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**Novel approach to characterize
developmental changes in pharmacokinetics
across the human lifespan: application to the
prediction of clearance in children**



Chenguang Wang



NOVEL APPROACH TO CHARACTERIZE DEVELOPMENTAL CHANGES IN
PHARMACOKINETICS ACROSS THE HUMAN LIFESPAN: APPLICATION TO THE
PREDICTION OF CLEARANCE IN CHILDREN

Chenguang Wang

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CHANGES IN PHARMACOKINETICS ACROSS THE HUMAN
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CLEARANCE IN CHILDREN**

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To Dad and Mom
To NaiNai

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Chapter

1

**General introduction to a novel approach
to characterize developmental changes
in pharmacokinetics across the human
lifespan: application to the prediction of
clearance in children**

1.1. Introduction

Medicine is both an art and a science. In the beginning of Cecil's Textbook of Medicine, it says, "Medicine is a profession that incorporates science and the scientific method with the art of being a physician. The art of tending to the sick is as old as humanity itself. Compared with its long and generally distinguished history of caring and comforting, the scientific basis of medicine is remarkably recent." [1]. Individualized medicine or personalized medicine comprises up-to-date scientific methods that aim to match the right drug to the right patient according to individual characteristics. Dose selection is one of the important issues in individualized treatment, as it determines the efficacy and the safety in the patients who receive the drug.

In paediatrics, selection of the optimal dose is even more crucial, since children are more vulnerable and less tolerant than adults to the drug treatment. However, the knowledge on the variation in the two major determinants of the drug effect, pharmacokinetics (PK) and pharmacodynamics (PD), is limited in paediatrics. Such reality makes that in paediatric clinical practice the dose selection is still empirical and based on mg/kg, despite profound differences in response between children and adults, and between children of different ages, that may or may not be scaling with bodyweight in a linear manner [2].

Pharmacokinetics (PK), which includes the processes of drug absorption, distribution, and elimination, may be referred to as the action of the body on drugs. Pharmacokinetic processes determine the time course of the drug concentration in the circulation, in tissues, and eventually at the target site. Obviously, the knowledge of the variation in pharmacokinetics in children is essential in determining the optimal dose to reach a certain target concentration or exposure.

In addition, the drug concentration-effect relationship, characterizing the pharmacodynamics (PD), determines the intensity of the pharmacological response resulting from the drug treatment. In paediatrics, the concentration-effect or the exposure-response relationship may differ from that in adults. Therefore knowledge of the pharmacodynamics in children is equally important in selecting the optimal dose.

It is not surprising that the PK-PD characteristics of a drug in paediatrics are not the same as in adults, due to physiological differences between adults and children. For example, the gastric pH is neutral (6 to 8) at birth and is acidic (2 to 3) in adults [3]; the total body water content is 80-90% of the bodyweight at birth and is 55-60% in adults [3]; the activity of CYP3A4 is extremely weak or absent in the fetus and begins to rise after birth to reach 30-40% of the adult activity after one month [3]. The differences in PK-PD between adults and children are not straightforward but rather unpredictable because of the complexity of the underlying physiological changes during growth and development. In order to describe how PK-PD characteristics change during the ontogeny process and ideally across the human age-span from birth to adults, mathematical and statistical modeling is needed.

Mathematical modeling must have been used in pharmacology for a long time, although it is hard to tell when the exact start was. The nature of the human learning process, from the observational learning towards the abstract reasoning, makes the application of mathematical modeling in pharmacology inevitable. After a thorough understanding of the mechanism of the underlying process, mathematical models constitute a scientific basis for quantifying and parameterizing the real biological, physiological and pathological processes, which enables the prediction and comparison of the drug effect and individualization of the drug treatment.

Legislation in the United States [4, 5] and in Europe [6] requires that clinical investigations be conducted in paediatric populations. Due to the ethical and practical limitations in paediatric clinical trials, the conduction of the clinical investigation is facing the challenge of evaluating drug efficacy and safety in the paediatric population on the basis of very limited information. Modeling and simulation was soon recognized by drug developers and regulators as the most optimal way to efficiently analyze data and to design clinical trials [4, 6-9]. A paradigm with scenarios for the use of modeling and simulation in paediatric medicines development was proposed, where modeling and simulation was identified as a tool to navigate in the paediatric decision tree and as a study optimization and data analysis tool [8].

Generally, there are two modeling approaches to investigate the changes of the pharmacokinetic characteristics of a drug in human. The first modeling approach is based on observed drug concentrations in blood samples taken in a clinical study and is referred to as the descriptive method or top-down method. The other approach is based on massive physiological and biochemical information, such as enzyme abundance, gastric pH, permeability, blood flow etc. and is referred to as the physiologically based (PB) method or the bottom-up method.

Although discussion and exploration of modeling and simulation (M&S) in paediatric drug development is still ongoing, there is no doubt about the necessity of M&S according to a vote in FDA's Pharmaceutical Science and Clinical Pharmacology Advisory Committee meeting in March 2012, where all voters supported the viewpoint that M&S should be considered in all paediatric drug development programs. As to the modeling and simulation in paediatric drug development, both "top-down" and "bottom-up" modeling approaches are used.

1.2. Top down approach

The top down approach is fitting observations. In pharmacokinetics, observations are the concentrations of the drug. Compartmental pharmacokinetic analysis is the most common top down modeling approach, which simplifies the description of the drug distribution in the human body as a distribution in a number of hypothetical compartments, each with different values of the equilibrium rate constants. This compartmental modeling approach has distinct advantages in predicting or simulating the time course of drug concentrations, since it describes the concentration versus time profile with parameterized differential equations.

Based on the assumption of compartments, two data analysis approaches can be applied: the standard two-stage (STS) approach and the population approach. With the STS approach, PK parameters are, in the first stage, estimated in each individual based on individual concentration time profiles. In the second stage, these parameters are summarized by calculating the mean or median of the parameters and the variability between subjects. A major

drawback of this methodology is that it requires a relatively large number of samples in each individual, while each individual has to contribute roughly the same number of samples. With the STS approach, it is very difficult to distinguish between inter-individual (variability between subjects) and intra-individual or residual variability (variability within one subject, measurement error and model misspecification) and as a result inter-individual variability is often overestimated [10].

In the second approach, the population approach, statistical random variables are added on its structural pharmacokinetic parameters, to account for the inter-individual variability within a population. With the help of these population pharmacokinetic (POPPK) models, we can attribute the variability between individuals in the observed drug concentrations to the variability between individuals in the values of different pharmacokinetic parameters, such as clearance, volume of distribution, absorption rate constant etc. Finally, the inter-individual variability of the pharmacokinetic parameters may be explained by some variables that are related to physiological volumes and functions, e.g. weight or age, in a so-called covariate analysis. In this population approach, the human body system is regarded as a whole in the beginning and is gradually unfolded searching for the physiological cause of the variation in the observed drug concentrations. The uppermost feature of this approach is that it describes or fits the facts, which are drug concentrations.

In paediatrics, clinical studies are more difficult to conduct than they are in adults. Due to ethical and practical issues [11], typically fewer PK samples per individual can be collected during paediatric trials, which results in sparse data for modeling. The population approach is usually preferred for analyzing paediatric PK data because of its capability in dealing with sparse data and in identifying inter-individual variability in paediatric population. Moreover, the population pharmacokinetic approach has been proposed in aiding dose selection in early paediatric development [12]. Besides, Clinical Trial Simulation (CTS) based on available population pharmacokinetic models has been used in the development of an adaptive paediatric clinical trial design [13]. Furthermore, sampling schemes were optimized according to the non-linear mixed effects model based simulation [14] and maximization of the Fisher Information Matrix (FIM) [15] tilde.

One of the most important applications of the top down modeling approach in paediatrics is scaling drug pharmacokinetic parameters (e.g. clearance and volume of distribution etc.) from adults to children. In general, there are three methods for scaling drug pharmacokinetic parameters: allometric scaling, $\frac{3}{4}$ allometric scaling with maturation function, and systematic covariate analysis.

1.2.1. Allometric scaling

Scaling is a term borrowed from engineering, which refers to adaptation of a functional system to operate at different production scales [16]. Similar adaptations of biological function between species and within the human lifespan are needed in pharmacokinetics. Allometry is the study of size and its consequences. The scaling of biological functional systems can be studied and mathematically described on the basis of allometric equations.

$$P = a \times BW^b \quad (1)$$

In the allometric equation, P is the physiological parameter of interest, BW is the bodyweight. The parameters a and b are the allometric constant and the allometric exponent, respectively. This simple equation has been found to be a robust and powerful basis of scientific theory when applied to the analysis of the ecological implications of body size [17]. This theory was thought to be realistic as allometric equations are built empirically using actual observations [18]. Even though there are some biological explanations on this allometric phenomenon [19-21], it is important to realize that the constant b , which is referred to as the allometric exponent, has no physiological meaning by itself [16]. This allometric function has been studied widely in inter-species metabolic rate scaling [22-25] and has been brought into the area of pharmacokinetic modeling for the scaling drug clearance and volume of distribution [26]. In the population pharmacokinetic modeling, the allometric equation is adapted as follows [18]

$$P_i = P_{std} \times \left(\frac{BW_i}{BW_{std}} \right)^b \quad (2)$$

in which P_i is the individual PK parameter in a subject with a bodyweight of BW_i and P_{std} is the PK parameter of a standardized individual with a bodyweight of BW_{std} . The adapted allometric equation (equation 2) is the most frequently

used covariate model structure in population pharmacokinetic modeling for describing changes in PK parameters between adults and children.

For paediatric studies, it has been proposed that the allometric exponent b should be fixed to the value of 0.75 for the scaling of drug clearance and to the value of 1 for volume of distribution to account for differences in size, no matter whether it is applied to adults or children [18]. This view is based on the allegedly substantial experimental evidence for these values of the allometric exponent in biology. Indeed many studies have reported values of the estimated allometric exponent for clearance that are close to 0.75. However, the allometric equation with the fixed value of 0.75 for clearance, referred to as $\frac{3}{4}$ allometric equation, has been challenged by different researchers [27, 28]. The challenges are mostly on the basis of the argument that the value of 0.75 should be the universal allometric exponent for clearance, while many studies have shown that use of the $\frac{3}{4}$ allometric equation yields discrepancies between observed versus predicted concentrations in very young children [29-31].

In this respect, Mahmood systematically investigated the predictive performance of scaling methods with different allometric exponent values (0.75, 0.8, 0.85 and 1) across the whole paediatric age-span for 41 drugs [27]. Clearances reported from literature were compared with allometric model predicted clearances with different exponent values. He found that all methods exhibited uncertainty in the prediction of the drug clearance in children and that no single method was suitable for all drugs for all age groups. The study also indicates that a single exponent may not be suitable for the prediction of drug clearance in children of all ages from adult data; therefore, a combination of methods is recommended in order to improve the prediction. It appears that for children ≤ 1 year old, a better approach is to use no exponent (exponent = 1) on the ratio of children and adult body weight. Specifically, allometric exponent values close to or above 1 were estimated for scaling clearances in young children [27]. After age 5, one can use any of the three exponents (0.75, 0.80 and 0.85) to achieve a reasonably good prediction of clearance in children [27].

1.2.2. $\frac{3}{4}$ Allometric scaling with maturation functions

In paediatric POPPK modeling, age may also have an influence on the inter-individual variability of drug clearance, especially in young children, infants and

neonates. This influence can be explained as the reflection of changes during the ontogeny in the physiological mechanism related with maturation instead of size. However the distinction between age and bodyweight as the basis for the scaling is complicated by the fact that age and bodyweight are correlated in a highly nonlinear manner. Consequently, in the covariate analysis of POPPK modeling, the apparent influence of age on the value of clearance may be contaminated by the influence from bodyweight and vice versa. Therefore, it has been proposed to separate the influence of bodyweight and age effect by using the $\frac{3}{4}$ allometric equation in the covariate analysis for clearance to account for size [26]. To account for the influence of maturation, an asymptotic exponential maturation function for clearance, incorporating postnatal age, has been proposed, which is incorporated as a multiplicative factor in the $\frac{3}{4}$ allometric equation based on body weight [32].

$$CL_i = CL_{std} \times \left(\frac{BW_i}{70}\right)^{0.75} \times \left(1 + \beta \times e^{\left(-AGE_i \times \frac{\ln 2}{t}\right)}\right) \quad (3)$$

$$MF = 1 + \beta \times e^{\left(-AGE_i \times \frac{\ln 2}{t}\right)} \quad (4)$$

In this equation β is the parameter reflecting fractional difference from the CL_{std} at birth. The parameter t is the parameter describing half-times of age-related changes in clearance. It is worthy to note that parameter β should be constrained between -1 and 0 so that the maturation factor (MF) will start from $1 + \beta$, which is less than 1, at birth and gradually approach to 1 with adult age.

A modified version of equation 3 was used to describe acetaminophen (paracetamol) age-related clearance maturation, in the basis of postconception age (PCA) [33].

$$CL_i = CL_{std} \times \left(\frac{BW_i}{70}\right)^{0.75} \times \left(1 + \beta \times e^{\left(-(PCA_i - 28) \times \frac{\ln 2}{t}\right)}\right) \quad (5)$$

In this equation, β is the parameter reflecting fractional difference from the CL_{std} at postconception age of 28 weeks. Postconception age rather than postnatal age was regarded to be a better predictor because the maturation of clearance begins before birth. Postmenstrual age (PCA) can also be used in the maturation function and the differences in PMA and PCA are expected to be 2 weeks in average [34].

As the asymptotic exponential maturation function has poor properties for extrapolation, later on a hyperbolic function has been proposed as the maturation function in the $\frac{3}{4}$ allometric scaling model [35].

$$MF_{PMA} = \frac{PMA^{Hill}}{PMA^{Hill} + MATCL_{50}^{Hill}} \quad (6)$$

Maturation functions on the basis of age as covariate seem to help to account for the discrepancy from the $\frac{3}{4}$ allometric equation in young children and can be used to describe the changes in clearance across a large age-span. However, it was also reported to have a poor predictive power of clearance in preterm and term neonates, infants and very young children [36, 37]. In addition, incorporating the combination of two correlated covariates in the nonlinear mixed effect model may result in bias in parameter estimates [38]. Recently, it has been shown that when one of two correlated covariates that contain information about a model parameter is pre-selected over the other, the predictive performance of the resulting model may be diminished, unless the pre-selected covariate relationship reflects the true biological relationship [39]. Although there are examples of the successful use of the $\frac{3}{4}$ allometric equation in the adult population, it has been shown to be unreliable in young children [27-31]. The colinearity between the bodyweight and age covariates and its consequences, therefore, become a concern when it is used as a descriptive reference model for clearance.

1.2.3. Systematic covariate analysis

In population pharmacokinetic modeling, a covariate analysis does not only comprise the identification of covariates that significantly influence the PK parameters but also includes the shape of the relationships between covariates and parameters. In the $\frac{3}{4}$ allometric scaling approach with maturation functions, the shape of the relationship between bodyweight and clearance is not determined by the data but is fixed *a priori*. In contrast to this approach, a data-driven systematic covariate analysis approach without any *a priori* assumption was proposed in paediatric modeling [40]. In the systematic covariate analysis, covariates are plotted independently against the empirical Bayesian estimates (EBE) of PK parameters, which provides graphical aids in identifying the most appropriate covariate equation. Covariate equations can be tested from the simplest linear function to complicated nonlinear functions such as Generalized Additive Model (GAM). Potential covariates are separately

tested and are considered statistically significant if the objective function decreased 7.9 points [40]. When more than one significant covariate for the simple model is found, the covariate-adjusted model with the largest decrease in objective function is chosen as a basis to subsequently explore the influence of additional covariates by means of the same criteria [40]. Covariates can be stepwise included into or excluded from the model depending on the model building strategy and the number of the candidate covariates. However, the final covariate model should be confirmed by the absence of unexplained inter-individual variability from covariates, which can be illustrated by the absence of a trend in the plot of EBE inter-individual (η) against the covariate [41].

An example of such a systematic covariate analysis approach was reported in a study of morphine glucuronidation clearance in preterm neonates, infants and children younger than 3 years [40]. The final model was reported to be the best model for describing the changes in morphine glucuronidation clearance within the study age-span [42]. Later on, this model was externally validated [43] and proved to be applicable across drugs that share same metabolism pathway mediated by UGT2B7 enzymes [44]. Paci *et al.* also applied such systematic covariate analysis in a research for busulfan in 205 children from 10 days to 15 years [45]. After testing different covariate models for clearance, they found that the allometric scaling model with the exponent value of 1.25 for bodyweight less than 9 kg and 0.76 for greater than 9 kg was the best covariate model [45].

1.3. Bottom up approach

In contrast with the top-down approach, the physiologically based approach looks at the pharmacokinetics from the underlying mechanism. The general concept of PBPK modeling is to mathematically describe relevant physiological, physicochemical, and biochemical processes that determine the pharmacokinetic behavior of a compound in as much detail as is appropriate or needed [46]. In paediatrics, unlike top-down approaches that require the availability of age-appropriate observed data, bottom-up approaches are used at a stage when these data have yet to be generated. Knowledge-driven or bottom-up approaches are based on the integration of various types of information (e.g. *in vitro* data, physiological and anthropometric information,

and drug physicochemical properties) with a model that structurally mimics the functioning of the biological system including relevant organ system(s) [47]. Chen and Gross [48] and Himmelstein and Lutz [49] discuss the rationale and history behind the development of PBPK models and report examples of the first PBPK models. Khalil and Läer provided a general overview of the structure of PBPK models as well as their applications and weaknesses [46]. Most recently, Barrett *et al.* gave a review of the PBPK modeling specialized on its application in paediatric drug research [50].

Physiologically Based Pharmacokinetic (PBPK) models have been applied for the selection of the first-in-children dose in paediatric drug development. Edginton reviewed the knowledge-driven PBPK approaches for the guidance of first-in-children dosing [47] and proposed a workflow of the application of PBPK in paediatric drug development together with Maharaj and Barrett [51]. Additionally, Johnson and Rostami-Hodjegan pointed out other potential applications of the PBPK model, where the conduct of studies is not feasible (e.g. drug-drug interactions in paediatric populations, particularly those with varying genotypes or renal function compared to average individuals) [52].

Interest in PBPK modeling in the field of paediatric drug development has been increasing recently. However, there are still skepticisms about the predictive performance of the PBPK models in paediatric drug development. The main concerns about the PBPK models are on the reliability of the resources to build PBPK models and the knowledge gap between *in vitro* and *in vivo*, between adults and children. Specifically, the PBPK model prediction in young children is less confident as some physiological information, such as enzyme activity, in those populations is not available. Leong *et al.* reviewed data relating to PBPK modeling and simulation in submissions to FDA made during the years 2009 - 2011 with respect to four paediatric drugs, as well as research on the use of PBPK tools with respect to several model agents used across varying age groups [53]. They found that PBPK models could not accurately predict weight normalized clearance values (CL_{BWnorm}) for all paediatric groups [53]. They urged that there is a critical need to refine paediatric PBPK models [53]. The request of the refinement of PBPK models not only comes from regulators but also arose from clinicians, who reminded us not to get PBPK modeling too disconnected from the "*in vivo*" world of paediatric developmental pharmacology [54].

1.4. Descriptive and predictive performance of paediatric models

For all pharmacokinetics models, either descriptive or mechanistic, the performance in describing observations should be demonstrated. In nonlinear mixed effect models, a large number of methods have been proposed for diagnosing, evaluating and validating various aspects of the model performance. Where possible all these methods should be considered in building a descriptive model. Generally, the more validations a model goes through, the more reliable the model is. External validation, incorporating data that have not been used to build the model, is preferred over internal validation. However, we should be aware that a model is always developed based on data collected in distinct study settings, which are determined by factors such as the inclusion criteria of patients, the design of the study and the distribution of covariates. For the external data these settings may be different, leading to models that differ from the original. One can never make sure that a model is valid in the next new dataset even if the model has been validated internally and externally before. Model validation results are always reliable within certain constraints. Having noticed the limitation of the model validation, we should always be aware of the importance of the necessary model validations as they are helping us to determine whether the model is appropriate for the data it is built on.

In paediatrics, evaluation of pharmacokinetic models are especially important due to the heterogeneity in paediatric populations and the fact that paediatric datasets are typically sparse [11]. However, paediatric models were seldom evaluated adequately in the past [55]. Amongst all model validation methods, some aspects are especially important. This concerns in particular the model for scaling of the PK parameters over a large age-span. Krekels *et al.* have proposed a scheme of systematic model evaluation approaches for paediatric pharmacokinetic modeling, which covers all the important aspects [42].

The fit of the model to the drug concentration is always the first aspect to be evaluated in pharmacokinetic modeling, since good concentration prediction is the basic requirement for a PK model. Usually, the goodness-of-fit is evaluated through the visual inspection of graphs [56] including i) observed (OBS) versus

individual predicted (IPRED) concentrations, ii) OBS versus population predicted (PRED) concentrations, iii) conditional weighted residual error (CWRES) versus time, and iv) CWRES versus PRED. The OBS versus PRED plot is specifically important as it reflects whether the whole population model (comprising structure model and covariate model) is adequate. In a model covering a large age-span, the OBS versus PRED plot needs to be stratified between age groups for further examination of whether the adequacy is the same for individuals in different age ranges. However, the stratified OBS versus PRED plot cannot help in identifying the cause of the bias in itself, as it reflects sum effects of all pharmacokinetic parameters. In population pharmacokinetic modeling with the First Order approximation Conditional Estimation (FOCE) algorithm, empirical Bayesian estimates (EBE) provide post hoc individual parameters of pharmacokinetic parameters (clearance or volume of distribution). This EBE is not only useful for predicting individual concentrations but is also helpful in covariate model building and diagnosis. Specifically, plots of *post hoc* inter-individual variability of a PK parameter versus covariates may be used to check whether there is a covariate relationship. Moreover, plots of *post hoc* estimates of a PK parameter versus covariates facilitate the identification the shape of the covariate relation. Eventually, an adequate covariate model should be confirmed by the absence of correlation between covariates and inter-individual variability of the concerned PK parameters [41]. However, the evaluation based on the EBE should be done with caution if there is a high shrinkage of the EBE inter-individual random effect, defined as η -shrinkage [57]. High η -shrinkage usually occurs when few data are available from an individual [41] and may mislead the covariate relationships finding [58]. This is particularly important in paediatric investigations, as they may typically contain sparse data.

Simulation based model validations or evaluations play an important role in POPPK modeling. A visual predictive check (VPC) is a commonly used for model validation in adults [59], which illustrates lines for the median values and their 90% prediction interval based on 100 to 1,000 times simulation of concentration-time profiles. The observed values in the internal or external dataset are subsequently plotted on top of this. It can then be visually checked whether 90% of the observations are within the indicated prediction interval and whether there is no bias in the observations compared with the prediction

interval. The VPC is a simulation-based diagnostic that can be used when the PK or PD profiles for all individuals in the dataset are similar and it allows for easy interpretation of the result. For this diagnostic tool, there are no statistical tests and all evaluations are based on visual assessment.

Although the VPC can also be used for the evaluation of paediatric models, when data are obtained during routine clinical practice and variability in individual dosing and sampling schemes are high, a (normalized prediction distribution error) NPDE methodology [60] is often easier to perform and interpret. This method yields information on how accurate the model predicts the median value of the observations and the variability within them. The interpretation of this diagnostic is less straightforward than for the VPC, but the advantage of this method is that it can be used when the variability in dosing regimen (both in time, amounts and rates) is high or when there is a large number of covariates in the model. This can for instance be the case for data obtained during routine paediatric clinical practice.

Model predictive performance usually refers to the capability of a model in predicting true measurements [61]. In paediatric pharmacokinetic models, true measurements are concentration profiles. Therefore, the goodness-of-fit plot is an evaluation method of the predictive performance of the whole pharmacokinetic model, which comprises the structure model, statistical models and covariate models. Besides, another predictive performance may be evaluated if the empirical Bayesian estimates (EBE) of pharmacokinetic parameters are regarded as the true individual measurements predicted by covariates. Actually, the predictive performance of the covariate models can be visually inspected by the plot of individual *post hoc* parameters against covariates superimposed with covariate model predicted curves. However, two numerical quantities of this predictive performance, mean squared prediction error (MSE) and root mean squared prediction error (RMSE), are more preferable in comparing different covariate models [61].

$$MSE = \frac{1}{n} \sum_{i=1}^n (predicted_i - true_i)^2 \quad (7)$$

$$RMSE = \sqrt{MSE} \quad (8)$$

The definitions of those two quantities are expressed in equation 7 and equation 8. The root mean squared prediction error (RMSE) can be expressed as percent of mean true measurements (%RMSE) using equation 9 as follows [37].

$$\%RMSE = \frac{RMSE \times 100}{\frac{1}{n} \sum_{i=1}^n true_i} \quad (9)$$

1.5. Discussion and conclusion

Drug prescription in the paediatric population is still very empirical while there is an urgent need for evidence based dosing in children. In pharmacokinetics, both population pharmacokinetics (POPPK) models and physiologically based pharmacokinetics (PBPK) models can describe and/or predict drug concentration time profiles and facilitate drug development and clinical practice.

The population pharmacokinetic approach is aiming to fit clinical observations. Paediatric POPPK model is usually based on a relatively large clinical dataset from a large number of different individuals, while the paediatric physiologically based model (PBPK) is based on relatively limited physiological data coming from published or unpublished *in vitro* studies. Although clinical samples are difficult to collect in paediatric populations because of the ethical and practical reasons, POPPK model can still utilize these sparse data efficiently and derive reliable results. Not only can POPPK model estimate the mean values of pharmacokinetic parameters over a population, it can also provide the distribution of those parameters, which is useful for clinical trial simulations. However, due to the absence of physiological mechanisms, POPPK model results are restricted to specific drugs or populations and can not be extrapolated to other drugs. A combination of the POPPK approach and PBPK approach is naturally the best solution. Krekels *et al.* elaborated on combining PBPK and POPPK approaches in order to identify the influence of changes in the system- and drug- specific parameters on the net maturation of *in vivo* UGT2B7-mediated glucuronidation [62]. In the end, they suggested that the maturation profile for UGT2B7 ontogeny in a PBPK model can be improved based on information obtained from a covariate model of a paediatric population model derived for a specific substrate for UGT2B7 on the basis of a systematic covariate analysis [62].

Such a combination of POPPK and PBPK approaches may be important particularly for paediatric drug development, where dose selection may be determined based on PBPK model simulation. The PBPK model for dose selection in the development of a new drug can be refined by the results of covariate functions from POPPK models of probe drugs sharing the same physiological mechanism as the new drug. Moreover, with the help of POPPK model, physiological parameters in PBPK models can be scaled to certain paediatric populations where no *in vitro* information is available. Therefore, developing a POPPK model covering the age-span from birth to adult with good descriptive performance all across is of great value. For this, a novel approach is proposed given the described limitations of the $\frac{3}{4}$ allometric scaling model and its derivative maturation model.

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Chapter

2

Scope and intent of the investigations

2.1. Background and introduction

In pharmacokinetics, drug clearance is an important parameter that characterizes the rate of elimination from the circulation. The value of clearance constitutes the basis for dose selection in clinical practice. During growth and development of a human being, there are many physiological and anatomic changes resulting in varying drug clearance in the paediatric population, with values typically differing substantially from drug clearance in the adult population. The need to optimize the dose in children warrants detailed investigations into the variation in drug clearance in the paediatric population.

Population modeling and simulation constitutes a scientific basis to characterize inter-individual variation in pharmacokinetics. An important feature of population modeling is the possibility to attribute the variability between individuals in the observed drug concentrations to the variability in the values of different pharmacokinetic parameters. The latter variability may in its turn be explained by variables that are related to physiological volumes and functions, e.g. weight or age, in a so-called covariate analysis. This data-driven modeling approach provides us pertinent information on the variation in clearances over the whole paediatric age-span based on clinical observations. A limitation of population pharmacokinetic analysis is that it does not enable predictions on the variation in clearance beyond the drugs and the conditions that have actually been studied.

At present, there is great interest in the use of physiologically based pharmacokinetic (PBPK) for the prediction of variation in drug clearance paediatric populations [1-3]. An important feature of PBPK models is their physiological basis. As a result they enable prediction of the variation in pharmacokinetics of drugs that have not been studied, based on known information on changes in physiological function. However, due to the difficulty of collecting *in vitro* data in children, e.g. enzyme activity in neonates, the knowledge of physiological processes is missing for some paediatric populations, making the PBPK model less reliable in simulating PK profiles in those populations.

Ideally, the best paediatric pharmacokinetic model should be able to not only fit the clinical observations but also explain the physiological mechanism. A combination of the POPPK and PBPK is naturally the best solution. It is proposed

that PBPK models are refined by the results of covariate functions from POPPK models of probe drugs sharing the same physiological mechanism. Moreover, with the help of POPPK model, physiological parameters in PBPK models can be scaled to certain paediatric populations where no *in vitro* information available. Therefore, developing a POPPK model covering the age-span from birth to adult with good descriptive performance all across is of great value.

Amongst currently used top-down population pharmacokinetic models, neither the $\frac{3}{4}$ allometric scaling model nor its derivative maturation model can describe the developmental changes in drug clearances with adequate statistical stability and predictive performance overall across the entire age-span. In this thesis we present a novel approach to describe the developmental changes in pharmacokinetic parameter estimates across the entire human lifespan, specifically focusing on drug clearances.

2.2. Outlines of the thesis

In Chapter 3, we studied the developmental changes in the clearance of propofol in various age groups, ranging from preterm neonates to adults. The emphasis was on estimation of the value of the allometric exponent in distinct age ranges. To this end, a large database was established comprising data obtained in neonates, infants, toddlers, children, adolescents and adults. Next, the allometric exponent was estimated when scaling clearance between two different paediatric subgroups or between one paediatric group and one adult group. With the pre-specified allometric equation, the values of the exponent were found to vary depending on the combination of the selected paediatric subgroups. Interestingly, the values of the allometric scaling exponent were larger than 1 when the subgroup with neonates was included in the analysis. From this analysis, it is concluded that there is no single value of the allometric exponent that describes variation in clearance across the entire human lifespan.

In Chapter 4, we introduced a novel approach to describe developmental changes in clearance across the human lifespan from preterm neonates to adults, based on the concept that “the allometric exponent varies with bodyweight”. To this end data on the clearance of propofol were analyzed with

different covariate models to describe developmental changes in clearance. The covariate models that were considered were: i) a mixture model, ii) a cut-point model and iii) a bodyweight dependent exponent (BDE) model. In this analysis the BDE model was found to yield the best description of the changes in clearance across the whole paediatric age-span.

In **Chapter 5** the BDE model was applied for the scaling of the total clearance of morphine, clearance to M3G and clearance of M3G across preterm neonates, children, adolescents and adults. It succeeded in describing those clearances without including additional age covariates, which were found to be necessary in model developed before. In this study, we also demonstrated the BDE model can be used not only for parent drug but also for its metabolites.

In **Chapter 6**, we further tested the BDE model in describing paracetamol clearance across whole paediatric age-span, which had been modeled with different covariates in the past. Again, it was clearly proved that for data across the human lifespan, the BDE model is able to describe the age-related changes in clearance very well without the need for additional age variables. Visual comparison among model predicted clearances from different models showed the BDE model had adequate descriptive performance of clearance throughout the whole paediatric age-span, whereas other models could only perform well in part. Based on the final model, simulations were performed to derive optimized dosing guidelines for paracetamol across the entire paediatric age range including preterm neonates.

In the meantime, a simplified version of the BDE model was successfully implemented for three other drugs: busulfan, midazolam and antibiotics. The results of these analyses are presented in the **appendices I - II**. The simplified BDE model is still based on the concept of using a monotonous continuously decreasing function of bodyweight to express age related changes in allometric exponent with bodyweight. The simplified BDE model may be better suited than the full BDE model in cases where preterm or term neonates are not included, or when the drug is metabolized by the CYP450 3A iso-enzymes or renally cleared by the kidney.

In **Chapter 7**, general conclusions and future perspectives of the work presented in this thesis were discussed.

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Chapter 3

The allometric exponent for scaling clearance varies with age: a study on seven propofol datasets ranging from preterm neonates to adults

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SUMMARY

Aim: For scaling clearance between adults and children, allometric scaling with a fixed exponent of 0.75 is often applied. In this analysis, we performed a systematic study on the allometric exponent for scaling propofol clearance between two subpopulations selected from neonates, infants, toddlers, children, adolescents and adults.

Methods: Seven propofol studies were included in the analysis (neonates, infants, toddlers, children, adolescents, adults¹, and adults²). In a systematic manner, two out of the six study populations were selected resulting in 15 combined datasets. In addition, the data of the seven studies were regrouped into five age-groups (FDA Guidance 1998), from which four combined datasets were prepared consisting of one paediatric age-group and the adult group. In each of these 19 combined datasets, the allometric scaling exponent for clearance was estimated using population pharmacokinetic modelling (NONMEM 7.2).

Results: The allometric exponent for propofol clearance varied between 1.11 and 2.01 in case the neonate dataset was included. When two paediatric datasets were analysed, the exponent varied between 0.2 and 2.01, while it varied between 0.56 and 0.81 when the adult population and a paediatric dataset except for neonates were selected. Scaling from adults to adolescents, children, infants and neonates resulted in exponents of 0.74, 0.70, 0.60 and 1.11 respectively.

Conclusions: For scaling clearance, $\frac{3}{4}$ allometric scaling may be of value for scaling between adults and adolescents or children, while it can neither be used for neonates nor for two paediatric populations. For scaling to neonates an exponent between 1 and 2 was identified.

3.1. Introduction

In paediatric pharmacokinetic (PK) modeling, scaling of pharmacokinetic parameters and in particular of clearance is a major issue, as it provides the rationale for tailoring suitable doses in children [1-3]. Scaling is required both in paediatric drug development for dose finding and selection of new drugs and in clinical practice where dose optimization and individualization is being performed when treating children. Accordingly, in paediatric drug development scaling of PK parameters between adults and the target paediatric population is highly relevant, while in clinical practice it may be relevant to scale within the paediatric population, i.e. a dose from children to neonates. For both purposes, currently the $\frac{3}{4}$ allometric scaling approach is nowadays propagated.

Allometric scaling was originally brought up to describe metabolic rates between different species [4]. The allometric scaling function for clearance can be described as

$$CL_i = CL_{std} \times \left(\frac{BW_i}{BW_{std}} \right)^{exp_{CL}} \quad (1)$$

where CL_i represents clearance for an individual with bodyweight BW_i , CL_{std} represents clearance for a standard individual with bodyweight BW_{std} , and exp_{CL} is the allometric scaling exponent which was proposed to be $\frac{3}{4}$ for metabolic rate [4]. Later on, for the purpose of scaling clearance from adults to children, this allometric exponent was also proposed to be fixed to a value of 0.75 [5], which leads to an over-prediction of clearance values for young children [6, 7]. In order to account for this discrepancy, a maturation function on the basis of age was proposed and applied in many studies [5]. More recently, a bodyweight-dependent exponent model was reported to cope with over-prediction of clearance at youngest age ranges by allowing the exponent value to vary with bodyweight [8]. Without the need for an age-based function, this approach was reported to capture changes in clearance from preterm neonates to adults for propofol [8] and from 1 month infants to adults for busulfan [9]. Even though the exact value for the exponents slightly varied between these drugs, typically higher values for the exponent were identified at younger age ranges, representing higher maturation rates at lower bodyweights.

In absence of specific information on the age-based maturation function required for the application of the allometric scaling theory [5] or on specific values of the bodyweight dependent exponent function [8, 9], allometric scaling based on bodyweight alone may be applied during paediatric drug development or when analysing data from clinical practice. In literature, both a fixed value of 0.75 [10-12] and an estimated value [13-15] for the allometric exponent have been reported for scaling clearance in children.

In this study, we did a series of hypothetical analyses by applying the allometric scaling function for clearance on two types of combined datasets from seven previously published propofol studies consisting of neonates, infants, toddlers, children, adolescents and adults. Combined datasets could consist of two study populations (type I models) or two age groups according to FDA Guidance 1998 [16] (type II models). In these combined datasets, the allometric exponents for clearance were estimated and the performances of the scaling function were evaluated in order to investigate the feasibility and boundary of the allometric scaling method without an age-based maturation function in both clinical practice situation and paediatric drug development situation.

3.2. Methods

3.2.1. *Subjects of the original studies*

A total of 174 subjects from seven previously published studies on propofol PK were included in the current study, including neonates (1-25 days) [17], infants (3.8-17.3 months) [18], toddlers (12-31 months) [19], children (3-11 years) [20], adolescents (9.8-20.1 years) [21], adults I (33-57 years) [22], adults II (26-81 years) [23]. Detailed information on the studies is summarized below.

Neonates [17]

Twenty-five cardiovascularly and respiratory stable neonates with a median of bodyweight of 2.82 (range 0.68-4.03) kilograms, postnatal age of 8 (1-25) days and gestational age of 37 (26-40) weeks were given an intravenous bolus dose of propofol ($3 \text{ mg} \times \text{kg}^{-1}$) for the elective removal of chest tubes, (semi)elective chest tube placement or endotracheal intubation.

Infants [18]

Twenty-two non-ventilated infants after major craniofacial surgery with a median bodyweight of 8.9 (4.8-12.5) kilograms, aged 10 (3.8-17.3) months received $2\text{--}4\text{ mg}\times\text{kg}^{-1}\cdot\text{h}^{-1}$ propofol during a median of 12.5 (6.0-18.1) hours.

Toddlers [19]

Twelve toddlers with minor burns, who had a median bodyweight of 11.2 (8.7-18.9) kilograms and age of 17.8 (12-31) months, were administered $4\text{ mg}\times\text{kg}^{-1}$ propofol just before bathing.

Children [20]

Fifty-three healthy unpremedicated children with a median bodyweight of 23.3 (15-60.5) kilograms and median age of 7 (3-11) years participated in this study. Twenty children received an intravenous loading dose of $3\text{ mg}\times\text{kg}^{-1}$ propofol. In the remaining 33 children, an intravenous loading dose of $3.5\text{ mg}\times\text{kg}^{-1}$ was followed by a maintenance infusion. In 18 of the 33 children, a single infusion rate of $0.15\text{ mg}\times\text{kg}^{-1}\times\text{min}^{-1}$ was administered, while 15 children received an infusion of $0.20\text{ mg}\times\text{kg}^{-1}\times\text{min}^{-1}$ for 30 minutes, followed by an infusion of $0.125\text{ mg}\times\text{kg}^{-1}\times\text{min}^{-1}$ until the end of the procedure.

Adolescents [21]

Fourteen adolescents with a median bodyweight of 51 (36.6-82) kilograms and median age of 14.7 (9.8-20.1) years were anaesthetized with propofol-remifentanyl ($2\text{--}10\text{ mg}\times\text{kg}^{-1}\cdot\text{h}^{-1}$) for scoliosis surgery during 6.8 (3.3-7.7) hours with an intra-operative wake-up test followed by re-induction of anaesthesia.

Adults I [22]

Twenty-four women undergoing gynaecological surgery, with a median bodyweight of 68.5 (55-80) kilograms and a median age of 45.5 (33-57) years, received $2.5\text{ mg}\times\text{kg}^{-1}$ propofol over 60 seconds for induction of anaesthesia.

Adults II [23]

Twenty-four healthy volunteers with a median bodyweight of 79.4 (44.4-122.7) kilograms and median age of 53 (26-81) years were administered a bolus dose of propofol, followed 1 hour later by a 60 minutes infusion with an infusion rate of 25, 50, 100, or $200\text{ mg}\times\text{kg}^{-1}\cdot\text{min}^{-1}$ in a study which investigated the influence

of the method of administration, infusion rate, patient covariates, and EDTA (ethylenediaminetetraacetic acid) on the pharmacokinetics of propofol.

3.2.2. Combined datasets that were analysed

Two types of combined datasets were prepared from the data of the seven previously published propofol studies [17-23]:

Type I models: For the type I models, six study populations, i.e. neonates [17], infants [18], toddlers [19], children [20], adolescents [21] and adults [22, 23], were identified from the data of the seven original studies by merging datasets Adults I [22] and Adults II [23] into one adult population. In a systematic manner, two out of these six study populations were selected resulting 15 combined datasets.

Type II models: For the type II models, the data of the seven propofol studies [17-23] were regrouped into five age groups as defined by FDA Guidance for industry of 1998 [16]:

- 1) neonates (birth to 1 month)
- 2) infants (1 month to 2 years)
- 3) children (2 to 12 years)
- 4) adolescents (12 years to 16 years)
- 5) adults (above 16 years)

Four combined datasets were then prepared consisting of one of the four paediatric age groups and the adult group.

In total, 19 models were built on those 19 datasets, each of which either comprised two study populations (type I models) or two FDA age groups (type II models).

3.2.3. Pharmacokinetic modelling

The population pharmacokinetic analysis was performed with the non-linear mixed effects modelling software NONMEM version 7.2. (ICON Development Solutions, Ellicott City, MD, USA) using the first-order conditional estimation method with the interaction option (FOCEI). Tools like S-PLUS interface for

NONMEM (LAP&P Consultants BV, Leiden, NL), S-Plus (version 8.1, Insightful Software, Seattle, WA, USA), XPose and R (version 2.10.0) were used to visualize the output and evaluate the models.

Model Building & Assessment

Propofol concentrations were logarithmically transformed and fitted simultaneously, since the range in concentrations was more than 1000 fold. Model building was performed in three steps: (1) selection of structural model, (2) selection of statistical sub-model, (3) covariate analysis. A difference in objective function (OFV) between models of more than 3.8 points was considered as statistically significant ($p < 0.05$ assuming a Chi-square distribution). Furthermore, the goodness-of-fit plots (observed *versus* individual predicted concentrations and *versus* population predicted concentrations, and conditional weighted residuals *versus* time and *versus* population prediction concentrations) were evaluated [24]. In addition, improvement of the individual concentration-time profiles, the confidence intervals of the parameter estimates, and the correlation matrix were assessed. Besides, stratified observed *versus* population predicted goodness-of-fit plot and *post hoc* clearance *versus* bodyweight plots were considered, as in each of the 19 analyses two populations with a different human age range were analysed [24]. According to Karlsson *et al.*, a high value for shrinkage of the inter-individual variability (η), named as η -shrinkage, may distort the true relationship between the parameters and covariates when empirical Bayes Estimates (EBE), sometimes referred as *post hoc* estimates of parameters, are used [25]. As the *post hoc* clearances were used in our study in the covariate analysis, we evaluated the η -shrinkage for clearance, for which a maximum percentage of 20% was considered acceptable.

Structural Model

Based on previous reports [26-28], the time-course of propofol concentrations in most combined datasets were modelled with a three-compartment model, which was parameterized in terms of total clearance (CL), volume of distribution of the central compartment (V1), volume of distribution of the rapid-equilibrating peripheral compartment (V2) and slow-equilibrating peripheral compartment (V3), and inter-compartmental clearances between central compartment and two peripheral compartments (Q2,Q3). In two models that were built on the datasets that included individuals from the infant study [18],

a two compartment model was the most suitable structural model because, due to the lack of samples in that period, the very fast distribution process could not be identified.

Statistical Model

Inter-individual variability in the pharmacokinetic parameters was tested in the model assuming log-normal distributions, expressed as

$$\theta_i = \theta_{TV} \times e^{\eta_i} \quad , \quad \eta_i \sim N(0, \omega^2) \quad (2)$$

where θ_i is the individual pharmacokinetic parameter value for the i th individual, θ_{TV} is the population pharmacokinetic parameter value or typical value, and η_i is a random variable for the i th individual from a normal distribution with mean zero and variance ω^2 . While the inclusion of inter-individual variability on the different PK parameters was tested, model improvement by inclusion of covariance between these variability parameters was tested as well.

For the residual error, an additive model for log-transformed concentrations was used which corresponds to proportional error on untransformed data, expressed as:

$$\log C_{ij} = \log C_{predij} \times \varepsilon_{ij} \quad , \quad \varepsilon_{ij} \sim N(0, \sigma^2) \quad (3)$$

where C_{ij} is the value of the observed propofol concentration of i th individual at time j , C_{predij} is the value of the predicted propofol concentration of the i th individual at time j , and ε_{ij} is a random variable for this observation from a normal distribution with mean zero and variance σ^2 .

Covariate Model

In all 19 combined datasets, *post hoc* propofol clearances were described by bodyweight using the allometric scaling function of equation (1). For the other pharmacokinetic parameters, bodyweight was also incorporated in an allometric manner (equation 4) if this would decrease the OFV significantly ($p < 0.005$).

$$P_i = P_{TV} \times \left(\frac{BW_i}{BW_{median}} \right)^k \quad (4)$$

In this equation, P_i is the individual parameter; P_{TV} is the population parameter; BW_i and BW_{median} are corresponding to the individual and median bodyweight; k is the allometric exponent. Allometric scaling functions for clearance and/or covariate functions for other parameters were accepted if the criteria described under Model Building & Assessment were met ($p < 0.05$).

Table 3-1 Characteristics of the six study populations used in the type I models

Study-population	Reference	Number of individuals	Weight (kg)	Age (yrs)	Samples per subject (range)
Neonates	[17]	25	2.82(0.68-4.03)	8(1-25) †	4-14
Infants	[18]	20	9(4.8-12.5)	10.2(3.8-17.3) ‡	4-15
Toddlers	[19]	12	11.2(8.74-18.9)	1.25(1-2.6)	11-12
Children	[20]	53	23.3(15-60.5)	7(3-11)	5-18
Adolescents	[21]	14	51(36.6-82)	14.5(9.6-19.8)	6-21
Adults	[22,23]	48	79.4(44.4-122.7)	53(26-81)	18-21

† age in days

‡ age in months

3.3. Results

An overview of the data of the seven propofol pharmacokinetic studies [17-23], which were merged into six study populations for the type I models and into five paediatric FDA age groups for the type II models, are summarized in Table 3-1 and Table 3-2, respectively.

Table 3-3 provides an overview of the results of all 19 models (15 Type I models and 4 Type II models) indexed by the model number with their estimated allometric exponent, including the relative standard error (RSE%) and corresponding ninety-five percent confidence interval (95%CI), for propofol clearance. Information on model structure, inter-individual variability on clearance and shrinkage values for the inter-individual variabilities on clearance are also listed in Table 3-3. Shrinkage values for clearance for all models were very low with values varying between 2.06% and 13.45%, indicating acceptable reliability of individual clearance values from the model. Given the designated allometric scaling model for clearance, all models were optimized in the covariate analysis with respect to covariates for pharmacokinetic parameters other than clearance in order to minimize the objective function and obtain

optimal goodness-of-fit plots. Diagnostic plots (observed versus population predicted plots) of the 15 type I models are shown in Figure 3-1, while the diagnostic plots for the 4 type II models are presented in Figure 3-2.

Table 3-2 Characteristics of age groups according to FDA guidance [16] used in the type II models

Age group	Number of individuals	Weight (kg)	Age (yrs)	Samples per subject (range)
Neonates	25	2.82(0.68-4.03)	8(1-25) †	4-14
Infants	31	9(4.8-14.2)	304.8(113.7-689) †	4-15
Children	58	36.6(11.2-74)	9.6(2-11.3)	5-18
Adolescents	9	53(40-82)	14.5(13.6-15.7)	6-21
Adults	48	79.4(44.4-122.7)	53(26-81)	18-21

† age in days

Of the type I models, 5 models (model 1-5) included the neonate population. Estimation of the allometric exponent for clearance in those five models resulted in values varying between 1.11 and 2.01 (Table 3-3). The performance of those models (model 1-5) in terms of goodness of fit were quite adequate as shown in Figure 3-1 although there was some bias left. In the log-log scaled *post hoc* clearances versus bodyweight plot (Figure 3-3 A), all *post hoc* individual clearances from model 1 to model 5 are shown, with the allometric scaling functions that resulted from these models (see Table 3-3 for estimated exponents). In addition, the $\frac{3}{4}$ fixed allometric scaling line that was extrapolated from the adult sub-population was inserted to Figure 3-3 as a reference line. Figure 3-3 A shows that for model 1-5, none of the allometric functions estimated in the models was able to capture the change in clearance within the preterm and term neonate subpopulation completely, independently from which other sub-population they were scaled from.

There were 10 type I models (model 1-4, 6-8,10-11,13, Table 3-3) which scaled clearance within two different paediatric populations. The estimated allometric exponent in those models varied largely with values between 0.20 (model 6) and 2.01 (model 1) without a trend (Table 3-3). The diagnostic plots of those 10 models were good except for some small bias when the infant population was included (Figure 3-1). In Figure 3-3, the panels B, C, D and E depict the *post hoc* individual clearance values and estimated scaling curves of the models scaling

to infants (model 6, 7, 8, 9, Figure 3-3 B), toddlers (Model 10, 11, 12, Figure 3-3 C), children (Model 13, 14, Figure 3-3 D) and adolescents (Model 15, Figure 3-3 E). These subfigures 3B-3E suggest that with increasing age of the target scaling population, the range in *post hoc* clearances was smaller. In addition, these subfigures show that with increasing age, the scaling lines deviate less from the $\frac{3}{4}$ allometric line.

Table 3-3 Model results including estimated allometric exponent for clearance (Exp_{cl}) for type I models on two study populations (model 1-15) and type II models on one paediatric FDA age group and the adult dataset (model 16-19)

Model number	Younger sub population	Older sub population	Structure Model	Exp_{cl} (RSE%)	95% CI	η_{cl} % (RSE%)	Shrink η_{cl} %
1	Neonate	Infant	3-COM	2.01 (15.20%)	1.41-2.61	79% (21.30%)	4.38%
2	Neonate	Toddler	3-COM	1.64 (12.40%)	1.24-2.04	81% (19.40%)	2.55%
3	Neonate	Child	3-COM	1.39 (6.80%)	1.20-1.58	54% (16.30%)	2.06%
4	Neonate	Adolescent	3-COM	1.13 (5.40%)	1.01-1.25	75% (17.40%)	2.31%
5	Neonate	Adult	3-COM	1.11 (5.50%)	0.99-1.23	46% (17.90%)	2.37%
6	Infant	Toddler	3-COM	0.20 (98%)	-0.18-0.57	34% (13.60%)	4.20%
7	Infant	Child	3-COM	0.46 (16.60%)	0.31-0.61	28% (11.60%)	7.43%
8	Infant	Adolescent	2-COM	0.32 (16.30%)	0.21-0.42	24% (13.90%)	8.25%
9	Infant	Adult	2-COM	0.56 (7.40%)	0.48-0.64	21% (10.70%)	12.08%
10	Toddler	Child	3-COM	0.88 (9.20%)	0.72-1.04	22% (10.60%)	9.90%
11	Toddler	Adolescent	3-COM	0.72 (6.20%)	0.63-0.81	17% (14.30%)	5.64%
12	Toddler	Adult	3-COM	0.81 (4.20%)	0.74-0.87	18% (9.90%)	6.88%
13	Child	Adolescent	3-COM	0.55 (11.10%)	0.43-0.67	21% (8.80%)	5.12%
14	Child	Adult	3-COM	0.69 (4.90%)	0.62-0.75	19% (8.50%)	7.46%
15	Adolescent	Adult	3-COM	0.84 (11.90%)	0.64-1.03	19% (9.40%)	4.77%
16	FDA neonate	Adult	3-COM	1.11 (5.50%)	0.99-1.23	46% (17.90%)	2.37%
17	FDA infant	Adult	2-COM	0.60 (6.00%)	0.53-0.67	22% (10.70%)	13.45%
18	FDA child	Adult	3-COM	0.70 (4.90%)	0.63-0.77	20% (7.80%)	7.15%
19	FDA adolescent	Adult	3-COM	0.74 (17.00%)	0.49-0.98	18% (10.20%)	5.23%

Younger subpopulation = the younger sub-population of the combined dataset of type I or type II models; Older sub-population = the older sub-population of the combined dataset of type I or type II models; 3-COM = three-compartment model; 2-COM = two-compartment model; Exp_{cl} (RSE%) = estimate of the allometric exponent for clearance (equation 1) and corresponding relative standard error in percentage; 95%CI = ninety-five percent confidence interval of the estimate of the allometric exponent for clearance; η_{cl} % (RSE%) = estimate of inter-individual variability of clearance in percentage and corresponding relative standard error in percentage; Shrink η_{cl} % = shrinkage of the inter-individual variability of clearance in percentage

In the four type II models (model 16-19), modelling was performed on combined datasets comprising data from the adult population and data

from one paediatric age group according to the FDA guideline [16] that was extracted from the available merged dataset.

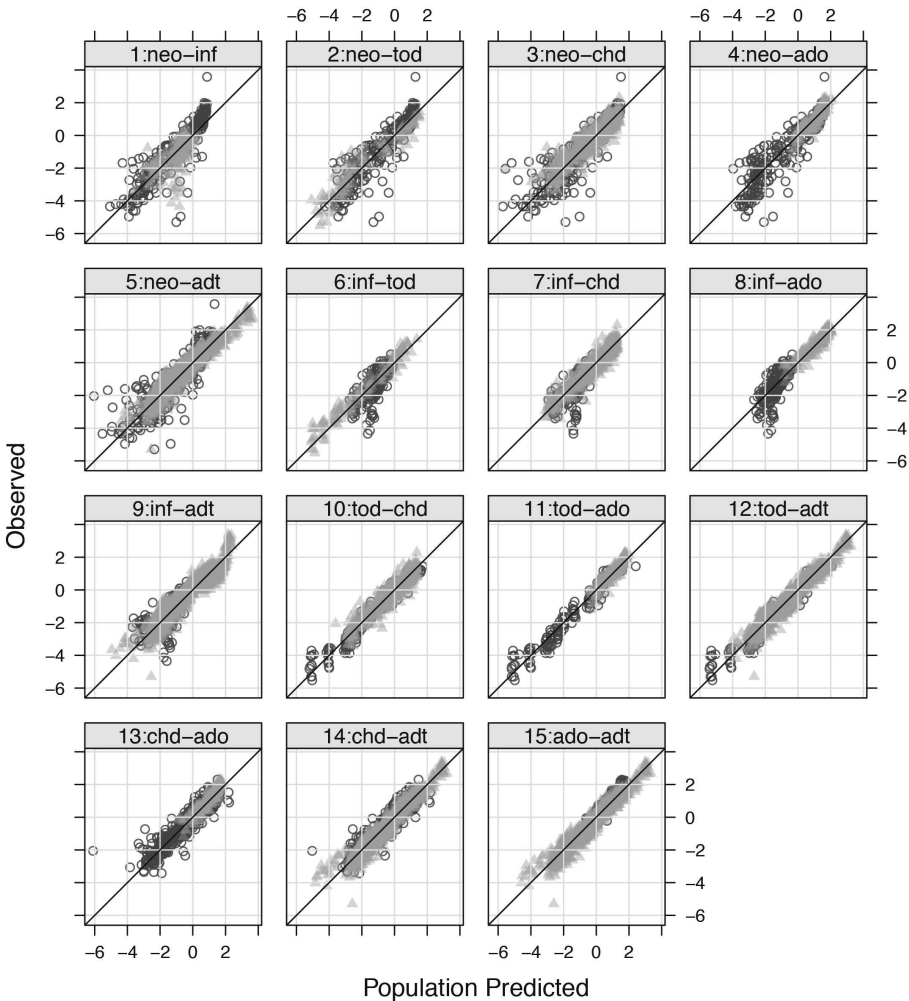


Figure 3-1 Observed versus population predicted concentration plots for type I models (model 1-15), each of which was based on a combined dataset comprising two out of the six study populations of Table 3-1 (neonates, infants, toddlers, children, adolescents, and adults).

Open circle: younger sub-population of the combined dataset (Table 3-3); Filled triangle: older sub-population of the combined dataset (Table 3-3); neo=neonates, inf=infants, tod=toddlers, chd=children, ado=adolescents, adt=adults.

The estimated allometric exponent values were relatively close to each other when scaling from FDA adults to infants, children and adolescents (0.60, 0.70

and 0.74, respectively), while for scaling from adults to neonates a value higher than 1 was identified (i.e. 1.11) (Table 3-3). Figure 3-3 F illustrates the results of these type II models 16-19 with *post hoc* clearances and scaling curves estimated in the models versus bodyweight. This figure shows that scaling to infants leads to the lowest value for the allometric exponent (i.e. 0.60) and scaling to neonates to the highest value for the allometric exponent (i.e. 1.11), with the latter having a wide variability in *post hoc* clearances.

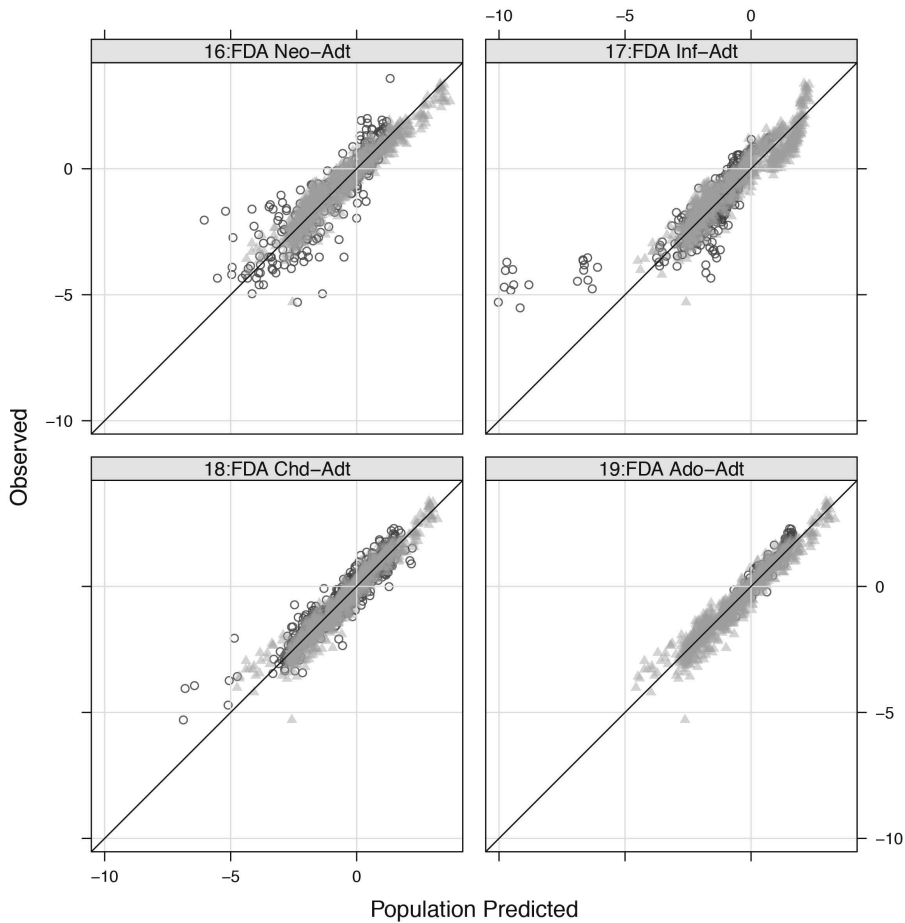


Figure 3-2 Observed versus population predicted concentration plots for type II models (model 16-19), each of which was based on a combined dataset comprising one paediatric FDA age group (neonates: birth-1 month, infants: 1 month- 2 years, children: 2 years-12 years, and adolescents: 12 years-16 years [16]) and one adult (above 16 years) age group (Table 3-2).

Open circle: younger sub-population of the combined dataset (Table 3-3); Filled triangle: older sub-population of the combined dataset (Table 3-3); neo=neonates, inf=infants, chd=children, ado=adolescents, adt=adults

3.4. Discussion

The allometric scaling method is often propagated when scaling for size in paediatric pharmacokinetic modeling [5] while there is more recently also interest for this scaling function when scaling for size in (morbid) obesity [29, 30]. Particularly in early drug development when based on adult data a first-time-in-kids dose needs to be selected, this $\frac{3}{4}$ allometric scaling approach for scaling clearance seems attractive. In addition, as the fixed value of 0.75 for the allometric exponent of clearance has also lead to acceptable results in children [12, 31-34], its use is increasingly popular for scaling between paediatric populations. However, as this allometric scaling theory is particularly based on the combination of the 0.75 fixed allometric equation together with an age-based maturation function [5], the question is how valid the value of the exponent of 0.75 is in absence of these age-based functions which are often not available. Therefore in this study, where relatively rich pharmacokinetic datasets of propofol were available across the entire human age range, a series of hypothetical analyses were performed to identify the allometric exponent for clearance between populations that varied in age.

The results of this study show that a large variety in the value for the allometric exponent for clearance can be expected ranging from 0.2 to 2.01, when two paediatric populations are analysed (model 1-4, 6-8, 10-11, 13, 15). While the lowest exponent of 0.2 was identified between infants and toddlers (model 6), the highest exponent of 2.01 was found between neonates and infants (model 1). These findings seem in accordance with previous reports stating that the fixed $\frac{3}{4}$ allometric function is inappropriate to describe and predict drug clearance in preterm and term neonates, infants and young children, as it systematically over-predicts clearance for neonates and under-predicts clearances for infants [6, 21, 35, 36]. In addition, for busulfan clearance across very young neonates to adolescents, Paci *et al.* also identified two exponents; an exponent of 1.25 for children < 9 kg and an exponent of 0.76 for children > 9 kg [37]. Concerning our finding of an exponent of 0.88 for toddlers and children (model 10), this value seems in good agreement with findings on oxycodone clearance in children 6 months to 7 years reporting a value of 0.875 [13]. It therefore seems from these findings that for scaling clearance between two paediatric populations, the allometric exponent needs to be estimated

instead of fixed to 0.75 in order to account for differences in maturation rates in different age groups. However, as with increasing age the estimated allometric scaling line moved slowly towards 0.75 (Figure 3-3 B to E), it may seem that $\frac{3}{4}$ allometric scaling function may be of value in older children (>3 or 4 yrs).

In drug development situations, paediatric pharmacokinetic information is necessary if the drug will be prescribed for paediatric population. A decision tree has been proposed by the FDA [38] to determine when and what kind of paediatric study (PK, PD, safety) should be conducted, depending on similarities in disease and response to treatment between children and adults [16, 38]. Adequate selection of the first-time-in-kids dose is thereby highly relevant, which is in early drug development based on results of adult PK studies. Our type II models mimic this situation by studying the allometric exponent between the adult group and one paediatric group defined according to the age range defined by FDA (0-1 month, 1 month-2 years, 2-12 years and 12-18 years) [16]. The results show that among FDA adolescents and children the exponent of the allometric scaling curve is close to 0.75 (0.74 and 0.70, respectively, Figure 3-3 F) at low inter-individual variability in clearance (18% and 20%, respectively). For adolescents, this result seems in accordance with the recent conclusion of the FDA advisory committee which agreed that dose(s) for adolescents (>12 years) can be derived from adults data on the basis of allometric scaling with a fixed exponent of 0.75 without the need for a dedicated PK study [39]. In contrast, the estimates of the allometric exponents in FDA defined groups of infants and neonates were found to deviate from 0.75 (0.60 and 1.11 respectively), while the resulting allometric functions were also not capable of describing all individuals across these two groups (Figure 3-3 F, infants and neonates). As such it seems that extrapolation from adults to infants and neonates is not possible using either $\frac{3}{4}$ allometric scaling or allometric scaling with another exponent, while scaling to adolescents and potentially children older than toddler age (3 or 4 yrs) could be considered.

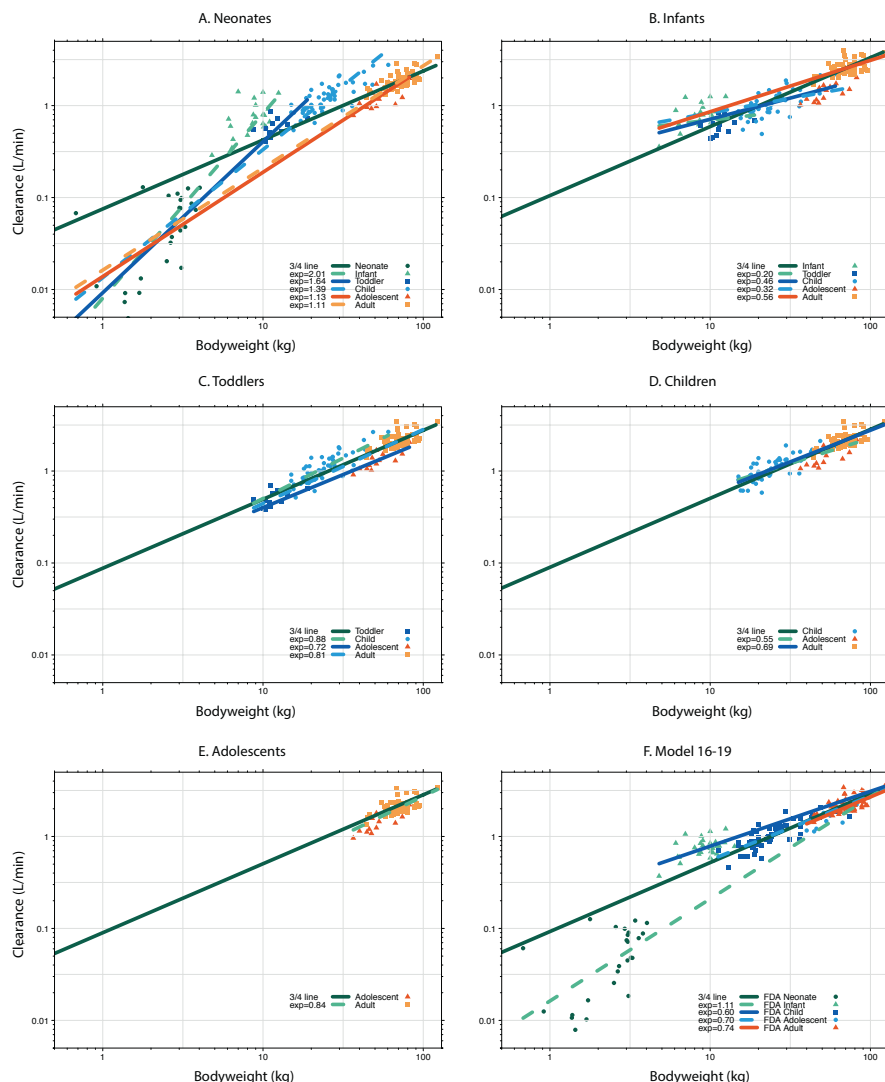


Figure 3-3 *Post hoc* individual clearance values (symbol) and estimated allometric function from the model (line) versus bodyweight plots for all 19 Type I and Type II models in log-log scale (with $\frac{3}{4}$ reference line).

A. Neonates): *Post hoc* clearances versus bodyweight from the five type I models that comprise the neonates sub-population (model 1-5, Table 3-3). Dark green filled circles are median *post hoc* clearances of neonates from the models 1-5; Light green filled triangle: *post hoc* clearances of infants from model 1; Dark blue filled square: *post hoc* clearances of toddlers from model 2; Light blue filled circle: *post hoc* clearances of children from model 3; Dark orange filled triangle: *post hoc* clearances of adolescents from model 4; Light orange filled square: *post hoc* clearances of adults from model 5; Dark green solid line: $\frac{3}{4}$ allometric scaling line that scales from the adult population; Light green dash line: the allometric scaling line with exponent value 2.01 from model 1; Dark blue solid line: the allometric scaling line with exponent value 1.64 from model 2; Light blue dash line: the allometric scaling line with exponent

value 1.39 from model 3; Dark orange solid line: the allometric scaling line with exponent value 1.13 from model 4; Light orange dash line: the allometric scaling line with exponent value 1.11 from model 5

B. Infants): *Post hoc* clearances versus bodyweight from the four type I models that comprise the infants population (model 6-9, Table 3-3). Light green filled triangles are median *post hoc* clearances of infants from the models 6-9; Dark blue filled square: *post hoc* clearances of toddlers from model 6; Light blue filled circle: *post hoc* clearances of children from model 7; Dark orange filled triangle: *post hoc* clearances of adolescents from model 8; Light orange filled square: *post hoc* clearances of adults from model 9; Dark green solid line: $\frac{3}{4}$ allometric scaling line that scales from the adult population; Light green dash line: the allometric scaling line with exponent value 0.20 from model 6; Dark blue solid line: the allometric scaling line with exponent value 0.46 from model 7; Light blue dash line: the allometric scaling line with exponent value 0.32 from model 8; Dark orange solid line: the allometric scaling line with exponent value 0.56 from model 9

C. Toddlers): *Post hoc* clearances versus bodyweight from the three type I models that comprise the toddlers population (model 10-12, Table 3-3). Dark blue filled squares are median *post hoc* clearances of toddlers from the models 10-12; Light blue filled circle: *post hoc* clearances of children from model 10; Dark orange filled triangle: *post hoc* clearances of adolescents from model 11; Light orange filled square: *post hoc* clearances of adults from model 12; Dark green solid line: $\frac{3}{4}$ allometric scaling line that scales from the adult population; Light green dash line: the allometric scaling line with exponent value 0.88 from model 10; Dark blue solid line: the allometric scaling line with exponent value 0.72 from model 11; Light blue dash line: the allometric scaling line with exponent value 0.81 from model 12

D. Children): *Post hoc* clearances versus bodyweight from the two type I models that comprise the children population (model 13-14, Table 3-3). Light blue filled circles are median *post hoc* clearances of children from the models 13 and 14; Dark orange filled triangle: *post hoc* clearances of adolescents from model 13; Light orange filled square: *post hoc* clearances of adults from model 14; Dark green solid line: $\frac{3}{4}$ allometric scaling line from the adult population; Light green dash line: the allometric scaling line with exponent value 0.55 from model 13; Dark blue solid line: the allometric scaling line with exponent value 0.69 from model 14

E. Adolescents): *Post hoc* clearances versus bodyweight from the type I model that comprises the adolescent population (model 15). Dark orange filled triangle: *post hoc* clearances of adolescents from model 15; Light orange filled square: *post hoc* clearances of adults from model 15; Dark green solid line: $\frac{3}{4}$ allometric scaling line that scales from the adults population; Light green dash line: the allometric scaling line with exponent value 0.84 from model 15

F. Model 16-19): *Post hoc* clearances versus bodyweight from the type II models, each of which was based on a combined datasets comprising one paediatric FDA age group (neonates: birth-1 month, infants: 1 month- 2 years, children: 2 years-12 years, and adolescent: 12 years-16 years) and one adult (above 16 years) age group (model 16-19, Table 3-3). Dark orange filled triangles are median *post hoc* clearances of adults from four models; Dark green filled circle: *post hoc* clearances of neonates from model 16; Light green filled triangle: *post hoc* clearances of infants from model 17; Dark blue filled square: *post hoc* clearances of children from model 18; Light blue filled circle: *post hoc* clearances of adolescents from model 19; Dark green solid line: $\frac{3}{4}$ allometric scaling line that scales from the adults population; Light green dash line: the allometric scaling line with exponent value 1.11 from model 16; Dark blue solid line: the allometric scaling line with exponent value 0.60 from model 17; Light blue dash line: the allometric scaling line with exponent value 0.70 from model 18; Dark orange solid line: the allometric scaling line with exponent value 0.74 from model 19

In the models analysing neonates as one of the two groups, we found in our study that estimates for the allometric exponent for clearance were larger than 1, and were larger than the estimates for the exponent in other paediatric groups. Beside propofol, an exponent larger than 1 has been reported before for

morpine in (preterm) neonates to children of 3 years of age [15]. Also, Mahmood reported that the exponent of 1.2 performs better compared to an exponent of 1.0 when predicting drug clearance in children < 3 months, while an exponent of 1.0 was superior over an exponent of 1.2 for children ≥ 3 months to 1 year [40]. In addition, Paci *et al.* found two different allometric exponents for busulfan clearance, with an exponent larger than 1 for children < 9 kg [37]. This finding that in neonates the value for the allometric exponent for clearance is high, while lower values are identified at higher age and bodyweight ranges, are captured in our recently developed bodyweight dependent exponent (BDE) model [8]. In this BDE model, changes in propofol clearance across the entire human life-span were very well described using an allometric function in which the exponent was allowed to vary with bodyweight (range 1.34 for neonates to 0.55 for adults), without the need for an additional age-base function [8]. Considering the results of the current study in relation to the full analysis of all datasets [8], it seems that fairly similar exponents are identified, i.e. values between 1 and 1.5 for neonates to values between 0.5 and 1 for older children and adults. More recently, this BDE function in a simplified manner was also applied with success to busulfan for children from 1 month to adults in which the exponent was found to vary from 1.21 to 0.54 [9]. Given the similarities in these exponents, it seems that this BDE model should be studied across different drugs for which data are available over the entire human age range including neonates, as it may capture in a continuous function changes in clearance from neonates to adults despite the fact that maturation rates may vary at different ages.

In this study, we investigated the allometric scaling approach for clearance of propofol. As propofol is a high hepatic extraction ratio drug and it is mainly metabolised by the UGT-1A9 iso-enzyme, the results may not be necessarily the same for other drugs which have medium or low extraction ratio or have different metabolism pathway. We also recognize the allometric scaling approach is not physiologically based and it cannot explain the physiological mechanisms, such as the maturation of enzyme capacity etc. Furthermore, it should be considered that the estimated allometric exponents for clearance in this study may be influenced by the inclusion of covariates for other parameters such as volume of distribution. Given such limitations, we can only assure our findings for propofol and the feasibility and boundary of the allometric scaling approach for other drugs remains to be investigated.

3.5. Conclusion

Different allometric exponents for propofol clearance were identified depending on the included age-range, with the largest difference in allometric exponent between neonates and infants and between infants and toddlers (2.01 versus 0.2, respectively). Our findings show that for scaling clearance, $\frac{3}{4}$ allometric scaling may be of value for scaling from adults to adolescents and perhaps children, while it can not be used for scaling from adults to neonates, within neonates or between two paediatric populations.

3.6. Acknowledgements

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Appendix: NONMEM code of the model

Example model with 3 compartments

```

$SUBROUTINE ADVAN11 TRANS4
$PK
TVCL = THETA(1)*(BW/68)**THETA(7)
CL    = TVCL*EXP(ETA(1))
Q2    = THETA(2)*EXP(ETA(2))
TVQ3  = THETA(3)*(BW/68)
Q3    = TVQ3*EXP(ETA(3))
TVV1  = THETA(4)*(BW/68)
V1    = TVV1*EXP(ETA(4))
TVV2  = THETA(5)*(BW/68)
V2    = TVV2*EXP(ETA(5))
TVV3  = THETA(6)*(BW/68)
V3    = TVV3*EXP(ETA(6))
S1    = V1
ETCL  = ETA(1)
ETQ2  = ETA(2)
ETQ3  = ETA(3)
ETV1  = ETA(4)
ETV2  = ETA(5)
ETV3  = ETA(6)

$ERROR
IPRE = LOG(.000001)
IF (F.GT.0) IPRE=LOG(F)
IRES=DV-IPRE
W = 1
IWRES=IRES/W
Y=IPRE+W*ERR(1)

$THETA
(0, 1.6, ) ; TH1 CL
(0, 1.7, ) ; TH2 Q2

```

```
( 0, 1.5, ) ; TH3 Q3
( 0, 4, ) ; TH4 V1
( 0, 5, ) ; TH5 V2
( 0, 100, ) ; TH6 V3
( 0, 1.3, ) ; TH7 EXP of BW on clearance
```

```
$OMEGA
0.2 ; OMEGA1 CL
0 FIX ; OMEGA2 Q2
0.2 ; OMEGA3 Q3
0.2 ; OMEGA4 V1
0 FIX ; OMEGA5 V2
0.3 ; OMEGA6 V3
```

```
$SIGMA
0.1 ; SIGMA1
```

```
$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION
$COV COMP PRINT=E
```

Example model with 2 compartments

```
$SUBROUTINE ADVAN3 TRANS4
$PK
TVCL = THETA(1)*(BW/63.5)**THETA(5)
CL = TVCL*EXP(ETA(1))
TVQ = THETA(2)*(BW/63.5)
Q = TVQ*EXP(ETA(2))
TVV1 = THETA(3)*(BW/63.5)
V1 = TVV1*EXP(ETA(3))
TVV2 = THETA(4)*(BW/63.5)
V2 = TVV2*EXP(ETA(4))
S1 = V1
ETCL = ETA(1)
ETQ = ETA(2)
```


ETV1 =ETA(3)

ETV2 =ETA(4)

;

\$ERROR

W=1

IPRE = LOG(.000001)

IF (F.GT.0) IPRE=LOG(F)

IRES=DV-IPRE

IWRES=IRES/W

*Y=IPRE+W*ERR(1)*

;

\$THETA

(0, 1.2,) ; TH1 CL

(0, 0.8,) ; TH2 Q

(0, 5,) ; TH3 V1

(0, 20,) ; TH4 V2

(0, 0.5,) ; TH5 EXP of BW on CL

\$OMEGA

0.15 ; OMEGA1 CL

0 FIX ; OMEGA2 Q

0 FIX ; OMEGA3 V1

0.1 ; OMEGA4 V2

\$SIGMA

0.1 ; SIGMA1

\$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION

\$COV COMP PRINT=E





Chapter 4

A bodyweight dependent allometric exponent for scaling clearance across the human life-span

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Abstract

Purpose: In this study, we explored different allometric equations for scaling clearance across the human life-span using propofol as a model drug.

Methods: Data from seven previously published propofol studies ((pre)term neonates, infants, toddlers, children, adolescents and adults) were analysed using NONMEM VI. To scale clearance, a bodyweight-based exponential equation with four different structures for the exponent was used: (I) $\frac{3}{4}$ allometric scaling model; (II) mixture model; (III) bodyweight-cut-point separated model; (IV) bodyweight-dependent exponent model.

Results: Model I adequately described clearance in adults and older children, but overestimated clearance of neonates and underestimated clearance of infants. Use of two different exponents in Model II and Model III showed significantly improved performance, but yielded ambiguities on the boundaries of the two subpopulations. This discontinuity was overcome in Model IV, in which the exponent changed sigmoidally from 1.35 at a hypothetical bodyweight of 0 kg to a value of 0.56 from 10 kg onwards, thereby describing clearance of all individuals best.

Conclusions: A model was developed for scaling clearance over the entire human life-span with a single continuous equation, in which the exponent of the bodyweight-based exponential equation varied with bodyweight.

4.1. Introduction

For scaling pharmacokinetics across the human life-span, the $\frac{3}{4}$ allometric scaling approach [1] has gained in popularity in the field of pediatrics. While the $\frac{3}{4}$ allometric scaling approach was originally designed to describe metabolic rates between different species covering a wide range in bodyweight [2], this function is now being applied to parameterize the influence of changes in body size on drug clearance parameters within the human weight-range.

In contrast with the reports supporting the $\frac{3}{4}$ allometric scaling principles [3-5], there is a number of theoretical arguments against these scaling principles in general [6-8]. Furthermore, the value of the allometric exponent is debated [9, 10], the existence of a unique and universal value for the exponent is thought to be unlikely [11, 12] and the application of allometric scaling principles to pharmacokinetics in the human weight-range is disputed [13, 14]. For paediatrics, the covering of changes in body size made by this bodyweight-based allometric equation with a fixed exponent of 0.75 for clearance are specifically insufficient to describe and predict drug clearance in preterm and term neonates, infants and young children [13, 15, 16]. When applying the $\frac{3}{4}$ allometric scaling method to different drugs, it has been found that the model systematically over-predicted clearances for neonates and under-predicted clearances for infants [15, 17].

In order to account for the discrepancy of the $\frac{3}{4}$ allometric method in young children, an augmentation of the $\frac{3}{4}$ allometric equation with an age-based sigmoidal equation has been proposed [18, 19]. However, this age-adjustment is unidirectional, whereas bi-directional changes from the $\frac{3}{4}$ allometric line have been reported in the papers aforementioned, that depend on the age of the children [15, 17]. Furthermore, introduction of this age-based maturation equation introduces age into the model in addition to bodyweight. While age and bodyweight are highly correlated in the paediatric population in a nonlinear manner, this may result in a collinearity problem [20]. In this respect, it is emphasized that recent research shows that *a priori* inclusion of a false predefined covariate relationship into a model may decrease the predictive performance of that model [21]. As a result of these limitations, in some reports

the exponent of the allometric scaling equation was estimated instead of fixed to 0.75, resulting in values higher than 1 in datasets of young infants [22, 23]. From these reports, it seems that an optimized and statistically sound scaling approach is needed for scaling of clearance in paediatrics.

The aim of this study was therefore to explore new approaches to scale drug clearance over the entire human life-span. Propofol was used as a model drug, and for this study seven datasets were available from preterm neonates with a median (range) gestational age of 37 (26-40) weeks to 81 year old adults [15, 24-29].

4.2. Materials and methods

4.2.1. Subjects

A total of 174 subjects from seven previously published propofol studies were included in the current study. These studies are described in detail elsewhere and are briefly discussed as relevant to the current analysis.

Neonates [24]

Twenty-five cardiovascularly and respiratory stable neonates with a median of bodyweight of 2.93 (range 0.68-4.03) kilograms, postnatal age of 8 (1-25) days and gestational age of 37 (26-40) weeks were given an intravenous bolus dose of propofol ($3 \text{ mg} \times \text{kg}^{-1}$) for the elective removal of chest tubes, (semi)elective chest tube placement or endotracheal intubation.

Infants [25]

Twenty-two non-ventilated infants after major craniofacial surgery with a median bodyweight of 8.9 (4.8-12.5) kilograms, aged 10 (3.8-17.3) months received $2\text{-}4 \text{ mg} \times \text{kg}^{-1} \cdot \text{h}^{-1}$ propofol during a median of 12.5 (6.0-18.1) hours.

Toddlers [26]

Twelve toddlers with minor burns, who had a median bodyweight of 11.2 (8.7-18.9) kilograms and age of 17.8 (12-31) months, were administered $4 \text{ mg} \times \text{kg}^{-1}$ propofol just before bathing.

Children [27]

Fifty-three healthy unmedicated children with a median bodyweight of 23.3 (15-60.5) kilograms and median age of 7 (3-11) years were studied. Twenty children received an intravenous loading dose of $3 \text{ mg} \times \text{kg}^{-1}$ propofol. In the remaining 33 children, an intravenous loading dose of $3.5 \text{ mg} \times \text{kg}^{-1}$ was followed by a maintenance infusion. In 18 of the 33 children, a single infusion rate of $0.15 \text{ mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ was administered, while 15 children received an infusion of $0.20 \text{ mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ for 30 minutes, followed by an infusion of $0.125 \text{ mg} \times \text{kg}^{-1} \times \text{min}^{-1}$ till the end of the procedure.

Adolescents [15]

Fourteen adolescents with a median bodyweight of 51 (36.6-82) kilograms and median age of 14.7 (9.8-20.1) years were anaesthetized with propofol-remifentanyl ($2\text{-}10 \text{ mg} \times \text{kg}^{-1} \cdot \text{h}^{-1}$) for scoliosis surgery during 6.8 (3.3-7.7) hours with an intra-operative wake-up test followed by re-induction of anesthesia.

Adults I [28]

Twenty-four women undergoing gynaecological surgery, with a median bodyweight of 68.5 (55-80) kilograms and a median age of 45.5 (33-57) years, received $2.5 \text{ mg} \times \text{kg}^{-1}$ propofol over 60 seconds for induction of anesthesia.

Adults II [29]

Twenty-four healthy volunteers with a median bodyweight of 79.4 (44.4-122.7) kilograms and median age of 53 (26-81) years were administered a bolus dose of propofol, followed 1 hour later by a 60 minutes infusion with an infusion rate of 25, 50, 100, or $200 \text{ mg} \times \text{kg}^{-1} \cdot \text{min}^{-1}$ in a study which investigated the influences of method of administration, infusion rate, patient covariates, and EDTA (ethylenediaminetetraacetic acid) on the pharmacokinetics of propofol.

4.2.2. Pharmacokinetic ModelingModel Building

The population pharmacokinetic analysis was performed with the non-linear mixed effects modeling software NONMEM version 6.2. (ICON Development Solutions, Ellicott City, MD, USA) using the first-order conditional estimation method with the interaction option (FOCEI). Tools like S-PLUS interface for NONMEM (LAP&P Consultants BV, Leiden, NL), S-Plus (version 8.1, Insightful

Software, Seattle, WA, USA), XPose and R (version 2.10.0) were used to visualize the output and evaluate the models.

Propofol concentrations were logarithmically transformed and fitted simultaneously, since the range in concentrations was more than 1000 fold. Model building was performed in four steps: (1) selection of structural model, (2) selection of statistical sub-model, (3) covariate analysis, (4) model validation. A difference in objective function (OFV) between models of more than 7.88 points was considered as statistically significant ($p < 0.005$ assuming a Chi-square distribution). Furthermore, the goodness-of-fit plots (observed *versus* individual predicted concentrations and *versus* population predicted concentrations, and conditional weighted residuals *versus* time and *versus* population prediction concentrations) were evaluated [30]. Finally, improvement of the individual concentration-time profiles, the confidence intervals of the parameter estimates and the correlation matrix were assessed.

Structural Model

Based on previous reports [31-33] the time-course of propofol concentrations was modeled with a three-compartment model, which was parameterized in terms of total clearance (CL), volume of distribution of the central compartment (V1), volume of distribution of the rapid-equilibrating peripheral compartment (V2) and slow-equilibrating peripheral compartment (V3), and inter-compartmental clearances between central compartment and two peripheral compartments (Q2,Q3). In addition, the performance of a two compartment model with the parameters CL, Q2, V1 and V2 was evaluated.

Statistical Model

Inter-individual variability in the pharmacokinetic parameters was tested in the model assuming log-normal distributions, expressed as

$$\theta_i = \theta_{TV} \times e^{\eta_i} \quad , \quad \eta_i \sim N(0, \omega^2) \quad (1)$$

where θ_i is the individual pharmacokinetic parameter value for the i th individual, θ_{TV} is the population pharmacokinetic parameter value or typical value, and η_i is a random variable for the i th individual from a normal distribution with mean zero and variance ω^2 . In addition to testing the inclusion of inter-individual

variability on individual parameters, model improvement by inclusion of covariance between these variability parameters was tested as well.

For the residual error, an additive model for log-transformed concentrations was used which corresponds to proportional error on untransformed data, expressed as:

$$\log C_{ij} = \log C_{pred_{ij}} \times \varepsilon_{ij} \quad , \quad \varepsilon_{ij} \sim N(0, \sigma^2) \quad (2)$$

where C_{ij} is the value of the observed propofol concentration of i th individual at time j , $C_{pred_{ij}}$ is the value of the predicted propofol concentration of the i th individual at time j , and ε_{ij} is a random variable for this observation from a normal distribution with mean zero and variance σ^2 .

Covariate Models

To visualize potential relationships, candidate covariates such as age and bodyweight were plotted independently *versus* the empirical Bayes *post hoc* estimates for all pharmacokinetic parameters. Potential covariates were separately implemented into the model using a linear or power equation over the studied covariate range:

$$\theta_i = \theta_{TV} \times \left(\frac{Cov_i}{Cov_{median}} \right)^k \quad (3)$$

In this equation, θ_i represents the individual parameter estimate of the i th subject, θ_{TV} represents the population parameter estimate, Cov is the covariate of interest with subscript *median* indicating the median value of the particular covariate and k is the exponent which was fixed to 1 for a linear function or estimated for a power function.

The significance of a covariate was statistically tested by use of the objective function. A p value < 0.005 was considered significant in the forward inclusion while on the other hand a more stringent p value of <0.001 was used in the backward deletion. In addition, individual and population parameter estimates were plotted against the most predictive covariate to evaluate whether the individual predicted parameters are evenly distributed around the population predicted values [30]. When two or more covariates were found to significantly

improve the model, the covariate causing the largest reduction in the objective function was retained in the model and served as the basis for subsequent inclusion of additional covariates. Furthermore, for the final covariate model, general criteria for model evaluation were considered as described under 'Model Building', while also the results of the model validation procedures were taken into account.

As bodyweight proved to be superior over age as a covariate for clearance, four covariate models based on bodyweight were tested. For propofol clearance, the common structure of these allometric models was:

$$Cl_i = Cl_p \times \left(\frac{BW_i}{70}\right)^k \quad (4)$$

in which Cl_i is clearance in the i th individual with bodyweight BW_i ; Cl_p is the clearance in a standardized adult with a bodyweight of 70 kg; and the exponent k has different values in the four different covariate models as described below:

Model I: The $3/4$ allometric model; in which k was fixed to 0.75.

Model II: The mixture model; in which different values for the exponent k were estimated for two sub-populations. The entire population was assumed to have two subpopulations: POP_1 and POP_2 . For each sub-population different k and Cl_p values were estimated, but the inter-individual variability on Cl was kept the same for both sub-populations. The assignment of individuals to one of the sub-populations was determined by a probability model [34]. Briefly, the probability model assumed two log-normal distributions for clearance with different mean values but common standard deviation for two sub-populations. Each individual has a probability of π belonging to POP_1 and has $1-\pi$ probability of belonging to POP_2 . The mixture model was implemented by the \$MIX option in NONMEM VI.

Model III: The bodyweight-cut-point separated model; in which different values for the exponent k were estimated for two sub-populations: $POP_{bw < d}$ and $POP_{bw \geq d}$. These sub-populations were determined by a cut-point bodyweight d which is a fixed effect parameter in NONMEM: individuals with bodyweight less than the cut-point belonged to $POP_{bw < d}$ and individuals with bodyweight greater than or equal to the cut-point belonged to $POP_{bw \geq d}$.

Model IV: The bodyweight-dependent exponent (BDE) model; in which the allometric exponent k changed in a sigmoidal fashion with bodyweight according to equation 5:

$$k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma} \quad (5)$$

in which BW_i is bodyweight of an individual i ; k_0 is the value of the exponent at a theoretical bodyweight of 0 kg; k_{max} is the maximum decrease of the exponent; k_{50} is the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained, and γ is the Hill coefficient determining the steepness of sigmoidal decline in the exponent.

4.2.3. Model Validation

Stratified bootstrap re-sampling was performed to obtain the same numbers of patients of each of the seven study populations in the new re-sampled datasets compared to the original dataset. This stratified bootstrap was implemented by the “bootstrap” command and the “-stratify_on” option in Perl-speaks-NONMEM (copyright by Mats Karlsson, Niclas Jonsson and Andrew Hooker). The median, standard deviation and the 90% confidence interval of the parameter estimates were calculated based on the results of the runs that minimized successfully.

In addition, the normalized prediction distribution errors (NPDE) method [35] was used as a simulation-based diagnostic. The dataset was simulated 2000 times in NONMEM, each observed concentration was subsequently compared to the simulated reference distribution using the NPDE add-on package in R. A histogram of the NPDE distribution in the total dataset and plots of NPDE *versus* individual predicted concentration and *versus* time were used to evaluate the final model.

In order to evaluate the precision of the population predicted clearances by the four different covariate models for clearance, we used the root mean square error (RMSE) which was calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (Cl_p + Cl_i)^2}{n}} \quad (6)$$

$$\%RMSE = \frac{RMSE \times 100}{\frac{1}{n} \sum_{i=1}^n Cl_i} \quad (7)$$

in which Cl_p is the population predicted clearance; Cl_i is the individual predicted clearance; n is the number of individuals.

4.3. Results

The analysis was based on a total number of 4396 observations from 174 individuals aged between 1 day to 81 years and with a bodyweight varying between 0.68 to 122.7 kilograms. An overview of the datasets used for model building is shown in Table 4-1.

Table 4-1 Overview of propofol pharmacokinetic datasets used in the current analysis (values expressed as median (range))

Population	N	Indication	Weight (kg)	Age (yrs)	Administration	Samples*
Neonates (24)	25	Removal of chest tubes	2.93(0.68-4.03)	8(1-25) †	Bolus	4-14
Infants (25)	20	Post-operative sedation after -Craniofacial surgery	9(4.8-12.5)	10.2(3.8-17.3) ‡	Infusion	4-15
Toddlers (26)	12	Bathing of patients with minor burn injuries	11.2(8.74-18.9)	1.25(1-2.6)	Bolus	11-12
Children (27)	53	Superficial body surface surgery	23.3(15-60.5)	7(3-11)	Bolus&infusion	5-18
Adolescents (15)	14	Scoliosis surgery	51(36.6-82)	14.5(9.6-19.8)	Bolus&infusion	6-21
Female adults(28)	24	Gynecological surgery	68.5(55-80)	45.5(33-57)	Bolus	18
Adults (29)	24	PK study in healthy volunteers	79.4(44.4-122.7)	53(26-81)	Infusion	20-21

† age in days

‡ age in months

* number of samples per subject (range)

A three compartment model performed better than a two compartment model. In this three compartment model, four different bodyweight-based allometric equations for clearance were separately tested and evaluated for their performance. All parameter estimates including their coefficients of variation (CV values) and objective functions of these four covariate models are listed in Table 4-2.

The $\frac{3}{4}$ allometric model (Model I) with a fixed allometric exponent of 0.75 had an OFV of -2339.7 (Table 4-2). With this model population values of propofol clearance in children weighing less than 15 kg, were both over-estimated (in children between 0.5 and 5 kg) and under-estimated (in children between 5 and 15 kg) (Figure 4-1).

For the mixture model (Model II) the OFV decreased significantly ($p < 0.001$) compared to Model I (Table II). The estimated allometric exponent value for clearance of population POP_1 was less than 1 (0.676), whereas its value for clearance of population POP_2 was greater than 1 (1.321) (Table II). The probability of an individual belonging to POP_1 (π) in Model II was found to be 0.2. Figure 4-1 shows that the population line of Model II described the *post hoc* propofol clearances better than Model I.

The bodyweight-cut-point separated model (Model III) had an OFV of -2464.4, which was an increase of 21.8 ($P < 0.001$) points compared to Model II and a decrease of 124.7 points ($p < 0.001$) compared to Model I (Table 4-2). The bodyweight cut-point was estimated to be 16.5 kg, which was fixed to this value later on to obtain a successful covariance step. The estimated exponents of populations $POP_{bw < d}$ and $POP_{bw \geq d}$ were 1.68 and 0.614, respectively (Table 4-2). Figure 4-1 shows that Model III results in improved the fit of the population line to the *post hoc* clearances compared to Model I.

The bodyweight-dependent exponent model (Model IV) had the lowest OFV of -2489 of the four allometric covariate models, which was significantly superior over the $\frac{3}{4}$ allometric model and the bodyweight-cut-point separated model ($p < 0.001$) (Table 4-2). The OFV of this model was not significantly different from the OFV of the mixture model. In order to get a successful covariance step, the exponent of bodyweight at 0 kg (k_0) and the hill factor (γ) were fixed to the estimated values from a successful run without covariance step, which were 1.35 and 5.24, respectively. Visually, Model IV had the best overall distribution of *post hoc* clearances versus population clearance (Figure 4-1). Finally, model IV had the lowest inter-individual variability in clearance (Table 4-2), indicating that this new equation indeed accounts for variability in clearance in the entire range of bodyweight of the subjects.

Table 4-2 Parameter values, corresponding coefficients of variation (CV%), and objective function for the four different covariate models for clearance.

Parameter	Model			
	Model I	Model II	Model III	Model IV
Fixed effect				
Cl (L/min)	$= Cl_p \times (bw/70)^{0.75}$	$= Cl_p \times (bw/70)^k$	$= Cl_p \times (bw/70)^k$	$= Cl_p \times (bw/70)^k$
Cl_p (L/min·70kg)	1.88(5.3%)			1.99(2.5%)
Cl_p (L/min·70kg) of POP ₁		2.52(9.7%)		
of POP ₂		2.13(3.8%)		
Cl_p (L/min·70kg) of POP _{bw<d}			15.4(29.3%)	
of POP _{bw<d}			2.03(2.8%)	
k				
of POP ₁		1.32(4.2%)		
of POP ₂		0.68(8.9%)		
k				
of POP _{bw<d}			1.68(8.4%)	
of POP _{bw<d}			0.61(5.9%)	
p (i=1)		0.2(27.7%)		
d (kg)			16.5 FIX	
$k = k_0 - k_{max} \cdot bw^g / (k_{50} + bw^g)$				
k_0				1.35 FIX
k_{max}				0.79(8.5%)
k_{50} (kg)				3.71(7.4%)
g				5.1 FIX
Q2(L/min)	1.81 (6.2%)	1.82 (6.3%)	1.8 (6.2%)	1.76 (6.6%)
Q3 (L/min)	$= Q3_p \times (bw/70)$	$= Q3_p \times (bw/70)$	$= Q3_p \times (bw/70)$	$= Q3_p \times (bw/70)$
$Q3_p$ (L/min·70kg)	1.55 (4.8%)	1.54(4.8%)	1.55 (4.8%)	1.59 (5.5%)
V1 (L)	5.18 (10.6%)	4.94 (11.4%)	5.06 (11.1%)	4.9 (11.8%)
V2 (L)	$= V2_p \cdot (bw/70)$	$= V2_p \cdot (bw/70)$	$= V2_p \cdot (bw/70)$	$= V2_p \cdot (bw/70)$
$V2_p$ (L/70kg)	23.9 (10.5%)	23.4 (10.8%)	23.1 (10.7%)	23.9 (10.4%)
V3 (L)	$= V3_p \times (bw/70)$	$= V3_p \times (bw/70)$	$= V3_p \times (bw/70)$	$= V3_p \times (bw/70)$
$V3_p$ (L/70kg)	228 (6.8%)	219 (7.0%)	216 (7.0%)	226 (8.6%)
Inter-individual variability				
ω^2 CL	0.44(20.7%)	0.11 (17.4%)	0.21 (24.5%)	0.08(17.1%)
ω^2 V1	1.55(15.2%)	1.52(16.0%)	1.53(15.9%)	1.53(16.3%)
ω^2 Q3	0.23(14.5%)	0.22(16.3%)	0.22(15.7%)	0.27(20.1%)
ω^2 V2	0.81(11%)	0.82(11.4%)	0.81(11.4%)	0.82(11.5%)
ω^2 V3	0.3(17.4%)	0.31(17.8%)	0.32(17.4%)	0.76(39.3%)
Residual error				
σ^2 additive	0.06(10.1%)	0.06(10.2%)	0.06(10.3%)	0.06(10.3%)
OFV	-2339.7	-2486.2	-2464.4	-2489

Model I: $\frac{3}{4}$ allometric model; Model II: the mixture model; Model III: the bodyweight-cut-point separated model; Model IV: the bodyweight-dependent exponent (BDE) model. bw=bodyweight; π = the probability of the individuals belonging to *POP1* in the mixture model (Model II); d = the cut-point in bodyweight dividing the two sub-populations in the bodyweight-cut-point separated model (Model III); k_0 = value of the exponent at a theoretical bodyweight of 0 kg; k_{max} = the maximum decrease of the exponent; k_{50} = the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained; γ = the Hill coefficient determining the steepness of sigmoidal decline in the exponent.

Table 4-3 lists the %RMSE values for the different paediatric subpopulations for all four covariate models. The precision of clearance prediction shows a trend of decrease from adult population to neonate population for the four models. In neonates, infants, and children, the bodyweight-dependent exponent model (Model IV) had the lowest %RMSE, and thus the highest precision, of the four models. In toddlers, the mixture model (Model II) had the lowest %RMSE value compared to other models.

Table 4-3 %RMSE of the four bodyweight-based exponential equations for the different human subpopulations, Model I: $\frac{3}{4}$ allometric model; Model II: the mixture model; Model III: the bodyweight-cut-point separated model; Model IV: the bodyweight-dependent exponent (BDE) model.

Population	Model I (%)	Model II (%)	Model III (%)	Model IV (%)
Neonates	160	149	61	49
Infants	63	41	53	40
Toddlers	25	37	38	33
Children	32	26	26	23
Adolescents	24	53	30	27
Adults I & II	24	26	21	20

Based on these results, Model IV was selected as covariate model and further optimized. The parameter estimates of the final PK model are listed in **Table 4-4**. **Figure 4-2** shows how the bodyweight dependent exponent for clearance (k , equation 5) changes with bodyweight according to the estimated parameters of equation 5 in **Table 4-4**. The figure shows that k decreased from a highest value of 1.35 (k_0) at the theoretical bodyweight zero to a lowest value of 0.65 ($k_0 - k_{max}$). At a bodyweight of 3.78 kg (k_{50}), half of the maximum decrease was reached (**Table 4-4**). The Hill coefficient (γ) of 5.24 reflected the rapid decrease in the exponent (k) with bodyweight (**Figure 4-2**).

Table 4-4 Parameter estimates, bootstrap results and their corresponding coefficients of variation (CV%) values for the final PK model.

Parameter	Final model	Bootstrap [†]
Fixed effect		
Cl (L/min)	$= Cl_p \cdot (bw/70)^k$	$= Cl_p \cdot (bw/70)^k$
Cl_p (L/min·70kg)	2.02(2.6%)	2.02(2.5%)
$k = k_0 - k_{max} \cdot bw^9 / (k_{50}^9 + bw^9)$		
k_0	1.34 FIX	1.35(6.2%)
k_{max}	0.79(12.2%)	0.80(10.7%)
k_{50} (kg)	3.78(15.1%)	3.75(7.5%)
g	5.24 FIX	5.25(41.6%)
Q2(L/min)	1.77(6.3%)	1.72(7.9%)
Q3 (L/min)	$= Q3_p \times (bw/70)$	$= Q3_p \times (bw/70)$
$Q3_p$ (L/min·70kg)	1.65(5.0%)	1.64(4.8%)
V1 (L)	if $PNA \geq 100$ then $= V1_p$, if $PNA < 100$ then $= V1_p \times (bw/70)$	
$V1_p$ (L)	7.58(12.4%)	7.69(10.1%)
V2 (L)	$= V2_p \cdot (bw/70)^m$	$= V2_p \cdot (bw/70)^m$
$V2_p$ (L/70kg)	15.5(14.7%)	15.7 (11.4%)
m	0.55(17.5%)	0.55(10.8%)
V3 (L)	$= V3_p \times (bw/70)$	$= V3_p \times (bw/70)$
$V3_p$ (L/70kg)	221(8.9%)	225(8.9%)
Inter-individual variability		
ω^2 CL	0.09(18.0%)	0.08(19.4%)
ω^2 V1	1.19(41.3%)	1.22 (18.3%)
ω^2 Q3	0.25(17.0%)	0.26(19.9%)
ω^2 V2	0.52(40.8%)	0.54(16.6%)
ω^2 V3	0.71(44.0%)	0.75(46%)
Residual error		
σ^2 additive	0.06(10.3%)	0.06(10.1%)
OFV	-2580.7	

† Mean estimated parameter values and their coefficient of variation in percentage from 200 stratified bootstrapping re-samples. bw=bodyweight; k_0 = the exponent at a theoretical bodyweight of 0 kg; k_{max} = the maximum decrease of the exponent; k_{50} = the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained; γ = the Hill coefficient determining the steepness of sigmoidal decline in the exponent; m = the exponent of bodyweight on V2.

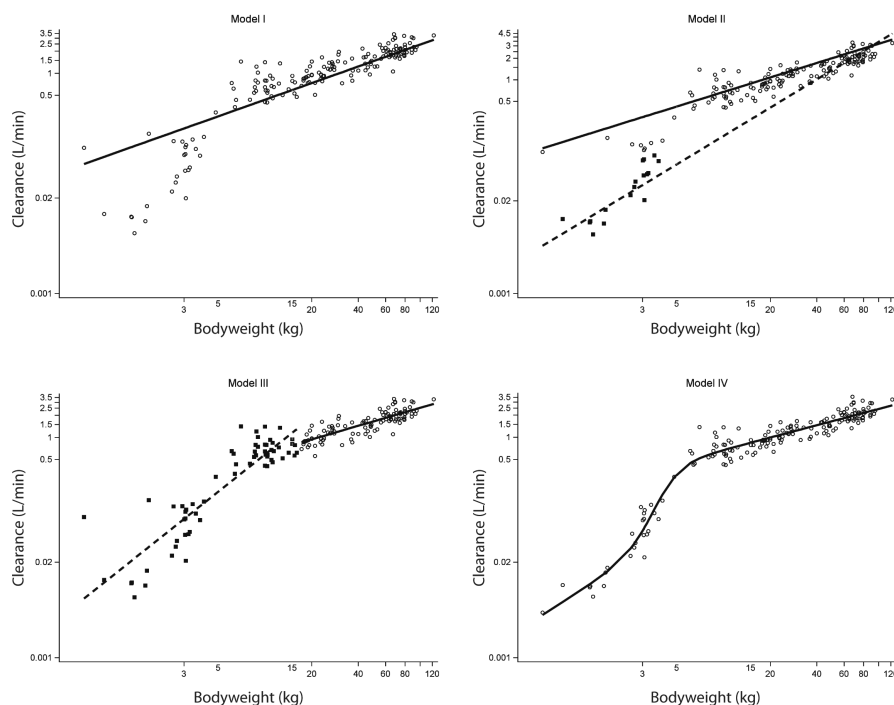


Figure 4-1 Plots of post hoc and population clearance values versus bodyweight.

For the 3/4 allometric model (Model I), ○: individual *post hoc* clearances, solid line: population predicted clearance curve; the mixture model (Model II), ■: individual *post hoc* clearances of POP₁, ○: individual *post hoc* clearances of POP₂, dashed line: population predicted clearance curve of the POP₁, solid line: population predicted clearance curve of POP₂; the bodyweight-cut-point separated model (Model III), ■: individual *post hoc* clearances of POP_{bw<df}, ○: individual *post hoc* clearances of POP_{bw≥df}, dashed line: population predicted clearance curve of the POP_{bw<df}, solid line: population predicted clearance curve of POP_{bw≥df}; the bodyweight-dependent exponent (BDE) model (Model IV), ○: individual *post hoc* clearances, solid line: population predicted clearance curve.

Concerning other parameters than clearance, a linear function for bodyweight on V1 for the children who were younger than 100 days was identified, while for V2 a power equation based on bodyweight was found. Inclusion of these covariates resulted in a further decrease of 91.7 point ($p < 0.001$) in the OFV compared to Model IV. The observed *versus* population predicted plots stratified by study in Figure 4-3 confirm that the final model not only describes the study population as a whole, but also describes the individual study populations without bias. Results of the bootstrap analysis show that the median estimated values based on resampled data were close to the estimated values from the final model fit of the original data and that all CV percentages are below 50%. This suggests the final model to be stable and the estimated

parameter values to be accurate and precise. The results of the bootstrap are of particular relevance for the parameters that were fixed in model IV, i.e. k_0 and γ , as they justify the actual value these parameters were fixed to. Furthermore, the results from the normalized prediction distribution errors (NPDE) analysis in **Figure 4-4** show that the model can predict the median concentration in the overall dataset accurately, even though there is a slight over-prediction of the variability in the model. Finally, no trend in the plots of NPDE *versus* time and predicted concentration were observed.

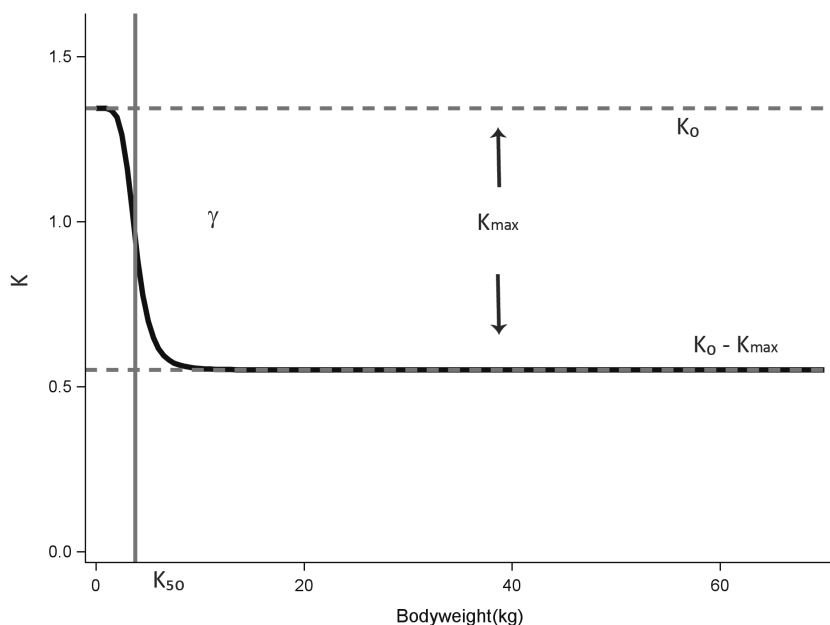


Figure 4-2 The relationship between the allometric exponent k (equation 5) for clearance and bodyweight in the bodyweight-dependent exponent (BDE) model (Model IV).

The parameter k_0 represents the value of the exponent at a theoretical bodyweight of 0 kg, k_{max} represents the maximum decrease of the exponent, k_{50} represents bodyweight at which 50% of the maximum decrease in the exponent is attained, and γ represents the Hill coefficient that determines the steepness of the sigmoidal decline.

Figure 4-5 shows how variation in the parameters k_0 , k_{max} , k_{50} , and γ of the bodyweight dependent exponent k of equation 5 results in different curves for clearance *versus* bodyweight, including the observed curve in the current analysis on propofol clearance. This figure illustrates that the developed bodyweight-dependent exponent model is capable of capturing different

maturation profiles of clearance *versus* bodyweight allowing in principle to be applied to different drugs and/or different metabolic pathways.

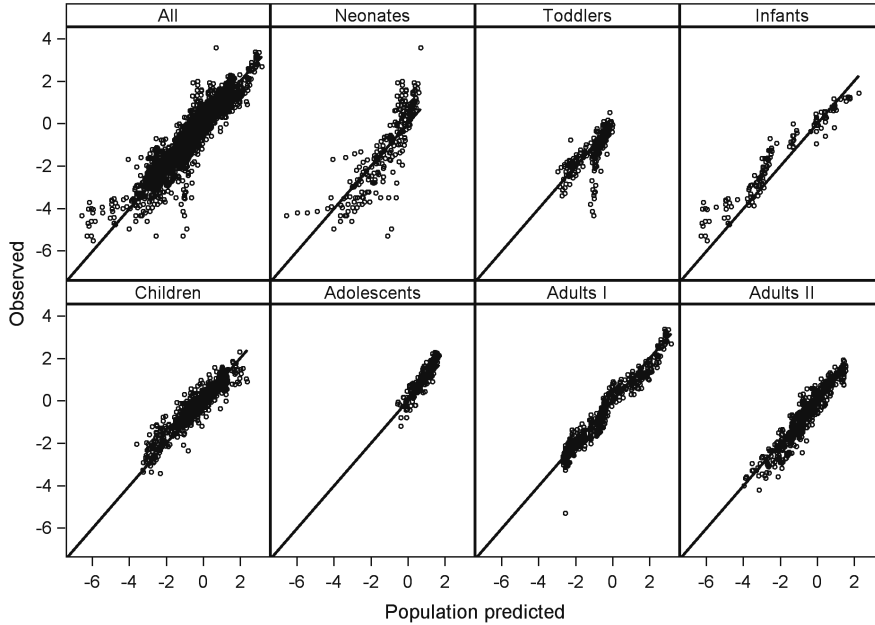


Figure 4-3 Observed versus population predicted propofol concentrations of the bodyweight-dependent exponent model (Model IV).

'All' presents data of all datasets together. Other panels represent data of neonates [24], infants [25], toddlers [26], children [27], adolescents [15], adults I [28] and adults II [29].

4.4. Discussion

In this study, we tested four different allometric equations to capture changes in propofol clearance in seven datasets comprising almost every stage of human life. We found that fixing the allometric exponent to 0.75 (the $\frac{3}{4}$ allometric model, Model I) resulted in adequate description of clearance in adults, adolescents, children and toddlers, but yielded significant under-prediction in infants and over-prediction in term and preterm neonates. Results of the mixture model (Model II) and the bodyweight-cut-point separated model (Model III) showed that an allometric exponent other than 0.75 was more suitable for

neonates and infants. In fact, both models identified an allometric exponent greater than 1 for the subpopulation that was composed of mainly neonates and infants resulting in improved description of clearance in these youngest and lightest patient groups in comparison with the $\frac{3}{4}$ allometric model. This has been reported before for morphine clearance in children younger than 3 years of age, where an exponent of 1.44 was found to best describe the developmental changes in clearance [22]. Similarly, Mahmood reported that the error in the prediction of clearance decreased when the scaling exponent increased from 0.75 towards 1 when studying different drugs in children less than 1 year of age [13]. Even though the overall performance improved significantly, the use of two different allometric exponents for different human subpopulations as implemented in Model II and Model III resulted in ambiguous clearance predictions for the individuals on the boundaries of the paediatric subpopulations. In the current study, the observed issue of different exponents at different weight ranges was overcome by implementing an allometric equation with an exponent that varies with bodyweight. This bodyweight-dependent exponent (BDE) model contains a continuous bodyweight-based equation which allowed for the best description of maturational changes in propofol clearance in individuals covering the entire human life-span. While the current model needs to be further evaluated in an external validation procedure, according to the advanced model evaluation criteria for pediatric population models [30], the descriptive and predictive performances of this model were affirmed by stratified diagnostics (Figure 4-3), bootstrap validation (Table 4-4), NPDE (Figure 4-4), and %RMSE results (Table 4-3).

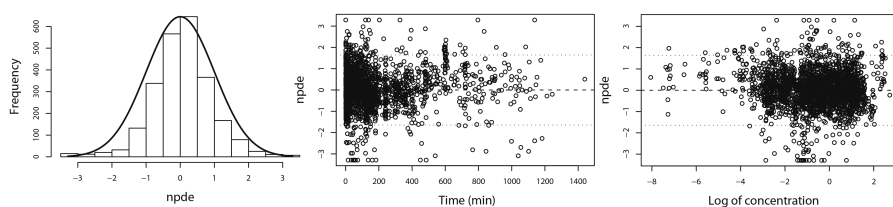


Figure 4-4 NPDE results of the final PK model for propofol. *Left panel:* histogram of NPDE distribution in the full dataset with the solid line representing a normal distribution as a reference; *Middle panel:* NPDE versus time; *Right panel:* NPDE versus log transformed concentration.

An important question is whether this bodyweight-dependent exponent may reflect underlying physiological maturation processes. Unfortunately, we were

not able to establish a direct link between the change in exponent and the physiological maturation process, due to the lack of physiological information in our data. The results of this study are therefore to be compared to other studies. The reported high value of the exponent at very low bodyweights (Figure 4-2), is the result of rapid changes in propofol clearance with bodyweight at these young age ranges (Figure 4-1, model IV). The lower value for the exponent at higher weight ranges, results from the accomplishment of the maturation process of propofol clearance (Figure 4-2). According to the well-stirred model, hepatic clearance is determined by hepatic extraction ratio and liver blood flow. As propofol is a drug with a high extraction ratio, systemic clearance is driven by liver blood flow [36, 37]. While there are no good data on hepatic blood flow in relation to age, hepatic blood flow in infants was suggested to be comparable to adult values. Therefore maturation of metabolic capacity could be considered the cause of the rapid changes in clearance in the young individuals [38]. For propofol, glucuronidation by the UGT1A9 isoenzyme is the major elimination pathway. UGT1A9 was reported to undergo an age-dependent quantitative differential regulation extending up to 24 months of age [39], and it was found that only part of the reduced glucuronidation rate was compensated by hydroxylation in neonates [40]. Even though this cannot be proven, it can be speculated that the rapid change in the bodyweight-dependent exponent at low bodyweight ranges may be the result of the change in the capacity of the UGT1A9 isoenzyme.

In recent years, there has been a debate on how bodyweight and age, which are two correlated covariates in the paediatric population, should be included in population pharmacokinetic models. It has been proposed that the $\frac{3}{4}$ allometric equation can be used to standardize drug clearance to the average adult bodyweight of 70 kg, after which age-based equations can be added to account for maturational differences in the younger populations compared to the older ones [1, 41]. These age based functions are needed because poor prediction performance of the $\frac{3}{4}$ allometric model can be expected when scaling clearance to children under a certain age [15-17]. However, this addition of age to the $\frac{3}{4}$ allometric bodyweight based scaling function may result in collinearity. The effect of the collinearity on parameter estimates in nonlinear mixed effect models has already been studied and it was found that high collinearity between predictors, defined as data collinearity,

increased the bias of the parameter estimates and enlarged the corresponding standard errors [20]. More recently, it has been shown that when one of two correlated covariates that contain information about a model parameter is pre-selected over the other, the predictive performance of the resulting model may be diminished, unless the pre-selected covariate relationship reflects the true biological relationship [21]. With the great amount of theoretical and experimental evidence against the $\frac{3}{4}$ allometric model [6-12, 14], and the risk of collinearity when introducing age to correct for deviations from this model, new scaling approaches are needed. As such, data-driven approaches, as applied in the current analysis, are more suitable for the development of population pharmacokinetic models thereby resulting in a continuous function using only bodyweight as a covariate.

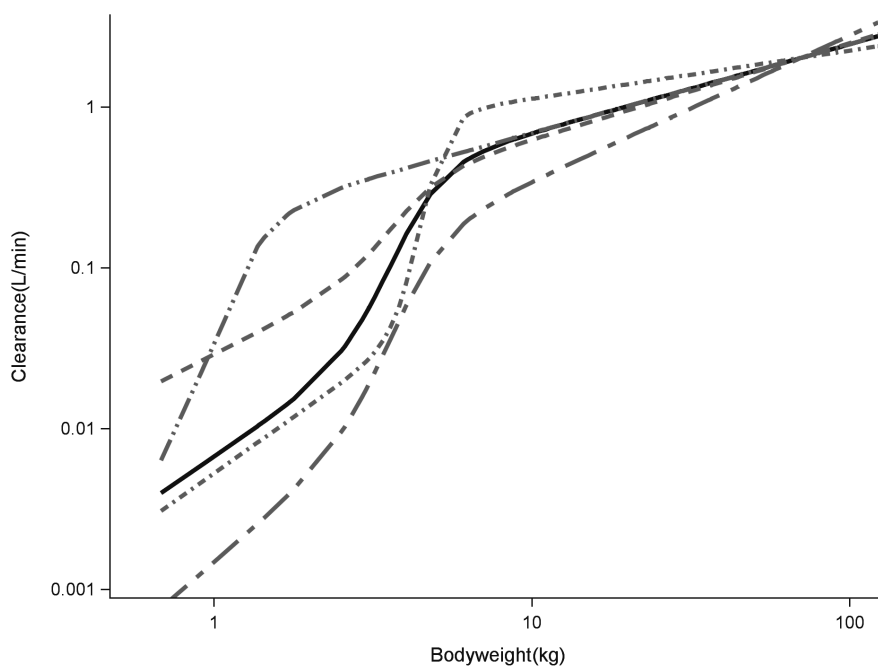


Figure 4-5 Simulations for the bodyweight-dependent exponent (BDE) model (Model IV) on the basis of different values for the parameters of Model IV (equation 5).

Solid black curve: BDE curve with $k_0=1.34$, $k_{max}=0.79$, $k_{50}=3.78$, $\gamma=5.24$ (final PK model); dashed grey curve: BDE curve with $k_0=1$, $k_{max}=0.4$, $k_{50}=3.78$, $\gamma=5$; long dash short dash grey curve: BDE curve with $k_0=1.7$, $k_{max}=0.8$, $k_{50}=3.78$, $\gamma=5$; short dash dot grey curve: BDE curve with $k_0=1.4$, $k_{max}=1.1$, $k_{50}=4.5$, $\gamma=10$; medium dash double dot grey curve: BDE curve with $k_0=1.34$, $k_{max}=0.8$, $k_{50}=1$, $\gamma=5$.

The bodyweight-dependent exponent model we described in this paper is of particular relevance for scaling clearance parameters to the youngest age ranges including infants, term and preterm neonates. Since in these age groups changes in the pharmacokinetics may be expected to be large, there is currently great interest in scaling clearance parameters from older populations to neonates or infants younger than 2 years of age [15, 16, 42]. It is however uncertain whether the parameters of the bodyweight-dependent exponent model we developed for propofol can be generalized to other drugs. Propofol has very specific characteristics such as a high extraction ratio, direct glucuronidation and high lipophilicity. However, as Figure 4-5 shows, the function is very flexible, and in our opinion the proposed model can be applied to other drugs provided data in young children are available to estimate the exact parameters of equation 5 of the bodyweight dependent exponent model for that drug.

4.5. Conclusion

In this study, we have developed a model for scaling propofol clearance over the entire human life-span with a single continuous bodyweight based equation, in which the exponent of the equation varies with bodyweight. The flexibility of this bodyweight-dependent exponent (BDE) model may increase the applicability of this type of models to scale clearance of other drugs over large parts of the human life-span. This function may provide an alternative for allometric scaling approaches in the extrapolation of drug clearances from older to younger human age-ranges.

4.6. Acknowledgements

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Appendix: NONMEM code of the model

Model I: The $\frac{3}{4}$ allometric model

```
$SUBROUTINE ADVAN11 TRANS4
```

```
$PK
```

```
TVCL = THETA(1)*(BW/70)**0.75
```

```
CL = TVCL*EXP(ETA(1))
```

```
Q2 = THETA(2)*EXP(ETA(2))
```

```
Q3 = THETA(3)*(BW/70)*EXP(ETA(3))
```

```
V1 = THETA(4)*EXP(ETA(4))
```

```
TVV2 = THETA(5)*(BW/70)
```

```
V2 = TVV2*EXP(ETA(5))
```

```
V3 = THETA(6)*(BW/70)*EXP(ETA(6))
```

```
S1 = V1
```

```
ETCL =ETA(1)
```

```
ETQ2 =ETA(2)
```

```
ETQ3 =ETA(3)
```

```
ETV1 =ETA(4)
```

```
ETV2 =ETA(5)
```

```
ETV3 =ETA(6)
```

```
;------
```

```
$ERROR
```

```
W=1
```

```
IPRED = LOG(.000001)
```

```
IF (F.GT.0) IPRED=LOG(F)
```

```
IRES=DV-IPRED
```

```
IWRES=IRES/W
```

```
Y=IPRED+W*ERR(1)
```

```
;------
```

```
$THETA
```

```
( 0, 1.6, ) ; TH1 CL
```

```
( 0, 1.7, ) ; TH2 Q2
```

```
( 0, 1.5, ) ; TH3 Q3
```

```
( 0, 4, ) ; TH4 V1
```

```
( 0, 15, ) ; TH5 V2
```

(0, 200,) ; TH6 V3

\$OMEGA

0.4 ; OMEGA1 CL

0 FIX ; OMEGA2 Q2

0.2 ; OMEGA3 Q3

1 ; OMEGA4 V1

0.6 ; OMEGA5 V2

0.3 ; OMEGA6 V3

\$SIGMA

0.1 ; SIGMA1

\$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION

\$COV COMP PRINT=E

Model II: The mixture model

\$DATA analysis.csv IGNORE=#

\$SUBROUTINE ADVAN11 TRANS4

\$PK

IF (MIXNUM.EQ. 1) THEN

TVCL = THETA(1)*(BW/70)**THETA(7)

ELSE

TVCL = THETA(8)*(BW/70)**THETA(9)

ENDIF

CL = TVCL*EXP(ETA(1))

Q2 = THETA(2)*EXP(ETA(2))

Q3 = THETA(3)*(BW/70)*EXP(ETA(3))

V1 = THETA(4)*EXP(ETA(4))

TVV2 = THETA(5)*(BW/70)

V2 = TVV2*EXP(ETA(5))

V3 = THETA(6)*(BW/70)*EXP(ETA(6))

S1 = V1

ETCL = ETA(1)

```

ETQ2 = ETA(2)
ETQ3 = ETA(3)
ETV1 = ETA(4)
ETV2 = ETA(5)
ETV3 = ETA(6)
EST = MIXEST
;-----
$MIX
NSPOP=2
P(1)=THETA(10)
P(2)=1-P(1)
;-----
$ERROR
W=1
IPRED = LOG(.000001)
IF (F.GT.0) IPRED=LOG(F)
IRES=DV-IPRED
IWRES=IRES/W
Y=IPRED+W*ERR(1)
;-----
$THETA
(0, 2, ) ; TH1 CL OF SUBPOP2 WITH HIGH EXP
(0, 1, ) ; TH2 Q2
(0, 1.5, ) ; TH3 Q3
(0, 4, ) ; TH4 V1
(0, 15, ) ; TH5 V2
(0, 200, ) ; TH6 V3
(0, 1.3, ) ; TH7 BW EXPONENT OF SUBPOP1 WITH HIGH EXP
(0, 1.5, ) ; TH8 CL OF SUBPOP2 WITH LOW EXP
(0, 0.6, ) ; TH9 BW EXPONENT OF SUBPOP2 WITH LOW EXP
(0, 0.2, 1) ; TH10 PORTION OF SUBPOP1

$OMEGA
0.1 ; OMEGA1 CL
0 FIX ; OMEGA2 Q2
0.2 ; OMEGA3 Q3

```

0.5 ; OMEGA4 V1

0.5 ; OMEGA5 V2

0.3 ; OMEGA6 V3

\$SIGMA

0.1 ; SIGMA1

\$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION

\$COV COMP PRINT=E

Model III: The bodyweight-cut-point separated model

\$SUBROUTINE ADVAN11 TRANS4

\$PK

CUTP= THETA(10)

IF (BW.GT. CUTP)

THEN

TVCL = THETA(1)*(BW/70)**THETA(7)

ELSE

TVCL = THETA(8)*(BW/70)**THETA(9)

ENDIF

CL = TVCL*EXP(ETA(1))

Q2 = THETA(2)*EXP(ETA(2))

Q3 = THETA(3)*(BW/70)*EXP(ETA(3))

V1 = THETA(4)*EXP(ETA(4))

TVV2 = THETA(5)*(BW/70)

V2 = TVV2*EXP(ETA(5))

V3 = THETA(6)*(BW/70)*EXP(ETA(6))

S1 = V1

ETCL = ETA(1)

ETQ2 = ETA(2)

ETQ3 = ETA(3)

ETV1 = ETA(4)

ETV2 = ETA(5)

ETV3 = ETA(6)

```

;-----
$ERROR
W=1
IPRED = LOG(.000001)
IF (F.GT.0)
IPRED=LOG(F)
IRES=DV-IPRED
IWRES=IRES/W
Y=IPRED+W*ERR(1)
;-----
$THETA
(0, 2.113, ) ;TH1 CL OF SUBPOP1
(0, 1.869, ) ;TH2 Q2
(0, 1.877, ) ;TH3 Q3
(0, 6.216, ) ;TH4 V1
(0, 20.59, ) ;TH5 V2
(0, 194.2, ) ;TH6 V3
(0, 0.5096, ) ;TH7 BW EXPONENT OF SUBPOP1 WITH LOW EXP
(0, 18.01, ) ;TH8 CL OF SUBPOP2
(0, 1.527, ) ;TH9 BW EXPONENT OF SUBPOP2 WITH HIGH EXP
16.5 FIX ;TH10 CUT POINT OF BW

$OMEGA
0.193 ;OMEGA1 CL
0 FIX ;OMEGA2 Q2
0.247 ;OMEGA3 Q3
0.617 ;OMEGA4 V1
0.781 ;OMEGA5 V2
0.207 ;OMEGA6 V3

$SIGMA 0.1 ;SIGMA1
$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION
$COV COMP PRINT=E

```

Model IV: The bodyweight-dependent exponent (BDE) model

```

$SUBROUTINE ADVAN11 TRANS4
$PK
MDEX = THETA(7) ;MSEX is the Maximum Decrease of Exponent
EX0 = 0.556+MDEX ;EX0 is the starting exponent
EB50 = THETA(8)
HILL = THETA(9)
EX = EX0-(MDEX*BW**HILL)/(EB50**HILL+BW**HILL)
TVCL = THETA(1)*(BW/70)**EX
CL = TVCL*EXP(ETA(1))
Q2 = THETA(2)*EXP(ETA(2))
Q3 = THETA(3)*(BW/70)*EXP(ETA(3))
V1 = THETA(4)*EXP(ETA(4))
TVV2 = THETA(5)*(BW/70)
V2 = TVV2*EXP(ETA(5))
V3 = THETA(6)*(BW/70)*EXP(ETA(6))
S1 = V1
;-----
$ERROR
W=1
IPRED = LOG(.000001)
IF (F.GT.0) IPRED=LOG(F)
IRES=DV-IPRED
IWRES=IRES/W
Y=IPRED+W*ERR(1)
;-----
$THETA
(0, 1.3, ) ;TH1 CL
(0, 0.5, ) ;TH2 Q2
(0, 0.8, ) ;TH3 Q3
(0, 5, ) ;TH4 V1
(0, 5, ) ;TH5 V2
(0, 50, ) ;TH6 V3
(0, 0.8, ) ;TH7 MDEX
(1.5, 3.7, ) ;TH8 EB50

```


5.1 FIX ;TH9 HILL

;------

\$OMEGA

0.154 ;OMEGA1 CL

0 FIX ;OMEGA2 Q2

0.199 ;OMEGA3 Q3

0.5 ;OMEGA4 V1

0.607 ;OMEGA5 V2

0.304 ;OMEGA6 V3

\$\$SIGMA 0.15 ;ERROR

\$EST NOABORT PRINT=15 MAXEVALS=9999 METHOD=1 INTERACTION

\$COV COMP PRINT=E





Chapter 5

Developmental changes in morphine clearance across the entire paediatric age range are best described by a bodyweight-dependent exponent model

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Abstract

Introduction: Morphine clearance has been successfully scaled from preterm neonates to 3-year-old children on the basis of a bodyweight-based exponential function and age younger or older than 10 days. The aim of current study is to characterize the developmental changes in morphine clearance across the entire paediatric age-range.

Methods: Morphine and morphine-3-glucuronide (M3G) concentration data from 358 (pre)term neonates, infants, children and adults, and morphine concentration data from 117 adolescents were analyzed using NONMEM 7.2. Based on available data, two models were developed: I. using morphine data; II. using morphine and M3G data.

Results: In model I, morphine clearance across the paediatric age range was very well described by a bodyweight-based exponential function in which the allometric exponent decreased in a sigmoidal manner with bodyweight (BDE model) from 1.47 to 0.88, with half the decrease in exponent reached at 4.01 kg. In model II, the exponent for the formation and elimination clearance of M3G was found to decrease from 1.56 to 0.89 and from 1.06 to 0.61, with half the decrease reached at 3.89 and 4.87 kg, respectively. Using the BDE model, there was no need to use additional measures for size or age.

Conclusion: The BDE model was able to scale both total morphine clearance and glucuronidation clearance through the M3G pathway across all age-ranges between (pre)term neonates and adults by allowing the allometric exponent to decrease across the paediatric age range from values higher than 1 for neonates to values lower than 1 for infants and children.

5.1. Introduction

The pharmacokinetics of morphine have been widely studied in the paediatric population using different approaches and modeling techniques [1]. In paediatric population pharmacokinetic models, bodyweight was reported the most significant covariate for morphine clearance [2-4]. While a variety of bodyweight-based functions has been used, i.e. exponential equations using a 0.75 fixed exponent or an estimated exponent of 1.44, in all models additional age-related variables were needed to adequately describe clearance across paediatric age-ranges [1-5]. This may be explained by the fact that single exponent functions based on body weight may not be expected to be suitable for the prediction of drug clearance in children of all ages [6, 7]. However, as bodyweight and age are correlated in a complex and highly nonlinear manner as part of a child's growth and development, the use of both bodyweight and age as covariates on a single parameter may harm the predictive performance of the resulting model [8, 9]. Additionally, many studies on morphine clearance in paediatrics are limited to small age-ranges [2-4, 10] and no study has proven adequate extrapolation potential outside the studied age-range. This highly limits the development of unambiguous continuous dosing guidelines for children.

Recently, a bodyweight dependent exponent (BDE) model was developed to scale clearance from preterm neonates to adults [11]. Using this function, clearance scales with bodyweight on the basis of an allometric function. However, because the allometric exponent is allowed to vary with bodyweight, the BDE function offers maximal flexibility to capture different maturation rates at varying stages of pediatric development [11]. Typically, this exponent k has a certain value k_0 at a hypothetical bodyweight of 0 kg after which it decreases with bodyweight sigmoidally according to an Emax model [11]. More recently, also simplified decreasing functions on the basis of a power function have been proposed when a smaller weight range is concerned (i.e. lack of data of preterm neonates) [12]. In both analyses, the BDE function proved to optimally describe the changes in clearance between neonates and adults using bodyweight without of the need for a secondary age-related covariate [11, 12].

Therefore, in the current study, we analyzed morphine concentration – time profiles from 475 preterm and term neonates, infants, children, adolescents

and adults with the aim to characterize developmental changes in morphine clearance across the entire human lifespan. Given the strong evidence for a high maturation rate (exponent of 1.44) in children under the age of 3 years [4, 10] and the need to reach a plateau for the maturation rate at older age ranges with a lower value for the exponent, the recently developed BDE model was applied [11]. This analysis also allows us to study whether the changes in clearance of morphine and its metabolite can be described by the BDE function without subsequent need for additional age-related covariates.

5.2. Methods

5.2.1. Subjects

Morphine concentration – time data from a total of 475 subjects participating in eight different clinical studies [13-20] were included in the current analysis. Studies represented three age groups: neonates and young children (0-3y), older children and adolescents (6-15y), adults (18-36y) (Table 5-1). The studies were performed at different centers in different countries resulting in the administration of two different morphine salts. To compare the administered doses, the amount of administered morphine base was calculated for each individual in each study.

Neonates and Young children [13-18]

Morphine and morphine-3-glucuronide (M3G) metabolite concentrations in 338 pediatric patients (age 0.1-1070 days; bodyweight 0.57-16.8 kg) of six different studies [13-18] were included in our analysis. Detailed demographic and clinical information on the patients in the six studies can be found in original publications [13-18]. In Table 5-1, a summary of patient demographics is presented of these six studies.

Older children and Adolescents [19]

The study in older children and adolescents was a prospective, genotype blinded, clinical observational study to investigate the impact of race and genotype on morphine clearance [19]. Children of all races aged 6-15 years scheduled for elective adenotonsillectomy with American Society of Anesthesiologists (ASA) physiological status 1 or 2 were included. As African-American children

were found to have higher morphine clearance than Caucasian children [19], we excluded 29 African subjects out of the total of 146 subjects, leaving 117 patients aged between 6 – 15 years with a bodyweight between 17.9 – 79.5 kg) for our modeling analysis (Table 5-1).

Table 5-1 Overview of the datasets used to develop the population PK model for parent morphine (Model I) and for parent morphine and M3G metabolite (Model II).

Age group	Population	N	Weight (kg)	Age	Samples	Reference
Neonates and young children	Postoperative term neonates, infants and children	185	1.9–16.8	0.1–1070 days	Morphine: 618 M3G: 512	[13]
	Preterm and term neonates on artificial ventilation	63	0.56–3.87	0.1–6.7 days	Morphine: 110 M3G: 132	[14]
	Preterm neonates on artificial ventilation	41	0.64–3.55	0.1–13 days	Morphine: 88 M3G: 111	[15]
	Postoperative term neonates and infants	28	1.7–9.3	0.1–294 days	Morphine: 98 M3G: 122	[16]
	Postoperative term neonates and infants	9	2.64–8.1	1–271 days	Morphine: 16	[17]
	Term neonates and infants on artificial ventilation	12	2.2–8.7	3–354 days	Morphine: 8 M3G: 12	[18]
Older children and adolescents	Older children and Adolescents after adenotonsillectomy	117	17.9–79.5	6–15 years	Morphine: 264	[19]
Adults	Healthy adults	20	56–85	20–36 years	Morphine: 300 M3G: 300	[20]

M; morphine; M3G: morphine-3-glucuronide

Adults [20]

This prospective study compared the analgesic effects of a bolus and short infusion of morphine in healthy male and female volunteers [20]. Twenty healthy non-obese adults were given 0.1 mg/kg intravenous bolus of morphine followed by an infusion of $0.03 \text{ mg} \times \text{kg}^{-1} \times \text{h}^{-1}$ for 1 hour after which 15 samples per individual were collected.

5.2.2. Pharmacokinetic Modeling

The population pharmacokinetic analysis was performed with the non-linear mixed effects modeling software NONMEM version 7.2. (ICON Development Solutions, Ellicott City, MD, USA) using the first-order conditional estimation

method with the interaction option (FOCEI). The S-PLUS interface for NONMEM (LAP&P Consultants BV, Leiden, NL), S-Plus (version 8.1, Insightful Software, Seattle, WA, USA), PsN, Pirana and R (version 2.14.2) were used to visualize the output and evaluate the models.

Structural model

As morphine concentrations were available for all three age groups whereas M3G metabolite concentrations were only available in neonates and young children and adults (not in older children and adolescents), two different structural models were used in our pharmacokinetic analysis.

Parent morphine model (Model I)

A two-compartment structural model [4] was applied to the parent morphine concentration data for all three age groups depicted in Table 5-1.

Parent morphine and M3G metabolite model (Model II)

A two-compartment structural model for parent morphine and a one compartment structural model for M3G [4] was applied to parent morphine and M3G metabolite concentration data that were available in datasets of neonates and young children and the adult population (Table 5-1).

Statistical model

The inter-individual variability on morphine and M3G clearance and volumes of distribution was assumed to be log-normal distributed, and expressed as:

$$\theta_i = \theta_{TV} \times e^{\eta_i} \quad , \quad \eta_i \sim N(0, \omega^2) \quad (\text{eq. 1})$$

where θ_i is the individual parameter value for i-th individual, θ_{TV} is the population parameter value, and η_i is a random variable from a normal distribution with mean zero and variance ω^2 .

All concentration data were log-transformed in the analysis. An additive residual error model was applied on the log-transformed data, which corresponds to the proportional error on the linear scale, expressed as:

$$\log C_{ij} = \log C_{pred_{ij}} \times \varepsilon_{ij} \quad , \quad \varepsilon_{ij} \sim N(0, \sigma^2) \quad (\text{eq. 2})$$

where C_{ij} is the observed concentration of i-th individual at time j and C_{predij} is the corresponding predicted concentration. ε_{ij} is a random variable from a normal distribution with mean zero and variance σ^2 .

Covariate model

The BDE function, as shown in equation 3 [eq. 3], was applied to the total morphine clearance in Model I and the formation clearance of morphine-3-glucuronide and the elimination clearance of the morphine-3-glucuronide in Model II:

$$Cl_i = Cl_p \times \left(\frac{BW_i}{70} \right)^k, \quad k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma} \quad (\text{eq. 3})$$

in which Cl_i is clearance in the i-th individual with bodyweight BW_i ; Cl_p is the clearance in a standardized adult with a bodyweight of 70 kg; BW_i is bodyweight of an individual i; k is the exponent; k_0 is the value of the exponent at a theoretical bodyweight of 0 kg; k_{max} is the maximum decrease of the exponent; k_{50} is the bodyweight at which a 50% decrease in the maximum decrease of exponent value is attained, and γ is the Hill coefficient determining the steepness of sigmoidal decline in the exponent.

Beside the BDE function for bodyweight that was tested on the different clearance parameters, bodyweight was tested in a linear or power function on other pharmacokinetic parameters, as shown in equation 4 [eq. 4]:

$$\theta_i = \theta_p \times \left(\frac{BW_i}{70} \right)^m \quad (\text{eq. 4})$$

In this equation, θ_i is the parameter of i-th individual with bodyweight BW_i ; θ_p is the parameter standardized adult with a bodyweight of 70kg; BW_i is bodyweight of an individual i. In case of a power function, m represents the exponent value, while for a linear relationship m is fixed to 1.

The covariate was included in the model if the decrease in objective function value (OFV) was greater than 7.88 points, which corresponds to $p < 0.005$ in the Chi-square test. In addition, criteria as defined under Model Validation were considered.

5.2.3. Model Validation

The two models were validated internally using five criteria that were recently proposed for pediatric population model evaluation [5]. (i) It was checked whether the coefficient of variation (CV) of the parameter estimates either from the covariance step in NONMEM or from stratified bootstrap resampling results was less than 50%. (ii) The basic diagnostic plots and particularly the plots of the observed versus population predicted concentrations stratified for age, were visually assessed for bias. (iii) The η -shrinkage was calculated according to Karlsson and Savic was considered [21]. (iv) The individual and population predicted parameters were plotted against bodyweight to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters. (v) The simulation-based normalized prediction distribution error (NPDE) proposed by Brendel *et al.* [22], was calculated based on 2,000 simulations of the entire dataset and were evaluated visually for bias and precision.

5.3. Results

For the analysis, data of 475 subjects varying from preterm and term neonates to adults were available from eight different clinical studies (Table 5-1). Data of all 475 subjects were used in the model describing the time-course of the parent drug concentration (parent morphine model; Model I), whereas data of 358 individuals in which both morphine and M3G concentrations were available, were used to describe the time-course of both morphine and M3G concentration (parent morphine and M3G metabolite model; Model II). A summary of the available datasets is given in Table 5-1.

A BDE model in which the exponent decreased with bodyweight in a sigmoidal manner [eq. 3] very well described the developmental changes in total clearance of morphine (CLT) in the parent morphine model (Model I). Similarly, a BDE model well described changes in the formation clearance of M3G (CL_{M3G}) and the elimination clearance of M3G ($CL_{E_{M3G}}$) across all ages in the parent morphine and M3G metabolite model (Model II). Figure 5-1 (upper panels) shows the *post hoc* estimates of total morphine clearance, formation clearance of M3G and elimination clearance of M3G *versus* bodyweight (η -shrinkage values being

24.9%, 18.9% and 20.4%, respectively). The lower panels in Figure 5-1 show how the bodyweight-dependent exponent (k) of total morphine clearance, formation clearance of M3G and elimination clearance of M3G was found to change with bodyweight. For the parent morphine model (Model I), the value of k for CLT dropped from 1.47 (k_0) at the theoretical bodyweight of zero kilogram to 0.88 ($k_0 - k_{max}$) and reached half this decrease at 4.01 kilogram (k_{50}) (see Table 5-2 for estimated parameters for the exponent k). For parent morphine and M3G metabolite model (Model II), the value of k for CLM_{M3G} dropped from 1.56 (k_0) at the theoretical bodyweight of zero kilogram to 0.89 ($k_0 - k_{max}$) and reached half this decrease at 3.89 kilogram (k_{50}), while the k -value for CLE_{M3G} dropped from 1.06 (k_0) to 0.61 ($k_0 - k_{max}$) and reached half this decrease at 4.87 kilogram (k_{50}) (see Table 5-3 for estimated parameters for the exponent k).

Table 5-2 Parameter estimates of the parent morphine model (Model I).

Parameter	Estimated value	Bootstrap [§]
Fixed Effect		
CLT (L/min)	CLT = TVCLT × (BW/70) k	
TVCLT (L/min·70kg)	1.62 (5.3%)	1.63 (6.1%)
k	$k = k_0 - k_{max} \cdot BW^\gamma / (k_{50}^\gamma + BW^\gamma)$	
k_0	1.47 (3.7%)	1.47 (5.8%)
k_{max}	0.59 (4.7%)	0.59 (9.3%)
k_{50} (kg)	4.01 (3.9%)	4 (4.1%)
γ	4.62 (9.5%)	6.4 (88.5%)

Q (L/min)	Q = TVQ × (BW/70)	
TVQ (L/min·70kg)		
Pop ≠ 2	1.9 (9.6%)	1.95 (11.9%)
Pop = 2	0.5 (19%)	0.49 (16.1%)

Vc (L)	Vc = TVVc × (BW/70)	
TVVc (L/70kg)		
Pop ≠ 2	81.2 (7.8%)	79.16 (6.4%)
Pop = 2	46 (5.1%)	45.44 (3.8%)

Vp (L)	Vp = TVVp × (BW/70)	
TVVp (L/70kg)	128 (8%)	129.91 (7.2%)
Inter-individual variability		
ω^2 (CLT)	0.16 (6.9%)	0.156 (12.9%)
ω^2 (Vc)	0.25 (27.5%)	0.24 (43.6%)
Residual error		
σ^2	0.19 (8.7%)	0.19 (7.7%)
σ^2 for time>1900min	0.46 (113.2%)	0.79 (76.9%)

CLT: total morphine clearance; TVCLT: CLT normalized to bodyweight value of 70kg; BW: bodyweight in kilogram; k : bodyweight dependent exponent (BDE) on BW for total clearance; k_0 : BDE at the theoretical bodyweight of zero; k_{max} : maximum decrease of the exponent; k_{50} : the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained; γ is the Hill coefficient determining the steepness of sigmoidal decline in the exponent; Q : inter-compartmental clearance; TVQ: Q normalized to bodyweight value of 70kg V_c : volume of distribution of the central compartment of morphine; TVVc: V_c normalized to bodyweight value of 70kg; Pop=2: population of older children and adolescents; Pop $\neq$ 2: population of neonates and young children or adults; V_p : the volume of distribution of the peripheral compartment of morphine; TVVp: V_p normalized to bodyweight value of 70kg; ω^2 variance of the normal distribution that quantifies the inter-individual variability on the designated parameter according to equation 1; σ^2 : variance of the normal distribution that quantifies the residual error of the morphine observations according to equation 2; σ^2 for time>1900min: variance of the normal distribution that quantifies the residual error of extra additive error for concentrations of morphine when the time after dose is beyond 1900 minutes [4]

§ Bootstrap mean and CV percentage

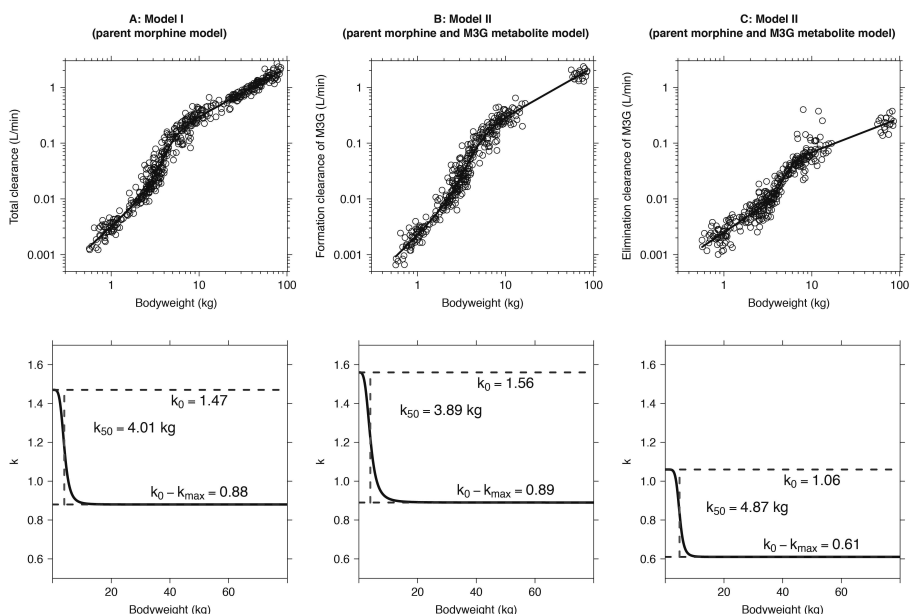


Figure 5-1 Post hoc clearance values of total clearance, formation clearance of M3G, and elimination clearance of M3G and values of the corresponding bodyweight dependent exponent (k) versus bodyweight from Model I (parent morphine model) and Model II (parent morphine and M3G metabolite model).

Upper panels: open circles are post hoc values of total clearance (A), formation clearance of M3G (B), or elimination clearance of M3G (C); solid curves are corresponding model predicted values.

Lower panels: k is the bodyweight dependent allometric exponent (eq.3) of total clearance (A), formation clearance of M3G (B), or elimination clearance of M3G (C); k_0 is the value of the exponent at a theoretical bodyweight of 0 kg; k_{max} is the maximum decrease of the exponent; k_{50} is the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained; upper blue dash line is the reference line of k_0 ; lower blue dash line is the reference line of $k_0 - k_{max}$; red vertical dash line is the reference line of k_{50} .

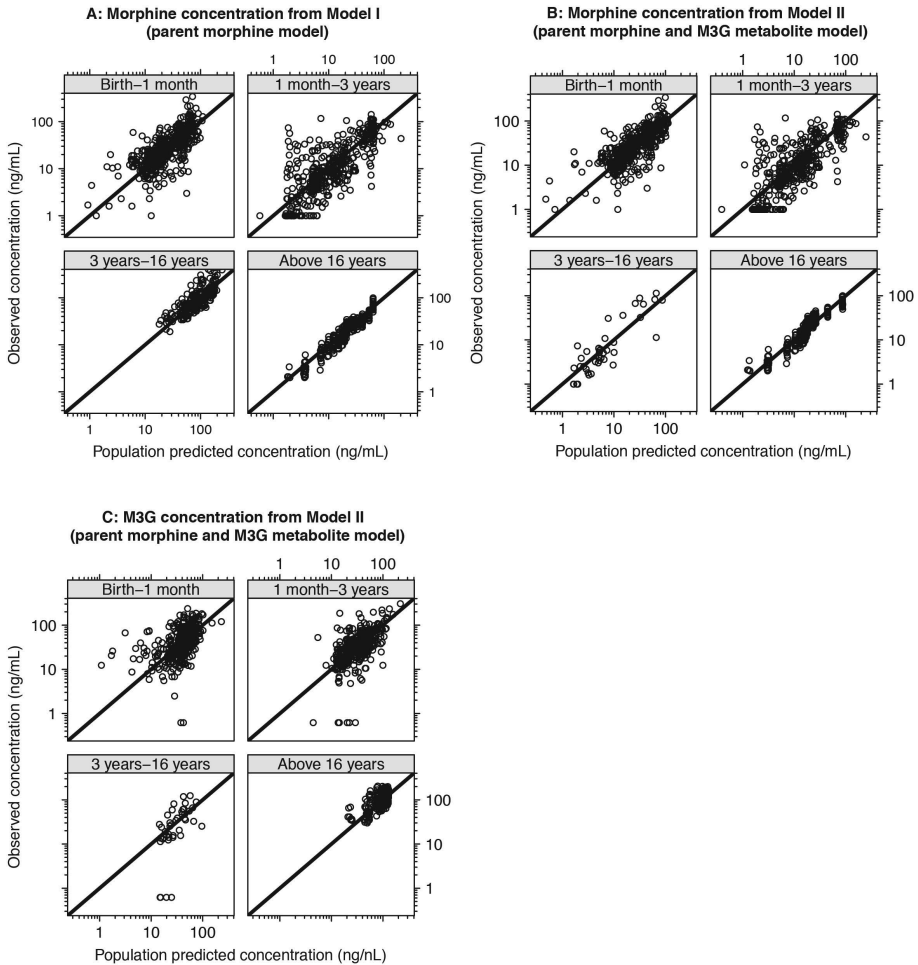


Figure 5-2 Age-stratified observed versus population predicted log-transformed concentrations of morphine from Model I (parent morphine model) and of parent morphine and M3G metabolite from Model II (parent morphine and M3G metabolite model)

For CLT of the parent morphine model (Model I) and CLM_{M3G} and CLE_{M3G} of the parent morphine and M3G metabolite model (Model II), no additional covariates could be identified based on visual inspection of the corresponding inter-individual variability against covariate plot and given the criteria as defined under Methods (Covariate Model and Model Validation). In the parent morphine model (Model I), bodyweight was identified as a covariate in a linear equation for volume of distribution of the central compartment of morphine (V_c), volume of distribution of the peripheral compartments of morphine

(Vp), and inter-compartmental clearance (Q) (Table 5-2). In addition, lower bodyweight normalized population values of Q and Vc were identified for the older children and adolescents (0.071 L/kg/min and 0.66 L/kg) compared to children younger than 3 years and adults (0.027 L/kg/min and 1.16 L/kg) (Table 5-2). In the parent morphine and M3G metabolite model (Model II), bodyweight was identified as a covariate in a linear equation for clearance of morphine through other routes than M3G (CL_o), volume of distribution of the central compartment of morphine (Vc), volume of distribution of the peripheral compartments of morphine (Vp), and inter-compartmental clearance (Q) (Table 5-3). For the volume of distribution of M3G (V_{M3G}), a population value of 20L was estimated, which proved in accordance with literature [23] and which was later on fixed to this value in order to achieve successful minimization with a covariance step. V_{M3G} was found to vary with bodyweight, which was best described by a power function with an estimated exponent value of 0.71. In both the parent and the parent and metabolite model (Model I and Model II respectively), no other covariates were identified on any of the other parameters based on the criteria as described in section Methods (Covariate Model and Model Validation).

Figure 5-2 shows that both the parent morphine model (Model I) and the parent morphine and M3G metabolite model (Model II) described the morphine and M3G concentration data in all different age groups well. The NPDE analysis as a simulation based validation method, shows that morphine and M3G concentrations in the models were normally distributed around the median prediction and that there was no trend in the NPDE versus TIME and versus the log-transformed individual predicted concentrations (Figure 5-3). All parameter estimates and results of the bootstrap validation of the parent morphine model (Model I) and the parent morphine and M3G metabolite model (Model II) are listed in Table 5-2 and Table 5-3, respectively.

Figure 5-4 illustrates that postnatal age (PNA) younger or older than 10 days, which was reported as a covariate for morphine glucuronidation clearance in a previous study in children younger than 3 years of age [4], was not a covariate for clearance in the final model of the current study.

Table 5-3 Parameter estimates of the parent morphine and M3G metabolite model (Model II (based on morphine and M3G concentrations)).

Parameter	Estimated value	Bootstrap ^s
Fixed Effect		
CLM_{M3G} (L/min)	$CLM_{M3G} = TVCLM_{M3G} \times (BW/70)^k$	
$TVCLM_{M3G}$ (L/min•70kg)	1.67	1.66 (5.2%)
k of CLM_{M3G}	$k = k_0 - k_{max} \cdot BW^\gamma / (k_{50}^\gamma + BW^\gamma)$	
k_0 of CLM_{M3G}	1.56	1.56 (4.1%)
k_{max} of CLM_{M3G}	0.67	0.67 (6.8%)
k_{50} of CLM_{M3G} (kg)	3.89	3.91 (3.8%)
γ of CLM_{M3G}	3.61	3.94 (21%)
CLE_{M3G} (L/min)	$CLE_{M3G} = TVCLE_{M3G} \times (BW/70)^k$	
$TVCLE_{M3G}$ (L/min•70kg)	0.23	0.22 (7.1%)
k of CLE_{M3G}	$k = k_0 - k_{max} \cdot BW^\gamma / (k_{50}^\gamma + BW^\gamma)$	
k_0 of CLE_{M3G}	1.06	1.07 (9%)
k_{max} of CLE_{M3G}	0.45	0.45 (11.8%)
k_{50} of CLE_{M3G} (kg)	4.87	4.68 (6.4%)
γ of CLE_{M3G}	6.84	9.49 (78.3%)
CL_0 (L/min)	$CL_0 = TVCL_0 \times (BW/70)$	
$TVCL_0$ (L/min•70kg)	0.06	0.06 (40.3%)
Q (L/min)	$Q = TVQ \times (BW/70)$	
TVQ (L/min•70kg)	4.2	4.12 (4.9%)
V_c (L)	$V_c = TVV_c \times (BW/70)$	
TVV_c (L/70kg)	29.3	27.67 (13.5%)
V_p (L)	$V_p = TVV_p \times (BW/70)$	
TVV_p (L/70kg)	155	155.29 (7.1%)
V_{M3G} (L)	$V_{M3G} = TVV_{M3G} \times (BW/70)^p$	
V_{M3G} (L/70kg)	20 FIX	20 FIX
p	0.71	0.71 (6.2%)
Inter-individual variability		
$\omega^2 CLM_{M3G}$	0.20	0.20 (15%)
$\omega^2 CLE_{M3G}$	0.19	0.18 (20.5%)
$\omega^2 CL_0$	0.07	0.26 (173.3%)
$\omega^2 V_c$	0.51	0.47 (26.4%)
$\omega^2 V_p$	0.31	0.31 (43.6%)
$\omega^2 V_{M3G}$	0.37	0.39 (37.7%)
Residual error		
σ^2 additive morphine	0.20	0.19 (8.1%)
σ^2 additive M3G	0.14	0.13 (10.1%)
σ^2 for time>1900min	1.85	1.92 (32.5%)

CLM_{M3G}: formation clearance of morphine-3-glucuronide; TVCLM_{M3G}: CLM_{M3G} normalized to bodyweight value of 70kg; BW: bodyweight in kilogram; CLE_{M3G}: elimination clearance of morphine-3-glucuronide; TVCLE_{M3G}: CLE_{M3G} normalized to bodyweight value of 70kg; k: bodyweight dependent exponent (BDE) of BW CLM_{M3G} or CLE_{M3G}; k₀: BDE at the theoretical bodyweight of zero; k_{max}: maximum decrease of the exponent; k₅₀: the bodyweight at which a 50% decrease in the maximum decrease of exponent is attained; γ is the Hill coefficient determining the steepness of sigmoidal decline in the exponent; CL₀: clearance of morphine via other elimination routes; TVCL₀: CL₀ normalized to bodyweight value of 70kg; Q: inter-compartmental clearance; TVQ: Q normalized to bodyweight value of 70kg; Vc: the volume of distribution of the central compartment of morphine; TVVc: Vc normalized to bodyweight value of 70kg; Vp: the volume of distribution of the peripheral compartment of morphine; TVVp: Vp normalized to bodyweight value of 70kg; V_{M3G}: volume of distribution of the morphine-3-glucuronide; TVV_{M3G}: V_{M3G} normalized to bodyweight value of 70kg; p: exponent value of the power function of BW for V_{M3G}; ω^2 variance of the normal distribution that quantifies the inter-individual variability on the designated parameter according to equation 1; σ^2 : variance of the normal distribution that quantifies the residual error of the morphine or morphine-3-glucuronide observation according to equation 2; σ^2 for time>1900min: variance of the normal distribution that quantifies the residual error of extra additive error for concentrations of morphine or morphine-3-glucuronide when the time after dose is beyond 1900 minutes [4]

§ Bootstrap mean and CV percentage

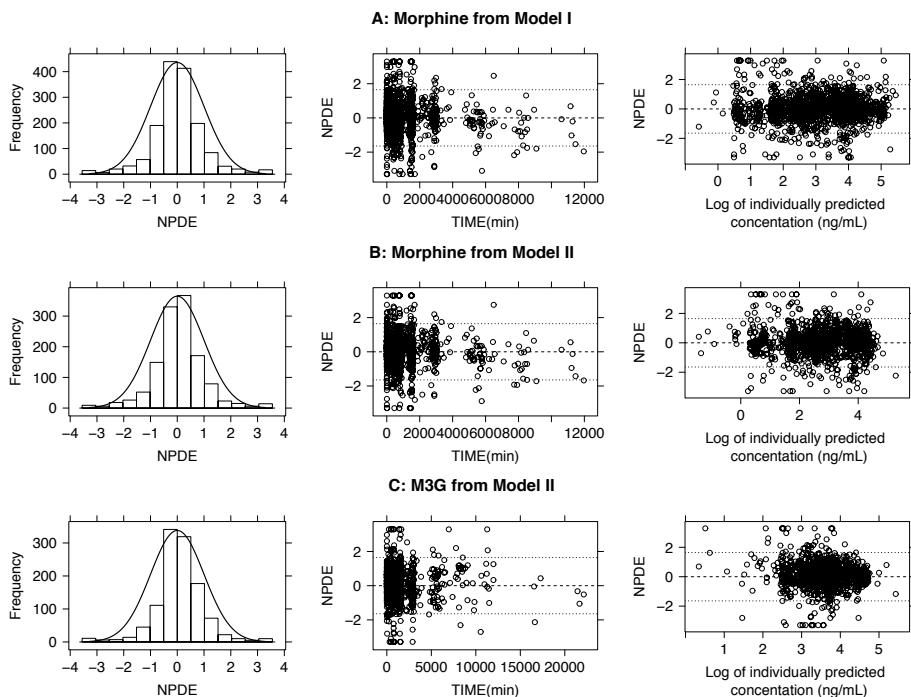


Figure 5-3 NPDE results of morphine concentrations from Model I (parent morphine model) and parent morphine and M3G metabolite concentrations from Model II (parent morphine and M3G metabolite model).

5.4. Discussion

Morphine is metabolized mainly through glucuronidation mediated by the enzyme UDP-glucuronosyltransferase 2B7 (UGT2B7), which was reported to be expressed at very low levels in early life [24-26]. In the past, several models have been developed to describe the changes in glucuronidation clearance of morphine and to predict its clearance in children for the purpose of dosing guidance [2-4]. Among those models, a model was developed for pediatric patients aged less than 3 years including preterm and term neonates [4], in which an allometric exponent value of 1.44 for morphine clearance was identified. Additional extensive investigations confirmed this finding using external data [10] and data from another UGT2B7 substrate [27]. Upon these studies, the allometric exponent of 1.44 for UGT2B7-mediated glucuronidation in children under the age of 3 years was proposed to be a system-specific parameter reflecting the maturation of the UGT2B7 enzyme in humans [27, 28]. The current study confirms not only the validity of the exponent value as high as 1.44 in neonates and young infants given the estimated exponent at a hypothetical bodyweight of 0 kg of 1.56 in this study, but also provides a basis for extrapolation to older age-ranges by the quantification of the maturation of glucuronidation across the entire pediatric age-range with the estimation of a lower exponent for higher bodyweight ranges.

In this study, we successfully scaled morphine clearance from preterm and term neonates to infants, children, adolescents and adults using an allometric function, in which the exponent (k) was allowed to vary with bodyweight in a bodyweight-dependent exponent (BDE) function (eq.3). In both Model I and Model II of our study, the BDE function was able to capture the changes in the clearance parameters (total morphine clearance, formation of M3G, and elimination of M3G), such despite the fact that they were highly nonlinear in nature (**Figure 5-1, upper panels**). According to Karlsson and Savic [21], diagnostics based on the empirical Bayes estimates (EBE) should be assessed in combination with corresponding η -shrinkages as they may distort covariate relationships. Based on a simulation study, it was reported that EBE-based diagnostics generally lose their power with false indications starting to appear at a level of 20-30% [29]. In our study, the η -shrinkages of total clearance, formation clearance of M3G and elimination clearance of M3G were all below

25%, which is on the border of what is acceptable. In addition, both the age-stratified goodness-of-fit diagnostic plots (Figure 5-2) and simulation based NPDE diagnostics (Figure 5-3) demonstrate good population and individual prediction performance of the final bodyweight-dependent exponent (BDE) models for concentrations of morphine and its M3G metabolite. Based on these results, it is concluded that the bodyweight-dependent exponent (BDE) model allows for the description of maturational changes in morphine glucuronidation clearance using a single continuous function, which has not been possible in previous attempts based on the use of allometric equations with single exponents [2-5].

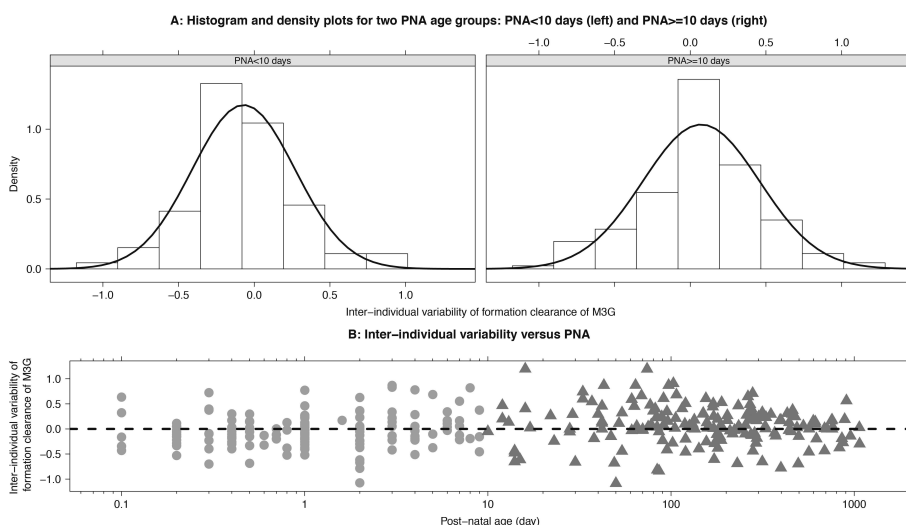


Figure 5-4 Inter-individual variability of formation clearance of M3G from Model II (parent morphine and M3G metabolite model) stratified by postnatal age (PNA) of 10 days

filled circle: individuals PNA < 10 days; triangle: individuals PNA >= 10 days

The parameter values of the bodyweight-dependent exponent (BDE) function, i.e. k_0 , $k_{\max}$, k_{50} and γ , were found to be similar for total morphine clearance (parent morphine model; Model I) and formation clearance of M3G (parent morphine and M3G metabolite model; Model II). This result can in our opinion be explained by the fact that morphine-3-glucuronide is the major metabolite of morphine and glucuronidation of morphine is the rate-limiting step in the clearance of morphine. On the contrary, these sigmoidal equations describing the changes in the exponent k differed between the formation and elimination

of M3G (Figure 5-1, lower panel A and B *versus* C). In our view, these results can be explained by differences in maturation of the glucuronidation of morphine *versus* the renal elimination of M3G. Even though we do not intend to enforce any physiological meaning on the parameters in the BDE function, as the aim of this analysis was primarily to most optimally describe the observations from preterm neonates to adults, this limitation does in our opinion not preclude studies in which the parameters of the BDE function reported for morphine glucuronidation in this study are explored for the prediction of maturational changes in clearance of morphine or other drugs that are glucuronidated. In a similar manner, the parameters of the BDE function for the renal excretion of the M3G metabolite can be explored for its predictive value for the maturation in excretion of other renally excreted compounds as this approach may largely accelerate paediatric data analysis [27, 28].

Previously, for children younger than 3 years of age, postnatal age (PNA) of less than 10 days was identified as a separate covariate for formation clearance of M3G, M6G and their corresponding elimination clearances in addition to the allometric scaling function with an exponent of 1.44 [4]. While it has been suggested before that single allometric exponent functions would not be suitable for the prediction of drug clearance in children of different age-groups [6], different publications have confirmed this conclusion by reporting that an additional covariate function on the basis of an age-related covariate was needed when using single exponent functions [2-4]. In our study, we found an exponent that changed with bodyweight from an initial value at a hypothetical bodyweight of 0 kg of 1.47 and 1.56 for total clearance and formation clearance of M3G, respectively. While the initial value is in good agreement with the previously obtained value of 1.44, in the current analysis, no additional age or weight related covariates could be identified after inclusion of the (BDE) covariate model. From these results, it seems that the changes that were accounted for by the inclusion of the additional covariate relationship based on PNA [4] are now captured by the BDE function, in which the exponent was allowed to change with bodyweight being of specific relevance in the youngest age ranges (Figure 5-4). In this respect, Figure 5-5 illustrates these findings with a graphical comparison of *post hoc* values for glucuronidation clearance of morphine to M3G *versus* bodyweight between the previous model in children younger than 3 years [4] and Model II. In the figure, two parallel lines are placed

with different intercepts for subjects with $PNA < 10$ days and $PNA \geq 10$ days at the lower end of the bodyweight range from our study (Figure 5-5 B), which were found to be quite similar to the patterns described by the previous model (Figure 5-5 A). The two simulated lines in Figure 5-5 B have slope values of 1.56, which corresponds with k_0 in the BDE function for CL_{m3g} , and can roughly describe the changes in M3G formation clearance in children in two subgroups ($PNA > 10$ days and $PNA < 10$ days) up to a bodyweight of 10 kg. From this figure, it seems that applying an allometric function in which the exponent is allowed to vary with bodyweight itself results in an optimal description of the varying rates of maturation of glucuronidation clearance of morphine across all age ranges without the need for additional age-based covariates.

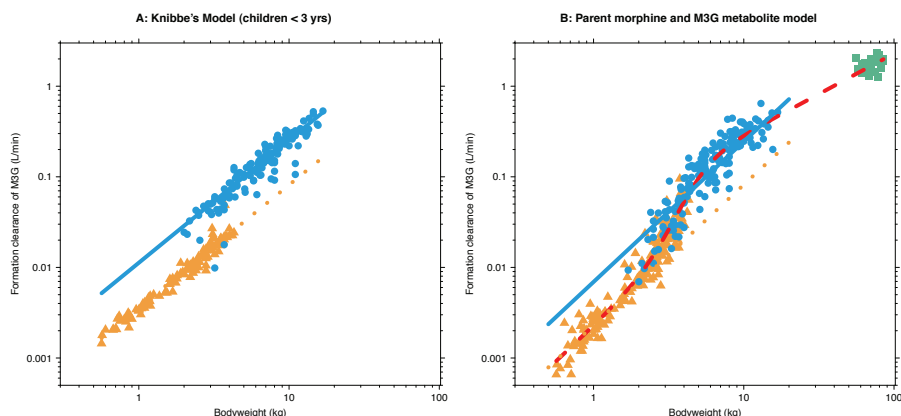


Figure 5-5 Comparison of formation clearance of M3G versus bodyweight in log-log scale between current Model II (parent morphine and M3G metabolite model) that included all age ranges except for older children and adolescents (B) and a previously published population model for morphine in children younger than 3 years by Knibbe *et al.* [4] (A). **PNA=postnatal age.**

(A): Orange filled triangle: children with $PNA < 10$ days; Blue filled circle: children with $PNA \geq 10$ days; Orange dotted line: Knibbe's model [4] predicted clearance curve for $PNA < 10$ days ($CL \text{ (mL/min)} = 3.48 * BW^{1.44}$); Blue solid line: Knibbe's model [4] predicted clearance curve for $PNA \geq 10$ days ($CL \text{ (mL/min)} = 8.62 * BW^{1.44}$) (B): Orange filled triangle = children with $PNA < 10$ days; Blue filled circle: children with $PNA \geq 10$ days; Green filled square: adults; Orange dotted line: simulated population clearance curve for $PNA < 10$ days ($CL \text{ (L/min)} = 0.0023 * BW^{1.56}$); Blue solid line: simulated population clearance curve for $PNA \geq 10$ days ($CL \text{ (L/min)} = 0.0069 * BW^{1.56}$); Red dash line: the BDE model predicted clearance curve of Model II in which, $CL_i = 1.67 * \left(\frac{BW_i}{70}\right)^k$, $k = 1.56 - \frac{0.67 * BW_i^{3.61}}{3.89^{3.61} + BW_i^{3.61}}$

The development of the bodyweight dependent exponent model was triggered by the reports that single exponent functions are not suitable for the prediction of

drug clearance in children of all age ranges [6] and the idea of using a continuous function describing clearance across a large age-span without the need for an additional age based function [11]. Beside application to propofol [11], this BDE model has been successfully applied to busulfan [12] and midazolam [30], albeit in a simplified power equation (). However, in the current analysis on morphine glucuronidation clearance between preterm neonates and adults, the full sigmoidal BDE model was more appropriate. This was the result of the S-shape in the double log plot of clearance versus bodyweight (Figure 5-1), which can be captured by the Emax function with Hill factor of the full BDE model [11], but not by the simplified function that consists of a power function [12, 30]. From these results it seems that the choice for a full BDE model which was applied in this study and for propofol, or for a simplified BDE model as applied for busulfan and midazolam is related to both the age range studied and the properties of the drug. Further study of the BDE model on datasets of other drugs across the entire paediatric age range will demonstrate in which cases the simplified or full BDE model is applicable. In any case, the choice for the final model should depend on the observed data in this data-driven approach whereby the model with the lowest number of parameters should be chosen (the principle of parsimony).

5.5. Conclusion

In this study, developmental changes in total morphine clearance were described in 475 preterm and term neonates, infants, children, adolescents and adults using an allometric function, in which the exponent decreased with bodyweight in a sigmoidal manner from 1.47 for preterm neonates to 0.88 in adults, with no need to use other body size or age-based measures. Similarly, we identified values for the exponent for formation clearance of M3G to vary from 1.56 to 0.89 while these values varied from 1.06 to 0.61 for elimination of M3G. From these results, it can be concluded that an allometric function with a bodyweight-dependent exponent (BDE) may be of great value when scaling clearance of drugs across the entire pediatric age-range.

5.6. Acknowledgements

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Appendix: NONMEM codes

Model I: Parent morphine model

```

$SUBROUTINES ADVAN5
;-----
$MODEL NCOMPARTMENTS=2
COMP (CENTRAL, DEFDOSE) ;MORPHINE CENTRAL
COMP =(2) ;PERIPHERAL COM OF MORHINE
;-----
$PK
KDEC = THETA(1) ; DECREASE OF EXPONENT FOR CLM1
KMAX = THETA(2)+KDEC ; MAXIMUM EXPONENT OF CLM1
KHAL = THETA(3) ; K50 OF CLM1
GAMMA = THETA(4) ; GAMMA OF CLM1
KBDE = KMAX-KDEC*(BW**GAMMA)/(KHAL**GAMMA+BW**GAMMA)
;-----
TVCL = THETA(5)*(BW/70)**KBDE ; POPULATION CLEARANCE OF MORPHINE
CL = TVCL*EXP(ETA(1)) ; INDIVIDUAL ...
TVQ2 = THETA(6)*(BW/70) ; POPULATION INTERCOMPARTMENTAL
CLEARANCE OF MORHPINE
IF (POP.EQ.2) TVQ2= THETA(10)
Q2 = TVQ2*EXP(ETA(2)) ; INDIVIDUAL ...
;-----
TVV1 = THETA(7)*(BW/70) ; POPULATION VOLUME OF MORPHINE CENTRAL
COMPARTMENT
IF (POP.EQ.2) TVV1=THETA(11)*(BW/70);
V1 = TVV1 * EXP(ETA(3)) ; INDIVIDUAL ...
TVV2 = THETA(8)*(BW/70) ; POPULATION VOLUME OF MORPHINE PEREPHERAL
COMPARTMENT
V2 = TVV2*EXP(ETA(4)) ; INDIVIDUAL ...
;-----
S1=V1
;-----
K10 = CL/V1
K12 = Q2/V1

```

$$K21 = Q2/V2$$

$$F1 = 1$$

$$IF (POP.EQ.3) F1 = 0.88$$

;------

\$ERROR

$$IPRED = LOG(0.000001)$$

$$IF (F.GT.0) IPRED = LOG(F)$$

$$W = THETA(9)$$

$$IRES = IPRED - DV$$

$$IWRES = IRES / W$$

$$TEH = 0$$

$$IF (TIME.GT.1900.AND.NKOD.EQ.1) TEH = 1$$

$$IF (TIME.GT.1900.AND.NKOD.EQ.2) TEH = 1$$

$$Y = IPRED + ERR(1)*W + TEH*ERR(2) \quad ; MORPHINE$$

;------

$$\$THETA (0.1, 0.5,) \quad ; KDEC$$

$$(0.2, 0.9,) \quad ; KMAX - KDEC \text{ OR MINIMUM EXP (TH2)}$$

$$(0.05, 4, 20) \quad ; KHAL$$

$$(1, 5,) \quad ; GAMMA$$

$$(0.001, 1.5,) \quad ; CL$$

$$(0.01, 1.7,) \quad ; Q2$$

$$(0.1, 70,) \quad ; V1$$

$$(0.1, 100,) \quad ; V2$$

$$(0, 0.33,) \quad ; ERR1$$

$$(0.1, 1.1,) \quad ; Q2 \text{ ADO}$$

$$(0.1, 50,) \quad ; V1 \text{ ADO}$$

;------

$$\$OMEGA 0.15 \quad ; CL$$

$$0 \text{ FIX} \quad ; Q2$$

$$0.1 \quad ; V1$$

$$0 \text{ FIX} \quad ; V2$$

;------

\$\$SIGMA

$$1 \text{ FIX} \quad ; ERR1$$

$$2 \quad ; ERR2 > 1900 \text{ min}$$

;------

```

$EST NOABORT SIGDIG=3 PRINT=15 MAXEVAL=9999 METHOD=1 INTERACTION
POSTHOC
$COV COMP PRINT=E

```

Model II: Parent morphine and M3G metabolite model

```

$SUBROUTINES ADVAN5
;-----
$MODEL
NCOMPARTMENTS=3
COMP (CENTRAL, DEFDOSE);MORPHINE CENTRAL
COMP=(2);M3G
COMP=(3);PERIPHERAL COM OF MORHINE
;-----
$PK
KDEC1 = THETA(1)           ; DECREASE OF EXPONENT FOR CLM1
KMAX1 = THETA(2)+KDEC1      ; MAXIMUM EXPONENT OF CLM1
KHAL1 = THETA(3)           ; K50 OF CLM1
GAMMA1 = THETA(4)          ; GAMMA OF CLM1
KBDE1 = KMAX1-KDEC1*(BW**GAMMA1)/(KHAL1**GAMMA1+BW**GAMMA1)

KDEC2 = THETA(5)           ; DECREASE OF EXPONENT FOR CLE2
KMAX2 = THETA(6)+KDEC2      ; MAXIMUM EXPONENT OF CLE2
KHAL2 = THETA(7)           ; K50 OF CLE2
GAMMA2 = THETA(8)          ; GAMMA OF CLE2
KBDE2 = KMAX2-KDEC2*(BW**GAMMA2)/(KHAL2**GAMMA2+BW**GAMMA2)

;-----
TVCLM1 = THETA(9)*(BW/70)**KBDE1      ; POPULATION METABOLISM OF
MORPHINE TO M3G
CLM1 = TVCLM1*EXP(ETA(1))      ; INDIVIDUAL ...
TVCLE1 = THETA(10)*(BW/70)      ; POPULATION EXCRETION OF MORPHINE +
METABOLISM TO M6G
CLE1 = TVCLE1*EXP(ETA(2))      ; INDIVIDUAL ...
TVCLE2 = THETA(11)*(BW/70)**KBDE2    ; POPULATION EXCRETION OF M3G

```

```

CLE2 = TVCLE2*EXP(ETA(3))      ; INDIVIDUAL ...
;-----
TVV1 = THETA(12)*(BW/70)      ; POPULATION VOLUME OF MORPHINE CENTRAL
COMPARTMENT
V1 = TVV1 * EXP(ETA(4))      ; INDIVIDUAL ...
TVV2 = THETA(13)*(BW/70)**THETA(18) ; POPULATION VOLUME OF M3G
V2 = TVV2 * EXP(ETA(5))      ; INDIVIDUAL ...
TVQ2 = THETA(14)*(BW/70)      ; POPULATION INTERCOMPARTMENTAL
CLEARANCE OF MORHPINE
Q2 = TVQ2*EXP(ETA(6))      ; INDIVIDUAL ...
TVV3 = THETA(15)*(BW/70)      ; POPULATION VOLUME OF MORPHINE
PEREPHERAL COMPARTMENT
V3 = TVV3*EXP(ETA(7))      ; INDIVIDUAL ...

F1 = 1
IF (POP.EQ.3) F1 = 0.88
;-----
S1=V1
S2=V2
;-----
K10 = CLE1/V1
K12 = CLM1/V1
K13 = Q2/V1
K20 = CLE2/V2
K31 = Q2/V3

;-----
$ERROR
COM1=0
IF (CMT.EQ.1) COM1=1
COM2=0
IF (CMT.EQ.2) COM2=1
;
IPRED=LOG(0.000001)
IF (F.GT.0) IPRED = LOG(F)
W1 = THETA(16)      ;ERR Morphine

```

W2 = THETA(17) ;ERR M3G

IRES = IPRED-DV

IWRES = IRES/(COM1*W1+COM2*W2)

TEH=0

IF (TIME.GT.1900.AND.NKOD.EQ.1) TEH = 1

IF (TIME.GT.1900.AND.NKOD.EQ.2) TEH = 1

Y1 = IPRED + ERR(1)*W1 + TEH*ERR(3) ; MORPHINE

Y2 = IPRED + ERR(2)*W2 + TEH*ERR(3) ; M3G

Y=COM1*Y1+COM2*Y2

;------

\$THETA

(0.1, 0.74,) ;KDEC1

(0.4, 0.7,) ;KMAX1-KDEC1 OR MINMUM EXP1 (TH2)

(0.05, 4, 20) ;KHAL1

(1, 3,) ;GAMMA1

;------

(0.1, 0.47,) ;KDEC2

(0.4, 0.6,) ;KMAX2-KDEC2 OR MINMUM EXP2 (TH15)

(0.05, 5, 20) ;KHAL2

(1, 6,) ;GAMMA2

;------

(0.001, 1.4,) ;CLM1

(0.001, 0.1,) ;CLE1

(0.001, 0.23,) ;CLE2

(0.01, 30,) ;V1

20 FIX ;V2

(0.001, 4,) ;Q2

(0.01, 180,) ;V4

(0, 0.435,) ;ERR MORP

(0, 0.362,) ;ERR M3G

(0.1, 0.7,) ;EXP V2

;------

\$OMEGA

0.2 ;CLM1

0.1 ;CLE1

0.19 ;CLE2

0.2 ;V1

0.2 ;V2

0 FIX ;Q2

0.3 ;V3

;-----

\$SIGMA

1 FIX

1 FIX

2 ;ERR3

;-----

\$EST NOABORT SIGDIG=3 PRINT=15 MAXEVAL=9999 METHOD=1 INTERACTION

POSTHOC

\$COV COMP PRINT=E





Chapter 6

Population pharmacokinetics of paracetamol across the human age-range from (pre)term neonates, infants, children to adults

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Abstract

Introduction: In order to characterize the variation in pharmacokinetics of paracetamol across the human age span, we performed a population pharmacokinetic analysis from preterm neonates to adults with specific focus on clearance.

Methods: A total of 220 (pre)term neonates, infants, children and adults from eight previously published studies on paracetamol pharmacokinetics were analysed using NONMEM 7.2. In the covariate analysis, covariates were tested in linear functions, power functions and a power function with a bodyweight-dependent exponent.

Results: Between preterm neonates and adults, linear bodyweight functions were identified for Q2, Q3, V1, V2 and V3, while for CL a power function with a bodyweight-dependent exponent k was identified ($CL_i = CL_p \cdot (BW/70)^k$). The exponent k was found to decrease in a sigmoidal manner with bodyweight from 1.2 to 0.75, with half the decrease in exponent reached at 12.2 kg. No other covariates such as age were identified.

Conclusions: A pharmacokinetic model for paracetamol characterising changes in pharmacokinetic parameters across the pediatric age-range was developed in which clearance was found to change in a highly nonlinear manner with bodyweight. Based on the final model, dosing guidelines are proposed from preterm neonates to adolescents resulting in similar exposure across all age ranges.

6.1. Introduction

Paracetamol or acetaminophen is an antipyretic and analgesic agent widely used in both adult and pediatric populations. Typically, paracetamol is given to patients orally or rectally¹. Recently, an intravenous formulation of paracetamol has become available, which is also regularly used as analgesic in neonates even though the USFDA only approved its use for the treatment of acute pain and fever in children aged 2 years and older and adults. In latest years, there has also been great interest in intravenous paracetamol in preterm neonates for closure of neonatal ductus²⁻⁴ at even higher dosages than when used as an analgesic.

To derive evidence based dosing guidelines for intravenous paracetamol across all age ranges including neonates and infants, information is needed on the pharmacokinetics. While the pharmacokinetics of paracetamol have been modeled in neonates and infants⁵⁻⁷, different models have been derived upon paracetamol or its prodrug given orally, rectally or intravenously⁸⁻¹⁰. Regardless of whether in these analyses data from preterm and term neonates were available⁸ or not available^{9,10}, in all these studies, paracetamol clearance was modeled on the basis of bodyweight with a $\frac{3}{4}$ allometric function, after which an age-based maturation function was added⁸⁻¹⁰. As the use of both bodyweight and age as covariates on a single parameter was recently reported to potentially harm the predictive performance of the resulting model because bodyweight and age are correlated in a complex and highly nonlinear manner as part of a child's growth and development^{11,12}, further study on the influence of bodyweight on the pharmacokinetics of paracetamol across the entire human lifespan with inclusion of data from (pre)term neonates seems important. Preferably in this analysis, the influence of bodyweight on clearance is optimized in a way that the inclusion of an additional maturation functions based on age is not required^{11,12}. For this purpose, an allometric function on the basis of bodyweight in which the allometric exponent was allowed to vary with bodyweight was proposed for scaling clearance across the human age range¹³. Following the successful application of this so called bodyweight dependent exponent (BDE) model to the scaling of the clearances of propofol¹³, morphine¹⁴, busulfan¹⁵ and midazolam¹⁶, the current analysis focuses on the utilization of this model for scaling paracetamol clearance over a large lifespan including

preterm neonates. Given the fact that dosing in children including preterm and term neonates is typically performed on the basis of bodyweight, the results can also be used to derive bodyweight-based dosing guidelines for this drug in all pediatric subpopulations aiming to reach similar paracetamol exposure on the assumption of an identical exposure-effect relationship in pediatrics as in adults.¹⁷

Therefore, in the current study, we included pharmacokinetic data from eight previously published paracetamol studies^{7,18-24} in order to characterize its pharmacokinetics across the entire human lifespan. In the covariate analysis, there was specific focus on the optimization of the influence of bodyweight as predictor of inter-individual variability in clearance.

6.2. Methods

6.2.1. *Subjects of the original studies*

A total of 220 subjects from eight previously published studies^{7,18-24} on paracetamol pharmacokinetics were included in the current study, including neonates (1- 76 days), infants (0.11-1.33 years), children (2-7 years) and adults (19-34 years). Detailed information on the studies is given below and summarized in Table 6-1.

Neonates^{7,18,19}

*Study 1: Single dose intravenous propacetamol in neonates*¹⁸

Thirty preterm (gestational age 27 – 35 weeks) and term (gestational age 36 – 40 weeks) neonates aged 1 day and weighing 0.5-4 kg, who received single dose of intravenous propacetamol, were included in the current analysis. Propacetamol dose was 20 mg/kg (10 mg/kg paracetamol) for the first 15 neonates and 40 mg/kg (20 mg/kg paracetamol) for the next 15 neonates. Propacetamol bolus infusion was prepared from a 1 gram propacetamol (500 mg paracetamol) powder-containing vial diluted in 50 ml normal saline. Samples were collected 30, 60, 90, 120, 180, 240, and 600 minutes after the start of intravenous administration.

*Study 2: Repeated dose intravenous propacetamol in neonates*¹⁹

Eighteen preterm and term neonates aged 1-76 days (postconceptual age 27 – 42 weeks) and weighing 0.84-4 kg, who received repeated doses of intravenous propacetamol, were included in the current analysis. The dosing regimen consisted of a loading dose (20 mg/kg paracetamol) with a maintenance dosing of 10 mg/kg/dose every 12, 8 or 6 hours for extreme preterm, preterm and term neonates, respectively. Samples were collected 30, 60, 90, 120, 180, 240 and 600 min after initiation of intravenous administration.

*Study 3: Repeated dose intravenous paracetamol in neonates*⁷

Sixty preterm and term neonates aged 1-28 days and weighing 0.6-4.8 kg, who received repeated dose intravenous paracetamol, were included in the current analysis. The dosing regimen consisted of a loading dose (20 mg/kg) followed by a maintenance dose of 5, 7.5 or 10 mg/kg every 6 hours for extreme preterm, preterm and term neonates, respectively. Samples were collected from an arterial line in the first 48 hours after the paracetamol loading dose.

Infants^{20,23}*Study 4: Repeated dose intravenous propacetamol and rectal paracetamol in infants*²⁰

Twenty-six infants aged 0.11 – 1.33 years and weighing 7.5-12.2 kg after major craniofacial surgery were included in the current analysis. During surgery, all infants received a rectal loading dose of 40 mg/kg paracetamol 2 hours before anticipated extubation. On admittance to the pediatric surgical ICU, the children were randomized to receive either a 15 minutes intravenous infusion of 40 mg/kg propacetamol or 20 mg/kg paracetamol rectally every 6 hours. Blood samples were taken from the arterial catheter at 15 minutes, 1 and 6 hours after the first dose, at 5 minutes, 4 and 6 hours after the third dose, and at 30 minutes, 2 and 3 hours after the fourth dose.

*Study 5: Repeated dose rectal acetaminophen in infants*²³

Twenty rectally dosed infants out of forty infants in the original study, aged 0.67 -1.25 years and weighing 7.9-12.2 kg, were included in the current analysis. Approximately 2 hours before anticipated extubation, a loading dose of acetaminophen (40 mg/kg) was administered rectally. Two hours after arrival

in the pediatric surgical ICU, 20-mg/kg acetaminophen was administered rectally at $t = 6$, $t = 12$, and $t = 18$ hours. Blood samples were taken at 30, 60, and 90 minutes after the rectal loading dose and at 5, 8, 11, 14, 17 and 20 hours after arrival in pediatric surgical ICU. One individual was excluded from the population pharmacokinetic analysis because specific dosage information was missing.

Children

*Study 6: Repeated dose rectal acetaminophen in children*²⁴

Twenty-nine children, aged 2-7 years and weighing 14-33 kg, received a loading dose of 40 mg/kg rectally resulting in a dose of 600, 700, 800, 900, 1000, 1100, 1200 or 1300 mg when the child was in weight class 14 - 16 kg, 17 - 18 kg, 19 - 21 kg, 22 - 23 kg, 24 - 26 kg, 27 - 28 kg, 29 - 31 kg or in weight class 32 - 33 kg, respectively. The maintenance dose of 30 mg/kg was given rectally every 8 hours and was a dose of 450, 525, 600, 675, 750, 825, 900 or 975 mg when the child was in weight class 14 - 16 kg, 17 - 18 kg, 19 - 21 kg, 22 - 23 kg, 24 - 26 kg, 27 - 28 kg, 29 - 31 kg or 32 - 33 kg, respectively. Samples were taken at the start and at the end of surgery and 1, 2 and 3 hours postoperatively.

Adults^{21,22}

*Study 7: Intravenous infusion of propacetamol in healthy male adults*²¹

Data from twelve healthy male volunteers aged 21-25 years and weighting 63-83 kg from one sub-branch (Study I, Occasion B) in the original study were included in the current analysis. Each subject was given 1 gram of propacetamol HCL (=500 mg paracetamol) intravenously at a constant infusion rate over 15 minutes. Samples were taken before the administration and at 15, 20, 30, 45 minutes, 1, 2, 3, 4, 6, 8, 10, 12, 24 hours after administration.

*Study 8: Intravenous infusion of paracetamol in healthy adults*²²

Twenty-six healthy volunteers aged 19-34 years and weighing 49.2-94 kg were included in the current analysis. Each subject received first a 15 minutes infusion of 2 gram of paracetamol (Perfalgan), followed by four additional 15 minutes infusions of 1 gram paracetamol each, at 6 hours intervals. Blood samples were collected before dosing and at 15 minute (the end of infusion), 30 minutes and

1 hour, 1.1 hour, 2, 3, 4, and 6 hour after the 2 gram dose and the first 1 gram dose; before dosing and at the end of infusion of the second and third 1 gram doses; before dosing and at 15 minutes (end of infusion), 30 minutes and 1 hour, 1.5 hours, 2, 3, 4, 6, 8, 10, 12, and 24 hours after the last 1 gram dose.

6.2.2. Pharmacokinetic modelling

Model Building

The population pharmacokinetic analysis was performed with the non-linear mixed effects modeling software NONMEM version 7.2. (ICON Development Solutions, Ellicott City, MD, USA) using the first-order conditional estimation method with the interaction option (FOCEI). Tools like S-PLUS interface for NONMEM (LAP&P Consultants BV, Leiden, NL), S-Plus (version 8.1, Insightful Software, Seattle, WA, USA), XPose and R (version 2.15.1) were used to visualize the output and evaluate the models.

Paracetamol concentrations were logarithmically transformed and fitted simultaneously, since the range in concentrations was more than 1000 fold. Model building was performed in four steps: (1) selection of structural model, (2) selection of statistical sub-model, (3) systematic covariate analysis, (4) model validation. For selection of a structural or statistical model, a difference in objective function (OFV) between models of more than 3.84 points was considered as statistically significant ($p < 0.05$ assuming a Chi-square distribution). Furthermore, the goodness-of-fit plots (observed *versus* individual predicted concentrations and *versus* population predicted concentrations, and conditional weighted residuals *versus* time and *versus* population prediction concentrations) of all data and data stratified per age category were evaluated. Finally, the total number of parameters, improvement of the individual concentration-time profiles, the confidence intervals of the parameter estimates and the correlation matrix were assessed. In case the subjects were given propacetamol intravenously, 1 unit propacetamol dose was converted to 0.5 unit of paracetamol as dosing input for the pharmacokinetic analysis.

Structural Model

Based on previous reports^{9,20}, the distribution and elimination pharmacokinetics of paracetamol were primarily modelled with a two-compartment model, which was parameterized in terms of total clearance (CL), volume of

distribution of the central compartment (V1), volume of distribution of the peripheral compartment (V2) and inter-compartmental clearances between central compartment and peripheral compartment (Q2). To describe the process of rectal absorption for individuals who were given paracetamol rectally, a rectal deposit compartment was incorporated into the model with first-order absorption rate constant (Ka), bioavailability (F), and lag time (Tlag) as parameters. Since we had data upon bolus infusion of paracetamol in our analysis²¹, we also explored the performance of a three-compartment model for distribution and elimination of paracetamol, which was parameterized in terms of total clearance (CL), volume of distribution of the central compartment (V1), volume of distribution of the rapidly-equilibrating peripheral compartment (V2) and slowly-equilibrating peripheral compartment (V3), and inter-compartmental clearances between central compartment and two peripheral compartments (Q2, Q3).

Because in some of the studies included in this analysis propacetamol, an intravenous prodrug of paracetamol, was given, we tested a deposit compartment for propacetamol and its first order hydrolysis rate in order to describe the process of conversion from propacetamol to paracetamol. As the half-life of the hydrolysis rate turned out to be very short (<0.0001 hour), this compartment was neglected in the final model.

Statistical Model

Interindividual variability in the pharmacokinetic parameters was tested in the model assuming log-normal distributions, expressed as

$$\theta_i = \theta_{TV} \times e^{\eta_i} \quad , \quad \eta_i \sim N(0, \omega^2) \quad (1)$$

where θ_i is the individual pharmacokinetic parameter value for the i th individual, θ_{TV} is the population pharmacokinetic parameter value or typical value, and η_i is a random variable for the i th individual from a normal distribution with mean zero and variance ω^2 . In addition to testing of the inclusion of interindividual variability on individual parameters, model improvement by inclusion of covariance between these variability parameters was tested as well.

As bioavailability should be constrained between 0 and 1, interindividual variability in bioavailability was parameterized in a model proposed by Karlsson *et al.*²⁵ using the following function:

$$F_i = \frac{e^{\ln\left(\frac{TVF}{1-TVF}\right) + \eta_i}}{1 + e^{\ln\left(\frac{TVF}{1-TVF}\right) + \eta_i}} \quad (2)$$

For the residual error, an additive model for log-transformed concentrations was used which corresponds to proportional error on untransformed data, expressed as:

$$\log C_{ij} = \log C_{pred_{ij}} + \varepsilon_{ij} \quad , \quad \varepsilon_{ij} \sim N(0, \sigma^2) \quad (3)$$

where C_{ij} is the value of the observed paracetamol concentration of i th individual at time j , $C_{pred_{ij}}$ is the value of the predicted paracetamol concentration of the i th individual at time j , and ε_{ij} is a random variable for this observation from a normal distribution with mean zero and variance σ^2 .

Covariate Model

The covariates bodyweight and age were tested on the pharmacokinetic parameters in a linear function, power function or a power function with a bodyweight dependent exponent. This power function with a bodyweight dependent exponent is called a bodyweight dependent exponent (BDE) model¹³ and is shown in equation 4, where it is applied to paracetamol clearance.

$$CL_i = CL_p \times \left(\frac{BW_i}{70}\right)^k \quad , \quad k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma} \quad (4)$$

In equation 4, CL_i is clearance in the i th individual with bodyweight BW_i ; CL_p is the clearance in a standardized adult with a bodyweight of 70 kg; BW_i is bodyweight of an individual i ; k is the exponent; k_0 is the value of the exponent at a theoretical bodyweight of 0 kg; k_{max} is the maximum decrease of the exponent; k_{50} is the bodyweight at which a 50% decrease in the maximum decrease of exponent value is attained, and γ is the Hill coefficient determining the steepness of sigmoidal decline in the exponent¹³.

The significance of a covariate for a parameter in the model was statistically evaluated by the use of the objective function. In the forward inclusion a p

value <0.005 was considered as statistically significant while a more stringent p value <0.001 was used in the backward deletion. When two or more covariates were found to significantly improve the model, the covariate that reduces the objective function the most was retained into the model and served as a basis for subsequent inclusion of additional covariates. The choice of the covariate model was further evaluated as described under Model Building whereby the results of the model validation were also considered.

6.2.3. Model Validation

The model was validated internally using five criteria that were recently proposed for pediatric population model evaluation²⁶. (i) The coefficient of variation (CV) of the parameter estimates either from the covariance step in NONMEM or from 200 stratified bootstrap resampling results is less than 50%, (ii) The basic diagnostic plots and particularly the plots of the observed versus population predicted concentrations stratified for age, are visually assessed for bias and precision, (iii) The η -shrinkage is calculated and evaluated according to Karlsson and Savic²⁷, (iv) The individual and population predicted parameters are plotted against the primary covariate to evaluate whether the individual predicted parameters are equally distributed around the population predicted parameters, (v) The simulation based normalized prediction distribution error (NPDE) proposed by Brendel *et al.* is calculated based on at least 2,000 simulations of the entire dataset and is evaluated visually for bias and precision²⁸.

Simulations on the basis of the final model

Based on the final model paracetamol concentration-time profiles were simulated for children with bodyweight of 0.5, 1, 1.5, 2, 3, 5, 8, 9, 15, 20, 35 and 50 kg. Two intravenous administration dosing regimens were simulated, i.e. a traditional dosing regimen and a model-based dosing regimen.

The traditional dosing regimen for prematurely born neonates and children was based on the Dutch Children's Formulary [5mg/kg every 6 hours (group 1 with weight 0.5, 1, 1.5 and 2 kg)]²⁹ while for term neonates and children it was based on the official labeling information in the Netherlands (for term neonates and children weighing less than 10 kg 7.5 mg/kg every 6 hours (group 2 with weight 3, 5, 8 and 9 kg) and for children weighing between 10 and 50 kg 15mg/kg every 6 hours (group 3 with weight 15, 20, 35 and 50 kg).

In the model-based dosing regimen, model based doses were derived for children of all bodyweights on the basis of a loading dose and a maintenance dose every 6 hours while aiming for steady state concentrations attained in group 3 using the traditional dosing regimen.

6.3. Results

For the analysis, data of 220 subjects varying from preterm and term neonates, infants, children and adults receiving paracetamol intravenously or rectally or propacetamol intravenously were available. An overview of the data of the eight paracetamol studies was summarized in Table 6-1.

Table 6-1 Summary of studies included in this analysis

Study	Population	Administration	N	Age	Weight	Reference
1	preterm and term neonates	i.v. propacetamol	30	1 day	0.505–4 kg	(18)
2	preterm and term neonates	i.v. propacetamol	18	1–76 days	0.81–4 kg	(19)
3	preterm and term neonates	i.v. paracetamol	60	1–28 days	0.6–4.3 kg	(7)
4	infants	rectal paracetamol i.v. propacetamol	26	0.11–1.33 yrs	7.5–12.2 kg	(20)
5	infants	rectal paracetamol	19	0.67–1.25 yrs	7.9–12.2 kg	(23)
6	children	rectal paracetamol	29	2–7 yrs	14–33 kg	(24)
7	Male adults	i.v. propacetamol	12	21–25 yrs	63–83 kg	(21)
8	adults	i.v. paracetamol	26	19–34 yrs	49.2–94 kg	(22)

Table 6-2 presents the results of the final model. The results demonstrated that a three-compartment structural model for distribution and elimination of paracetamol was superior over a two-compartment model, based on the improvement of the goodness of fit plots and a decrease of 639 points ($P < 0.0001$) in objective function value. The population lag time (Tlag) of the rectal dose was estimated to be 0.409 hour without interindividual variability. The rectal bioavailability was estimated to be 0.962 with a large interindividual variability of 205.9%. The rectal absorption rate constant (Ka) was estimated to be 0.28 (1/hour) with an interindividual variability of 86.7%. The population value for clearance in a typical adult with a bodyweight of 70kg was estimated

17.6 L/hr and the corresponding population volume of distribution of the central compartment was 25.1 L (Table 6-2).

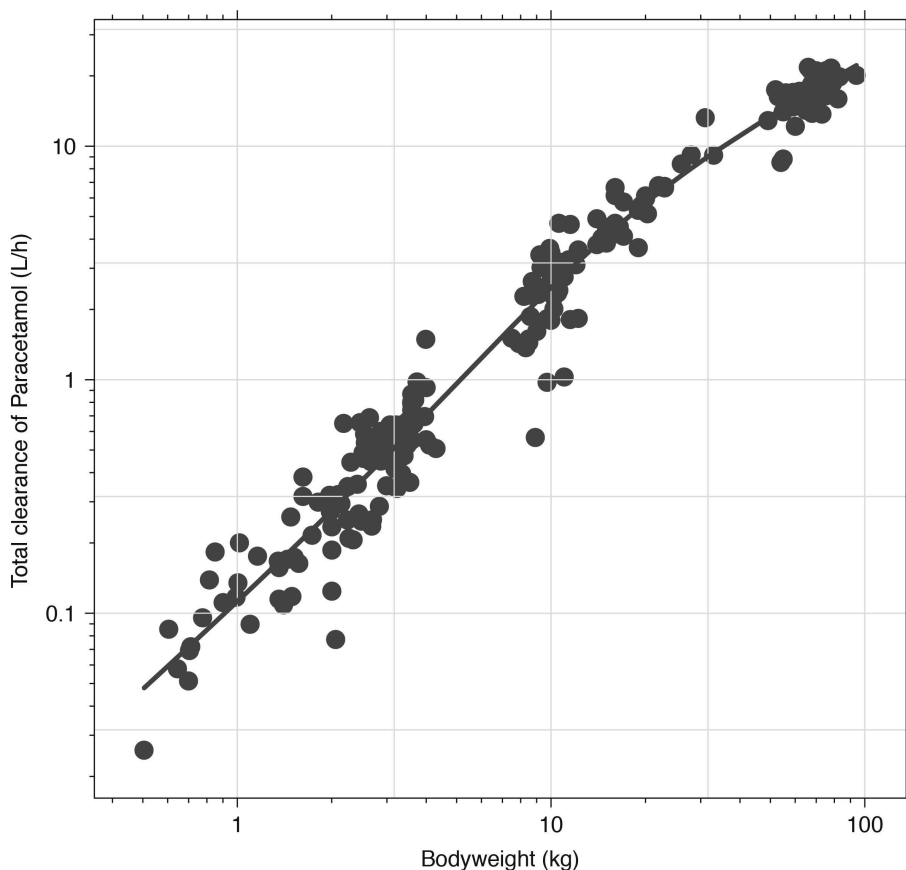


Figure 6-1 Plot of paracetamol clearance of all individuals (*post hoc* parameter estimates) against bodyweight in log-log scale.

Filled circles: *post hoc* individual paracetamol clearance; Solid line: model predicted paracetamol clearance as defined by $CL_i = CL_p \times (BW/70)^k$ with $k = k_0 - k_{max} \times BW^\gamma / (k_{50}^\gamma + BW^\gamma)$ with parameters as shown in Table 6-2.

Table 6-2 Parameter estimates of the final model

Parameter	Model Estimates	RSE	Bootstrap Mean	Bootstrap RSE
Fixed effect				
CL	$= TVCL \times (BW/70)^k$			
TVCL (L/hr•70kg)	17.6	3.2%	17.5	2.8%
k	$= k_0 - k_{max} \times BW^\gamma / (k_{50}^\gamma + BW^\gamma)$			
k_{max}	0.454	5.4%	0.44	5.7%
$k_0 - k_{max}^S$	0.75 FIX	-	-	-
k_{50} (kg)	12.2	27.2%	28.3	202.9%
γ	1.4 FIX	-	-	-
V1	$= TVVc \times (BW/70)$			
TVV1 (L/70kg)	25.1	11.2%	24.4	16.1%
Q2	$= TVQ \times (BW/70)$			
TVQ2 (L/hr•70kg)	96.8	12.6%	103.7	19.5%
V2	$= TVVp \times (BW/70)$			
TVV2 (L/70kg)	36.1	5.5%	36.8	7.3%
Q3	$= TVQ \times (BW/70)$			
TVQ3 (L/hr•70kg)	1.36	9.8%	1.37	8.1%
V3	$= TVV3 \times (BW/70)$			
TVV3 (L/70kg)	21.6	12.8%	22.2	13.9%
Ka (1/hr)	0.252	13.8%	0.29	25.9%
Tlag (h)	0.409	5.7%	0.409	6.4%
F	0.962	8%	0.9	11.7%
Random effect (%)				
ω CL	35.4%	9.1%	34.6%	17.6%
ω Vc	63.6%	12.6%	68.4%	36.6%
ω Ka	86.7%	14%	95.7%	42.5%
ω F	205.9%	76.4%	311%	110%
σ	21.4%	8.8%	21.4%	15.9%

k = bodyweight dependent exponent ; k_{max} = maximum decrease of k ; k_0 = value of k at a theoretical bodyweight of 0 kg; k_{50} = the bodyweight at which a 50% decrease in the maximum decrease of k is attained; γ = the Hill coefficient determining the steepness of sigmoidal decline in k ; Ka = rectal absorption constant rate; Tlag = lag time in rectal absorption; F= rectal bioavailability; ω = standard deviation of the inter-individual variability (η); σ = standard deviation of the proportional residual error (ϵ); $k_0 - k_{max}^S$ was parameterized as a fixed effect parameter (THETA) in NONMEM

Concerning the covariate analysis, bodyweight was identified a significant covariate for all structural pharmacokinetic parameters except for Ka, F and Tlag. For the parameters Q2, Q3, V1, V2 and V3, the influence of bodyweight was best described by a linear function (Table 6-2). For clearance, a power function in which the exponent k varied with bodyweight in a sigmoidal function (BDE model, eq.5) proved superior over a linear or a power function with a fixed exponent k . The bodyweight-dependent exponent k was estimated to decrease

from a value of 1.2 (k_0) at the theoretical bodyweight of zero kilogram to 0.75 (k_0-k_{max}) and reached half this decrease at 12.2 kilogram (k_{50}) (Table 6-2). The minimum exponent, which equals k_0-k_{max} , was fixed to 0.75, since this exponent proved successful in a separate pre-analysis based on adult data only. The Hill factor (γ) was found to be 1.4 and was fixed to this value in the final model in order to get the covariance step (Table 6-2). Figure 6-1 shows that on the basis of this BDE model, the change in individual paracetamol clearance over bodyweight of all individuals of this analysis was very well captured. As η -shrinkage was low (12.8%), the reliability of the individual clearance values in the subjects can be considered high²⁷. Concerning the evaluation of the implementation of the covariate model for clearance in the final model by the bodyweight-dependent exponent (BDE) model, Figure 6-2 shows that there were no remaining trends in the *post hoc* eta of clearance versus bodyweight, postnatal age (PNA), postmenstrual age (PMA) and gestational age (GA) plots. As the data of PMA and GA were only available in neonates, corresponding plots were limited to 50 weeks in the x-axes.

With respect to model validation, goodness of fit plots stratified by age (1. birth to 1 month; 2. 1 month to 2 years; 3. 2-12 years; 4 12-16 years) showed that the population predicted paracetamol concentrations of the final model were in good agreement with the observed paracetamol concentrations for the different age ranges (Supplemental Figure 6-S1). As a simulation based validation method, the normalized prediction distribution error (NPDE) showed that paracetamol concentrations in the models were normally distributed (mean=-0.02462, variance= 0.7756, $p<0.001$) around the median prediction and that there was no trend in the NPDE versus TIME and *versus* the logarithm of the individual predicted concentrations (Supplemental Figure 6-S2). In addition, the results of the bootstrap validation of the final model are listed in Table 6-2.

Table 6-3 Model-based dosing regimen of intravenous paracetamol aiming for a target paracetamol concentration of 9 mg/L in individuals weighing between 0.5 and 50 kg. Both maintenance doses administered 4 times daily (MD) and loading doses (LD) are presented. For the maintenance dose expressed per kg (MD/kg) the difference with a traditional dosing regimen is given.

BW (kg)	CL (L/h)	V (L)	$t_{1/2}$ (h)	Target Cavg (mg/L)	MD (mg)	MD/kg (mg/kg)	Traditional MD/kg (mg/kg)	% DIFF MD/kg	L/M Ratio	LD (mg)	LD/kg (mg/kg)
0.5	0.047	0.179	2.641	9	2.5	5.1	5	+2%	2.2	5.6	11.2
1	0.112	0.359	2.223	9	6.0	6.0	5	+20%	2	12.1	12.1
1.5	0.188	0.538	1.982	9	10.2	6.8	5	+36%	1.8	18.3	12.2
2	0.274	0.717	1.813	9	14.8	7.4	5	+48%	1.8	26.7	13.3
3	0.473	1.076	1.576	9	25.5	8.5	7.5	+13%	1.5	38.3	12.8
5	0.958	1.793	1.297	9	51.8	10.4	7.5	+39%	1.3	67.3	13.5
8	1.836	2.869	1.083	9	99.1	12.4	7.5	+65%	1.3	128.9	16.1
9	2.151	3.227	1.039	9	116.2	12.9	7.5	+72%	1.3	151.0	16.8
15	4.109	5.379	0.907	9	221.9	14.8	15	-1%	1.3	288.4	19.2
20	5.689	7.171	0.874	9	307.2	15.4	15	+3%	1.2	368.7	18.4
35	9.870	12.550	0.881	9	533.0	15.2	15	+1%	1.2	639.6	18.3
50	13.422	17.929	0.926	9	724.8	14.5	15	-3%	1.2	869.8	17.4

BW is body weight; CL is the predicted clearance from final model; V is the predicted volume of distribution from final model; $t_{1/2} = \ln(2) / (CL/V)$; Target Cavg is the target average concentration used for the model-based dosing regimen which was taken from the average steady state concentrations observed in individuals weighing 15, 20, 35, 50 kg upon the traditional dosing regimen; MD is the final model optimized Maintenance Dose = Target Cavg*CL*6h; MD/kg is the final model optimized Maintenance Dose Per Kilogram = MD/BW; TraditionalMD/kg is the Maintenance Dose per kilogram of the traditional dosing regimen; % DIFF MD/kg is the change of model based MD/kg from the TraditionalMD/kg in percentage = $100\% * (MD/kg - TraditionalMD/kg) / TraditionalMD/kg$; L/M Ratio is the ratio of loading dose to maintenance dose in the model-based dosing regimen; LD is the model-based Loading Dose = MD * L/M Ratio; LD/kg is the final model optimized Loading Dose per kilogram

Simulations on the basis of the final model for both a traditional dosing regimen and a model-based dosing regimen are presented in Figure 6-3. For the traditional dosing regimen, 3 different maintenance doses were used 6 hourly, i.e. 5 mg/kg, 7.5 mg/kg and 15 mg/kg for neonates with a body weight between 0.5 - 3 kg, 3 - 10 kg and children with weight 10 - 50 kg, respectively, according to the official label and the Dutch Childrens Formularium²⁹. The simulations upon the traditional dosing regimen in group 3 (Figure 6-3 a, bottom row, 10-50 kg) show that on average a concentration of 9 mg/L was attained (Figure 6-3 a, dotted reference line). For the other groups, a variety of average concentrations was attained upon this traditional mg/kg dosing regimen (Figure 6-3 a, upper two rows).

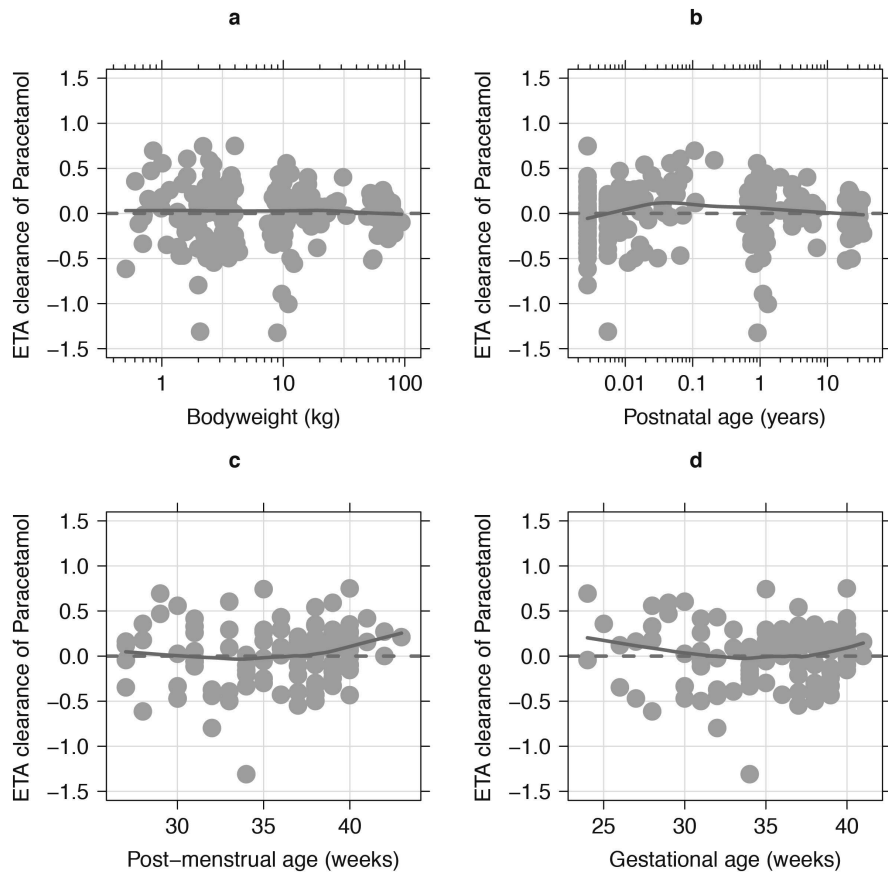


Figure 6-2 Plot of interindividual variability (ETA) in paracetamol clearance of the final model versus candidate covariates, including bodyweight, postnatal age, postmenstrual age (only for neonates) and gestational age (only for neonates).

Table 6-3 shows the loading and maintenance doses for the model-based dosing regimen aiming for a concentration of 9 mg/L in all groups with individuals varying in bodyweight between 0.5 and 50 kg. Figure 6-3 b shows the simulated concentrations in all individuals upon this model-based dosing regimen.

6.4. Discussion

In this meta-pharmacokinetic analysis we studied the pharmacokinetics of paracetamol using data from eight different clinical studies in (pre)term neonates, infants, children and adults. The results show that, whereas changes in most pharmacokinetic parameters were linearly related to bodyweight, developmental changes in clearance were best described on the basis of a power function with an exponent that varied with bodyweight. This exponent was found to vary from the value of 1.2 for neonates to 0.75 for older children and adults. Using this function, the influence of bodyweight on clearance was implemented in an optimal way which was confirmed by advanced internal validation procedures, being particularly important for pediatric studies where datasets are often sparse and variability due to age is large²⁶. Most importantly, the Empirical Bayes Estimates (EBE) based *post hoc* interindividual variability of clearance had no trend against either bodyweight or age (including PNA, PMA and GA) (Figure 6-2), confirming that an appropriate final model has been reached³⁰. These results demonstrate that the influence of bodyweight on clearance as described by the highly nonlinear bodyweight dependent exponent (BDE) function also takes the influence of age (PNA, PMA or GA) into account. However, caution is needed in case of obese individuals. In our datasets, there were no obese individuals, and therefore the derived function should not be used in obesity. A graphical comparison of clearance predictions between the BDE model and literature models⁸⁻¹⁰ shows that the predictions of these four models were in reasonable agreement with each other, such despite the fact that different functions and covariates were used (Supplemental Table 6-S1, Figure 6-S3).

The bodyweight-dependent exponent (BDE) function was originally developed on the basis of a very large and rich dataset from preterm neonates to adults on propofol¹³. Later on, it was applied for scaling morphine across the entire human lifespan¹⁴ and in the current study. Concerning the results from propofol, morphine and paracetamol from the current study, it seems that relatively similar values for k were found (exponent of 1.35 to 0.56, 1.47 to 0.88 and 1.2 to 0.75, respectively). We think that this may be explained by the fact that glucuronidation is the main pathway for these drugs upon which the question rises whether this function could be seen as a semiphysiological function for glucuronidation in humans.

For a previously developed function for morphine glucuronidation from preterm neonates to 3 year old children, this has recently been proposed and evaluated using physiologically based modelling principles^{31,32}. On the other hand, the current function for paracetamol differs from propofol and morphine by the k_{50} being 12.2 kg for paracetamol vs 3.78 and 4.01 kg for propofol and morphine, respectively, indicating that paracetamol clearance matures at a slower rate than glucuronidation of propofol and morphine. An explanation for this finding is that paracetamol is not entirely metabolised through glucuronidation as sulphation plays an important role, particularly in neonates⁹. Therefore the currently derived function is probably a mixture of glucuronidation and sulphation which each mature at their own rate. At least, it seems that the BDE model in which the exponent is allowed to vary with bodyweight is of relevance for scaling clearance across a large range in bodyweight on the basis of a single covariate.

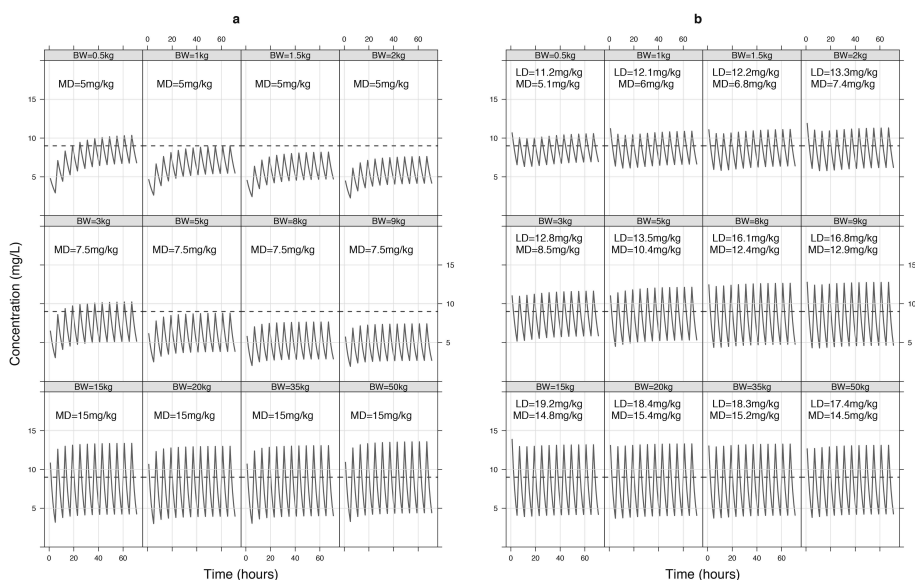


Figure 6-3 Simulated paracetamol concentration profiles of intravenously administered paracetamol 4 times daily based on a traditional regimen as described in section Methods (panel a) and a final model optimized regimen (panel b, Table 6-3) for neonates with a body weight between 0.5 - 3 kg (upper row), neonates and infants weighing between 3 - 10 kg (middle row) and children with a weight between 10 - 50 kg (lower row).

Solid curves are paracetamol concentration profiles; horizontal dotted line is the target average paracetamol concentration (9 mg/L) used for the final model optimized regimen (panel b) which was calculated from the average steady state concentration obtained upon the traditional dosing regimen in individuals weighing 15, 20, 35, 50 kg (panel a); MD is the maintenance dose per kilogram; LD is the loading dose per kilogram.

Intravenous paracetamol is not licensed for children younger than 2 years of age in the United States. Although intravenous paracetamol is licensed in the EU for children of all age ranges except prematurely born neonates, there is a large debate on the labelled dose for term neonates and children weighing less than 10 kg while doses for preterm neonates are based on expert opinion only ²⁹. In our study, we used the final model that was derived on the basis of eight different datasets across the entire human lifespan to simulate concentrations both upon a traditional dosing regimen and a model-based dosing regimen. For the traditional dosing regimen, 3 different maintenance doses were used 6 hourly, i.e. 5 mg/kg, 7.5 mg/kg and 15 mg/kg for neonates with a body weight between 0.5 - 3 kg, 3 - 10 kg and children with weight 10 - 50 kg, respectively. For the last two categories these doses are based on the official label, whereas the dose for preterm neonates is based on the Dutch Children's Formulary ²⁹. For the model-based dosing regimen a target paracetamol concentration was needed. Since the target therapeutic average paracetamol concentration for neonates is not known and the 6-hourly 15 mg/kg dose is widely accepted for children weighing more than 10 kg, we took the average paracetamol concentration reached in this dose group (i.e. 9 mg/L) as the target concentration for the model-based dosing regimen. On the basis of the 9 mg/L concentration as the target concentration, loading doses and maintenance doses of intravenous paracetamol were optimized based on clearance and volume of distribution as estimated using the final model (Table 6-3). Simulation results confirmed that 15 mg/kg maintenance doses is sufficient for children with weight 10 - 50 kg (Figure 6-3 a and b). However, 7.5 mg/kg was not enough to reach 9 mg/L average paracetamol concentration in neonates or infants weighing 5, 8 or 9kg, while 5 mg/kg proved too low of a dose for a prematurely born neonate weighing 1, 1.5 or 2 kg (Figure 6-3 a). Therefore bodyweight adjusted doses were proposed for these two categories as presented in Table 6-3, which proved to be in accordance with previous calls for adjustment of the labelled dose in infants and neonates. Palmer *et al.* recommended 10 mg/kg 6 hourly for preterm neonates with postmenstrual age (PMA) of 28-32 weeks, 12.5 mg/kg 6 hourly for neonates with PMA of 32-36 weeks and 15 mg/kg 6 hourly for neonates with PMA more than 36 weeks ³³. Allegaert *et al.* suggested a dosing regimen of 10 mg/kg every 6 hours within the age range 32-44 weeks postmenstrual age (PMA) ⁷. Although final dose recommendations should consider not only the pharmacokinetics but also

the pharmacodynamics and safety of paracetamol in neonates and infants, we think that our model is a first step towards an evidence based dosing in children of all ages including preterm and term neonates. In addition, our model can also be used to derive adjusted pediatric doses by use of Table 6-3 in which another target concentration (higher or lower than 9 mg/L) can easily be filled in, for instance when another therapeutic target concentration for a specific age category would be identified.

6.5. Conclusion

A pharmacokinetic model for paracetamol characterising changes in pharmacokinetic parameters across the entire human lifespan was developed in which clearance was found to change in a highly nonlinear manner with bodyweight. The results may provide insight in the exact relation between weight and clearance and as such provide a guide for individualised dosing in children varying between preterm neonates and adults. Once the therapeutic target concentration is known, corresponding appropriate doses can be easily calculated based on this model.

6.6. Acknowledgements

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Appendix

6.7.1 Notes for Figure 6-S1 and Figure 6-S2

The results obtained in this study were internally validated using advanced methods, which is highly important in particular for paediatric studies where datasets are often sparse and variability due to age is large ⁴. From the population predicted versus observed diagnostic plots (Figure 6-S1), it can be concluded that the model had adequate predictive value for each of the four different age groups, indicating that the model accounted for the changes in pharmacokinetics across the entire age span from preterm neonates to adults. In addition, simulation based NPDE validation results proved the model without significant deficiency or model misspecification (Figure 6-S2).

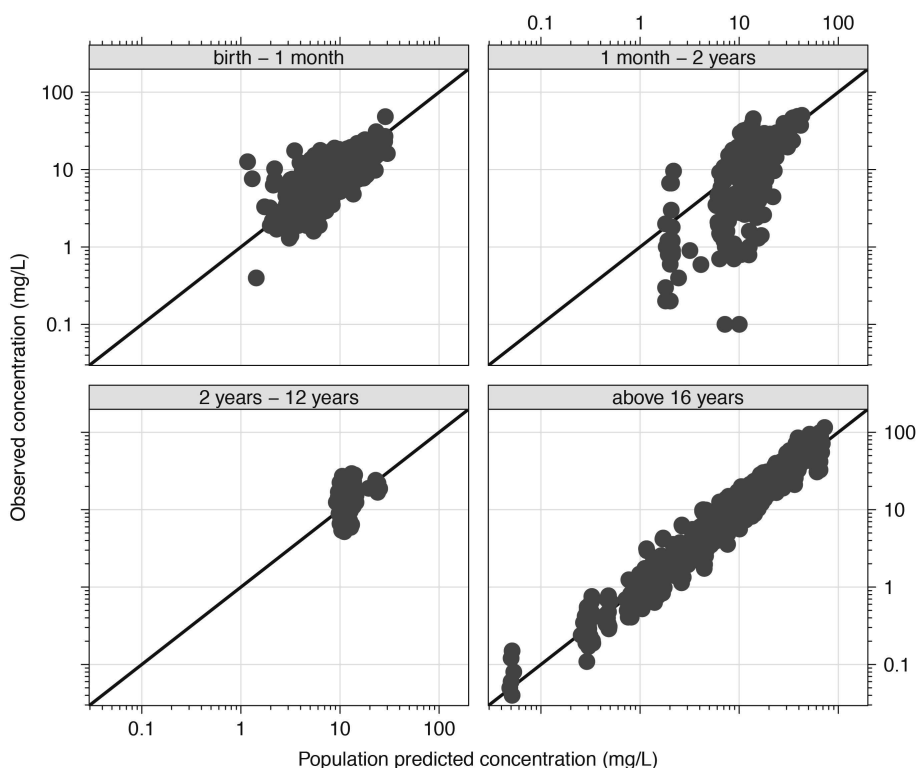


Figure 6-S1: Observed versus population predicted paracetamol concentrations stratified for four different age groups

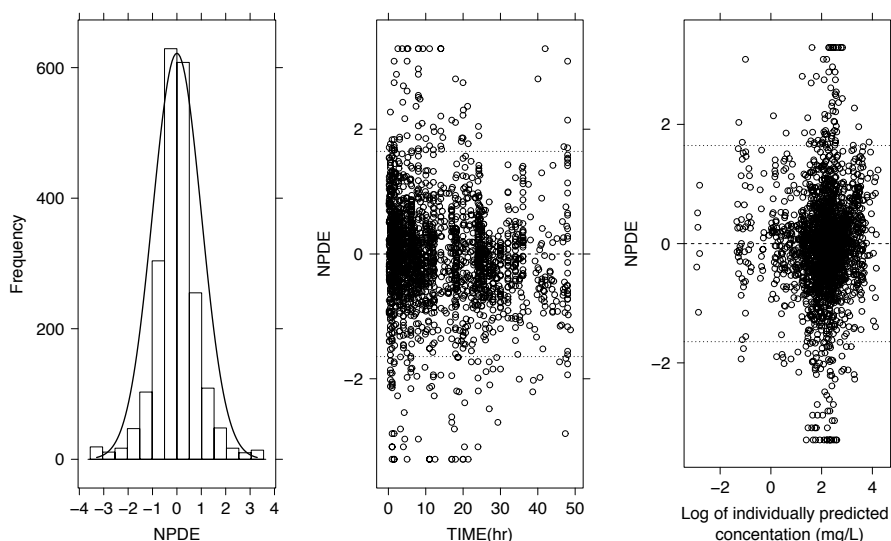


Figure 6-S2: Results of the Normalized Prediction Distribution Error (NPDE) validation with a histogram showing the NPDE distribution of paracetamol concentrations with a solid line indicating a normal distribution (left), distribution of NPDE versus time (middle) and distribution of NPDE versus the log of the paracetamol concentration (right).

6.7.2 Notes for Table 6-S1 and Figure 6-S3

Supplemental Table 6-S1 summarizes previously published functions to describe changes in paracetamol clearance in various age ranges across whole age span¹⁻³. From the age of 1.8 years onwards typically an allometric function with an exponent of 0.75 suffices to describe the change in clearance. However when including neonates^{1,2}, advanced functions and multiple covariates bodyweight and age (postconceptional age or postnatal age) are needed to describe the variation in clearance (Table 6-S1). In Figure 6-S3, we plotted the model predicted clearances from the three previously published models¹⁻³ and from our model, against the individual bodyweights of all our individuals. The model-predicted paracetamol clearances from these three models were derived by applying the respective maturation functions (Table 6-S1) to the individual data from our analysis. The predictions for these models were limited to the age range of the original studies (Table 6-S1) in order to prevent unintended extrapolations of these models. Figure 6-S3 shows that the predictions of these four models were in reasonable agreement with each other, such despite the fact that different functions and covariates were used. While Zuppa *et al.*² did not study neonates and had mainly access to children of 7 kg onwards without

reporting bodyweight and Mohammed *et al.*³ studied children from 1.8 years onwards, Anderson *et al.* did have a large number of preterm and term neonates in the dataset¹. For the neonatal range, the performance of the latter model¹ seemed in good agreement with the current model, such despite the fact that the models varied largely in functions and number of covariates (Table 6-S1). From neonates to infants of about 20 kg years, it seems that the models by Zuppa *et al.*², by Anderson *et al.*¹ and by Mohammed *et al.*³ slightly overestimated clearance compared to the values in our study. Finally, none of these analyses had adult data included even though predictions up to 30 kg were fairly similar to the predictions of our model which did have access to adult data.

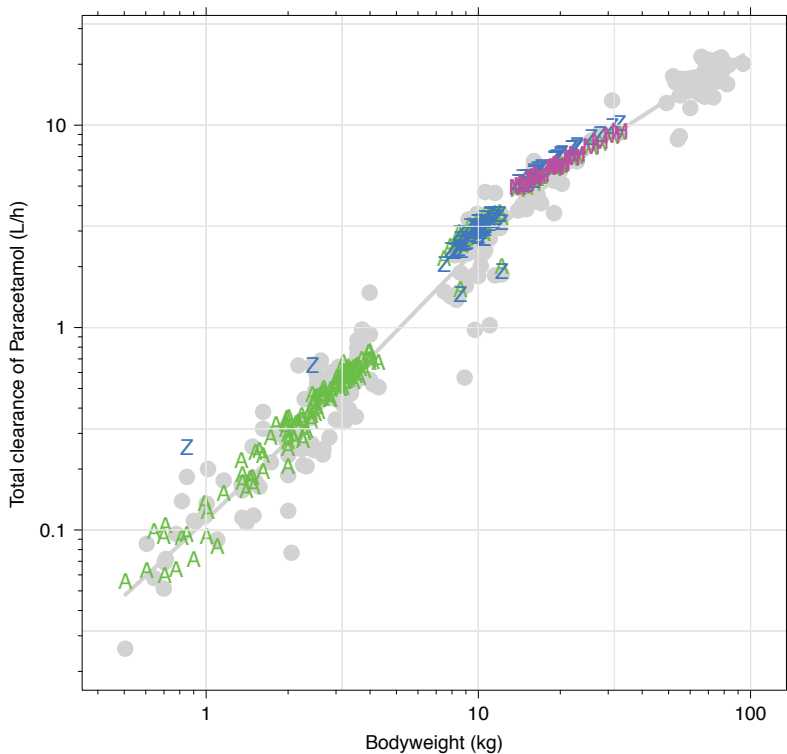


Figure 6-S3: Graphical comparison of predictions of paracetamol clearance versus bodyweight between the final model of the current analysis (gray circles and gray line) and three previously reported models (colored letters) in log-log scale. Gray circles: post hoc individual clearances estimated from the current model; Gray line: final model predicted clearance as defined by $(CL_i = CL_p \cdot (BW/70)^k$ with $k = k_0 - k_{\max} \cdot BW^y / (k50^y + BW^y)$, see Table 6-2 for parameter estimates); Green 'A': predicted clearance with model from Anderson *et al.*¹; Blue 'Z': predicted clearance with model from Zuppa *et al.*²; Pink 'M': predicted clearance with model from Mohammed *et al.*³

Table 6-S1: Paracetamol clearance across the human age span: comparison between previously published reports and current study

Year	Author	Weight	Age-span	Covariate model for clearance
2005	Anderson <i>et al.</i> ¹	0.5 – 55 kg	postconception age 27 weeks - 14 years	$CL = 16.3 \times (BW/70)^{0.75} \times (1 - 0.885 \times \exp(-PCA \text{ in weeks} \times \ln(2)/26.6))$
2011	Zuppa <i>et al.</i> ²	not reported	29 days - less than 18 years	$CL = 18.4 \times (BW/70)^{0.75} \times (1 - 0.678 \times \exp(-PNA \text{ in weeks} \times \ln(2)/41))$
2012	Mohammed <i>et al.</i> ³	13.7 - 56 kg	1.8 - 15 years	$CL = 16.51 \times (BW/70)^{0.75}$
	This study	0.5 – 94 kg	postconception age 27 weeks - 34 years	$CL = 17.6 \times (BW/70)^k$; $k = 1.204 - 0.454 \times BW^{1.4} / (12.2^{1.4} + BW^{1.4})^{\S}$

CL= paracetamol clearance; BW=bodyweight; PCA=postconception age; PNA=postnatal age
 $\S$ see table 2 for parameter estimates

6.7.3 NONMEM codes

```
$$SUBROUTINES ADVAN5 TRANS1
```

```
;------
```

```
$MODEL
```

```
NCOMPARTMENTS=4
```

```
COMP=(DEPOT);RECTAL DOSE
```

```
COMP=(CENTRAL DEFDOSE DEFOBS);CENTRAL PLASMA COMPARTMENT
```

```
COMP=(PERIPH1);PERIPHERAL COMPARTMENT1
```

```
COMP=(PERIPH2);PERIPHERAL COMPARTMENT2
```

```
;------
```

```
$PK
```

```
KDEC = THETA(10) ; DECREASE OF EXPONENT
```

```
KMAX = THETA(11)+KDEC ; MAXIMUM EXPONENT
```

```
KHAL = THETA(12) ; K50
```

```
GAMMA = THETA(13) ; GAMMA
```

```
KBDE = KMAX-KDEC*(BW**GAMMA)/(KHAL**GAMMA+BW**GAMMA)
```

```
TVCL = THETA(1)*(BW/70)**KBDE
```

```
CL = TVCL*EXP(ETA(1))
```

```
TVVC = THETA(2)*(BW/70)
```

```
VC = TVVC*EXP(ETA(2))
```

$$Q2 = \text{THETA}(3) * (\text{BW}/70)$$

$$\text{TVVP1} = \text{THETA}(4) * (\text{BW}/70)$$

$$\text{VP1} = \text{TVVP1} * \text{EXP}(\text{ETA}(3))$$

$$Q3 = \text{THETA}(5) * (\text{BW}/70)$$

$$\text{TVVP2} = \text{THETA}(6) * (\text{BW}/70)$$

$$\text{VP2} = \text{TVVP2} * \text{EXP}(\text{ETA}(4))$$

$$\text{KA} = \text{THETA}(7) * \text{EXP}(\text{ETA}(5))$$

$$\text{ALAG1} = \text{THETA}(8)$$

$$\text{TVF1} = \text{THETA}(9)$$

$$\text{F1} = \text{EXP}(\text{LOG}(\text{TVF1}/(1-\text{TVF1}))+\text{ETA}(6))/(1+\text{EXP}(\text{LOG}(\text{TVF1}/(1-\text{TVF1}))+\text{ETA}(6)))$$

;------

$$\text{ET1} = \text{ETA}(1)$$

$$\text{ET2} = \text{ETA}(2)$$

$$\text{ET3} = \text{ETA}(3)$$

$$\text{ET4} = \text{ETA}(4)$$

$$\text{ET5} = \text{ETA}(5)$$

$$\text{ET6} = \text{ETA}(6)$$

;------

$$\text{S2} = \text{VC}$$

;------

$$\text{K12} = \text{KA}$$

$$\text{K23} = \text{Q2}/\text{VC}$$

$$\text{K32} = \text{Q2}/\text{VP1}$$

$$\text{K24} = \text{Q3}/\text{VC}$$

$$\text{K42} = \text{Q3}/\text{VP2}$$

$$\text{K20} = \text{CL}/\text{VC}$$

;------

\$ERROR

$$\text{IPRED} = \text{LOG}(0.000001)$$

$$\text{IF (F.GT.0) IPRED} = \text{LOG}(F)$$

$$Y = \text{IPRED} + \text{EPS}(1)$$

```

;Y = IPRED + SQRT(THETA(10)**2+THETA(11)**2/F**2)*ERR(1)
;-----
$THETA
(0.01, 5, ) ;TH1 CL
(2, 10, ) ;TH2 VC
(0.01, 70, ) ;TH3 Q2
(0.1, 30, ) ;TH4 VP1
(0.01, 2, ) ;TH5 Q3
(0.1, 30, ) ;TH6 VP2
(0.01, 0.13, ) ;TH7 KA
(0.01, 0.2, ) ;TH8 ALAG
(0.01, 0.93, 1) ;TH9 F1

(0.1, 0.4, 1) ;TH10 KDEC
0.75 FIX ;TH11 KMAX-KDEC OR KMIN
(0.05, 3.5, ) ;TH12 K50
1.4 FIX ;TH13 GAMMA
;-----
$OMEGA
0.12 ;CL
0.12 ;VC
0 FIX ;VP1
0 FIX ;VP2
0.11 ;KA
0.2 ;F1
;-----
$SIGMA
0.1
;-----
$EST NOABORT SIGDIG=3 PRINT=15 MAXEVAL=9999 METHOD=1 INTERACTION
POSTHOC
$COV COMP PRINT=E

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Chapter

7

**Scaling of clearance across the human
life span from neonates to adults:
conclusions and perspectives**

7.1. Introduction

Drug treatment is more difficult in children than in adults. The wide variation in various physiological processes in children leads to a wide variation in treatment response and this warrants an individualized approach to drug treatment in this vulnerable group of patients. Identifying the optimal dose is the key element of a good drug treatment practice for children. At present dose adjustment on the basis of milligram per kilogram of body weight is still widely used. However, dose selection in children should be based on pertinent information with regard to the variation in pharmacokinetics and pharmacodynamics as the key determinants of the drug effect. In that regard also disease and other factors need to be taken into account [1-4].

Interindividual variation in pharmacokinetics and pharmacodynamics in the paediatric population is the result of complex physiological changes during growth and development. Clearance is one of the most important pharmacokinetic parameters of which the value needs to be known for rational dose adjustment. However, collecting blood samples for pharmacokinetic studies in children is not easy and the information available for estimation of drug clearance in children is typically scarce. Therefore, scaling of drug clearance with pharmacokinetic modeling techniques (between adults and children or within paediatric populations) is of great interest for both clinical practice and drug development in pediatrics [4]. In general, there are two modeling approaches to the description of pharmacokinetics: the population pharmacokinetic (POPPK) modeling approach and the physiologically based pharmacokinetic (PBPK) modeling approach. POPPK modeling is a data-driven method aiming at the fitting of pharmacokinetic models to observed drug concentrations, while PBPK modeling is a physiological knowledge based method aiming to predict pharmacokinetics based on pertinent information on the variation in the underlying processes.

To date, for the scaling of drug clearances under the POPPK approach three modeling approaches are used: i) allometric scaling, ii) allometric scaling with a fixed exponent value of 0.75 combined with an age based maturation function, and iii) systematic covariate analysis. As neither allometric scaling nor allometric scaling plus an age-based maturation model can describe the developmental

changes in drug clearance with adequate statistical stability and predictive performance across the entire age-span, we explored, in a systematic covariate analysis, the utility of novel functions to describe developmental changes in clearance across the entire human life span. As bodyweight was found to be the best descriptor for drug clearance across different pediatric age groups in many studies, the research in this thesis focused on the identification of novel functions to describe the changes in drug clearance across the whole life span on the basis of bodyweight without additional covariates.

7.2. The observation of different values for the allometric exponent

The objective of the research described in Chapter 3 was to explore the variation in the value of the allometric exponent for children in different age groups in a systematic covariate analysis. To this end a large database with pertinent information on the pharmacokinetics of propofol across the human lifespan was established. Data from seven propofol studies were included in the analysis (neonates, infants, toddlers, children, adolescents, adults¹, and adults²). In a systematic manner, two out of the six study populations were randomly combined resulting in 15 combined datasets. In addition, the data of the seven studies were regrouped into five age-groups (according to FDA Guidance 1998), from which four combined datasets were prepared consisting of one paediatric age-group and the adult group. In each of these 19 combined datasets, the allometric scaling exponent for clearance was estimated using population pharmacokinetic modelling (NONMEM 7.2).

The results of this study show a large variety in the value of the allometric exponent for clearance with values ranging from 0.2 to 2.01 when analyzing combined datasets of two paediatric populations. While the lowest values of the exponent of 0.2 was identified between infants and toddlers, the highest exponent of 2.01 was found between neonates and infants. These findings show that there is no single unique value of the allometric exponent that can describe the variation in clearance across the human age-span. This implies that especially in the pediatric age range, the widely applied $\frac{3}{4}$ allometric function is inappropriate to describe and predict drug clearance in preterm

and term neonates, infants and young children. Also in literature, this function was found to systematically over-predict clearance for neonates and under-predict clearances for infants [5-8]. There are indications that the results found for propofol in this chapter, apply to other drugs as well. For example, the value of the exponent of 0.88 for propofol in toddlers and children (model 10), appears to be in good agreement with findings on oxycodone clearance in children aged between 6 months to 7 years, where a value of the allometric exponent of 0.875 has been reported [9]. It is concluded that for the scaling of clearance between two paediatric populations, the allometric exponent needs to be estimated rather than fixed to the value 0.75 in order to account for differences in maturation between different age groups, since the values appear to be highly variable. However, as with increasing age the estimated allometric scaling line moved slowly towards 0.75 (Chapter 3, Figure 3-3 B to E), it seems that $\frac{3}{4}$ allometric scaling function may be of value in older children (>4 years) towards adults.

7.3. Exploring a novel descriptive model for the scaling of the clearance of propofol

In Chapter 4, we tested four different models based on different allometric equations, to capture changes in propofol clearance in the complete dataset from chapter 3 comprising almost every stage of human life. The four different models were: model I - $\frac{3}{4}$ allometric scaling model; model II- mixture model; model III - bodyweight-cut-point separated model and model IV - bodyweight-dependent exponent model. We found that fixing of the allometric exponent to the value of 0.75 (the $\frac{3}{4}$ allometric model; model I) resulted in an adequate description of the variation in clearance in adults, adolescents, children and toddlers, but that it yielded a significant under-prediction in infants and an over-prediction in term and preterm neonates. The results of the mixture model (model II) and the bodyweight-cut-point separated model (model III) showed that a value of the allometric exponent different from 0.75 is more suitable for neonates and infants. In fact, both models identified an allometric exponent larger than 1 for the subpopulation that was composed of mainly neonates and infants, resulting in improved description of clearance in these youngest patients with the lowest body weight in comparison to the $\frac{3}{4}$ allometric model.

This has been reported before for morphine clearance in children younger than 3 years of age, where a value of the allometric exponent of 1.44 was found to best describe the developmental changes in clearance [10]. Similarly, Mahmood reported that the error in the prediction of clearance decreased when the scaling exponent increased from 0.75 towards 1 when studying a range of different drugs in children younger than 1 year of age [11].

Even though the overall performance improved significantly, the use of two different allometric exponents for different human subpopulations as implemented in model II and model III resulted in ambiguous clearance predictions for the individuals on the boundaries of the pediatric subpopulations. Therefore, in this study, a novel approach to the description of the variation in clearance across the entire life span was introduced, which is based on an allometric equation with an exponent that varies in a continuous manner with bodyweight (model IV). This bodyweight-dependent exponent (BDE) model contains a sigmoidal function to describe the change in the value of the allometric exponent with bodyweight. It is shown that this function allows for the best description of maturational changes in propofol clearance across the entire human life span. For propofol clearance, the exponent was found to decrease in a sigmoidal manner with bodyweight from 1.34 to 0.55, with half the decrease in exponent reached at a body weight of 3.78 kg. It was also illustrated that the BDE function is flexible in capturing very different shapes of the relation between clearance and bodyweight (Figure 7-1).

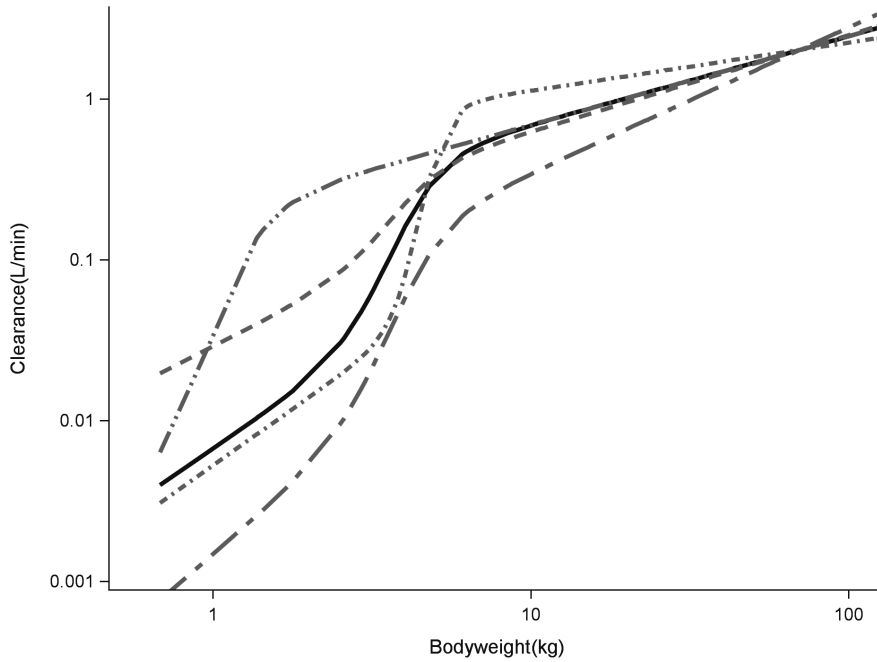


Figure 7-1 Simulations for the bodyweight-dependent exponent (BDE) model for scaling clearance ($Cl_i = Cl_p \times \left(\frac{BW_i}{70}\right)^k$ $k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma}$) on the basis of different values for the parameters of the bodyweight-dependent exponent ($k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma}$).

Solid black curve: BDE model predicted clearance curve with $k_0 = 1.34$, $k_{max} = 0.79$, $k_{50} = 3.78$, $\gamma = 5.24$ (final PK model of Chapter 4); dashed grey curve: BDE model predicted clearance curve with $k_0 = 1$, $k_{max} = 0.4$, $k_{50} = 3.78$, $\gamma = 5$; long dash short dash grey curve: BDE model predicted clearance curve with $k_0 = 1.7$, $k_{max} = 0.8$, $k_{50} = 3.78$, $\gamma = 5$; short dash dot grey curve: BDE model predicted clearance curve with $k_0 = 1.4$, $k_{max} = 1.1$, $k_{50} = 4.5$, $\gamma = 10$; medium dash double dot grey curve: BDE model predicted clearance curve with $k_0 = 1.34$, $k_{max} = 0.8$, $k_{50} = 1$, $\gamma = 5$.

7.4. Applying the novel BDE model to morphine and morphine-3-glucuronide

In Chapter 5, the novel bodyweight-dependent exponent model was applied to characterize the developmental changes in morphine clearance across the entire pediatric age-range. To this end, morphine and morphine-3-glucuronide (M3G) concentration data from 358 (pre)term neonates, infants, children and adults, and morphine concentration data from 117 adolescents were analyzed using NONMEM 7.2. Based on available data, two models were developed: i) a

model using morphine data only (model 1) and ii) using morphine and M3G data (model 2). In model 1, morphine clearance across the pediatric age range was very well described by a bodyweight-based exponential function in which the allometric exponent decreased in a sigmoidal manner with bodyweight (BDE model) from 1.47 to 0.88, with half the decrease in the value of the exponent reached at a body weight of 4.01 kg. In model 2, the exponent for the formation and elimination clearance of M3G was found to decrease from 1.56 to 0.89 and from 1.06 to 0.61, with half the decrease reached at body weights of 3.89 and 4.87 kg, respectively. Using the BDE model, there was no need to use additional covariates to account for size or age. These study results not only confirmed the model results based on children less than 3 years in which an exponent of 1.44 was identified [10], but also provides a basis for extrapolation to older age-ranges by the quantification of the maturation of glucuronidation across the entire pediatric age-range with the estimation of a lower exponent for higher bodyweight ranges. Moreover, it seems that applying an allometric function in which the exponent is allowed to vary with bodyweight itself results in an optimal description of the varying rates of maturation of glucuronidation clearance of morphine across all age ranges without the need for additional age-based covariates (PNA $\leq$ 10 days) that were reported when a single allometric exponent is used [10, 12, 13] (Chapter 5, Figure 5-5).

7.5. Using the novel BDE model for paracetamol

In Chapter 6, we further tested the BDE model for describing variation in paracetamol clearance across whole paediatric age-span. A total of 220 subjects from eight previously published studies on paracetamol pharmacokinetics were included in the analysis, including (pre)term neonates, infants, children and adults. Population pharmacokinetic modelling was performed using NONMEM 7.2. In the covariate analysis for clearance, linear functions, power functions and a power function with a bodyweight-dependent exponent were tested. It was clearly shown that the BDE model describes the age-related changes in clearance of paracetamol across the human life span best, and without the need for inclusion of additional age variables as a covariate. For clearance, the exponent was found to decrease in a sigmoidal manner with bodyweight from 1.2 to 0.75, with half the decrease in exponent reached at a

body weight of 12.2 kg. Based on the final model, simulations were performed to derive optimized dosing guidelines across the entire paediatric age range. In this respect, a model based intravenous paracetamol dose calculation table for children was provided aiming for similar paracetamol exposure across the paediatric age range. This is of particular relevance because there is yet no dosing information on paracetamol iv in preterm neonates (Europe) or in children younger than 2 years of age (US).

7.6. Perspectives

7.6.1. *The best descriptive predictor for clearance*

In this thesis, an important question was how to accurately describe maturation in clearance across the human life span. A complicating factor in the analysis of age-related changes in pharmacokinetics is the correlation between covariates that can be used as the basis for the description of maturation. Specifically the covariates bodyweight and age are correlated during growth and development of a child. Bodyweight is often referred to as measure of size only, whereas age is referred to as a measure of maturation. The relationship between age and bodyweight has been investigated by demographic surveys. Based on these survey results, growth charts have been developed. In **Figure 7-2**, data from the CDC growth chart percentiles table (<http://www.cdc.gov/growthcharts/data/zscore/>) are plotted in four panels demonstrating the relationship between weight and age for boys and girls younger or older than 2-3 years of age. These graphs show that between birth and adulthood the relation between age and bodyweight is highly non-linear.

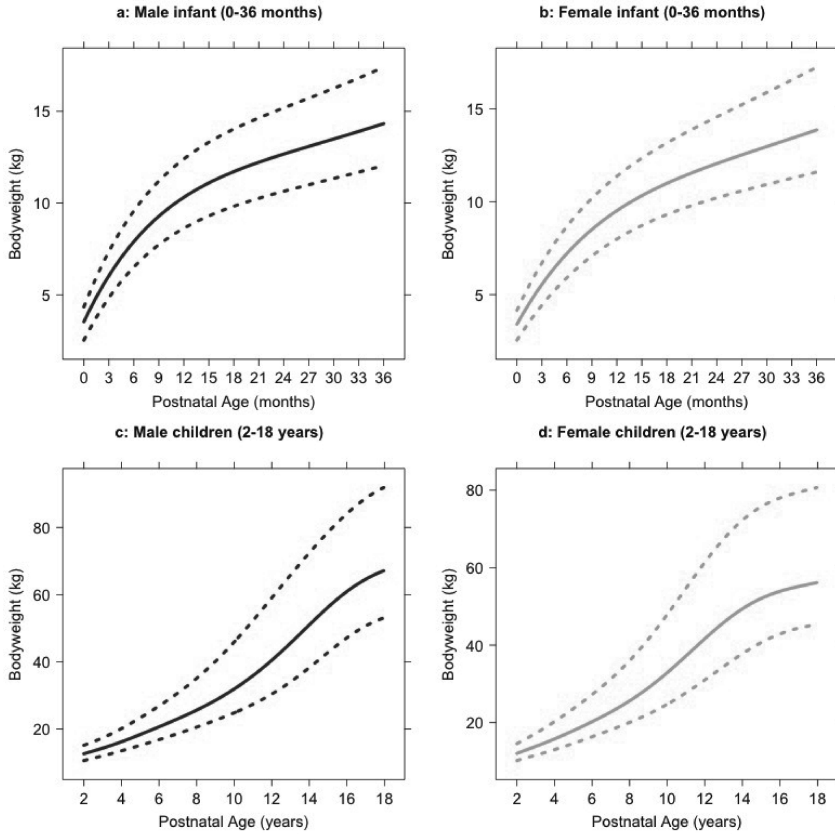


Figure 7-2 CDC growth chart plot (5%, 50% and 95% percentile) for different age-ranges divided by gender based on CDC percentiles table (<http://www.cdc.gov/growthcharts/data/zscore/>).

However, for neonates, in particular when both prematurely born neonates and term neonates are considered, the correlation between weight and postnatal age may not be that obvious. Figure 7-3 shows a plot of body weight against postnatal age (PNA), postmenstrual age (PMA), gestational age (GA) with the corresponding Pearson's Correlation Coefficient values in 874 preterm and term neonates from a previous publication of our group [14]. The plot shows that bodyweight is highly correlated with PMA or GA, and that there is less correlation between bodyweight and PNA. In PBPK models, postnatal age instead of bodyweight is typically used as a basis for maturation functions in paediatrics. However, it seems from these results that in neonates, bodyweight, PMA or GA instead of PNA is preferred to describe maturation in clearance across the human age range.

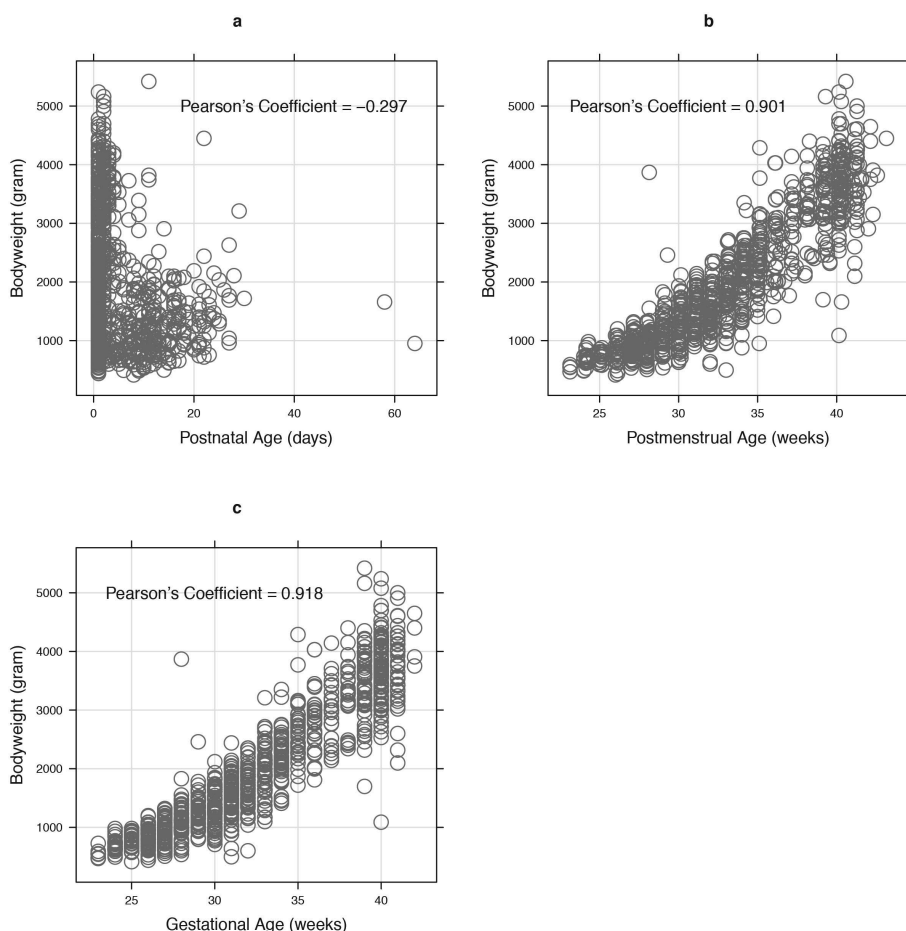


Figure 7-3 Scatter plots of bodyweight against postnatal age (PNA), postmenstrual age (PMA) and gestational age (GA) in 874 preterm and term neonates [14].

For scaling clearance across the pediatric age-range with inclusion of preterm and term neonates, we found in this thesis, that the bodyweight based scaling approach (BDE model) can describe the changes in drug clearance without the need for additional (age-related) covariates. We think that this can be explained by the fact that the BDE model is highly flexible in nature and can capture many different nonlinear relations between bodyweight and clearance (Figure 7-1). Describing changes in clearance across the human age range on the basis of one covariate only seems of importance as the implementation of two (correlated) covariates on one parameter may lead to bias in parameter estimates [15]. After

implementation of the BDE function it was found that age variables have no influence on inter-individual variability in clearance of propofol, morphine and paracetamol. In previous studies in which a fixed exponent was used, very often other covariates were needed to obtain an optimal description of the data, as was reported for morphine under the age of 3 years for instance [10, 16]. From these results, it is concluded that body weight may play a role as a surrogate of different age variables (PNA, PMA, or GA) across the whole pediatric age-span. Eventually, the influence from both weight and age on clearance is explained by an advanced BDE equation with bodyweight as the only covariate. Results from this thesis on propofol (Chapter 3 and Chapter 4), morphine (Chapter 5), paracetamol (Chapter 6), busulfan (Appendix I) and midazolam (Appendix II) show that the BDE model can result in adequate prediction of clearance across the entire pediatric age-span. From a model diagnostic point of view, the BDE model has adequate goodness-of-fit properties for different age groups and has good empirical Bayes estimates (EBE) diagnostics. Besides, the BDE model also allows for a weight based dose recommendation, as was shown for paracetamol in Chapter 6. Therefore, we conclude that bodyweight alone can be used as descriptor for clearance across the human age range, provided advanced functions such as the BDE model are applied, and the results are confirmed by proper validations [17].

7.6.2. Full or simplified BDE function?

In this thesis we present the BDE model to describe maturation of clearance across the human life span for three drugs propofol (Chapter 4), morphine (Chapter 5), paracetamol (Chapter 6). In the meantime the model has also been applied to busulfan (Appendix I), midazolam (Appendix II) and a number of antibiotics (gentamicin, tobramycin and vancomycin; unpublished observations). The BDE model was developed based on a sigmoidal equation that was initially derived for propofol (Chapter 4). Later on, it was simplified to a power equation for busulfan, midazolam and antibiotics. Table 7-1 shows the BDE equations and the corresponding parameter estimates that were identified in those studies. In studies of propofol, morphine and paracetamol, for the exponent BDE or k a sigmoidal equation

$$Cl_i = Cl_p \times \left(\frac{BW_i}{70} \right)^k, \quad k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma} \quad (1)$$

was used in the BDE model, albeit with the identification of different parameter estimates for k (or the bodyweight dependent exponent). Table 7-1 shows that all estimates of the parameter k_0 were found to be greater than 1 in studies for propofol, morphine and paracetamol (1.35, 1.56 and 1.2 respectively). Besides, k_{max} , which represents the decrease in k from k_0 at the hypothetical body weight of 0 kg, was found to vary between drugs (0.79, 0.67 and 0.45 for propofol, morphine and paracetamol, respectively) even though at adulthood similar values for k were observed (0.56, 0.89, and 0.75 at a weight of 70 kg) (Table 7-1, Figure 7-4 a). Concerning the parameters k_{50} and γ , for propofol and morphine, we observed an early and steep decrease in k with corresponding estimates of $k_{50} = 3.71$ kg, $\gamma = 5.1$ and $k_{50} = 3.89$ kg, $\gamma = 3.61$, respectively (Figure 7-4 a), which results in a stair-climbing pattern of increase in clearance over bodyweight from newborn babies to adults on a log-log scale (Figure 7-4 b). However, this decrease in k appears to be much slower for paracetamol with corresponding estimates for $k_{50} = 12.2$ kg and $\gamma = 1.2$ (Figure 7-4 a), resulting in a water-overflowing pattern of increase in clearance over bodyweight from newborn babies to adults in a log-log scale (Figure 7-4 b).

In studies on busulfan, midazolam and antibiotics, a power equation

$$Cl_i = Cl_p \times \left(\frac{BW_i}{70}\right)^k, k = L \times BW^M \quad (2)$$

was used in the BDE model (Table 7-1, Figure 7-4 c). The decrease in k over bodyweight for those drugs is rather slow compared to previous three drugs, although the magnitude in decrease varies very much among those five drugs (Figure 7-4 c). In contrast to the sigmoidal BDE function, which is highly flexible (Figure 7-1), the simplified power BDE equation lacks the flexibility of capturing different shapes with a trade-off of stable model estimates. Obviously, the simplified BDE model is preferred because of the parsimonious rule (estimation of 2 parameters instead of 4 parameters for k , Table 7-1). In our analysis, the simplified model was selected for busulphan for which no data from preterm and term neonates were available (Figure 7-4 c,d, Table 7-1). However, also for midazolam the simplified BDE equation was able to capture changes in clearance, even though the study covered the whole life span including preterm and term neonates. We think that this may be explained by the pathway that is involved, which is CYP3A oxidation in case of midazolam.

As such, beside the age range studied, the pathway involved may determine whether a simplified or a sigmoidal BDE function is needed. The study of three antibiotics is another example of the identification of a simplified or power BDE equation across the whole age-span, in this case for drugs that are all eliminated through Glomerular Filtration Rate (GFR). Although the three curves of k and clearance versus bodyweight in the antibiotics study were different from those identified for midazolam and busulfan, no large differences were found between these three antibiotics (Figure 7-4 c,d, Table 7-1). Given the fact that these three antibiotics are all eliminated through the same pathway (GFR), this finding may provide the basis for the extrapolation of this simplified BDE model to other drugs eliminated through GFR. This is an approach that has been proposed before [3] and was applied for glucuronidation as well [18].

Table 7-1 Summary of all studies in which the bodyweight-dependent exponent (BDE) model ($Cl_i = Cl_p \times \left(\frac{BW_i}{70}\right)^k$) was used for scaling clearance from adults to children with either a sigmoidal or a power equation for k .

Drug	Equation for BDE (k)	k_0	k_{max}	$k_{50}(kg)$	γ	L	M	Elimination pathway	Age and BW
propofol ¹		1.35	0.79	3.71	5.1			glucuronidation UGT1A9 & sulfation	PNA: Birth – 81 yrs BW: 0.68 – 122.7 kg
morphine ²	$k = k_0 - \frac{k_{max} \times BW_i^\gamma}{k_{50}^\gamma + BW_i^\gamma}$	1.56	0.67	3.89	3.61			glucuronidation UGT2B7	PNA: Birth – 36 yrs BW: 0.56 – 85 kg
paracetamol ³		1.2	0.45	12.2	1.2			glucuronidation UGT1A6 & sulfation	PNA: Birth – 34 yrs BW: 0.505 – 94 kg
busulfan ⁴						1.56	-0.226	glutathione S-transferase	PNA: 0.1 – 26 yrs BW: 3 – 65 kg
midazolam ⁵						0.81	-0.135	CYP3A	PNA: Birth – 31 yrs BW: 0.77 – 89 kg
gentamicin ⁶	$k = L \times BW^M$					2.6	-0.093	Glomerular Filtration Rate (GFR)	PNA: Birth – 15 yrs BW: 0.44 – 80 kg
tobramycin ⁶						2.67	-0.079	Glomerular Filtration Rate (GFR)	PNA: 2 days – 18 yrs BW: 0.485 – 85 kg
vancomycin ⁶						2.12	-0.065	Glomerular Filtration Rate (GFR)	PNA: Birth – 17 yrs BW: 0.415 – 85 kg

¹ Chapter 4 in this thesis

² Chapter 5 in this thesis

³ Chapter 6 in this thesis

⁴ Appendix I in this thesis

⁵ Appendix II in this thesis

⁶ Unpublished results

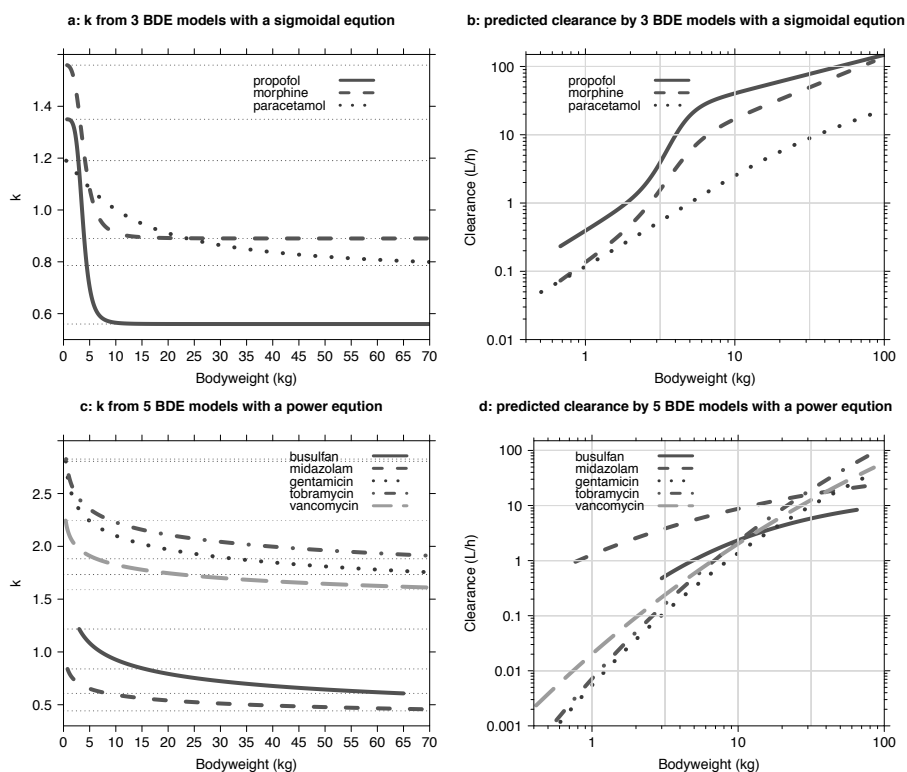


Figure 7-4 Bodyweight-dependent exponent (BDE or k) (panels a and c) and clearance ($Cl_i = Cl_p \times \left(\frac{BW_i}{70}\right)^k$) (panel b and d) versus bodyweight for the models derived for propofol, morphine and paracetamol in which a sigmoidal function ($k = k_0 - \frac{k_{\max} \times BW_i^Y}{k_{50}^Y + BW_i^Y}$) for k was used (panel a and b) and for the models derived for busulfan, midazolam, gentamicin, tobramycin and vancomycin in which a simplified (or power) function ($k = L \times BW^M$) for k was used (panel c and d). For specific estimates see Table 7-1.

7.6.3. Physiologically based pharmacokinetic modeling in pediatrics

At present there is an increasing interest in the application of physiologically based pharmacokinetic (PBPK) modeling in paediatric drug development. In PBPK modeling, the human body is considered to be composed of multi-compartments representing actual organs and other physiological spaces. Mass balance equations for each organ describe drug distribution in the organ from arterial blood and its exit into venous blood. The PBPK model is parameterized in relevant drug specific parameters (e.g. LogP and pKa) and biological system

specific parameters (e.g. tissue volumes, the perfusion rates of these tissue compartments, the concentration of binding proteins, the values of the activities of drug metabolizing enzymes, the expression and function of transporters in the excretion). The use of PBPK models for prediction of pharmacokinetics in children from information in adults requires information on the change in the values of the system specific parameters across the paediatric life span. The common approach is to modify a PBPK model that has been validated with adult PK data and then to incorporate the differences in growth and maturation that can affect all system specific parameters. In PBPK modeling, information on the values of the changes in system specific parameters is typically obtained from *in vitro* studies, which is often not available for children of all age ranges including preterm and term neonates. Here we propose to use information from our descriptive BDE models for different metabolic or elimination pathways on the change in for example intrinsic clearance as a measure of developmental changes in system specific parameter in PBPK models. Given the fact that the *in vitro* data are extremely scarce in preterm neonates, the information from BDE model may be specifically helpful for this population. Recently, Krekels *et al.* explored such application of the POPPK model in describing developmental changes of UGT2B7-mediated glucuronidation parameters [19]. In the end, they suggested that the maturation profile for UGT2B7 ontogeny in a PBPK model can be improved based on information obtained from a covariate model of a paediatric population model derived for a specific substrate for UGT2B7 on the basis of a systematic covariate analysis [19]. Further studies on using POPPK (covariate) models in adjusting PBPK models for improving the simulation performance of PBPK models in pediatric drug development are encouraged.

7.7. Conclusion

In this thesis, we propose the body weight dependent exponent (BDE) model as novel approach for describing drug clearance over the entire pediatric age-span. This provides us an alternative approach to the previous “weight plus age” approach with better statistical properties. The model offers the advantage of much improved clearance estimates of drugs in clinical practice enabling the prediction of the individualized dose in children, particularly neonates. Moreover, the model provides a basis for the incorporation of information on

the maturation of clearance from *in vivo* studies into physiologically-based pharmacokinetic models for the prediction of developmental changes in clearance for novel drugs. These predictions constitute the scientific basis for optimized clinical trial designs for studies in pediatrics.

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Chapter

8



**Nederlandse Samenvatting:
Schaling van de klaring van
geneesmiddelen over de
levensduur van een mens van
pasgeborenen tot volwassenen:
conclusies en perspectieven**

Introductie

Medicamenteuze behandeling is moeilijker bij kinderen dan bij volwassenen. De grote variatie in de verschillende fysiologische processen bij kinderen leidt tot een grote variatie in de respons op de behandeling en dit maakt een geïndividualiseerde benadering van de behandeling met geneesmiddelen in deze kwetsbare groep patiënten noodzakelijk. Het identificeren van de optimale dosis is het belangrijkste onderdeel van een goede behandeling met geneesmiddelen voor kinderen. Momenteel wordt in de klinische praktijk de dosis veelal aangepast op basis van kilogram lichaamsgewicht. Echter, de dosering bij kinderen zou moeten worden aangepast op basis van relevante informatie met betrekking tot de variatie in farmacokinetiek en farmacodynamiek als de belangrijkste factoren die bepalend zijn voor de werking van het geneesmiddel. In dit verband moeten ook ziekte en andere factoren in aanmerking worden genomen.

Interindividuele variatie in farmacokinetiek en farmacodynamiek van geneesmiddelen in de pediatrische populatie is het resultaat van complexe fysiologische veranderingen tijdens groei en ontwikkeling. Klaring is een van de belangrijkste farmacokinetische parameters waarvan de waarde bekend moet zijn voor rationele dosisaanpassing. Echter, het verzamelen van bloedmonsters voor farmacokinetische studies bij kinderen is niet eenvoudig en de beschikbare informatie over de klaring van specifieke geneesmiddelen bij kinderen is meestal schaars. Daarom is schaling van geneesmiddelklaring met farmacokinetische modelleertechnieken tussen volwassenen en kinderen of binnen de pediatrische populatie van groot belang voor zowel de klinische kinderpraktijk als voor de ontwikkeling van nieuwe geneesmiddelen in de kindergeneeskunde. In het algemeen zijn er twee modelmatige benaderingen voor de beschrijving van de farmacokinetiek: populatie farmacokinetische modellering (POPPK) en fysiologisch farmacokinetische modellering (PBPK). POPPK modellering is een data - gedreven benadering gericht op het beschrijven van variatie in de farmacokinetiek, terwijl PBPK modellering gebaseerd is op de fysiologische processen die ten grondslag liggen aan veranderingen in farmacokinetiek.

Tot voor kort werden bij de POPPK benadering voor de schaling van de klaring driemethoden toegepast: i) allometrische schaling op basis van lichaamsgewicht, ii) allometric scaling op basis van lichaamsgewicht met een vaste exponent waarde van 0,75 in combinatie met een leeftijd gebaseerde rijping functie, en iii) de systematische covariaat analyse. Omdat noch allometrische schaling op basis van lichaamsgewicht noch allometrische schaling op basis van lichaamsgewicht plus een op leeftijd gebaseerde rijping functie de ontwikkelingsgerelateerde verandering in klaring goed kan beschrijven met voldoende statistische stabiliteit en voorspellende waarde over het gehele leeftijdsbereik, hebben we in een systematische covariaat analyse de betekenis van nieuwe functies voor het beschrijven van veranderingen in de klaring over de gehele levensduur de mens onderzocht. Aangezien lichaamsgewicht de beste descriptor voor verandering in geneesmiddelklaring over verschillende pediatrische leeftijdsgroepen is gebleken, richt het onderzoek in dit proefschrift zich op de identificatie van nieuwe functies op basis van het lichaamsgewicht, die gebruikt kunnen worden om de veranderingen in geneesmiddelklaring over de hele levenscyclus van de mens zonder bijkomende covariaten te beschrijven. Deze functies kunnen vervolgens gebruikt worden als basis om geïndividualiseerd te kunnen doseren in kinderen.

De waarneming van verschillende waarden voor de allometrische exponent

Het doel van het in hoofdstuk 3 beschreven onderzoek was om de variatie in de waarde van de allometrische exponent voor kinderen in verschillende leeftijdsgroepen te bepalen in een systematische covariaat analyse. Hiertoe is een grote databasis met relevante informatie over de farmacokinetiek van propofol over het menselijke leeftijdsbereik opgezet. Gegevens uit 7 propofol studies die betrekking hebben op 6 verschillende leeftijdsgroepen werden in de analyse betrokken: pasgeborenen, zuigelingen, peuters, kinderen, adolescenten, en volwassenen. Op een systematische manier werden twee van de zes studie populaties willekeurig gecombineerd wat resulteerde in 15 gecombineerde datasets. Bovendien zijn de gegevens van de zeven studies gegroepeerd in vijf leeftijdsgroepen volgens FDA Guidance 1998, waaruit vervolgens vier gecombineerde datasets werden gemaakt bestaande uit de volwassengroep en

een van de vier pediatrie leeftijdsgroepen. In elk van deze 19 gecombineerde datasets, werd de allometrische exponent voor de klaring van propofol geschat met behulp van populatie farmacokinetisch modellering (NONMEM 7.2).

De resultaten van deze studie laten een grote variatie zien in de waarde van de exponent voor allometrische schaling met waarden variërend van 0,2 tot 2,01 wanneer gecombineerde datasets van twee pediatrie populaties worden bestudeerd. De laagste waarde van de exponent van 0,2 werd vastgesteld bij de combinatie van gegevens in baby's met zuigelingen tot 2 jaar, terwijl de hoogste waarde van de exponent van 2,01 werd gevonden bij de combinatie van gegevens in pasgeborenen met zuigelingen tot 2 jaar. Deze bevindingen laten zien dat er niet een enkele unieke waarde van de allometrische exponent is waarmee de variatie in de klaring over het menselijk leeftijdsbereik kan worden beschreven. Dit betekent dat de in kinderen op grote schaal toegepaste $\frac{3}{4}$ allometrische functie niet kan worden gebruikt voor het voorspellen van de klaring bij (premature) pasgeborenen, zuigelingen en jonge kinderen tot 2-4 jaar. Omdat er aanwijzingen zijn dat de resultaten zoals gevonden voor propofol in dit hoofdstuk, ook gelden voor andere geneesmiddelen, wordt geconcludeerd dat bij de schaling van de klaring tussen twee pediatrie populaties, de waarde van de allometrische exponent moet worden geschat om rekening te houden met verschillen in rijpingssnelheid tussen verschillende leeftijdsgroepen. Echter, er werd ook gevonden dat met toenemende leeftijd de waarde van de allometrische exponent zich steeds meer in de richting van de waarde van 0,75 beweegt (hoofdstuk 3, figuur 3B tot 3E). Het lijkt er daarom op dat de $\frac{3}{4}$ allometrische scaling functie van waarde kan zijn bij oudere kinderen (> 4 jaar) en volwassenen.

Een nieuw beschrijvend model voor de schaling van de klaring van propofol

Het onderzoek in hoofdstuk 4 heeft betrekking op de vergelijking van vier verschillende, op allometrie gebaseerde vergelijkingen, voor de beschrijving van de veranderingen in de klaring van propofol in de volledige dataset van hoofdstuk 3 met gegevens over het volledige leeftijdsbereik van prematuren tot volwassenen. De vier verschillende modellen waren: model I – $\frac{3}{4}$ allometrische

schaling-model; model II - gemengd allometrisch schalingsmodel van twee subgroepen; model III - lichaamsgewicht-gescheiden allometrisch schalingsmodel van twee subgroepen en model IV - lichaamsgewicht-afhankelijk exponent model. We vonden dat de beschrijving van de data van het allometrisch schalingsmodel met een vaste waarde van de exponent van 0,75 (de $\frac{3}{4}$ allometrische model; model I) weliswaar een goede beschrijving gaf van de variatie in de klaring bij volwassenen, adolescenten, kinderen en peuters, maar dat de klaring werd onderschat in jonge kinderen tussen de 1 en 2 jaar en werd overschat in prematuren en a terme pasgeborenen. De resultaten van het gemengd model met twee subgroepen (model II) en het lichaamsgewicht-gescheiden model van twee subgroepen (model III) toonden aan dat een waarde van de allometrische exponent anders dan 0,75 meer geschikt is voor pasgeborenen en zuigelingen. Met een waarde van de allometrische exponent die groter is dan 1 voor de subpopulatie die voornamelijk bestaat uit pasgeborenen en zuigelingen, geven beide modellen een sterk verbeterde beschrijving van de klaring bij deze jongste patiënten met het laagste lichaamsgewicht. Dit is in overeenstemming met de waarnemingen voor morfine, waar bij kinderen jonger dan 3 jaar, een waarde van de allometrische exponent van 1,44 werd gevonden voor de beschrijving van de verandering in de klaring. Mahmood meldde ook dat bij kinderen jonger dan 1 jaar de voorspelling van de klaring van een scala van verbindingen verbetert wanneer een waarde van de allometrische exponent van 1 wordt gebruikt in vergelijking met 0.75.

Hoewel de voorspelling van de klaring door de leeftijd heen aanzienlijk verbetert, door het gebruik van twee verschillende allometrische exponenten voor twee verschillende leeftijdsgroepen in de populatie (modellen II en III), kennen ook deze modellen beperkingen. Een belangrijke beperking heeft betrekking op de schatting van de klaring in individuen met een leeftijd in het grensgebied van de twee subpopulaties. In deze individuen levert de extrapolatie op basis van de twee modellen, twee verschillende waarden van de klaring op. Daarom is in dit hoofdstuk, een nieuwe benadering voor de beschrijving van de verschillen in klaring over de gehele levensduur voorgesteld, die is gebaseerd op een allometrische vergelijking met een exponent die varieert met het lichaamsgewicht (model IV). Dit zgn. "lichaamsgewicht afhankelijke exponent (BDE) model" bevat een continue sigmoïdale functie om de verandering in

de waarde van de allometrische exponent van lichaamsgewicht beschrijven. Er wordt aangetoond dat deze functie de beste beschrijving geeft van de veranderingen in de klaring van propofol over de gehele leeftijdsbereik van de mens. Voor de klaring van propofol werd gevonden dat met een toenemend lichaamsgewicht, de waarde van de allometrische exponent op een sigmoïdale wijze afneemt van 1,34-0,55, waarbij de helft van de daling werd bereikt bij een lichaamsgewicht van 3,78 kg. Ook werd aangetoond dat de BDE functie een flexibele functie is waarmee heel verschillende vormen van de relatie tussen klaring en lichaamsgewicht kunnen worden beschreven.

Het toepassen van het nieuwe BDE-model op morfine klaring

In hoofdstuk 5 werd het lichaamsgewicht-afhankelijke exponent model (BDE model) toegepast om leeftijdsafhankelijke veranderingen in morfine klaring over het gehele pediatrie leeftijdsgebied te karakteriseren. Daartoe werden morfine en morfine-3-glucuronide (M3G) concentraties in 358 prematuren, pasgeborenen, zuigelingen, kinderen en volwassenen, en morfine concentraties in 117 adolescenten geanalyseerd met NONMEM 7.2. Op basis van deze gegevens, werden twee modellen ontwikkeld: i) een model op basis van uitsluitend morfine concentraties (model 1) en ii) een model op basis van zowel morfine en M3G concentraties (model 2). In model 1 werd de verandering in de morfine klaring zeer goed beschreven met een op lichaamsgewicht gebaseerde exponentiële functie waarbij de waarde van de allometrische exponent op een sigmoïdale wijze afneemt met lichaamsgewicht (BDE-model) van 1,47 tot 0,88. Hierbij wordt de helft van de daling in de waarde van de exponent bereikt bij een gewicht van 4,01 kg. In model 2 werd gevonden dat de waarde van de exponent voor de vorming en eliminatieklaring van M3G afneemt van 1,56 tot 0,89 en van 1,06 tot 0,61, waarbij de helft van de afname werd bereikt bij een lichaamsgewicht van 3,89 en 4,87 kg, respectievelijk. Met het BDE model was er geen noodzaak om extra covariaten gebruiken om de waargenomen variabiliteit in de klaring te beschrijven. Deze studie resultaten bevestigen niet alleen de reeds bekende modelresultaten in kinderen jonger dan 3 jaar waarin een exponent van 1,44 werd vastgesteld, maar vormen ook een basis voor extrapolatie naar

oudere leeftijd. Bovendien blijkt dat toepassing van een allometrische functie waarin de exponent kan variëren met lichaamsgewicht leidt tot een optimale beschrijving van de uiteenlopende rijping van de glucuronidering klaring van morfine in alle leeftijdsgroepen zonder dat extra leeftijdsgebaseerde covariaten (PNA < 10 dagen) nodig zijn.

Toepassing van het nieuwe BDE-model op paracetamolklaring

In hoofdstuk 6 hebben we het BDE-model toegepast voor het beschrijven van variatie in de klaring van paracetamol over het gehele pediatrische leeftijdsbereik. Plasma concentraties in 220 patiënten uit acht eerder gepubliceerde studies over de farmacokinetiek van paracetamol werden opgenomen in de analyse, met inbegrip van (premature) pasgeborenen, zuigelingen, (jonge) kinderen en volwassenen. Populatie farmacokinetische modellering werd uitgevoerd met behulp van NONMEM 7.2. In de covariaat analyse voor de klaring werden lineaire functies, power functies en een power-functie met een lichaamsgewicht-afhankelijke exponent (BDE model) getest. Er werd aangetoond dat het BDE model de leeftijd gerelateerde veranderingen in de klaring van paracetamol in het menselijke leeftijdsbereik goed beschrijft en dat het niet nodig is om aanvullende leeftijd variabelen als covariaat mee te nemen. Voor klaring werd gevonden dat met toenemend lichaamsgewicht de waarde van de allometrische exponent op een sigmoïdale wijze afneemt van 1,2-0,75, waarbij de helft van de daling werd bereikt bij een lichaamsgewicht van 12,2 kg. Op basis van het uiteindelijke model werden simulaties uitgevoerd voor het opstellen van geoptimaliseerde doseringsrichtlijnen voor paracetamol over het gehele pediatrische leeftijdsbereik. In dit verband werd met behulp van het model een intraveneuze paracetamol dosis berekend voor kinderen van alle leeftijden waarbij naar ongeveer gelijke paracetamol blootstelling werd gestreefd. Dit is met name van belang omdat er nog geen informatie is over de intraveneuze dosering paracetamol bij premature neonaten (Europa) of bij kinderen jonger dan 2 jaar (VS).

Conclusie

In dit proefschrift stellen we het lichaamsgewicht afhankelijke exponent (BDE) model voor als nieuwe benadering voor het beschrijven van veranderingen in geneesmiddelklaring over het gehele pediatrische leeftijdsbereik. Dit geeft een alternatieve benadering voor de schatting van de klaring met betere statistische eigenschappen. Het model heeft als voordeel dat betere ramingen van de klaring van geneesmiddelen kunnen worden verkregen, waardoor de voorspelling van de juiste individuele dosering bij kinderen, in het bijzonder bij pasgeborenen, kan worden geoptimaliseerd. Bovendien kan het model een basis zijn voor fysiologisch gebaseerde farmacokinetische modellen, met name daar waar *in vitro* of andere data ontbreken. Hiermee kunnen mogelijk in de toekomst ontwikkelingsveranderingen in klaring van nieuwe geneesmiddelen worden voorspeld. Deze voorspellingen vormen de wetenschappelijke basis voor geoptimaliseerde trial designs voor studies in kinderen.





Appendix



Body weight-dependent pharmacokinetics of busulfan in pediatric hematopoietic stem cell transplantation patients: towards individualized dosing

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Abstract

Background and Objectives: The wide variability in pharmacokinetics of busulfan in children is one factor influencing outcomes such as toxicity and event free survival. A meta-analysis was conducted to describe the pharmacokinetics of busulfan in patients from 0.1 to 26 years of age, to elucidate patient characteristics that explain the variability in exposure between patients and to optimize dosing accordingly.

Patients and Methods: Data were collected from 245 consecutive patients (from 3 to 100 kg) who underwent HSCT in four participating centers. The inter-patient, inter-occasion and residual variability in the pharmacokinetics of busulfan were estimated with a population analysis with the software program NONMEM VI (Globomaxx LLC, Hanover, MD, USA). Covariates were selected on the basis of their known or theoretical relationships with busulfan PK and were plotted independently against the individual PK-parameters and the weighted residuals of the model without covariates to visualize relations. Potential covariates were formally tested in the model.

Results: In a two-compartment model, body weight was the most predictive covariate for clearance, volume of distribution and inter-compartmental clearance and explained 65%, 75% and 40% of the observed variability, respectively. The relation between body weight and clearance was characterized best using an allometric equation with a scaling exponent that changed with body weight from 1.2 in neonates to 0.55 in young adults. This implies that an increase in body weight in neonates results in a larger increase in busulfan clearance than an increase in body weight in older children or adults. Clearance on the first day was 12% higher than that of subsequent days ($p < 0.001$). Inter-occasion variability on clearance was 15% between the four days. Based on the final PK-model, an individualized dosing nomogram was developed.

Conclusions: The model-based individual dosing nomogram is expected to result in predictive busulfan exposures in patients ranging between 3 and 65 kg and thereby to a safer and more effective conditioning regimen for hematopoietic stem cell transplantation in children.

I.1. Introduction

Children may differ from adults in drug pharmacokinetics (PK), in response to treatment (efficacy) and their susceptibility to side effects (safety). Variability in pharmacokinetics is one factor which alters drug exposure and this in turn might explain differences in the (un)wanted responses (Pharmacodynamics, PD) between children. Characterization of variability in PK between neonates, infants, children and adolescents is therefore important especially for drugs with a small therapeutic window such as busulfan. Busulfan is a chemotherapeutic drug which is standard of care in preparative chemotherapy in patients undergoing hematopoietic stem cell transplantation (HSCT) for a variety of malignant and non-malignant diseases [1]. Since the 1970s, total body irradiation (TBI) based conditioning regimens have been used [2]. Unfortunately, TBI is complicated by cataracts, endocrine disorders (including stunted growth), delayed intellectual development and secondary tumors. As an alternative to TBI, in the 1980s, chemotherapy-based conditioning (high-dose oral busulfan in combination with cyclophosphamide) was introduced [3, 4]. The therapeutic potential of oral busulfan was limited by the large PK-variability [5-8]. This PK-variability has major implications for outcomes of treatment; a low total exposure (expressed as the total area under the concentration curve using all administrations from day 0 to day 4, $AUC_{\text{day0-4}}$) to busulfan is associated with an increased risk of graft failure and relapse [9, 10],^{9;10} whereas a high $AUC_{\text{day0-4}}$ is associated with an increased risk of toxicity like veno-occlusive disease (VOD) and graft versus host disease [9, 10]. To reduce the variability in exposure and to improve safety of the regimen, intravenous (IV) busulfan was introduced in 2000 [11]. Although this excludes differences in absorption, wide inter-individual variability in busulfan pharmacokinetics is still observed with the IV formulation, particularly in children [12-17]. Therapeutic drug monitoring to guide IV busulfan dosing was therefore introduced [1, 14], which resulted in further improved of the event free survival in children [18]. It may be anticipated that patient-outcomes after HSCT may further benefit from an optimal starting dose for each individual, especially for centers where TDM is not available. Previous studies used body surface area (BSA) or body weight-based functions to characterize the variable PK of busulfan in children [17, 19, 20]. In addition some of the variability was suggested to be explained by the underlying disease of the patient [21-23]. However, most of these results

originated from relatively small studies. We describe a pharmacokinetic meta-analysis of 245 patients, in whom a large number of covariates related to the PK of busulfan, such as body weight, underlying disease and liver function tests, were measured. The meta-analysis was conducted with the objective of describing the pharmacokinetics of busulfan in patients from 0.1 to 26 years of age and to elucidate patient characteristics that explain the variability in exposure between patients. The resulting PK-model was used to derive an individualized dosing algorithm aiming for an optimal exposure of busulfan in each patient.

I.2. Methods

Setting and study population

In this prospective meta-analysis, patients were enrolled in the research protocol after the patient and/or their parents (the latter in patients under 12 years of age) provided written informed consent. The study was approved by the local ethics committees. Data were collected from all 245 consecutive patients who underwent HSCT between August 2000 to September 2009 in four centers and of whom concentration measurements after intravenous busulfan administration were available. The four centers were: the University Medical Center Utrecht and the Leiden University Medical Center in the Netherlands, the Universitätsklinikum at Münster, Germany, and the Children's Hospital at Westmead, Sydney, Australia. Part of the data (135 of the 245 patients) has been described in previous publications [17, 21, 24, 25]. The characteristics of the settings, busulfan administration and analysis and samples schemes are shown in table 1. Busulfan was administered during 3 to 4 consecutive days. In 98% of patients blood samples were collected on day 1 and in 68% of patients, blood samples were also collected on day 2-4.

Table 1: Participating centers.

	Leiden	Münster	Sydney	Utrecht
Busulfan preparation/formulation	Busilvex® Pierre Fabre Medicament, France	Busilvex® Pierre Fabre Medicament, France	Busulfex® Orphan, Australia	Busilvex® Pierre Fabre Medicament, France
Busulfan dosing algorithm (day1)	until 2003: <4 years: 4mg / kg in 4 doses ≥4 years: 3.2 mg / kg in 4 doses from 2003 <1 years: 80 mg / m2 in 1 dose ≥1years: 120 mg / m2 in 1 dose	3.2 mg / kg in 2 doses 120mg / m2 in 2 doses	120 mg / m2 in 1 dose 130 mg / m2 in 1 dose 3.2 mg / kg in 4 doses	until 2008: <1 years: 80 mg / m2 in 1 dose ≥1years: 120 mg / m2 in 1 dose from 2008: <0.5 years: 80 mg / m2 in 1 dose 0.5-1years: 120 mg / m2 in 1 dose ≥1years: 130 mg / m2 in 1 dose
Infusion duration day 1 (h)	median (range) 3 (2-3)	4	2(1-3.75)	3(2.75-3.25)
Dose adjustments based on TDM	yes	no	yes	yes
Sample scheme	predose, 1, 2, 4 hours after inf.	pre-dose, during infusion, 1, 1.5, 2, 3, 6 and 7.5 hours after inf.	4-8 samples following the dose between 0.08 to 7 hours after inf.	0.08, 1, 2 and 4 hours after inf.
Method of determination ^a	HPLC	LC-MS	GC	HPLC/LC-MS
LOQ	50 µg/L	5 µg/L	30 µg/L	50 µg/L
Precision within/between runs (% variation)	3.5%, 0.8%	<11%, <11%	<9%, <10%	2.3%, 0.2%

a. The methods Leiden, Utrecht (HPLC and LC-MS) and Münster were successfully cross validated.

HPLC = high pressure liquid chromatography, LCMS = liquid chromatography-mass spectrometry, GC = gas chromatography, CV = Coefficient of Variation (%), LOQ = limit of quantification, TDM = therapeutic drug monitoring

Transplantation details and other patient characteristics are shown in table 2. All patients received IV busulfan-based myeloablative conditioning according to the applicable (inter)national protocols. All patients were cared for in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Gut decontamination and infection prophylaxis was given according to the institutional protocol. Patients received anti-emetic drugs and prophylactic anticonvulsive therapy (clobazam, clonazepam, diazepam and in 10 cases phenytoin) during busulfan treatment.

Pharmacokinetic analysis

Model building and evaluation

The nonlinear mixed-effects modeling software NONMEM VI using ADVAN subroutines with first-order conditional estimation (FOCE) with η - ϵ interaction (Globomaxx LLC, Hanover, MD, USA) was used. Log-transformed busulfan concentrations were used for analysis. Twenty-nine of a total of 1775 busulfan concentrations were below the limit of quantification (LOQ). The values of these samples were set at $\frac{1}{2}$ the LOQ [26]. Mixed effects models consist of a structural model (e.g. a one or two-compartment model) describing the relationship between dose and concentrations, and a stochastic model describing the random variability in the PK-parameters of the structural model. Random variability consisted of inter-individual in PK-parameters, inter-occasion variability (inter-occasion variability, IOV) in PK-parameters and intra-individual variability. Inter-individual and inter-occasion variability were modeled assuming a log-normal

distribution (eq. 1, appendix 1) [27]. Intra-individual variability was modeled using an additive error (eq. 2, appendix 1) which is equivalent to a proportional error model in the untransformed scale. Discrimination between different models was made by comparison of the objective function (-2 log likelihood). A value of $p < 0.005$, $df = 1$, representing a decrease in objective function of 7.8 on a χ^2 distribution, was considered statistically significant. In addition, goodness-of-fit plots (individual predicted *versus* observed concentrations, population predicted *versus* observed concentrations, conditional weighted residuals *versus* time and conditional weighted residuals *versus* population PK-parameters) were used for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual inspection of the distribution of the model parameters, were used to evaluate the model.

In order to determine whether the model was over-parameterized, the condition number of the final PK-model was calculated. The condition number should not exceed the critical value of 1000 [28].²⁹ Finally, η - and ϵ -shrinkage as defined by Karlsson *et al.* [29]³⁰ was calculated for all model parameters for which inter-individual variability was estimated. A shrinkage-value below 20% was considered acceptable.

Covariate analysis

The covariates depicted in table 1 and 2 were selected on the basis of their known or theoretical relationships with busulfan PK. As described before, BSA and body weight are associated with busulfan PK [17, 19, 20]. Many drugs may interact with busulfan [30-32]. Therefore, all concomitant medications used by more than 10% of patients were included as a covariate. Underlying disease and clinical chemical parameters like liver function have been reported to influence busulfan clearance [13, 21-23, 33], while also blood counts may relate to variability in busulfan-PK.

All covariates were plotted independently against the individual PK-parameters (post hoc values) and the weighted residuals of the model without covariates to visualize relations. Potential covariates were formally tested in the model as follows. For continuous covariates such as body weight, age or BSA, the influence of the covariate on each PK-parameter was tested using a linear (eq. 4, appendix 1) or allometric function (eq. 5, appendix 1). In addition, other allometric functions were explored for the PK parameters of which the plot

of inter-individual variability of the PK-parameter *versus* the covariate showed that neither a linear nor an allometric function results in adequate description of the data. I.e., the plot of inter-individual variability of *Cl* *versus* body weight showed that an allometric function over-predicted patients < 10 kg and > 40 kg and under-predicted patients between 10 and 40 kg. In this respect, it has been reported in studies using small cohorts of children that the scaling exponent of the allometric function for clearance is larger in neonates and young children (i.e. a scaling exponent > 1) in comparison with older children (i.e. a scaling exponent < 1) [34-36]. Wang *et al.* have used an allometric function with a scaling exponent that varied with body weight between 1.35 in neonates to 0.57 in adults, when studying propofol clearance [35]. Therefore, beside a linear or standard allometric function, as a third approach, an allometric function was tested with a scaling exponent that varied with bodyweight, age or BSA (eq. 6, appendix 1). For categorical covariates, typical values of the PK-parameters were compared between categories (eq. 7, appendix 1).

Statistical evaluation of the incorporated covariate relationships was performed by forward inclusion and backward deletion [37]. A p-value < 0.005 was applied to evaluate the covariates in the forward inclusion (decrease of objective function of at least 7.8 points), while the backward deletion procedure used a stricter criterion (objective function > 10.83, $p < 0.001$). When two or more covariates were found to significantly improve the model, the covariate causing the largest reduction in objective function was left in the model. Additional covariates had to reduce this objective function further to be retained in the model. Moreover, to accept a covariate, a reduction in inter-individual variability of the PK parameter involved was required. In addition, individual and population PK-parameters were plotted against the most predictive covariate to evaluate whether the individual PK-parameters were equally distributed around the population parameters [38]. The choice of the model was further evaluated as discussed under model building and evaluation, whereby the results of the internal validation procedure were also considered.

Internal validation

The robustness of the population pharmacokinetic model was assessed by the bootstrap re-sampling method throughout the model-building process and on the final PK-model, using 1000 replicate datasets per bootstrap [39]. The mean

value, 95%-confidence intervals and covariance of all parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original data set.

Derivation of model-based dosing regimen

The parameters of the final PK-model were used as a basis to determine an individualized dosing regimen in patients from 3-65 kg. The new dosing regimen aimed to reach a target exposure (defined as $AUC_{\text{day0-4}}$) of 90 mg*h/L ($\approx 5400 \mu\text{M}\cdot\text{min}/\text{day}$) in myeloablative regimens and an $AUC_{\text{day0-3}}$ of 60 mg*h/L for non-myeloablative conditioning regimens, based on the current literature of optimal exposures of busulfan in children [12, 15, 22, 24, 40, 41]. For each of the 245 patients, $AUC_{\text{day0-4}}$ values were simulated in each patient upon four consecutive once daily IV doses, using ONLYSIM-option as implemented in NONMEM. All PK-parameters were fixed to the final PK-parameters and all covariate relations were included. The amounts were integrated into a dummy AUC-compartment. For each patient, an individual dose was derived for each of the two target-AUCs, which was plotted versus bodyweight resulting in two model-based dosing nomograms.

I.3. Results

Busulfan PK-data on 245 patients were obtained from the four participating centers. Patient characteristics such as body weight, underlying disease and concomitant medications were rather evenly distributed between centers and within the total dataset (table 2).

Table 2: patient characteristics.

		Leiden	Münster	Sydney	Utrecht	Total
Nr. of patients	n	102	18	55	70	245
Age (years)	median (range)	3.58 (0.2-17)	3.88 (0.8-17)	4.25 (0.2-18)	2.5 (0.1-26)	3.33 (0.1-26)
Body weight (kg)	median (range)	15.4 (4-73)	16.5 (9.5-74)	16.7 (3.1-109)	14.8 (3.7-107)	15.3 (3.1-109)
BSA (m ²)	median (range)	0.65 (0.3-1.8)	0.70 (0.42-2.0)	0.69 (0.2-2.3)	0.61 (0.2 - 2.4)	0.65 (0.2-2.4)
Gender	male	66%	56%	56%	50%	58%
Number of samples	n (mean per patient)	472 (5)	205 (11)	466 (8)	632 (9)	1775 (7)
Frequency of Bu adm	n (4dd, 2dd, 1dd)	(39,-63)	(-,18,-)	(4,-51)	(-,70)	(43,18,184)
Underlying disease	malignancy / MDS	52	13	24	25	114
	bone marrow failure syndrome	7	1	7	6	21
	inborn errors (1)	13	4	7	20	44
	immunodef. + HLH	29	0	17	19	65
Chemotherapeutic regimen (2)	Bu + Cy	41	8	8	37	94
	Bu + Cy + Mel	44	8	0	8	60
	Bu + Flu	7	0	11	12	30
	Bu + Mel	1	1	6	0	8
	Bu + Cy + Eto	5	1	3	8	17
	Bu + Cy + Flu	4	0	19	4	27
	Bu + Mel + Flu	0	0	8	1	9
Timing chemotherapy	Chemo before or during Bu	10	0	36	21	67
	Chemo after Bu	92	18	19	49	178
Serotherapy (3)	No serotherapy	39%	50%	60%	20%	40%
Nr of transplant	1	102	18	55	67	242
	>1				3	3
Type of transplants	CB,BM,PBSC (N)	(13,73,17)	(-,19)	(20,18,16)	(28,33,8)	(61,124,60)
GGT (u/L) (4)	median (range)	14 (6-870)		21 (4-275)	21 (7-1990)	18.5 (4-1990)
ALAT (u/L) (4)	median (range)	23 (6-253)	11 (4-41)	32 (11-840)	26 (11-550)	26 (4-840)
ASAT (u/L) (4)	median (range)	37 (9-145)	14 (6-366)	35 (16-166)	29 (12-350)	32.5 (6-366)
Hemoglobuline (mmol/L) (4)	median (range)	6.3 (4.4-8.5)	5.6 (3.7-8.6)	5.9 (3.9-8.6)	5.8 (4.1-9.6)	6.1 (3.7-9.6)
Leucocytes (*10E9/L) (4)	median (range)	5.4 (0.3-45)	3.3 (0.4-81)	3.1 (0.2-16.4)	2.9 (0.1-16.6)	3.9 (0.1-81)
Thrombocytes (*10E9/L) (4)	median (range)	121 (1-688)	141 (13-615)	192 (12-629)	109 (7-661)	127 (1-688)
Erythrocytes (*10E12/L) (4)	median (range)	3.5 (2.5-5.4)		3.5 (2.2-4.6)	3.1 (1.9-5.2)	3.5 (1.9-5.4)
Albumine (g/L) (4)	median (range)	40 (29-510)	39	37 (24-47)	31 (13-45)	37 (13-51)
Concomitant medications	median nr. (range)	2 (0-8)	8 (6-11)	5 (1-9)	6 (0-13)	5 (0-13)
Glucocorticoids (5)	yes	39%	78%	15%	36%	36%
Antibiotics (5)	yes	40%	100%	22%	99%	57%
Trimethoprim/cotrimoxazol (5)	yes	0%	89%	5%	0%	8%
Antivirals (5)	yes	2%	17%	11%	60%	22%
Omeprazol/pantoprazol (5)	yes	2%	28%	5%	24%	11%
Antimycotics (5)	yes	2%	19%	25%	32%	17%
Fluconazol (5)	yes	5%	39%	73%	89%	47%
Alizapride	yes	12%	0%	0%	4%	6%
Ondansetron (5)	yes	58%	72%	100%	97%	80%
Opiates (5)	yes	11%	0%	13%	0%	7%
Antihistaminics (5)	yes	10%	22%	11%	34%	18%
Diuretics (5)	yes	8%	61%	5%	19%	14%
Ursodeoxychol acid (5)	yes	0%	6%	75%	0%	17%
Benzodiazepines (5)	yes	21%	78%	73%	96%	58%
> 1 Drug cleared via renal clearance (6)	yes	54%	100%	80%	99%	76%
> 1 Drug cleared via P450 metabolism (7)	yes	33%	100%	76%	99%	67%
> 1 Drug cleared via phase 2 metabolism (8)	yes	79%	100%	100%	99%	91%

Bu = busulfan, MDS = myelodysplastic syndrome, immunodef. = immune deficiencies, HLH = hemophagocytic lymphohistiocytosis, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, JMML = juvenile myelomonocytic leukemia, CML = chronic myeloid leukemia, Cy = cyclophosphamide, Mel = melphalan, Flu = fludarabine, P450 = cytochrome P450 enzymes, BSA = body surface area.

- 1) Inborn errors were inborn errors of metabolism and hemoglobinopathies.
- 2) In general the dose of melphalan was 140 mg/m² once daily, etoposide 1200 mg/m² once daily, cyclophosphamide: 60 mg/kg once daily for 4 days, fludarabine 40 mg/m² for 4 days.
- 3) Patients with unrelated donors received serotherapy, either anti-thymoglobulin (ATG)-rabbit (Genzyme/Fresenius) or alemtuzumab (Genzyme).
- 4) These covariates were measured at day 1 or just before the day of the first busulfan administration. These covariates were also explored in relation to their age-related reference-values.
- 5) Covariate selection of concomitant medications used in these patients, based on the route of metabolism of the drugs (selections were made of medications which were used by more than 10% of patients).
- 6) Drugs cleared primarily via renal clearance were antibiotics, trimethoprim, antivirals, fluconazole, alizapride, metoclopramide, diuretics.
- 7) Drugs cleared primarily via P450 metabolism were phenytoin, antimycotics, proton-pump inhibitors, benzodiazepines, opiates.
- 8) Drugs cleared primarily via phase 2 metabolism were acetaminophen (if needed medication of acetaminophen was not included), metronidazole, co-trimoxazole, corticosteroids, metoclopramide, ondansetron, antihistaminics, diuretics, ursodeoxycholic acid.

Busulfan concentrations could adequately be described using a two-compartment model parameterized in terms of clearance (CL), inter-compartmental clearance (Q), volume of distribution of central compartment (V1) and peripheral compartment (V2, estimated as a factor times V1). The two-compartment model was superior over a one-compartment model for statistical reasons (decrease in objective function of 132 points ($p < 0.001$)) and improved goodness of fit plots. Inter-occasion variability on clearance in the structural model resulted in a large reduction of objective function of -309 ($p < 0.001$). The addition of inter-occasion variability on V1 did not result in a significant improvement of the model (objective function -5.9). In the final PK-model, clearance at day 2-4 was estimated as a fraction of clearance at day 1 (eq 3, appendix 1) and was 12% lower in comparison with day 1 (objective function -45, $p < 0.001$).

Figure 1a (left panel) shows that the two-compartment PK-model without covariates could adequately describe the observed concentrations. This model showed poor predictive performance, however, as shown by the population predicted *versus* observed concentrations (figure 1a, right panel). Particularly in the extremities of the body weight range (3-7 kg and >30 kg) concentrations were over- and under-predicted, respectively.

The covariate analysis identified body weight, BSA and age as most important covariates related to volume of distribution and clearance. The introduction of body weight as a covariate for V1 using a linear function (eq.4, appendix 1) resulted in a decrease in objective function of -442 ($p < 0.001$). An allometric function for V1 (eq. 5, appendix 1) with a single scaling exponent of 0.89 was superior over a linear function (objective function -26 points, $p < 0.001$) and resulted in an equal distribution of the individual V1 parameter estimates of all body weights around the population V1 parameter estimates. As a result, the inter-individual variability on V1 decreased from 76% to 20% in comparison with the PK-model without covariates. For clearance, inclusion of body weight using a linear equation (eq. 4, appendix 1) further improved the model (objective function -457, $p < 0.001$). An allometric function for clearance (eq. 5, appendix 1) with a single scaling exponent of 0.91 was superior over a linear function (objective function -11 compared to a linear function, $p < 0.001$), but the population predicted values of both models were biased compared to

the individual predicted PK-parameters. A linear or allometric function over-predicted patients < 10 kg and > 40 kg and under-predicted patients between 10 and 40 kg. An allometric equation with a scaling exponent, that changed with body weight from 1.2 in neonates to 0.55 in young adults (eq. 6, appendix 1) described the relation between bodyweight and clearance significantly better compared to the allometric equation with a single scaling exponent (decrease in objective function of 35, $p < 0.001$). The population predicted values of this model described individual predicted PK-parameters without bias. This model resulted in an adequate distribution of the individual CI parameter estimates over the entire body weight-range and resulted in a decrease in inter-individual variability on CI from 99% to 27% in comparison with the PK-model without covariates. Body weight was linearly related to Q (objective function -26, $p < 0.001$) and resulted in a decrease in inter-individual variability from 148 to 88% in comparison with the PK-model without covariates.

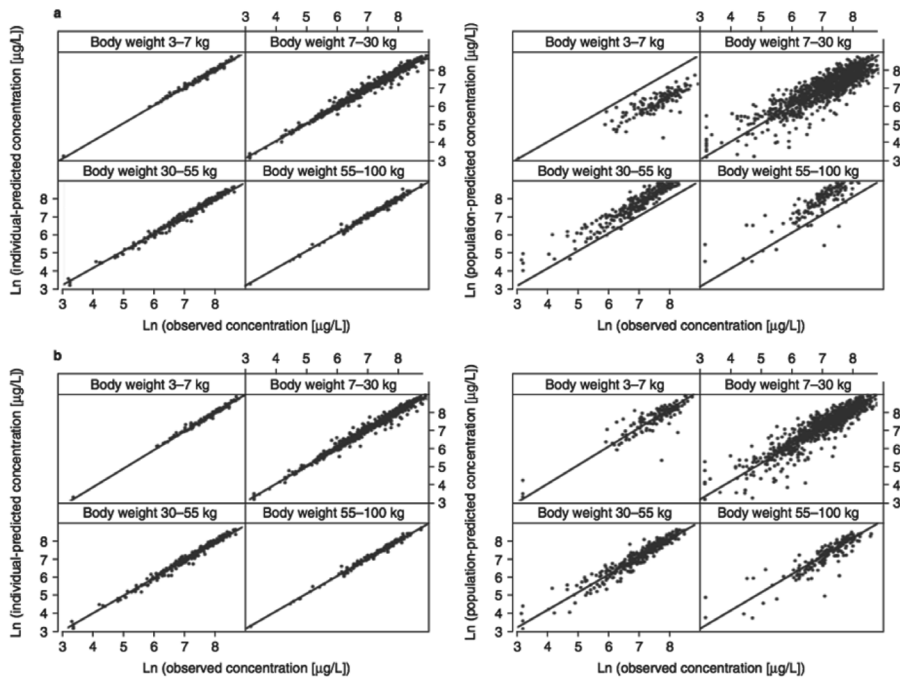


Figure1: Individual predicted concentrations vs observed concentrations (leftpanels) and population predicted concentrations vs observed concentrations (right panels) of the pharmacokinetic model without covariates (a) and the final pharmacokinetic-model (b). Data of four body weight categories (kg) are shown separately. Ln = lognormal.

The inclusion of body weight on the PK-parameters resulted in a more significant reduction of the objective function in comparison with age on all parameters (objective function +108), while the model using BSA showed similar results in comparison with body weight-model given the criteria as defined under Methods.

The systematic covariate analysis did not identify any other covariates. No differences in the diagnostic-plots were observed between the participating centers (appendix 2), frequency of busulfan administration, concomitant medications or any of the other covariates.

For the final PK-model, the individual and population predicted *versus* observed concentrations per body weight-category are depicted in figure 1b. The right panel demonstrates that the predictive performance of the model is similar in all four bodyweight groups. The individual and population PK-parameters (Cl and V1) of the final PK-model *versus* body weight are shown in figure 2. The figure shows an equal distribution of the individual PK-parameter estimates of all body weights around the population PK-parameter estimates. All parameter estimates of the final PK-model and the results of the statistical evaluation (bootstrap validation, shrinkage and condition number) are presented in table 3. These statistical evaluation tools were within the limits, given the criteria as defined under Methods, except for shrinkage on Q and inter-occasion variability which were both >20%.

Based on the final PK-model, the model-based dosing nomogram was derived as depicted in figure 3. With this nomogram, a dose for each individual between 3-65 kg can be obtained, aiming for a myeloablative ($AUC_{\text{day0-4}}$ of 90 mg*h/L) or non-myeloablative conditioning regimen ($AUC_{\text{day0-3}}$ of 60 mg*h/L). Table 4 shows the model-based nomogram in mg/kg of patients between 3 and 65 kg and the deviations of their simulated AUC's in comparison with the target- $AUC_{\text{day0-4}}$ of 90 mg*h/L. Deviations in the expected AUCs upon the currently approved dose in the summary of product characteristics by EMA [30] and a BSA based dosing regimen of 130 mg/m² are also shown in table 4. Both regimens are used in pediatric clinical practice by different institutes. The first dosing algorithm was based on a population-pharmacokinetic study of 24 children by Nguyen *et al.* in 2004 [20], aiming at a target AUC of 78mg*h/L (59-98mg*h/L in combination

with cyclophosphamide), which was prospectively validated in 55 children by Vassal *et al.* in 2008 [42]. The BSA-based dosing regimen was used in children by Nath *et al.* and Gordon *et al.* [22, 43] This dosing regimen was derived from a studies using oral busulfan using 130-150mg/m² per day in children [5, 44] and from a study of IV busulfan in adults [45].

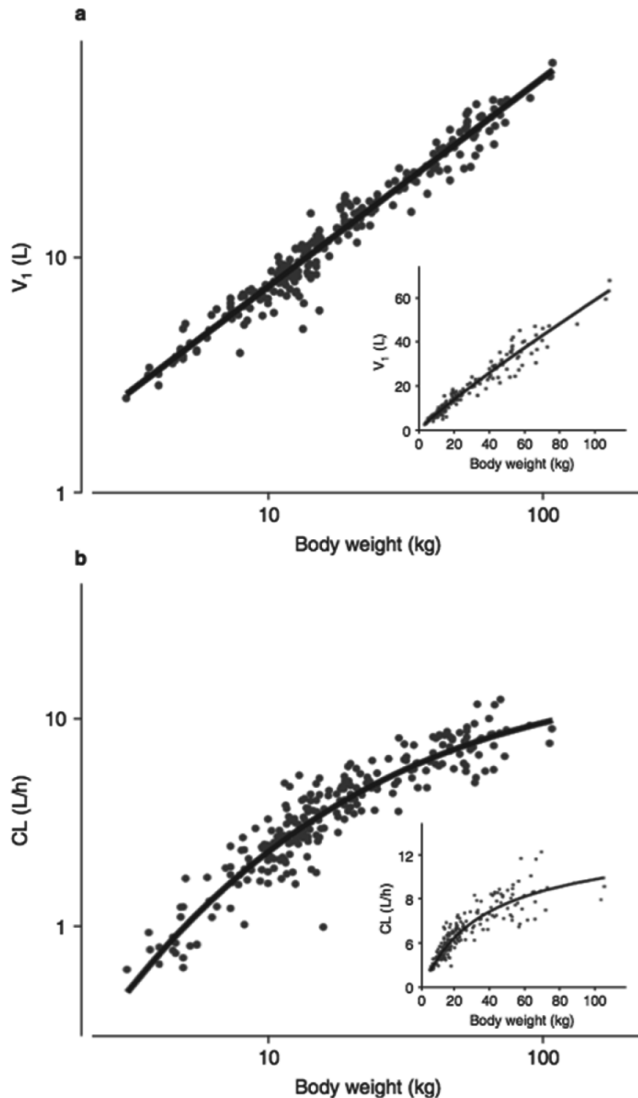


Figure 2: Individual predicted pharmacokinetic parameters (post hoc, pre- sented as dots) and population predicted pharmacokinetic parameters (black line) of volume of distribution of the central compartment (a) and clearance (b) vs body weight of the final pharmacokinetic model. The data are presented on log-scale and on normal scale (insert). CL = clearance; V_1 = volume of dis- tribution of the central compartment.

Table 3: Population PK-parameter estimates, shrinkage and PK-parameter estimates obtained after bootstrap of the final PK-model.

Parameter	Dataset		Shrinkage	1000 Bootstrap replicates	
	Estimate	CV %		Median	5-95 percentile
Structural model					
Cl _{15.3kg} (L/h) ^a	3.47	3.0		3.44	3.24-3.72
V1 _{15.3kg} (L) ^b	11.1	1.6		11.1	10.8-11.4
V2 expressed as factor (times V1)	6.92	6		6.89	1.56-13.5
Q _{15.3kg} (L/h) ^c	0.495	12.2		0.509	0.29-0.67
L1 in: $Cl = Cl_{15.3kg} \times (BW / 15.3)^{L1 \times BW^M}$	1.56	10		1.55	1.31-1.88
M in: $Cl = Cl_{15.3kg} \times (BW / 15.3)^{L1 \times BW^M}$	-0.226	15		-0.224	-0.29--0.17
L2 in: $V1 = V1_{15.3kg} \times (BW / 15.3)^{L2}$	0.890	2.0		0.889	0.86-0.92
Cl _{day2-4} expressed as reduction factor of day ₁	0.12	14		0.12	0.08-0.15
Random variability					
Inter-individual variability on Cl (%)	27%	17	16%	27%	23%-31%
Inter-individual variability on V1 (%)	20%	21	19%	20%	17%-24%
Inter-individual variability on Q (%)	88%	24	50%	87%	64%-119%
Corr. IIV ClxV1	0.52	21		0.53	0.39-0.66
Inter-occasion variability Cl (%)	15%	21	42%	15%	12%-18%
Proportional residual error (%)	14%	12	18%	14%	12%-15%
Objective function	-3671			-3702	

$Cl_{15.3kg}$ = clearance for a typical individual of 15.3kg, $V1_{15.3kg}$ = volume of distribution of the central compartment for a typical individual of 15.3kg, $V2_{15.3kg}$ = volume of distribution of the peripheral compartment for a typical individual of 15.3kg, $Q_{15.3kg}$ = inter-compartmental clearance for a typical individual of 15.3kg, 15.3 kg was the mean value of the body weights in the dataset. In the allometric function, L1 represents the intercept and M is the exponent, which allows the scaling exponent to change with body weight. L2 represents the single scaling exponent. BW= body weight (kg) CV= coefficient of variation (%), inter-individual variability was calculated as the square root of the exponential variance -1).

a. Clearance was described, according to the following equations:

$$Cl = Cl_{15.3kg} \times \left(\frac{BW}{15.3} \right)^{L1 \times BW^M}$$

b. V1 was described as volume of distribution of the first compartment, according to the following equations:

$$V1 = V1_{15.3kg} \times \left(\frac{BW}{15.3} \right)^{L2}$$

c. Q was described as inter-compartmental clearance, according to the following equations:

$$Q = Q_{15.3kg} \times \left(\frac{BW}{15.3} \right)^1$$

Table 4: The model-based individualized dosing nomogram of busulfan, expressed in mg/kg for 20 patients with body weight ranging between 3 and 65 kg (aiming at a target-AUC_{day0-4} of 90 mg*h/L^a in combination with fludarabine). The deviations of their corresponding AUC_{day0-4}-values in relation with this target-AUC_{day0-4} are shown (left column). For comparison, the dosing nomogram of the currently approved dose in the EMA-summary of product characteristics (SPC) (43) and deviations in their corresponding AUC_{day0-4} values of the target-AUC_{day0-4} of 90mg*h/L^a are shown (middle column). Doses in mg/kg derived from a BSA based dosing regimen of 130mg/m² 1dd and their concurrent deviations of the target-AUC_{day0-4} of 90mg*h/L^a, are also shown (right column).

Body weight kg	Model-based individualized dosing nomogram Myeloablative dose 4 days, 1dd, mg/kg target AUC _{day0-4} 90mg*h/L ^a		Approved dose in SPC 4 days, 1dd, mg/kg		Dose based on 130 mg/m ² ^b 4days, 1dd, mg/kg	
	Dose (mg/kg)	± % deviation of target AUC	Dose (mg/kg)	± % deviation of target AUC	Dose (mg/kg)	± % deviation of target AUC
3	3.8	0%	4.0	5%	8.7	128%
5	4.7	0%	4.0	-15%	7.0	49%
7	5.1	0%	4.0	-22%	6.5	28%
8	5.2	0%	4.0	-23%	6.2	19%
9	5.2	0%	4.8	-8%	6.2	19%
11	5.2	0%	4.8	-9%	7.0	33%
13	5.2	0%	4.8	-8%	5.5	6%
15	5.1	0%	4.8	-6%	5.2	2%
16	5.1	0%	4.4	-13%	5.4	7%
20	4.9	0%	4.4	-9%	5.2	7%
23	4.7	0%	3.8	-19%	4.9	4%
25	4.6	0%	3.8	-17%	4.9	8%
30	4.3	0%	3.8	-12%	4.3	0%
35	4.1	0%	3.2	-22%	3.9	-4%
40	3.9	0%	3.2	-18%	3.9	0%
45	3.7	0%	3.2	-14%	3.9	6%
50	3.5	0%	3.2	-10%	3.8	8%
55	3.4	0%	3.2	-6%	3.5	5%
60	3.3	0%	3.2	-2%	3.7	12%
65	3.1	0%	3.2	2%	3.4	8%

a. A total exposure of 90 mg*h/L to 21.6 mM*min total, or 5400 µM*min/day

b. Doses in mg/kg of the BSA based dosing regimen were extracted from a BSA-for-body weight plot of all patients.

I.4. Discussion

This international pharmacokinetic meta-analysis of 245 patients was conducted to characterize the pharmacokinetics of busulfan from 1 month to 26 years of age. Body weight was the most predictive covariate for clearance (Cl), volume of distribution (V1) and inter-compartmental clearance (Q) and explained 65%, 75% and 40% of the observed inter-individual variability, respectively. The relation between body weight and clearance was clearly non-linear and was described using an allometric function with a scaling exponent

that varied between 1.2 in neonates and 0.55 in young adults. This precise relation could be identified as a result of the large range in body weights and extensive number of patients and resulted in an individualized dosing nomogram for patients between 3-65 kg.

The PK-model was built based on a large dataset obtained from a multi-center setting. The dataset contained multiple treatment regimens, a wide range of age, bodyweights, underlying diseases and a large number of other covariates. The model adequately described the data and only 13% residual variability remained in the final PK-model. Statistical evaluation tools (CV of parameter estimates, bootstrap) show that this is a robust model. No differences were seen between the participating centers, even though the centers had different settings. This indicates that the results of the model may be extrapolated to other pediatric HSCT centers elsewhere, if patient characteristics are comparable to the characteristics in this dataset (like similar concomitant medications). The quality of the structural model (figure 1b, left panel) indicates that this PK-model can reliably predict exposures in new patients, ranging from 3-65 kg.

Body weight, rather than age, was the most predictive covariate that explained the variability in exposure between patients from 3-100 kg. Because BSA is a composite parameter taking account both length and body weight, and the BSA model did not result in improvement of the description of the data in comparison with body weight as the only parameter, the final body weight model was preferred over the BSA model. The non-linear relation between body weight and clearance reflects that an increase in body weight in neonates results in a larger increase in busulfan clearance than an increase in bodyweight in older children or adults. The maturation of activity and expression of glutathione S-transferase have been studied in enterocytes and after oral administration of busulfan [13, 46, 47]. Assuming that the expression and activity of liver and enterocyte enzymes show similar developmental patterns, the non-linear relation of body weight and clearance could be related to changes in maturation rate of glutathione S-transferase as described in these articles. The effect might also relate to differences in liver volume, blood flow and biliary functions in young infants as compared to adults [48, 49]. This relation between body weight and clearance could not be estimated using an allometric function with a single scaling exponent, an approach that

is commonly applied - also in busulfan PK studies [17, 19, 20]. In this dataset, the allometric function with a single scaling exponent of 0.89 on body weight overestimated the clearance of busulfan up to 1.5 times in patients < 10 kg and > 40 kg, and underestimated the clearance up to 1.25 times in patients between 10-40 kg. In this meta-analysis we characterized the clearance of busulfan using an allometric function with a scaling exponent that varied with body weight from 1.2 in neonates to 0.55 in young adults. These values are very similar to a recent study of busulfan in children which showed a single scaling exponents 1.25 in children <9 kg and 0.76 >9kg [50]. In studies using other compounds varying (single) scaling exponents are published of 1.3-1.5 in neonates to 0.56 in adults [34-36], such despite the fact that different routes of metabolism are concerned. Literature data suggest that scaling of PK-parameters between children could be performed using an allometric scaling function with a fixed exponent of 0.75 or 1 for bodyweight and subsequently estimating a function that describes maturation processes as a function of age [51]. However, postnatal age and body weight are highly correlated. Therefore, in the current analysis, one single function based on bodyweight was identified, which would adequately describe the pharmacokinetics of busulfan from neonates to young adults. It would be of interest to test this function for other drugs when studying the effects of maturation in the whole pediatric age range including neonates.

After inclusion of body weight in the model, the disease-group (e.g. immune deficiencies) was not a significant covariate while also the inter-individual variability was similar between the four groups. In our cohort, the patients treated for malignancies or bone marrow failures were older and heavier in comparison with immune deficiencies, or inborn errors of metabolism. While in other studies, the disease group (i.e. diseases like immune deficiencies or lysosomal storage disease) has been reported a covariate for busulfan PK [13, 21-23, 33] we suggest that body weight may be an effect modifier in the relation between busulfan-PK [21-23]. As Glutathione S-transferases play an essential protective role against reactive oxygen species [52], many drugs could interact with busulfan. However, no significant interactions were identified in this large dataset, but drugs like metronidazole and phenytoin which have shown to influence busulfan PK in previous studies [30-32], could not be studied in full extent in this dataset due to use in <10% of patients. Moreover, neither clinical

chemistry data nor blood counts showed any biomarkers which could predict variability between patients. This analysis shows that the nature of the body weight-dependent pharmacokinetics of busulfan, including the smallest and heaviest patients, should be adequately characterized first. Only thereafter, other less influential covariate relationships like disease can be studied, to overcome confounding by maturation.

In this study, 15% day-to-day variability of busulfan clearance between the four days of administration was shown, which is only slightly higher than 5-10% shown in adults [19, 49]. In addition we showed that busulfan clearance was 12% lower at day 2-4 compared to day 1. This finding is consistent with results of a study in children with thalassemia in which a decrease of 11% in clearance between the first and subsequent doses was found [41]. Other studies did not report a significant decrease in busulfan AUC on following days [15, 16]. Yeh *et al.* reported that the concurrent use of fludarabine might decrease the busulfan clearance from day 1 to day 4 [53]. However, in the current study, the difference in clearance between consecutive days was not significantly altered in fludarabine as compared to non-fludarabine users. While the decrease in clearance deserves further study, it should be accounted for in the design of new dosing algorithms and when performing TDM.

Based on the final PK-model, a body weight dependent-dosing nomogram was defined (figure 3). The nomogram leads to a smaller range in predicted AUC's between patients of different body weights than the currently approved dose in the EMA-summary of product characteristics [30] or BSA-based dosing nomogram of 130mg/m² as shown in table 4. The currently approved dose leads to deviations in exposures, especially in children near the edges of each dosing category. A limitation of our meta-analysis is however, that only 12 patients >65 kg were included and therefore dose recommendations for patients >65 kg could not be provided based on these analyses.

Table 4 also demonstrates that a dose of 130 mg/m² [22, 43] results in overdosing up to 220% in patients < 0.5 m² and should therefore not be used. Even though the model-based nomogram takes into account variation from differences in bodyweight, the remaining unexplained inter-occasion variability in apparent clearance in the final PK-model is 15% and the unexplained inter-individual

variability 27%. Validation studies are needed to establish the predictive performance of the model and dosing regimen. Based on these considerations, we advocate that combined with the new dosing nomogram, TDM remains needed.

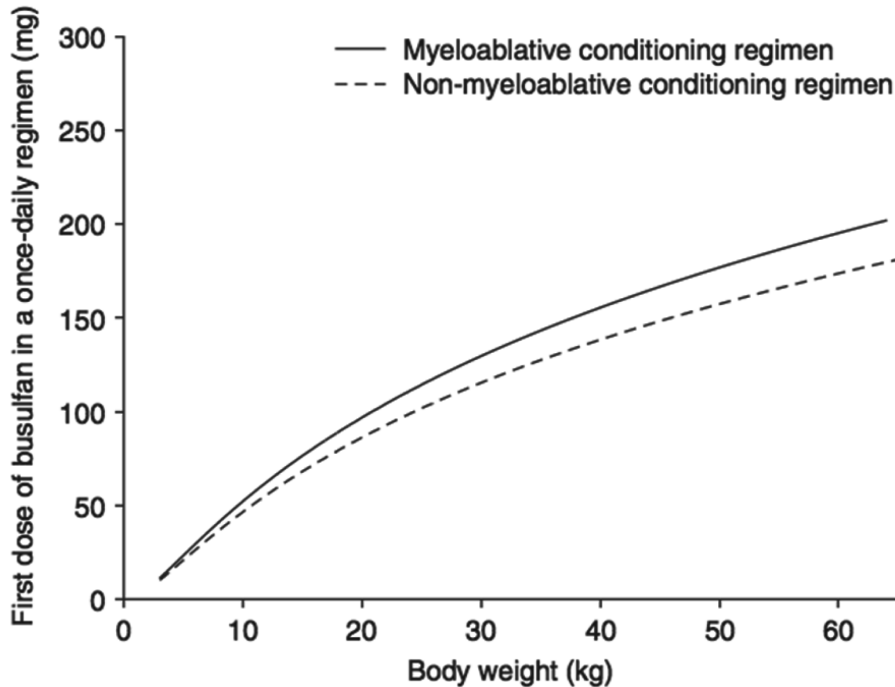


Figure 3: Model-based individualized dosing nomogram of busulfan related to the body weight of the patient, aiming for a myeloablative ($AUC_{\text{day0-4}}$ of 90 $\text{mg}\cdot\text{h}/\text{L}$ in combination with fludarabine) (grey line) and a non-myeloablative conditioning regimen ($AUC_{\text{day0-3}}$ of 60 $\text{mg}\cdot\text{h}/\text{L}$) (dotted black line).

The model-based dosing nomogram described in this article targets a narrow $AUC_{\text{day0-4}}$ of 90 $\text{mg}\cdot\text{h}/\text{L}$ ($\approx 5400 \mu\text{M}\cdot\text{min}/\text{day}$) in myeloablative and 60 $\text{mg}\cdot\text{h}/\text{L}$ in non-myeloablative conditioning regimens, in a platform with a (preferably non-alkylating) immunosuppressive agent (e.g. fludarabine) [45, 54, 55]. In the literature, different values for optimal AUC's have been proposed for patients with varying underlying diseases, disease severity and differences in concomitant medications given during the conditioning regimens [12, 15, 22, 24, 40, 41]. Comparing busulfan exposures between studies should be performed with care, as AUC's may have been calculated using different

sampling schedules and different methods. Most pediatric literature data are based on conditioning regimens containing busulfan and cyclophosphamide, resulting in a total $AUC_{\text{day0-4}}$ of approximately 80 mg*h/L in children [24, 41]. We recently showed that busulfan with a target $AUC_{\text{day0-4}}$ between 80 and 100 mg*h/L combined with fludarabine (n=40) was as effective, but less toxic in comparison with Busulfan-cyclophosphamide-melphalan (n=45) [56]. Also adult data shows that busulfan-fludarabine targeted to an $AUC_{\text{day0-4}} < 100$ mg*h/L results in optimal outcome [55, 57]. In children, a target $AUC_{\text{day0-4}}$ of approximately 45-65 mg*h/L has been published using busulfan-fludarabine as a non-myeloablative conditioning regimen [40, 58]. Perhaps in the future, additional agents may be added to this busulfan-fludarabine combination to enhance the anti-leukemia effect (e.g. clofarabine, which was shown *in vitro* to have a synergistic anti-leukemic effect) [59, 60]. These developments and optimizations will lead to further individualization of the target exposures to busulfan. Using the structural parameters of this PK-model, simulations can be performed leading to a dosing nomogram that can target any desired AUC for busulfan.

In conclusion, in this population pharmacokinetic model for busulfan in patients ranging between 1 month and 26 years of age, body-weight was the most predictive covariate for all PK-parameters of busulfan and explained a major part of the observed inter-individual variability. The model-derived individualized dosing nomogram is expected to result in predictive busulfan exposures in patients ranging between 3 and 65 kg when combined with TDM, resulting in a safer and more effective HSCT in children.

I.5. Acknowledgements

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Appendix:

Appendix 1: Equations:

Model of random variability:

$$P_{ig} = P_{pop} \times e^{\eta i + \kappa g} \quad (\text{eq. 1})$$

Equation 1 describes the inter-individual variability and day-to day (inter-occasion) variability²⁷ of the structural parameters within the population, in which lognormal distribution was assumed. P_{ig} represents the individual PK-parameter for subject i on occasion g . P_{pop} is the typical value of the population PK-parameter. An occasion (g) was defined as all measurements performed in 1 day, κ_{ig} is the random effect between days. η and κ are random variables that follow the normal distribution with a mean value of 0 and variance ω^2 and π^2 , respectively.

$$\log C_{ij} = \log C_{pred\ ij} + \varepsilon \quad (\text{eq. 2})$$

Equation 2 describes the intra-individual variability; the differences between the observed and predicted concentrations. This residual error includes among other factors, model misspecification and measurement errors. The intra-individual variability was modeled using an additive error, equivalent to a proportional error model in the untransformed scale. C_{ij} is the observed concentration for subject i at time j , and $C_{pred\ ij}$ is the predicted concentration for individual i at time j . ε is a random variable that follows the normal distribution with a mean value of 0 and variance σ^2 .

Other model-equations.

$$Cl_{day_1} = Cl_{pop} \times (1 - fraction_{day_{2-4}}) \quad (\text{eq. 3})$$

Equation 3 describes the clearance at day₂₋₄, estimated as a fraction of clearance at day₁.

Cl_{day1} is the typical value of clearance at day 1. Cl_{pop} is the typical value of clearance. Fraction day2-4 is the clearance at day₂₋₄ expressed as reduction factor of day₁.

Covariate functions:

The nature of the influence of continuous covariates on each PK parameter was tested using a linear (eq.4) and allometric (eq. 5) function:

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^1 \quad (\text{eq. 4})$$

In equation 4, P_i is the individual parameter for subject i with Cov_i . P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate.

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^{L1} \quad (\text{eq. 5})$$

In equation 5, P_i is the individual parameter for subject i with Cov_i . P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate. $L1$ represents the scaling exponent of the allometric function, which is one fixed estimated value in case of an allometric function with a single scaling exponent.

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^{L2 \times Cov_i^M} \quad (\text{eq. 6})$$

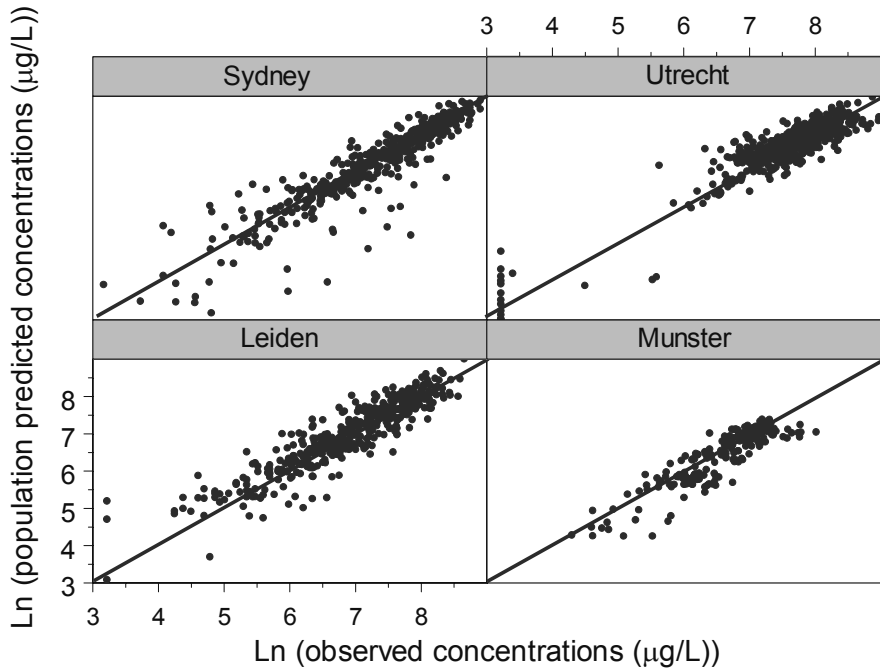
In equation 6, the allometric function with a scaling exponent that varies with body weight, BSA or age is shown, in which P_i is the individual parameter for subject i with Cov_i . P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate. In the scaling exponent, $L2$ represents the intercept and M is the exponent, which allows the scaling exponent to change with the covariate body weight, BSA or age.

Potential categorical variables were modeled using:

$$P_i = P_{pop} \times P_c^{CCov} \quad (\text{eq. 7})$$

In equation 7, CCov is the categorical covariate, P_i is the individual parameter for subject i , P_{pop} is the typical value of the population PK-parameter in absence of the covariate of interest (CCov=0) and P_c is the fractional change in the typical value of the PK-parameter caused by the covariate.

Appendix 2: Population predicted concentrations versus observed concentrations of the four participating centers.



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Appendix

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A novel maturation function for clearance of the CYP3A substrate midazolam from preterm neonates to adults

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Abstract

Background and objective: Major changes in Cytochrome P450 (CYP) 3A may be expected in the first months of life with later on relatively limited changes. In this analysis we studied the maturation of *in vivo* CYP3A mediated clearance using midazolam as model drug, from preterm neonates of 26 weeks gestational age (GA) onwards to adults. To investigate the maturation of *in vivo* CYP3A mediated clearance of drugs from prematurely born neonates with gestational ages (GAs) of 26 weeks throughout adulthood using midazolam as a model drug.

Methods: Pharmacokinetic data after intravenous midazolam were obtained from 6 previously reported studies. Subjects were prematurely born neonates (n=24; GA 26-33.5 weeks, PNA 3-11 days, and n=24; GA 26-37 weeks GA, PNA 0-1 days), 23 children after elective major craniofacial surgery (3-23 months), 18 pediatric intensive care patients (2 days to 17 years), 18 pediatric oncology patients (3-16 years) and 20 healthy male adults (20-31 years). Population pharmacokinetic modeling with a systematic covariate analysis was performed using NONMEM v6.2.

Results: Across the entire lifespan from prematurely born neonates to adults bodyweight was a significant covariate for midazolam clearance. The influence of body weight was best described using an allometric equation with an exponent changing with bodyweight in an exponential manner from 0.84 in preterm neonates (0.77kg) to 0.44 in adults (89kg) showing that the most rapid maturation occurs during the youngest age range.

Conclusions: An *in vivo* maturation function for midazolam clearance from prematurely born neonates to adults has been developed. This function can be used to derive evidence-based doses for children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic or physiologically based pharmacokinetic (PBPK) models.

II.1. Background

Cytochrome P4503A (CYP3A) is the most abundant CYP enzyme in the human liver. [1] It is involved in the metabolism of over half of all metabolized drugs. [2] Large inter-individual and intra-individual variation has been shown for CYP3A, resulting in inter-individual differences in the clearance of CYP3A substrate drugs. An important factor explaining variation in CYP3A activity is age. However, the exact ontogenetic pattern of the CYP3A isoforms CYP3A4 and CYP3A5 *in vivo* is still unclear. [3-7] This information gap hampers the development of individualized dosing guidelines of CYP3A4/5 substrate drugs in children. To define the *in vivo* activity of CYP3A4/5, surrogate probes that correlate with actual enzyme activity can be used. [8] The best validated CYP3A4/5 probe is midazolam, which has been used extensively in adults and children. [9, 10]

For CYP3A4/5 mediated clearance of midazolam in children between one month and 17 years of age, critical illness proved a more significant covariate than bodyweight. [11] However, particularly in the first days and weeks of life, CYP3A4/5 activity may be expected to be low on the basis of *in vitro* data and *in vivo* reports on reduced clearance of CYP3A substrates in neonates [6, 7, 12-15]. Therefore, we report here on the maturation of *in vivo* CYP3A mediated clearance of midazolam across the entire human life span, using a dataset consisting of 6 midazolam studies in which the pharmacokinetics were studied in populations ranging in age from prematurely born neonates to adults. The resulting maturation function may be used as a basis for evidence based dosing of midazolam and potentially other CYP3A substrates in population pharmacokinetic or physiology based pharmacokinetic (PBPK) models.

II.2. Methods

Patients and data

Individual data from six previous studies were included in the analysis. [3, 6, 16-19] Individuals were prematurely born neonates (two datasets: n=32; gestational age (GA) 26-33.5 weeks, postnatal age (PNA) 3-11 days, and n=24; GA 26-37 weeks, PNA 0-1 days), 23 children after elective major craniofacial

surgery (3-23 months), 18 pediatric intensive care patients (2 days to 17 years), 18 pediatric oncology patients (3-17 years) and 20 healthy male adults (20-31 years). Details on the datasets are provided in table 1.

Pharmacokinetic data analysis

Population pharmacokinetic analysis was performed using the first-order conditional estimation (FOCE) with η - ϵ interaction in NONMEM version 6.2, release 1.1 (GloboMax LLC, Hanover, MD, USA) [20]. S-plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands) was used to visualize the data. Model development was performed in four steps: (1) choice of the structural model, (2) choice of the error model, (3) covariate analysis, and (4) validation of the model. Discrimination between different models was done by comparison of the objective function. A decrease in the objective function of 3.8, corresponding to a value of $p < 0.05$, was considered statistically significant. Goodness-of-fit plots (observed *versus* individually predicted concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* population predictions) of all data and stratified per dataset, were used for diagnostic purposes. In addition, the confidence interval of the parameter estimates, the correlation matrix, and visual improvement of the individual plots were used to evaluate the model. Furthermore, η -shrinkage as defined by Karlsson *et al.* [21] was calculated for all model parameters for which inter-individual variability was estimated, and over-parameterization (ill-conditioning) of the model was tested by calculating the condition number. [22]

Model development

For the structural model of midazolam, a one, two and three compartment model was tested. In the model, the previously reported influence of critical illness on CYP3A mediated clearance of midazolam [11] was incorporated, implying a reduction in midazolam clearance in critically ill patients (datasets of prematurely born neonates and pediatric intensive care patients) compared to non-critically ill children and adults (datasets of children after major craniofacial surgery, pediatric oncology patients, and adults). The individual value (*post hoc* value) of the parameters of the i th subject was modeled using equation 1, where P_i equals the individual or *post hoc* value of the parameters

of the i th subject, and P_{pop} is the population value of the parameter. Random variable (RV) is assumed to be a gaussian random variable with mean zero and variance of ω^2 , assuming log-normal distribution.

$$P_i = P_{pop} \times e^{RV} \quad (1)$$

The intra-individual variability was described with a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range, shown in equation 2, where j is the observed midazolam concentration (Y) of the i th individual, C_{pred} is the predicted midazolam concentration and RV_{ij} is the random variable for midazolam, with mean zero and variance σ^2 .

$$Y_{ij} = C_{pred,ij} \times (1 + RV_{ij}) \quad (2)$$

Covariate analysis

Individual post hoc parameter estimates were plotted independently against the covariates to visualize potential covariate relationships. The following covariates were tested for all parameters: bodyweight, postnatal age, gestational age, study population, and mechanical ventilation (yes/no). Continuous covariates were separately entered into the model using a linear function (equation 3), or an allometric equation (equation 4), where P_i equals the individual or post hoc value of the parameters of the i th subject, P_{pop} is the population value of the parameter, COV is the concerned covariate, K is the exponent which may be estimated or fixed to 0.75.

$$P_i = P_{pop} \times (COV_i / COV_{median}) \quad (3)$$

$$P_i = P_{pop} \times (COV_i / COV_{median})^K \quad (4)$$

Alternatively, an allometric equation with an exponent that changes with bodyweight [23] was tested (equation 5) in which BDE represents the body weight dependent exponent. For BDE , a sigmoidal function [23] and an exponential function (equation 6) with $Coeff$ as a coefficient, BW as body weight and $exp1$ as an exponent) [24] were tested.

$$P_{pop} \times (COV_i / COV_{median})^{BDE} \quad (5)$$

$$BDE = Coeff \times BW^{\exp 1} \quad (6)$$

Categorical covariates (e.g. mechanical ventilation (yes/no) or study population (critically ill / healthy)) were tested using 'IF' statements, in which the parameter for one subgroup was estimated as a multiple of the parameter estimate for the other subgroup, shown in equations 7 and 8, in which *factor* is the multiplication factor, and *y* equals one of the two subgroups.

$$P_i = P_{pop} \quad (7)$$

$$IF \ variable = y, P_i = P_{pop} \times (factor) \quad (8)$$

The influence of a covariate was statistically tested for significance by use of the objective function. P-values < 0.005 (decrease of objective function of 7.8 points) were applied to evaluate the covariates in the forward inclusion, while a more stringent p value of <0.001 (decrease of objective function of 10.83 points) was used in the backwards deletion step. When two or more covariates were found to significantly improve the model, the covariate that showed the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was further evaluated as discussed under 'pharmacokinetic data analysis' and 'model development'. This included also individual and population parameter estimates versus the most predictive covariate in the model. [25]

Validation of the model

For the internal validation of the final model, a bootstrap analysis was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP. interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). Two hundred datasets were resampled from the combined original datasets, and refitted to the model. [26]

Secondly, Normalized Prediction Distribution Errors (NPDE) were calculated [27] using the NPDE package in R. [28] For this method, the dataset used for building the model was simulated 1000 times with inclusion of the inter-individual variability and residual error.

Simulation dosing regimen

Simulations were performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NONMEM SP:interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). The parameter estimates of the final Pharmacokinetic model were used to simulate midazolam concentrations in critically ill children ranging from prematurely born neonates to adolescents varying in body weight between 0.75 and 60 kg (0.75, 1, 2, 3, 5, 10, 20, 45, and 60 kg) upon a bolus injection and an infusion duration of 24 hrs. Both dosing schedules used in clinical practice (traditional dosing schemes) and model based dosing schedules were simulated. The traditional dosing scheme for prematurely born neonates was an iv bolus of 0.05 mg/kg and an infusion of 0.06 mg/kg/hr [29-31] while for children and adolescents an iv bolus of 0.1 mg/kg and an infusion of 0.1 mg/kg/hr (conscious sedation in critically ill patients [32]) was chosen. The model based dosing regimen consisted of a dosing regimen that would result in therapeutic midazolam concentrations 0.25-0.37 µg/mL for conscious sedation in children in pediatric intensive care (PICU) patients. [32] As therapeutic midazolam concentrations for prematurely born neonates are unknown, these were set at 0.2 µg/ml being the lowest target concentration aimed for in a prematurely born neonate study. [18]

II.3. Results

Patients and data

1105 midazolam concentrations from 136 individuals of six different datasets, [3, 6, 16-19] with a postnatal age varying between 0 days and 31 years of age, and a body weight between 0.77 kg and 89 kg were included for analysis. None of the patients received CYP3A inhibitors or inducers. A summary of the patient and data characteristics of the six studies is shown in table 1.

Table 1 Overview of datasets.

Parameter	de Wildt SN <i>et al.</i> ^[6] (2001)	Jacqz-Aigrain E. <i>et al.</i> ^[18] (1994)	Peeters, M.Y. <i>et al.</i> ^[2] (2006)	de Wildt SN <i>et al.</i> ^[16] (2003)	de Wildt SN <i>et al.</i> ^[17] (2000)	van Gerven J.M.A. <i>et al.</i> ^[19] (1997)
Patient Population	Preterm neonates	Preterm neonates with respiratory distress syndrome	Children after elective major craniofacial surgery	Pediatric intensive care patients	Oncology patients	Male adults
Indication for midazolam sedation	Sedation for invasive procedure in Intensive Care	Mechanical ventilation in Intensive Care	Postoperative sedation	Conscious sedation in Intensive Care	Sedation for invasive procedure	Healthy volunteers
Number of Patients	24	24	23	18	18	20
Postnatal Age median (range)	5 days (2.9-11)	0 days (0-1)	11.5 months (3.2 - 24.7)	38.5 months (0.03-203.5)	6.1 years (3.2 - 16.2)	24 years (20-31)
Gestational Age median (range)	28.3 weeks (26-33.6)	32 weeks (26-37)	-	-	-	-
Bodyweight median (range)	1.07 kg (0.77-1.6)	1.64 kg (0.96-3.7)	9.6 kg (5.1- 12)	14 kg (2.8-60)	22.5 kg (12.6- 60.1)	72.5 kg (64-89)
Mechanical ventilation N/ N _{TOTAL}	13 / 24	24 / 24	2 / 23	15 / 18	0 / 18	0 / 20
Midazolam Dose median (range)	0.1 mg/kg iv infusion in 30 minutes	60 µg/kg/hr iv infusion If GA < 33w → after t > 24hr 30 µg/kg/hr	0.1 mg/kg iv loading dose, 0.05- 0.2 mg/kg/hr infusion	0.1 mg/kg loading dose, 0.05-0.4 mg/kg/hr infusion	0.1 (0.03-0.53) mg/kg iv bolus dose	0.1 mg/kg iv infusion in 20 minutes
Number of Samples	155	63	198	233	82	336

Model development and covariate analysis

The pharmacokinetics of midazolam was best described with a two-compartment model with a proportional error model. In the model, the lower midazolam clearance due to critical illness [13] was incorporated for the datasets with prematurely born neonates [6, 18] and the dataset obtained in children admitted to an intensive care [16]. Introducing inter-individual variance for CL, Q, V₁, and V₂, significantly improved model performance with a total decrease in objective function (-2LL) of 3276.7 (p < 0.001).

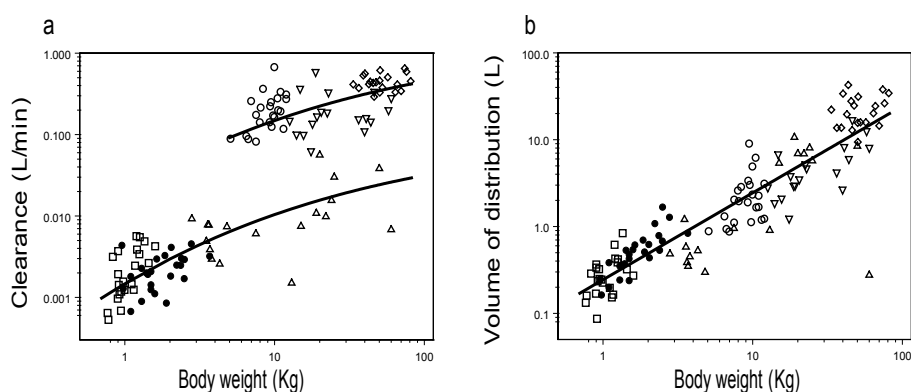


Figure 1 Posthoc estimates of CYP3A4 mediated clearance of midazolam *versus* body weight (a), and central volume of distribution *versus* body weight (b) in the final model. □ = preterm neonates, ● = preterm neonates with RDS syndrome, ○ = children after elective major craniofacial surgery, △ = pediatric intensive care patients, ▽ = pediatric oncology patients, and ◇ = male adults, with a black line as their post-hoc population predicted values, respectively.

In the covariate analysis, body weight was the most significant covariate for CL resulting in a decrease in objective function of 149.7 points ($p < 0.001$) and explained 65.4% of the inter-individual variability for CL (a decrease from 202.58% to 70.13%). The relation between bodyweight and CL was best described using an allometric function with an exponent that varies with bodyweight (body weight dependent exponent (BDE) [23]), as shown in figure 1 (*left*). The BDE was defined as $\text{Coeff} \times \text{BW}^{\text{exp1}}$, in which BW represents body weight, Coeff proved 0.81 (CV of 8.2%) and $\text{exp1} = 0.135$ (CV of 31.4%) (table 2), resulting in a BDE which decreased from 0.84 in a preterm neonate of 0.77kg to 0.44 in adult of 89 kg in an exponential manner. The covariate analysis also revealed that bodyweight was of significant influence on V_1 (delta -2LL of -52.8, $p < 0.001$). The nature of this influence was best described using a linear function (figure 1, *right*), because the exponent was found to not differ significantly from 1 when using an allometric function. For V_2 , in the postoperative craniofacial surgery patients [3] a 6-fold higher peripheral volume of distribution compared to the five other datasets was found. A stable model could only be achieved when estimating this peripheral volume of distribution for the craniofacial surgery patients simultaneously with the addition of bodyweight in an allometric function to V_2 . This resulted in a significantly improved model (-2LL of -184.6, $p < 0.001$). A separate addition resulted in model termination, with a significant decrease in objective function for each covariate. For Q, there was no significant influence of age, bodyweight or population. None of the other tested covariates were of influence on any of the pharmacokinetic parameters.

The pharmacokinetic parameters of the final model along with their confidence intervals, inter-individual variability, and the results of the bootstrap validation are shown in table 2. This table also shows that after the covariate analysis, the inter-individual variance for Q could be fixed to 0 in the backwards deletion step without deterioration of the model while a successful covariance step could be achieved.

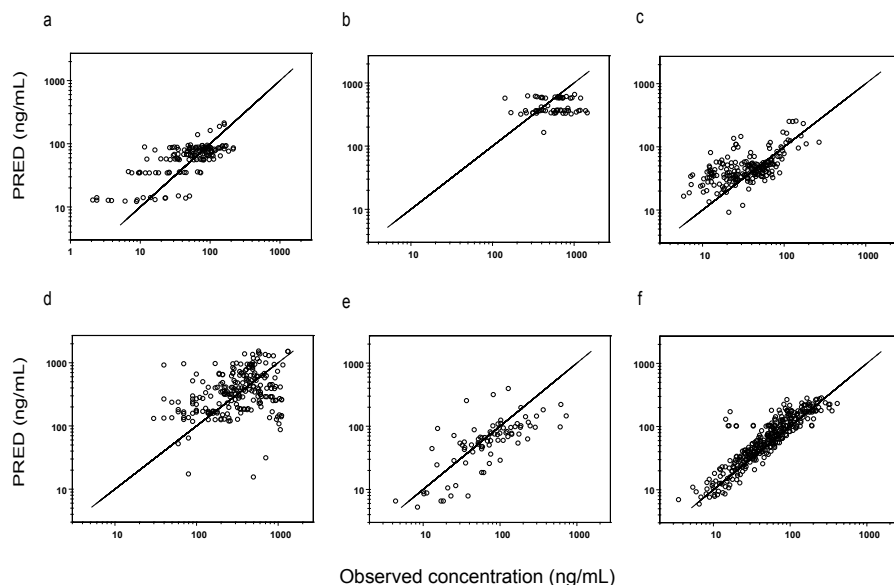


Figure 2 Visual diagnostics of the final model per dataset. Population predicted concentrations by the model versus the observed concentrations of midazolam. (a) de Wildt SN *et al.* [6]: preterm neonates; (b) Jacqz-Aigrain E. *et al.* [18]: preterm neonates with respiratory distress syndrome; (c) Peeters, M.Y. *et al.* [3]: children after elective major craniofacial surgery; (d) de Wildt SN *et al.* [16]: pediatric intensive care patients; (e) de Wildt SN *et al.* [17]: oncology patients; and (f) van Gerven J.M.A. *et al.* [19]: Male adults. PRED predicted concentration. The solid line indicates the line of unity.

Figure 2a-f illustrates the population predicted concentrations *versus* observed concentrations for each dataset separately. These diagnostic plots (in log-scale) indicate that overall the concentrations are predicted by the developed pharmacokinetic model without bias, as the concentrations are evenly distributed around the line of unity, except for the lower concentrations from the craniofacial surgery patients (Figure 2c), for which a slight under-prediction was observed.

Validation of the final model using NPDE simulations (figure 3) illustrate the distribution of the NPDEs, with a mean close to zero (-0.04 for the mean, and 0.98 for the variances, respectively), and with no observed trends in time or concentration. In the final model, all values of η -shrinkage were below 20% (CL: 5.1%; V_1 : 17.7%; V_2 14.3%), which indicates that the individual parameter estimates are reliable. [21] The calculated condition number of 195 of the final model was well below the critical value for the indication of ill-conditioning of 1000. [22]

Table 2 Population parameter estimates obtained for the final midazolam pharmacokinetic model

Parameter	Model fit		Bootstrap results		Explanation
	Value	(CV%)	Value	(CV%)	
$CL_i = f \cdot CL_{13kg} \cdot (BW/13)^{BDE}$ $BDE = Coeff \cdot BW^{exp1}$					CYP3A4/5 mediated clearance of midazolam BW dependent exponent of allometric exponent function on CL
CL_{13kg} (L/min)	0.17	(8.4)	0.17	(8.2)	CL for a median individual of the population with a BW of 13 kg
Coeff	0.81	(8.2)	0.81	(8.0)	Coefficient of the BDE function
exp1	- 0.135	(31.4)	- 0.138	(31.6)	Exponent of the BDE function
Q (L/min)	0.69	(18.1)	0.71	(20.4)	Inter-compartmental clearance between central and peripheral compartment
$V_{ti} = V_{1,13kg} \cdot (BW/13)$ $V_{1,13kg}$ (L)	2.7	(18.3)	2.7	(18.4)	Distribution volume of central compartment for a median individual of the population with a BW of 13 kg
$V_{2i} = g \cdot V_{2,13kg} \cdot (BW/13)^{exp2}$ $V_{2,13kg}$ (L)	6.38	(5.9)	6.38	(6.2)	Distribution volume of peripheral compartment (V_2) V_2 for a median individual of the population with a BW of 13 kg
exp2	0.76	(7.6)	0.76	(6.1)	Allometric exponent of V_2 in: $V_{2i} \cdot V_2 \cdot (BW/13)^{exp2}$
g	6.0	(18)	6.2	(19.4)	Multiple of V_2 for children after elective major craniofacial surgery ^[28] ; g=6 children after elective major craniofacial surgery, g=1 all other patients
ω^2_{CL}	0.37	(17.6)	0.35	(16.8)	Variance in CL
$\omega^2_{V_1}$	0.58	(32.4)	0.38	(46.3)	Variance in V_1
$\omega^2_{V_2}$	0.37	(37.5)	0.36	(37.7)	Variance in V_2
$\omega^2_{(V_2 / V_1)}$	0.41	(41.3)	0.56	(41.4)	Correlation between the Variance in V_2 and V_1
$\sigma^2_{proportional}$	0.12	(14.5)	0.12	(11.7)	Residual error (proportional error)

f: fraction to estimate CL in intensive care patients compared to CL (f=1 healthy patients, f=0.07 intensive care patients), **BW** : body weight, **BDE** : bodyweight dependent exponent. Coeff : coefficient of the BDE function, g : multiple of distribution volume of peripheral compartment for children after elective major craniofacial surgery, CL_{13kg} : midazolam clearance for a median individual of the population with a body weight of 13 kg, $V_{1,13kg}$: distribution volume of central compartment for a median individual of the population with a body weight of 13 kg, $V_{2,13kg}$: distribution volume of peripheral compartment for a median individual of the population with a body weight of 13 kg. [11]

Simulations to derive a model based dosing regimen

Simulations have been performed using the typical value of the parameters with the aim to show the exact influence of the covariates on the overall concentration-time relationships for different typical individuals. Figure 4 (left panels) illustrates that based on the traditional dosing scheme for conscious sedation in prematurely born neonates and critically ill children large differences in midazolam concentrations between individuals can be anticipated, particularly in the pediatric subpopulation. This results from the fact that the traditional dosing regimen is expressed in mg/kg/h while the results of this study show that clearance relates nonlinearly to body weight. Upon a model based dosing regimen aiming for predefined midazolam concentration ranges over a 24 hour period (table 3) similar midazolam profiles are anticipated across the human life span (Figure 4, right panels). This model-based dosing regimen that resulted from the simulations consists for preterm

neonates of 0.05 mg/kg bolus and 0.06mg/kg^{0.85}/h for 7 hours followed by 0.03mg/kg^{0.85}/h for 17 hours (table 3). For children in the Intensive Care (>5 kg) requiring conscious sedation, the model based dosing regimen consists of 0.1 mg/kg bolus and 0.1 mg/kg/h for 3 hours followed by 0.05 mg/kg^{0.65}/h for 21 hours (table 3). In table 3, both traditional and model-based guidelines are presented for conscious sedation with midazolam in preterm neonates and children in the Intensive Care ranging in bodyweight between 0.75 and 60 kg.

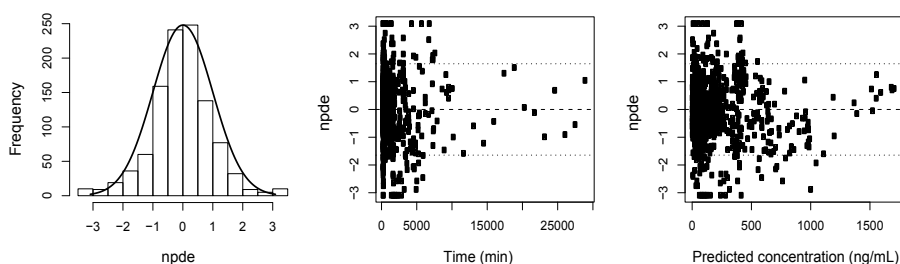


Figure 2 Internal validation, Normalised Prediction Distribution Errors (NPDE) of the final model. The histogram illustrates the distribution of the NPDE's for midazolam in which the solid line represents a normal distribution. In addition, the distribution of NPDE's in time after first dose and against the observed concentrations is illustrated.

II.4. Discussion

We have developed a novel maturation function for midazolam clearance based on a dataset consisting of data obtained in six different clinical studies performed with midazolam in (prematurely born) neonates, infants, toddlers, children, adolescents, and adults. This model provides a quantitative insight in the developmental pattern of *in vivo* CYP3A activity across the pediatric age range, including prematurely born neonates. This model may provide guidance to dosing of midazolam in children across all age ranges and potentially other CYP3A substrates, i.e. by applying this function that describes the increase in midazolam clearance over weight, in population pharmacokinetic and physiologically based models, as *a priori* information.

Table 3 Traditional and model-based dosing schemes for conscious sedation with midazolam in preterm neonates and children in the intensive care

Patient group	Weight kg	Infusion duration over 24 hrs	Traditional dose ^{\$}		Model-based dose ^{\$}	
			mg/hr	mg/kg/hr	mg/hr	mg/kg/hr
Preterm Neonates	0.75	0 – 7 hrs	0.045	0.06	0.047	0.063
		7 – 24 hrs	0.045	0.06	0.023	0.031
	1	0 – 7 hrs	0.06	0.06	0.06	0.06
		7 – 24 hrs	0.06	0.06	0.03	0.03
	2	0 – 7 hrs	0.12	0.06	0.11	0.054
		7 – 24 hrs	0.12	0.06	0.054	0.027
	3	0 – 7 hrs	0.18	0.06	0.15	0.051
		7 – 24 hrs	0.18	0.06	0.076	0.025
Children	5	0 – 3 hrs	0.5	0.1	0.5	0.1
		3 – 24 hrs	0.5	0.1	0.14	0.028
	10	0 – 3 hrs	1	0.1	1	0.1
		3 – 24 hrs	1	0.1	0.22	0.022
	20	0 – 3 hrs	2	0.1	2	0.1
		3 – 24 hrs	2	0.1	0.35	0.018
	45	0 – 3 hrs	4.5	0.1	4.5	0.1
		3 – 24 hrs	4.5	0.1	0.59	0.013
	60	0 – 3 hrs	6	0.1	6	0.1
		3 – 24 hrs	6	0.1	0.72	0.012

*Model-based doses aim for reported therapeutic midazolam concentrations of 0.25–0.37 µg/mL [32] for children and 0.2 µg/mL [29–31] for preterm neonates, and are 0.06mg/kg^{0.85}/h for 7 hours followed by 0.03mg/kg^{0.85}/h for 17 hours for preterm neonates and 0.1 mg/kg/h for 3 hours and 0.05 mg/kg^{0.65}/h for 21 hours for intensive care children. \$ Depicted doses are maintenance doses after a bolus dose of 0.05 mg/kg for preterm neonates and 0.1 mg/kg for intensive care children.

Previously, we have shown in children between 1 month and 17 years of age that critical illness was a more important covariate for CYP3A mediated clearance of midazolam than bodyweight. [11] As CYP3A is known to mature particularly in the first days and weeks of life, we included in the current analysis midazolam clearance values spanning the age range from prematurely born neonates to adults. This allowed to describe the change in CYP3A activity from birth onwards to adults. The current analysis suggests that the major change in midazolam clearance occurs in the first year of age. (figure 1). More specifically, in prematurely born neonates between 0.5 and 4 kg, midazolam clearance was estimated to be only 2.6% to 21.8% of adult values. These results confirm a very low CYP3A4/5 activity following birth [14] as midazolam is only slightly metabolized by CYP3A7. [33] Our finding that the largest change in clearance occurs in the first weeks of life is in line with CYP3A4/5 *in vitro* and *in vivo* phenotyping data. CYP3A4 mRNA levels in human fetal liver microsomes (gestational age 11–30 weeks) were reported to be on average 10% of that in

adults. [14, 34] At the age of one to three months, Lacroix *et al.* reported an increase in CYP3A4 activity *in vitro* reaching 30-40% of adult levels [14], which approximates our *in vivo* findings of 25-35% of adult activity.

Data on CYP3A4 activity after the first year of life, as derived from *in vitro* and different *in vivo* studies are highly discrepant [35-38]. The *in vitro* finding by Stevens *et al.* reporting CYP3A4 protein content at age 5–15 years to be only about 20% of adult levels [15] is not in agreement with the *in vivo* data on total clearance of midazolam. Based on reported average midazolam clearance data from the literature, Johnson *et al.* summarized that CYP3A4/5 mediated midazolam clearance expressed in L/hr reaches approximately 18% and 40% of the clearance in adults at the age of 1 year, [39] and 5 years, [39] respectively. Interestingly, Anderson *et al.* using average *in vivo* clearance values from literature data estimated midazolam clearance at birth in a term neonate to be 14% of an adult, reaching 64% of that in adults at one year of age and 90% at two years of age. [40] Additionally, using the urinary ratio of dextromethorphan and its metabolite 3-hydroxymorphan, Johnson *et al.* simulated an increase in CYP3A4 activity that reaches 72% of that in adults at the age of 1 year based on a phenotyping study by Blake *et al.* [37] An explanation for the large variation reported in CYP3A activity in *in vivo* and *in vitro* studies may relate to the sub-optimal quality of post-mortem tissue used for the *in vitro* studies, resulting in erroneously low CYP3A activity levels. In addition, the studies by Johnson *et al.* [39] and Anderson *et al.* [40] both used average clearance values instead of individual doses and concentrations to model the maturation of midazolam clearance. Furthermore, the number of underlying individual data of children between 0 and 1 year of age in these pooled studies was very low. which could have resulted in erroneous estimations for this age range [40] For our analysis we had access to all raw data on demographics, doses, concentrations, and covariates of 136 individuals varying in age between prematurely born neonates and adults. We therefore suggest that our analysis was less prone to the mentioned drawbacks, and increases the validity of the value of the estimated maturation function for *in vivo* CYP3A4/5 activity. However, as the estimated CYP3A4/5 abundance by Johnson *et al.*, and Anderson *et al.* [39, 40] was related to age, and not to body weight, the exact difference with our model based on body weight, is difficult to derive, and should be kept in mind when comparing the results.

The maturation of CYP3A as assessed by the clearance of midazolam was found to be highly nonlinear (figure 1), and could be described best by an allometric function with a body weight dependent exponent (BDE). This conforms with the report of Wang *et al.* who first reported that an exponent changing with bodyweight that is accounted for with an allometric function, best described the changes in propofol clearance occurring between neonatal to adult age. [23] In their model, the allometric exponent changed in a sigmoidal manner ($E_{\max}$ model) with bodyweight, and had four parameters to be estimated.[23] For propofol clearance, this exponent was found to change from 1.43 for a hypothetical bodyweight of 0 kg, to 0.55 for 10 kg upwards.^[23] In our model, we used a simplified version of the model of Wang *et al.* [23] by allowing the exponent to change in an exponential manner, thereby reducing the degrees of freedom from four to two, which was previously applied for busulphan in children. [24] This approach allowed us to adequately estimate all pharmacokinetic parameters without overparameterization of the model, and to describe the midazolam clearance from prematurely born neonates to adults without bias over the entire range of bodyweight. This contrasts to an allometric function with an estimated exponent or a fixed exponent of 0.75, as this resulted in failure to minimize successfully or in inadequate description of clearance throughout the entire weight range of the population, without adding more age related information in the model. Using those models, especially in the lowest weight range, midazolam clearances were overestimated. While the addition of an age-based function to capture maturation in the youngest age ranges correcting for this overestimation could be considered, we decided to use a bodyweight-dependent exponent model instead. With the BDE model, the use of two different functions of covariates that are correlated (i.e. age and bodyweight) which may potentially lead to inappropriate functions, is prevented. [41] Interestingly, using the BDE model in which the exponent was found to vary between 0.84 in neonates to 0.44 in adults, midazolam clearance, was well estimated across the entire age range. The fact that maturation of CYP3A activity appears less steep than reported for propofol (highest exponent of 0.91 versus 1.44, respectively) may reflect differences in pathways involved in metabolism of midazolam and propofol, being CYP3A oxidation and glucuronidation, respectively. [23] As such it seems that pathway-specific functions need to be developed across the age range for different common metabolic and elimination pathways that can be used to

predict changes in drug metabolism and elimination in children. Particularly, in view of the growing interest in pediatric PBPK models, [42] it is important to quantify the maturational *in vivo* behavior of all metabolic enzyme systems that are clinically relevant.

In this analysis, the results of the current function for CYP3A mediated clearance of midazolam have been used to derive a model based dosing scheme for conscious sedation in prematurely born neonates and children in the Intensive Care. Table 3 shows that instead of empiric dosing in mg/kg/h, it is proposed to dose the maintenance dose in a nonlinear manner. In accordance with the exponent that was found for changes in clearance within the pediatric range, an exponent of 0.85 is proposed for prematurely born neonates while an exponent of 0.65 is used for older children. In order to prevent dosing errors, a table is provided to guide dosing according to these non-linear functions. Figure 4 shows that upon this nonlinear dosing scheme, similar concentrations can be expected across a wide range in bodyweight, while in mg/kg/h dosing schedule large variations may be expected due to the nonlinear nature of clearance across bodyweight. The target midazolam concentration chosen from literature [18, 32] may, however, be arbitrary, as it is conceivable that some children may require higher concentrations than others. Therefore the dosing table (table 3) is only a guide for dose adjustments between children, while the absolute value (e.g. 0.06 in $0.06 \text{ mg/kg}^{0.85}/\text{hr}$ for prematurely born neonates) may be adjusted at the discretion of the attending physician. A previous study of our group has shown that it is indeed feasible to apply a nonlinear dosing scheme in the context of a clinical trial. [43] In that study, morphine was dosed according to a function with an exponent of 1.44, implying that neonates require less and older children require more than what is currently administered based on mg/kg/h dosing schedule.

No dosing implications have been derived on the basis of the results of this study for non-critically ill patients. We have reported previously that absolute midazolam clearance in critically ill patients is much lower (figure 1 left) compared to relatively healthy children [11]. We hypothesized this phenomenon to be due to critical illness related inflammation response causing CYP3A4 gene repression. [44] Due to the absence of midazolam data in non-critically ill neonates it was not possible to quantify the maturational behavior of CYP3A

in healthy neonates, using midazolam clearance as surrogate. However, as healthy neonates and children most often receive bolus doses of midazolam for a diagnostic or therapeutic intervention, changes in volume of distribution as a result of age may be more important than changes in clearance for this age group. While volume of distribution was found to scale linearly with bodyweight, there are no clear reasons to change the current practice of dosing a bolus in mg/kg across the pediatric age range when midazolam is given for procedural sedation. For V2, a 6-fold higher peripheral volume of distribution was found in the postoperative craniofacial surgery patients [3] compared to the five other datasets. As blood loss during craniofacial surgery may increase up to average values of 24% of the total volume of blood, [45] blood transfusion is often required to maintain the blood cell-, thrombocyte-, and plasma levels at normal values. This may have eventually have led to an increase of peripheral volume of distribution of midazolam in these patients, compared to the other patients.

As we described in this study, the maturation of hepatic CYP3A activity using midazolam as an *in vivo* probe, the impact of ontogeny on intestinal CYP3A activity is still unknown. It has become clear over the years that CYP3A activity in the intestine is of considerable importance, thereby necessity the investigation of the maturation of CYP3A mediated first pass-elimination of midazolam in children across different ages. Another limitation is that external validation of a pharmacokinetic model using independent data, and ultimately prospective clinical evaluation, which are important steps following internal validation of model still needs to be performed. [46] An important issue in the validation process of pediatric modeling, is the lack of sufficient data to perform the external validation, and the ethical constraints for performing prospective clinical evaluation of a drug in children. However, although only 17% of published pediatric pharmacokinetic and pharmacodynamic models are validated, [47] we believe that all models should be at least fully internally validated. [46]

In addition, extrapolation of the maturation model for the CYP3A substrate midazolam, will test the ability of the model to predict the system specific properties of other drugs that share, at least partly, the similar pharmacokinetic pathway. [48] Another CYP3A substrate from the literature that has been

studied quite adequately during the first months of life is cisapride. [49] Using the maturation model for midazolam to describe cisapride pharmacokinetics in the youngest infants would be another step in the validation process of the semi-physiological pharmacokinetic model.

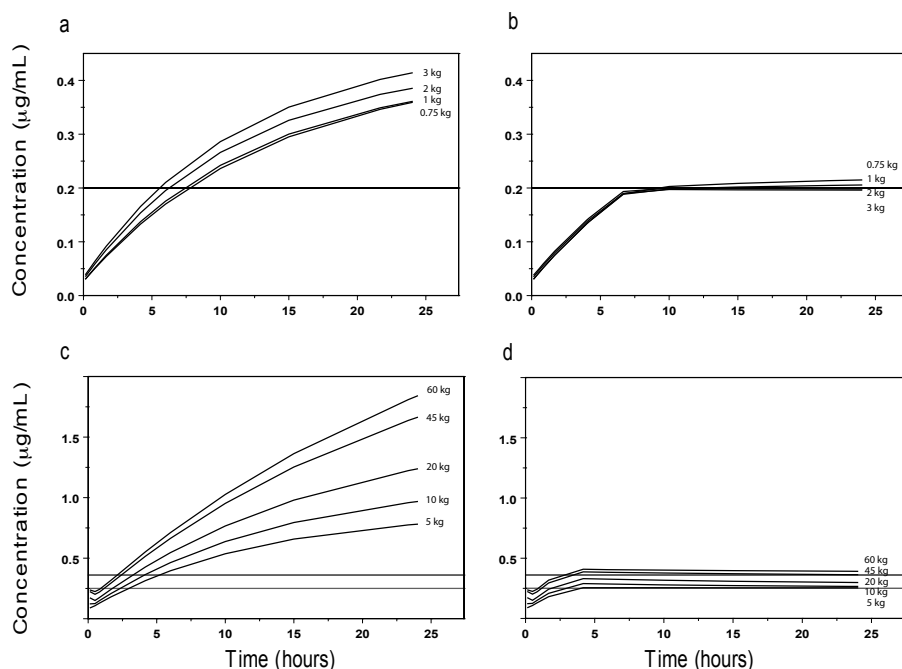


Figure 4 Simulated midazolam concentrations versus time in preterm neonates (a, b) and children (c, d) in intensive care with traditional dosing regimen (a, c) and model-based dosing regimen (b, d) aiming for reported therapeutic midazolam concentrations (horizontal grey lines). (a, b): midazolam concentrations in preterm neonates (0.75, 1, 2, 3 kg body weight) after traditional dosing regimen [bolus dose of 0.05 mg/kg followed by 0.06 mg/kg/h for 24 hours (a)], and after model-based dosing regimen as shown in table 3 (b). (c, d): midazolam concentrations in critically ill children (5, 10, 20, 45, and 60 kg body weight) after traditional dosing regimen [0.1 mg/kg bolus dose followed by 0.1 mg/kg/h for 24 hours (c)], and after model-based dosing regimen as shown in table 3 (d).

II.5. Conclusion

In conclusion, an *in vivo* maturation function for CYP3A mediated clearance of midazolam from prematurely born neonates to adults has been developed. This maturation function can be used to derive evidence-based doses for prematurely born neonates, infants and children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic or physiologically based pharmacokinetic (PBPK) models.

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NaiNai: I miss you every day!

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Wang C, Peeters MY, Allegaert K, Blusse van Oud-Alblas HJ, Krekels EH, Tibboel D, Danhof M, Knibbe CA. A bodyweight-dependent allometric exponent for scaling clearance across the human life-span. *Pharm Res* 2012; 29: 1570-81. doi: 10.1007/s11095-012-0668-x.

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Curriculum Vitae

Chenguang Wang was born on 17 August 1980 in Beijing. In 1998, he graduated from Beijing 101 middle school and started his study in the Department of Mathematics in Beijing University of Technology. He got his Bachelor of Applied Science degree in 2002 and Bachelor of Business Administration degree in 2004. In 2005, he joined the Clinical Pharmacology Research Center in Peking Union Medical College Hospital as a biostatistician. In 2008, he began his Ph.D. study in the Division of Pharmacology of the Leiden Academic Centre for Drug Research at Leiden University in the Netherlands under the supervision of Prof. Catherijne Knibbe, Prof. Meindert Danhof and Prof. Dick Tibboel focusing on PK/PD modeling in paediatric population. In the spring of 2013, he joined in the Clinical Pharmacometrics group in Bayer HealthCare in Wuppertal, Germany.

