Size does matter : drug glucuronidation in children

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Elke H.J. Krekels

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Elke H.J. Krekels
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Table of Contents

For everything you missed, you have gained something else.
And for everything you gain, you loose something else.
- Ralph Waldo Emerson

Section I

Background and Introduction

Chapter 1

Scope and Intent of Investigation

1.1 Background and Introduction.

There are many sources of variability in drug response that are unique to the paediatric population. Firstly, there are marked increases in body size as well as significant changes in the expression and function of drug metabolizing enzymes and transporters. Additionally, differences in cardiac output and blood flow influence the perfusion of drug eliminating organs, and differences in the acid-base balance, the concentration and composition of drug-binding plasma proteins and other blood components potentially influence plasma protein binding. Moreover, the relative size of organs vary, as well as body composition, with the amount of total body water and extracellular water decreasing with age. All these factors can alter drug exposure in paediatric patients [1]. Furthermore, developmental changes in drug pharmacodynamics influence the variability in paediatric drug response. Changes in the function and expression of receptors and target proteins can alter the pharmacological response to drug exposure, while disease states may also affect the physiological system and physiological feedback mechanisms, making diseases that are unique to the paediatric population or diseases with a different progression in children compared to adults, unique contributors to the variability in paediatric drug response [2].

Unfortunately, the sources of variability in drug exposure and response in children have not been studied in as much detail as they have been in adults. As a result, evidence-based drug dosing algorithms that account for functional differences between children and adults, as well as for functional differences between children of different ages are often lacking. Between 1995 and 2005, off-label and unlicensed paediatric drug prescription was high around the world with the number of paediatric patients receiving at least one off-label or unlicensed drug ranging between 80% to 93% in the neonatal intensive care units, between 36% to 100% in paediatric wards, and between 3.3% to 56% in non-hospital settings [3], without any apparent difference between university hospitals and general hospitals in hospital setting [4,5]. At the same time, out of all the new drugs licensed by the European Medicines Evaluation Agency (EMEA) only 33% was licensed for use in children, 23% for infants and 9% for neonates and while attention to drug licensing for the paediatric population was increased, an increasing trend in drug licensing for the full paediatric population could not be observed during this period [6,7].

When evidence-based dosing information is missing, paediatric drug doses are often empirically extrapolated from adult doses. Consensus-based paediatric drug dosing guidelines are sometimes formalized in paediatric formularies after years of clinical experience [8,9]. To improve paediatric drug dosing, it is essential to study the influence of developmental changes on drug pharmacokinetics and pharmacodynamics

in an integrated manner and to determine the relevance to and interaction with other factors like for instance diseases status, (concomitant) therapy, and genetics. Laws in the US and Europe like the Pediatric Rule (FDA – 1998), the Best Pharmaceuticals for Children Act (FDA – 2002), and the Paediatric Regulation (EMEA – 2007) have been introduced to encourage or compel pharmaceutical companies to perform paediatric studies for new chemical entities. However, to date laws that apply to marketed off-patent drugs are lacking.

The objective of the research described in this thesis was to develop a novel model-based approach to derive drug dosing algorithms for the paediatric population, which account for developmental changes in drug response in this population. Naturally, this approach should take into account changes in drug pharmacokinetics, including absorption, distribution, metabolism and elimination (ADME), as well as drug pharmacodynamics, including effect site distribution, target activation, and signal transduction. However, as a first step in the research on paediatric pharmacology, the studies in this thesis are limited to the developmental changes in drug pharmacokinetics. For many drugs, the developmental differences in drug clearance are thought to be the major cause of age-dependent differences in dose requirements [10]. Specific focus is on uridine 5′-diphosphate glucuronosyltransferase (UGT) 2B7-mediated drug glucuronidation in neonates and young children. The UGT enzyme family is responsible for the glucuronidation of various endogenous en exogenous compounds in humans, but has been studied less extensively than the cytochrome P450 enzyme family, which is involved in the oxidation of the majority of the currently marketed drugs [11]. Inappropriate dose adjustments of UGT substrates in the paediatric population may lead to therapeutic failure or to overdosing, which may cause serious side-effects and even fatalities [12]. In this chapter an outline is presented of the various investigations that are described in this thesis.

Chapter 2 provides an overview of the various *in vitro* and *in vivo* methodologies to study the onset and development, the so-called ontogeny, of hepatic enzyme systems. Special emphasis is on the results obtained with these methods for UGTs. Different endpoints representing different parts of the physiological system that can be studied to determine enzyme ontogeny were identified. Since the number of components interacting with each part of the physiological system increases in going from mRNA transcription, to enzyme expression, *in vitro* enzyme activity, and *in vivo* glucuronidation clearance, conclusions on hepatic enzyme ontogeny may differ when different endpoints are used to characterize the changes in the physiological system. Additionally, different techniques to obtain and/or analyze data from each part of the physiological system may further diversify the results. Based on literature results it could however be concluded that even

though the ontogeny profiles of the different UGT isoenzymes may vary significantly, the onset and development of UGT enzyme expression and activity generally occur after 20 weeks of gestation with a boost in expression and activity occurring in the first weeks of life. Since many other physiological changes also occur within this timeframe and since children are encountered most frequently in hospital settings during the first weeks to months of life, detailed information on clinical changes in drug pharmacokinetic is particularly relevant for the youngest patients. Therefore the focus of the research in the current thesis was on preterm and term neonates to children up to three years of age.

1.2 Paediatric Morphine Glucuronidation Model for Individualized Dosing

An *in vivo* approach was used to study clinically relevant differences in drug disposition and exposure in early childhood. Traditional compartmental and non-compartmental *in vivo* approaches either require dense concentration-time information from each individual, obtained according to a stringent study design to ensure similar drug dosing and blood sampling in each individual, or they rely on imprecise measurements of steady state concentrations. A further drawback of traditional approaches is that they do not allow for the identification of the sources of variability within a population. Therefore 'population modeling', also known as non-linear mixed effect modelling, was the preferred tool for the studies in the current thesis. Population modeling not only allows for the analysis of dense, sparse, and/or unbalanced data, and for the identification and quantification of the sources of variability in a population, it also allows for the identification of significant predictors of this variability, known as covariates [13]. The information obtained on these covariates can subsequently be used as the basis for evidence-based dosing algorithms. A population modeling approach was applied in **Section II** to describe and quantify developmental changes in the glucuronidation of the selective UGT2B7 probe morphine [14,15] in preterm and term neonates, and children up to three years of age. The resulting model, including the model covariates, was used to develop a dosing algorithm for this population.

In **Chapter 3**, sparse and unbalanced concentration-time data on morphine and its two major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), that were obtained during routine clinical practice from 248 term and preterm neonates to children younger than three years, were analyzed. In a comprehensive covariate analysis, all potential covariates were tested for significance and included in the model when they were sufficiently predictive of variability in morphine disposition to significantly improve the model fit according to predefined criteria. This yielded a

population model that described the developmental changes in morphine clearance and distribution in this young population with a bodyweight-based function. To ascertain that the model indeed described the data without bias, the final model was validated internally using advanced methods like a normalized prediction distribution error (NPDE) analysis, after which simulations were performed to obtain an optimized, individualized morphine dosing algorithm that yields similar steady state concentrations for morphine, M3G and M6G throughout the population of preterm and term neonates to three-year-old children.

Before applying the model-derived morphine dosing algorithm obtained in Chapter 3 in clinical practice, the predictive performance of the paediatric population pharmacokinetic model for morphine and its metabolites was corroborated using six external datasets in **Chapter 4**. This ascertained that the model could not only accurately predict morphine and metabolite concentrations in independent datasets of postoperative and ventilated patients with similar characteristics as the patients in the internal dataset used for model building in Chapter 3, but also in datasets of patients on very invasive extracorporeal membrane oxygenation (ECMO) treatment. The results from this study justified the next step in developing an evidence-based paediatric dosing algorithm for morphine, namely the prospective evaluation of the algorithm in a clinical trial [16].

Chapter 5 describes the results obtained with the prospective validation of the novel paediatric dosing algorithm of morphine. In a randomized controlled trial that compared postoperative analgesic efficacy of morphine and paracetamol in patients under the age of 1 year, the patients in the morphine arm were dosed according to the optimized and individualized paediatric dosing algorithm obtained with the population pharmacokinetic model developed and validated in Chapter 3 and Chapter 4. According to this highly non-linear dosing regimen, neonates younger than ten days received morphine maintenance doses that were between 25% and 50% of the traditional, linear, and consensus-based morphine regimen, whereas older children received up to about 150% of this traditional dose. In this proof-of-principle study, it was assessed whether the proposed morphine doses based on the pharmacokinetic differences quantified in the population model, sufficed to obtain a satisfactory clinical response throughout the population, or whether further dose adjustments based on age-related differences in pharmacodynamics were necessary. The clinical response to morphine was assessed by analyzing the nurse-controlled morphine rescue medication that was administered based on a standardized pain-protocol using validated, age-appropriate COMFORT-behaviour [17] and VAS [18] scores to assess pain. Morphine and metabolite plasma concentrations were measured to ascertain that the concentration predictions by the population model were still accurate in patients dosed according to the novel algorithm, thereby confirming that the model-derived dosing algorithm indeed corrected for age-related differences in morphine pharmacokinetics.

1.3 Semi-Physiological Covariate Model for Paediatric Glucuronidation

It would require a tremendous amount of resources to develop and thoroughly validate paediatric population models for each individual drug in every population in a manner similar to what was described for morphine in Section II. Hence a novel approach is proposed in **Section III**, to limit the amount of resources and expedite the development of population pharmacokinetic models for the paediatric population. This approach is based on the hypothesis that paediatric covariate models for drug elimination describe system-specific properties rather than drug-specific properties [19] and can therefore be directly extrapolated from one drug to another drug that shares a common elimination pathway. The drug-specific parameter values in the population model of the new drug are on the other hand still estimated in a population analysis based on concentration-time data of this drug. As such, this approach can be considered a semi-physiological hybrid between population pharmacokinetic modeling, called top-down modeling, and physiologically-based pharmacokinetic modeling, called bottom-up modeling.

The new semi-physiological approach for the development of paediatric population models is introduced in **Chapter 6**. In this proof-of-concept study, the paediatric covariate model for morphine glucuronidation that was developed and validated in Section II, was directly extrapolated to the glucuronidation of zidovudine, a drug which is also predominantly eliminated through UGT2B7-mediated glucuronidation [15,20]. The descriptive and predictive performance of this semi-physiological model was found to be similar to the descriptive and predictive performance of a reference model that was developed using a comprehensive covariate analysis of the zidovudine data to provide the best description of these data based on statistical criteria.

In **Chapter 7** the physiological and physicochemical basis of the developed semi-physiological paediatric covariate model that quantifies the developmental changes in UGT2B7-mediated glucuronidation clearance for morphine and zidovudine in neonates and young children was investigated. The physiology-based modeling software Simcyp (Simcyp Ltd, Sheffield, UK) allowed for the determination, in strictly quantitative manner, of the influence of distinct system-specific and drug-specific parameters on the ontogeny pattern of the clearance of existing and hypothetical drugs in various populations, including the paediatric population. Using Simcyp the underlying maturational changes in liver volume, milligram microsomal protein per gram of liver, hepatic blood flow, plasma protein concentration, and ontogeny of UGT2B7 expression and function were disentangled and the main drivers of the net observed changes in UGT2B7-mediated glucuronidation in early childhood were identified. Additionally,

it was uncovered how physicochemical drug properties like the molecular mass, the octanol/water partition coefficient (logP), the acid dissociation constant (pKa), influence the magnitude and the ontogeny pattern of UGT2B7-mediated clearance in order to define specific drug properties that would preclude the direct application of the semi-physiological developmental model for paediatric drug glucuronidation.

1.4 Paediatric Model Evaluation

Population modeling allows for the analysis of sparse data, but when data are limited there are risks of drawing erroneous conclusions. This could have far-reaching consequences, especially when population models are used for simulation purposes, for instance to optimize clinical trials in drug development, to derive dosing algorithms for application in clinical practice as was illustrated in Section II, or to extrapolate paediatric covariate models between drugs as was illustrated in Section III of this thesis. Under these circumstances proper assessment of both the descriptive and predictive properties of a model is imperative. This is however often neglected both in the adult and the paediatric population [21,22]. **Section IV** of this thesis therefore focuses on the evaluation of paediatric population models.

Numerous tools for the evaluation of population models currently exist. However, these tools may not always directly suffice for the evaluation of paediatric population models, due to distinctive patient and study characteristics in this special population. The paediatric population is for instance relatively small and can be regarded as a population consisting of multiple subpopulations due to the heterogeneity in maturational status. Additionally, since data in this population are usually obtained during routine clinical practice the variability in dosing and sampling schemes is high, while limitations in sampling size and frequency may cause data to be sparse. A new framework was therefore developed in **Chapter 8** for the systematic assessment of the descriptive and predictive properties of paediatric covariate models. In this framework, existing numerical diagnostics, prediction-based diagnostics, and simulation-based diagnostics are placed into context and adjusted for application to paediatric population models where necessary. Additionally, a new tool to specifically evaluate paediatric covariate models is presented. As an illustration, this new framework was applied to two peer-reviewed, published, paediatric population models for morphine and its two major glucuronides that were based on an identical dataset, but developed with fundamentally different covariate analysis approaches.

In recent years, several studies on the maturation of morphine clearance, using a wide array of different data analysis approaches leading to different pharmacokinetic models, have been published. This raises important questions with regard to the model that best predicts morphine concentrations in the pediatric population. In **Chapter 9** advantages and disadvantages of different data analysis techniques to describe and quantify the *in vivo* maturation of morphine clearance in the paediatric population, like traditional methods, population pharmacokinetic modeling and physiologically-based pharmacokinetic modeling, are discussed. Subsequently, the accuracy of morphine clearance predictions by multiple published paediatric pharmacokinetic models that were based on a variety of datasets and modeling approaches were reviewed. This is important because the value of paediatric pharmacokinetic models mostly depends on clearance predictions and population concentration predictions. Special attention was paid to the accuracy of morphine concentration predictions across different age-groups and the level of evidence supporting each model either in the original publication or in succeeding publications.

1.5 Summary, Conclusion and Perspectives

Finally, in **Chapter 10** of **Section V**, the results of the studies presented in this thesis are discussed in conjunction with each other and perspectives of future research are presented.

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Chapter 2

Ontogeny of Hepatic Glucuronidation; Methods and Results

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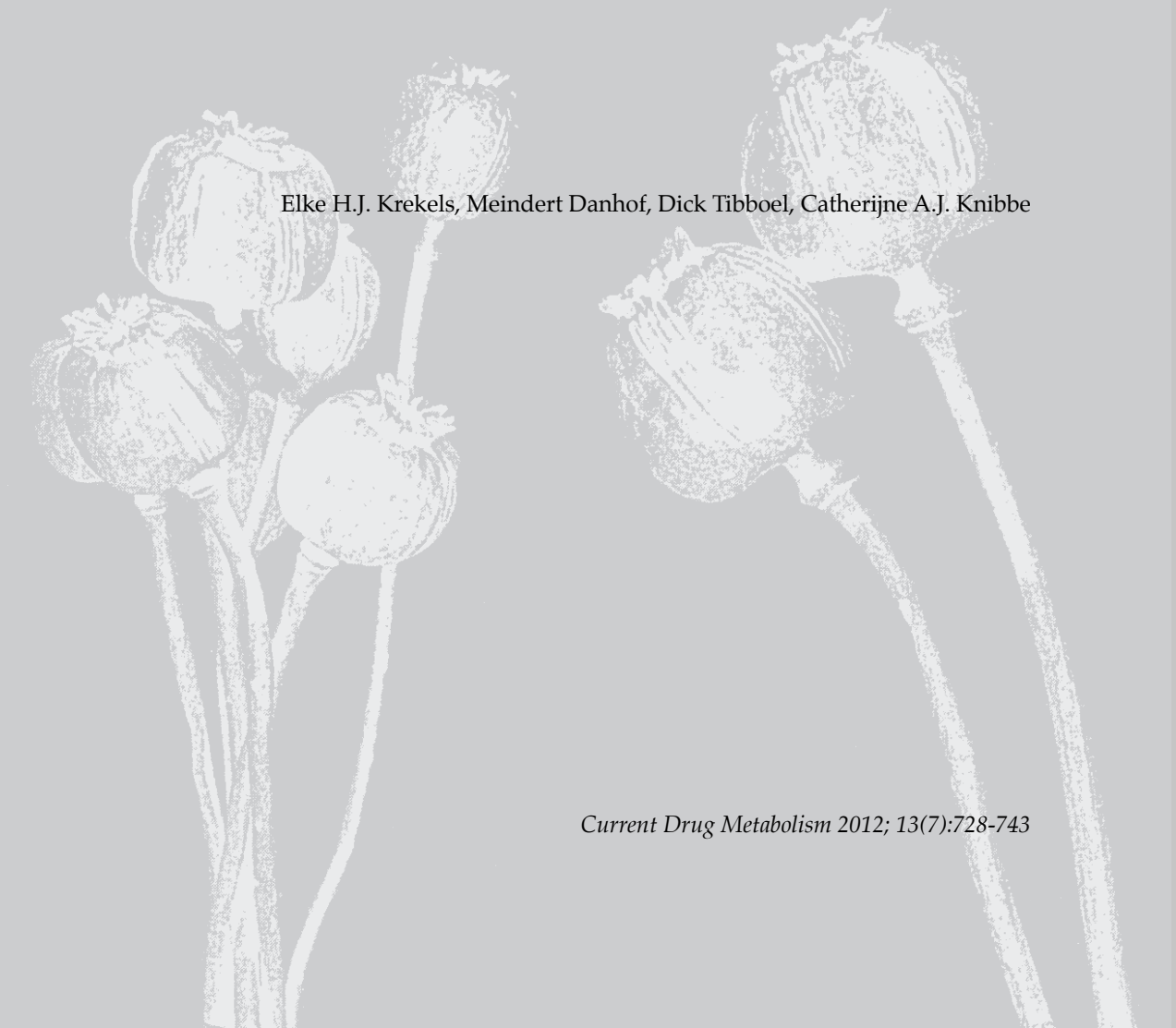

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Abstract

The onset and maturation, so-called ontogeny, of hepatic glucuronidation is important for the clearance of a number of drugs in children. The current review discusses methods for studying the ontogeny of liver enzyme systems and specifically focuses on the results obtained with these methods for uridine 5'-diphosphate glucuronosyltransferases (UGTs). The number of contributing components in the biological system increases in going from mRNA transcription, to enzyme expression, *in vitro* enzyme activity, and *in vivo* glucuronidation clearance. This may result in different conclusions on UGT ontogeny when different methods are used. Various metrics to quantify glucuronidation activity, like linear or allometric scaling based on bodyweight, further disperse the conclusions on UGT ontogeny. Generally, it can be concluded that the onset of UGT expression and activity occurs after 20 weeks of gestation with a boost in expression and activity occurring in the first weeks of life. Maturation rates vary between the UGTs, but may well extend beyond the age of two years. Compared to adults, absolute doses of drugs eliminated via glucuronidation should be reduced in children. However, since the UGT isoenzymes mature differently, since substrate specificities are overlapping and since many external factors influence drug glucuronidation, it is not possible to derive general dosing recommendations for the paediatric population for these drugs. This can be improved by obtaining system specific information on each UGT isoenzyme on the basis of validated *in vivo* models that describe the ontogeny of glucuronidation and the influence of other patient characteristics like genetic polymorphisms and co-morbidities on the (intrinsic) clearance of isoenzyme specific probe drugs.

2.1 Introduction

Hepatic metabolic plasma clearance is to various extents influenced by 1) intrinsic clearance, 2) hepatic blood flow and perfusion, 3) plasma protein binding, and 4) active hepatic influx and efflux mechanisms, and intra-cellular transport processes. Intrinsic clearance is the maximum capacity to eliminate drugs in the absence of rate-limiting factors. Intrinsic clearance through hepatic biotransformation is determined by enzyme expression and enzyme activity in the liver.

Numerous enzymes responsible for the biotransformation of endogenous and exogenous compounds are present in the liver. Phase I metabolism, which entails the oxidation, reduction or hydrolysis of compounds to make them (more) polar, is carried out by various enzyme systems. Of these, the cytochrome P450 monooxygenases (CYPs) are oxidizing enzymes that have been extensively studied, since they are responsible for the majority of drug metabolism. Phase II metabolism comprises various conjugation reactions, like UDP-glucuronic acid, sulfate, glutathione, methyl and acetyl conjugation. Conjugation promotes passive renal elimination by increasing the solubility of compounds as well as active excretion of compounds through renal tubular secretion. These conjugation reactions usually result in the biological deactivation of endogenous compounds, drugs or phase I metabolites, although some examples of pharmacological active phase II metabolites are known, like for instance the morphine glucuronides [1]. The uridine 5'-diphosphate glucuronosyltransferases (UGTs) are the most important phase II enzymes in humans and they are the focus of the current review.

In the human genome four UGT families with glucuronidation capacity have been identified, namely the UGT1, UGT2, UGT3, and UGT8 families. The members of the UGT1 and UGT2 family are predominantly involved in detoxification of compounds and in humans the UGT1A and UGT2B subfamily have been studied most extensively in this respect. Within these two subfamilies 16 functional isoforms have been identified (see for latest nomenclature update Mackenzie *et al.* [2]). The UGTs are high-capacity, low-affinity enzymes that are mainly expressed in the liver, however isoforms have also found to be expressed extrahepatically [3–6]. Together, the UGTs glucuronidate many endogenous and exogenous compounds. Substrate specificities of the UGTs are broad and they may overlap, indicating that one isoform may glucuronidate a wide range of compounds and that one compound may be metabolized by multiple isoforms. Table I gives an overview of some of the UGT substrates and substrate specificities.

Table I. *Overview of UGT substrates and substrate specificities.*

UGT isoform	substrate	UGT specific substrate	references
1A1	paracetamol, 1-naphthol, thyroxine, 4-methylumbelliferone, carvediol	bilirubin, SN-38 (irinotecan metabolite)	[7–12]
1A3	1-naphthol, 4-methylumbelliferone, thyroxine	R-lorazepam	[10,12,13]
1A4	valproic acid	trifluoperazine, lamotrigine, imipramine	[13–16]
1A5	So far no substrates have been identified for this isoenzyme.		
1A6	paracetamol, 1-naphthol, valproic acid, 4-methylumbelliferone, chloramphenicol	serotonin	[9,10,17–19]
1A7	1-naphthol, 4-methylumbelliferone		[10]
1A8	1-naphthol, 4-methylumbelliferone, valproic acid		[10,15]
1A9	paracetamol, 4-methylumbelliferone, 1-naphthol, indomethacin, valproic acid, propofol, R-oxazepam, chloramphenicol		[9,10,13,18–21]
1A10	1-naphthol, 4-methylumbelliferone, valproic acid		[10,15]
2B4	androsterone, carvediol		[11,22]
2B7	1-naphthol, 4-methylumbelliferone, valproic acid, indomethacin, testosterone, androsterone, estradiol, lorazepam, carbamazepine, R-oxazepam, epirubicin, carvediol, chloramphenicol	morphine, zidovudine	[10,11,15,19–29]
2B10	amitriptyline, imipramine, clomipramine, trimipramine		[30]
2B11	4-methylumbelliferone, 1-naphthol, 4-nitrophenol, 4-hydroxyesterone, 4-hydroxybiphenyl, methol, estriol, 2-aminophenol, 2-hydroxyesteriol.		[31]
2B15	4-methylumbelliferone, testosterone	S-oxazepam, S-lorazepam	[10,13,22,28,32]
2B17	4-methylumbelliferone, testosterone, dihydrotestosterone, androsterone		[10,22]
2B28	eugenol, 1-naphthol, testosterone, 4-methylumbelliferone		[33]

The UGT isoforms 1A2, 1A11, 1A12, 1A13, 2B24, 2B25, 2B26, 2B27, 2B29 are considered to be pseudogenes [2].

The ontogeny of the hepatic glucuronidation system, which in the current review refers to the onset and maturation of hepatic glucuronidation activity in the paediatric population, is considered to be an important determinant for the hepatic clearance of a number of drugs in children. Detailed description of the ontogeny of the hepatic glucuronidation system will therefore facilitate the accurate prescription of these drugs in the paediatric population. The current review discusses the age-related changes in drug glucuronidation clearance and in the underlying physiological processes of drug glucuronidation in the paediatric population. It is specified what part of the causal chain between gene expression and phenotypical hepatic clearance is studied with available techniques and various aspects that influence these techniques and the physiological processes on the causal chain of events are discussed to provide insight in factors that can cause discrepancies between findings obtained using different techniques.

2.2 Methods to Determine the Ontogeny of Enzyme Systems

The ontogeny of enzyme systems can be determined at different levels ranging from *in vitro* mRNA transcription to the intrinsic hepatic clearance of a model compound, also known as a probe, *in vivo*. Figure 1 gives an overview of the processes that are studied at the different levels. Going from left to right in the diagram, the complexity of and variability in the studied biological systems increase due to the increasing number of factors that contribute to the functionality of the enzymes, and therefore the ultimate contribution of each process to *in vivo* drug clearance decreases going from right to left.

Studying enzyme ontogeny at different levels enables a mechanistic interpretation of the clinically observed developmental changes in the functionality of drug metabolizing enzymes. By going backwards on the causal chain of events different characteristics of the biological system are studied and the number of factors that contribute to the functionality of metabolizing enzymes are reduced. By simplifying the system noise is reduced and physiological insight in the characteristics of the system is obtained. However, due to the influence of contributing factors further down the causal chain, the contribution of a particular process to the overall biological system may be negligible. These contributing factors may also result in different findings when studying ontogeny at different levels. By studying more complex systems only the net influence of all underlying physiological processes are taken into account and they might therefore represent clinical observations of the drug clearance process better. However, due to the high complexity, more data and advanced statistical tools are required to identify significant descriptors for the maturation of drug clearance capacity.

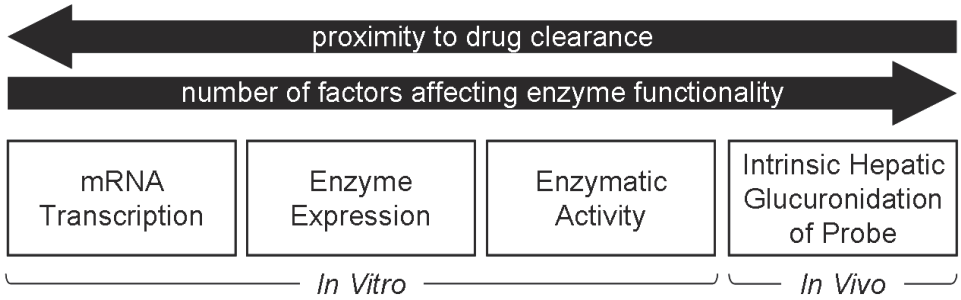

Figure 1. *Schematic representation of the processes that can be studied to determine the ontogeny of enzyme systems.*

2.2.1 *In Vitro* Methods

The *in vitro* methods to study enzyme ontogeny include the determination of RNA transcription, enzyme expression, and enzyme activity using human liver tissue obtained from academic or commercial liver banks. Metabolism of compounds takes place in hepatocytes, which are specialized cells that make up 70 to 80% of the liver's mass. When these cells are lysed in the laboratory, vesicles called microsomes are formed from the endoplasmic reticulum in which CYP and UGT enzymes are located. Liver homogenates, isolated hepatocytes, and microsomes can serve as experimental systems for *in vitro* studies of ontogeny of hepatic drug metabolism.

The origin of the liver tissue samples used in *in vitro* studies varies. In the past they were mostly obtained *post mortem* from liver donors that could not be matched to a recipient. Whereas currently paediatric liver tissue material is still predominantly obtained from deceased children, adult liver tissue material nowadays originates more often from tissue adjacent to removed (cancer) lesions [34]. Since only a couple of hundred milligrams of liver tissue are necessary for these *in vitro* techniques, multiple experiments can be performed with the samples of one individual. This not only allows for replication of studies or for the testing of different conditions in the same individual, it also allows for the study of various processes like RNA transcription, enzyme expression and enzyme activity within tissue of the same individual.

Prior to using the liver tissue samples in the *in vitro* studies, they are checked for morphological and histological anomalies and often other biological and serological tests are performed on donor material to prevent the use of diseased liver samples, although this is not always possible in the paediatric population due to the limited number and size of the samples. In addition to pathophysiological changes of the liver, numerous other factors may still cause high variability in the results obtained with these samples.

These factors include 1) demographics and characteristics of the donor, like for instance age, gender, ethnicity, and genetic polymorphisms, 2) life style of the donor, such as alcohol use, smoking habits, general drug use, and dietary preferences, 3) *perimortem* or procedure related drug use and disease or trauma status in the donor prior to obtaining the tissue sample, and 4) harvesting, storage, and experimental conditions. The first two represent factors that are present in the clinical situation as well and therefore give a reflection of the variability that can be expected within a population, the second two factors mainly add noise to the data. Pooling *in vitro* material from various donors allows for the description of average trends in enzyme ontogeny, this approach will however not allow for the quantification of variability in the population nor will it allow for identification of the sources of the variability. Information on variability is as important as information on general trends and can be obtained by performing experiments in multiple samples of different individuals to obtain a measurement range rather than a single value. This can also be used to identify patient characteristics (covariates) that influence the ontogeny process.

2.2.1.1 RNA Transcription

The beginning of every physiological process is the transcription of the DNA sequence that encodes the enzymes that catalyze a process, into messenger RNA (mRNA). Studies on the onset of UGT mRNA transcription and on age-related changes in mRNA transcript levels are therefore an obvious approach to determine UGT ontogeny. The UGT1A isoforms are formed by alternative splicing of an mRNA transcript originating from a single gene, whereas the UGT2B isoforms are all encoded by independent genes [2]. mRNA samples therefore contain distinct mRNA copies for each UGT isoform that can be uniquely identified. But it is important to keep in mind that UGT mRNA levels have been reported to be reduced under hepatic inflammation conditions [35].

There are various techniques to detect and quantify gene transcription of which Northern blotting and quantitative real time polymerase chain reaction (qrt-PCR) are most frequently used. Northern blotting is an older technique that separates the different mRNAs in an isolated hepatic mRNA mixture by length on an agarose gel. A radio-labeled RNA probe that is complementary to the mRNA sequence of the UGT of interest will hybridize with the mRNA transcript of the UGT and the band strength of the labeled RNA on the image of the gel is then measured by autoradiography and used as a quantitative measure for specific mRNA expression.

With qrt-PCR, complementary DNA (cDNA) templates are created from an isolated hepatic RNA sample by reverse transcription. The cDNA template of the UGT of interest is subsequently amplified in successive cycles of the polymerase chain reaction in the presence of probes that give a fluorescent signal when bound to formed DNA

strands. With the amount of DNA being doubled in every cycle, the cycle in which a certain threshold in fluorescence is reached can be used to quantify the amount of mRNA in the initial sample. The DNA amplification cycles allow qrt-PCR to detect much smaller mRNA amounts than Northern blotting.

PCR analysis has revealed that there are no mRNA transcripts of UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, and 2B15 present in fetal liver at 20 weeks of gestation. In livers of infants with a postnatal age of 6 months, transcripts of UGT1A1, 1A3, 1A4, 1A6, 2B7, 2B10 and 2B15 were detected at levels similar to adults. At 6 months of age detectable levels of transcripts of UGT1A9 and 2B4 were present, but at lower levels than in adults. These levels further increased with age, with UGT1A9 transcript levels reaching adult values around 1.5 years and UGT2B4 transcript levels not reaching adult levels before the age of 2 years [36].

2.2.1.2 Enzyme Expression

mRNA transcripts are translated into amino acid chains that subsequently fold into active enzymes. However, not necessarily all mRNA transcripts are translated into enzymes, as some mRNA may be intracellularly degraded before being translated. Additionally, degradation of formed enzyme also influences the net amount of enzymes available for catalyzing cellular processes. By measuring the ontogeny of UGT enzyme expression one is therefore one step closer to the ontogeny of *in vivo* drug glucuronidation on the causal chain of events.

Techniques to detect and quantify enzyme expression, like Western blotting (also known as protein immunoblotting) and enzyme-linked immunosorbent essay (ELISA), involve the use of antibodies against the specific enzyme of interest in an isolated enzyme sample from the liver sample. Antibodies usually have very specific targets to which they bind, allowing for the detection of specific enzymes. Quantification occurs by using a label (e.g. radioactive, fluorescent) on the antibody that is detected after binding of the antibody to the enzyme. Quantification with ELISA is more precise than with Western blotting. Currently, the availability of UGT specific antibodies is scarce, which hampers the quantification of certain specific UGT isoenzymes in an enzyme sample.

Using rat antibodies that recognize a broad range of UGTs in a Western Blot analysis of human liver microsomes, it was revealed that at 18 and 27 weeks of gestation a UGT of 53 kDa is present. At term birth 3 isoforms could be observed and all isoforms that could be identified in adults with this experimental setup were found to be present at three months of age, albeit at only approximately 25% of the adult levels [37]. Unfortunately it is difficult to derive which UGT isoforms are described in this study as at the time

the different UGTs had not been identified and classified the way they are currently classified.

More recently, differences were found in the enzyme expression levels of UGT1A1, 1A6 and 2B7 between adults and children ranging from 7 months to 2 years of age with Western blotting. These results for enzyme expression were in agreement with finding on mRNA transcription in the same study [36]. Another study using a similar experimental setup showed an age-dependent increase in UGT2B7 enzyme expression in infants and young children. Adult expression levels of UGT2B7 enzymes in this study were only reached in the oldest paediatric age category ranging from 12 to 17 years [38].

2.2.1.3 Enzymatic Activity

After translation, enzymes can be further modified, for instance by phosphorylation and N-glycosylation, which may either enhance or inhibit the activity of an enzyme. It has for instance been suggested that UGT1A1 requires phosphorylation for the metabolism of some, but not all, of its substrates [39] and that N-glycosylation may influence the enzymatic activity of some of the enzymes of the UGT2B subfamily [40], but not on all of them [41]. Additionally, as reviewed by Ishii *et al.* UGTs can form homo- or heterodimers with each other or form complexes with other enzymes [42], and UGT activity is also influenced by the lipid composition of the membrane in which they are integrated [43]. Post-translational modifications and enzyme and lipid interactions can be studied independently, but this is not commonly done in studies of the ontogeny of enzyme systems. Rather, the net influence of gene expression, post-translational modifications, enzyme interactions and other possible contributing factors on enzymatic activity is often studied.

Contrary to the more quantitative measurements of mRNA and enzyme abundance, enzyme activity studies are of a more qualitative nature. These methods depend on the measure of either the formation of an enzyme product or the depletion of an enzyme substrate in a hepatocyte or microsome sample or liver homogenate. Individual UGTs can be investigated by using substrates specific for the UGT of interest, however due to the overlapping substrate specificities of the UGTs, few isoform selective substrates have been identified (see table I). Additionally, activity findings are neither absolute nor generalizable in that the results are specific for a particular combination of a certain enzyme and substrate and may be different for another substrate of the same enzyme. With enzyme kinetics generally being non-linear, different results are often obtained at different substrate concentrations, so it is also important to use physiologically relevant substrate concentrations in the experimental set up.

By tracking the initial formation rate of a metabolite at various substrate concentrations, the Michaelis-Menten parameters V_{max} (maximum reaction rate) and K_m (Michaelis-Menten constant) can be determined and equation 1 can subsequently

be used to determine the intrinsic clearance (Cl_{int}), which is the maximum metabolic capacity in the absence of rate-limiting factors:

$$Cl_{\text{int}} = \frac{V_{\max}}{K_m} \qquad \text{(Equation 1)}$$

It is important to characterize the relationship between substrate concentration and enzyme reaction rate in detail, since the enzyme kinetic parameters that describe this relationship are directly correlated with the intrinsic clearance.

There is increasing evidence that some UGTs do not follow the typical non-linear behavior described by the Michaelis-Menten equation with some of their substrates [44–46]. This is indicative of the presence of allosteric effector sites, simultaneous binding of two substrate molecules to the enzymes active site, or cooperation with other enzymes and proteins in complexes. Technically, different methodologies should be used to accurately determine intrinsic clearance for enzymes that follow non-typical behavior, but this is not often done. Additionally, it is not clear how to correlate such non-typical *in vitro* behavior to the *in vivo* situation.

As an alternative, substrate depletion rates can be measured at a low concentrations (below K_m) as kinetics can then be assumed to be linear and measurements at a single substrate concentration suffice. The elimination rate constant (k_e) is used together with the distribution volume (V) to determine the intrinsic clearance according to equation 2:

$$Cl_{\text{int}} = k_e \cdot V \qquad \text{(Equation 2)}$$

The elimination rate constant is derived either from the elimination half-life of the substrate, or from the ratio between substrate concentrations at the beginning and at the end of the experiment. The distribution volume is composed of the volume of the incubation medium, the volume of the cells ($4 \cdot 10^{-9}$ mL per cell [47]), and the binding of the substrate to various components of the experimental system. In these studies, unspecific binding of the substrate to the experimental system or cellular uptake of the substrate without metabolism of the substrate, will increase the substrate disappearance rate at the beginning of the incubation period and will also result in over-prediction of the distribution volume. Since only one substrate concentration is used in this methodology it is very important to use relevant substrate concentrations to get physiologically meaningful results.

In the *in vitro* activity studies described above, the use of microsomes is easier and cheaper than the use of hepatocytes, however experimental protocols for microsomes are

often optimized for CYP enzymes and not for UGTs [48]. Numerous incubation conditions influence measured glucuronidation rates [49] and it has proven to be difficult to get good results for glucuronidation processes using microsomes [50]. Other, general advantages of using hepatocytes over microsomes for these studies are that all cofactors needed in the metabolic process are present at physiological concentrations and that the physical structure of the hepatocyte, including drug-binding cell compartments, cell membranes and transporters, is still in tact. Disadvantages of hepatocytes are that the expression and activity of many enzymes and transporters decline within hours.

The hepatocytes used in *in vitro* studies usually either originate from fresh liver samples or from cryopreserved samples. Various steps in the cryopreservation process influence the results obtained with cryopreserved hepatocytes, as reviewed by Hengstler *et al*. [51]. This may influence the findings on the correlation between results obtained in fresh and cryopreserved hepatocytes. Some found a good correlation for CYPs and UGTs [52], whereas others obtained ambiguous results for the correlation of UGT activity between cryopreserved hepatocytes and fresh hepatocytes [53].

In the late seventies and eighties of the 20th century, it was found that glucuronidation activity per gram of liver towards bilirubin and 2-aminophenol in fetal and neonatal liver homogenates was about 1% of the activity observed in adult liver homogenates. This study described an exponential increase in glucuronidation activity with age, to reach adult values three months postnatally. No differences were observed between term and preterm neonates [54]. More detailed investigation of the perinatal development of human hepatic UGT1A1 glucuronidation towards bilirubin revealed glucuronidation activity to be at 0.1% of adult activity levels between 17 and 30 weeks of gestation. Between 30 and 40 weeks of gestation activity levels increased to 1% of adult activity levels and after birth a rapid increase was observed to reach adult levels at 14 weeks of age. This postnatal increase was again found to be independent from gestational age, indicating that birth-related factors and not age-related factors are the main driving force for this increase [55]. In another study using microsomes the findings for bilirubin were substantiated. In term and preterm infants glucuronidation activity towards bilirubin was low and at 8 to 15 weeks activity values close to adult values were observed [37].

Experiments in microsomes on general glucuronidation activity have yielded inconsistent results. Glucuronidation of 4-methylumbelliferone, a compound glucuronidated by multiple UGT isoforms, was found to reach adult levels at 20 months of age [14]. Yet, in another study, the rate of glucuronidation of a series of 18 compounds including steroids, antidepressants, analgesics, opioids, flavones and coumarines, was found to not have reached adult values at 2 years of age and the difference between activity levels of 2 year-olds and adults could reach up to 40-fold [36]. The latter study

did however find mRNA transcription levels and enzyme expression of most UGTs to have reached adult values by the age of 6 months, underscoring the importance of other contribution factors of enzyme activity. Glucuronidation activity in microsomes towards testosterone and 1-naphthol, both compounds that are glucuronidated by a range of UGT isoforms, was found to be low at preterm and term birth and to slowly increase. At 1 year of age adult values were found not to have been reached [37]. Another study also found the glucuronidation capacity towards testosterone and 1-naphthol as well as towards bilirubin, androsterone, oestrone, 2-aminophenol and 4-nitrophenol to be low in the fetus and neonate [56].

Interestingly, in microsomes glucuronidation capacity towards serotonin, a substrate for UGT1A6, was found to be higher than adult levels in both fetal and neonatal liver [56]. A study in liver cells, however showed no detectable paracetamol glucuronidation in fetal liver [57], while paracetamol is also metabolized by UGT1A6, in addition to the isoforms 1A1 and 1A9 [9]. One explanation for these observations is that enzyme activity not solely depends on the enzyme, but on the combination of the enzyme and substrate together. However, based on the limited mRNA expression for all UGTs in the fetus, it is more likely that alternative hepatic elimination routes are available for serotonin in fetuses and neonates that are not or less abundantly present in adults livers.

Morphine is considered to be a specific substrate for UGT2B7 [23,25]. Microsomal glucuronidation activity towards morphine was found to be 6 tot 10 times lower in fetal liver samples obtained between 25 to 27 weeks of gestation, than in liver samples from adults. Within this age-range a correlation with gestational age was lacking [58].

2.2.2 Genetic Variation in UGT Expression and Activity

Even before gene transcription and translation, the causal chain of events for drug glucuronidation starts with a genetic code on a chromosome. Mutations can occur in the promoter region of a UGT gene, potentially influencing the transcription of the gene and thereby the enzyme abundance. Additionally, mutations in genes of transcription factors that regulate UGT gene expression may also influence DNA transcription and enzyme abundance. Regulation of UGT gene expression and the influence of polymorphisms on this process have been reviewed by Mackenzie *et al.* [59].

Polymorphisms in the coding regions of UGT enzymes have been identified as well. Such mutations may or may not influence enzyme activity. Mutations involved in the catabolism of endogenous compounds may lead to congenital diseases. A wide range of different mutations in the UGT1A1 isoform leads for instance to various degrees of unconjugated hyperbilirubinaemia as observed in Crigler Najjar syndrome and Gilbert's syndrome [60], which in newborns may also lead to kernicterus. In case a mutation leads to functional changes, the k_m or V_{max} of an enzyme for a specific substrate may be altered,

or both. Therefore, other than the presence or absence of functional changes in mutant enzymes, the extent of the functional changes may also be substrate specific [8,11,28,29,61–73]. Additionally, some mutations may cause functional changes in glucuronidation activity by impacting the ability of UGT enzymes to interact with other membrane components to form complexes [74]. Since UGT1 isoforms are alternatively spliced from the same mRNA transcript, the isoforms of this subfamily share part of their genetic code. A certain polymorphism can therefore affect multiple UGT1 isoforms [75].

Genetic differences in elimination capacity prevail throughout life and studies on the impact of genetic variation on UGT enzyme expression and activity are generally performed in (tissue from) the adult population or in artificially synthesized *in vitro* enzyme systems. It is possible that (unknown) mutations in UGT genes have influenced some of the findings on UGT ontogeny, this is however not further discussed or considered in the current review.

2.2.3 Prediction of *In Vivo* Hepatic Clearance Based on *In Vitro* Enzyme Activity

Enzyme ontogeny is predominantly studied to make inferences about the maturation rate of *in vivo* drug elimination in the paediatric population. Various approaches are available to make inferences on *in vivo* hepatic clearance based on *in vitro* data obtained in hepatic material, these approaches do however not take into account extra-hepatic sources of drug glucuronidation and elimination.

It can be envisioned that *in vitro* enzyme behavior in a non-physiological medium that may lack necessary co-factors is different from the enzyme behavior *in vivo*. However when predicting *in vivo* clearance from *in vitro* data, *in vivo* and *in vitro* intrinsic clearance per unit of enzyme are assumed to be the same. Subsequently, *milligram of microsomal protein per gram of liver* (MPPGL) and *number of hepatocytes per gram of liver* (HPGL) can be used in a straightforward manner to determine the rate of metabolism per gram of liver from microsomal or hepatocyte *in vitro* clearance respectively. This in turn can be multiplied by liver weight to give an estimate of total hepatic intrinsic clearance.

For MPPGL most commonly a value of 45 mg/g is used, however a recent meta-analysis, showed geometric mean MPPGL values to be 32 mg/g in Caucasian adults with high inter-individual variability and a weak negative correlation with age [76]. No differences were found between microsomes from fresh and frozen liver samples [77]. A later analysis included data from 4 Caucasian paediatric livers (age 2, 4, 9, and 13 years) and 11 fetal livers. The geometric mean of MPPGL in fetal livers was found to be 26 mg/g. The geometric mean of MPPGL in the paediatric livers was 28 mg/g and increased to 40 mg/g at the age of 28 years, after which it slowly decreased again [77]. It should however be noted that this age-effect could only explain 10% of the observed variability observed in MPPGL.

In Caucasian adults the geometric mean of HPGL values was found to be $99 \cdot 10^6$ cells/g, with high inter-individual variability [76]. For HPGL there was also a negative correlation observed with age in adults, however no information is currently available on HPGL in paediatric livers.

As reviewed by Johnson *et al.* [78], many models exist to describe liver volume or liver weight as a function of body surface area (BSA), bodyweight, age or other covariates. Meta-analysis of these data revealed that the median liver weight in children younger than 2 years was 3.5% of the total bodyweight, whereas in adults this was only 2.2%. The authors propose the following equation to predict liver volume (LV) in the paediatric population:

$$LV = 0.722 \cdot BSA^{1.176} \qquad \text{(Equation 3)}$$

In this study a value of 1.08 kg/L for liver density was used to derive liver weight from liver volume [78]. Reference values of the International Commission on Radiological Protection (ICRP) for paediatric liver weights are 130, 330, 570, 830, and 1300 gram for respectively neonates and children of the ages of 1, 5, 10, and 15 years [79].

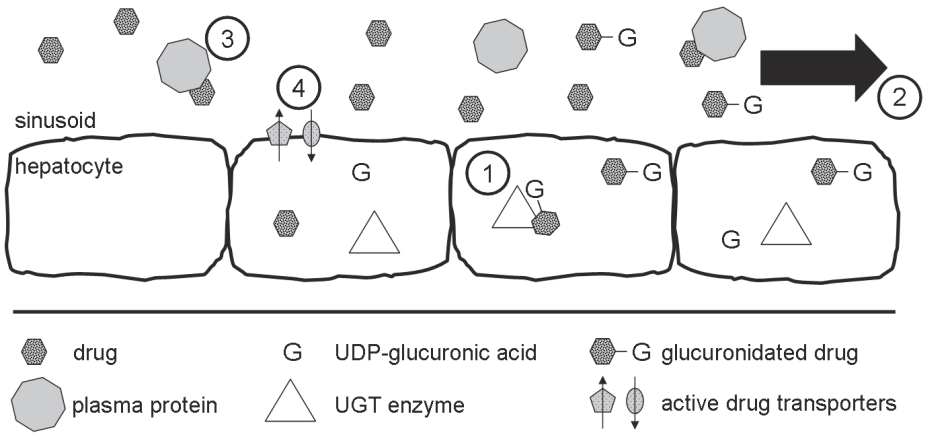

Figure 2. *Schematic representation of the mechanisms involved in in vivo drug glucuronidation by the liver: (1) intrinsic clearance by the enzymes, (2) blood flow and perfusion, (3) plasma protein binding, and (4) active hepatic influx and efflux mechanisms.*

In addition to scaling *in vitro* obtained intrinsic clearance per gram of enzyme to *in vivo* intrinsic clearance of the full liver, other contributing factors can be taken into account to derive *in vivo* hepatic clearance. Figure 2 provides a schematic representation of the

mechanisms involved in *in vivo* hepatic drug metabolism that were already mentioned in the introduction. *In vitro-in vivo* extrapolation methods that consider the different mechanisms described in figure 2 are available to make quantitative predictions of *in vivo* hepatic clearance based on *in vitro* intrinsic clearance obtained with microsomes, hepatocytes or liver homogenates.

Physiology-based pharmacokinetic software (e.g. Simcyp, PK-sim, PKQuest, GastroPlus) can integrate a wide range of *in vitro* and *in vivo* data on drug parameters and physiological parameters to aid the prediction of hepatic clearance based on historic and experimental data, first attempts to include pathological conditions like liver cirrhosis have been made as well [80]. In addition to the derived intrinsic hepatic clearance, physiology-based models may also use information on the age-related changes in hepatic blood flow and plasma protein binding.

Commonly used models to derive whole-blood hepatic drug clearance ($Cl_{H,B}$) are the well-stirred model (Equation 4) and the parallel-tube model (Equation 5).

$$Cl_{H,B} = \frac{(Q_{H,B} \cdot Clu_{\text{int}} \cdot f_{u,B})}{(Q_{H,B} + Clu_{\text{int}} \cdot f_{u,B})} \qquad \text{(Equation 4)}$$

$$Cl_{H,B} = Q_{H,B} \cdot (1 - e^{-(Clu_{\text{int}} \cdot f_{u,B} / Q_{H,B})}) \qquad \text{(Equation 5)}$$

In addition to the intrinsic clearance of the full liver, which should be based on unbound drug concentrations (Clu_{int}), these models also take the unbound drug fraction in blood ($f_{u,B}$) and hepatic blood flow ($Q_{H,B}$) into consideration. Modifications of these equations are necessary to determine the more commonly used plasma drug clearance instead of whole-blood drug clearance [81].

Both the well-stirred model and the parallel-tube model grossly divide the liver into sinusoids, vascular channels in which arterial blood and blood from the portal vein combine, and hepatocytes, the cells that surround the sinusoids and that are responsible for the drug metabolism. The two models assume the physiological extremes in the extent of mixing of drug concentration in the sinusoids and hepatocytes. The well-stirred model assumes drug concentrations in the sinusoid and hepatocytes to be equal, whereas the parallel-tube model assumes the liver to be composed of parallel tubes along which the drug concentration decreases and the concentration driving the hepatic drug uptake to be the logarithmic mean sinusoidal concentration [82]. Both models assume that plasma protein binding is at steady state, that there is no diffusion delay and that no active transport systems are involved [81]. When the hepatic drug extraction ratio is low (<0.5) both models give similar results, but for drugs with an intermediate or high extraction

ratio predictions with the well-stirred model are generally lower than with the parallel-tube model.

In these models, commonly used adult values for hepatic blood flow are 1.35 L/min for females and 1.5 L/min for males. It has however hardly been studied whether and how hepatic blood flow changes with age in the paediatric population. Some have suggested that there are no major differences in hepatic blood flow between neonates and adults [83], this seems however unlikely, since the relative liver size decreases with age, which would imply liver blood perfusion per kilogram of liver to be lower in children compared to adults. Other possible assumptions that are often made in this case are: 1) hepatic blood flow per kilogram bodyweight is the same in children and adults, 2) hepatic blood flow per kilogram of liver is the same in children and adults, 3) hepatic blood flow is proportional to metabolic rate (parameterized by BSA), and 4) the percentage of cardiac output directed to the liver remains constant with age. Opportunely, the decreased metabolic capacity of the liver in young children decreases the drug extraction ratio, thereby making hepatic clearance less dependent on hepatic blood flow, making knowledge of paediatric hepatic blood flow less vital in the predictions of paediatric hepatic clearance. Finally, it has to be remembered that an unclosed ductus venosus can impair hepatic drug clearance in neonates, by allowing part of the blood flow to bypass the liver.

The unbound drug fraction in the paediatric population is also (potentially) influenced by various factors, namely 1) increases in the concentration of drug-binding plasma proteins from 76.7% of the adult value in neonates for human serum albumin (HSA) and 53.4% of the adult value in neonates for α 1-acid glycoprotein (AGP). The increases in the concentrations of these proteins with age have been described by various functions [84,85], 2) increases in bilirubin and free fatty acid concentrations shortly after birth, and 3) differences in the protein binding affinity of drug between children and adults [86,87]. Therefore, the value for the unbound drug fraction in blood obtained in adults cannot automatically be used in these equations for children.

With information on paediatric hepatic blood flow and plasma protein binding lacking, there is another approach that is sometimes applied to derive *in vivo* paediatric hepatic clearance based on *in vitro* data by the used of the well-stirred or parallel-tube model. In this approach *in vitro* intrinsic clearance is determined in paediatric hepatic material, this value together with adult values of the other parameters in the well-stirred or parallel-tube model are used to obtain a value for hepatic clearance. Paediatric hepatic clearance ($Cl_{H,B,paed}$) is subsequently derived from this value by the use of allometric scaling as illustrated in equation 5:

$$Cl_{H,B,paed} = Cl_{H,B} \cdot (\frac{W_i}{W_{std}})^{0.75} \qquad \text{(Equation 6)}$$

In this model, the bodyweight of the individual (W_i) is divided by the bodyweight of a 'standard adult' (W_{std}) usually taken to be 70 kg. When the individual weight of the paediatric liver donor is unknown, a value can be derived from growth charts based on the age and sex of the donor. The application of allometric scaling on the organ level is however controversial [88].

So far it has proven to be difficult to characterize the influence of hepatic uptake and transporter mechanisms on drug clearance, even in adults [89]. This information is therefore rarely incorporated in physiology-based models. Pgp efflux transporters were found to be expressed in the human hepatocytes and this expression was suggested to be initiated by birth [90], however quantitative information on age-related changes in expression of hepatic uptake and transporter proteins is missing. Since hepatic transporters are already present in hepatocytes, the influence of ontogeny of transporter proteins on clearance only becomes relevant when dealing with intrinsic clearance values obtained with microsomes.

Both the well-stirred and parallel-tube model generally yield under-predictions of *in vivo* intrinsic clearance of unbound drugs up to ten-fold or even higher [49,91,92]. The assumption in both models that plasma protein binding is at steady state conditions instead of in a dynamic state has been proposed as an explanation for this under-prediction. This under-prediction is less when data obtained in hepatocytes are used compared to data obtained in microsomes, possibly because cellular uptake proteins are functionally present in hepatocytes but not in microsomes. Recently it has been found that the accuracy of the predictions of *in vivo* clearance with hepatocytes could be improved to some extent by incubating the cells with serum instead of buffer solution [93]. This system approaches the physiological situation better as drug binding to plasma proteins is already incorporated in the experimental setup, thereby eliminating the need for separate studies of the unbound drug fraction in blood. In microsomal studies the use of serum is however thought to be un-physiological, since the unbound drug fraction in microsome incubation is not an analogue for the unbound drug fraction that is available for *in vivo* metabolism. It has been suggested that the under-predictions with microsomal data could be corrected by an empirical scaling factor [46,92].

Microsomal studies on UGT1A4 activity in which trifluoperazine depletion was used as a marker for product formation to obtain Michaelis-Menten parameters, showed UGT1A4 activity to reach adult levels by 1.4 years. However, calculating hepatic clearances from the obtained *in vitro* intrinsic clearances by the use of both the well-stirred and parallel-tube model and subsequent allometic scaling, suggested *in vivo* UGT1A4 mediated hepatic glucuronidation to reach adult values only at the age of 18.9 years [14], which was however not corroborated with experimental data.

Using epirubicin as specific substrate for UGT2B7 in a study determining Michaelis-Menten parameters with human microsomes, a small age-dependent increase in UGT2B7 activity was found over the paediatric age-range, but activity in all paediatric age categories was lower than in adults. UGT2B7 enzyme expression levels in this study showed a good correlation with UGT2B7 activity levels, however adult expression levels were already reached at 12 – 17 years. By applying the allometric model directly to the *in vitro* clearances, the differences between the different paediatric age categories and between adults and the paediatric categories disappeared [38].

2.2.4 *In Vivo* Methods

To make reliable predictions of *in vivo* clearance based on *in vitro* methods requires information on a large number of parameters, not all of which can be determined experimentally in adults, let alone in children. In addition to that, *in vitro* hepatocytes have lost normal cell attachment, polarity and have a bigger surface area than in the *in vivo* situation and the architecture of the liver also changes with age. Therefore the ontogeny of *in vivo* drug glucuronidation may be best determined *in vivo*. *In vivo* studies can be performed in both animals and humans, however animal models are not considered in the current review, as the extrapolation potential from animals to humans in ontogeny studies is unknown. Also, differences in substrate specificity between animals and humans have been reported [94].

In vivo enzyme ontogeny studies can be performed by determining drug metabolism in neonates, infants and children of various postnatal and postmenstrual ages. By looking at changes in total drug elimination from plasma, the influence of maturation in all clearance routes combined (i.e. glucuronidation, metabolism other than glucuronidation, biliary excretion and unchanged renal clearance) is determined. It is therefore important to either ensure that glucuronidation is the major route of elimination for the drug of interest or to determine the contribution of each route to the overall clearance. It should also be considered, that *in vivo* drug metabolism is not restricted to hepatic metabolism and that metabolism in organs other than the liver is also measured. The latter does not necessarily pose a problem, since the reason to study drug metabolism is generally to determine how quickly a parent drug is cleared, for instance to determine the dosing regimen, or how quickly certain metabolites are formed, which is especially important when the metabolites are pharmacologically active or toxic.

In vivo glucuronidation clearance can be derived from concentration-time profiles. With traditional methods, clearances are calculated by dividing dose by the area under the concentration-time curve (AUC). AUCs can only be derived from full concentration-time profiles, which cannot be obtained from very small children for practical and ethical reasons. Additionally, AUC calculations are sensitive to sampling times. Alternatively,

clearance can be obtained by dividing the drug infusion rate by the steady state plasma concentration, however it may take a while for the parent drug to reach steady state concentrations and even longer for drug metabolites. Additionally, determining whether steady state has been reached may proof to be difficult. Other drawbacks of traditional studies are that often only a limited number of patients are included and that usually differences in clearance capacity between groups instead of individuals are quantified, making the results on maturation processes partially dependent on the stratification of the study population.

In the past few decades advanced statistical tools have been developed to aid in the analysis of sparse and unbalanced data that are often obtained in paediatric pharmacology studies. As described by De Cock *et al.*, the population approach using non-linear mixed effects modeling is currently the preferred tool, as it analyses individuals as constituents of a population, thereby allowing for the analysis of sparse and unbalanced data and also for the identification of both within- and between-subject variability [95]. By investigating trends in the glucuronidation clearance of different individuals in the population, the best descriptor for the maturation rate can be identified. This allows for the continuous quantification of changes occurring throughout the paediatric age-range. Since incorrect conclusions can be easily drawn from sparse data, thorough model validation is imperative to ensure that the models obtained with this method are reliable.

When a drug is metabolized through multiple routes, the contribution of the glucuronidation to the total systemic clearance has to be determined. With the traditional methods the recovery of the metabolites of a drug dose in urine would be determined to establish the contribution of each route to the overall systemic drug clearance. Alternatively the drug:metabolite ratio in blood or urine would be traced in time. The latter is however not recommended as this ratio not only depends on the glucuronidation rate, but also largely depends on the fate of the metabolite. With the population approach information on the metabolites can be incorporated into the model, describing all pharmacokinetic parameters relating to both the parent drug and the metabolite, thereby allowing for the quantification of each individual elimination route, including glucuronidation.

One of the first and archetypical examples of the impact of UGT ontogeny on paediatric pharmacology was the observation in the fifties of last century that lack of UGT activity causes the grey baby syndrome in neonates that are treated with chloramphenicol [96]. Around that time it was also observed that the recovery of the acetanilide glucuronide in urine is significantly reduced in neonates and that the recovery in preterm neonates is lower than the recovery in the term neonates. The glucuronide recovery was found to increase with age, with faster maturation rates for term neonates compared to their preterm counterparts. Adult recovery levels of the glucuronide were reached around 3 months of age [97].

Studies on paracetamol [98–100] and salicylamide [99] in the seventies of the last century, showed the ratio of sulphate and glucuronide metabolites in urine to differ with age, while the total drug elimination did not increase dramatically. The sulphate fraction was found to be higher in children for both drugs, probably to compensate for the reduced glucuronidation capacity in this age-group. In 7 to 10 year old children glucuronidation fractions where found not to have reached adult values [99], whereas in 12 year-old children adult fractions appeared to have been reached [98]. In more recent studies paracetamol glucuronidation was found to increase with both postmenstrual and postnatal age [101–108]. These changes were quantified in different ways. Glucuronide:sulphate ratios were found to increase with gestational and postnatal age [101,106] and to reach adult values at 3 years of age [103]. The maturation half-life of total paracetamol clearance was found to be close to 3 months for term and preterm neonates [102,104], whereas the maturation half-life of paracetamol glucuronidation in specific was found to be 8.09 months [103]. It is however important to note that these half-lives do not reflect the maturation of absolute clearances, but of the clearances that are allometrically scaled to average adult bodyweights of 70 kg. Therefore, when after 4 to 6 half-lives adult clearance values are assumed to have been reached in a child, it means that clearance would be at adult values had the child been of average adult bodyweight. However, absolute clearance values in this child are still low and increasing due to the physiological increase in size (bodyweight). According to the Centers for Disease Control and Prevention (CDC) in the US, males on average reach a bodyweight of 70 kg around 18 years of age, whereas the average female does not reach this bodyweight [109]. Based on urine recovery studies in neonates, it has been suggested that paracetamol glucuronidation is up-regulated with multiple doses [106], something that has also been observed in adults [110], However, our group has suggested that this observation is a possible artifact of a slower glucuronidation rate compared to the elimination rates of other routes, since this slower glucuronidation rate causes the fraction of a drugs glucuronide recovered in urine to increase at later time-intervals [111].

Morphine is a UGT2B7 specific substrate and the maturation of its glucuronide formation in neonates, infants and children has been widely studied. Studies on morphine and its glucuronides using traditional methodologies were not unambiguous. It is generally recognized that even at a gestational age as young as 24 weeks, neonates can glucuronidate morphine [112–114]. In neonates, total morphine clearance per kilogram bodyweight was reported to be positively correlated with birth weight and gestational age [113,115], however others could not identify an effect of gestational age on morphine pharmacokinetic parameters in neonates [114]. Morphine clearance per kilogram bodyweight is lower in neonates compared to infants, children and adolescents [112,116,117]. Adult clearance values were reported to be reached between 1 to 3 months [118] or between 6 months and 2.5 years [116]. The contribution of sulphation to morphine elimination was

found to be minor in neonates and to decrease further with age [119]. It is suggested that the increase in the fraction that is glucuronidated is predominantly responsible for the observed increases in morphine clearances. The fraction of morphine that is cleared through glucuronidation was found to be lower in neonates than in older patients [112,120] and in these neonates this fraction was found to increase with birth weight [113]. Others reported however that sulphation and unchanged renal excretion are still important morphine elimination routes in infants and children [116] and yet another group found the contribution of each elimination pathway to total morphine clearance to already be at adult ratio's in preterm neonates [114].

The maturation of morphine glucuronidation in the first three years of life has been quantified in different ways with the use of population modeling. In all studies absolute morphine glucuronidation clearance was found to continuously increase in this population. Two studies scaled clearances allometrically to 70 kg and subsequently described this maturation either with an exponential equation based on postnatal age [121] or with a sigmoidal equation based on postmenstrual age that was different for term and preterm neonates [122]. A third study by our group found that the changes in glucuronidation clearance was best described by a bodyweight-based exponential equation with an estimated exponent of 1.44 and that glucuronidation was decreased by about 50% in neonates younger than 10 days of age while there were no differences between term and preterm neonates except those resulting from the differences in bodyweight (Chapter 3).

For zidovudine, which is also mainly metabolized by UGT2B7, clearance has been reported to mature rapidly with postnatal age in the first weeks of life [123,124]. Adult clearance values have been reported to be reached after two to eight weeks [123,124], whereas others report a slower increase in clearance for a subsequent two years, which is then followed by an even slower increase of absolute clearance rates during the rest of childhood and adolescence [125]. In preterm neonates clearance is lower [126,127] and maturation is slower [124] than in their term counterparts. In the preterms both bodyweight [126,127] and gestational age [127] have been reported to correlate with clearance rates.

The pharmacokinetics of propofol, which is mainly eliminated through glucuronidation by UGT1A9, has also been broadly studied in the paediatric and neonatal population. Studies have shown propofol metabolism in neonates to be different from adults and mainly suggest age-related changes in glucuronidation capacity for this drug in early life [128,129]. Kataria *et al.* have found propofol clearance per kg bodyweight to increase linearly with bodyweight in children ranging between the ages of 3 and 11 years [130], and Wang *et al.* described multi-directional age-related changes in total propofol clearance over the entire human age-range [131]. However, cautions is warranted when using propofol as an *in vivo* probe for glucuronidation, since this drug has a high

extraction ratio causing its clearance to be limited by liver blood flow rather than enzyme capacity, especially after maturation of the metabolic clearance is complete around the age of 2 years [132].

Additionally, some of the drugs used for the treatment of epilepsy (e.g. lamotrigine, carbamazepine, valproic acid) are also, at least partially, glucuronidated. Since these drug are often used in combination with each other and since issues with UGT-(auto) induction or inhibition are frequently encountered in this drug class [16], these drugs are also considered not to be very suitable as *in vivo* probes for glucuronidation.

2.2.4.1 Other Factors that Potentially Influence Pharmacokinetics *In Vivo*

When deriving paediatric drug dosages from enzyme maturation rates, it is important to consider that drug concentrations after single bolus doses or after loading doses not only depend on elimination rates but also on distribution volume. Additionally, age-related changes in the unbound drug fraction may influence the hepatic drug extraction ratio and thereby elimination rates, as well as drug effects.

In addition to maturational changes in drug glucuronidation rates and genetics, environmental factors, comedication, comorbidities and medical treatments and procedures may influence drug glucuronidation capacity. The latter three are especially important in paediatrics since ethically it is difficult to perform studies in healthy subjects. Maternal smoking for instance may influence glucuronidation capacity in the first few days of life [133]. A number of compounds have been identified to induce the activity of a variety of UGT isoenzymes [134], whereas on the other hand phenobarbital and phenotoin were found to decrease paracetamol glucuronidation in the offspring [135]. Hypothermia to treat hypoxic ischemic encephalopathy was found to reduce morphine clearance in neonates [136]. Additionally, morphine clearance was found to be different in infants undergoing cardiac surgery *versus* patients undergoing non-cardiac surgery [137] and the type of cardiac surgery was found to further influence morphine clearance [138]. Extracorporeal membrane oxygenation (ECMO) generally influences drug pharmacokinetics in numerous ways [139]. In neonates and infants morphine clearance was reported to be reduced in patients on ECMO [140–142], however others have reported no change in morphine concentration after initiation of ECMO treatment [143]. In neonates, maturation rates of morphine glucuronidation were found to be faster in patients on ECMO compared to non-cardiac post-operative infants, with a combination of bodyweight and postnatal age as descriptors for this process [140,141]. It cannot be excluded that changes in maturation rates observed during medical treatments are the result of improvements in the medical status of the patient rather than changes in maturation. Finally, clinical studies on the maturation rates of metabolic pathways are generally restricted to patients with unimpaired liver function, leaving the influence of liver failure on drug glucuronidation in the paediatric population largely unidentified.

2.3 Discussion and Conclusion

The different methodologies discussed in the current review measure different characteristics of the developing biological system. The *in vitro* methods provide mechanistic insight in different underlying processes of the clinically observed developmental changes in drug glucuronidation in the paediatric population and all methods should be regarded as complementary to each other. Table II gives a summary of the findings on maturation of the specific UGT isoenzymes with the various techniques, as discussed in the current review.

It can be seen in table II that the physiological processes in gene expression may have their own unique pattern of onset and maturation, this may be due to differences in contributing factors. Therefore different *in vitro* techniques may yield different answers to the onset and maturation of hepatic glucuronidation, even when the samples used with the various techniques are obtained from the same individual. This is nicely illustrated in the study by Strassburg *et al.*, where differences in glucuronidation activity were observed between paediatric and adult samples, while mRNA and enzyme levels in the same paediatric samples where already indistinguishable from adult values [36]. Also Zaya *et al*. showed the age-related increase in the enzyme expression of UGT2B7 to be more pronounced than the increase in UGT2B7 activity, and adult expression levels to be reached at 12-17 years of age, whereas activity levels remained below adult values throughout the paediatric age-range [38]. These findings imply that the ontogeny of UGT activity does not solely depend on the ontogeny of UGT gene expression (i.e. transcription and translation). Since UGT activity is influenced by post-translational modifications [39–41], maturation of UGT activity is therefore also dependent on the age-related changes in these processes. Additionally, UGT activity was found to be dependent on the formation of complexes with other UGTs, other enzymes and other proteins and on the lipid composition of the membrane it is integrated in [42,43], age-related changes in the expression of these components may thus further influence the ontogeny of the UGT enzyme activity. Moreover, genetic polymorphisms have also been found to influence UGT activity and alter glucuronidation capacity in adults in a substrate specific way [144–146].

It is also important to consider the fundamental differences between the methods that measure mRNA and enzyme quantities and the more qualitative methods that measure enzyme activity. As long as primers and antibodies are specific enough, absolute amounts of mRNA and enzyme can be quantified relatively unambiguously. The difference in the qualitative measurement of enzyme activity is that it is substrate-dependent. Findings on *in vitro* and *in vivo* enzyme activity not necessarily reflect

absolute enzyme activity, they rather describe the characteristics of a specific enzyme-substrate combination. This may limit the potential to extrapolate findings on enzyme activity from one enzyme-substrate combination to a combination of the same enzyme and another substrate. Likewise, genetic mutations may cause functional changes of an enzyme towards one substrate and not towards another.

Table II also shows that the methods that are used to derive *in vivo* clearances from *in vitro* results by incorporating various contributing components in different ways further diversify the findings on glucuronidation ontogeny. In *in vivo* studies, where external factors also influence drug glucuronidation capacity, the influence of contributing components is largest, but this setup resembles the clinical situation best. Here different scaling techniques to quantify glucuronidation capacity disperse the conclusions on glucuronidation ontogeny. In the paediatric population clearance is often expressed per kilogram or it is allometrically scaled to values of a 70 kg 'standard adult'. When clearance values expressed per kilogram or per 70 kg have reached adult values, absolute clearance values are often still low and increasing. In the study of Zaya *et al*. *in vitro* glucuronidation activity expressed per mg enzyme did not reach adult values before adulthood, whereas none of the paediatric activity levels could be discerned from adult levels when the activity levels were allometrically scaled to *in vivo* values of a 70 kg individual [38]. When expressed per 70 kg, morphine glucuronidation has reached 80% of adult levels at the age of 6 months and at 1 year adult values have been reached [121], whereas absolute glucuronidation rates have been found to be still low and exponentially increasing at least up to the age of 3 years and probably even further (Chapter 3).

Most findings suggest that absolute doses of drugs that are glucuronidated should be reduced in neonates, infants, and children and possibly also in adolescents. It is however not possible to derive more general dosing guidelines, as various UGT isoenzymes mature differently, as substrate specificities overlap and as there are many external factors that may influence drug glucuronidation. A proposed method to improve this situation is to develop validated population pharmacokinetic models for probe drugs that are metabolized by specific UGT isoenzymes. From these models information that is describing the biological system can be derived, which may include information on the maturation rate of glucuronidation in the population but also information on the influence of other factors that significantly contribute to the variability in glucuronidation capacity. It is hypothesized that this information of the biological system can be extrapolated between drugs that are metabolized by the same UGT isoenzyme [147]. Due to the fact that enzyme activity is a property of both the enzyme and its substrate, absolute clearance values can most likely not be extrapolated between drugs, however the extrapolation potential of maturational changes in clearance from one enzyme specific substrate to another specific substrate of the same enzyme is currently being investigated (Chapter 6)

[148]. The feasibility of these types of studies relies on the identification of suitable probes. Since it is largely unknown how age-related changes in the biological system affect enzyme specificities the assumption is often made that enzyme specificities are similar between artificial or adult-derived enzyme sources *in vitro* and in children *in vivo*.

In conclusion, gaps in our knowledge on UGT ontogeny at the various *in vitro* and *in vivo* levels still exist. Enzyme expression and *in vitro* and *in vivo* enzyme activity have been studied more frequently in fetuses, neonates and infants than in older children and adolescents. The various studies that investigated UGT ontogeny at different levels generally suggest that the onset of UGT expression and hepatic glucuronidation occurs after 20 weeks of gestation, with a boost in expression and activity in the first weeks of life. The maturation rate of glucuronidation varies between the different UGT isoforms, but maturation of some UGTs may extend well beyond the age of two years.

Table II. *Findings for the ontogeny of various UGT isoenzymes as determined by the various techniques discussed in this review. A. findings for the UGT1A subfamily. B. Findings for the UGT2B subfamily and C. Findings for unspecific or unspecified UGTs.*

A.

UGT isoenzyme	level of ontogeny study	findings on ontogeny
1A1	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	Expression levels have reached adult values between 7 months and 2 years [36].
	enzymatic activity (*in vitro*)	The glucuronidation rate of bilirubin at a GA of 17 to 30 weeks is at 0.1% of adult levels [55], at a GA of 30 to 40 weeks this is at 1% of adult levels [54,55] and after birth there is a rapid age-dependent increase that is independent of GA, to reach adult values at 8 to 15 weeks [37,54,55].
	glucuronidation clearance (*in vivo*)	-
1A3	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	-
	enzymatic activity (*in vitro*)	-
	glucuronidation clearance (*in vivo*)	-
1A4	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	-
	enzymatic activity (*in vitro*)	Glucuronidation rates reach adult levels at 1.4 years, after deriving *in vivo* activity levels using the well-stirred and parallel-tube model adult levels are reached at 18.9 years [14].
	glucuronidation clearance (*in vivo*)	-

1A6	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	Expression levels have reached adult values between 7 months and 2 years [36].
	enzymatic activity (*in vitro*)	Serotonin glucuronidation is higher than adult levels in fetal and neonatal livers [56], however no paracetamol glucuronidation was detectable in fetal liver [57].
	glucuronidation clearance (*in vivo*)	-
1A9	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, transcripts are detectable at a PNA of 6 months, adult transcript levels of have been reached at 1.5 years [36].
	enzyme expression	-
	enzymatic activity (*in vitro*)	Glucuronidation of 2-aminophenol in fetal and neonatal liver is about 1% of adult levels. Activity levels exponentially increase to reach adult values at a PNA of 3 months, with no differences in term and preterm born neonates [54]. Glucuronidation of 4-methylumbelliferone has reached adult values at a PNA of 20 months [14]. Glucuronidation of testosterone and 1-naphthol is low at birth and increases slowly. At a PNA of 1 year adult values have not been reached [37]. Glucuronidation rates of 18 different compounds has not reached adult values at 2 years of age, for some compounds the glucuronidation rates are up to 40-fold lower than in adults [36].
	glucuronidation clearance (*in vivo*)	Acetanilide glucuronidation is reduced in neonates and this reduction is more pronounced in preterm neonates compared to their term counterparts. The age-dependent increase in glucuronidation capacity is more pronounced in term neonates compared to preterms. Adult levels are reached around 3 months [97]. For paracetamol and salicylamide the fraction of drugs eliminated through glucuronidation has not reached adult values at 7 to 10 years [99], at 12 years adult levels have been reached [98].

GA = gestational age, PNA = postnatal age

B.

UGT isoenzyme	level of ontogeny study	findings on ontogeny
2B4	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, transcripts are detectable at a PNA age of 6 months, adult transcript levels have not been reached at 2 years [36].
	enzyme expression	-
	enzymatic activity (*in vitro*)	-
	glucuronidation clearance (*in vivo*)	-
2B7	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	Expression levels have been found to reach adult values between 7 months and 2 years [36] or between 12 to 17 years [38].
	enzymatic activity (*in vitro*)	Epirubicin glucuronidation capacity showed a small age-dependent increase with PNA, but in all pediatric age-ranges activity is lower then adults. After allometric scaling, *in vivo* adult clearance levels are predicted to be reached between 12 and 17 years [38]. Morphine glucuronidation can be detected at a GA of 24 to 27 weeks and is then 6 to 10 times lower than in adults [112–114]. No trends with age are observed in this group [58]. Morphine glucuronidation in neonates is lower than in older paediatric patients [112,116,117] and this fraction was found to increase with birth weight [113] . Others however report the ratio's in elimination routes of *in vivo* morphine clearance to already be at adult levels in preterm neonates [114].
	glucuronidation clearance (*in vivo*)	Absolute morphine glucuronidation increases in the first three years of life. Clearances allometrically scaled to 70 kg mature exponentially with PNA [121] or sigmoidally with PMA with slower maturation in preterm neonates compared to term neonates [122]. Absolute morphine glucuronidation rates increase with bodyweight to the power of 1.44, with a reduction in neonates younger than 10 days and no differences between term and preterm neonates (Chapter 3). Zidovudine clearance rapidly increases in the first weeks of life [123,124]. Thereafter some report that adult clearance rates are reached at 2 to 8 weeks [123,124] , whereas others report a slower increase to the age of 2 years, followed by an even slower increase in absolute clearance values till adulthood [125]. In preterm neonates clearance was lower [126,127] and maturation was slower [124] than in term neonates.

2B10 / 2B15	mRNA transcription	No transcripts are detectable at a GA of 20 weeks, adult levels of transcripts have been reached at a PNA of 6 months [36].
	enzyme expression	-
	enzymatic activity (*in vitro*)	-
	glucuronidation clearance (*in vivo*)	-

GA = gestational age, PNA = postnatal age

C.

UGT isoenzyme	level of ontogeny study	findings on ontogeny
Unspecific / unspecified	mRNA transcription	-
	enzyme expression	One unidentified UGT isoenzyme could be detected at a GA of 18 to 27 weeks, at term birth 3 isoforms could be detected and, at a PNA of 3 months all detectable adult isoforms are present at about 25% of adult level [37].
		Glucuronidation of 2-aminophenol in fetal and neonatal liver is about 1% of adult levels. Activity levels exponentially increase to reach adult values at a PNA of 3 months, with no differences in term and preterm born neonates [54].
	enzymatic activity (*in vitro*)	Glucuronidation of 4-methylumbelliferone has reached adult values at a PNA of 20 months [10].
		Glucuronidation of testosterone and 1-naphthol is low at birth and increases slowly. At a PNA of 1 year adult values have not been reached [56].
		Glucuronidation rates of 18 different compounds has not reached adult values at 2 years of age, for some compounds the glucuronidation rates are up to 40-fold lower than in adults [36] .
		Acetanilide glucuronidation is reduced in neonates and this reduction is more pronounced in preterm neonates compared to their term counterparts. The age-dependent increase in glucuronidation capacity is more pronounced in term neonates compared to preterms. Adult levels are reached around 3 months [97].
	glucuronidation clearance (*in vivo*)	For paracetamol and salicylamide the fraction of drugs eliminated through glucuronidation has not reached adult values at 7 to 10 years [99], at 12 years adult levels have been reached [98].
		For paracetamol the glucuronidation/sulphation fraction increases with PNA and GA [101,103] to reach adult values at an age of 3 years [103]. When allometrically expressed per 70 kg, the maturation half-life of paracetamol glucuronidation is 8.09 months [103][144].

GA = gestational age, PNA = postnatal age

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Section II

Paediatric Morphine Glucuronidation Model for Individualized Dosing

Chapter 3

Morphine Glucuronidation in Preterm Neonates, Infants and Children Younger than Three Years

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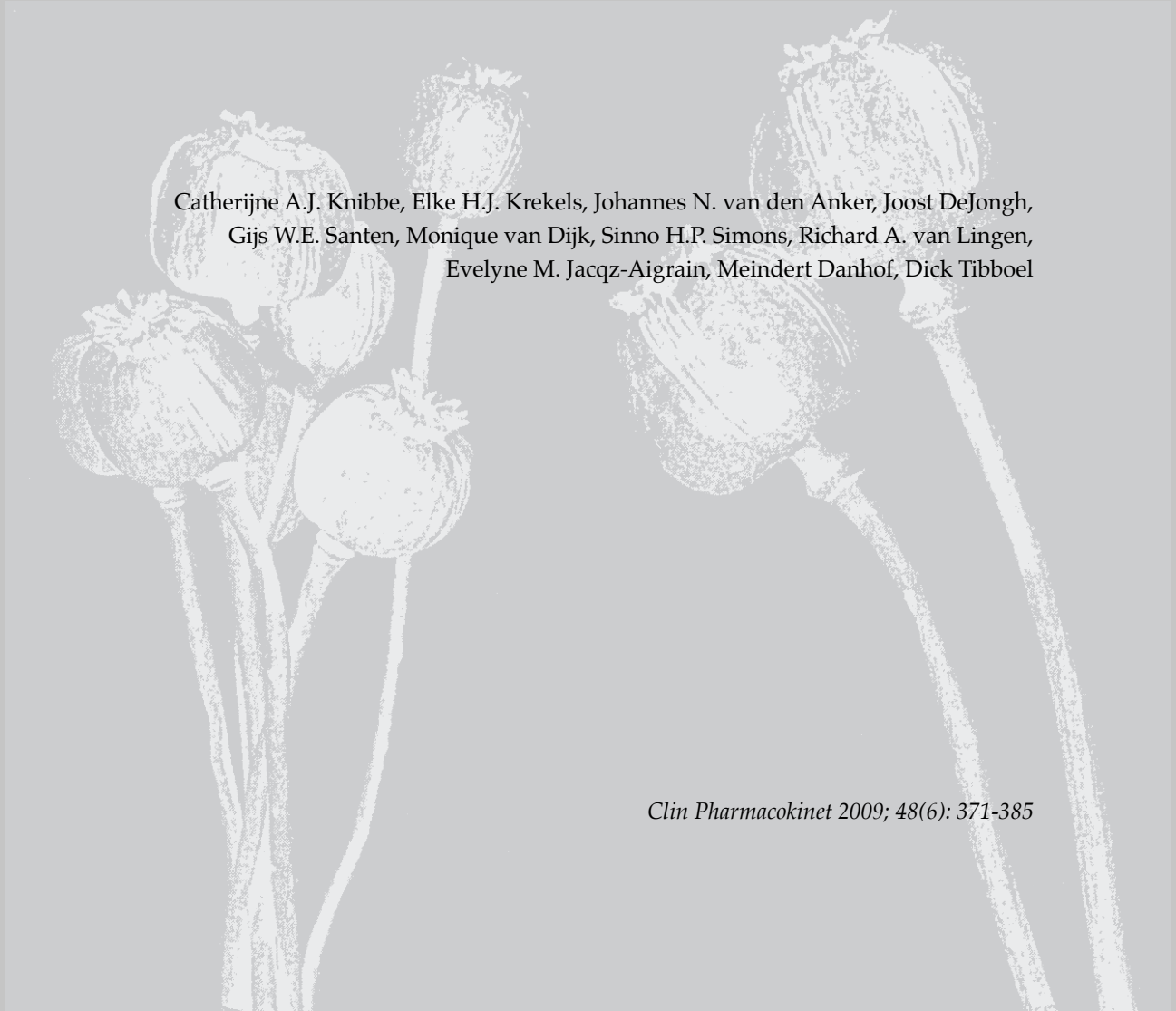

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Abstract

Background and objective: A considerable amount of drug use in children is still unlicensed or off-label. In order to derive rational dosing schemes, the influence of aging on glucuronidation capacity in newborns, including preterms, infants and children under the age of three years was studied using morphine and its major metabolites as a model drug.

Methods: A population pharmacokinetic model was developed with the nonlinear mixed-effects modeling software NONMEM V, on the basis of 2159 concentrations of morphine and its glucuronides from 248 infants receiving intravenous morphine ranging in bodyweight from 500 g to 18 kg (median 2.8 kg). The model was internally validated using normalized prediction distribution errors.

Results: Formation clearances of morphine to its glucuronides and elimination clearances of the glucuronides were found to be primarily influenced by bodyweight, which was parameterized using an allometric equation with an estimated exponential scaling factor of 1.44. Additionally, a postnatal age of less than ten days was identified as a covariate for formation clearance to the glucuronides, independent of birthweight or postmenstrual age. Distribution volumes scaled linearly with bodyweight.

Conclusions: Model-based simulations show that in newborns, including preterms, infants and children under the age of three years, a loading dose in mg/kg and a maintenance dose expressed in $mg/kg^{1.5}/h$, with a 50% reduction of the maintenance dose in newborns younger than ten days, results in a narrow range of morphine and metabolite serum concentrations throughout the studied age range. Future pharmacodynamic investigations are needed to reveal target concentrations in this population, after which final dosing recommendations can be made.

3.1 Background

Despite initiatives of both the US and the European Union (i.e. Pediatric Rule (FDA, 1998), Best Pharmaceuticals for Children Act (FDA, 2002), Paediatric Regulation (EMEA, 2007), and 7th Research Framework Programme (EU, 2007 - 2013), a considerable number of drugs prescribed in children are still unlicensed or used in an off-label manner, in a newborn intensive care setting this even amounts to 90% of the prescriptions [1]. Although it is often stated that 'children are not small adults', dosing schemes for this population are frequently empirically derived from studies restricted to adult patient groups, using linear extrapolations on the basis of bodyweight. To account for differences in drug disposition and/or drug response between children and adults and between children of different ages, higher or lower dosages per kilogram bodyweight are regularly recommended in different age-groups. While labeled information for children and (preterm) neonates in particular, is often lacking, investigations into developmental changes in pharmacokinetics (PK) and pharmacodynamics (PD) in the growing child are of utmost importance [2].

Our group aims at developing a series of PK-PD models that describe the influence of developmental changes on drug disposition, efficacy and safety, which will ultimately be used to develop rational dosing schemes with a predictable efficacy and safety profile for the individual child of varying age. In the current, study the influence of age on glucuronidation capacity of the UGT2B7 enzyme in newborns, including preterms, and infants up to three years was studied using morphine and its two major metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) as model drugs. Although it concerns many endogenous and exogenous substrates [3–8], the maturation of conjugation catalyzed by uridine diphosphate glucuronosyltransferases (UGT), has historically received less attention than oxygenation by the cytochrome P450 enzyme system [3,4]. We hypothesize that information obtained for morphine glucuronidation may be of value for other substrates metabolized through this route.

3.2 Methods

Study Design. This analysis was performed based on observations obtained in preterm and term neonates, infants and toddlers from two different blind randomized controlled trials evaluating the analgesic effects of morphine. Both study protocols were approved by local ethics committees and written informed consent was obtained from the parents.

The study designs are described in detail in the articles of 1) Simons *et al.* [9], and 2) Van Dijk *et al.* [10], and are shortly repeated as relevant to this article.

1) Preterm and term neonates with a postnatal age of less than three days that were on artificial ventilation for less than eight hours and had an indwelling arterial catheter were eligible for inclusion. Exclusion criteria were severe asphyxia, severe intraventricular hemorrhage, major congenital of facial malformations, neurological disorders or use of neuromuscular blockers. Patients were randomly allocated to receive a loading dose of 100 μg/kg morphine followed by a 10 μg/kg/h morphine infusion or sodium chloride infusion. COMFORT-B and VAS scores [11] were obtained twice daily. When patients were judged to be in pain or distress they were given an additional morphine dose. Arterial blood samples were obtained once or twice daily where possible during routine clinical monitoring.

2) Neonates with a postmenstrual age (PMA) > 37 weeks and a bodyweight ^{3}1500 g and infants aged up to three years undergoing major thoracic or abdominal surgery were eligible for inclusion. Exclusion criteria were use of analgesic or sedative co-medication, use of neuromuscular blockers, hepatic or renal dysfunction, seriously compromised neurological status or altered muscle tone. At the end of surgery all patients received an intravenous loading dose of 100 μg/kg morphine. Patients were randomly allocated to receive either a continuous morphine infusions of 10 μg/kg/h or three-hourly iv boluses of 30 μg/kg. Additional morphine doses were given if patients were judged to be in pain or distress based on COMFORT-B and VAS scores [11] that were assessed every three hours. Arterial blood samples were taken at baseline, 5-10 minutes after the loading dose and at 6, 12, and 24 hours after surgery. An additional sample was taken 24 hours after the last morphine dose or after discontinuation of the morphine infusion.

Analytical Method. Morphine, M3G and M6G serum concentrations were determined using an HPLC-MS method as described by Van der Marel *et al.* [12]. Intra- and inter-assay variability were lower than 10%.

Pharmacokinetic Analysis. NONMEM V (Globomax LLC, Hanover, MD) with Splus (version 6.2; Insightful software, Seattle, WA) for the visualization of the data, was used. The concentrations of morphine, M3G and M6G were expressed as μg morphine units per L, logarithmically transformed, and fitted simultaneously. Missing data were omitted from the modeling procedure. Model development was performed in four steps: 1) choice of the structural model, 2) choice of the error model 3) covariate analysis 4) validation of the model.

A decrease in objective function of more than 7.9 points between different (sub) models was considered to be statistically significant: this correlates with a value of $p<0.005$ based on a χ^2 distribution. In addition, the following plots were used for diagnostic purposes: A) observed *versus* individually predicted, B) observed *versus* population-predicted, C) time *versus* weighted residuals, D) population predictions *versus* weighted residuals. As the model was developed for prospective use, special focus was on plot B instead of the most commonly used A. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual improvement of the diagnostic plots were used to evaluate the model.

Covariate Analysis. Covariates were plotted independently against the individual post-hoc parameter estimates and the weighted residuals to visualize potential relationships. Covariates were tested in linear or allometric equations (equation 1 with k fixed to 1 or estimated) or as subpopulations in which a separate parameter is estimated for two or more subpopulations.

$$P_i = P_p \cdot (Cov / Cov_{standard})^k \qquad \text{(Equation 1)}$$

In this equation P_i and P_p represent individual and population parameter estimates respectively, *Cov* represents the covariate and $Cov_{standard}$ represents the standard value of the covariate. *k* represents the exponential scaling factor.

Based on the post-hoc plots the following covariates were tested: bodyweight, bodyweight at birth, body surface area, gender, postnatal age, postmenstrual age, bilirubin serum concentration, creatinine clearance, and mechanical ventilation (linear, allometric or subpopulations). Additionally, trial number (1 or 2), surgery *versus* non-surgery, and type of surgery were investigated as covariates (subpopulations). Missing information on covariates was indicated with a "."(dot) in the data file.

Potential covariates were separately incorporated into the model and considered statistically significant if the objective function decreased 7.9 points or more and the 95% confidence interval of the additional parameter did not include 0 (assuming normal distribution). When more than one significant covariate for the simple model was found, the covariate-adjusted model with the largest decrease in objection function was chosen as a basis to sequentially explore the influence of additional covariates with the use of the same criteria.

Internal Validation. There was a wide range in number and time points of drug administrations, and drug dosing for the individuals. Additionally observations were sparse. To validate a model based on a complicated dataset like this, the Normalized

Prediction Distribution Errors (NPDE) method recently developed by Brendel *et al.* [13,14] is very suitable. This method was implemented using the NPDE add-on software package that was run in R. Each observation was simulated 2000 times. The software then assembled the predictions in a cumulative distribution function (F) and determined the prediction discrepancy, which is defined as the value of F at the observed concentration. Prediction distribution errors were obtained by making decorrelations for multiple observations in one patient. These prediction distribution errors are expected to follow a uniform distribution over the interval [0,1]. Applying the inverse function of the normal cumulative density function subsequently yielded normalized prediction distribution errors which are expected to follow a normal distribution. The software performed standard statistical tests on the normal distribution. The Wilcoxon signed rank test indicates whether the mean of the NPDE is significantly different from 0, with the Fisher test for variance it is determined whether the variance is significantly different from 1.

Simulations. Generally, morphine is dosed on a μg per kg basis. With the developed pharmacokinetic model, it was simulated to what serum concentrations of morphine, M3G and M6G this practice leads in children with a postnatal age less than ten days weighing 0.5, 1, 2, 2.5, or 4 kg, and children with a postnatal age of ten days or older weighing 0.5, 1, 2, 2.5, 4, 10, or 17 kg, after they received a loading dose of 100 μg/kg morphine followed by a 10 μg/kg/h infusion, as was the case in the two studies.

Additional simulations were performed to establish morphine dosing regimens for children in these age and weight ranges that lead to more predictable serum concentrations of morphine and its metabolites. Serum concentrations were simulated in the same set of individuals that received a loading dose of 100 μg/kg followed by a 10 $\mu g/kg^{1.5}/h$ infusion. As target concentrations in the population are yet unknown, this amount for the infusion was chosen arbitrarily. To determine what dose reduction in neonates was needed to obtain similar morphine and metabolite concentrations in children above and below the age of ten days, simulations were performed in which the children with a postnatal age below ten days received reduced maintenance doses.

3.3 Results

Patients. The analysis was based on 792 morphine, 644 M3G and 722 M6G serum concentrations obtained from 248 newborns, including preterms, and infants. Patient characteristics are summarized in Table I.

Table I. *Demographic and clinical characteristics of the studied patient population*

	Preterm neonates 0 – 1 month **n = 64**	Term neonates 0-1 month **n = 59**	Infants & Toddlers 1 – 24 months **n = 113**	Children 2 – 3 years **n = 12**	**Total** **n = 248**
Age[a]					
PNA (days)	0.4 (0.2 – 0.7)	2 (1 – 6)	178 (91 – 315)	863 (763 – 919)	**33 (0.95 – 203)**
PMA (weeks)	30.1 (27.9 – 32.5)	40.4 (38.6 – 41.4)	60.4 (50.0 – 75.8) 13 unknown	161 (151 – 169) 2 unknown	**41.9 (35.6 – 62.6) 15 unknown**
Born preterm	64 (100)	0 (0)	33 (29) 13 unknown	1 (8) 2 unknown	**98 (39) 15 unknown**
Bodyweight[a]					
At birth (g)	1180 (863 – 1793)	3160 (2795 – 3510)	2857 (2040 – 3552) 19 unknown	3125 (2338 – 3573) 2 unknown	**2600 (1523 – 3328) 21 unknown**
At time of study (g)	1180 (863 – 1793)	3150 (2800 – 3580)	6500 (4500 – 8400)	12100 (11000 – 13750)	**3580 (2200 – 7000)**
Sex[a]					
Boy	36 (56)	38 (64)	66 (58)	4 (33)	**144 (58)**
Girl	28 (44)	21 (36)	47 (42)	8 (67)	**104 (42)**
Number of Available Samples					
Morphine	199	142	406	45	**792**
M3G	156	82	362	44	**644**
M6G	182	127	370	43	**722**
Blood Chemistry[a]					
Creatinine plasma concentration [μmol/l]	60 (43.8 – 77.8) 56 unknown	39.5 (28.0 – 52.8) 11 unknown	21.0 (14.0 – 36.5) 2 unknown	14.0 (12.0 – 21.3)	**25 (17 – 46)**
Bilirubin plasma concentration [μmol/l]	170 (134 – 234) 3 unknown	99 (41 – 145) 3 unknown	6.5 (5.0 – 11.3) 5 unknown	6.0 (5.0 – 7.3)	**38 (6 – 140)**
Ventilated[a]					
Yes	64 (100)	42 (71)	23 (20)	2 (17)	**131 (53)**
No	0 (0)	17 (29)	90 (80)	10 (83)	**117 (47)**
Surgical[a]					
No	55 (86)	8 (14)	0 (0)	0 (0)	**63 (25)**
Yes (superficial)	0 (0)	4 (7)	8 (7)	1 (8)	**13 (5)**
(thoracic)	3 (5)	10 (17)	13 (12)	2 (17)	**28 (11)**
(abdominal)	6 (9)	35 (59)	89 (78)	8 (58)	**138 (56)**
(thoracic & abdominal)	0 (0)	2 (3)	3 (3)	1 (8)	**6 (3)**

[a] Data are represented as median (25% - 75% percentile) or as n (%).
PNA = postnatal age, PMA = postmenstrual age, M3G = morphine 3-glucuronide, M6G = morphine 6-glucuronide

Model Optimization. The time course of the morphine serum concentrations was best described by a two-compartment model (V_1, V_2, and Q) and glucuronidation clearances Cl_1 and Cl_2. Morphine elimination through other routes (Cl_0) was found to be not significantly different from zero. V_1 and V_2 were initially estimated separately, and found to be not significantly different from each other. They were therefore fixed to be equal. The PK of the formed metabolites M3G and M6G was described by one-compartment models with volumes of distribution V_3 and V_4, and elimination clearances Cl_3 and Cl_4, respectively. The volumes of distribution V_3 and V_4 were estimated as a fraction of V_1 and found not to be significantly different from each other. They were therefore also fixed to be equal. In figure 1 a schematic representation of this model is shown.

Concerning inter-individual variability, log-normal distribution was found to describe the data most adequately. For the residual or intra-individual variability, a proportional error model was found, with a different error for morphine, M3G and M6G. On the concentrations that were determined 24 hours after discontinuation of the infusion an additional additive error with a similar value for morphine, M3G and M6G was found.

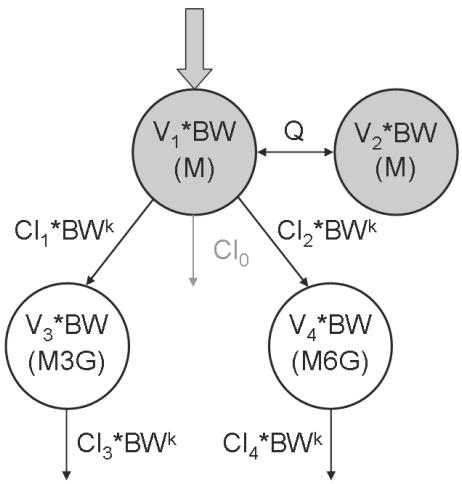


Figure 1. *Schematic representation of the pharmacokinetic model for morphine and its glucuronides. M=morphine, M3G=morphine-3-glucuronide, M6G=morphine-6-glucuronide, V_1=volume of distribution of central compartment of M, V_2= volume of distribution of peripheral compartment of M, V_3=volume of distribution of M3G, V_4=volume of distribution of M6G, Q=inter-compartmental clearance of M, Cl_0= M excretion by routes other than glucuronidation (not observed), Cl_1=formation clearance of M3G, Cl_2=formation clearance of M6G, Cl_3=elimination clearance of M3G, Cl_4=elimination clearance of M6G.*

Covariate Analysis. In the covariate analysis, bodyweight proved to be the most predictive for the formation clearances to M3G (Cl_1) and M6G (Cl_2), the elimination clearances of M3G (Cl_3) and M6G (Cl_4) and for the volumes of distribution. The influence of bodyweight on the clearances was best described using an allometric equation in which the exponential scaling factor (k) was estimated to be 1.44. Estimating different values for k for the different clearance parameters did not result in a significant decrease in objective function. The exponential scaling factor of the volumes of distribution was not significantly different from 1, indicating a linear relationship between bodyweight and volume of distribution.

Table II. *Population pharmacokinetic parameter estimates*

Parameter	Value	CV (%)
Fixed Effects		
k = exponential scaling factor	1.44	2.92
$Cl_{1\ PNA<10d}$ (ml/min/kg^k)	3.48	5.89
$Cl_{1\ PNA>10d}$ (ml/min/kg^k)	8.62	8.82
$Cl_{2\ PNA<10d}$ (ml/min/kg^k)	0.426	11.1
$Cl_{2\ PNA>10d}$ (ml/min/kg^k)	0.67	12.6
Cl_3 (ml/min/kg^k)	2.02	6.68
Cl_4 (ml/min/kg^k)	1.05	11.2
Q (ml/min)	29.6	17.8
$V_1 = V_2$ (l/kg)	1.81	7.62
$V_3 = V_4$ (fraction of V_1)	0.121	18.2
Inter-individual Variability		
$\omega^2(Cl_1)$	0.0671	25.9
$\omega^2(Cl_3)$	0.253	20.1
$\omega^2(Cl_4)$	0.146	13.9
$\omega^2(V_1)$	0.196	17.4
$\omega^2(Cl_3\text{-}Cl_4)$ interaction	0.164	13.7
Residual Error		
σ^2(morphine)	0.406	13.3
σ^2(morphine-3-glucuronide)	0.217	24.7
σ^2(morphine-6-glucuronide)	0.0844	13.6
$\sigma^{2,add}$(post infusion sample)	10.3	31.2

Cl=clearance, Q=inter-compartmental clearance, V=volume of distribution, PNA=postnatal age, ω^2=variance, σ^2= proportional intra-individual variance; $\sigma^{2,add}$= additive intra-individual variance; CV=coefficient of variation

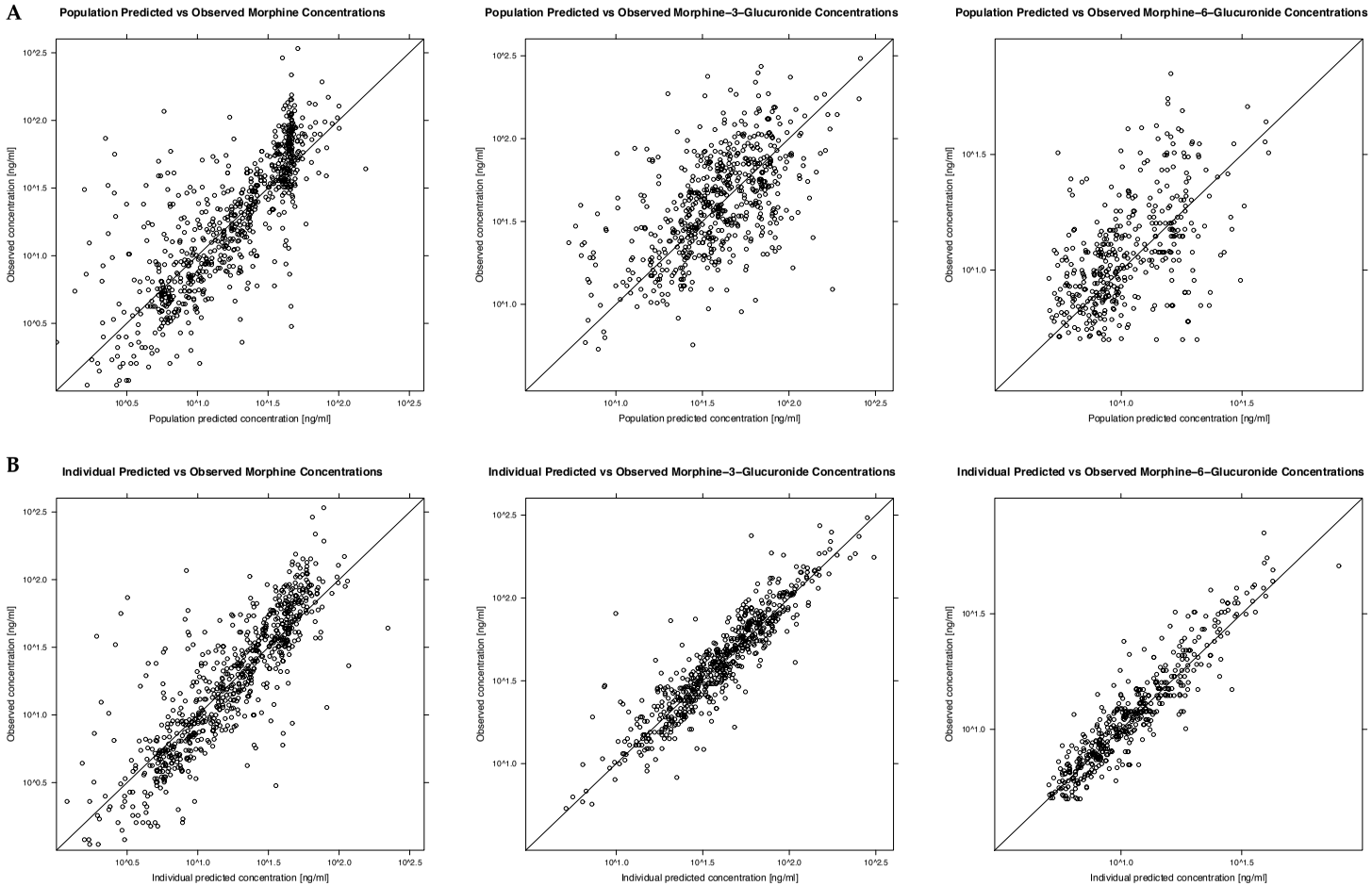


Figure 2. *Observed versus population predicted (a) and individual predicted (b) morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations.*

Postnatal age less than ten days proved to be an additional covariate for formation clearance to M3G and M6G, which was found to be independent of birth weight or postmenstrual age. Defining postnatal age as a continuous variable resulted in minimization difficulties. Selecting a period of ten days resulted in a lower objective function compared to 3, 7, 14 and 21 days. No other covariates could be identified. In table II all the parameter estimates obtained with the FOCE method are listed.

Figure 2 depicts the observed concentrations *versus* individually predicted (A) and model-predicted (B) concentrations of morphine and its glucuronides for the final model. Plots of weighted residuals *versus* PNA and PMA for term and preterm neonates are depicted in figure 3. In figure 4 estimated individual and population formation clearances to M3G (Cl_1) are plotted against bodyweight for children with a postnatal age more or less than ten days. After incorporation of the covariates inter-individual variability in the formation clearance to M6G (Cl_2) was not significantly different from zero. Figure 5 shows the estimated individual and population predictions of the elimination clearances of M3G (Cl_4) and M6G (Cl_5) *versus* bodyweight.

Validation. Figure 6 depicts the histograms of the NPDE for morphine and its metabolites. The lines indicate the normal distribution. The value of the mean and variance are given below each graph, with * and ** indicating a significant difference from 0 and 1 at respectively the $p<0.05$ and $p<0.01$ level as determined by the Wilcoxon signed rank test and Fisher test for variance. Plots of NPDE *versus* time after first dose and NPDE *versus* the log of the concentration for morphine and its metabolites are also shown.

Simulations. The model-based simulations depicted in figure 7a show the range of morphine, M3G and M6G serum concentrations predicted in children with a bodyweight varying between 0.5 and 17 kg and postnatal ages above (solid line) or below (dotted line) ten days that received a loading dose of 100 μg/kg morphine followed by a maintenance dose of 10 μg/kg/h.

A considerably narrower range of serum concentrations of morphine and it metabolites are predicted in this population when maintenance doses are given in $\mu g/kg^{1.5}/h$. Nevertheless, due to the lower glucuronide formation rates in children less than ten days of age, the concentrations obtained in these children are noticeably different from the concentrations obtained in children older than ten days (data not shown). A 50% reduction of the maintenance dose in the children younger than ten days resulted in an even narrower range of morphine and metabolite serum concentrations. Figure 7b shows these results of this simulation.

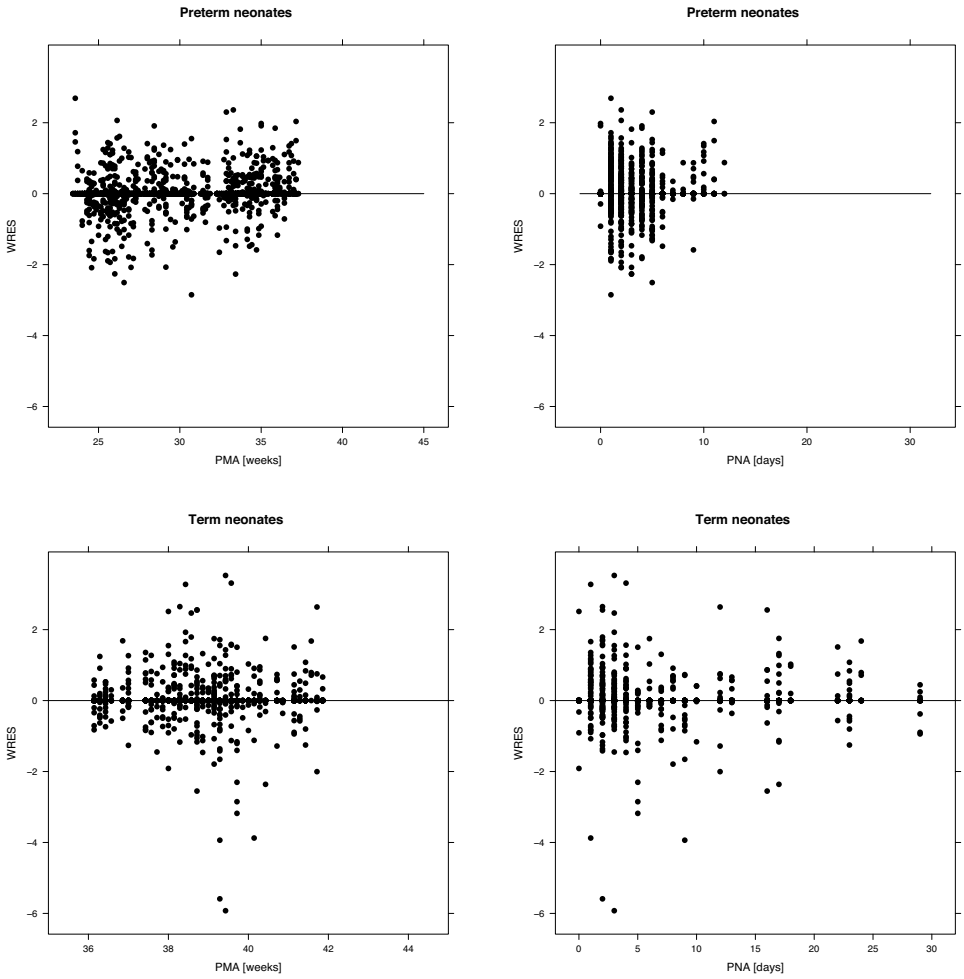


Figure 3. *Weighted residuals (WRES) for term and preterm neonates plotted versus postnatal age (PNA) and postmenstrual age (PMA).*

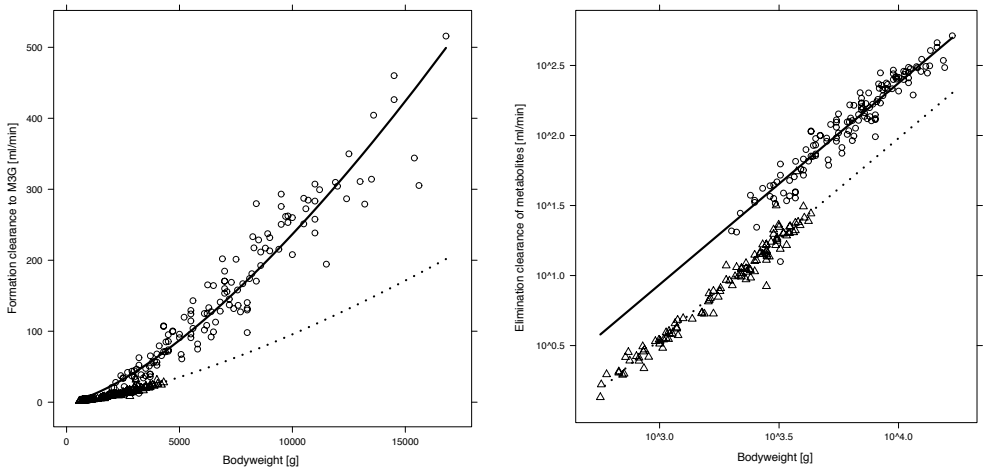


Figure 4. *Morphine formation clearance to morphine-3-glucuronide (M3G) (=Cl_1) versus bodyweight. Population prediction in children younger than ten days (dotted line) and older than ten days (solid line) and individual obtained values in children younger than ten days (triangles) and older than ten days (circles), on linear scale (left) and log scale (right).*

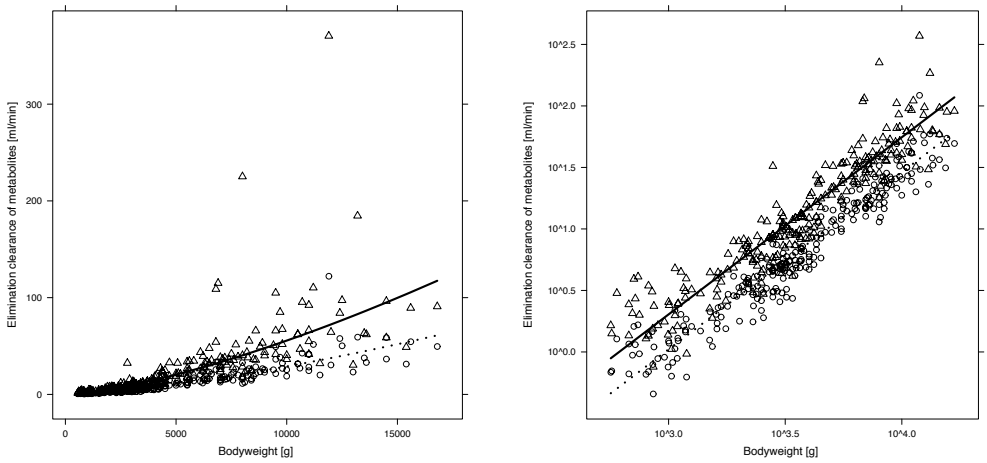
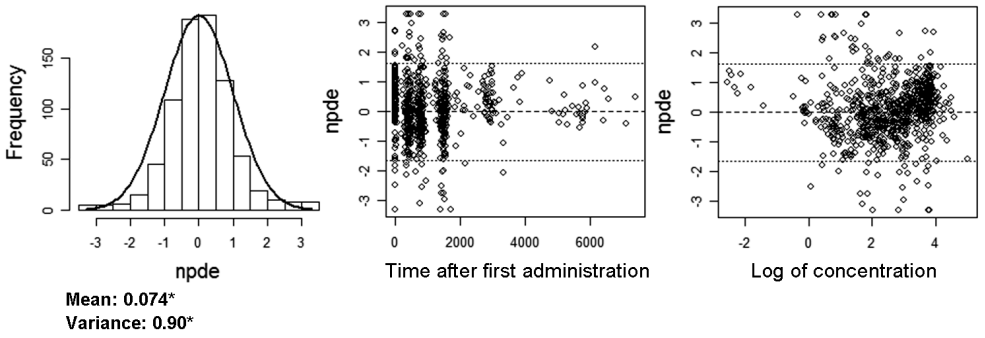
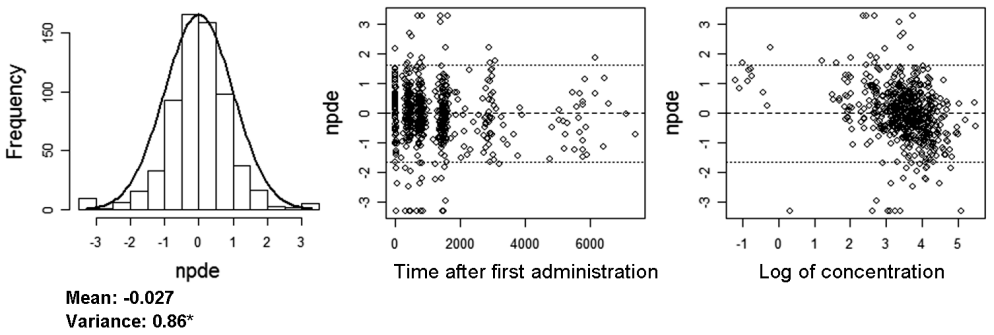


Figure 5. *Elimination clearance of morphine-3-glucuronide (M3G) (=Cl_3) and morphine-6-glucuronide (M6G) (=Cl_4) versus bodyweight. Lines are population predicted (solid for CL_3 and dotted for CL_4), symbols are individual obtained values (triangles for CL_3 and circles for CL_4), on linear scale (left) and log scale (right).*

morphine



morphine-3-glucuronide



morphine-6-glucuronide

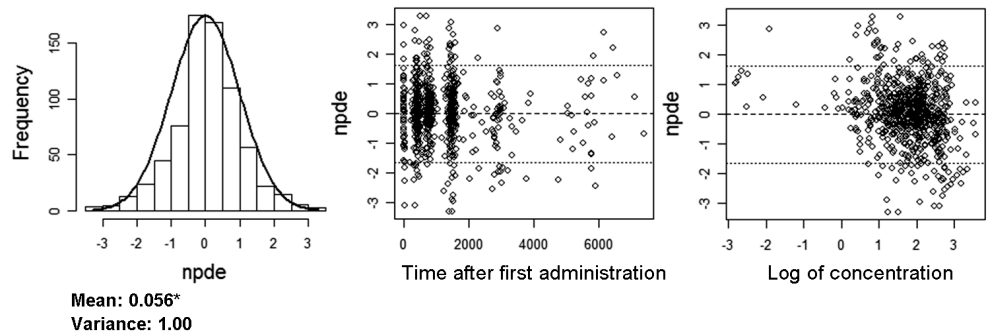


Figure 6. *Results of the internal validation with the Normalized Prediction Distribution Error (NPDE) method. The histograms show the NPDE distribution for morphine (top), morphine-3-glucuronide (middle), and morphine-6-glucuronide (bottom) and the solid line indicates a normal distribution. The value for the mean and variance of the NPDE distribution are given below each graph, with * indicating a significant difference of a mean of 0 and a variance of 1 at the p<0.05 level. The distribution of NPDE versus time after first dose and NPDE versus the log of the concentration are also shown.*

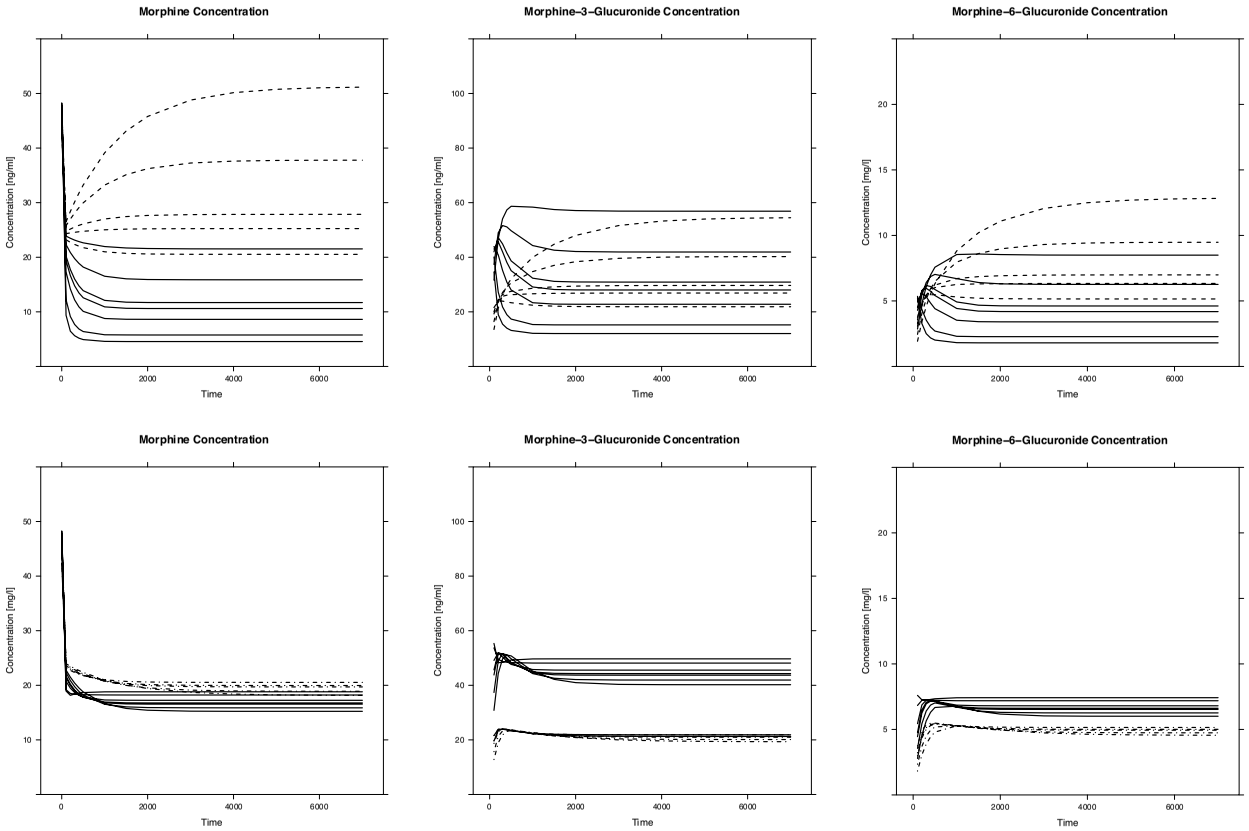


Figure 7. *Morphine, morphine-3-glucuronide, and morphine-6-glucuronide concentrations predicted in model-based simulations in children of 0.5, 1, 2, 2.5, 4, 10 and 17 kg and a postnatal age of less than ten days (dotted lines) or more than ten days (solid lines) based on a dosing regimen with a loading dose of 100 µg/kg and maintenance dose of 10 µg/kg/h (a) and based on a regimen with a loading dose of 100 µg/kg followed by an infusion of 10 µg/kg$^{1.5}$/h with a 50% reduction in maintenance dose for children younger than ten days (b).*

3.4 Discussion

To our knowledge, the population PK model developed in this study is the first that simultaneously describes and predicts morphine and its main metabolite concentrations in children ranging from preterm and term neonates up to infants approximately three years of age. Herewith, the model covers a wide and common age-group of the neonatal and paediatric intensive care, which is characterized by large maturational and developmental changes. The influences of these changes on the PK of morphine are now described in a quantitative manner. This study also proves that an adequate PK model can be developed based on sparse and unbalanced data obtained from routine clinical practice. Whereas often, both in adult and paediatric population pharmacokinetic models, proper internal validation is lacking [15,16], the model presented here is internally validated using an advanced tool for model validation.

Because the ontogenesis of clearance is believed to be the most critical determinant of a pharmacological response in infants and children [17], there is a specific interest in this parameter. An overview of morphine clearances in neonates and infants reported in the past two decades is given in table III.

It was found in this study that bodyweight, rather than body surface area or age, is the most predictive covariate for glucuronidation capacity of morphine under the age of three years. This glucuronidation capacity increases more than linearly with bodyweight and is best parameterized by a bodyweight-based power equation with an exponential scaling factor of 1.44. Recently, the allometric equation based on bodyweight with an exponential scaling factor of 0.75 has gained in popularity in the field of paediatrics. Originally designed to describe metabolic rates between different species covering a range of bodyweight of many orders of magnitude [32], this function is now being applied to parameterize the influence of changes in body size on clearance parameters within the human weight-range. After clearances are expressed as per 70 kg bodyweight an additional age-based equation needs to be estimated to describe maturation [33]. This method has been applied to morphine in a previous study that did not include preterm neonates. This model required, in addition to age, the use of two additional parameters (i.e. creatinine and bilirubin concentrations) to describe the time course of morphine across the whole age range [19]. Rather than incorporating a scaling function for bodyweight and subsequently estimating a function that describes maturation processes as a function of age, in the current study one single function based on bodyweight was estimated to describe the influence of all maturational and developmental changes on morphine elimination in children below the age of three years. By optimizing the

influence of bodyweight on glucuronidation clearance with an estimated scaling factor of 1.44, the influence of other covariates such as age, renal function and liver function became not significant, except for the influence of age on glucuronidation capacity in the first ten days of life.

For clearance we found the value of the allometric scaling factor to be higher than 1. Functions with an exponential scaling factor below 1 are characterized by a relatively high initial slope that levels-off (dome-shaped curve), whereas functions with an exponential scaling factor above 1 are characterized by a relatively low initial slope that increases (concave-shaped curve, see figure 4 and 5). Contrary to a previous study on morphine PK in young children [19], in the current study data from preterm neonates with a very low bodyweight were included. In preterm neonates, the initial maturation rate of elimination pathways is supposed to be very slow [34], a bodyweight allometric equation with an exponential scaling factor higher than 1 can therefore be expected across this population. By optimizing the influence of bodyweight with an exponential scaling factor of 1.44 the influence of other covariates was limited to a decreased glucuronidation rate in neonates with a postnatal age of less than ten days.

The increase in glucuronidation capacity will level-off at a certain age and bodyweight. This is not incorporated in the current model and therefore one of the limitations of the model is that no extrapolations can be made beyond the upper boundaries in bodyweight of our studied population.

The UGT2B7 isoenzyme is thought to be the major contributor to morphine glucuronidation [6,7,35]. The fact that the same scaling factor was found for formation clearance to both M3G and M6G appears to confirm that these metabolic routes mature at the same rate, which has been suggested before by others [36,37]. We found morphine metabolism to increase exponentially with bodyweight in the first three years of life, additionally we found a major increase ten days after birth. Interestingly, in concordance with these results, studies on zidovudine, the first antiviral drug approved for the treatment of HIV and AIDS in the paediatric population that is also predominantly glucuronidated by UGT2B7 [7,8], showed that its glucuronidation capacity increases dramatically in the first two weeks of life followed by a period of slower capacity increase of two years [38,39]. This suggests that the influence of maturational changes on morphine metabolism found in this study can be extrapolated to other exogenous and possibly endogenous substrates metabolized by the UGT2B7 enzyme, although this requires further study.

According to our model maturation of morphine glucuronidation is independent of PMA. Table III shows that reports on the influence of gestation on the maturation rate of morphine are ambiguous [18,21,29]. Additionally, Capparelli *et al.* [40] found zidovudine clearance to be reduced in preterm neonates compared to term neonates.

Table III. *Overview of morphine clearances in neonates and infants reported in the past 20 years.*

Publication	Population	Structural Model	Cl	Unit	Comments
Current model	0 – 3 yr, including term and preterm neonates	2 comp for morphine, 1 comp for M3G and 1 comp for M6G.	Formation M3G 8.62 M6G 0.67 Elimination M3G 2.02 M6G 1.05	ml/min/kg$^{1.44}$	BW is covariate for formation and elimination clearances of metabolites and for distribution volume. Formation of metabolites reduced in children with PNA <10 days
Anand et al. [18]	0 – 3 yr, including term and preterm neonates	1 comp for morphine, no metabolites.	Total body clearance 84.2 (1403)	l/h/70 kg$^{0.75}$ (ml/min/70kg$^{0.75}$)	BW is a covariate for clearance and volume of distribution. PMA is a covariate for clearance the influence of which is different in term and preterm neonates. PNA is a covariate for distribution volume, the correlation is similar for term and preterm neonates.
Bouwmeester et al. [19]	0 – 3 yr, only term neonates	1 comp for morphine, M3G & M6G. Includes unaccounted morphine elimination (Cl_{ex})	Formation M3G 64.3 (1071) M6G 3.63 (60.5) Elimination M3G 17.4 (290) M6G 5.8 (97) Cl_{ex} 3.12 (52)	l/h/70 kg$^{0.75}$ (ml/min/70kg$^{0.75}$)	BW is covariate for formation and elimination clearances of metabolites and for distribution volume. Bilirubin & creatinine clearance covariates for respectively formation and elimination of metabolites. PNA is a covariate for formation and elimination clearances of metabolites and on distribution volume.
Saarenmaa et al. [20]	Term & preterm neonates	Non-parametric	Total body clearance 2.4	ml/min/kg	Moderate influence of PMA on clearance. Also influence of BW on clearance.
Scott et al. [21]	Preterm neonates	Non-parametric	Total body clearance 2.27 – 7.80	ml/min/kg	There is a positive correlation between PMA and clearance
Lynn et al. [22]	0 – 1 yr, only term neonates	Non-parametric	Total body clearance 9.2 – 48.9	ml/min/kg	Clearance increased with PNA

Barrett et al. [23]	Neonates, mainly preterm	Non-parametric	Formation M3G 2.5 M6G 0.46 Elimination M3G 0.46 M6G 0.71	ml/min/kg	Diamorphine was administered. No influence of PMA and PK parameters could be identified
Mikkelsen et al. [24]	Term en preterm neonates	Non-parametric	Total body clearance 1.75 – 6.61	ml/min/kg	No influence of PMA on clearance but there is a negative correlation between PMA and elimination half-life
Hartley et al. [25]	Preterm neonates	Non-parametric	Total body clearance 2.4	ml/min/kg	Trend of increase in clearance with increase in birth weight and PMA
Pokela et al. [26]	0 – 6 mo	Non-parametric	Total body clearance 8.7 – 28.0	ml/min/kg	Clearance increases with PNA.
McRorie et al. [27]	0 – 2.5 yr	Non-parametric	Total body clearance 5 - 21	ml/min/kg	Morphine glucuronidation increased with PNA. Elimination through sulphation and unchanged renal clearance was indentified and found to be independent of PNA
Choonara et al. [28]	0 – 11 mo mostly term	Non-parameteric	Total body clearance neonates: 20.1 Infants: 23.4	ml/min/kg	M3G/morphine ratio in term neonates is higher than in preterm neonates, but lower than in infants.
Chay et al. [29]	Term & preterm neonates	Non-parametric	Total body clearance 1.65 – 2.09	ml/min/kg	PK parameters not significantly different in term and preterm neonates
Choonara et al. [30]	0 – 10 days & 1 – 16 yr	Non-parametric	Total body clearance neonates: 0.8 – 9.6	ml/min/kg	Formation of both metabolites increases after neonatal period in a parallel manner.
Olkkola et al. [31]	0 – 6 yr	Non-parameteric	Total body clearance neonate & infant < 3 mo: 5.2 Children: 25.8 – 75.6	ml/min/kg	Maturation of morphine PK is in first 6 months of life.

Figure 3 shows no trend in weighted residuals *versus* PNA and PMA for term and preterm neonates in our model, corroborating that with the current model morphine glucuronidation can be accurately predicted based on bodyweight and postnatal age alone.

Although reports are inconclusive, it has been suggested that especially in neonates, morphine is also partially cleared through sulphation [27,30] and unchanged renal excretion [19]. In this study Cl_0, which represents all elimination pathways other than glucuronidation to M3G and M6G, was found not to be statistically different from 0. Even in the preterm or very young neonates this parameter was not found to have any significance, suggesting that these pathways do not play a significant role in morphine clearance in the studied population.

In most publications, observed concentrations of a drug are often compared to individual model predictions, however especially when PK-PD models are developed for prospective simulations, it is important to also have accurate and unbiased population predictions. As the metabolites of morphine also possess pharmacological properties, it is not sufficient to be able to only predict morphine concentrations accurately, it is imperative to be also able to accurately predict the concentrations of the metabolites. Figure 2 shows accurate and unbiased distribution of both the individual and population predictions of morphine and its metabolites with the current model.

The internal validation procedure further corroborates the predictive value of the model developed in this article. Even though the statistical tests indicate a significant difference from the mean of 0 and variance of 1, this can be the result of the NPDE method not being fully optimized yet. The developers indicate that especially for large datasets the graphic output should be considered as well to determine whether the model sufficiently describes the data [14]. According to the histograms in figure 6 the model can quite accurately predict median concentrations in the population, however the variability appears to be slightly over-predicted by the model. Additionally figure 6 shows no trend in NPDE over time or over the concentration range. Considering that on average only four samples were available for each individual, we believe the results to be remarkable. Since for the simulations in this article only population predictions were used, this model deficiency has no substantial effect on the inference made here. Future inferences made on model based simulations could be influenced by the over-prediction of the variability, however we believe model based predictions would always be on the conservative side, as the actual variability is expected to be slightly less than what is predicted.

The model based simulations in figure 7a demonstrate that a wide concentration range is predicted for both morphine and its glucuronides, when dosing morphine in mg per kg bodyweight in children weighing less than 17 kg. Because distribution volume was found to change linearly with bodyweight and clearances was found to change exponentially with a scaling factor close to 1.5, a dosing regimen with a loading dose in μg/kg and a maintenance dose in $\mu g/kg^{1.5}/h$ was expected to yield drug and metabolite serum concentrations in a narrower range for all the children in this weight range. Indeed, simulations prove this to be the case. The influence of the reduced glucuronidation capacity on morphine serum concentrations in the first ten days of life can be compensated by a 50% reduction of the maintenance dose in patients with a postnatal age of less than ten days. This dose reduction also results in more similar M3G and M6G serum concentrations between patients younger and older than ten days, although there is still a marked difference in the metabolite serum concentrations between these patient groups (figure 7b).

One should bear in mind that the optimal dosing regimen should result in safe and effective pharmacological responses which may not *per se* mean a similar drug serum concentration across the whole population. Therefore the influence of the developmental stage of a child on the relationship between drug concentration and drug effect needs to be determined. As M6G, like morphine, is believed to exert analgesic actions with high potency through binding to the μ-opioid receptor [41–43] and as there is some evidence that M3G may functionally antagonize the analgesic effect of morphine [44,45], both metabolites need to be incorporated in this PD analysis. This investigation will be part of future studies of our group and will yield evidence-based and age-specific target concentrations of morphine for our study population. The current PK model can then be used to define final dosing recommendations. Additionally, the clinical importance of the differences in metabolite concentrations that still exist in the current model between the patients older and younger than ten days after the 50% dose reduction can be determined based on the PD investigation.

3.5 Conclusion

Based on an analysis of sparse data in newborns, including preterms, and infants under the age of three years, using morphine as a model drug, maturation of glucuronidation by the UGT2B7 enzyme was described. It was found that this glucuronidation capacity as well as elimination clearance of morphine glucuronides can be best described by a bodyweight-based power equation with an exponential scaling factor of 1.44 in this population. Within this power equation clearances to glucuronides are decreased in neonates younger than ten days. Model-based simulations showed that a narrow range of morphine and metabolite concentrations is obtained across the studied population when morphine infusions are administered per $kg^{1.5}$ per hour with a 50% reduction in neonates younger than ten days. Definitive dosing recommendations for morphine can be made after safe and effective target concentrations of morphine and its metabolites in this population are determined and after prospective studies have been performed. Additionally, the investigation of the possibility to extrapolate the findings on UGT2B7 maturation to other drugs metabolized by the same enzyme is part of future investigations.

Acknowledgments

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Appendix.

Equations for the final model in NONMEM ADVAN5.
For morphine:

$$\begin{cases} \dfrac{dA_{M1}}{dt} = R_{\text{inf }usion} - k_{12} \cdot A_{M1} + k_{21} \cdot A_{M2} - k_{13} \cdot A_{M1`} - k_{14} \cdot A_{M1} \\ \dfrac{dA_{M2}}{dt} = k_{12} \cdot A_{M1} - k_{21} \cdot A_{M2} \end{cases}$$

For morphine-3-glucuronide

$$\frac{dA_{M3G}}{dt} = k_{13} \cdot A_{M1} - k_{30} \cdot A_{M3G}$$

For morphine-6-glucuronide

$$\frac{dA_{M6G}}{dt} = k_{14} \cdot A_{M1} - k_{40} \cdot A_{M6G}$$

in which:

$$k_{12} = Q / V_{M1\_ind}$$

$$k_{21} = Q / V_{M2\_ind}$$

$$k_{13} = Cl_{1\_ind} / V_{M1\_ind}$$

$$k_{30} = Cl_{3\_ind} / V_{M3G\_ind}$$

$$k_{14} = Cl_{2\_ind} / V_{M1\_ind}$$

$$k_{40} = Cl_{4\_ind} / V_{M6G\_ind}$$

and:

$$Cl_{1\_ind} = (Cl_{1\_pop} \cdot BW^{k}) \cdot \exp(\omega^2_{Cl1})$$

$$Cl_{2\_ind} = Cl_{2\_pop} \cdot BW^{k}$$

$$Cl_{3\_ind} = (Cl_{3\_pop} \cdot BW^{k}) \cdot \exp(\omega^2_{Cl3})$$

$$Cl_{4\_ind} = (Cl_{4\_pop} \cdot BW^{k}) \cdot \exp(\omega^2_{Cl4})$$

$$V_{M1\_ind} = V_{M2\_ind} = (V_{M1\_pop} \cdot BW) * \exp(\omega^2_{v1})$$

$$V_{M3G\_ind} = V_{M6G\_ind} = V_{M1\_pop} \cdot BW \cdot fraction\_of\_V_{M1}$$

In which *A* represents the amount of morphine equivalents in the designated compartment (*M1* and *M2* for the central and peripheral compartment of morphine respectively, *M3G* for morphine-3-glucuronide and *M6G* for morphine-6-glucuronide). *t* represents time, $R_{infusion}$ represents the morphine infusion rate, and *Q* represents the equilibrium constant for the two morphine compartments. Clearances are indicated by *Cl*, subscripts 1 and 2 indicate formation clearances of M3G and M6G respectively and subscripts 3 and 4 indicate elimination clearances of M3G and M6G. For the formation of the metabolites a different value for CL_{pop} is calculated for children older and younger than ten days. Distribution volumes are indicated by V, subscripts M1 and M2 indicating respectively the central and peripheral compartment for morphine and M3G and M6G indicating the metabolite compartments. Individual and population parameters are indicated by subscripts *ind* and *pop* respectively. ω indicates the inter-individual variability on the

designated parameter. *BW* stands for bodyweight and *k* is the exponential scaling factor. The distribution volumes of the metabolites are calculated as a fraction of V_{M1} the value of which is estimated and represented by *fraction_of_* V_{M1}.

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Chapter 4

Predictive Performance of a Recently Developed Population Pharmacokinetic Model for Morphine and its Metabolites in New Datasets of (Preterm) Neonates, Infants and Children

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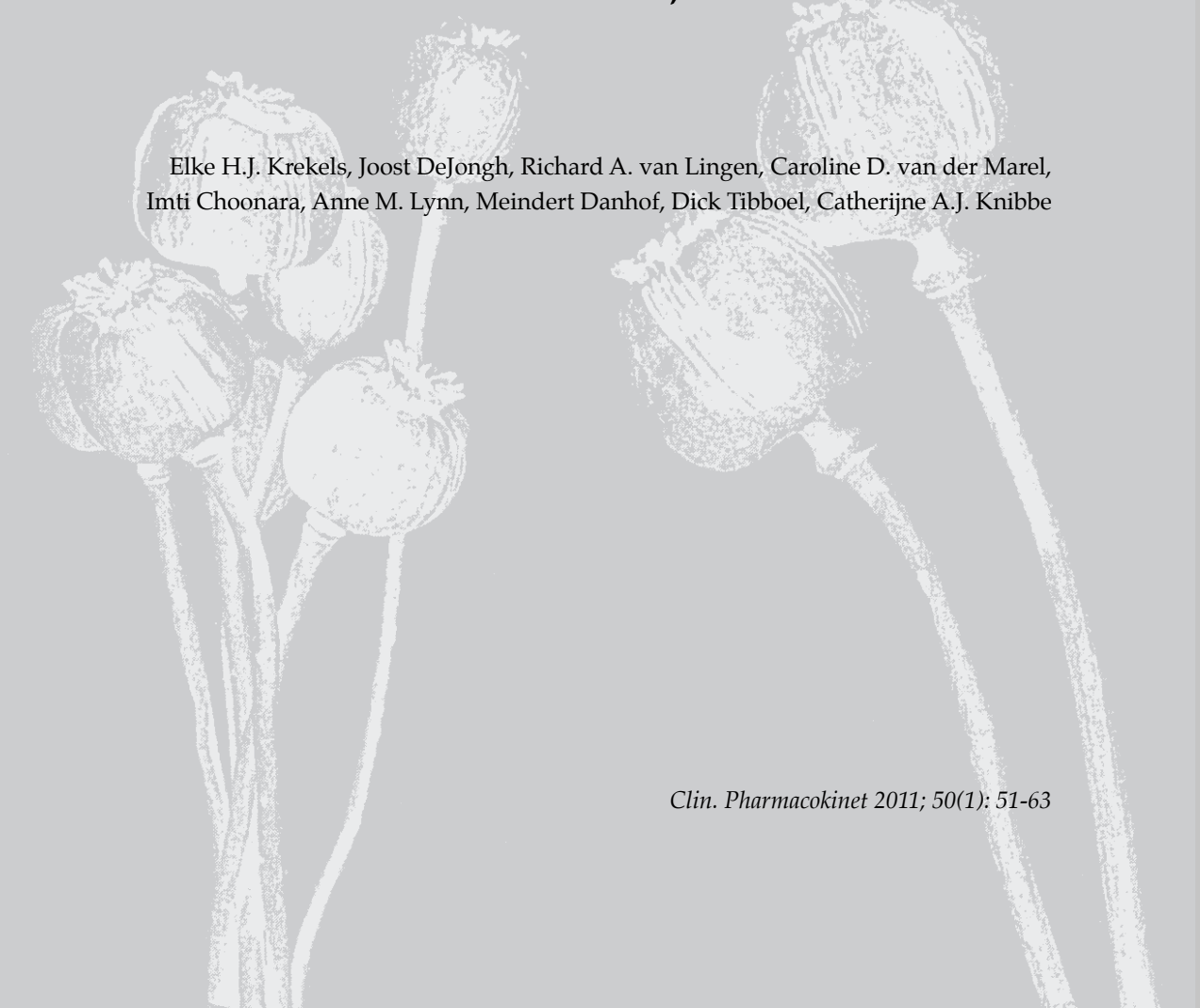

Clin. Pharmacokinet 2011; 50(1): 51-63

Abstract

Background and Objective: Model validation procedures are crucial when models are to be used to develop new dosing algorithms. In this study, the predictive performance of a previously published paediatric population pharmacokinetic model for morphine and its metabolites in children younger than three years (original model) is studied in new datasets that were not used to develop the original model.

Methods: Six external datasets including neonates and infants up to one year were obtained from four different research centres. These datasets contained postoperative patients, ventilated patients and patients on extracorporeal membrane oxygenation (ECMO) treatment. Basic observed *versus* predicted plots, normalized prediction distribution error analysis, model refitting, bootstrap analysis, subpopulation analysis and a literature comparison of clearance predictions were performed with the new datasets to evaluate the predictive performance of the original morphine pharmacokinetic model.

Results: The original model was found to be stable and the parameter estimates were found to be precise. The concentrations predicted by the original model were in good agreement with the observed concentrations in the four datasets from postoperative and ventilated patients, and the model-predicted clearances in these datasets were in agreement with literature values. In the datasets from patients on ECMO treatment with continuous venovenous haemofiltration (CVVH) the predictive performance of the model was good as well, whereas underprediction occurred, particularly for the metabolites, in patients on ECMO treatment without CVVH.

Conclusion: The predictive value of the original morphine pharmacokinetic model is demonstrated in new datasets by the use of six different validation and evaluation tools. It is herewith justified to undertake a proof-of-principle approach in the development of rational dosing recommendations – namely, performing a prospective clinical trial in which the model-based dosing algorithm is clinically evaluated.

4.1 Background

Adequate validation studies to establish the predictive performance of population pharmacokinetic (PK) and/or pharmacodynamic (PD) models are often lacking both in the adult and paediatric population [1,2]. Validation procedures are crucial when models are to be used for simulation exercises. Model simulations can for instance be used to optimize dosing algorithms that take individual characteristics such as bodyweight and age into account. Additionally, simulations can be useful in setting-up clinical trials optimizing the information that is obtained while minimizing the burden to each individual in the trial by reducing the number of blood samples that need to be obtained. Without proper validation a model can only be regarded descriptive, limiting the safe use of these models for clinical and research applications.

Validation methods have been classified into three categories [2]: (i) basic internal methods (e.g. basic goodness-of-fit plots, uncertainty in parameter estimates and model sensitivity to outliers); (ii) advanced internal methods (e.g. data splitting, resampling techniques and Monte Carlo simulations); (iii) external validation (comparing observations in a new external dataset to predictions obtained using the model that was built on an internal dataset). Additionally, the aptness of model-based dosing algorithms should be assessed in confirmatory prospective clinical trials [3].

Ethical and practical constraints in paediatric studies may complicate the validation steps of paediatric models. Firstly, paediatric studies are often performed during routine clinical practice leading to high variability in drug administration due to different individual needs. Standard validation tools such as a visual predictive check may then not suffice and more sophisticated tools are required. Additionally, the paediatric population is relatively diverse due to the many maturational changes between preterm newborns and 18 year old adolescents, therefore diagnostic tools should not only be applied to the dataset of the population as a whole but also to various (age) subgroups in a dataset. Finally, the limited number of studies performed in this population makes external datasets less available and due to limited numbers of patients in paediatric studies the use of part of the dataset for model building and the other part for the external validation is often not viable either.

Recently a population PK model for morphine and its two major metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) was developed based on data from postoperative and ventilated neonates (including preterms), infants and children up to the age of three years (Chapter 3). This model was validated internally using basic and advanced validation methods and will be referred to as the 'original model'. In the current study the predictive performance of this model and its suitability

for simulation purposes is assessed in an external validation study with six new external datasets using basic and advanced validation methods.

4.2 Methods

Patients and Data

For the external validation study six datasets were available [4–10]. All studies had been approved by local ethic committees and informed parental consent was obtained. The datasets contained patient data not linked to identifiable patient information. An overview of the internal and external datasets is given in table I.

The studies were performed at four different centers, in three different countries and two different morphine salts were administered. To compare the administered doses the amount of administered morphine base was calculated for each individual.

Original Model

A schematic representation of the original model is shown in figure 1. In this model distribution volumes are scaled linearly with bodyweight. Formation clearances (CL_1 and CL_2) and elimination clearances (CL_3 and CL_4) of the morphine metabolites were best described by a bodyweight-based allometric equation with an estimated exponential scaling factor of 1.44. Additionally, within this power-function formation of the metabolites (CL_1 and CL_2) is reduced in neonates younger than ten days.

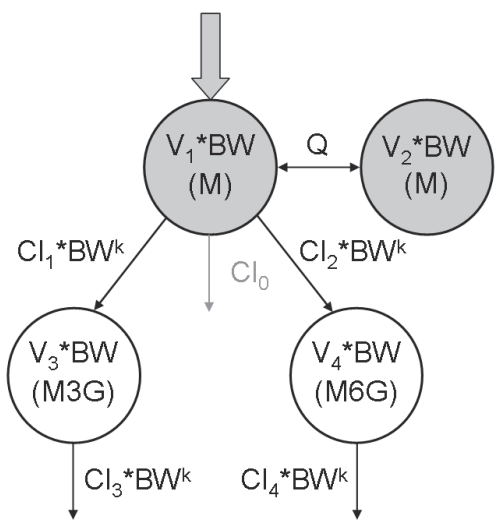

Figure 1. *Schematic representation of the original peadiatric population PK model for morphine and its glucuronides in children younger than three years.*

Table I. *Overview of the internal datasets (Int.) used to develop the original morphine model and external datasets (Ext) in the current external validation of the original morphine model.*

Dataset	Patient Population	Research Center	Number of Patients	Number of Samples	Postnatal Age in days (median, range)	Bodyweight in g (median, range)	Administered Morphine Salt
Int.1. [11]	Postoperative term neonates, infants and children.	Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands)	185	M: 618 M3G: 512 M6G: 594	97 (0.1 - 1070)	4700 (1900 – 16800)	Morphine hydrochloride
Int.2. [12]	Preterm and term neonates on artificial ventilation	Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands) Isala Clinics (Zwolle, The Netherlands)	63	M: 110 M3G: 132 M6G: 128	0.4 (0.1 – 6.7)	1180 (565 – 3875)	Morphine hydrochloride
Ext.1. [4]	Preterm neonates on artificial ventilation	Isala Clinics (Zwolle, The Netherlands)	41	M: 88 M3G: 111 M6G: 65	1 (0.1 – 13)	1035 (640 – 3550)	Morphine hydrochloride
Ext.2. [5]	Postoperative term neonates and infants	Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands)	28	M: 98 M3G: 122 M6G: 115	14 (0.1 – 294)	3100 (1700 – 9300)	Morphine hydrochloride
Ext.3. [6]	Postoperative term neonates and infants	Children's Hospital and Regional Medical Center, (Seattle, WA, USA)	9	M: 16	10.5 (1 – 271)	3800 (2640 – 8100)	Morphine sulphate
Ext.4. [7]	Term neonates and infants on artificial ventilation	Alder Hey Children's hospital (Liverpool, UK)	12	M: 8 M3G: 12 M6G: 10	13 (3 – 354)	3050 (2200 – 8700)	Morphine sulphate
Ext.5. [8,9]	Term neonates on ECMO treatment without CVVH	Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands)	14	M: 328 M3G: 326 M6G: 296	1.1 (0.1 – 26.1)	3220 (2150 – 4520)	Morphine hydrochloride
Ext.6. [10]	Term neonates on ECMO treatment with CVVH	Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands)	16	M: 167 M3G: 197 M6G: 195	0.5 (0 – 7)	3250 (2700 – 4000)	Morphine hydrochloride

ECMO = extracorporeal membrane oxygenation
CVVH = continuous venovenous hemofiltration

Model Validation and Evaluation

NONMEM VI (ICON, Ellicott City, MD) was used for all model-based simulations and model fitting in the current study.

Simulations were performed based on dosing regimen, bodyweight and postnatal age of the children in the new external datasets, to obtain model-based population predicted concentrations. These predicted concentrations were then plotted *versus* the concentrations that were actually observed in these datasets. As population predicted concentrations are based on the fixed effects of the model, this analysis allows for the assessment of the predictive performance of both the structural model, encompassing the parent drug and metabolite model, and the covariate model, encompassing the relationships between the patient characteristics bodyweight and age and the model parameters clearance and distribution volume.

Additionally, a normalized prediction distribution error (NPDE) analysis was performed using the add-on software package that was run in R [13,14]. One-thousand model-predicted concentrations were generated for each observation in the external datasets with simulations based on dosing regimen, bodyweight and age of the patients and with the parameter values (including the inter-individual and residual variability) that were obtained for the original model. The observed concentrations in the external datasets were subsequently compared to these 1000 predicted concentrations [13,14]. In addition to assessing the structural and covariate model, this validation tool also allows for the assessment of how well the model predicts variability within the population.

When the plots of the predicted *versus* observed concentrations and the NPDE analysis showed no trends or bias in the external datasets of the postoperative and ventilated patients (Ext.1 – 4), these datasets were merged and analyzed together. These four external datasets were then combined with the internal datasets used to develop the original model and refitted to this model simultaneously. The resulting parameters could then be compared to the parameters obtained in the original model fit. Additionally, this combined dataset was used in a bootstrap analysis using the PSN software package [15]. For the bootstrap analysis the combined dataset was resampled 500 times and these resampled datasets were subsequently refitted to the model. All parameter estimates were then summarized as means and standard errors and could be compared to the results of the original model fit. Both the model refit and the bootstrap analysis give insight into model stability and the uncertainty of the parameter estimates of the model.

The performance of the covariate model was evaluated in the combined internal and external dataset by investigating subpopulations. It was examined whether bodyweight or age is the best descriptor for the maturation of the PK parameters by plotting *post hoc* PK parameter values obtained from the simultaneous refit *versus* bodyweight and age (both postnatal and postmenstrual) for small for gestational age

(SGA) and appropriate for gestational age (AGA) neonates. Neonates are considered to be SGA when their birth weight is below two standard deviations of the mean birth weight at that gestational age. All other neonates are considered to be AGA.

Lastly, model-predicted total morphine clearances for both the internal and merged external datasets were compared to morphine clearances published in the past twenty years. These reference values were derived from population PK models [16,17] or obtained non-parametrically [7,18–27]. For the original morphine model, total morphine clearances were calculated for each individual in the combined internal and external dataset as the sum of both metabolite formation clearances (CL_1 and CL_2) obtained in the simultaneous model refit. This was done for both population parameter estimates and individual *post hoc* parameter estimates. Population clearance parameters from publications that used a model-based approach and average clearances from publications that used a non-parametric approach were used together with individual patient characteristics (i.e. bodyweight, age and bilirubin concentrations), to calculate the reference total morphine clearances. This was only calculated for individuals in the combined internal and external dataset that met the inclusion criteria of the study described in a particular reference publication. All obtained clearance parameters were subsequently plotted *versus* bodyweight.

Although the original model was not based on data of patients on extracorporeal membrane oxygenation (ECMO) treatment, the predictive performance of the original model was also tested in two datasets with ECMO patients (Ext. 5 & 6). The datasets were evaluated individually by making plots of population predicted concentration *versus* observed concentrations and by performing an NPDE analysis as described above. Due to the inconclusive results on the predictive performance of the model in these two datasets, they were not merged or combined with the internal or other external datasets.

4.3 Results

The external datasets included a total of 37 non-cardiac postoperative patients, 53 ventilated patients, and 30 patients on ECMO treatment, with a total of 705 morphine, 668 M3G, and 681 M6G concentrations. Detailed information on the internal datasets (Int. 1 & 2) and the new external datasets (Ext. 1 – 6) is given in table I.

No trends or biases were observed in the predicted *versus* observed plots and the NPDE results of the four external datasets with postoperative and ventilated patients (Ext. 1 – 4). Figure 2a depicts a plot of the model-based population predicted concentrations *versus* the observed concentrations in these dataset using different symbols for the different datasets. In figure 3 the results of the NPDE analysis are shown, including the NPDE frequency distribution with the mean and standard deviation of this distribution, the NPDE distribution in time and the NPDE distribution *versus* the log value of the concentration. The plots show limited trends or biases in the predictions by the model.

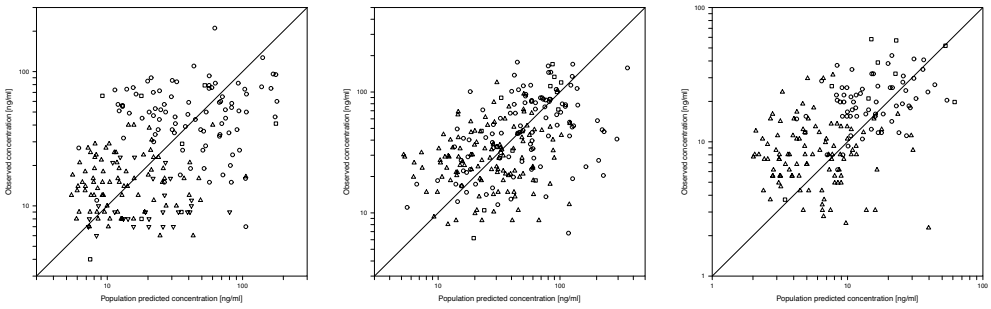

Figure 2. *Results of the external validation representing the concentration predicted by the original model versus the concentrations observed in the external datasets of postoperative and ventilated patients (Ext.1 – Ext.4) for morphine, morphine-3-glucuronide and morphine-6-glucuronide.* ○ *= Ext.1 preterm neonates on artificial ventilation* [4], △ *= Ext.2 non-cardiac postoperative term neonates and infants* [5], ▽ *= Ext.3 non-cardiac postoperative term neonates and infants* [6], □ *= Ext.4 term neonates and infants on artificial ventilation* [7].

Table II. *Parameter estimates obtained in the original fit of the model based on the internal dataset (Int. 1 & 2), and the refit and bootstrap of the combined internal and external datasets for postoperative and ventilated patients (Int. 1 & 2 and Ext. 1 – 4).*

Parameters	**original model fit** internal dataset	**refit of the model** internal & external dataset	**Bootstrap** internal & external dataset
	Value (CV%)	*Value (CV%)*	*Value (CV%)*
Fixed effects			
k = exponential scaling factor	1.44 (2.92)	1.44 (2.69)	1.44 (2.62)
$Cl_{1\ PNA < 10\ d}$ (ml/min/kg^k)	3.48 (5.89)	3.09 (5.15)	3.07 (8.37)
$Cl_{1\ PNA > 10d}$ (ml/min/kg^k)	8.62 (8.82)	8.25 (8.18)	8.27 (8.09)
$Cl_{2\ PNA < 10d}$ (ml/min/kg^k)	0.426 (11.1)	0.408 (11.4)	0.410 (10.8)
$Cl_{2\ PNA > 10d}$ (ml/min/kg^k)	0.67 (12.6)	0.699 (12.4)	0.714 (11.8)
Cl_3 (ml/min/kg^k)	2.02 (6.68)	2.19 (5.43)	2.19 (5.53)
Cl_4 (ml/min/kg^k)	1.05 (11.2)	1.11 (11.5)	1.12 (11.0)
Q_{eq} (ml/min)	29.6 (17.8)	28.9 (16.6)	29.7 (16.1)
$V_1 = V_4$ (l/kg)	1.81 (7.62)	1.99 (6.48)	1.99 (6.43)
$V_2 = V_3$ (fraction of V_1)	0.121 (18.2)	0.119 (17.2)	1.22 (17.1)
Inter-individual variability			
ω^2 Cl_1	0.0671 (25.9)	0.104 (18.4)	0.103 (18.5)
ω^2 V_1	0.196 (17.4)	0.23 (18.1)	0.223 (17.9)
ω^2 Cl_3	0.253 (20.1)	0.258 (16.7)	0.253 (16.4)
ω^2 Cl_4	0.146 (13.9)	0.185 (14.2)	0.184 (14.2)
ω^2 Cl_3-Cl_4 interaction	0.164 (13.7)	0.178 (13.4)	0.177 (13.2)
Residual error			
$\sigma^{2,\ prop}$ (morphine)	0.406 (13.3)	0.371 (5.94)	0.368 (11.7)
$\sigma^{2,\ prop}$ (M3G)	0.217 (24.7)	0.206 (19..4)	0.204 (19.0)
$\sigma^{2,\ prop}$ (M6G)	0.0844 (13.6)	0.0967 (12.0)	0.0959 (12.0)
$\sigma^{2,\ add}$ (24 hr post-infusion samples)	10.3 (31.2)	9.36 (30.2)	9.08 (29.5)

Table II gives an overview of (i) parameter estimates obtained in the original model fit (Chapter 3); (ii) parameter estimates of the model refit of the combined internal and four merged external datasets (Int. 1 & 2 and Ext. 1 – 4); (iii) the parameter estimates obtained in the bootstrap of the model with the combined internal and external dataset.

In the combined internal and external datasets, there were 168 neonates of which birthweight and gestational age at birth was known. Of these neonates 24 (=14%) were SGA. In figure 4 the individual *post hoc* parameter estimates of four model parameters are plotted *versus* bodyweight and postmenstrual age using different symbols for SGA and AGA neonates. The figure shows that when distribution volume (V_1) and M3G formation (CL_1) are plotted *versus* bodyweight, the individual *post hoc* estimates for SGA neonates are in line with the *post hoc* estimates for the AGA neonates, which is not the case when they are plotted *versus* postmenstrual age. Additionally the individual *post hoc* parameter estimates of the elimination clearance of M3G and M6G (CL_3 and CL_4) of the SGA neonates are shifted to the left compared to the *post hoc* estimates of the AGA neonates when plotted *versus* bodyweight. When plotted *versus* postmenstrual age the *post hoc* estimates of the elimination of the morphine metabolites of the SGA neonates are more in line with the values of the AGA neonates.

Figure 5 shows total morphine clearance values *versus* bodyweight. Population and individual *post hoc* predictions from the original model are shown in solid and open black circles respectively. The two lines represent children that are older and younger then ten days. Clearances calculated based on previously published clearance values are depicted with grey solid circles.

Figure 6 shows the plot of population predicted concentrations *versus* observed concentrations for the two datasets that included children on ECMO treatment (Ext.5 & 6) using different symbols for the two datasets. Predictions for dataset 6 are less biased than for dataset 5, particularly for the metabolites. In figure 7 the results of the NPDE analysis using only external dataset 6 are shown, showing limited trends or biases in the NPDE distributions.

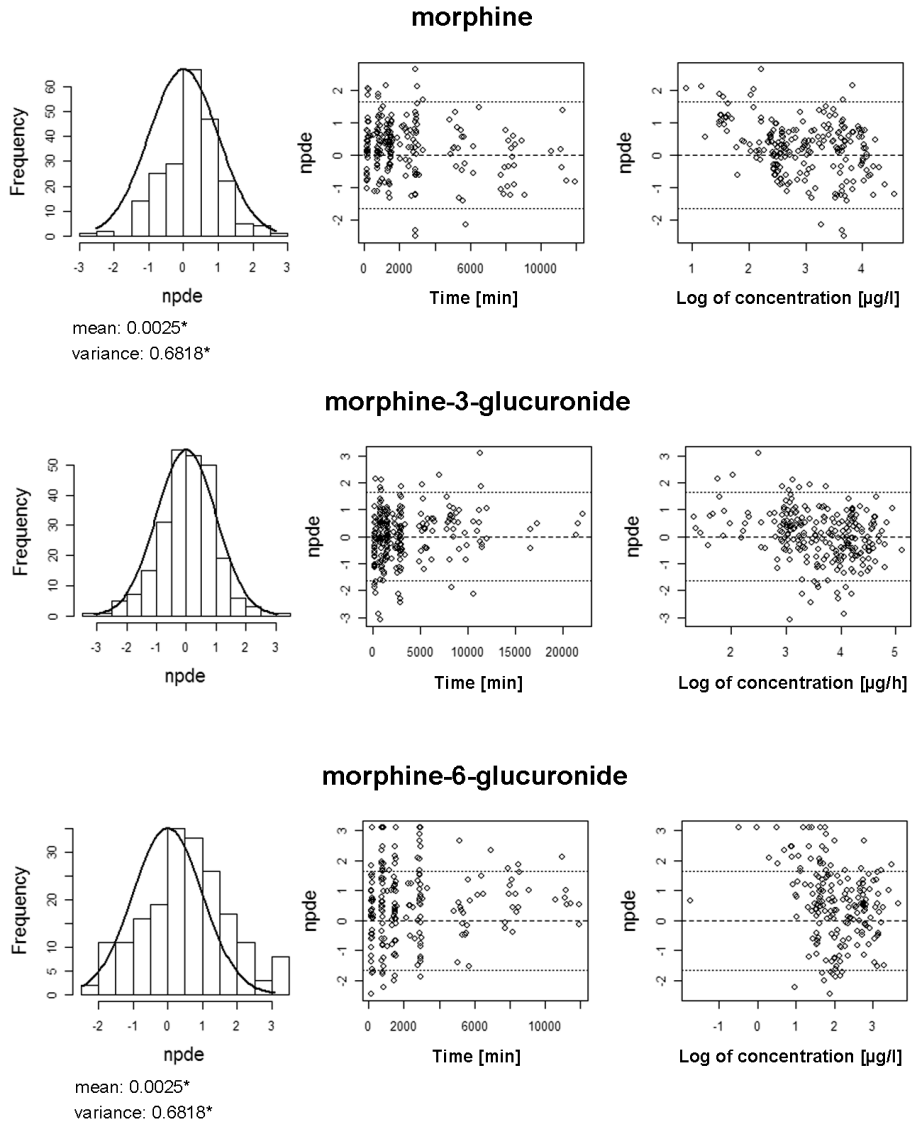

Figure 3. *Results of the external validation with the NPDE method using external datasets of postoperative and ventilated patients (Ext.1 – Ext.4). The histograms show the NPDE frequency distribution in the merged external dataset for morphine, morphine-3-glucuronide and morphine-6-glucuronide, the solid line indicates a normal distribution. The values for the mean and variance of the NPDE distribution are given below each histogram with * indicating a significant difference of a mean of 0 and a variance of 1 at the $p < 0.05$ level as determined by the Wilcoxon signed rank test and the Fisher test of variance. The distribution of NPDE versus time after first dose and NPDE versus the log of the concentration are also shown. The dotted lines represent the 90% distribution of the NPDE.*

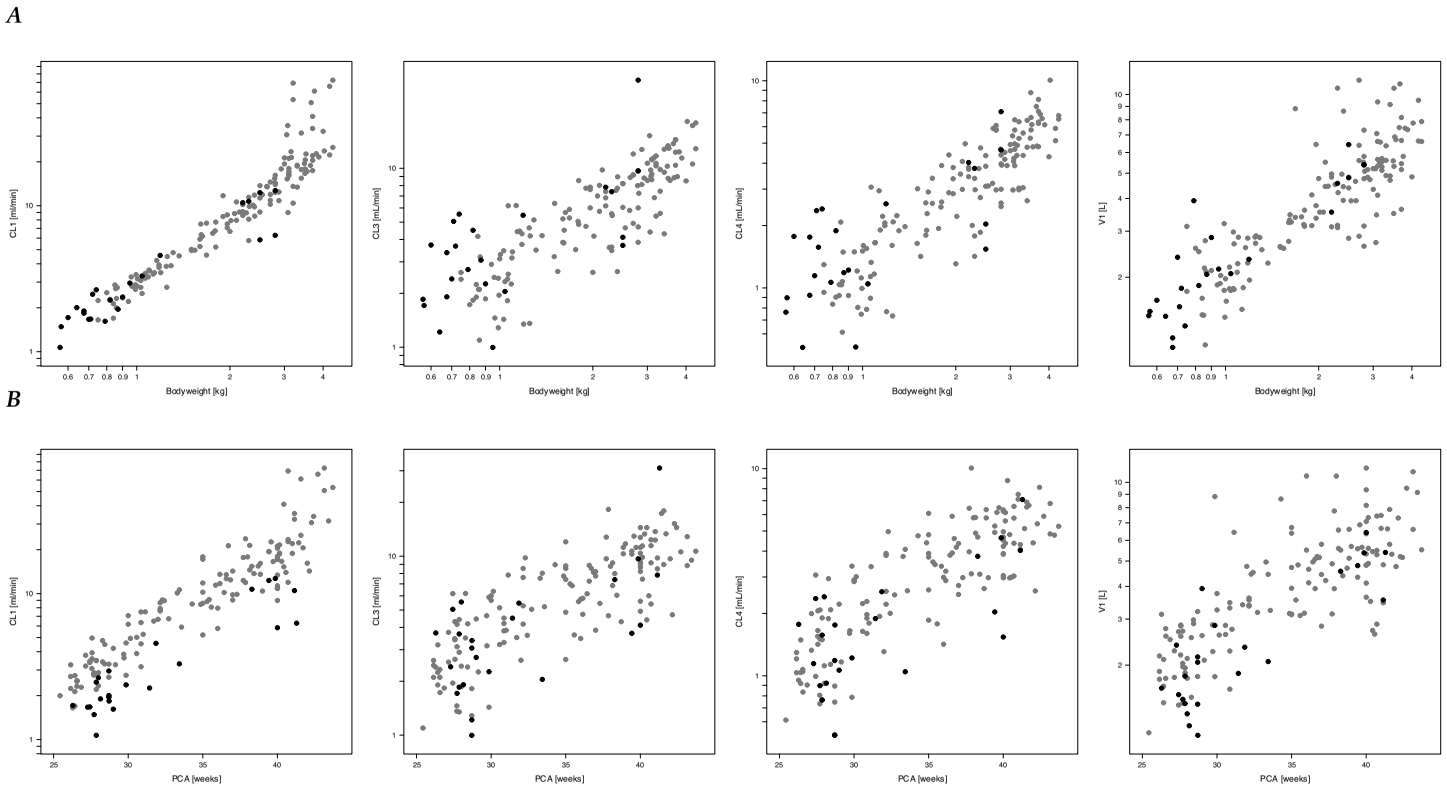

Figure 4. *Plots of individual post hoc parameter estimates of glucuronidation clearance of morphine to morphine-3-glucuronide (CL_1), distribution volume (V_1), elimination clearance of morphine-3-glucuronide (CL_3) and morphine-6-glucuronide (CL_4) versus A) bodyweight and B) postconceptual age (PCA) in neonates.* ● *= neonates appropriate for gestational age (AGA)* ● *= neonates small for gestational age (SGA).*

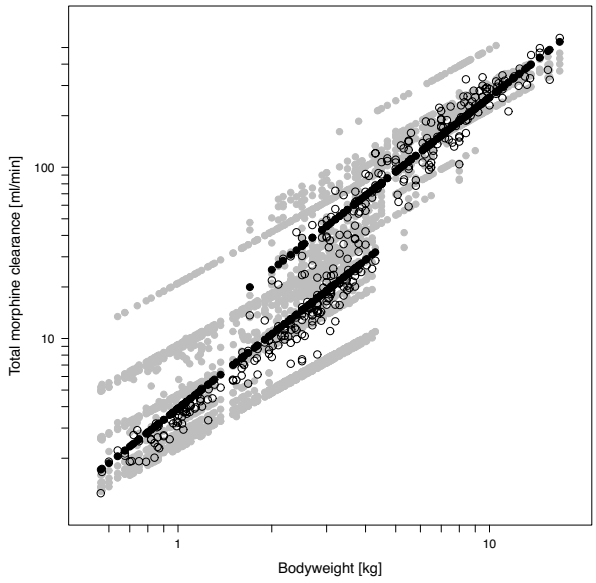

Figure 5. *Total morphine clearance values versus bodyweight for the patients in the internal and external datasets (Int. 1 & 2 and Ext. 1 – 4). ● = population predictions from the original model, ○ = individual post hoc estimates from the original model. ● = values reported in literature over the past 20 years [7,18–27].*

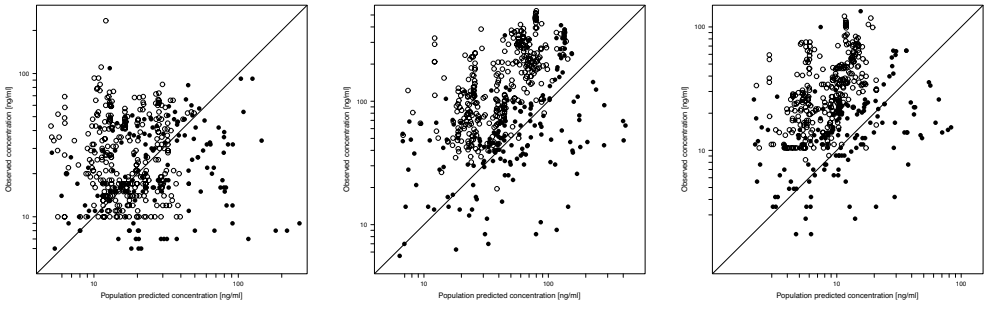

Figure 6. *Results of the external validation representing the concentrations predicted by the original model versus observed concentrations in the external datasets of patient receiving ECMO treatment for morphine, morphine-3-glucuronide, and morphine-6-glucuronide. ○ = Ext.5 term neonates on ECMO treatment without CHHV [8,9], and ● = Ext.6 term neonates on ECMO treatment with CVVH [10].*

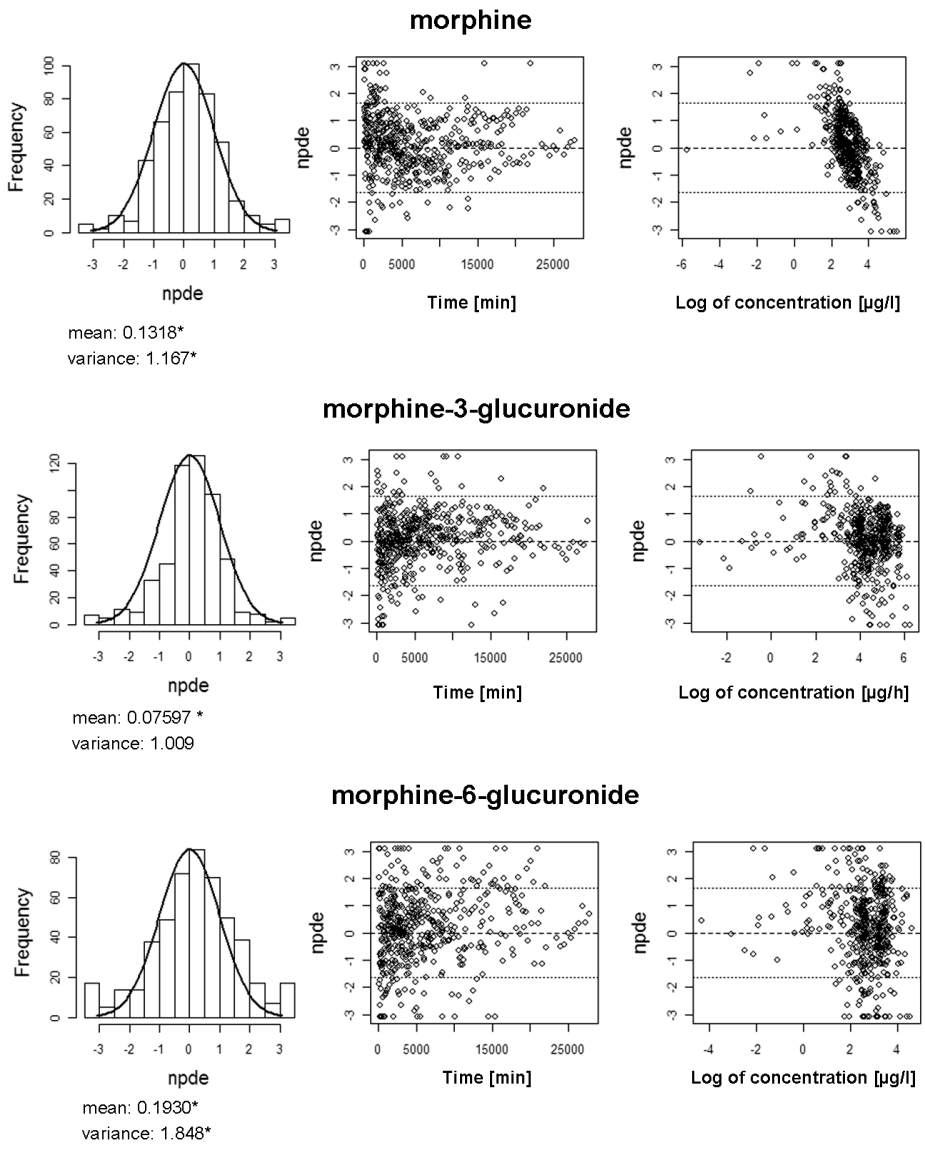

Figure 7. *Results of the external validation with the NPDE method using external dataset 6 (neonates on ECMO treatment with CVVH). The histograms show the NPDE frequency distribution in the merged external dataset for morphine, morphine-3-glucuronide and morphine-6-glucuronide, the solid line indicates a normal distribution. The values for the mean and variance of the NPDE distribution are given below each histogram with * indicating a significant difference of a mean of 0 and a variance of 1 at the $p < 0.05$ level as determined by the Wilcoxon signed rank test and the Fisher test of variance. The distribution of NPDE versus time after first dose and NPDE versus the log of the concentration are also shown. The dotted lines represent the 90% distribution of the NPDE.*

4.4 Discussion

In many publications on population PK and PD models results of even basic validation procedures are lacking. In adults only 28% of the population PK models and 26% of the population PD models were found to be adequately evaluated [1]. In the paediatric population advanced internal evaluations are performed on merely 16% of the models and external validations are performed on only 9% of the models [2]. Our group recently developed a population PK model for morphine glucuronidation in neonates (including preterms) infants and children up to three years which was validated internally using basic and advanced methods (Chapter 3). The internal validation procedure showed that the model can adequately describe the dataset used to develop the model, but when a model is to be used to derive dosing algorithms, descriptive properties are not enough. Therefore the predictive performance of the original model and thereby the validity of the use of the original morphine PK model for simulation purposes in this age group needed to be established as well. This can only be done in an external validation procedure for which in the current analysis six external datasets were available and basic and advanced validation methods were used. Moreover, the predicted morphine clearances are compared to literature values published in the past twenty years.

Two of the external datasets (Ext.3 & 4) originate from medical centers other than the centers from which the internal datasets were obtained. Irrespective of the center at which the studies were performed or the morphine salt that was administered, the predictive performance of the original morphine PK model was found to be good in the external datasets of postoperative and ventilated patients younger than one year. Figure 2 shows that the model can predict morphine and metabolite concentrations without bias in all individuals in the external datasets on the basis of dose, bodyweight and postnatal age alone. The spread in the observed data, which reflects the variability within the overall population, is equally large above and below the line of unity. The NPDE analysis in figure 3 confirms that the original model predicts morphine and metabolite concentrations accurately and detects only a slight over-estimation of the variability which was also observed in the internal validation procedure. The refit of the combined internal and merged external datasets (Int. 1 & 2 and Ext. 1 – 4) and the bootstrap analysis performed with this entire dataset show the original PK model for morphine in these young patients to be stable and the estimated parameters to be precise (table II). This means that the concentrations that were measured upon blood collections in these external datasets could have been adequately predicted based on individual characteristics that are readily available in clinical practice (bodyweight and age), thereby reducing the need for (extensive) blood sampling in drug monitoring.

The results of this external validation also strengthen the confidence in the obtained covariate relationships. This is important because in the paediatric population there is a strong correlation between bodyweight and age, leading to an ongoing debate on which of these characteristics to use as a descriptor for maturational changes in population PK models in this population. Some incorporate bodyweight *a priori* as a covariate using a bodyweight based allometric equation with fixed exponents of 0.75 for clearance and 1 for distribution volume [28]. The paediatric population PK model that is evaluated in the current study was developed by regarding bodyweight and age as conventional covariates in a systematic covariate analysis. In model development bodyweight was found to be a better descriptor of the maturation of morphine PK parameters than age and its influence was best described by a bodyweight based allometric equation with an estimated exponent of 1.44 for clearance and 1 for distribution volume (Chapter 3). Additionally, within this power-function metabolite formation was found to be reduced in neonates younger than ten days. In this external validation procedure, the original model showed to generate adequate predictions in a patient population up to one year of age.

The current analysis also demonstrates that the correlation between bodyweight and age is different in neonates that are SGA compared to their AGA counterparts. Therefore insights into the use of bodyweight or age as descriptors for maturational changes on drug PK can be obtained by studying these two subpopulations. For M3G formation clearance (CL_1) and distribution volume (V_1), the same relationship for SGA and AGA neonates was found when plotted *versus* bodyweight, which is not the case when plotted *versus* postmenstrual age (figure 4 a and b). Although shrinkage was 26.3% and 31.7% respectively, which renders plots using *post hoc* parameter estimates less reliable, this suggests that that bodyweight is indeed the most appropriate descriptor to describe maturational changes in the distribution volume and glucuronidation of morphine. For the elimination clearance of M3G and M6G (CL_3 and CL_4) the relationship with bodyweight is different for SGA and AGA neonates while the relationship between CL_3 and CL_4 and postmenstrual age in SGA and AGA neonates is more similar (figure 4 c and d). With 14.6% and 13.0% respectively, shrinkage was sufficiently low for the *post hoc* estimates of these parameters to be reliable. Bodyweight appears not to be the most optimal descriptor of the maturation rate of the elimination of the morphine metabolites and age may be a better descriptor. Based on these results an age-based exponential equation for the elimination clearances was tested, but this did not significantly improve the model (data not shown). According to the rule of parsimony this was therefore not incorporated into the model. An explanation for this could be the strong correlation between bodyweight and age in this population. Possibly the use of either of the two covariates results in maturation profiles that are very similar over the entire age-range.

The influence of the limited number of SGA neonates at the low end of the age and weight-range is then too small to significantly affect the overall model fit of the entire population.

This example illustrates that in paediatrics, in addition to looking at the population as a whole, subsets of the population should also be investigated. Despite the fact that postmenstrual age was not included into the final PK model for statistical reasons the subpopulation analysis in this study demonstrates that the best physiological descriptor for maturational changes (e.g. bodyweight or age) may be different for different PK parameters of the same drug, providing evidence against the *a priori* inclusion of bodyweight as a covariate in paediatric PK models. As it cannot be known beforehand what the best descriptor for maturational changes in PK parameters is, a systematic covariate analysis is always required.

As dosing algorithms are predominantly derived from clearance parameters it is important to assess how well a model can predict these parameters. A direct comparison between model-predicted clearances and 'actual clearances' is however difficult, since clearances can only be derived indirectly from population models or through non-parametric methods that require either steady state or dense data. As an alternative the model-predicted clearances were compared to previously reported values from literature. All but two previously published clearance values scaled linearly with bodyweight, which is reflected in figure 5 by identical slopes in the lines of published clearance parameters. Figure 5 also shows these lines to shift upwards with increasing bodyweight, indicating an increased clearance with increased bodyweight. The clearances predicted by the original morphine model increase exponentially with bodyweight, resulting in a different slope. Also this model predicts higher clearances for children older than ten days compared to their younger counterparts. The model predicted clearances fall nicely within the range of previously published clearances, increasing the confidence in the model-predicted clearances, although the ultimate validation is a prospective clinical study.

The relatively limited availability of suitable data in the paediatric population may hamper the validation of population models in this population. By using existing data, as was done in the current analysis, the number of unnecessary studies in this vulnerable population can be significantly reduced [29,30]. This often requires data sharing but unfortunately there are limiting ethical and practical issues that at present still need to be addressed by the scientific community and society as a whole [31]. Compared to neonates and infants younger than one year, children between one and three years of age are encountered relatively infrequently in paediatric ICUs, and they are less often included in clinical trials. For this population no datasets were available for the external validation of the original morphine model evaluated in this study, leaving this model externally largely unvalidated in this age-range.

Two external datasets (Ext.5 & 6) included a patient population that was not included in the learning dataset for the original model, namely neonates undergoing ECMO treatment. ECMO is an invasive procedure that may influence PK parameters, most often increasing distribution volume and decreasing clearance [32–35]. Additionally, morphine doses were on average higher in these studies compared to the studies of the internal and other external datasets (Ext. 1 – 4). The predictive performance of the original model was investigated in this patient population as well to determine whether, despite these differences, the dosing algorithm in $\mu g/kg^{1.5}/h$ derived from the covariate relationships in the original morphine PK model could still be beneficial in patients on ECMO treatment. The predictive performance of the original model proved to be good for external ECMO dataset 6, although considerable bias towards under prediction, particularly for the morphine metabolites, was observed in external ECMO dataset 5. The duration of the study of dataset 5 was longer than the study of dataset 6 and therefore accumulation was expected, however the NPDE analysis of this individual dataset showed no such trend in time (data not shown). An important difference between the two studies was that the study of dataset 6 was a more recent study and at that time the augmentation of ECMO treatment with continuous venovenous hemofiltration (CVVH) was routine practice, in contrast to the study of dataset 5. Morphine metabolites are eliminated through renal clearance and since CVVH complements renal function this could explain the under-prediction of morphine metabolites by the original model in dataset 5. In fact previous analysis of this dataset showed CVVH augmentation to increase metabolite elimination but not morphine elimination in ECMO treated neonates [9].

Augmentation of ECMO treatment with CVVH has been shown to improve clinical outcome of the treatment and reduce costs [36], therefore CVVH during ECMO has become standard clinical practice in our institution. Since the two ECMO datasets could not be merged due to the conflicting results and as the conditions seen in the study of dataset 6 resemble current clinical practice best, only the NPDE analysis performed with this dataset is shown (figure 7). The NPDE analysis of this dataset indicates a reasonable prediction of median morphine and metabolite concentrations with limited bias over time and over the log value of the concentration range in patients on ECMO treatment with CVVH. Bearing in mind the considerable difference in the patient populations in the internal dataset and in this dataset this result is quite remarkable.

Previously two population PK models for morphine in patients on ECMO treatment were developed based on dataset 5 [8,9]. These studies showed ECMO treatment to affect some of the clearance parameters and the distribution volumes. Also the maturation rates of some of these parameters were shown to be different from postoperative and ventilated patients. Possibly the influence of changes in clearance and distribution volume counterbalance each other during ECMO treatment, resulting

in morphine and metabolite concentrations that are similar to those observed in postoperative and ventilated neonates.

The validation of the predictive performance of the original morphine PK model in these datasets does not imply this model to be optimal in this population, as such optimization would require a laborious separate analysis. The sole purpose of performing this validation procedure was to investigate whether the model-derived dosing algorithms, which are based on the covariate model, could also be suitable in patients on ECMO treatment. By confirming the models predictive performance in neonates on ECMO treatment augmented by CVVH, it is suggested that dosing morphine in $\mu g/kg^{1.5}/h$ could be appropriate in these patients as well.

4.5 Conclusion

In the current analysis the predictive performance of a previously published paediatric PK model for morphine and its metabolites was tested externally in new datasets from postoperative patients, patients on artificial ventilation and patients on ECMO treatment ranging from preterm and SGA neonates to infants of one year. The predictive performance was found to be good in the postoperative and ventilated patients and in patients on ECMO treatment with CVVH. Herewith the suitability of the original model for simulation purposes is confirmed. The establishment of the predictive performance of the model in this study justifies the next step in developing new dosing recommendations, namely a prospective clinical trial. Dosing algorithms previously derived from the original model (Chapter 3) are currently being evaluated at our facilities in postoperative patients younger than one year (Dutch trial registration number NTR1438 [37]) and in neonates on ECMO treatment with CVVH (NTR2180 [37]).

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Chapter 5

Innovative Paediatric Pharmacology; Validation of an Evidence-Based Dosing Algorithm for Morphine in Neonates and Infants

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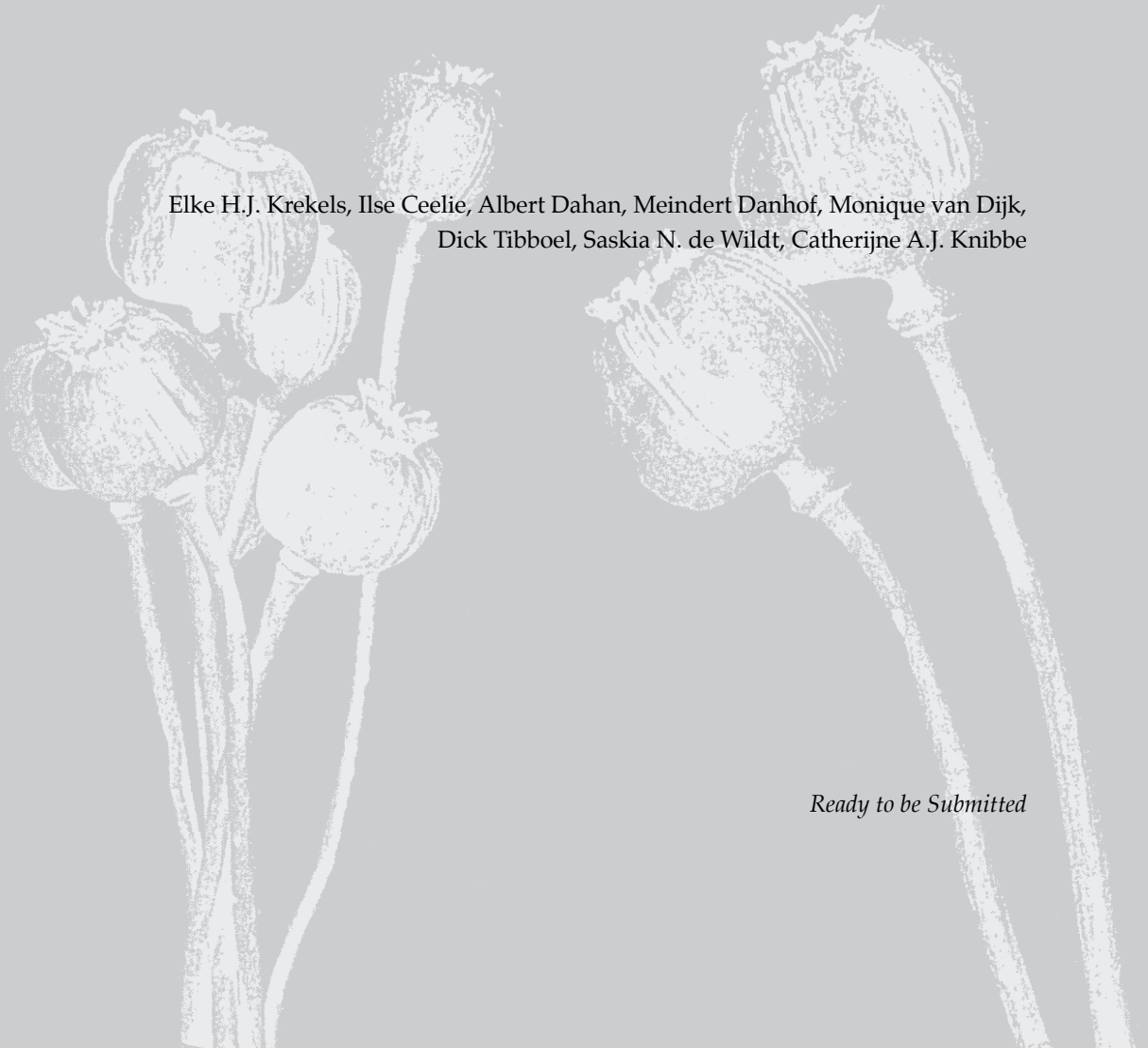

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Abstract

Context: Validated morphine dosing algorithms are lacking for neonates and infants. A paediatric population pharmacokinetic model showed morphine clearance to non-linearly increase with bodyweight and be reduced by 50% in neonates younger than ten days.

Objective: Prospectively evaluate a morphine IV dosing algorithm that was derived from a population pharmacokinetic model, in term neonates up to infants of one year of age.

Design: Single-centre, prospective, study (March 2008 – July 2010, 48 hrs follow-up, www.trialregister.nl: number NTR1438).

Setting: Level III pediatric intensive care unit.

Patients: 38 patients after major non-cardiac surgery, including 18 term neonates younger than ten days and 20 older patients.

Interventions: Postoperative continuous morphine IV infusion of 2.5 $\mu g/kg^{1.5}/h$ in neonates younger than ten days and 5 $\mu g/kg^{1.5}/h$ in older patients. Morphine IV rescue according to a validated age-appropriate pain protocol.

Main outcome measures: 1) morphine rescue dose; 2) average actual morphine infusion rate; 3) morphine and metabolite concentrations.

Results: For young neonates compared to older patients, patients needing morphine rescue was 27.8% *vs.* 90% ($p<0.001$) and total rescue dose was 0 (0 - 539) $\mu g/kg$ *vs.* 193 (0-1183) $\mu g/kg$ (median (range), $p<0.001$). Median actual morphine infusion rate was 4.4 (3.6 – 5.0) $\mu g/kg/h$ *vs* 14.4 (7.4 – 15.7) $\mu g/kg/h$ (median (range), $p<0.001$), and the number of patients needing more than 125% of the initial model-derived infusion rate was 17% *vs* 55% ($p<0.05$). Morphine and metabolite concentrations were accurately predicted by the paediatric population morphine model.

Conclusions: Compared to traditional morphine dosing in $\mu g/kg/h$, the proposed dose in young neonates was lower but still efficacious, while the higher dose in older patients still yielded a substantial need for rescue medication. Improvements seem possible, but the model-based dosing algorithm correcting for age-related differences in morphine pharmacokinetics may prevent overdosing in the youngest neonates and reduce suboptimal dosing in infants.

Introduction

Lack of paediatric dosing information has caused unlicensed and off-label drug prescription to be common practice in the paediatric population, despite the increased risk of suboptimal dosing or adverse drug effects [1]. Population pharmacokinetic and/or pharmacodynamic modeling approaches, also known as non-linear mixed-effects modeling, strongly facilitates the development of evidence-based rather than empiric or consensus-based paediatric drug dosing algorithms [2]. In population analyses, pharmacokinetic and/or pharmacodynamic parameters can be derived from sparse, dense and/or unbalanced data obtained from patients during routine clinical practice. Moreover, the sources of variability in the population can be quantified, and in a covariate analysis patient characteristics that are predictive of this variability, such as for instance bodyweight or age, can be identified [3]. These key patient characteristics form the basis of model-derived dosing algorithms. Before these dosing algorithms are implemented in clinical practice, they should however be prospectively evaluated to ascertain that the observed endpoints obtained with the new dosing algorithm are in agreement with the model-based predictions [2].

A drug that is commonly used in paediatrics is the opioid morphine. Interestingly, although this drug has been used in clinical settings for a very long time, validated dosing guidelines across the paediatric age-range are lacking. Under or overdosing of morphine should be prevented to avoid inadequate pain relief, opioid-related safety issues and opioid-withdrawal symptoms. Recently, a population model for the pharmacokinetics of morphine and its two major metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) was developed for a population of postoperative and ventilated preterm and term neonates up to children of three years of age (Chapter 3). In this model, clearance proved to nonlinearly increase with bodyweight. Additionally, a 50% reduction in morphine glucuronidation was observed in neonates with a postnatal age younger than ten days, which was independent the gestational age. This is similar to what is observed for bilirubin glucuronidation after birth. To ascertain good predictive model performance, this model was extensively validated, both internally and externally (Chapters 3 and 4). From this model it was subsequently derived that dosing morphine maintenance doses on the basis of $\mu g/kg^{1.5}/h$ with a 50% dose reduction in neonates younger than ten days, would yield similar morphine and metabolite concentrations across this population (Chapter 3). Compared to a traditional dosing scheme of 10 $\mu g/kg/h$, this nonlinear dosing algorithm leads to a substantial reduction in morphine infusion rates in neonates (e.g. 4.3 *vs* 10.0 $\mu g/kg/h$ for a young neonate of 3 kg), while infants would receive a higher dose (e.g. 14.1 *vs* 10.0 $\mu g/kg/h$ for an infant of 8 kg). The

current proof-of-principle study prospectively evaluates this model-derived paediatric dosing regimen for morphine over 48 hours in postoperative patients under the age of one year, using the required morphine rescue dose administered according to a validated age-appropriate pain protocol as primary endpoint. Furthermore the average actual morphine infusion rate was evaluated and measured morphine and metabolite concentrations in the patients were compared to concentration predictions by the paediatric morphine pharmacokinetic model (Chapter 3).

Methods

Study Design

In a single-center, double-blind, randomized controlled trial comparing the postoperative analgesic efficacy of morphine and paracetamol over 48 hours in term neonates and infants younger than 1 year [4], the patients allocated to receive morphine as primary analgesic agent were dosed according to the model-derived dosing algorithm (Chapter 3). The current analysis evaluates the efficacy of this new algorithm across the age-range in the morphine arm of this study in terms of required morphine rescue doses and actual average morphine infusion rates. Blood samples for the evaluation of model predicted morphine and metabolite concentrations were obtained from patients in both the morphine and paracetamol arm of the study. As the comparison between the analgesic efficacy of morphine and paracetamol and full details of this study where published elsewhere [4], in the present publication details are summarized as relevant to the current analysis.

Patients

Term neonates and infants under the age of one year, undergoing major abdominal or non-cardiac thoracic surgery between March 2008 and July 2010 at the Erasmus MC – Sophia Children's Hospital in Rotterdam, The Netherlands, were eligible for inclusion. Exclusion criteria were: 1) postconceptual age younger than 36 weeks; 2) bodyweight less than 1.5 kg; 3) extra corporeal membrane oxygenation (ECMO) treatment; 4) neurological or hepatic dysfunction or renal insufficiency; 5) pre- or postnatal administration of opioids or psychotropic drugs for more than 24 hours; 6) known allergy or intolerance for paracetamol or morphine; 7) administration of opioids in the 24 hours prior to surgery.

The study was approved by the Erasmus MC ethics review board and was registered in the Netherlands Trial Register under the number NTR1438 [5]. Written informed consent was obtained from the parents or legal guardians before inclusion of the patients.

Interventions

Patients were stratified by age into one group that was younger than ten days and one group that was older than ten days, as patients in these groups would receive different morphine dosages. All patients received a 100 μg/kg morphine IV bolus dose 30 minutes before the anticipated end of the surgical procedure. In the morphine arm of the study, patients with a postnatal age less than ten days received a postoperative continuous morphine IV infusion of 2.5 $\mu g/kg^{1.5}/h$ and older patients received 5 $\mu g/kg^{1.5}/h$, which was implemented using the dosing table depicted in table I. To maintain the blinding of the morphine *versus* paracetamol study, the patients allocated to the morphine arm of the study also received four times daily placebo saline infusions of the same volume as the paracetamol bolus dose a patient would receive in the paracetamol arm of the study.

Table I. *Dosing table used to implement the model-derived morphine dosing algorithm for continuous infusions in clinical practice. Patients younger than ten days received a dose of 2.5 $\mu g/kg^{1.5}/h$ and patients older than ten days received 5 $\mu g/kg^{1.5}/h$.*

	Model-derived dosing algorithm	
	PNA < 10 days *2.5 $\mu g/kg^{1.5}/h$*	*PNA > 10 days* *5 $\mu g/kg^{1.5}/h$*
Bodyweight (kg)	**Infusion rate μg/kg/h**	**Infusion rate μg/kg/h**
1.5 – 2	3.1	6.1
2 – 2.5	3.5	7.1
2.5 – 3	4.0	7.9
3 – 3.5	4.3	8.7
3.5 – 4	4.7	9.4
4 – 4.5	5.0	10.0
4.5 – 5	5.3	10.6
5 – 5.5	5.6	11.2
5.5 – 6	.	11.7
6 – 6.5	.	12.3
6.5 – 7	.	12.8
7 – 7.5	.	13.2
7.5 – 8	.	13.7
8 – 8.5	.	14.1
8.5 – 9	.	14.6
9 – 9.5	.	15.0
9.5 – 10	.	15.4
10 – 10.5	.	15.8

PNA = postnatal age

During the first 48 hours of postoperative recovery at the intensive care unit, trained nurses assessed patient's pain levels every 2 hours or when the patients appeared to be in discomfort, according to an age-appropriate standardized pain protocol [6], based on COMFORT-behavior scores [7] and Numeric Rating Scale (NRS) pain scores [8]. Open-label morphine rescue medication was administered to patients of both the morphine and paracetamol arm when NRS ≥ 4. Patients younger than ten days received a bolus dose of 10 μg/kg and older patients received 15 μg/kg. Patients were reassessed after 10 minutes and received additional bolus doses when necessary. If analgesia was not adequate after three bolus doses within one hour, the patients received an additional loading dose of 100 μg/kg after which the morphine infusion rate was increased by 1.25 $\mu g/kg^{1.5}/h$ in neonates younger than ten days and 2.5 $\mu g/kg^{1.5}/h$ in older children. When after this increase the patient needed again more than 3 rescue bolus doses within one hour, another loading dose was administered and infusion rates were increased by the same amount again. The morphine infusion was stopped or reduced in case of morphine related adverse events or after 12 hours of adequate analgesia, indicated by an NRS score < 4. In case of discomfort, indicated by COMFORT-behaviour scores of >17 and NRS pain scores of < 4, midazolam was administered.

Outcomes

The nurse-controlled open-label morphine rescue medication in the first 48 postoperative hours was used as the primary efficacy outcome measure. This was expressed as percentage of patients in need of rescue medication, the number of rescue events with an event being an administration of a morphine bolus dose, an additional morphine loading dose or an increase of morphine infusion rate, and the total morphine rescue dose. The two age-groups were analyzed separately and compared. Additionally, the average actual morphine infusion rate over the duration of the postoperative infusion for each patient was compared between these groups. Since there were bodyweight and age-related differences in the model-derived morphine dosing algorithm, the average morphine infusion rate in each individual patient was also compared to the initial model-derived infusion rate for that patient, by calculating the percentage of patients that had an average morphine infusion rate within or outside 25% of the prescribed dose (as in bioequivalence studies, this is considered to be clinically significant) and the percentage of patients that required more than double the initial model-derived infusion rate.

Blood samples to determine morphine and metabolite concentrations were obtained to determine the accuracy of the concentration predictions by the pharmacokinetic model (Chapter 3) in the current set of patients that were dosed according to the model-derived dosing algorithm in table I. A maximum of eight blood samples or a total 5% of the blood volume of a patient, were obtained from an indwelling arterial catheter

when present. When possible a blood sample was taken prior to a morphine rescue dose or prior to a scheduled paracetamol or placebo bolus dose. Additional samples were taken when possible at various times, to obtain information on a wide range of the concentration-time curve. Blood samples were centrifuged at 3000 rpm and plasma was subsequently stored at -80°C till further analysis.

Statistical Analysis

To statistically compare the analgesic efficacy of the model-derived morphine dosing algorithm between neonates younger than ten days and patients of ten days or older, the Fisher exact test was used for the dichotomous endpoints (need for rescue mediation, actual morphine infusion rate within or outside 25% of the prescribed dose, actual morphine infusion rate more than 200% of the prescribed dose). The Mann-Whitney test was used for the other categorical and continuous endpoints (number of rescue events per patient, morphine rescue dose, average actual morphine infusion rate).

To ascertain that the patients of whom pharmacokinetic samples were obtained could be regarded as a representative sample of the patients that were analyzed for the analgesic efficacy, the demographics and clinical characteristics of the patients in these groups were statistically compared. For this also the Fisher exact test was used for the dichotomous data (sex, location of surgery, need for postoperative ventilation) and the Mann-Whitney test was used for the continuous data (postnatal age, bodyweight, duration of surgery).

Pharmacokinetic Blood Sample Analysis

The frozen plasma samples were allowed to thaw at room temperature. Proteins in 200 μl samples were precipitated with 700 μl acetonitrile which contained ^{2}H3-Morphine ($^{2}H_3$-M), ^{2}H3-Morphine-3-glucuronide ($^{2}H_3$-M3G), and ^{2}H3-Morphine-6-glucuronide ($^{2}H_3$-M6G) (Cerilliant, Texas, USA) as internal standards and 100 μl 1 mM zinc sulphate. The samples were mixed for 2 minutes and centrifuged for 5 minutes at 13000 rpm. 200 μl of the supernatant was dried under a gentle stream of nitrogen at 50°C. The residues were reconstituted in 100 μl of 0.1 % (v/v) formic acid in water and 20 μl of this sample was injected into the HPLC system, which contained an Ultimate 3000 autosampler (Dionex, Amsterdam, The Netherlands), a HPG680 pump (Dionex, Amsterdam, The Netherlands), and a 3 μm, 120Å, 50 x 2.1 mm YMC-pack ODS-AQ column (YMC Inacom, Overberg, The Netherlands) with an ODS precolumn (Phenomenex, Utrecht, The Netherlands) at 30°C. The mobile phase consisted of 0.1 % formic acid in water with 3% acetonitril (Lichosolv) (Merck B.V., Amsterdam, The Netherlands) as modifier and the flow rate was 0.5 ml/min. The system was controlled by Chromeleon (Dionex, Amsterdam, The Netherlands). The eluent of the HPLC system was monitored by a Quattro micro API tandem mass

spectrometer (Waters, Etten-Leur, The Netherlands). Peak areas of reaction ions from morphine, M3G, M6G and the internal standards $^{2}H_{3}$-M, $^{2}H_{3}$-M3G and $^{2}H_{3}$-M6G were obtained in the multiple reaction mode and integrated by data software Masslynx 4.1 (Waters, Etten-Leur, The Netherlands). m/z was 165.0 (285.9>165.0) for morphine and 286.0 (461.9 >286.0) for M3G and M6G. For the internal standards m/z was 165.0 (288.9>165.0) for $^{2}H_{3}$-M and m/z (464.9>289.0) for $^{2}H_{3}$-M3G and $^{2}H_{3}$-M6G. All analytes could be analyzed in one run and all samples were analyzed in triplo. The sample concentrations were calculated by the internal standard method with weighing factor $1/(Y^{2})$.

Blank pooled human serum was used for control samples and serum spiked with morphine, M3G, and M6G (Cerilliant, Texas, USA) in methanol/water were used for the calibration curve and quality controls.

Model-Based Pharmacokinetic Predictions

NONMEM VI (ICON, Ellicott City, MD, USA) was used to obtain model-based concentration predictions. In predicted *versus* observed plots, the available morphine and metabolite concentrations were visually compared to both the individual and population concentration predictions by the paediatric population pharmacokinetic model for morphine (Chapter 3). Individual predicted concentrations were based on a model fit of the individually observed concentrations to the model, based on the administered dose, bodyweight and postnatal age of the patient. Population predicted concentrations were obtained using the population parameter values of a typical individual to simulate concentrations based on the administered dose, specified bodyweight and postnatal age of the patient.

Results

Patients and Sampling

Figure 1 shows the inclusion diagram for this study. Thirty-nine patients were allocated to receive morphine as primary analgesic (morphine arm) of which 38 patients actually received continuous morphine infusions according to the model-derived dosing algorithm. These 38 patients were included in the current analysis of the analgesic efficacy of the new morphine dosing algorithm. Morphine and metabolite concentrations were available for 8 of these 38 patients, due to the limited number of patients with an arterial line. In the paracetamol arm, 33 patients were included, of which the analgesic efficacy was assessed in a separate study [4]. For a total of 7 of the 33 patients in the paracetamol

arm, morphine concentrations were available resulting upon the standard loading dose of morphine that was administered in all patients at the end of surgery and morphine rescue boluses when indicated by the standardized pain protocol.

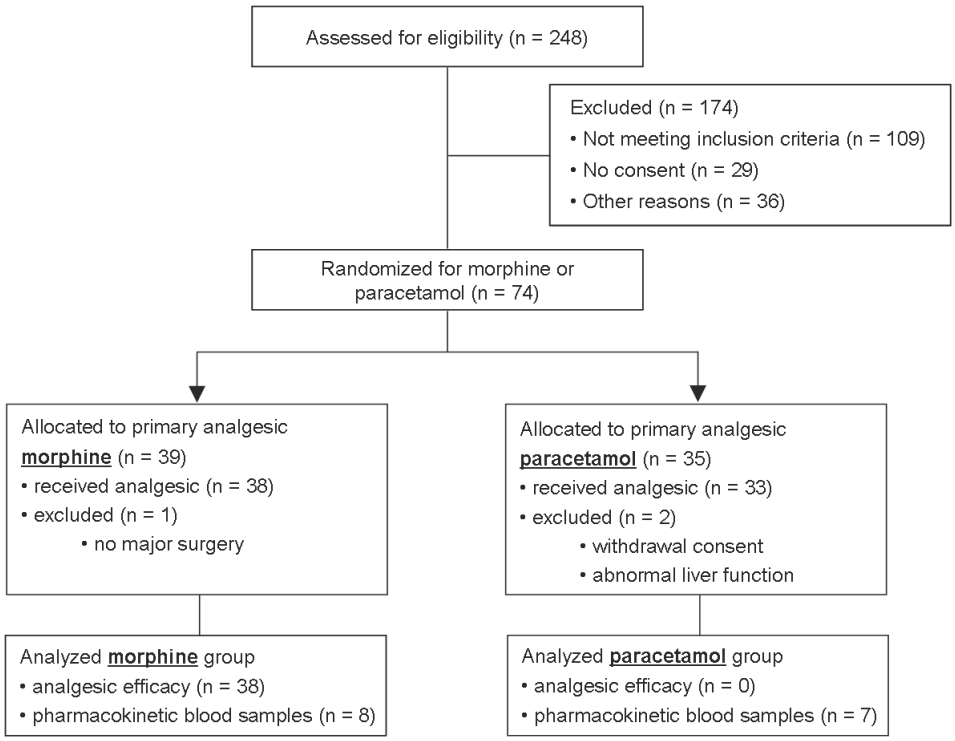

Figure 1. *Inclusion diagram for the current analysis.*

Table II summarizes the demographics and clinical characteristics of the patients in the group analyzed for analgesic efficacy and in the group analyzed for model predictions of morphine and metabolite concentrations. The table shows that the group of patients of whom blood samples were obtained represents the overall group analyzed for morphine efficacy well, as there were no statistically significant differences in demographics and patient characteristics between these groups.

Table II. *Demographics and clinical characteristics of the patients in the analysis of the analgesic efficacy of the morphine dosing algorithm and the patients in the analysis of the pharmacokinetic model predictions of morphine and its metabolite concentrations in the current study.*

	Patients analyzed for analgesic efficacy of the morphine dosing algorithm (n = 38)	**Patients analyzed for pharmacokinetics of morphine (n = 15)**	**p-value**
Postnatal age (days, median (range))	20 (0 – 355)	3 (0 – 200)	0.56 †
Bodyweight (kg, median (range))	3.6 (2.0 – 9.8)	3.3 (1.6 – 8.0)	0.42 †
Sex (n, M / F)	26 / 12	7 / 8	0.21 ‡
Location of surgery (n, thorax / abdomen)	11 / 27	5 / 10	0.75 ‡
Duration of surgery (min, median (range))	139 (27 - 480)	149 (69 – 480)	0.30 †
Postoperative ventilation (n, %)	14 (37%)	7 (47%)	0.55 ‡

† Mann-Whitney test, ‡ Fisher exact test

Analgesic Efficacy

An overview of the need for morphine rescue medication in patients younger and older than ten days dosed according to the model-derived morphine dosing algorithm is provided in table III.

Table III. *Analgesic efficacy of the model-derived morphine dosing algorithm, expressed as need for rescue medication.*

Patient group	**n**	**Patients in need of rescue medication**	**Rescue events #**	**Total rescue dose (μg/kg) #**
Total	38	23 (60.5%)	2 (0 – 14)	20 (0 – 1183)
0 – 10 days	18	5 (27.8%)	0 (0 – 10) ‡	0 (0 – 539) †
10 – 365 days	20	18 (90.0%)	4.5 (0 – 14) ‡	193 (0 - 1183) †

median (range), † $p < 0.05$, ‡ $p < 0.001$

Overall, 60.5% of all patients needed rescue medication, with four out of 18 neonates younger than ten days (27.8%) needing rescue medication, compared to 18 out of 20 patients (90%) who were ten days or older ($p<0.001$, Fisher exact test). In neonates younger than ten days compared to the older children, the number of rescue events per

patient (median (range)) were 0 (0-10) *versus* 4.5 (0 – 14) (p<0.001, Mann-Whitney test) and the total rescue dose (median (range)) was 0 (0-539) μg/kg *versus* 193 (0 – 1183) μg/kg (p <0.001, Mann-Whitney test). Considering only the patients in need of rescue medication, the average number of rescue events per patient was 6 (2 – 10) *versus* 5.5 (1 – 14) in patients younger and older than ten days (p=0.97, Mann-Whitney test). While the median rescue dose in neonates younger than ten days who were in need of rescue medication was lower than the median rescue dose in the older children that needed rescue medication 140 (20 – 539) μg/kg *versus* 228 (15 – 1183) μg/kg, this difference did not reach statistical significance (p=0.53, Mann-Whitney test).

The average actual morphine infusion rate (median (range)) calculated over the duration of the post-operative infusion, consisting of the initial model-derived morphine infusion rate according to table I and additional morphine rescue doses that where required according to the standardized pain protocol, was 4.4 (2.5 – 24.6) μg/kg/h in neonates younger than ten days and 14.4 (7.9 – 39.3) μg/kg/h in older patients (p<0.001, Mann-Whitney test). In figure 2, the average morphine infusion rate during the duration of postoperative infusion is depicted for each individual patient (symbols) together with the model-derived morphine infusion rates which differentiates between children older and younger than ten days (solid lines). Different symbols are used for patients younger and older than ten days.

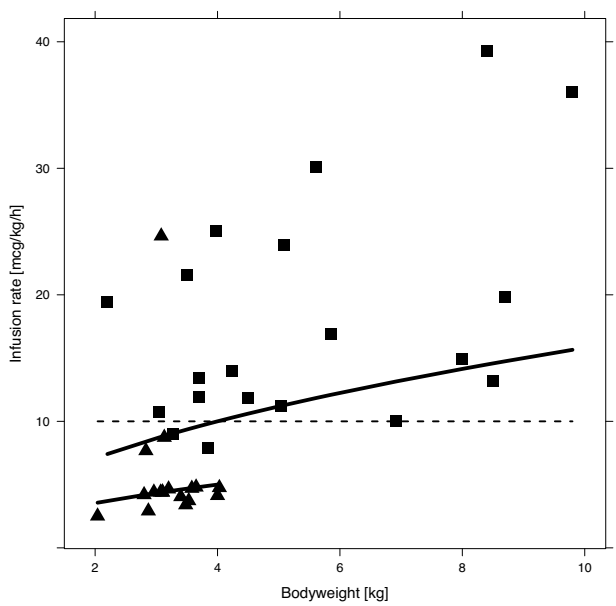

Figure 2. *Average actual postoperative morphine infusion rates during the postoperative infusion* time

for each individual patient with triangles (▲) representing neonates younger than ten days and squares (■) representing older children, and initial infusion rates according to the model-derived dosing algorithm (solid lines) in patients younger than ten days (2.5 $\mu g/kg^{1.5}/h$) and older than ten days (5 $\mu g/kg^{1.5}/h$). The dashed line represents the infusion rates according to the traditional dosing regimen in our facilities.

Three out of 18 neonates (17%) required less than 75% of the initial infusion rate *versus* none of the 20 older patients (p=0.10, Fisher exact test), 12 out of 18 neonates (67%) *versus* 9 out of 20 older patients (45%) had actual infusion rates within 25% of the initial dose (p=0.21, Fisher exact test), 3 out of 18 neonates (17%) required more than 125% of the initial infusion rate *versus* 11 out of 20 older patients (55%) ($p<0.05$, Fisher exact test), and 1 out of 18 neonates required more than twice the initial infusion rate *versus* 7 out of 20 older patients ($p<0.05$, Fisher exact test). For unknown reasons, a 1 day old boy required on average 5.6 times the initial morphine infusion rate during his 38 hour postoperative infusion. Of the older patients that need more than twice the initial morphine infusion rate none required more than three times the initial infusion rate.

In figure 2 a reference line representing the traditional intravenous morphine dose for the patient population in the current study in our unit (10 ug/kg/h – dashed line) was also added. This shows that initial morphine IV infusion rates were lower than the traditional dose in neonates younger than ten days and higher than the traditional infusion rate for infants older than ten days and heavier than 4 kg.

Morphine and Metabolite Concentrations

Figure 3 shows the plots of predicted *versus* observed concentrations of morphine (left), M3G (middle), and M6G (right). In the upper panels of figure 3, individual predicted *versus* observed concentrations are shown, which are based on the model fitting to the data. These plots indicate no bias and adequate precision of the concentration predictions. The graphs of the population predicted *versus* observed concentrations (lower panels) indicate no bias around the line of unity, suggesting the simulated predictions to be accurate in this patient population. The spread of data points around the line of unity in these plots indicates that the inter-individual variability in morphine pharmacokinetics in the population is considerable.

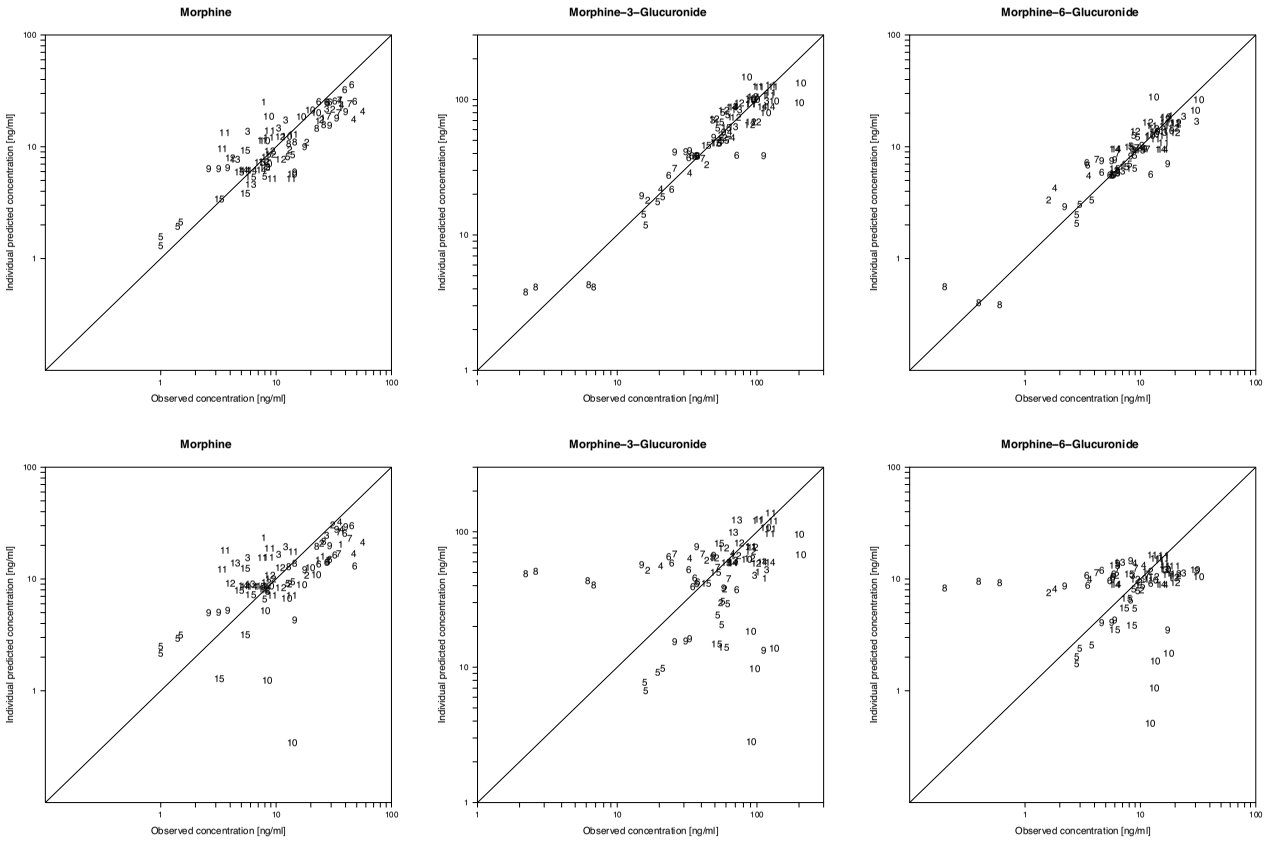

Figure 3. *Model predicted versus observed concentrations for morphine (left), morphine-3-glucuronide (middle), and morphine-6-glucuronide (right). Individual predictions (top) are based on the individual model fit of the observed concentrations, whereas population predictions (bottom) are simulated concentrations for a typical patient with a specified bodyweight and age. Different numbers are used to indicate data points of different individuals.*

Discussion

The current study prospectively evaluated a model-derived dosing algorithm for morphine in term neonates to infants of one year of age. According to this algorithm initial morphine maintenance infusion rates were 2.5 $\mu g/kg^{1.5}/h$ for neonates younger than ten days and 5 $\mu g/kg^{1.5}/h$ in older patients (table I), resulting in a reduction between 50% and 75% in neonates younger than ten days compared to the traditional morphine dose in our unit of 10 $\mu g/kg/h$, while children older than ten days and heavier than four kilograms received up to 150% of our traditional morphine dose. The limited need for rescue medication in the very young suggests the reduced morphine dose in this age-group to still be efficacious, while the increased dose in the older patients still does not appear to yield adequate analgesia in most of these patients (table III). Figure 2 further illustrates that the morphine infusion rate according to the model-derived dosing algorithm gives a good reflection of the actual morphine need in children younger than ten days. In older patients, the variability in average morphine consumption is higher, and in this group the percentage of patients requiring more than twice the prescribed morphine dose is also higher (35% compared to 6% in the young neonates, $p<0.05$). Moreover, figure 2 proves the morphine IV infusion rates according to the traditional dosing guideline to be too high for young neonates.

The morphine dosing algorithm that was evaluated in the current study was derived from a population pharmacokinetic model in which bodyweight and a postnatal age of less than ten days were identified as key patient characteristics that are predictive of the inter-individual variability in the clearance and distribution of morphine and its main metabolites (Chapter 3). Thorough external evaluation of the population pharmacokinetic model with four independent datasets from four different centers previously established confidence in the predictions by this model (Chapter 4). Blood samples obtained from a limited number of patients in the current study further confirm the accuracy of the model predictions in patients that were dosed according to the new dosing regimen (figure 3). It is thereby confirmed that the model-derived dosing algorithm indeed corrects for the developmental changes in morphine pharmacokinetics in this population, yielding similar steady state concentrations for morphine and its metabolites across the full age- and weight-range in the current study.

According to the pharmacokinetic model, continuous morphine IV infusion rates should be dosed on the basis of $\mu g/kg^{1.5}/h$, with a 50% reduction in neonates younger than ten days, to correct for age-related differences in morphine clearance. However, target concentrations needed to determine the infusion rate have not been firmly established for morphine in the paediatric population, although concentrations

around 20 ng/ml have been suggested in postoperative neonates and infants [9,10]. The infusion rate in the current study was derived from clinical experience in our own unit. Our traditional dosing guideline for continuous morphine IV infusions for neonates and children up to three years of age is 10 μg/kg/h irrespective of the age of the patient, which is in the lower range of literature reported postoperative morphine IV infusion rates of 10 – 40 μg/kg/h [11]. Previous research suggested this infusion rate to be relatively high for neonates and young children, but often insufficient for older patients [12]. Based on these results a morphine dose amount of 2.5 μg/kg$^{1.5}$/h in neonates younger than ten days and 5 μg/kg$^{1.5}$/h in older patients was selected for the current study as this leads to a reduced dose in neonates and an increased dose in older heavier patients, compared to our traditional dosing guideline. Upon these doses, average steady state concentrations of approximately 10 ng/ml are anticipated throughout the entire study population from term neonates and infants to the age of one year. Even though this concentration is lower than the previously suggested 20 ng/ml, the results show that this target concentration is sufficient, particularly for neonates.

To our knowledge no prospectively validated paediatric morphine dosing algorithms have been published that are based on a thorough investigation and understanding of the developmental changes in the pharmacology of morphine in this population. However, when pharmacokinetic models are used as the sole basis for paediatric dosing corrections, it is implicitly assumed that the pharmacodynamics of that drug remain constant within this population. This assumption is acceptable when: 1) pathophysiological processes are similar throughout the population, 2) the exposure-effect relationship can be assumed independent of age based on the mechanism of action, and 3) the same clinical endpoints for treatment are used throughout the populations [13]. It has not been proven that morphine meets the first two criteria in the current study population.

The pain protocol in the current study has been specifically developed and validated for the population included in the current study [7,8], thereby making rescue medication and total morphine consumption an appropriate and objective endpoints for analgesic efficacy in the current study. The model-derived morphine dosing algorithm evaluated in the current study corrects for age-related difference in morphine pharmacokinetics leading to similar concentrations throughout the population. The observed difference in rescue medication and morphine consumption between younger and older patients therefore suggests age-related differences in either pain perception or analgesic efficacy of morphine. The latter can for instance be caused by differences in effect-site distribution and/or differences in sensitivity to morphine and its pharmacologically active metabolites. Although evidence-based corrections for age-related differences in morphine pharmacokinetics already contributed to improving

paediatric morphine dosing, an investigation into morphine pharmacodynamics in this population could be used to further refine the morphine dosing algorithm by defining age-specific target concentrations.

Medicine always strives to expose patients to the lowest possible drug doses that are clinically effective. It is now recognized that neonates, even extreme premature ones, are able to experience pain that requires treatment [14–18]. With respect to short-term outcome measures expressed using various clinical endpoints like mortality, pain, duration of ventilation, or general clinical outcome measures, results on the potential benefit of morphine or opioid administration in general have been contradictory, while acute respiratory, gastro-intestinal, cardiovascular, and neurological side effects as well as the occurrence of tolerance, dependence and withdrawal symptoms are well known. Animals studies have raised concerns about the long-term effects of both pain and morphine exposure in neonates on endpoints like brain structure, (hormonal) stress response, and behaviour later in life [19–21]. Data in humans are scarce, however recent long-term follow-up studies in cohorts of children that were exposed to morphine for the treatment of pain or for ventilatory support during the neonatal period showed limited to no difference between these children and controls on outcome measures like intelligence, behaviour, motor skills, memory, chronic pain, and health-related quality of life [22–24].

The number of patients in the current study is too small to establish the influence of the reduced morphine dose in young neonates on the occurrence of acute morphine-related side-effects, dependence or withdrawal, but it is expected that a morphine dose reduction positively influence these endpoints. Similarly, it could not be established whether the increased morphine dose in older children, yielded significant increases in the occurrence of acute morphine side-effects, dependence or withdrawal, however exposing these patients to ineffective or suboptimal doses of morphine can be regarded as equally unethical as over-dosing. It is also expected that optimization of the paediatric morphine dosing algorithm positively influences potential long-term effects that morphine exposure or untreated pain may have.

In conclusion, the development of evidence-based dosing regimens in the paediatric population is complicated by practical, ethical, and legal constraints. However population modeling now makes it possible to obtain drug dosing algorithms for the paediatric population with a similar level of scientific evidence as has been the standard requirement for the adult population for a long time [3]. It is envisioned that this methodology can be extended to other vulnerable patient populations as well.

For morphine, the development of a paediatric dosing algorithm that corrects for developmental changes in the pharmacokinetics yielded a 50% to 75% dose reduction in initial infusion rates in neonates younger than ten days that was still efficacious for the

majority of the patients. For children of ten days or older, the developed dosing algorithm prescribed an increased dose compared to the traditional dose, that still required rescue dosing in the majority of patients, although the total morphine requirement was still within 25% of the prescribed dose for almost half of the patients. Overall, the new morphine dosing algorithm reduces the risk of over-exposure in the youngest neonates as well as the risk of exposing older patients to suboptimal doses.

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Section III

Semi-Physiological Covariate Model for Paediatric Glucuronidation

Chapter 6

Semi-Physiological Model for Glucuronidation in Neonates and Infants; Application to Zidovudine

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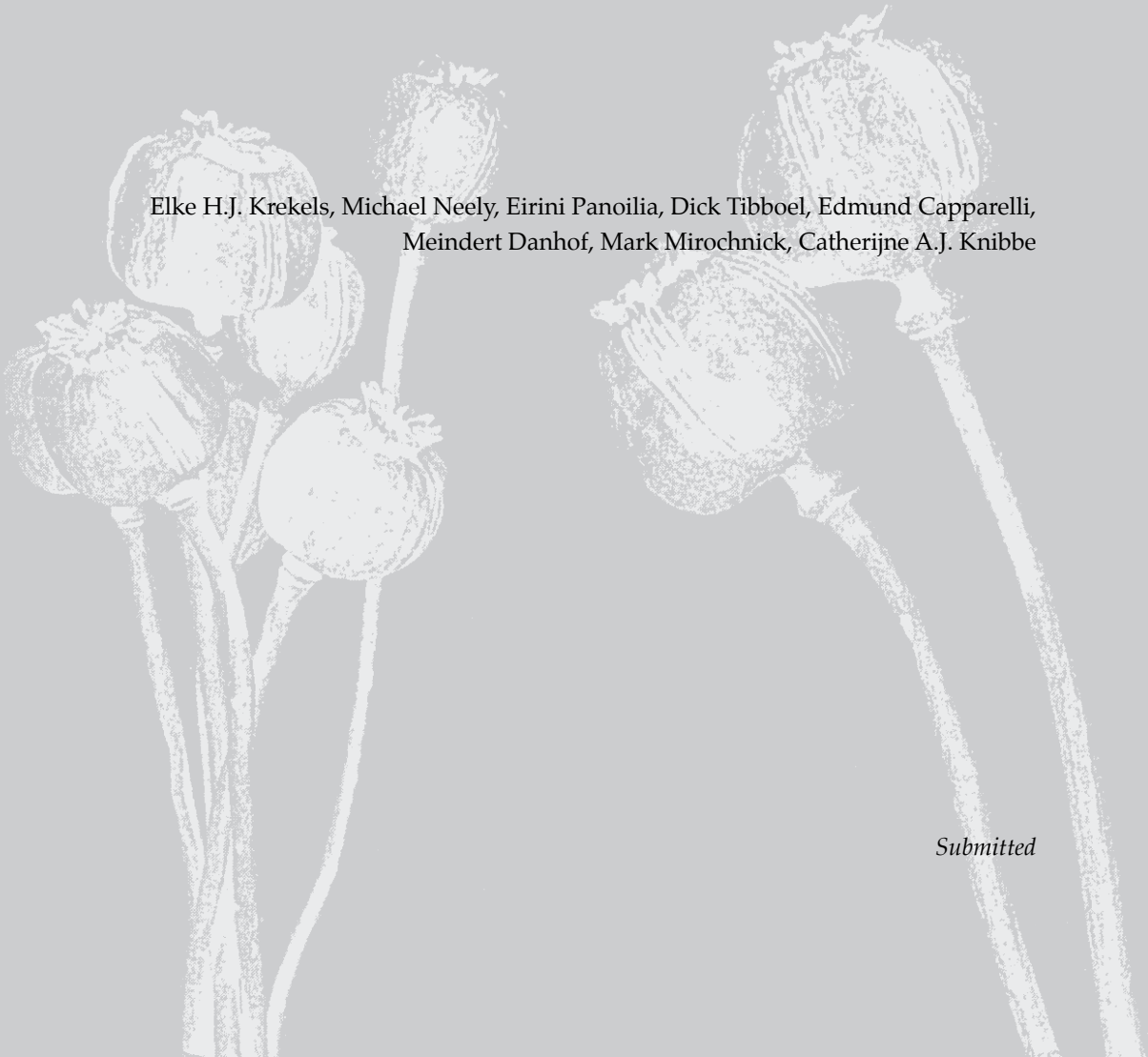

Submitted

Abstract

Aim. New approaches to expedite the development of safe and effective paediatric dosing regimens are necessary. We test the hypothesis that paediatric pharmacokinetic covariate models describe developmental changes in the physiological system and can therefore be extrapolated between drugs that share elimination pathways. Morphine and zidovudine, both primarily eliminated through UGT2B7-mediated glucuronidation, were used as paradigm compounds.

Method. Two population pharmacokinetic models were developed for a dataset of zidovudine and zidovudine-glucuronide in neonates and infants. One model was based on a comprehensive covariate analysis and served as a reference model. In the second model, a validated covariate model for morphine glucuronidation was directly incorporated. The performance of this system-specific model was compared to the reference model.

Results. In the reference model, developmental changes in glucuronidation clearance were best described by postnatal age in a sigmoidal function, while the system-specific model used a bodyweight-based exponential equation. Nevertheless, both models predicted similar population clearance values for the individuals in the dataset. The descriptive performances of both models were good and similar between the models, as expressed by a difference in objective function of only 13 points and similar goodness-of-fit plots. The predictive performance assessed by normalized prediction distribution errors, were good and similar as well for both models.

Conclusion. This proof-of-concept study supports our hypothesis that paediatric covariate models describe the physiological system quantitatively and can be considered to be semi-physiological. This approach may benefit paediatric pharmacokinetic analyses, the development of paediatric dosing algorithms and first-in-child studies.

6.1 Introduction

It is currently well established that the clearance of many drugs differ between adults and children and between children of different ages, and that these differences are a major cause of age-dependent differences in dose requirements [1]. However, the pharmacological properties of many drugs that are commonly prescribed for children have often not been properly investigated in this vulnerable population [2]. Therefore evidence-based dosing recommendations are often lacking for this patient group.

Pharmacokinetic studies in the paediatric population are complicated by ethical, practical and legal constraints. These constraints can be addressed by the application of novel data-analysis approaches [3]. Population pharmacokinetic modelling approaches are based on the simultaneous analysis of data from an entire population, while still taking into account that different observations come from different patients. They allow for the simultaneous analysis of sparse and/or dense data or unbalanced data. Population models not only yield pharmacokinetic parameter values for the population as a whole, but also quantify and differentiate sources of variability in the population. By identifying which patient characteristics (e.g. bodyweight, age, gender, race, genetics, disease status etc.) are predictors of the variability in model parameters, trends in the population can be described. Such predictors are called covariates; the equations describing the relationship between a covariate and a model parameter are called covariate relationships; and a set of covariate relationships in a population model is referred to as a covariate model. Pharmacokinetic covariate relationships can serve as a basis for evidence-based dosing guidelines, as drug doses should be adjusted according to changes in pharmacokinetic parameters.

It would require tremendous resources to develop and thoroughly validate pharmacokinetic covariate models for every new and existing drug prescribed for the paediatric population. Therefore smarter and more efficient approaches to expedite the development of safe and effective paediatric dosing regimens are necessary. We have hypothesized before that validated paediatric covariate models contain quantitative information about the developmental changes in the underlying physiological system in children. This implies that covariate relationships describing the developmental changes in the clearance of a specific drug can be extrapolated to another drug that is cleared through the same pathway [4]. The extrapolation of covariate models between drugs would expedite the development of paediatric population models, which could serve in optimizing drug dosing in first-in-child studies and in facilitating the development of evidence-based paediatric dosing recommendations.

In this analysis, morphine and zidovudine are used as paradigm compounds, as morphine and zidovudine are both prescribed for children of all ages and are predominantly metabolized through glucuronidation by the UGT2B7 isoenzyme [5–8]. The current proof-of-concept study shows that paediatric pharmacokinetic covariate models for a given metabolic pathway are semi-physiological and can therefore be extrapolated from one drug to another.

6.2 Methods

6.2.1 Study Design

To test the between-drug extrapolation potential of paediatric pharmacokinetic covariate models, two population pharmacokinetic models were developed for a single dataset of zidovudine (also known as 3'-azido-3'-deoxythimidine or azidothymidine) and its glucuronide metabolite:

A. Reference Model: For this model a comprehensive covariate analysis was performed yielding a pharmacokinetic model with a set of covariate relationships that best described the current data according to statistical criteria. This model will be referred to as the 'reference model'.
B. System-Specific Model: In this model the internally and externally validated covariate model from a population pharmacokinetic model for morphine glucuronidation in patients under the age of three years (Chapters 3 and 4) was directly incorporated. This semi-physiological covariate model will be referred to as the 'developmental covariate model' and the full population model that is based on the developmental covariate model will be referred to as the 'system-specific model'.

The descriptive and predictive properties of the system-specific model (B) were assessed by comparing them to the descriptive and predictive properties of the fully optimized reference model (A).

6.2.2 Patients and Data

Zidovudine

The current analysis is based on 473 zidovudine concentrations and 173 zidovudine-glucuronide concentrations collected on 68 occasions from 29 individuals varying from term neonates to infants up to five months of age (PACTG 049 [9]). These data were obtained from a multicenter study to evaluate the safety, tolerability and pharmacokinetics of zidovudine as a prophylaxis to prevent mother-to-child HIV transmission in healthy neonates and infants born to HIV infected women. The study protocol was approved

by institutional review boards of the participating institutions and written informed consent was obtained from the parents or legal guardians of each patient.

For each patient dense data were available from multiple occasions that were days or weeks apart. Zidovudine was administered both intravenously and orally to each patient. Data were obtained after single dose administrations on separate occasions and for eight patients data from administrations that were part of a long-term oral dosing regimen were available as well. Dosing started at 2 mg/kg but could be increased to 4 mg/kg during the course of the study as deemed appropriate by the treating physician.

Morphine

A dataset of morphine and its glucuronides in 248 preterm and term neonates to three year old infants was used to obtain the developmental covariate model used in the system-specific model (Chapter 3). In table I study and patient characteristics for the zidovudine dataset used for both models in the current analysis and the morphine datasets used to obtain the developmental covariate model are shown for comparison.

Table I. *Patient and study characteristics of the zidovudine dataset that was the basis for the reference model and the system-specific model in the current analysis and the morphine dataset that was the basis for the developmental covariate relationships applied in the system-specific model.*

Characteristic	**Zidovudine dataset [9]**	**Morphine dataset (Chapter 3)**
Number of patients	29	248
Number of samples of parent compound	473	792
Number of samples of glucuronide	173 (G-ZDV)	664 (M3G) 722 (M6G)
Administration route	oral and short-term iv	short-term and continuous iv
Duration	multiple occasions days or weeks apart	single occasion of up to 5 days
Sampling	dense	sparse
Population	healthy patients	ventilated and post-operative (non-cardiac surgery) patients
Postnatal age (range, days)	2 – 145	0 – 1071
Postmenstrual age (range, weeks)	36 – 57	25 – 193
Bodyweight (range, kg)	1.9 – 6	0.5 – 16.8
Sex (M/F)	18 / 11 (62% / 38%)	144 / 104 (58% / 42%)

G-ZDV = zidovudine glucuronide, M3G = morphine-3-glucuronide, M6G = morphine-6-glucuronide

6.2.3 Model Development

NONMEM VI (ICON, Ellicott City, MD, USA) was used to perform the data analysis, with PLT Tools version 3.0.0 [10] in combination with R version 2.10.0 for the visualization of the data. All parameter estimates were obtained with the first-order conditional estimation method with interaction (FOCE-I).

Model development for the reference model and the system-specific model was performed in three steps:

1. choice of structural model
2. choice of error model
3. choice of the covariate model

For the reference model and the system-specific model, the first two steps in the model development process (i.e. the choice of the structural and error model) were the same. One- and two-compartment models were tested for the structural model. For the error model inter-individual variability on the model parameters was tested assuming a log-normal distribution described by an exponential distribution model depicted in equation 1. For bioavailability (F) inter-individual variability was described using equation 2 to avoid individual bioavailability estimates of more than 100%.

$$P_i = \theta * \exp(\eta_i) \qquad \text{(Equation 1)}$$

$$P_i = \frac{e^{\theta+\eta_i}}{1+e^{\theta+\eta_i}} \qquad \text{(Equation 2)}$$

In these equations P_i is the individual parameter estimate for the ith individual, θ represents the population parameter estimate for parameter P, and η_i is a random variable for the ith individual from a normal distribution with a mean of zero and estimated variance of ω^2. For the intra-individual variability and residual error in the observed zidovudine and zidovudine-glucuronide concentrations proportional (equation 3), additive (equation 4), and combination (equation 5) error models were tested:

$$C_{obs,ij} = C_{pred,ij}(1+\varepsilon_{ij}) \qquad \text{(equation 3)}$$

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{(equation 4)}$$

$$C_{obs,ij} = C_{pred,ij}(1+\varepsilon_{1,ij}) + \varepsilon_{2,ij} \qquad \text{(equation 5)}$$

where $C_{obs,ij}$ is the jth observation in the ith individual, $C_{pred,ij}$ is the predicted value of that observation and ε_{ij} is a random variable from a normal distribution with a mean of zero and estimated variance of σ^2.

The Likelihood Ratio, which was assumed to be χ^2 distributed, was used to assess whether the difference between (sub)models was statistically significant. A decrease in the objective function corresponding to $p < 0.01$ was considered to be significant. In addition, the following basic goodness-of-fit plots were used for diagnostic purposes: (a) observed *versus* individually predicted concentrations, (b) observed *versus* population predicted concentrations, (c) conditional weighted residuals *versus* time, and (d) conditional weighted residuals *versus* population predicted concentrations. Furthermore, the 95% confidence intervals of the model parameters and the correlation matrix were assessed.

The third and final step of the model development process (i.e. choice of the covariate model) was different for the reference model and the system-specific model:

A. *Reference model:* A comprehensive covariate analysis with forward inclusion and backward deletion of covariates was performed to obtain a covariate model with the best description of the current zidovudine data according to statistical criteria. The following covariates were tested for significance: postnatal age, postmenstrual age, gestational age at birth, bodyweight, sex, and creatinine clearance. The continuous covariates were tested in linear equations, exponential equations with estimated exponents, or sigmoidal equations. A decrease in the objective function corresponding to $p < 0.01$ for the forward inclusion of covariates was considered to be significant. Additionally, the aforementioned diagnostic criteria were used. When more than 1 significant covariate was identified, the most significant covariate was included in the model and the resulting model served as the basis for the subsequent exploration of additional covariate effects. For the backward deletion of covariates an increase in objective function corresponding to $p < 0.001$ was considered to be significant.

B. *System-specific model:* The previously obtained and internally and externally validated covariate model for morphine glucuronidation in children younger than three years (Chapters 3 and 4) was directly incorporated into the model for zidovudine. Specifically, a bodyweight-based exponential equation with an exponent of 1.44 for the formation and elimination of zidovudine-glucuronide with a reduced formation clearance of zidovudine-glucuronide in neonates younger than ten days was included, as was a linear correlation for distribution volume of the parent compound and metabolite (see figure 1 for equations). While this developmental covariate model describes the rate of developmental changes in clearance and distribution volume, the population values that describe the absolute values of these parameters for zidovudine were still estimated by NONMEM.

6.2.4 Model Evaluation

Model performance of the reference model and the system-specific model were evaluated and compared. Although the reference model and system-specific model are not nested, they are based on the exact same patients and data. Therefore the -2 log likelihood, by means of the NONMEM objective function, was used as a measure to statistically compare the description of the zidovudine data by the system-specific model to the description of the zidovudine data by the reference model. To directly compare clearance predictions between the two models, population clearance predictions from the reference model were plotted *versus* population clearance predictions from the system-specific model. As age and bodyweight change rapidly in this young population, estimated parameter values did not remain constant between the occasions, yielding one prediction per patient per occasion.

Furthermore, the descriptive properties of the models were assessed and compared by inspecting the basic goodness-of-fit plots of the models. These plots were stratified by age into a group that was younger and a group that was older than 38 days (the median age of the individuals at the different occasions) to ascertain that the entire age-range was described equally well. In addition, the covariate relationships describing the population predicted zidovudine clearances and the individual *post hoc* clearance estimates of each individual at each separate study occasion were plotted in one graph for each model, to visually assess the description of the individual zidovudine glucuronidation clearances by the covariate relationships. Finally, bias and precision of the individual zidovudine glucuronidation clearance values compared to the population predicted clearances described by the covariate relationships were quantified by calculating the percentage mean prediction error (%MPE, equation 6) and the root mean square error (RMSE, equation 7) respectively.

$$\%MPE = \frac{\sum \frac{(populationCL - individualCL)}{individualCL}}{n} \cdot 100 \qquad \text{(equation 6)}$$

$$RMSE = \sqrt{\frac{\sum (populationCL - individualCL)^2}{n}} \qquad \text{(equation 7)}$$

To compare the predictive properties of both models, a normalized prediction distribution error (NPDE) analysis [11] which is a simulation-based diagnostic, was used. The entire dataset was simulated 1000 times in NONMEM and subsequently each observed concentration was compared to the reference distribution of the simulated data points using the NPDE add-on package in R [12].

6.3 Results

6.3.1 Model Development

In the first step of model development (i.e. choice of structural model) a two-compartment model was found to best describe the time-course of zidovudine, and a one-compartment model was used to describe the time-course of the zidovudine-glucuronide, as is depicted in figure 1. Zidovudine absorption from the oral depot compartment was described by first-order absorption (k_a) and the oral bioavailability (F) was estimated. Zidovudine clearance through pathways other than glucuronidation was found to be not significantly different from 0. When estimated, the values of the distribution volume of the central (V_1) and peripheral (V_2) compartment of zidovudine were not significantly different from each other, these values were therefore fixed to be equal. The distribution volume of the glucuronide (V_3) was estimated as a fraction of the central compartment of zidovudine (θ_{V3}).

In the second step (i.e. choice of error model) significant inter-individual variability could be identified for the absorption rate constant (k_a), the formation (Cl_1) and elimination (Cl_2) clearance of zidovudine-glucuronide, the distribution volume of the central compartment (V_1), and the bioavailability (F). Additionally, in the reference model a correlation between the inter-individual variability of the distribution volume of the central compartment (V_1) and the formation clearance of zidovudine-glucuronide (Cl_1) was identified. The inter-individual variability and residual error for the reference model and the system-specific model were best described by a proportional error model (equation 3).

The third step (i.e. choice of covariate model) was different for the reference model and the system-specific model.

A. *Reference model*: In the comprehensive covariate analysis, age (either postnatal or postmenstrual age) and bodyweight were readily identified as predictive and statistically significant covariates for the clearance parameters. Due to the relatively small range in bodyweight and age of the patients in the current zidovudine dataset (see table I), only small differences in objective function and diagnostics between models using either of the three covariates or between models using these covariates in different equations (i.e. linear, exponential or sigmoidal) were obtained. Based on the objective function postnatal age was found to be the slightly superior covariate for the formation clearance (Cl_1) and the elimination clearance (Cl_2) of zidovudine-glucuronide. The inclusion of this covariate in the reference model was most optimal in a sigmoidal relationship on Cl_1 and in a linear relationship with estimated y-intercept on Cl_2.

Based on the statistical criteria no covariates were identified for the distribution volumes.

B. *System-specific model*: as mentioned in the methods section, the developmental covariate model included in the system-specific model consisted of bodyweight-based exponential equations with an exponent of 1.44 for the formation and elimination of zidovudine-glucuronide with a reduced formation clearance of zidovudine-glucuronide in neonates younger than ten days, and linear relationships between bodyweight and distribution volumes (Chapter 3).

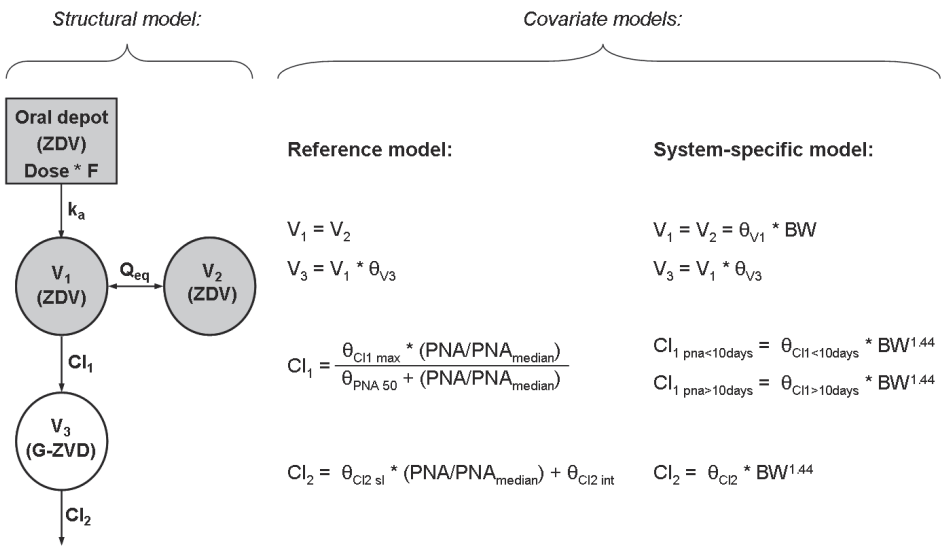

Figure 1. *Schematic representation of the structural model for the zidovudine models (left) and equations of the covariate relationships in the reference model (middle) and the system-specific model (right). ZDV = zidovudine, G-ZDV = zidovudine-glucuronide, F = bioavailability, k_a = absorption rate constant, V = distribution volume of designated compartment, Cl = clearance of designated route, Q= inter-compartmental clearance, θ_V = distribution volume of designated compartment as fraction of V_1, PNA = postnatal age with subscript 'median' indicating the median value of the individuals at the different occasions, θ_{Cl1} max = maximum value of the zidovudine glucuronidation clearance, $\theta_{PNA\ 50}$ = postnatal age at which half the maximum value of zidovudine glucuronidation clearance is reached, $\theta_{Cl2\ sl}$ = slope of the line describing age-related changes in zidovudine glucuronide elimination clearance, $\theta_{Cl2\ int}$ = y-intercept of the line describing age-related changes in zidovudine glucuronide elimination clearance, BW = bodyweight, $\theta_{Cl1<10days}$ = population value of zidovudine glucuronidation clearance value in children younger than ten days of age, $\theta_{Cl1>10days}$ = population value of zidovudine glucuronidation clearance value in children older than ten days of age, θ_{Cl2} = population value of zidovudine glucuronide elimination clearance value.*

Figure 1 shows the equations of the covariate relationships in the reference model and the system-specific model, in addition to providing a schematic representation of the structural model for both models.

Table II. *Final parameter estimates of the reference model and the system-specific model for zidovudine glucuronidation.*

Pharmacokinetic parameter [unit]	**Reference model**		**System-specific model**	
	model parameter [unit]	**value (CV%)**	**model parameter [unit]**	**value (CV%)**
Fixed effects				
F	θ	1.56 (21.0)	θ	1.55 (30.6)
	F [%]	82.6	F [%]	82.5
k_a [min^{-1}]		0.0307 (0.9)		0.031 (17.7)
$V_1 = V_2$	[l]	4.02 (1.3)	[l/kg]	1.08 (11.4)
V_3 [fraction of V_1]		0.226 (20.4)		0.211 (18.3)
Cl_1	$Cl_{1\ max}$ [l/min]	0.116 (7.6)	$Cl_{1<10days}$ [$l/min/kg^{1.44}$]	0.00435 (12.1)
	$Cl_{PNA\ 50}$ [days]	1.63 (17.9)	$Cl_{1>10days}$ [$l/min/kg^{1.44}$]	0.00853 (11.7)
Cl_2	$Cl_{2\ sl}$ [l/min/day]	0.00257 (17.5)	Cl_2 [$l/min/kg^{1.44}$]	0.00231 (10.7)
	$Cl_{2\ int}$ [l/min]	0.00911 (11.64)		
Q_{eq} [l/min]		0.0275 (11.8)		0.0289 (11.7)
Inter-individual variability				
ω^2 (F)		2.78 (42.5)		2.82 (45.0)
ω^2 (k_a)		0.625 (36.8)		0.607 (39.9)
ω^2 (V_1)		0.443 (56.2)		0.366 (49.7)
ω^2 (Cl_1)		0.328 (38.7)		0.255 (54.51)
ω^2 (Cl_2)		0.142 (46.3)		0.112 (70.3)
ω^2 (V_1-Cl_1) interaction		0.312 (54.5)		-
Residual variance				
σ^2 (ZDV)		0.11 (7.2)		0.11 (11.5)
σ^2 (G-ZDV)		0.158 (13.3)		0.152 (15.5)

F = bioavailability presented as value of θ in eq. 2 and population value of F calculated with eq. 2, ω^2 = variance of the normal distribution that quantifies the inter-individual variability on the designated parameter according to eq. 1 or eq. 2 for bioavailability, σ^2 = variance of the normal distribution that quantifies the residual error of the designated observation according to eq. 3. See figure 1 for explanation of other symbols.

In table II the model parameter estimates obtained for the models are shown. The values for structural parameters as well as for parameters of the error model are similar between the reference model and the system-specific model. Additionally, for both models the coefficient of variation of the fixed effects remain well below 50%, indicating that 0 was not in the 95% confidence interval of the parameter estimates and that the parameters can therefore be considered significant and estimated with acceptable precision. The coefficient of variation of some of the variance estimates of the inter-individual variability did exceed 50% indicating that the information in the dataset was uninformative for precise estimation of these parameter values. Interestingly, as shown in figure 2, both models estimate similar population clearance values for each individual at each occasion, despite the differences in covariate models.

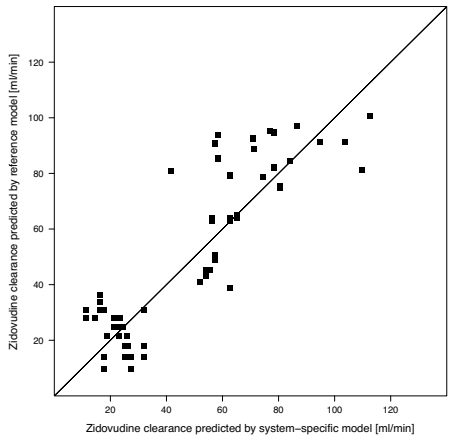

Figure 2. *Population predicted zidovudine clearances (Cl_1) for the reference model versus the system-specific model for each individual at each separate study occasion.*

6.3.2 Model Evaluation

The reference model was statistically superior over the system-specific model in describing the zidovudine data, as demonstrated by a difference in objective function of 13 points and a 2 point difference in degrees of freedom. Although statistically significant, this difference is small suggesting only a small difference in the description of the data between the two models. This is corroborated by the goodness-of-fit plots in figure 3. Visual inspection of the graphs shows that both models can describe the observed concentrations in children older and younger than the median age of children at the different occasions without bias and that the difference in the plots of the two models is negligible.

In addition to an unbiased description of the concentrations, the plots in figure 4 show that both models can also describe individual glucuronidation clearances for

zidovudine (Cl_1) in the population without bias, despite the use of different primary covariates as descriptors for the developmental changes. Accuracy of the individual zidovudine clearance values compared to the population values described by the covariate relationships was numerically quantified as mean percentage error and was 20.5% for the reference model compared to 11.3% for the system-specific model. The precision, numerically quantified as root mean square error, was 19.2 for both the reference model and the system-specific model.

In terms of predictive performance, the two models perform similar as well, as expressed by the results of the normalized prediction distribution error analysis shown in figure 5. The reference model and the system-specific model can accurately predict the median zidovudine concentrations, but they slightly over-estimate the variability in the observations. In addition, there is no bias in normalized prediction distribution errors in time or across the concentration range for any of the models.

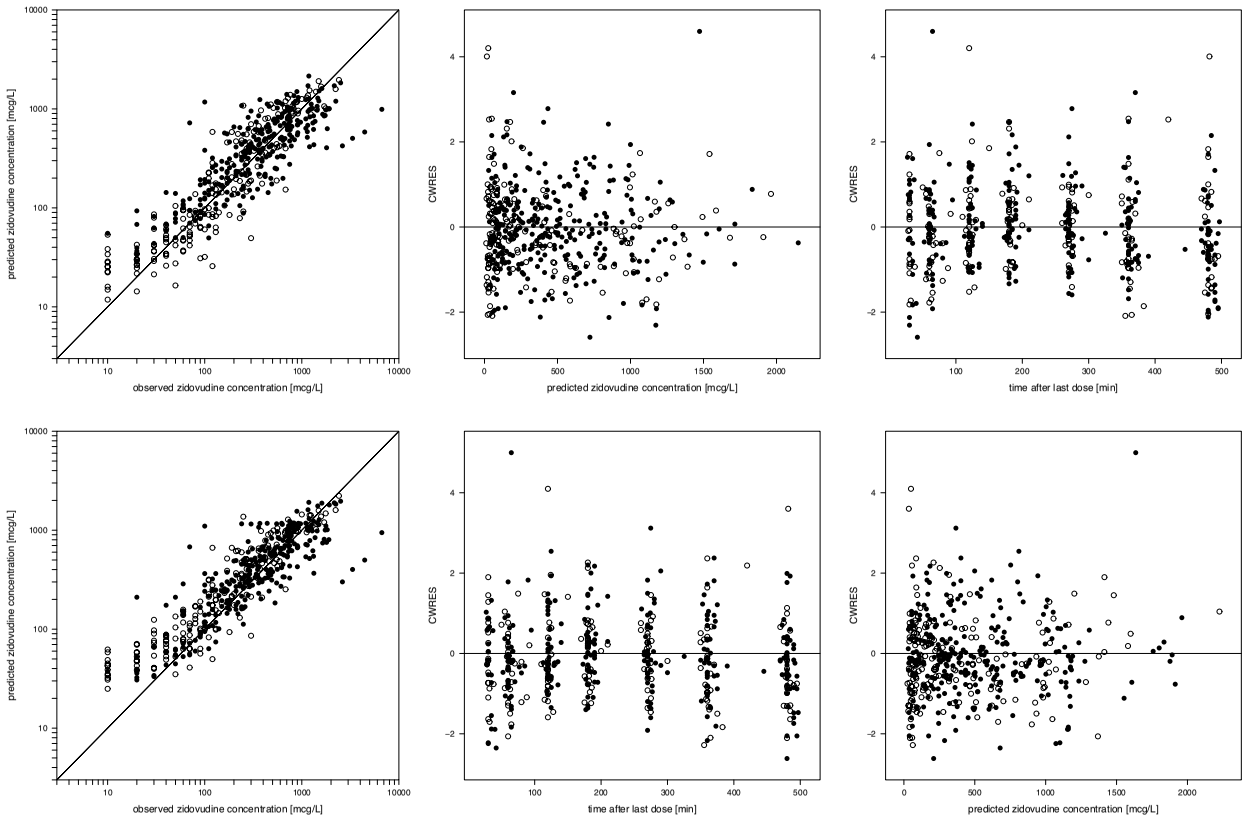

Figure 3. *Basic goodness-of-fit plots for zidovudine of the reference model (top) and the system-specific model (bottom). On the left are population predicted concentrations versus observed concentrations, in the middle are conditional weighted residuals (CWRES) versus time after last dose, and on the right are conditional weighted residuals (CWRES) versus population predicted concentrations. The plots are stratified by age, with solid circles indicating observations in children on occasions that they were younger than the median age of the individuals at the different occasions and open circles indicating observations in children on occasions that they were older than this median age.*

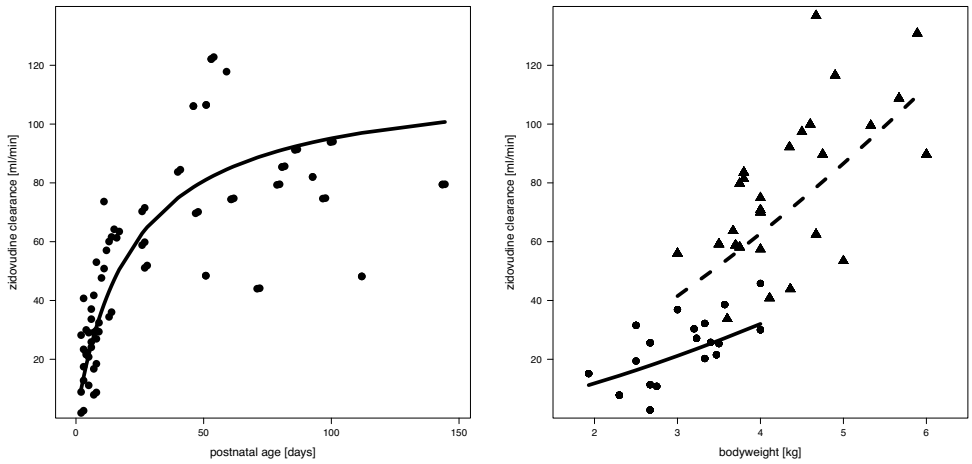

Figure 4. *Individual post hoc parameter values of the glucuronidation clearance to zidovudine-glucuronide (Cl_1) for each individual at each separate study occasion versus the most predictive covariate, which is postnatal age for the reference model (left) and bodyweight for the system-specific model (right). The covariate relationship describing the population clearance values are indicated with lines. For the plot of the system-specific model (right) individual post hoc parameter estimates and population estimates of children younger than ten days are indicated with circles and a solid line respectively, for children older than ten days triangles and a dotted line are used respectively.*

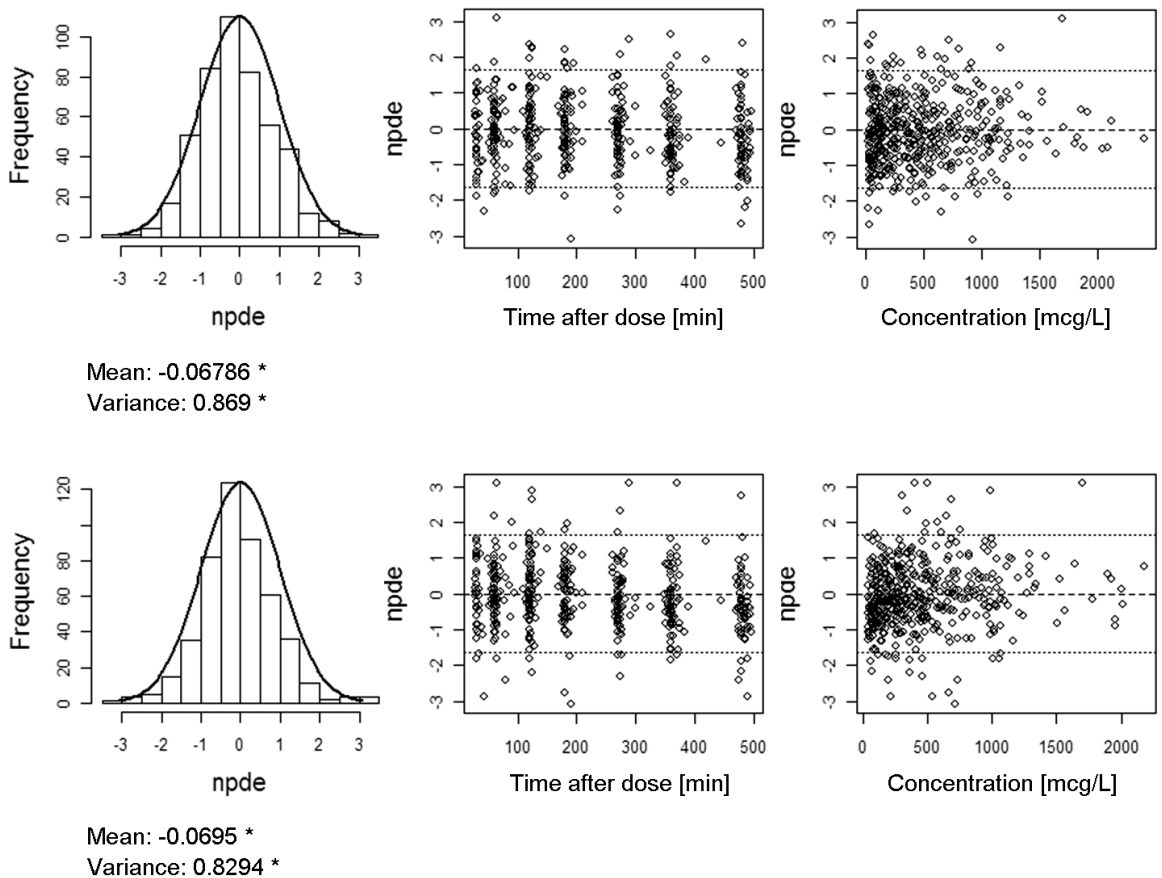

Figure 5. *Results of the NPDE analysis for zidovudine from the reference model (top) and the system-specific model (bottom). In the histograms the distributions of the NPDEs in the overall dataset are shown with the solid line depicting a normal distribution and the values below specifying the mean and variance of the npde distribution in the histogram. A significant ($p < 0.05$) deviation of the distribution from a mean of 0 and a variance of 1 is indicated with an asterisk (*). The NPDE distribution versus time after last dose (middle) and versus the observed concentrations (right) are also shown.*

6.4 Discussion

The current investigation is a proof-of-concept study to examine the hypothesis that paediatric covariate models for drug clearance describe changes in the underlying physiological system and can therefore be extrapolated from one drug to another drug that is eliminated through the same pathway. Our focus was on clearance in particular, because the ontogeny of clearance is considered to be the main driver of differences in pharmacological drug response in the paediatric population [1]. Covariate models that describe the developmental changes of clearance pathways in paediatric population pharmacokinetic models are crucial to determine first-in-child or evidence-based dosing regimen, as the covariate relationship describing these changes can be directly used in drug dosing algorithms.

Morphine and zidovudine were used as paradigm drugs in this investigation because they are both primarily eliminated through glucuronidation by the UGT2B7 isoenzyme [5–8] . The developmental glucuronidation model, an internally and externally validated paediatric covariate model for morphine glucuronidation (Chapters 3 and 4), was directly incorporated into the pharmacokinetic model for the glucuronidation of zidovudine. The descriptive and predictive properties of this system-specific model were compared to a reference model. The covariate model of the reference model was developed by a comprehensive covariate analysis of the same dataset to obtain a model that provided the best description of the data according to statistical criteria. The results of this analysis show these two models to have similar descriptive and predictive performances. Given that the difference in time it took to develop both models is measured in weeks, the system-specific model performed remarkably well.

Observed pharmacokinetic profiles are the result of the interaction between a drug and the physiological system. The parameters used to describe pharmacokinetic profiles therefore represent drug-specific and/or system-specific aspects of this interaction. The results from the current study suggest that developmental changes in drug glucuronidation are drug-independent and are therefore indeed likely to reflect changes in the underlying physiological system. Other studies suggest the same to be applicable to glomerular filtration as well [13]. Our group previously described and defined a distinction between drug-specific and system-specific parameters in population models for pharmacokinetic and pharmacodynamic processes [14]. Based on the current analysis the context of system-specific properties can be extended to not only include static descriptors of the physiological system, but to also include temporal changes in the physiological system as a result of developmental changes in the paediatric population. We therefore denote

the zidovudine model that was based on the developmental covariate model obtained with morphine as the 'system-specific model'.

With the incorporation of system-specific information into population models, the methodology proposed here is moving away from the empiricism of population modelling, towards the mechanistic approach of physiologically-based pharmacokinetic modelling. We envision that the developmental covariate models not necessarily only include the influence of age-related changes on drug pharmacokinetics, but that they may also include significant influences of other static and/or dynamic covariates (e.g. genetics, disease status etc.) on the underlying physiological system that is driving pharmacokinetics. It is however a prerequisite that these paediatric covariate models are extensively validated and that the population to which the covariate model applies is well defined in terms of other potentially important covariates, like for instance genetics or disease status.

Paediatric population pharmacokinetic models of hitherto unstudied drugs can be developed in a time-efficient manner and with limited resources, by the between-drug extrapolation of these semi-physiological paediatric covariate models. This methodology allows for the use of denser information or information from a wider age-range than may be available for the analysis of an unstudied drug. The developmental covariate model in the system-specific model of the current analysis was for instance based on the analysis of morphine glucuronidation in 248 patients ranging from preterm neonates to infants of three years, whereas in the current zidovudine analysis data from only 29 patients ranging from term neonates to infants of five months were available (see table I). Due to the small range in age and bodyweight in this zidovudine dataset, the difference in descriptive and predictive properties of models with different covariate relationships was small. In the comprehensive covariate analysis inclusion of postnatal age, postmenstrual age or bodyweight in either linear, exponential or sigmoidal relationships yielded models with similar objective functions and diagnostics, however based on statistical criteria postnatal age in a sigmoidal equation was selected as the final covariate model for the maturation of zidovudine glucuronidation. The bodyweight-based exponential covariate relationship identified for morphine glucuronidation was not identified for zidovudine in the comprehensive covariate analysis of the current zidovudine data. This is probably due to the indistinctive curvature of this relationship in the bodyweight-range of the zidovudine dataset. Nonetheless, direct incorporation of the developmental covariate model into the zidovudine model did provide a good description of the population and individual zidovudine clearance parameters as shown in figure 4. As such, information from one drug seems indeed to be of value for the analysis of a similar drug, which is especially important in the paediatric population where often only limited data are available.

The clinically observed developmental changes in drug pharmacokinetics represent the net result of the developmental changes in a number of processes in the underlying biological system. This may include changes in expression and function of drug metabolizing enzymes and active transporters, changes in body composition, changes in cardiac output and organ perfusion, changes in acid-base balance, and changes in the amount and composition of drug-binding plasma proteins and the presence of other blood components that may influence plasma protein binding [15]. The weight that each individual process has on the net observed changes in drug pharmacokinetics may be different for drugs with different molecular and pharmacokinetic properties. Morphine and zidovudine are quite similar with respect to these properties. Their molecular masses are 285 g/mol and 267 g/mol respectively. Plasma protein binding in adults ranges between 25% and 40% for both drugs [16,17] and their hepatic extraction ratios in adults range between 0.5 and 0.65 [18–20]. The pKa value for morphine is around 7.9 [21] and for zidovudine this value is around 9.5 [22]. Finally, $\log P_{octanol/water}$ values for these compounds were reported to be 0.75 [23] and 0.05 [24] respectively. It remains to be investigated how and to what extent differences in physicochemical and pharmacokinetic drug properties influence the between-drug extrapolation potential of the semi-physiological paediatric covariate models.

One of the drawbacks of the method applied in the current analysis is that model development for the new drug (zidovudine in this case) still relies on the availability of at least a limited amount of paediatric data to determine the population value of the clearance, which is mainly determined by the drug-specific parameters K_m and V_{max}. This does not pose a problem when a marketed drug that is unstudied in the paediatric population is already being used off-label in that population. However, when in drug development a drug has never been used in a paediatric age-range before, a methodology that does not rely on *in vivo* paediatric data of the drug under investigation is required. To date there is no suitable methodology based on population pharmacokinetic modelling available to extrapolate paediatric pharmacokinetic parameters from older to younger age-ranges in the drug development process [25]. With physiologically-based pharmacokinetic modelling the absolute value of drug clearance could be predicted without prior paediatric *in vivo* data. Unfortunately knowledge on all underlying physiological processes is currently incomplete especially for the paediatric population, which potentially impedes paediatric clearance predictions by physiologically-based pharmacokinetic modelling. Therefore the approach proposed here is combining the physiological insight from physiologically-based pharmacokinetic modelling with the descriptive approach of population modelling. If system-specific profiles on developmental changes in certain metabolic pathways were available over the entire paediatric age-range, these profiles could be used to design successive studies

in children of decreasing ages for unstudied drugs. These studies could then be of a confirmative rather than an explorative nature.

In conclusion, this proof-of-concept study supports our hypothesis that paediatric covariate models that describe the developmental changes in drug elimination pathways constitute system-specific rather than drug-specific information and can therefore be used for extrapolation between drugs that share an elimination pathway. This approach can be considered a semi-mechanistic hybrid between empirical population modelling and physiologically-based pharmacokinetic modelling. Between-drug extrapolation of semi-physiological covariate models can expedite the development of paediatric population pharmacokinetic models that can in turn be used to derive first-in-child and evidence-based dosing recommendations for this population.

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Chapter 7

Top-Down and Bottom-Up Modeling; The Physiological and Physicochemical Basis for the Maturation of UGT2B7-Mediated Drug Glucuronidation

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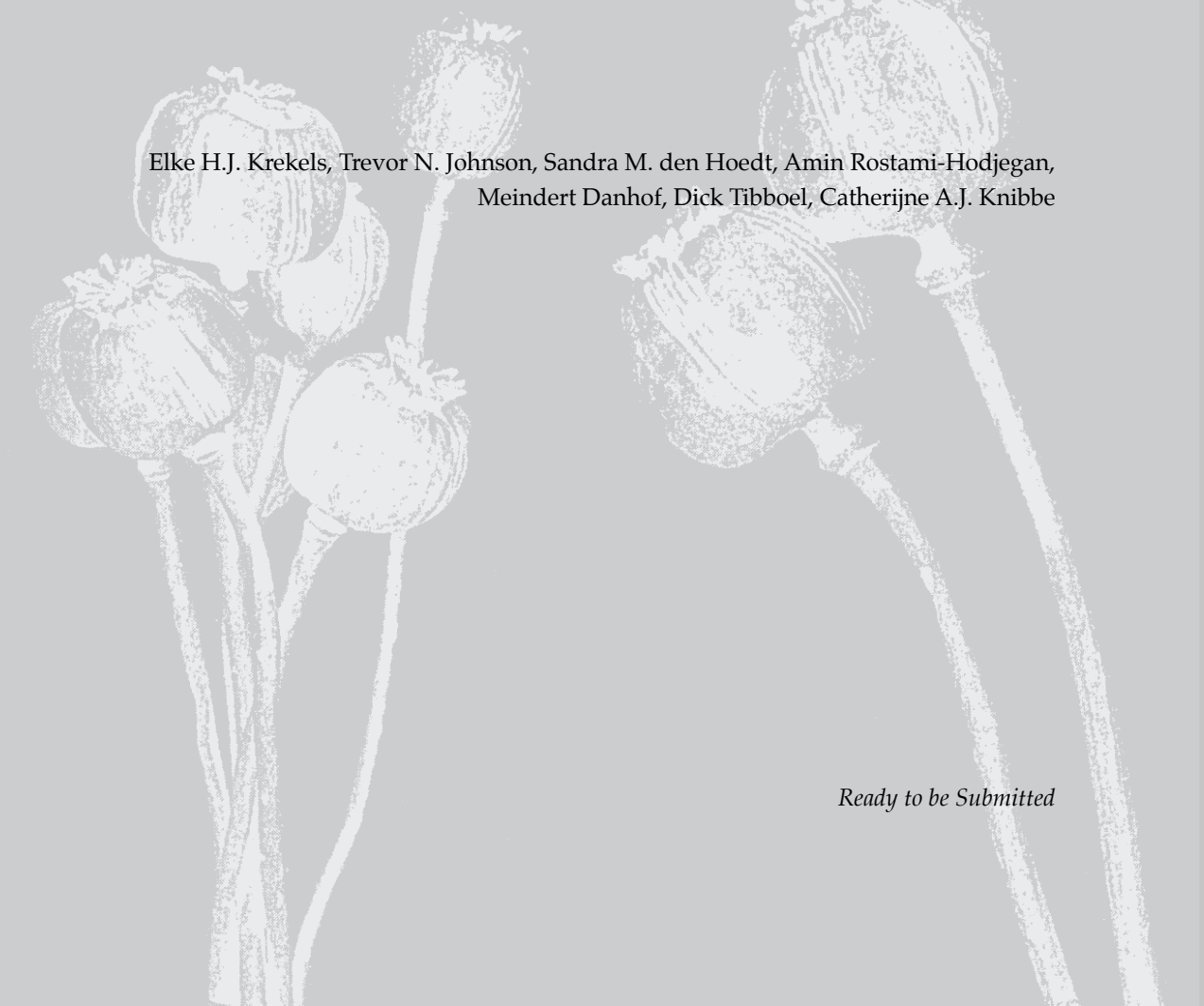

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Abstract

There is little insight in the extrapolation potential of paediatric covariate models between drugs that share a common elimination pathway. In this study, the physiological and physicochemical basis of a semi-physiological covariate model quantifying the developmental changes in *in vivo* UGT2B7-mediated drug glucuronidation in children younger than three years in population models (top-down models), was untangled using a physiologically-based model (bottom-up model).

Simcyp version 11 was used to simulate *in vivo* clearance values for morphine and zidovudine, both selective UGT2B7 substrates, in children younger than three years. The contribution of changes in system-specific parameters (hepatic blood flow, liver volume, microsomal protein per gram of liver, UGT2B7 ontogeny, unbound drug fraction) to changes in *in vivo* drug clearances were quantified. Additionally, the influence of physicochemical drug properties (molecular mass, logP, pKa) on the *in vivo* clearance of hypothetical UGT2B7-substrates was determined.

Using currently available *in vitro* data, morphine and zidovudine clearances were under-predicted by the physiologically-based model. However, the predicted developmental profile in glucuronidation clearance was similar to the clinically observed profile across the first three years of life, with the exception of the first two weeks of life. Changes in system-specific parameters explained 79% and 41% of the increases in *in vivo* morphine and zidovudine clearance, respectively, with the influence of liver size and UGT2B7 ontogeny being most pronounced. Physicochemical drug parameters did not affect the developmental glucuronidation profile, although logP and pKa did both influence the absolute value of clearance.

In conclusion, liver size and UGT2B7 ontogeny were identified as the main physiological drivers of the increases in UGT2B7-mediated clearance in the first three years of life. As physicochemical drug parameters only alter the absolute value of paediatric *in vivo* glucuronidation, the semi-physiological paediatric covariate model for drug glucuronidation can be used to predict the developmental clearance profile of other UGT2B7 substrates. Situations involving non-linear clearance as well as blood flow dependence due to high drug extraction ratios need further investigation.

7.1 Introduction

Research to develop evidence-based rather than empiric or consensus-based dosing algorithms for the paediatric population is complicated by practical, ethical, and legal constraints. These issues can be overcome by the application of population modeling approaches as is also recommended by the FDA and EMA [1,2]. Population modeling relies on outcome measures (i.e. observed concentrations) that can be obtained during routine clinical practice and allows for data analysis on the basis of sparse samples [3]. However, despite the multi-factorial nature of the ontogeny of drug clearance, paediatric population models only describe net observed changes in ontogeny with a limited number of covariate relationships. Therefore these models are only applicable to specific drugs in a specified population, requiring collection of the same type of data and full analysis of these data for every drug in every population of interest. Recently physiologically-based modeling has gained in popularity. These models use *in vitro* data on drug kinetics in combination with anatomical measurements, physiological parameters and mathematical equations to quantify physiological processes and the interaction of a molecule with certain physicochemical properties with this system. The usefulness of this type of modeling in paediatric pharmacokinetics has also been recognized by regulatory agencies [4]. Not all parameters in the physiologically-based models can be easily obtained, especially in the paediatric population. However, most parameters are 'system specific' and therefore not restricted to specific drugs or populations. As a result physiologically-based models are more generalizable.

Only rarely have the advantages of physiologically-based modeling, known as the bottom-up approach, been combined with the top-down approach of population modeling to augment each other. We have recently developed and validated a population pharmacokinetic model for morphine glucuronidation in children younger than three years (Chapters 3 and 4). It was found that the paediatric covariate model that quantifies the developmental changes in morphine clearance can be directly incorporated in the paediatric population model for the clearance of zidovudine (Chapter 6). Since morphine and zidovudine are both primarily eliminated through UGT2B7-mediated glucuronidation, this confirmed our hypothesis that covariate relationships in paediatric population models quantify developmental changes in the underlying physiological system, and thereby constitute system-specific information that can be extrapolated between drugs that share a common elimination pathway. Pharmacokinetic modeling based on this concept was called semi-physiological modeling, as it combines analyzing outcome measures with population modeling and the mechanistic insight of physiologically-based modeling.

The current study focuses on the semi-physiological developmental glucuronidation model for UGT2B7-mediated glucuronidation clearance in preterm and term neonates to children younger than three years, which was developed using morphine and zidovudine as model drugs (Chapters 3, 4, and 6). We aimed to dissect the various sources of developmental changes in the physiological system to identify which changes are the main drivers of the net observed changes in UGT2B7-mediated glucuronidation in neonates and infants, thereby also identifying patient characteristics that potentially limit the applicability of the semi-physiological developmental glucuronidation model. Additionally, we investigated whether certain physicochemical drug properties could affect the utility of the semi-physiological developmental model for compounds that share the UGT2B7-mediated elimination pathway.

7.2. Materials and Methods

7.2.1 Physiologically-Based Simulations

The physiologically-based pharmacokinetic modeling software Simcyp version 11 (Simcyp Ltd, Sheffield, UK) was used to investigate the influence of system-specific and drug-specific parameters on the ontogeny of *in vivo* UGT2B7-mediated drug glucuronidation in the first three years of life.

The paediatric database in Simcyp was selected for the physiologically-based pharmacokinetic simulations and parameters were set to include patients with a maximum age of three years. One thousand individuals were simulated and the same random number generation seed was used for repeated simulations. This yielded exactly the same set of individuals for each simulation and allowed for a direct comparison of clearance predictions between individuals in simulations with varying model parameter values. A uniform age distribution was used and the male/female ratio was set to 1. For each of the 1000 simulated individuals, age and sex appropriate bodyweight and height were determined based on UK reference growth charts taking inter-individual variability into account [5]. Body surface area was calculated according to Haycock *et al.* [6] for children with a bodyweight less than 15 kg and according to DuBois and DuBois [7] for heavier children. The parallel-tube model was used to derive hepatic drug clearances from *in vitro* intrinsic clearances.

In the current study, morphine and zidovudine were used as model compounds. Both drugs are specific substrates for the UGT2B7 isoenzyme [8–10] and used in the proof-of-concept studies for the development of the semi-physiological paediatric model (Chapters 3 and 6). In the simulations, intravenous bolus doses of 0.1 mg/kg morphine or 3 mg/kg zidovudine were administered, representing clinically relevant doses.

7.2.1.1 System-Specific Parameters

Hepatic blood flow, liver volume, milligram microsomal protein per gram of liver (MPPGL), UGT2B7 ontogeny and unbound drug fraction were the five system-specific parameters of the physiologically-based pharmacokinetic model that were investigated in the current study. The term UGT2B7 ontogeny is used to describe the fractional expression and function of the UGT2B7 isoenzyme in children compared to adults. The percentage change of each of the five parameters was calculated for patients in i) the first three months of life (0 – 3 months, group I), ii) the second three months (3 – 6 months, group II), iii) the second half year (6 – 12 months, group III), iv) the second year (1 – 2 years, group IV), and v) the third year (2 – 3 years, group V). This was done by calculating the mean parameter value of all individuals with an age in the first two weeks and the last two weeks of each interval and determining the percentage increase in these values per group.

Subsequently, one at a time, the values of each of the five system-specific parameters were increased within a physiologically relevant range, based on the mean increase in the parameter values in each of the five age-groups. For hepatic blood flow and liver volume this was 23%, for MPPGL this was 2%, for the UGT2B7 ontogeny factor this was 21%, and for the unbound drug fraction this was 0.51% for morphine and 0.33% for zidovudine. To quantify the influence of these changes on the *in vivo* drug clearance a 'sensitivity ratio' was calculated for each individual by dividing the difference in physiologically-based morphine or zidovudine clearance prediction by the percentage difference in the system-specific parameter value. This sensitivity ratio quantifies how sensitive *in vivo* drug clearance is to changes in the underlying system-specific parameters. For example, a sensitivity ratio of 0.80 indicates that a 10% increase in a system-specific parameter, would increase *in vivo* drug clearance by 8%. Mean sensitivity ratios were calculated for each system-specific parameter in each of the age-groups described above. By multiplying the percentage change in a system-specific parameter in each age-group by the mean sensitivity ratio for that age-group, the percentage change in *in vivo* glucuronidation clearance as a result of the changes in the underlying parameter value in that age-range was determined.

Since the user cannot alter UGT2B7 ontogeny in the Simcyp software package, an alternative scenario was simulated that represents a situation with a 21% increase in the UGT2B7 ontogeny factor. This ontogeny factor is a scalar for the intrinsic glucuronidation clearance that takes place in both the liver and the kidneys. Similarly, liver density is a scalar for intrinsic UGT2B7-mediated glucuronidation in the liver, while milligram microsomal protein per gram of kidney (MPPGK) is a scalar of intrinsic UGT2B7-mediated glucuronidation clearance in the kidney. Therefore simulation of a scenario in which both liver density and MPPGK are increased by 21%, were used to represent a situation with a 21% increased UGT2B7 ontogeny factor.

7.2.1.2 Drug-Specific Parameters

Morphine and zidovudine were added to the compound database in Simcyp by obtaining their drug-specific parameters from literature. In the simulations the assumption was made that there is no morphine or zidovudine elimination through other pathways than UGT2B7-mediated glucuronidation, that there is no biliary clearance of these drugs, and that there is no active drug transport into or out of hepatocytes.

The obtained drug-specific parameters for each drug are presented in Table I. With respect to the Michaelis-Menten parameters, values for the formation of the two morphine glucuronides were obtained from a study using human adult liver microsomes from five separate individuals [20] and values for the glucuronidation of zidovudine were obtained from a study using liver microsomes from four adults [14]. To verify the obtained drug-specific parameters, morphine and zidovudine clearances were predicted by the physiologically-based model for 1000 healthy adult volunteers and compared to reported clearance values in adults of 93 L/h for morphine [21–23] and 91.2 L/h for zidovudine [15,24,25]. This yielded a 75% mean under-prediction for morphine and a 71% mean under-prediction for zidovudine. Based on literature reports that showed that for UGTs the presence or absence of albumin and fatty acids in *in vitro* assays influence K_m values but not V_{max} values [26,27], the physiologically-based clearance predictions for morphine and zidovudine in adults were optimized by adjusting the K_m values of these drugs. For morphine the optimized K_m value was 115.8 μM for the formation of both M3G and M6G, and for zidovudine this was a value of 4 μM. These were the K_m values that were used in the subsequent paediatric simulations.

To identify how physicochemical drug properties influence paediatric UGT2B7-mediated glucuronidation, *in vivo* clearance values of hypothetical small molecular UGT2B7-specific substrates with various physicochemical properties, were simulated with Simcyp. In these simulations, the implicit assumption was made that the changes in physicochemical properties influenced neither the active transport of the hypothetical drug into or out of the hepatocytes nor the interaction of the hypothetical drug with the UGT2B7 isoenzyme. For the hypothetical drugs, molecular weights of 100, 200, 500, 800, and 1000 g/mol were used in combination with octanol/water partition coefficients (logP) of 0.01, 1, and 5.5. Neutral compounds were simulated as well as monoprotic acids and bases with acid dissociation constants (pKa) of 2 or 5 and 8.5 or 12 respectively, and diprotic acids and bases with pKa values of 2 and 5 and 8.5 and 10 respectively. An ampholyte was used with a pKa of 5 and 9. These values are summarized in table I. The Simcyp toolbox was used to calculate the blood/plasma ratio and unbound drug fraction for each hypothetical drug based on logP and pKa values. The Michaelis-Menten parameters obtained for morphine were used and in the simulations an intravenous drug dose of 0.1 mg/kg was administered.

Table I. *Drug-specific parameters for morphine, zidovudine, and hypothetical drugs used in the physiologically-based simulations.*

Parameter [unit]	**Parameter values**			**references**
Physicochemical parameters	*Morphine*	*Zidovudine*	*Hypothetical drug*	
molecular mass [g/mol]	285.34	267.24	100 - 1000	
logP	0.77	0.05	0.01 - 5.5	[11–13]
pKa1	7.93	9.68	2 - 12	[14,15]
pKa2	9.63	-		
Blood binding parameters				
blood / plasma ratio	1.08	0.86	derived	[13,16,17]
fraction unbound in adults	0.62	0.77	derived	[13,18,19]
Enzyme kinetic parameters				
K_m [μM]	115.8	4	115.8	
V_{max} [pmol/min/mg protein]	9250, M3G 1917, M6G	1166	9250 1917	[14,20]

logP = octanol/water partition coefficient, pKa = acid dissociation constant, K_m = Michaelis-Menten constant, V_{max} = maximum formation rate, NA = not applicable

The influence of the changes in physicochemical drug parameters on the predicted *in vivo* clearance were assessed by changing one parameter value while keeping all other parameter values constant and calculating the percentage difference between the clearance predictions between two simulations. When the highest individual prediction difference was less than 5%, the parameter was classified as not significantly influencing drug glucuronidation. When the highest individual prediction difference was more than 5% and the difference between mean prediction difference of individuals in the first month of life and individuals in the 35th month of life was less than 5% a constant was classified as influencing the absolute value of drug glucuronidation. When the difference between mean prediction difference of individuals in the first month of life and individuals in the 35th month of life was more than 5%, the parameter was classified as influencing the ontogeny profile in addition to influencing the absolute value of drug glucuronidation clearance.

7.2.2 Semi-Physiological Developmental Glucuronidation Model

The semi-physiological developmental glucuronidation model is represented by the covariate model quantifying the net observed developmental changes in drug glucuronidation clearance in children under the age of three years including preterm

and term neonates. This is the model obtained and validated in a previous population analysis of paediatric morphine data (Chapters 3 and 4) which was also directly extrapolated to zidovudine (Chapter 6). In this model the overall developmental changes in drug glucuronidation in children younger than three years are quantified according to equation 1:

$$CL = a \cdot f_{neonate<10} \cdot BW^{1.44} \qquad \text{(equation 1)}$$

in which *CL* represents the drug glucuronidation clearance, *a* is a constant that represents the absolute value of clearance, $f_{neonate<10}$ represents a reduced glucuronidation fraction in neonates younger than ten days, and *BW* represents the bodyweight of an individual paediatric patient in kilograms. The absolute value of clearance for each drug (i.e. value of *a* in equation 1) is estimated from concentration-time data in a population analysis. The reduction in glucuronidation clearance in neonates with a postnatal age younger than ten days ($f_{neonate<10}$) is 50% and is independent from gestational age. The final element of equation 1 quantifies the overall ontogeny of *in vivo* drug glucuronidation in this young population using bodyweight as a surrogate descriptor in an exponential equation with an exponent of 1.44.

Using the currently available *in vitro* data as input parameters, the morphine and zidovudine clearance predictions by the physiologically-based pharmacokinetic model in Simcyp were compared to the clearance values according to the semi-physiological developmental glucuronidation model. This was done by plotting clearance values from both models *versus* bodyweight, which is the primary covariate in the semi-physiological developmental glucuronidation model, and by plotting the prediction difference between the physiologically-based clearance values and the semi-physiological clearance values for each of the 1000 simulated individuals *versus* bodyweight. Additionally, for each of the 1000 individuals in the simulation dataset of the current study, the morphine and zidovudine clearances according to the semi-physiological model were determined as well. The percentage increase in morphine and zidovudine clearance predictions in each of the five age-groups according to the semi-physiological developmental glucuronidation model were calculated as described above.

7.3. Results

7.3.1 System-Specific Parameters

Table II ranks the five system-specific parameters by their relative contribution to the developmental changes in *in vivo* drug glucuronidation, as depicted in the last two

columns. The percentage change in clearance as a result of developmental changes in the underlying system-specific parameters is calculated from the percentage change in the system-specific parameter according to the physiologically-based model in Simcyp and the sensitivity ratio quantifying the sensitivity of drug clearance to these changes. It can be seen that the contribution of each system-specific parameter to the developmental changes in clearance is different for morphine and zidovudine. With respect to the different age-groups, the contribution of the parameters to developmental changes in *in vivo* clearance is highly non-linear and may even be bi-directional. Despite these differences, liver volume can overall be regarded as the main driver of developmental changes in UGT2B7-mediated glucuronidation, by causing an increase in *in vivo* clearance in the different age-groups between 13% and 31% for morphine and 7.3% and 22% for zidovudine, with an especially large contribution in the first three months of life. The increase in *in vivo* morphine and zidovudine clearance as a result of UGT2B7 ontogeny in the different age-groups ranges between 10% and 29%, and 7.4% and 18% respectively. The influence of hepatic blood flow on developmental changes in morphine clearance is below 5% in all age-groups and can therefore be regarded negligible, while for zidovudine clearance the contribution of changes in hepatic blood flow to increases in *in vivo* clearance ranges between 3.7% and 7.9%. For both drugs, the contribution of changes in MPPGL and unbound drug fraction is negligible in all age-groups.

7.3.2 Drug-Specific Parameters

Simulations with hypothetical small molecular UGT2B7 substrates with physicochemical properties in the ranges depicted in table I revealed that physicochemical drug properties do not influence the ontogeny profile of *in vivo* UGT2B7-medidated glucuronidation clearance. It was found that molecular mass, in the range between 100 g/mol and 1000 g/mol, did not influence UGT2B-mediated glucuronidation clearance of the simulated hypothetical drugs at all, assuming that the increase in mass did not alter the uptake or efflux of the drug by hepatocytes or the interaction of the drug molecule with the UGT2B7 isoenzyme. Increasing the octanol/water partition coefficient (logP) between 0.01 and 5.5 while keeping all other parameters constant, yielded a slight decreasing trend in the predicted absolute value of drug glucuronidation. On the other hand, increasing the acid dissociation constant (pKa) between 2 and 12 while keeping other parameters constant, yielded a trend towards an increasing predicted absolute value of drug glucuronidation. No strong relationship was observed between the physicochemical properties of the hypothetical drugs and the absolute value of glucuronidation clearance, nor was there a relationship between the derived blood to plasma ratio of the hypothetical drugs and the absolute value of drug glucuronidation. There was however a strong linear correlation ($r = 0.978$) between the mean glucuronidation clearance predicted by the physiologically-

based model for the hypothetical drugs in each of the 1000 simulated individuals and the unbound drug fraction of the hypothetical drug in plasma, which was derived from the logP and pKa value using the Simcyp toolbox. According to this relationship, while keeping the Michaelis-Menten parameters constant at the values obtained for morphine, every 0.1 increase in unbound drug fraction of the hypothetical drug resulted in an increase in *in vivo* drug clearance of 1.5 L/h.

Table II. *System-specific parameters investigated in the current study. The percentage increase in parameter value in each of the five age-groups is given, as well as the mean sensitivity ratios of the clearance of morphine and zidovudine in each group. The calculated percentage change in in vivo morphine and zidovudine clearance as a result of the change in the underlying system-specific parameters are also presented.*

Parameter	Percentage change in parameter value	Mean sensitivity ratio		Percentage change in clearance as a result of changes in parameter	
		morphine	zidovudine	morphine	zidovudine
Liver volume	I: 38% II: 18% III: 19% IV: 17% V: 21%	I: 0.82 II: 0.81 III: 0.79 IV: 0.76 V: 0.72	I: 0.58 II: 0.56 III: 0.50 IV: 0.43 V: 0.35	I: 31% II: 15% III: 15% IV: 13% V: 15%	I: 22% II: 10% III: 9.5% IV: 7.3% V: 7.4%
UGT2B7 ontogeny	I: 12.7% II: 11.4% III: 20.2% IV: 33.8% V: 25.2%	I: 0.90 II: 0.90 III: 0.88 IV: 0.85 V: 0.81	I: 0.66 II: 0.65 III: 0.60 IV: 0.52 V: 0.45	I: 11% II: 10% III: 18% IV: 29% V: 20%	I: 8.4% II: 7.4% III: 12% IV: 18% V: 11%
Hepatic blood flow	I: 33% II: 17% III: 19% IV: 22% V: 24%	I: 0.059 II: 0.061 III: 0.081 IV: 0.103 V: 0.127	I: 0.24 II: 0.22 III: 0.29 IV: 0.22 V: 0.29	I: 1.9% II: 1.0% III: 1.5% IV: 2.3% V: 3.0%	I: 7.9% II: 3.7% III: 5.5% IV: 4.8% V: 7.0%
Milligram microsomal protein per gram of liver	I: 0.71 % II: 0.70% III: 1.63% IV: 3.3 % V: 3.1%	I: 0.83 II: 0.83 III: 0.81 IV: 0.78 V: 0.75	I: 0.62 II: 0.60 III: 0.55 IV: 0.47 V: 0.39	I: 0.6% II: 0.6% III: 1.3% IV: 2.6% V: 2.3%	I: 0.4% II: 0.4% III: 0.9% IV: 1.6% V: 1.2%
Unbound drug fraction	Morphine / zidovudine I: -1.6% / I: -0.93% II: -0.72% / II: -0.45% III: -1.5% / III: - 0.89% IV: -0.05% / IV: -0.008% V: 1.2% / V: 0.74%	I: 0.92 II: 0.92 III: 0.90 IV: 0.87 V: 0.85	I: 0.71 II: 0.70 III:0.65 IV:0.58 V: 0.51	I: -1.5% II: -0.67% III:-1.4% IV: -0.04% V: 1.02%	I: -0.66% II: -0.32% III:-0.58% IV: -0.005% V: 0.38%

Age-groups: I: 0 – 3 months, II: 3 – 6 months, III: 6 – 12 months, IV: 1 – 2 years, V: 2 – 3 years.

7.3.3 Semi-Physiological Developmental Glucuronidation Model

Figure 1 shows the total morphine glucuronidation clearance and zidovudine glucuronidation clearance values in children younger than three years according to the semi-physiological developmental glucuronidation model and the physiologically-based pharmacokinetic model in Simcyp. The graphs in figure 1 indicate an under-prediction of the morphine and zidovudine glucuronidation in children older than ten days (solid circles) by the physiologically-based model, which is reflected in a mean percentage difference for this subpopulation of -68.3% for morphine and -19.1% for zidovudine in the bottom graph. In children younger than ten days (asterisks), the reduction in glucuronidation capacity as quantified by the semi-physiological developmental glucuronidation model, is not observed in the predictions by the physiologically-based pharmacokinetic model. This yields a mean percentage difference of -19.4% for morphine and 105% for zidovudine. These results illustrate large differences in the prediction of developmental changes in *in vivo* glucuronidation clearance between the semi-physiological model and the physiologically-based model in the first two weeks of life. In older infants and children, the prediction difference remains constant throughout the bodyweight-range, suggesting that in this older subpopulation the ontogeny profile predicted by the physiologically-based model mainly differs from the semi-physiological developmental glucuronidation model in absolute value while it is rather similar in shape.

Developmental changes in *in vivo* morphine and zidovudine clearance relative to birth are depicted in figure 2 for the semi-physiological model (grey lines) and the physiologically-based model (black lines), including the individual contribution of each system-specific parameter in the physiologically-based model (non-solid black lines). It can be seen that for both morphine and zidovudine, the largest contribution to the increase in *in vivo* glucuronidation is coming from the increase in liver volume (dotted black line) and the ontogeny of the UGT2B7 isoenzyme (long dashed black line). When not taking into account the rapid increase in drug glucuronidation predicted by the semi-physiological model at the age of ten days (dashed grey line), the combined influence of the changes in the five system-specific parameters investigated in the current study explains 79% of the clinically observed increases in morphine and 41% of the clinically observed increases in zidovudine clearance in the first three years of life.

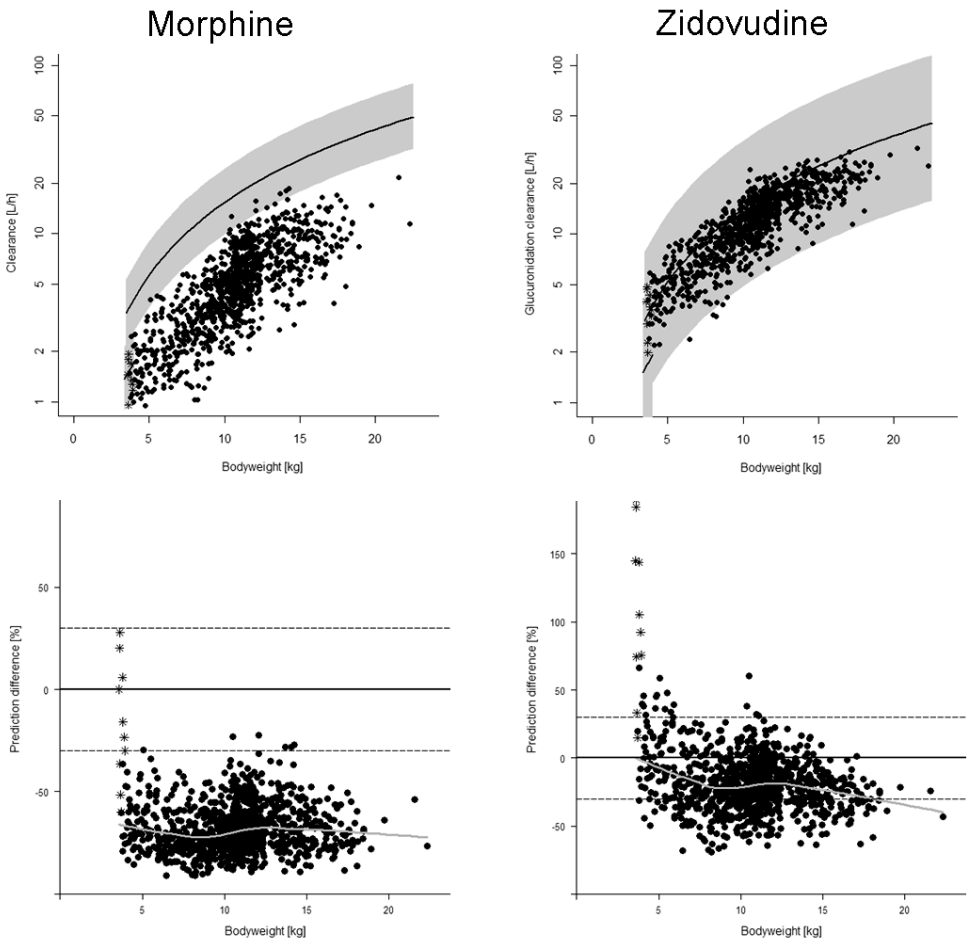

Figure 1. *Predicted in vivo morphine clearance (top left) and zidovudine clearance (top right) versus bodyweight in children younger than three years by the physiologically-based pharmacokinetic model (asterisk for neonates younger than ten days, and solid dots for children older than ten days) and the semi-physiological developmental glucuronidation model (lines are population predictions and shaded area indicates the 95% prediction interval). The prediction difference between the two models is depicted versus bodyweight for both drugs (bottom). The horizontal lines in these graphs show 0% prediction difference (solid line) and ± 30% prediction difference (dotted lines) and the grey line represents the loess curve of the data.*

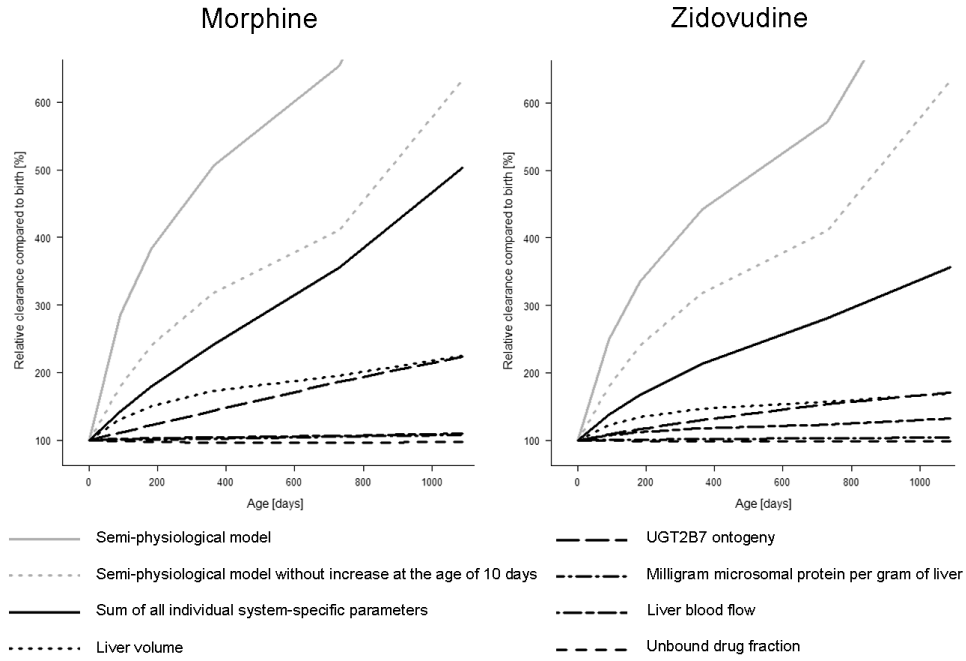

Figure 2. *Developmental changes in in vivo morphine and zidovudine clearance in the first three years of life relative to birth. The solid grey line represents the total increase predicted by the semi-physiological model and the dotted grey line represents the clearance increase predicted by the semi-physiological model without taking the rapid increase at the age of ten days into account. The solid black line represents the sum of the changes by all five system-specific parameters with the non-solid black lines representing the individual contribution of each system-specific parameter.*

7.4. Discussion

The clinically observed *in vivo* maturation pattern for morphine glucuronidation in children has been extensively studied and quantified in a paediatric covariate model before (Chapters 3 and 4) and it was shown that this covariate model could be directly extrapolated to the glucuronidation of zidovudine in a semi-physiological modelling concept (Chapter 6). It is of interest to investigate to what extent this paediatric developmental glucuronidation model can be extrapolated to other patient populations or other UGT2B7 substrates. Therefore the current study investigated the physiological and physicochemical basis of the semi-physiological developmental covariate model for UGT2B7-mediated glucuronidation clearance, using the physiologically-based

modelling software Simcyp. Detailed investigation of the influence of individual system-specific and drug-specific parameters on the ontogeny pattern of *in vivo* glucuronidation revealed that increases in liver volume and in the ontogeny of UGT2B7 isoenzymes are the main physiological drivers of the developmental changes in drug glucuronidation (table II and figure 2). The logP and pKa of a drug, but not the molecular mass, influence the absolute value of the drug glucuronidation clearance without however influencing the pattern of developmental changes.

In figure 1 it can be observed that the clearance predictions by the physiologically-based model (symbols), using the currently available *in vitro* information on morphine and zidovudine clearance, are generally lower than the clearance values obtained from the semi-physiological developmental glucuronidation model. Additionally, as can be observed in figure 2, the combined influence of the age-related increases in the five system-specific parameters investigated in the current study (solid black line) do not fully explain the clinically observed increase in morphine and zidovudine clearance (solid grey line), not even when the rapid increase in glucuronidation clearance predicted by the semi-physiological model at the age of ten days are not taken into account (dashed grey line). There are a number of possible explanations for the discrepancies in clearance values obtained by the physiologically-based model and the semi-physiological model.

The UGT enzyme kinetic parameters for morphine and zidovudine were obtained from two studies using liver microsomes described in literature. Confidence that these values accurately represent *in vivo* enzyme kinetic values is limited by the fact that numerous incubation conditions influence measured UGT enzyme kinetics, causing difficulties in obtaining good predictions on *in vivo* UGT enzyme kinetics from microsome studies [26–30]. In fact, in the current study the reported K_m values had to be adjusted to values that yielded accurate clearance predictions by the physiologically-based model in adults. In a sensitivity analysis, when changing V_{max} and K_m values from values ten-fold lower to ten-fold higher than the values used in the current analysis, predicted paediatric glucuronidation clearances changed with a factor two for M6G formation and a factor twelve for both zidovudine glucuronidation and M3G formation. This illustrates, that imprecise *in vitro* values for enzyme kinetics may influence clearance predications by the physiologically-based model significantly.

Further discrepancies may be the result of the assumption made in the simulations with the physiologically-based model. For instance, morphine and zidovudine were both assumed to be solely eliminated through glucuronidation. While glucuronidation is believed to be the major elimination pathway for these drugs, a small contribution of other elimination pathways cannot be excluded. Some paediatric morphine studies have for instance suggested that morphine is to a small degree eliminated through sulphation

or unchanged elimination in the very young [31,32]. Additionally, in the physiologically-based model active drug uptake or efflux by hepatocytes and biliary clearance of morphine and zidovudine were assumed to be zero. Although it is unclear how accurately this reflects the clinical situation, animal studies with morphine have shown energy-dependent carrier-mediated uptake of morphine in hepatocytes [33,34], while others found active hepatic uptake to not limit hepatic morphine metabolism [35]. Additionally, biliary clearance of morphine was reported in rat livers [35].

Of the system-specific parameters in the physiologically-based model, the ontogeny profile for liver volume is based on a large number of observations [36], while the paediatric information on other system-specific parameters is more limited, decreasing the level of confidence for the ontogeny profiles of these parameters in the physiologically-based model. Especially for the ontogeny of UGT2B7, expression and function of this isoenzyme in the physiologically-based model increases linearly with age from 8.9% from adult values at birth to adult values at the age of 20 years. Review of literature data however, suggests the expression and function of UGT isoenzymes to increase rapidly in the first few weeks of life (Chapter 2). Given that morphine and zidovudine clearances were found to be rather sensitive to changes in the UGT2B7 ontogeny factor, which is expressed in average sensitivity ratios of 0.87 and 0.58 respectively, a suboptimal representation of this ontogeny profile may explain the discrepancy in the morphine and zidovudine clearance values according to the semi-physiological model and physiologically-based model in figure 1 and the discrepancy between the predicted increases in morphine and zidovudine clearances by the physiologically-based model (solid grey line) and the semi-physiological model (solid black line) in figure 2. Therefore, improving the UGT2B7 ontogeny profile in the physiologically-based model may improve the predictions for paediatric UGT2B7-mediated glucuronidation.

The relatively large impact of changes in liver volume on *in vivo* drug glucuronidation observed in this study, may limit the applicability of the semi-physiological developmental glucuronidation model in patients with a reduced liver size, as a result of for instance liver resection, or a reduced liver function, for instance in patients with hepatic dysfunction as a result of virus associated hepatic disease or liver cirrhosis. Literature reports have indeed shown morphine and zidovudine clearance to be significantly reduced in adult patients with cirrhosis [37–40]. Reduced liver size or liver function may have clinical implications for dosing UGT2B7 substrates in these patients. Interestingly, hepatic blood flow was found have a limited impact on morphine glucuronidation in the current study, while cardiac surgery was found to have a clinically significant influence on paediatric morphine clearance, which was attributed to changes in hepatic blood flow resulting from changes in cardiac output [41,42].

Physicochemical parameters that influence the ontogeny profile of drug glucuronidation clearance in children would limit the application of the semi-physiological developmental glucuronidation model to UGT2B7 substrates with these properties in a population modeling approach. However, assuming that changes in the physicochemical properties of drugs do not influence drug uptake or efflux by hepatocytes or the interaction of the drug molecule with the UGT2B7 isoenzyme, the molecular weight of hypothetical drugs did not influence drug glucuronidation, while the logP and pKa of the hypothetical drug molecule only influenced the absolute value of the drug glucuronidation clearance. The absolute value of the drug glucuronidation clearance is reflected in the value of *a* in equation 1, these results thereby further support the hypothesis that this is a drug-specific constant. Since in a semi-physiological modeling approach for new UGT2B7 substrates the value of this constant has to be estimated based on the population analysis of outcome measures, these results suggest that the semi-physiological developmental glucuronidation model can predict developmental changes in glucuronidation clearance of all small molecular substrates for UGT2B7.

However, although results showed that the unbound drug concentration of hypothetical drugs in plasma is the main driver of the absolute value of drug glucuronidation, the Michaelis-Menten parameters were kept constant in all simulations with hypothetical drugs, in this case at the values used in the simulations for morphine. Michaelis-Menten parameters are major drivers of the absolute value of drug metabolism, but due to the non-linear correlation between substrate concentration and intrinsic drug clearance, interpretation of the results from simulations with varying Michaelis-Menten constants is complex. These simulations were not performed in the current study, however such simulations are necessary to further investigate the applicability of the semi-physiological developmental glucuronidation model for drugs with saturable glucuronidation kinetics.

Additionally, the application of the semi-physiologically-based developmental glucuronidation model in scenarios of drugs with varying extraction ratios needs to be further investigated. The ontogeny of UGT isoenzymes may have a one to one effect on low extraction UGT substrates since in this case *in vivo* clearance closely reflects intrinsic clearance. High extraction UGT substrates on the other hand, may be affected less by an increased level in UGT activity as their clearance will be limited by hepatic blood flow. Morphine and zidovudine have similar extraction ratios of 0.5 and 0.65 respectively [43–45]. The influence of changes in the underlying physiological system is therefore expected to be rather similar for both drugs, which resulted in the same ranking of system-specific parameters for both drugs in table II and the possibility to extrapolate the semi-physiological developmental glucuronidation model between these drugs (Chapter 6). The small difference in extraction ratio that does exist between these two drugs may

explain why in table II the percentages change in morphine and zidovudine *in vivo* clearance as a result of the changes in the underlying system-specific parameters are not the same, and why the influence of hepatic blood flow is for example more pronounced for zidovudine than for morphine. It is expected that direct extrapolation of paediatric covariate models in a semi-physiological modelling approach between UGT2B7 substrates is possible when both substrates that have similar extraction ratio's, however extrapolation between drugs with different hepatic extraction ratio's may require further deconvolution of the maturational changes in the underlying physiological processes.

5. Conclusion

The current analysis illustrates that the key physiological driver of the maturation of UGT2B7-mediated hepatic morphine and zidovudine glucuronidation in children younger than three years, as quantified by the semi-physiological developmental glucuronidation model, are liver blood flow and ontogeny of UGT2B7 expression and function. The logP and pKa are two important physicochemical drug properties that influence the absolute value of the glucuronidation clearance, but not the maturation profile, with a strong correlation between the unbound drug fraction and the absolute clearance value for drugs with similar Michaelis-Menten parameters. The results of this study suggest that in patients with normal liver function, the ontogeny pattern in clearance of new UGT2B7 substrates in children under the age of three years can be predicted by the semi-physiological developmental glucuronidation model and that only the absolute clearance value of the new substrate needs to be estimated. The generalizability of the semi-physiological modelling concept to patients with reduced liver size or liver function and to scenarios with non-linear clearance or large differences in hepatic drug extraction rations requires further investigation.

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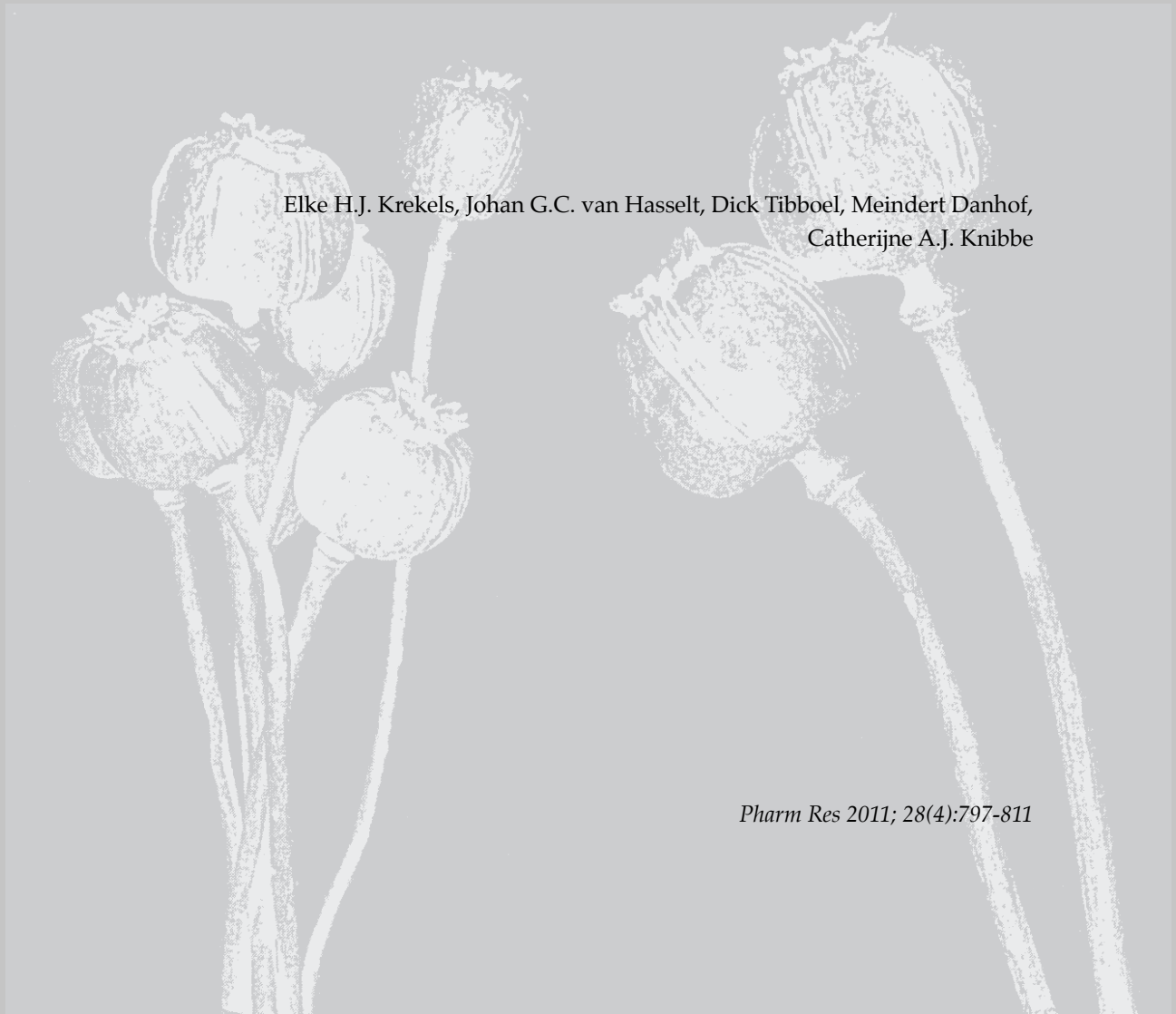
Section IV

Paediatric Model Evaluation

Chapter 8

Systematic Evaluation of the Descriptive and Predictive Performance of Paediatric Morphine Population Models



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Abstract

Purpose: A framework for the evaluation of paediatric population models is proposed and applied to two different paediatric population pharmacokinetic models for morphine. One covariate model was based on a systematic covariate analysis, the other on fixed allometric scaling principles.

Methods: The six evaluation criteria in the framework were 1) number of parameters and condition number, 2) numerical diagnostics, 3) prediction-based diagnostics, 4) η-shrinkage, 5) simulation-based diagnostics, 6) diagnostics of individual and population parameter estimates *versus* covariates, including measurements of bias and precision of the population values compared to the observed individual values. The framework entails both an internal and external model evaluation procedure.

Results: The application of the framework to the two models resulted in the detection of over-parameterization and misleading diagnostics based on individual predictions caused by high shrinkage. The diagnostic of individual and population parameter estimates *versus* covariates proved to be highly informative in assessing obtained covariate relationships. Based on the framework, the systematic covariate model proved to be superior over the fixed allometric model in terms of predictive performance.

Conclusions: The proposed framework is suitable for the evaluation of paediatric (covariate) models and should be applied to corroborate the descriptive and predictive properties of these models.

8.1 Introduction

Whereas many diagnostic and validation tools are available for the evaluation of population models in the adult population, these tools may not always directly suffice in the paediatric population due to the heterogeneity of this special population and the scarcity of the datasets. In this study a framework including six evaluation criteria is presented for the systematic assessment of the descriptive and predictive properties of paediatric (covariate) models that takes these specific issues into consideration.

In paediatric population pharmacokinetic (PK) models, the influence of the many physiological changes that take place in the paediatric age-range are reflected in covariate relationships that are usually based on bodyweight and/or age. However, since bodyweight and age are naturally correlated in the paediatric population there is a debate on how to incorporate the influence of the physiological changes in paediatric population PK models. Bodyweight and age can either be regarded as regular covariates whose predictive properties on PK parameters are evaluated together with other covariates in a systematic covariate analysis by formally testing them for significance and only retaining them in the model if they statistically improve the model fit (Chapter 3) [1,2]. Alternatively, bodyweight can be included *a priori* into paediatric PK models by the use of a bodyweight-based allometric equation with a fixed exponent of 0.75 for clearance and 1 for distribution volume. These equations can subsequently be augmented by estimated age-based functions of various forms [2–4].

In recent years two different population PK models for morphine and its two major pharmacologically active metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in children younger than three years have been published (Chapter 3)[3]. The model by Knibbe *et al.* (Chapter 3) was developed using a systematic covariate analysis. The model by Bouwmeester *et al.* [3] was developed using fixed allometric scaling principles in conjunction with estimated age-based functions. As these models were developed using similar datasets, they provide both an example for the assessment of the developed framework for the evaluation of paediatric (covariate) models, as well as an opportunity to directly compare the performance of these two fundamentally different paediatric covariate models.

8.2 Materials and Methods

8.2.1 Models and Data

Figure 1 shows a schematic representation of the two models that are evaluated in the current analysis. In the model by Knibbe *et al.* (Chapter 3) the maturation of the formation and elimination clearances of the morphine glucuronides was found to be best described by a bodyweight-based exponential equation with an estimated exponent of 1.44. Within this exponential equation the formation clearance of the glucuronides was found to be significantly reduced in neonates younger than ten days. Distribution volumes were estimated to scale linearly with bodyweight. This model will be referred to as the systematic covariate model. In the model by Bouwmeester *et al.* [3] bodyweight was included *a priori* using an allometric equation with fixed exponents of 0.75 for clearance and 1 for distribution volume. Three exponential equations based on postnatal age (PNA) augmented the model, one equation for distribution volumes, one for the formation of the morphine metabolites and one for the elimination of the metabolites. Bilirubin concentration and creatinine concentrations were also incorporated to the model as covariates for the formation and elimination of the glucuronides respectively. This model will be referred to as the fixed allometric model.

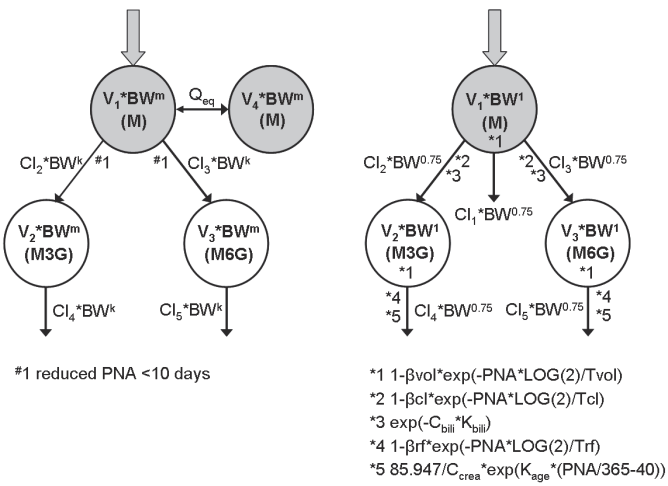

Figure 1. *Schematic representation of the systematic covariate model (Chapter 3) (A) and the fixed allometric model [3] (B). M = morphine, M3G = morphine-3-glucuronide, M6G = morphine-6-glucuronide, V = distribution volume of the designated compartment, Cl = clearance of designated route, Q = intercompartimental clearance, PNA = postnatal age, k and m = exponential scaling constants, β = fraction below adult values at birth and T = maturation half-life for distribution volume (vol), formation clearance of the metabolites (cl), and elimination clearance of the metabolites (rf), C = plasma concentration and K = scaling constant for bilirubin (bili) and creatinine (crea).*

The systematic covariate model (Knibbe *et al.* (Chapter 3)) was developed using two datasets [5,6], while the fixed allometric model (Bouwmeester *et al.* [3]) was developed using only one of these two datasets [5]. To allow for a direct comparison, the systematic covariate model was refit with the data from the one common dataset. This dataset will be referred to as the internal dataset of this study and consists of postoperative term neonates, infants and children up to the age of three years on a continuous or intermitted intravenous morphine regimen [5].

For the external evaluation of the two paediatric covariate models in the current analysis, five previously published datasets [6–10] were used. These external datasets included the same patient population as the internal dataset with the exception that their PNA ranged up to only one year instead of three years. In addition, two external datasets included preterm neonates [6][7], which is a younger age-range than the age-range in the internal dataset [5] used for model building. An overview of all datasets is given in table I.

Table I. *Overview of the internal dataset (Int.1) and the external datasets (Ext.1-5) used for model building and external model evaluation.*

Dataset	Patient Population	Number of Patients	Postnatal Age in days (median, IQR)	Bodyweight in g (median, IQR)
Int.1. [5]	Post-operative term neonates, infants and children.	183	97 (8 – 286)	4700 (3100 – 8000)
Ext.1. [8]	Post-operative term neonates and infants	28	14 (0 – 70)	3100 (2550 – 4000)
Ext.2. [9]	Post-operative term neonates and infants	9	10.5 (3 – 135)	3800 (3000 - 5000)
Ext.3. [10]	Term neonates and infants on artificial ventilation	12	13 (6 – 80)	3050 (2675 – 6900)
Ext.4. [6]	Preterm and term neonates on artificial ventilation	63	0.4 (0.2 – 0.5)	1180 (862.5 – 1760)
Ext.5. [7]	Preterm neonates on artificial ventilation	41	1 (1 – 2)	1035 (892.5 – 1295)

Int. = internal dataset, Ext. = external dataset, M = morphine, M3G = morphine-3-glucuronide, M6G = morphine-6-glucuronide

8.2.2 Model Evaluation

All model fitting and model-based simulations in the current study were performed using NONMEM VI (ICON, Ellicott City, MD).

The framework for the evaluation of paediatric population models is composed of the following six evaluation criteria and tools:

1) total number of parameters and condition number of the model. The latter was obtained by taking the ratio of the largest and smallest eigenvalue of the covariance matrix of the estimate from the NONMEM output.
2) numerical diagnostics by means of a bootstrap analysis using the PsN software package [11]. One hundred datasets were resampled with replacement from the internal dataset and refit to the models. The parameter estimates for fixed and random effects obtained in every separate run were summarized in terms of mean and relative standard errors (RSE) for each parameter. Runs that did not minimize successfully were excluded from the analysis.
3) prediction-based diagnostics by means of basic goodness-of-fit plots. Both the individual and population predicted concentrations were plotted *versus* the concentrations that were actually observed in these datasets. Plots were made for both the internal and external datasets and for the population as a whole as well as for stratified subsets based on age (0-1 month, 1 month – 1 year, 1-3 years). Mirror plots were created to serve as a reference for these predicted *versus* observed plots [12].
4) η-shrinkage as defined by Karlsson and Savic. [12], which was calculated for all model parameters for which inter-individual variability was estimated.
5) simulation-based diagnostics by means of normalized prediction distribution errors (NPDE) [13]. Both the internal and merged external datasets were simulated 1000 times with inclusion of the inter-individual variability and residual error. Using the NPDE add-on package for R (version 1.2) [14] a cumulative distribution was assembled for each observation with the 1000 simulated concentrations and subsequently the value of the cumulative distribution at the observed concentration was determined. An inverse function of the normal cumulative density function was then applied to these data to obtain what are called the normalize prediction distribution errors. The NPDEs are presented in a total distribution, *versus* time and *versus* the concentration. This analysis was also performed on the population as a whole and on the stratified subsets described in item 3.
6) Individual and population parameter estimates *versus* the most predictive covariate in the model. In both models, bodyweight was the most predictive covariate. Total morphine clearance was defined as the sum of Cl_1 and Cl_2 for the systematic covariate model (see figure 1A) and of Cl_0, Cl_1, and Cl_2 for the fixed allometric model (see figure 1B). The elimination clearances of the metabolites (Cl_3 and Cl_4) and the distribution volume of the central morphine compartment (V_1) were directly compared between the two models. To numerically quantify the bias and precision of the model predicted parameter values compared to the observed parameter values in the internal datasets, Mean Prediction Error (MPE,

equation 1) and the Root Mean Square Error (RMSE, equation 2) were calculated respectively for both models.

$$MPE = \frac{\sum \frac{(predicted - observed) * 100}{observed}}{n} \qquad \text{(equation 1)}$$

$$RMSE = \sqrt{\frac{\sum (predicted - observed)^2}{n}} \qquad \text{(equation 2)}$$

In these equations predicted parameter values were the population predicted values for each individual by both models and the observed parameter values were the individually observed *post hoc* parameter values for that individual. MPE and RMSE were calculated separately for the strata described before. A table with MPE and RMSE was also constructed for the external datasets. This table was also stratified based on age with the following strata: preterm neonates (PNA < 1 month and postmenstrual age (PMA) at birth < 36 weeks), term neonates (PNA <1 month and PMA at birth ≥36 weeks), toddlers (PNA 1 month – 1 year).

8.3 Results

Table II A and B lists the parameter estimates as obtained with the fit of the internal dataset by the systematic covariate model and the fixed allometric model, respectively. With a total of 18 model parameters, the systematic covariate model described the fixed and random effects with fewer parameters than the fixed allometric model, which contains 35 model parameters. The condition number of the systematic covariate model was 293, which is well below the critical value for the indication of serious ill-conditioning of 1000 [15]. For the fixed allometric model the condition number was 10698, which is more then ten-fold higher than the critical value.

Table II. *Parameter estimates from the model fit, number of model parameters and parameter estimates from the bootstrap procedure for the systematic covariate model (A) and for the fixed allometric model (B) obtained with the internal dataset. Parameter names are explained in figure 1.*

A.

Parameters *Systematic covariate model*	***Model fit***	***Bootstrap*** *(98 out of 100 successful)*
	Value (RSE%)	*Mean value (RSE%)*
Fixed effects (n=10)		
k = exponential scaling factor on clearance	1.49 (3.6)	1.49 (3.42)
m = exponential scaling factor on distribution volume	1 fixed	1 fixed
$Cl_{1\ PNA<10\ d}$ (ml/min/kg^k)	3.68 (9.0)	3.68 (8.15)
$Cl_{1\ PNA>10d}$ (ml/min/kg^k)	8.04 (11.0)	8.01 (10.1)
$Cl_{2\ PNA<10d}$ (ml/min/kg^k)	0.423 (11.1)	0.42 (14.4)
$Cl_{2\ PNA>10d}$ (ml/min/kg^k)	0.623 (10.2)	0.62 (13.0)
Cl_3 (ml/min/kg^k)	1.84 (9.9)	1.85 (9.73)
Cl_4 (ml/min/kg^k)	0.955 (9.0)	0.95 (12.6)
Q_{eq} (ml/min)	40.8 (24.1)	43.8 (42.7)
$V_1 = V_4$ (l/kg)	1.64 (8.2)	1.65 (9.44)
$V_2 = V_3$ (fraction of V_1)	0.157 (21.2)	0.161 (27.0)
Inter-individual variability (n=5)		
ω^2 Cl_1	0.0809 (24.0)	0.0774 (22.9)
ω^2 Cl_3	0.256 (27.1)	0.263 (29.1)
ω^2 Cl_4	0.110 (15.2)	0.110 (15.5)
ω^2 Cl_3-Cl_4 covariance	0.128 (18.2)	0.126 (19.8)
ω^2 V_1	0.162 (17.9)	0.168 (20.7)
Residual variance (n=3)		
$\sigma^{2,\ prop}$ (morphine)	0.440 (14.7)	0.431 (14.0)
$\sigma^{2,\ prop}$ (M3G)	0.261 (27.2)	0.243 (26.0)
$\sigma^{2,\ prop}$ (M6G)	0.0894 (15.7)	0.0894 (16.4)

B.

Parameters *Fixed allometric model*	***Model fit***	***Bootstrap*** *(46 out of 100 successful)*
	Value (RSE%)	*Value (RSE%)*
Fixed effects (n=14)		
Cl_0 (l/h/70kg$^{0.75}$)	3.12 (117)	2.59 (43.4)
Cl_1 (l/h/70kg$^{0.75}$)	64.3 (18.0)	55.2 (32.3)
Cl_2 (l/h/70kg$^{0.75}$)	3.63 (14.0)	3.99 (65.6)
Cl_3 (l/h/70kg$^{0.75}$)	17.4 (16.0)	7.23 (38.0)
Cl_4 (l/h/70kg$^{0.75}$)	5.8 (20.2)	5.43 (23.4)
V_1 (l/70kg)	136 (59.3)	147 (34.9)
V_2 (l/70kg)	23 fixed	23 fixed
V_3 (l/70kg)	30 fixed	30 fixed
Bcl	0.834 (6.41)	0.894 (8.35)
Tcl (days)	88.3 (37.4)	65.2 (176)
Brf	0.832 (9.74)	0.814 (10.7)
Trf (days)	129 (49.8)	136 (45.4)
Bvol	0.391 (28.4)	0.388 (38.1)
Tvol (days)	26.3 (72.2)	26.7 (58.9)
K_{age}	0.0141 (140)	0.0201 (36.3)
K_{bili}	-0.00203 (33.2)	-0.00207 (35.3)
Inter-individual variability (n=16)		
ω^2 Cl_0	1.37 (104)	1.80 (69.9)
ω^2 Cl_1	0.346 (20.9)	0.916 (74.9)
ω^2 Cl_2	0.675 (29.3)	1.21 (75.8)
ω^2 Cl_3	0.185 (20.8)	0.764 (41.7)
ω^2 Cl_4	0.545 (32.1)	1.39 (87.6)
ω^2 V_1	0.351 (29.1)	1.54 (121)
full omega block on all eta's except Cl_0	Data not shown	Data not shown
Residual variance (n=5)		
$\sigma^{2,\text{ prop morphine}}$	0.128 (11.6)	0.503 (105)
$\sigma^{2,\text{ add M3G}}$ (ng/ml)	50.3 (36.2)	136 (217)
$\sigma^{2,\text{ prop M3G}}$	0.118 (27.0)	2.21 (80.0)
$\sigma^{2,\text{ add M6G}}$ (ng/ml)	0.198 (26.0)	1.31 (148)
$\sigma^{2,\text{ prop M6G}}$	0.0925 (16.9)	0.249 (53.0)

In tables II A and B the parameter estimates obtained with the bootstrap analyses are presented as well. The parameter estimates of the bootstrap deviated more from the values obtained in the initial model fit for the fixed allometric model compared to the systematic covariate model. For both the model fit and the bootstrap procedure with the fixed allometric model, the overall precision of the parameter estimates was lower than the systematic covariate model as expressed by the higher relative standard error (RSE) of the parameter estimates. Additionally, for the bootstrap, using the fixed allometric model only 46 out of 100 model refits minimized successfully, whereas 98 out of 100 model refits successfully minimized using the systematic covariate model.

Figure 2 shows the individual predicted concentrations *versus* observed concentrations for morphine and its metabolites as obtained with the internal dataset for both the systematic covariate model (A) and the fixed allometric model (B). This figure shows a slightly better description of individual concentrations by the fixed allometric model compared to the systematic covariate model, especially for the mother compound morphine.

Figure 3 shows the population predicted concentrations *versus* observed concentrations obtained with the internal as well as the five external datasets. This figure shows the systematic covariate model to be superior over the fixed allometric model in the predictions of population concentrations in the datasets. The predictions for the systematic covariate model are only slightly biased and since this bias is also observed in the mirror plots (data not shown) this is not indicative of model misspecification. For the fixed allometric model on the other hand significant bias towards under-prediction can be observed, that did not correspond to trends observed in the mirror plots (data not shown).

For both models, stratification of the plots of the predicted *versus* observed concentrations into the different age-groups showed no differences in model performance (data not shown).

Table III shows the percentage of η-shrinkage for the parameters for which inter-individual variability was identified in each of the two models. Both models have parameters for which shrinkage is relatively high (>20%), indicating that the individual data in the internal dataset is not rich in information about these parameters.

Table III. *Percentage η-shrinkage in both models for the parameters for which inter-individual variability was identified.*

	Cl_0	Cl_1	Cl_2	Cl_3	Cl_4	V_1
Systematic covariate model (%)	-	29.6	-	8.26	5.76	30.3
Fixed allometric model (%)	52.3	10.8	13.2	21.0	18.9	17.7

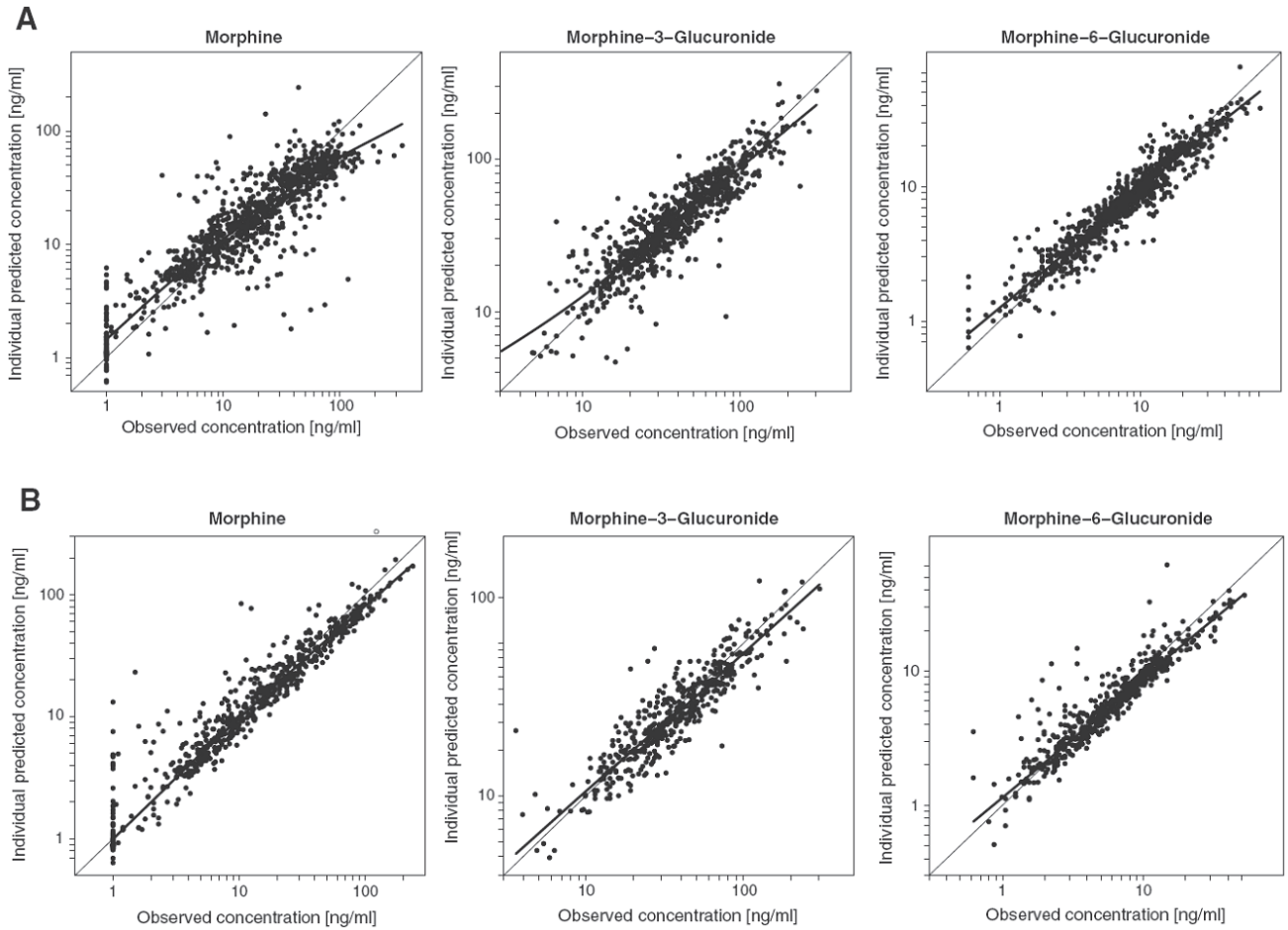

Figure 2. *Individual predicted concentrations versus observed concentrations including a loess curve of morphine and its metabolites in the internal dataset Predictions by the systematic covariate model are depicted in panel A and predictions by the fixed allometric model in panel B.*

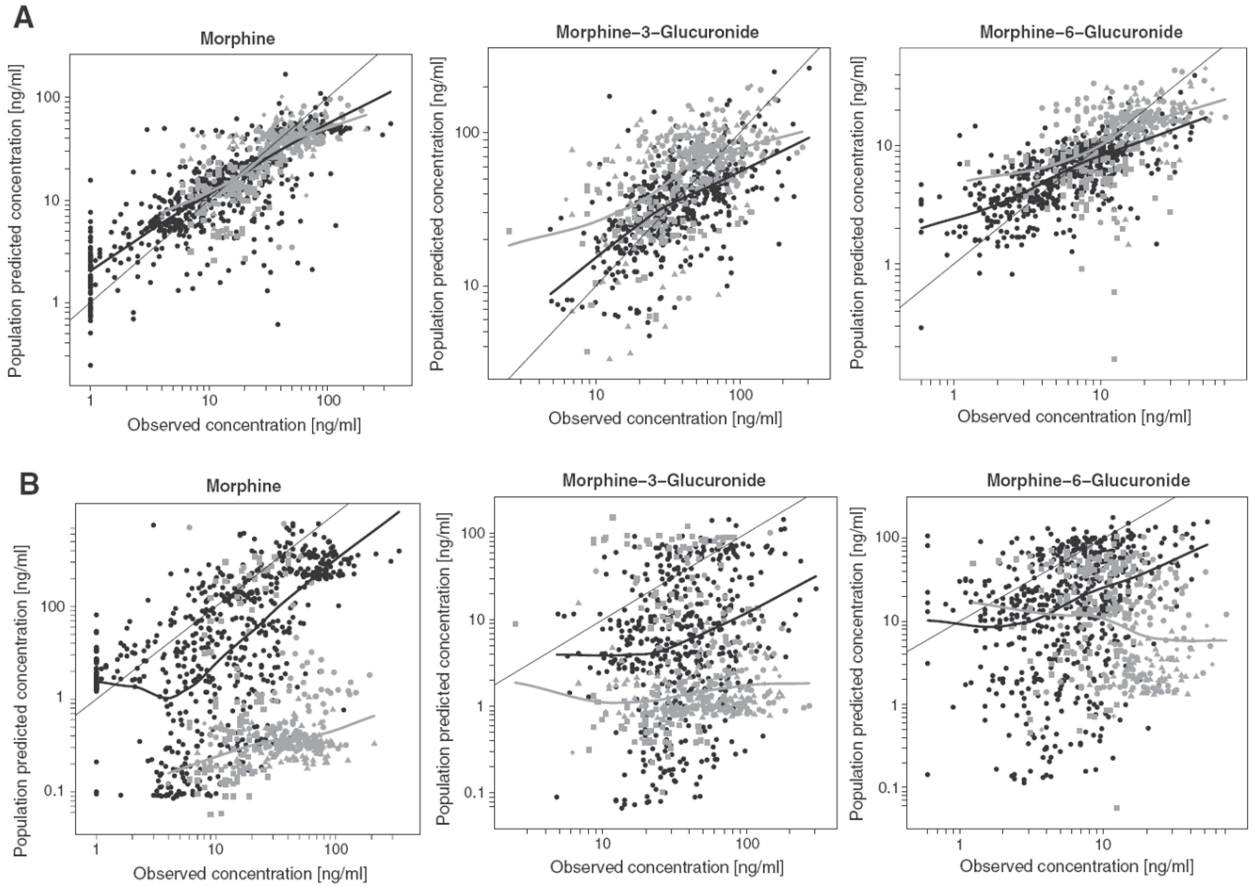

Figure 3. *Population predicted concentrations versus observed concentrations including loess curves of morphine and its metabolites for the systematic covariate model (A) the fixed allometric model (B). Data points in black originate from the internal dataset and data points in grey from the external datasets. Different symbols are used for different external datasets:* ■ *= Ext. 1* [8] , ● *=Ext 2* [9], ♦ *= Ext 3* [10],* *Ext 4* [6], ▲ *= Ext 5* [7].

For both models the results of the NPDE analysis with the internal dataset are depicted in figure 4. For the systematic covariate model no trends in time or concentration are observed and the mean of the distribution of NPDEs is close to 0 while the variance is slightly lower than 1. The trends observed in the plots for the fixed allometric model are indicative of an under-prediction, which appears to be relatively constant over time but to increase with decreasing concentrations.

The results of the NPDE analysis with the external datasets confirmed the results obtained with the internal dataset for both models (data not shown). Stratification into different age-groups also revealed similar results for the three age-groups for each of the models (data not shown).

The plots of the individual *post hoc* parameter estimates and population predicted parameter estimates for total morphine clearance, the clearances of the metabolites and distribution volume of the central morphine compartment *versus* bodyweight for both models are shown in figure 5. For the systematic covariate model, total morphine clearance is composed of Cl_1 and Cl_2 (see figure 1A) which both have different population values for children older and younger than ten days resulting in two different lines of population parameter estimates. For the fixed allometric model, total morphine clearance is composed of Cl_0, Cl_1 and Cl_2 (see figure 1B). The larger number of additional covariates (age, and bilirubin and creatinine concentration) on the structural parameters in the fixed allometric model results in scattered lines for the population parameter estimates of this model. This figure shows that for the systematic covariate model the population predicted values describe the individual *post hoc* values without bias for all parameters, whereas for the fixed allometric model the population predicted values are biased compared to the individual *post hoc* values for all parameters.

Table IV numerically quantifies the bias (MPE) in the population predicted parameter values compared to the individually observed parameter values for both models. The RMSE in this table quantifies the precision of the population prediction. It can be seen that for the systematic covariate model, mean bias in the population predictions stays well below 25% and remains relatively constant over the age-ranges for all parameters. For the fixed allometric model, bias in the population predictions reaches up to 250% and especially in the clearance of the metabolites an increasing trend towards over-prediction is observed with increasing age.

Table V shows the mean bias and precision in the predictions of the model parameters in the external dataset stratified in three age-groups. As can be expected, for both models the bias in the parameter predictions of the external datasets is generally larger than for the internal dataset, however for the systematic covariate model it still remains below 35.6%, whereas for the systematic covariate model the values are between 25% and 300%.

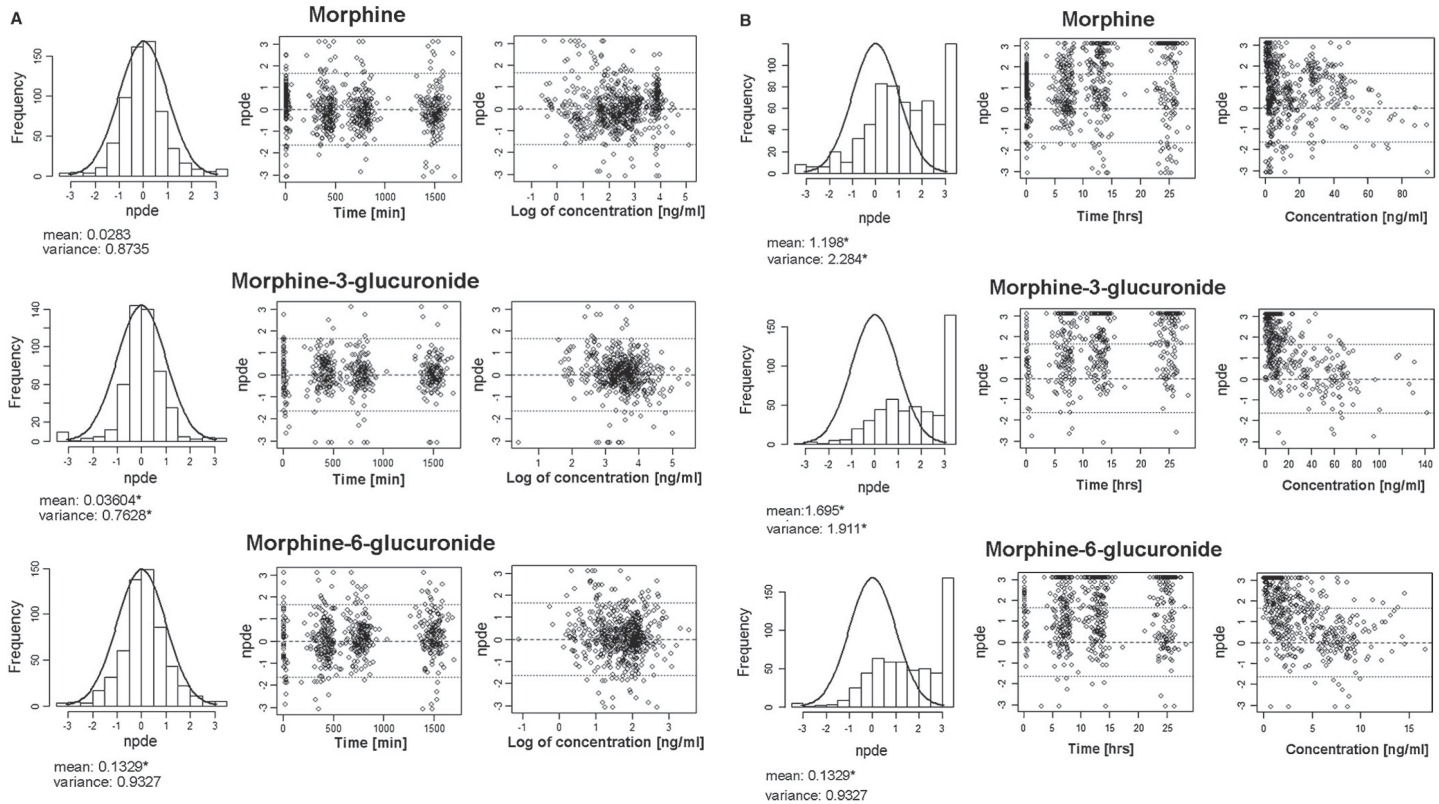

Figure 4. *Result of the NPDE analysis for morphine and its metabolites using the internal dataset with the systematic covariate model (A) and the fixed allometric model (B). In the histograms the distributions of the NPDEs for morphine and its metabolites in the total dataset are shown. The solid line depicts a normal distribution and the values below specify the mean and standard deviation of the observed NPDE distribution in the histogram. A statistically significant deviation of the distribution from a mean of 0 and a standard deviation of 1 is indicated with an asterisk (*). The distributions of NPDEs in time after first dose and against the observed concentrations are also shown. As for the systematic covariate model log-transformed data have been used, the last plot shows the NPDE against the log-value of the observed concentration.*

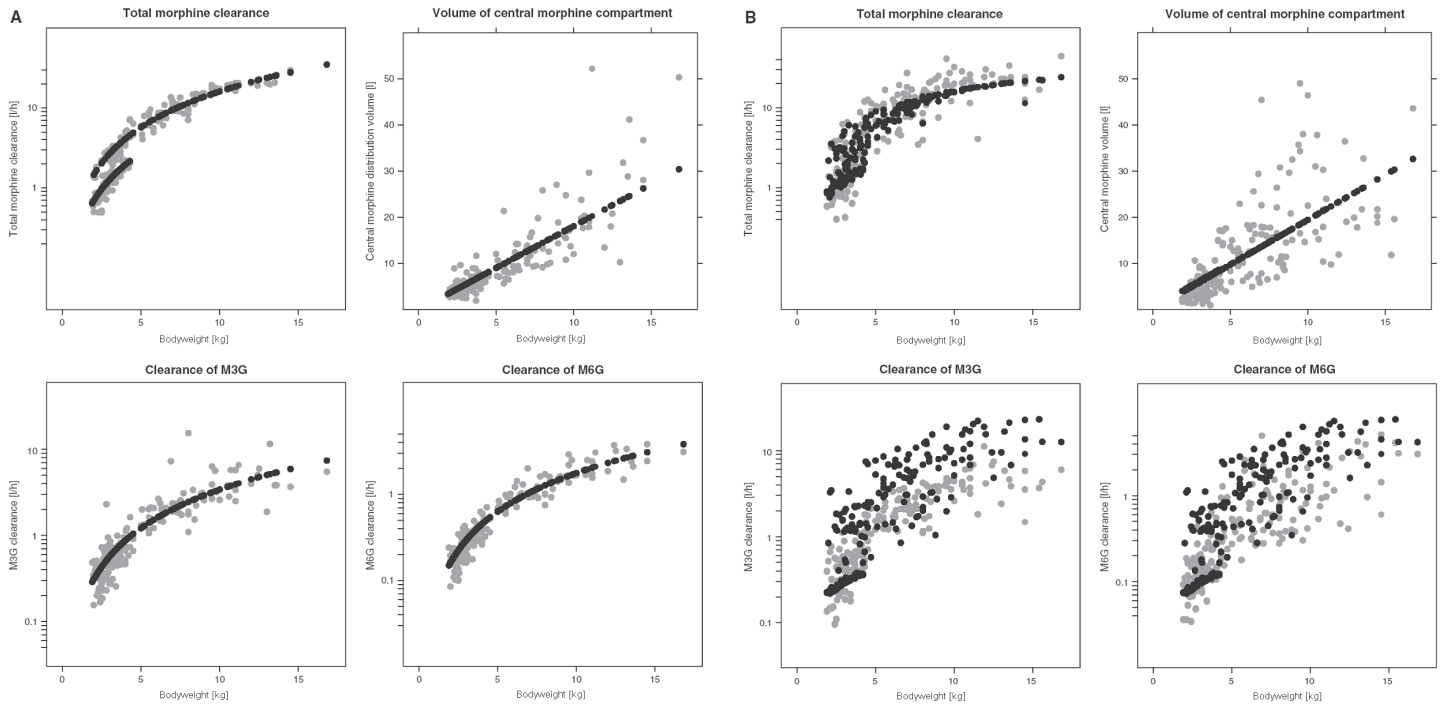

Figure 5. *Individual post hoc parameter estimates (grey) and population predicted parameter estimates (black) for total morphine clearance (Cl_1+Cl_2 for the systematic covariate model and Cl_0+Cl_1+Cl_2 for the fixed allometric model), the elimination clearances of the metabolites (Cl_3 and Cl_4) and distribution volume of the central morphine compartment (V_1) versus bodyweight for the systematic covariate model (A) and the fixed allometric model (B).*

Table IV. *Bias (MPE) and precision (RMSE) of the predicted parameter values in the internal datasets stratified by age-group.*

parameter	Age-group	Systematic covariate model		Fixed allometric model	
		MPE	RMSE	MPE	RMSE
Total morphine clearance	neonates*	4.26	0.426	10.8	0.922
	toddlers†	-0.74	1.65	12.7	5.00
	infants‡	4.63	3.28	-2.56	7.90
M3G clearance	neonates*	19.7	0.291	-19.5	2.50
	toddlers†	1.85	0.888	23.8	15.5
	infants‡	14.5	3.82	188	9.03
M6G clearance	neonates*	12.5	0.0784	-69.0	0.825
	toddlers†	0.262	0.230	100	5.27
	infants‡	4.93	0.852	253	2.84
Distribution volume of central morphine compartment	neonates*	23.2	1.30	90.6	2.93
	toddlers†	15.9	3.43	22.4	7.26
	infants‡	5.90	9.27	9.35	13.4

MPE = mean prediction error, RMSE = root mean square error,
* neonates, PNA<30 days, n= 61,
† toddlers, PNA=1 month-1 year, n= 87,
‡ infants, PNA>1 year n= 35

Table V. *Bias (MPE) and precision (RMSE) of the predicted parameter values in the external datasets stratified by age-group.*

parameter	age-group	systematic covariate model		fixed allometric model	
		MPE	RMSE	MPE	RMSE
Total morphine clearance	preterm neonates*	17.4	0.0667	192	0.444
	term neonates†	29.74	0.274	85.5	3.91
	toddlers‡	29.3	1.64	-26.6	4.22
M3G clearance	preterm neonates*	-16.3	0.102	114	0.225
	term neonates†	31.0	0.463	108	0.928
	toddlers‡	5.48	0.818	172	1.34
M6G clearance	preterm neonates*	-12.5	0.0447	46.8	0.779
	term neonates†	35.6	0.0818	31.1	0.502
	toddlers‡	12.5	0.285	93.5	0.490
Distribution volume of central morphine compartment	preterm neonates*	-12.9	0.945	-115	1.46
	term neonates†	-10.3	2.03	-296	3.50
	toddlers‡	3.45	3.47	-93.1	3.22

MPE = mean prediction error, RMSE = root mean square error
* preterm neonates, (PNA < 30 days and PMA at birth < 36 weeks), n=80
† term neonates, (PNA < 30 days and PMA at birth ≥ 36 weeks), n=40
‡ infants, PNA = 1 month – 1 year, n=33

8.4 Discussion

As in childhood many physiological changes take place in quick succession, the paediatric population is very heterogeneous. Additionally, studies in this population are often performed during routine clinical practice, which increases the variability in both dosing and sampling schemes, while due to limitations in sample size and frequency often only sparse data are obtained. All these factors influence the evaluation of population and covariate models for this young population. In the current study a framework of six different evaluation criteria is proposed for the evaluation of paediatric models. Most tools in the framework are not necessarily new, but in the context of paediatric model evaluation adaptations to the standard methods are sometimes required or a shift in emphasize on the various tools is essential.

As an example, two previously published paediatric population PK models for morphine that were based on the same dataset but fundamentally different covariate models, were evaluated with this framework. The systematic covariate model was developed by regarding bodyweight and age as regular covariates in a systematic covariate analysis (Chapter 3). The fixed allometric model was based on allometric principles including bodyweight *a priori* using exponential functions with fixed exponents and estimating an age-based function [3].

In itself the number of parameters in a model is not an evaluation criterion, however according to the rule of parsimony a model should have the lowest possible number of parameters. Large deviations of bootstrap parameter values from the original value, low precision in parameter estimates as expressed by high RSE values in the model fit and bootstrap procedures, small number of successful bootstrap runs and a condition number higher than 1000 are all generally indicative of model inaccuracy, model instability, and ill-conditioning [15–18]. Testing covariates for significance and only retaining a covariate when it significantly improves the model adheres to the rule of parsimony, does not introduce specific assumptions into the model, and the resulting model is always supported by data. In the current example, it is shown that the systematic covariate model performs well on all the criteria of ill-conditioning. In case covariates in a model are not formally tested for significance there is a risk of over-parameterization. The results for the fixed allometric model in this example suggest the fixed allometric model to be an unstable model for which precise parameter estimates cannot be obtained. It should also be noted in this respect that with the increased number of fixed and random model parameters in the fixed allometric model, the degrees of freedom in this model are increased as well. More degrees of freedom improve the description of data by a model, but do not necessarily improve the predictions by that model.

As stated before, paediatric PK analyses are often based on sparse data. This is an important aspect to consider when evaluating paediatric population PK models, because when data are uninformative due to the scarcity of the data, the estimated variability parameters may shrink to zero causing the individual *post hoc* parameter estimates to move towards the population predictions. This shrinkage phenomenon makes individual parameter estimates and the diagnostics based on them less reliable or even misleading [12,19]. Population predictions however, are solely based on the fixed effects described in the structural and covariate model. As the random effects are not considered in the population predictions, diagnostics based on population predictions are not sensitive to shrinkage and therefore more reliable then diagnostics based on individual predictions. The two models in the current study illustrate how diagnostics based on individual predictions can be misleading when shrinkage is high, as, according to table III, was the case for some of the parameters in both models. The plots of the individual predicted *versus* observed concentrations in figure 2 inadvertently suggest the fixed allometric model to perform better than the systematic covariate model, since especially for morphine the data points are closer to the line of unity. However, in figure 3 large differences in the predictive performance between the two models are revealed for both the internal and external datasets when considering population predicted concentrations instead of the individual predicted concentrations. For the systematic covariate model there are no signs of model misspecification. The fixed allometric model on the other hand shows significant bias towards under-prediction throughout the total concentration range. Since diagnostics based on population predictions are generally more reliable, these should always be included in the evaluation of paediatric (covariate) models.

Simulation-based diagnostics known as posterior predictive checks (PPC) are diagnostics that create a reference distribution of an observation of interest by performing multiple model simulations with inclusion of both fixed and random effects and subsequently compare the actual observations to this reference distribution [20]. A visual predictive check (VPC) is a commonly used and easily interpretable form of a PPC that graphically presents the reference distribution and observed data [21]. A VPC can be used for the evaluation of paediatric models as well, however when data are obtained during routine clinical practice and variability in individual dosing and sampling schemes are high, the NPDE methodology [13] is often easier to perform and interpret. Shrinkage does not influence the results of simulation-based diagnostics [12]. The results of the NPDE analysis of the models in the current example demonstrate that the systematic covariate model can quite accurately predict median concentrations for morphine and the glucuronides, but that it slightly over-predicts the variability in the overall dataset. This over-prediction of the variability is constant over time and over the concentration range. If this model

were to be used in simulation exercises the predictions would be unbiased and the inferences made on the variability in the population would be on the conservative side as the variability is predicted to be higher than it actually is. Based on the trend towards under-prediction by fixed allometric model, it can be concluded that significant bias in the predictions would occur if this model would be used in simulation exercises. The under-prediction of concentrations by the fixed allometric model increases the risk of overdosing when deriving morphine doses based on this model. These NPDE results substantiate the results obtained in the population predicted *versus* observed plots in figure 3.

Due to the heterogeneity in the paediatric population it is very important to not only perform diagnostics on the population as a whole, but to also look at various subpopulations by stratifying the datasets based on bodyweight or age.

For both models in the example, stratification showed the same descriptive and predictive performance in all age-groups. For the systematic covariate model the predictive performance of the model was adequate in all age-ranges. The trends towards under-prediction identified for the fixed allometric model was also similar across all age-ranges. Despite the fact that stratification of the diagnostics did not reveal new information in the current examples, this adjustment of the various validation tools remains imperative for the detection of previously unidentified age-related misspecifications.

To corroborate the obtained covariate relationships in paediatric models, the plots of individual and population parameter values *versus* the covariate presented here in figure 5, together with a numerical representation of bias and precision in table IV, have proven to be highly informative. Even in case of high shrinkage this diagnostic will enable the identification of bias in the population predictions of parameters. In this study, in both models bodyweight was the most important covariate for clearances and distribution volumes. For the systematic covariate model, population predicted parameter values are adequately centred in the range of individual predicted values for all parameters and across the entire bodyweight- and age-range. However, for the fixed allometric model, the population predicted parameter values are biased compared to the individual predicted values. For some parameters this bias exists over the total weight and age-range, for others only over part of these ranges. The results in the plots in figure 5 and table IV provide an explanation for the adequate individual concentration predictions by the fixed allometric model and the highly biased population predictions by this model, as observed with the other evaluation tools. Structural model misspecifications in the population parameter values result in biased population predictions that are corrected by the error models to yield good individual predictions. This type of diagnostic is hardly

ever published, however the information contained in these plots and tables is crucial and should become a standard diagnostic tool with paediatric population PK models.

Especially in drug development, population PK models in paediatric subpopulations are often used for extrapolations to younger age-ranges. When a model is used for this purpose, the obtained covariate relationships should be thoroughly evaluated, for instance by using the diagnostic in figure5 and table IV. In the current study, two out of five external datasets (Ext 4 and Ext 5) include preterm patients, a younger and smaller population than the population in the internal dataset. For both models the results of the various tools in the framework were similar irrespective of the age-range in the dataset that was used, indicating that the inclusion of a new patient population in the external validation did not influence the overall results

A systematic covariate analysis is a data driven approach, therefore the extrapolation potential of the resulting model cannot be known *a priori*. It is clear that for the systematic covariate model in the current analysis extrapolations to older (heavier) children is not possible as the bodyweight based exponential equation for clearance predicts rapidly increasing clearances at higher weight-ranges. Figure 5 indicates that for this model, population parameter predictions are unbiased in the lower weight-ranges, suggesting that extrapolation to smaller children could be possible. The extensive evaluation procedures in the current example prove this to be the case in this particular example.

It is claimed that the allometric equations used in the fixed allometric model are based on 'sound biological principles' [22] and that the methodology based on these equations therefore yields mechanistic models that can be used for extrapolations outside the studied age- or weight-range. It is argued that the influence of size (parameterized by bodyweight) and maturation (parameterized by age) on the parameters in paediatric population PK models are disentangled by using the fixed allometric equations augmented by age-based functions [4,23]. However, the theory of allometry is based on the empirical observation that over a wide weight-range, metabolic rates of animal species increase with bodyweight to the power of 0.75 [24]. The fixed allometric exponents have no biological or physiological meaning, although reports exist that propose possible physiological explanations [25–27]. Conversely, a large body of evidence exists against the existence of one unique value for the allometric clearance exponent [28–34] and against the application of these allometric equations in human paediatric PK models [35,36]. Additionally, the maturation function based on age, only reflects a mathematical residue of the age-effect that remains after the inclusion of the correlated covariate bodyweight. In the current study, in the fixed allometric model the model-predicted increase in clearance comes to a plateau with increasing bodyweight. Therefore, it can not be excluded that

this model can be used for extrapolations to higher weight-ranges. The prospective properties of this model in preterm neonates is however very poor, albeit comparable to the predictions in the older children that comprised the learning dataset. As a result, the extrapolation potential of fixed allometric models in general can neither be confirmed nor disputed based on the results in the current study.

8.5 Conclusion

The framework of six evaluation criteria proposed in the current study takes into consideration the specific issues encountered in the evaluation of paediatric population models. The application of this framework to two models for morphine and its two major metabolites in children younger than three years with fundamentally different covariate models demonstrates how to detect over-parameterization, which is a risk with models based on sparse data. Additionally it illustrates the importance of diagnostics that are based on population predictions rather than individual predictions, as high shrinkage due to sparse data may yield misleading individual prediction-based diagnostics. Finally, the diagnostic comparing population parameter predictions with individually observed parameter values proved to be highly informative in assessing obtained covariate relationships as in the current example it detected the cause of model misspecification by the fixed allometric model. Stratification of the various diagnostics did not yield much additional information in the current examples, however due to the heterogeneity of the paediatric population this adaption of standard validation tools may be of value for other paediatric models.

The differences observed in model performance between the systematic covariate model and the fixed allometric model in the current study do not imply that any of the two methodologies for covariate model development is superior over the other. The current study does however highlight the importance of corroborating results in evaluation procedures. It also illustrates that information in data should not be ignored and that one should never be guided by theories alone.

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Chapter 9

Prediction of Morphine Clearance in the Paediatric Population: How Accurate are the Available Pharmacokinetic Models?

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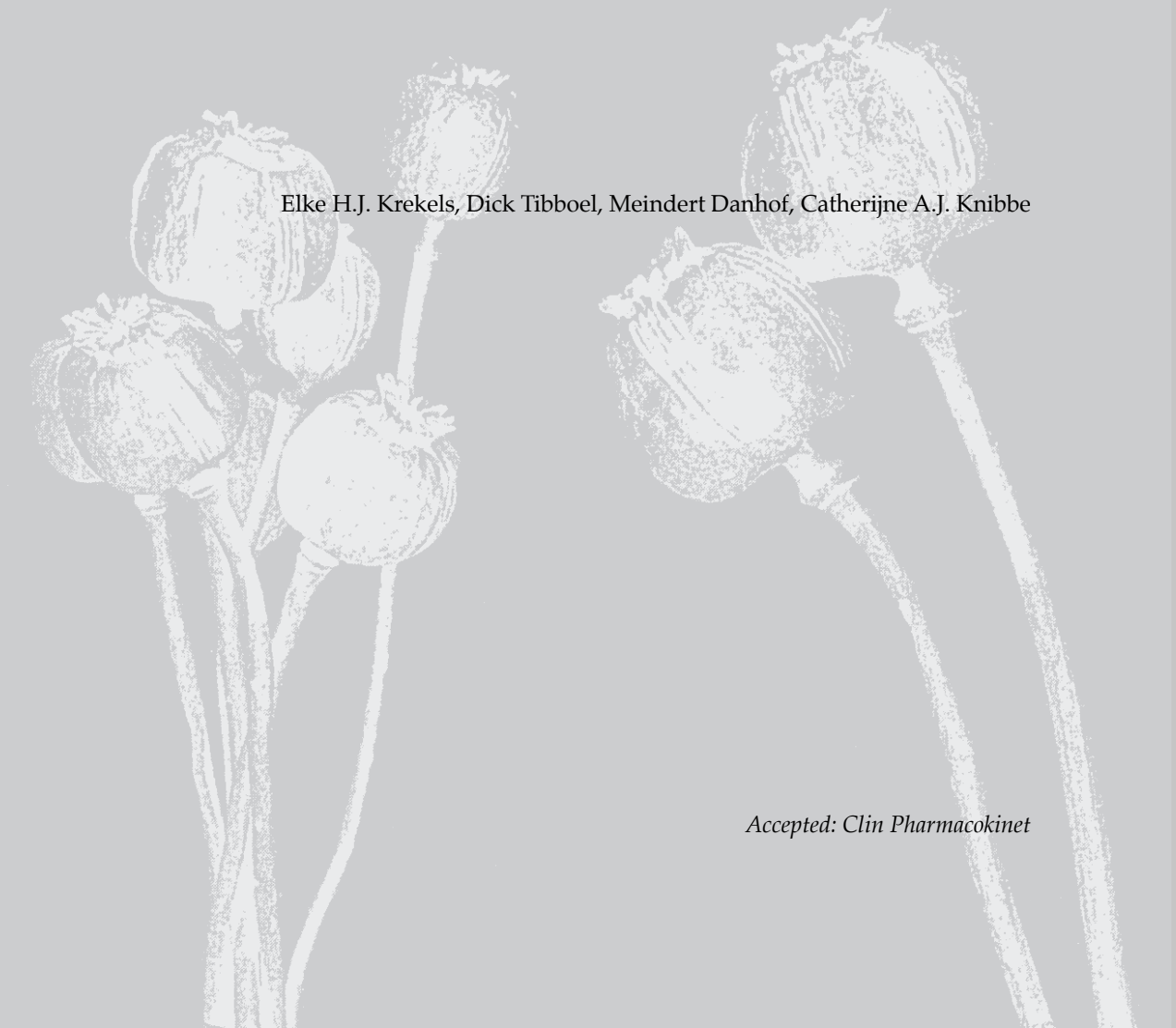

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Abstract

The pharmacokinetics of morphine in paediatrics have been widely studied using different approaches and modeling techniques. In this review, we explore advantages and disadvantages of the different data analysis techniques that have been applied, with specific focus on the accuracy of morphine clearance predictions by reported paediatric pharmacokinetic models.

Twenty paediatric studies reported a wide range in morphine clearance values using traditional rather descriptive methods. Clearance values were expressed per kilogram bodyweight, while maturation in clearance was described by comparing mean clearance per kg bodyweight between age-stratified subgroups. Population modeling allows for the analysis of sparse data thereby limiting the burden to individual patients. Using this technique, continuous maturation profiles can be obtained on the basis of either fixed allometric scaling or comprehensive covariate analysis. While the models based on fixed allometric scaling resulted in complex maturation functions, all three paediatric population models for morphine yielded quite similar clearance predictions. The largest difference in clearance predictions between these three population models occurred in the first months of life, particularly in preterm neonates. Morphine clearance predictions by a physiologically-based pharmacokinetic model were based on many continuous equations describing changes in underlying physiological processes across the full paediatric age-range, and resulted in similar clearance predictions as well. Preterm neonates could however not be integrated in this model.

In conclusion, the value of paediatric pharmacokinetic models is mostly dependent on clearance predictions and population concentration predictions, rather than on the individual description of data. For most pharmacokinetic models however, the assessment of model performance was very limited and for only one model was the accuracy of morphine clearance predictions as well as population concentration predictions confirmed by formal evaluation and validation procedures.

9.1 Background

Dosing guidelines for children have originally been scaled from adult doses using functions related to body size (i.e. bodyweight). After years of clinical experience, these dosing guidelines are often formalized in (national) formularies. Research necessary to develop evidence-based, rather than consensus-based dosing algorithms for the paediatric population is complicated by practical, ethical, and legal constraints. However, advances in pharmacokinetic and pharmacodynamic analyses and the enormous increase in computing capacities of processors over the past few decades have opened up new possibilities in data analysis and data aggregation yielding novel opportunities for paediatric pharmacological investigations.

Morphine is commonly prescribed for the paediatric population in hospital settings. Morphine clearance, its variability, and the maturation in this parameter have been extensively studied across the paediatric population. This has lead to the publication of a wide range of paediatric morphine clearance values, obtained with traditional methods as well as with the new computing-intensive modeling methodologies. Irrespective of the methodology used, reported clearance values should be representative for the studied population, because they provide the basis for paediatric dose adjustments and clinical decision making. Therefore, it is crucial that these values are both accurate and predictive for the next unstudied individual represented by the studied population.

Morphine is predominantly eliminated through glucuronidation by UGT2B7 [1–3], thus morphine clearance directly reflects the formation of its two major metabolites morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). The metabolites are cleared through renal elimination and reduced renal function may result in accumulation of the metabolites. Since M3G and M6G are considered to be pharmacologically active [4–6], the fate of the metabolites after formation through morphine glucuronidation of is of clinical importance. However, as only a limited number of publications have addressed the pharmacokinetics of the morphine metabolites in addition to the pharmacokinetics of morphine, the current review is limited to total morphine clearance.

In this review, reported paediatric morphine clearance values and the maturation in this parameter in the paediatric population are evaluated. Morphine clearance values obtained using the traditional methods will be discussed after which the focus will shift to results obtained with the more recent computing-intensive modeling approaches such as population pharmacokinetic modeling and physiologically-based pharmacokinetic modeling. General advantages and disadvantages of the different analysis approaches are explored and details of the different published pharmacokinetic morphine models are discussed. The predictive value of the models for a 'new' patient represented by the

studied population, and thus the suitability of the models for simulation purposes for a population of patients with similar characteristics as the studied population, is assessed in particular. The assessment of the accuracy of model predictions is mainly based on the visual comparison of population concentration predictions *versus* clinically observed concentrations, while the accuracy of clearance predictions is based on the Mean Prediction Errors (MPE) of the population clearance values compared to individual clearances.

9.2 Methods

Pubmed was searched in November 2011 for original research on morphine clearance in the paediatric population. The search was limited to the last 20 years, including publications from January 1991 onwards. The following key words were used: 'morphine clearance', 'morphine metabolism', 'morphine glucuronidation', 'morphine elimination', or 'morphine pharmacokinetics'. Limits were set for age to include children between 0 – 18 years. Case reports were excluded. Only studies with intravenous administration were selected, to avoid confounding issues with bioavailability in the reported clearance values. Since the pharmacokinetics of drugs in patients on extracorporeal membrane oxygenation (ECMO) may depend on various components of the ECMO circuit itself [7,8], studies in these patients were excluded as well. The obtained publications were categorized as analyzed according to 1) traditional methods, 2) population pharmacokinetic modeling, or 3) physiologically-based pharmacokinetic modeling.

9.3 Clearance Estimates Obtained With Traditional Methods

9.3.1. Traditional Methods

Traditional methods to determine pharmacokinetic drug parameters in a population rely on firstly determining individual parameter values, using either compartmental or non-compartmental analysis techniques, after which each parameter is summarized as mean and standard deviation. As such, this yields for each pharmacokinetic parameter a point estimate (mean value) for the population and a measure of variability (standard deviation). This may be useful in early drug development, when data of a very limited number of patients are available. However, since intra-individual variability, measurement error and model misspecification, cannot be distinguished from inter-

individual variability with this method, other methods are preferred to describe and quantify trends in a population when more data become available.

As the determination of individual drug clearance values with compartmental methods relies on densely sampled concentration-time profiles for each subject, this method may not always be feasible especially in the very young. Similarly, non-compartmental methods may not be feasible as they rely either on the area under the concentration-time curve, which also requires dense sampling per individual, or on imprecise measurements of steady state concentrations. Using traditional methods, the maturation patterns in drug clearance are usually studied by expressing individual clearances per kilogram bodyweight, stratifying patients into age-groups, and comparing mean clearance values per kilogram bodyweight between the age-groups. This allows for easy comparisons between studies and between age groups, but this makes findings on maturation dependent on the stratification and precludes the development of continuous maturation profiles. Additionally, it assumes clearance to scale linearly with bodyweight within the age-groups, which may be a practical approximation when the range in bodyweight within each stratum is small, but it may not accurately reflect the underlying physiological changes across the entire human life-span.

9.3.2. Morphine Clearance Determined With Traditional Methods.

Table I provides an overview of paediatric morphine clearance values obtained with traditional methods. The reported morphine clearance values in neonates with a postnatal age from 0 – 30 days range from about 0.58 ml/min/kg [9] to about 16 ml/min/kg [19], which is more than a twenty-fold difference. In infants aged 1 month to 1 year, morphine clearances were reported to range between 7.8 ml/min/kg [16] to 69.4 ml/min/kg [19,20], while in children from 1 to 18 years the range in morphine clearance was reported to vary from about 12 ml/min/kg [23] to about 60 ml/min/kg [27]. The wide ranges in reported clearance values within each age-group may in part be explained by the differences between studies in terms of patient characteristics, sampling schemes or dosing schemes, but are probably mainly caused by the relative imprecision of the applied data analysis methods. Additionally, most studies are based on a relatively small number of individuals (table I), limiting the precision of each finding.

Table I. *Overview of paediatric morphine clearance values reported over the past 20 years, obtained with traditional methods.*

Population	Age	Bodyweight [kg]	Number of patients	Morphine clearance [ml/min/kg]	Reference
term ischemic neonates (normotherm)	< 6 hr	3.3 (2.4 – 4.0)[a]	6	0.89 (0.65-1.33)[a]	Róka, *et al.* 2008 [9]
term ischemic neonates (hypotherm)	< 6 hr	3.4 (2.5 – 4.0)[a]	10	0.69 (0.58 – 1.2)[a]	
preterm neonates on artificial ventilation	< 24 hr	1.3 ± 0.38[c]	9	2.4[b]	Hartley *et al.* 1993 [10]
term and preterm neonates on artificial ventilation	< 24 hr	1.3 (0.77 – 4.0)[a]	31	2.4 ± 1.1[c]	Saarenmaa *et al.* 2000 [11]
term and preterm neonates on artificial ventilation	1 – 2 d	1.4 ± 0.6[c]	19	total: 4.6 ± 3.2[c] formation M3G: 2.5 ±1.8[c] formation M6G: 0.46 ± 0.32[c]	Barrett *et al.* 1996 [12]
preterm and term neonates on artificial ventilation	1 – 4 d	2.6 (1.3 – 3.6)[a]	19	2.55 ± 1.65[c] 2.09 ± 1.19[c]	Chay *et al.* 1992 [13]
preterm neonates	1 – 18 d	1.1 (0.6 – 1.6)[a]	8	2.82 (1.88 – 6.60)[a]	Mikkelsen *et al.* 1994 [14]
term neonates	1 – 18 d	3.4 (2.3 – 4.0)[a]	5	4.73 (1.75 – 6.61)[a]	
preterm neonates on artificial ventilation	1 – 37 d	0.88 – 1.46[d]	26	3.6 ± 0.9[c]	Barrett *et al.* 1991 [15]
preterm neonates	1.1 ± 0.3[b] wk GA 26.6 ± 0.7[b] wk	birth weight 1.0 ± 0.17[c]	10	2.27 ± 1.07[c]	Scott *et al.* 1999 [16]
	1.3 ± 0.6[b] wk GA 29.5 ± 1.3[b] wk	birth weight 1.4 ± 0.24[c]	16	3.21 ± 1.57[c]	
	6.1 ± 9.1[b] wk GA 32.5 ± 1.6[b] wk	birth weight 2.1 ± 0.41[c]	15	4.51 ± 1.97[c]	
	16.4 ± 31.6[b] wk GA 35.4 ± 4.8[b] wk	birth weight 3.3 ± 0.46[c]	7	7.80 ± 2.67[c]	
postoperative or artificially ventilated patients	1 – 7 d	-	10	8.7 ± 5.8[c]	Pokela *et al.* 1993 [17]
	8 – 60 d	-	10	11.9 ± 5.1[c]	
	61 – 180 d	-	7	28.0 ± 8.9[c]	
term neonates and infants on artificial ventilation	3 d – 11 mo	2.2 – 8.7[d]	12	23.4 ± 18[c]	Choonara *et al.* 1992 [18]

postoperative term neonates and infants, non-cardiac surgery	1 – 7 d	7.3 ± 5.0[c]	10	9.8 (6.3 – 16)[a]	Lynn *et al.* 2000 [19]
	8 – 30 d		4	13.3	
	31 – 90 d		14	23.9 (16.7 – 33.3)[a]	
	91 – 180 d		25	32.3 (18.5 – 52.1)[a]	
	181 – 365 d		30	38.1 (18 – 69.4)[a]	
postoperative term neonates and infants, non-cardiac surgery	1 – 7 d	6.6 ± 2.0[c]	4	9.2 (6.3 – 10.4)[a]	Lynn *et al.* 1998 [20]
	31 – 90 d		6	25.3 (21.7 – 33.3)[a]	
	91 – 180 d		6	31.0 (18.9 – 59.5)[a]	
	181 – 380 d		10	48.9 (34.7 – 69.4)[a]	
postoperative patients, non-cardiac surgery	3 – 12 mo	8.1 ± 1.0[c]	6	19.8 ± 5.9[c]	Haberkern *et al.* 1996 [21]
postoperative term neonates and infants	1 – 7 d	3.2 (2.5 – 3.6)[a]	9	5.5 (3.2 – 8.4)[a]	McRorie *et al.* 1992 [22]
	8 – 30 d	3.9 (3.2 – 4.6)[a]	5	7.4 (3.4 – 13.8)[a]	
	31 – 90 d	4.3 (3.5 – 5.2)[a]	7	10.5 (9.8 – 20.1)[a]	
	91 – 180 d	5.1 (4.3 – 8)[a]	11	13.9 (8.3 – 24.1)[a]	
	181 d – 2.5 y	7.2 (5.5 – 13.8)[a]	17	21.7 (5.8 – 28.6)[a]	
post-operative patients, cardiac surgery	8 mo – 7 yr	-	21	19.2 ± 7.0[c]	Dagan *et al.* 1993 [23]
patients with leukemia	1.4 – 15.9 yr	20.0 (9.3 – 54.5)[a]	17	35[b] [ml/min]	Hain *et al.* 1999 [24]
patients with cancer	2.6 – 16.42 yr	32.4 ± 21.4[c]	7	24.8[b]	Mashayekhi *et al.* 2009 [25]
patients with sickle cell disease	5 – 17 yr	34.6 ± 7.6[c]	11	1600 ± 700[c] [l/min]	Kopecky *et al.* 2004 [26]
patients with sickle cell disease	6 – 19 yr	-	18	35.5 ± 12.4[c] 34.4 ± 14.3[c]	Dampier *et al.* 1995 [27]
patients with sickle cell disease	pre-pubertal children	14 – 72[d]	11	40.4 ± 10[c]	Robieux *et al.* 1992 [28]
	pubertal children		5	37.1 ± 9[c]	
	post-pubertal children		8	28.0 ± 11[c]	

[a] median (range), [b] mean, [c] mean ± SD, [d] range
hr = hours, d = days, wk = weeks, mo = months, yr = years, GA = gestational age

9.4 Clearance Estimates Obtained With Population Modeling

9.4.1. Population Modeling

Increases in computing power now allow for the analysis of concentration-time measurements from a population as a whole while considering individuals as constituents of this population. This method is called population or non-linear mixed effects modeling. As long as data are sufficiently informative, population modeling can be used for the analysis of dense, sparse, and/or unbalanced data. This is especially beneficial for the vulnerable paediatric population as it allows for the analysis of a limited number of blood samples per patient and for the analysis of data obtained during routine clinical practice. Additionally, it may allow for the meta-analysis of data from multiple studies with different designs, thereby reducing the burden for individual paediatric patients. A proper covariate analysis does however require information on the same set of covariates in each individual dataset. Since data from various sources can be analyzed simultaneously, the precision of the findings may also increase.

Population modeling can also distinguish inter-individual variability from intra-individual variability, measurement error, and model misspecification. By identifying which patient characteristics (e.g. age, bodyweight, gender, race, genetics, disease status) are predictors of the inter-individual variability in model parameters, trends in the population can be identified and quantified. These predictors are known as covariates and the relationship between a covariate and a model parameter is known as covariate relationship. Typically, population pharmacokinetic modeling relies on outcome measures and information on covariates. Concentration data for pharmacokinetic models can be obtained relatively easily from blood samples. Covariate relationships in the population models generally include patient information that can be obtained from medical records or from routine clinical measurements. An important feature of population pharmacokinetic modelling is that it allows for the identification of continuous maturation profiles that do not depend on stratifications and that, when pharmacodynamic relationships remain constant with age, the covariate relationships describing this maturation can be directly used as the basis of evidence-based dosing algorithms. Since steady state drug concentrations are solely dependent on drug clearance and peak concentrations heavily dependent on distribution volume, the covariate relationships for these parameters can be directly incorporated in the algorithms of paediatric maintenance or loading doses respectively. However, since the use of sparse data may increase the risk of drawing wrong conclusions, population models require an advanced level of evaluation and validation before a model can be accepted [29].

One of the approaches that can be applied for paediatric population covariate modeling is fixed allometric scaling [30]. Using this approach, bodyweight is included *a priori* in the model as a covariate on clearance (*Cl*) according to the following allometric equation:

$$Cl = a \cdot (BW_i / 70)^b$$

in which BW_i is the bodyweight of the individual paediatric patient in kg, that is normalized to an average adult bodyweight of 70 kg and the value of the exponent *b* is fixed to 0.75 for clearance. The value of *a* that represents the magnitude of clearance in adults, is estimated. This fixed allometric equation describes the influence of changes in body size on drug clearance and on average predicts paediatric drug clearances with a fair degree of accuracy in children older than five years [31]. In younger children the allometric equation is augmented with an age-based function called 'maturation model', to describe the remaining influence of developmental changes on drug clearance. Additional covariate relationships that reflect the influence of altered function of elimination organs (i.e. liver or kidneys) may be incorporated as well [30].

The fixed allometric scaling approach is frequently applied, despite theoretical and data-driven studies challenging the hypothesis that the allometric equations accurately describe the influence of body size on pharmacokinetic processes [32–39]. Additionally, with the inclusion of bodyweight, part of the influence of age is included as well, due to the strong correlation between bodyweight and age in the paediatric population. This makes the maturation model a mathematical residue of the influence of age that remains after the inclusion of the correlated covariate bodyweight, rather than a descriptor of maturation *per se*. Moreover, since bodyweight and age are included without formal testing for significance, there is a risk of over-parameterizing the models, leading to imprecise parameter estimates. Finally, special attention is required for the interpretation of these models. Due to the separation of the influence of body size (expressed by bodyweight) and maturation (expressed by age), the statement that maturation is completed at a certain age does in this context not mean that absolute clearance has reached adult values, as body size is usually still increasing. Misinterpretation of such results can lead to over-dosing when used for paediatric dose adjustments, and therefore the expression of the pharmacokinetic parameters per 70 kg may be unwarranted particularly in neonates.

Another approach in paediatric population pharmacokinetic modelling is the application of a comprehensive covariate analysis, in which all potential covariates for pharmacokinetic parameter are tested in various relationships and are included into the model based on statistical significance. This procedure can be used to identify demographic factors or

co-morbidities that significantly influence drug clearance. In the paediatric population it can also be used to identify covariate relationships that describe functional maturational changes in drug clearance [40]. The paediatric covariate relationships, are usually based on bodyweight, age or a combination of both, and may vary in nature (e.g. exponential or linear). It should be noted however that these covariate relationship are empirical and that bodyweight or age should not be regarded as the drivers of the observed changes in drug pharmacokinetics, but as surrogate descriptors of the net changes in the underlying physiological system. The descriptive nature of these covariate relationships explicitly precludes extrapolations outside the covariate range in the learning dataset.

9.4.2 Morphine Clearance Determined With Population Modeling

9.4.2.1. Bouwmeester *et al.* (2004)

The model by Bouwmeester *et al.* [41] comprises morphine as well as its two main metabolites M3G and M6G. The model is based on data from 184 term neonates to infants up to the age of three years from Van Dijk *et al.* [42].

The Bouwemeester-model was developed using fixed allometric scaling principles described in section 9.4.1. The maturation model for the formation of morphine glucuronides was an exponential model based on postnatal age and serum bilirubin concentrations were included as a covariate on morphine glucuronidation. The set of equations below shows how total morphine clearance is described by the Bouwmeester-model:

$$Cl_{tot} = Cl_{M3G} + Cl_{M6G} + Cl_R$$

$$Cl_{M3G} = 64.3 \cdot (BW_i / 70)^{0.75} \cdot (1 - 0.834 \cdot EXP(-PNA \cdot (\ln 2 / 88.3))) \cdot EXP(C_{bili} \cdot -0.00203)$$

$$Cl_{M6G} = 3.63 \cdot (BW_i / 70)^{0.75} \cdot (1 - 0.834 \cdot EXP(-PNA \cdot (\ln 2 / 88.3))) \cdot EXP(C_{bili} \cdot -0.00203)$$

$$Cl_R = 3.12 \cdot (BW_i / 70)^{0.75}$$

In these equations, Cl_{tot} is total morphine clearance in l/h, Cl_{M3G} and Cl_{M6G} are the formation clearance of M3G and M6G in l/h, and Cl_R is the residual clearance through alternative pathways in l/h. BW_i is the bodyweight of the individual paediatric patient in kg, PNA is the postnatal age in days, and C_{bili} is the serum bilirubin concentration in μmol/l. Total morphine clearance is 71.1 l/h/70kg$^{0.75}$, and from the maturation model it can be derived that the adult value of morphine glucuronidation is reached between the age of 6 to 12 months. Absolute morphine glucuronidation is however still increasing after that age, as a result of changes in bodyweight, which is described by the allometric function.

Model performance was corroborated by plots of the ratio of observed and individual morphine and metabolite concentrations *versus* time, which showed limited bias. Results of other diagnostics, in particular plots of population predicted concentrations *versus* observed concentrations, were not reported. More recently, this model has been evaluated by our group using both the learning dataset and external datasets (Chapter 8). With a condition number of 10698 the model was shown to be over-parameterized, resulting in imprecise parameter estimates that caused the bootstrapped parameter value for a number of parameters to deviate more than 10% from the originally reported values. Plots of predicted concentrations *versus* observed concentrations revealed accurate individual concentration predictions, suggesting that morphine concentrations can be described accurately when at least one observation per individual is available, although high shrinkage values render the diagnostics based on individual predictions to be potentially misleading. Population concentration predictions were found to be biased. This suggests that model-based concentration predictions based on age, weight, and bilirubin concentrations of a child alone are inaccurate. Additionally, simulation-based diagnostic showed bias towards over-prediction of morphine concentrations in the population as a whole. The cause of this bias was diagnosed to originate from structural model misspecification, since plots of individual and population parameter estimates *versus* the primary covariate bodyweight revealed that the covariate relationships describe the maturational changes in model parameters with bias, which was reflected in MPEs for the predictions of total morphine clearance in the external dataset of 86% and -27% in term neonates and toddlers respectively. A claimed advantage of the application of fixed allometric scaling principles is that it allows for predictions outside the studied age-range, however clearance predictions in preterm neonates were found to have an MPE of 192% (Chapter 8), while clearance predictions in older children have never been assessed.

9.4.2.2. Anand *et al.* (2008)

The population pharmacokinetic model by Anand *et al.* [43] was based on morphine concentrations obtained from 875 preterm neonates as well as on the data from the 184 term neonates and infants from Van Dijk *et al.* [42] that were previously analyzed by Bouwmeester *et al.*. The pharmacologically active morphine metabolites were not included in this model.

The Anand-model continued to build on the concepts introduced in the publication by Bouwmeester *et al.* Fixed allometric scaling was augmented by a maturation model, in which the best fit was obtained with a sigmoidal model based on postmenstrual age, compared to an exponential model. Covariates based on organ function (i.e. serum bilirubin concentrations to reflect hepatic function) were not included,

but a scaling factor to adjust morphine clearance in preterm neonates in comparison to term neonates was included. There is some ambiguity on which parameter this fraction for preterm neonates is applied, as both the standard adult value of morphine clearance and the postmenstrual age at which half the allometric adult value of morphine clearance is reached, are mentioned. Most probably, the preterm factor was applied to the standard value of morphine clearance, indicating that morphine clearance in preterm neonates is 61% of the clearance in term neonates. This reduction remains constant throughout the full age-range described in the model. The equations below show how total paediatric morphine clearance is described by the Anand-model:

$$Cl_{tot,term} = 84.2 \cdot (\frac{BW_i}{70})^{0.75} \cdot (\frac{PMA^{3.92}}{PMA^{3.92} + 54.2^{3.92}}) \text{ or}$$

$$Cl_{tot,preterm} = 51.4 \cdot (\frac{BW_i}{70})^{0.75} \cdot (\frac{PMA^{3.92}}{PMA^{3.92} + 54.2^{3.92}})$$

In these equations, $Cl_{tot,term}$ and $Cl_{tot,preterm}$ represent total morphine clearance in l/h in term and preterm patients, respectively, BW_i is the bodyweight of the individual paediatric patient in kg, and *PMA* is the postmenstrual age in weeks. According to the maturation model, half the standard adult value of morphine clearance is reached at a postmenstrual age of 54 weeks. Around the postnatal age of one year, the influence of the maturation models becomes negligible, after which the increase in absolute clearance is described solely by bodyweight in the allometric equation.

In terms of model evaluation and validation procedures, diagnostics based on individual as well as population concentration predictions are reported, although due to their layout it is difficult to assess the accuracy of the predictions from these plots. No other results on model evaluation and validation were reported. The Anand-model was further evaluated by Mahmood using external data [44]. The MPE in total morphine clearance ranged between 8% in preterm neonates, 19% in term neonates, and 21% in toddlers between one week and two months of age, while the MPE was 1.5% in toddlers between two to ten months of age. The MPE in clearance predictions in children between the age of 3 to 5 years, which was older than the age-range in internal dataset, was 17%.

9.4.2.3. Knibbe *et al.* (2009)

The model by Knibbe *et al.* (Chapter 3) was also based on data from the 184 term neonates and infants of Van Dijk *et al.* [42]. Additionally data from Simons *et al.* on 64 preterm and term neonates [45] were added. Both morphine and its main metabolites were included in the model.

Model development of the Knibbe-model was based on a comprehensive covariate analysis. Bodyweight, bodyweight at birth, body surface area, sex, postnatal age, postmenstrual age, serum bilirubin concentration, creatinine clearance, mechanical ventilation, surgery *versus* non-surgery and type of surgery were investigated as potential covariates on clearance in equations of various forms. Differences in morphine glucuronidation were best described by a bodyweight-based exponential equation with an estimated exponent of 1.44. Within this equation the formation clearance of the morphine glucuronides was found to be reduced in neonates younger than ten days. This discontinuity did not result from stratification of the data, but from the observed differences in morphine clearance between young neonates and older patients after inclusion of the bodyweight-based covariate relationship. Compared to inclusion of age in a continuous relationship or to age cut-points at 3, 7, 14 or 21 days, inclusion of a discontinuity at the postnatal age of ten days provided the best mathematical description of this observed difference according to predefined statistical criteria. Physiologically a rapid but continuous change is however more probable. Clearance through pathways other than glucuronidation was found to be not significant and therefore not included in the model. The set of equations below shows how total morphine clearance is described by the Knibbe-model.

$$Cl_{tot} = Cl_{M3G} + Cl_{M6G}$$

$$Cl_{M3G(d<10)} = 3.48 * BW_i^{1.44} \quad \text{or} \quad Cl_{M3G(d>10)} = 8.62 * BW_i^{1.44}$$

$$Cl_{M6G(d<10)} = 0.426 * BW_i^{1.44} \quad \text{or} \quad Cl_{M6G(d>10)} = 0.67 * BW_i^{1.44}$$

In these equations Cl_{tot} is total morphine clearance in ml/min, Cl_{M3G} and Cl_{M6G} are the formation clearance of M3G and M6G in ml/min with different values for neonates younger than ten days and older patients, and BW_i is the bodyweight of the individual paediatric patient in kg.

The model was evaluated using various methods with the learning dataset and later also with external datasets (Chapter 4). With a condition number of 293 the model was found to be not over-parameterized, which resulted in precise parameter estimates causing the bootstrapped parameter values also to be within 10% of the originally reported value for all parameters. Plots of individual predicted morphine concentrations *versus* observed concentrations were minimally biased, although the value of diagnostics based on individual predictions is limited due to high shrinkage. Population predicted concentrations showed limited bias. Simulation-based diagnostics further confirmed that the model could accurately predict morphine concentrations based on bodyweight and age alone in children under the age of three years that, similar to the patients in the learning dataset had undergone major non-cardiac surgery or were mechanically

ventilated. Additionally, it was confirmed that covariate relationships describe individual parameter values accurately, with MPEs for total morphine clearance in the external datasets of 17% for preterm neonates and 30% for term neonates and toddlers (Chapter 4). The exponential increase in morphine clearance with bodyweight explicitly precludes this model from making clearance predictions in children older than three years.

Table II summarizes the details of the pharmacokinetic population models discussed above. For comparison, table III lists the absolute clearance values and clearance values per kg bodyweight for nine hypothetical patients, predicted by each of these three population models. The largest differences in predicted morphine clearance values between the models are observed at the extremes of the age-ranges of the models, with a difference of almost a factor 2 in the first month of life and around a 30% difference at the age of three years. Particularly large differences were found for preterm neonates aged 1 day to 2 weeks, and term neonates aged 2 weeks.

Table III. *Overview of morphine clearance predicted by the three population pharmacokinetic models for morphine in children with normal hepatic function.*

	Clearance prediction by the Bouwmeester-model [41]	**Clearance prediction by the Anand-model** [43]	**Clearance prediction by the Knibbe-model (Chapter 3)**
preterm neonate 1 day, 0.5 kg (GA 32 wk)	n.a.	2.37 ml/min 4.73 ml/min/kg	1.44 ml/min 2.88 ml/min/kg
preterm neonate 2 weeks, 1.0 kg (GA 34 wk)	n.a.	4.90 ml/min 4.90 ml/min/kg	9.29 ml/min 9.29 ml/min/kg
term neonate 1 day, 3.5 kg (GA 38 wk)	26.2 ml/min 7.47 ml/min/kg	29.5 ml/min 8.44 ml/min/kg	23.7 ml/min 6.78 ml/min/kg
term neonate 2 weeks, 4 kg (GA 40 wk)	39.5 ml/min 9.88 ml/min/kg	38.2 ml/min 9.56 ml/min/kg	68.4 ml/min 17.1 ml/min/kg
infant 3 monts, 6 kg	114 ml/min 19.0 ml/min/kg	98.0 ml/min 16.3 ml/min/kg	123 ml/min 20.4 ml/min/kg
infant 6 months, 7.5 kg	179 ml/min 23.9 ml/min/kg	173 ml/min 23.0 ml/min/kg	169 ml/min 22.5 ml/min/kg
infant 1 year, 10 kg	263 ml/min 26.3 ml/min/kg	287 ml/min 28.7 ml/min/kg	256 ml/min 25.6 ml/min/kg
infant 2 years, 13 kg	334 ml/min 25.7 ml/min/kg	388 ml/min 29.9 ml/min/kg	373 ml/min 28.7 ml/min/kg
infant 3 years, 17 kg	410 ml/min 24.1 ml/min/kg	482 ml/min 28.4 ml/min/kg	549 ml/min 32.3 ml/min/kg

GA = gestational age

Table II. *Details of the three paediatric population pharmacokinetic models on morphine.*

Model	**Bouwmeester *et al.*** **2004** [41]	**Anand *et al.*** **2008** [43]	**Knibbe *et al.*** **2009 (Chapter 3)**
Population and number of patients	- 184 term neonates to infants of three years [Van Dijk *et al.* [42]]	- 184 term neonates to infants of three years [Van Dijk *et al.* [42]] - 875 preterm neonates	- 184 term neonates to infants of three years [Van Dijk *et al.* [42]] - 64 preterm and term neonates [Simons *et al.* [45]]
Age in overall dataset	PNA: 195 (0 - 1070)[a] days	Preterm neonates: PNA: 0.27 (0 – 2.84)[a] weeks PMA: 27.4 (0.42 – 2.4)[a] weeks Term neonates and infants: PNA: 195 (0 - 1070)[a] days	PNA: 33 (0 – 203)[b] days PMA: 41.9 (35.6 – 62.6)[b] weeks
Bodyweight in overall dataset	5.9 (1.9 – 16.8)[a] kg	Preterm neonates: 1.04 (0.42 – 2.44)[a] kg Term neonates and infants: 5.9 (1.9 – 16.8)[a] kg	3.5 (2.2 – 7.0)[b]
Pharmacologically active metabolites	Included	Not included	Included
Covariate modeling	Fixed allometric scaling principles, including covariates *a priori*.	Fixed allometric scaling principles, including covariates *a priori*.	Comprehensive covariate analysis, inclusion of covariates based on statistical criteria.
Model	- Fixed allometric equation for size. - Age-based exponential equation for maturation. - Bilirubin serum concentrations for organ function.	- Fixed allometric equation for size. - Age-based sigmoidal equation for maturation. - Fraction of clearance values in preterm compared to term neonates.	- Bodyweight-based exponential equation with estimated exponent of 1.44. - Reduction of glucuronidation in neonates younger than 10 days.
Internal model evaluation and validation by authors	- Observed/individually predicted concentration *versus* time. - Individual parameter values and covariate relationships describing population parameter values *versus* included covariates.	- Observed *versus* individual predicted concentration. - Observed *versus* population predicted concentration. - Observed/individually predicted concentration *versus* time. - Observed/population predicted concentration *versus* time. - Individual parameter values and covariate relationships describing population parameter values *versus* included covariates.	- Observed *versus* population predicted concentration. - Observed *versus* individual predicted concentration. - Weighted residuals *versus* various covariates. - Simulation based diagnostics (NPDE) - Individual parameter values and covariate relationships describing population parameter values *versus* most predictive covariate.

External evaluation and validation by authors	None.	None.	In separate publication (Chapter 4): - 120 new patients, from preterm neonates to 1 year old infants [18,19,46,47]. - Refit using all data, and bootstrap. - Observed *versus* population predicted concentrations. - Simulation based diagnostics (NPDE). - Investigation of covariate relationships using subpopulations. - Also confirmed predictive value in neonates on ECMO treatment [48–50].
Model evaluation and validation by others than the authors of the original publication	Krekels *et al*. (Chapter 8) using internal data and data from 153 external patients [18,19,45–47], from preterm neonates to 1 year old infants. - Bootstrap using internal dataset. - Observed *versus* population predicted concentrations. - Simulation based diagnostics (NPDE). - Individual and population parameter values *versus* most predictive covariate. - Mean percentage error and root mean square error in clearance predictions. - Condition number (as measure for over-parameterization).	Mahmood et al. [44] using data from 147 external patients [10,12–15,17,18,51–53] from preterm neonates to 5 year old infants: - Percentage error, mean percentage error and root mean square error in clearance predictions. - Population predicted *versus* observed concentrations.	-
Conclusion of all evaluation and validation procedures	The model can accurately describe data based on at least one blood sample. In terms of predictions, the model shows an over-prediction of morphine concentrations, especially in the very young. The model is over-parameterized and parameter estimates are not precise.	The prediction error is large especially in the very young, but this reflects properties of the population not of the model. There is a small trend towards over-prediction for clearance especially in the first 3 months of life.	Model can describe and predict morphine and metabolite concentrations without bias. The parameter estimates are precise.

PNA = postnatal age, PMA = postmenstrual age, NPDE = normalized prediction distribution error, ECMO = extracorporeal membrane oxygenation, [a] = mean (range), [b] = median (inter-quartile range).

9.5 Clearance Estimates Obtained With Physiologically-Based Pharmacokinetic Modeling

9.5.1. Physiologically-Based Pharmacokinetic Modeling

In physiologically-based pharmacokinetic models, an exhaustive set of mathematical equations mechanistically describe and quantify the interaction between a drug molecule with specific physicochemical properties and the underlying physiological system. Additionally, interactions within the physiological system are described and quantified as well. These equations and the constants within these equations thereby aggregate compound-specific information with anatomical measurements and *in vitro* or *in vivo* physiological information. So while population modeling yields models for a specific drug in a specified population, physiologically-based models are more generalizable and non-specific for particular drugs.

Physiologically-based pharmacokinetic models require a wider variety of information compared to population modeling. Some of this information may be difficult to obtain, but since a substantial part of this information relates to underlying (patho)physiological processes, rather than to specific drugs, this information needs to be obtained only once. With the current gaps in our knowledge on human physiology and maturation, years of research are still required to properly describe and quantify all physiological parameters and interactions. However, the influence of some parameters or interactions on the overall drug pharmacokinetics may be negligible and with the major physiological determinants of pharmacokinetic processes currently being well described, physiologically-based models have already been proven useful to make inferences about the changes in pharmacokinetics of drugs that have not yet been studied in a particular population [54–56]. The additional research in this area is successively refining these models or extending their application to special populations.

The paediatric population can be included into this approach by integrating information on maturational changes in the physiological system into the model. Maturation of drug clearance is not defined for specific drugs, but for specific elimination routes, like glomerular filtration or biotransformation through various phase I and phase II enzymes. As morphine is mainly eliminated through hepatic glucuronidation by the UGT2B7 isoenzyme [1–3], information on ontogeny (i.e. expression and function) of this enzyme system is required, as well as maturational changes in liver size, hepatic blood flow and perfusion, plasma protein binding, and active hepatic transport mechanisms. As maturation profiles in physiologically-based pharmacokinetic models are established for all the underlying physiological changes, the developmental changes in pharmacokinetic parameters are described by a wide variety of mostly non-linear equations. This enables

the determination of pharmacokinetic parameters for drugs with specific properties in individuals for which certain key demographics (e.g. bodyweight and age) are known, which may be helpful in the development of first-in-child doses. However, the net maturation profile of pharmacokinetic parameters in a population as a whole cannot be directly derived. This complicates the establishment of evidence-based dosing guidelines from physiologically-based pharmacokinetic models.

5.2. Morphine Clearance Determined With Physiologically-Based Pharmacokinetic Modeling

9.5.2.1. Edginton et al. (2006)

The publication by Edginton *et al.* [56] is the only retrieved publication that compares overall *in vivo* morphine clearance predictions by a physiologically-based pharmacokinetic model to observed *in vivo* morphine clearances in the paediatric population. In the Edginton-model, hepatic UGT2B7 ontogeny profiles were derived from literature values of *in vivo* clearance as well as from *in vitro* determinations of enzyme activity for morphine and lorazepam. First, *in vitro* determinations of paediatric UGT2B7 enzyme activity were expressed as percentage of adult activity. This information was subsequently combined with maturational changes in the underlying physiological processes and *in vivo* adult morphine clearance values, to obtain model predicted *in vivo* paediatric clearance parameters. The *in vivo* maturation profile of morphine glucuronidation over the entire paediatric age-range was obtained by determining mean morphine clearance per kilogram of bodyweight at 17 distinct ages and generating a cubic spline of mean morphine clearance *versus* age. Available paediatric *in vivo* clearance values for morphine and lorazepam were used to further adjust the UGT2B7 ontogeny profile to provide the best visual fit of *in vivo* predicted drug clearances to the observed clearances. This yielded a bi-phasic maturation profile describing the net influence of underlying physiological changes on *in vivo* morphine clearance expressed per kilogram of bodyweight.

Optimization of the *in vivo* maturation profiles was based on visual improvement of how well the predicted profile described *in vivo* literature data, but this model fit was not numerically quantified. Age was selected as descriptor for the UGT2B7 ontogeny profile in the Edginton-model, but the ambiguity about how to quantify maturation in the first few days of life, especially comparing preterm and term neonates, could not be resolved. Therefore this model used one single clearance value for all premature neonates irrespective of postnatal or postmenstrual age. Additionally, since the maturation profile was not compared to individual clearance data, but to mean study values in stratified

age-ranges, the quality of model fit could not be assessed properly. In the manuscript, the predictive performances of the enzyme ontogeny models are tested on paediatric data from test compounds that are eliminated through multiple elimination pathways. However, this is not an ideal method to test the prediction of the clearance profiles of individual elimination routes. Alternatively, the UGT2B7 ontogeny profile was later used in a full physiologically-based pharmacokinetic model to assess the accuracy of morphine concentration predictions [55]. It was found that the predicted morphine concentrations were on average within a factor 2.06 from the observed value. However, in preterm neonates, a clear trend towards under-prediction of concentrations, and thus over-prediction of clearance, was observed.

9.6 Discussion

Morphine pharmacokinetics has been widely studied in the paediatric population, with a relatively large amount of this research being performed in children in the first few days to months of life. The majority of traditional pharmacokinetic studies in section 3 were performed in the younger age-ranges and the three population pharmacokinetic models in section 4 only included patients up to the age of three years. This is probably not only because most developmental changes occur in the early life-stages, but also because these very young patients are encountered most frequently in hospitals settings and paediatric intensive care units. Only the physiologically-based pharmacokinetic model in section 5 covers the entire paediatric age-range.

As can be seen in table I, there is a twenty-fold difference in the reported morphine clearances by traditional methods in neonates which narrows down to about a three-fold difference in older children and adolescents. The predicted clearance values by the three population models fall within the range of morphine clearance values obtained with the traditional methods. When the three population models are compared, the difference in morphine clearance predictions is most prominent in preterm neonates (which were not included in the Bouwmeester model) and in patients in the first few months of life, as illustrated in table III. The morphine clearance predictions by the Edginton-model are in the same range as the other studies as well, but an explicit relationship describing the developmental changes in morphine clearance in the paediatric population is lacking as the maturation of underlying physiologically processes instead of clearance are quantified.

The three paediatric population pharmacokinetic models for morphine discussed in section 4, were assessed for the accuracy of both their population concentration predictions and clearance predictions. The model by Knibbe *et al.* was the only model for

which accurate concentration predictions were confirmed as bias in individual as well as population concentration predicted *versus* observed concentration plots was found to be minimal. Especially the Bouwmeester-model proved to have poor population concentration predictions, while the population concentration predictions by the Anand-model were difficult to assess. With regards to MPEs of the population predictions of total morphine clearance, the error of the Bouwmeester model reached up to 85% (Chapter 8). For the Anand-model the MPE of total morphine clearance ranged between 8% and 21% [44], while for the Knibbe-model this ranged between 17% and 30% (Chapter 8). The MPEs reported for the Anand-model cannot be directly compared to the reported MPEs of the Bouwmeester-model and the Knibbe-model, as different external data were used as well as different age-ranges of the paediatric subsets and different methods to determine individual morphine clearances. These results however suggest the accuracy of total morphine clearance predictions by the Anand-model and the Knibbe-model to be in a similar range, despite the fact that the Anand-model was based on data from a larger number of preterm neonates than the Knibbe-model. This illustrates that model performance not only dependents on data density, but also on the quality of that data, showing that data should be obtained at time points that are informative for the various pharmacokinetic processes.

Concerning the physiologically-based model by Edginton *et al.*, the method to assess the model predictions was not quantitative and the visual tools were not optimal [56], making it difficult to assess the morphine clearance predictions by this model. However, morphine concentration predictions by this model were on average within a factor 2 from the observed value, which could be regarded acceptable for determining first-in-child doses or inter-drug scaling of new drugs in the paediatric population.

As biased clearance predictions can be harmful when used for paediatric dose adjustments or clinical decision making, we would like to emphasize that proper model evaluation and validation for all paediatric population pharmacokinetic models is of utmost importance. It should however be noted that most evaluation and validation procedure assess the accuracy of model predictions for a population as a whole. As mentioned by Mahmood [44], the inter-individual variability in paediatric morphine clearance is high, causing the prediction error in individual clearances to be high even with the most accurate population model. As a result, clinical monitoring is still important in paediatric patients on morphine treatment.

As illustrated in figure 1, population modeling and physiologically-based modeling approach the study of a drug's pharmacokinetics from opposite perspectives, and are therefore often referred to as the 'top-down' and 'bottom-up' approach, respectively. Inherent to these different perspectives is a difference in the nature of the data that are required for these models. Physiologically-based pharmacokinetic

models require a vast amount of data, which is generalizable but may not always prove to significantly influence net pharmacokinetic parameters, while population pharmacokinetic models only allow for the quantification of rate limiting processes that are not always generalizable and have to be repeated for every new drug studied in every new population. Future endeavors in paediatric pharmacology will therefore benefit from using the physiological insight and generalizability of physiologically-based models while restricting the focus to significant and rate limiting processes, as is done with population modeling. This will yield hybrid models that meet in-between the top-down and bottom-up approach and expedited paediatric model development (Chapter 6)[57].

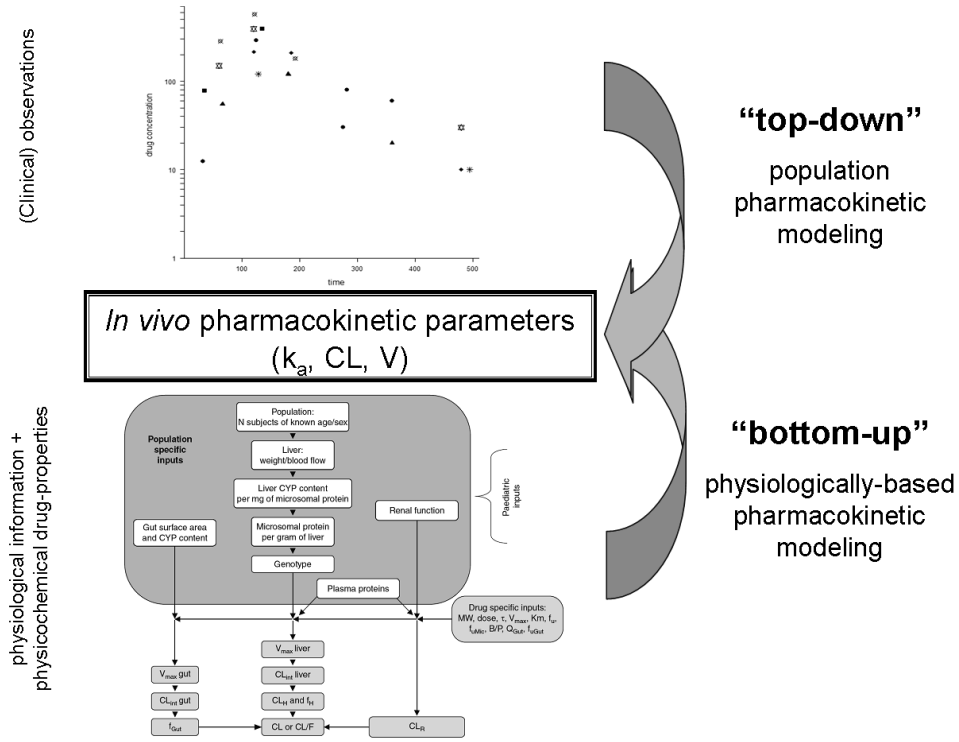

Figure 1. *Population pharmacokinetic modeling and physiologically-based pharmacokinetic modeling are often referred to as 'top-down' and 'bottom-up' approach respectively. Population modeling derives in vivo pharmacokinetic parameters from clinically observed drug concentrations, whereas physiologically-based modeling derives this information by aggregating physiochemical information of the drug with anatomical and physiological information of the biological system.*

One of the disadvantages of both population modeling and physiologically-based modeling is that they require specialized software and skilled professionals to design studies and perform the analyses. Additionally, with the mathematical equations that describe and quantify maturational changes in clearance values in the paediatric population becoming more complex, reported clearance values also become more difficult to interpret and compare. Particularly, the use of fixed allometric scaling principles in combination with age-based maturation functions [30] results in a combined function of two highly non-linear relationships for the maturation of morphine clearance in the Bouwmeester- and Anand-model. Since the analysis of data generated in population pharmacokinetic studies often yield complex covariate relationships, evidence-based dosing algorithms also grow increasingly complex. As dosing algorithms should be as simple as possible, but not simpler, special attention is required to implement these regimens in clinical practice. This may require a closer involvement of the hospital pharmacist in patient care to optimize and individualize drug dosing and to avoid dosing errors [58].

To date, most paediatric pharmacological research has focuses on drug pharmacokinetics. This is because clearance is generally believed to be the main driver of required dose adjustments in the paediatric population [59]. When pharmacokinetic models are used to derive evidence-based paediatric dosing algorithms, it is implicitly assumed that the pharmacodynamics remain constant. This assumption is acceptable when: 1) pathophysiological processes are similar in adults and children, 2) the exposure-effect relationship can be assumed independent of age based on the mechanism of action, and 3) the clinical endpoints for treatment are the same in both populations [57]. Morphine does not meet these criteria as the expression of the mu-opioid receptor may differ between age groups, and as the clinical endpoints for pain differ in adults and children. This implies that morphine pharmacodynamics needs to be studied as well, to establish age appropriate target concentrations. Future paediatric pharmacodynamic studies are therefore necessary to derive final dosing algorithms in this population that account for both pharmacokinetic and pharmacodynamic changes.

9.7 Conclusion

Traditional compartmental and non-compartmental analysis approaches, population modeling and physiologically-based pharmacokinetic modeling have been applied to study morphine clearance and the maturational changes in this parameter in the paediatric population. This has lead to a variety of reported values for paediatric morphine clearance and functions for the maturation profiles of this parameter. However, absolute predicted clearance values obtained with the different methods seem to be in good agreement, except in preterm and term born neonates and infants in the first three months of life. The predictive value of models is determined by accurate clearance predictions (quantified by MPE values) and concentration predictions (assessed in population predicted *versus* observed plots). The Knibbe-model was the only model for which accurate concentration predictions on the individual as well as population level were corroborated throughout the full age-range of the model and for both morphine and its metabolites. With regards to the prediction of total morphine clearance the Anand-model and Knibbe-model have similar accuracies, although the Anand-model did not include the pharmacologically-active metabolites.

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Section V

Summary, Conclusions, and Perspectives

Chapter 10

Drug Glucuronidation in Children – Summary, Conclusions and Perspectives

It is common practice for paediatricians to prescribe drug doses per kilogram bodyweight, while scientific evidence supporting these dose prescriptions are often lacking for the majority of drugs used in children. As a result the number of paediatric patients receiving at least one off-label or unlicensed drug ranges between 80% to 93% in neonatal intensive care units, between 36% to 100% in paediatric wards, and between 3.3% to 56% in non-hospital settings [1]. There are many factors that may contribute to age-related changes in drug pharmacokinetics or drug pharmacodynamics [2,3]. It is imperative that these changes are taken into account when designing drug dosing algorithms for children, because although for most drugs clinical experience will in time lead to consensus-based dosing guidelines that reduce fatalities, suboptimal dosing algorithms may lead to unnecessary therapy failure or to adverse drug reactions resulting from overdosing. Recently, the need for optimizing paediatric drug therapy has been recognized by regulatory agencies as well. By introducing new legislation to encourage or compel paediatric pharmacological studies (e.g. Pediatric Rule (FDA – 1998), the Best Pharmaceuticals for Children Act (FDA – 2002), and the Paediatric Regulation (EMEA – 2007)), these agencies are now key players in the process of making paediatric drug dosing safer.

The research in the current thesis focuses on a novel model-based approach to develop drug dosing algorithms for the paediatric population by accounting for developmental changes in pharmacokinetics. Pharmacokinetic changes are believed to be a major cause of age-dependent differences in paediatric dose requirements [4], although in daily clinical practice not many clinicians are aware of this, as they are more concerned with pharmacodynamic endpoints. Specific focus in this thesis was on uridine 5'-diphosphate glucuronosyltransferase (UGT) 2B7-mediated drug glucuronidation.

Studies on developmental changes in pharmacokinetics often start in the adult population. Subsequently age-related changes in clearance are studied in different paediatric subpopulations with successively decreasing age-ranges, often with a limited number of patients per subpopulation. Alternatively, the research in this thesis started in children under the age of three years, with the inclusion of preterm and term neonates. This approach was chosen because the treatment of patients from this young population presents a major challenge. Specifically, these young children may require drug treatment, for instance after major surgery to correct major congenital anomalies, while numerous and profound physiological changes take place of which the influence on drug pharmacology is still largely unknown. Morphine and zidovudine are regularly prescribed for children and are both predominantly eliminated through UGT2B7-mediated glucuronidation [5–7], these two compounds were therefore used as probes in the development of a population modeling approach and a semi-physiological modeling approach, used for deriving paediatric dosing algorithms.

This thesis started by discussing methods to study ontogeny of liver enzyme systems and reviewing results reported for the UGT enzymes in **Section I**. Subsequently, in **Section II** the developmental changes in morphine glucuronidation in preterm and term neonates up to three-year-old children were quantified using a population modeling approach. After corroborating the descriptive and predictive properties of this population model in internal and external validation procedures, the morphine dosing algorithm derived from this model was prospectively evaluated in terms of analgesic efficacy in a clinical trial. In **Section III**, a novel approach to develop paediatric population models used for optimized dosing regimens was explored using zidovudine. This novel approach is based on the concept that the pattern of developmental changes in clearance can be considered a system-specific property, which can be extrapolated from one drug to another, provided that these drugs share a common elimination pathway. Application of this concept may lead to a reduction of time and resources needed for paediatric model development. So far, this semi-physiological approach has only been applied to compounds with similar physicochemical properties. As a first step towards a more universal modeling concept, the utility of semi-physiological pharmacokinetic models towards the prediction of developmental changes in clearance was investigated using a physiologically-based pharmacokinetic model. Finally, **Section IV** focused on the evaluation and validation of paediatric population models. This is important since with sparse paediatric datasets there is an increased risk of drawing erroneous conclusions which may have far-reaching consequences. Commonly used diagnostic tools were found to not always suffice for the evaluation of paediatric models, due to specific patient and study characteristics in this population. Therefore, a framework was developed for the systematic evaluation of paediatric population models that takes these characteristics into account. Additionally, the level of scientific evidence supporting the predictive value of different published paediatric pharmacokinetic models for morphine was evaluated.

10.1 Paediatric Morphine Glucuronidation Model for Individualized Dosing

Clinically relevant inter-individual variability in glucuronidation clearance forms the basis of paediatric dose adjustments of UGT2B7 substrates. Therefore *in vivo* clearance of UGT2B7 substrates was selected as an endpoint in the studies of the current thesis. Such research is however complicated by a unique set of challenges with regards to ethical, legal, and practical constraints.

Since children cannot consent, it is unethical to perform studies in healthy children. Therefore paediatric studies are typically performed in patients that require medical treatment, which may increase the heterogeneity of the patient population due to differences in the nature and the severity of illness, while the polypharmacy in most of these patients may introduce drug-drug interactions. Practical constraints lay in the small blood volume of the paediatric patients especially in the very young, limiting the volume and number of blood samples that can be obtained from a single patient. Additionally, without an indwelling arterial line, arterial blood sampling for the sole purpose of pharmacokinetic studies is not allowed. This implies that blood sampling can often not adhere to a stringent design. Likewise drug dosing can often not adhere to a stringent design, as drug dosing may be clinically titrated to individual medical needs, which is guided by both efficacy and possible side effects.

In neonates, practical constraints with regards to limitations in obtainable blood volume can be overcome by advanced and sensitive analysis methods like LC-MS or electrophoresis that can detect drug concentrations in low-volume samples [8,9]. Ethical issues with regards to invasive arterial blood sampling for pharmacokinetic measurements can be overcome by using scavenged samples (i.e. samples discarded from clinical specimens) [10], by developing methods to determine drug concentrations from dried blood spots [11], or by investigating the use of other biological matrices like for instance saliva [8]. In **Section II**, the issues with regards to the analysis of data from a heterogeneous population, with sparse observations per patient and irregular dosing and sampling times were overcome by using a population modeling approach. This approach not only allows for the analysis of sparse, dense and/or unbalanced data, it also allows for the identification and quantification of the sources of variability in a population, and more importantly for the identification of significant predictors of this variability, known as covariates [12]. As was shown for morphine in this thesis, covariate relationships describing the relationship between a predictor of variability (e.g. bodyweight) and a pharmacokinetic parameter like clearance can directly serve as the basis of drug dosing algorithms.

In **Chapter 3** a population pharmacokinetic analysis was performed based on concentration-time data on morphine, morphine-3-glucuronide (M3G), and morphine-6-glucuronide (M6G) obtained from 248 patients with sparse sampling per patients (on average 4 samples per patient). These samples were obtained from postoperative or mechanically ventilated preterm or term neonates to children up to the age of three years. The analysis revealed that in this young population the developmental changes in the pharmacokinetics of morphine are best described by bodyweight-based covariate relationships. Increases in formation and elimination clearances of the morphine metabolites could be best quantified by a bodyweight-based exponential equation with

an exponent of 1.44. Within this exponential equation the formation of the metabolites was found to be reduced by approximately 50% in neonates with a postnatal age of less than ten days, which was independent from gestational age. The elimination of morphine through other pathways was found to be not statistically significantly different from zero throughout the entire population. The distribution volume of morphine and its metabolites increased linearly with bodyweight. From these covariate relationships it was derived that for children under the age of three years morphine IV maintenance doses are best dosed on the basis of $\mu g/kg^{1.5}/h$ with a 50% dose reduction in neonates younger than ten days, while IV loading doses and bolus doses are best dosed linearly with bodyweight. Model-based simulations indeed confirmed that similar morphine and metabolite concentrations would be obtained throughout this entire population with this dosing algorithm.

Although the novel model-derived dosing algorithm for morphine covers a relatively large age-range in early life, in which many physiological changes occur, like many other paediatric drug dosing guidelines it is limited to a specific age-range, in this case to preterm and term neonates up to three-year-old children. Due to the exponential increase at the higher extreme of the bodyweight-range, this algorithm cannot be used for extrapolations to older children. It would therefore be interesting to expand the morphine dataset to older paediatric patients up to adults, enabling quantification of the developmental changes in glucuronidation clearance over the entire paediatric age-range. Recently, a bodyweight-dependent exponential covariate model was developed to scale propofol clearance from preterm neonates to adult patients with a single continuous covariate relationship [13]. This bodyweight-dependent exponential covariate model consists of a bodyweight-based allometric equation in which the exponent decreases in a sigmoidal fashion with bodyweight. Interestingly, the developmental changes in propofol clearance in early life were found to be best described with an exponent close to 1.44, namely 1.35.The flexible nature of the bodyweight-dependent exponential covariate model, makes the model potentially useful for different elimination pathways like the UGT2B7-mediated glucuronidation of morphine.

A single continuous function to describe developmental changes in drug glucuronidation would also yield a single dosing algorithm for the entire paediatric population. This would resolve issues concerning drug dosing in patients with characteristics close to the extremes of the range for which a specific dosing algorithm is defined and dosing errors arising from using an incorrect algorithm for a particular patient. For instance, when there are two different algorithms for patients younger than three years and for patients of three years and older, two different algorithms may be used for dosing in children around their third birthday, each leading to a different dose. Additionally, a treating physician may erroneously calculate the dose for a two-year-

old patient using the algorithm for children of three years and older or *vice versa*. In daily practice, dosing errors from miscalculations of highly non-linear algorithms for a specific patient at the bedside, may be resolved by a close involvement of the hospital pharmacist in clinical practice [14] and the use of dosing tables (Chapter 5) or by the development of applications for smartphones or tablets [15,16] and the introduction of electronic prescription systems.

In Chapter 3, the descriptive and predictive properties of the developed paediatric morphine pharmacokinetic model were evaluated in an internal validation procedure, using the data that were used to develop the model. To further corroborate the predictive value of this model, extensive evaluation and validation procedures were performed in **Chapter 4**, using independent external data from preterm and term neonates up to infants of one year, from four different centers. In these procedures, the model was found to accurately predict morphine and metabolite concentrations based on dose, bodyweight, and age alone, in a population that, like the internal dataset, consisted of ventilated neonates and postoperative patients after major non-cardiac surgery.

The paediatric pharmacokinetic morphine model developed in Chapter 3 and dosing guidelines derived from this model should not be applied to other patient population until the accuracy of model predictions have been established in this population. Literature reports for instance suggest paediatric morphine pharmacokinetics to be affected by cardiac surgery [17,18]. These effects should therefore be further analyzed and quantified in a population analysis. Similarly, critical illness was found to severely reduce the CYP3A4-mediated clearance of midazolam [19], since it has not been established whether and how critical illness affects UGT-mediated drug clearance, caution is warranted when applying the paediatric morphine pharmacokinetics model and the model-derived dosing algorithm for this patient population. In Chapter 4, the model was shown to make accurate concentration predictions in neonates on extracorporeal membrane oxygenation (ECMO) treatment with continuous venovenous haemofiltration (CVVH). This is remarkable as the very invasive ECMO treatment had been shown to influence pharmacokinetic parameters of various drugs [20–23]. It is unlikely that ECMO does not influence pharmacokinetic parameters for morphine, in fact pharmacokinetic changes have been reported to occur for morphine in patients on ECMO treatment [24,25], but our results suggest that the various changes may have counteracting influences on the morphine and metabolite concentrations. This would imply that the model-derived dosing algorithm for morphine may also be advantageous for patients on ECMO treatment, which is being prospectively studied [26].

With the predictive performance of the paediatric morphine pharmacokinetic model confirmed for postoperative patients, analgesic efficacy of the model-derived

morphine dosing algorithm was prospectively evaluated in postoperative patients in **Chapter 5**. According to this algorithm neonates younger than ten days received a morphine IV infusion of 2.5 $\mu g/kg^{1.5}/h$, while older patients received 5 $\mu g/kg^{1.5}/h$. Compared to a traditional dosing regimen of 10 $\mu g/kg/h$, the model-derived algorithm resulted in a 50% to 75% dose reduction in neonates younger than ten days, while children older than ten days and bodyweights of more than four kilograms received up to 150% of the traditional morphine dose. Based on the percentage of patients needing rescue medication, the reduced dose in young neonates proved to be efficacious, whereas in older children a relatively high need for rescue medication remained despite the increased dose for these patients. In both groups, for the patients that did require rescue medication the morphine rescue dose per kilogram bodyweight did however not significantly differ statistically. As the model-derived dosing algorithm corrects for age-related differences in morphine pharmacokinetics, yielding steady state plasma concentrations of about 10 ng/ml in all patients, the observed age-related differences in the efficacy of the morphine regimen are likely the result of age-related differences in the distribution of morphine to its effect site or differences in the morphine pharmacodynamics. It can therefore be concluded, that adjusting the morphine dose to differences in the pharmacokinetics leads to significant improvements in paediatric morphine therapy as it reduces the risk of over-exposing young neonates and exposing older children to inefficacious doses. Future studies on the pharmacodynamics of morphine and its pharmacologically active metabolites in this patient population may further improve the dosing algorithm, by defining age-appropriate target concentrations.

Morphine and its metabolites exert their pharmacological effect by binding to μ-opioid receptors in the brain. To reach this effect site the compounds need to pass the blood-brain barrier and diffuse through the brain tissue. Age-related changes in the functionality of the blood-brain barrier may cause age-related differences in effect site distribution, which may clinically manifest in differences in morphine efficacy. It has been demonstrated that multiple processes are involved in the transport of morphine across the blood-brain barrier. In addition to passive diffusion, this includes carrier mediated and active transport mechanisms. In a number of studies it has been shown that the passive diffusion of morphine across the blood-brain barrier is slow and that the capacity for active uptake is limited, while active efflux of morphine from the brain via P-glycoprotein is considerable [27]. As a result, the morphine concentrations in plasma and at the site of action are at best in dynamic equilibrium, which complicates the interpretation of plasma concentrations in terms of active therapeutic concentrations. In the developing brain the various barrier and transport functions mature at different rates [28], therefore it is not possible to describe a single maturation pattern for blood-brain barrier penetration of drugs in paediatric patients, but it is conceivable that developmental changes in

blood-brain barrier distribution cause the age-related differences in the morphine exposure-effect relationship observed in Chapter 5. It is in this respect important to note that blood-brain barrier functionality may also be compromised in patients with certain medical conditions like meningitis [29] or epilepsy / seizures [30]. On the other hand, the age-related differences in the response to morphine could be the result of differences in the sensitivity of the developing biological system to morphine and its metabolites or differences in the sensitivity of the developing physiological system to pain stimuli. This could be caused by changes in the organization of the central nervous system and the expression and differentiation of opioid receptors in the brain, or by changes in downstream signal transduction resulting from opioid receptor activation and the integration of this signaling pathway with the pathways that process pain [31].

Pharmacodynamic studies on analgesics in children are associated with several methodological complexities. An important factor is that these studies require an age-appropriate endpoint to quantify pain. Measurements of pain and analgesia are complicated by the subjective nature of pain perception, therefore the gold standard for quantifying pain is self-report by means of a visual analogue scale (VAS) or numeric rating scale (NRS). In children between the age of four and twelve years a "faces pain scale" may be used [32,33], but in preverbal-verbal children under the age of three to four years self-report is not possible. The COMFORT-behaviour scale has been developed to assess postoperative pain in preverbal children up to the age of three years and this score has been extensively validated for this purpose [34] ,which showed that the pain intensity quantified the by behavioral items in COMFORT score are in agreement with pain intensity quantified from physiological measurements [35]. In addition to the COMFORT-behaviour scale, a wide range of other pain scoring instruments have been developed for preverbal patients. Most of these pain assessment scales rely on behavioral items, with facial expression, crying, and body movement included in most instruments [33,36–38]. So far no methods have been developed for the population modeling of a single endpoint (e.g. pain) using different quantification methods for that endpoint (e.g. different pain rating scales). This impedes the retrospective analysis of data from multiple centers that use different pain scores. To analyze developmental changes in the pharmacodynamics of analgesic drugs across the paediatric population, a validated biomarker that objectively quantifies pain in patients of all ages, is necessary. Potential biomarkers include cardiovascular, hormonal, or other physiological parameters, but hitherto no biomarker has been identified that can quantify pain, discriminate between pain and stress, anxiety or discomfort, and has an acceptable inter-individual variability [39–42]. As an alternative, the use of pain scoring instruments for the different paediatric subpopulations that all have the same scale (e.g. a range from 0 to 10) could be explored.

Finally, medicine strives to optimize the balance between minimal drug exposure

and maximum efficacy, to yield an optimal risk/benefit ratio. Neonates, especially premature neonates, can experience pain which may have both short-term and long-term effects that could potentially be reversed by morphine [43–46]. Morphine exposure is however also associated with acute respiratory, gastro-intestinal, cardiovascular, and neurological side effects as well as the occurrence of tolerance, dependence and withdrawal. Additionally, animal and *in vitro* studies raised concerns about the long-term effects of exposure of the developing brain to morphine [46–48], which includes enhanced neuronal apoptosis [49–51]. Long-term follow-up studies however suggest the influence of any potential changes in the central nervous system of human neonates on behavior and performance to be small to negligible [52–54]. Nevertheless, studies are being performed to evaluate the efficacy of paracetamol or, in infants and children older than three months, non-steroidal anti-inflammatory drugs (NSAIDs) as alternative or adjuvant analgesic agents to opioids [55–58]. In addition to this, non-pharmacological interventions may also reduce (procedural) pain, distress, and opioid consumption in various clinical settings and should always be applied before pharmacological interventions. This may include none-nutritive sucking or administration of non-nutritive glucose, sucrose or other sweet tasting solutions, kangaroo care, holding, or parental presence, auditory or olfactory stimulation, swaddling or facilitated tucking, and reducing environmental stimuli [59–62], although except for non-nutritive sucrose administration the evidence supporting these non-pharmacological interventions is limited.

Conclusions and Recommendations:

- The maturation of morphine glucuronidation in the first three years of life is best described by a bodyweight-based exponential equation with an exponent of 1.44 and a 50% reduction in preterm neonates younger than ten days, which is independent from gestational age.
- A paediatric dosing algorithm that corrects for differences in morphine pharmacokinetics reduces the risk of over-exposing young neonates while also reducing suboptimal morphine exposure in older patients.
- Extension of the current morphine pharmacokinetic study to include older children up to adults may yield a single continuous dosing algorithm that covers the entire paediatric age-range.
- Studies on morphine pharmacodynamics may further improve the dosing algorithm. A large number of validated pain assessment instruments in specific paediatric age-ranges are available for these studies, however uniform endpoints that allow for the quantification of pain across the entire paediatric age-range are still lacking.

- Alternative pharmacological and non-pharmacological methods to effectively treat pain while reducing opioid consumption should be considered and investigated to optimize the risk/benefit ratio of paediatric pain management.

10.2 Semi-Physiological Covariate Model for Paediatric Glucuronidation

The approach used in section II to develop validated model-derived dosing algorithms that correct for developmental changes in the pharmacokinetics for each drug in every paediatric age-group, would require much time and resources. It was therefore explored to what extent the covariate relationship for UGT2B7-mediated glucuronidation of morphine could be regarded as descriptor of developmental changes in the underlying physiological system rather than as a specific descriptor of changes in the pharmacokinetics of morphine *per se*, and how this information could be used in population model development of other drugs.

In **Chapter 6** the paediatric covariate model for morphine glucuronidation was directly incorporated into a population pharmacokinetic model for zidovudine, as both drugs are predominantly eliminated through the same pathway of UGT2B7-mediated glucuronidation. This yielded a model with descriptive and predictive properties that were similar to a reference model that was based on a comprehensive covariate analysis of the same dataset and that provided the statistically best description of the data according to predefined criteria. The proposed modeling approach in which paediatric covariate models are extrapolated between drugs that share a common elimination pathway combines population modeling with the mechanistic insight of physiologically-based modeling. This approach was therefore called a semi-physiological modeling approach and the covariate model for UGT2B7-mediated glucuronidation clearance that was extrapolated from morphine to zidovudine was called a semi-physiological developmental glucuronidation model.

The findings in Chapter 6 support our hypothesis that paediatric covariate models describe system-specific rather than drug-specific properties. This would imply that the context of system-specific properties [63] can be extended to not only include static descriptors of the physiological system, but to also include age-related developmental changes in the physiological system in the paediatric population. This would mean that the developmental profile of clearance of a new drug in children can be predicted based on findings on a probe compound that is eliminated through the same pathway. The generalizability of the semi-physiological modeling approach does

however require further investigation. This approach has now been successfully applied to the glucuronidation of morphine and zidovudine in the current thesis as well as to the glomerular filtration of vancomycin and netilmicin [64], thereby supporting this approach for two drug eliminating pathways using compounds with similar physicochemical properties and extraction ratios. It should be further investigated how well this approach performs when extrapolating covariate models between drugs that have very different physicochemical properties and/or extraction ratios. Additionally, the applicability of this approach should be tested on drugs with non-linear pharmacokinetics or drugs that are eliminated through multiple pathways.

The study described in **Chapter 7** explored the physiological and physicochemical basis of the semi-physiological developmental glucuronidation model from Chapter 6. This was done to investigate whether the applicability of the semi-physiological developmental glucuronidation model was limited by specific patient or drug characteristics. For this, the influence of system-specific and drug-specific parameters on *in vivo* drug glucuronidation was quantified using physiologically-based modeling. The study illustrated that developmental changes in liver volume and UGT2B7 ontogeny, rather than the changes in milligram microsomal protein per gram of liver, hepatic blood flow or plasma protein binding, are the main drivers of the clinically observed developmental increases in drug glucuronidation in the first three years of life. This implies that the pharmacokinetics of drugs that are eliminated through UGT2B7-mediated glucuronidation may be affected in patients with hepatic dysfunction as a result of for instance virus associated hepatic disease.

Bodyweight, which was identified as the main covariate for the developmental changes in morphine clearance in the population model developed in Chapter 3, should be regarded as a surrogate descriptor of the sum of all underlying changes in physiology instead of being regarded as the driver of the observed changes in glucuronidation clearance in mechanistic terms. In children under the age of three years, bodyweight is closely correlated with age. The evaluation procedure in Chapter 4 in premature neonates and neonates small for gestational age, proved bodyweight to be a better predictor of inter-individual variability of morphine glucuronidation than age in these patients. It remains to be investigated whether bodyweight is also the best descriptor of this inter-individual variability in toddlers at the extremes of their age-appropriate weight-range (i.e. underweight / malnourished or overweight / obese toddlers).

The study in **Chapter 7** also illustrated that, provided that changes in drug-specific parameters do not influence the uptake of the drug into hepatocytes or the interaction of drug molecules with the UGT2B7 isoenzyme, physicochemical drug parameters only influence the absolute value of the drug glucuronidation clearance and

not the pattern of developmental changes in glucuronidation clearance. This suggests that the semi-physiological modeling approach can be applied to all small molecular substrates of the UGT2B7 isoenzyme.

Using currently available *in vitro* data on UGT2B7 enzyme kinetics, the physiologically-based model used in Chapter 7 yielded under-predictions for *in vivo* morphine and zidovudine clearances. This discrepancy may result from inaccuracies in the values of the *in vitro* enzyme kinetic parameters obtained from literature. Another factor may be the applied assumption that morphine and zidovudine are solely eliminated through UGT2B7-mediated clearance. The developmental profile of *in vivo* drug glucuronidation on the other hand was well predicted by the physiologically-based model in Chapter 7 in infants and toddlers older than approximately two weeks. However, the predictions of developmental changes in glucuronidation clearance in term neonates in the first two weeks of life were less accurate. This could be explained by the fact that the UGT2B7 ontogeny profile in the physiologically-based model used in Chapter 7 increased linearly throughout childhood, while literature reports on *in vitro* studies showed a rapid increase in UGT2B7 expression and function in the first few weeks in life, which is also reflected in the clinical observations. Accurate prediction of clearance in the first few days of life has proven to be difficult with physiologically-based models in general and predictions for preterm neonates are often not even possible with these models. For this purpose, prenatal and postnatal maturation processes in the various physiological parameters need better quantification. To improve the availability of liver and kidney samples for this purpose, it would be beneficial to always snap-freeze liver and kidney samples of diseased paediatric patients and obtain informed consent for such studies.

Physiologically-based models largely depend on reliable *in vitro* enzyme kinetic parameters. As reviewed in **Chapter 2**, *in vitro* methods to study ontogeny patterns of UGT isoenzymes use various endpoints that represent different parts of the physiological system. A number of factors contribute to differences in findings between studies on hepatic UGT ontogeny:

1) Inter-individual variability in UGT enzyme expression and activity is high and influenced by numerous (patho)physiological [65,66] and experimental [65–69] conditions, while the limited availability of paediatric hepatic donor tissue prevents the accurate characterization of developmental changes and associated variability.
2) The diversity of endpoints that can be studied may affect the conclusions on hepatic enzyme ontogeny. For instance, mRNA and protein expression for many UGT isoenzymes did not correlate well with the *in vitro* activity of the isoenzymes [70], possibly because post-translational modifications and interactions with other microsomal membrane bound components influence UGT activity [71–75].

3) Measured enzyme activity is never absolute nor generalizable, because the rates of biotransformation are non-linearly dependent on substrate concentrations and specific for a given enzyme-substrate combination. Additionally, substrate specificities of the UGT isoenzymes are broad and they may overlap, meaning that one isoform may glucuronidate a wide range of compounds and that one compound may be metabolized by multiple isoforms, limiting the availability of suitable isoenzyme specific substrates to investigate developmental changes in the activity of a single UGT isoenzyme.

With respect to the UGT2B7 isoenzyme, as reviewed in **Chapter 2**, mRNA expression is undetectable in fetuses at a gestational age of 20 weeks, while UGT2B7 mRNA expression was found to have reached adult values at a postnatal age of six months [70]. For UGT2B7 enzyme expression on the other hand, adult values were reached at a postnatal age between seven months and two years according to one study [70], or between the ages of 12 and 17 years according to another study [76]. Absolute *in vitro* activity of the UGT2B7 isoenzyme as measured on the basis of the rate of biotransformation of epirubicin, showed a small age-dependent increase throughout the total paediatric age-range, with activity levels being lower than adult values in all age-ranges [76].

Due to the variability in study outcomes, incorporation of *in vitro* data on enzyme kinetics into physiologically-based models is not straightforward. Studies to obtain enzyme kinetic parameters can be based on microsomes or hepatocytes, with hepatocytes being the preferred experimental system. This is because the current protocols for studies in microsomes are optimized to study the activity of cytochrome P450 (CYP) enzymes rather than UGT activities [65]. Additionally, in hepatocytes the structural integrity, including drug-binding cell compartments, cell membranes and transporters, is still intact and co-factors are present at physiological concentrations thereby improving determination of physiologically relevant *in vivo* enzyme kinetic values.

The proposed semi-physiological modeling approach in Chapter 6 combines concepts of physiologically-based pharmacokinetic modeling with population pharmacokinetic modeling. Future endeavors to predict developmental changes in drug metabolism could greatly benefit from a closer integration of these two approaches. Population pharmacokinetic modeling offers the advantage of obtaining the best possible description of the developmental changes in clearance based on outcome measures. As such, it allows for the quantification of net effects of all underlying physiological changes that contribute to the observed changes in clearance. This is usually expressed with a limited number of covariate relationships that can be directly used as the basis for dosing algorithms. Physiologically-based pharmacokinetic modeling on the other hand

provides a detailed and generalizable description and quantification of the functioning of the physiological system and the interaction of drug molecules with this system. This can be of great benefit to make predictions for new drugs or drugs in new populations. Some of the parameters in physiologically-based models can be obtained with great accuracy from *in vitro* studies or physiological measurements, while others are more difficult to obtain experimentally. The combination of population modeling and physiologically-based modeling may allow for the quantification and characterization of developmental changes of a limited number of parameters in a physiologically-based model, based on outcome measures.

Conclusions and Recommendations:

- Proof-of-concept studies with a semi-physiological modeling approach for UGT2B7-mediated paediatric drug glucuronidation support the hypothesis that paediatric covariate models describe developmental changes in the physiological system and can be extrapolated between drugs that share a common elimination pathway.
- By applying physiologically-based pharmacokinetic modeling concepts, liver volume and UGT2B7 ontogeny were identified as the main drivers of developmental changes in *in vivo* UGT2B7-mediated drug glucuronidation in children younger than three years.
- Bodyweight to the power 1.44 should be regarded a surrogate descriptor of the sum of all developmental changes in the underlying physiological system on drug glucuronidation in children. The descriptive value of this covariate in toddlers at the extremes of their age-appropriate bodyweight range needs further investigation.
- Physicochemical drug properties only influence the absolute value of *in vivo* glucuronidation clearance, not the maturation pattern. Therefore, the semi-physiological modeling approach can be used to predict the developmental changes in the clearance of other UGT2B7 substrates.
- The proposed semi-physiological paediatric pharmacokinetic modeling approach is promising. Future studies to investigate to what extent this approach is universally applicable should focus on other elimination pathways, on extrapolations between drugs with different physicochemical drug properties, and on drugs with non-linear or blood-flow dependent kinetics.

10.3 Paediatric Model Evaluation

It has been recognized before that population models are often not adequately evaluated and validated, both for the adult and paediatric population [77,78]. Model misspecification could have far-reaching consequences when pharmacokinetic models are used as the basis for dosing algorithms in children. When paediatric covariate models are extrapolated to other compounds in a semi-physiological modeling approach these consequences are even perpetuated. During the evaluation and validation of the morphine pharmacokinetic model in Chapter 3 and Chapter 4 it was discovered that, due to some unique properties of paediatric datasets, the evaluation and validation of paediatric models requires special attention.

Chapter 8 identified how paediatric patient and study characteristics influence the structure of paediatric datasets. It was also shown how to deal with complexities arising from these data characteristics in the evaluation and validation of paediatric population models. Specific features of paediatric datasets are: the heterogeneity in developmental status of the patients, the high variation in drug dosing and blood sampling, and sparse and unbalanced sampling per patient. These features require validation procedures to be performed on age-stratified subsets of the data, the use of advanced validation methodologies and focus on population predictions rather than individual predictions. The framework for the validation of pediatric population pharmacokinetic models proposed in Chapter 8 takes these requirements into account. In addition a novel validation tool to investigate the accuracy of paediatric covariate relationship across the entire range in covariate values was also introduced. This framework is based on six diagnostics, which include 1) number of parameters and condition number, 2) numerical diagnostics, 3) prediction-based diagnostics, 4) η-shrinkage, 5) advanced simulation-based diagnostics, 6) diagnostics of individual and population parameter estimates *versus* covariates. These diagnostics *per se* were not necessarily new, but were sometimes slightly adjusted (e.g. stratified) or required a shift in emphasis (e.g. focus on population predictions instead of individual predictions). The validation framework was applied to two paediatric population models for morphine in children younger than three years, which were based on the same dataset. This revealed that the proposed paediatric model validation framework can identify model over-parameterization, model instability and structural model misspecification leading to poor predictive model performance.

Model misspecification may lead to serious consequences when conclusions based on these models are used for clinical decision making. As peer-reviewed publications reach a large audience and may be considered to be accurate by this audience, it is imperative that paediatric pharmacokinetic models are not accepted for

publication without confirming their results with proper model evaluation and validation procedures as described in Chapter 8.

Morphine pharmacokinetics has been widely studied in the paediatric population. The reported morphine clearance values in children are reviewed in **Chapter 9** with specific focus on model-based results. Since the results of Chapter 8 raised questions with regards to the accuracy of published pharmacokinetic models, special attention was paid to the evidence supporting the accuracy and precision of the pharmacokinetic predictions obtained with these models. Three population models in children younger than three years used different clearance parameterizations and expressions for the quantification of developmental changes in clearance. This mainly led to differences in clearance predictions in patients in the first few weeks of life. The three population models included the model developed in Chapter 3 of this thesis and two other models based on fixed allometric scaling principles [79,80]. Of these models, the model from Chapter 3 was the only model for which accurate concentration predictions on the individual as well as population level were corroborated throughout the full age-range of the model and for both morphine and its metabolites. With regards to the prediction of total morphine clearance this model had similar accuracies as the model by Anand *et al.* [80] , although the Anand-model did not include the pharmacologically-active metabolites. Moreover, serious model misspecification was reported for neonates by the third model (Chapter 8). No adequate evaluation and validation was performed on the accuracy of morphine clearance predictions by a published physiologically-based pharmacokinetic model [81].

The results from Chapter 8 and Chapter 9 could suggest that population models based on fixed allometric scaling principles [82] generally have poor predictive performances. This is however not directly evident from these results, although the incorporation of covariates without formally testing them for significance does increase the risk of obtaining a model that is not supported by data. Provided that a model is supported by results from evaluation and validation procedures, results from paediatric population models that are based on fixed allometric scaling principles may be equally suitable for clinical decision making as any other properly validated model. For the research in the current thesis a comprehensive covariate modeling approach was applied rather than a fixed allometric scaling approach. This was based on a wide range of theoretical and experimental evidence against the application of allometric scaling with a universal fixed exponent of 0.75 in paediatric pharmacokinetics [83–90]. More recent publications on the development of population models with strongly correlated covariates (e.g. bodyweight and age in young paediatric patients) support this approach [91,92].

The findings on developmental changes in drug glucuronidation in children obtained in the current thesis may appear to be very different from previous findings, but they are not. Morphine pharmacokinetics has been widely studied in the paediatric population using traditional methods for the analysis of *in vivo* data, which was reviewed amongst others in Chapter 2 and Chapter 9. In line with our findings, *in vivo* pharmacokinetic studies of morphine and its metabolites in literature also showed that preterm neonates with a gestational age as young as 24 weeks already metabolize morphine by glucuronidation [93]. In most published studies morphine clearance is parameterized per kilogram bodyweight and investigated in age-strata with a limited range. The thus reported morphine clearance values cover a wide range [18,94–100], with different studies reporting clearance to remain either constant [93,101,102] or to change with postconceptual age [103–106], postnatal age [17,107–111], or bodyweight [103,104,112]. However, figure 5 of Chapter 4 illustrates how the highly non-linear covariate relationship for morphine glucuronidation clearance developed in the current thesis accurately describes the trend observed in reported morphine clearances over the entire age-range from preterm neonates to three year old children. Although the pharmacokinetics of zidovudine has been studied less extensively in children than morphine, literature findings on this drug are also in line with the findings on developmental changes in glucuronidation in the current thesis. Zidovudine clearance was shown to increase rapidly in the first two weeks of life. The subsequent increase in zidovudine clearance was reported to be slower, with studies reporting adult values to be reached within two months [113,114], while others reported a two year period of slower increase to be followed by an even slower increase to reach adult values in late adolescence [115].

Compared to the traditional methods, population model-based analysis approaches to study drug pharmacokinetics offer several advantages as discussed in Chapter 9. With population modeling each individual is regarded as constituent of the overall population. By analyzing the population as a whole, fewer samples per individual are required and the precision of findings may be improved as it is possible to simultaneous analyze data from multiple studies with different study designs, as long as detailed records are available on the exact time of drug dosing and blood sampling. Moreover, inter-individual variability can be distinguished from other sources of variability like intra-individual variability, measurement error and model misspecification. Descriptors that can explain (part of) the inter-individual variability can be identified and quantified in continuous relationships, without the need for stratification of the population based on age. One of the biggest advantages of the covariate relationships in population models is that they quantify the net changes in pharmacokinetic parameters that can directly serve as the basis for paediatric drug dosing algorithms.

In conclusion, the approach described in Section II of this thesis should be the basis for the development of evidence-based paediatric dosing algorithms for off-patent drugs that are already regularly prescribed for children. This approach entails 1) population pharmacokinetic and/or pharmacodynamic model development which can be based on data from various (existing) sources, 2) internal and external model validation according to the framework developed in Chapter 8 to assess the predictive performance of the population model, and 3) a prospective clinical trial as proof-of-principle to confirm the efficacy and safety profile of the model-derived dosing algorithm. Although the broader applicability and generalizability of the semi-physiological modeling concepts proposed in Section III need further investigation, our initial results are promising and show that semi-physiological modeling could limit the time and resources needed for paediatric population model development. This approach could also be expanded to derive first-in-child doses in the drug development process, based on *in vitro* and adult information on the elimination pathways of a new chemical entity.

Conclusion and recommendations:

- Proper model evaluation and validation based on appropriate tools should be a standard requirement for the publication of all population models.
- Patient and study characteristics in the paediatric population differ from the adult population and require special considerations in model evaluation and validation procedures that are taken into account in the framework developed in Chapter 8.
- Only when a model is proven to be supported by clinical data can it be considered to provide an accurate reflection of clinical reality, therefore only validated (covariate) models should be used for clinical decision making, for deriving dosing algorithms or for application in a semi-physiological modeling approach.
- A theory-based modeling approach rather than a data-driven approach may lead to models that are not supported by clinical evidence which may limit the predictive value of these models.
- Population pharmacokinetic modeling is ideally suitable for quantifying net developmental changes in drug clearance. The model-based approach described in Section II of this thesis should be the basis for the development of evidence-based paediatric dosing algorithms.

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Appendix

Nederlandse Samenvatting:

Grootte is een Factor van Betekenis:

Glucuronidering van Geneesmiddelen in Kinderen

I. Achtergrond en Doel van het Onderzoek

Voor de meeste geneesmiddelen zijn geen wetenschappelijk onderbouwde doseervoorschriften voor kinderen beschikbaar, dat geldt in het bijzonder voor pasgeborenen (neonaten) en jonge kinderen. Als gevolg hiervan komen zowel onderdosering als overdosering vaak voor. De voornaamste oorzaak hiervan is dat vaak niet is onderzocht welke factoren bij kinderen bepalend zijn voor de variatie in de werking van geneesmiddelen.

De intensiteit en duur van de werking van geneesmiddelen wordt bepaald door twee processen, te weten de farmacokinetiek (het verloop van de concentratie van het geneesmiddel in het lichaam in de tijd) en de farmacodynamiek (de verandering in het biologische systeem die een geneesmiddel bij een bepaalde concentratie te weeg brengt). Beide kunnen in kinderen veranderen met de leeftijd. Factoren die bij kinderen de farmacokinetiek van een geneesmiddel, en daarmee de blootstelling, beïnvloeden zijn: i) de grootte en groei van het lichaam en ii) de veranderingen in de processen die bepalend zijn voor de opname en de verdeling van stoffen in het lichaam en de eliminatie uit het lichaam. Deze veranderingen kunnen het gevolg zijn van veranderingen in a) de expressie en functie van enzymen en transporteiwitten, b) het hartminuutvolume en de doorbloeding van organen, c) de pH van het bloed en d) de concentratie van geneesmiddelbindende plasma-eiwitten en andere componenten in het bloed. Bovendien veranderen ook de relatieve grootte van de organen, de verhouding tussen water en vetweefsel en de verhouding tussen tussen intra- en extracellulair water. Naast de veranderingen in farmacokinetiek kunnen veranderingen in de expressie en functie van receptoren of andere'targeteiwitten' leiden tot een veranderde farmacodynamiek bij kinderen in vergelijking met volwassenen en tussen kinderen van verschillende leeftijden.

Wanneer er geen rekening wordt gehouden met leeftijdsafhankelijke verschillen in de farmacokinetiek en farmacodynamiek van geneesmiddelen kan dit leiden tot het falen van de behandeling of tot bijwerkingen met soms zelfs fatale afloop. Het is daarom van groot belang dat er onderzoek plaatsvindt naar de factoren die bij kinderen de farmacokinetiek en de farmacodynamiek van geneesmiddelen kunnen beïnvloeden. Recente wet- en regelgeving in Europa en de Verenigde Staten is er op gericht om geneesmiddelonderzoek in kinderen te bevorderen. Dat heeft ertoe geleid dat onderzoek in kinderen verplicht is voor nieuwe, nog op de markt te brengen geneesmiddelen. Er zijn vooralsnog geen wetten die betrekking hebben op onderzoek voor geneesmiddelen die al op de markt zijn en die niet meer onder een patent vallen.

Het doel van het onderzoek in dit proefschrift was om nieuwe methodes te ontwikkelen die gebruikt kunnen worden voor het vaststellen van wetenschappelijk onderbouwde doseervoorschriften voor geneesmiddelen in kinderen. De gebruikte methoden zijn gebaseerd op zogenaamde "populatie" analyses. Bij deze analyses worden wiskundige en statistische modellen gebruikt om farmacokinetische en farmacodynamische processen te beschrijven op basis van waargenomen geneesmiddelconcentraties of geneesmiddeleffecten. Daarnaast wordt ook de variabiliteit in de verschillende processen tussen individuen geanalyseerd. Hierdoor is het mogelijk om patiëntkarakteristieken te identificeren op basis waarvan de variabiliteit in de verschillende processen tussen individuen kan worden voorspeld. Deze voorspellende patiëntkarakteristieken worden covariaten genoemd. Covariaten vormen de basis voor geïndividualiseerde doseervoorschriften, of te wel doseervoorschriften die op de individuele patiënt zijn toegesneden.

Het onderzoek in dit proefcshrift is vooral gericht op kinderen jonger dan drie jaar, omdat de grootste en belangrijkste veranderingen plaatsvinden in de eerste levensjaren. Omdat wordt aangenomen dat leeftijdsafhankelijke verschillen in geneesmiddeleffecten bij kinderen voornamelijk het gevolg zijn van veranderingen in de farmacokinetiek, is het onderzoek in dit proefschrift hierop gericht. De belangrijkste farmacokinetische parameter in dit verband is de klaring. Dit is de parameter die beschrijft hoe snel een geneesmiddel uit het lichaam wordt geëlimineerd. Bij chronische toediening wordt bij een gegeven doseringsschema de hoogte van de gemiddelde plasmaconcentratie uitsluitend bepaald door de klaring. Geneesmiddelen kunnen geklaard worden door onveranderde uitscheiding in de nieren of door metabolisme in de lever. Bij metabolisme worden geneesmiddelen door enzymen omgezet in metabolieten die gemakkelijker door de nieren kunnen worden uitgescheiden dan het geneesmiddel zelf. Het onderzoek in dit proefschrift richt zich op de klaring van geneesmiddelen via metabolisme door het enzym uridine 5′-diphosphate glucuronosyltransferase (UGT) 2B7. Afbraak van geneesmiddelmoleculen door dit type enzym wordt glucuronidering genoemd. Morfine en zidovudine zijn twee geneesmiddelen die regelmatig aan kinderen worden voorgeschreven en beiden worden voornamelijk via glucuronidering door het UGT2B7 enzym geklaard. Deze twee geneesmiddelen zijn daarom gebruikt als modelstoffen om de rijping (het patroon waarmee de snelheid van geneesmiddelmetabolisme zich in kinderen ontwikkelt) van deze klaringsroute in kinderen te bepalen.

In **Hoofdstuk 2** wordt een overzicht gegeven van beschikbare *in vitro* en *in vivo* methodes om de rijping van enzymen in de lever te bestuderen. Hierbij worden ook de resultaten die met deze methodes voor de verschillende UGT enzymen zijn verkregen besproken.

De rijping van enzymen kan op verschillende niveaus onderzocht worden, waarbij telkens specifieke onderdelen van het biologische systeem worden uitgelicht. Hierbij moet gedacht worden aan i) mRNA transcriptie, ii) expressie van enzymen, iii) de activiteit van enzymen *in vitro* en iv) *in vivo* klaring. Deze verschillende onderdelen van het biologische systeem worden op verschillende manieren door de overige processen in dat biologische systeem beïnvloed. Daarnaast bestaan er vaak meerdere technieken om het biologische systeem op één specifiek niveau te bestuderen. Afhankelijk van de gebruikte techniek en het niveau van het biologische systeem dat wordt bestudeerd, kunnen de conclusies met betrekking tot enzym rijping dus verschillen.

Op basis van literatuurgegevens kon worden vastgesteld dat de rijping van de verschillende UGT enzymen erg varieert. Over het algemeen kan expressie en activiteit van deze enzymen al worden waargenomen in foetussen na een draagtijd van 20 weken en vindt er een snelle toename in expressie en activiteit plaats in de eerste weken na de geboorte. De gerapporteerde leeftijd waarop de rijping van de verschillende UGT enzymen is voltooid verschilt tussen 6 maanden en 18 jaar.

Van alle technieken die beschikbaar zijn om rijping van UGT enzymen te bestuderen is de klaring via glucuronidering in de mens de meest relevante parameter voor het vaststellen van het doseervoorschrift. Daarom is er in dit proefschrift voor gekozen om klaring via glucuronidering te gebruiken als eindpunt in de studies.

II. Morfine Glucuronidering in Kinderen.

In **Sectie II** van dit proefschrift wordt het onderzoek naar de glucuronidering van morfine in kinderen jonger dan drie jaar beschreven. Het doel van dit onderzoek was om een wetenschappelijk onderbouwd doseervoorschrift voor dit geneesmiddel in deze kinderen te ontwikkelen. Bij het klinisch geneesmiddelonderzoek dat daarvoor nodig is, spelen specifieke ethische, wettelijke en praktische factoren een rol.

Omdat (jonge) kinderen zelf niet kunnen instemmen met deelname aan studies, is het onethisch geneesmiddelstudies uit te voeren in gezonde kinderen. Om die reden is geneesmiddelonderzoek in gezonde kinderen wettelijk niet toegestaan. Geneesmiddelstudies vinden daarom alleen plaats in kinderen die een middel als onderdeel van hun medische behandeling voorgeschreven krijgen. Dit vergroot de variabiliteit tussen de patiënten die worden onderzocht, omdat de ernst van de ziekte en het aantal en soort van de overige voorgeschreven geneesmiddelen sterk kunnen verschillen. Praktische beperkingen vinden hun oorzaak in het kleine bloedvolume van (jonge) kinderen. Hierdoor kan per patiënt slechts een beperkt aantal bloedmonsters

worden afgenomen voor de bepaling van geneesmiddelconcentraties. Daarnaast mogen kinderen niet worden blootgesteld aan de stress van bloedafname wanneer dit alleen voor onderzoeksdoeleinden wordt gedaan. Bloedmonsters kunnen daarom alleen verkregen worden van patiënten die een permanente arterielijn hebben. Wanneer er geen permanente arterielijn aanwezig is, mogen er alleen bloedmonsters worden afgenomen op tijdstippen dat er bloed wordt verzameld voor behandeldoeleinden. Hierdoor kan het tijdstip van bloedmonsters afname niet altijd vooraf in het studieprotocol worden vastgelegd. Ook de hoogte en het tijdstip van dosering kan vaak niet vooraf worden vastgesteld, omdat dit door de behandelend arts wordt voorgeschreven op basis van de individuele behoefte van een patiënt. Voor de analyse van data met grote variatie in patiëntkarakteristieken, in het tijdstip en de hoogte van de dosering en in het tijdstip en de frequentie van de bloedmonsterafname, is de populatie analyse die in dit proefschrift is toegepast uitermate geschikt.

Het onderzoek dat wordt beschreven in **Hoofdstuk 3** heeft betrekking op de farmacokinetiek van morfine, inclusief de twee belangrijkste metabolieten morfine-3-glucuronide (M3G) en morfine-6-glucuronide (M6G), in kinderen jonger dan drie jaar. Dit onderzoek is uitgevoerd op basis van een populatie analyse van de bloedconcentraties in 248 patiënten. Per patiënt waren gemiddeld 4 bloedconcentraties beschikbaar. Deze patiënten kregen morfine toegediend voor pijnbestrijding na een zware operatie (met uitzondering van hartoperaties) of tijdens mechanische beademing. Op basis van deze analyse kon worden vastgesteld, dat lichaamsgewicht de meeste voorspellende covariaat is om de verandering in morfine klaring ten gevolge van groei en ontwikkeling in deze jonge kinderen te beschrijven. De verandering in morfine klaring werd het beste beschreven met een exponentiële functie op basis van lichaamsgewicht met een exponent van ongeveer 1.5. Daarnaast bleek dat de snelheid van glucuronidering van morfine ongeveer 50% lager is in neonaten die jonger zijn dan tien dagen, ongeacht de draagtijd.

De voorspellende waarde van het ontwikkelde model is uitvoerig getest door te bepalen of het model de morfine, M3G en M6G concentraties die gebruikt zijn voor de ontwikkeling van het model ook inderdaad accuraat kan voorspellen. Een goede voorspelling van de concentraties van de twee metabolieten is daarbij net zo belangrijk als een goede voorspelling van de morfine concentraties, omdat beide metabolieten bijdragen aan de (bij)werkingen van morfine. In deze analyse zijn de voorspellingen van de concentraties gedaan op basis van het lichaamsgewicht en leeftijd van de patiënt, het tijdstip van toediening en het tijdstip van de concentratiebepaling. De voorspelde concentraties zijn vervolgens vergeleken met de gemeten concentraties. Hieruit bleek dat de voorspellingen van de concentraties van morfine en de twee metabolieten tijdens en na beëindiging van de infusie accuraat waren voor alle patiënten.

De evenwichtsconcentraties van geneesmiddelen in het bloed zijn bij continue infusies uitsluitend afhankelijk van de klaring van het geneesmiddel. Op basis van het ontwikkelde model kon daarom worden vastgesteld dat continue morfine infusies het best gedoseerd kunnen worden in microgram per kilogram tot de macht 1.5 per uur ($\mu g/kg^{1.5}$/uur), in plaats van in de meer gebruikelijke dosering in microgram per kilogram per uur ($\mu g/kg$/uur), en dat de dosering in neonaten jonger dan tien dagen met 50% moet worden gehalveerd. Simulaties met het model lieten inderdaad zien dat de variabiliteit in concentraties van morfine, M3G en M6G in pasgeborenen tot peuters van drie jaar met dit nieuwe doseringsregime lager is dan de variabiliteit in de concentraties die voorspeld worden bij dosering volgens het traditionele doseringsschema.

Voordat het bovengenoemde doseervoorschrift in de praktijk kon worden getest, is in **Hoofdstuk 4** bestudeerd of het model ook accurate concentratievoorspellingen kan doen voor externe data. Hiermee worden data bedoeld die afkomstig zijn van patiënten die geen onderdeel vormden van de dataset waarmee het model is ontwikkeld. Voor deze analyses waren morfine en metaboliet concentraties beschikbaar van 90 patiënten uit vier verschillende studies die waren uitgevoerd in vier verschillende ziekenhuizen. Deze studies waren uitgevoerd in neonaten tot peuters van één jaar oud die een zware operatie hadden ondergaan (met uitzondering van hartoperaties) of die mechanisch beademd werden. Met behulp van verschillende methodes is vastgesteld dat de morfine en metaboliet concentraties in deze externe patiënten met behulp van het model kunnen worden voorspeld op basis van het lichaamsgewicht, de leeftijd en de morfine dosering. Bovendien zijn de waarden van de klaringen via glucuronidering van morfine die door het model worden voorspeld vergeleken met literatuurwaardes. Ondanks de grote spreiding in de gerapporteerde waardes voor deze jonge populatie, bleek het model de trend in de resultaten van verschillende studies goed te beschrijven.

Nadat was vastgesteld dat het model goede voorspellingen voor de concentraties van morfine en de metabolieten oplevert, is in **Hoofdstuk 5** de effectiviteit van het nieuwe doseervoorschrift voor morfine geëvalueerd in de klinische praktijk. In een studie in neonaten tot peuters van één jaar die een morfine infusie kregen voor pijnbestrijding na een zware operatie (met uitzondering van hartoperaties), kregen neonaten jonger dan tien dagen een morfine infusie van 2.5 $\mu g/kg^{1.5}$/uur terwijl oudere kinderen een infusie kregen van 5 $\mu g/kg^{1.5}$/uur. Ten opzichte van traditioneel gebruikte morfine infusiesnelheid van 10 $\mu g/kg$/uur, betekende dit een dosisvermindering van 50% tot 75% voor neonaten jonger dan tien dagen, en een dosis verhoging tot 150% in oudere kinderen van meer dan vier kilogram.

De effectiviteit van de nieuwe dosering is geëvalueerd op basis van een analyse van de zogenaamde 'rescue doses'. Dit zijn extra morfine doses die worden toegediend wanneer de pijnbestrijding onvoldoende is op basis van objectieve methodes voor het vaststellen van pijn in deze jonge patiënten. In de neonaten jonger dan tien dagen had maar 27% van de patiënten rescue doses nodig, ten opzichte van 90% in de oudere patiënten. Ook het aantal rescue doses en de gemiddelde rescue dosis was beduidend lager in neonaten jonger dan tien dagen ten opzichte van de oudere kinderen. Deze resultaten tonen aan dat de gereduceerde dosis in jonge neonaten nog steeds voldoende effectief is, terwijl de verhoogde dosering in oudere patiënten nog niet hoog genoeg is. Ten opzichte van de traditioneel gebruikte morfine doseringen, leidt het nieuwe morfine doseervoorschrift dus tot een verbetering van de behandeling, omdat het de blootstelling aan te hoge doses in jonge neonaten vermindert, terwijl het ook, weliswaar in minder mate, de blootstelling aan ineffectieve doses in oudere patiënten tot één jaar vermindert.

Waar mogelijk waren er in deze studie ook bloedmonsters afgenomen om de concentraties van morfine, M3G en M6G te bepalen. Deze concentraties zijn vergeleken met de voorspellingen van het populatie model uit Hoofdstuk 3, om vast te stellen of de voorspellingen van dit model ook accuraat zijn bij doseringen volgens het nieuwe doseervoorschrift. Dit bleek het geval te zijn. Dit bevestigt dat het ontwikkelde doseervoorschrift corrigeert voor verschillen in de klaring van morfine in deze patiëntengroep. Er kan worden aangenomen dat doseervoorschriften die corrigeren voor verschillen in klaring voldoende zijn wanneer de farmacodynamiek van het geneesmiddel gelijk is in kinderen van alle leeftijden. Dit is niet waarschijnlijk voor morfine. Morfine werkt door binding aan specifieke opiaat receptoren in de hersenen. Het transport van morfine van het bloed naar deze receptoren in hersenen en de expressie en functie van deze receptoren in de hersenen zijn mogelijk beide onderhevig aan leeftijdsverschillen. Deze verschillen verklaren mogelijk het verschil in effectiviteit van het nieuwe morfine doseervoorschrift in oudere en jongere kinderen in deze studie. In vervolg onderzoek kan een populatie analyse van de farmacodynamiek van morfine in deze jonge kinderen gebruikt worden om voor elke leeftijdsgroep te bepalen welke concentratie tot het gewenste effect leidt. Simulaties met het huidige farmacokinetische model kunnen dan worden gebruikt om vast te stellen welke dosering tot de gewenste concentraties zal leiden. Hierdoor kan het doseervoorschrift van morfine verder geoptimaliseerd worden.

III. Semi-Fysiologisch Model voor de Glucuronidering van Geneesmiddelen in Kinderen.

De methode die in Sectie II van de dit proefschrift is gebruikt voor het vaststellen van wetenschappelijk onderbouwde doseervoorschriften voor morfine in kinderen, is tijdrovend en intensief. Het is onmogelijk om een soortgelijke analyse uit te voeren voor elk geneesmiddel en in elke leeftijdsgroep. In **Sectie III** is daarom gezocht naar een algemene methode voor de ontwikkeling van populatie modellen voor kinderen, die gebruikt kunnen worden voor het vaststellen van wetenschappelijk onderbouwde doseervoorschriften. De ontwikkelde methode is gebaseerd op de hypothese dat het rijpingspatroon van geneesmiddelklaring door metaboliserende enzymen een systeem-specifieke eigenschap is. Dit betekent dat het rijpingspatroon van toepassing is op alle geneesmiddelen die via een specifiek enzym worden geëlimineerd. Met andere woorden, als voor één geneesmiddel de covariaatrelatie voor de rijping van de klaring is vastgesteld, kan deze relatie ook worden gebruikt om de leeftijdsafhankelijke verandering in klaring van andere geneesmiddelen die via hetzelfde enzym worden gemetaboliseerd te voorspellen. De absolute waarde van de klaring kan daarbij wel verschillend zijn, omdat dit een geneesmiddel-specifieke eigenschap is.

De modellen die in deze sectie beschreven worden, worden semi-fysiologische modellen genoemd, omdat ze zijn afgeleid van fysiologische modellen. Fysiologische modellen zijn mechanistische modellen. Deze modellen gaan niet, zoals populatie modellen, uit van gemeten concentraties, maar zijn gebaseerd op een beschrijving en kwantificering van alle onderliggende biologische processen die bepalend zijn voor het verloop van de concentratie van een geneesmiddel in het lichaam (de absorptie, de verdeling, het metabolisme en de eliminatie) en de interacties tussen geneesmiddelen en het biologische systeem. Een fundamentele eigenschap van fysiologische modellen is het strikte onderscheid tussen systeem-specifieke eigenschappen (b.v. doorbloeding van het lever, concentratie van plasma-eiwitten, expressie en functie van enzymen en transport-eiwitten) en geneesmiddel-specifieke eigenschappen (b.v. lipofiliciteit, en affiniteit voor bindingseiwitten, enzymen en transporters). Deze modellen worden in de praktijk onder meer toegepast om de absolute waarden van de klaring van nieuwe stoffen in de mens te voorspellen op basis van pre-klinische gegevens. In de semi-fysiologische modellen in dit proefschrift, combineren eerder opgedane kennis van het biologische system met een populatie analyse. Zo wordt de covariaatrelatie die het rijpingspatroon voor het metabolisme van een bepaald geneesmiddel beschrijft, rechtstreeks gebruikt om de veranderingen in klaring van een nieuw geneesmiddel dat via hetzelfde enzym wordt gemetaboliseerd te beschrijven. Vervolgens wordt een populatie analysis op

basis van gemeten concentraties gebruikt om de absolute waarde van de klaring (een geneesmiddel-specifieke eigenschap) vast te stellen.

In **Hoofdstuk 6** is onderzocht of covariaatrelaties voor de rijping van specifieke klaringsroutes in kinderen uitwisselbaar zijn tussen geneesmiddelen die via hetzelfde enzym worden gemetaboliseerd. In deze studie zijn twee populatie modellen voor een dataset met concentraties van zidovudine en zidovudine-glucuronide met elkaar vergeleken. De data waren verkregen in een studie met gezonde kinderen tussen nul en vijf maanden, waarin werd onderzocht of zidovudine een geschikt middel is om de overdracht van het Hiv-virus tussen moeder en kind te voorkomen.

Zidovudine wordt, net als morfine, voornamelijk geklaard via metabolisme door het UGT2B7 enzym. In het eerste model is daarom de covariaatrelatie voor de rijping van de activiteit van UGT2B7 die in Hoofdstuk 3 is gevonden voor de glucuronidering van morfine, onveranderd toegepast op de glucuronidering van zidovudine. Vervolgens is de absolute waarde van de klaring voor de glucuronidering van zidovudine bepaald op basis van de zidovudine en zidovudine-glucuronide concentraties. Dit model is het semi-fysiologische model. Om vast te kunnen stellen hoe goed de beschrijvende en voorspellende waarde van het semi-fysiologische model is, is er tevens een referentie model ontwikkeld dat statistisch gezien de beste beschrijving van de zidovudine data geeft. In dit referentie model is een populatie analyse gebruikt om zowel de absolute glucuronideringsklaring van zidovudine vast te stellen, als de covariaatrelatie die het patroon van de leeftijdsafhankelijke verandering in de klaring beschrijft in deze groep kinderen.

De covariaatrelatie voor de glucuronidering van zidovudine die in het referentie model is gevonden verschilde van de relatie die in Hoofdsuk 3 is gevonden voor de glucuronidering van morfine. Dit komt waarschijnlijk omdat het leeftijdsverschil van de patiënten in de zidovudine dataset een stuk kleiner is (voldragen neonaten tot peuters van vijf maanden) dan het leeftijdsverschil van de patiënten in de morfine dataset (te vroeg geboren (onvoldragen) en voldragen neonaten tot kleuters van drie jaar). Ondanks dit verschil waren de beschrijvingen van zowel individuele zidovudine klaringen als individuele zidovudine en zidovudine-glucuronide concentraties door beide modellen vergelijkbaar. Bovendien waren voor beide modellen ook de voorspelde concentraties die werden verkregen op basis van informatie over de leeftijd, het gewicht en de dosis vergelijkbaar. Deze resultaten ondersteunen de hypothese dat covariaatrelaties voor de klaring van geneesmiddelen in kinderen systeem-specifieke eigenschappen zijn, die uitwisselbaar zijn tussen stoffen die via hetzelfde enzym worden gemetaboliseerd.

Door het gebruik van eerder opgedane kennis over de ontwikkeling van glucuronideringklaring, kon het semi-fysiologische model voor zidovudine vele malen

sneller ontwikkeld worden dan het referentie model. Dit toont aan dat deze methode de ontwikkeling van populatie farmacokinetische modellen voor kinderen, en daarmee de ontwikkeling van wetenschappelijk onderbouwde doseervoorschriften, kan bevorderen.

In Hoofdstuk 6 is voor het eerst aangetoond dat covariaatrelaties in een kinderpopulatie uitgewisseld kunnen worden tussen twee geneesmiddelen die via dezelfde route uit het lichaam worden geklaard. Omdat morfine en zidovudine echter veel op elkaar lijken met betrekking tot hun moleculaire eigenschappen, zal verder onderzocht moeten worden of het semi-fysiologische model voor glucuronidering ook gebruikt kan worden voor twee stoffen die weliswaar door hetzelfde enzym worden gemetaboliseerd, maar verder erg verschillen in hun moleculaire eigenschappen. Daarnaast is het model voor de glucuronidering van morfine gebaseerd op data van postoperatieve kinderen en kinderen aan de mechanische beademing, terwijl de zidovudine data verkregen zijn in gezonde kinderen. Het is belangrijk om te weten welke onderliggende biologische veranderingen verantwoordelijk zijn voor de verandering in klaring in kinderen, om vast te stellen of patiëntkarakteristieken het gebruik van het semi-fysiologische model voor glucuronidering in een bepaalde patiëntenpopulatie kunnen beperken.

Het onderzoek dat betrekking heeft op de invloed van zowel verschillende eigenschappen van het geneesmiddel als eigenschappen van het biologische systeem op het rijpingspatroon van UGT2B7 metabolisme wordt beschreven in **Hoofdstuk 7**. In dit onderzoek is gebruik gemaakt van simulaties met een fysiologisch model. Bij simulaties wordt de werkelijkheid nagebootst met behulp van modellen van die werkelijkheid. Er zijn verschillende scenario's gesimuleerd, waarin de waardes van geneesmiddel-specifieke parameters zoals moleculair gewicht, logP en pKa, en biologische systeem-specifieke parameters zoals lever volume, milligram eiwit per gram lever, plasma-eiwit binding, doorbloeding van de lever en UGT expressie en activiteit, één voor één zijn veranderd. Door te bepalen wat de invloed van deze veranderingen is op de voorspelde glucuronidering in kinderen, kon worden vastgesteld of en hoe de glucuronidering door de individuele parameters wordt beïnvloed.

Deze theoretische studie liet zien dat logP en pKa de absolute waarde van de klaring door glucuronidering kunnen beïnvloeden. Deze moleculaire eigenschappen van het geneesmiddel hadden echter geen invloed op het patroon waarmee de glucuronidering zich in kinderen ontwikkelt. Dit betekent dat de covariaatrelatie die deze ontwikkeling beschrijft, voor alle substraten hetzelfde zou zijn, wat suggereert dat geneesmiddeleigenschappen het gebruik van het semi-fysiologische model voor glucuronidering niet beperken. Deze bevindingen zijn echter gebaseerd op de aanname dat de geneesmiddeleigenschappen geen invloed hebben op de opname van de geneesmiddelen in de lever en de interactie tussen de geneesmiddelmoleculen en

het UGT2B7 enzym. Daarnaast zal verder onderzocht moeten worden of het semi-fysiologische model voor glucuronidering gebruikt kan worden voor geneesmiddelen met een lever extractie-ratio die anders is dan de extractie-ratio voor morfine en zidovudine en voor geneesmiddelen met verzadigbare glucuronidering.

Met betrekking tot the systeem-specifieke eigenschappen toonde deze theoretische studie aan dat veranderingen in het volume van de lever en in de expressie en activiteit van de UGT2B7 enzymen in de eerste drie levensjaren voor het grootste deel verantwoordelijke zijn voor de waargenomen veranderingen in glucuronidering door UGT2B7. Dit suggereert dat glucuronideringsnelheid in patiënten met insufficiënte leverfunctie mogelijk is afgenomen, wat zou kunnen betekenen dat de semi-fysiologische functie voor glucuronidering niet gebruikt kan worden in een patiëntenpopulatie met lever insufficiënties. Dit zal echter nog verder onderzocht moeten worden.

IV. Evaluatie van Farmacokinetische Modellen voor Kinderen

Een belangrijke eigenschap van de populatiebenadring voor de analyse van farmacokinetische data is dat het kan worden toegepast wanneer er een beperkt aantal waarnemingen per individu beschikbaar is. Vooral in zeer jonge kinderen komt dat vaak voor. Echter, hoe beperkter de data, hoe groter de kans op mis-gespecificeerde of 'onjuiste' modellen. Wanneer zulke 'onjuiste' modellen worden gebruikt als basis voor klinische beslissingen (bijvoorbeeld voor het bepalen van doseringen) kan dit grote gevolgen hebben. Uitgebreide evaluatie en validatie van een model, waarbij wordt vastgesteld of een model juiste voorspellingen geeft, is daarom heel belangrijk. In Hoofdstuk 3 en Hoofdstuk 4 is gebleken dat het evalueren en valideren van populatie farmacokinetische modellen voor kinderen op sommige punten aanpassingen vereist ten opzichte van de evaluatie en validatie van populatie farmacokinetische modellen voor volwassenen.

In **Hoofdstuk 8** zijn patiënt- en studiekarakteristieken geïdentificeerd die de eigenschappen van farmacokinetische datasets van kinderen beïnvloeden. Omdat (jonge) kinderen zich snel ontwikkelen, is de variatie in de ontwikkelingsstadia tussen patiënten bijvoorbeeld vaak groot. Daarnaast is, zoals eerder genoemd, de variabiliteit in geneesmiddeldosering en bloedmonster afname vaak groot omdat deze sterk beïnvloed worden door de conditie van de patiënt, en is het aantal bloedmonsters per patiënt vaak beperkt. Als gevolg van deze factoren zijn er voor de evaluatie en validatie van populatie modellen in kinderen vaak aanpassingen nodig van al bestaande validatietechnieken. Een belangrijk punt is bijvoorbeeld om vast te stellen of het model de data in alle leeftijdscategorieën juist voorspelt. Om die reden is het noodzakelijk een dataset niet

alleen in zijn geheel te bekijken, maar de dataset ook op te splitsen in verschillende leeftijdscategorieën. Daarnaast zijn soms geavanceerde evaluatie technieken nodig ter vervanging van meer traditionele technieken. Een zogenaamde "normalized prediction distribution error" (NPDE) analyse is bijvoorbeeld een meer geschikte evaluatie methode op basis van simulaties, dan de meer traditionele visual predictive check (VPC), omdat het flexibeler kan omgaan met grote variabiliteit in dosering en afname schema en in covariaatdistributies. Tot slot is in Hoofdstuk 8 ook een nieuwe evaluatie techniek gepresenteerd om vast te stellen of een gevonden covariaatrelatie de patronen in de variabiliteit in parameterwaardes tussen individuen goed beschrijft.

Het onderzoek dat is beschreven in **Hoofdstuk 8** heeft betrekking op de ontwikkeling van een theoretisch raamwerk voor de systematische evaluatie en validatie van populatiemodellen in kinderen. Dit raamwerk omvat zes verschillende onderdelen. Het raamwerk werd toegepast in de evaluatie van twee verschillende populatie farmacokinetische modellen voor morfine in kinderen. Deze twee modellen zijn op verschillende manieren ontwikkeld. Het eerste model is gebaseerd op het model dat is beschreven in Hoofdstuk 3, wat tot stand is gekomen op basis van een uitvoerige covariaat analyse. Het tweede model is gebaseerd op specifieke theoretische aannames met betrekking tot de samenhang tussen lichaamsgewicht en klaring. Omdat voor de ontwikkeling van beide modellen gebruik was gemaakt van exact dezelfde dataset, was een directe vergelijking tussen de twee modellen mogelijk. De resultaten van de analyse laten zien dat het ontwikkelde evaluatie en validatie raamwerk in staat is om problemen met de beschrijvende en voorspellende waarde van populatie modellen voor kinderen te identificeren. Daarnaast kan het ook gebruikt worden om vast te stellen waardoor gebrekkige beschrijvingen en voorspellingen van deze modellen worden veroorzaakt.

De farmacokinetiek en klaring van morfine in kinderen zijn uitgebreid bestudeerd in de literatuur. In **Hoofdstuk 9** worden de verschillende analysemethodes die hiervoor gebruikt zijn beschreven en zijn de voor- en nadelen van elke methode in kaart gebracht. Daarnaast zijn de gerapporteerde klaringswaardes met elkaar vergeleken en is de voorspellende waarde van gepubliceerde modellen (zowel populatie modellen als fysiologische modellen) geëvalueerd.

In de afgelopen twintig jaar zijn twintig studies naar de klaring van morfine in kinderen gerapporteerd waarin gebruik werd gemaakt van traditionele farmacokinetische analyse technieken. Deze studies zijn veelal beschrijvend en gebaseerd op waarnemingen in een klein aantal kinderen. De gerapporteerde waarden voor de klaring van morfine lopen ver uiteen, waarschijnlijk doordat de metingen met traditionele technieken minder nauwkeurig zijn en het aantal patiënten in de studies vaak klein is. Daarnaast is een nadeel van deze studies dat de klaring in kinderen vaak wordt uitgedrukt per kilogram

lichaamsgewicht en dat de leeftijdsafhankelijke veranderingen alleen maar beschreven kunnen worden door kinderen in leeftijdscategorieën in te delen en een gemiddelde klaring per groep te berekenen. Het patroon van leeftijdsafhankelijke verschillen in klaring hangt daarmee af van de meestal willekeurig gekozen leeftijdscategorieën.

De beschrijving van leeftijdsafhankelijke verschillen in klaring in populatie modellen en fysiologische modellen is niet afhankelijk van een indeling van de patiënten in leeftijdsgroepen. Daarnaast kunnen met deze technieken data van verschillende studies gecombineerd worden, wat de nauwkeurigheid van de bevindingen kan vergroten. Voor morfine zijn drie populatie farmacokinetische modellen beschreven in de literatuur, waarbij verschillende technieken voor de covariaatanalyse zijn gebruikt. Alle drie de modellen zijn voor kinderen die jonger zijn dan drie jaar waarbij in twee modellen ook de farmacokinetiek in onvoldragen neonaten wordt beschreven. De verschillende functies voor de verandering van morfineklaring in deze patiëntengroep in deze modellen, leveren ongeveer gelijke klaringswaardes op, behalve in neonaten die jonger zijn dan één maand en dan vooral in onvoldragen neonaten. Voor één fysiologisch model is gekeken naar de voorspellingen van morfine klaringen in kinderen en ook deze voorspellingen waren in dezelfde grootte orde dan de overige gerapporteerde klaringswaardes. Helaas is het vooralsnog niet mogelijk om met fysiologische modellen voorspellingen te doen in te vroeg geboren neonaten.

Met betrekking tot de validatie van de populatie modellen en het fysiologische model, kon worden vastgesteld dat niet voor alle modellen voldoende is bewezen dat ze de werkelijkheid accuraat kunnen beschrijven en voorspellen. Voor één populatiemodel is zelfs bewezen dat het model een te hoge morfineklaring in neonaten voorspelt. Wanneer dit model zou worden gebruikt voor het bepalen van morfine doseringen, zou dat kunnen leiden tot ernstige overdosering.

V. Conclusie

In de eerste paar levensjaren vindt er een groot aantal veranderingen plaats in het lichaam van een kind. Deze veranderingen kunnen de blootstelling aan en werking van geneesmiddelen beïnvloeden. Dit proefschrift beschrijft hoe wetenschappelijk onderbouwde doseervoorschriften voor geneesmiddelen in kinderen kunnen worden ontwikkeld waarbij rekening wordt gehouden met veranderingen in farmacokinetische processen in deze populatie. Deze aanpak omvat de volgende stappen:

1) Ontwikkeling van populatie farmacokinetische modellen in kinderen. Deze modellen kunnen worden ontwikkeld door middel van een uitgebreide analyse

zoals beschreven in Hoofdstuk 3, of door middel van een semi-fysiologische aanpak zoals beschreven in Hoofdstuk 6, hoewel de bredere toepasbaarheid van de semi-fysiologische methode nog verder onderzocht dient te worden.

2) Evaluatie en validatie van het ontwikkelde model met behulp van het raamwerk dat in Hoofddstuk 8 is ontwikkeld. De evaluatie en validatie dienen plaats te vinden op basis van zowel interne data (data die gebruikt zijn voor de ontwikkeling van het model), als externe data (data die niet gebruikt zijn voor de ontwikkeling van het model of van externe bronnen afkomstig zijn).
3) Een prospectieve klinische studie om de veiligheid en effectiviteit van het doseerregime dat van het populatiemodel is afgeleid, vast te stellen, zoals voor morfine is gedaan in Hoofdstuk 5.

Voor morfine heeft deze aanpak een onderbouwd doseervoorschrift opgeleverd dat overdosering van neonaten en blootstelling van ineffectieve doses in oudere kinderen vermindert.

Appendix

List of publications

Krekels EH, Tibboel D, Danhof M, Knibbe CA. Prediction of Morphine Clearance in the Paediatric Population: How Accurate are the Available Pharmacokinetic Models? Accepted *Clin Pharmacokinet*. **2012**

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Appendix

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