J. Clin. Pharmacol.* **54**, 619–29 (2014).

3. Krekels, E. H. J., Danhof, M., Tibboel, D. & Knibbe, C. a J. Ontogeny of hepatic glucuronidation; methods and results. *Curr. Drug Metab.* **13**, 728–43 (2012).

4. Kivistö, K. T. & Kroemer, H. K. Use of probe drugs as predictors of drug metabolism in humans. *J. Clin. Pharmacol.* **37**, 40S–48S (1997).

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Supplementary Material 2 R code PFIM model file

```
# PFIM model code
formED<-function(t,y,p){
# Estimated parameters
VA <- p[1]          # 4 L = Volume of distribution for drug A
CLINTA <- p[2]      # Whole liver intrinsic clearance of drug A
QH <- p[3]          # Hepatic blood flow
VB <- p[4]          # 4 L = Volume of distribution for drug B

# Fixed parameters
K0A <- p[5]         # Infusion rate of drug A
K0B <- p[6]         # Infusion rate of drug B
CLINTRATIO <- p[7]  # Ratio CLINTB/CLINTA



FuA <- 1 # Fraction unbound of drug A (fixed to 1)
FuB <- 1 # Fraction unbound of drug B (fixed to 1)
BPA <- 1 # Blood to plasma ration of drug A (fixed to 1)
BPB <- 1 # Blood to plasma ration of drug B (fixed to 1)


Dn <- 0.17 # Dispersion number

# Plasma clearance of drug A
RnA <- FuA*CLINTA/BPA/QH
aA <- sqrt(1+4*RnA*Dn)
CLA <-(QH*(1-(4*aA/((1+aA)**2*exp((aA-1)/(2*Dn))-(1-aA)**2*exp(-
(aA+1)/(2*Dn))))))*BPA

# Plasma clearance of drug B
RnB <- FuB*CLINTA*CLINTRATIO/BPB/QH
aB <- sqrt(1+4*RnB*Dn)
CLB <-(QH*(1-(4*aB/((1+aB)**2*exp((aB-1)/(2*Dn))-(1-aB)**2*exp(-
(aB+1)/(2*Dn))))))*BPB

# Plasma concentration time profile of drug A
yd1 <- K0A-CLA/VA*y[1]
# Plasma concentration time profile of drug B
yd2 <- K0B-CLB/VB*y[2]



list(c(yd1,yd2),c(y[1]/VA,y[2]/VB))

}
```

Supplementary Material 3 R code PFIM input file

```
#########################################################################
####                    INPUT FILE FOR PFIM 4.0                      ####
#########################################################################


#Name of the project
#--------------------
project<-"OMEGA QH = 0.1 & OMEGA CLINT = 0.1"

#Name of the file containing the PK or PD model
#-----------------------------------------------
file.model<-"model.R"

#Name of the output file for the results and for the Fisher information
matrix: names after the drug combination
#---------------------------------------
output<-paste("StdoutA",DrugA,"B",DrugB,".r",sep="");
outputFIM<-paste("FIMA",DrugA,"B",DrugB,".txt",sep="");


#FIM: Population (P) or Individual (I) or Bayesian (B) Fisher
information matrix
#---------------------------------------
FIM<-"P"

#Previous information for population design (FIM<-"P") only:
#If previous information is available, please specify below the file
name;
#otherwise leave it as the default
#-------------------------------------------------------
previous.FIM<-""

#RUN:  Evaluation (EVAL) or Optimisation (OPT)
#-------------------------------------------------------
run<-"EVAL"

#To display only graphs of models and/or sensitivity functions before
evaluating the Fisher Information matrix
graph.only<-F

#Block diagonal Fisher information matrix (option<-1) or complete
Fisher information matrix (option<-2)
#-------------------------------------------------------------
option<-1

#Number of responses
#-----------------------------------------------------------------
nr<-2
```

```
############################ MODEL OPTION ############################

#Model form: Differential equations (DE) or analytical form (AF)
#-------------------------------------------------------------
modelform<-"DE"


############## DIFFERENTIAL EQUATION OPTION ##############

#Initial time for which initial conditions are given
#---------------------------------------------------
time.condinit<-0
#Identical initial conditions in each elementary design (Yes=T, No=F)
#-------------------------------------------------------------
condinit.identical<-T

# If 'Yes', enter once the expression of the initial values of the
system at the initial time
# else, enter the vectors of the initial conditions for each elementary
design
# If initial values depend on the parameters to be estimated,
# enter this parameter into the expression without any quotation marks
#-----------------------------------------------------------
condinit<-expression(c(0,0))

# Error tolerance for solving differential equations
#---------------------------------------------------
RtolEQ<-1e-10
AtolEQ<-1e-10
Hmax<-Inf

###### END DIFFERENTIAL EQUATION OPTION ######



#Name of the fixed effects parameters
#------------------------------------
parameters<-c("VA","CLINTA","QH","VB","K0A","K0B","CLINTRATIO")

#Fixed effects parameters values
#-------------------------------
beta<-
c(4,param_Drug[DrugA,1],0.338351,4,param_Drug[DrugA,3],param_Drug[DrugB
,3],(param_Drug[DrugB,1]/param_Drug[DrugA,1]))

#Some parameters may not be estimated (not estimated = T, estimated =
F)
#-------------------------------
beta.fixed<-c(F,F,F,F,T,T,T)

#Number of occasions
#-------------------------------------------------------------------------
n_occ<-1

#Random effect model (1) = additive  (2) = exponential
#-----------------------------------------------------------------
Trand<-2;
```

```
#Diagonal Matrix of variance for inter-subject random effects:
#-------------------------------------------------
omega<-diag(c(0.25,0.1,0.1,0.25,0,0,0))

#Diagonal Matrix of variance for inter-occasion random effects:
#-------------------------------------------------
gamma<-diag(c(0,0,0,0,0,0,0))

#Standard deviation of residual error (sig.inter+sig.slope*f)^2:
#-----------------------------------------------------------------
sig.interA<-0
sig.slopeA<-0.1
sig.interB<-0
sig.slopeB<-0.1


#List of the vectors of sampling times for each elementary design
#You can specify that a group has no sampling time by writing NULL
#(ONLY if you have several response)
#-----------------------------------------------------------------
# vector of sampling times:sampling every half lifes, from 1st to 7th
half life
Vi <- 4 # Volume of distribution of drug A and drug B
CLAi <- param_Drug[DrugA,2] # CLearance of drug A
CLBi <- param_Drug[DrugB,2] # Clearance of drug B

protA<-list(seq(log(2)/(CLAi/Vi),log(2)/(CLAi/Vi)*7,length.out
=7),NULL)
protB<-list(NULL,seq(log(2)/(CLBi/Vi),log(2)/(CLBi/Vi)*7,length.out
=7))


#Vector of initial proportions or numbers of subjects for each
elementary design
#-----------------------------------------------------------
subjects<-c(45,45)

#Subjects input: (1) for number of subjects (2) for proportions of
subjects
#-------------------------------------------------------------------
subjects.input<-1


#####################################################################
#                                                                   #
#                        Covariate model                            #
#                                                                   #
#####################################################################

##########################################
# Covariates not changing with occasion  #
##########################################

#Add covariate to the model  (Yes==T No==F)
#-------------------------------------------------------------------
covariate.model<-F
```

```
#####################################
# Covariates changing with occasion #
#####################################


#Add covariate to the model   (Yes==T No==F)
#-------------------------------------------------------------------------
covariate_occ.model<-F



############## GRAPH SPECIFICATION OPTION ###############

#graphical representation of the model (Yes=T, No=F)
#-------------------------------------
graph.logical<-F

#graphical representation of sensitivity functions (Yes=T, No=F)
#-------------------------------------
graphsensi.logical<-F


############# END OF GRAPH SPECIFICATION OPTION ###############
```

Supplementary material 4 R command to launch the PFIM evaluation runs for all combinations of hypothetical drugs for one uncertainty scenario

```
# Set working directory
# open PFIM.r in R studio and clic "source"


# Table containing drug properties for all hypothetical drugs.
# Each row contains the drug properties for one specific drug.
# 1st column = Whole liver intrinsic clearance, 2nd column = CL, 3rd
column = infusion rate, 4th column = ER.

param_Drug <- read.csv("EC05_PBPKOD_ODPFIM_HDproperies.csv")  #
Table corresponding to supplementary table 1


combAB <- combn(nrow(param_Drug),2) # Generation of all possible
combinations of drugs from the pool of hypothetical drugs in
param_Drug (without repetitions)


# Run PFIM evaluation for each hypothetical drug combination
for (i in 1:ncol(combAB)){
DrugA <- combAB[1,i] # Row index in param_Drug containing the drug
prarameter values for the selected drug A
DrugB <- combAB[2,i] # Row index in param_Drug containing the drug
prarameter values for the selected drug B


tryCatch({

PFIM()
}, error=function(e){})
}
```

Supplementary Table 1 excel table containing the drug properties used for the PFIM runs

CLint	CL	k0	ER
0.001697	0.001691	0.118403	0.004999
0.003405	0.003383	0.236814	0.009999
0.006855	0.006767	0.473678	0.019999
0.01035	0.01015	0.710515	0.029999
0.013892	0.013534	0.94739	0.04
0.017481	0.016918	1.18423	0.05
0.021119	0.020301	1.421094	0.060001
0.024806	0.023684	1.657906	0.069999
0.028545	0.027068	1.894782	0.080001
0.032336	0.030452	2.131643	0.090001
0.03618	0.033835	2.368471	0.1
0.040079	0.037219	2.605308	0.11
0.044034	0.040602	2.842132	0.119999
0.048047	0.043985	3.078976	0.129999
0.05212	0.04737	3.31587	0.140001
0.056252	0.050752	3.552667	0.149999
0.060448	0.054137	3.789561	0.160001
0.064707	0.05752	4.026403	0.170001
0.069031	0.060903	4.263208	0.179999
0.073424	0.064287	4.500099	0.190001
0.077885	0.06767	4.736918	0.2
0.082418	0.071054	4.973778	0.21
0.087024	0.074437	5.210625	0.22
0.091705	0.077821	5.447453	0.23
0.096464	0.081204	5.684305	0.24
0.101303	0.084588	5.921165	0.250001
0.106224	0.087972	6.158015	0.260001
0.111229	0.091355	6.394834	0.27
0.116322	0.094738	6.631687	0.28
0.121505	0.098122	6.868542	0.29
0.12678	0.101505	7.105361	0.299999
0.132152	0.104889	7.342235	0.31
0.137622	0.108272	7.579072	0.32
0.143194	0.111656	7.815906	0.329999
0.148872	0.115039	8.052761	0.34
0.154659	0.118423	8.289614	0.35
0.160559	0.121807	8.526475	0.360001
0.166575	0.12519	8.763307	0.37
0.172712	0.128574	9.000146	0.38
0.178974	0.131957	9.236978	0.389999
0.185367	0.135341	9.473856	0.400001

CLint	CL	k0	ER
0.191893	0.138724	9.710675	0.409999
0.19856	0.142108	9.947543	0.42
0.205371	0.145491	10.18437	0.43
0.212333	0.148875	10.42122	0.44
0.219452	0.152258	10.65807	0.45
0.226734	0.155642	10.89492	0.46
0.234185	0.159025	11.13175	0.469999
0.241814	0.162409	11.36862	0.48
0.249627	0.165792	11.60547	0.49
0.257632	0.169176	11.8423	0.5
0.265838	0.172559	12.07913	0.509999
0.274255	0.175943	12.31599	0.52
0.282892	0.179326	12.55285	0.53
0.291758	0.18271	12.78967	0.539999
0.300867	0.186093	13.02654	0.55
0.310229	0.189477	13.26339	0.56
0.319857	0.19286	13.50023	0.57
0.329765	0.196244	13.73708	0.58
0.339967	0.199627	13.97391	0.59
0.350481	0.203011	14.21076	0.6
0.361323	0.206394	14.44761	0.61
0.372511	0.209778	14.68445	0.62
0.384067	0.213161	14.9213	0.63
0.396012	0.216545	15.15815	0.64
0.40837	0.219929	15.395	0.65
0.421167	0.223312	15.63183	0.66
0.434433	0.226695	15.86868	0.67
0.448199	0.230079	16.10554	0.68
0.462499	0.233463	16.34238	0.69
0.477372	0.236846	16.57923	0.7
0.49286	0.24023	16.81607	0.71
0.509011	0.243613	17.05291	0.72
0.525878	0.246997	17.28976	0.73
0.543519	0.25038	17.52661	0.74
0.562	0.253764	17.76345	0.75
0.581398	0.257147	18.0003	0.76
0.601796	0.260531	18.23714	0.77
0.623292	0.263914	18.47399	0.78
0.645998	0.267298	18.71084	0.79
0.670042	0.270681	18.94768	0.8
0.695574	0.274065	19.18453	0.81
0.722768	0.277448	19.42137	0.82
0.751833	0.280832	19.65822	0.83

CLint	CL	k0	ER
0.783016	0.284215	19.89507	0.84
0.816612	0.287599	20.13191	0.85
0.852986	0.290982	20.36876	0.86
0.892586	0.294366	20.60561	0.87
0.935971	0.297749	20.84245	0.88
0.98386	0.301133	21.0793	0.89
1.037185	0.304516	21.31614	0.9
1.097192	0.3079	21.55299	0.91
1.165593	0.311283	21.78984	0.92
1.244824	0.314667	22.02668	0.93
1.338523	0.31805	22.26353	0.94
1.452439	0.321434	22.50037	0.95
1.596435	0.324817	22.73722	0.96
1.789531	0.328201	22.97407	0.97
2.075989	0.331584	23.21091	0.98
2.604656	0.334968	23.44776	0.99

Supplementary Figure 1

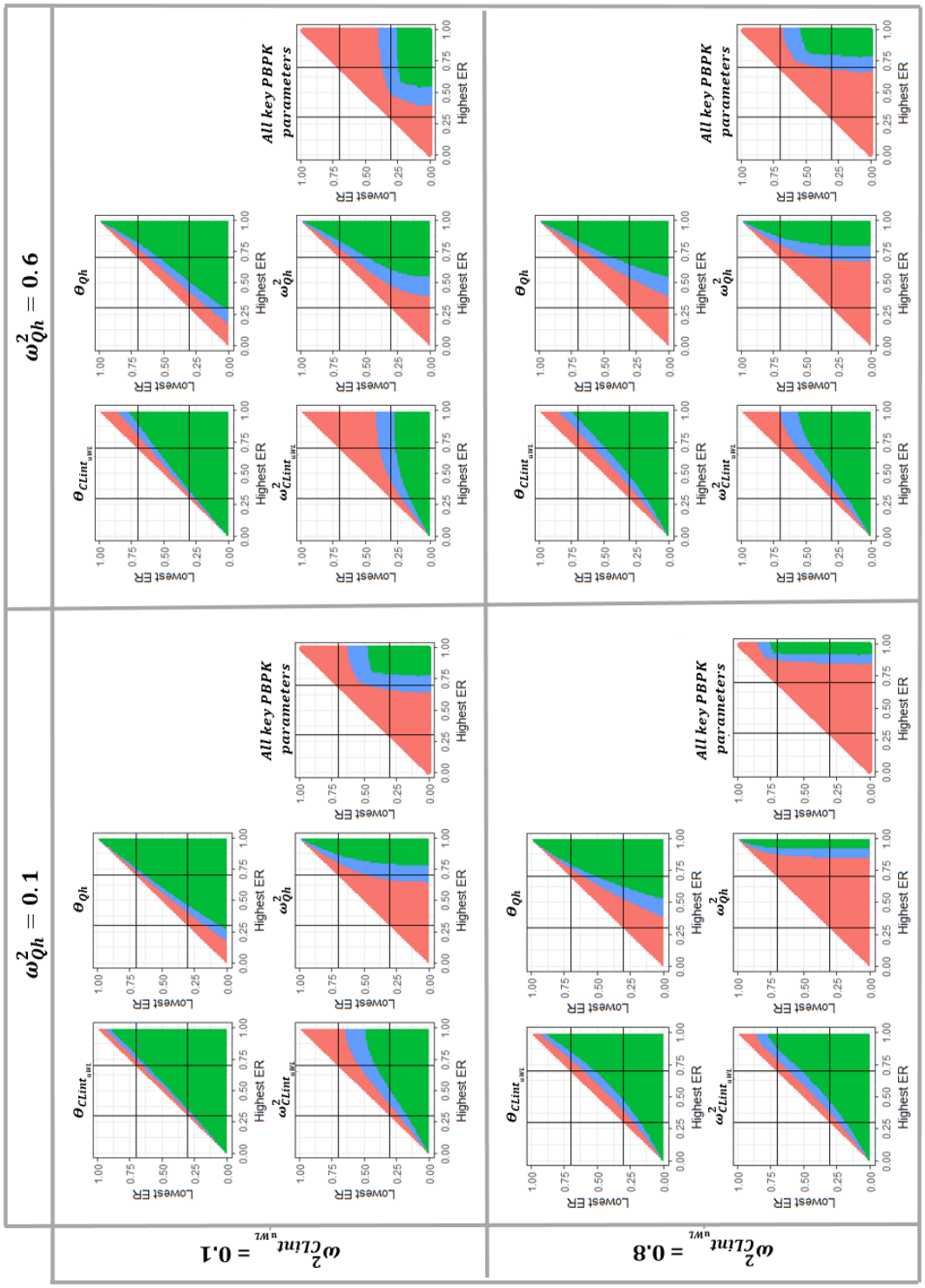
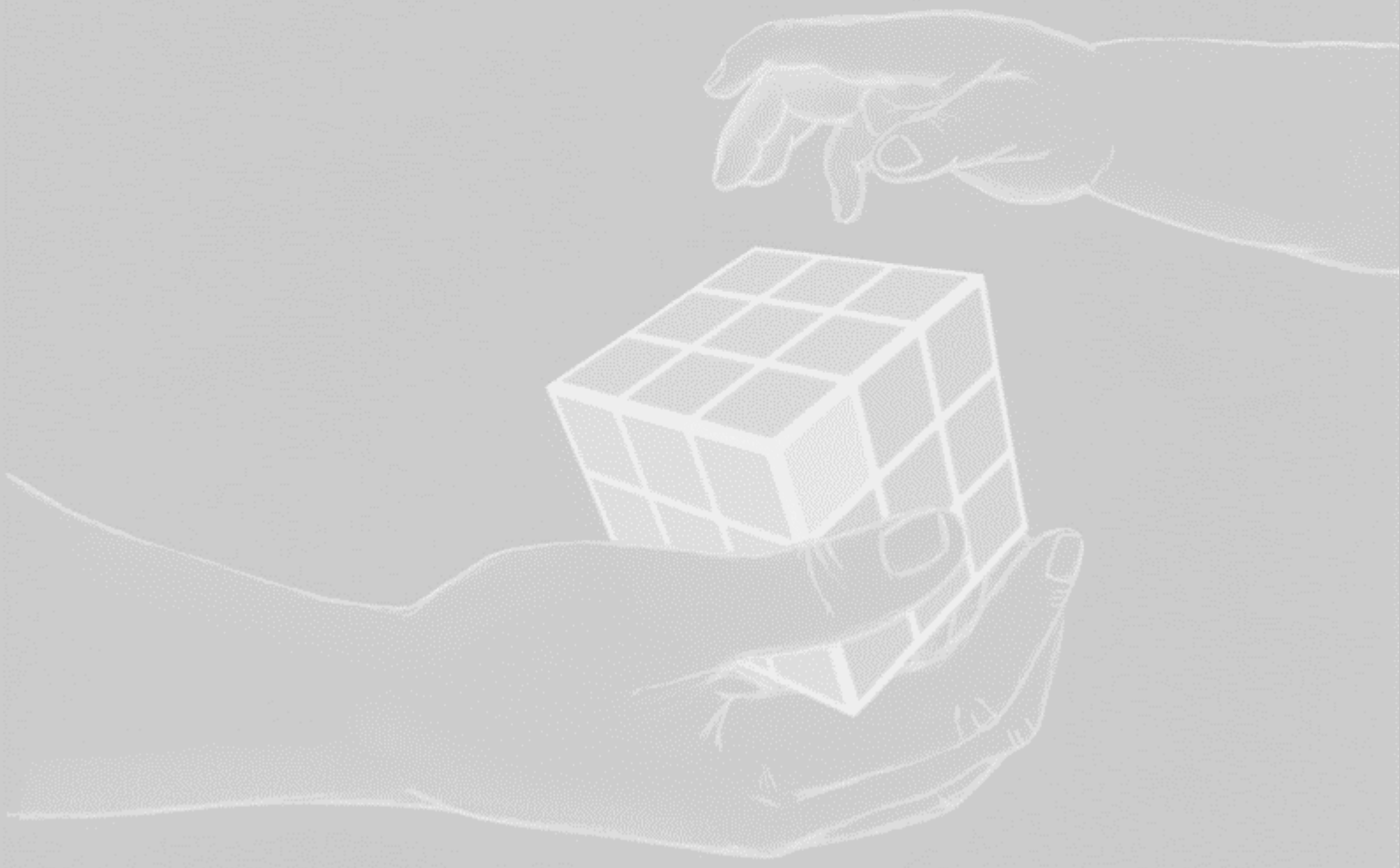


Figure S1 *Parameter precision as a function of the extraction ratios (ER) of the two drugs included in the clinical trial design, separated by variance scenario. Each pixel represents a tested drug combination, with the precision of the parameters being summarized as rse% ≤ 30 (green). 30% < rse% ≤ 50% (blue). and rse% > 50% (red).*

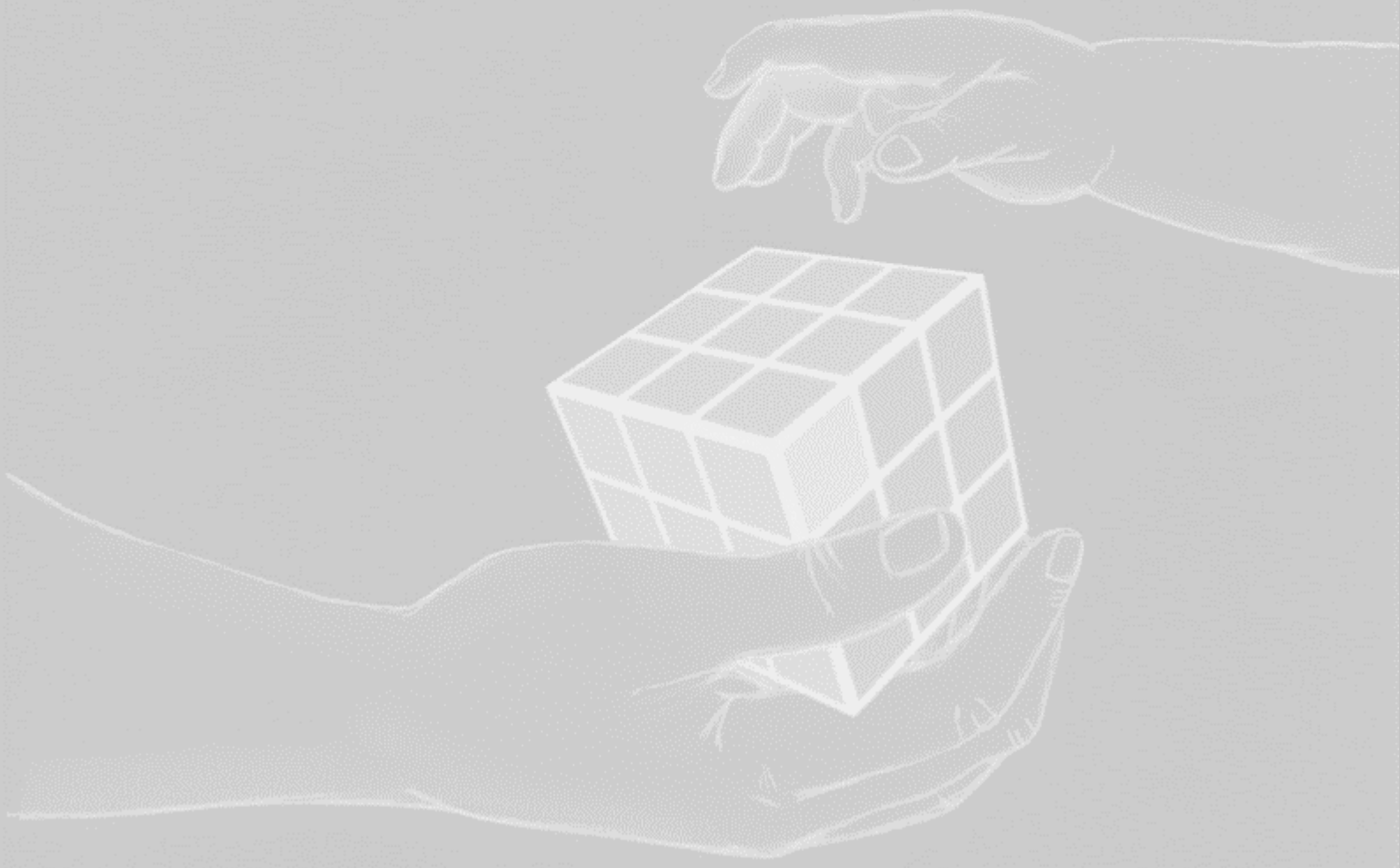
Section V

Summary, conclusions and perspectives



Chapter 8

Scaling clearance from adults to the paediatric population: Summary, conclusions and perspectives



8.1. Summary

Clearance scaling allows to derive clearance values across the paediatric age range based on adult clearance values and, ultimately, to derive paediatric dosing regimen in absence of (sufficient) paediatric clinical data. Accuracy of clearance scaling methods depends on their ability to aggregate the influence of ontogeny in diverse system-specific parameters impacting paediatric clearance for diverse drugs. Because Physiologically-Based PharmacoKinetic (PBPK) models allow to link ontogeny in system-specific parameters and drug properties with clearance ontogeny, in **section II** and **section III** of this thesis, using a new PBPK-based simulation workflow, the systematic accuracy of scaling methods was investigated across the paediatric age range for a wide range of drugs. This workflow unravelled paediatric age ranges and drug properties for which the different scaling methods efficiently aggregate important mechanistic information, thereby leading to systematically accurate paediatric clearance predictions. The investigations of **section II** and **section III** have thereby unravelled how clearance can be accurately scaled from adults to children with a minimum but necessary amount of information, with a focus on drugs undergoing hepatic metabolism, which is summarized by a paediatric clearance scaling decision tree in this concluding chapter. Moreover, we challenged beliefs about the universality of an allometric exponent to scale clearance across the paediatric age range (**chapter 3, 4** and **6**), and translated these findings into clearance ontogeny good scaling practice in this chapter.

PBPK models are essential to scale clearance of specific drugs in different paediatric ages and allow for the investigation of the accuracy of other scaling methods as illustrated in **section II** and **section III**. However, PBPK models may not be comprehensively available and/or reliable for all paediatric sub-populations, such as critically ill children, preterm neonates or obese children. In addition, these models require extensive *in vitro*, *ex vivo* and/or *in vivo* data for their development as well as a time consuming learn and confirm cycle for their validation [1,2]. In order to overcome these hurdles and ultimately develop PBPK models for understudied populations, we propose, in **section IV**, the direct estimation of PBPK parameters based on clinical PK data. Because of the challenges related to the application of this method, we developed in this section a new analysis framework based on optimal design principles, in order to investigate the feasibility and design requirements to estimate PBPK parameters based on clinical data using population PK (popPBPK) modelling.

8.1.1. Scaling methods based on bodyweight to extrapolate adult clearance to paediatric patients

In **chapter 3**, a systematic assessment of the applicability of allometric scaling on the basis of bodyweight raised to the power of 0.75 (AS0.75) was undertaken for scenarios considering size-related changes with and without maturation processes. These two scenarios were investigated since, based on strong beliefs, AS0.75 is frequently used to scale size-related changes in total (i.e., bound and unbound) plasma clearance (CLp) from adults to children and important prediction bias reported in young children with this scaling method is often attributed to the maturational processes occurring simultaneously with growth at young ages. A PBPK simulation workflow was developed in R for 12,620 hypothetical drugs. In scenario one, only size-related changes in liver weight, hepatic blood flow, and glomerular filtration were included in simulations of 'true' paediatric CLp. In a second scenario, maturation in unbound microsomal intrinsic clearance (CLint,mic), plasma protein concentration, and haematocrit were also included in these simulated 'true' paediatric CLp values. For both scenarios, the prediction error (PE) of AS0.75-based paediatric CLp predictions was assessed, while, for the first scenario, an allometric exponent was also estimated based on 'true' CLp. In the first scenario, the PE of AS0.75-based paediatric CLp predictions reached up to 278% in neonates, and the allometric exponent was estimated to range from 0.50 to 1.20 depending on age and drug properties. In the second scenario, the PE sensitivity to drug properties and maturation was highest in the youngest children, with AS0.75 resulting in accurate CLp predictions above 5 years of age. Below the age of 5 years, the PE is dependent on the drug properties and predictions are accurate for drugs with a low extraction ratio (ER) that bind to albumin and are hepatically cleared by an enzyme that is mature. For drugs that are excreted through glomerular filtration, predictions are accurate (PE within ±30% as compared to PBPK predictions) in children as young as one year of age for all drugs, except for drugs highly bound (i.e., adult unbound fraction in plasma ≤ 0.12) to α1-acid glycoprotein (AAG). Using PBPK principles, there is no evidence for one unique allometric exponent in paediatric patients, even in scenarios that consider size-related changes only. As PE is most sensitive to the allometric exponent, drug properties and maturation in the youngest children, AS0.75 leads to increasingly worse predictions with decreasing age.

In **chapter 4**, we evaluated the accuracy of linear scaling using the PBPK workflow developed in **chapter 3**, and for situations where linear scaling does not lead to accurate predictions, we compared the results with AS0.75. Currently there is renewed interest in linear

scaling because there seem to be only limited differences compared to commonly used AS0.75. We found that linear CLp scaling is accurate down to the age of 1 month for drugs undergoing glomerular filtration, except for drugs highly bound to AAG. For hepatically cleared drugs, linear scaling is reasonably accurate down the age of 2 years, except for AAG bound drugs with a low ER and isoenzyme activity similar to or higher than in adults. In neonates, linear scaling outperforms AS0.75 for drugs excreted through glomerular filtration, irrespective of whether they are bound to human serum albumin (HSA) or AAG. These results suggest that paediatric patients can in many cases be treated as small adults.

8.1.2. Scaling methods accounting for isoenzyme maturation for prediction of clearance in children

In **chapter 5**, we identified conditions for which extrapolations of population pharmacokinetic (PopPK) covariate models between drugs sharing an elimination pathway consistently lead to accurate pathway-specific CLp scaling from adults to children for drugs undergoing hepatic metabolism. This scaling approach has enabled accelerated development of paediatric models and dosing recommendations. For the purpose of this study, the PBPK simulation workflow utilizing mechanistic equations defining hepatic metabolism developed in the previous chapters was used. We found that drugs eliminated via the same pathway require similar paediatric dose adjustments only in a limited number of specific cases, depending on the ER of the drugs, unbound fraction, type of binding plasma protein, and the fraction metabolized by the isoenzyme pathway for which CLp is scaled. Overall, between-drug extrapolation of paediatric covariate functions for CLp is mostly applicable to low and intermediate ER drugs eliminated by one isoenzyme and binding to HSA in children older than 1 month.

In **chapter 6**, we evaluated currently used simple methods to scale clearance from adults to young children. To date, scaling from adults to children using allometric exponents that vary with age (age-dependent exponent (ADE)) and AS0.75 with isoenzyme maturation functions similar to those implemented in PBPK models (AS0.75+MF_{PBPK}) represent potentially valuable scaling methods, but an assessment of their applicability is lacking. The aim of this study was therefore to systematically assess the scaling accuracy of ADE and AS0.75+MF_{PBPK} in children younger than five years for drugs undergoing hepatic metabolism. This was performed using the previously developed PBPK-based simulation workflow

including hypothetical drugs with a wide range of properties and metabolized by different isoenzymes. In this platform, the impact of drug properties and isoenzyme maturation on clearance maturation and the accuracy of each scaling method was investigated to define scenarios for which these methods systematically lead to accurate paediatric CLp scaling. The drug properties that were most relevant in predicting the systematic accuracy of the scaling methods were the ER in adults and the type of plasma proteins bound (HSA or AAG). We found that these drug properties and isoenzyme maturation highly impact clearance maturation in all children younger than five years, leading to a wide range of paediatric clearance values, which ultimately prevents the systematic accuracy of scaling methods solely accounting for age and bodyweight such as ADE. In contrast, paediatric clearance for all low and intermediate ER drugs can be accurately scaled using AS0.75+MF_{PBPK} except for drugs binding to AAG in neonates. For other drugs, no simple scaling method is yet accurate.

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

In **chapter 7**, we developed a methodology to investigate the feasibility and requirements for precise and accurate estimation of PBPK parameters using population modelling of clinical data and illustrate this for two key PBPK parameters for hepatic metabolic clearance, namely whole liver unbound intrinsic clearance ($CLint_{u,WL}$) and hepatic blood flow (Qh) in children. Estimation of PBPK parameters using clinical data is highly relevant, since the parameters that are required for PBPK models that are essential in drug development, cannot always be directly measured. First, structural identifiability was enabled through re-parametrization and the definition of essential trial design components. Subsequently, requirements for the trial components to yield precise estimation of the PBPK parameters and their inter-individual variability were established using a novel application of population optimal design theory. Finally, the performance of the proposed trial design was assessed using stochastic simulation and estimation. The results show that precise estimation of $CLint_{u,WL}$ and Qh and their inter-individual variability requires a trial with two drugs, of which one has an ER ≤ 0.27 and the other has an ER ≥ 0.93. The proposed clinical trial design was found to lead to precise and accurate parameter estimates and was robust to parameter uncertainty. The proposed framework can be applied to other PBPK parameters and facilitate the development of PBPK models.

8.2. Conclusions and perspectives

8.2.1. Clearance scaling from adults to children: a decision tree

As described in **chapter 1**, accurate clearance scaling from adults to children is key to improve and expedite paediatric dose tailoring. Although PBPK models represent the most accurate scaling method available to date, their use requires specific expertise and data from various and complex *in vitro* or *in vivo* experiments, leading to a need for simpler but accurate scaling methods. Through **section II** and **section III**, we developed and applied a PBPK-based simulation workflow in order to define scenarios for which different simple scaling methods are systematically accurate. In **section II**, we investigated two scaling methods that solely use bodyweight as scaling variable, namely AS0.75 and linear scaling. In **section III**, we investigated scaling methods relying on more information, accounting for isoenzyme ontogeny and demographic measurements (i.e., bodyweight alone or together with age). These methods require the knowledge of not only demographic values of the population for which the predictions are performed, but also of the ontogeny of the eliminating enzymes of the investigated drug and the fraction metabolized in adults by each of the isoenzymes involved in drug clearance. Based on the applicability of these different investigated scaling methods, a clearance scaling decision tree is proposed in Figure 1, which allows for the first time (clinical) pharmacologists to select scaling method(s) that require a minimum but sufficient amount of information to accurately scale clearance according to the paediatric age range and properties of the drugs under investigation.

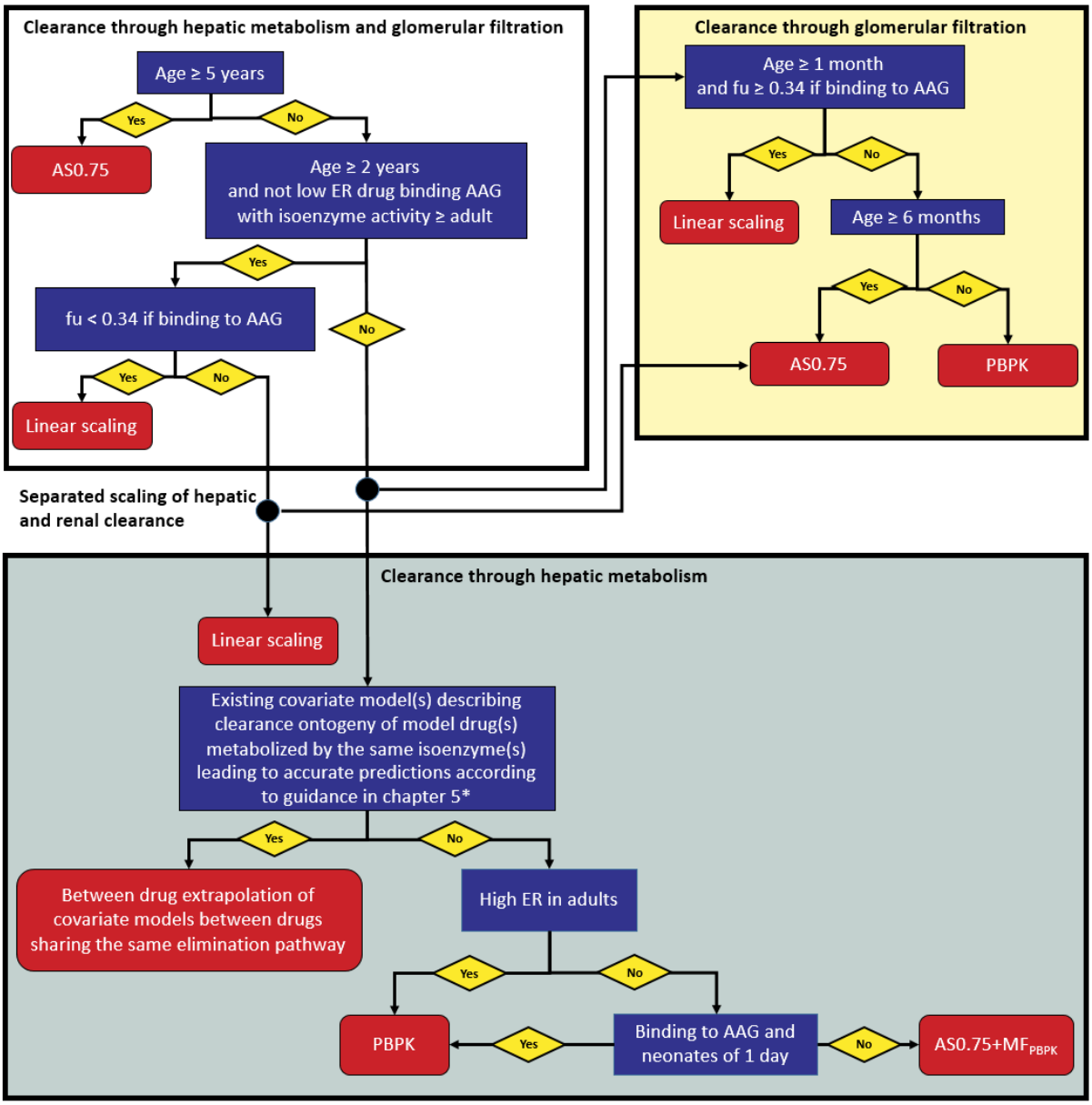


Figure 1 *Paediatric clearance scaling decision tree based on elimination pathway, drug properties and age. The white block indicates how to scale total drug clearance for drugs eliminated by hepatic metabolism and glomerular filtration. The yellow and grey blocks indicate how to scale either clearance due to glomerular filtration or due to hepatic metabolism respectively and need to be combined in case the situation of the white block does not apply. AS0.75; allometric scaling on the basis of bodyweight to the power of 0.75, PBPK: physiologically-based pharmacokinetic modelling, ER: extraction ratio in adults, AS0.75+MF$_{PBPK}$: fixed allometric exponent of 0.75 combined with functions similar to those implemented in physiologically-based pharmacokinetic (PBPK) models accounting for both isoenzyme and MPPGL maturation (**chapter 6**), AAG: α1-acid glycoprotein, fu: adult unbound drug fraction in plasma. * **Chapter 5** shows that between-drug extrapolation of paediatric covariate functions using this approach is mostly applicable to low and intermediate extraction ratio drugs eliminated by one isoenzyme and binding to human serum albumin in children older than 1 month, because its use is restricted by the degree of similarity of the fu and ER between the model and test drug, as well as the fraction of the model and test drug metabolized by the different isoenzymes. Supplementary material 2 and 3 of **chapter 5** presents model-test drug scenarios that for drugs binding to human serum albumin or the alpha-1 glycoprotein respectively, lead to accurate pathway specific plasma clearance predictions for a test drug after between-drug extrapolation of a pathway specific paediatric covariate function.*

The paediatric scaling decision tree in Figure 1 is composed of three blocks that contain scaling methods (red rectangles) and requirements for their accuracy (blue rectangles), each block corresponding to different drug elimination pathways that can be scaled with reasonable accuracy (±50% prediction error as compared to PBPK-based simulations) by the methods they contain. The use of scaling methods outside the defined requirements in the decision tree does not mean that scaled CLp values will be inaccurate, but that accuracy cannot *a priori* be guaranteed.

The white block indicates how to scale total clearance of drugs eliminated by hepatic metabolism and glomerular filtration. The yellow and grey blocks indicate how to scale either clearance due to glomerular filtration or due to hepatic metabolism respectively. For drugs undergoing both hepatic metabolism and glomerular filtration when requirements for total clearance scaling using a single method (white block) are not met, separate scaling of hepatic and renal clearance is required (grey and yellow blocks).

The different requirements for scaling accuracy in the decision tree are based on the paediatric age range for the predictions, the isoenzyme activity compared to adults (maturation), whether the drug binds to AAG, the adult unbound drug fraction in plasma (fu), and the adult ER.

Isoenzyme activity corresponds to the unbound intrinsic clearance per gram of microsomes expressed as percentage of adult values. While AAG, a plasma protein to which drugs can bind, can limit the applicability of the different scaling methods, the decision tree is also applicable to drugs not binding to plasma protein and to drugs binding to HSA. The fu and ER in the decision tree are respectively the unbound drug fraction in plasma and the extraction ratio, as defined in adults since the value of these parameters can change with age and is often solely known in adults. The unbound drug fraction in plasma can undergo elimination unlike the fraction bound to plasma proteins. ER reflects to which extent drug clearance is driven by hepatic blood flow and intrinsic clearance, with clearance of low ER drugs (ER $\leq$ 0.3) mainly driven by intrinsic clearance, clearance of high ER drugs (ER > 0.7) mainly driven by hepatic blood flow, and clearance of intermediate ER drugs (ER between 0.3 and 0.7) driven by both hepatic blood flow and intrinsic clearance. For extrapolation of covariate models between drugs sharing the same elimination pathway, **chapter 5** provides guidance on the requirements for accurate scaling with this method, which is based on a combination of the aforementioned drug properties (i.e., the ER of the model drug, the type of plasma protein bound (either HSA or AAG) and the difference in ER and fu between the model

drug and the test drug) and additionally also based on the isoenzyme pathways involved in the drug clearance (e.g., SULT1A1 and CYP1A2) as well as the fraction of the metabolic clearance due to each of these isoenzyme pathways.

As an example, to scale CLp from adults down to children as young as 1 month for a drug that has a low ER in adults, binds to AAG with an fu of 0.6 and undergoes both hepatic metabolism and glomerular filtration, AS0.75 can be used to scale total clearance down 5 years of age (white block). For younger children, metabolic clearance (the fraction of the total drug clearance due to hepatic metabolism) and glomerular filtration are separately scaled and added up in order to scale total CLp. Hepatic clearance (grey block), can be scaled using AS0.75+MF_{PBPK} or between drug extrapolation of covariate models provided the guidance of **chapter 5** *a priori* predicts accurate CLp prediction for that drug. The part of drug clearance due to glomerular filtration for that drug can be scaled using linear scaling (yellow block).

As shown in Figure 1, for drugs undergoing glomerular filtration (yellow block), linear scaling can be used in children as young as one month of age, except for drugs that are highly bound to AAG, with an fu in adults < 0.34. For these drugs highly bound to AAG, AS0.75 can be used but only in children no younger than 6 months of age.

Disregarding the drug properties and the routes of drug elimination (white block), CLp can be accurately scaled from adults to children of 5 years and older using AS0.75, which solely requires information on bodyweight. For hepatically cleared drugs, linear scaling is reasonably accurate down the age of 2 years, except for AAG bound drugs with a low ER and isoenzyme activity similar or superior to adult values (white block) and, if the drug also undergoes glomerular filtration, except for drugs highly bound to AAG for which separate scaling of hepatic (linear scaling in grey block) and renal clearance (AS0.75 in yellow block) is required.

For CLp scaling to younger children involving drugs undergoing hepatic metabolism the use of scaling methods accounting for isoenzyme ontogeny such as extrapolation of covariate models between drugs sharing the same elimination pathway (**chapter 5**) or AS0.75+MF_{PBPK} (**chapter 6**) is required (Figure 1, grey block). Indeed, the wide difference in maturation patterns between different isoenzymes in those very young ages leads to a wide variation in CLp ontogeny between drugs, which cannot be solely described based on demographic information, as found in **chapter 6**. However, the wide variation in clearance ontogeny across different drugs in young children is not solely due to isoenzyme maturation,

but also to other variables underlying drug clearance, impacting plasma clearance maturation in a drug-specific manner, such as the ER, type of plasma protein bound and maturation of its concentration. Therefore, the applicability of scaling methods accounting for isoenzyme maturation (i.e., AS0.75+MF$_{PBPK}$ in **chapter 6** and between drug extrapolation in **chapter 5**) is also dependent on the drug properties, and more specifically on the drug ER and type of plasma protein the drug binds to. As a result, the accuracy of these methods is mostly limited to low and intermediate ER drugs since clearance ontogeny of these drugs is mostly impacted by isoenzyme maturation. Additionally, for drugs binding to AAG in neonates, the steep maturation of AAG leads to a high impact of fu on clearance ontogeny, preventing the systematic accuracy or limit the applicability of these methods for these drugs.

To scale CLp using between drug extrapolation of covariate models, CLp through each isoenzyme pathway of the drug (test drug) is scaled from adults to children using a covariate model that describes CLp ontogeny of another drug (model drug) due to the respective isoenzyme pathway (**chapter 5**). This method is illustrated in Figure 2 for model and test drugs eliminated by one isoenzyme.

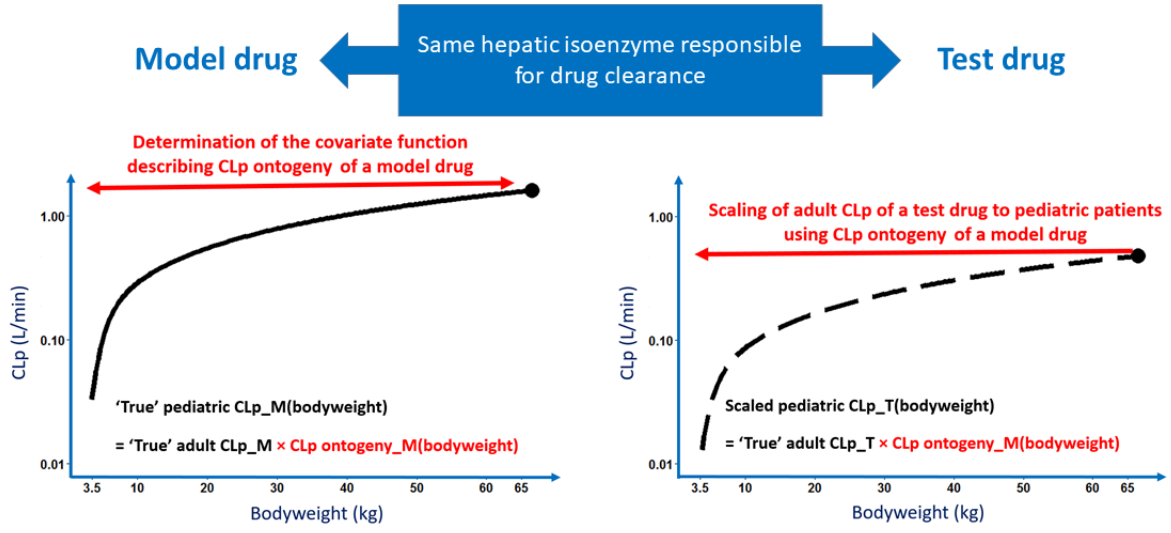


Figure 2 *Illustration of between-drug extrapolation of paediatric covariate functions to scale hepatic plasma clearance (CLp) from adults to paediatric patients for drugs eliminated by the same isoenzyme. The black dot in both graphs shows the adult hepatic CLp value for the model drug ('true' adult CLp_M) and the test drug ('true' adult CLp_T). The solid black line represents the change in CLp of the model drug throughout the paediatric age range, which is described by a paediatric covariate function based, in this example, on bodyweight (CLp ontogeny_M (bodyweight)). The dashed black line represents the scaled paediatric CLp predicted for the test drug by between-drug extrapolation of the paediatric covariate function obtained for the model drug (CLp ontogeny_M (bodyweight)). Figure taken from* ***chapter 5*** [3].

With this method, the scaling functions (i.e., covariate functions describing plasma clearance ontogeny of the model drugs) aggregate information not only on the ontogeny of the scaled isoenzyme pathways but also on drug properties and, in case of elimination through multiple pathways, on the ontogeny of other isoenzyme pathways responsible for the model drug elimination. Therefore, the applicability of this method is restricted by the degree of similarity of the fu and ER between the model and test drug, as well as the fraction of the model and test drug metabolized by the different isoenzymes. This method is mostly applicable to low and intermediate ER drugs that are mainly metabolized by one isoenzyme and binding to HSA in children older than one month. However, unlike AS0.75+MF_{PBPK}, this method can be applied to predict clearance of high ER drugs and drugs binding to AAG in neonates of one day, albeit in very specific cases. Scenarios where this method leads to accurate CLp predictions (defined as ±30% prediction error compared to PBPK simulations) can be found in supplementary materials 2 and 3 of **chapter 5**. An illustration of the use of this framework to '*predict*' whether between-drug extrapolation is possible to '*scale*' CLp for commonly used CYP3A substrates in children has been provided [submitted for publication [4]]. In this work, a maturation function derived from midazolam (model drug) was used to scale clearance across the entire paediatric age range or parts of this range for diverse CYP3A substrates (test drugs) for which the framework predicted accurate CLp scaling based on the ER, fu and the fraction of the drugs metabolized by CYP3A of both the model drug and test drugs. Alternatively, AS0.75+MF_{PBPK} (**chapter 6**) can be used for low (PE within ±30% compared to PBPK predictions) and intermediate ER drugs (PE within ±50% compared to PBPK predictions), except for drugs binding to AAG in neonates of one day. With this method, the maturation function(s), which are similar to those implemented in PBPK models, should account for isoenzyme maturation per gram of liver (e.g., both maturation in isoenzymes in microsomes and maturation in microsomal protein per gram of liver or MPPGL) for each isoenzyme involved in drug clearance. Indeed, isoenzyme maturation functions that do not take MPPGL maturation into account was found to lead to important prediction bias (**chapter 6**) and therefore should not be used when scaling clearance with AS0.75+MF_{PBPK}.

For drugs that have a high ER in adults, and for drugs binding to AAG in neonates of one day, either accurate scaling using extrapolation of covariate models between drugs sharing the same elimination pathway can be ensured *a priori* (see **chapter 5**), or no simple scaling method will be systematically accurate, and, as a result, clearance should be predicted using PBPK modelling approaches. It is emphasized here that, in young children, clearance of drugs

that have a high ER in adults, might not solely reflect hepatic blood flow like in adults, but also reflect isoenzyme activity. This is due to the change in ER in young children with isoenzyme maturation [5] that is often overlooked. Therefore, clearance ontogeny of these drugs reflects hepatic blood flow and isoenzyme activity to a different extent, depending on the isoenzyme maturation (with age) and the ER of these drugs in adults, ultimately leading to a wide variation in clearance ontogeny across drugs that have a high ER in adults (**chapter 6**). Because this shift in the contribution of hepatic blood flow to hepatic clearance is not accounted for in any of the scaling methods that are based on bodyweight and/or age only, even when accounting for isoenzyme ontogeny, their accuracy is hindered for these drugs.

8.2.2. The paediatric clearance scaling decision tree in practice

By unravelling the minimum amount of information required for accurate scaling of clearance from adults to children of various ages, the paediatric decision tree (Figure 1) enables the selection of the simplest scaling methods that can be used instead of PBPK models, thereby decreasing computational efforts and required information collection. Scaling methods selected based on this decision tree can be used in order to optimize paediatric clinical trials through the definition of the paediatric dosing regimen(s), informative sampling times [6], and inclusion criteria based on the scaling variables [7], to ensure accurate and precise PK parameter estimates. Additionally, these scaling methods can be implemented as priors during population PK analysis in order to inform the analysis of underpowered PK studies, such as for some specific diseases for which only small numbers of paediatric patients can be enrolled.

In children of 5 years and older, only adult clearance values and bodyweight of the paediatric patients are needed for accurate clearance scaling using AS0.75, with an absolute prediction error of less than 50%, and when isoenzymes are mature, of less than 30%. This information might also be sufficient to scale clearance with reasonable accuracy (PE within ±50%) in children as young as 2 years or one month of age using linear scaling for drugs undergoing hepatic metabolism or glomerular filtration respectively. However, this will require knowledge of the type of plasma protein bound and, for drugs undergoing hepatic metabolism binding to AAG, of the ER, fu and the maturity of the isoenzymes involved in drug clearance, since applicability of linear scaling depends on these parameters in children between 2 and 5 years of age (Figure 1). Similar for drugs undergoing glomerular filtration, linear scaling for instance only applies down to one month of age if the drug does not bind to

AAG with an fu ≥ 0.34. For hepatically cleared drugs which are the main focus of this thesis, in younger children, while the proposed scaling methods require less information on drug- and system-specific properties compared to PBPK models, they require, similarly as for PBPK models, accurate estimates of the fraction of the drug metabolized in adults and the availability of maturation functions or covariate models for each isoenzymes involved in the drug clearance (see **chapter 5** and **chapter 6**).

The fraction of drug metabolized by diverse isoenzymes is often available from adult ADME (Absorption Distribution Metabolism Excretion) studies, but might not be available for old drugs due for instance to lack of specificity, sensitivity and efficiency of quantitative analytical methods [8,9], a lack of method selectivity for metabolic route qualification [10,11] or unawareness of pharmacokinetic differences between enantiomers (stereo selectivity in drug metabolism) [12,13]. In case this information is missing, it could be collected in children older than 5 years, for which accurate scaling solely requires bodyweight measurements, and then used to support accurate scaling of clearance in younger children. This necessitates the conduct of a mass balance study, which may require the collection of urines, faeces in addition to blood samples. However, this type of study is feasible in children of that age.

When information on isoenzyme maturation for part of the isoenzymes involved in drug clearance is missing, the lowest reported isoenzyme maturation value in the different age groups could be used instead, which would favour under-predictions over potential over-predictions of clearance, thereby reducing risk of adverse events.

For drugs undergoing both hepatic metabolic clearance and glomerular filtration, in young children each route of elimination can be scaled using the paediatric decision tree (i.e., combination of information in the grey block with information in the yellow block), since glomerular filtration does not impact hepatic metabolic clearance nor its ontogeny. For these drugs, additional information on the fraction of the drug eliminated through glomerular filtration in adults is required so as to separately scale hepatic metabolic clearance and renal clearance.

For drugs that are transporter substrates, bind to lipoproteins and for situations where clearance ontogeny might be impacted by disease or drug effect, the paediatric decision tree might not hold true, since these situations were not considered in this thesis due to a lack of available information to implement in PBPK models. In those cases, valuable alternatives could be microdosing studies [14,15], or adaptive design studies [16] with first a sub-therapeutic

dose. This first dose can be predicted taking the minimum dose predicted using PBPK models with inferences on the processes for which data are currently lacking (e.g., transporter ontogeny, impact of disease on physiological parameters, etc) and applying a safety factor as performed in adults [17].

8.2.3. The paediatric clearance scaling decision tree: future development

Although the paediatric decision tree allows for the identification of clearance scaling methods for a wide range of drugs, further investigations are needed for paediatric sub-populations and drugs subject to or driving processes potentially impacting clearance that were not investigated in this thesis. In many cases, this would first require the quantification of these processes and/or of system-specific parameters of these populations before they can be implemented in PBPK models, since they are currently poorly characterized. This is for instance the case of preterm neonates [18], for which drug clearance is known to potentially differ from term neonates, drugs that are transporter substrate [19] and renally cleared drugs undergoing active tubular absorption and/or secretion. Once sufficient data (e.g., on transporters abundance in the liver, in the renal tubules or reabsorption) across the paediatric age range has been collected, implemented and validated in PBPK models, the impact of these processes could be investigated using a PBPK simulation workflow similarly as in **section II** and **III**.

For some diseases and/or drugs, physiological parameters which drive drug clearance and/or relationship between demographic values and clearance ontogeny can be altered, either directly or through drug-drug interaction. For instance, plasma protein binding can be reduced due to uraemia, hypoalbuminemia or drug-drug interaction, which can significantly alter clearance of drugs with high protein binding and low ER or undergoing glomerular filtration [20–22]. Another example is obese children, for which clearance ontogeny as well as its relationship with total bodyweight are likely to differ from patients with normal weight [23,24]. Therefore, the relevant system-specific parameters for these paediatric sub-populations that have already been characterized could be implemented in the PBPK simulation workflows developed in this thesis in order to assess whether and how the decision tree in Figure 1 should be adapted.

Mechanistic characterization of some of the scenarios that were not investigated in this thesis has already been recognized as important and is currently under research, such as for

active drug transporters [25–27], pre-term neonates [18] and obese children [23,24]. However, the relevance of the mechanistic characterization for other scenarios remains unclear. Discrepancies between CLp ontogeny as quantified based on paediatric clinical data and the scaled CLp using the relevant method defined in the decision tree could help to pinpoint specific scenarios for which such investigations are highly relevant.

While accurate scaling of clearance from adults to paediatric patients is of utmost importance to define first-in-child doses and plan informative sampling times and patient recruitment for paediatric clinical trials, accurate prediction of the drug absorption rate and bioavailability can also be crucial when the drug is administered through extra vascular routes. Ontogeny in drug bioavailability will impact the dose to be administered to reach the target exposure in paediatric patients. In case drug peak concentrations drive drug toxicity and/or efficacy and in case of slower absorption rate than elimination rate, the ontogeny in the drug absorption rate should be accurately predicted to ensure efficacious and safe dose tailoring. This also applies to estimation of volume of distribution in children which may be of relevance for instance for drugs given intravenously and where peak concentrations determine the effect like anaesthetics or muscle relaxants and for drugs with a prolonged half-life in order to determine a loading dose.

8.2.4. The use of popPBPK modelling to extend predictions to subpopulations

PBPK models are instrumental in understanding and predicting PK of diverse drugs but require for their development extensive data collection of *in vitro*, *ex vivo* and/or *in vivo* measurements, which are not always accurate, or hypotheses on system-specific parameters that are not directly measurable, such as hepatic blood flow in paediatric sub-populations such as (preterm) neonates. Therefore, time consuming iterative learn and confirm cycles are needed for the validation of these models. Direct estimation of PBPK parameters based on clinical data has the potential to overcome some of these problems and may represent a valuable method to expedite the development of PBPK models for different understudied sub-populations, such as preterm neonates or obese children and adults, or understudied PK processes, such as first pass metabolism. Moreover, this method has been found superior to *in vitro* estimation. For instance it has been shown that isoenzyme maturation functions developed based on clinical data perform markedly better than those developed based on *in vitro* data [28,29]. Since system-specific parameters are often structurally non-identifiable, that is

to say not all parameters can be estimated from the available data, currently some of the model parameters are fixed to measured values, usually those that can be accurately and directly measured. This is however problematic when the parameters that should be fixed cannot be accurately measured, as bias in their measurement is likely to lead to bias in the estimated parameter(s). Alternatively, one can develop (semi-)physiological covariate models for model drugs that describe clearance ontogeny via a specific pathway, thereby characterizing the overall influence of system- and drug- specific parameters on the process described. Such work has been performed for the characterisation of first-pass metabolism in preterm neonates [30] or clearance ontogeny in obese patients driven by CYP3A using CYP3A probe drugs [31]. Whereas this method allows to accurately predict processes of other drugs undergoing the same processes, we found in **chapter 5** that it can be limited to situations where some drug properties of the model drug (drug for which the process has been described) and the test drug (drug for which predictions are performed) are similar.

Therefore, new methodologies are needed to overcome structural identifiability problems of PBPK models in order to allow for the accurate and precise estimation of all system specific parameters. Indeed, being able to identify PBPK parameters that cannot be obtained by direct experimental measurements in a time and cost-efficient manner would greatly improve and expedite the development of PBPK models and their predictive performance, without putting too great a burden on the population involved. To this end, we proposed in **chapter 7** of this thesis a new methodology to investigate the feasibility and requirements for precise and accurate estimation of PBPK parameters using population modelling of clinical data and illustrate this for two key PBPK parameters for hepatic metabolic clearance, namely whole liver unbound intrinsic clearance and hepatic blood flow in children. In this chapter, the use of clinical data of different drugs with specific properties enables the identification of parameters that are unidentifiable based on data of one drug. Moreover, using popPK for parameter estimation enables the estimation of the parameter value in the studied population as well as its variability. The results show that trial requirements to perform such analysis are difficult to meet, with a high number of patients and drugs with very specific properties needed. This highlights the importance of assessing the clinical trial design requirements before conducting clinical trials or gathering information for a meta-analysis when aiming at estimating PBPK parameters based on clinical data. Defining *a priori* clinical trial requirements enables to assess the feasibility of the analysis and, when possible, to only select drugs whose PK profile is informative enough for such studies.

Although in **chapter 7** the proposed method is illustrated with the estimation of hepatic blood flow and whole blood intrinsic clearance in paediatric patients and adults, it could be used to estimate the same or other key PBPK parameters in different sub-populations, such as critically ill or obese patients of various ages or even in different species. To apply this method to a different population (or specie), similar as in the example given in **chapter 7**, a range of likely values for each of the system specific parameters of the PBPK model that are to be estimated should be tested. For the application of this method to a different set of parameters, structural identifiability based on the simultaneous analysis of different drug should be ensured, which might require model re-parametrization, as illustrated in **chapter 7**, and/or additional clinical samples. This could be the case for instance of PBPK parameters describing first pass metabolism which is responsible for drug metabolism in the gastro-intestinal tract before the drug can reach the blood stream. Particularly in specific populations, it may be of relevance to understand the changes in first pass metabolism of drugs [32]. An often used PBPK model for first pass metabolism is the Qgut model, which is parametrized with both enterocytic blood flow and permeability clearance [33]. To allow for structural identifiability of these parameters, reparameterization can be done as described in **chapter 7**, but additionally, both iv and oral data of the parent compound and metabolites would be required. Indeed, while in **chapter 7** hepatic clearance is identifiable based on clinical data of drugs administered through a unique route (provided there is no flip flop phenomenon), first-pass metabolism requires the above-mentioned PK data to ensure its identifiability, which would require a cross over study or micro tracer study design.

As found in **chapter 7**, PBPK parameters that are unidentifiable based on the data of one drug can be simultaneously estimated based on clinical PK data of diverse specific drugs and a number of patients that is higher than that of classical non-mechanistic population PK analysis studies. Because of these specific requirements, the use of the analysis framework proposed in this chapter or similar approach, is crucial in order to identify *a priori* the feasibility and the requirements of clinical trials for such an approach, thereby guiding data collection for meta-analysis and/or the design of new clinical trials. Meeting these requirements will likely involve data sharing, which might be a major drawback of this approach given the current efforts that need to be made before data can actually be shared. In light of the need of clinical data from diverse drugs to improve PK predictions for first-in-child doses, and other understudied populations and improve the understanding of mechanisms impacting drug PK, failure in sharing data appears unethical, which questions current

regulations on data ownership. Currently, the importance of data sharing is more and more recognized, and data sharing projects have been developed in order to improve mechanistic understanding and quantitative modelling of drugs PK [34]. Examples of data sharing projects are the OrBiTo IMI project that is designed to improve the understanding and modelling of how drugs are absorbed [35] or the mechanism-based PK-PD modelling platform of TI pharma which aimed at the development of novel mechanism-based PK-PD modelling concepts for stationary and non-stationary biological systems [36].

8.2.5. Good modelling practice for the description of clearance ontogeny

In case data of children of different ages are available for analysis, the simplest covariate model, which solely relies on bodyweight as covariate, is allometric scaling with an estimated allometric exponent. Unlike theoretical allometry which aims at only accounting for size-related changes in clearance, the allometric equation with estimation of the exponent captures overall clearance ontogeny, accounting for both size- and age-related changes since bodyweight and age are highly correlated in children. Often, the estimated allometric exponent is confused with the theory-based exponent which only aims at scaling size-related changes in clearance, and values differing from 0.75 are misinterpreted as erroneous and as allegedly due to bias in the estimate of the allometric exponent arising from unpowered studies. For instance, it has been erroneously claimed that the confidence interval of the allometric exponent estimate should always include the value of 0.75 [37]. In **chapter 3**, we showed that the use of an exponent of 0.75 (AS0.75, the claimed theoretical allometric exponent) in order to predict overall clearance ontogeny can lead to highly biased predictions in children younger than 5 years (scenario 2 of **chapter 3**). In **chapter 6**, we found that values of the estimated exponent (accounting for both size- and age-related changes) can widely differ from 0.75 in children younger than 5 years, ranging from 0.57 to 2.06 for drugs undergoing hepatic metabolism, and varies with age, drug properties and the elimination pathway. Values found in that range are realistic, since these values were derived from PBPK knowledge and a similar range in the allometric exponent have been reported in popPK data analyses [38–41]. This wide range of estimated exponents reported in literature does not arise from estimation bias, as recently shown by Sinha *et al.* [42]. In this paper, the authors showed that 20 patients (from 3 years old up to adults), which is less than the number included in typical clinical studies, is sufficient to reach a study power of 80% or more, which is the power required for unbiased estimates of

the allometric exponent, even in case of high PK variability. Moreover, other values outside the range found in **chapter 6** could be expected when adult data is not included, since the allometric exponent depends on the age range studied, or in situations that have not been studied yet (e.g., drug transport).

Therefore, in order to estimate paediatric clearance in children younger than 5 years, in our opinion the allometric exponent should be estimated and not fixed to 0.75, and values differing from 0.75 should not *a priori* be interpreted as clinically implausible. In cases where the number of patients is small (< 20), which could for instance be the case for rare diseases, the study power to obtain an unbiased estimate of the allometric exponent is likely to be smaller than 80% and bias in the estimate might arise. In such situation one should consider fixing the covariate model parameters describing clearance ontogeny. If the allometric exponent estimate is however uncertain, then prior knowledge of clearance ontogeny would be required. While using a fixed allometric exponent of 0.75 is a reasonable approach for children of 5 years and older, as shown in **chapter 3** of this thesis, other scaling functions, e.g., linear scaling, should be selected in younger children, for which the paediatric clearance scaling decision tree can be used. This does not mean that AS0.75 cannot be accurate in children younger than 5 years, but it reflects that accuracy cannot be guaranteed in that age range since AS0.75 can lead to important bias in clearance predictions depending on the age and drug properties.

8.2.5.1. Assessing the bias and precision of the allometric exponent

The assessment of the bias and precision of the allometric exponent estimate is often poorly performed. Difference in sensitivity of the accuracy of clearance ontogeny predictions to the bias in allometric exponent has been shown in **chapter 3**. Indeed, in this chapter, we found that the younger the child, the more clearance predictions are sensitive to the bias in the bodyweight-based exponent. This means that the same difference between the true allometric exponent and the chosen fixed allometric exponent used to scale clearance can lead to a variety of differences in clearance prediction bias in different ages. This bias increases with decreasing age, which is further illustrated in Figure 3, where the changes in bias of the allometric exponent (exponent used for the predictions minus true exponent) with bodyweight (BW) leading to ±30% or ±50% prediction error in clearance (CLp PE) is displayed for scenarios where clearance is scaled from adult values and a median adult bodyweight of 70kg (see

equation 1). This figure shows that for younger children (lower bodyweights), to reach the same clearance prediction bias as in older children, the bias in allometric exponent should be much lower.

$$\text{Exponent bias (prediction – true)} = \frac{\log(\text{CLp PE} + 1)}{\log(\frac{\text{BW}}{70})} \quad (1)$$

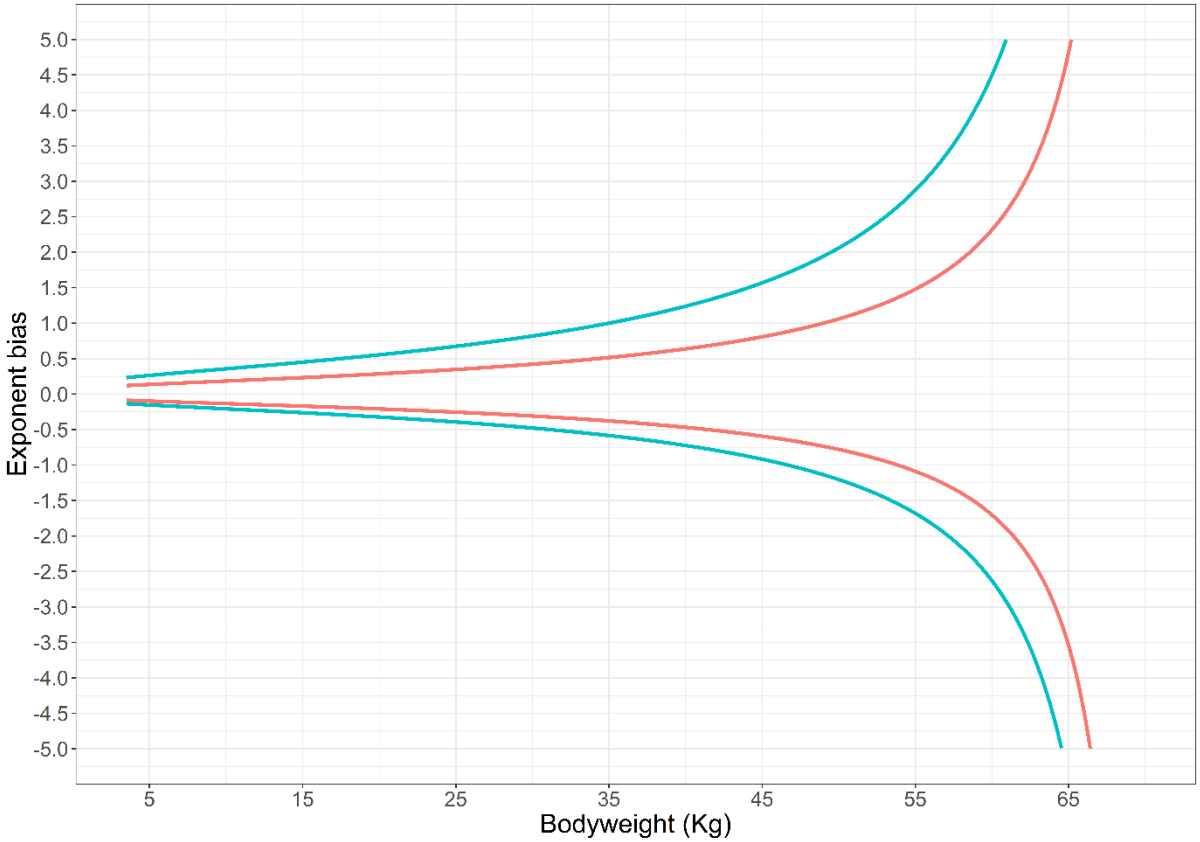


Figure 3 *Exponent bias (equation 1) leading to ±30% (red) or to ±50% (green) clearance prediction error versus bodyweight*

Since the aim of estimating the allometric exponent during clinical PK data analysis is not to estimate the exact true exponent, but to estimate an exponent that allows for accurate clearance predictions, bias in the allometric exponent should be assessed based on its impact on clearance accuracy. However, often the bias or uncertainty in the allometric exponent estimate is assessed based on fixed criteria, such as an accepted percentage of parameter bias or uncertainty. This is an important aspect that needs attention when finalising a (covariate) model together with standard model evaluation methods [43].

Although the allometric exponent was found to vary with age, drug properties and isoenzyme maturation in our PBPK-based simulation platform (**chapter 6**), this does not mean that a single allometric exponent cannot describe clearance ontogeny for a specific drug across a specific paediatric age range. Indeed, as shown in Figure 3, in the oldest children, clearance predictions are not sensitive to bias in the allometric exponent, and therefore one single exponent can accurately scale clearance across that paediatric age range. This also can be the case in younger children when changes in clearance with bodyweight follows the allometric equation, which is the case when the combination of the drug properties and maturation of isoenzymes involved in drug clearance leads to similar allometric exponents in that age range. When the variations in the allometric exponent across the studied age range are greater than the bias in allometric exponent leading to acceptable bias in clearance (see Figure 3) , more flexible functions should be used to capture clearance ontogeny, such as a bodyweight-dependent exponent [39,40].

8.3. Conclusion

Throughout this thesis, we developed a PBPK-based simulation workflow allowing to unravel the conditions for accurate scaling of drug clearance from adults to children as young as term neonates of one day for diverse methods. Based on these results, we proposed a clearance scaling decision tree, which allows for the first time (clinical) pharmacologists to select scaling method(s) that require a minimum but still sufficient amount of information to accurately scale clearance according to the paediatric age range and properties of the drugs under investigation. Moreover, the PBPK-based simulation workflow provides a mechanistic understanding of the diverse challenges in scaling drug clearance, as for instance the steep maturation of AAG in young children or the changes in ER with age due to isoenzyme maturation, which hinders the accuracy of most clearance scaling methods for drugs binding to AAG and for high ER drugs respectively. Moreover, we provided an analysis framework to assess the feasibility and clinical trial requirements for the estimation of PBPK parameters using population pharmacokinetic modelling (popPBPK), which has the potential to expedite development of PBPK models for understudied paediatric subpopulations. Such framework is crucial to guide clinical trial design or data collection for meta-analysis, as we found that trial requirements to perform popPBPK modelling are difficult to meet, as a high number of patients and drugs with very specific properties are needed. Finally, we broke through the beliefs and

misconceptions around allometric scaling using a bodyweight-based exponential relationship with a fixed exponent of 0.75, showing that there is no universal allometric exponent to scale size-related changes in clearance across the paediatric age range and providing good modelling practice for the estimation of the allometric exponent to scale both size- and age-related changes in clearance.

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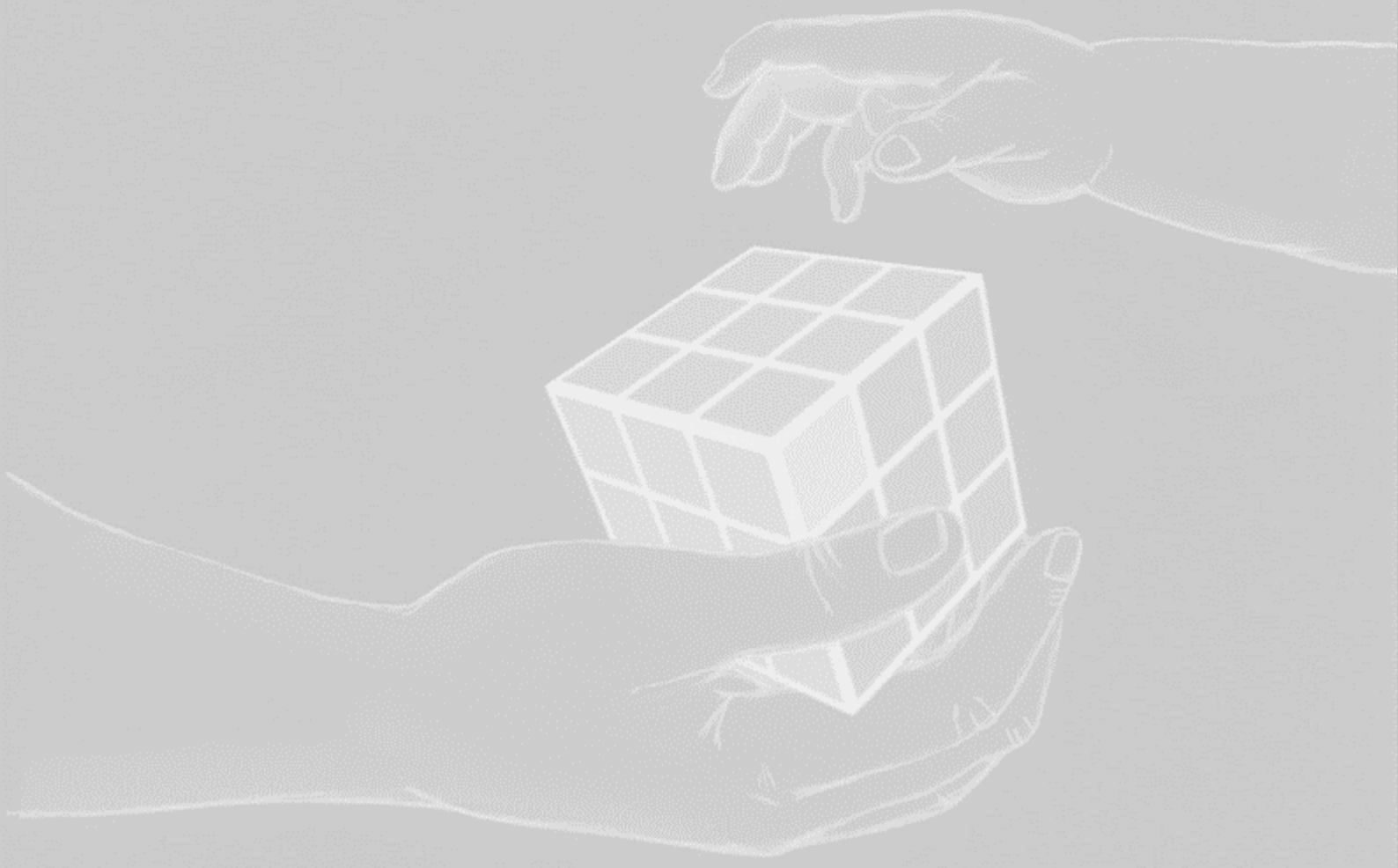
Appendix

Nederlandse samenvatting



Hoe kan de klaring van geneesmiddelen die in de lever gemetaboliseerd worden, geschaald worden van volwassenen naar kinderen?

Inzichten verkregen met geavanceerde PBPK modellen

Het schalen van geneesmiddelklaring maakt het mogelijk om klaringswaarden voor alle pediatrische leeftijden te bepalen gebaseerd op klaringswaarden van volwassenen en, uiteindelijk, om pediatrische doseringsvoorschriften te ontwikkelen in afwezigheid van (voldoende) klinische data. De nauwkeurigheid van klaringsschalingsmethodes is afhankelijk van hun vermogen om de invloed van ontogenie binnen diverse systeem-specifieke parameters die pediatrische klaring van verschillende geneesmiddelen beïnvloeden, te aggregeren. Omdat fysiologisch-gebaseerde farmacokinetische (PBPK) modellen het mogelijk maken om de ontogenie binnen systeem-specifieke parameters en geneesmiddel-eigenschappen te koppelen aan klaringsontogenie is in **sectie II** en **III** van dit proefschrift, door een nieuwe op PBPK-gebaseerde simulatie platform te gebruiken, de systematische nauwkeurigheid van schalingsmethodes onderzocht voor verschillende pediatrische leeftijden, voor een breed scala aan geneesmiddelen. Dit platform ontrafelde pediatrische leeftijden en eigenschappen van geneesmiddelen waarvoor de verschillende schalingsmethodes belangrijke mechanistische informatie efficiënt aggregeren, en daardoor leiden tot systematisch nauwkeurige pediatrische klaringsvoorspellingen. De onderzoeken in **sectie II** en **III** hebben daarbij ontrafeld hoe de klaring nauwkeurig van volwassenen naar kinderen kan worden geschaald met een minimale doch voldoende hoeveelheid informatie, waarbij de focus lag op geneesmiddelen die hepatisch gemetaboliseerd worden, wat is samengevat in een pediatrische klaringsschaling beslissingsboom in **hoofdstuk 8.** Daarnaast hebben we overtuigingen over de universaliteit van een allometrische exponent om klaring te schalen voor alle pediatrische leeftijden aangevochten (**hoofdstuk 3, 4, en 6**), en deze bevindingen vertaald naar richtlijnen voor het schalen van de klaringsontogenie in **hoofdstuk 8**.

PBPK modellen zijn essentieel voor het schalen van de klaring van specifieke geneesmiddelen in verschillende pediatrische leeftijden en maken het mogelijk om de nauwkeurigheid van simpele schalingsmethodes te onderzoeken, zoals toegelicht in **sectie II** en **III**. Echter kan het voorkomen dat PBPK modellen niet volledig beschikbaar zijn en/of niet voor alle pediatrische sub-populaties betrouwbaar zijn, zoals bijvoorbeeld het geval is voor ernstig zieke kinderen, premature neonaten of zwaarlijvige kinderen. Hier komt bij dat voor

de ontwikkeling van deze modellen uitgebreide *in vitro, ex vivo* en/of *in vivo* data nodig zijn, naast een tijdrovende leer- en bevestigingscyclus voor validatie[1,2]. Om deze hindernissen te overwinnen en uiteindelijk PBPK modellen te ontwikkelen voor onderbestudeerde populaties stellen we in **sectie IV** voor om PBPK parameters direct te schatten op basis van klinische PK data. Voor de uitdagingen die bij het toepassen van deze methode komen kijken, hebben we in deze sectie een nieuw analysekader ontwikkeld gebaseerd op *optimal design* principes, met als doel de toepasbaarheid en studieontwerpeisen voor het schatten van PBPK parameters gebaseerd op klinische data met gebruik van populatie PK (popPK) methodes te onderzoeken.

Schalingsmethodes op basis van lichaamsgewicht om klaringswaardes van volwassenen naar pediatrische patiënten te extrapoleren

In **hoofdstuk 3** is een systematische analyse uitgevoerd, waarbij allometrisch schalen gebaseerd op lichaamsgewicht met een macht van 0.75 (AS0.75) onderzocht is voor scenario's met en zonder maturatie. Deze scenario's zijn onderzocht omdat, gebaseerd op sterke overtuigingen, AS0.75 regelmatig wordt gebruikt om grootte-gerelateerde veranderingen in totale (d.w.z. gebonden en ongebonden) plasma klaring (CLp) te schalen tussen volwassenen en kinderen. Hierbij wordt een belangrijke afwijking gerapporteerd voor de voorspellingen bij jonge kinderen, wie wordt toegeschreven aan het maturatieproces dat tegelijk met groei plaatsvindt op jonge leeftijd. Een PBPK-gebaseerde simulatie platform is ontwikkeld in R voor 12,620 hypothetische geneesmiddelen. In scenario één zijn alleen grootte-gerelateerde veranderingen van levergewicht, hepatische bloedstroom en glomerulaire filtratie geïncludeerd in simulaties van 'ware' pediatrische CLp. In een tweede scenario zijn ook maturatie van ongebonden microsomale intrinsieke klaring (CLint,mic), plasma eiwit concentraties en hematocriet geïncludeerd in de gesimuleerde 'ware' pediatrische CLp waardes. Voor beide scenario's is de voorspellingsfout (PE) voor AS0.75-gebaseerde pediatrische CLp voorspellingen berekend, waarbij voor het eerste scenario ook een allometrische exponent is geschat gebaseerd op de 'ware' pediatrische CLp. In het eerste scenario bereikten de PE van AS0.75-gebaseerde pediatrische CLp voorspellingen 278% in neonaten, en varieerde schatting van de allometrische exponent tussen 0.50 en 1.20, afhankelijk van leeftijd en geneesmiddeleigenschappen. In het tweede scenario gaf AS0.75 accurate CLp voorspellingen voor kinderen ouder dan 5 jaar, maar was de PE-gevoeligheid voor geneesmiddeleigenschappen en maturatie hoog in de jongste kinderen. Onder de 5 jaar werden accurate voorspellingen gedaan voor geneesmiddelen met een lage extractie ratio (ER)

die binden aan albumine en hepatisch geklaard worden door een rijp enzym. Voor geneesmiddelen die worden uitgescheiden door glomerulaire filtratie waren voorspellingen accuraat (PE tussen ±30%) voor kinderen vanaf 1 jaar, voor alle geneesmiddelen behalve voor geneesmiddelen met een hoge binding aan α1-acid glycoproteine (AAG) (b.v. ongebonden fractie in plasma in volwassenen van ≤ 0.12). Het gebruik van PBPK principes toont aan dat er geen bewijs is voor het bestaan van een unieke allometrische exponent in pediatrische patiënten, zelfs niet bij scenario's waarbij alleen grootte-gerelateerde veranderingen werden meegenomen. Omdat PE het meest gevoelig is voor de allometrische exponent, geneesmiddeleigenschappen en maturatie in jongere kinderen, worden voorspellingen slechter met jongere leeftijd bij het gebruik van AS0.75.

In **hoofdstuk 4** hebben wij de nauwkeurigheid van lineair schalen geëvalueerd m.b.v. de PKPB-gebaseerd simulatie platform ontwikkeld in hoofdstuk 3. In situaties waarbij lineair schalen niet leidde tot nauwkeurige voorspellingen hebben wij de resultaten vergeleken met AS0.75. Momenteel is er hernieuwde interesse in lineaire schaling, omdat de verschillen met het vaak gebruikte 0.75 allometrische schalen klein lijken te zijn. Wij hebben aangetoond dat lineaire CLp schaling accuraat is vanaf een leeftijd van 1 maand voor geneesmiddelen die glomerulair gefiltreerd worden, behalve voor geneesmiddelen met een hoge binding aan AAG. Voor hepatisch geklaarde geneesmiddelen bleek lineaire schaling redelijk nauwkeurig voor kinderen vanaf 2 jaar, behalve voor AAG-gebonden geneesmiddelen met een lage ER en iso-enzymactiviteit gelijk aan of hoger dan volwassen waardes. In neonaten presteert lineaire schaling beter dan AS0.75 voor geneesmiddelen die worden uitgescheiden door glomerulaire filtratie, onafhankelijk van binding aan HSA of AAG. Deze resultaten suggereren dat pediatrische patiënten in veel gevallen gezien kunnen worden als kleine volwassenen.

Schalingsmethodes om klaringswaardes van kinderen te voorspellen op basis van iso-enzymmaturatie

In **hoofdstuk 5** hebben we condities geïdentificeerd waarbij extrapolaties van populatie farmacokinetische (popPK) covariaat modellen tussen geneesmiddelen met eenzelfde eliminatie route consistent resulteren in een accurate route-specifieke CLp schaling van volwassenen naar kinderen voor geneesmiddelen die hepatisch gemetaboliseerd worden. Deze methode maakte een versnelde ontwikkeling van pediatrische modellen en doseringsvoorschriften mogelijk. Voor deze studie is gebruik gemaakt van de PBPK-

gebaseerde simulatie platform die met mechanistische vergelijkingen het hepatisch metabolisme definieert en ontwikkeld is in voorgaande hoofdstukken. We toonden aan dat geneesmiddelen die via dezelfde route geëlimineerd worden slechts in een beperkt aantal specifieke gevallen dezelfde pediatrische doseringsaanpassingen nodig hebben, afhankelijk van de ER van de geneesmiddelen, ongebonden fractie van het geneesmiddel, type plasmaproteïne binding, en de fractie gemetaboliseerd door de iso-enzym route waarvoor CLp geschaald is. In het algemeen is de extrapolatie van pediatrische covariaat functies voor CLp tussen geneesmiddelen vooral toepasbaar voor geneesmiddelen met lage en gemiddelde ER die geëlimineerd worden door één iso-enzym en binden aan serumalbumine (HSA) in kinderen ouder dan 1 maand.

In **hoofdstuk 6** zijn simpele methodes geëvalueerd die momenteel gebruikt worden om klaring te schalen van volwassenen naar kinderen. Tot op heden wordt schaling van volwassenen naar kinderen met behulp van een allometrische exponent die varieert met leeftijd (leeftijdsafhankelijke exponent (ADE)) en allometrische schaling waarbij een exponent van 0.75 met iso-enzymmaturatie functies gelijk aan functies geïmplementeerd in PBPK modellen (AS0.75+MF_{PBPK}) gezien als potentieel waardevolle schalingsmethodes, maar ontbreekt een beoordeling van hun toepasbaarheid. Het doel van deze studie was dan ook om de nauwkeurigheid van ADE en AS0.75+MF_{PBPK} systematisch te beoordelen in kinderen jonger dan 5 jaar, voor geneesmiddelen die hepatisch gemetaboliseerd worden. Hiervoor is de eerder ontwikkelde PBPK-gebaseerde simulatie platform gebruikt, inclusief hypothetische geneesmiddelen met een breed scala aan eigenschappen die gemetaboliseerd worden door verschillende iso-enzymen. In dit platform zijn de impact van geneesmiddeleigenschappen en maturatie van iso-enzymen op klaringsmaturatie en de nauwkeurigheid van elke schalingsmethode onderzocht om scenario's te definiëren waarbij deze methodes systematisch resulteren in accurate pediatrische CLp schaling. De geneesmiddeleigenschappen die het meest relevant bleken voor het voorspellen van systematische nauwkeurigheid van de schalingsmethodes waren ER in volwassenen en plasmaproteïne binding aan HSA of AAG. We toonden aan dat deze geneesmiddeleigenschappen en iso-enzymmaturatie een groot effect hebben op klaringsmaturatie in alle kinderen jonger dan 5 jaar, wat resulteert in een breed scala aan pediatrische klaringswaardes, wat uiteindelijk belemmerend is voor systematische nauwkeurigheid van schalingsmethodes waarbij alleen leeftijd en lichaamsgewicht worden beschouwd, zoals ADE. Daarentegen kan pediatrische klaring voor alle geneesmiddelen met lage- en gemiddelde ER nauwkeurig geschaald worden met behulp van AS0.75+ MF_{PBPK}, met

uitzondering van geneesmiddelen met binding aan AAG in neonaten. Voor andere geneesmiddelen is er nog geen nauwkeurige simpele schalingsmethode en zijn PBPK methodes nodig voor accurate schaling.

Verbeterde voorspellingen van pediatrische klaring door het gecombineerde gebruik van populatie PK en PBPK modellen

In **hoofdstuk 7** hebben we een methodologie ontwikkeld om de haalbaarheid en eisen voor precieze en accurate schatting van PBPK parameters te onderzoeken, door middel van een popPK analyses van klinische data. We hebben dit geïllustreerd voor twee belangrijke PBPK parameters voor metabolische leverklaring, namelijk gehele lever ongebonden intrinsieke klaring ($CLint_{,u,WL}$) en bloedtoevoer van de lever (Qh) in kinderen. Schatting van PBPK parameters met gebruik van klinische data is hoogst relevant, aangezien de vereiste parameters voor PBPK modellen niet altijd direct gemeten kunnen worden. Eerst werd identificeerbaarheid mogelijk gemaakt door her-parameterisatie en het definiëren van essentiële onderdelen van het studieontwerp. Vervolgens werden de eisen voor het de opzet van de studie vastgesteld, die de exacte schatting van de PBPK parameters en hun interindividuele variabiliteit moesten opleveren met gebruik van een nieuwe toepassing van *optimal design* theorie. Tenslotte werd de uitvoering van voorgestelde studieopzet beoordeeld met stochastische simulaties en schattingen. De resultaten lieten zien dat een precieze schatting van $CLint_{,u,WL}$ en Qh en hun interindividuele variabiliteit een studie met twee geneesmiddelen vereist, waarvan er één een ER van ≤ 0.27 heeft en de andere van ≥ 0.93. Het voorgestelde klinische studieontwerp bleek te leiden naar precieze en accurate parameterschattingen en was robuust voor parameteronzekerheid. Het voorgestelde kader kan worden toegepast voor andere PBPK parameters en vergemakkelijkt de ontwikkeling van PBPK modellen.

Conclusies en perspectieven

Schalen van klaring van volwassenen naar kinderen: Een beslissingsboom

Door dit gehele proefschrift heen ontwikkelden we een PBPK-gebaseerde simulatie platform die ons in staat stelde om voor verschillende methodes de voorwaarden te ontrafelen voor accurate schaling van geneesmiddelklaring van volwassenen naar kinderen, waarbij de jongste leeftijd een voldragen neonaat van een dag oud is. Gebaseerd op deze resultaten introduceerden we in **hoofdstuk 8** een klaringsschaal beslissingsboom (zie **hoofdstuk 8**,

figuur 1), welke (klinisch) farmacologen voor het eerst in staat stelt om schalingsmethode(s) te selecteren die een minimum maar wel voldoende hoeveelheid van informatie vereisen om accuraat klaring te schalen afhankelijk van de leeftijd en de eigenschappen van het te schalen geneesmiddelen. Door het ontrafelen van de minimale hoeveelheid informatie die nodig is voor accurate schaling van de klaring van volwassenen naar kinderen van verschillende leeftijden, maakt de beslissingsboom de selectie van eenvoudige methodes mogelijk die kunnen worden gebuikt in plaats van PBPK modellen, waardoor de vereiste rekenkracht en informatieverzameling verminderend word. Geselecteerde schalingsmethodes gebaseerd op deze beslissingsboom kunnen worden gebruikt om de klinische studies in kinderen te optimaliseren door de definitie van de doseringsvoorschrift(en), tijdstippen van metingen[3], en inclusiecriteria gebaseerd op de schalingsvariabelen, om accurate en nauwkeurige klaringparameterschattingen te verzekeren. Daarnaast kunnen deze schalingsmethodes worden gebruikt ter informatie tijdens popPK analyses om deze analyses bij kleine PK studies, zoals voor sommige specifieke ziektes waarvoor slechts een klein aantal kinderpatiënten kan worden opgenomen, te informeren.

De ontwikkeling van de schalingsbeslissingsboom zoals geïllustreerd in dit proefschrift zou kunnen worden uitgebreid naar pediatrische subpopulaties zoals premature neonaten of zwaarlijvige kinderen, naar geneesmiddelen die beïnvloed worden of een invloed hebben op drijvende processen die mogelijk een impact hebben op klaring die niet werden onderzocht in dit proefschrift alsmede naar schaling van andere PK parameters zoals biobeschikbaarheid welke samen met klaring een sleutelparameter is voor de dosisafstemming voor niet-intraveneus toegediende geneesmiddelen. In veel gevallen zou dit eerst de kwantificatie van deze processen en/of van systeem-specifieke parameters van deze populaties vereisen alvorens zij kunnen worden geïmplementeerd in PBPK modellen, aangezien zij momenteel slecht zijn gekarakteriseerd.

Dit is bijvoorbeeld het geval voor premature neonaten[5], waarvan bekend is dat geneesmiddelklaring mogelijk verschilt van voldragen neonaten, geneesmiddelen die substraten zijn voor transporters, en geneesmiddelen die door de nieren geklaard worden en die actieve tubulaire absorptie en/of uitscheiding ondergaan. Wanneer eenmaal voldoende data (bijvoorbeeld over de hoeveelheid transporters in de lever, of in de renale tubuli) voor iedere pediatrische leeftijd verzameld, geïmplementeerd en gevalideerd is in PBPK modellen, zou de impact van deze processen kunnen worden onderzocht met gebruik van een PBPK simulatiewerkstroom zoals in **sectie II** en **III**.

Het gebruik van populatie PBPK modelleren om voorspellingen uit te breiden naar subpopulaties

Er is behoefte aan de ontwikkeling van PBPK modellen voor pediatrische subpopulaties en voor specifieke geneesmiddelen die mogelijk processen aansturen die klaring of andere PK parameters beïnvloeden. Echter, niet alle relevante parameters voor zulke modellen zijn gekwantificeerd. Het verkrijgen van PBPK parameters die niet op een tijds- en kosteneffectieve manier gekwantificeerd kunnen worden door directe experimentele metingen zou in hoge mate de ontwikkeling van zulke PBPK modellen en hun voorspellende waarde verbeteren en versnellen, zonder de betrokken populatie te veel te belasten. Daartoe stelden we in **hoofdstuk 7** van dit proefschrift een nieuwe methodologie voor om de haalbaarheid en studievereisten te onderzoeken om PBPK parameters in speciale populaties te schatten op basis van klinische PK data. Vanwege de erg specifieke vereisten aan klinische data voor PBPK parameter schatting die in **hoofdstuk 7** zijn geïdentificeerd, is het gebruik van een analytisch kader zoals voorgesteld cruciaal om *a priori* de haalbaarheid en vereisten van de klinische studie voor zo'n benadering te identificeren, om aan de hand daarvan data te verzamelen voor meta-analyse en/of de nieuwe klinische studies te ontwerpen. Bij het voldoen aan deze criteria zal het waarschijnlijk zijn dat data gedeeld moeten worden, wat een belangrijk nadeel van deze benadering kan zijn gegeven de huidige inspanningen die gemaakt moeten worden voordat data daadwerkelijk gedeeld kunnen worden. In het licht van de klinische data van verschillende geneesmiddelen die nodig is voor het verbeteren van zowel de voorspellingen van de meeste geschikte doseringen in kinderen en andere onder-bestudeerde populaties, als van het begrip van de mechanismen die invloed hebben op geneesmiddel PK, lijkt het niet delen van data onethisch. Dit trekt de huidige regelgeving over dataeigenaarschap in twijfel. Momenteel wordt het belang van het delen van data meer en meer erkend, en projecten waarbij data gedeeld wordt, zijn ontwikkeld om het mechanistische begrip en het kwantitatieve modelleren van geneesmiddelen PK[7] te verbeteren. Voorbeelden van projecten waarbij data gedeeld wordt, zijn het OrBiTo IMI project dat ontworpen is om het begrip en modelleren van hoe geneesmiddelen worden geabsorbeerd te verbeteren of het mechanisme-gebaseerde PK-PD modelleerplatform van TI-pharma welke de ontwikkeling beoogt van nieuwe mechanisme-gebaseerde PK-PD modelleerconcepten voor stationaire en niet-stationaire biologische systemen[9].

Richtlijnen voor modellen voor het beschrijven van de ontogenie van klaring

Wanneer er voor een analyse data beschikbaar is van kinderen van verschillende leeftijden, dan is het simpelste covariaat model dat alleen lichaamsgewicht als covariaat gebruikt, allometrische schaling met een geschatte allometrische exponent. In tegenstelling tot theoretische allometrie, waarbij alleen geprobeerd wordt om rekening te houden met grootte-gerelateerde veranderingen in de klaring, kan het gebruik van een allometrische vergelijking met een geschatte exponent de gehele ontogenie van de klaring beschrijven, hierbij rekening houdend met zowel grootte- als leeftijd gerelateerde veranderingen, omdat lichaamsgewicht en leeftijd sterk gecorreleerd zijn in kinderen. Vaak wordt de geschatte allometrische exponent verward met de op theorie gebaseerde exponent die alleen probeert om de aan grootte gerelateerde veranderingen van klaring te schalen. Waardes die anders dan 0.75 zijn, worden dan onterecht ingeschat als zijnde fout en als het gevolg van een afwijking in de schatting van de allometrische exponent uit een studie met te weinig statistische power. Er is bijvoorbeeld ten onrechte beweerd dat het betrouwbaarheidsinterval van de allometrische exponent altijd de waarde van 0.75 moet bevatten[10]. In **hoofdstuk 3**, hebben we laten zien dat het gebruik van een exponent van 0.75 (AS0.75, de beweerde theoretische allometrische exponent) om de gehele ontogenie van klaring te voospellen kan leiden tot zeer afwijkende voorspellingen in kinderen jonger dan 5 jaar (scenario 2 in **hoofdstuk 3**). In **hoofdstuk 6**, vonden wij dat de waarde van de geschatte exponent die rekening houdt met zowel grootte- als leeftijdsgerelateerde veranderingen veel kan verschillen van 0.75 in kinderen jonger dan 5 jaar, van wel 0.57 tot 2.06 voor geneesmiddelen die in de lever afgebroken worden, en dat deze exponent varieert met leeftijd, geneesmiddeleigenschappen en de eliminatieroute. De waardes in dit interval zijn realistisch, aangezien deze zijn afgeleid van PBPK kennis, en een vergelijkbaar interval van de allometrische exponent is gerapporteerd in popPK data analyses[11-14]. Dit wijde interval van geschatte exponenten in de literatuur komt niet door een afwijking in de schatting, zoals recentelijk is aangetoond door Sinha *et al*[15]. Bovendien kunnen waardes buiten het in **hoofdstuk 6** gevonden interval ook verwacht worden wanneer er geen data van volwassenen wordt meegenomen, aangezien de allometrische exponent afhangt van het leeftijdsinterval dat bestudeerd wordt, of in situaties die nog niet zijn bestudeerd (b.v., geneesmiddel transport).

Daarom moet de allometrische exponent in onze mening geschat worden om de klaring te schatten van kinderen jonger dan 5 jaar, en niet vastgezet op 0.75. Ook moeten waardes anders dan 0.75 niet op voorhand als onwaarschijnlijk worden geïnterpreteerd. In gevallen

waarin het aantal patiënten klein is (<20), bijvoorbeeld in het geval van zeldzame ziektes, is de power van de studie voor het vinden van een niet-afwijkende schatting van de allometrische exponent waarschijnlijk kleiner dan 80%, kan er een afwijking in de schatting ontstaan, en zou men moeten overwegen om de parameters vast te zetten in het covariaat model dat de ontogenie van de klaring beschrijft. Als de schatting van de allometrische exponent daarentegen onzeker is, dan is bestaande kennis van de ontogenie van de klaring noodzakelijk. Hoewel de vastgezette allometrische exponent van 0.75 een redelijke aanpak is voor kinderen van 5 jaar of ouder, zoals aangetoond in **hoofdstuk 3** van dit proefschrift, moeten andere schalingsfuncties zoals lineaire schaling gebruikt worden in jongere kinderen. Hiervoor kan de beslissingsboom voor de schaling van de pediatrische klaring gebruikt worden. Dit betekent niet dat AS0.75 niet nauwkeurig kan zijn in kinderen onder de 5 jaar, maar wel dat de nauwkeurigheid niet gegarandeerd kan worden in deze leeftijdsgroep omdat AS0.75 tot een belangrijke afwijking kan leiden van de klaringsvoorspellingen, afhankelijk van de leeftijd en de geneesmiddeleigenschappen.

Het beoordelen van de afwijking en precisie van de allometrische exponent

De beoordeling van de afwijking en precisie van de schatting van de allometrische exponent wordt vaak slecht uitgevoerd. Een verschil in de gevoeligheid van de nauwkeurigheid van voorspellingen van de ontogenie van de klaring voor de afwijking in de allometrische exponent is aangetoond in **hoofdstuk 3**. In dit hoofdstuk, vonden wij namelijk dat hoe jonger het kind, hoe gevoeliger de klaringsvoorspellingen waren voor afwijkingen in de exponent van de functie die de relatie tussen lichaamsgewicht en klaring beschrijft. Dat betekent dat hetzelfde verschil tussen de echte allometrische exponent en de gekozen vastgezette exponent die gebruikt wordt om de klaring te schalen, kan leiden tot verschillende afwijkingen in klaringsvoorspellingen voor verschillende leeftijden. Deze afwijking neemt toe met jongere leeftijd, dus voor jongere kinderen met een lager lichaamsgewicht kan de afwijking in de allometrische exponent veel lager zijn, om dezelfde afwijking in de klaringsvoorspellingen te hebben als bij oudere kinderen.

Omdat het doel van het schatten van de allometrische exponent tijdens een klinische PK data analyse niet het schatten van de exacte exponent is, maar het schatten van een exponent die zorgt voor nauwkeurige klaringsvoorspellingen, moet de afwijking in de allometrische schaling bepaald worden aan de hand van diens impact op de nauwkeurigheid

van de voorspelling van de klaring. Echter, de afwijking of de onzekerheid van de schatting van de allometrische exponent wordt vaak beoordeeld aan de hand van vaste criteria, zoals een bepaald percentage van parameterafwijking of onzekerheid dat geaccepteerd wordt. Dit is een belangrijk aspect dat de aandacht verdient wanneer een (covariaat) model wordt gefinaliseerd, naast de standaard modelevaluatiemethodes.

Hoewel er is gevonden dat de allometrische exponent varieert met leeftijd, geneesmiddeleigenschappen en iso-enzymmaturatie in ons PBPK-gebaseerde simulatie platform (**hoofdstuk 6**), betekent dit niet dat één specifieke allometrische exponent niet de ontogenie van de klaring kan beschrijven voor een specifiek geneesmiddel in een bepaalde pediatrische leeftijdsgroep. In de oudste kinderen zijn de klaringsvoorspellingen niet gevoelig voor de afwijking in de allometrische exponent, en daarom kan één allometrische exponent de klaring van alle geneesmiddelen nauwkeurig schalen binnen deze pediatrische leeftijdsgroep. Dit kan ook het geval zijn in jongere kinderen wanneer de veranderingen in klaring met lichaamsgewicht de allometrische vergelijking volgt. Dit is het geval wanneer de combinatie van geneesmiddeleigenschappen en de maturatie van de iso-enzymen die betrokken zijn bij de klaring leiden tot vergelijkbare allometrische exponenten in een bepaalde leeftijdsgroep. Als de variaties in de allometrische exponent binnen de bestudeerde leeftijdsgroep groter zijn dan de afwijking in de allometrische exponent die nog een acceptabele afwijking in de klaring zou geven, dan moeten er flexibelere functies gebruikt worden om de ontogenie van de klaring te beschrijven, zoals een lichaamsgewichtafhankelijke exponent[12,13].

Conclusie

In dit proefschrift, gebruikten wij een op PBPK-gebaseerde simulatie platform om voor diverse methodes de voorwaarden te ontrafelen voor een nauwkeurige schaling van de geneesmiddelklaring van volwassenen naar kinderen, waarbij de jongste leeftijd een voldragen neonaat van één dag oud is. Op basis van deze resultaten, introduceerden wij een beslissingsboom (**Hoofdstuk 8**, Figuur 1) waarmee (klinisch) farmacologen voor het eerst de schalingsmethode(s) kunnen selecteren die een minimale doch voldoende hoeveelheid informatie benutten om de klaring nauwkeurig te kunnen schalen op basis van de kinderleeftijdreeks en de eigenschappen van de onderzochte geneesmiddelen. Daarnaast biedt het PBPK-gebaseerde simulatie platform een mechanistisch begrip van de diverse uitdagingen bij het schalen van geneesmiddelklaring. Dit proefschrift biedt daarnaast een analysekader om

de haalbaarheid en de vereisten van een klinische studie te bepalen, die ons in staat stellen PBPK parameters te schatten door het gebruik van popPBPK modelleren. Dit heeft de potentie om de ontwikkeling van PBPK modellen voor weinig onderzochte pediatrische subpopulaties te versnellen. Dit kader is cruciaal voor het ondersteunen van het ontwerp van de opzet van klinische studies of het verzamelen van data voor een meta-analyse, aangezien wij vonden dat de data vereisten voor het ontwikkelen van popPBPK modellen moeilijk te vervullen zijn, omdat hoge aantallen patiënten en geneesmiddelen met hele specifieke eigenschappen hiervoor nodig waren. Tenslotte, doorbraken wij de overtuigingen en misvattingen rond het schalen van klaring met exponentiele functies gebaseerd op lichaamsgewicht met een vastgezette exponent van 0.75, door te laten zien dat er geen universele allometrische exponent is om aan grootte gerelateerde veranderingen in klaring te schalen voor iedere pediatrische leeftijd en bieden wij richtlijnen voor het schatten van de allometrische exponent om te schalen voor zowel grootte- als leeftijd gerelateerde veranderingen in klaring.

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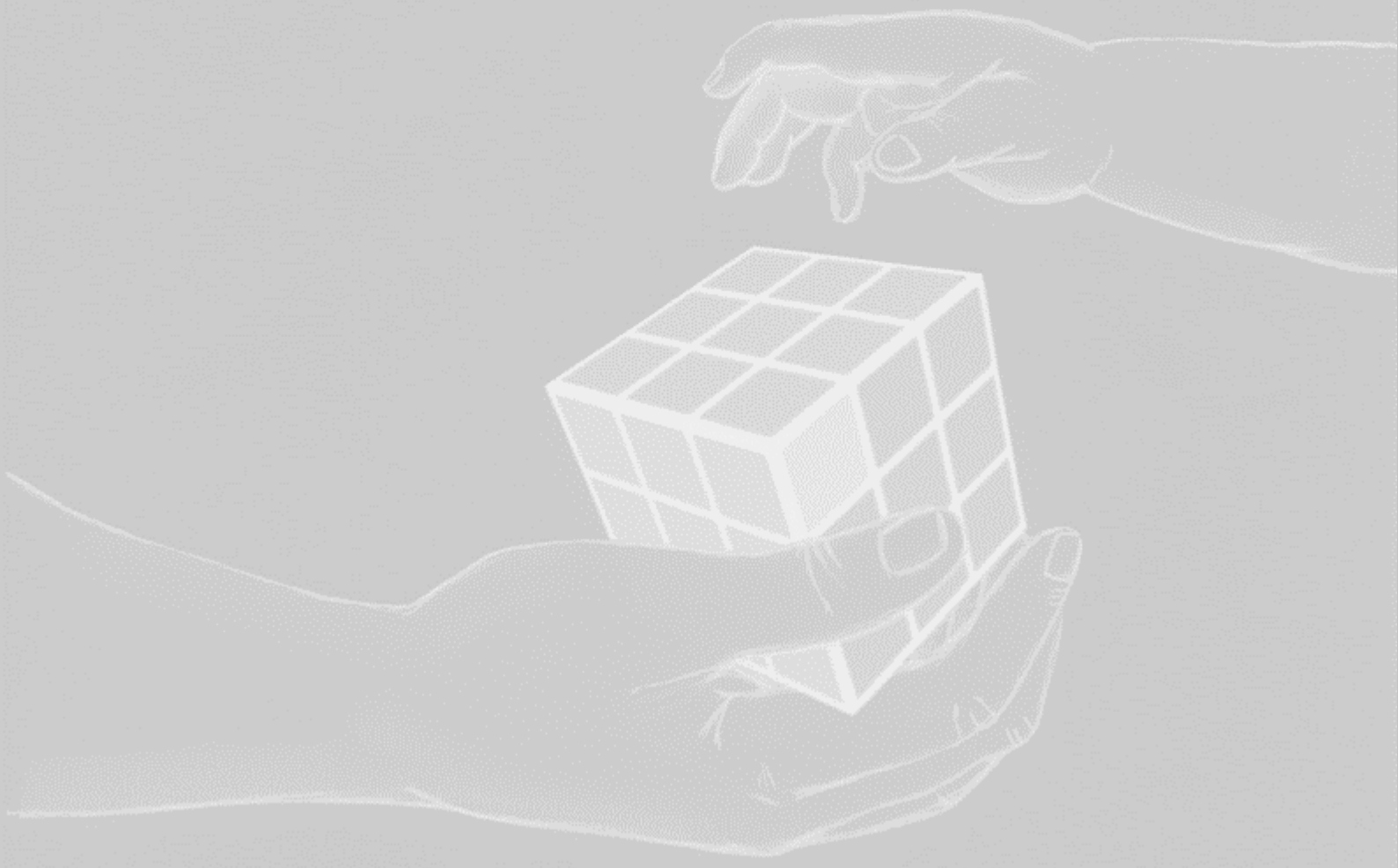
Appendix

Acknowledgments



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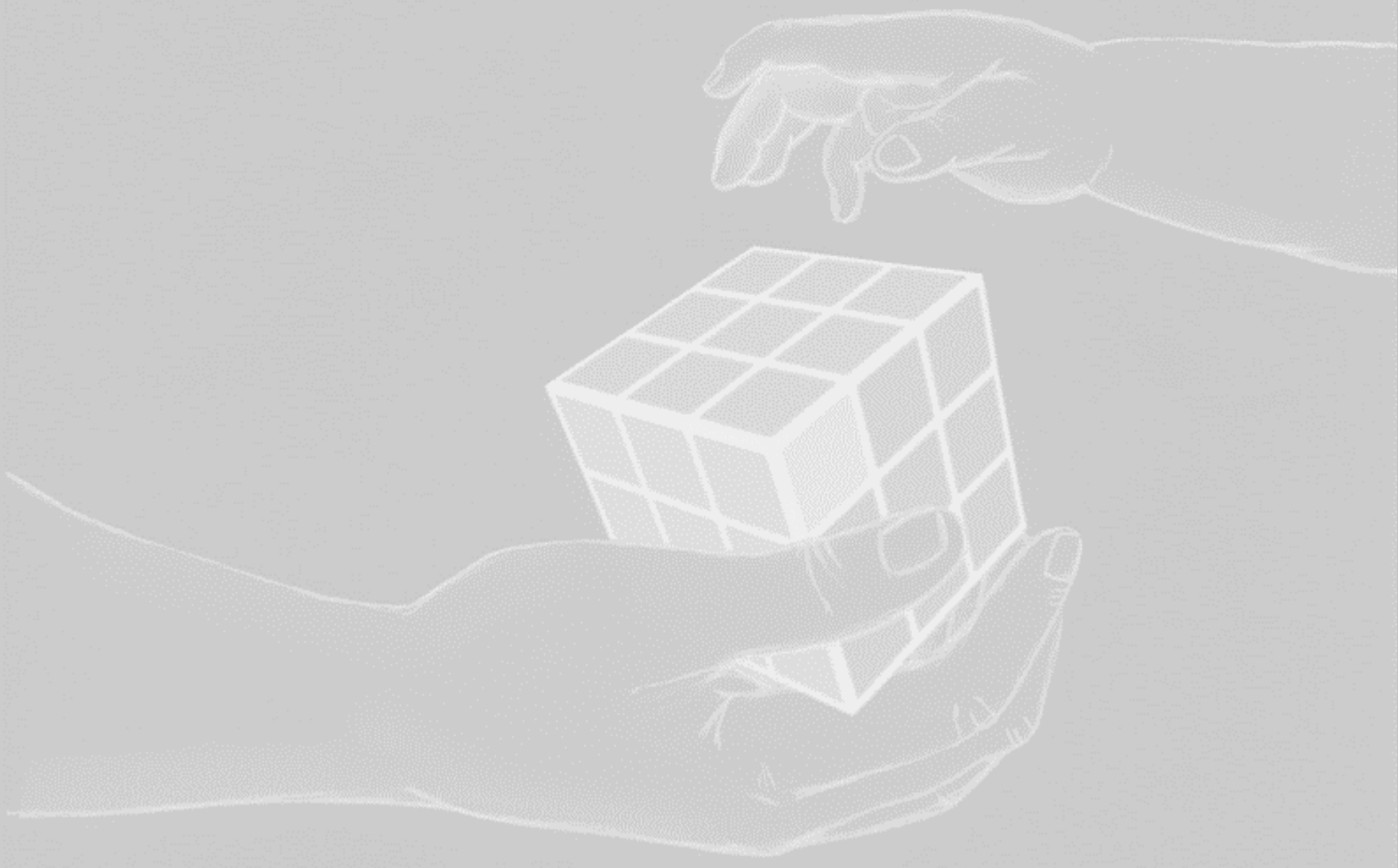
Appendix

Curriculum Vitae

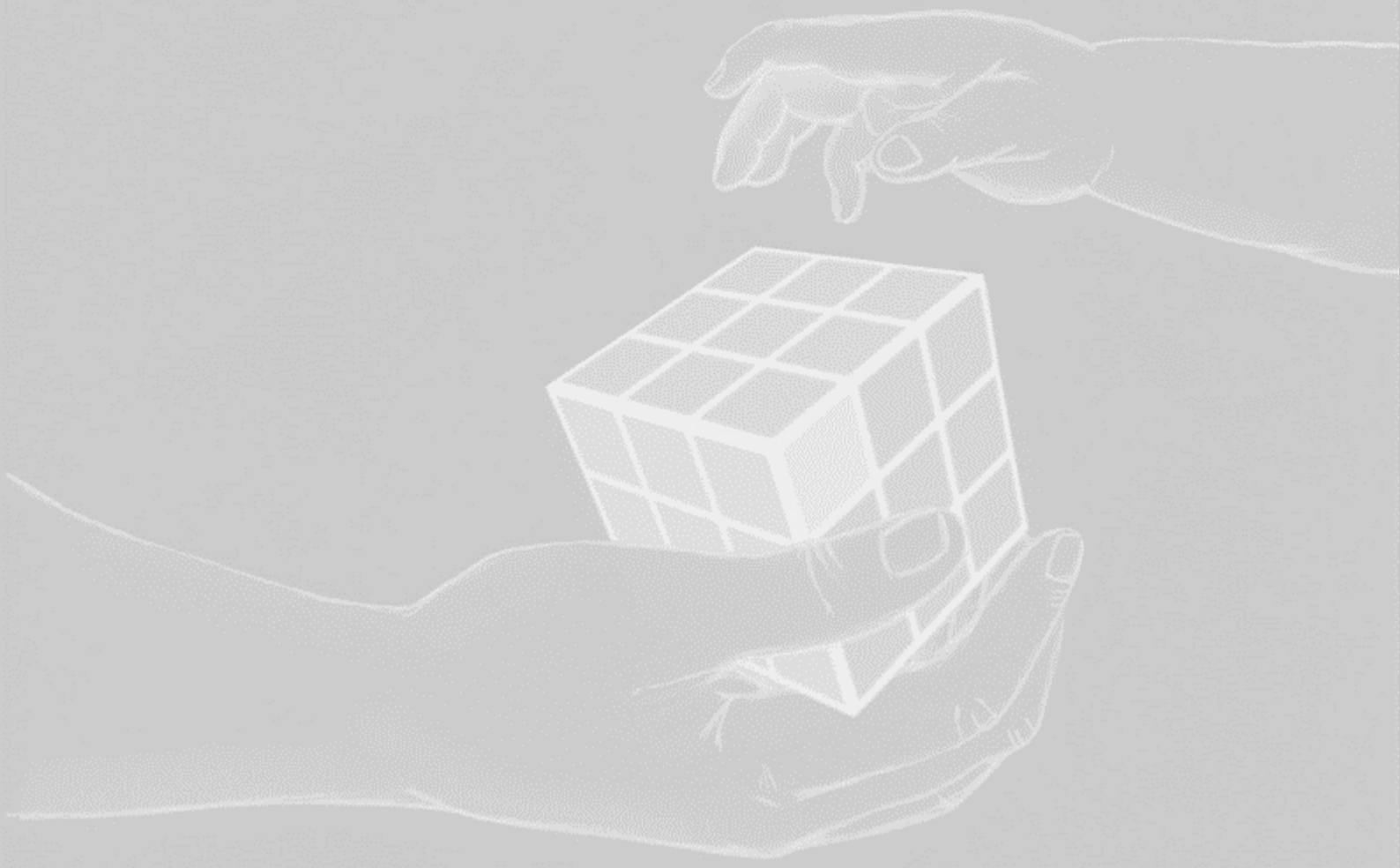


Curriculum Vitae Elisa Calvier

Elisa Calvier was born on the 23rd of September 1987, in Montélimar, France. She completed her A levels in 2005 at Alain Borne secondary school in Montélimar, before studying pharmaceutical sciences and graduating with a PharmD in 2014 at the University of Montpellier I, France. She specialized in pharmacometrics through an MSc in pharmacokinetics at the faculty of pharmacy of Paris Descartes University in 2013 as well as internships in the pharmacokinetic department of the faculty of pharmacy of Montpellier, in the Modelling and Simulation group of Sanofi Montpellier, and in the pharmacometrics group of Uppsala University. In 2013, she started her PhD research at Leiden Academic Centre for Drug Research at Leiden Universtiy under the supervision of Professor Catherijne Knibbe, Professor Dick Tibboel and Doctor Elke Krekels. Her research aimed at expediting and ensuring the systematic accuracy of clearance scaling from adults to paediatric patients, with a special focus on drugs undergoing hepatic metabolism, which resulted in this thesis. This research was founded by the Vidi Grant of Catherijne Knibbe, a grant from the NWO, the Netherlands Organisation for Scientific Research. In 2018 she started to work as a pharmacometrician in the Modelling and Simulation group of Sanofi Montpellier.

Appendix

List of publications



List of publications

Publications related to this thesis

Calvier, E. A. M., Krekels, E. H. J., Johnson, T. N., Rostami-Hodjegan, A., Tibboel, D. *et al*. Scaling drug clearance from adults to the young children for drugs undergoing hepatic metabolism: Striving for a simple solution. *The AAPS journal*, 2018. [Manuscript under revision]

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*Both authors contributed equally