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Size does matter : drug glucuronidation in children

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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 (EMA – 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 $\mu\text{g}/\text{kg}^{1.5}/\text{h}$ 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 $2.5 \mu\text{g}/\text{kg}^{1.5}/\text{h}$, while older patients received $5 \mu\text{g}/\text{kg}^{1.5}/\text{h}$. Compared to a traditional dosing regimen of $10 \mu\text{g}/\text{kg}/\text{h}$, 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 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 ^[117,107–111], 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 simultaneously 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.

References

1. Cuzzolin L, Atzei A, Fanos V. Off-label and unlicensed prescribing for newborns and children in different settings: a review of the literature and a consideration about drug safety. *Expert Opin. Drug Saf* **5**, 703-718 (2006).
2. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N.Engl.J.Med.* **349**, 1157-1167 (2003).
3. Stephenson T. How children's responses to drugs differ from adults. *Br.J.Clin.Pharmacol.* **59**, 670-673 (2005).
4. Alcorn J, McNamara PJ. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clin.Pharmacokinet.* **41**, 959-998 (2002).
5. Court MH *et al.* Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the UGT2B7*2 polymorphism. *Drug Metab Dispos.* **31**, 1125-1133 (2003).
6. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos.* **25**, 1-4 (1997).
7. Barbier O *et al.* 3'-azido-3'-deoxythymidine (AZT) is glucuronidated by human UDP-glucuronosyltransferase 2B7 (UGT2B7). *Drug Metab Dispos.* **28**, 497-502 (2000).
8. Rauh M, Stachel D, Kuhlen M, Groschl M, Holter W, Rascher W. Quantification of busulfan in saliva and plasma in haematopoietic stem cell transplantation in children: validation of liquid chromatography tandem mass spectrometry method. *Clin.Pharmacokinet.* **45**, 305-316 (2006).
9. Jabeen R, Payne D, Wiktorowicz J, Mohammad A, Petersen J. Capillary electrophoresis and the clinical laboratory. *Electrophoresis* **27**, 2413-2438 (2006).
10. Cohen-Wolkowicz M *et al.* Population pharmacokinetics of metronidazole evaluated using scavenged samples from preterm infants. *Antimicrob.Agents Chemother.* **56**, 1828-1837 (2012).
11. Edelbroek PM, Van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. *Ther.Drug Monit.* **31**, 327-336 (2009).
12. De Cock RF, Piana C, Krekels EH, Danhof M, Allegaert K, Knibbe CA. The role of population PK-PD modelling in paediatric clinical research. *Eur.J.Clin.Pharmacol.* **67**, 5-16 (2011).
13. Wang C *et al.* A Bodyweight-Dependent Allometric Exponent for Scaling Clearance Across the Human Life-Span. *Pharm.Res.* (2012).
14. Knibbe CA, Danhof M. Individualized dosing regimens in children based on population PKPD modelling: are we ready for it? *Int.J.Pharm.* **415**, 9-14 (2011).
15. Flannigan C, McAloon J. Students prescribing emergency drug infusions utilising smartphones outperform consultants using BNFCs. *Resuscitation* **82**, 1424-1427 (2011).
16. Franko OI, Tirrell TF. Smartphone App Use Among Medical Providers in ACGME Training Programs. *J.Med.Syst.* (2011).
17. Lynn A, Nespeca MK, Bratton SL, Strauss SG, Shen DD. Clearance of morphine in postoperative infants during intravenous infusion: the influence of age and surgery. *Anesth.Analg.* **86**, 958-963 (1998).
18. Dagan O, Klein J, Bohn D, Barker G, Koren G. Morphine pharmacokinetics in children following cardiac surgery: effects of disease and inotropic support. *J.Cardiothorac.Vasc.Anesth.* **7**, 396-398 (1993).

19. Ince I *et al.* Critical Illness Is a Major Determinant of Midazolam Clearance in Children Aged 1 Month to 17 Years. *Ther.Drug Monit.* (2012).
20. Mulla H, Pooboni S. Population pharmacokinetics of vancomycin in patients receiving extracorporeal membrane oxygenation. *Br.J.Clin.Pharmacol.* **60**, 265-275 (2005).
21. Buck ML. Pharmacokinetic changes during extracorporeal membrane oxygenation: implications for drug therapy of neonates. *Clin.Pharmacokinet.* **42**, 403-417 (2003).
22. Mulla H, McCormack P, Lawson G, Firmin RK, Upton DR. Pharmacokinetics of midazolam in neonates undergoing extracorporeal membrane oxygenation. *Anesthesiology* **99**, 275-282 (2003).
23. Mulla H, Nabi F, Nichani S, Lawson G, Firmin RK, Upton DR. Population pharmacokinetics of theophylline during paediatric extracorporeal membrane oxygenation. *Br.J.Clin.Pharmacol.* **55**, 23-31 (2003).
24. Peters JW, Anderson BJ, Simons SH, Uges DR, Tibboel D. Morphine pharmacokinetics during venoarterial extracorporeal membrane oxygenation in neonates. *Intensive Care Med.* **31**, 257-263 (2005).
25. Peters JW, Anderson BJ, Simons SH, Uges DR, Tibboel D. Morphine metabolite pharmacokinetics during venoarterial extra corporeal membrane oxygenation in neonates. *Clin.Pharmacokinet.* **45**, 705-714 (2006).
26. Intravenous morphine versus intravenous paracetamol in children on ECMO [Netherlands Trialregister Identifier NTR2180]. <http://www.trialregister.nl> (2012).
27. Groenendaal D, Freijer J, De Mik D, Bouw MR, Danhof M, De Lange EC. Population pharmacokinetic modelling of non-linear brain distribution of morphine: influence of active saturable influx and P-glycoprotein mediated efflux. *Br.J.Pharmacol.* **151**, 701-712 (2007).
28. Saunders NR, Habgood MD, Dziegielewska KM. Barrier mechanisms in the brain, II. Immature brain. *Clin.Exp.Pharmacol.Physiol* **26**, 85-91 (1999).
29. Schubert-Unkmeir A, Konrad C, Slanina H, Czapek F, Hebling S, Frosch M. Neisseria meningitidis induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8. *PLoS.Pathog.* **6**, e1000874 (2010).
30. Zimmermann A, Domoki F, Bari F. Seizure-induced alterations in cerebrovascular function in the neonate. *Dev.Neurosci.* **30**, 293-305 (2008).
31. Nandi R, Fitzgerald M. Opioid analgesia in the newborn. *Eur.J.Pain* **9**, 105-108 (2005).
32. Tomlinson D, Von Baeyer CL, Stinson JN, Sung L. A systematic review of faces scales for the self-report of pain intensity in children. *Pediatrics* **126**, e1168-e1198 (2010).
33. McGrath PJ *et al.* Core outcome domains and measures for pediatric acute and chronic/recurrent pain clinical trials: PedIMMPACT recommendations. *J.Pain* **9**, 771-783 (2008).
34. Van Dijk M, De Boer JB, Koot HM, Tibboel D, Passchier J, Duivenvoorden HJ. The reliability and validity of the COMFORT scale as a postoperative pain instrument in 0 to 3-year-old infants. *Pain* **84**, 367-377 (2000).
35. Van Dijk M *et al.* The association between physiological and behavioral pain measures in 0- to 3-year-old infants after major surgery. *J.Pain Symptom.Manage.* **22**, 600-609 (2001).
36. Van Dijk M, Peters JW, Bouwmeester NJ, Tibboel D. Are postoperative pain instruments useful for specific groups of vulnerable infants? *Clin.Perinatol.* **29**, 469-91, x (2002).
37. Stevens BJ, Johnston CC, Gibbins S. Pain assessment in neonates. *Pain in neonates* **2nd revise**, 101-134 (2012).
38. Franck LS, Greenberg CS, Stevens B. Pain assessment in infants and children. *Pediatr.Clin.North Am.* **47**, 487-512 (2000).

39. Oberlander T, Saul JP. Methodological considerations for the use of heart rate variability as a measure of pain reactivity in vulnerable infants. *Clin.Perinatol.* **29**, 427-443 (2002).
40. Simons SH *et al.* Randomised controlled trial evaluating effects of morphine on plasma adrenaline/ noradrenaline concentrations in newborns. *Arch.Dis.Child Fetal Neonatal Ed* **90**, F36-F40 (2005).
41. Bouwmeester NJ, Anand KJ, Van Dijk M, Hop WC, Boomsma F, Tibboel D. Hormonal and metabolic stress responses after major surgery in children aged 0-3 years: a double-blind, randomized trial comparing the effects of continuous versus intermittent morphine. *Br.J.Anaesth.* **87**, 390-399 (2001).
42. Harrison D, Boyce S, Loughnan P, Dargaville P, Storm H, Johnston L. Skin conductance as a measure of pain and stress in hospitalised infants. *Early Hum.Dev.* **82**, 603-608 (2006).
43. Anand KJ *et al.* Analgesia and sedation in preterm neonates who require ventilatory support: results from the NOPAIN trial. Neonatal Outcome and Prolonged Analgesia in Neonates. *Arch.Pediatr. Adolesc.Med.* **153**, 331-338 (1999).
44. Puchalski M, Hummel P. The reality of neonatal pain. *Adv.Neonatal Care* **2**, 233-244 (2002).
45. McPherson RJ, Gleason C, Mascher-Denen M, Chan M, Kellert B, Juul SE. A new model of neonatal stress which produces lasting neurobehavioral effects in adult rats. *Neonatology.* **92**, 33-41 (2007).
46. Boasen JF, McPherson RJ, Hays SL, Juul SE, Gleason CA. Neonatal stress or morphine treatment alters adult mouse conditioned place preference. *Neonatology.* **95**, 230-239 (2009).
47. Traudt CM, Tkac I, Ennis KM, Sutton LM, Mammel DM, Rao R. Postnatal morphine administration alters hippocampal development in rats. *J.Neurosci.Res.* **90**, 307-314 (2012).
48. Zhang GH, Sweitzer SM. Neonatal morphine enhances nociception and decreases analgesia in young rats. *Brain Res.* **1199**, 82-90 (2008).
49. Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology* **42**, 829-836 (2002).
50. Mao J, Sung B, Ji RR, Lim G. Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. *J.Neurosci.* **22**, 7650-7661 (2002).
51. Bryant L *et al.* Spinal ceramide and neuronal apoptosis in morphine antinociceptive tolerance. *Neurosci.Lett.* **463**, 49-53 (2009).
52. Ferguson SA, Ward WL, Paule MG, Hall RW, Anand KJ. A pilot study of preemptive morphine analgesia in preterm neonates: effects on head circumference, social behavior, and response latencies in early childhood. *Neurotoxicol.Teratol.* **34**, 47-55 (2012).
53. De Graaf J *et al.* Long-term effects of routine morphine infusion in mechanically ventilated neonates on children's functioning: five-year follow-up of a randomized controlled trial. *Pain* **152**, 1391-1397 (2011).
54. MacGregor R, Evans D, Sugden D, Gaussen T, Levene M. Outcome at 5-6 years of prematurely born children who received morphine as neonates. *Arch.Dis.Child Fetal Neonatal Ed* **79**, F40-F43 (1998).
55. Prins SA *et al.* Pharmacokinetics and analgesic effects of intravenous propacetamol vs rectal paracetamol in children after major craniofacial surgery. *Paediatr.Anaesth.* **18**, 582-592 (2008).
56. Van der Marel CD, Peters JW, Bouwmeester NJ, Jacqz-Aigrain E, Van den Anker JN, Tibboel D. Rectal acetaminophen does not reduce morphine consumption after major surgery in young infants. *Br.J.Anaesth.* **98**, 372-379 (2007).
57. Ceelie I. Pain; Postoperative analgesia in infants and neonates. 69-86 (2011).
58. Michelet D *et al.* A meta-analysis of the use of nonsteroidal antiinflammatory drugs for pediatric postoperative pain. *Anesth.Analg.* **114**, 393-406 (2012).

59. Stevens B, Yamada J, Ohlsson A. Sucrose for analgesia in newborn infants undergoing painful procedures. *Cochrane.Database.Syst.Rev.* CD001069 (2010).
60. Harrison D, Yamada J, Adams-Webber T, Ohlsson A, Beyene J, Stevens B. Sweet tasting solutions for reduction of needle-related procedural pain in children aged one to 16 years. *Cochrane.Database.Syst.Rev.* CD008408 (2011).
61. Pillai Riddell RR *et al.* Non-pharmacological management of infant and young child procedural pain. *Cochrane.Database.Syst.Rev.* CD006275 (2011).
62. Lago P *et al.* Guidelines for procedural pain in the newborn. *Acta Paediatr.* **98**, 932-939 (2009).
63. Danhof M, De Lange EC, Della Pasqua OE, Ploeger BA, Voskuyl RA. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modeling in translational drug research. *Trends Pharmacol.Sci.* **29**, 186-191 (2008).
64. De Cock RFW *et al.* Maturation of GFR in preterm and term neonates reflected by clearance of different antibiotics. *PAGE 20 Abstr* **2096**, (2011).
65. Miners JO, Knights KM, Houston JB, Mackenzie PI. In vitro-in vivo correlation for drugs and other compounds eliminated by glucuronidation in humans: pitfalls and promises. *Biochem.Pharmacol.* **71**, 1531-1539 (2006).
66. Hengstler JG *et al.* Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metab Rev.* **32**, 81-118 (2000).
67. McGinnity DF, Soars MG, Urbanowicz RA, Riley RJ. Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. *Drug Metab Dispos.* **32**, 1247-1253 (2004).
68. Steinberg P *et al.* Drug metabolizing capacity of cryopreserved human, rat, and mouse liver parenchymal cells in suspension. *Drug Metab Dispos.* **27**, 1415-1422 (1999).
69. Li AP. Human hepatocytes: isolation, cryopreservation and applications in drug development. *Chem.Biol.Interact.* **168**, 16-29 (2007).
70. Strassburg CP *et al.* Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut* **50**, 259-265 (2002).
71. Basu NK, Kole L, Owens IS. Evidence for phosphorylation requirement for human bilirubin UDP-glucuronosyltransferase (UGT1A1) activity. *Biochem.Biophys.Res.Comm.* **303**, 98-104 (2003).
72. Barbier O, Girard C, Breton R, Belanger A, Hum DW. N-glycosylation and residue 96 are involved in the functional properties of UDP-glucuronosyltransferase enzymes. *Biochemistry* **39**, 11540-11552 (2000).
73. Mackenzie PI. The effect of N-linked glycosylation on the substrate preferences of UDP glucuronosyltransferases. *Biochem.Biophys.Res.Comm.* **166**, 1293-1299 (1990).
74. Ishii Y, Takeda S, Yamada H. Modulation of UDP-glucuronosyltransferase activity by protein-protein association. *Drug Metab Rev.* **42**, 140-153 (2010).
75. Castuma CE, Brenner RR. The influence of fatty acid unsaturation and physical properties of microsomal membrane phospholipids on UDP-glucuronosyltransferase activity. *Biochem.J.* **258**, 723-731 (1989).
76. Zaya MJ, Hines RN, Stevens JC. Epirubicin glucuronidation and UGT2B7 developmental expression. *Drug Metab Dispos.* **34**, 2097-2101 (2006).
77. Brendel K *et al.* Are population pharmacokinetic and/or pharmacodynamic models adequately evaluated? A survey of the literature from 2002 to 2004. *Clin.Pharmacokinet.* **46**, 221-234 (2007).
78. Tod M, Jullien V, Pons G. Facilitation of drug evaluation in children by population methods and modelling. *Clin.Pharmacokinet.* **47**, 231-243 (2008).

79. Bouwmeester NJ, Anderson BJ, Tibboel D, Holford NH. Developmental pharmacokinetics of morphine and its metabolites in neonates, infants and young children. *Br.J.Anaesth.* **92**, 208-217 (2004).
80. Anand KJ *et al.* Morphine pharmacokinetics and pharmacodynamics in preterm and term neonates: secondary results from the NEOPAIN trial. *Br.J.Anaesth.* **101**, 680-689 (2008).
81. Edginton AN, Schmitt W, Voith B, Willmann S. A mechanistic approach for the scaling of clearance in children. *Clin.Pharmacokinet.* **45**, 683-704 (2006).
82. Anderson BJ, Holford NH. Mechanistic basis of using body size and maturation to predict clearance in humans. *Drug Metab Pharmacokinet.* **24**, 25-36 (2009).
83. Dodds PS, Rothman DH, Weitz JS. Re-examination of the “3/4-law” of metabolism. *J.Theor.Biol.* **209**, 9-27 (2001).
84. Agutter PS, Wheatley DN. Metabolic scaling: consensus or controversy? *Theor.Biol.Med.Model.* **1**, 13 (2004).
85. Bokma F. Evidence against universal metabolic allometry. *Functional Ecology* **18**, 184-187 (2004).
86. Glazier DS. Beyond the “3/4-power law”: variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews* **80**, 611-662 (2005).
87. White CR, Cassey P, Blackburn TM. Allometric exponents do not support a universal metabolic allometry. *Ecology* **88**, 315-323 (2007).
88. Kolokotronis T, Van S, Deeds EJ, Fontana W. Curvature in metabolic scaling. *Nature* **464**, 753-756 (2010).
89. Mahmood I. Prediction of drug clearance in children from adults: a comparison of several allometric methods. *Br.J.Clin.Pharmacol.* **61**, 545-557 (2006).
90. Mahmood I. Prediction of drug clearance in children: impact of allometric exponents, body weight, and age. *Ther.Drug Monit.* **29**, 271-278 (2007).
91. Ivaturi VD, Hooker AC, Karlsson MO. Selection bias in pre-specified covariate models. *PAGE 20 Abstr* **2228**, (2012).
92. Khandelwal A, Hooker AC, Karlsson MO. Influence of correlated covariates on predictive performance for different models. *PAGE 20 Abstr* **2220**, (2012).
93. Barrett DA, Barker DP, Rutter N, Pawula M, Shaw PN. Morphine, morphine-6-glucuronide and morphine-3-glucuronide pharmacokinetics in newborn infants receiving diamorphine infusions. *Br.J.Clin.Pharmacol.* **41**, 531-537 (1996).
94. Roka A, Melinda KT, Vasarhelyi B, Machay T, Azzopardi D, Szabo M. Elevated morphine concentrations in neonates treated with morphine and prolonged hypothermia for hypoxic ischemic encephalopathy. *Pediatrics* **121**, e844-e849 (2008).
95. Choonara I, Lawrence A, Michalkiewicz A, Bowhay A, Ratcliffe J. Morphine metabolism in neonates and infants. *Br.J.Clin.Pharmacol.* **34**, 434-437 (1992).
96. Haberkern CM. *et al.* Epidural and intravenous bolus morphine for postoperative analgesia in infants. *Can.J.Anaesth.* **43**, 1203-1210 (1996).
97. Hain RD, Hardcastle A, Pinkerton CR, & Aherne GW. Morphine and morphine-6-glucuronide in the plasma and cerebrospinal fluid of children. *Br.J.Clin.Pharmacol.* **48**, 37-42 (1999).
98. Mashayekhi SO, Ghandforoush-Sattari M, Routledge PA, Hain RD. Pharmacokinetic and pharmacodynamic study of morphine and morphine 6-glucuronide after oral and intravenous administration of morphine in children with cancer. *Biopharm.Drug Dispos.* **30**, 99-106 (2009).
99. Kopecky EA, Jacobson S, Joshi P, Koren G. Systemic exposure to morphine and the risk of acute chest syndrome in sickle cell disease. *Clin.Pharmacol.Ther.* **75**, 140-146 (2004).

100. Dampier CD, Setty BN, Logan J, Ioli JG, Dean R. Intravenous morphine pharmacokinetics in pediatric patients with sickle cell disease. *J.Pediatr.* **126**, 461-467 (1995).
101. Chay PC, Duffy BJ, Walker JS. Pharmacokinetic-pharmacodynamic relationships of morphine in neonates. *Clin.Pharmacol.Ther.* **51**, 334-342 (1992).
102. Mikkelsen S, Feilberg VL, Christensen CB, Lundstrom KE. Morphine pharmacokinetics in premature and mature newborn infants. *Acta Paediatr.* **83**, 1025-1028 (1994).
103. Hartley R, Green M, Quinn M, Levene MI. Pharmacokinetics of morphine infusion in premature neonates. *Arch.Dis.Child* **69**, 55-58 (1993).
104. Saarenmaa E, Neuvonen PJ, Rosenberg P, Fellman V. Morphine clearance and effects in newborn infants in relation to gestational age. *Clin.Pharmacol.Ther.* **68**, 160-166 (2000).
105. Barrett DA, Elias-Jones AC, Rutter N, Shaw PN, Davis SS. Morphine kinetics after diamorphine infusion in premature neonates. *Br.J.Clin.Pharmacol.* **32**, 31-37 (1991).
106. Scott CS *et al.* Morphine pharmacokinetics and pain assessment in premature newborns. *J.Pediatr.* **135**, 423-429 (1999).
107. Pokela ML, Olkkola KT, Seppala T, Koivisto M. Age-related morphine kinetics in infants. *Dev. Pharmacol.Ther.* **20**, 26-34 (1993).
108. Lynn AM, Nespeca MK, Bratton SL, Shen DD. Intravenous morphine in postoperative infants: intermittent bolus dosing versus targeted continuous infusions. *Pain* **88**, 89-95 (2000).
109. McRorie TI, Lynn AM, Nespeca MK, Opheim KE, Slattery JT. The maturation of morphine clearance and metabolism. *Am.J.Dis.Child* **146**, 972-976 (1992).
110. Robieux IC *et al.* Analgesia in children with sickle cell crisis: comparison of intermittent opioids vs. continuous intravenous infusion of morphine and placebo-controlled study of oxygen inhalation. *Pediatr.Hematol.Oncol.* **9**, 317-326 (1992).
111. Choonara IA, McKay P, Hain R, Rane A. Morphine metabolism in children. *Br.J.Clin.Pharmacol.* **28**, 599-604 (1989).
112. Hartley R, Green M, Quinn MW, Rushforth JA, Levene MI. Development of morphine glucuronidation in premature neonates. *Biol.Neonate* **66**, 1-9 (1994).
113. Boucher FD *et al.* Phase I evaluation of zidovudine administered to infants exposed at birth to the human immunodeficiency virus. *J.Pediatr.* **122**, 137-144 (1993).
114. Mirochnick M, Capparelli E, Connor J. Pharmacokinetics of zidovudine in infants: a population analysis across studies. *Clin.Pharmacol.Ther.* **66**, 16-24 (1999).
115. Capparelli EV *et al.* Population pharmacokinetics and pharmacodynamics of zidovudine in HIV-infected infants and children. *J.Clin.Pharmacol.* **43**, 133-140 (2003).