



Universiteit
Leiden
The Netherlands

First-pass and systemic metabolism of cytochrome P450 3A substrates in neonates, infants, and children

Brussee, J.M.

Citation

Brussee, J. M. (2018, December 4). *First-pass and systemic metabolism of cytochrome P450 3A substrates in neonates, infants, and children*. Retrieved from <https://hdl.handle.net/1887/67291>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/67291>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/67291> holds various files of this Leiden University dissertation.

Author: Brussee, J.M.

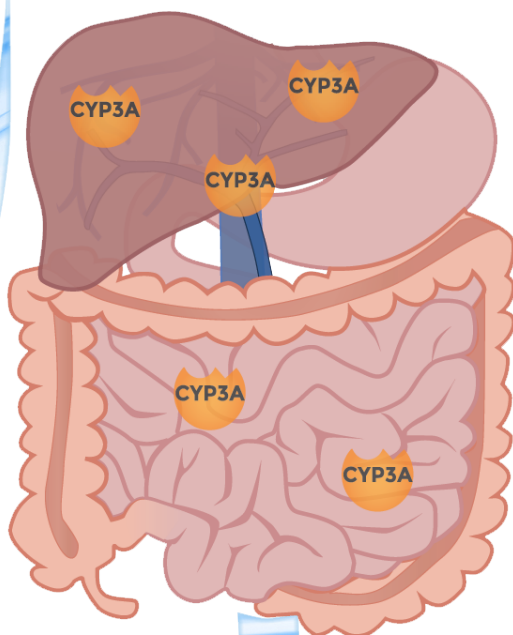
Title: First-pass and systemic metabolism of cytochrome P450 3A substrates in neonates, infants, and children

Issue Date: 2018-12-04



Section IV

Midazolam as probe drug for other
CYP3A substrates



Chapter 7

A pediatric covariate function for CYP3A-mediated midazolam clearance to scale clearance of selected CYP3A substrates in children

Janneke M Brussee¹, Elke HJ Krekels¹, Elisa AM Calvier¹, Semra Palić^{1,2},
Amin Rostami-Hodjegan^{3,4}, Meindert Danhof¹, Jeffrey S Barrett^{5,6},
Saskia N de Wildt^{7,8}, Catherijne AJ Knibbe^{1,9}

¹Division of Systems Biomedicine and Pharmacology, Leiden Academic Centre for Drug Research (LACDR), Leiden University, Leiden, the Netherlands; ²Current affiliation: Dutch Cancer Institute (NKI), Amsterdam, the Netherlands; ³Centre for Applied Pharmacokinetic Research, University of Manchester, Manchester, UK; ⁴Simcyp Limited (A Certara Company), Sheffield, UK; ⁵Translational Informatics, Sanofi, Bridgewater, NJ, USA; ⁶Department of Pediatrics, Division of Clinical Pharmacology & Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA, USA; ⁷Department of Pharmacology and Toxicology, Radboud University Medical Centre, Nijmegen, the Netherlands; ⁸Intensive Care and Department of Pediatric Surgery, Erasmus MC - Sophia Children's Hospital, Rotterdam, the Netherlands; ⁹Department of Clinical Pharmacy, St. Antonius Hospital, Nieuwegein, the Netherlands

Manuscript in preparation

ABSTRACT

Recently a framework was presented to assess, based on drug properties, whether pediatric covariate models for clearance can be extrapolated between drugs sharing elimination pathways. Our aim is to evaluate when a pediatric covariate function for midazolam clearance can be used to scale clearance of other CYP3A substrates.

A population PK model including a covariate function for clearance was developed for midazolam in children aged 1-17 years. Commonly used CYP3A substrates were selected and using the framework, it was assessed whether the midazolam covariate function can accurately scale their clearance. For eight substrates, reported adult and pediatric clearance values were compared numerically and graphically with clearance values scaled using the midazolam covariate function. For sildenafil clearance values obtained with population PK modeling based on pediatric concentration-time data were compared to those obtained with the midazolam covariate function.

According to the framework, a covariate function for midazolam clearance will at least yield systematically accurate (absolute prediction error (PE) <30%) clearance scaling in children of all ages for CYP3A substrates with an extraction ratio of 0.35-0.65 or 0.05-0.55 for drugs binding <10% or >90% to albumin in adults, respectively. The results show that scaled clearance values for eight commonly used CYP3A substrates were reasonably accurate (PE <50%). Scaling of sildenafil clearance was accurate (PE <30%).

We defined for which CYP3A substrates a pediatric covariate function for midazolam clearance can accurately scale plasma clearance in children. This scaling approach is useful for CYP3A substrates with scarce or no available pediatric PK information.

Keywords

Pediatrics, clearance, CYP3A ontogeny, scaling function, population pharmacokinetics

INTRODUCTION

To define the optimal first-in-child dose during drug development and to develop pediatric dose recommendations for clinical practice, accurate scaling of the plasma clearance of drugs is essential (1-3). This is of particular relevance as it would take many resources to perform dedicated pharmacokinetic (PK) studies for all drugs in all pediatric (sub)populations and as this is even unethical when other methods are possible. One proposed approach shares PK information of drugs eliminated by the same pathway by extrapolating covariate relationships for clearance between drugs (4). This has been successfully applied for scaling pediatric clearance for drugs glucuronidated by UGT2B7 and drugs eliminated by glomerular filtration (4-6).

Within this context, recently, a framework was presented for hepatically metabolized drugs identifying the conditions for which between-drug extrapolation is systematically accurate (7). This framework takes into account changes in physiological parameters with age, including changes in (hepatic) blood flow, plasma protein concentrations, hematocrit, liver size, the amount of microsomal protein per gram of liver, and the ontogeny of isoenzyme expression (the microsomal intrinsic clearance)(7). Based on this framework, it was shown that the accuracy of this scaling method depends on the fraction metabolized by the isoenzyme pathway for which plasma clearance is scaled, on the hepatic extraction ratio of both drugs in adults, on the type of binding plasma protein, and on the unbound drug fraction (f_u) in adults (7).

As many drugs are eliminated by the cytochrome P450 (CYP) 3A enzyme family (8, 9), a pediatric covariate function for CYP3A-mediated clearance may aid in scaling clearance of CYP3A substrates. Midazolam is an established probe drug for CYP3A-mediated clearance (10, 11) and has an intermediate extraction ratio (12). Our aim is to evaluate when a pediatric covariate function for midazolam clearance can be used to scale clearance of other CYP3A substrates in children, taking into account the recent insights of the developed framework (7).

METHODS

Overall approach

A population PK model for midazolam in children was developed based on concentration-time data, to establish a pediatric covariate function for midazolam clearance. Next, we selected a range of drugs that are CYP3A substrates that are

commonly prescribed in children, covering compounds prescribed for varying indications in different therapeutic areas, with oral or intravenous administration, and with different drug properties, i.e. alprazolam (13), atorvastatin (14), cisapride (15), domperidone (16), quinidine (17), sildenafil (18), simvastatin (19), solifenacin (20), sufentanil (21), sirolimus (22), tacrolimus (23), tamsulosin (24), and vincristine (25). Based on the drug properties of these CYP3A substrates, we used the framework of Calvier *et al.*(7) to define to which age the covariate function for midazolam can be used for accurate scaling of pediatric clearance of the CYP3A substrates from adult clearance values. For eight of the selected CYP3A substrates pediatric and adult clearance values were available in literature, allowing for the assessment of the accuracy of the scaling function by comparing pediatric clearance values that were scaled from adult clearance values using the covariate function for midazolam to the published literature clearance values in children. Furthermore, for sildenafil, concentration-time data were available from 156 children (26). Using these data, we developed two pediatric population PK models for sildenafil; one using the pediatric covariate function of midazolam clearance directly and one in which the covariate relationship for clearance was optimized using a data-driven analysis, after which the performance of both models, as well as the estimated and scaled clearance values, were compared.

Table I. Study and patient characteristics of the midazolam and sildenafil PK studies

	Midazolam	Sildenafil
Indication	Pre-operatively	Pulmonary arterial hypertension
Number of patients	31	156
Number of samples	327	591
Samples/patient*	10 (8-11)	4 (1-4)
Age (years)*	8 (1-17)	10 (1-17)
Body weight (kg)*	30.2 (9.5-83.2)	28.0 (8.2-106.0)
Male/female, n (%)	15/16 (48/52%)	57/99 (37/63%)
Dose (mg)*	12.5 (3-15)	20 (10-80)

*median (range)

Midazolam population PK model

Midazolam PK data were available from 31 patients (15 male, 16 female) from the Children's Hospital of Philadelphia, PA (Table I), with a median age of 8 years of age (range 1-17 years) and a median body weight of 30.2 kg (range 9.5-83.2 kg) (27). Before participation, signed informed consent by the subject's parents or guardian, and assent were obtained. Children undergoing surgery were included if they met the criteria I or II of the American Society of Anesthesiologist's (ASA) classification. A median dose of 12.5 mg (range 3-15 mg) of midazolam was administered as oral

suspension (5 mg/mL, Roche Laboratories) to the patients pre-operatively. Blood was densely sampled for midazolam plasma concentrations around 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 22 hours after dose administration, with a median of 10 samples per patient (range 8-11). Blood was centrifuged and plasma samples stored at <-20°C until midazolam plasma concentrations were determined using LC/MS (27).

A population PK model was developed using non-linear mixed effects modelling (NONMEM version 7.3, ICON, Globomax LLC, Ellicott, MD, USA; Perl-speaks-NONMEM (PsN) version 4.2.0, Uppsala, Sweden; and Pirana 2.9.0, Pirana Software & Consulting BV, Denekamp, the Netherlands) based on first-order conditional estimation with interaction. R (version 3.3.1) and R-studio (version 0.98.1078) were used for data visualization. Several structural models were considered, including 1, 2 and 3-compartmental models, and evaluated based on criteria for model stability, goodness-of-fit, and parameter precision, and on comparisons of the objective function values (OFV, $-2 \times \log\text{-likelihood}$), using a significance level of $p < 0.05$. The absorption rate could not be estimated and was therefore fixed at 3.5 h^{-1} , which results in a $t_{\max}$ around 0.5 hours post-dose, which is in agreement with known values.

Inter-individual variability in the estimated parameters for clearance and central volume of distribution was included in the model by the following equation:

$$P_i = \theta_{TV} \times e^{\eta_i} \quad (\text{Eq. 1})$$

In which P_i is the individual parameter estimate for individual i , θ_{TV} is the typical value of the parameter in the studied population and η_i is a random variable for the i th individual from a normal distribution with a mean of zero and variance of ω^2 , assuming a log-normal distribution for the parameter value in the population.

To describe residual unexplained variability, a proportional error model, an additive error model, and a combination of the proportional and additive error were considered. The j th observed concentration of the i th individual (Y_{ij}) was modeled according to:

$$Y_{ij} = C_{\text{pred},ij} \times (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad (\text{Eq. 2})$$

where $C_{\text{pred},ij}$ is the j th predicted midazolam concentration of the i th individual, and ε_{ij} is a random variable from a normal distribution with a mean of zero and variance of σ^2 , with ε_1 the proportional error and ε_2 the additive error.

A systematic covariate analysis was performed for the estimated model parameters in which age, body weight, and sex were tested for statistical significance. For sex, the typical value (θ_{TV}) for girls was estimated relative to the value for boys. The remaining continuous covariates body weight and age were tested using a power (Eq. 3) function:

$$P_i = \theta_{TV} \times \left(\frac{COV_i}{COV_{med}} \right)^{\theta_{cov}} \times e^{\eta_i} \quad (\text{Eq. 3})$$

where P_i is individual parameter estimate for individual i with a covariate value of COV_i , θ_{TV} is the parameter value for a typical individual with a median covariate value (COV_{med}), θ_{COV} is the estimated exponent and η_i is a random variable as described above (eq. 1). For the forward inclusion of a covariate, a drop in OFV by at least 6.64 points ($p < 0.01$) was considered statistically significant, while for the backward deletion a more stringent p value ($p < 0.005$, $\Delta OFV > 7.88$) was used. In addition, the inter-individual variability in the PK parameter or the residual variability should decrease for a covariate to be retained in the model.

To evaluate whether the model described the observed concentrations well, goodness-of-fit plots were assessed. These diagnostic plots include observed versus population- and individual-predicted concentrations and conditional weighted residuals (CWRES) versus population predicted concentrations and versus time. To evaluate model stability and parameter precision, a bootstrap analysis ($n=250$) was performed. Finally, a normalized prediction distribution error (NPDE) analysis was performed using the NPDE package in R (28), with $n=1000$ simulations to evaluate whether the model can accurately predict the concentration and captures the observed variability.

Between-drug extrapolation potential of midazolam clearance to other CYP3A substrates

The previously published framework on between drug extrapolation of covariate functions (7) was used to assess, based on the drug properties of CYP3A substrates, whether between-drug extrapolation of the covariate relationship for midazolam would lead to accurate scaling of the pediatric clearance of the selected CYP3A substrates. For this, the relevant drug properties, i.e. the extraction ratio, the plasma protein to which the drug is binding, and the f_u for midazolam and the selected drugs were obtained from literature. In this analysis, the selected drugs were assumed to exclusively bind to either human serum albumin (HSA) or $\alpha 1$ -acid glycoprotein (AAG), while midazolam was either assumed to bind to HSA (for comparison with HSA-binding drugs) or to AAG (for comparison with AAG-binding drugs).

Using the difference in extraction ratio and the f_u between midazolam and the selected CYP3A-substrates that were considered within the results from the framework (7), it was assessed to what age clearance scaling with the covariate function of midazolam would certainly be accurate for the selected drugs. For this, we assumed CYP3A metabolism to be responsible for $\geq 75\%$ of the total metabolism for both midazolam and all selected substrates. Based on the extraction ratio and f_u from midazolam and the difference in these values that, according to the framework, are known to lead to systematically accurate scaling across the entire

pediatric age range, we also derived general criteria for accurate clearance scaling for CYP3A substrates using the covariate function for midazolam clearance.

Comparison of scaled versus reported pediatric clearance values

For the selected CYP3A substrates for which both pediatric and adult clearance values were reported in literature, we applied the pediatric covariate function for midazolam clearance to the reported adult clearance values to scale to pediatric clearance values. For this we assumed that typical adults have a body weight of 70 kg. We graphically compared the scaled typical clearance values with the reported pediatric clearance values. Moreover, we calculated the prediction error (PE) for three typical subjects (an infant of 10 kg, a child of 20 kg, and an adolescent of 50 kg) based on literature values for pediatric clearance using equation 4:

$$PE = \frac{CL_{scaled} - CL_{ref}}{CL_{ref}} \times 100\% \quad (\text{Eq. 4})$$

with CL_{scaled} the scaled clearance value and CL_{ref} the reported pediatric clearance. An absolute PE of <30% was considered accurate, an absolute PE of 30-50% reasonably accurate, and an absolute PE of $\geq 50\%$ inaccurate.

Sildenafil population PK models

Sildenafil PK data from a previously published study (26) were made available by Pfizer Inc. In this study, sildenafil PK data were collected from 156 (57 male, 99 female) patients in a randomized, double-blind, placebo controlled, dose ranging, parallel group study of oral sildenafil for the treatment of children with pulmonary arterial hypertension (26, 29). Subjects included children ranging in age from 1-17 years (median 10 years), with a median body weight of 28.0 kg (range 8.2-106 kg) (Table I). A median of 4 samples per patient (range 1-4) were available, with a total of 591 measurements available for analysis. Samples were taken at steady-state at through and around 3, 6 and 8 hours post-dose. Patients were randomly assigned to a low, medium or high dose group (n=39, n=48 and n=69, respectively), and the dosages were weight-stratified, with a medium dose of 10, 20 and 40 mg and a high dose of 20, 40 and 80 mg for patients of 8-20 kg, 20-45 kg or >45 kg, respectively. The low dose was 10 mg for all patients >20kg, and patients with a body weight ≤ 20 kg received either a medium or high dose, as no drug effect was expected with a lower dose than 10 mg (26, 29). In the population PK analysis, the samples without recorded sampling times were excluded.

Based on these data, a 'reference model' was developed in the same manner as described for midazolam. The absorption rate constant could not be estimated and was therefore fixed at 1 h^{-1} , leading to a maximum concentration around 2 hours post-dose, which was before the first sample was taken.

The extrapolation potential of the covariate function for midazolam clearance was evaluated in a second population PK model referred to as the ‘extrapolation model’. This model was kept the same as the reference model, except the clearance was not estimated, but scaled from an apparent CL/F value of 100 L/h for adults, which was derived from reported systemic clearance and oral bioavailability values of 41 L/h and 0.41, respectively (30), using the covariate function for midazolam clearance. We assumed the same bioavailability in adults and pediatric patients.

The reference and extrapolation model were evaluated in the same manner as the midazolam PK model (see under Midazolam population PK model).

Sildenafil clearance values from the sildenafil ‘reference model’ (CL_{ref}) and the sildenafil ‘extrapolation model’ (CL_{scaled}) were compared graphically. For a numerical comparison of both sildenafil models, typical clearance values for three typical subjects (an infant of 10 kg, a child of 20 kg, and an adolescent of 50 kg) were calculated, and a PE for clearance was calculated using equation 4.

RESULTS

Midazolam population PK model

For midazolam, a two-compartmental model with body weight included in an exponential covariate relationship on clearance, volumes of distribution, and

Table II. Model parameter estimates for the midazolam PK model and the bootstrap results based on n=250 resampling

Parameter		Model estimate (RSE)	Bootstrap median (90 CI)
Midazolam clearance [†]	$CL_{30.2 \text{ kg}}$ (L/h)	102.6 (9%)	101.4 (89.1-118.2)
$CL_i = CL_{30.2 \text{ kg}} * (WT_i/30.2)^{k1}$	k1	0.874 (13%)	0.901 (0.698-1.11)
Volume of distribution [†]	$V_{c30.2 \text{ kg}}$ (L)	156 (25%)	141 (76.5-210)
$V_{c,i} = V_{30.2 \text{ kg}} * (WT_i/30.2)^{k2}$	k2	1.88 (17%)	2.15 (1.43-3.30)
Peripheral volume [†]	$V_{p,30.2 \text{ kg}}$ (L)	255 (14%)	252 (197-338)
$V_{p,i} = V_{30.2 \text{ kg}} * (WT_i/30.2)^{k3}$	k3	0.91 (23%)	0.88 (0.60-1.21)
Intercompartmental clearance [†]	$Q_{30.2 \text{ kg}}$ (L/h)	121.8 (21%)	115.6 (73.7-163)
$Q_i = Q_{30.2 \text{ kg}} * (WT_i/30.2)^{k4}$	k4	0.75 fix	0.75 fix
Absorption rate constant	k_a (h ⁻¹)	3.5 fix	3.5 fix
IIV Clearance	ω^2 CL	0.158 (41%)	0.145 (0.063-0.259)
IIV Volume of distribution	ω^2 V_c	1.19 (27%)	1.06 (0.64-1.71)
Proportional error	σ^2	0.283 (13%)	0.272 (0.222-0.346)

RSE is the relative standard error, and 90 CI is the 90% confidence interval representing the 5th and 95th percentiles. Inter-individual and residual variability values are shown as variance estimates.

[†]Parameters are apparent parameters, as only oral data was included.

inter-compartmental clearance, best described the data (table II, figure S1, S2). As midazolam was administered orally, apparent parameters for clearance and volume of distribution were obtained. For a typical individual of 30.2 kg, apparent clearance was 102.6 L/h, and the exponent in the exponential equation relating body weight and clearance, was found to be 0.874 (table II). As a result, this pediatric covariate function was used to scale CYP3A-mediated clearance in the between-drug extrapolation:

$$CL_{\text{pediatric}} = CL_{\text{adult}} \times \left(\frac{WT}{70}\right)^{0.874} \quad (\text{Eq. 5})$$

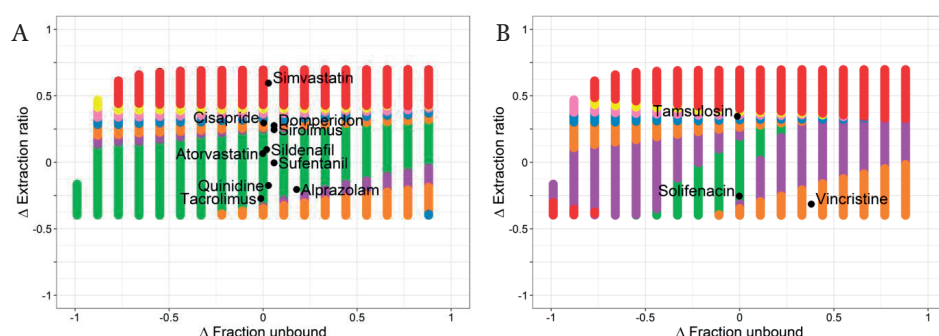


Figure 1. Prediction of the age down to which the pediatric covariate function for CYP3A-mediated midazolam clearance can be used to accurately scale clearance of CYP3A substrates with specific drug properties. Difference in extraction ratio between CYP3A substrates (test drugs) and midazolam (model drug) is plotted versus difference in fraction unbound (f_u) between these drugs. The color scheme was obtained from the published framework (7) and represent hypothetical model-test drug combinations that lead to systematically accurate scaling of clearance in children down to 1 day (green), 1 month (purple), 6 months (orange), 1 year (blue), 2 years (pink), and 5 years of age (yellow). Red indicates that scaling is not systematically accurate in children of 5 years and younger. The black data points represent the included test drugs and their parameter values relative to midazolam. Panel A shows drugs binding to albumin (HSA), while panel B shows drugs binding to α 1-acid glycoprotein (AAG). Modified from Calvier *et al.* (7) (with permission).

Between-drug extrapolation potential of midazolam clearance to other CYP3A substrates

The obtained drug properties of midazolam and the selected CYP3A substrates required for between-drug extrapolation of clearance are listed in Table SI (31-52). Figure 1 shows down to what age the clearance of the selected substrates can at least be extrapolated from adult values with the covariate relationships for midazolam clearance, based on the differences in extraction ratio and f_u according to the framework that was previously reported (7). This figure shows that this method will accurately scale pediatric clearance values down to neonates of 1 day of age

for alprazolam, atorvastatin, quinidine, sildenafil, solifenacin, sufentanil, and tacrolimus, while for the other drugs clearance will be at least accurately scaled down to infants of 1 month (sirolimus), and 6 months of age (cisapride, domperidon, and vincristine). Tamsulosin clearance scaling will be accurate down to at least 2 years of age, while for simvastatin accurate scaling down to 5 years of age may not even be possible (figure 1).

From figure 1 it can also be derived that a pediatric covariate function for midazolam can be used to scale CYP3A-mediated clearance of HSA-bound substrates which are highly protein bound ($>90\%$, $f_u \leq 0.1$) when the extraction ratio in adults that does not differ more than $+0.20$ or -0.30 from the extraction ratio of midazolam (assuming $\geq 75\%$ metabolism by CYP3A enzymes for both drugs), resulting in required extraction ratio values between 0.05 and 0.55. In addition, for substrates with low protein binding ($<10\%$, $f_u \geq 0.9$) the drug to which the covariate function is extrapolated should have an extraction ratio between 0.35 and 0.65. For AGP-bound drugs, fewer combinations of drug properties lead to accurate scaling based on a midazolam pediatric covariate function, with no scenarios for drugs with low protein binding ($f_u \geq 0.9$), but an extraction ratio of 0.4-0.6 or 0.1-0.5 in adults lead to accurate scaling for drugs that are 90% or $\geq 97.5\%$ bound, respectively (figure 1).

Comparison of scaled versus reported pediatric clearance values

Obtained pediatric and adult clearance values of CYP3A substrates are summarized in table SI (30, 33, 53-63). In figure 2, the scaled clearance values are shown together with the reported pediatric clearance values for the various substrates versus body weight. Table IV lists the calculated prediction errors for the three typical pediatric individuals. For most drugs, the scaled covariate relationships fall within the range of observed values, except for sirolimus and vincristine. The calculated PE values also show that scaled vincristine and sirolimus clearance values are inaccurate, although with a PE value of 64.3% and 58.3%, respectively, this inaccuracy is not extreme. The PE values for all other drugs are $<50\%$, indicating accurate or reasonably accurate scaling of clearance in infants, children, and adolescents.

Table III. Model parameter estimates for the sildenafil ‘reference model’ versus the sildenafil ‘extrapolation model’, and the bootstrap results for both models based on n=250 resampling

Parameter		Reference model		Extrapolation model	
		Model estimate (RSE)	Bootstrap (90 CI)	Model estimate (RSE)	Bootstrap (90 CI)
Sildenafil clearance [†] $CL_i = CL_{70\text{ kg}} * (WT_i/70)^{k1}$	$CL_{70\text{ kg}}$ (L/h)	113 (13%)	112 (84.6-149)	100 fix	100 fix
	k1	1.08 (11%)	1.05 (0.82-1.30)	0.874 fix	0.874 fix
Volume of distribution [†] $V_i = V_{28\text{ kg}} * (WT_i/28)^{k2}$	$V_{28\text{ kg}}$ (L)	540 (33%)	561 (311-1424)	590 (29%)	574 (389-1134)
	k2	3.18 (10%)	3.17 (2.41-4.27)	3.18 (10%)	3.16 (2.49-4.01)
Absorption rate constant	k_a (h ⁻¹)	1 fix	1 fix	1 fix	1 fix
IIV Clearance	ω^2 CL	0.493 (14%)	0.487 (0.363-0.631)	0.510 (13%)	0.512 (0.397-0.650)
Proportional error	σ^2	0.627 (7%)	0.616 (0.538-0.703)	0.651 (8%)	0.646 (0.564-0.738)

RSE is the relative standard error, and 90 CI is the 90% confidence interval representing the 5th and 95th percentiles. Inter-individual and residual variability values are shown as variance estimates.

[†]Parameters are apparent parameters, as only oral data was included.

Sildenafil population PK models

The reference model and extrapolation model for sildenafil, described the sildenafil concentrations with a one-compartmental model. Table III presents model parameters and bootstrap values for both models and the goodness-of-fit plots and results from the NPDE analyses are presented in figure S3 and figure S4, respectively. These results show that descriptive and predictive properties of both models are similar.

In the reference model, apparent sildenafil clearance for a typical individual of 28 kg was found to be 41.9 L/h, and clearance increased exponentially with increasing body weight (exponent of 1.08 [RSE 11%]), leading to an apparent clearance of 113 L/h for a 70-kg individual. In the extrapolation model, apparent clearance was scaled using eq. 5, with a CL_{adult} of 100 L/h for a 70-kg individual, leading to a scaled apparent clearance of 44.9 L/h for a 28-kg individual. As shown in a graphical comparison in figure 3A, both covariate relationships are very similar, with only a small difference in clearance values between the two models for children with the lowest body weight. Figure 3B shows that when individual clearance predictions by both models are plotted versus age, the loess functions for these relationships are also similar with small difference in the youngest age range. These small discrepancies may be

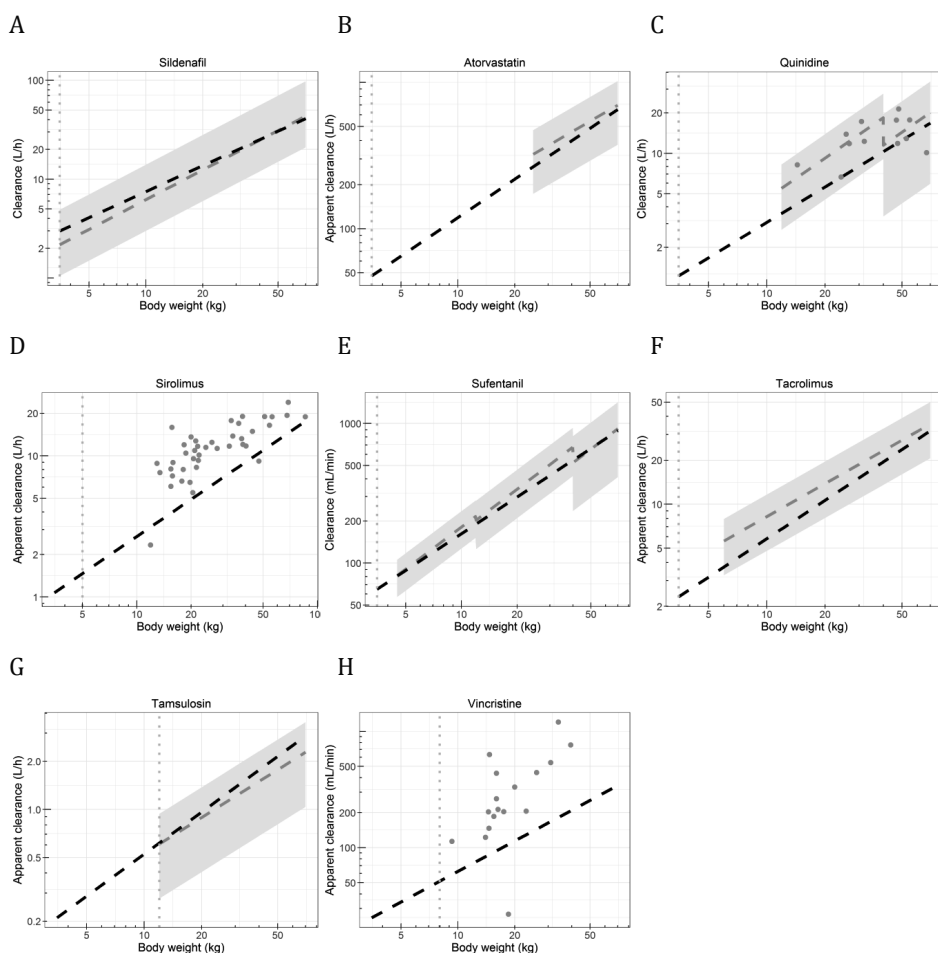


Figure 2. Scaled and reported clearance values versus body weight for various CYP3A substrates. Clearance (or apparent clearance) values are based on the between-drug extrapolation of the pediatric covariate function for CYP3A-mediated midazolam clearance and reported adult clearance values (**black**), and based on reported pediatric clearance values in literature (**grey**), for sildenafil (A), atorvastatin (B), quinidine (C), sirolimus (D), sufentanil (E), tacrolimus (F), tamsulosin (G) and vincristine (H). The vertical dotted line (grey) indicates the body weight down to which systemically accurate clearance scaling is predicted to be possible according to the framework (7). For the reported clearance values the following is depicted: A) Mean sildenafil clearance (line) with minimal and maximal reported values (grey area). B) Typical atorvastatin clearance (line) $\pm 46.3\%$ (%CV, grey area). C) Mean quinidine clearance (line) ± 2 SD (grey area), and individual reported clearances (closed circles). D) Individual reported sirolimus clearances (closed circles). E) Mean sufentanil clearance (line) ± 2 SD (grey area). F) Typical tacrolimus clearance (line) $\pm 41.6\%$ (%CV, with $CV = \sqrt{e^{\sigma^2} - 1}$, and $\sigma^2 = 0.16$, grey area). G) Typical tamsulosin clearance (line) $\pm 54.4\%$ (%CV, grey area). H) Individual reported vincristine clearances, corrected for body surface area (closed circles).

due to the small number of individuals in the youngest age group (1-2 years of age) in the population receiving midazolam used for establishing the pediatric covariate function.

The PE for clearance increases with decreasing age, with a PE of -5.2%, 14.6%, and 32.1%, in an adolescent, child, and infant, respectively, indicating that with decreasing age and body weight, the extrapolation model yields a larger overprediction of clearance. However, the scaled clearance values are within the range of observed clearance values, which show a high variability throughout the pediatric age range (figure 3B).

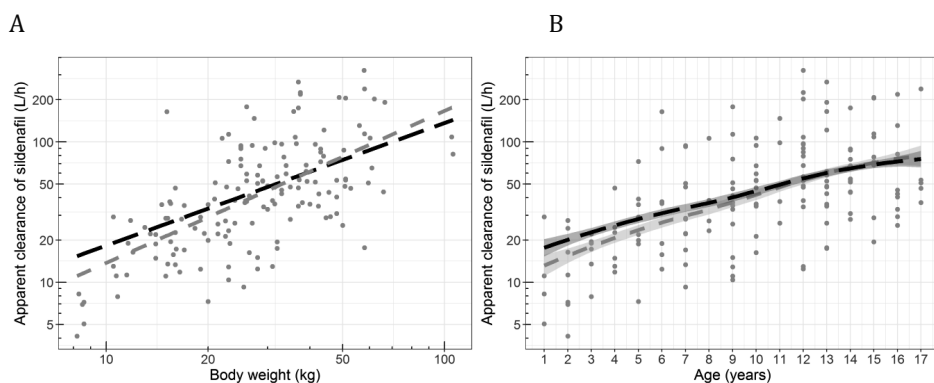


Figure 3. Sildenafil apparent clearance versus body weight (A) and versus age (B) for the sildenafil reference model (grey) and based on between-drug extrapolation of clearance (black) with points representing the individual predicted clearance by the reference model. In panel A the lines represent population predicted clearance values directly derived from the bodyweight-based covariate relationship, while in panel B the lines represent the loess function summarizing the population predicted clearance values with a 95% confidence interval (shaded area).

DISCUSSION

Accurate scaling of plasma clearance is essential to establish optimal first-in-child doses during drug development and for the development of pediatric dose recommendations. As many drugs are metabolized by CYP3A enzymes and midazolam is a commonly accepted probe drug for CYP3A, we aimed to evaluate when a pediatric covariate function for CYP3A-mediated midazolam clearance can be used to scale pediatric clearance of CYP3A substrates given the recently reported guidance on between-drug extrapolation of covariate models.

Whether scaling of pediatric clearance of CYP3A substrates based on a covariate function from a pediatric population PK model for midazolam is accurate, depends

on the drug properties (7). We used a previously developed framework (7) to assess for which of the selected CYP3A substrates scaling with the pediatric covariate function from midazolam will lead to accurate clearance values (figure 1). The color code in this framework indicates down to which age scaling of clearance is expected to be systemically accurate based on differences in extraction ratio and f_u of the test and model drug only. Each colored dot in this graph represents multiple drugs with differences in the remaining drug properties (i.e. blood-to-plasma partitioning and affinity to isoenzymes) and it should be noted that when the framework predicts that scaling of clearance is not systemically accurate for all drugs with the indicated combination of drug properties, there may still be drugs within the set of drugs represented by a data point for which this scaling is accurate. In those cases, it can however not be predicted *a priori* whether this will be the case for each of the individual drugs (7).

For the selected CYP3A substrates alprazolam, atorvastatin, cisapride, domperidone, quinidine, sildenafil, solifenacin, sufentanil, sirolimus, tacrolimus, and vincristine, scaling of clearance with the covariate function of midazolam is expected to be accurate down to children of at least 1 year of age and for some drugs even to neonates and infants (figure 1).

Several approaches and methods for scaling of clearance in children have been described in literature, including scaling of clearance using a bodyweight-based exponential function with exponents of e.g. 0.67, 0.75 or 1. In a systematic assessment of the applicability of bodyweight-based scaling with a fixed exponent of 0.75, it was found that this approach leads to increasingly inaccurate scaled values with decreasing age, reaching prediction errors of up to 278% in neonates (64). Extrapolation of pediatric covariate functions for drugs sharing elimination pathways were therefore considered as an alternative approach, and this method had already been successful in scaling pediatric clearance for UGT2B7 substrates and for drugs eliminated through glomerular filtration (4-6). A systematic assessment of this method provided the prerequisites for this scaling technique (7). In the current work we illustrate how the previously obtained knowledge can be applied. Moreover, we add CYP3A metabolism to the list of elimination pathways for which between-drug extrapolation of pediatric covariate relationships for clearance have been successfully applied.

Between-drug extrapolation of clearance on the basis of a pediatric covariate function for CYP3A-mediated midazolam clearance, indeed led to accurate or reasonably accurate scaling of pediatric clearance of most of the selected CYP3A

substrates in children (table IV, figure 2). This confirms that the pediatric covariate function for midazolam clearance can accurately scale pediatric clearance of CYP3A substrates down to at least one year of age for a large number of relevant substrates including sildenafil, atorvastatin, quinidine, sufentanil, tacrolimus and tamsulosin. In addition to reported clearance values, for sildenafil concentration-time data were available as well. With these data it was further confirmed that the between-drug extrapolation of the covariate relationship of midazolam clearance yields accurate clearance predictions.

Table IV. Prediction error (PE) of scaled clearance values using the pediatric covariate function for CYP3A-mediated midazolam clearance *versus* reported pediatric clearance values for three representative pediatric subjects of 10, 20 and 50 kg (eq. 4). Colors indicate an accurate prediction (<30%, green), a reasonably accurate prediction (30-50%, orange) and an inaccurate prediction (≥50%, red).

	Infant (10 kg)	Child (20 kg)	Adolescent (50 kg)
Atorvastatin	-26.7%	-20.1%	-10.5%
Quinidine	-33.5%	-39.0%	-12.8%
Sildenafil	20.7%	10.6%	-1.4%
Sirolimus	x	-58.3%	-31.5%
Sufentanil	-10.3%	-12.0%	1.1%
Tacrolimus	-44.6%	-39.6%	-32.3%
Tamsulosin	x	8.1%	21.2%
Vincristine	-64.3%	x	x

x: No pediatric or adult clearance values reported in literature.

Contrary to what was expected based on the theoretical framework, scaled clearance values of sirolimus and vincristine were inaccurate compared to reported literature values (PE >50%, table IV). For sirolimus, this may be due to the known induction of hepatic CYP3A activity and possibly altered hepatic P-glycoprotein expression (65), which impacts its plasma clearance, which is not accounted for in the scaling method. The scaling of vincristine may be inaccurate, because it is predominantly metabolized by CYP3A5 (66), with a relative smaller contribution of CYP3A4-mediated metabolism compared to midazolam. Other factors that may affect the accuracy of our pathway-specific scaling approach, apart from the hepatic extraction ratio and f_u in adults, include that the fraction eliminated by a certain pathway may be different from the ≥75% assumed here. It has for instance been shown that the age down to which clearance can accurately be scaled, increases when the contribution of CYP3A metabolism to the overall hepatic metabolism is decreasing (7). Additionally, the contribution of minor elimination pathways to overall drug clearance has been ignored in the current analysis. Moreover, scenarios

for the between-drug extrapolation of pediatric covariate functions for clearance of HSA-bound drugs to AAG-bound drugs have not been investigated; therefore we assumed midazolam to be AAG-bound when using its covariate function to scale the clearance of AAG-bound CYP3A substrates. The impact of this assumption would be largest in neonates and in the youngest children <1 year of age, as in these age groups the concentration of AAG is known to vary more with age than the concentration of HSA, due to the more rapid maturation of AAG-concentrations (67). Lastly, as in the sildenafil PK study not many samples were taken shortly after administration, absorption rate constants for sildenafil could not be estimated and were therefore fixed it at 1 h^{-1} , which is close to reported values for k_a 0.34 h^{-1} (68). A sensitivity analysis showed that fixing it at different values had no impact on the scaled clearance values.

In this analysis we only included midazolam PK data from children >1 year of age. Therefore, the pediatric covariate function for midazolam clearance we developed in this analysis cannot be used to scale clearance values of CYP3A substrates in children <1 year of age, as extrapolation of the covariate relationship to (premature) neonates and infants is anticipated to yield overprediction, due to the large impact of maturation in the first weeks and months of life (69). To be able to apply this covariate function to scale CYP3A-mediated clearance in neonates and young infants up to 1 year of age, the midazolam covariate function should be extended with children <1 year of age.

CONCLUSION

This analysis showed that a pediatric covariate relationship describing how midazolam clearance changes throughout the pediatric age range can be used to scale adult clearance values for many other CYP3A substrates to pediatric clearance values. Specifically, it was found that this approach is applicable to accurately scale clearances of drugs that are mainly eliminated by CYP3A-mediated metabolism with for example high protein binding to HSA (>90%) and a low to intermediate extraction ratio of <0.55 in adults. Furthermore, clearances of CYP3A substrates with low binding to HSA (<10%) and an extraction ratio of 0.35-0.65 can be accurately scaled from adult values, while scaling of clearance of AGP-bound drugs is accurate for fewer combinations of drug properties. The ability to scale clearance of CYP3A substrates in children from adult clearance using a pediatric covariate function for CYP3A-mediated clearance will significantly enhance the development of dosing guidelines of CYP3A substrates for clinical practice and aid in determining the dose in first-in-child studies involving new CYP3A substrates. This may be especially useful for CYP3A substrates in which scarce or no pediatric PK information is available, for example for alprazolam, domperidone, and solifenacin.

ACKNOWLEDGEMENTS

The authors would like to thank Pfizer Inc. for kindly sharing their data on sildenafil PK in children of 1-16 years of age. Drs. Jeff Galinken and Peter Adamson were the PIs on the original midazolam PK study conducted at CHOP, and the study was supported by NIH / NICHD, Pediatric Pharmacology Research Unit, Grant # HD037255-06. The authors would like to thank Bas Goulouze (LACDR, Leiden University) for reviewing all scripts involved in this analysis. CAJ Knibbe is supported by a NWO Vidi grant (Vidi Knibbe, June 2013).

REFERENCES

1. EMA. EMA (EMA/CHMP/EWP/147013/2004 Committee for Medicinal Products for Human Use [CHMP]). Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population. 2007.
2. FDA. General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products, Guidance for Industry, December 2014. Available from: <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM425885.pdf>.
3. Zisowsky J, Krause A, Dingemanse J. Drug Development for Pediatric Populations: Regulatory Aspects. *Pharmaceutics*. 2010;2(4):364-88.

4. Krekels EH, Neely M, Panoilia E, Tibboel D, Capparelli E, Danhof M, et al. From pediatric covariate model to semiphysiological function for maturation: part I-extrapolation of a covariate model from morphine to Zidovudine. *CPT Pharmacometrics Syst Pharmacol*. 2012;1:e9.
5. Zhao W, Biran V, Jacqz-Aigrain E. Amikacin maturation model as a marker of renal maturation to predict glomerular filtration rate and vancomycin clearance in neonates. *Clin Pharmacokinet*. 2013;52(12):1127-34.
6. De Cock RF, Allegaert K, Sherwin CM, Nielsen EI, de Hoog M, van den Anker JN, et al. A neonatal amikacin covariate model can be used to predict ontogeny of other drugs eliminated through glomerular filtration in neonates. *Pharmaceutical research*. 2014;31(3):754-67.
7. Calvier EAM, Krekels EHJ, Yu H, Valitalo PAJ, Johnson TN, Rostami-Hodjegan A, et al. Drugs Being Eliminated via the Same Pathway Will Not Always Require Similar Pediatric Dose Adjustments. *CPT Pharmacometrics Syst Pharmacol*. 2018.
8. Michaels S, Wang MZ. The revised human liver cytochrome P450 "Pie": absolute protein quantification of CYP4F and CYP3A enzymes using targeted quantitative proteomics. *Drug Metab Dispos*. 2014;42(8):1241-51.
9. Ince I, Knibbe CA, Danhof M, de Wildt SN. Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from in vitro and in vivo investigations. *Clin Pharmacokinet*. 2013;52(5):333-45.
10. Gorski JC, Hall SD, Jones DR, VandenBranden M, Wrighton SA. Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem Pharmacol*. 1994;47(9):1643-53.
11. Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, et al. Use of midazolam as a human cytochrome P450 3A probe: I. In vitro-in vivo correlations in liver transplant patients. *J Pharmacol Exp Ther*. 1994;271(1):549-56.
12. Dundee JW, Collier PS, Carlisle RJ, Harper KW. Prolonged midazolam elimination half-life. *Br J Clin Pharmacol*. 1986;21(4):425-9.
13. von Moltke LL, Greenblatt DJ, Harmatz JS, Shader RI. Alprazolam metabolism in vitro: studies of human, monkey, mouse, and rat liver microsomes. *Pharmacology*. 1993;47(4):268-76.
14. Jacobsen W, Kuhn B, Soldner A, Kirchner G, Sewing KF, Kollman PA, et al. Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. *Drug Metab Dispos*. 2000;28(11):1369-78.
15. Bedford TA, Rowbotham DJ. Cisapride. Drug interactions of clinical significance. *Drug Saf*. 1996;15(3):167-75.
16. Simard C, Michaud V, Gibbs B, Masse R, Lessard E, Turgeon J. Identification of the cytochrome P450 enzymes involved in the metabolism of domperidone. *Xenobiotica*. 2004;34(11-12):1013-23.
17. Guengerich FP, Muller-Enoch D, Blair IA. Oxidation of quinidine by human liver cytochrome P-450. *Mol Pharmacol*. 1986;30(3):287-95.
18. Walker DK, Ackland MJ, James GC, Muirhead GJ, Rance DJ, Wastall P, et al. Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica*. 1999;29(3):297-310.

19. Prueksaritanont T, Gorham LM, Ma B, Liu L, Yu X, Zhao JJ, et al. In vitro metabolism of simvastatin in humans [SBT]identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos.* 1997;25(10):1191-9.
20. Swart PJ, Krauwinkel WJ, Smulders RA, Smith NN. Pharmacokinetic effect of ketoconazole on solifenacin in healthy volunteers. *Basic Clin Pharmacol Toxicol.* 2006;99(1):33-6.
21. Tateishi T, Krivoruk Y, Ueng YF, Wood AJ, Guengerich FP, Wood M. Identification of human liver cytochrome P-450 3A4 as the enzyme responsible for fentanyl and sufentanil N-dealkylation. *Anesth Analg.* 1996;82(1):167-72.
22. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metab Dispos.* 1992;20(5):753-61.
23. Vincent SH, Karanam BV, Painter SK, Chiu SH. In vitro metabolism of FK-506 in rat, rabbit, and human liver microsomes: identification of a major metabolite and of cytochrome P450 3A as the major enzymes responsible for its metabolism. *Arch Biochem Biophys.* 1992;294(2):454-60.
24. Kamimura H, Oishi S, Matsushima H, Watanabe T, Higuchi S, Hall M, et al. Identification of cytochrome P450 isozymes involved in metabolism of the α 1-adrenoceptor blocker tamsulosin in human liver microsomes. *Xenobiotica.* 1998;28(10):909-22.
25. Zhou XJ, Zhou-Pan XR, Gauthier T, Placidi M, Maurel P, Rahmani R. Human liver microsomal cytochrome P450 3A isozymes mediated vindesine biotransformation. *Metabolic drug interactions. Biochem Pharmacol.* 1993;45(4):853-61.
26. Barst RJ, Ivy DD, Gaitan G, Szatmari A, Rudzinski A, Garcia AE, et al. A randomized, double-blind, placebo-controlled, dose-ranging study of oral sildenafil citrate in treatment-naïve children with pulmonary arterial hypertension. *Circulation.* 2012;125(2):324-34.
27. Gupta M EA, Willmann S, Adamson PC, Galinkin JL, Barrett JS. Model-based Approaches to Investigate Pharmacogenetic and Developmental Sources of Variation in the Pharmacokinetics of Midazolam after Oral administration in Children. 2006 [Available from: AAPS 2006. Abstract 003255. <https://abstracts.aaps.org/Published/Browse.aspx>.
28. Comets E, Brendel K, Mentre F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. *Comput Methods Programs Biomed.* 2008;90(2):154-66.
29. Pfizer. Study protocol 'A Randomized, Double-Blind, Placebo-Controlled Study of Sildenafil in Children With Pulmonary Arterial Hypertension'. <https://clinicaltrials.gov/ct2/show/NCT00159913>.
30. European Medicines Agency. Revatio Product Information. Accessed on 18th December 2017. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000638/WC500055840.pdf
31. Frechen S, Junge L, Saari TI, Suleiman AA, Rokitta D, Neuvonen PJ, et al. A semiphysiological population pharmacokinetic model for dynamic inhibition of liver and gut wall cytochrome P450 3A by voriconazole. *Clin Pharmacokinet.* 2013;52(9):763-81.
32. Lown KS, Thummel KE, Benedict PE, Shen DD, Turgeon DK, Berent S, et al. The erythromycin breath test predicts the clearance of midazolam. *Clin Pharmacol Ther.* 1995;57(1):16-24.
33. DrugBank Canada, accessed July 17th 2017. Available from <https://www.drugbank.ca>.
34. Lennernas H. Clinical pharmacokinetics of atorvastatin. *Clin Pharmacokinet.* 2003;42(13):1141-60.

35. CVZ. College voor zorgverzekeringen Nederland; Farmacotherapeutisch Kompas (Dutch Pharmacotherapeutic Compass) 2013 [updated April 1, 2013. <http://www.fk.cvz.nl/>].
36. Rampe D, Roy ML, Dennis A, Brown AM. A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett.* 1997;417(1):28-32.
37. Heykants J, Hendriks R, Meuldermans W, Michiels M, Scheygrond H, Reyntjens H. On the pharmacokinetics of domperidone in animals and man. IV. The pharmacokinetics of intravenous domperidone and its bioavailability in man following intramuscular, oral and rectal administration. *Eur J Drug Metab Pharmacokinet.* 1981;6(1):61-70.
38. Simcyp (R) Simulator version 15.1. Certara, Sheffield, United Kingdom. Available from: <https://www.certara.com/software/physiologically-based-pharmacokinetic-modeling-and-simulation/simcyp-simulator/> [
39. Bressler R, Bahl JJ. Principles of drug therapy for the elderly patient. *Mayo Clin Proc.* 2003;78(12):1564-77.
40. Zhao P, Zhang L, Grillo JA, Liu Q, Bullock JM, Moon YJ, et al. Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin Pharmacol Ther.* 2011;89(2):259-67.
41. Mehrotra N, Gupta M, Kovar A, Meibohm B. The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy. *Int J Impot Res.* 2007;19(3):253-64.
42. Gruer PJ, Vega JM, Mercuri MF, Dobrinska MR, Tobert JA. Concomitant use of cytochrome P450 3A4 inhibitors and simvastatin. *Am J Cardiol.* 1999;84(7):811-5.
43. Benet LZ, Cummins CL, Wu CY. Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharm.* 2004;277(1-2):3-9.
44. Osmulski PA, Gaczynska M. Rapamycin allosterically inhibits the proteasome. *Mol Pharmacol.* 2013;84(1):104-13.
45. Doroshenko O, Fuhr U. Clinical pharmacokinetics and pharmacodynamics of solifenacin. *Clin Pharmacokinet.* 2009;48(5):281-302.
46. Raucoules-Aime M, Kaidomar M, Levron JC, Le Moing JP, Goubaux B, Gugenheim J, et al. Hepatic disposition of alfentanil and sufentanil in patients undergoing orthotopic liver transplantation. *Anesth Analg.* 1997;84(5):1019-24.
47. Saari TI, Ihmsen H, Mell J, Frohlich K, Fechner J, Schuttler J, et al. Influence of intensive care treatment on the protein binding of sufentanil and hydromorphone during pain therapy in postoperative cardiac surgery patients. *Br J Anaesth.* 2014;113(4):677-87.
48. Tuteja S, Alloway RR, Johnson JA, Gaber AO. The effect of gut metabolism on tacrolimus bioavailability in renal transplant recipients. *Transplantation.* 2001;71(9):1303-7.
49. Piekoszewski W, Jusko WJ. Plasma protein binding of tacrolimus in humans. *J Pharm Sci.* 1993;82(3):340-1.
50. Matsushima H, Kamimura H, Soeishi Y, Watanabe T, Higuchi S, Tsunoo M. Pharmacokinetics and plasma protein binding of tamsulosin hydrochloride in rats, dogs, and humans. *Drug Metab Dispos.* 1998;26(3):240-5.
51. Dennison JB, Mohutsky MA, Barbuch RJ, Wrighton SA, Hall SD. Apparent high CYP3A5 expression is required for significant metabolism of vincristine by human cryopreserved hepatocytes. *J Pharmacol Exp Ther.* 2008;327(1):248-57.
52. Chan JD. Pharmacokinetic drug interactions of vinca alkaloids: summary of case reports. *Pharmacotherapy.* 1998;18(6):1304-7.

53. Knebel W, Gastonguay MR, Malhotra B, El-Tahtawy A, Jen F, Gandelman K. Population pharmacokinetics of atorvastatin and its active metabolites in children and adolescents with heterozygous familial hypercholesterolemia: selective use of informative prior distributions from adults. *J Clin Pharmacol*. 2013;53(5):505-16.
54. Dansirikul C, Morris RG, Tett SE, Duffull SB. A Bayesian approach for population pharmacokinetic modelling of sirolimus. *Br J Clin Pharmacol*. 2006;62(4):420-34.
55. Scholz J, Steinfath M, Schulz M. Clinical pharmacokinetics of alfentanil, fentanyl and sufentanil. An update. *Clin Pharmacokinet*. 1996;31(4):275-92.
56. Saeves I, Line PD, Bremer S, Vethe NT, Tveit RG, Meltevik TJ, et al. Tacrolimus exposure and mycophenolate pharmacokinetics and pharmacodynamics early after liver transplantation. *Ther Drug Monit*. 2014;36(1):46-53.
57. Tsuda Y, Tatami S, Yamamura N, Tadayasu Y, Sarashina A, Liesenfeld KH, et al. Population pharmacokinetics of tamsulosin hydrochloride in paediatric patients with neuropathic and non-neuropathic bladder. *Br J Clin Pharmacol*. 2010;70(1):88-101.
58. de Graaf SS, Bloemhof H, Vendrig DE, Uges DR. Vincristine disposition in children with acute lymphoblastic leukemia. *Med Pediatr Oncol*. 1995;24(4):235-40.
59. Hill KD, Sampson MR, Li JS, Tunks RD, Schulman SR, Cohen-Wolkowicz M. Pharmacokinetics of intravenous sildenafil in children with palliated single ventricle heart defects: effect of elevated hepatic pressures. *Cardiol Young*. 2016;26(2):354-62.
60. Szeffler SJ, Pieroni DR, Gingell RL, Shen DD. Rapid elimination of quinidine in pediatric patients. *Pediatrics*. 1982;70(3):370-5.
61. Scott JR, Courter JD, Saldana SN, Widemann BC, Fisher M, Weiss B, et al. Population pharmacokinetics of sirolimus in pediatric patients with neurofibromatosis type 1. *Ther Drug Monit*. 2013;35(3):332-7.
62. Greeley WJ, de Bruijn NP, Davis DP. Sufentanil pharmacokinetics in pediatric cardiovascular patients. *Anesth Analg*. 1987;66(11):1067-72.
63. Jalil MH, Hawwa AF, McKiernan PJ, Shields MD, McElnay JC. Population pharmacokinetic and pharmacogenetic analysis of tacrolimus in paediatric liver transplant patients. *Br J Clin Pharmacol*. 2014;77(1):130-40.
64. Calvier EA, Krekels EH, Valitalo PA, Rostami-Hodjegan A, Tibboel D, Danhof M, et al. Allometric Scaling of Clearance in Paediatric Patients: When Does the Magic of 0.75 Fade? *Clin Pharmacokinet*. 2017;56(3):273-85.
65. Bai S, Stepkowski SM, Kahan BD, Brunner LJ. Metabolic interaction between cyclosporine and sirolimus. *Transplantation*. 2004;77(10):1507-12.
66. Dennison JB, Jones DR, Renbarger JL, Hall SD. Effect of CYP3A5 expression on vincristine metabolism with human liver microsomes. *J Pharmacol Exp Ther*. 2007;321(2):553-63.
67. McNamara PJ, Alcorn J. Protein binding predictions in infants. *AAPS PharmSci*. 2002;4(1):E4.
68. Olguin HJ, Martinez HO, Perez CF, Mendiola BR, Espinosa LR, Pacheco JLC, et al. Pharmacokinetics of sildenafil in children with pulmonary arterial hypertension. *World J Pediatr*. 2017;13(6):588-92.
69. Brussee JM, Vet NJ, Krekels EHJ, Valkenburg AJ, Jacqz-Aigrain E, van Gerven JMA, et al. Predicting CYP3A-mediated midazolam metabolism in critically ill neonates, infants, children, and adults with inflammation and organ failure. *Br J Clin Pharmacol*. 2017.
70. Brocks DR, Mehvar R. Stereoselectivity in the pharmacodynamics and pharmacokinetics of the chiral antimalarial drugs. *Clin Pharmacokinet*. 2003;42(15):1359-82.

SUPPLEMENTAL MATERIAL (CHAPTER 7)

Table SI. Drug properties of selected CYP3A substrates

Drug	ER _{adult}	Ref	Protein binding	Ref	F _{u,adult}	Ref	Reported CL _{adult,70kg}	Ref	Reported CL _{child}	Ref
Midazolam	0.354	(31)	HSA	(33)	0.022	(32)	-		-	
Alprazolam	0.15	(33)	HSA	(33)	0.20	(33)	-		-	
Atorvastatin	0.42	(34)	HSA	(34)	0.02	(33, 35)	652 L/h [#]	(53)	$699 \cdot \left(\frac{WT}{70}\right)^{0.75}$ L/h [#]	(53)
Cisapride	0.65	(33)	HSA	(36)	0.025	(36)	-		-	
Domperidone	0.63	(37)	HSA	(37)	0.08	(37)	-		-	
Quinidine	0.18	(38)	HSA	(39)	0.05*	(35)	4 mL/min/kg	(33)	Individual values, 0.461 L/h/kg 0.287 L/h/kg [†]	(60)
Sildenafil	0.45	(40)	HSA	(41)	0.04	(41)	41 L/h	(30)	0.62 L/h/kg	(59)
Simvastatin	0.95	(42)	HSA	(34)	0.05	(33, 35)	-		-	
Sirolimus	0.60	(43)	HSA	(44)	0.08	(35)	0.210 L/h/kg [#]	(54)	Individual values	(61)
Solifenacin	0.10	(45)	AAG	(45)	0.02	(35, 45)	-		-	
Sufentanil	0.35	(46)	HSA	(47)	0.08	(35)	0.762 L/h/kg	(55)	18.1 mL/min/kg 16.9 mL/min/kg 13.1 mL/min/kg [†]	(62)
Tacrolimus	0.082	(48)	HSA	(49)	0.01	(33, 35)	31.8 L/h ^{##}	(56)	$12.9 \cdot \left(\frac{WT}{12.2}\right)^{0.75}$ L/h [#]	(63)
Tamsulosin	0.70 [^]	(50)	AAG	(50)	0.01	(35, 50)	2.88 L/h	(57)	$2.28 \cdot \left(\frac{WT}{70}\right)^{0.75}$ L/h [#]	(57)
Vincristine	0.04	(51)	AAG	(52)	0.402	(52)	0.293 L/h/kg	(58)	Individual values, 1.049 L/h/kg	(58)

ER: extraction ratio. F_u: fraction unbound. Ref: reference.

HSA: human serum albumin. AAG: α 1-acid glycoprotein

[#]Reported clearances are apparent clearances (CL/F).

^{*}Brooks *et al.* describe a different value of f_u of 0.23 (70), but both f_u values (0.23 or 0.05) lead to the same conclusion down to which age accurate scaling is possible (down to ≤ 1 day of age).

[†]Quinidine clearance in children was reported for children 3.7-12 years and ≥ 12 years of age.

[†]Sufentanil clearance in children was reported for 3 different age groups of 1-24 months of age, 2-12 years of age, and 12-18 years of age, with assumed weight ranges (based on WHO guidelines) of 4.5-12, 12-40 and 40-70 kg respectively.

[†]Tacrolimus clearance was derived from dose and exposure.

[^]Vincristine is reported to be a high extraction ratio drug. Therefore, the extraction ratio was assumed 0.7, but this may be higher.

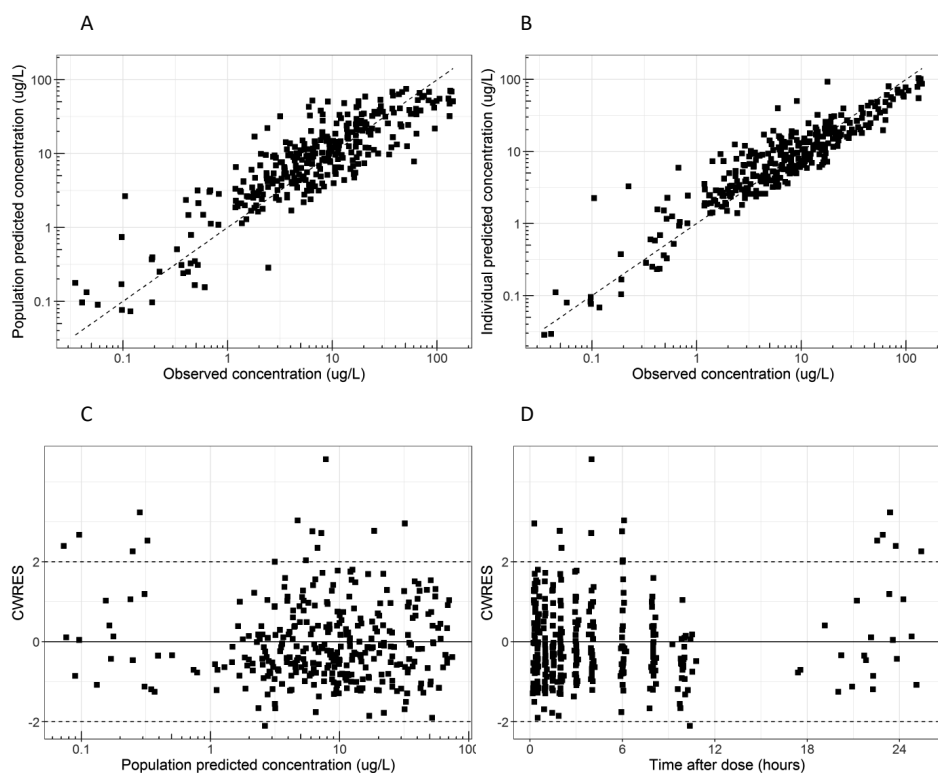


Figure S1. Goodness-of-fit plots for the pediatric population PK model of midazolam (model drug). Plots include individual and population predicted concentration versus observed concentration (A,B) and conditionally weighted residuals (CWRES) versus predicted concentration (C) and versus time after dose (D).

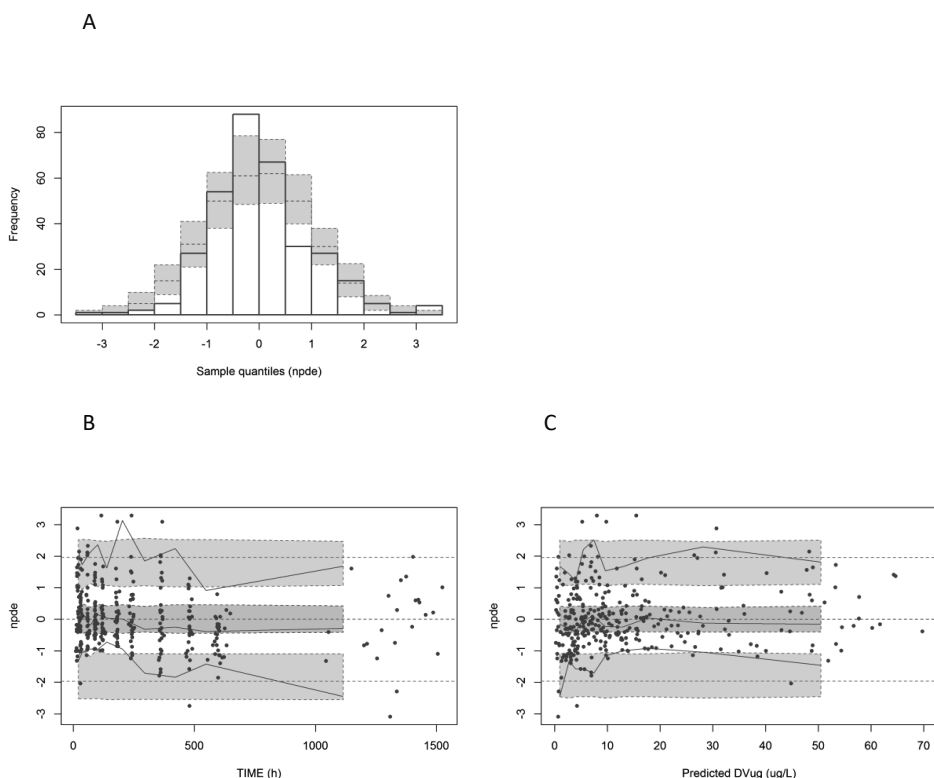
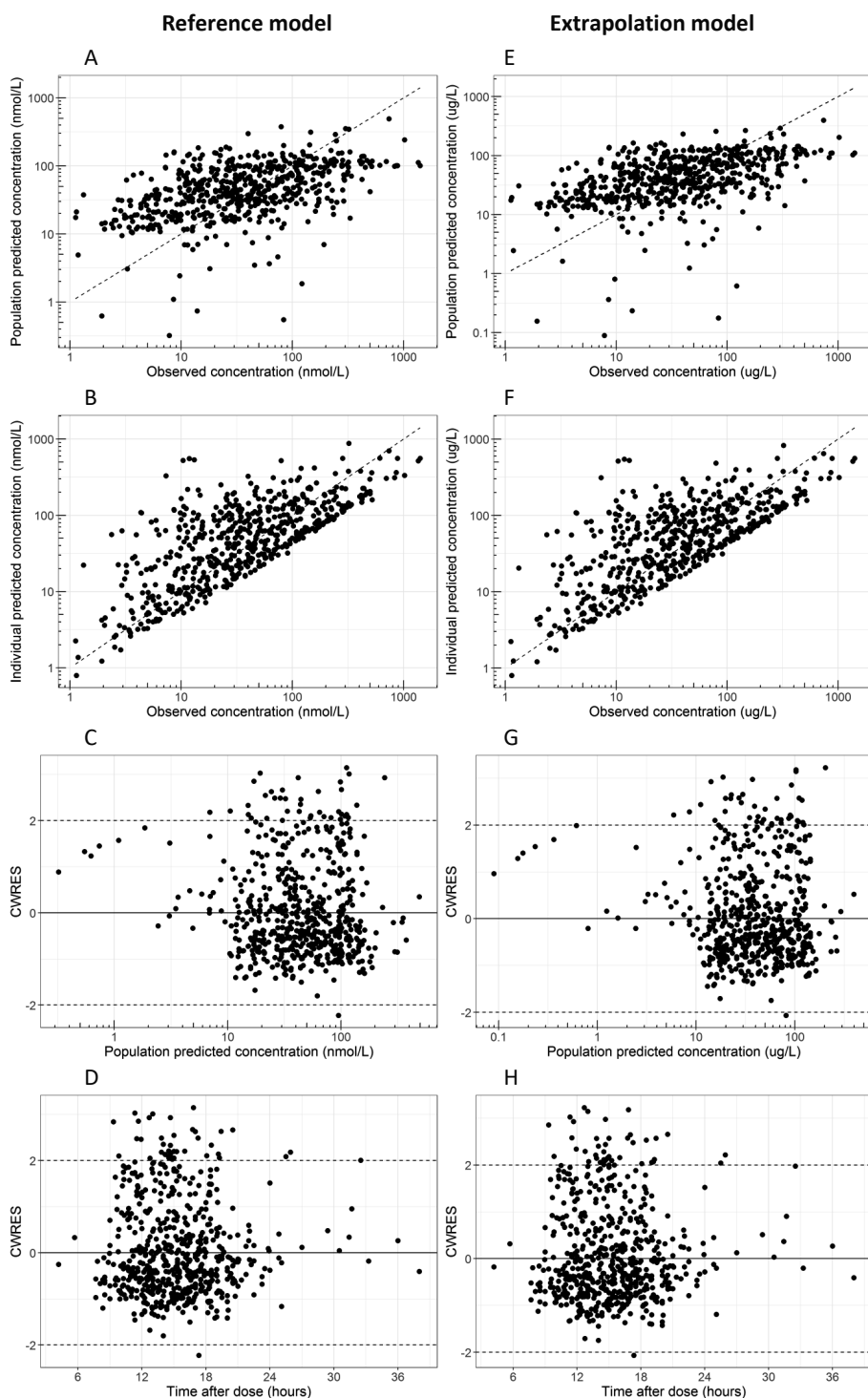


Figure S2. Visualization of the normalized prediction distribution error (NPDE) results for midazolam (E-G) showed that the mean NPDE of 0.016 was not significantly different from 0 ($p > 0.1$, Wilcoxon signed rank test), and the variance for midazolam of 0.92 was not significantly different from 1 ($p > 0.1$, Fisher variance test). A) Histogram of NPDEs, with the observed frequency of sample quantiles of the NPDEs (white bars), overlaid with the density of the standard normal distribution (blue bars). B) NPDE versus time, with the NPDE for each observation (dots), and the lines indicate the mean (red) and the 5th and 95th percentiles (blue) of the NPDEs, and the shaded areas are the simulated 95% confidence intervals of the NPDE median (red) and 5th and 95th percentiles (blue). C) NPDE versus predicted concentration, with dots and lines as described for panel B.

Figure S3. Goodness-of-fit plots for the two pediatric population PK models for the test drug (sildenafil). In the reference model clearance was estimated from the data (A-D) and in the extrapolation model the covariate model obtained with midazolam was used to describe the change in clearance in this population (E-H).



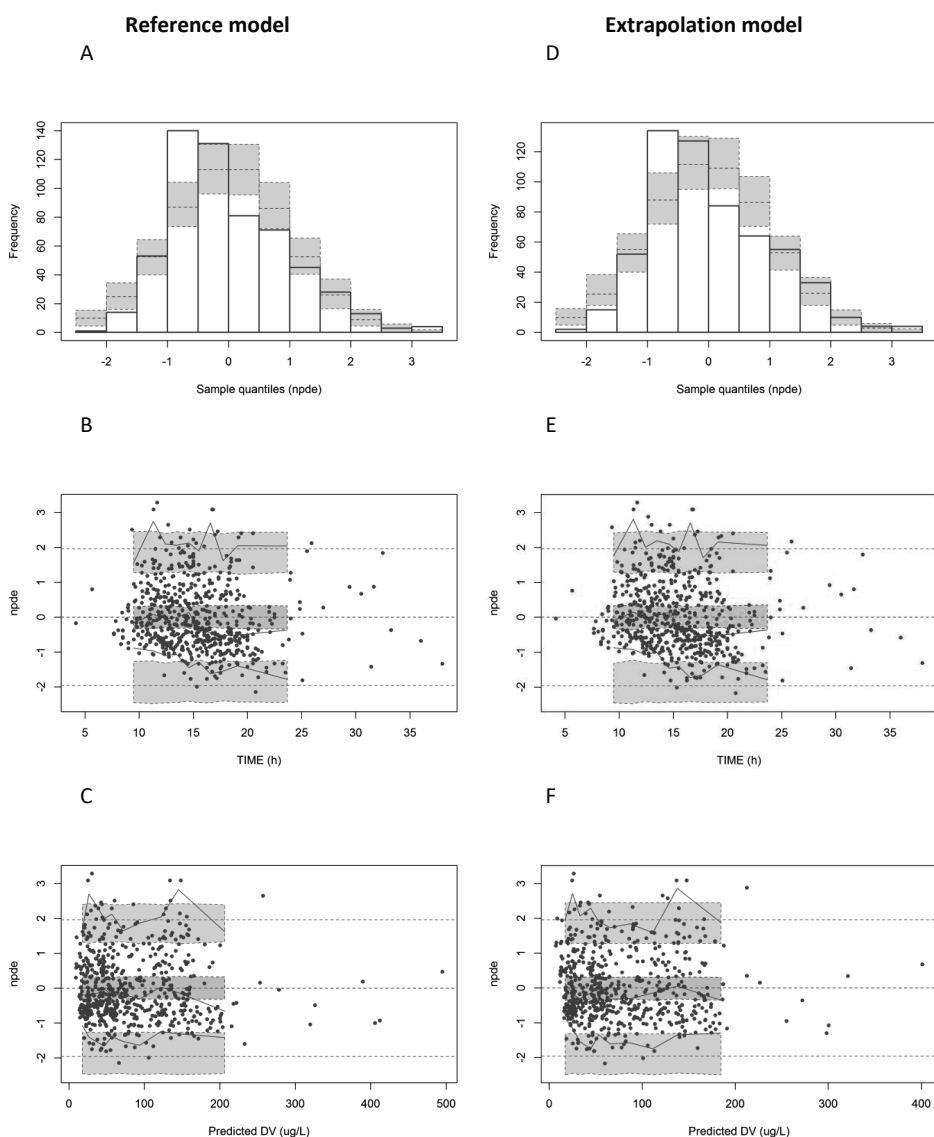


Figure S4. Visualization of the normalized prediction distribution error (NPDE) results for the sildenafil reference model (A-C) and the model based on between-drug extrapolation (D-F). The mean NPDE for the reference and extrapolated model were with 0.004 and 0.031, respectively, not significantly different from 0 ($p > 0.1$, Wilcoxon signed rank test), and the variances were with 0.92 and 0.97, respectively, not significantly different from 1 ($p > 0.1$, Fisher variance test). First row: Histogram of NPDEs, with the observed frequency of sample quantiles of the NPDEs (white bars), overlaid with the density of the standard normal distribution (blue bars). Second and third row: NPDE versus time and predicted concentration respectively, with the NPDE for each observation (dots), and the lines indicate the mean (red) and the 5th and 95th percentiles (blue) of the NPDEs, and the shaded areas are the simulated 95% confidence intervals of the NPDE median (red) and 5th and 95th percentiles (blue).

