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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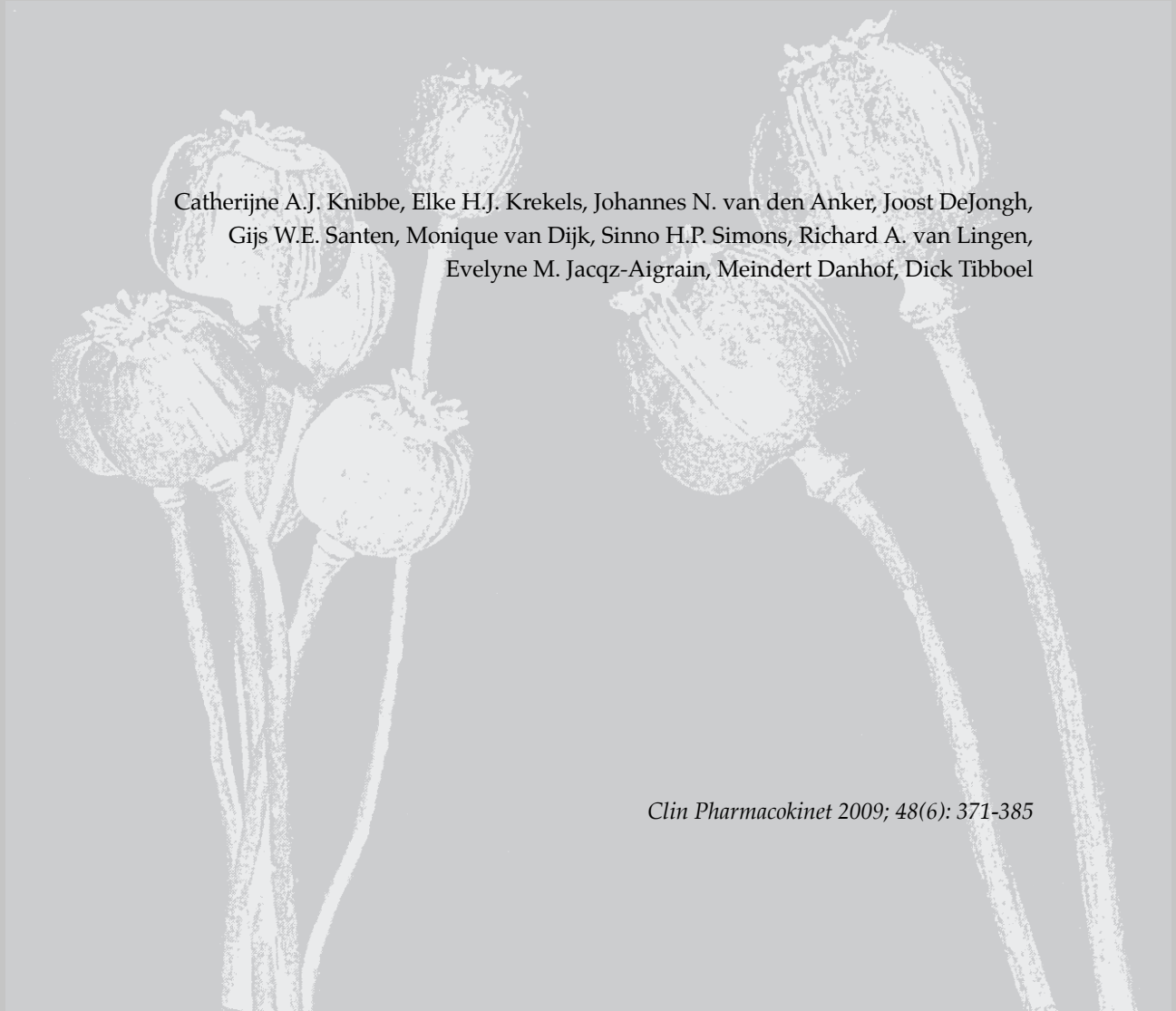

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Abstract

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.

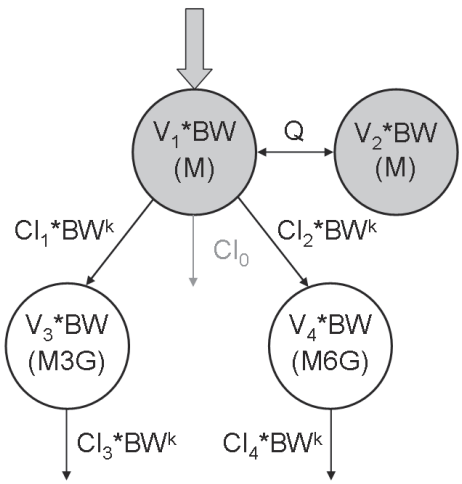


Figure 1. *Schematic representation of the pharmacokinetic model for morphine and its glucuronides. M=morphine, M3G=morphine-3-glucuronide, M6G=morphine-6-glucuronide, V_1=volume of distribution of central compartment of M, V_2= volume of distribution of peripheral compartment of M, V_3=volume of distribution 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

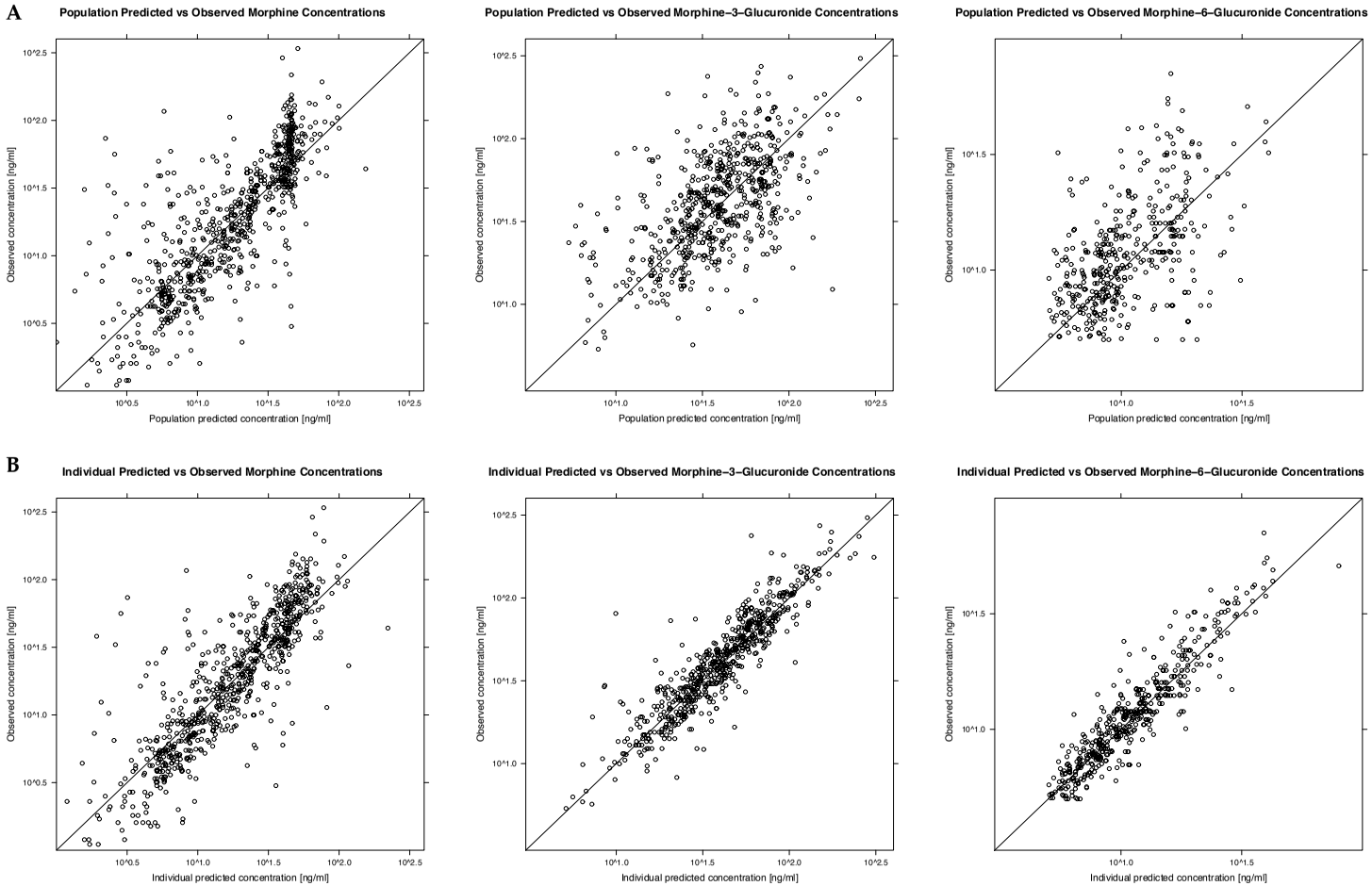


Figure 2. *Observed versus population predicted (a) and individual predicted (b) morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations.*

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.

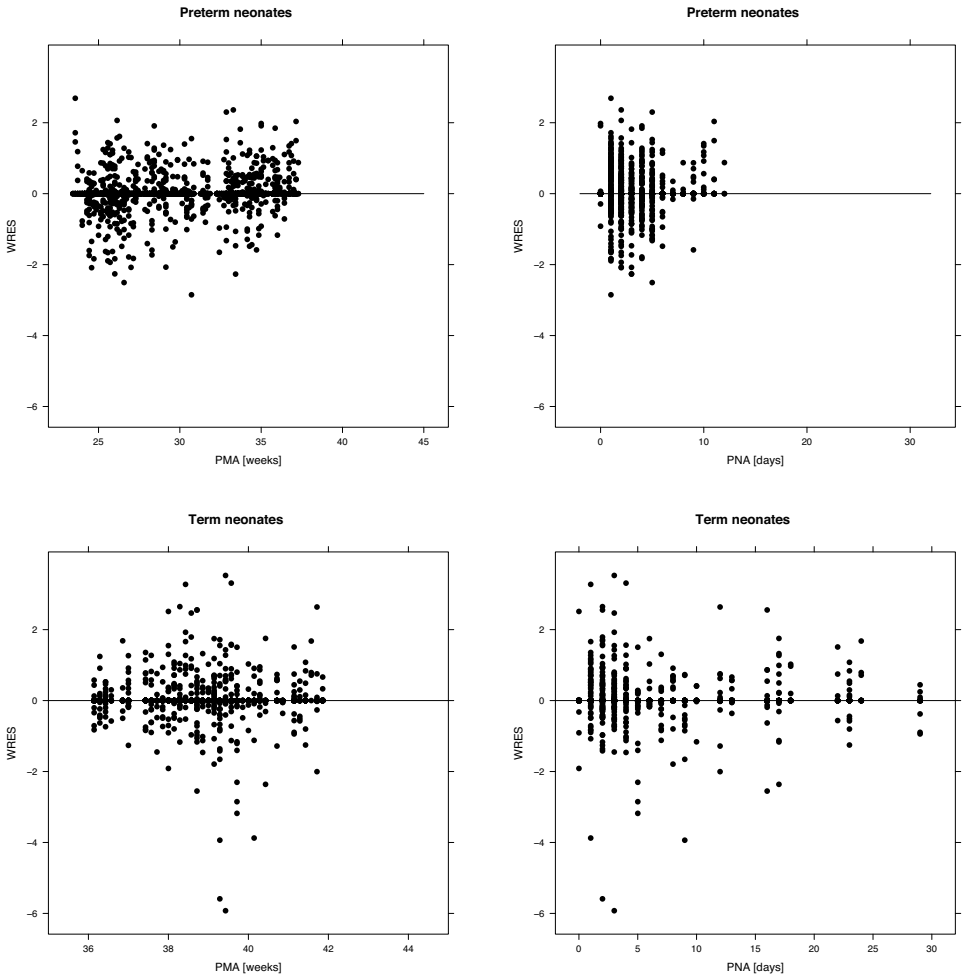


Figure 3. *Weighted residuals (WRES) for term and preterm neonates plotted versus postnatal age (PNA) and postmenstrual age (PMA).*

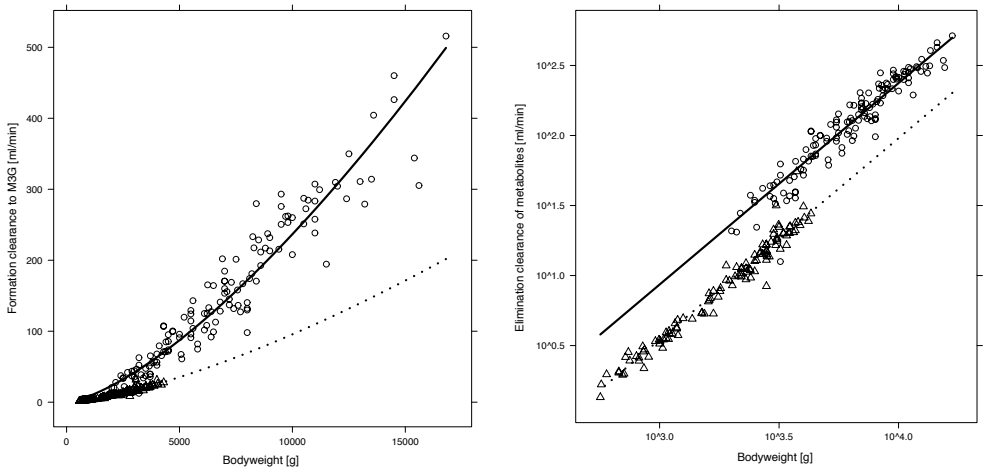


Figure 4. *Morphine formation clearance to morphine-3-glucuronide (M3G) (=Cl_1) versus bodyweight. Population prediction in children younger than ten days (dotted line) and older than ten days (solid line) and individual obtained values in children younger than ten days (triangles) and older than ten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

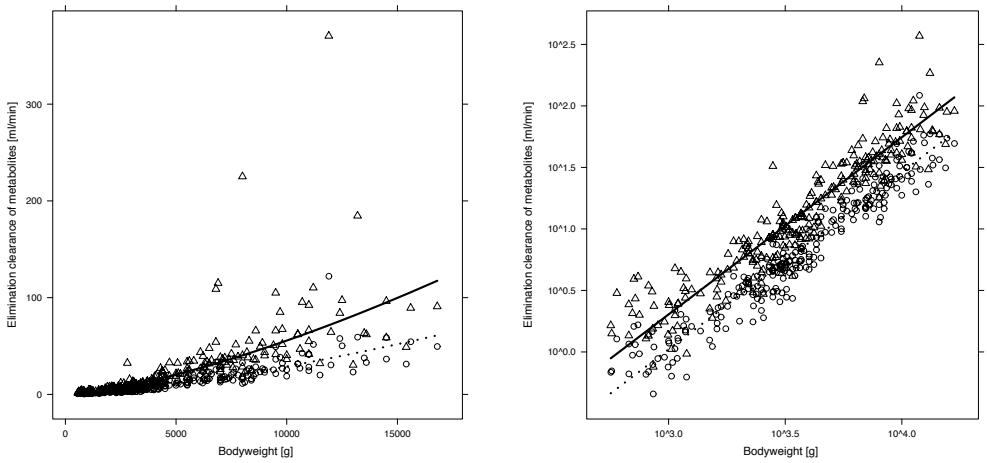
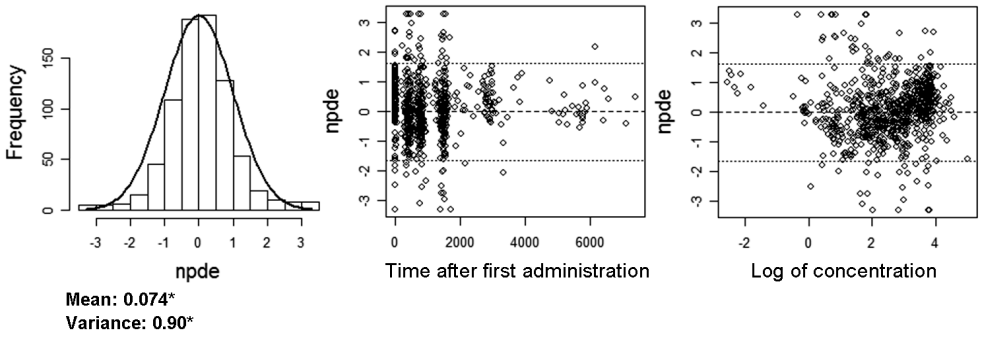
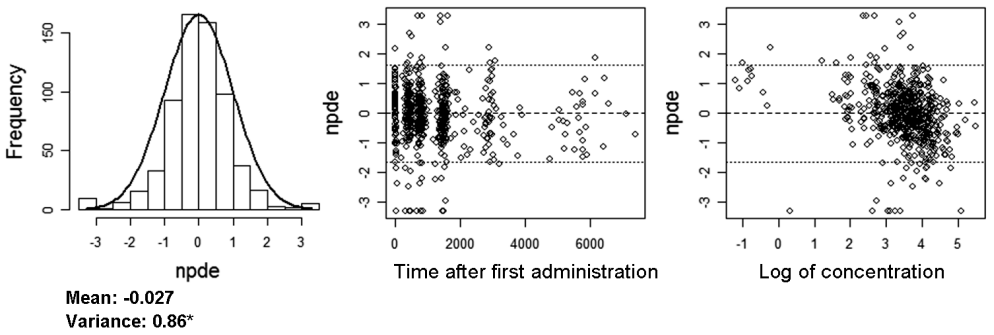


Figure 5. *Elimination clearance of morphine-3-glucuronide (M3G) (=Cl_3) and morphine-6-glucuronide (M6G) (=Cl_4) versus bodyweight. Lines are population predicted (solid for CL_3 and dotted for CL_4), symbols are individual obtained values (triangles for CL_3 and circles for CL_4), on linear scale (left)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

morphine



morphine-3-glucuronide



morphine-6-glucuronide

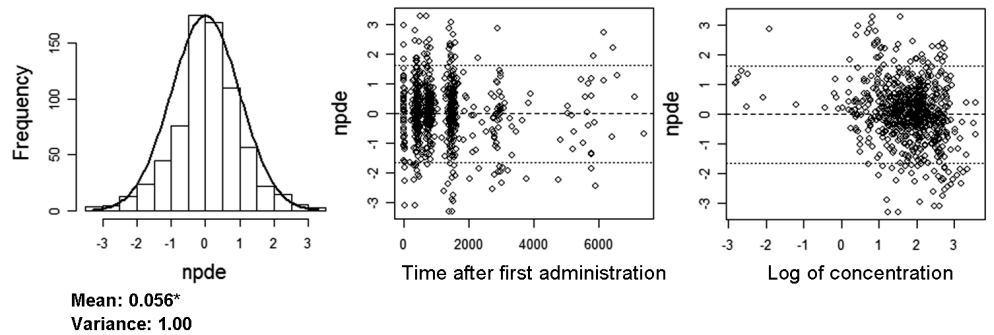


Figure 6. *Results of the internal validation with the Normalized Prediction Distribution Error (NPDE) method. The histograms show the NPDE distribution for morphine (top), morphine-3-glucuronide (middle), and morphine-6-glucuronide (bottom) and the solid line indicates a normal distribution. The value 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.*

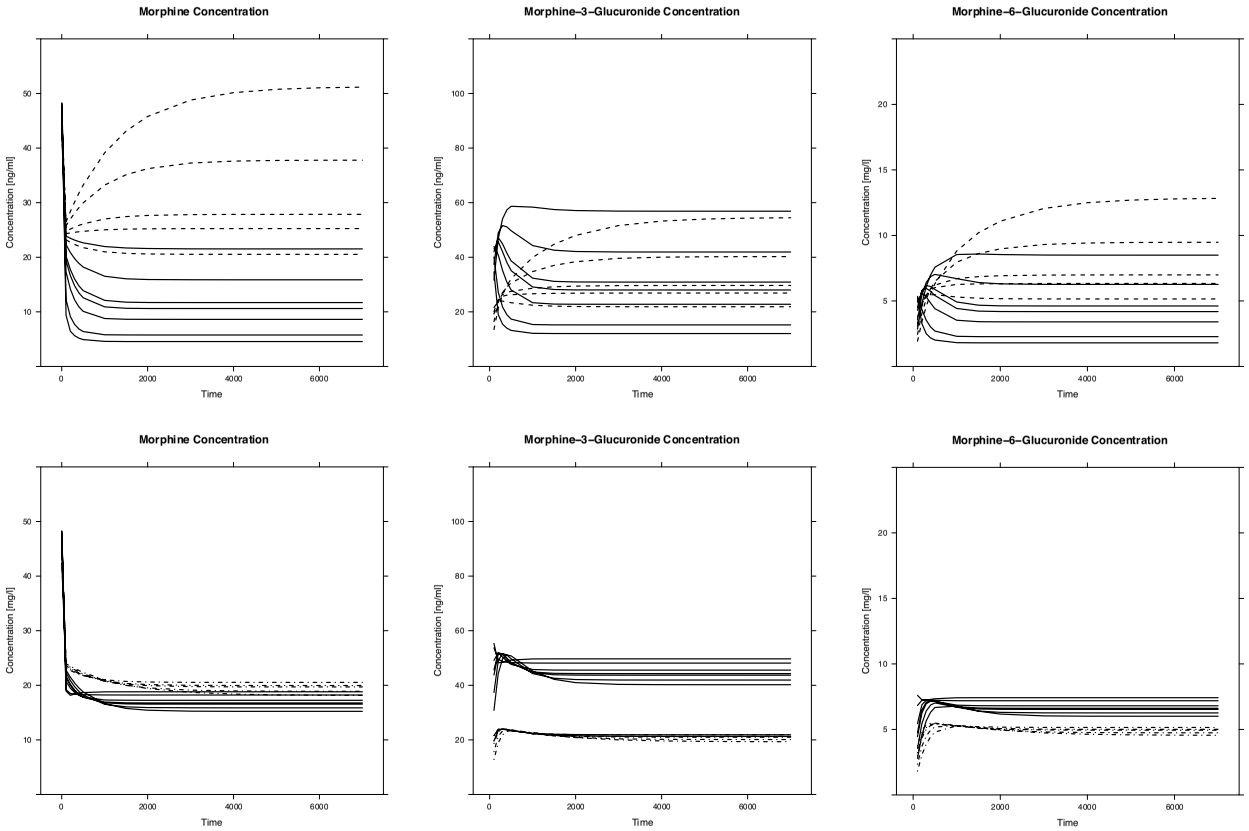


Figure 7. *Morphine, morphine-3-glucuronide, and morphine-6-glucuronide concentrations predicted in model-based simulations in children of 0.5, 1, 2, 2.5, 4, 10 and 17 kg and a postnatal age of less than ten days (dotted lines) or more than ten days (solid lines) based on a dosing regimen with a loading 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.

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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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