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

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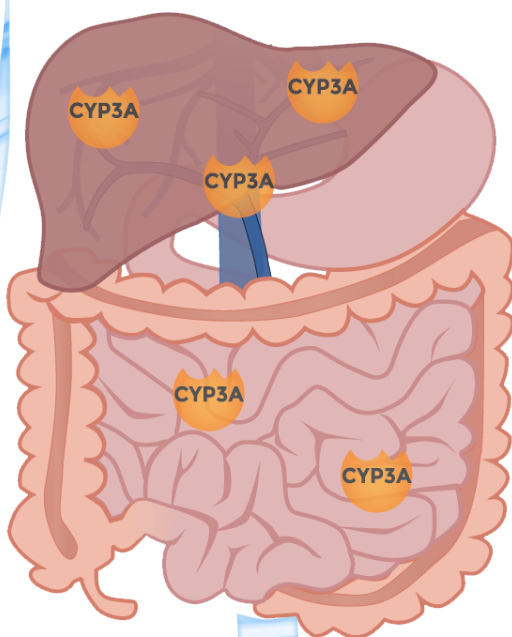
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Section I

General introduction and background



Chapter 1

**First-pass and systemic metabolism
of cytochrome P450 3A substrates
in neonates, infants, and children
– General Introduction, Scope, and
Outline**

Pediatric pharmacology

“Children are not just small adults” (1) as, in addition to growth, many developmental physiological changes occur with increasing age. The physiological differences in children compared to adults and in children of different ages, are especially pronounced in neonates and young infants (2). The developmental changes, together with growth, may impact the pharmacokinetics (PK) of drugs administered to the pediatric population and increase the observed interindividual variability in absorption, distribution, metabolism, and excretion (ADME) in children (3).

Drug absorption after oral administration may differ between neonates, infants, and children due to differences in e.g. intestinal surface area, permeability, gastric emptying time and intestinal transit, gastric and intestinal pH, bile fluid, (pancreatic) enzyme production, membrane transporters, and drug metabolizing enzymes in gut and liver (4, 5). Distribution of the drug may differ because of the developmental changes in e.g. body composition, tissue/organ weight and size compared to total body weight (6), the cardiac output and tissue blood flows (7), plasma protein binding (8) and the tissue: plasma partitioning (7). Furthermore, kidney growth (7) and age-related changes in glomerular filtration rate and active tubular processes (3) impact renal excretion, and, finally, hepatic blood flow (7), plasma protein binding (8), liver growth (9), and maturation of enzyme expression and activity (3, 7) influence the rate of hepatic metabolic clearance of drugs (10).

Cytochrome P450 3A enzymes

Cytochrome P450 (CYP) enzymes form an enzyme family involved in phase I metabolism of many drugs, of which the CYP3A subfamily, with its isoforms CYP3A4, CYP3A5, and CYP3A7, metabolizes many drugs. Profound changes occur in the activity of the CYP3A isoforms, with the CYP3A4 activity increasing with age, the CYP3A5 activity remaining relatively constant with age, while CYP3A7 is mostly expressed in fetal and neonatal tissue and decreases very fast in the first month after birth (11-13). For hepatic CYP3A4 enzymes, which are mainly responsible for the systemic metabolism of CYP3A substrates, the abundance is known to increase with age (12, 14), as well as the liver size (7), while the amount of microsomal protein in the liver is believed to remain constant with age (15). CYP3A enzyme activity per gram of liver has been studied *in vitro* and *in vivo* (11-13, 16), but many questions remain on the maturation of CYP3A-mediated drug clearance in children in a clinical setting, relevant to individualized dosing of CYP3A substrates in this population (17). For example, inflammation, sepsis, and organ failure, appear to impact CYP3A expression and activity (18-23), but what is the contribution of disease severity and inflammation, and is this consistent across the pediatric age range?

In addition to inflammation, age-related differences in clearance (24) and oral bioavailability of midazolam have been reported (25). Is this due to changes in intestinal and/or hepatic CYP3A activity with age? CYP3A enzymes reside in both the gut wall and the liver (16), for which different maturation profiles may be anticipated, leading to different activity in neonates, infants, and children compared to adults, but also to different maturation profiles for presystemic and systemic metabolism (26).

After oral administration of a CYP3A substrate, part of the initial dose does not enter the systemic blood circulation, as the total bioavailability (F_{total}) depends on the fraction of the drug that gets absorbed (F_a), and the fraction escaping gut wall (F_g) and hepatic (F_h) metabolism (Figure 1)(27). It is therefore of interest to distinguish between intestinal and hepatic CYP3A activity with respect to their roles in first-pass and systemic metabolism, as the fraction escaping gut wall and hepatic metabolism may differ with age, resulting in a different total bioavailability and total plasma clearance throughout the pediatric age range.

Furthermore, it has been assumed that clearance of drugs can be predicted from clearance of another drug sharing an elimination pathway, as shown for some drugs eliminated by glomerular filtration and drugs glucuronidated by UGT2B7 (28-30). But is this also the case for drugs that are mainly metabolized by CYP3A, and can this approach be used to predict clearance of CYP3A substrates in neonates, infants, and children?

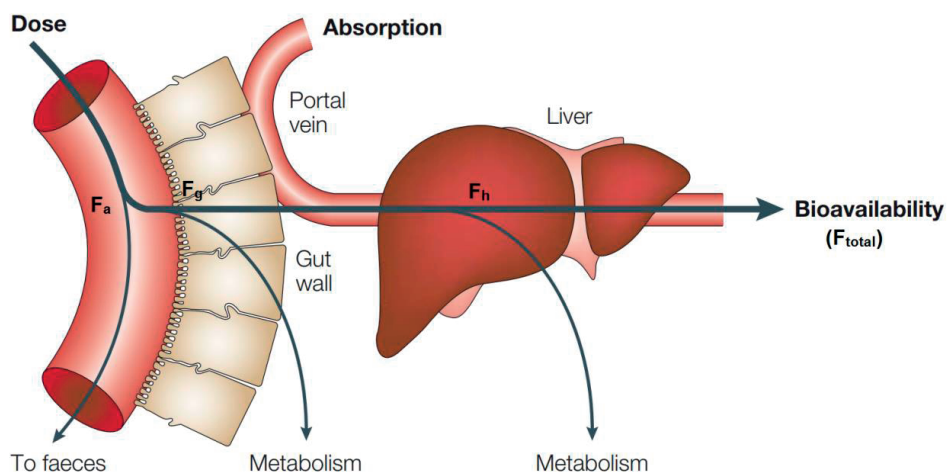


Figure 1. Presystemic metabolism upon oral drug administration. The total bioavailability (F_{total}) depends on the fraction absorbed of the initial dose (F_a), the fraction appearing in the portal vein escaping gut wall metabolism (F_g), and the fraction reaching the blood circulation after the first-pass through the liver (F_h) with $F_{total} = F_a \times F_g \times F_h$, adapted and modified from Waterbeemd et al. (27) with permission.

CYP3A-mediated midazolam clearance in children

To predict plasma clearance of CYP3A substrates in children, the hydroxylation of drugs by CYP3A enzymes should be studied across a wide age range. Combining the knowledge of CYP3A-mediated drug clearance with the intended therapeutic window will enable the development of guidelines for dosing of CYP3A substrates in children. To describe CYP3A-mediated metabolism, midazolam is commonly used as probe drug, as its clearance is believed to reflect CYP3A activity (31, 32). Midazolam is a benzodiazepine, often used for sedation in the neonatal and pediatric intensive care unit (33, 34). Midazolam is considered a good probe drug for CYP3A4/5 activity (35), as midazolam is mainly metabolized by CYP3A4, with limited metabolism by CYP3A5 and CYP3A7 (36). Midazolam is an intermediate extraction ratio drug (37), and its metabolism depends mostly on intrinsic clearance by CYP3A enzymes rather than hepatic blood flow. That midazolam clearance is a good marker for (hepatic) CYP3A expression, was first proven by Thummel *et al.* who showed a significant *in vitro*–*in vivo* correlation ($p < 0.01$) between CYP3A content in liver biopsy specimens and formation of 1-OH-midazolam *in vitro* with the measured midazolam clearance *in vivo* in patients who received a liver transplant (31). Furthermore, midazolam clearance adequately reflects the induction and inhibition of CYP3A4/5 activity by known CYP3A inducers and inhibitors (17), making midazolam a widely accepted probe drug for CYP3A activity.

Therefore, data on midazolam PK after intravenous administration from preterm neonates up to adolescents, would allow for the characterization of total systemic CYP3A activity at different stages of development. Moreover, midazolam PK data after oral administration will also allow for studying the proportional contribution of intestinal and hepatic CYP3A enzymes to the CYP3A-mediated first-pass metabolism in neonates, infants, and children, when added to results upon intravenous midazolam administration.

Model-based approach to describe drug pharmacokinetics

To quantify CYP3A-mediated metabolism using clearance of midazolam or another CYP3A substrate as surrogate marker, a clinical trial may be performed to estimate PK parameters including clearance from densely sampled individual concentration-time profiles. Based on these PK profiles, the parameters can be estimated in each individual, after which summary statistics are used to describe the distribution of parameter values in the study population (the two-stage approach). However, this requires many samples per individual, and this is, especially in pediatric studies, not always feasible due to ethical and practical constraints (38). Therefore, a population approach is preferably applied, which requires less concentration measurements per individual and allows for simultaneous analysis of data from the

whole population, while taking into account the individual differences (39). This model-based approach, also known as non-linear mixed effects modeling, can be used to estimate PK parameters on the basis of a limited number of PK samples per child, and interindividual and residual variability can be described separately (39). This allows for a covariate analysis, in which covariates, e.g. body weight or age, can be identified within the studied pediatric population to (partly) explain the interindividual variability. These covariates can subsequently be used to guide dosing.

Several types of PK models can be developed, based on availability of data and the model's purpose (40), ranging from relatively simple empirical models to more complex (semi-) physiologically-based models. To describe clearance in a pediatric population, an empirical model may be sufficient, and after proper evaluation (41), these PK models can be used for the development of dosing guidelines in the studied population.

However, an empirical model might not be sufficient to obtain insight into drug independent systems information (42), for example to study what role intestinal and hepatic CYP3A play in the first-pass metabolism of CYP3A substrates like midazolam (43, 44). A combination of mechanistic and empirical models can be used to enable incorporation of physiological knowledge on the gastro-intestinal tract, while obtaining more insight into the system by parameter estimation of for example gut wall and hepatic intrinsic clearance based on reverse translation of the observed clinical data (42).

Pediatric clearance of various CYP3A substrates

To obtain information on the pediatric clearance of different CYP3A substrates, dedicated PK studies in patients from all ages for all drugs should be performed. This requires a large burden and many resources. Therefore, other approaches have been proposed, including an approach in which a covariate relationship for clearance from one drug is extrapolated to another drug that shares the same elimination pathway (28). This has been shown to result in accurate pediatric clearance predictions for some drugs eliminated by glomerular filtration and drugs glucuronidated by UGT2B7 (28-30). In order to explore the potential use of this approach in a more general way, a framework was developed by Calvier *et al.* to identify the conditions in which clearance can accurately be scaled from adult clearance based on between-drug extrapolation of a pediatric covariate function for clearance from a model drug to a test drug (45). This framework can be applied to evaluate down to which age systemically accurate scaling of clearance is possible, based on drug properties including the fraction metabolized by the isoenzyme pathway for which plasma clearance is scaled, the hepatic extraction ratio of the drugs in adults, the type of

binding plasma protein, and the unbound fraction (45). This framework is useful to predict to which drugs a pediatric covariate function for CYP3A-mediated midazolam clearance can be extrapolated.

Scope and intent of the investigations

A critical information gap exists with regard to the metabolism of CYP3A substrates in neonates, infants, and children, and CYP3A-mediated clearance of drugs cannot yet be predicted throughout the pediatric age range. Therefore, the aim of this thesis is to predict first-pass and systemic metabolism of CYP3A substrates in neonates, infants, and children, by development of pediatric (physiological) population PK models using the probe drug midazolam. For this purpose, the population approach is used to study systemic CYP3A-mediated midazolam clearance after intravenous administration, and a more physiological population approach is used to study the role of gut wall and hepatic CYP3A enzymes in presystemic metabolism when midazolam is administered orally. Lastly, we evaluate the application of a previously developed framework (45) to scale clearance of commonly used CYP3A substrates in children, using a pediatric covariate function for CYP3A-mediated midazolam clearance. This chapter provides an outline of the investigations that are described in this thesis.

Section I (Chapter 2) describes the general trend towards evidence-based pharmacotherapy in the pediatric population using pharmacokinetic-pharmacodynamic modeling. As many drugs, including many CYP3A substrates, are still used off-label in neonates, infants, and children, PK and PD studies are urgently needed in this population. We describe the different steps to take to come to an individualized dosing regimen, using PK and PKPD models, and how these models should be validated both internally and externally before a new dosing regimen can be proposed. Such new dosing regimens can be used to improve clinical practice, and guidelines can be updated accordingly. We foresee that properly designed clinical PKPD studies will remain the backbone of pediatric pharmacological research, and explaining the variability in PK is the first step towards individualized pharmacotherapy of CYP3A substrates and other drugs in children.

Section II focuses on the systemic metabolism by CYP3A enzymes in neonates, infants, and children, using intravenous midazolam clearance to reflect CYP3A-mediated metabolism, and aims to quantify the impact of maturation, critical illness, and inflammation on midazolam clearance in critically ill children. **Chapter 3** describes midazolam PK after intravenous administration in critically ill children aged between 1 day and 16.8 years of age. A PK model is developed to describe

clearance of midazolam in critically ill children and a covariate analysis is performed to explain the inter- and intra-individual variability within the population. The most important studied factors include body weight and age, reflecting the growth and maturation of the children, as well as critical illness and inflammation. Other pediatric studies suggested an important influence of these factors, but this has not been elucidated before. As it is important to evaluate the PK model both internally and externally before it is to be used to guide dosing in clinical practice or to derive dosing recommendations, in **Chapter 4** the predictive performance of the developed population PK model is evaluated in independent clinical datasets including data from similar populations (postoperative and critically ill term neonates, infants, and children) and other populations (including preterm neonates, healthy adults, and critically ill adults).

In **Section III** a novel physiological population PK modeling approach is used to delineate the contribution of enzymes in the gut wall and the liver to first-pass metabolism by CYP3A after oral administration of midazolam. The model includes physiological compartments representing the gastro-intestinal tract and the liver, and also empirical central and peripheral compartments for midazolam and 1-OH-midazolam distribution. Pediatric physiological information from literature is included to account for differences in relevant physiological parameters such as hematocrit, intestinal surface area, organ size, tissue blood flows, and plasma protein binding in neonates, infants, and children, compared to adults. Based on this physiological information and by using PK data from preterm neonates and children (25, 46, 47), the intrinsic intestinal and hepatic clearances are estimated to derive values for bioavailability and plasma clearance. **Chapter 5** focuses on intestinal and hepatic CYP3A activity in preterm neonates, while **Chapter 6** describes the characterization of intestinal and hepatic CYP3A-mediated metabolism in children from 1 – 18 years of age.

Section IV discusses how information on CYP3A-mediated clearance of the probe drug midazolam in children can be used for the scaling of plasma clearance of other commonly used CYP3A substrates in the pediatric population. Based on the developed framework by Calvier *et al.* which takes into account drug properties like hepatic extraction ratio and fraction unbound (45), **Chapter 7** describes the application of the between-drug extrapolation of a covariate relationship for clearance based on midazolam to scale clearance of other CYP3A substrates in children. Chapter 7 also evaluates whether a pediatric covariate function for CYP3A-mediated midazolam clearance can be used to scale clearance of various commonly used CYP3A substrates in children, by comparison of scaled clearance values with

reported pediatric clearance values. In addition, the pediatric covariate function for CYP3A-mediated clearance is applied to scale pediatric clearance of sildenafil, and these scaled clearance values are then compared with clearance values which are estimated based on sildenafil PK data in children 1-17 years of age.

Lastly, **Section V (Chapter 8)** summarizes the results presented in this thesis on the maturation of systemic and first-pass CYP3A-mediated metabolism reflected by midazolam clearance in neonates, infants, and children, and on the between-drug extrapolation of a pediatric covariate function for clearance from midazolam to other commonly used CYP3A substrates. Furthermore, it provides future perspectives on the translation of our results to the clinic, on the use of physiological modeling approaches, and on how information from midazolam PK models can be used to predict drug clearance of other CYP3A substrates in the pediatric population.

REFERENCES

1. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology–drug disposition, action, and therapy in infants and children. *N Engl J Med*. 2003;349(12):1157-67.
2. Johnson PJ. Neonatal pharmacology–pharmacokinetics. *Neonatal Netw*. 2011;30(1):54-61.
3. Lu H, Rosenbaum S. Developmental pharmacokinetics in pediatric populations. *J Pediatr Pharmacol Ther*. 2014;19(4):262-76.
4. Debotton N, Dahan A. A mechanistic approach to understanding oral drug absorption in pediatrics: an overview of fundamentals. *Drug Discov Today*. 2014;19(9):1322-36.
5. Mooij MG, de Koning BA, Huijsman ML, de Wildt SN. Ontogeny of oral drug absorption processes in children. *Expert Opin Drug Metab Toxicol*. 2012;8(10):1293-303.
6. Haddad S, Restieri C, Krishnan K. Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. *J Toxicol Environ Health A*. 2001;64(6):453-64.
7. Bjorkman S. Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: theophylline and midazolam as model drugs. *Br J Clin Pharmacol*. 2005;59(6):691-704.
8. McNamara PJ, Alcorn J. Protein binding predictions in infants. *AAPS PharmSci*. 2002;4(1):E4.
9. Johnson TN, Tucker GT, Tanner MS, Rostami-Hodjegan A. Changes in liver volume from birth to adulthood: a meta-analysis. *Liver Transpl*. 2005;11(12):1481-93.
10. Alcorn J, McNamara PJ. Ontogeny of hepatic and renal systemic clearance pathways in infants: part II. *Clin Pharmacokinet*. 2002;41(13):1077-94.
11. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: ontogeny and drug disposition. *Clin Pharmacokinet*. 1999;37(6):485-505.

12. Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J Pharmacol Exp Ther.* 2002;300(2):355-60.
13. Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, et al. Developmental expression of the major human hepatic CYP3A enzymes. *J Pharmacol Exp Ther.* 2003;307(2):573-82.
14. Treluyer JM, Bowers G, Cazali N, Sonnier M, Rey E, Pons G, et al. Oxidative metabolism of amprenavir in the human liver. Effect of the CYP3A maturation. *Drug Metab Dispos.* 2003;31(3):275-81.
15. Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, et al. Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. *Curr Drug Metab.* 2007;8(1):33-45.
16. 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.
17. de Wildt SN, Ito S, Koren G. Challenges for drug studies in children: CYP3A phenotyping as example. *Drug Discov Today.* 2009;14(1-2):6-15.
18. Vet NJ, de Hoog M, Tibboel D, de Wildt SN. The effect of inflammation on drug metabolism: a focus on pediatrics. *Drug Discov Today.* 2011;16(9-10):435-42.
19. Carcillo JA, Doughty L, Kofos D, Frye RF, Kaplan SS, Sasser H, et al. Cytochrome P450 mediated-drug metabolism is reduced in children with sepsis-induced multiple organ failure. *Intensive Care Med.* 2003;29(6):980-4.
20. Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. *Drug Metab Dispos.* 2007;35(9):1687-93.
21. Aitken AE, Richardson TA, Morgan ET. Regulation of drug-metabolizing enzymes and transporters in inflammation. *Annu Rev Pharmacol Toxicol.* 2006;46:123-49.
22. Kirwan CJ, MacPhee IA, Lee T, Holt DW, Philips BJ. Acute kidney injury reduces the hepatic metabolism of midazolam in critically ill patients. *Intensive Care Med.* 2012;38(1):76-84.
23. Pacifici GM. Clinical pharmacology of midazolam in neonates and children: effect of disease-a review. *Int J Pediatr.* 2014;2014:309342.
24. Anderson BJ, Larsson P. A maturation model for midazolam clearance. *Paediatr Anaesth.* 2011;21(3):302-8.
25. de Wildt SN, Kearns GL, Hop WC, Murry DJ, Abdel-Rahman SM, van den Anker JN. Pharmacokinetics and metabolism of oral midazolam in preterm infants. *Br J Clin Pharmacol.* 2002;53(4):390-2.
26. de Wildt SN. Profound changes in drug metabolism enzymes and possible effects on drug therapy in neonates and children. *Expert Opin Drug Metab Toxicol.* 2011;7(8):935-48.
27. van de Waterbeemd H, Gifford E. ADMET in silico modelling: towards prediction paradise? *Nat Rev Drug Discov.* 2003;2(3):192-204.
28. 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.

29. 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.
30. 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.
31. 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.
32. 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.
33. Hall RW, Shbarou RM. Drugs of choice for sedation and analgesia in the neonatal ICU. *Clin Perinatol*. 2009;36(1):15-26.
34. Vet NJ, Ista E, de Wildt SN, van Dijk M, Tibboel D, de Hoog M. Optimal sedation in pediatric intensive care patients: a systematic review. *Intensive Care Med*. 2013;39(9):1524-34.
35. Watkins PB. Noninvasive tests of CYP3A enzymes. *Pharmacogenetics*. 1994;4(4):171-84.
36. Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, et al. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos*. 2002;30(8):883-91.
37. Dundee JW, Collier PS, Carlisle RJ, Harper KW. Prolonged midazolam elimination half-life. *Br J Clin Pharmacol*. 1986;21(4):425-9.
38. Shaddy RE, Denne SC, Committee on D, Committee on Pediatric R. Clinical report-guidelines for the ethical conduct of studies to evaluate drugs in pediatric populations. *Pediatrics*. 2010;125(4):850-60.
39. 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>.
40. Tsamandouras N, Rostami-Hodjegan A, Aarons L. Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. *Br J Clin Pharmacol*. 2015;79(1):48-55.
41. Krekels EH, van Hasselt JG, Tibboel D, Danhof M, Knibbe CA. Systematic evaluation of the descriptive and predictive performance of paediatric morphine population models. *Pharmaceutical research*. 2011;28(4):797-811.
42. Rostami-Hodjegan A. Reverse Translation in PBPK and QSP: Going Backwards in Order to Go Forward With Confidence. *Clin Pharmacol Ther*. 2017.
43. Brill MJ, Valitalo PA, Darwich AS, van Ramshorst B, van Dongen HP, Rostami-Hodjegan A, et al. Semiphysiologically based pharmacokinetic model for midazolam and CYP3A mediated metabolite 1-OH-midazolam in morbidly obese and weight loss surgery patients. *CPT Pharmacometrics Syst Pharmacol*. 2016;5(1):20-30.
44. 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.

45. 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.
46. de Wildt SN, Kearns GL, Hop WC, Murry DJ, Abdel-Rahman SM, van den Anker JN. Pharmacokinetics and metabolism of intravenous midazolam in preterm infants. *Clin Pharmacol Ther*. 2001;70(6):525-31.
47. 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>.