APPLICATIONS OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELLING TO PREDICTION OF THE LIKELIHOOD OF METABOLIC DRUG INTERACTIONS IN PAEDIATRIC POPULATION AND STUDYING DISPARITIES IN PHARMACOKINETICS BETWEEN CHILDREN AND ADULTS

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Abstract

Anticipation of drug-drug interactions (DDIs) in the paediatric population are merely based on data generated in adults. Hence decision on avoiding certain combinations or attempts to adjust and manage the doses under combination-therapy are mainly speculative from the knowledge of what occurs in adults. However, due to developmental changes in elimination pathways from birth to adolescents, the assumption of DDIs being similar in adults and children might not be correct. This thesis firstly identifies and quantitatively compares the reported DDIs in paediatric and adult populations through a systematic literature review of DDIs reported in paediatric subjects. The study highlights the clear paucity of the data in children younger than 2 years. Therefore, the logical approach to test this hypothesis has been through modelling and simulation and incorporation of the biological knowledge on ontogeny of various enzymes and other elimination routes. The magnitude of any metabolic DDI depends on fractional importance of inhibited pathway which may not necessarily be the same in young children when compared to adults. To show this disparity between rate of ontogeny for metabolic pathways, the ontogeny pattern of CYP enzymes and renal function were analysed systematically. Bootstrap methodology was used to account for variability, and to define the age range over which a statistical difference is likely between each pair of specific pathways. A number of DDIs were simulated for virtual compounds to highlight the possibility that the magnitude of DDI can be influenced by age. Depending on the extent of contribution of metabolic pathways, neonates could be more sensitive to DDI than adults in certain scenarios or vice versa. Thus, extrapolation from adult DDI data may not be applicable across paediatric age groups. The uncertainty around the ontogeny functions based on *in vitro* information led us to carry out comprehensive performance verification for *in vivo* data on probe substrates of CYP1A2, -2C9 and 3A4 and assess the predictions of clearance (CL) by monitoring AUC. Although the evaluation showed that in most cases predictions were within two fold of observed data in adult and paediatric studies, the outcome suggests that the current ontogeny profiles result in under-prediction of CL values compared to clinical studies in infants and children and there is a need for better ontogeny models. Therefore, we derived novel ontogeny functions for CYP1A2 and CYP3A based on caffeine-theophylline and midazolam *in vivo* data. Age related CL data for caffeine, theophylline and midazolam were reconstructed back to intrinsic CL values per milligram of microsomal protein and best fit ontogeny models for CYP1A2 and CYP3A were derived from these data. The function for CYP1A2 describes an increase in relative intrinsic metabolic CL from birth to 3 years followed by a decrease to adult values. The function for CYP3A4 describes a continuous rise in relative intrinsic metabolic CL, reaching the adult value at about 2 years of age. The new models were validated by showing improved predictions of the systemic CL of ropivacaine (major CYP1A2 substrate; minor CYP3A4 substrate) and alfentanil (major CYP3A4 substrate) compared to those using a previous ontogeny function based on *in vitro* data. When implementing enzyme ontogeny functions it is important to consider potential confounding factors related to disease, anaesthesia and surgery that may affect the prediction of net *in vivo* CL. Finally, we demonstrated the application of paediatric physiologically-based pharmacokinetic (p-PBPK) models for calculation of sample size in paediatric clinical pharmacokinetic (PK) studies in a methodology suggested by Wang *et al*., based on desired precision for a PK parameter of interest. We obtained estimates of variability for CL, volume of distribution and area under the plasma concentration-time curve for 5 different drugs from (i) adult and paediatric classic clinical PK studies, and (ii) p-PBPK combined with *in vitro-in vivo* extrapolation. The estimates were applied to the sample size calculation proposal methodology for non-compartmental analysis. There were clear and drug dependent differences in calculated sample size based on various estimates of variability and overall, there was no consistent discrepancy in the sample size calculated according to the source of variability used for sample size calculations. The results are discussed in terms of their potential impact on the clinical PK studies in children. In general, considering the sensitivity of paediatric clinical PK studies and paucity of data in this group of patients, the use of p-PBPK models may offer an interim solution to uncovering age bands with potential higher vulnerability to DDI. However, these models require further refinements and testing before widely used in clinical practice with confidence.

Declaration

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Publications

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Poster presentations

1. **Salem F**, Barter ZE, Johnson TN, Rostami-Hodjegan A. ”Theoretical Assessment of Metabolic Drug Interactions in Paediatric Population: The Impact of Age Related Fractional Metabolism (fm) and Its Disparity between adults and Neonates”, PKUK November 2008, Hilton Hotel London Stansted Airport

2. **Salem F**, Barter ZE, Johnson TN, Rostami-Hodjegan A., "Taking Guesswork Out of Paediatric Drug Development: Using the Knowledge of Biology in the Prediction ADME in Children", Japan Society of Developmental Pharmacology & Therapeutics, 5-6th December 2008, Tokyo, Japan

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model " Drug Metabolism Discussion Group (DMDG), Robinson College, 23rd- 25th September 2009, Cambridge, UK

4. **Salem F**, Johnson TN, Rostami-Hodjegan A. "Can the "in silico Child" help to develop better medicines for our children?", 28th-29th September, 2010, Genova–Quarto, Italy

5. **Salem F**, Johnson TN, Rostami-Hodjegan A, "Prediction of Drug-Drug Interactions in the Paediatric Population" Neonatal and Paediatric Pharmacists Group 16th Annual Conference & Exhibition, 13th-14th November, 2010, Sheffield, UK

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7. **Salem F**, Johnson TN, Rostami-Hodjegan A, "Challenges in design of paediatric studies: DDI risk and decisions *on sample size* ", Centre for Applied Pharmacokinetic Research (CAPKR), Chancellors, 30th June 2011, Manchester, UK

8. **Salem F**, Johnson TN, Rostami-Hodjegan A, "Metabolic drug-drug interactions (DDI) in Paediatric *vs*. Adults ", Pharmacokinetic UK (PKUK), Radisson Blu Hotel, 9-11th November 2011, Durham, UK

9. **Salem F**, Johnson TN, Rostami-Hodjegan A, "Mapping in vitro and *in vivo* derived CYP3A ontogeny function: A critical comparison between various ontogeny models", Population Approach Discussion Group (PAGE), Palazzo del Casinò, 5-8th June 2012, Venice Lido, Italy

10. **Salem F**, Johnson TN, Rostami-Hodjegan A, "Mapping *in vitro* and *in vivo* derived CYP3A ontogeny function: A critical comparison between various ontogeny models", CAPKR, Chancellors, 5-6th July 2012, Manchester, UK

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12. **Salem F**, Johnson TN, Rostami-Hodjegan A, “A Critical Comparison between CYP1A2 and 3A4 Ontogeny Profiles Used in Paediatric PBPK Models”, World Conference on Pharmacometrics (WCoP), 5-7th September 2012, Grand Hilton Hotel, Seoul, Korea

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1. “In Silico child in drug development”, Informa Life Sciences, Rubens at the Palace, 29th -30th September, 2009, London, UK
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3. “Do children have the same vulnerability to metabolic drug-drug interactions as adults?”, University of North Carolina, July 2012
4. “Is drug-drug interaction in paediatrics similar to that in adults?”, Manchester Royal Infirmary Children`s Hospital, July 2012
5. “Prediction of Drug-Drug Interactions in Paediatric Populations”, Manchester Royal Infirmary Children`s Hospital, March 2012
6. “Improvement of PK predictions in the Simcyp paediatric simulator by new ontogeny models and potential applications of paediatric PBPK models in trial design”, The university of Nottingham, Medical School, Royal Derby Hospital, July 2013

List of abbreviations

ADME	Absorption, distribution, metabolism and elimination
AUC	Total area under the plasma drug concentration-time curve between time zero and time infinity
B:P	Blood to plasma drug concentration ratio
BD	Twice daily
BSA	Body surface area
BW	Body weight
BW(paed)	Paediatric body weight
CI	Confidence intervals
CL	Total systemic clearance based on plasma drug concentration
CL_B	Total systemic clearance based on blood drug concentration
$CL_{H,B}$	Hepatic metabolic clearance based on blood drug concentration
CL_{int}	Intrinsic clearance
$CL_{int,pe}$	Intrinsic metabolic clearance per enzyme for individual pathways
CL_{iv}	Intravenous clearance
CL_{perm}	Permeability clearance through the enterocyte
CL_{po}	Oral clearance
CL_R	Renal plasma clearance of drug
CL_R(adult)	Renal plasma clearance of drug in adults
CL_R(paed)	Renal plasma clearance of drug in paediatric subjects
$CL_{R,B}$	Renal blood clearance of drug
$CLu_{int,G}$	Gut intrinsic clearance of unbound drug
$CLu_{int,H}$	Hepatic intrinsic clearance of unbound drug
$CL_{u,int,H,pe}$	Hepatic intrinsic clearance of unbound drug per each pathway
CsA	Cyclosporin
C_{ss}	Plasma drug concentration at steady state
CV	Coefficient of variation

CYP	Cytochrome P450
DDI	Drug-drug interaction
DM	Dextromethorphan
DX	Dextrorphan
E_G	Intestinal extraction ratio of drug
E_H	Hepatic extraction ratio of drug
EMA	European medicine agency
E:P	Erythrocyte to plasma drug concentration ratio
F	Total availability of unchanged drug
f_a	Fraction of dose entering the cellular space of the enterocytes from the gut lumen
FDA	Food and drug administration
Fe	Fraction of dose in systemic circulation excreted unchanged in the urine
F_G	Fraction of the drug enterocyte which escapes first pass gut wall metabolism
F_H	Fraction of dose entering the liver that escapes hepatic first pass hepatic metabolism and biliary secretion
fm	Fraction of dose in systemic circulation which is eliminated metabolically
f_u	Fraction of drug in plasma unbound
fu(adults)	Fraction of drug unbound in plasma of adults
fu(paed)	Fraction of drug unbound in the plasma of paediatric subjects
fu_B	Fraction of drug unbound in blood
fu_{inc}	Fraction of drug unbound in incubations
fu_G	Fraction of unbound drug in the enterocyte
GFR	Glomerular filtration rate
GFR(adult)	Glomerular filtration rate in adults
GFR(paed)	Glomerular filtration rate in paediatric subjects
GSA	Gestational age
Hct	Haematocrit
HLM	Human liver microsomes
ISEF	Inter system extrapolating factor

IVIVE	*In vitro-in vivo* extrapolation
K_i	Inhibitory constant
K_m	Michaelis- Menten constant
$K_{m,pe}$	Michaelis- Menten constant for individual metabolic pathways
IV	Intravenous
Intravenous	Octanol/buffer partition coefficient
MPPGI	Milligram of microsomal protein per gram of intestine
MPPGL	Milligram of microsomal protein per gram of liver
NG	Nasogastric
[P]	Protein concentration
$[P]_{adult}$	Plasma protein concentration in adults
$[P]_{paed}$	Plasma protein concentration in paediatric subjects
PBPK	Physiologically-based pharmacokinetic
PD	Pharmacodynamic
P_{eff}	Effective permeability in human
PEJ	Percutaneous endoscopic jejunostomy
PIP	Paediatric investigation plan
PK	Pharmacokinetic
POPPK	Population pharmacokinetic
p-PBPK	Paediatric physiologically-based pharmacokinetic
PMA	Postmenstrual age
po	By mouth
PPI	Proton pump inhibitor
PUMA	Paediatric-use marketing authorisation
Q.D.S	Four times a day
Q_{gut}	Gut blood flow
$Q_{H,B}$	Hepatic blood flow
Q_{villi}	Villous blood flow
rCYP	Recombinantly expressed Cytochrome P450
SD	Standard deviation

TDM	Therapeutic drug monitoring
TDS	Three times a day
UGT	Uridine glucuronyl transferase
V_d	Volume of distribution
V_{max}	Maximum velocity of metabolism by an enzymatically mediated reaction (amount/time)
$V_{max,pe}$	Maximum velocity of metabolism by an enzymatically mediated reaction (amount/time) for individual enzymatic pathways
VPA	Valproic acid
V_{ss}	Volume of distribution at steady state, based upon measurement of drug in plasma
WHO	World health organisation

Chapter 1. Introduction

1.1 Aspects of paediatric clinical pharmacology

Paediatric clinical pharmacology aims to overcome diseases and clinical conditions in children through rational evidence based drug therapy. Administration of appropriate medicines, dosage forms and dosing regimens require the knowledge of the disease and the pharmacokinetics or pharmacodynamics of drugs.

1.1.1 Pharmacotherapy in paediatric group

In pharmacotherapy, safe and effective exposure of subjects to drugs is desirable. There are several aspects in paediatric pharmacotherapy that should be considered. These include disease state, choice of drugs and appropriate dose of drugs.

1.1.1.1 Disease

Children may suffer from diseases that are specific to their own age group or diseases that occur in adult populations (FDA, 2001). The course of many diseases might be different between paediatrics and adults (Zimmet et al., 2007; Pigneur et al., 2010). Even common diseases between paediatrics and adults such as cancer have different characterisation in paediatric patients due to physiological differences and especially due to the development of the immunological system. The biomarkers for diagnosis of disease may also follow an age dependent pattern such as anaemia (a common childhood disease) hypertension and hepatitis C (Goldman et al., 2011).

The course and clinical characterisation of diseases in children may be:

- similar to adult patients e.g. many infectious diseases
- similar but follow a different pattern and require different treatment e.g. childhood leukaemia and pneumonia (bronchopulmonary dysplasia, persisted pulmonary hypertension)
- unique but limited to a specific age group e.g. neonatal sepsis, patent ductus arteriosus.
- unique to the paediatric population due to limited survival e.g. lennox gastaux syndrome, various metabolic diseases or due to a limited window of importance e.g.

short stature due to lack of growth hormone. Due to improved treatments some diseases that were unique to children including cystic fibrosis or attention deficit hyperactivity disorder (ADHD) are now being seen in adulthood

Food and drug administration (FDA) tries to promote the evidence based safety and efficacy of medical treatment in paediatric patients by requiring clinical studies at all age groups. Based on paediatric rule 1994, if the course of disease and drug effects are comparable in paediatric and adult populations, this similarity will allow extrapolation of adult efficiency and pharmacokinetics data of a drug to paediatric patients (FDA, 1998). In the latter case specific drug evaluations may not be required in children; however, if there are differences in disease and disease progression, extrapolation from adult data might not be appropriate and therefore, conducting clinical pharmacology studies in the relevant age groups becomes necessary (Meijer et al., 2009; Goldman et al., 2011). The FDA centre for drug evaluation and research have published a decision tree to guide drug developers regarding the likely need to conduct both pharmacokinetic (PK) and efficacy studies in children (Figure 1.1-1) (FDA, 2003).

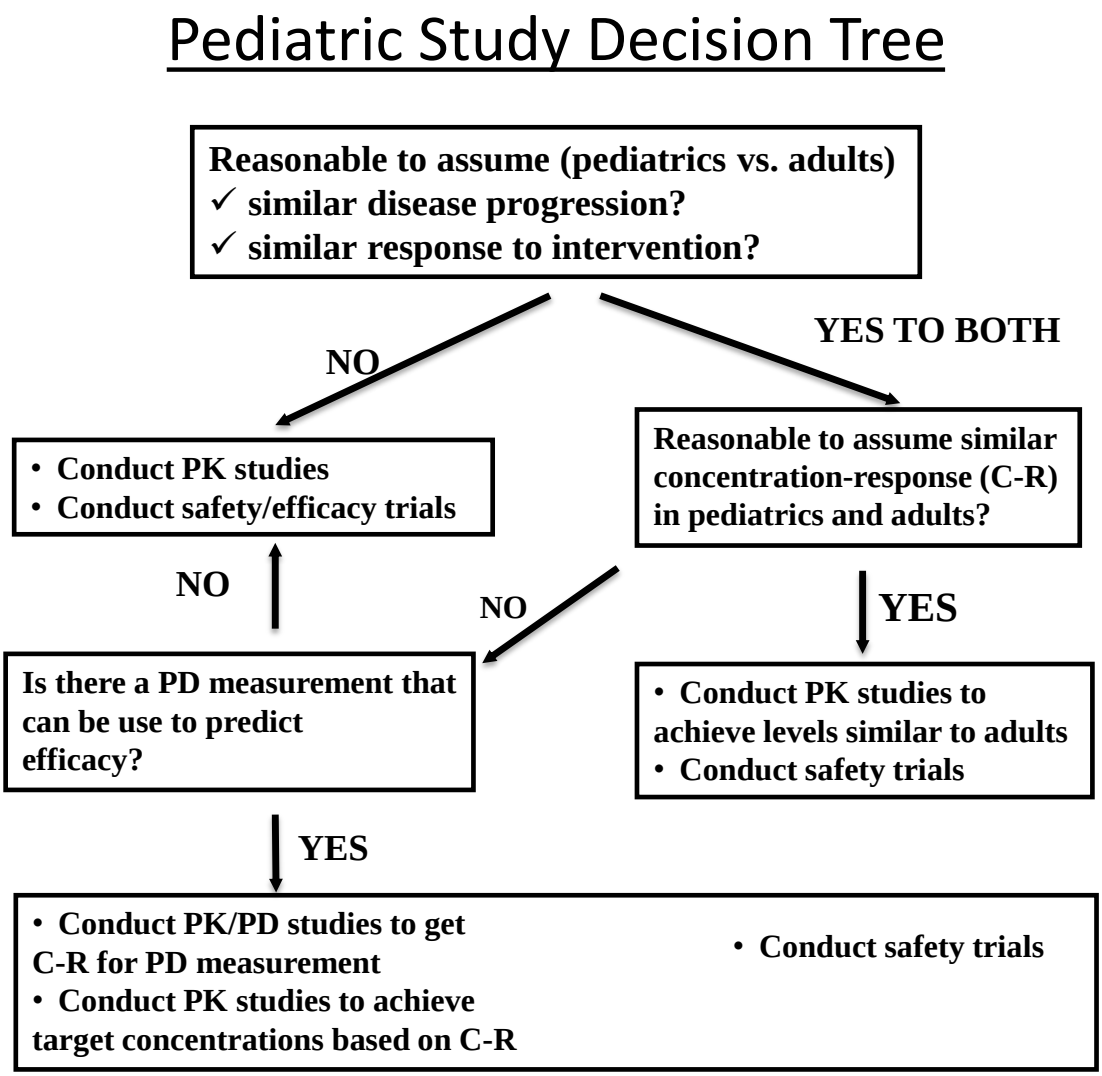

Figure 1.1-1 Paediatric decision tree integration of PK-PD adapted from (FDA, 2003).

1.1.1.2 Drugs

Due to the limited number of drugs being studied in paediatric age groups, currently there is lack of adequate evidence that the same treatment as in adults can be applied to paediatrics (Vloet and Hagenah, 2009); therefore, by necessity, children are often treated with the same drugs as those used to treat adults (FDA, 2001). In clinical practice appropriate dose is often estimated or arrived at by consensus in children. Historically, paediatric doses have been scaled from adult dose based on age, body weight, body surface area and allometric scaling. Examples of these methods are Young's rule (Equation 1.1-1) and Clark's rule (Equation 1.1-2 and Equation 1.1-3) based on scaling dose using body weight or body surface area (BSA) (Johnson, 2008). However, these models have limitations, for example, BSA

overestimates the dose for neonates and infants (Johnson, 2008), consequently, to avoid the risk of overdose when scaling based on body size, body weight is preferred to calculate the dose in neonates and infants.

$$\mathrm{Dose_p} = \mathrm{Dose_A} \times \frac{\mathrm{Age_p\,(years)}}{\mathrm{Age_A\,(years)} + 12} \qquad \textbf{Equation 1.1-1}$$

$$\mathrm{Dose_p} = \mathrm{Dose_A} \times \frac{\mathrm{BW_p}}{\mathrm{BW_A}} \qquad \textbf{Equation 1.1-2}$$

where $\mathrm{Dose_p}$ and $\mathrm{Dose_A}$ are paediatric and adult doses and $\mathrm{BW_p}$ and $\mathrm{BW_A}$ paediatric and adult body weight, respectively.

$$\mathrm{Dose_p} = \mathrm{Dose_A} \times \frac{\mathrm{BSA_p}}{\mathrm{BSA_A}} \qquad \textbf{Equation 1.1-3}$$

where $\mathrm{BSA_p}$ and $\mathrm{BSA_A}$ are paediatric and adult body surface area, respectively.

Allometric scaling using allometric exponent is another method of dose scaling from adults (Equation 1.1-4). This approach applies an allometric exponent that could be variable in different studies. This exponent is usually equals to 0.75.

$$\mathrm{Dose_p} = \mathrm{Dose_A} \times \left(\frac{\mathrm{BW_p}}{\mathrm{BW_A}} \right)^{0.75} \qquad \textbf{Equation 1.1-4}$$

Overall, the scaling of doses from adults to children is not recommended except as a last resort when no published dose is available and it is imperative that the child receives the drug. A conservative approach starting with a low dose and titrating based on response is encouraged. Selection of the appropriate dose in paediatric patients requires an understanding of PK and PD of that drug as well as age related physiological changes during neonatal and infancy periods. More recently, evidence based dosing regimens are suggested based on PK and PD of drugs. This approach allows individual dosing regimens

to be determined through simulation of drug concentration based on estimated PK parameters in the population PK model (Admiraal et al., 2014).

1.1.2 Knowledge gap on PK of drugs in paediatric subjects

Due to difficulties in conducting clinical studies in children, PK characteristics of many drugs are not well-understood and pharmacokinetics and safety of many drugs in paediatric population is not tested. Most medicines given to children are used off label or unlicensed (Conroy et al., 2000; Conroy and McIntyre, 2005; Aagaard and Hansen, 2011) and this off-label or unlicensed use of drugs has raised safety concerns in using these drugs in children (Turner et al., 1999; Horen et al., 2002; Aagaard and Hansen, 2011). However, the association between increase incidence of adverse effects and off-label or unlicensed use of drugs in paediatric patients was not seen in some studies (Schirm et al., 2004; Phan et al., 2010). Around25% of drugs that have been approved in adults are not approved to be used in any paediatric age group (FDA, 2011). Not only is there insufficient information on paediatric use for most drugs but also there are examples that show children occasionally require larger doses of drugs based on body weight compared to adults for effective pharmacotherapy (Kelly, 1987; Blanco et al., 2000; Bartelink et al., 2006) due to higher clearance (CL) per kg of drugs from the body. This higher dose may have potential risks of toxicity in over-weight children and neonates; especially in preterm neonates. In chronic use of drugs to treat conditions such as seizures, the drug might be used from the neonatal period through to adulthood. Due to rapid physiological changes in the body from birth onwards, the PK of these drugs may undergo changes; therefore, maintenance dose for chronic treatment in paediatrics should be individualised and monitored regularly. However, this procedure can be costly and complicated for many drugs.

1.1.3 Polypharmacy

It is common that patients are administered more than one drug in order to manage one or independent clinical complications. The concomitant administration of two or more therapeutic entities to manage patient's disease is called polypharmacy (Martinbiancho et al., 2007). For example, a combination of anti-epileptic drugs is intended to establish seizure free episodes for the patients or a combination of antibiotics are administered in HIV or tuberculosis for more effective therapy and prevention of drug resistance. These combination

therapies are desirable and well-studied. However, occasionally combination therapy leads to suboptimal therapeutic effect manifested by adverse drug effects or lack of effect or even toxicities.

In 2013, Barrett et al., reported that 19% of paediatric cancer patients in Children's Hospital of Philadelphia showed at least one out of twenty forms of toxicities reported in this article. These toxicities ranged in severity from grade 1 (least) to 4 (most). The highest incidence of toxicities attributed to neutrophil count decrease and alanine aminotransferase increase following the cancer treatment (Barrett et al., 2013). These adverse drug effects might be attributed to changes in PK or PD of drugs following polypharmacy however, such association has not been established.

1.1.3.1 Importance of DDIs in clinical pharmacology

Drug-drug interactions (DDIs) are an important and avoidable cause of variability in drug response and occur when one drug changes the effectiveness or toxicity of another drug when administered together to a patient. DDIs are considered as the major cause of hospitalisation and death amongst patients and increased cost of treatments (Martinbiancho et al., 2007). It is expected that 3 to 5% of patients experience a DDI when a few drugs are co-administered. This number reaches up to 20% when 10 to 20 drugs are taken by the patient (Martinbiancho et al., 2007). Examples of DDIs were reported by (Barrett et al., 2013) in paediatrics cancer patients. These included acetaminophen-diphenhydramine and methotrexate –vincristine. In that study, the authors concluded that some forms the frequent toxicities including hepatotoxicity as a result of fentanyl-midazolam co-administration can simply be avoided if the drugs are administered in dosing intervals over 30 minutes (Barrett et al., 2013). This finding perhaps shows role of dose staggering in prevention of these DDIs.

Undesirable DDIs are categorised by (Martinbiancho et al., 2007) as;

- severe with a risk to life or permanent damages
- moderate with a need for additional treatment
- mild that do not significantly alter the effect of other drug therapy.

According to European Medicines Agency (EMA) an interaction is “clinically relevant” when the therapeutic activity and or toxicity of a drug is changed to such an extent that a dosage adjustment of a medication or medical intervention may be required (CHMP, 1997). DDIs not only result in unwanted clinical consequences but also cause additional costs to healthcare. The reason for the latter is that DDIs are one of the important causes of hospitalisation and they are a burden on drug development especially when they result in the drug being withdrawn from the market.

In the past two decades new prospects have been sought in paediatric clinical pharmacology for safe and effective administration of drugs and to avoid the risk of adverse drug effects that is higher in patients receiving polytherapy. Paediatric patients and especially neonates are expected to have a lower capacity for drug excretion and elimination and therefore may respond differently to DDIs (Qorraj-Bytyqi et al., 2012). DDI for many drug combinations is known in adult population; however, there is a knowledge gap with regard to the risk of DDIs in the paediatric age group.

1.1.3.2 Pharmacokinetic (PK) and pharmacodynamic (PD) DDIs

DDI may be classified as pharmacokinetic or pharmacodynamic DDIs. Pharmacokinetic DDIs occur when the perpetrator alters the absorption, distribution, metabolism or excretion of a victim drug and leads to altered plasma concentration of the victim drug. An example of this type of DDIs is inhibition or induction of relevant metabolic pathways by a perpetrator. The perpetrator affects the concentration and consequently exposure to the victim drug.

If the DDI occurs through changes in metabolism of the victim drug, levels of metabolic DDI could be quantified using *in vitro* data during drug development. *In vitro* data on metabolic DDIs need to be extrapolated to *in vivo* conditions. The fold change in drug exposure after DDI for an orally administered drug that undergoes first-pass and systematic hepatic metabolism is calculated from Equation 1.1-5 (Rostami-Hodjegan and Tucker, 2004). The original form this equation was proposed by (Rowland, 1973).

$$\frac{AUC_{inhibited}}{AUC_{uninhibited}} = \frac{1}{\sum_{j=1}^{n} \frac{fm_j}{1+\frac{[I]}{K_i}} + (1-\sum_{j=1}^{n} fm_j)}$$ **Equation 1.1-5**

where $AUC_{inhibited}$ is exposure to victim drug after inhibition and $AUC_{uninhibited}$ is exposure to victim drug in absence of inhibitor, fm_j is the fraction of victim drug eliminated by the inhibited metabolic pathway ''j'', [I] is the inhibitor's concentration and K_i is the inhibitory constant. However, most of the information to calculate the level of DDI is not available until early stages of drug development. Equation 1.1-6 presents a simplified form of Equation 1.1-5 with the assumption that fm=1 i.e. the drug is metabolised only by one metabolic pathway.

$$\frac{AUC_{inhibited}}{AUC_{uninhibited}} = 1 + \frac{[I]}{K_i}$$ **Equation 1.1-6**

There are other factors contributing to the level of DDI that make their quantification more complex such as active uptake of inhibitor into liver, inhibition of gut wall metabolism, mechanism based inactivation of enzyme and impact of genetic differences (Rostami-Hodjegan and Tucker, 2004).

Another type of DDI occurs at the site of action through synergism or antagonism of victim drug's effect as considered as pharmacodynamic DDI. DDI between benzodiazepines and flumazenil is an example of PD interactions. In this type of DDI, victim dug and perpetrator compete for occupation of GABA receptors.

Another category of DDIs is called pharmaceutical DDIs referring to drug incompatibilities ex-vivo. However, this type of DDI is not in the scope of this investigation.

1.1.4 Defining paediatric population

In general, children are the group of population from birth to 18 years of age. Within this population there are subgroups. Food and Drug Administration (FDA, 1998) defines these subgroups within the paediatric population as:

- Neonate: birth to 1 month
- Infant: 1 month to 2 years

- Children: 2 to 12 years
- Adolescent: 12 years to 16 years.

A further age category for the paediatric population defined by EMA (EMA, 2006) is as following;

- Preterm new-born infants
- Term new-born infants: 0 to 27 days
- Infants and toddlers: 1 month to 23 months
- Children: 2 to 11 years
- Adolescents: 12 to 16 or 18 years

In another report World Health Organisation (WHO) defines children as “Young Child” if they are between 2 – 6 years and “Child” if they are 6 to 12 years old (Knoppert et al., 2007).

1.2 Paediatric drug development

1.2.1 Regulatory aspects

Despite the fact many drugs are used in paediatric patients without adequate evidence on their safety, the overall aim of regulators and scientists is to enhance the safety of pharmacotherapy in children. From a regulatory view point, paediatric clinical studies for the development of medicinal products for paediatric use are essential and are now a legal obligation for industries.

United States: The FDA has made attempts to enhance the knowledge of pharmacotherapy in paediatric patients by requiring labelling for safe and effective paediatric use and providing incentives to sponsors of paediatric clinical studies. According to FDA rule in 1994, safe and effective use of drugs in paediatrics is accepted by evidence from adequate and well-controlled studies that investigate (1) a specific paediatric indication different from the indication(s) approved for adults and (2) the same indication in children as approved for adults.

From the FDA’s point of view, paediatric clinical PK studies should be performed in all paediatric age groups based on the purpose of drug administration. If the drug has linear PK

in adults, single-dose studies are often adequate for PK assessment in the paediatric population but if the drug has nonlinear PK in any aspect of ADME (i.e. absorption, distribution, and elimination) in adults, and any duration-of-effect related changes, it is recommended to conduct paediatric clinical PK studies at steady state to better understand the PK of the drug (FDA, 1998).

Under Paediatric Research Equity Act 2007, any paediatric submission should contain data using appropriate formulations for the age group to ensure safety and effectiveness of the drug or biological product for the indication in question for all the relevant age groups (FDA, 2008).

In 2008, Rodriguez et al., used CL as the most important parameter influencing dose for 108 drugs and showed subsequent changes to paediatric labelling of these 108 drugs following recommendations from the FDA Modernisation Act 1997 and Best Pharmaceuticals for Children Act in 2002. The revised labelling consisted of dosing changes (23 cases), new safety information (34 cases), information concerning lack of efficacy (19 cases), new paediatric formulation (12 cases) and extended age limits (77 cases) (Rodriguez et al., 2008). In response to the FDA's Best Pharmaceuticals for Children Act, this organisation reported that 131 drug products were studied or reviewed in paediatrics. The list of these drugs is available at FDA's website http://www.fda.gov/Drugs/DevelopmentApproval Process/DevelopmentResources/UCM049872 (FDA, 2012).

Europe: As part of the requirement for new drug development, the EMA in conjunction with the European commission established the requirement for new drug applications to include a Paediatric Investigation Plan (PIP). The PIP requires paediatric data be provided in all applications for the authorisation of new medicinal products prior to the study by pharmaceutical companies, unless a deferral or a waiver has been granted by EMA. They also provide requirements, rewards and incentives as well as funding to encourage pharmaceutical companies to test their drugs in the paediatric population (Dunne, 2007; Lehmann, 2008). The PIP aims to ensure that the necessary data is obtained from clinical studies in paediatrics. European Commission also started advocating paediatric medicinal product development in 2002 and called this programme "better medicine for children". This

programme aimed to increase availability of authorised medicine in children by introducing incentives, funding and facilitating access to existing experience of paediatric use of medicine and creating a European union working party with specific responsibility for paediatric medicine (Conroy and McIntyre, 2005).

EMA recently published a report on the success over the period of 2007 to 2012 as a consequence of the above programs. According to the report there were 1) more high quality research in paediatric medicines 2) more information on medicines 3) more medicines for children, with age-appropriate forms following enforcement of paediatric regulations in Europe (EMA, 2013). Paediatric-use marketing authorisations (PUMAs) may be sought for new formulations and indications for existing drugs that are no longer on patent, these are granted to companies after they have submitted a PIP and performed the necessary clinical studies and ensure them 10 years of marketing protection for the new indication or formulation.

1.2.1.1 Consideration in conduct of clinical studies from regulatory view point

In paediatric clinical pharmacology, in order to achieve the same level of systemic exposure that is considered safe and effective in adults, paediatric dose must be adjusted. Initial doses as described above are usually calculated based on mg/kg of BW or mg/m^2 of BSA, extrapolated from adult doses. To refine the initial dose estimate, it is recommended to combine knowledge on the physiological development of the target paediatric study population with knowledge of ADME in an adult population and any paediatric experience or data. Subsequent clinical observations, regular monitoring and assay of biological fluids for the drug and/or its metabolites should be considered to further adjust the paediatric dose (FDA, 1998).

The FDA also recommends consideration of a number of factors in initial paediatric studies, particularly in younger subgroups i.e. neonates and infants. These considerations include:

- Relative bioavailability of the formulation compared to adults;
- Age of the paediatric population;
- Therapeutic index of the drug;

- PK data from the adult population;
- Body size of the paediatric study population.

Another important aspect of PK clinical studies is to determine the appropriate sample size. In designing any experiment it is important to relate the sample size to a specific degree of precision (Armitage et al., 2002). The FDA 1998 draft guidance (FDA, 1998) advocates the use of 6-12 paediatric subjects in clinical trials, irrespective of the PK of the compound. Recently, a new approach to calculate the sample size for the conduct of clinical PK studies in paediatric population based on estimates of the standard deviation (SD) is suggested. In the proposed methodology, it is stated that "The study must be prospectively powered to target a 95% CI (confidence interval) within 60% and 140% of the geometric mean estimates of clearance (CL) and volume of distribution (V_d) for DRUG NAME in each paediatric sub-group with at least 80% power" (Wang et al., 2012). Implications of this approach will be examined and discussed in future chapters.

1.3 Physiological differences between children and adults leading to PK differences

In early neonatal life, important physiological changes occur that reflect on all aspects of drug ADME and make drug PK an age dependant process. These differences are described in terms of body composition (lipid and water), organ weights, and organ blood flows, as well as functionality of hepatic and renal systems to eliminate drugs and toxicants from the body. These physiological differences and subsequently PK differences contribute to variation in therapeutic efficacy and adverse drug reactions (Kearns et al., 2003; Kennedy, 2008). There are many examples of changing PK parameters with age in paediatric population. For example, the plasma CL of midazolam follows the pattern: adults> full-term neonates> pre-term neonates (Jacqz-Aigrain et al., 1992). However, the elimination/CL of many drugs when expressed on a body weight basis ($L.kg^{-1}.h^{-1}$) is higher in children of age 2 to 6 years than in adults. As a consequence, children in this age range can have lower body burdens than adults for the same daily (mg/kg) intake of a chemical (Gibbs et al., 1997; Renwick, 1998) and will require higher per kg doses of same drugs to achieve similar exposure to adults. On the contrary, neonates and in particular preterm neonates have a lower CL per kg of body weight. In 2004, Ginsberg et al compared the PK parameters (including half-life, CL, V_d,

AUC, C_{max}) of 45 drugs in paediatrics of all age groups with adults. The most abundant data from these analyses were available for half-life. Their results indicate that neonates and infants younger than 6 months have longer half-life than adults and there is a tendency for shorter drug half-life at about 6 months to 2 years of ages (Ginsberg et al., 2002). The variability in half-life seems to be significantly larger for drugs primarily cleared by oxidative metabolism in the neonates. Some drugs have a comparable or shorter half-life in infants and young children. For example, renally cleared substrates (ampicilline, cimetidine, furosemide, piperacillin, ticarcillin, tobramycin and vancomycin), CYP1A2 substrates (caffeine, theophylline), CYP3A4 substrate, carbamazepine, showed shorter half-life (and increased CL relative to adults). Some drugs such as phenytoin, omeprazole, and sirolimus are metabolised by hepatic CYP2C9/19. In the case of omeprazole, faster apparent CL for children than adults is due to a higher metabolic capacity in children as well as differences in the extent of protein pump inhibitors (PPI) bioavailability (Litalien et al., 2005). The higher CL value in paediatrics has also been reported from *in vitro* studies. Voriconcazole, shows higher CL in children between 2 and 10 years of age than in adults (Yanni et al., 2010).

The PK differences between paediatrics and adults are briefly discussed below;

1.3.1 Oral Absorption

The oral route is a preferred method of drug administration in the paediatric populations. Neonates have relatively higher gastric pH that becomes acidic within a few hours after birth (Miclat et al., 1978). The higher pH can affect the amount of drug dose available for absorption resulting in higher or lower bioavailability of drugs in neonates (Huang and High, 1953; Morselli, 1974; van den Anker et al., 1993). There is evidence that gastric emptying time and intestinal motility will develop from neonatal period to infancy to reach full maturation (Huang and High, 1953; Morselli, 1974; Gupta and Brans, 1978; Ittmann et al., 1992). However, a recent analysis of literature data suggests that the gastric emptying time does not change with age (Bonner et al., Under prepration). A number of studies have measured the fasted gastric fluid volume in children. They have reported the fluid volumes as ml per kg of body weight. However, the utility of these average body-size normalised volumes is limited due to the wide age range evaluated and different study designs including the duration of fasting before the measurement (Malhotra et al., 1992; Cook-Sather et al.,

1997; Ingebo et al., 1997; Schmitz et al., 2011; Schmitz et al., 2012). Other factors including intestinal surface area, organ blood flow, intestinal metabolic enzymes and efflux transporters which will affect the absorption of drugs will further develop in neonates.

1.3.2 Distribution

Neonates and infants have different percentages of body content of water and lipid than older children and adults (Kearns and Reed, 1989). At birth, there is a greater percentage of body water and less body lipid which can increase the volume of distribution (V_d) of water-soluble chemicals because of expanded water volume. The higher water proportion may also decrease the partitioning and thus retention of lipid soluble chemicals. Body lipid rises steadily after birth for the first nine months of life but then decreases steadily until preadolescence, which marks a second period of increasing body lipid (Kearns and Reed, 1989; Hattis et al., 2003). The changes in body composition can affect half-life and V_d of drugs.

Some tissues such as liver, kidney, and lung undergo rapid growth during the first 2 years (Haddad et al., 2001). In contrast, reproductive tissues are generally small per body weight during this period.

Another factor that can affect the distribution of chemicals is the binding capacity of plasma proteins. Some drugs and chemicals are highly bound to plasma proteins and therefore little free drug (i.e. the active form for drug) is available. Since only free drug is capable of crossing barriers such as the placenta, blood brain barrier, or get into tissues, extensive protein binding will tend to prolong the half-life and reduce elimination that can occur at these sites. Neonates have lower plasma protein concentration and consequently have protein-binding levels (Besunder et al., 1988; Kearns and Reed, 1989).

1.3.3 Metabolism

Liver is the major organ responsible for metabolism of drugs and physiological changes in liver affects the extent of drug metabolism by different pathways. As reported by (Gibbs et al., 1997), liver weight in paediatrics aged between 1 week to 4 years was approximately 1.7-fold higher than in older children and adults. This fact leads to higher CL per kg of some drugs in paediatric population than in adults. The maturation of metabolic pathways has an

important impact on the elimination of endogenous and exogenous compounds from the body. Most drugs are metabolised by phase I (mainly cytochromP450) and phase II enzymes (mainly UGTs). The ontogeny of major CYPs will be discussed later in this chapter.

1.3.4 Renal elimination

The kidneys are a major organ for the excretion of both drugs and metabolites. Renal CL (CL_R) occurs through glomerular filtration and active tubular secretion and is generally still developing in the new born and matures after birth. Renal CL of drugs is not commonly measured in PK studies in young children but there are models available that predict the CL_R with post menstrual age (Rhodin et al., 2009). In this model, Rhodin et al., applied allometrical scaling to glomerular filtration rate (GFR) and according to their model 90% of adult GFR is achieved by the first year of life. Another model that describes the ontogeny of renal function is by Johnson et al., (Johnson et al., 2006). This model is based on the relative GFR in paediatrics to GFR in adults. GFR in this model is calculated from BSA which is a function of body weight and body height. The resulted renal CL from these models is in good agreement (in house comparison).

Hayton et al., developed a function accounting for maturation and growth of renal function parameters including GFR, active tubular secretion and renal plasma flow. The Hayton model is based on data from mannitol and para-aminohippuric acid assuming an exponential maturation with age (Hayton, 2000). Glomerular filtration rate and tubular secretion calculated from the models developed by Hayton et al., can be applied in the Johnson model (Johnson et al., 2006) to calculate paediatric CL_R.

De Cock et al., developed a maturation function for GFR based on amikacin CL through a POPPK study in paediatric subjects (De Cock et al., 2012). Performance of this model was later evaluated for gentamicin, vancomycin, netilmicin and tobramycin (De Cock et al., 2013).

1.4 Introduction to physiologically based pharmacokinetic (PBPK) models

The objective of physiologically based pharmacokinetic (PBPK) models is to establish a relationship between patient demographic and genetic characteristics and the dose exposure. These models allow prediction of changes in ADME as a result of age, genetic or

disease state in patients. The application of PBPK models in drug development especially in medicines for children is becoming increasingly popular (Barrett et al., 2012; FDA, 2012; Leong et al., 2012). These models allow predicting the PK of drugs while considering the factors such as organ dysfunction, age, genetic, drug or disease interactions (Zhao et al., 2011). PBPK models are a combination of three major components: 1) system properties (including organ mass or volume, blood flow, and tissue composition), 2) drug properties (including tissue affinity, plasma-protein binding affinity, membrane permeability, enzymatic stability, and transporter activities, Pk_a), and 3) trial design.

PBPK models are more mechanistic than other types of modelling (i.e., classic compartmental) and population pharmacokinetic (POPPK) studies and can be scaled between and within species based on real physiological data. PBPK models permit simulation of complex ADME scenarios, DDIs and parent and metabolite drug profiles. Based on system's information, they also provide a platform for the simulation of virtual patient populations with liver cirrhosis, obesity, pregnancy and genetically distinguished groups such as Japanese and Chinese (Jamei et al., 2009a). Rowland et al, 2011 published a comprehensive report on PBPK models. Figure 1.4-1 presents an example of a full PBPK model (Rowland et al., 2011). PBPK models may also use *in vitro-in vivo* extrapolation (IVIVE) to predict the PK parameters of drugs from *in vitro* systems such as human liver microsomes or recombinant systems (Khalil and Laer, 2011).

As described by the FDA's office of clinical pharmacology 25 submissions to this office were based on PBPK models between 2008 and 2011 for investigational new drug, new drug applications and to support regulatory policies. Of the 25 submissions the highest proportions belonged to DDI and paediatrics (Zhao et al., 2012a).

1.4.1 Paediatric PBPK models

Paediatric PBPK (p-PBPK) models are the extension of full PBPK models and use the knowledge on ontogeny of biological processes such as organ growth, blood flow, binding to plasma proteins and most importantly ontogeny of metabolic and elimination pathways, to predict age-dependant drug ADME in paediatric population (Johnson and Rostami-Hodjegan, 2011). There are a number of published p-PBPK models but they share the same

principle in prediction of PK parameters (Ginsberg et al., 2004; Bjorkman, 2005; Edginton et al., 2006b; Johnson et al., 2006).

These models reflect the age related PK differences as in paediatric subjects through relevant algorithms and can bridge the knowledge gap between paediatric and adult PK parameters. This approach utilises mechanistic modelling and simulation to account for all known parameters and predict exposure to unbound drug concentrations in paediatric patients compared to adults. The most important age-dependant system parameters in p-PBPK models include cardiac output and organ blood flow, organ weight, plasma protein binding, haematocrit, hepatic metabolic pathways and renal function. Further development and validation of p-PBPK models will help to explore or predict the PK characteristics of drugs in understudied populations as well as exploring the PK of drugs that are not well-studied in paediatric subjects.

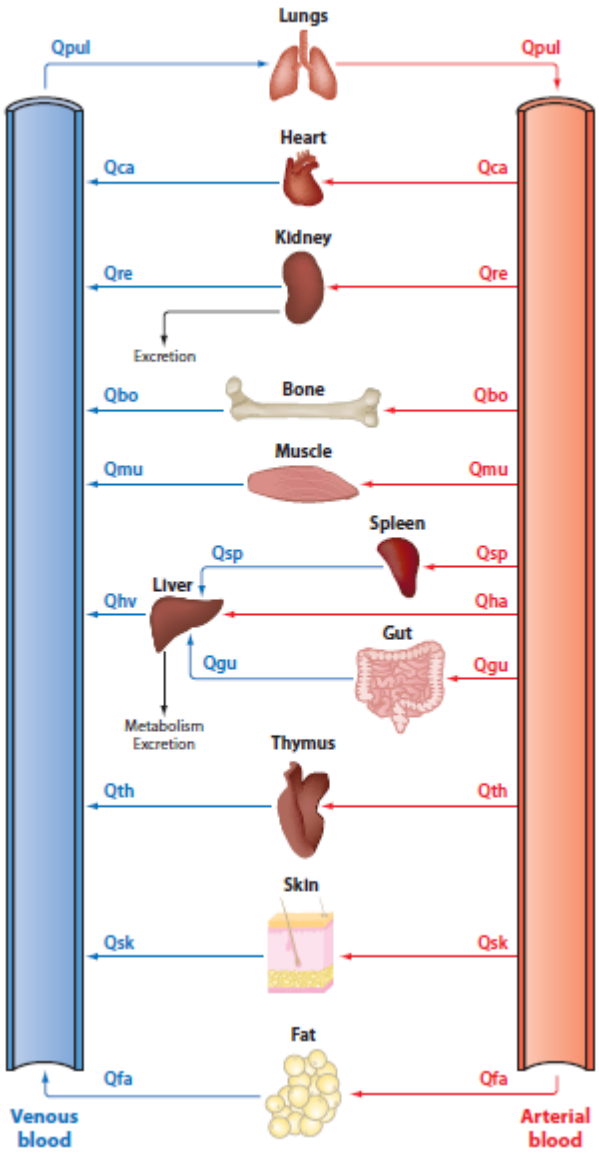

Figure 1.4-1 An example of whole body PBPK model.
Q = blood flow: to the lungs (Qpul), the heart (Qca), the kidneys (Qre), the bones (Qbo), the muscles (Qmu), the spleen (Qsp), the liver (Qha), the hepatic vein (Qhv), the gut (Qgu), the thymus (Qth), the skin (Qsk), and the fat (Qfa). The figure is adapted from (Rowland et al., 2011)

Simcyp p-PBPK: Simcyp paediatric is an example of p-PBPK models. Applications of Simcyp paediatric in drug development are as following;

- doses to give equivalent exposure in adults and children.
- dose linearity at different ages.
- drug interaction potential at different ages.
- different route of administration – buccal administration with different % swallowed.

- absorption form sustained release formulations in paediatrics compared to adults.

1.4.2 Ontogeny of hepatic metabolic pathways for prediction of CL in p-PBPK models

Perhaps the most important factor that determines the age-dependant CL in p-PBPK models is the ontogeny of metabolic pathways. These pathways are mainly present in liver and to a lesser extent are expressed in intestine and other organs. The *in vitro* data on enzyme abundance, activity or mRNA expression for major CYPs are available in the literature. These data are used to derive ontogeny profiles for some pathways which are then incorporated into p-PBPK models (Bjorkman, 2005; Edginton et al., 2006a; Johnson et al., 2006). These ontogeny models are used to scale the enzyme abundance from adult value (i.e. different for each CYP) to children at different ages. This information in conjunction with other age dependant parameters such as milligram of microsomal protein per gram of liver (MPPGL), fraction of unbound drug (f_u), blood to plasma ratio (B:P) and hepatic blood flow ($Q_{H,B}$) are used in p-PBPK models to calculate intrinsic CL (CL_{int}) and hepatic metabolic CL ($CL_{H,B}$).

1.4.2.1 Developmental changes of expression and activity for major CYPs

The Cytochrome P450 family is the most important group of enzymes contributing in phase I metabolism. The enzymes within this family are stratified into three categories during development (Hines, 2007);

- enzymes expressed at their highest levels during the first trimester, decrease during gestation and are silenced or expressed at low levels within 2 years after birth such as CYP3A7,
- enzymes expressed at a relatively constant level during gestation such as CYP2C19 and CYP3A5
- enzymes such as CYP3A4, CYP2C9 and CYP2E1 which are absent or expressed at low levels in foetus with the onset of expression in second or third trimester.

Following is a brief description of development of individual CYP pathways that are used in this dissertation. Table 1.4.1 and Table 1.4.2 show the activity and abundance of these pathways at different ages.

1.4.2.1.1 Hepatic CYP1A2

CYP1A2 is the major isoform from the CYP1A family and is responsible for the metabolism of drugs and chemicals including phenacetin, theophylline, caffeine and ropivacaine. CYP1A2 is the last major CYP isoform to develop during the first three months after birth. There is a large interindividual variability in caffeine and theophylline CL as suggested by clinical PK studies in children (Ellis et al., 1976; Giacoia et al., 1976; Loughnan et al., 1976; Leung et al., 1977; Walson et al., 1977; Simons and Simons, 1978; Mulla et al., 2003). CYP1A2 protein was absent in microsomes prepared from foetal and neonatal livers and its levels increased in infants aged 1-3 months to attain 50% of the adult value at one year (Sonnier and Cresteil, 1998). The ontogeny of CYP1A2 activity is presented in Figure 1.4-2.

In another study, the expression of CYP1A2 between prenatal and infancy (under one year old) and over one year olds was investigated and showed the expression and catalytic activity of CYP1A2 is lower in prenatal period and infancy (Tateishi et al., 1997).

CYP1A2 activity has been shown to be affected by environmental factors such as nutrition (Le Guennec and Billon, 1987), smoking as well as sex differences due to hormonal changes in puberty (Lambert et al., 1986a; Levitsky et al., 1989).

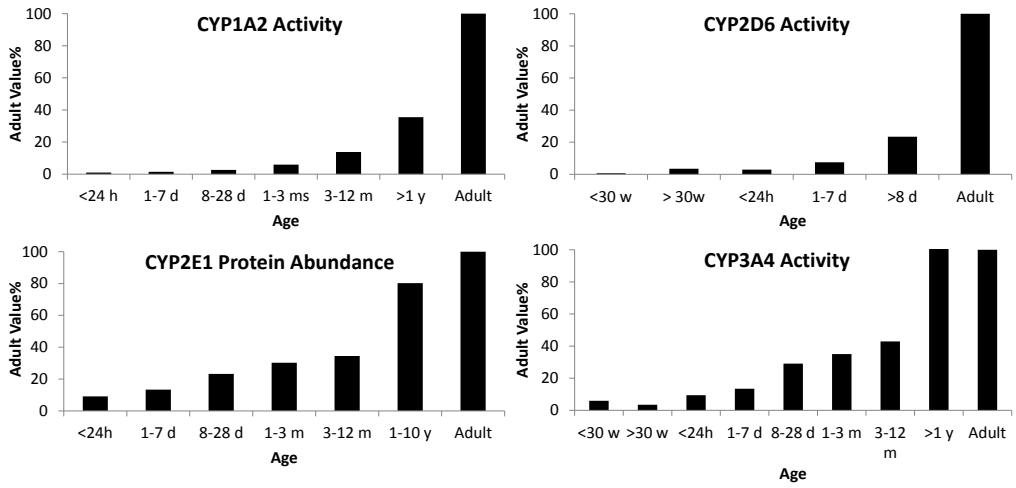

Figure 1.4-2 Graph to show the development of CYP1A2, -2D6, -2E1 and -3A4 activity or abundance with age relative to adults.
Data taken from (Sonnier and Cresteil, 1998), (Treluyer et al., 1991), (Vieira et al., 1996) and (Alcorn and McNamara, 2002), respectively. In this graph w=weeks of gestational age, h=hours, d=days, m=months and y=years.

1.4.2.1.2 Hepatic CYP2B6

CYP2B6 participates in the oxidative metabolism of a number of pharmaceuticals, including bupropion, propofol and lidocaine, cyclophosphamide, ifosfamide and tamoxifen, efavirenz and artemesinin (Croom et al., 2009). Foetal livers fail to express CYP2B6 protein (Hakkola et al., 1994; Shimada et al., 1996) and it is likely that CYP2B6 protein expression achieves adult capacity only after first year of life (Hakkola et al., 1994). Higher levels of CYP2B6 protein in infants greater than 1 year of age as compared with younger infants suggests a delay in CYP2B6 development (Tateishi et al., 1997). More recent data (Croom et al., 2009) shows the median CYP2B6 level in samples over 30 postnatal days to 17 years of age (1.3 pmol/mgmicrosomal protein) was lower than previously reported adult levels (2.2–22 pmol /mg microsomal protein).

1.4.2.1.3 Hepatic CYP2C

Many clinically important drugs such as warfarin, proton pump inhibitors such as omeprazole, pantoprazole, lansoprazole, phenytoin and tolbutaminde are metabolised by CYP2C subfamily. This subfamily contains four highly homologous genes, CYP2C8, -2C9, -2C18 and -2C19 (Koukouritaki et al., 2004). The CYP2C subfamily accounts for about 18% of the total adult liver cytochrome P450 content (Shimada et al., 1994). The major form is CYP2C9 followed by CYP2C19 and CYP2C8 (Edwards et al., 1998; Goldstein, 2001).

CYP2C9 expression shows significant variability. This drug metabolising enzyme is not expressed or expressed at low levels during gestational period. CYP2C9 is expressed only 1% of adult value in the first trimester. During the third trimester CYP2C9 expression increased substantially and reached 10% of adult expression. In the neonatal period this expression reached approximately 25% of that seen in adults (Treluyer et al., 1997; Hines, 2007) and remains almost constant in between 1 month and 1 year. Between 1 and 10 years CYP2C9 expression remained at around 40 to 50% of adult expression.

CYP2C19 is reported to be expressed at about 10% of adult value during the first trimester and this value increases to about 20% of the adult value in the second trimester. CYP2C19 expression increases by approximately 2-fold in infants and reaches nearly 50% of observed value in adults and remained relatively constant until 10 years (Treluyer et al., 1997; Hines, 2007).

1.4.2.1.4 Hepatic CYP2D6

CYP2D6 contributes to the biotransformation of drugs such as opioids like codeine, dextromethorphan, tramadol, β-blockers and tricyclic antidepressants. Only 2% of adult liver CYP content is made by CYP2D6 (Shimada et al., 1994; Alcorn and McNamara, 2002). Foetal liver may express very low levels of CYP2D6 (Shimada et al., 1996; Treluyer et al., 1997) and the rise in protein expression occurs within the first week following birth, regardless of gestational age at birth. This finding is in agreement with the results from another study where the authors report CYP2D6 activity in foetal liver microsomes was not detected until birth but CYP2D6 mRNA was identifiable (Jacqz-Aigrain and Cresteil, 1992). By the first month of life, CYP2D6 enzyme activity reaches around 30% of adult levels (Treluyer et al., 1991). Figure 1.4-2 shows the ontogeny of CYP2D6 activity.

CYP2D6 is affected by genetic polymorphism. An *in vivo* study investigated the plasma and urinary concentrations of tramadol and its metabolite O-demethyl tramadol in neonates and showed that the CYP2D6 activity was observed in very early neonatal life but it shows a large inter-individual variability in CYP2D6 activity which might be related to enzyme polymorphism (Allegaert et al., 2005). Finally, CYP2D6 metabolite activity was investigated by measuring dextromethorphan (DM):dextrorphan (DX) ratios in urine samples from different ages of children in a study by (Blake et al., 2007). This report apparently showed no age related change in CYP2D6 activity *in vivo* as measured by the DM/DX ratio, however following a correction for renal function with age by Johnson et al, (Johnson et al., 2008), results showed that CYP2D6 activity was in close agreement with the ontogeny profile reported by (Johnson et al., 2006) which is based on *in vitro* date.

1.4.2.1.5 Hepatic CYP2E1

CYP2E1 is important for the oxidative metabolism of acetaminophen, halothane and chlorzoxazone (Tanaka et al., 2000). It is moderately abundant (7%) in adult livers (Alcorn and McNamara, 2002). CYP2E1 protein is absent from the foetal liver with levels increasing immediately in the first few hours after birth, regardless of the gestational age at birth. As shown in Figure 1.4-2 the protein gradually increases during the first year to reach adult value in infants aged 1-10 years (Vieira et al., 1996).

1.4.2.1.6 CYP3A

CYP3A family is the most abundant CYP in the liver. This family includes four enzymes: CYP3A4, -3A5, -3A7 and -3A43. CYP3A4 contributes to metabolism of most drugs and xenobiotics. CYP3A4 activity was detected in second trimester but its abundance remains low during the third trimester, neonatal life and infancy. CYP3A4 activity is about 10% of adults in foetal livers and reaches about 30-40% of adult activity by first month of postnatal age. CYP3A4 activity reaches adult level around 1 year of old (Figure 1.4-2). Hines et al., reported adult levels of CYP3A4 at around 2 to 3 years old (Hines, 2007).

In liver samples used by Hines, CYP3A5 was only detected in a few samples in the first trimester and its levels remain constant during the second and third trimester and neonatal period. Little change is reported in infancy (Hines, 2007). A large inter-individual variability exists in CYP3A5 expression and activity during all stages of development. CYP3A5 in 83% homologous to CYP3A4 (de Wildt et al., 1999). Only 17% of Caucasian adults express CYP3A5 (Simcyp in house data analysis) but no apparent developmental pattern of CYP3A5 activity has been identified (Stevens et al., 2003).

CYP3A7 is abundant in foetal and neonatal livers. CYP3A7 abundance in the first trimester is two or three times higher than adult CYP3A4 level (Hines, 2007) and no significant change in CYP3A7 was observed during the second and third trimester compared to the first trimester. During the neonatal age, CYP3A7 decreased by about 50% and its content decreased during the first year after birth. CYP3A7 remained greater than the average CYP3A4 levels until at least six months postnatal age (Stevens, 2006). Although mean CYP3A7 protein decreases by 50% in the neonate, it still remains substantially higher than any other of the CYPs and certainly remained the dominant CYP3A enzyme expressed during the neonatal stage (Stevens et al., 2003; Hines, 2007). CYP3A7 which is 90% homologous to CYP3A4 (de Wildt et al., 1999) is detectable in adult liver, but at much lower levels than CYP3A4.

1.4.2.1.6.1 Factors affecting CYP3A activity

CYP3A activity may be affected by the ethnicity. African-American subjects have different 3A5 polymorphism compared to Caucasians (Roy et al., 2005) and this differences may

explain some of the CL differences between two groups (Min et al., 2004). By contrast, despite genetic polymorphism and metabolic differences *in vitro*, some authors did not find any differences in midazolam and alfentanil CL between African-American and Caucasian healthy volunteers *in vivo* (Kharasch et al., 2007; Miao et al., 2009). Mancielli et al., reported no differences between these ethnic groups after intravenous administration of tacrolimus but they did observed significant differences in PK parameters after oral administration of tacrolimus (Mancinelli et al., 2001).

Recently, it has been shown that CYP3A4 activity is affected by the clinical condition of paediatric patients (Machavaram et al., 2013). Premature neonates and critically ill neonates or infants in intensive care have lower CYP3A4 activity. As shown by Ince et al., midazolam CYP3A4 mediated CL is lower in this group of patients compared to non-critically ill patients (Ince et al., 2013). According to this paper, critical illness is a more significant covariate than body weight to explain interdividaul variability in midazolam CL (Ince et al., 2012). They reported a 93% reduction in CYP3A4/5-mediated midazolam CL in critically ill patients as well as an 86% reduction in uridine diposphate glucuronosyltransferase (UGT).

Critical illness seems to elevate cytokine levels as a result of inflammation and infection which will reduce the activity of CYP3A4 (Aitken et al., 2006; Morgan, 2009). Machavaram *et al*., applied IVIVE and PBPK to quantitatively demonstrate the effect of cytokines on simvastatin in rheumatoid arthritis patients and cyclosporine in bone marrow transplant patients through modelling and simulation (Machavaram et al., 2013).

CYP3A is a major consumer of oxygen. Tissue hypoxia is common in critically ill patients. Perk et al., investigated the impact of hypoxia *in vitro* on reduction of CYP3A induction after exposure to rifampicin. In their study, oxygen demands increase and may exceed oxygen supply in a hypoxic atmosphere. This in turn may result in the reduction in cytochrome synthesis (Park et al., 1994).

Table 1.4.1 Protein expression of major CYPs at different ages.

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d	8-28 d	1-3 m	3-12 m	1-5 y	5-15 y	20-50 y		
CYP1A2											42[1]	pmol/mg	(Shimada et al., 1994)
				0.003	0.02	0.03	0.11	0.23	0.5		0.96	Absorbance units/mg protein	(Sonnier and Cresteil, 1998)
CYP2B6											Low		(Hakkola et al., 1998)
											11	pmol/mg	(Shimada et al., 1994)
				2.7[2]					19.4[3]			pmol/mg	(Croom et al., 2009)
				2.654					19.365			pmol/mg	(Tateishi et al., 1997)
Total CYP2C											601	pmol/mg	(Shimada et al., 1994)
	<0.5										83	pmol/mg	(Shimada et al., 1996)
				0.04	0.24	0.35	0.436		0.36[4]		1.12	Absorbance units/mg protein	(Treluyer et al., 1996)

[1] Data from 60 Caucasian and Japanese samples
[2] Data from donors >37 GSA but <10 months PNA
[3] Data from donors age 2 to 72 years
[4] Data from subjects over 9 months

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d	8-28 d	1-3 m	3-12 m	1-5 y	5-15 y	20-50 y		
CYP2C9	0.3		5	11.8[5]					18[6]			pmol/mg	(Koukouritaki et al., 2004)
	0.4	0.4	5.0	11.3					15.55[7]		29.58	pmol/mg	(Hines, 2007)
CYP2C19	1.7	4.0	4.7	4.1		8.9			12.5[7]		14	pmol/mg	(Hines, 2007)
CYP2D6											51	pmol/mg	(Shimada et al., 1994)
	0.29		0.55	0.12	1	2	4.2				7.19	Absorbance units/mg protein	(Treluyer et al., 1991)
	<0.5										10	pmol/mg	(Shimada et al., 1996)
	0.6		2.11	1.96		9.35						pmol/mg	(Stevens et al., 2008)

[5] 0 to 5 months
[6] >5 months to 18 years
[7] 1 to 10 years

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d			3-12 m	1-5 y	5-15 y			
CYP2E1											221	pmol/mg	(Shimada et al., 1994)
				0.55	0.76	1.3	1.7	1.9		4.3	5.4	Absorbance units/mg protein	(Vieira et al., 1996)
				94.32[8]					136.91[9]			pmol/mg	(Tateishi et al., 1997)
		0.35	5.8					48.7[10]				pmol/mg	(Johnsrud et al., 2003)
	0.51	0.3	7.52	8.84			28.2		43.1[7]		50.35	pmol/mg	(Hines, 2007)
				0.39	0.47	0.93	1.6[11]		3.8		4.9	Absorbance units/mg protein	(Treluyer et al., 1996)
	<0.5										33	pmol/mg	(Shimada et al., 1996)
Total CYP3A											961	pmol/mg	(Shimada et al., 1994)
	97										147	pmol/mg	(Shimada et al., 1996)
	0.66		0.84	0.72	0.68	0.72	0.74	0.74	0.67		0.81	Absorbance units/mg protein	(Lacroix et al., 1997)

[8] Data from Japanese subjects from 37 weeks GSA to 10 months
[9] Data from Japanese subjects age 2 to 72 years
[10] First year
[11] Samples from subjects age 1 to 9 months

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d	8-28 d	1-3 m	3-12 m	1-5 y	5-15 y	20-50 y		
CYP3A4				4.17				10.42[12]	14.58[13]	6.25	58.33	pmol/mg	(Stevens et al., 2003)
	4.5	23.93	10.5	6.5			8.77[14]				93.49	pmol/mg	(Hines, 2007)
CYP3A5	0	5.2	9.7	5.8			1.02[13]				1.13	pmol/mg	(Hines, 2007)
		4.21	11.02	4.46				7.43[12]	5.56[13]	5.96	14.51	pmol/mg	(Stevens et al., 2003)
CYP3A7		313	200	97.83				12.83[12]	4.12[13]	4.15	1.77	pmol/mg	(Stevens et al., 2003)
	260.55	380.71	200.9	142.2			1.53[13]					pmol/mg	(Hines, 2007)

[12] 0.5 to 1 years
[13] 2 to 5 years
[14] 1 to 18 years

Table 1.4.2 Developmental changes in CYP activity with age.

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d	8-28 d	1-3 m	3-12 m	1-5 y	5-15 y	20-50 y		
1A2	0.02		0.017	0.048	0.039	0.083	0.16	0.19		0.46	0.41	Imipramine nmol/min/mg protein	(Berthou et al., 1988)
				1.2	1.8	3.3	6.6	14		35	100	methoxyreseorufin nmol/min/mg protein	(Berthou et al., 1988)
	0.87				0.58			2.2			24	Caffeine 3-demethylation ρmol /min/mg protein	(Cazeneuve et al., 1994)
				0.83	1.26	2.17	5.99	13.20	34.46		98.34	Phenoxazone nmol/min/mg protein	(Sonnier and Cresteil, 1998)
2C9					0.44	11	8.4	7.4			27	Tolbutamide nmol/min/mg protein	(Treluyer et al., 1997)
			37–735	53–4600[10]				60–1996[15]				Diclofenac ρmol /min/mg protein	(Koukouritaki et al., 2004)
2C19	0.1–345.5			0.1–1516.5[16]				0.1–656.5[17]		0.4–101.7		s-mephenytoin ρmol l/min/mg protein	(Koukouritaki et al., 2004)
2D6	0.22		0.32	0.37	1.1	3.0					10	Dextorphan nmol/min/mg protein	(Jacqz-Aigrain et al., 1992)
	0.12		0.37	0.41	0.85	2.5	4.2				10	Dextorphan nmol/min/mg protein	(Treluyer et al., 1991)
2E1	0		0.006	0.007		0.04[18]						Dextromethorphan nmol/min/mg protein	(Stevens et al., 2008)
	0.031			0.20	0.30	0.35	0.44	0.37		0.78	0.96	Chlozoxazone nmol/min/mg protein	(Vieira et al., 1996)

[15] >5 months to 18 years
[16] 0 to 5 months
[17] >5 months to 10 years
[18] >7 days- 18 years post natal age

CYP Enzyme	Foetus			Neonates		Infants			Children		Adults	Unit	Reference
	1st trimester	2nd trimester	3rd trimester	<24 h	1-7 d	8-28 d	1-3 m	3-12 m	1-5 y	5-15 y	20-50 y		
3A4	173										2756	Testosterone pmol/min/mg protein	(Shimada et al., 1996)
	0.01		0.004	0.01	0.02	0.03	0.04	0.05	0.13		0.12	Testosterone nmol/min/mg protein	(Lacroix et al., 1997)
3A7	0.75		0.49	0.98	1.3	0.57	0.37	0.19			0.082	Dehydroepiandrosterone nmol/min/mg protein	(Lacroix et al., 1997)

1.4.3 Some applications of modelling and simulation in paediatric clinical pharmacology

One application of p-PBPK models is to predict/simulate real case scenarios in clinical practice. For example prior to prescribing drugs especially when polytherapy is required and there is a risk of DDI, p-PBPK models can give some insight about the plasma concentration of drugs following co-administration of prescribed drugs. Johnson et al, 2011 present an example of simulation of a complex case of DDI in an infant and predict the relevant plasma-concentration time profile of midazolam in presence of several concurrently administered interacting drugs including rifampicin, fluconazole and clarithromycin (Johnson and Rostami-Hodjegan, 2011).

1.4.3.1 Initial dose recommendation

One possible application of modelling and simulation especially in neonates (i.e. a more vulnerable population) is to estimate initial dose (Johnson et al., 2006; Jacqz-Aigrain et al., 2013). Another example as suggested by (Khalil and Laer, 2011) is predicting the age-specific dose of sildenafil. In this example the initial dose of sildenafil is not known and the simulations use different doses to predict the equivalent AUC of sildenafil at given ages.

PBPK modelling is considered a superior method of dose estimation than traditional allometric scaling. These models aim to achieve adult exposure in paediatric group through predicting the concentration-time profile, PK parameters and variability around them.

1.4.3.2 Suggest sampling times

One major difficulty in conducting clinical PK studies in children and particularly neonates is the amount of blood that needs to be taken for drug measurements. With the limited sampling time point, the ultimate information on the concentration of drug is desirable which means no sampling should be below the limit of quantification. Khalil and Laer have used the PBPK to identify the optimal sampling times in neonates, three-year old children and adults for a drug. These optimal sampling times can potentially reduce the volume of blood required and presumably the number of sampling times and the sample size for the study (Khalil and Laer, 2011).

Optimal sampling time design in children was also investigated by Dumont et al. Simulation of plasma concentration-time profile was performed using a p-PBPK model and simulated

data were used to build a four compartment model for the parent drug and its metabolite in NONMEM. Sampling time design was then optimised from the parent-metabolite PK model in 82 paediatric subjects (Dumont et al., 2013).

1.4.3.3 Modelling and Simulation in DDI

In 1998, for the conduct of paediatric PK studies, the FDA recommended use of POPPK studies. This approach relies on infrequent (sparse) sampling of blood from a larger population that would be used in a standard PK study to determine PK parameters (FDA, 1998). POPPK analysis is the posteriori application of modelling and simulation. A priori application of modelling and simulation is through PBPK models (Barrett et al., 2012). The difficulties in conducting clinical studies in paediatric population have led to increasing popularity of modelling and simulation techniques.

1.4.3.4 Prediction of drug exposure in vulnerable populations

The tragic death of a healthy new-born as a result of morphine toxicity was reported (Koren et al., 2006). The nursing mother was administered high codeine for pain relief. Codeine is metabolised by CYP2D6 to morphine which can then pass through the breast milk to the neonate. Post-mortem investigation showed that the neonate was a CYP2D6 extensive metaboliser, the enzyme responsible for N-demethylation of codeine to morphine and the mother happened to be CYP2D6 ultra-rapid metaboliser.

PBPK simulations combining the knowledge of genotype and phenotype with the dosing regimen have been used to predict the potential morphine concentration ingested by the neonate through breast milk (Willmann et al., 2009). In these simulations milk morphine concentration was simulated successfully; however, the relevant plasma drug concentration was under-predicted. Despite this under-prediction the authors highlighted both the application of PBPK models in lactation and the need to further refine these models.

1.4.3.5 Assisting with drug development for challenging patient populations

Parrott et al., built an animal and human PBPK model for oselamivir for treatment of influenza based on physiochemical and *in vitro* data. They also investigated application of juvenile animal PBPK model in predicting PK parameters and then developed this model in human neonates and infants for oseltamivir and its active metabolite, oseltamivir

carboxylate. They also explored intravenous oseltamivir formulation for use in very young or critically ill patients with difficulty to swallow or tolerate oral dosage from (Parrott et al., 2011). They were able to demonstrate that despite potentially low human carboxyesterase expression in neonates, sufficient oseltamivir carboxylate was likely to be produced to result in an anti-viral effect (Yang et al., 2009).

Recently, the Association of Pharmaceutical Scientists has highlighted the need for child-friendly oral dosage forms. Due to the large tablet or capsule size and poor palatability, adult oral formulations fail to be used in paediatric patients. This association aims to develop child-friendly oral dosage forms (Zajicek et al., 2013). In this publication modelling and simulation approaches in general and specifically PBPK models are suggested to facilitate testing different scenarios.

1.4.4 Regulatory views on paediatric PBPK models

Although p-PBPK models are confined by some data limitations, in 2012, FDA recommended the routine use of PBPK models in paediatric drug development when possible with the view that p-PBPK models will help to understand the observed variability in paediatric populations. However, due to the existing knowledge gap, a “best practice guideline” for these models seems to be necessary. FDA strongly supported and recommended modelling and simulations in all paediatric drug development programmes, including for assessing potential DDIs in paediatric subjects (FDA, 2012).

Recently, the office of Clinical Pharmacology in the Centre for Drug Evaluation and Research of FDA applied PBPK models in reviewing and decision making on the submissions to the office with regard to the need for conducting clinical PK studies, study design and labelling (Zhao et al., 2011). The examples provided in this regulatory report suggest that the regulators have been able to make more informed decisions on these submissions.

Recently, the application of modelling and simulation in drug development has been highlighted by the EMA to investigate the potential for DDIs. This guideline recommends that high quality data on inhibition of major CYPs and UGTs from *in vitro* data is utilised in PBPK models and that the model assumptions and input parameters are justifiable and any

uncertain parameter be subject to sensitivity analysis. The guideline go on to say that ‘An in vivo study should be considered if a drug combination suspected or known to interact is common in the paediatric population and there is a need for clear dosing recommendations. If an interaction study is needed, a sparse sampling and population pharmacokinetic approach could be considered. The applicant is invited to find ways of providing satisfactory supportive data, such as drug interaction simulations (using PBPK) provided that the simulations successfully quantify the observed interaction in adults and the data on enzyme abundance and other physiological parameters in the paediatric population are reliable’.

This guideline also recommends verification of PK parameters from simulations against observed PK profile (CHMP, 2012).

In summary, absorption and disposition processes are not fully developed during first year of age and the PK parameters are different between paediatrics and adults. These PK differences range from extended half-life of drugs (accumulation and toxicity) to higher CL values (and larger required doses) or reduced excretion and toxicity risk. Therefore, the level of DDI at each age may or may not be similar to that is expected in adults. It is difficult for paediatricians to know what kind and to which degree the interaction can occur. Paediatric patients require special attention as they could react to drugs differently from adults.

Modelling and simulation can be used to verify the risk of potential drug interactions and hence prevent administration of drug pairs that cause important and severe interactions and minimise the risk of patient's exposure to these harmful interactions.

The Aims of the thesis

1. Investigate records of DDI in children for which there are corresponding adults, find DDIs in paediatrics and find similar DDIs if possible in adults and then analyse and compare the level of DDI
2. Hypothesize expectations for paediatric DDI on the basis of integration of information on ontogeny of enzymes and disposition of drugs in adults inputted in a p-PBPK models

3. Evaluating performance of a p-PBPK model in prediction of plasma concentration-time profile and exposure to substrates of CYP enzymes in paediatric and adult populations with the view of reviewing ontogeny functions
4. Compare the *in vitro* and *in vivo* derived ontogeny models in prediction of CL
5. Investigate application of PBPK models to estimate sample size for a potential paediatric clinical study

Chapter 2. Do children have the same vulnerability to metabolic drug-drug interactions as adults? A critical analysis of the literature.

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Declaration

This chapter constitutes a published article;

"Do children have the same vulnerability to metabolic drug-drug interactions as adults? A critical analysis of the literature"

The Journal of Clinical Pharmacology, 2013; 53 (5): 559-66

F. Salem: Lead of study design, literature search, data and statistical analysis, preparation of manuscript

A. Rostami-Hodjegan: Supervision of research and input on manuscript

T.N. Johnson: Supervision of data collection and analysis and detailed editing of manuscript

The bioavailability section and audit from hospitals present in this chapter are not part of the published paper and are added for further clarification. Supplemental material of the paper appears in the main text here to facilitate reading.

2.1 Abstract

Many drug-drug interactions (DDIs) in the paediatric population are managed based on data generated in adults. However, due to developmental changes in elimination pathways from birth to adolescents, and variable weight-adjusted dose of interacting drugs, the assumption of DDIs being similar in adults and paediatrics might not be correct. This study compares the magnitude of reported DDIs in paediatric and adult populations. A systematic literature review was undertaken to identify reports of DDIs in paediatric subjects. A total of 145 reports of DDIs were identified over the age range of birth to 20 years. The magnitude of DDIs for 24 drug pairs from 31 different paediatric studies could be assessed and compared

with those in adults where corresponding data existed. The magnitude of the DDI, as measured by inhibited/non inhibited parameter defining exposure in paediatrics, were higher (>1.25-fold), similar (0.8 to 1.25-fold) or lower (<0.8-fold) than the same ratio in adults in 10, 15 and 8 cases respectively. An age-related trend in the magnitude of DDIs could not be established. However, the study highlighted the clear paucity of the data in children younger than 2 years. Care should be exercised when applying the knowledge of DDIs from adults to children younger than 2 years of age.

2.2 Introduction

Drug-drug interactions (DDI) are an important and avoidable cause of variability in drug response and occur when one drug changes the effectiveness or toxicity of another drug in a patient, due to pharmacokinetic (PK) or pharmacodynamic (PD) reasons. The accurate prediction of DDIs can be complex as there are many factors that may be contributing to the observed changes (Rostami-Hodjegan and Tucker, 2004). These include induction or inhibition of metabolic enzymes (Christensen and Skovsted, 1969; Koup et al., 1978; Landay et al., 1978; Saccar et al., 1985), drug induced physiological alterations such as changes in hepatic blood flow or cardiac output (Conrad et al., 1983; Schneck et al., 1984), the involvement of transporters (Brown et al., 2002; Kuypers et al., 2009), changes in absorption (Gendrel et al., 1990) and antagonism / enhancement of pharmacological effect (Danhof and Mandema, 1992; Belani et al., 1999; van den Berg et al., 1999).

Many studies investigating specific drug interactions are performed in healthy volunteers as part of the drug development process. However, the extent and subsequent clinical impact of a DDI can be influenced by altered physiology and biochemistry in patients. Factors that increase the likelihood of an adverse effect due to a DDI include the use of polypharmacy (Gronlund et al., 2010), impaired renal (Zhao et al., 2012b) or hepatic function (Orlando et al., 2009) and genetics (Collins et al., 2006) and poor metaboliser phenotype for the non-inhibited pathway. High levels of polypharmacy in any population whether that be in elderly medicine (Qato et al., 2008) or paediatric intensive care (Johnson and Rostami-Hodjegan, 2011) may lead to a higher rates of DDI in that population which could be mistaken for direct effect of age on DDI. To our knowledge, very little is known via systematic analysis of

available data regarding the severity of DDI in paediatric patients compared to adults. This is not an easy area to investigate as carrying out DDI studies in younger children is fraught with ethical and practical problems and consequently, such studies are sparse. Examples include a study by Delgado (Delgado, 1994) who investigate the effects of introducing felbamate therapy as adjunctive therapy for the treatment of epilepsy in 14 children (2 – 14 years) already receiving valproic acid (VPA). Serum morning trough VPA concentrations rose from 108 to 126 μg/ml during the first week of felbamate therapy despite a 26% decrease in VPA dose on initiating the drug. The authors suggest that the drug interaction between felbamate and VPA might be stronger in children compared to adults and that children may need a more aggressive reduction in VPA dose. In another study, Crocker et al, (Crocker et al., 1994) investigated the effects of nifedipine on cyclosporin (CsA) half-life in five children (3 – 18 years). The mean half-life of CsA was increased from 2.5 h to 4.1 h ($p<0.04$) on commencing the nifedipine therapy in this study but the authors noted no such effects in adults based on literature reports. Their overall conclusion was that nifedipine has the capacity to cause a serious drug interaction with CsA in children resulting in possible nephrotoxicity and that careful monitoring is necessary.

Although DDI differences between adult and paediatric patients is likely to be attributed to metabolic capacity of drugs in paediatric and adult patients, the extent that orally administered drugs reach the systemic circulation may contribute in these potential differences. Due to the physiological changes after birth, bioavailability of drugs may not necessarily be similar between two groups. A relatively large bioavailability study including 580 children by Heimann suggests adult bioavailability data cannot be assumed for paediatrics without further investigation (Heimann, 1980) and highlights a need for better understanding any differences in bioavailability between paediatrics and adult subjects.

The level of interaction in a paediatric population might be influenced by growth and developmental factors leading to a higher or lower DDI potential compared to adults. Thus, data generated in adults cannot be necessarily extrapolated to paediatrics with adequate confidence. A recent European Medicines Agency, Committee for Human Medicinal Products guidance document (CHMP, 1997) on investigation of drug interactions suggests that in certain circumstances clinical DDI studies in children may be needed.

The overall aim of the current study is to perform a systematic review of the literature on paediatric DDIs and where possible to compare the magnitude of reported DDIs in paediatrics with those in adult populations. The secondary aim is to perform an audit on the DDIs reported in three UK children hospitals. Although not directly designed, age-related differences in bioavailability between paediatric and adults subjects were investigated with a view to assessing the potential effect on DDI differences seen between the two populations.

2.3 Methods

2.3.1 Data collection

Separate literature searches were conducted to identify reports of DDIs and bioavailability of drugs in paediatric population and adult healthy volunteers. Details of search strategies for these data collections are explained below.

2.3.1.1 Data collection on DDI from the literature

A comprehensive literature search was carried out to identify the studies that report any kind of DDI in paediatric populations and included the age range birth to 20 years. Various sources such as Pubmed, Scopus, Medline and ISI Web of Knowledge were searched using the relevant combination of keywords “drug interaction” plus age group for example “paediatric” or “neonate” or “infant” or “child” or “children” plus type of DDI using key words “pharmacokinetic” or “pharmacodynamic”. To capture the case reports of DDI, the keyword “drug interaction” and “toxicity” was used in combination with “girl” or “boy” or “neonate” or “infant”. To identify studies that investigate the pharmacokinetic or pharmacodynamic of specific drugs and crossover studies in paediatrics, the key words “effect on” was used together with “pharmacokinetic” or “clearance” or “exposure” or “pharmacodynamic” or “disposition” plus any keywords signifying the age group. In the latter, article titles and abstracts were screened to maintain the focus of the search upon paediatric subjects. Some references were also found by exploring the Stockley’s Drug Interaction electronic database (Baxter, 2010) by searching for keywords “(neonate), (infant), (child) and (paediatric / paediatric)”. References in all retrieved reports were scrutinised for further sources of published data on paediatric DDIs. Authors were traced and contacted directly if additional information was required and if the publication date was not earlier than 1995. For all DDI combinations recorded in paediatrics a further literature search was undertaken for reports of

the same DDIs reported in adults, using keywords with “drug interaction” plus “drug names” plus, “adult” or “healthy volunteers” or “healthy subjects”. The key words “effect on” was used plus “pharmacokinetic” or “exposure” or “clearance” or “pharmacodynamic” or “disposition”. Only two studies (Russian and Hebrew languages) were excluded because of difficulties in extracting relevant data. Extracted information from other sources included subject characteristics such as age, body weight and disease; number of subjects; drug information such as name and dose of victim and interacting drugs; and finally, any PK parameters for victim drugs if reported in presence and absence of interacting drugs. Studies were classified, based on whether the PK parameter is measurable or not.

2.3.1.2 Data collection on Bioavailability from the literature

Drugs that are available in both oral and intravenous preparations and are administered to paediatrics were recorded from the British National Formulary for children 2011-2012 (Fowlie et al., 2011-2012). The literature was searched for studies reporting bioavailability values for these drugs in paediatric patients and corresponding bioavailability values in adult healthy volunteers. Sources included Pubmed, Scopus, Medline and the ISI Web of Knowledge. Key words, in addition to the drug names were “bioavailability”, in all combinations with “paediatric”, “neonate, “infant”, “child” and “adolescents”. All of the articles retrieved were screened for relevance and reference to other relevant articles. Studies were excluded if administration of drugs was through routes other than oral and intravenous. Then, search terms were expanded to include studies in healthy adult volunteers using a combination of “bioavailability” with “healthy”, and “volunteer” or “subjects”.

2.3.1.3 Data collection on DDI from hospital records

Three children hospitals in the UK were selected to conduct an audit. These sites included Great Ormond street children’s hospital, Manchester Royal Infirmary children’s hospital and Sheffield Children’s Hospital. For this audit, pharmacists in these sites were contacted and provisionally approval for this collaboration was acquired. The study was approved by Integrated Research Application System (IRAS) in the UK for ethics. Copies of ethics and applications were sent to the pharmacists in hospital sites. The data was obtained through completing forms by the pharmacists in charge during their visits to the wards or at pharmacy. DDIs were identified and reported in these forms by the pharmacy team during

filling the prescriptions or if clinical symptoms or biological tests indicated the risk of a DDI upon visits to the wards.

The data obtained in the forms included demographic information (age, body weight and sex), name, dose and frequency of administration for the interacting drugs as well as start and stop date of the drugs. A sample form is presented in Appendix 2.

2.3.2 Calculation of bioavailability

Bioavailability gives an estimate of the amount of unchanged drug that reaches the systemic circulation after the drug is administered via routes other than intravenous. Bioavailability is partially responsible for the observed inter-individual variability in concentration-time profiles following administration of similar doses especially in drugs with low bioavailability (Hellriegel et al., 1996). Bioavailability is calculated by comparing area under the plasma concentration-time profile of drugs following intravenous and oral administration.. Equation 2.3-1 is used to calculate the bioavailability considering the dose normalisation if intravenous and oral doses were not the same:

$$F_{oral} = \left(\frac{AUC_{po}}{AUC_{iv}}\right) * \left(\frac{Dose_{iv}}{Dose_{Oral}}\right) \qquad \textbf{Equation 2.3-1}$$

For orally administered drugs, bioavailability is influenced by both drug's physiochemical characteristics and physiological parameters of the target population. For commonly used drugs in paediatrics, similar bioavailability as in adults is assumed when administering the drug to children. Moreover, the effect of formulation differences is assumed to be similar between adults and paediatrics and hence the bioequivalence of paediatric formulations are often measured in adult population. However, this assumption may not necessarily be valid due to rapid physiological developments after birth that may alter bioavailability of drugs. These physiological developments include changes in gastric pH, absorption surface area, blood flow to intestine and permeability of drugs through gut wall.

As shown in Equation 2.3-2**,** absolute bioavailability of drugs is the running product of the fraction of dose liberating from the formulation and entering the cellular space of the enterocyte from the gut lumen (f_a), fraction of the dose entering gut wall and escaping first-

pass gut wall metabolism (F_G) and fraction of dose that enters portal vein and escapes hepatic first pass metabolism and biliary secretion (F_H).

$$F_{oral} = f_a . F_G . F_H$$ **Equation 2.3-2**

2.3.2.1 Factors affecting fa, F_G and F_H

Factors affecting fa are a combination of formulation characteristics, physiochemical properties of the drug, physiological variables and study design (fasted or fed conditions, type of diet). These factors are explained in details by Jamei et al., (Jamei et al., 2009b); Some of differences between adult and paediatric subjects in physiological variables contributing to fa (gastric emptying rate, intestinal transit and motility, gastrointestinal fluid pH, gastric fluid volume) are briefly explained in section 1.3. Despite physiological developments after birth, there is little evidence that suggests small intestinal transit time will change with age (Sankaran et al., 1982; Fallingborg et al., 1989; Fallingborg et al., 1990; Mihatsch et al., 2001).

F_G is affected by the abundance and location of enzymes and transporters in gastrointestinal tract (Jamei et al., 2009b), villi blood flow, gut surface area and effective permeability in man ($P_{eff,man}$) (Yang et al., 2007).F_G generally can be calculated from Equation 2.3-3.

$$F_G = 1 - E_G$$ **Equation 2.3-3**

where E_G is intestinal extraction ratio of drug.

The final parameter in Equation 2.3-2, F_H, can be calculated from Equation 2.3-4;

$$F_H = 1 - E_H$$ **Equation 2.3-4**

where E_H is hepatic extraction ratio and can be calculated from Equation 2.3-5;

$$E_H = \frac{CL_{H,B}}{Q_{H,B}}$$ **Equation 2.3-5**

where $CL_{H,B}$ is hepatic metabolic CL and $Q_{H,B}$ is liver blood flow. Liver blood flow is 25.5 percentage of cardiac output (Ann ICRP, 2002) and the latter is an age-dependant parameter. $CL_{H,B}$, as will be shown in chapter 5, depends on liver weight, hepatic blood flow and unbound fraction of drug in blood (fu_B) and all these parameters will change with age. Unless the age-dependant changes in $CL_{H,B}$ and $Q_{H,B}$ occur in parallel with the same rate, E_H will be an age-dependant parameter.

The above relationships suggest that bioavailability can change with age in paediatric subjects and ideally bioavailability-related differences should be considered when DDI pharmacokinetic comparisons are made between adult and paediatric subjects after oral administration of drugs.

2.3.3 Data analysis

2.3.3.1 Age related DDIs

Reports on paediatric DDIs were analysed by study type:

- Prospective DDI studies which were specifically planned and designed to investigate a particular drug-drug interaction in the paediatric population e.g. the effects of co-administering prednisolone and methylprednisolone with antiepileptic drugs (Bartoszek et al., 1987). The end points of these studies were either PK parameters before and after co-administering the interacting drug or PD measures e.g. in the case above the frequency of seizure episodes before and after the interacting drug. Included in this group were prospective population pharmacokinetic (POPPK) studies performed in children.

- Retrospective studies which investigated a DDI using data such as that generated from therapeutic drug monitoring, interactions could be identified by viewing these data alongside patient medication histories e.g. inhibition of lamotrigine by sodium valproate using TDM data from the latter (Armijo et al., 1999). This type of study includes POPPK studies where retrospective PK or TDM data was analysed alongside information on covariates such as co-administered drugs.

- Case studies include reports where either lack of effect or toxicity are observed in individuals or groups of up to 5 individuals and are suspected to be as a result of the co-administration of another drug, e.g. toxicity from carbamazepine due to co-administration of erythromycin (Kessler, 1985). In some cases plasma concentrations of the victim drug are measured after administration of the interacting drug and shown to be outside of the therapeutic range.

Where relevant data were available for both paediatric and adult studies, the fold interaction based on the area under the concentration-time curve (AUC) ratio, or steady state plasma concentration (C_{ss}) or changes in clearance (CL) was compared (Equation 2.3-9).

$$\text{Changes to exposure} = \frac{AUC'}{AUC} \text{ or } \frac{C_{ss}'}{C_{ss}} \text{ or } \frac{CL}{CL'} \qquad \textbf{Equation 2.3-9}$$

Where AUC' and AUC is exposure to substrate in presence and absence of the perpetrator, C_{ss}' and C_{ss} is average or trough steady state concentration in presence and absence of inhibitor and CL' and CL drug clearance in presence and absence of inhibitor.

The results were expressed as a change in exposure in paediatric subjects divided by the change in exposure in adults and stratified into discreet age bands as suggested by ICH guidelines, 0 – 1 month, >1 month to 2 year, >2 year to 12 years and >12 years to 18 years (Hoog-Labouret). In these comparisons indifferent bioavailability between paediatric and adult subjects was assumed due to a lack of any systematically gathered evidence in relation to age-dependent bioavailability in the literature (see section 2.2.3 for age related changes in bioavailability). In the final analysis both victim and inhibitor drug would be indicated in the data. Where possible all comparisons between paediatric and adult populations have been performed for the different drug combinations even where for one of the populations n = 1 from a case study, such more limited comparisons are highlighted in the data analysis. The limits used to define bioequivalence have been used to define whether the magnitude of an interaction is similar (0.8-1.25), greater (>1.25) or less (<0.8) in paediatrics compared to adults. Where there were several studies an attempt has been made to combine them and perform a meta-analysis to generate a weighted mean and standard deviation value (Johnson et al., 2006). Where PK studies in healthy adults were available, these were used for comparison in preference to studies performed in patients or the results of combined analysis from all studies.

For two of the drug combinations, "carbamazepine-erythromycin" and "digoxin-amiodarone", there were multiple adult studies with similar design and dosing regimen (3 in each case) to enable assessment of between-study variability in the adult population. A multidirectional comparison between all reported DDI levels of carbamazepine in presence of erythromycin

was carried out (12 comparisons). The 5th and 95th percentile for these ratios was derived using “Percentile” command within Microsoft Excel to enable visualisation of the between study variations in adults. These were used as a reference point for corresponding paediatric study. This helped with the assessment of the reported level of DDI in paediatrics and whether it is within the expected between-study variability observed in adults. A similar approach was used for the 12 possible combinations from the digoxin-amiodarone studies.

2.3.3.2 Age related bioavailability

Bioavailability was compared as a ratio of paediatric to adult values. Meta-analysis on bioavailability values was performed where more than one value on bioavailability of a drug was available from the literature and weighted mean value of the reported values was used in the final ratios. Due to the limited number of studies reporting bioavailability values in paediatric subjects, in this analysis formulation differences between adults and paediatrics were not considered.

2.4 Results

2.4.1 Outcome of the Paediatric vs. adult DDIs comparison

2.4.1.1 Published reports on DDIs in paediatrics

A total of 145 reports on DDIs in paediatric patients were found from the literature review over the age range birth to 20 years. Of these reports, 9 gave information in neonates, 38 in infants, 120 in children and 52 in adolescents. The majority (89%) of DDI reports were from children with a variety of clinical conditions, however the rest were from either healthy children undergoing minor procedures or being treated for minor illnesses. In total 104 different DDIs were reported in paediatric subjects and of these 27 were not also reported in adults. Table 2.4.1 shows the DDIs reported in a paediatric population but similar interaction is not reported in adults.

In terms of study type, 74 (49%) were prospective studies, 16 (11%) retrospective studies and 60 (40%) were case reports. The literature results including outcome measures and references are summarised in a supplementary table.

2.4.1.2 Magnitude of DDIs compared to corresponding values in adults

Comparison of the interactions at the PK level between paediatric and adult studies was possible for 24 drug pairs involving 31 different paediatric studies and 33 adult studies. Certain drug combinations had more than one paediatric report including 4 for carbamazepine / erythromycin, 2 for theophylline / phenobarbitone, 2 for theophylline / salbutamol, 2 for etoposide / ciclosporin, 2 and 2 for the carbamazepine / valproic acid interaction. Most comparisons were based on multiple subjects and just 6 were based on single subject case studies in paediatric subjects. The number of studies in the meta-analysis for the drug pairs carbamazepine / erythromycin, theophylline / terbutaline, lidocaine / clonidine, phenytoin / chloramphenicol, zidovudine / didanosine, digoxine / amiodarone, CsA / ketoconazole and phenytoine / co-trimoxazole were 3, 3, 2, 2, 2, 2, 2 and 2, respectively. Only 1 study was in the neonatal age group, however 3 were in the infant group, 26 in the children group and 3 in the adolescent group. The comparison between paediatric and adult DDI studies for the drug pairs are shown in the Supplemental Table S 1 in appendix 1. The relative magnitude of DDI in paediatrics to those in adults and the variability around the adult carbamazepine-erythromycin and digoxin-amiodarone studies are presented in Figure 2.4-1.

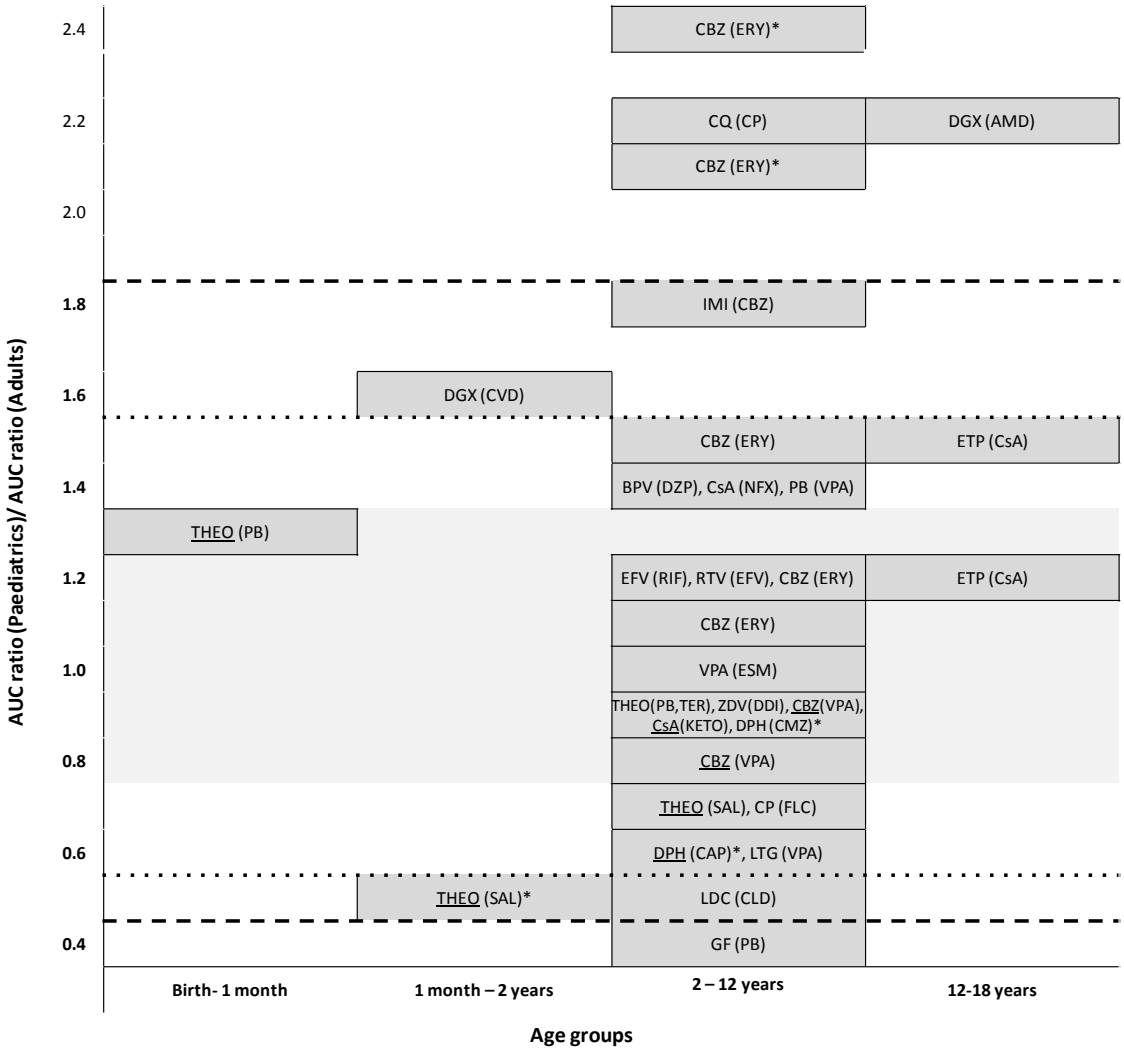

Figure 2.4-1 Comparison of the changes in exposure to substrate following drug interaction in paediatrics relative to corresponding changes in adults.

The underlined compounds are those substrates reported in multiple paediatric studies (and multiple occurrences can be found in the figure for the pair), the drug pairs highlighted with an asterisk (*) indicate that the comparison is made on the basis of a single case report (either in paediatrics or adults arm of the analysis). Inhibiting drugs are shown in parenthesis. The shaded area shows the 0.8 and 1.25 fold of the relative DDI ratios. The broken lines (- - -) and dotted lines (...) indicate the between study variability in adult DDI ratios (based on reported changes in AUC) for carbamazepine (erythromycin) and digoxin (amiodarone), respectively. The latter are based on calculated 5 and 95 percentiles of all combination of various DDI ratios reported for each pair in different studies (see text for details).Key: Amiodarone:AMD, Bupivacaine:BPV, Carvedilol:CVD, CBZ: Carbamazepine, Chloramphenicol:CAP, Chloroquine:CQ, Chlorpheniramine:CP, Clonidine:CLD, Co-trimoxazole:CMZ, Cyclophosphamide:CP, Diazepam:DZP, Didanosine:DDI, Digoxin:DGX, Efavirenz:EFV, Erythromycin: ERY, Ethosuximide:ESM, Etopside:ETP, Fluconazole:FLC, Griseofulvin:GF, Imipramine:IMI, Ketoconazole:KETO, Lamotrigine:LTG, Lidocaine:LDC, Norfloxacin:NFX, Phenobarbitone:PB, Phenytoin:DPH, Rifampicin:RIF, Ritanovir:RTV, Salbutamol:SAL, Terbutaline:TER, Theophylline:THEO, Zidovidine: ZD.

Table 2.4.1 DDIs reported in paediatrics but not in adults.

Victim drug	Interacting drug	References	Outcome
6- mercaptopurine	Methotrexate	(Balis et al., 1987; Innocenti et al., 1996; Andersen et al., 1998)	Increased exposure & ADR[19]
Azithromycin	Atovaquone	(Ngo et al., 1999)	Lower exposure
Carbamazepine	Methylphenidate	(Schaller and Behar, 1999)	Suboptimal effect
Chloramphenicol	Phenytoin	(Powell et al., 1981; Krasinski et al., 1982; Kamaluddin et al., 2001)	Lower plasma concentration with phenobarbital and higher olasma concentration with phenytoin
Digoxin	Josamycine	(Cambonie et al., 2006)	Higher exposure
Etoposide	Prednisone	(Kishi et al., 2004)	Increased CL
Ifosfamide	Phenytoin	(Ducharme et al., 1997)	increase therapeutic efficiency
Imipramine	Propranolol	(Gillett et al., 2005)	Increased exposure
Methylphenidate	Carbamazepine	(Behar et al., 1998; Schaller and Behar, 1999)	Suboptimal effect and subtherapeutic concentrations
Methyl prednisolone	Phenobarbitone/ carbamazepine/ phenytoin	(Bartoszek et al., 1987)	Increased CL
Oxcarbazepine	Antiepileptic drugs	(Sallas et al., 2003)	Increased CL
Phenytoin	Halothane	(Karlin and Kutt, 1970)	ADR

[19] Adverse Drug Reaction

	Diazoxide	(Roe et al., 1975)	Subtherapeutic concentrations
	Dipropylacetate	(Windorfer et al., 1975)	Increased exposure
Primidone	Dipropylacetate	(Windorfer et al., 1975)	Increased exposure
Salicylate	Griseofulvin	(Phillips et al., 1993)	Subtherapeutic concentrations
Sodium valproate	Isoniazid	(Dockweiler, 1987; Jonville et al., 1991)	ADR
Temozolomide	O6-benzylguanine	(Meany et al., 2009)	No significant DDI in paediatrics
Theophylline	Cefaclor	(Hammond and Abate, 1989)	ADR
	Isoproterenol	(Hemstreet et al., 1982; O'Rourke and Crone, 1984; Griffith and Kozloski, 1990)	Increased clearance, ADR, decreased exposure
	Pyrantel	(Hecht and Murray, 1989)	Increased exposure
	Secobarbitone	(Paladino et al., 1983)	Increased clearance
Thiamylal	Clonidine	(Nishina et al., 1994)	Increase therapeutic efficiency
Tolazoline	Cimetidine and ranitidine	(Huang and Huang, 1996)	ADR
Vancomycin	Indometacin	(Spivey and Gal, 1986; Asbury et al., 1993)	Decreased CL

Table 2.4.2 Victim drugs and perpetrators presented in Figure 2.4-1

Victim (Interacting drug)	Age (Years)	Paediatric AUC ratio	Adult AUC ratio	Fold DDI	Paediatric References
Bupivacaine (Diazepam)	2-10	1.70	1.25	1.36	(Giaufre et al., 1988)
Carbamazepine (Erythromycin)	6	2.17	1.49	1.46	(Zitelli et al., 1987)
	9	3.17		2.13	(Kessler, 1985)
	8	2.00		1.34	(Hedrick et al., 1983)
	9	1.63		1.09	(Stafstrom et al., 1995)
Carbamazepine (Valproate)	9	0.92	1.07	0.86	(Schoeman et al., 1984)
	9	0.82		0.77	(Liu and Delgado, 1995)
Chloroquine (Chlorpheniramine)	6-12	1.73	0.79	2.2	(Okonkwo et al., 1999)
Cyclofosfamide (Fluconazole)	2 months-18	1.29	1.79	0.72	(Yule et al., 1999)
CsA (Ketoconazole)	3	1 .01	1.15	0.88	(El-Husseini et al., 2006)
CsA (Norfloxacine)	10	1.64	1.16	1.41	(McLellan et al., 1995)
Digoxin (Amiodarone)	0.5-18	3.53	1.62	2.18	(Koren et al., 1984)
Digoxin (Carvedilol)	2 weeks-7.8	1.90	1.16	1.63	(Ratnapalan et al., 2003)
Efavirenz (Rifampicin)	3-15	0.97	0.97	1.00	(Eiden et al., 2009)
Etopside (CsA)	8 months-17	1.47	1.25	1.52	(Lacayo et al., 2002)
	4.4 -20.7	1.90	1.25	1.18	(Bisogno et al., 1998)
Griseofulvin (Phenobarbitone)	7 & 8	0.24	0.69	0.35	(Beurey et al., 1982)
Imipramine (Carbamazepine)	6 -16	1.06	1.06	1.83	(Brown et al., 1990)
Lamotrigine (Valpoate)	0.8 -19	0.99	1.71	0.58	(Battino et al., 2001)
Lidocain (Clonidine)	1-9	0.64	1.21	0.53	(Inomata et al., 2001)
Phenobarbital (Valproate)	NA	2.13	1.46	1.45	(Fernandez de Gatta et al., 1986)
Phenytoin (Chloramphenicol)	10-108 months	0.94	1.60	0.58	(Ogutu et al., 2002)
phenytoin (Co-trimoxazole)	4	1.27	1.52	0.84	(Gillman and Sandyk, 1985)
Ritanovir (Efavirenz)	5.7-16.3	1.27	1.09	1.16	(Bergshoeff et al., 2005)

Sodium valproate (ethosuximide)	2.1-16.8	0.72	0.70	1.02	(Salke-Kellermann et al., 1997)
Theophylline (Terbutaline)	7-11	0.79	0.93	0.85	(Danziger et al., 1985)
Theophylline (Salbutamol)	19 months	0.42	0.82	0.50	(Amirav et al., 1988)
	5-13	0.54	0.83	0.65	(Dawson and Fergusson, 1982)
Theophylline(phenobarbitone)	8.86	0.70	0.88	0.80	(Saccar et al., 1985)
	29 weeks GSA	0.94		1.08	(Kandrotas et al., 1990)
Zidovudine (Didanosine)	3-14	1.11	1.21	0.92	(Gibb et al., 1995)

Figure 2.4-1 shows the interaction ratio in paediatrics relative to those observed in adults, the values above the shaded area indicate more interaction in paediatrics than in adults and values below the shaded area indicate more interaction in adults than in paediatrics for the same compounds. The magnitude of the DDI, as measured by parameter in presence/absence of inhibitor in paediatrics, were classified as higher (>1.25-fold) in 10 cases, similar (0.8 to 1.25-fold) in 15 cases and lower (<0.8-fold) in 8 cases. In the case of carbamazepine-erythromycin two of the paediatric studies fall above the 95th percentile for the expected between study variation in adult population and in the case of digoxin-amiodarone one paediatric study is above the 95th percentile for expected between study variation in adults. There was no observable age related pattern for the magnitude of paediatric DDIs compared to adults. There were three drug combinations where a comparison of the level of DDI was possible between more than one paediatric age range and adults. These pairs were theophylline & phenobarbitone, theophylline & salbutamol and carbamazepine and erythromycin.

Kandrotas et al. report theophylline clearance in the presence and absence of phenobarbital in a premature neonate with a gestational age of 29 weeks (Kandrotas et al., 1990), the results of this study were compared against those in children aged 6 to 12 and adults. The detected level of interaction was 0.94-fold in neonates compared to 0.71-fold in children and 0.77-fold in adults. A brief description of data presented in Figure 2.4-1 is shown in Table 2.4.2 to make comparison between pairs simpler. The summary of the studies are presented in the Supplemental Table S 1 in appendix 1.

2.4.2 Outcome of Bioavailability comparison

2.4.2.1 Published reports on bioavailability in paediatrics

Overall, 132 drugs in British National Formulary 2011-2012 were identified for which oral and intravenous preparations were available in paediatrics. Bioavailability for 16 drugs was reported in the paediatric age range in the literature. Corresponding adult value for 6 drugs were not identified from the literature (Table 2.4.5). Of these reports, children bioavailability data were retrieved in 15 occasions. As shown in Table 2.4.3, Bioavailability in neonates was reported in 4 studies for carnitine, midazolam, digoxin and theophylline. In infants,

bioavailability was reported for midazolam and busulfan and in children for alendronate, busulfan, etoposide, midazolam, fluconazole and methotrexate.

2.4.2.2 Magnitude of bioavailability compared to corresponding values in adults

Table 2.4.3 and Table 2.4.4 show the summary of studies and corresponding bioavailability values in paediatric and healthy adult subjects. Figure 2.4-2 shows the ratio of paediatric to adult bioavailability values for seven drugs. The results suggest that ratio of paediatric to adult bioavailability were within two-folds in 80% of the occasions. Table 2.4.5 presents the bioavailability values in paediatric group where comparable bioavailability in adults was not found.

Table 2.4.3 Reported bioavailability after oral and intravenous administration of drugs in paediatric subjects

Drug	N	Age (Y)	Oral dose	Bioavailability (%)	Route	Reference
Alendronate	25	4 to 16	35 mg	0.43	Oral	(Ward et al., 2005)
	12	4 to 11	35 mg	0.43	Oral	(Nakhla et al., 2011)
	12	12 to 17	35 mg	0.39		
Busulfan	8	1.5 to 6	2 mg	68	Oral	(Hassan et al., 1994)
Carnitine	5	27 weeks gestation and 2.9 days postnatal age	20 mg/kg/d	83.7	Enteral (week 8)	(Crill et al., 2006)
Etoposide	9	2 to 16	100 to 150 mg/m^2	48	Oral	(Pinkerton et al., 1993)
Fluconazole	10	7.4	3 mg/kg	92	Oral	(Seay et al., 1995)
Methotrexate	11	13.5	between 7.5 and 40 mg/m2	84	Oral	(Stephens et al., 2005)
Midazolam	8	5.81	0.15 mg/kg	27	Oral syrup	(Payne et al., 1989)
	8	5.48	0.45 mg/kg	16		
	8	5.97	1.0 mg/kg	15		
	15	3 to 13 days	0.1 mg/kg	49		(de Wildt et al., 2002)
	3	0.5 to 2	0.5 mg/kg	37		(Reed et al., 2001)
	3	2 to 12	0.5 mg/kg	35		
Theophylline	14	5 to 13	20 mg/kg	67.9	Oral	(Coulthard et al., 1983)

Table 2.4.4 Reported biavailability in healthy adult volunteers after oran and intravenous administration of drugs

Drug	N	Age (Y)	Oral dose	Bioavailability (%)	Route	Reference
Alendronate	132	60	5 to 80 mg	0.76	Oral	(Gertz et al., 1995)
Busulfan	8	37	2 mg	80	Oral	(Hassan et al., 1994)
Busulfan	11	39	0.5-0.6 mg/kg	69	Oral	(Schuler et al., 1998)
Carnitine	6	38	2g	16	Oral	(Harper et al., 1988)
			6 g	5		
Etoposide	11		80 mg/m^2	50	Oral	(Carney, 1991)
Fluconazole[a]	16	>18	200 mg	51 and 63	Oral	(Barquist et al., 2007)
Midazolam	12	21 to 32	7.5 mg	41	Oral	(Palkama et al., 1999)
	7	44.3	15 mg	38	Oral	(Pentikainen et al., 1989)
	6	22 to 27	10, 20, 40	31 to 72	Oral	(Heizmann et al., 1983)
Theophylline[b]	20	30	7.3 mg/kg	99	Oral (solution)	(Hendeles et al., 1977)
				96	Oral (tablet)	

a critically ill patients

b asthmatic patients

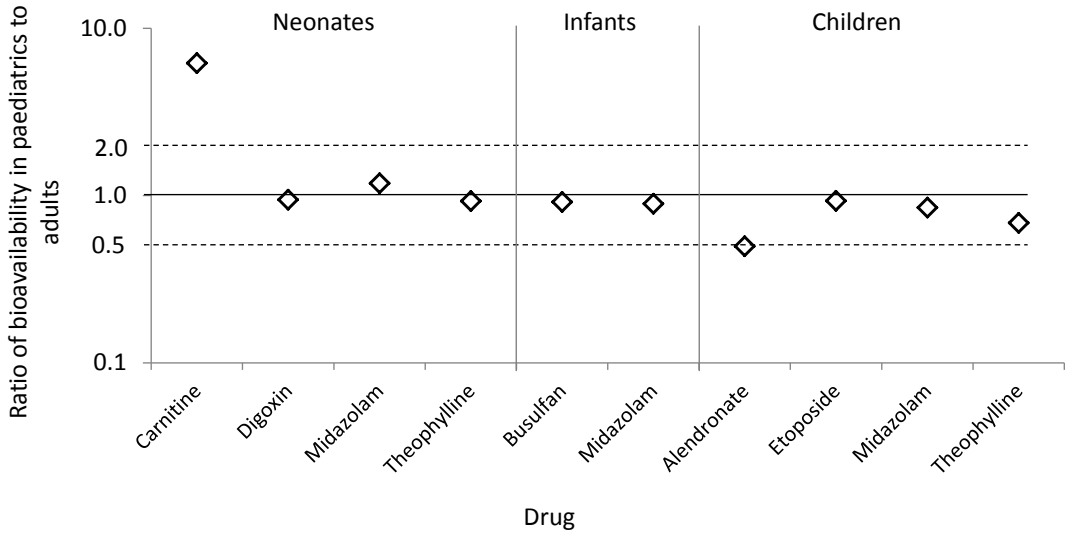

Figure 2.4-2 Ratio of bioavailability in paediatric to adult subjects for a carnitine, digoxin, midazolam, theophylline, busulfan, alendronate and etoposide.
Solid black line crossing one indicates when paediatric and adult bioavailability values are equal. Two Dashed lines show two folds higher and lower ratios.

Table 2.4.5 Bioavailability values in paediatric subjects for a number of drugs where comparable bioavailability value in adults was not retrieved from the literature.

Drug	Age (Y)	Bioavailability (F)%	Reference
Clonidine	6.5	55.4	(Larsson et al., 2011)
Desmethylsildenafil	NA	62 and 26	(Ahsman et al., 2010)
Etoposide	9	48	(Pentikainen et al., 1989)
Flurbiprofen	3 months to 13 year	81	(Kumpulainen et al., 2010)
Hydrocortisone	10.9	94.2	(Charmandari et al., 2001)
Zolpidem	NA	70	(Salva and Costa, 1995)

2.4.3 Outcome of audit from hospital records

Overall, 19 DDI cases were identified by the pharmacists in Manchester Royal Infirmary and Sheffield Children's Hospital. Great Ormond Street withdrew from the study due to technical problems in electronic data base and staff change. The identified DDIs in Manchester Royal Infirmary and Sheffield Children's Hospital are presented in Table 2.4.6. The results indicate that most DDIs are reported in subjects taking anticonvulsant and immunosuppressant drugs. There are no DDIs reported in neonates.

Table 2.4.6 DDIs reported by pharmacists in Manchester Royal Infirmary and Sheffield Children's Hospital.

Age	Weight	Sex	Drug 1	Dose	Route	Duration	Drug 2	Dose	Route	Duration	Drug 2	Dose	Route	Duration
0.4	5.6	F[1]	Phenobarbitone	30 mg BD	NG[2]		Phenytoin	16.3 mg BD	NG					
1	8.8.	F	Phenytoin	33 mg BD	NG	Ongoing	Phenobarbitone	10 mg BD	NG	Ongoing	Erythromycin	24 mg QDS	NG	2 days
1.3	12.7	M[3]	Sodium valproate	280 mg BD	Oral		Phenobarbitone	50 mg BD	Oral					
1.8	12	F	Tacrolimus	1mg BD	NG		Sirolimus	0.5 mg OD	NG					
2[4]	13.1	M	Methotrexate	85 mg/m2		1 day	Gentamicin	6 mg/kg/d	IV	1 day	Calcium leucovorin	8.25 mg 3 hourly for 5 doses and then 6 hourly	IV and Oral	when methotrexate level <0.1
2	9	M	Rifampicicn	90 mg OD	NG		Voriconazole	63 mg BD	IV	less than 1 day				
3	12	F	Ceftriaxone	30 mg BD	IV		Calcium gluconate	25 meq	IV					
3.5	11.85	M	Clarithromycin	90 mg BD	IV		Midazolam	71 mg in 50 ml (100 mcg /kg/h)	IV					
4	12.2	M	Cyclosporin	35 mg BD	Oral	Ongoing	Voriconazole[5]	50 mg BD	Oral	Ongoing	Carvedilol	700 mi mcg BD	Oral	Ongoing
8	31	M	Budesonide	0.5 mg/ 2ml BD	inhalatio n	2 days	Clarythromycin	250 mg BD	Oral	2 days				
10	26	F	Phenobarbitone	100 mg BD	NG	Ongoing	Levetiracetam	100 mg BD	NG	Ongoing				
11	57	F	Fluconazole	180 mg OD	Oral		Nifedipine	10 mg	Oral					
14[6]	50.9	F	Cyclosporin	50 mg BD	Oral		Voriconazole	200 mg BD	Oral	7 days	Metronidazole	400 mg TDS	Oral	
14	35.85	F	Sodium valproate	600 mg OM, 680 mg ON	Oral/PE G[7]	Ongoing	Meropeenem	710 mg TDS	IV					

[1] Female
[2] nasogastric
[3] Male
[4] additional drug was ceftazidine 975 mg BD intravenous that was continued.
[5] Omitted occasionally when non-by-mouth and a caspofungin used instead
[6] additional drug was Methylprednisolone 100 mg BD intravenous

14	50.9	F	Mycophenolate	500 mg BD	Oral		Metronidazole	400 mg BD	Oral					
14	57.7	M	Phenytoin	150 mg BD	Oral		Warfarin	10 mg ON	Oral					
16	50	F	Phenytoin	100 mg BD	NG		Abacavir	300 mg BD	NG					
16	50	F	Phenytoin	100 mg TDS	IV	7 days	Itraconazole	5 mg/kg/d	IV	1 day				
16	54	F	Phenytoin	280 mg BD	Oral	Ongoing	Clarythromycin	250 mg BD	PEJ[8]	5 days				

[7] Percutaneous endoscopic gastrostomy

[8] percutaneous endoscopic jejunostomy

2.5 Discussion

To our knowledge, the current study is the first critical analysis of the literature in relation to DDI in paediatrics and a comparison with corresponding values in adults. There are a number of DDIs reported in paediatrics which are not reported in adults and this could be due to a number of reasons:

- Differences in the fraction eliminated by various pathways with age: this could be metabolic such as cytochrome P450 or non-metabolic such as renal.
- Differences in dosage where a relatively higher dose is given in children resulting in bigger interaction potential: this is especially true where the same dose is administered across an age range e.g. erythromycin 250mg four times a day from 2 to 8 years of age.
- Particular drug combinations are only used in paediatric drug treatment e.g. 6-mercaptopurine and methotrexate for acute lymphoblastic leukaemia.
- Random chance that the interaction has only been reported in paediatrics.
- Pharmacodynamic differences between adults and children (concentration-response relationship not the same), hence children are more susceptible to the side effects of drugs e.g. cyclosporine (Marshall and Kearns, 1999).
- Different pathologies affecting drug eliminating pathways.

Some of the reasons presented above can equally be used to explain why some interactions are reported in adults but not in paediatrics. Amongst all of the paediatric DDI reports in the literature, few give details of PK parameters that enable a comparison of the magnitude of a particular interaction e.g. AUC ratio between paediatrics and adults. A positive example of such a study in paediatrics was conducted by Ngo et al. in HIV infected children (Ngo et al., 1999). In this study the PK parameters of azithromycin were investigated in the presence and absence of atovaquone and the results were compared to previously reported non-HIV infected children. Only a relatively small number of paediatric DDI reports could be compared against corresponding studies conducted in adults. Most of these were performed in children aged 1 -12 years, with three studies found in the infant and adolescent age

groups and just one in neonates. The lack of PK data is not surprising and is related to ethical and practical reasons surrounding blood sampling and undertaking PK studies particularly in young children. In many paediatric studies the clinical symptoms and pharmacological effects (including adverse drug reactions) in the presence and absence of an inhibitor were reported rather than PK parameters.

There were limited data on the bioavailability of drugs in paediatrics. Comparison of these data with corresponding adult value does not show significant differences between bioavailability of two groups. There was no consistent trend in bioavailability of investigated drugs with age. However, theses comparisons are confounded by differences in study designs and formulations between paediatric and adult studies. Some of observed differences can be explained by physiological differences in absorption process of drugs. For example, for a relatively large size molecule such as carnitine the significant difference (over 6 folds) between preterm neonates and adults might be due to development of the intestinal permeability. Using lactoluse and mannitol solution, higher permeability especially in preterm neonates during the first two days after birth is reported (Weaver et al., 1984; Van Elburg et al., 2003; Riezzo et al., 2009). Two studies reported bioavailability of midazolam across paediatrics age range (Reed et al., 2001; de Wildt et al., 2002). Although there seems to be a decline in observed bioavailability values with age, these bioavailability values were not significantly different from those in adults. Interestingly, bioavailability in south African children seems be lower which is consistent with higher expression of CYP3A5 and p-gp in this ethnic group (Payne et al., 1989). Oral absorption of many drugs is transporter mediated and therefore saturable at high doses. Increasing the dose in these cases reduces the bioavailability and hence the increase in the absorbed dose would be less than proportional to the increase in the dose. In absence of adequate data in all age groups, explicit conclusion on the age-related changes in bioavailability is difficult. It is surprising that despite all known physiological changes relatively small research has been placed on measurement of bioavailability in paediatric subjects. This pilot study highlights the need for a systematic investigation and analysis of data through well-established studies in adults and children.

There are a number of limitations in the current study not least of which is that some comparisons involved a limited number of subjects and could not account for differences in

study design and disease pathology. In cases where the fold DDI was compared across age ranges an important factor in comparison is the relative dose and consequent exposure to the inhibitor. In the case of the erythromycin interaction with carbamazepine the dose of inhibitor was 250 mg qid in the 2 year and adult groups. The average body weight in a 2 year old is 13 kg and of an adult is 68 kg, consequently the 2 year old could be exposed to higher plasma concentrations of the inhibitor leading to a larger interaction compared to adults. This is an important clinical issue for some drugs where doses are banded into ages because, should the drug interact with other drugs, these interactions are likely to be greater in the younger and smaller children within a dose age band.

There are many covariates in addition to dosage to consider when trying to compare the magnitude of a DDI in different age groups using unrelated studies. These include the timing of victim and interacting drugs in the studies and whether the fold-interactions were determined at steady state or following a single dose. Only when the study design differences are accounted for can a true comparison of the level of interaction be determined, and other biochemical and physiological factors (e.g. ontogeny of drug metabolising enzymes) put forward as reasons for a different magnitude of interaction in children compared to adults. Subsequently, this has been one of the difficulties in this investigation. Bearing all these factors in mind the current DDI comparison between paediatrics and adults can only be viewed as a crude attempt and points to the need to develop other approaches to assess DDIs in neonates, infants and children compared to adults.

The differential ontogeny of drug metabolising enzymes may be an important cause of different magnitudes of DDIs between adults and children. Comparison of DDI between theophylline and phenobarbital shows a fairly similar level of DDI in paediatrics (0.71 fold) and adults (0.77 fold) but a less significant interaction in premature neonates (0.94 fold). The major enzyme responsible for theophylline metabolism is CYP1A2 - this pathway is not fully developed in premature neonates. CYP1A2 mRNA and protein are not traceable in the foetus and become detectable between 1-3 months, corresponding enzyme activity during foetal stages is negligible. The inability of the new-born and young infant to metabolise caffeine to paraxanthine, a CYP1A2 pathway (Alcorn and McNamara, 2002) has been

reported before. The net consequence of the reduced involvement of CYP1A2 in theophylline metabolism in the neonate is a lesser risk of DDI for inducers and inhibitors of CYP1A2 in this age group compared to older children and adults. One area where there is little research is on the expression of the nuclear receptors, pregnane X receptor (PXR) and constitutive androstane receptor (CAR) with age. These receptors induce CYP gene expression in response to xenobiotics such as phenobarbitone. Their ontogeny will have an effect on this mechanism and the magnitude of drug-drug interaction observed with age (Vyhlidal et al., 2006).

Modelling and simulation tools incorporating information on system ontogeny can open a new approach for assessing DDIs in paediatric populations without the need for the conduct of clinical studies. These can help to visualize the effects of interacting drugs in young children and how may requires a dose adjustment. The objections to such an approach (in place of real clinical observations) are based on the presumption that these models are replacing the clinical studies whilst as it has been argued recently (Rostami-Hodjegan, 2012) they are alternatives to 'guesswork'. Further use and validation of such models will give confidence in dosing commonly used drugs in paediatrics as well as giving an assessment of the likely magnitude of a DDI in this vulnerable population. A recent paper (Leong et al., 2012) on the regulatory experience with physiologically based pharmacokinetic (PBPK) modelling for paediatric drug trails highlights their utility. The report puts emphasis on the need to refine existing PBPK models with respect to obtaining new data on the age dependent processes that govern ADME and also the increased application of these PBPK models. CHMP guidelines (CHMP, 2010) recommend that a clinical DDI study should be considered for new drugs in children where they are likely to be used in combination with a potentially interacting second drug. They also suggest that evidence from a paediatric modelling and simulation approach may provide satisfactory supportive data provided the approach is successful in predicting the DDI in adults, and data on enzyme abundance and other physiological parameters in paediatrics are reliable.

Finally, data collection from three different centres showed potential DDIs may occur frequently in paediatric wards. However, due to lack of infrastructure for provision of research time by pharmacists in these centres, adequate data were not collected and

therefore, it was not feasible to explicitly conclude DDI between which drug categories occur most frequently.

2.6 Conclusion

Neonates and infants are expected to show the highest disparity in the magnitude of DDI based on information known on variable ontogeny of enzymes and excretory elimination pathways. However, there is not enough data to confirm or refute this hypothesis. The current PK data in the literature indicates that higher, similar and lower levels of DDI are possible in children compared to corresponding DDI reported in adults. Some of these differences could be related to variations in study designs and no obvious trends could be established based on currently available data. Further non-interventional studies for assessing the magnitude of DDIs in neonates, infants and children compared to adults are needed.

Chapter 3. Age related changes in fractional elimination pathways for drugs: Assessing the impact of variable ontogeny on metabolic drug-drug interactions

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Declaration

This chapter constitutes a published article;

"Age related changes in fractional elimination pathways for drugs: Assessing the impact of variable ontogeny on metabolic drug-drug interactions"

The Journal of Clinical Pharmacology, 2013; 53 (8): 857-865

F. Salem: Lead for study design, data and statistical analysis, modelling and simulation in Matlab and preparation of manuscript

T.N. Johnson: Supervision of data analysis and detailed editing of manuscript

Z. E. Barter: Supervision of data analysis

J. S. Leeder: Expert opinion on pharmacogenetic aspect and advice on removal of specific sections

A. Rostami-Hodjegan: Supervision of research and analysis and input on manuscript

We acknowledge Dr Kayode Ogungbenro for his expert opinion on bootstrapping methods and help in modelling the data in Matlab.

Supplemental material of the paper appears in the main text here to facilitate reading.

3.1 Abstract

The magnitude of any metabolic drug-drug interactions (DDI) depends on fractional importance of inhibited pathway which may not necessarily be the same in young children when compared to adults. The ontogeny pattern of CYP enzymes (CYPs 1A2, 2B6, 2C8, 2C9, 2C18/19, 2D6, 2E1, 3A4) and renal function were analysed systematically and

bootstrap methodology was used to account for variability, and to define the age range over which a statistical difference existed between each pair of specific pathways. A number of DDIs were simulated (Simcyp Paediatric v12) for real and virtual compounds to highlight the effects of age on fractional elimination and consequent magnitude of DDI.

For a drug metabolized 50% by each of CYP2D6 and CYP3A4 pathways at birth, co-administration of ketoconazole (3mg/kg) resulted in a 1.65-fold higher level of AUC ratio, as opposed to a 2.4-fold in 1 year olds and 3.2-fold higher levels of DDI in adults. Conversely, neonates could be more sensitive to DDI than adults in certain scenarios. Thus, extrapolation from adult data may not be applicable across all paediatric age groups. The use of paediatric PBPK models may offer an interim solution to uncovering potential periods of vulnerability to DDI where there are no existing clinical data derived from children.

3.2 Introduction

Most of the data within the drug-drug interaction (DDI) databases are taken from adult clinical studies and case reports. Hence, the knowledge of DDIs in the paediatric population is limited. Extrapolation of adult data to infer DDI in paediatric populations is the default action in the absence of any other clinical information. However, this might not always be appropriate knowing variable ontogeny of elimination pathways.

Assessment of potential DDIs might be even more complex if genetic polymorphisms of metabolic pathways are considered. Therefore, DDI studies in adult healthy volunteer and patient populations may serve only as the 'starting point' for postulating about the likelihood of a DDI in paediatric patients knowing that these may not represent the clinical significance of DDI across the developmental spectrum. For example, in a study by Crocker et al., (Crocker et al., 1994) nifedipine inhibited CsA elimination in five children but in separate studies no effect was seen in adults.

A systematic review of DDIs in paediatric patients, with emphasis on metabolic interactions, has recently been published in this journal (Salem et al., 2013) which revealed the paucity of clinical data to support or refute the concept that the potential for DDIs might be different in younger children than in adults. Developmental changes at multiple levels influence drug pharmacokinetics (PK) during growth and development, (Kearns et al., 2003) but two major

factors determining drug exposure are renal and hepatic clearance. Rowland and Matin (Rowland, 1973) have explained the relationship between fold change in exposure to victim drug following a metabolic inhibition and the fraction of victim drug cleared by the inhibited route of interest prior to that interaction (fm). The latter is a function of enzyme activity and abundance relative to contributions made by other pathways. The original model of Rowland and Matin (Rowland, 1973) was expanded by Rostami and Tucker (Rostami-Hodjegan and Tucker, 2004) to include gut wall metabolism which plays a role in first-pass metabolism and is subject to ontogeny (Johnson et al., 2001). This adds another dimension of variability regarding the possibility of differential risk of DDI in paediatrics compared to adults.

The developmental trajectories of several elimination pathways are now established. For example, CYP3A7 expression in foetal liver increases as gestation proceeds and decreases after birth, whereas CYP2C19 is expressed at constant low levels during gestation and increases markedly after birth. The consequence of this differential ontogeny is not just lower total clearance at birth (beyond the effect of size), but it also leads to an age-related variation of fm by different pathways and altered risk of DDIs during development. For example, the risk of DDIs could be negligible if the inhibited pathways are not postnatally expressed in neonate where alternative routes are responsible for elimination of a victim drug. Conversely, the potential for DDI will be higher if alternative pathways to that of inhibited route are not developed adequately. The aims of the current study were:

1) To compare the maturation rate of the most important hepatic metabolic pathways and define the age ranges where a disparity exists.

2) To simulate and compare DDI examples in paediatric and adult populations.

3.3 Methods

3.3.1 Comparison of the ontogeny of drug elimination pathways

Published models describing the relative ontogeny of CYP enzymes from birth onwards were used in this study (Johnson et al., 2006). These are essential models for estimating the absolute values of clearance and some of the models are now updated using additional information becoming available since the original publication. These are presented in Table 3.3.1. The models were derived using *in vitro* expression and activity data collated from a

number of studies. The best fit model was derived in each case using the general form shown in Equation 3.3-1.

$$\text{Fraction of adult} = \frac{(\text{Adult}_{\text{Max}} - \text{F}_{\text{Birth}}) * \text{Age}^{n}}{\text{Age}_{\text{mid}}{}^{n} + \text{Age}^{n}} + \text{F}_{\text{Birth}}$$

Equation 3.3-1

where $\text{Adult}_{\text{Max}}$ is the maximum adult relative expression (1 at younger adults but with relevant corrections if the reference group were older), F_{birth} is the relative expression of enzyme (based on *in vitro* catalytic activity or immunoreactive protein) at birth and Age_{mid} is the age at which the fractional expression (/activity) is in the middle of the birth and adult values, n is the exponent related to the sigmoidicity of the developmental curve. For the renal model we used a linear model based on the relationship between glomerular filtration rate (GFR) and body surface area although other models based on post-menstrual age are now published (Rhodin et al., 2009) which give similar predictions.

Table 3.3.1 Data used to generate hyperbolic functions describing the development of individual cytochrome P450.

Enzyme	Location	Fractional expression at birth relative to adult	Time to half adult expression (Y)	Hyperbolic function (fraction of adult CYP abundance)	References
CYP1A2	Hepatic	Negligible	1.8	$CYP1A2 = \frac{1.05 - 0.08 * Age^{1.1}}{1.69^{1.1} + Age^{1.1}} + 0.08$	(Tateishi et al., 1997; Sonnier and Cresteil, 1998)
CYP2B6	Hepatic	0.15	1.2	$CYP2B6 = \frac{1 - 0.1 * Age}{1 + Age} + 0.1$	(Tateishi et al., 1997; Croom et al., 2009)
CYP2C8	Hepatic	0.3	0.008	$CYP2C8 = \frac{1 - 0.3 * Age}{0.02 + Age} + 0.3$	(Tateishi et al., 1997; Treluyer et al., 1997)
CYP2C9	Hepatic	0.17	0.005	$CYP2C9 = \frac{1 - 0.17 * Age^{0.53}}{0.016^{0.53} + Age^{0.53}} + 0.17$	(Tateishi et al., 1997; Treluyer et al., 1997; Koukouritaki et al., 2004; Hines, 2007)
CYP2C18/19	Hepatic	0.3	0.2	$CYP2C19 = \frac{1 - 0.3 * Age^{2.44}}{0.28^{2.44} + Age^{2.44}} + 0.3$	(Treluyer et al., 1997; Koukouritaki et al., 2004; Hines, 2007)
CYP2D6	Hepatic	0.036	0.08	$CYP2D6 = \frac{1.0 - 0.036 * Age}{0.1 + Age} + 0.036$	(Treluyer et al., 1991; Tateishi et al., 1997; Stevens et al., 2008)
CYP2E1	Hepatic	0.086	0.1	$CYP2E1 = \frac{1.074 - 0.086 * Age^{0.496}}{0.226^{0.496} + Age^{0.496}} + 0.086$	(Vieira et al., 1996; Tateishi et al., 1997; Johnsrud et al., 2003; Hines, 2007)
CYP3A4/5	Hepatic	Negligible	0.6	$CYP3A4/5 = \frac{1.061 * Age^{0.78}}{0.66^{0.78} + Age^{0.78}}$	(Lacroix et al., 1997; Tateishi et al., 1997; Stevens et al., 2003; Blake et al., 2007; Hines, 2007)
CYP3A4/5	Gut	0.42	0.3	$CYP3A4/5 = \frac{1 - 0.42 * Age}{2.357 + Age} + 0.42$	(Johnson et al., 2001)

Renal function	Renal	Male: 0.15 Female:0.14	5.5	$\text{Renal function} = \frac{(-0.61604 \times \text{BSA}^2) + (99.054 \times \text{BSA}) - 17.74}{120}$	(Johnson et al., 2006)

Since the magnitude of DDI depends on fractional (rather than absolute) value of each metabolic pathway, these models were applied to compare the 'relative rates of elimination pathway maturation' as defined in Equation 3.3-2.

$$\text{Ratio of pathway(i) to pathway(j) relative to adults} = \frac{\text{Paediatric}_i / \text{Adult}_i}{\text{Paediatric}_j / \text{Adult}_j} \quad \textbf{Equation 3.3-2}$$

This shows the ratio of the function of one pathway (i) relative to a potential competing pathway (j). So a constant value (of 1) should not be mistaken for lack of ontogeny but it is indicative of 'similar' ontogeny of the pathways of interest; even both of the pathways may have much lower absolute values compared to adult. Comparisons were undertaken between the major CYP enzymes and between these and renal elimination related to glomerular filtration rate.

Interindividual variability exists in the activity of metabolic enzymes as shown previously in several publications (Blake et al., 2007; Allegaert et al., 2008; Mukherjee et al., 2009); however, calculation of confidence interval (CI) around the ontogeny profiles from the *in vitro* data in the literature is often not feasible because of sparsity and variability across the whole age range and limited age groups within each study. To overcome this confinement a bootstrapping approach was used. Bootstrapping aims to use the data of a sample study (i.e. regression line for the ontogeny of each pathway in this study) as a "surrogate population", for the purpose of approximating the sampling distribution of a statistic in the overall population. This is done by re-sampling (with replacement) from the sample data at hand to create a large number of "phantom samples" known as bootstrap samples.

Firstly, the variance S_0^2 about the regression line was calculated using Equation 3.3-3 (Armitage).

$$S_0^{\,2} = \frac{\sum (y - Y)^2}{n - 2} \quad \textbf{Equation 3.3-3}$$

In this equation numerator represents the residual sum of squares, where y is an independent observation e.g. literature value for fraction of CYP ontogeny at a given age and Y is the predicted value from the ontogeny model (regression line).

Secondly, the confidence intervals (CI) around the 'best fit' ontogeny regression lines were calculated in MATLAB 7.12 using Equation 3.3-4.

$$Y \pm t_{n-2,0.05} S_0 \sqrt{\left[\frac{1}{n} + \frac{(x_0 - \bar{x})^2}{\sum (x - \bar{x})^2}\right]} \qquad \textbf{Equation 3.3-4}$$

Where Y is the regression line from the best fit ontogeny model, the term $t_{n-2,0.05}$ is the t-distribution on n-2 degree of freedom and 5 and 95% confidence limits for the predicted mean, *n* is the number of iterations in the bootstrap (10,000 in this study), $\bar{x}$ is mean value for observed ages (the mean age for which data was taken from the literature to build the ontogeny function), x_0 is a given value of x (the increments are defined in MATLAB with intervals of 0.1 year up to 20 years of age in order to give a smooth curve), x are the individual observed incremental ages for which data was taken from the literature) and S_0 is standard deviation about regression. Ten thousand iterations were performed for each pathway at each age point to generate data to calculate the 5th and 95th CI of the model.

Finally, the ratio of 10,000 simulated data points for pathways *i* and *j* was calculated. The median ratio, upper and lower 5% boundaries were calculated. Lower 5% boundary and the points at which this dips below the ratio of one were used to report the ages at which disparity between enzyme activities is observed.

3.3.2 Hypothetical age related changes in DDI

A number of theoretical simulations to show age related changes in the magnitude of DDIs were performed in the Simcyp Paediatric simulator. To show the potentially variable contribution of metabolic pathways on the elimination of substrates with age, two hypothetical compounds (COMP 1 & 2) have been created in Simcyp v12. For simplicity and to minimize the complexity of the system being modelled, the initial assessment was restricted to intravenous drug administration and the drugs were mainly metabolized hepatically and assumed to have negligible renal elimination. This also helped to avoid added complexity of the ontogeny at the level of gut wall metabolism. The selection of metabolic pathways for these compounds was based on the maximum disparity in the rate of

differential ontogeny or expression level at birth and thus likely to demonstrate age related changes in the magnitude of likely DDIs and creation of scenarios where the DDI could be lower or higher in a paediatric population. Therefore, COMP 1 is assumed to be metabolized by CYPs 1A2 and 2C9 and COMP 2 by CYPs 2D6 and 3A4. Both compounds possessed physiochemical and PK properties similar to alprazolam Table 3.3.2 and the metabolic parameters (CL_{int}) for each are shown in Table 3.3.3. The compounds were designed in such a way that the fm by each enzyme is approximately 50% at birth. The Simcyp Simulator gives the possibility to output fm by each pathway at given age and incorporates all other sources of ontogeny that may influence absolute values of clearance (e.g. liver size, liver blood flow, change in fraction unbound).

Table 3.3.2 Drug parameters that have been used in simulations.

Physiochemical Properties for Alprazolam like compounds	
Molecular Weight	308.8
Log $P_{O:W}$	2.12
Compound Type	Monoprotic Base
Pk_a	2.4
Blood to Plasma Ratio	0.825
Fraction unbound (f_u)	0.29
Pharmacokinetic Properties	
V_{ss} (L/kg)	0.99
CL_{int} (CYP2D6) (µl/min/pmol of isoform)	1.35
CL_{int} (CYP3A4)(µl/min/pmol of isoform)	0.09
CL_R (L/h)	0

Table 3.3.3 Drug related kinetic parameters entered to Simcyp for two hypothetical compounds.

	Pathway	CL_{int} (µl/min/mg)
COMP 1	CYP1A2	0.058
	CYP2C9	0.003
COMP 2	CYP2D6	0.089
	CYP3A4	0.018

To investigate the impact of DDI on fm, a strong inhibitor of CYP2C9 (sulfaphenazole) and CYP3A4 (ketoconazole) was introduced respectively for COMP 1 and COMP 2. One hundred simulations with 10 trials and 10 subjects were performed with fraction of females set as 0.5 for each of three age bands, 1 day (0.00274 – 0.00276 years), 1 year (0.99 – 1.01 years) and 20 years (19.99 – 20.01 years). For COMP 1 an intravenous dose of 0.5 mg/kg at steady state and a 28.5 mg/kg dose of the potent CYP2C9 inhibitor sulfaphenazole was co-

administered and for COMP 2 an intravenous dose of 0.5 mg/kg at steady state and a 3 mg/kg dose of the potent CYP3A inhibitor ketoconazole was co-administered. The magnitude of the interaction in each of the ages was recorded from the output from the Simcyp paediatric simulator.

Further simulations were performed based on COMP 3, this was similar to COMP 2 but in this instance the fm for CYP2D6 and CYP3A4 were changed so that each was approximately 50% in adult subjects rather than in neonates. All other parameter values and model assumptions were the same as above simulations apart from the CL_{int} values for CYP2D6 and CYP3A4 which were 0.09 and 1.35 μl/min/mg, respectively. One hundred simulations with 10 trials and 10 subjects were performed with fraction of females set as 0.5 for each of six age bands 1 day, 7 day, 1 month, 1 year, 2 years and 20 years. All simulations were run with and without a 3 mg/kg dose of ketoconazole and again the magnitude of the interaction in each of the ages was recorded from the Simcyp paediatric outputs.

3.4 Results

3.4.1 Development of drug elimination pathways

Maturation of hepatic metabolic pathways was investigated relative to adults for major CYPs. Figure 3.4-1 compares the updated models by Johnson et al. (Johnson et al., 2006) based on age for CYPs 1A2 & 2C9 and CYPs 2D6 & 3A4 and shows a higher activity at each age (relative to adults) for 2C9 and 2D6. A more comprehensive visualization of the variability in the rate of maturation of the different pathways relative to each other is shown in Figure 3.4-2 and Figure 3.4-3. In the graph of other pathways *vs.* CYP1A2 it is evident that all other CYP enzymes have a faster ontogeny compared to this enzyme and hence the ratio of relative abundance is greater than 1. Taking an example from the graph of other pathways *vs.* CYP2C9 and viewing CYP1A2 compared to CYP2C9, the former has a slow rate of ontogeny and consequently the ratio is less than 1 across the whole age range. The age at which maximum disparity in activity of the ratio of these metabolic routes occurs are presented in Table 3.4.1. The maximum pathway discrepancy relative to adult for most of major CYPs or renal elimination occurs at 1 day of age but there are cases that this

discrepancy happens in the first few weeks or months e.g. the maximum fold difference between CYP2B6 and CYP2C18/19 is 2.4 and occurs at month 5.

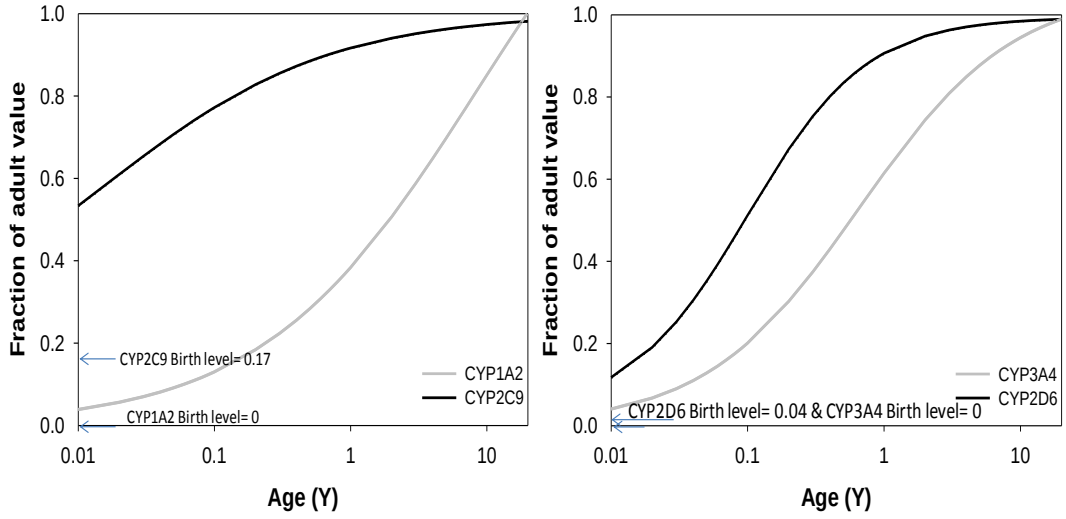


Figure 3.4-1 *In vitro* protein expression or activity relative to adults for CYPs1A2, 2C9, 2D6 and 3A4 (reproduced using the equations in Table 3.2.1).
Note: These patterns reflect the developmental trajectories of each CYP in relation to their own corresponding value in adults. Hence, they do not reflect the absolute abundance or activity of microsomal CYP3A4 vs. CYP2D6.

The age range where a significant difference was shown between specific pathways is presented in Figure 3.4-4. The ratios of the metabolic pathways with the 95% CI about the median value are shown for three sets of CYP enzymes in Figure 3.4-4. The age span over which the 5% lower confidence interval stay above a value of 1 represents the age band at which there is most confidence that the maturation of the pathways is different. For example, the maturation rate of CYP1A2 and CYP3A4 are different with 95% confidence between the ages of 1.4 to 11.6 years, for CYPs 2C9 and 1A2 from birth to 10.5 years and for CYPs 2B6 and 3A4 between 0.4 and 7.2 years.

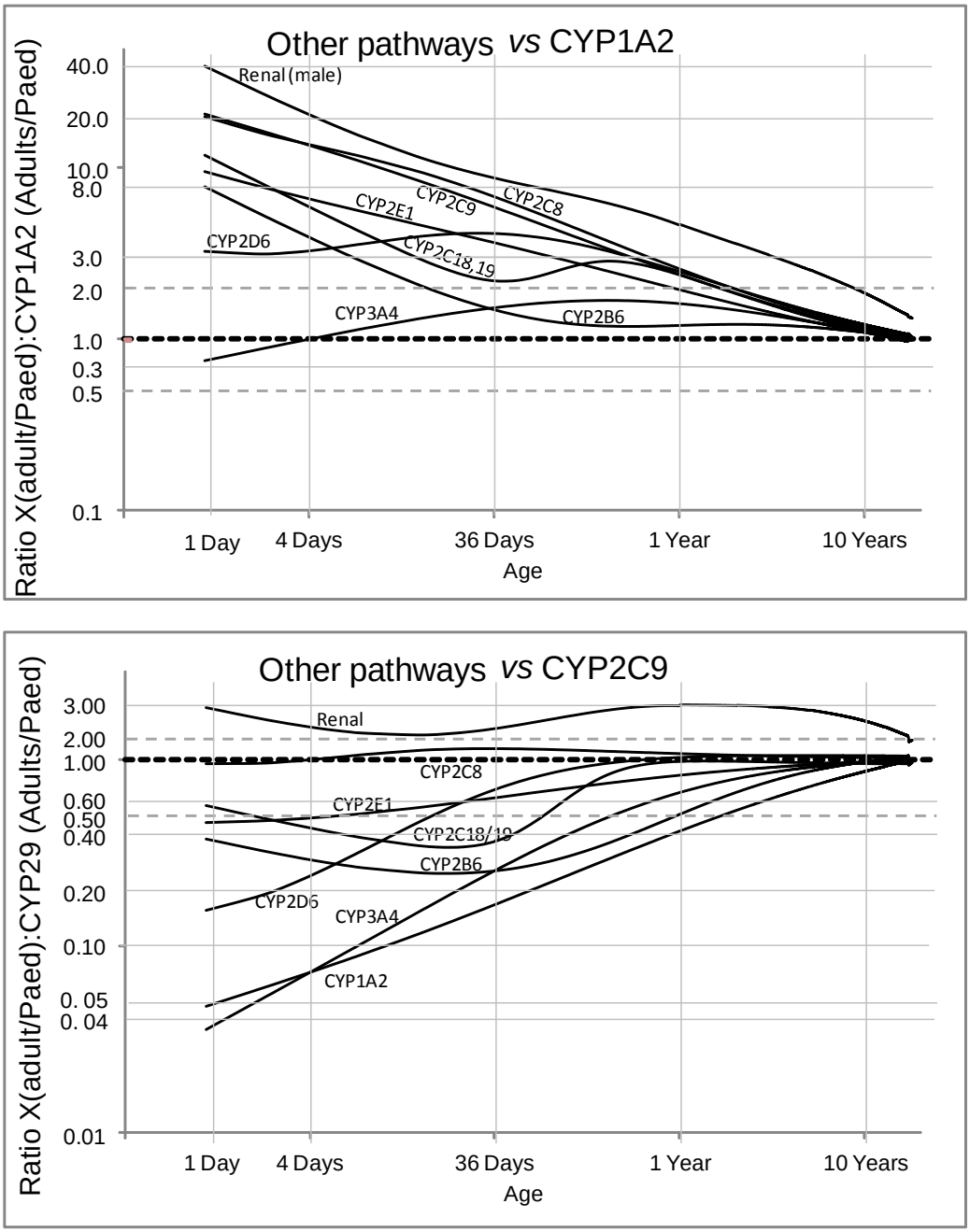
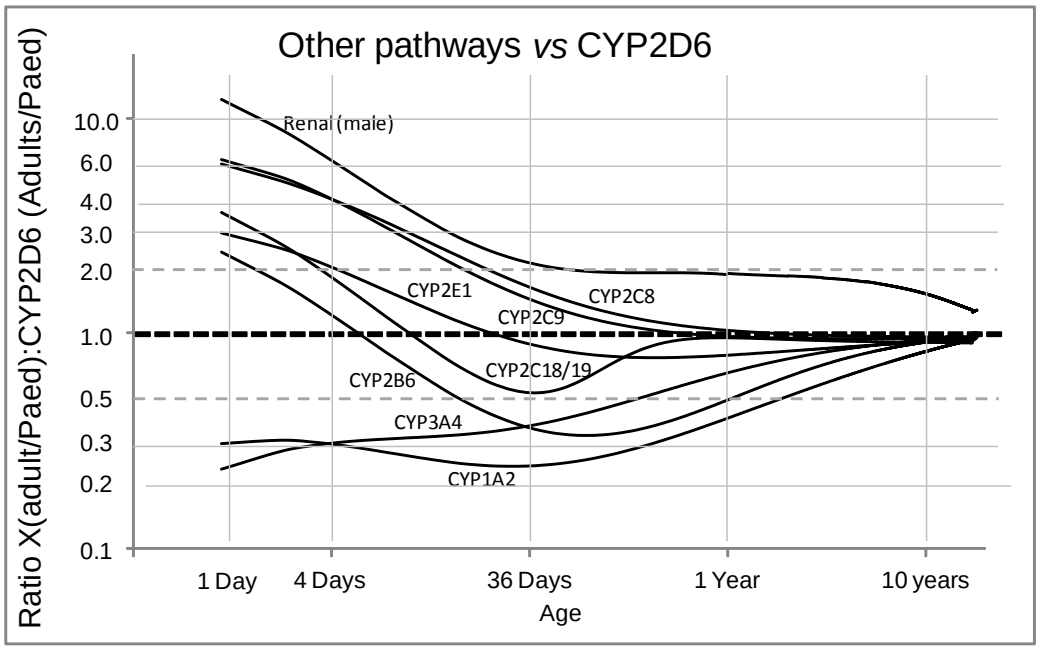


Figure 3.4-2 Ratio of paediatric to adult changes in the ontogeny of different enzymes relative to CYP2D6 (top) and CYP3A4 (bottom).
Line across 1 indicates where abundance of two pathways reaches adult level and broken line across 0.5 and 2 indicate two fold differences and when the trajectories are distant from one, it means that the activity/abundance of pathway X is higher (above) or lower (below).

The age range where a significant difference was shown between specific pathways for the hypothetical compounds is presented in Figure 3.4-4B and the full comparison between CYP pairs is presented in Figure 3.4-5. For some enzyme pairs such as CYP2C18/19 *vs.* CYP2D6 or CYP1A2 *vs.* CYP2B6 a 95% confidence always included zero by means of bootstrapping. Thus there was no indication for any statistically significant differences at any age range and graphs are not presented.



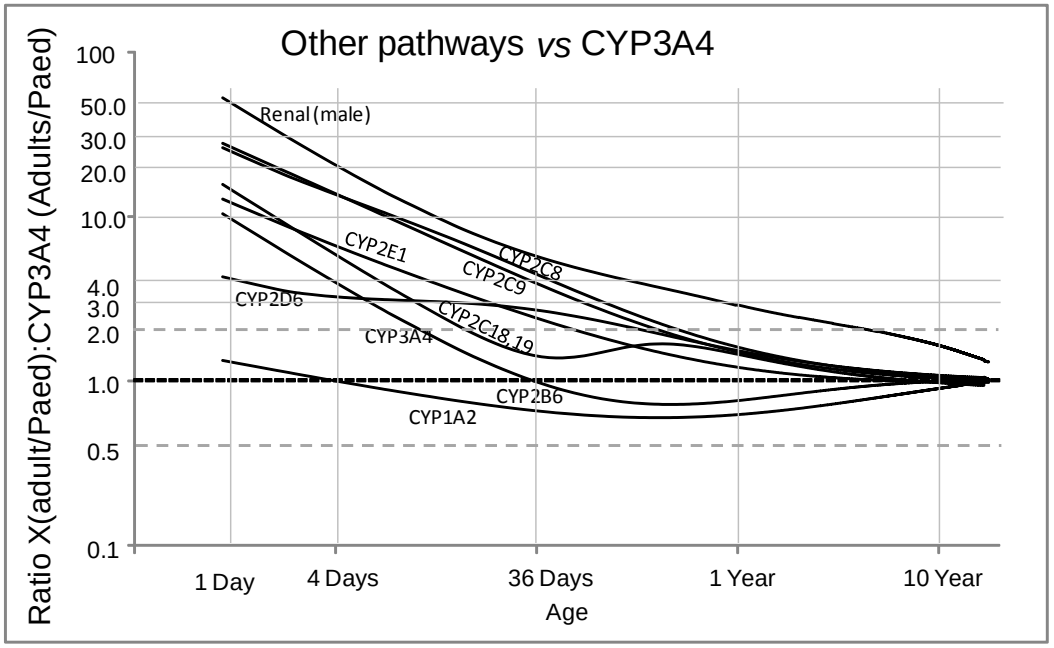


Figure 3.4-3 Variable rates of development for different pathways relative to adults for each CYP2D6 and CYP3A4 vs. other pathways.

Line across 1 indicates where abundance of two pathways reaches adult level and broken line across 0.5 and 2 indicate two fold differences.

Table 3.4.1 Maximum fold difference between in vitro expression/activity of pathways and age at which this happens.

Ratio	CYP1A2 vs	CYP2B6 vs	CYP2E1 vs	CYP2D6 vs	CYP2C8 vs	CYP2C9 vs	CYP2C18/19 vs	Renal vs
CYP3A4	5 Months 0.6	Day 1 10.4	Day 1 12.8	Day 1 4.3	Day 1 26.4	Day 1 27.8	Day 1 15.8	Day 1 52.9
CYP1A2		Day 1 7.8	Day 1 9.6	Day 28 4.1	Day 1 19.9	Day 1 20.9	Day 1 11.9	Day 1 39.7
CYP2B6			Day 42 2.5	Day 70 3.0	Day 21 4.6	Day 21 4.1	Month 5 2.4	Day 50 5.9
CYP2E1				Day 1 3.0	Week 1 2.1	Day 1 2.2	Day 28 1.7	Week 1 4.1
CYP2D6					Day 1 6.1	Day 1 6.4	Day 1 3.7	Day 1 12.3
CYP2C8						Day 35 1.1	Day 21 3.4	Day 1 2.0
CYP2C9							Day 21 2.9	Year 1 2.0
CYP2C18/19								Month 1 4.1

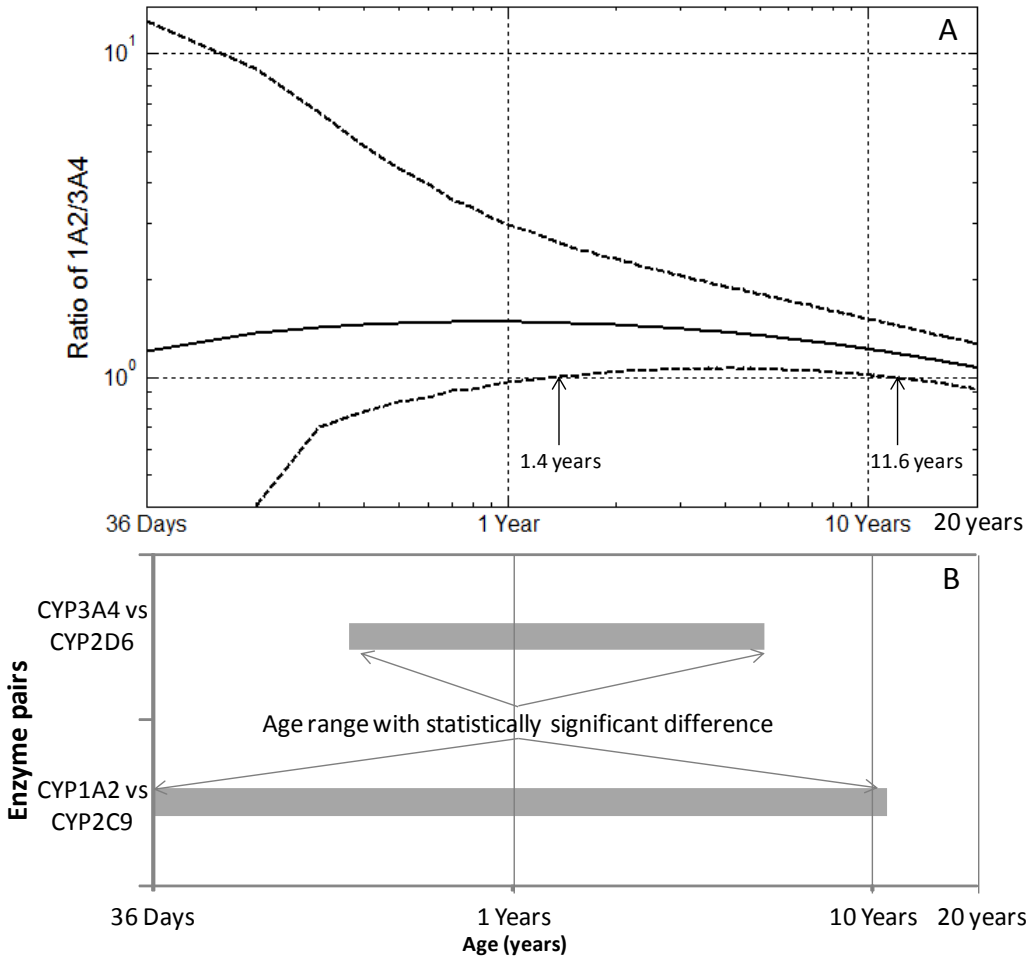


Figure 3.4-4 Median (solid line) and 5th and 95th centile for confidence interval (broken lines) surrounding the ratio of two pathways relative to adults are constructed (see text) for various pairs (e.g. CYP1A2/3A4 in part (A)) and subsequently age ranges at which there is statistically significant disparity between the activity or protein expression of pairs were identified.

If the drug is metabolised via these pairs and a potent perpetrator is introduced, a more serious DDI is expected if the subject is within these age ranges (see two examples for CYP3A4 vs. CYP2D6 and CYP1A2 vs. CYP2C9 in part (B) and the Figure 3.3-5 for the full figure).

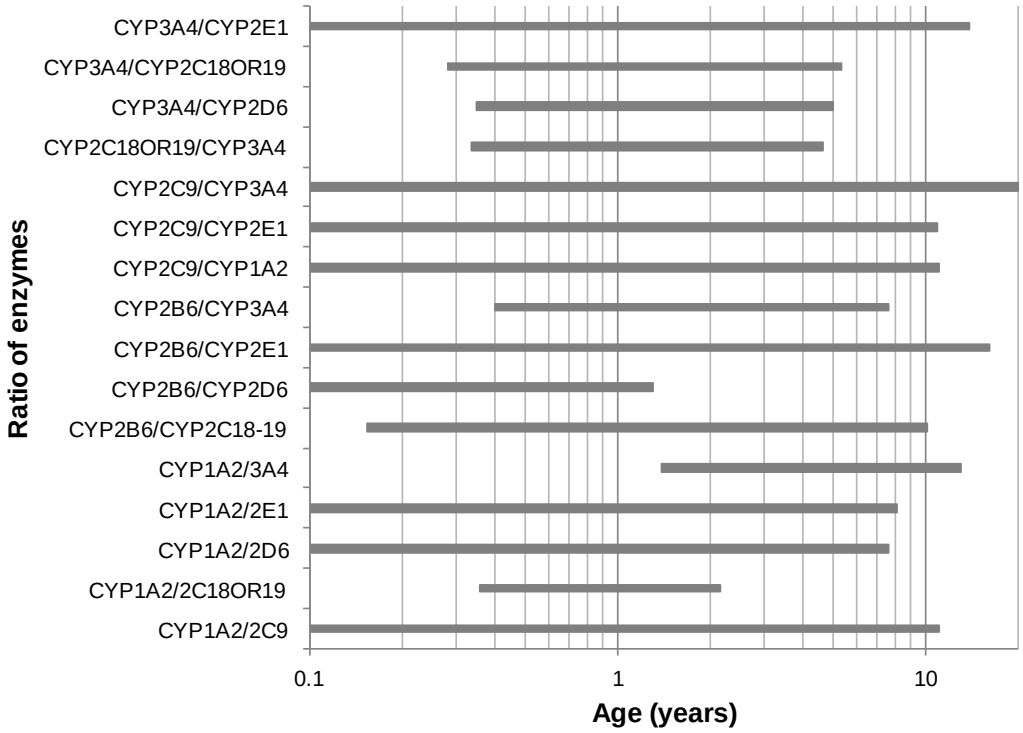


Figure 3.4-5 Age ranges at which there is significant disparity between metabolic pathways for various CYP enzyme combinations.

3.4.2 Age related changes in DDI

The age related changes in fm and median AUC ratio for COMP 1 and COMP 2 are shown in Table 3.4.2. Both theoretical compounds were designed to have approximately 50% fm by each enzyme in neonates. For COMP 1 because the CYP1A2 has a slower ontogeny than CYP2C9, the latter has a much more significant role in neonates compared to adults, consequently when this enzyme is strongly inhibited an interaction is seen in neonates (2.1-fold) but not in adults (1.05-fold). Conversely, for COMP 2, CYP2D6 has a faster ontogeny compared to CYP3A4 and thus when the latter is strongly inhibited by a drug such as ketoconazole, a more severe interaction is seen in adults (4.7-fold) compared to neonates (1.9-fold). These hypothetical interactions demonstrate that a potentially non-significant interaction in adults may cause a more important interaction in neonates and *vice versa*.

Table 3.4.2 Comparison of the level of drug-drug interaction with age for two theoretical drugs metabolised by different CYP enzymes.

	fm% for COMP 1			fm% for COMP 2		
Age	**CYP1A2**	**CYP2C9**	**Median AUC Ratio**	**CYP3A4**	**CYP2D6**	**Median AUC Ratio**
Year 20	93	7	1.05	80	20	4.7
Year 1	86	14	1.17	74	26	3.1
Day 1	47	53	2.1	51	49	1.9

COMP 3 was designed to undergo 50% metabolism by CYP2D6 and CYP3A4 in adults. The fraction metabolized by each enzyme across the age bands studied in the absence and presence of CYP3A4 inhibition by ketoconazole is shown in Figure 3.4-6. The fold AUC ratio values are shown on Figure 3.4-6 (right panel) and ranged from 1.02 in 1 day old neonates up to 1.72-fold in adults, these results are similar to COMP 2 reflect the faster ontogeny of CYP2D6 compared to CYP3A4. Inspecting the results indicated that there is a similar 81% reduction in the fraction of COMP 3 metabolized by CYP3A4 across the age range when the inhibitor is introduced.

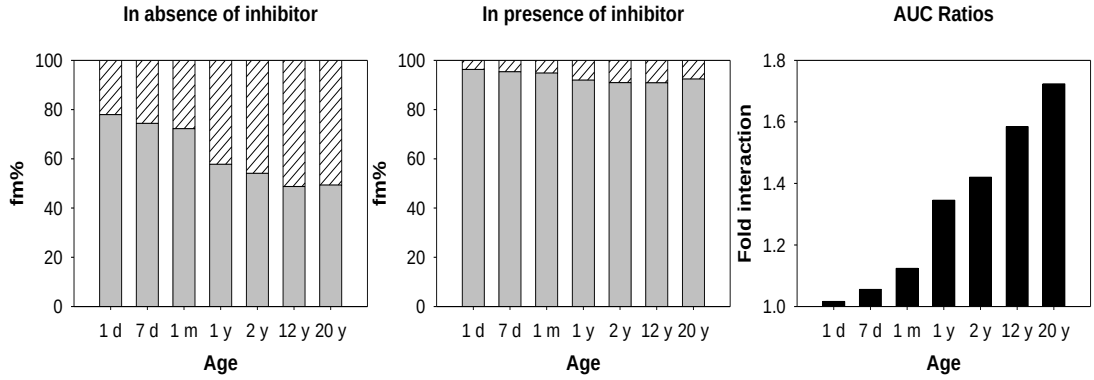


Figure 3.4-6 Changes in fm by CYPs2D6 (grey) and CYP3A4 (hashed) with age for COMP 3 before (left panel) and after (middle panel) inhibition with ketoconazole.

The corresponding fold AUC increases are shown in the right panel.

3.5 Discussion

Many drug elimination pathways e.g. CYP enzymes are detectable in the foetus in the second or third trimester and continue to develop up to a few months after birth (Hines, 2007). Each enzyme or elimination pathway has its own rate and pattern of development and consequently there is an age of maximum discrepancy between pathways and considering the inter-individual variability particularly between term and preterm neonates, there is an age range where there is a significant difference between them. This variability for each pathway is observed from *in vitro* and *in vivo* studies and is simulated by means of statistical methods. The *in vitro* data on activity or expression of enzymes and sometimes the variability around this data is lacking for certain ages. Therefore bootstrapping is used to overcome this limitation. Often the fraction of adult value for individuals is different from the mean trajectory and a variability to account for level of significance will help to identify the significant discrepancy in pathways. An example of this differential ontogeny is the rapid development of CYP2C9 (Koukouritaki et al., 2004) in contrast to the much slower development of CYP1A2 (Sonnier and Cresteil, 1998).The current study has shown a maximum 20.9-fold difference between these pathways at day 1 as well as an age range (0 – 10.5 years) over which there is a significant difference in expression due to the different ontogeny. Many other combinations of CYP enzymes as well as CYP enzymes/ renal function have a maximum discrepancy at 1 day old.

A large number of drugs are metabolized by a number of different pathways and the fraction of dose metabolized by these pathways in average adults may be known. From this information and also knowledge of the potency and dose of a co-administered inhibitor of one of the pathways and genetic data (as Rostami and Tucker showed in 2004 (Rostami-Hodjegan and Tucker, 2004)) it is possible to predict the likely mean level of a DDI in adult subjects and also its variability. However, prediction of the same DDI in the paediatric population requires additional knowledge on the differential ontogeny of the pathways and how this influences the fm values for the drug with age. An example is given in the results where a drug metabolized 50% by CYP3A4 and 50% by CYP2D6 in the neonates suggesting a maximum 2-fold interaction on potent inhibition of CYP3A4 (by ketoconazole) is in fact metabolized 76% by CYP3A4 in adults with a maximum possible 3.2-fold interaction.

Such interactions may be flagged by the DDI software and prevent co-administration of drugs in neonates and infants despite the fact that the risk of a clinically significant interaction is reduced in the latter group, and potentially deprives the young patient of the benefits of antifungal therapy.

In young children, if the major pathway is inhibited and an alternative route of elimination is not matured adequately, the drug may accumulate in system resulting in a more severe interaction compared to adults. However, there is very little information on how DDIs are likely to change with age in the literature. In a recent meta-analysis although a large number of reports of DDIs specifically in children were recovered, no studies were found comparing the magnitude of the same DDI across the paediatric age range, this is not surprising given the ethical and logistical constraints in performing such studies. Currently DDIs are managed in the paediatric population based on predominantly adult data with little consideration of how their magnitude is likely to change with age.

An understanding of how the fm by a specific pathway changes with age could be used clinically to assist in managing DDIs in young children based on data in adults especially where an interaction was found to be borderline in the latter. One way forward in terms of the prediction of DDIs in the paediatric population would be to calculate how the fm values change with age; this would give a visual idea of the likely severity of a DDI moving up or down the age bands. However, this would be very time consuming and requires knowledge on drug and system parameters. A much more logical and user friendly approach is to integrate the available information on patterns of ontogeny and physiological development and also drug data for the drug substrate and inhibitor into paediatric modelling and simulation tools.

A number of substantial studies on the *in vitro* ontogeny of a number of CYP enzymes have been published in the last few years (Tateishi et al., 1997; Hines, 2007; Croom et al., 2009), however, there are still uncertainties around some of the CYP ontogeny profiles (Leong et al., 2012). The different research groups involved in paediatric physiologically based pharmacokinetic (p-PBPK) modelling have generated similar ontogeny profiles for many of the enzymes and there is a growing body of evidence that some of these perform well at predicting PK parameters across the paediatric age range (Bjorkman, 2005; Edginton et al.,

2006b; Johnson et al., 2006; Johnson and Rostami-Hodjegan, 2011). In an evaluation of 'top down' (*in vivo*) vs. 'bottom up' (*in vitro*) ontogeny for CYP2D6 and CYP3A4 it has been demonstrated that there is close agreement between the shapes of the profiles (Johnson et al., 2008). Despite the available data some of the ontogeny profiles are based on relatively sparse *in vitro* data e.g. CYP2C8. More research needs to be undertaken both to evaluate *in vitro* ontogeny data against deconvoluted *in vivo* data from probe substrates used in children and also to fill in some of the gaps.

The p-PBPK models incorporating information on system ontogeny can open a new approach for assessing DDIs in paediatric populations without the need for the conduct of clinical studies. These can help to visualize the effects of interacting drugs in young children and how they may requires a dose adjustment. The objections to using such models (in place of real clinical observations) are based on the presumption that these models are replacing the clinical studies whilst as it has been argued recently (Rostami-Hodjegan, 2012) that they are alternatives to 'guesswork'. Further use and validation of such models will give confidence in dosing commonly used drugs in paediatrics as well as giving an assessment of the likely magnitude of a DDI in this vulnerable population. A recent paper (Leong et al., 2012) on the regulatory experience with p-PBPK modelling for paediatric drug trails highlights their utility especially in relation to the 'learn and confirm' approach utilized in current paediatric drug submissions. They emphasize the need to refine existing p-PBPK models, both as new data on the age dependent processes that govern ADME becomes available and also as more experience is gained through the increased application of the p-PBPK models to existing and new paediatric drugs. All p-PBPK models have to be validated in both adult and paediatric patients before they can be considered useful for paediatric DDI predictions. The CHMP guidelines (CHMP, 2012) recommend that a clinical DDI study should be considered for new drugs in children where they are likely to be used in combination with a potentially interacting second drug. They also suggest that evidence from a paediatric modelling and simulation approach may provide satisfactory supportive data provided the approach is successful in predicting the DDI in adults, and data on enzyme abundance and other physiological parameters in paediatrics are reliable. Likewise the recent FDA guidance (FDA, 2012) when discussing complex DDIs in paediatric and geriatric

subjects suggests that ‘simulations using system biology approaches such as p-PBPK may be helpful to predict drug interaction potential when the model can be constructed based on sufficient *in vitro* and clinical pharmacology and drug interaction data and incorporates development changes’.

3.6 Conclusion

There are a number of limitations in this study including the lack of reliable clinical DDI data in the paediatric population against which to compare the results of simulations in order to validate the models. More research is needed in these areas; however this paper illustrates how differences in ontogeny between elimination pathways may translate into a changing magnitude of DDIs with age for specific drugs. This has implications in the management of drug therapy in paediatric clinical practice but at the same time it is difficult to perform clinical studies for purpose of developing guidance. The use of p-PBPK models may offer a way to integrate our understanding in this area and may help in predicting the likely magnitude of DDIs in children.

Chapter 4. A critical evaluation of key system parameters in a paediatric PBPK model as a follow up to perdition of exposure in adult and paediatric patients following intravenous and oral administration of four commonly used drugs

4.1 Abstract

Accurate prediction of DDIs in children requires that the PBPK model adequately predicts the clearance of drugs in adults. Secondly, the exposure to the substrate and inhibitor and fold interaction should be quantified accurately in adults and then in paediatrics. Performance of Simcyp PBPK model in prediction of clearance for caffeine, theophylline, ibuprofen and midazolam in adults and paediatrics are investigated by simulation of a number of clinical studies. In these simulations the design of clinical studies was mimicked as closely as possible. Simulations were carried out using enzyme kinetics and retrograde options in Simcyp elimination interface. The weighted mean values for the ratio of predicted to observed area under the concentration-time profile were compared. Predicted to observed ratios in adult simulations from two approaches were in good agreement and reasonably close. However, predictions from paediatric studies revealed some discrepancies between predicted and observed values. Except for theophylline the best method of prediction in adults was the *in vitro* enzyme kinetic data. This investigation suggested some parameters within the p-PBPK models such as ontogeny require further investigations. New approaches should be sought to develop novel ontogeny models or correct the current models.

4.2 Introduction

Paediatric physiologically based PBPK (p-PBPK) models are an extension of adult models and allow understanding and evaluation of the factors affecting the absorption, distribution, metabolism and eliminations (ADME) of drugs at different ages.

As discussed in the previous chapter the fold drug interaction seen for a particular drug pair may change with age. However the accurate assessment of drug-drug interactions (DDI) in the paediatric age groups requires both estimation of the exposure to the substrate and perpetrator as well as prediction of the extent of interaction between co-administered drugs.

Hence reducing the uncertainty around both substrate and perpetrator exposure in children requires a thorough understanding of the development of system parameters particularly those factors pertaining to clearance (CL) prediction such as the ontogeny of metabolic enzymes, renal function, liver size, protein binding and liver blood flow. Accurate prediction of CL in children firstly relies on understanding adult CL mechanisms, and this is taken as a starting point for paediatric predictions, if the exposure to the substrate and inhibitor and fold interaction cannot be quantified accurately in adults, then there is little point in trying to predict an interaction in the paediatric population.

Simulation of previously undertaken clinical PK studies for known compounds is a conventional way of examining the performance of a PBPK model. In conducting performance verification studies, the three main factors that contribute to the quality of predictions are: 1) system data, 2) drug data and 3) trial design (Jamei et al., 2009a; Rowland et al., 2011). Successful prediction of PK parameters in adults prior to undertaking predictions in children is paramount and demonstrates that the system data, drug data and structural model in the model are correct. Expanding simulations to paediatrics virtual subjects allows for evaluation of the paediatric system data (e.g. ontogeny profiles) and model assumptions within the paediatric PBPK (p-PBPK) model. Trial design is an important part of simulations in both groups to facilitate the comparison of predicted *vs.* observed PK parameters.

One major PK parameter deriving the level of exposure to a compound and dose adjustment is CL. CL also accounts for the age-dependence of the PK parameters as well as the extent of DDI. There are several examples of previously undertaken validation of p-PBPK models in the literature (Edginton et al., 2006b; Johnson and Rostami-Hodjegan, 2011). These studies apply the available CL data for a number of studies in the literature for verification of CL predictions in paediatric ages. However, thorough evaluation of model requires examining the predictions for a larger number of studies and at various paediatric age groups.

In PBPK models CL from adult systemic CL or if the model is capable of *in vitro–in vivo* extrapolation (IVIVE) the data from *in vitro* experiments is used to predict *in vivo* CL. *In vitro* enzyme kinetic parameters (K_m and V_{max}) are extrapolated to children using knowledge of the factors affecting parameter values from different *in vitro* systems and ontogeny models.

There are several ontogeny models reported in the literature; however, there are discrepancies between these models (Bjorkman, 2006; Edginton et al., 2006a; Johnson et al., 2006). The ontogeny models used in p-PBPK models are based on measurement of *in vitro* activity, protein abundance or mRNA expression of enzyme that are quantified by different techniques such as reverse transcription polymerase reaction (RT-PCR), Western blotting, immunoassay, mass-spectrometry and depletion of probe substrates. Measurement of activity or expression of major CYPs on human liver tissues is carried out using post-mortem samples. These post-mortem samples are supplied from donors of various age groups, ethnicity and background diseases or history of drug. There are also technical issues such as post mortem time before the sampling and preparation of samples for experiments, variations in the methodology and quantification techniques between different laboratories, using a non-specific antibody or substrate for protein or activity measurements, binding to microsomes.

The aim of the current study is to evaluate the performance of paediatric Simcyp in prediction of area under the plasma concentration-time profiles (AUC) for substrates of CYP1A2 (caffeine and theophylline), -2C9 (ibuprofen), and -3A4 (midazolam) that were used for the prediction of DDI in previous chapter when CL is obtained from healthy volunteers or experimental enzyme kinetic data. In this evaluation two CL input options (*in vivo* CL and *in vitro* data for prediction of CL) are compared.

4.3 Methods

4.3.1 Selection of compounds and PK parameters

Compounds in this study are selected based on availability of PK parameters especially AUC in adults and children of various age groups in the literature. The reason for using AUC as an alternative to CL was that the literature data on AUC, particularly in paediatric age groups, was richer compared to other PK parameters. Since PBPK simulations for performance verification were carried out using Simcyp simulator (Simcyp Ltd, Sheffield, UK, http:www.simcyp.com), availability of compounds with pre-defined library within the simulator were considered to facilitate the study. Compounds selected to evaluate the model were probe substrates for major CYP enzymes used in the previous chapter for demonstrating the age related changes in DDI. The following compounds fulfilled these criteria: caffeine and

theophylline for CYP1A2, ibuprofen for CYP2C9 and midazolam for CYP3A4. The *in vivo* probes for CYP2D6 (dextromethorphan and tramadol) are not considered in this study. Dextromethorphan is not usually used in neonates and therefore the relevant PK data is not available and creating the compound file for tramadol was not feasible due to complex metabolism of this compound (see discussion).

4.3.2 Simcyp population-based simulator

Simcyp simulator is an example of PBPK models that provides different options for absorption, distribution and metabolism of drugs. Simcyp combines adult demographic, physiologic and genetic information with *in vitro* data through *IVIVE* by using appropriate models and high quality data and uses Monte Carlo methods (Johnson et al., 2006) and lognormal distribution of parameters assumption to generate the output.

In Simcyp, various options are available within each aspect of ADME that add different levels of complexity and more mechanistic characteristics to the model. For example, options within absorption include first order absorption, compartmental absorption and transit model (CAT) and advanced dissolution, absorption and metabolism model (ADAM). In terms of distribution, there are options of minimal and full PBPK model that account for different number of compartments. Minimal PBPK includes four compartments: small intestine, portal vein, liver and systemic compartment. There are two distribution method for calculating the tissue:plasma partitioning in each organ for the full PBPK including the Rodgers and Rowland or Poulin and Theil method (Jamei et al., 2009a), the former is preferably for basic compound and the latter for neutral or acid compounds. The elimination options include *in vivo* CL, whole organ metabolic CL and enzyme kinetics. Full description of the Simcyp model is provided elsewhere (Jamei et al., 2009a) .

4.3.2.1 Simcyp paediatric simulator

Simcyp paediatric simulator is an extension of adult simulator. In the paediatric simulator, the drug parameters in general remain the same as in adults but physiological (system) parameters change with age by incorporating information on developmental physiology and the ontogeny of major metabolic and elimination pathways (Johnson et al., 2006). The age related changes in system parameters are reflected on PK parameters using appropriate algorithms and scalars.

4.3.3 Input options for metabolic CL within Simcyp simulator

The elimination tab within paediatric Simcyp contains several options for CL. The current study concentrates on CL input options from purely *in vitro* (enzyme kinetic data) and from *in vivo* systemic CL using the retrograde model. The details on each CL input option to Simcyp is explained in the following section;

4.3.3.1 In vitro enzyme kinetics data (rhCYP/HLM, V_{max}, K_m)

In vitro metabolism data on Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) of CYP or UGT enzymes are used for the prediction of AUC through IVIVE. *In vitro* data becomes available in the early phase of drug development but these systems under-predict the CL to various extents for different drugs. IVIVE converts the CL_{int} (µl/min/ρmol) to CL_{int} (µl/min/g of liver) and scales the latter to hepatic metabolic CL ($CL_{H,B}$) using Equation 4.3-1 to Equation 4.3-3 (Johnson and Rostami-Hodjegan, 2011).

$$CL_{int,pe} = \frac{V_{max,pe}}{K_{m,pe}} \qquad \textbf{Equation 4.3-1}$$

where $CL_{int,pe}$ is intrinsic metabolic clearance per enzyme (e) for individual pathways (p), $V_{max,pe}$ is maximum rate of metabolism per enzyme for individual pathways and $K_{m,pe}$ is the Michaelis- Menten constant for individual pathways. $CL_{int,pe}$ is utilised to calculate the total unbound intrinsic hepatic clearance ($CLu_{int,H}$) for each enzymatic pathway ($CLu_{int,H,pe}$) using relevant scaling factors for recombinantly expressed CYP enzymes (rCYP) (Equation 4.3-2) and human liver microsomes (HLM) (Equation 4.3-3) data .

$$CL_{u,int,H,pe} = \frac{ISEF \times CL_{int,pe}}{f_{u,mic,pe}} \times Abundance \times Uptake \times MPPGL \times Liver\,weight \times 60 \times 10^{-6} \qquad \textbf{Equation 4.3-2}$$

$$CL_{u,int,H,pe} = \frac{CL_{int,pe}}{f_{u,mic,pe}} \times Uptake \times MPPGL \times Liver\ weight \times 60 \times 10^{-6} \qquad \textbf{Equation 4.3-3}$$

The data is derived from a meta-analysis of the literature and may include human adult subcellular liver fractions, HLM (µl/min/mg) or rCYP (µl/min/ ρmol). *In vitro* kinetic data (either V_{max} & K_m or CL_{int}) may then be scaled to a rate per whole organ (liver here) and other hepatic scaling factors such as milligram of microsomal protein per gram of liver (MPPGL) and liver size. HLM or rCYP are used in the simulator by applying the relevant scalars to

rCYP including unbound fraction in microsomal preparation (fu_{mic}) and inter system extrapolating factor (ISEF).

To scale the $CL_{u,int,H}$ to paediatrics of different ages additional models for MPPGL and appropriate enzyme ontogeny on activity or abundance for that CYP are applied using CYP ontogeny relationships as reported by (Johnson et al., 2006).

In vitro metabolism data in Simcyp is based on meta-analysis of the literature data. These values are presented in Table 4.3.1.

Table 4.3.1 Input options for K_m and V_{max} within elimination tab in Simcyp V12.

Compound	Enzyme	fm	Pathway	V_{max}	K_m
Caffeine	CYP1A2	0.99	N1-demethylation	0.56	157
			N3-demethylation	13.6	300
			N7-demethylation	0.21	245
			OH	0.36	265
	CYP2E1	0.01	N1-demethylation	0.03	1411
			N7-demethylation	0.02	823
			OH	0.18	1019
	CYP3A4	0.002	OH	1.8	45080
Theophylline	CYP1A2	0.9	N1-demethylation	2.47	1080
			N3-demethylation	6	377
			8-OH	4.11	394
	CYP2D6	0.003	N3-demethylation	1.8	6897
			8-OH	4.68	10709
	CYP2E1	0.1	8-OH	40.78	16855
	CYP3A4	0.001	8-OH	0.4	23393
Ibuprofen	CYP2C9	0.76	2-OH	16.5	35.5
			3-OH	29	42.8
	CYP2C8	0.01	2-hydroxy	7.4	285.6
	UGT1A9	0.004	Pathway1	98.4	219.4
	UGT2B7	0.16	Pathway2	371.7	21.2
	Other UGT	0.04			
Midazolam	CYP3A4	0.86	1-OH	5.23	2.16
			4-OH	5.2	31.8
	CYP3A5	0.09	1-OH	19.7	4.16
			4-OH	4.03	34.8
	UGT1A4	0.05	N-Gluc	445	40.3

4.3.3.2 In vivo systemic or oral CL data proportioned by in vitro enzyme contribution (retrograde model)

In the absence of *in vitro* metabolism data or in the event that the *in vitro* data fails to predict *in vivo* exposure, an alternative metabolic input option is back calculation of intravenous or oral CL to calculate the CL_{int}. This CL_{int} is proportioned by the knowledge of enzyme contribution to metabolism of drug. Systemic CL (CL_{iv}) values determined following intravenous administration of the drug are preferred in this "retrograde" model as no

assumptions on the F_G (fraction escapes the gut wall metabolism) and f_a (fraction of drug absorbed from the gut) of the compound are required (Equation 4.3-4). However, in the absence of qualitative literature data on CL_{iv}, back calculation of CL_{int} from oral CL (CL_{po}) is an alternative Equation 4.3-5.

$$CL_{int,H}(L/h) = \frac{Q_{H,B}(L) * CL_{H,B}(L/h)}{fu_B(Q_h(L) - CL_{H,B}(L/h))}$$ **Equation 4.3-4**

where $Q_{H,B}$ is hepatic blood flow=90.69 (L/h), fu_B is unbound fraction of the drug in blood. The data in these equations are taken from Simcyp library and are shown in Table 4.3.2. For caffeine, as shown in Equation 4.3-5 weighted mean for CL_{po} based on a number of *in vivo* studies is used to calculate the CL_{int} and complete absorption of caffeine from gut is assumed (F_G and f_a =1).

$$CL_{int,H}(L/h) = \frac{CL_{po} * F_G * f_a - CL_R}{fu_B\left(1 + \frac{CL_R}{Q_{H,B}}\right)}$$ **Equation 4.3-5**

In this method the *in vivo* CL_{int} from CL_{iv} or CL_{po} in clinical PK studies in healthy adult subjects was used to predict the paediatric AUC. Hepatic metabolic CL in blood ($CL_{H,B}$) is calculated from CL_{iv} using Equation 4.3-6.

$$CL_{H,B}(L/h) = \frac{CL_{iv}(L/h)}{B:P} - \frac{CL_R(L/h)}{B:P}$$ **Equation 4.3-6**

where CL_{iv} is based on meta-analysis of CL, CL_R is renal clearance and B:P is blood to plasma ratio. These parameter values are taken from Simcyp version 12 libraries.

Table 4.3.2 Parameter values from Simcyp version 12 used in retrograde approach to calculate CL_{int} based on *in vivo* (CL_{iv} or CL_{po}) data.

Compound	CL_{iv} (L/h)	CL_{po} (L/h)	CL_R (L/h)	B:P	fu_B
Caffeine	-	5.57	0	0.98	0.69
Theophylline	3.93	3.5	0.31	0.82	0.5
Ibuprofen	3.43	4	0	0.55	0.02
Midazolam	25.6	96.9	0.14	0.6	0.05

Equation 4.3-7 shows the unit transformation from CL_{int} (L/h) to (µl/min/pmol) for inputting the CL_{int} values in Simcyp elimination tab.

$$CL_{int}(L/min/pmol) = \frac{CL_{int}(L/h) * 1{,}000{,}000}{\text{liver weight}(g) * MPPGL * Enzym\,Abundance * 60}$$ **Equation 4.3-7**

where for in Simcyp library an average male liver weight is 1737 g, MPPGL is 39.79 mg (Barter et al., 2008) and enzyme abundance for CYP1A2= 52, CYP2C9=73, CYP2D6=8 and CYP3A4=137 ρmol.

The total CL_{int} (µL/min/ρmol) is proportioned between the relevant metabolic pathways by the knowledge of enzyme contribution from *in vitro* experiments.

For the compounds that are metabolized via uridine glucuronyl transferase (UGT), enzyme abundance was not available; therefore, CL_{int} was calculated per µl/min/MPPGL Equation 4.3-8:

$$CL_{int}(\mu l/min/MPPGL) = \frac{CL_{int}(L/h) * 1{,}000{,}000}{\text{liver weight}(g) * MPPGL * 60}$$ **Equation 4.3-8**

4.3.4 Identifying of clinical PK studies

A comprehensive literature search was carried out to identify the studies that report AUC or plasma concentration-time profile in paediatric group aged from birth to 20 years and for adults. Various sources such as Pubmed, Scopus, Medline and ISI Web of Knowledge were searched using the relevant combination of keywords “drug name (i.e. caffeine, theophylline, ibuprofen or midazolam)” “pharmacokinetic” or “disposition” or “clearance” or “biotransformation” plus age group, for example, “paediatric” or “neonate” or “infant” or “children” or “healthy volunteers” or “subjects”. Article titles and abstracts were screened to maintain the focus of the search upon PK in paediatric or healthy adult subjects. References in all retrieved reports were scrutinised for further sources of published data. Only data from intravenous and oral routes of administration were included. The adult studies were excluded if the systemic CL reported was used to calculate the weighted mean of CL in Simcyp library.

4.3.5 PBPK Simulations

Simulations were carried out in adults and paediatrics by inputting the CL_{int} from ***in vivo*** and from ***in vitro*** into the Simcyp elimination tab as explained above. Simulations trials were designed as closely as possible to the design of the clinical studies. The information in materials and method sections of the papers on the number of subjects, age, proportion of

females and information on drug administration including drug dose, route of administration, fasted or fed condition and duration of study were used to design the trial in Simcyp. Simulations were carried out with 100 subjects in each simulation.

4.3.6 Data Analysis

The Graphpad Prism 5 and GetData Graph Digitalizer 2.24 were used to obtain the $AUC_{0\text{-}inf}$ from the concentration-time profile presented in the articles if the AUC values were not originally reported in the papers. Predicted and observed AUCs were compared using Equation 4.3-9. Weighted mean and pooled standard deviation of the predicted to observed ratios ($R_{pred:obs}$) were used to identify the most predictive CL option based on the closeness to one.

$$R_{pred:obs} = \frac{AUC_{pred}}{AUC_{obs}} \qquad \textbf{Equation 4.3-9}$$

where AUC_{pred} is the AUC predicted from PBPK simulations and AUC_{obs} is the observed AUC reported from the PK study. The two-fold area (0.5 to 2) on the graphs was used to compare the ratios.

4.4 Results

4.4.1 Adults simulations

Figure 4.4-1 shows the $R_{pred:obs}$ values, weighted mean and pooled standard deviation for a number of studies in adults. The investigated compounds include caffeine (Newton et al., 1981; Blanchard and Sawers, 1983; Randinitis et al., 2001; Kamimori et al., 2002; Culm-Merdek et al., 2005), theophylline (Bowles et al., 1988; Oosterhuis et al., 1992; Pieniaszek et al., 1993; Haruta et al., 2002; Boot et al., 2008), ibuprofen (Evans et al., 1990; Smith et al., 1994; Fornasini et al., 1997; Stangier et al., 2000; Troconiz et al., 2000) and midazolam (Olkkola et al., 1994; Lam et al., 2003; Kokudai et al., 2009; Wermeling et al., 2009). Overall, weighted mean of predictions from purely enzyme kinetic *in vitro* data are marginally closer to one compared to the predictions from *in vivo* based CL_{int} from systemic CL proportioned by *in vitro* data. The exception is theophylline where the predictions are based on Simcyp SV-theophylline. A disparity is observed between the weighted mean predicted to observed

AUC ratios between two CL sources. Predicted *vs*. observed plasma concentration times for these compounds in adults are presented in Supplemental Figure S 1 to Supplemental Figure S 4 in appendix 3.

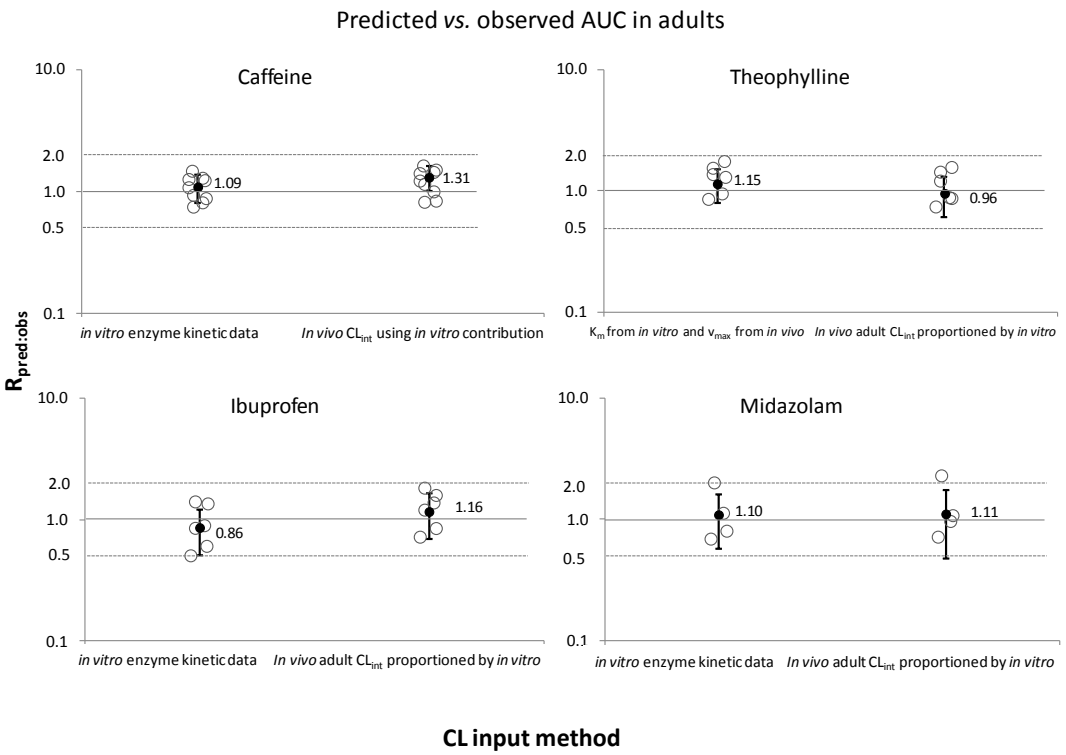


Figure 4.4-1 Comparison of AUC predicted *vs.* observed ratios in adults when the CL input into Simcyp is from purely *in vitro* enzyme kinetic data (i.e. K_m and V_{max}) and when the CL is from *in vivo*- based CL_{int} and *in vitro* enzyme contribution is used to proportion this CL_{int} between metabolic pathways.
In the latter method, CL_{int} is back calculated from CL_{iv} of theophylline, ibuprofen and midazolam and CL_{po} of caffeine. The open circles are the ratio of predicted to observed ratios for individual studies and closed circles are the weighted mean of the ratios. Error bars indicate the pooled standard deviation around the weighted mean. Upper and lower dotted lines indicate 2-fold prediction boundaries.

4.4.2 Paediatrics simulations

Figure 4.4-2 shows the paediatric ratio of predicted to observed AUCs for caffeine, theophylline, ibuprofen and midazolam from purely *in vitro* enzyme kinetic parameters k_m and V_{max} in Simcyp paediatric simulator. The results for retrograde method are presented in Figure 4.4-3. Predicted vs. observed plasma concentration times for these compounds in paediatrics are presented in Figure 4.4-4 to Figure 4.4-7.

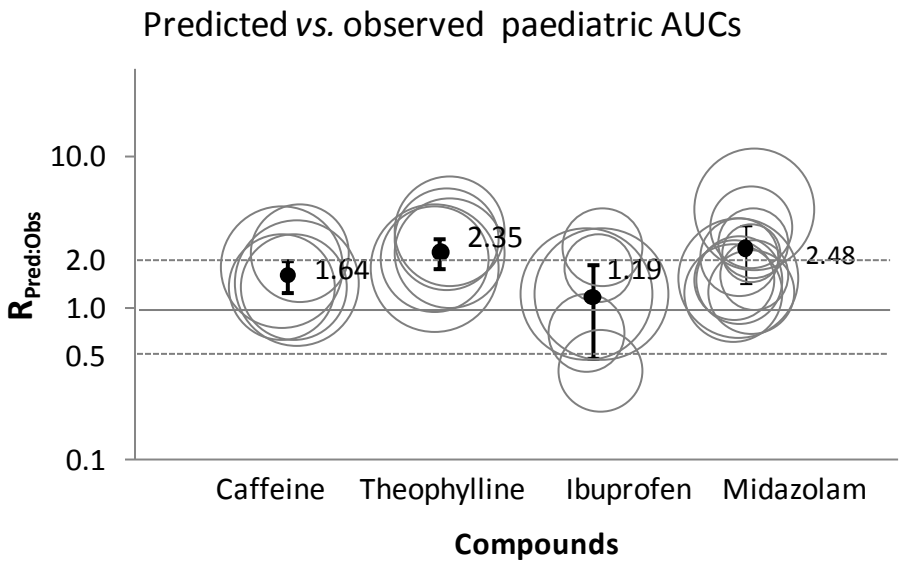


Figure 4.4-2 Ratio of predicted to observed paediatric AUCs from the purely *in vitro* CL input option.
The open circles are the ratio of predicted to observed data and the size of the circles reflects the study size. Closed circles are the weighted mean of the ratios. Error bars indicate the pooled standard deviation around the weighted mean. If the predicted and observed values are similar, the circles are close to the solid line, the upper dotted line indicates the two fold difference from one.

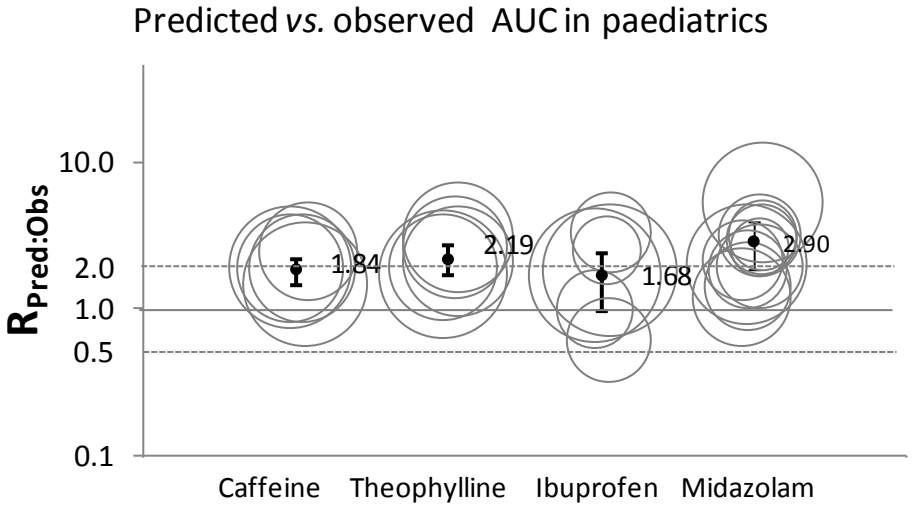


Figure 4.4-3 Ratio of predicted to observed paediatric AUCs from the *in vivo* systemic CL proportioned by in vitro data input option.
The open circles are the ratio of predicted to observed data and the size of the circles reflects the study size. Closed circles are the weighted mean of the ratios. Error bars indicate the pooled standard deviation around the weighted mean. If the predicted and observed values are similar, the circles are close to the solid line, the upper dotted line indicates the two fold difference from one.

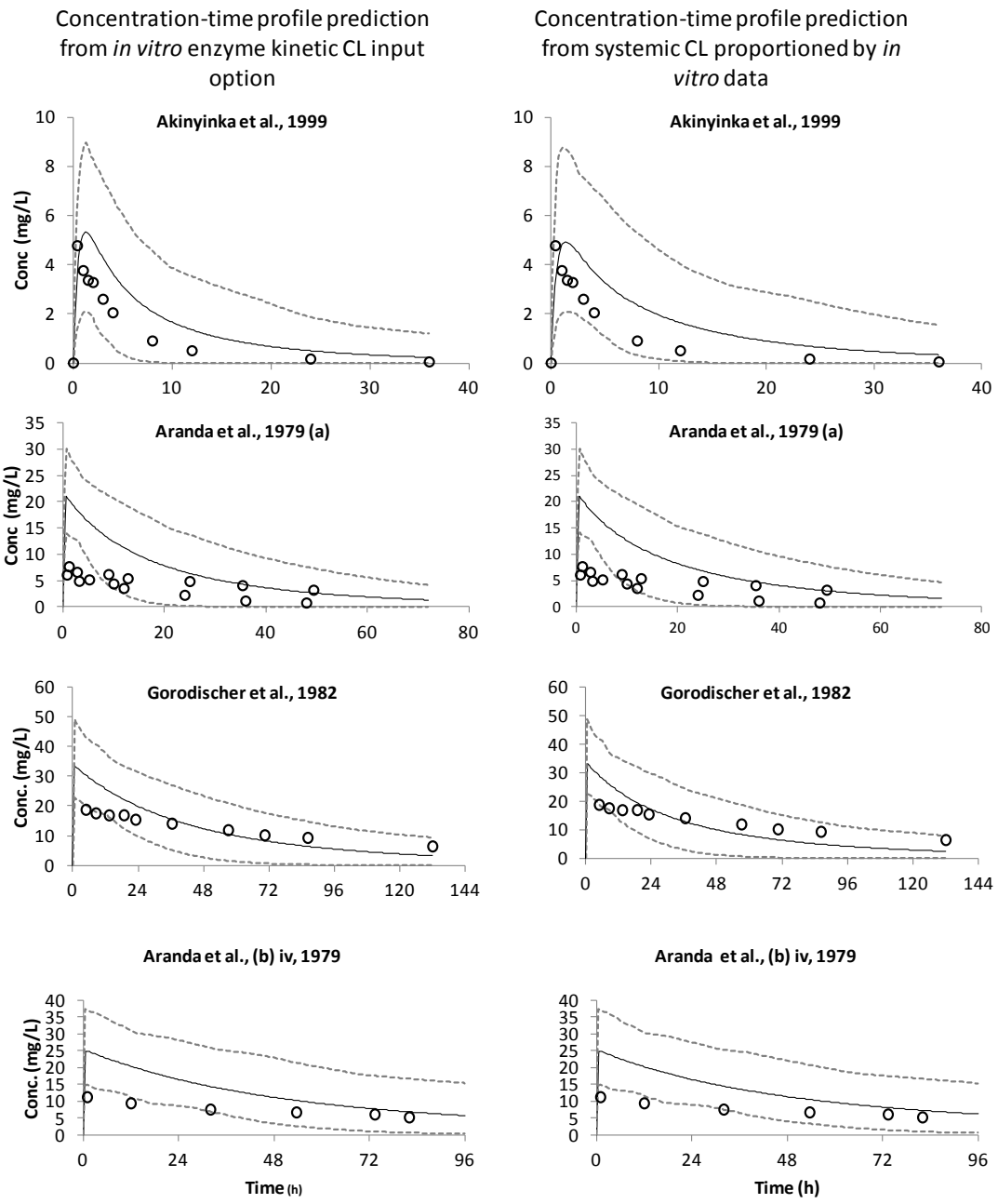


Figure 4.4-4 Comparison of predicted and observed plasma concentration-time profile for caffeine in children. Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.

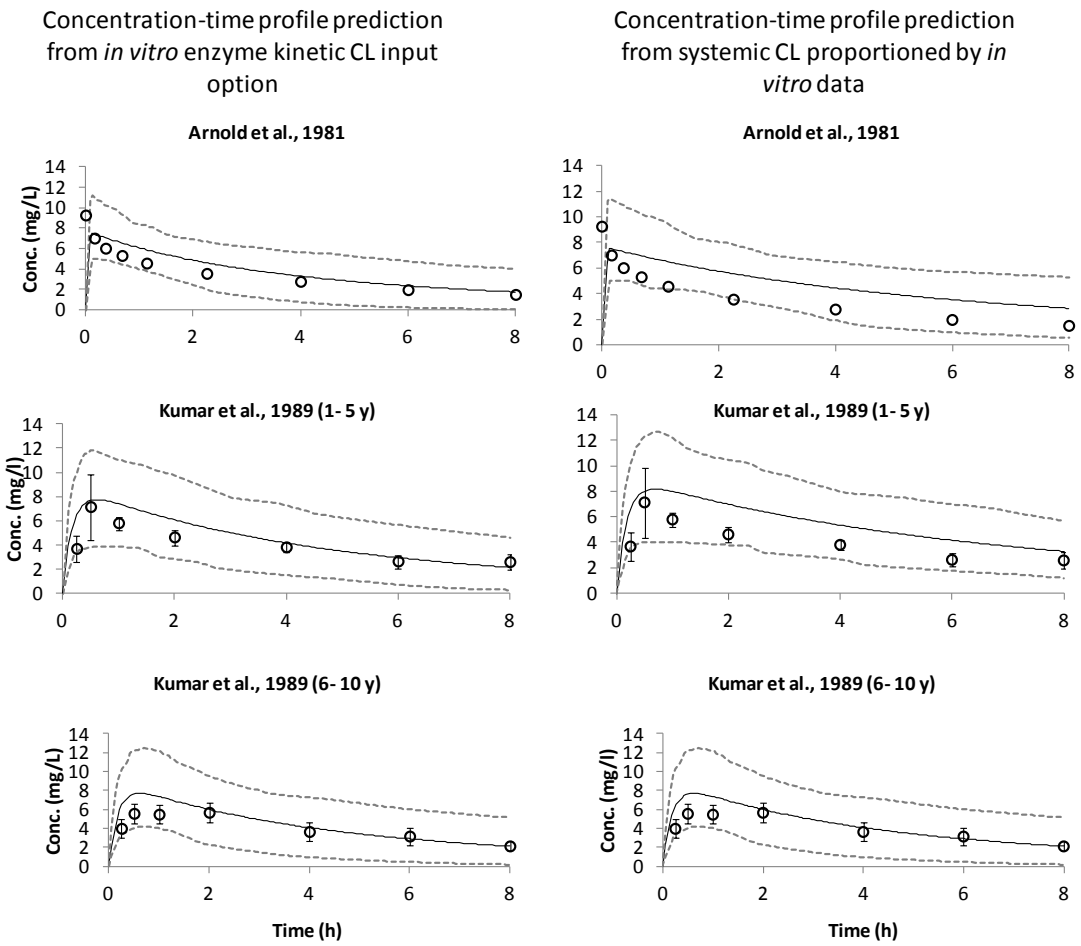


Figure 4.4-5 Comparison of predicted and observed plasma concentration-time profile for theophylline in children.
Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.

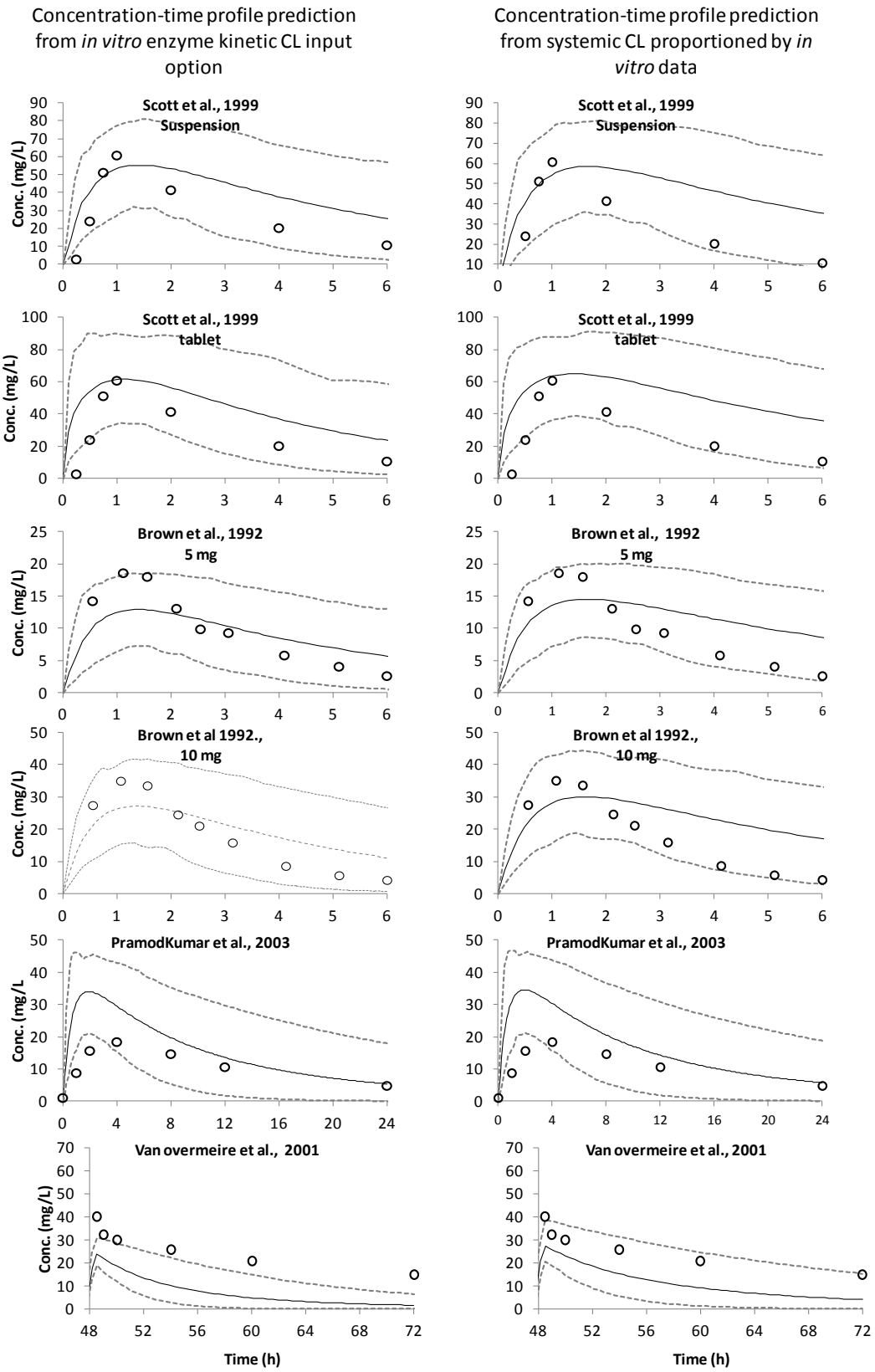


Figure 4.4-6 Comparison of predicted and observed plasma concentration-time profile for ibuprofen in children.

Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.

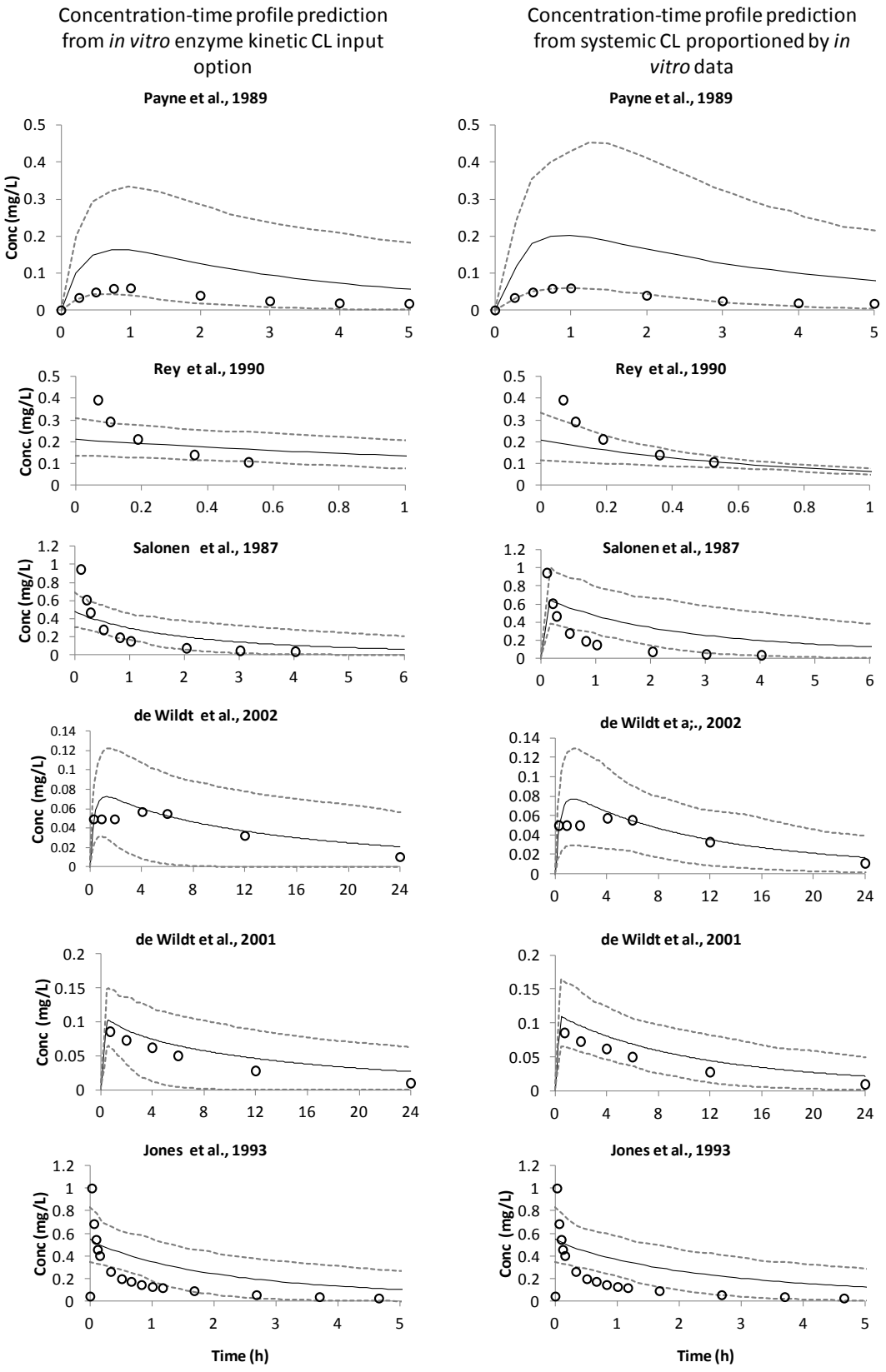


Figure 4.4-7 Comparison of predicted and observed plasma concentration-time profile for midazolam in children.
Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.

4.4.3 CYP1A2 substrates

A total of four studies (5 age groups) in premature neonates, infants and children for caffeine (Aranda et al., 1979a; Aranda et al., 1979b; Gorodischer and Karplus, 1982; Akinyinka et al., 2000) and three studies (5 age groups) for theophylline (Arnold et al., 1981; Lee and Ngiam, 1983; Kumar et al., 1989) were identified from the literature. These studies were used to compare the AUC predictions with observed AUC. Figure 4.4-8 presents the ratio of predicted AUC to observed AUC ($R_{pred:obs}$) with age in paediatric groups and illustrates the age related trend in these ratios. Both CYP1A2 probes reflect an over-prediction in AUC value due to an under-prediction in CL.

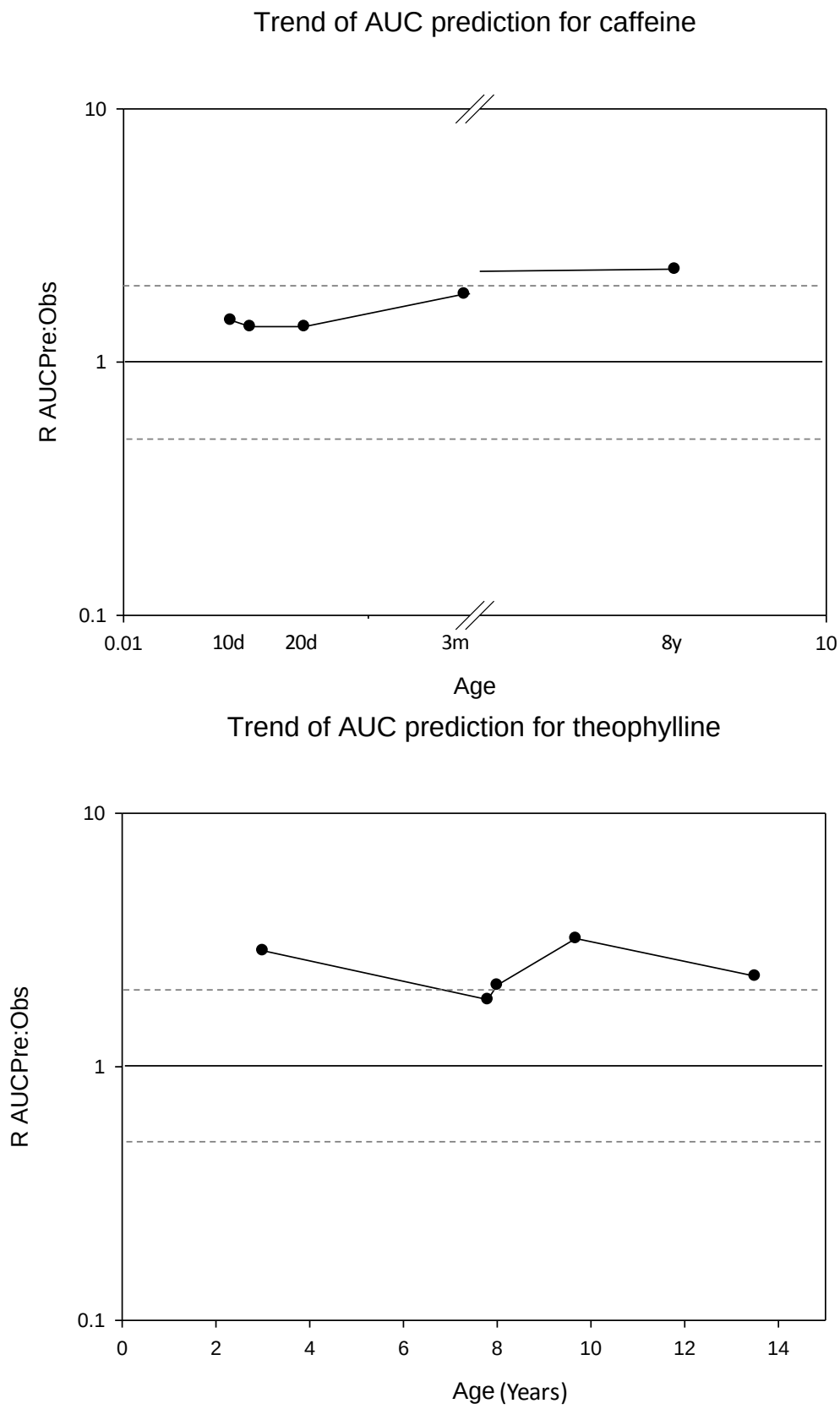


Figure 4.4-8 Ratio of predicted to observed AUC ($R_{pred:obs}$) for caffeine (top) and theophylline (bottom) with age.

Closed circles are the weighted mean of the ratios from several studies. Solid line indicates where the predicted and observed values are similar and broken lines indicate the two fold difference for $R_{pred:obs}$.

4.4.4 CYP2C9 substrate

A total of six paediatric studies (5 age groups) on ibuprofen were identified from the literature review (Walson et al., 1989; Aranda et al., 1997; Scott et al., 1999; Van Overmeire et al., 2001; Sharma et al., 2003; Brown et al., 2005). Figure 4.4-9 presents possible under-prediction of AUCs in neonates and over-prediction of this parameter in children for ibuprofen.

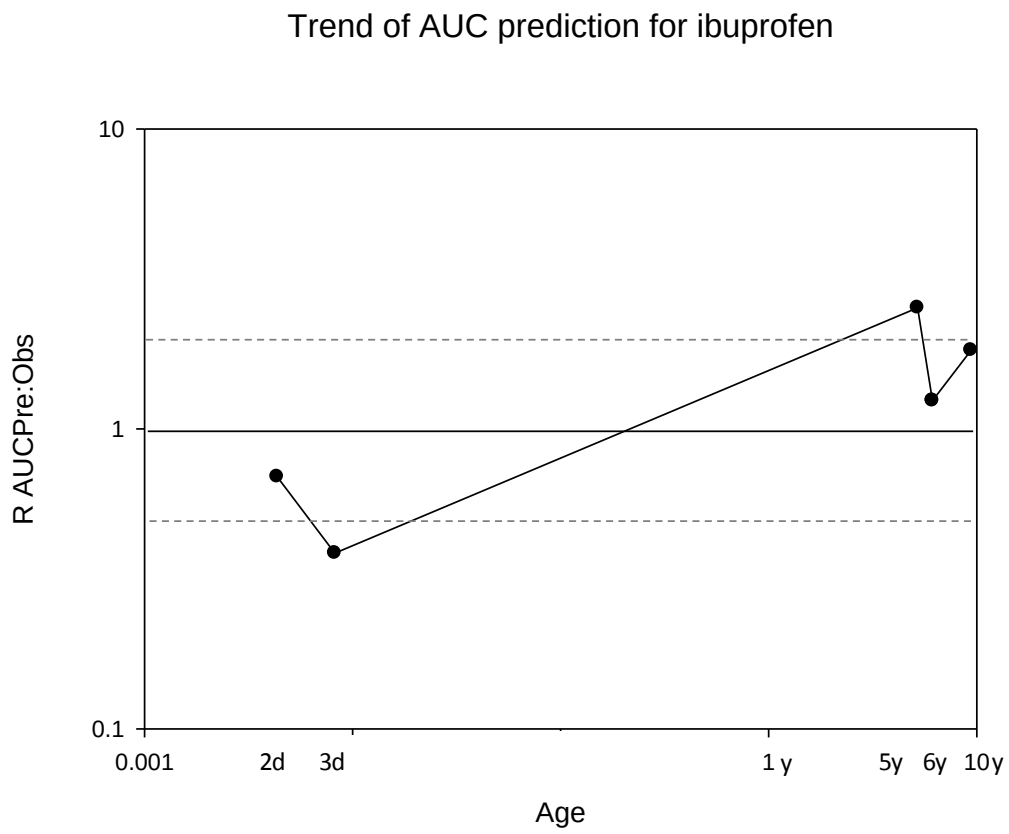


Figure 4.4-9 Ratio of predicted AUC to observed AUC ($R_{pred:obs}$) for ibuprofen with age.
Closed circles are the weighted mean of the ratios and solid line indicates where the predicted and observed values are similar. Broken lines indicate the two fold over and under-prediction.

4.4.5 CYP3A4 substrate

A total of 10 paediatric studies (11 age groups) were selected from the literature (Salonen et al., 1987; Payne et al., 1989; Jacqz-Aigrain et al., 1990; Rey et al., 1991; Jones et al., 1993; Lee et al., 1999; de Wildt et al., 2001; Reed et al., 2001; de Wildt et al., 2002; Muchohi et al., 2008). Figure 4.4-10 presents the variability in over-prediction of AUC (under-prediction of CL) for midazolam in paediatric groups.

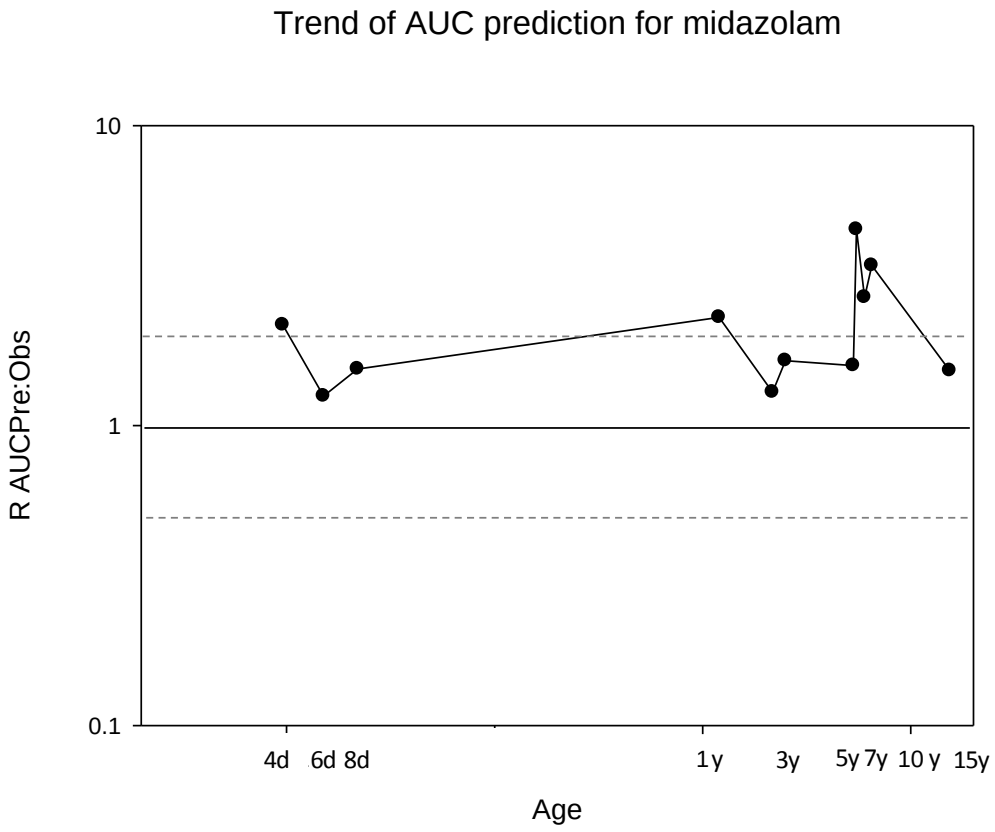


Figure 4.4-10 Ratio of predicted AUC to observed AUC ($R_{pred:obs}$) for midazolam with age.
Closed circles are the weighted mean of the ratios and solid line indicates where the predicted and observed values are similar. Broken lines indicate the two fold over and under-prediction.

4.5 Discussion

The ontogeny of metabolic pathways has a strong influence on the predicted DDI where a drug in metabolised by more than one enzyme. Enzyme ontogeny in most PBPK models is based on *in vitro* data; however, there are uncertainties around these models.

It is important to evaluate the performance of p-PBPK models for several compounds to provide evidence regarding the robustness of the ontogeny profiles the confidence that can be placed on the absolute results from Chapter 2. However, even after allowing for disparities between predicted and observed values; the principles of altered DDIs with age and the reasons why they change with ontogeny will still apply.

In this chapter, the paediatric data for four probe compounds covering three key enzymes allows us to show the performance of Simcyp paediatric in the prediction of AUC with specific consideration of ontogeny for one metabolic pathway.

Using different CL options, predicated AUCs in adults were generally within two fold for individual studies and very close to one for the overall weighted mean values regardless of

CL method used (range 0.86 to 1.15 for enzyme kinetic and IVIVE and 0.96 to 1.31 for systemic CL and back calculation) in three out of four cases; however, there is a marginal difference between the weighted mean of AUCs between two methods with the most predictive CL option is purely enzyme kinetic in adults. Further investigations suggest that the observed difference between weighted mean of two methods is due the log normal distribution of parameters being used to propagate the variability in the parameters used in the p-PBPK model and therefore the CL values are skewed. The back calculation of systemic CL method suggests that a slight difference exist between the starting CL and the predicted mean. This difference is more emphasised if the variability around the enzyme abundance is considered.

Predictions for $R_{pred:obs}$ from SV-theophylline were closer to one from *in vivo* source. The data in enzyme kinetic section of this compound is based on a combination of *in vitro* (k_m) and *in vivo* (V_{max}) data. Within Simcyp simulator, these compounds are called sim-*vivo* files. For this drug, ratio of predicted and observed AUC for CL_{int} from systemic CL is closer to one and therefore the *in vivo*- based method is a better predictor of theophylline exposure.

Simulations were extended to paediatric age groups using the most predictive CL input option, *in vitro* enzyme kinetics (and *in vivo* CL_{int} theophylline).

Reasonable prediction of adult AUCs and disparity between predicted and observed AUCs in paediatric subjects may suggest that some of the paediatric model system parameters may need re-visiting. Certain system parameters within the p-PBPK model are based on robust clinical data e.g. liver size, protein binding and cardiac output; however, other parameters such as enzyme ontogeny are often based on relatively sparse *in vitro* data. The trend of $R_{pred:obs}$ changes with age in these compounds shows that the ontogeny profiles within the model need further investigation.

The systematic departure and constant over-prediction of AUC for caffeine and theophylline and midazolam suggests that these ontogeny models may be resulting in the under-prediction of CL. The extent of AUC over-prediction for ibuprofen is not significant compared to CYP1A2 and -3A4 and due to limited number of CYP2C9 probes, complex PK of phenytoin and less frequent administration of CYP2C9 probes in neonates, further

investigation of CYP2C9 is not considered in this thesis. CYP2D6 ontogeny has similar confinements as CYP2C9 however, the *in vivo* activity data from Blake *et al.,* (Blake et al., 2007) and the clarification by Johnson *et al.,* demonstrated the validity of CYP2D6 ontogeny model (Johnson et al., 2008) and the agreement between *in vitro* and *in vivo* data for CYP2D6.

We also identified deviation of AUC ratios from the observed data when using systemic CL data as the starting point. This observation was investigated in Simcyp simulator and could be due to the assumption of log normal distribution within the simulator.

PBPK models are useful tools that have the potential in making clinical decisions, save unnecessary clinical trials and drug development expenses. These models will ideally find applications in clinical practice for dose adjustment and DDI predictions. Since p-PBPK models are an extension of adult PBPK models and use covariates such as age, body weight and body surface area, the reliability of predictions from these models relies on the accurate prediction of parameters in adult simulators. Therefore, validation of both adult and paediatric PBPK models is necessary prior to apply them in clinic.

In comparison of the predicted and observed parameters the limitations in observed clinical data should also be noted. Paediatric clinical studies usually have a small size, sampling is not rich due to ethical constrains and therefore the C_{max} could be easily missed. Demographic data are usually not available in full details and make the comparison between predicted and observed data difficult.

When comparing the outcome of simulations *vs.* observed data, the trial design of the simulations should be matched as closely as possible to the clinical design and a few points should be noted in interpreting the prediction results:

- The number of subjects in the observed study, the variability around the parameter
- Analytical methods in quantification of drug concentration
- Outliers in the observed data
- Genetic differences

- Difference in PK of drug isomers. For example R and S omeprazole have different PK but the active form is only the S form.
- Special conditions such as prematurity of neonates or critical illnesses

4.6 Conclusion

In summary, for adult simulations performance of two methods for the prediction of CL was reasonable but the *in vitro* enzyme kinetic option was marginally better for caffeine, ibuprofen and midazolam. This investigation suggested some parameters within the p-PBPK models such as ontogeny functions require further investigations as these play a substantial role in the prediction of PK parameters. New approaches should be sought to develop novel ontogeny models or improve the current models.

Chapter 5. A re-evaluation and validation of ontogeny functions for CYPs 1A2 and 3A4 based on *in vivo* data

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Clinical Pharmacokinetics, 2014; available online

Declaration

This chapter constitutes a published article;

"A re-evaluation and validation of ontogeny functions for CYPs 1A2 and 3A4 based on in vivo data"

Submitted to Clinical Pharmacokinetics

F. Salem: Lead of study design, literature search and data collection, statistical meta-analysis, modelling and simulation and preparation of manuscript

T.N. Johnson: Supervision of research and data analysis, input on drug metabolism and detailed editing of manuscript

K. Abduljalil: Expert opinion by performing NONMEM analysis as to considering critical illness as a covariant in analysis or not

G.T. Tucker: Expert opinion on drug metabolism and detailed editing of manuscript

A. Rostami-Hodjegan: Supervision of research, input on modelling and editing of manuscript

Supplemental material of the paper appears in the main text here to facilitate reading.

5.1 Abstract

Current CYP1A2 and CYP3A ontogeny profiles, which are derived mainly from *in vitro* studies and incorporated in paediatric physiologically-based pharmacokinetic models, have been reported to under-predict the *in vivo* clearances of some model substrates in neonates and infants. We report ontogeny functions for these enzymes, as paediatric to adult relative intrinsic clearance per mg of hepatic microsomal protein, based on the deconvolution of *in vivo* kinetic data for caffeine and theophylline as markers of CYP1A2 activity and for

midazolam as a marker of CYP3A4 activity. The function for CYP1A2 describes an increase in relative intrinsic metabolic clearance from birth to 3 years followed by a decrease to adult values. The function for CYP3A4 describes a continuous rise in relative intrinsic metabolic clearance, reaching the adult value at about 1.3 years of age. The new models were validated by showing improved predictions of the systemic clearances of alfentanil (major CYP3A4 substrate) and ropivacaine (major CYP1A2 substrate; minor CYP3A4 substrate) compared to those using a previous ontogeny function based on *in vitro* data (alfentanil: mean squared prediction error 3.0 *vs.* 6.8; ropivacaine: mean squared prediction error 2.3 *vs.*14.1). When implementing enzyme ontogeny functions it is important to consider potential confounding factors related to disease, anaesthesia and surgery that may affect the prediction of net *in vivo* clearance.

5.2 Introduction

Despite an increasing acceptance of the use of physiologically-based pharmacokinetic (PBPK) modelling in paediatric drug development (Barrett et al., 2012; Leong et al., 2012; Huang et al., 2013), uncertainties remain around some of the system parameters. Many of the algorithms describing system parameters relevant in the prediction of total systemic clearance based on plasma drug concentration (CL), (liver volume, liver blood flow, protein binding and renal function are based on large datasets and validated against external data (Johnson et al., 2006). However, information on the ontogeny of some metabolic enzymes is based on relatively sparse in vitro data. In evaluating current ontogeny models for both CYP1A2 and CYP3A4, Leong *et al.* (2012) reported under-prediction of observed clearance values, particularly in neonates and infants(Leong et al., 2012). In some of the cases, this might be related to discordance between the demographic characteristics of the simulated population and those of the actual subjects. However, the overall trend observed by Leong *et al* highlighted important issues in the elucidation of ontogeny functions in paediatric PBPK models. The information used to construct published paediatric PBPK models is derived from enzyme expression and activity data generated from *in vitro* studies (Bjorkman, 2005; Johnson et al., 2006) or a combination of such data with *in vivo* data (Edginton et al., 2006a).

As the mathematical formulae used to describe ontogeny functions have not been explicit in some of these publications, no direct comparisons have yet been made.

Combining the advantages of 'bottom-up' PBPK models, that rely heavily on prior systems information and *in vitro* data, with 'top-down' population pharmacokinetic models, that analyse observed clinical data, has been discussed recently (Tsamandouras et al., 2013). Hence, in this study, a novel approach was applied involving the creation of enzyme ontogeny models by deconvolution of *in vivo* drug clearance values across the paediatric age range. Deconvolution in this context involves removing the complicating effects of system parameters unrelated to enzyme function (e.g. body size, liver weight, microsomal protein per gram of liver (MPPGL), liver blood flow and plasma protein binding) from the *in vivo* clearance value in order to generate a value for hepatic unbound intrinsic clearance (CLuint,H) which represents enzyme activity per mg of microsomal protein. Although changes in the systemic clearance of a number of drugs in pre-term neonates to adults have been modelled previously (Levitsky et al., 1989; de Wildt et al., 2001; Anderson and Holford, 2009; Anderson and Holford, 2011; Anderson and Larsson, 2011), these models were specific for particular compounds Hence, they could not necessarily be applied to other drugs, especially where multiple metabolic pathways are involved with different proportional importance (e.g. paracetamol) or where the sensitivity of systemic clearance to age - related changes in organ blood flow or plasma binding is different.

CYP1A2 is primarily responsible for the metabolism of caffeine and theophylline and metabolism by CYP3A4 is the main determinant of midazolam clearance. Thus, age - related changes in the elimination of these compounds should reflect the ontogeny of CYP1A2 and CYP3A4 when allowances for changes in organ size, blood flows, plasma binding and physical condition or disease are made.

The aims of this study were to:

- Compare the performance of three published ontogeny profiles for both CYPs 1A2 and 3A (Bjorkman (Bjorkman, 2005), Johnson (Johnson et al., 2006) and Edginton (Edginton et al., 2006a) in recovering *in vivo* data.
- Derive novel ontogeny functions for CYP1A2 and CYP3A (µl/min per mg of microsomal protein) based on retrograde calculation of unbound intrinsic hepatic

clearances of caffeine, theophylline and midazolam from their observed *in vivo* systemic clearances.

- Evaluate the performance of the optimized ontogeny profiles for CYPs 1A2 and 3A in recovering the systemic clearances of theophylline and midazolam across the paediatric age range (model verification), and in predicting the clearances of ropivacaine (mostly metabolised by CYP1A2) and alfentanil (mostly metabolised by CYP3A4) (model validation).

5.3 Methods

5.3.1 Comparison of Existing CYP1A2 and CYP3A4 Ontogeny Models

Johnson *et al*, (Johnson et al., 2006) provided the mathematical formulae describing the ontogeny function in their publication. Edginton *et al* (Edginton et al., 2006a) and Bjorkman (Bjorkman, 2005) provided ontogeny values at given ages without explicit indication of the model and model parameters. The spline curve describing the model of Edginton *et al* (Edginton et al., 2006a) was obtained by contacting the authors (Andrea Edginton, personal communication). Bjorkman referred to the use of biexponential functions for his ontogeny model. Therefore, the ontogeny values in this report were fitted by such functions and the model parameter values were derived to describe the model used by the author. The three ontogeny models (Bjorkman (2005), Johnson (Johnson et al., 2006) and Edginton (Edginton et al., 2006a)) for CYP1A2 and CYP3A4 were compared with respect to description of enzyme activity in paediatric subjects relative to that in adults.

It should be noted that ontogeny data for all three models were based on measurements of protein expression (Western blotting) and activity (*in vitro* probes)) in human liver microsomes from reportedly histologically normal post-mortem samples (Lacroix et al., 1997; Tateishi et al., 1997; Sonnier and Cresteil, 1998; Stevens et al., 2003; Treluyer et al., 2003; Hines, 2007). However, Edginton et al., commented that their initial ontogeny models, developed based on *in vitro* data, were supplemented using observed (*in vivo*) clearance values of probe substrates, to arrive at the spline functions used to describe the ontogeny profiles (Edginton et al., 2006a).

5.3.2 Predictive Performance of Published Ontogeny Models

The ontogeny profiles for CYPs 1A2 and 3A4 from the three 3 published sources (Bjorkman, 2005; Edginton et al., 2006a; Johnson et al., 2006) were incorporated into the model population library within the Simcyp Paediatric Simulator (v12) and the outcomes of predicting observed clearances of theophylline and midazolam were compared. In the midazolam compound file in Simcyp (Sim-Midazolam), the value of systemic clearance was updated from 25.6 L/h (Simcyp V12) to 29.1 L/h, based on meta-analysis of adult data. For each ontogeny model, 1000 virtual paediatric subjects were split into sets of 250 subjects representing neonates (birth to 1 month (0.0027 - 0.083 years)), infants (1 month to two years), children (2.01 - 12 years) and adolescents (12.01 - 18 years), with equal proportions of males and females. Predictions of clearance were compared against observed data gathered by a literature survey as described below.

5.3.3 Construction of Ontogeny Models from *In Vivo* Data

5.3.3.1 Literature search

The literature was searched for studies reporting systemic clearance values for midazolam, caffeine and theophylline in adults and paediatric subjects. The clearances of caffeine were obtained from studies involving oral administration (with the assumption of 100% bioavailability), of theophylline from intravenous and oral studies (with the assumption of 100% bioavailability), and those of midazolam from intravenous data only. Sources of data included Pubmed, Scopus, Medline and the ISI Web of Knowledge. Key words, in addition to the drug names, were “clearance”, “disposition and “pharmacokinetic” in all combinations with “paediatric”, “neonate, “infant”, “children“, adults” or “subject”. All of the articles retrieved were screened for relevance and reference to other relevant articles. In particular, information on body weight per kilogram, postnatal age (PNA) per year and/or post-menstrual age (PMA) per weeks and numbers of subjects was extracted. Studies reporting data on preterm neonates included in the analysis; however, studies were excluded if patient management included extracorporeal membrane oxygenation, if the data were obtained in non-Caucasians and if the subjects received co-medication known to change the metabolic clearance of the drugs significantly.

5.3.3.2 Retrograde calculation of unbound intrinsic metabolic clearance from in vivo data

The steps for calculating unbound intrinsic metabolic clearance values are shown in Figure 5.3-1 and Figure 5.3-2. In the first instance, values for net hepatic metabolic clearance in paediatric subjects were determined from hepatic blood drug clearance observed *in vivo,* corrected for the ontogeny of haematocrit (based on the data of (Morse et al., 1947) and renal function (Rhodin et al., 2009) (Figure 5.3-2).

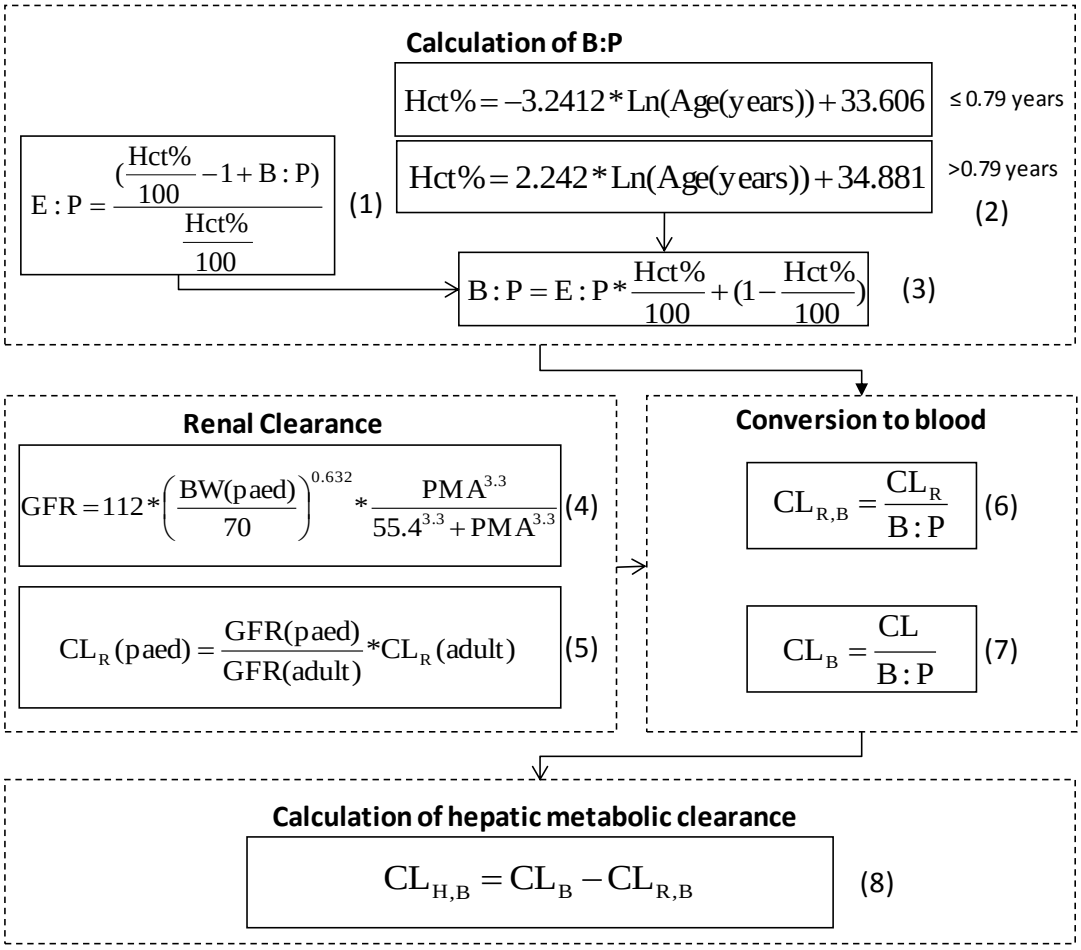

Figure 5.3-1 Steps in the calculation of net hepatic drug clearance in paediatric subjects based on an adult value of total blood clearance determined *in vivo* and ontology functions for haematocrit and renal drug clearance.
Blood to plasma drug concentration ratio (B:P), Haematocrit (Hct), Erythrocyte to plasma drug concentration ratio (E:P), Glomerular filtration rate (GFR), Paediatric body weight (BW(paed)), Post menstrual age (PMA), Renal Clearance (CL_R), Renal clearance in paediatric subjects (CL_R(paed)), Renal clearance in adults (CL_R(adult), Glomerular filtration rate in adults (GFR(adult)), Glomerular filtration rate in paediatric subjects (GFR(paed)), Renal clearance from blood ($CL_{R,B}$), Total systemic clearance based on blood drug concentration (CL_B)

Unbound intrinsic hepatic clearance values in paediatric subjects were then determined (Figure 5.3-2) from the net hepatic clearance values (Figure 5.3-1), estimates of the extent of plasma binding based on adult values and ontological changes in plasma albumin levels

(McNamara and Alcorn 2002; Johnson *et al*., 2006; Cartlidge and Ruttter, 1986; Reading *et al*., 1990), and estimates of hepatic blood flow based on ontological change in cardiac output (Johnson *et al*., 2006). The values of unbound intrinsic hepatic clearance were scaled for liver weight based on demographic information (body weight (cm), height, body surface are, age) provided in the reports from which *in vivo* clearance had been extracted. For pre-term neonates, liver weight was calculated using the model proposed by Laudy *et al*., (Laudy et al., 1998). Scaling to µl/min per mg microsomal protein was done using MPPGL values for full-term neonates, infants and children (Johnson et al., 2006; Barter et al., 2008), Information on MPPGL in pre-term neonates is scarce. It was assumed that the model developed by Barter *et al* can be back-extrapolated beyond the original age bands that were used to build the model. Hence, the PNA was entered into that model to estimate values of MPPGL based on the relationship developed for full-term babies of varying ages (Barter *et al*., 2008).

The ratios of paediatric to adult intrinsic clearances were plotted against PMA.

Clearly, the assumption is made that fraction metabolised by a particular enzyme (fm) (fm) values for metabolic clearance are invariant with age. Thus, the relationships will be confounded to a small extent by different ontogenies of metabolic pathways not mediated by the main enzyme. Developmental changes in renal clearance are accommodated. In the absence of definitive information, it was assumed that the ontogenies of CYPs 3A4 and 3A5 are similar (Johnson et al., 2006). Table 5.3.1 summarises the adult fm and fraction excreted unchanged in the urine (fe) values of the model substrates that were examined. Those for caffeine, theophyliline and midazolam were taken from the current Simcyp v12 compound file library; those for ropivacaine and alfentail fm's are based on data from drug interaction studies (Kharasch et al., 1997b; Arlander et al., 1998), respectively.

Table 5.3.1 Fractions of the drug substrates eliminated by particular pathways in adults

Substrate	fm_{1A2}	fm_{2D6}	fm_{2E1}	fm_{3A4}	fm_{UGT1A4}	fe	Reference	
Sim-Caffeine	0.98	-	0.01	0.003	-	0.01	Simcyp V12	
SV-Theophylline	0.76	0.004	0.10	0.002	-	0.13	Simcyp V12	
Sim-Midazolam	-	-	-	0.97*	0.03	0.004	Simcyp V12	
Ropivacaine	0.92	-	-	0.08	-	0.01	See the text	
Alfentanil	-	-	-	0.95	-	0.001	See the text	

† Sim and SV prefixes are compound files created in the Simcyp Simulator based on integration of data from several sources of in vitro (Sim) and combination of in vivo and in vitro (SV), respectively. For further information contact the authors

Fraction excreted unchanged in the urine (fe), Fraction metabolised by a particular enzyme (fm)

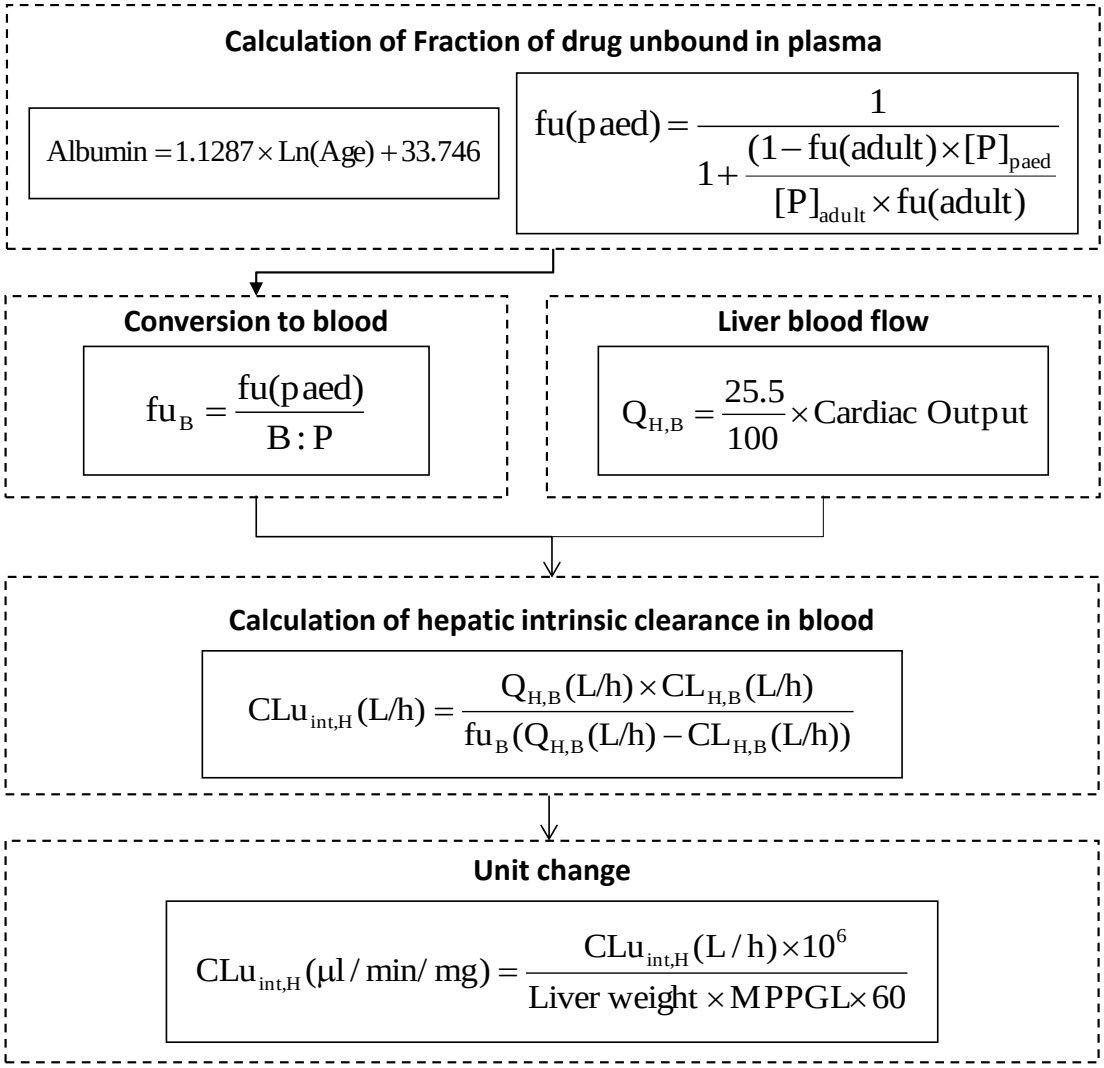

Figure 5.3-2 Steps in the calculation of unbound intrinsic hepatic clearance in paediatric subjects based on ontology functions for plasma protein levels and hepatic blood flow and correction for age-related changes in liver weight and MPPGL

Fraction of drug unbound in the plasma of paediatric subjects (fu(paed)), Fraction of drug unbound in adult plasma (fu(adult)), Plasma protein concentration in adults ($[P]_{adult}$), Plasma protein concentration in paediatric subjects ($[P]_{paed}$), Fraction of drug unbound in the plasma of paediatric subjects (fu(paed)), Fraction of drug unbound in blood (fu_B), Blood to plasma drug concentration ratio (B:P), Hepatic blood flow ($Q_{H,B}$), Hepatic unbound intrinsic clearance ($CLu_{int,H}$), Hepatic metabolic clearance based on blood drug concentration ($CL_{H,B}$)

Many of the published studies on midazolam kinetics in paediatric subjects refer to patients with critical illness under mechanical ventilation. Unfortunately, descriptions of disease severity were not reported in most articles. However, as far as possible, we attempted to correct for changes in system parameters (Hepatic blood flow (QH,B), protein concentration ([P]), fraction of drug unbound in the plasma of paediatric subjects (fu(paed))) associated with clinical condition when analysing these data. There is no consensus on the extent of hypoalbuminemia in critically ill children (Durward et al., 2003; Horowitz and Tai, 2007;

Ulldemolins et al., 2011). However, knowing that the average decrease in plasma albumin is 25% (from 45 to 35 g/L) in adults (Salive et al., 1992; Vincent et al., 2003), the same change was applied for paediatric patients across the age range. To accommodate a decrease in hepatic blood flow in adult and paediatric patients under mechanical ventilation, the relationship developed by Bonnet et al was used with modification. They showed a decrease in hepatic plasma flow with positive end-expiratory pressure (PEEP) in critically ill adults. Based on this relationship and the arbitrary but most common PEEP value reported in neonatal units a 4% decrease in hepatic blood flow was applied across the age range (Bonnet et al., 1982; Bamat et al., 2012).

5.3.4 Assessment of the Ontogeny Models

A variety of functions were assessed to obtain the best fit to the ontogeny profiles of the unbound intrinsic hepatic clearance ratios (paediatric relative to adult) with respect to CYPs 1A2 and 3A4. These included monotonic linear, exponential, logarithmic, polynomial, Gompertz, sigmoid, logistic, Weibull and Hill functions as well as discontinuous combination of these functions. The models were differentiated based on the AIC and the f-tests (Gabrielsson and Weiner, 2006) and most parsimonious model was selected as the best fit. Where possible, estimates of intrinsic clearance in individuals were used rather than mean data for each study. The data were weighted by the inverse of squared predicted value ($1/Y^2$) and by study size and were fitted by minimising the weighted least square value using Graphpad Prism 5. Dispersion of residuals was tested by calculating the numbers of data points above and below the fitted function, and by the runs test and visual inspection of residuals. Standard errors and 95% confidence intervals around mean parameter values were calculated.

The ability of the new models to capture the observed changes in the systemic clearances of theophylline (representing most of the CYP1A2-related data) and midazolam with post-menstrual age was then compared with predictions based on each of the existing ontogeny models based largely on *in vitro* data. This was done by inputting the ontogeny profiles into the Simcyp Paediatric Simulator (v.12). As the latter does not currently accommodate demographic data for pre-term infants, clearances were recovered only with respect to full-term infants. The performance of the models was assessed on the basis of weighted mean

fold error (wmfe) (Sheiner and Beal, 1981). Equation 5.3-1 was used if CL(predicted) > CL(observed) and Equation 5.3-2 if CL(predicted) < CL(observed).

$$wmfe = \frac{\sum (n_i * \left(CL_{pred,i} / CL_{obs,1} \right))}{N}$$

Equation 5.3-1

$$wmfe = \frac{\sum (n_i * \left(CL_{obs,i} / CL_{pred,i} \right))}{N}$$

Equation 5.3-2

Where n_i is the number of subjects in the i^{th} study, $CL_{obs,i}$ is the observed clearance value, $CL_{pred,i}$ is the corresponding predicted clearance value in the i^{th} study and N is the sum of number of subjects in all studies..

5.3.5 External Validation of the Ontogeny Models

The new ontogeny models were tested prospectively with respect to their ability to recover observed changes with PMA of the systemic clearances of ropivacaine and alfentanil, and in comparison with the previous model developed by (Johnson et al., 2006). For this purpose, the literature was surveyed for paediatric studies of alfenatanil and ropivacaine kinetics, and compound files for the two drugs were constructed in the Simcyp Simulator. In building these files it was particularly important to define fractions metabolised by CYPs 1A2 and 3A4 as accurately as possible. While an analysis of *in vitro* data for ropivacaine indicated a major role for CYP3A4 and a minor role for CYP1A2 (Ekstrom and Gunnarsson, 1996), an *in vivo* study of interactions with fluvoxamine (CYP1A2 inhibitor) and ketoconazole (CYP3A4 inhibitor) suggested the reverse order of involvement of the two enzymes (Arlander et al., 1998). Based on simulations of the *in vivo* data to recover this observed drug-drug interaction, adult fm values for CYPs 1A2 and 3A4 were set at 0.95 and 0.05, respectively. Since ropivacaine is bound in the plasma mainly to alpha1-acid glycoprotein (DrugBank), an ontogeny function for this protein (Johnson et al., 2006) was used rather than that for albumin. For alfentanil, there is consensus that most of its clearance is mediated by CYP3A4. Based on a simulation of a study of the interaction of troleandomycin (CYP3A4 inhibitor) with alfentanil (Kharasch et al., 1997b), the adult fm value for CYP3A4 was set at

0.95. All simulations were performed using the Simcyp Paediatric Simulator. The simulations mimicked the design of the clinical studies as closely as possible with respect to dose and age and sex of the patients. One hundred virtual subjects were simulated for each study and the mean ± SD predicted clearance values were compared with those for the observed data. The performance of the new models against the Johnson *et al* (Johnson et al., 2006) model was also assessed on the basis of weighted mean.

5.4 Results

5.4.1 Comparison of Existing CYP1A2 and -3A4 Ontogeny Models

The three published *in vitro* ontogeny models from Bjorkman, Edginton and Johnson are compared in Figure 5.4-1. For Bjorkman's models activity reaches 1.07 and 1.01 of the adult level at five years of age, and after 10 years an adult level is assumed. The Johnson models indicate a monotonic increase in activity with age for both enzymes reaching adult levers at 20 and 25 years for CYP1A2 and CYP3A4, respectively. The Edginton models indicate the adult level of activity of CYP1A2 being reached at eight years for CYP1A2, while CYP3A4 activity increases above the adult level, returning at ten years of age.

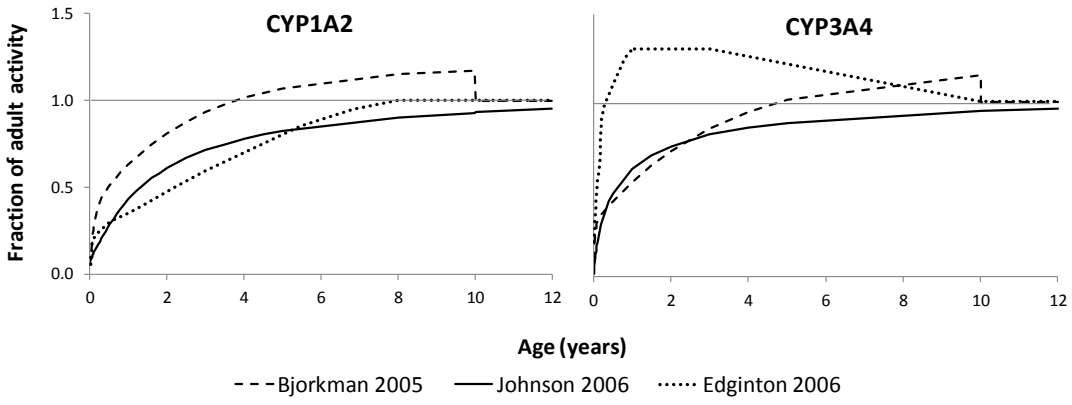

Figure 5.4-1 Comparison of published ontogeny profiles of CYPs 1A2 and 3A4 derived predominantly from expression and *in vitro* activity data

5.4.2 Literature Surveys

Meta-analysis of healthy adult data (Table 5.4.1 to Table 5.4.3) indicated mean net clearance values of 3.9, 8.1 and 29.1 L/h for caffeine (n = 76 subjects), theophylline (n = 305 subjects) and midazolam (n = 523 subjects), respectively.

Table 5.4.1 Studies reporting the clearance of caffeine in healthy adults (oral administration).

Study	n	Age range (years)	Mean body weight (kg)	CL (mL/min)
(Birkett and Miners, 1991)	6	20 to 22	63	71
(Blanchard and Sawers, 1983)	8	18.8 to 24	74	106
	84		76	166
(Kamimori et al., 2002)	84	18 to 35	76	146
	84		76	130
	5		69	77
(Newton et al., 1981)	6	21 to 36	69	68
	6		69	52
	6		69	75
(Soto and Alsar, 1997)	6	25 to 35	59	92
(Wietholtz et al., 1995)	10	21to 39	68	88

Table 5.4.2 Studies reporting the clearance of theophylline in healthy adults (intravenous and oral administration).

Study	N	Age range (Years)	Mean body weight (kg)	CL (L/h)
(Jonkman et al., 1989)	11	21to 26	69	4.2
(Jonkman et al., 1991)	8	20 to 26	77	4.1
(Knoppert et al., 1988)	15	20 to 29	68	3.4
(Ko et al., 1999)	6	25 to 30	74	4.8
(Korrapati et al., 1995)	8	23 to 49	69	4.1
(Lacarelle et al., 1994)	8	22 to 27	67	3.7
(Oosterhuis et al., 1992)	8	21 to 36	75	3.8
(Stringer et al., 1992)	12	22 to 35	81	3.9

Table 5.4.3 Studies reporting the clearance of midazolam in healthy adults (intravenous administration)

Study	N	Age range (Years)	Mean body weight (kg)	Mean (L/h)
(Albrecht et al., 1999)	9	24 to 28	66 to 89	23.9
(Allonen et al., 1981)	6	25 to 37	70	17.0
(Clausen et al., 1988)	8	23 to 32	68	25.6
(Darwish et al., 2008)	17	18 to 45		27.0
(Dingemanse et al., 1997)	10	21 to 26	71	25.2
(Ebert et al., 2000)	8	23.	80	29.7
(Farkas et al., 2007)	13	35	83	27.8
(Gorski et al., 1998)	16	34	78	27.8
(Gorski et al., 2003)	28	20 to 33	73.15	32.9
(Greenblatt et al., 1984)	20	24 to 37	63 - 65	32.0

(Greenblatt et al., 1989)	11	28 to 42		46.2
(Greenblatt et al., 2004)	8	32 to 44	78 -75	43.4
(Heizmann et al., 1983)	6	22 to 27	66	19.4
(Hotz et al., 2000)	6	26 to 32	70	29.0
(Ibrahim et al., 2002)	12	31	75	29.0
(Kharasch et al., 1997a)	9	20 to 43	74	14.65
(Kashuba et al., 1998)	20	34.8 and 38.2	77.85	37.7
(Kharasch et al., 1999)	11	18 to 32	65	26.2
(Kharasch et al., 2004)	12	20 to 39	75	32.8
(Kinirons et al., 1999)	20	24 to 44	84	22.2
(Klotz and Ziegler, 1982)	6	24 to 38		37.0
(Kupferschmidt et al., 1995)	8	25.1	70	26.3
(Lee et al., 2002)	12	19 to 42		28.6
(Liangpunsakul et al., 2005)	20	32.1	95	36.6
(Link et al., 2008)	8	27.7	70	15.9
(MacGilchrist et al., 1986)	8	37 to 42	61	37.8
(Mandema et al., 1992)	8	22	69	31.4
(Majumdar et al., 2007)	12	20 to 36		26.3
(Masica et al., 2004)	21	19 to 30	69	23.0
(Mould et al., 1995)	12	19 to 34	80	45.1
(Olkkola et al., 1996)	12	19 to 25	76	36.5
(Palkama et al., 1999)	12	21 to 31	69	32.3
(Platten et al., 1998)	12	30	72	26.8
(Rogers et al., 2002)	12	24 to 45	85	34.0
(Saari et al., 2006)	10	23 to 29	83	24.8
(Schwagmeier et al., 1998)	8	22 to 30	68	21.8
(Smith et al., 1981)	6	21 to 22	73	27.61
(Tateishi et al., 2001)	20	30.3	83	24.7
(Thummel et al., 1996)	20	26 to 42	78.	22.2
(Tsunoda et al., 1999)	9	19 to 41	69	32.6
(Van Gerven et al., 1997)	10	22 to 32	78	28.2
(Wandel et al., 2000)	15	24 to 44	86	18.
(Wang et al., 2001)	12	29.6	72	34.30

A total of 7 (3 combination of oral-intravenous and 4 intravenous) and 22 (7 oral and 15 intravenous) reports documenting caffeine and theophylline clearances, respectively, in paediatric subjects were found covering an age range from birth to 18 years (Supplemental Table S 2 and Supplemental Table S 3). Of these, 14 related to neonates (mostly pre-term), 10 to infants, 11 to children and 7 to adolescents. For midazolam, 15 paediatric studies were included in the analysis (Supplemental Table S 4). Of these, 6 referred to neonates (5 pre-term), 5 to infants, 9 to children, and 4 to adolescents.

Observed values for the clearances of ropivacaine and alfentanil in paediatric patients are summarised in Table 5.4.4 and Table 5.4.5, and data used in the compound files are summarised in Table 5.4.6 and Table 5.4.7. Only data obtained after IV administration were used for alfentanil; for ropivacaine the data refer to perineural administration.

Table 5.4.4 Studies reporting the clearance of alfentanil in paediatric subjects (iv administration).

Study	N	GSA (weeks)	Age (Years)	CL ($ml.min^{-1}.kg^{-1}$) ± SD
(Davis et al., 1989)	9		9 months to 10 years	5.6 ± 2.4
(Killian et al., 1990)	5	>36	1 to 3 days	1.7 ± 0.47
(Wiest et al., 1991)	9	>35	birth to 4 days	2.26 ± 0.75*
(Meistelman et al., 1987)	8		4 to 8	4.7 ± 1.7
(Goresky et al., 1987)	5		0.25 to 1	8.4 ± 1.6
	8		1 to 14	7.7 ± 1.6
	5		1.2 to 14	7.8 ± 1.8

* Two subjects that terminal data points were not detectable are removed from the analysis.

Table 5.4.5 Studies reporting the clearance of ropivacaine studies in paediatric subjects (perineural administration).

Study	n	Age	CL ($ml.min^{-1}.kg^{-1}$) ± SD
(Hansen et al., 2001)	15	Birth to 3 months	5.2 ± 2.6
(Hansen et al., 2000)	18		8.5
(Bosenberg et al., 2005)	11	Birth to 7 days	3.3 ± 0.7
	10	41 to 86 days	4.8 ± 1.9
	10	105 to 176 days	7.4 ± 3.2
	14	199 to 362 days	9.8 ± 5.2
(Lonnqvist et al., 2000)	5	1 to 2 years	6.4 ± 1.6
	9	3 to 4 years	7.1 ± 1.6
	6	5 to 8 years	8.8 ± 2
(McCann et al., 2001)	7	3 to 11 months	4.3 ± 0.4
	11	12 to 48 months	6.2 ± 0.7
(Rapp et al., 2004)	6	Birth to 30 days	3.5
	9	0.5 to 1	10.8

	35	Birth to 1	5.1
(Calder et al., 2012)	31	Birth to 0.44	4.8 ± 2.5
(Habre et al., 2000)	9	12 to71 month	7.6

Table 5.4.6 Summary of alfentanil compound file used in simulating alfentanil clinical studies

Parameter	Value	Reference
Molecular weight	416.52	(DrugBank)
$LogP_{o:w}$	2.16	(DrugBank)
pKa 1	7.5	(DrugBank)
pka 2	6.5	(DrugBank)
B:P	0.63	(Paixao et al., 2009)
fup	0.09	(Mather, 1983)
V_{ss} (L.kg-1)	0.36	(Bower and Hull, 1982; Chauvin et al., 1986; Kharasch et al., 1997b)
CL_{iv} (L.h-1)	19.64	(Bovill et al., 1982; Bower and Hull, 1982; Camu et al., 1982; McDonnell et al., 1982; Schuttler and Stoeckel, 1982; Chauvin et al., 1986; Meistelman et al., 1987; Roure et al., 1987; Kharasch et al., 1997a)
CL_R (L.h-1)	0.079	(Schuttler and Stoeckel, 1982)
$fmCYP_{3A4}$	95%	(Kharasch et al., 1997a)

Table 5.4.7 Summary of ropivacaine compound file used in simulating ropivacaine clinical studies

Parameter	Value	Reference
Molecular weight	274.40	(Chemicalize)
LogPo:w	2.85	(Chemicalize)
pKa	8.07	(DrugBank)
B:P	0.69	(Lee et al., 1989)
fu_p	0.06	(DrugBank)
V_{ss} ($L.kg^{-1}$)	0.68	(Lee et al., 1989; Halldin et al., 1996; Jokinen et al., 2001; Jokinen et al., 2003; Simon et al., 2006)
CL_{iv} ($L.h^{-1}$)	23.54	(Lee et al., 1989; Halldin et al., 1996; Arlander et al., 1998; Jokinen et al., 2001; Jokinen et al., 2003)
CL_R ($L.h^{-1}$)	0.12	(Ekstrom and Gunnarsson, 1996)
$fmCYP_{1A2}$	0.95	(Arlander et al., 1998)
$fmCYP_{3A4}$	0.05	(Arlander et al., 1998)

5.4.2.1 Ontogeny Profiles

Relationships between the ratios of unbound hepatic intrinsic clearances in paediatric subjects deconvoluted from *in vivo* data, relative to adult values for CYP1A2 and CYP3A4 mediated metabolism as a function of age are shown in Figure 5.4-2 and Figure 5.4-3, respectively. The best-fit to the CYP1A2 data required two equations; Equation 5.4-1

describing the data below 196 weeks PMA and Equation 5.4-2 describing the data above 196 weeks PMA.

$$\mathrm{CYP1A2} = \frac{1.6 * \mathrm{PMA}^{5.7}}{54.6^{5.7} + \mathrm{PMA}^{5.7}} \qquad \text{Equation 5.4-1}$$

$$\mathrm{CYP1A2} = 0.8 * \exp^{-0.001*(\mathrm{PMA}-196)} + 0.8 \qquad \text{Equation 5.4-2}$$

Thus, enzyme activity was represented as reaching adult levels at 63 weeks PMA, rising above adult values up to 196 weeks PMA (3 years) followed by a decline to adult levels by 25 years.

The best-fit to the CYP3A4 data was afforded by Equation 5.4-3, describing a monotonic increase in enzyme activity up to adult levels by 108 weeks PMA (1.3 years).

$$\mathrm{CYP3A4} = \frac{1 * \mathrm{PMA}^{3.9}}{71^{3.9} + \mathrm{PMA}^{3.9}} \qquad \text{Equation 5.4-3}$$

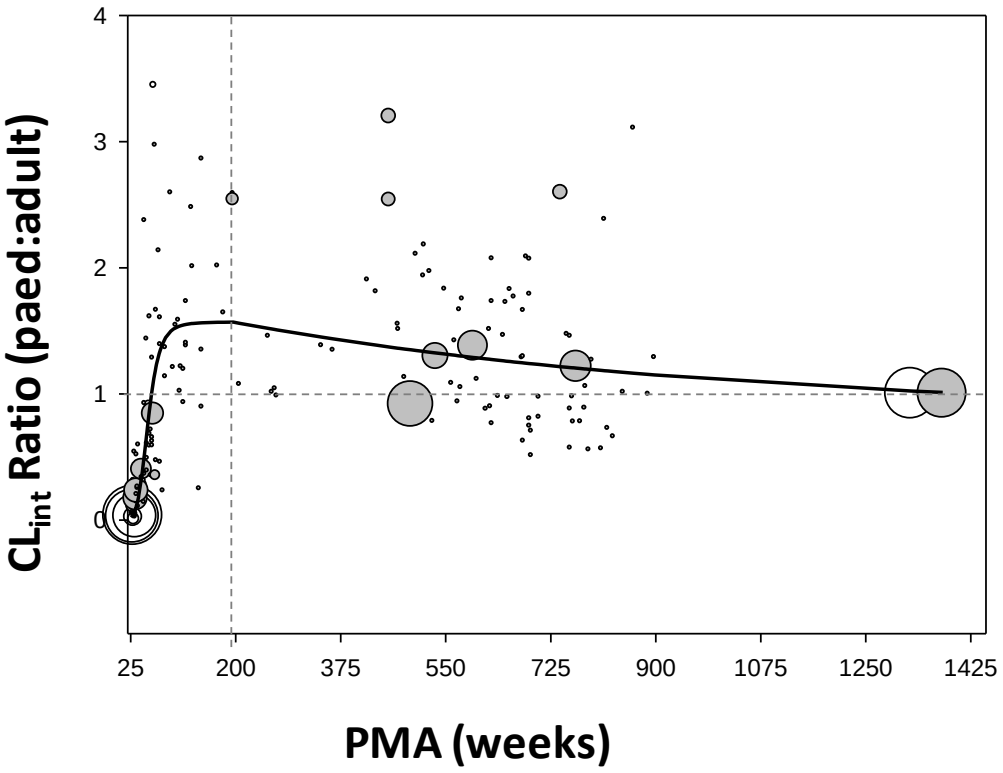

Figure 5.4-2 Relationship between the ratio of unbound intrinsic hepatic clearance mediated by CYP1A2 in paediatric subjects relative to adult values as a function of PMA.
(The circles represent mean back-extrapolated values from the *in vivo* studies; grey- theophylline: open-caffeine); the size of the circle indicating the relative numbers of subjects. The small data points refer to values in individuals calculated from observed clearances. The continuous line represents the two functions

used to describe the relationship; the vertical dashed line indicating the junction of the two functions and the horizontal dashed line indicating the adult level.)

5.4.2.2 Model Validation

The performance of the existing ontogeny models and the new models in recovering the relationships between theophylline and midazolam clearances (excluding those for pre-term infants) are indicated in Figure 5.4-4 and Figure 5.4-5, respectively.

With respect to the ontogeny of CYP1A2 clearance, visual inspection indicates the superiority of the new model in capturing the observed data for full term infants and older paediatric subjects. Weighted mean fold errors obtained with the Bjorkman (Bjorkman, 2005), Johnson *et al* (Johnson et al., 2006), Edginton *et al* (Edginton et al., 2006a) models and the new model were 2.27, 2.85, 1.17 and 0.89, respectively.

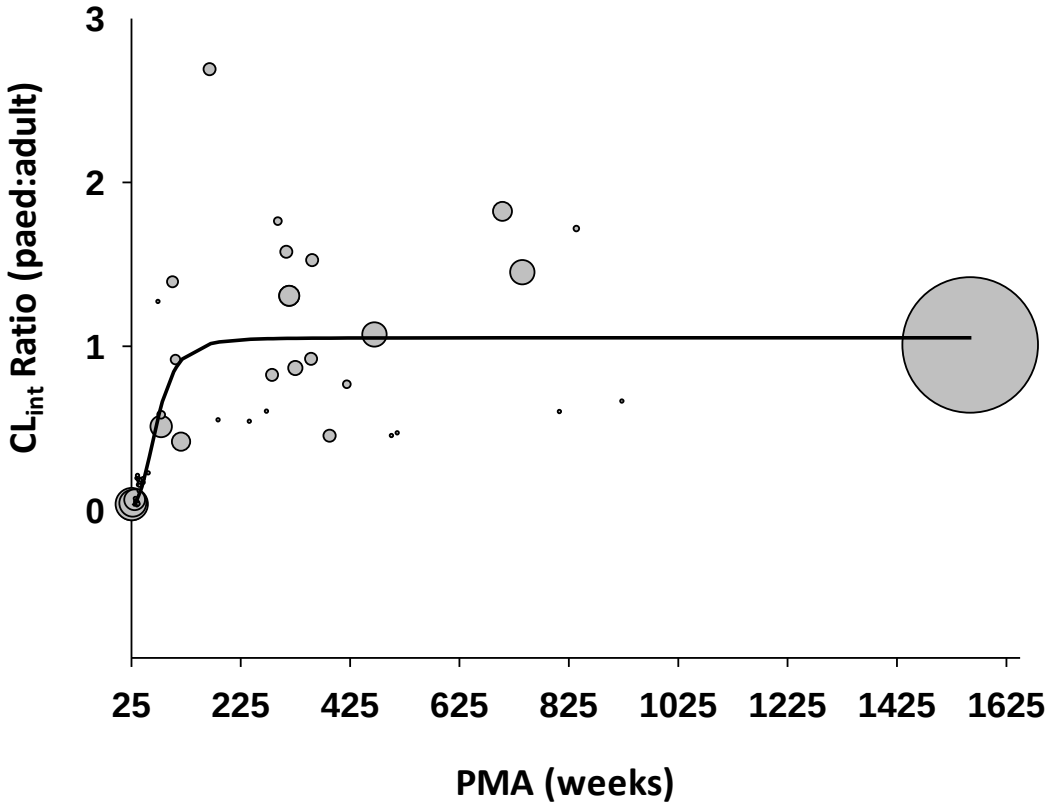

Figure 5.4-3 Relationship between the ratio of unbound intrinsic hepatic clearance mediated by CYP3A4 in paediatric subjects relative to adult values as a function of PMA.
(The circles represent mean data back-extrapolated values from the *in vivo* studies; the size of the circle indicating the relative numbers of subjects. The small circles represent values in individuals calculated from their clearance values. The continuous line represents the function used to describe the relationship.)

The new model of the ontogeny of CYP3A4 clearance captured the observed data marginally better than the other 3 models. Weighted mean fold errors obtained with the

Bjorkman (Bjorkman, 2005), Johnson (Johnson et al., 2006), Edginton (Edginton et al., 2006a) models and the new model were 1.51, 1.67, 1.51 and 1.40, respectively.

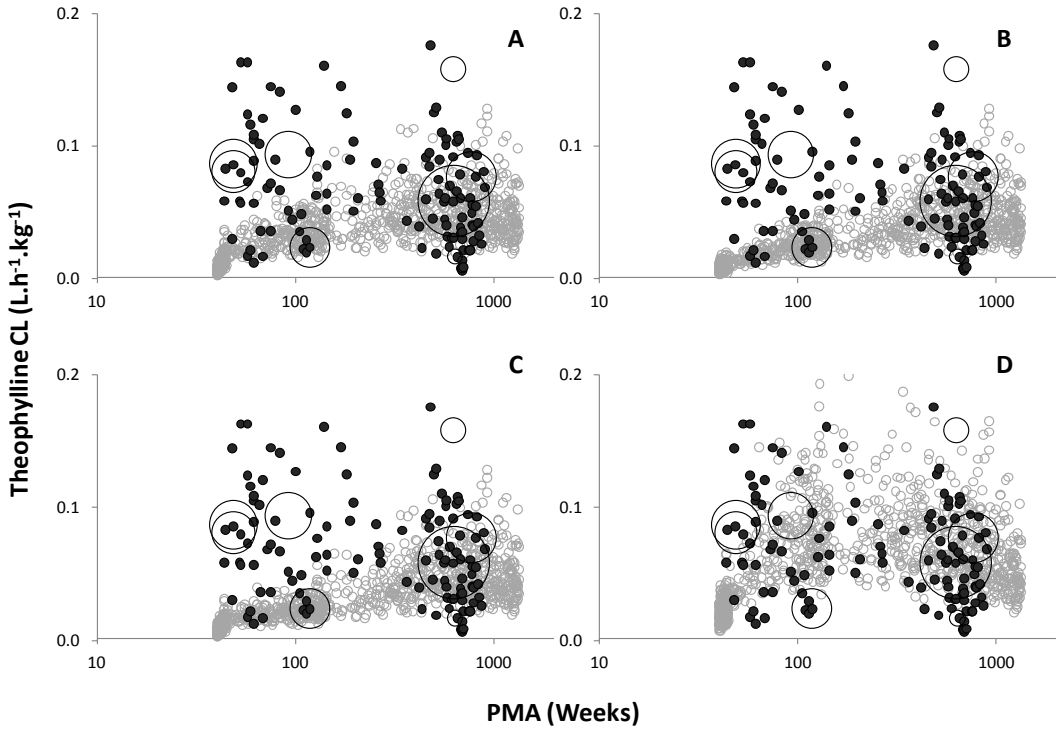

Figure 5.4-4 A comparison of the recovery of observed theophylline clearances as a function of PMA by the models of Bjorkman (2005) (A), Johnson et al., (2006) (B), Edginton et al.,(2006) (C), and the new model based on deconvolution of the *in vivo* data (D).
(The open circles indicate mean data from actual studies where individual data were not reported; the size of the circle indicating the relative number of subjects in each study. The filled black circles indicate data for individuals from actual studies where individual data were reported. The grey circles represent data predicted by the models using the Simcyp Simulator for individual virtual subjects).

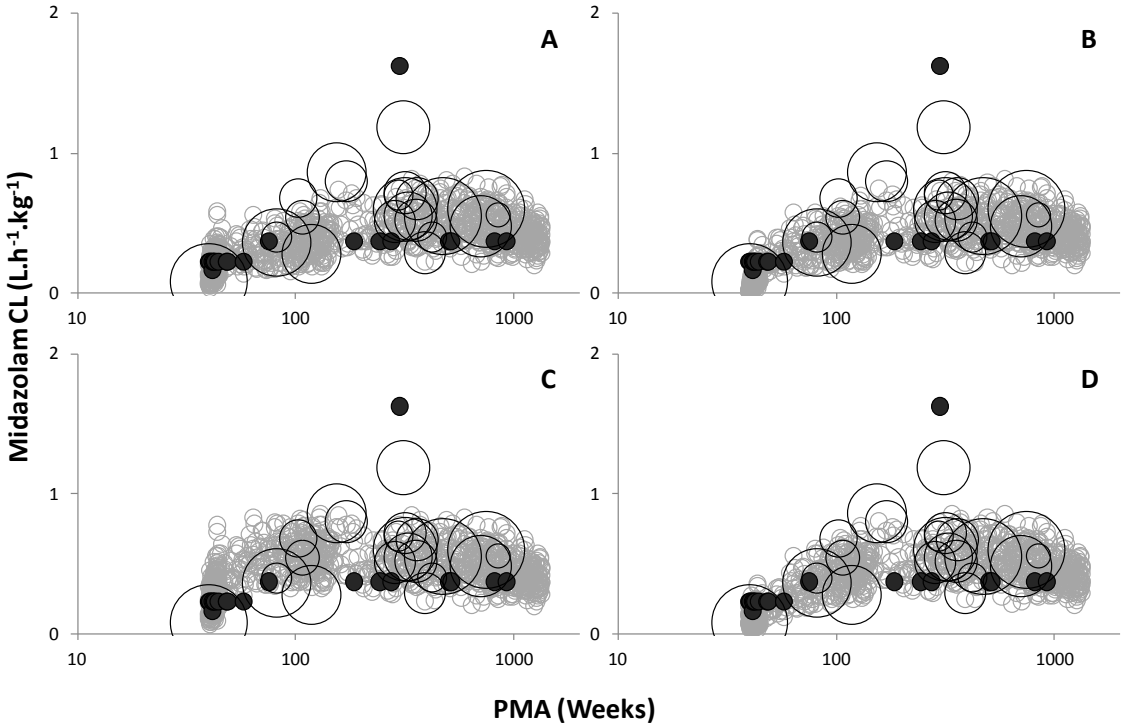

Figure 5.4-5 A comparison of the recovery of observed midazolam clearances as a function of PMA by the models of Bjorkman (2005) (A), Johnson et al., (2006) (B), Edginton et al.,(2006) (C), and the new model based on deconvolution of t *in vivo* data (D).
(The open circles indicate mean data from actual studies where individual data were not reported; the size of the circle indicating the relative number of subjects in. The closed black circles indicate data for individuals from actual studies where individual data were reported. The grey circles represent data predicted by the models using the Simcyp Simulator for individual virtual subjects).

A comparison of the performance of the new model against the model of Johnson *et al* (Johnson et al., 2006) with respect to prediction of the clearances of alfentanil and ropivacaine is shown in Figure 5.4-6. For all of the studies considered, the new model was superior. Absolute prediction by the new model was good except with respect to recovering the data for 1 study of alfentanil in infants. Mean squared prediction errors with respect to the prediction of alfentanil clearance from all studies were 3.03 and 6.78 for the new model and the Johnson model, respectively. Mean squared prediction errors with respect to the prediction of ropivacaine clearance from all studies were 2.35 and 14.19 for the new model and the Johnson *et al* (Johnson et al., 2006) model, respectively.

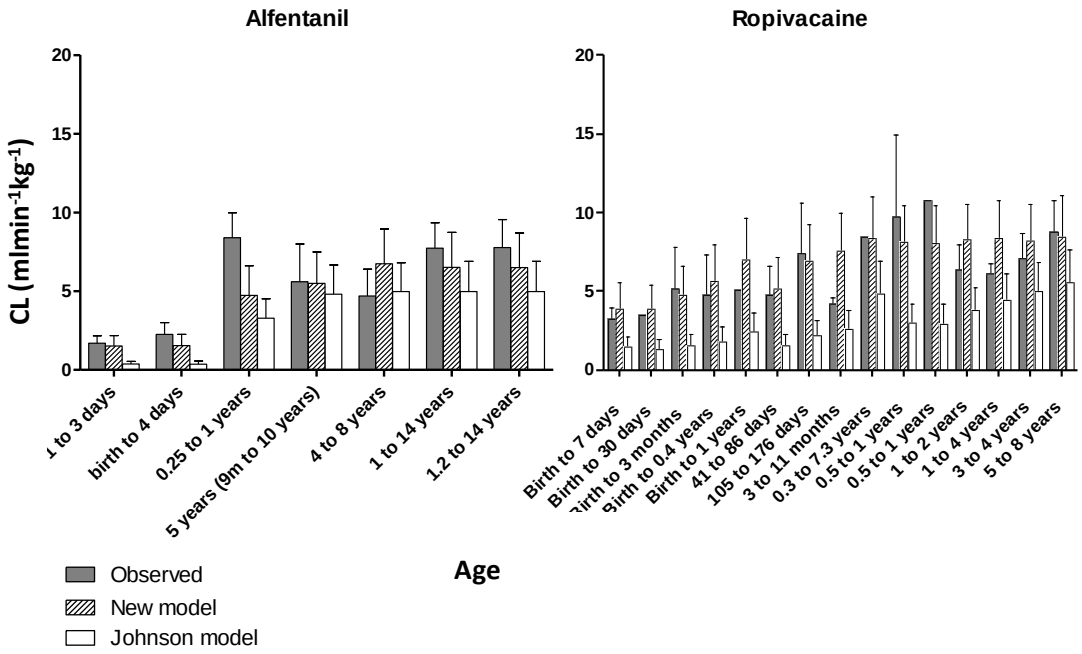

Figure 5.4-6 Comparison of mean (SD) predicted clearances of alfentanil and ropivacaine based on the new ontogeny models and the Johnson et al (2006) models against observed values reported in different studies

5.5 Discussion

5.5.1 Assessing the Performance of Paediatric PBPK Models

From our perspective, although the study by Leong et al. pointed out potential problems with some of the underlying systems parameters in paediatric PBPK, it also demonstrated the need for care when evaluating and interpreting comparisons of paediatric PBPK based simulations against data from clinical studies in paediatric patients (Leong et al., 2012). Several issues are relevant. For instance, in applying paediatric PBPK modelling an attempt should be made to obtain a reasonable prediction in adult populations for the test drug before performing simulations for paediatric subjects. With respect to one of the cases reported by Leong *et al.,* there was an appreciable under prediction of adult clearance (0.3 *vs.* 0.4 $L.h^{-1}.kg^{-1}$). Also, comparisons of mean values should be done with geometric means or medians when there are extreme outliers in the clinical data. Thus, in the 6 to <12 year age group for which Leong *et al.,* predicted the systemic clearance of omeprazole the observed arithmetic mean and geometric mean values were very discrepant (4.3 and 0.85 $L.h^{-1}.kg^{-1}$, respectively); indicating the presence of an extreme outlier. Hence, comparison of the geometric mean from the simulations (0.65 $L.h^{-1}.kg^{-1}$) with the geometric mean of the

observed values (0.85 $L.h^{-1}.kg^{-1}$) would have led to a different conclusion than that based on the arithmetic mean from simulation of 0.80 L.h-1.kg-1 per h and the observed value of 4.3 $L.h^{-1}.kg^{-1}$.

5.5.2 Potential Problems with Existing *In Vitro* Ontogeny Models

Despite the shortcomings listed above, the overall trend in the study by Leong *et al,*. highlighted the potential lack of predictive performance of some ontogeny models based on *in vitro* data (Leong et al., 2012). Such models have been constructed primarily with *in vitro* data obtained from histologically normal liver samples. However, it is known that disease states may influence the expression of enzymes and transporters through modulating effects of interleukins and cytokines (Machavaram et al., 2013; Azam et al., 2014). Therefore, although tissue samples may appear to be histologically normal, this may not reflect normal levels of enzyme activity. Other problems with the use of *in vitro* data include their sparse nature with respect to covering the complete age range, incomplete information on the tissue samples with respect to drug history, genotype and ethnicity and inappropriate age banding of samples in the final study. Hence, deriving ontogeny functions from *in vivo* data might have some advantages.

5.5.3 New *In Vivo* Ontogeny Models

In vivo-based ontogeny models can partially overcome some of the limitations of those based on *in vitro* data by offering selective collation of data with the knowledge of drug history, genotype and ethnicity. Prospective application of the new ontogeny models derived from *in vivo* data resulted in good prediction of the systemic clearance of ropivacaine and alfentanil in paediatric subjects. Clearly, further validation with more CYP1A2 and CYP3A4 substrates is desirable. A limitation of the use of *in vivo* data to define ontogeny profiles of specific enzymes, that is also shared by the approach using *in vitro* activity data, is that unless substrates are used that are only metabolised by the specific enzyme, the ontogeny functions will be confounded to some extent by the ontogenies of parallel elimination pathways. Thus fm values for metabolic clearance are assumed to be invariant with age (changes in renal clearance are accommodated in the simulations). With regard to this issue, substrates were chosen having either CYP1A2 or CYP3A as by far the dominant enzyme involved in their metabolism (Table 5.3.1). Also, when evaluating the ontogeny of CYP3A4 it

was assumed that any contribution of CYP3A5 and CYP3A7 to the metabolism of the substrates was nominal. Only 10-25% of Caucasians express CYP3A5, and the contribution of CYP3A7 to the metabolism of midazolam is relatively minor (Williams et al., 2002).

The reason for an apparent peak in CYP1A2 activity at 3 years of age is unknown but it may be associated with specific hormonal or dietary influences. It does not appear to be linked to breast-feeding, as the latter has been associated with a decrease in the clearance of caffeine (Le Guennec and Billon, 1987). There is some evidence suggesting an over-expression of CYP1A2 in children, mediated by growth hormone (Lambert et al., 1986b; Levitsky et al., 1989), but this might not be expected to be evident in the ontogeny profile until puberty,

Another difficulty in using *in vivo* data, especially in neonates and infants, to derive enzyme ontogeny functions is that reported data occasionally can lack adequate information on patient characteristics and in absence of such information particular clinical conditions are not considered. In addition, drug assay methods can vary between studies. The confounding influence of the clinical condition and management of the patient will add further complexity. In the current exercise this was a particular issue with the use of midazolam and alfentanil data, since many of the patients receiving this drug were critically ill. As far as possible we tried to allow for the impact of disease and clinical condition when developing the ontogeny profile for CYP3A4. It has been reported that the clearance of midazolam is decreased in premature neonates, especially those receiving care for respiratory distress syndrome or with inflammatory conditions and (Ince et al., 2013; Machavaram et al., 2013). Furthermore, when reviewing the midazolam database, we specifically excluded a study by Peeters *et al*., (Peeters et al., 2006) in paediatric patients after craniofacial surgery. In assessing these data it was estimated that values of hepatic clearance in 10 of the individuals were in excess of liver blood flow expected from their demographic characteristics. These patients were not ventilated and seem to have experienced some agitation and, as pointed out by the authors, their midazolam clearances were significantly greater than those reported for ventilated patients. This emphasises the need for caution when extrapolating enzyme ontogeny functions to modify dosage in paediatric patients with different clinical conditions from those to the patients used to compile the functions. Clearly, further work is also needed to establish

better dosage guidelines with respect to drug exposure in pre-term neonates when drug clearance is relatively low and highly variable.

5.6 Conclusion

The new ontogeny models for CYPs 1A2 and 3A4 are an improvement over previous models based primarily on *in vitro* data in recovering observed clearance values. As such, they reinforce the utility of physiologically-based pharmacokinetic modelling as a generic basis for predicting dosage requirements in paediatric patients with respect to substrates of specific enzymes. As knowledge of paediatric physiology, biochemistry and pharmacology increases, paediatric PBPK models will require continuous enhancement. The level of confidence in these models at any given timer reflects the state of existing knowledge from multiple sources (whether *in vitro* or clinical observations after deconvolution). Application of the methodology in this report to other enzymes is warranted.

Chapter 6. Precision criteria to derive sample size when designing paediatric pharmacokinetic studies: which measure of variability should be used?

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Declaration

This chapter constitutes a published article;

"Precision criteria to derive sample size when designing paediatric pharmacokinetic studies: which measure of variability should be used?"

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F. Salem: Data collection and statistical and meta-analysis, modelling and simulation using Matlab and PBPK and preparation of manuscript

K. Ogungbenro: Input on analysis in Matlab and expert opinion on POPPK side of analysis

P. Vajjah: Statistical advice

L. Aarons: Expert opinion on statistical analysis, supervision of analysis and detailed edition of manuscript

T.N. Johnson: Presentation of results, editing manuscript

A. Rostami-Hodjegan: Supervision of research, input in data analysis and input to manuscript

Supplemental material of the paper appears in the main text here to facilitate reading.

6.1 Abstract

A new approach for calculation of sample size in paediatric clinical pharmacokinetic studies was suggested based on desired precision for a pharmacokinetic parameter of interest. The estimate of variability for sample size calculations could be obtained from different sources. It is not known whether these sources constantly show higher/lower variability across compounds and age groups. We obtained estimates of variability for clearance, volume of

distribution and area under the plasma concentration-time curve for 5 drugs from adult/paediatric classic clinical pharmacokinetic studies, and physiologically based pharmacokinetics (PBPK) combined with *in vitro-in vivo* extrapolation. Estimates were applied to the proposal methodology for non-compartmental analysis. Sample size was different for each drug based on various estimates of variability from different pharmacokinetic parameters and depending on the age. Overall, there was no consistent discrepancy in sample size calculated according to the source of variability. A conservative approach should be taken when using 'precision based methodology' knowing that various sources of initial estimates of variability will not lead to similar sample size calculations. Although PBPK simulations could be used for estimating variability, further work is required to investigate the best approach to estimate variability of pharmacokinetic parameters in paediatric populations and hence sample size calculations.

6.2 Introduction

Recently Wang *et al* (Wang et al., 2012) suggested a criteria for calculating the sample size when conducting paediatric clinical pharmacokinetic (PK) studies based on estimates of the parameter precision. In the proposed methodology it is stated that "The study must be prospectively powered to target a 95% CI (confidence interval) within 60% and 140% of the geometric mean estimates of clearance (CL) and volume of distribution (V_d) for any given drug in each paediatric sub-group with at least 80% power" (Wang *et al*., 2012). Considering the surge of interest in paediatric clinical PK studies, due to new regulatory requirements, the suggested approach could have important implications for and introduce uniformity in the design of paediatric PK studies. The variability for the PK parameter of interest has important implications on estimating the study size. The estimates of the variability could be obtained from various sources such as;

- The standard deviation (SD) from prior PK studies undertaken in adults (SD_{Adult}),
- The SD reported in conventional paediatric PK studies with a limited sample size (SD_{Paed}),
- Predicted SD for PK parameters from physiologically based pharmacokinetic (PBPK) models combined with *in vitro in vivo* extrapolation (IVIVE) (SD_{PBPK}),

- The SD from drugs with similar physiochemical and metabolic characteristics

Wang et al did not provide formal guidance with respect to the selection of the optimal "source" of variability estimates. They suggest using variability estimates from adults or any prior relevant study or appropriate paediatric models that describe size, maturation and organ function (Wang et al., 2012). It is not clear whether using the SD from sources listed above will lead to significantly different estimates of sample size. Moreover, if the compound is a first-in-class drug, or it has not been studied in an adult population, then some of these sources for estimating variability will not be available. A recent study showed that for a number of drugs CL variability for children of 6 years and over is not significantly different from adults; however, they did not provide any information about neonates and infants (Edginton et al., 2013).

To the best of our knowledge the impact of the source of the variability on the estimation of sample size has not been evaluated. In addition, the potential disparities between the sample size calculations when different sources of variability estimates are age or compound-dependent are unknown. The aim of the current study was to use variability from different sources and compare the calculated sample size based on Wang's methodology.

6.3 Methods

Selection of compounds and PK parameters

Compounds in this study were selected based on availability of PK parameters in adults and children of various age groups in the literature. Since PBPK simulations for estimating SD were carried out using the Simcyp simulator (Simcyp Ltd, Sheffield, UK, http:www.simcyp.com), availability of compounds with a pre-defined library within the simulator was also considered to facilitate the study. The following compounds fulfilled these criteria: caffeine, ibuprofen, midazolam, itraconazole and theophylline.

Sample size was calculated for fundamental PK parameters which are important for clinical decisions related to loading and maintenance doses (i.e. V_d and CL). However, the area under plasma concentration-time curve (AUC) was also investigated as an alternative to CL or in parallel when appropriate.

6.3.1 Literature review and search strategy

A comprehensive literature search was carried out to identify the studies that report PK parameters for the five compounds in paediatric and adult groups. Various sources such as Pubmed, Scopus, Medline and ISI Web of Knowledge were searched using the relevant combination of keywords “drug name” plus age group for example “paediatric” or “neonate” or “infant” or “children” plus keywords “pharmacokinetic” or “disposition” or “clearance” or “biotransformation”. The selection of studies was based on whether they provided the PK parameters in question as well as variability around them. The studies were excluded if administration of drug was through routes other than intravenous or oral, the studies focused on PK parameters other than CL, V_d and AUC (for example half-life or plasma concentration), the variability around the parameters were reported in a form of statistical parameters (such as range) that were not convertible to SD.

6.3.2 Obtaining estimates of variability and calculation of sample size

An estimate of variability for PK parameters of interest was obtained by conducting meta-analysis on data from different sources (SD_{Adult} and SD_{Paed} , SD_{PBPK}) as explained in the following section. These variability estimates were then applied to Wang et al’s sample size calculation method. Calculations were done using the script provided by Wang *et al* for the R programming language (Wang et al., 2012). To determine whether the calculated sample sizes from SD_{Adult} - SD_{Paed} and SD_{Paed} - SD_{PBPK} were significantly different from each other, precision limits of 0.8 to 1.25 were used for the calculations. If the paediatric sample size were within 0.8 and 1.25 of adult or PBPK sources, sample size was taken to be not significantly different.

6.3.2.1 From NCA studies

An estimate of variability (SD_{Adult} and SD_{Paed}) for conventional adult and paediatric clinical PK studies was obtained from a meta-analysis of literature data. Where data were available from different literature reports for a given drug, they were recorded and analysed to obtain an overall measure of variability for CL ($L.h^{-1}.kg^{-1}$), V_d ($L.kg^{-1}$) and AUC [(mg.h/L)/(mg/kg)]. The AUC was normalised for mg of dose per kilogram of body weight. The analysis was conducted separately based on adult or paediatric age groups, as defined by the Food and

Drug Administration (FDA) classification (i.e. neonates (birth to 1 month), infants (1 month to 2 years) and children (2 to 12 years)). Meta-analysis was carried out to calculate the pooled variance (σ^2) from all studies within each age group (neonates, infants and children and adults) using Equation 6.3-1 and Equation 6.3-2 (Armitage et al., 2002; Johnson et al., 2006).

$$\sigma^2 = \frac{\sum_{i=1}^{N_S}(n_i - 1)S_i^2}{\sum_{i=1}^{N_S}(n_i - 1)}$$

Equation 6.3-1

where n_i and S_i are the number of subjects and the SD of the i^{th} study and N_s is the number of studies. If a study involved more than one age band, S_i from that study was incorporated in all the age groups comprising the study. A summary of the pharmacokinetic parameters from the clinical studies is presented in Table 6.3.1 to Table 6.3.6.

The standard deviation (δ) of the log-transformed PK parameter was obtained from Equation 6.3-2 and was used in the sample size calculation.

$$\delta = \sqrt{\ln(1 + CV^2)}$$

Equation 6.3-2

where CV is the coefficient of variation, and is calculated from σ and the weighted mean ($\bar{\mu}$) of the PK parameter. $\bar{\mu}$ was calculated from Equation 6.3-3.

$$\bar{\mu} = \frac{\sum_{i=1}^{N_s} n_i \times \mu_i}{N}$$

Equation 6.3-3

Table 6.3.1 Summary of clinical studies stratified into paediatric age bands for clearance ($L.h^{-1}.kg^{-1}$).
d=days, w=weeks, GSA=gestational age, m=months, y=years, h=hours.

Compounds	Paediatric group	Age	Number of subjects	Clearance ($L.h^{-1}.kg^{-1}$)			References
				Mean	SD	Overall SD	
Caffeine	Neonates	1- 42 d	13	0.01	0.001	0.004	(Gorodischer and Karplus, 1982)
		3-32 d	12	0.01	0.005		(Aranda et al., 1979b)
	Children	7 to 10	5	0.26	0.11	0.11	(Akinyinka et al., 2000)
Theophylline	Neonates	1 to 3 d (25-30 GSA w)	11	0.02	0.005	0.01	(Jones and Baillie, 1979)
		26 to 33 GSA w	7	0.01	0.003		(Latini et al., 1978)
		2.9 d	33	0.02	0.005		(Brazier et al., 1979)
		6 to 11 d	3	0.02	0.001		(Lonnerholm et al., 1983)
	Infants	1 to 5	6	0.2	0.08	0.17	(Kumar et al., 1989)
		3 w-6.5m	12	0.2	0.07		(Franko et al., 1982)
	Children	7 to 12	8	0.1	0.04	0.04	(Arnold et al., 1981)
		1 to 5	6	0.2	0.08		(Kumar et al., 1989)
		6 to 10	7	0.2	0.06		
		11 to 16	8	0.2	0.03		
		4 to 9	15	0.3	0.09		(Lee and Ngiam, 1983)
Ibuprofen	Neonates	3 d	27	0.01	0.01	0.01	(Van Overmeire et al., 2001)
		0-3 h	21	0.002	0.001		(Aranda et al., 1997)
	Infants	0.25- 2.5	38	0.11	0.09	0.1	(Brown et al., 1992)
			46	0.12	0.09		
	Children	2.9 -8.5	22	0.14	0.03	0.034	(Scott et al., 1999)
		4.9-13.2	12	0.1	0.04		
		2.5 -12	38	0.07	0.04		(Brown et al., 1992)
			46	0.09	0.03		
		4 to 11	24	0.1	0.02		(Gelotte et al., 2010)
Itraconazole	Infants	0.5 to 2	8	1.7	0.57	0.55	(de Repentigny et al., 1998)
		0.5 to 2	6	1.1	0.51		(Abdel-Rahman et al., 2007)
	Children	2 to 6	6	0.3	0.31	0.31	(Abdel-Rahman et al., 2007)

Midazolam	Neonates	2-5 d	10	0.13	0.10	0.10	(Jacqz-Aigrain et al., 1990)
			15	0.10	0.11		(Jacqz-Aigrain et al., 1992)
	Infants	1.75-4 y	6	0.80	0.26	0.20	(Rey et al., 1991)
		6 m- 13 y	13	0.86	0.16		(Muchohi et al., 2008)
		1.3 y	5	0.54	0.20		(Mathews et al., 1988)
	Children	1.75-4 y	6	0.84	0.26	0.24	(Rey et al., 1991)
		6 m- 13 y	13	0.86	0.16		(Muchohi et al., 2008)
		6 to 18	20	0.54	0.27		(Tolia et al., 1991)
		5.2 y	6	0.72	0.40		(Mathews et al., 1988)
		4.7 y	6	0.51	0.11		
		5 to 9	12	0.92	0.19		(Jones et al., 1993)

Table 6.3.2 Summary of clinical studies stratified into paediatric age groups for volume of distribution (L.kg^{-1})

Compounds	Paediatric group	Age	Number of subjects	V_d (L. kg^{-1})			References
				Mean	SD	Overall SD	
Caffeine	Neonates	1- 42 d	13	0.8	0.04	0.06	(Gorodischer and Karplus, 1982)
		3-32 d	12	0.9	0.1		(Aranda et al., 1979b)
Theophylline	Neonates	25 to 30 GSA w	15	0.70	0.18	0.33	(Jones and Baillie, 1979)
		26 to 32 GSA w	8	1.15	0.73		(Giacoia et al., 1976)
		2.9 d	20	1.03	0.20		(Brazier et al., 1979)
		2 to 26 d	7	0.94	0.23		(Ahn et al., 1999)
		26 to 33 GSA w	7	0.40	0.20		(Latini et al., 1978)
	Infants	1 to 5	6	0.8	0.1	0.14	(Kumar et al., 1989)
		2m-4y	6	0.5	0.1		(Bolme et al., 1979)
		3 w-6.5m	12	0.2	0.03		(Franko et al., 1982)
		3 to 23 m	15	0.34	0.20		(Simons and Simons, 1978)
		4 to 18 m	13	0.56	0.14		(Rosen et al., 1979)
		1.3 to 4.4 y	10	0.25	0.13		(Loughnan et al., 1976)
	Children	1 to 5	6	0.8	0.1	0.27	(Kumar et al., 1989)
		6 to 10	7	1	0.7		
		11 to 16	8	0.8	0.2		
		7 to 12	8	0.4	0.1		(Arnold et al., 1981)
		2m-4y	6	0.5	0.1		(Bolme et al., 1979)
		3 w-6.5m	12	0.2	0.03		(Franko et al., 1982)
		1.3 to 4.4 y	10	0.25	0.13		(Loughnan et al., 1976)
Ibuprofen	Neonates	0-3h	21	0.06	0.004	0.11	(Aranda et al., 1997)
		3 d	27	0.35	0.2		(Van Overmeire et al., 2001)
	Infants	1.78 - 45.8	98	0.17	0.04	0.14	(Murry et al., 1999)
		0.25 -2.5	38	0.22	0.16		(Brown et al., 1992)
			46	0.26	0.29		
	Children	1.78 - 45.8	98	0.2	0.04	0.11	(Murry et al., 1999)

		2.5 -12	38	0.13	0.08		. (Brown et al., 1992)
			46	0.19	0.21		
		2.9 -8.5	22	0.27	0.1		(Scott et al., 1999)
		4 to 11	24	0.15	0.04		(Gelotte et al., 2010)
Itraconazole	Infants	0.5- 2	6	23.6	15.20	15.20	(Abdel-Rahman et al., 2007)
	Children	2 to 6	6	8.3	7.10	5.64	(Abdel-Rahman et al., 2007)
		8 to 18	26	18.9	5.30		(Groll et al., 2002)
Midazolam	Infants	1.75-4 y	6	0.03	0.004	0.02	(Rey et al., 1991)
		6 m- 13 y	13	0.05	0.004		(Muchohi et al., 2008)
		6 m - 2 y	5	0.1	0.03		(Reed et al., 2001)
	Children	1.75-4 y	6	0.03	0.004	0.24	(Rey et al., 1991)
		6 m- 13 y	13	0.05	0.004		(Muchohi et al., 2008)
		2 - 12 y	14	0.1	0.04		(Reed et al., 2001)
		12- 16y	2	0.03	0.01		
		6 to 18	20	0.01	0.01		(Tolia et al., 1991)
		5 to 9	12	1.87	0.57		(Jones and Baillie, 1979)

Table 6.3.3 Summary of clinical studies stratified into paediatric age bands for area under the concentration-time curve (AUC)

Compounds	Paediatric group	Age	Number of subjects	AUC [(mg.h/L)/(mg/kg)]			References
				Mean	SD	Overall SD	
Caffeine	Children	7 to 10 y	5	4.59	3.2	3.2	(Akinyinka et al., 2000)
Thophylline	Infants	1 to 5	6	9.49	3.45	3.45	(Kumar et al., 1989)
	Children	1 to 5	6	9.49	3.45	2.39	(Kumar et al., 1989)
		6 to 10	7	10.22	4.91		
		11 to 16	8	8.16	1.53		
		7 to 12	8	0.69	0.37		(Arnold et al., 1981)
		4 to 9	15	1.01	0.33		(Lee and Ngiam, 1983)
Ibuprofen	Neonates	3 d	27	89.40	28.40	25.89	(Van Overmeire et al., 2001)
		0-3 h	21	12.83	8.17		(Aranda et al., 1997)
		4- 72 h	20	40.26	34.73		(Sharma et al., 2003)

		30.45 W GSA	20	40.26	24.15		(Sharma et al., 2003)
	Infants	0.25 to 2.5	38	11.45	5.34	5.42	(Brown et al., 1992)
			46	10.25	5.49		
	Children	0.25 -12	38	17.66	13.26	8.56	(Brown et al., 1992)
			46	12.60	4.72		
		4 to 11	24	13.24	2.81		(Gelotte et al., 2010)
Itraconazole	Infants	0.5 to 2	8	1.5	0.54	0.52	(de Repentigny et al., 1998)
		0.5 to 2	6	0.85	0.49		(Abdel-Rahman et al., 2007)
	Children	2 to 6	6	3.80	4.53	1.71	(Abdel-Rahman et al., 2007)
		8 to 18	26	1.49	0.26		(Groll et al., 2002)
		2 to 5	7	1.50	0.54		(de Repentigny et al., 1998)
Midazolam	Neonates	2-5 d	10	11.00	6	6	(Jacqz-Aigrain et al., 1990)
	Infants	1.75-4 y	6	1.42	0.53	0.56	(Rey et al., 1991)
		6 m - 2 y	3	1.88	1.02		(Reed et al., 2001)
		6 m- 13 y	13	1.99	0.46		(Muchohi et al., 2008)
	Children	3 - 10 y	56	0.29	0.11	0.51	(Payne et al., 1989)
		1.75-4 y	6	1.42	0.53		(Rey et al., 1991)
		6.11	6	0.63	0.24		(Salonen et al., 1987)
		2 - 12 y	54	1.87	0.68		(Reed et al., 2001)
		12- 16y	10	1.96	0.81		

Table 6.3.4 Summary of clinical studies in adults that reported clearance

Compounds	Number of subjects	Clearance ($L.h^{-1}.kg^{-1}$)			References
		Mean	SD	Overall SD	
Caffeine	7	0.1	0.03	0.1	(Culm-Merdek et al., 2005)
	12	0.1	0.03		(Randinitis et al., 2001)
	84	0.1	0.1		(Kamimori et al., 2002)50mg
	84	0.1	0.1		(Kamimori et al., 2002)100mg
	6	0.1	0.02		(Newton et al., 1981)
Theophylline	11	0.1	0.02	0.01	(Jonkman et al., 1989)
	12	0.04	0.01		(Sirmans et al., 1988)
	10	0.04	0.01		(Bowles et al., 1988)
	8	0.1	0.01		(Oosterhuis et al., 1992)
	12	0.04	0.01		(Gillum et al., 1996)
	6	0.1	0.002		(Ko et al., 1999)
Ibuprofen	12	0.1	0.01	0.05	(Smith et al., 1994)
	24	0.1	0.01		(Sadaba et al., 2006)
	16	0.05	0.02		(Tan et al., 2003)
	8	0.05	0.01		(Martin et al., 1990)
	13	0.05	0.004		(Ochs et al., 1985)
Itraconazole	6	0.2	0.1	0.7	(Mouton et al., 2006)
	6	0.3	0.1		(Heykants et al., 1989)
	13	1.1	0.8		(Ducharme et al., 1995)
	5	1.8	1		(Hardin et al., 1988)
Midazolam	11	1.1	0.6	0.7	(Kokudai et al., 2009)
	40	1.1	0.8		(Lam et al., 2003)
	9	2.2	1.5		(He et al., 2005)
	20	1.6	0.9		(Tateishi et al., 2001)
	21	1.3	0.7		(Masica et al., 2004)
	20	0.3	0.1		(Thummel et al., 1996)
	15	0.2	0.04		(Wandel et al., 2000)
	6	0.3	0.1		(Heizmann et al., 1983)
	13	0.4	0.1		(Ibrahim et al., 2002)

Table 6.3.5 Summary of clinical studies in adults that reported volume of distribution

Compounds	Number of subjects	V_d (L.kg^{-1})			References
		Mean	SD	Overall SD	
Caffeine	7	0.5	0.1	0.3	(Culm-Merdek et al., 2005)
	12	0.5	0.1		(Randinitis et al., 2001)
	84	0.8	0.2		(Kamimori et al., 2002)50mg
	84	0.8	0.4		(Kamimori et al., 2002)100mg
	6	0.6	0.1		(Newton et al., 1981)
Theophylline	11	0.5	0.1	0.06	(Jonkman et al., 1989)
	12	0.5	0.1		(Sirmans et al., 1988)
	8	0.5	0.1		(Oosterhuis et al., 1992)
	6	0.5	0.04		(Ko et al., 1999)
Ibuprofen	12	0.1	0.03	0.03	(Smith et al., 1994)
	13	0.1	0.01		(Ochs et al., 1985)
	8	0.1	0.04		(Martin et al., 1990)
	8	0.1	0.04		(Ceppi Monti et al., 1992)
Itraconazole	6	7.2	2.2	2.6	(Mouton et al., 2006)
	6	11.4	2.9		(Heykants et al., 1989)
Midazolam	11	3.8	0.9	2.4	(Kokudai et al., 2009)
	40	5.7	3.4		(Lam et al., 2003)
	20	0.9	0.3		(Tateishi et al., 2001)
	13	1.4	0.4		(Ibrahim et al., 2002)

Table 6.3.6 Summary of clinical studies in adults that reported AUC

Compounds	Number of subjects	AUC [(mg.h/L)/(mg/kg)]			References
		Mean	SD	Overall SD	
Caffeine	7	15.19	6.07	9.47	(Culm-Merdek et al., 2005)
	12	19.52	4.29		(Randinitis et al., 2001)
	84	14.54	12.99		(Kamimori et al., 2002)50mg
	84	11.10	5.48		(Kamimori et al., 2002)100mg
	8	11.43	4.31		(Blanchard and Sawers, 1983)
Thoephylline	11	18.77	8.32	5.20	(Jonkman et al., 1989)
	10	21.90	4.40		(Bowles et al., 1988)
	8	23.46	5.01		(Boot et al., 2008)
	8	3.75	0.88		(Oosterhuis et al., 1992)
	12	28.00	4.80		(Gillum et al., 1996)
	6	21.57	2.20		(Ko et al., 1999)
Ibuprofen	4	10.48	1.73	4.47	(Evans et al., 1990) (400 mg)
	4	12.07	3.01		(Evans et al., 1990) (200 mg)
	12	8.93	1.89		(Smith et al.,

					1994)
	7	14.10	4.17		(Fornasini et al., 1997)
	24	21.71	5.67		(Kapil et al., 2004)
Itraconazole	12	4.49	2.94	1.84	(Jaruratanasirikul and Sriwiriyajan, 2007)
	12	0.29	0.15		(Conway et al., 2004)
	6	5.83	2.10		(Mouton et al., 2006)
	12	0.67	0.39		(Lohitnavy et al., 2005)
	5	0.74	0.37		(Hardin et al., 1988)
	13	1.24	0.72		(Ducharme et al., 1995)
	6	3.22	0.76		Heykants et al. (1989)
Midazolam	17	3.39	0.40	0.67	(Wermeling et al., 2009)
	40	1.41	0.83		(Lam et al., 2003)
	9	0.59	0.26		(He et al., 2005)
	21	0.50	0.26		(Masica et al., 2004)
	13	2.89	0.99		(Ibrahim et al., 2002)

6.3.2.2 From IVIVE-PBPK:

PBPK models combined with *in vitro-in vivo* extrapolation (IVIVE) are used to predict PK parameters. IVIVE is used to predict the absorption, distribution, metabolism and excretion (ADME) of drugs from *in vitro* systems and extrapolate to *in vivo* situations. PBPK models provide appropriate models for IVIVE by combining the system-specific properties, drug properties, and the structural model (Brown et al., 1992; Gibson and Rostami-Hodjegan, 2007; Jamei et al., 2009a; Rowland et al., 2011). The Simcyp simulator is an example of such models (Yeo et al., 2013).

Within Simcyp the prediction of CL is based on the determination of intrinsic CL (CL_{int}) from *in vitro* systems (hepatocytes, microsomes, recombinant CYP) and scaling CL_{int} to whole liver using relevant scaling factors and finally converting CL_{int} to hepatic metabolic and systemic CL values (Rowland et al., 2011). The prediction of V_d is based on the method by Rodgers *et al* that relies on tissue composition and its covariates (Jamei et al., 2009a).

PK parameters (CL, V_d and AUC) were simulated in Simcyp paediatric v11 for 1000 subjects in each age group. Simulations were designed to capture the neonate (0 - 1 month), infant (1 month - 2 years) and child (2 - 12 years) groups with the fraction of females set to 0.5. Doses for clinical trials in the simulations were based on the British National Formulary for children (BNFC) (Fowlie et al., 2011-2012). If there were several indications for the drug in paediatric patients, the doses in Table 6.3.1 and Table 6.3.2 were used in the trial design of simulations.

6.4 Results

6.4.1.1 Studies with NCA approach

A total of 26 reports on PK parameters for five compounds were found from the literature review over the age range birth to 12 years, of these 3 gave information on caffeine and on itraconazole, 5 on theophylline, 8 on ibuprofen and 7 on midazolam. The CL, V_d and AUC and the δ from non-compartmental PK studies in the paediatric groups are presented in Table 6.3.1 to Table 6.3.3. Table 6.3.4 to Table 6.3.6 present data on the same parameters in adults.

Comparison of SD from different sources and PK parameters (CL,V_d and AUC) for the five compounds are presented in Table 6.4.1.

Table 6.4.1 comparison of SD from different sources for CL, V_d and AUC.
Details of the studies in this table are shown in Table 6.2.1 to Table 6.2.3.

	SD for CL				SD for V_d				SD for AUC			
Drugs	**Neonates**	**Infants**	**Children**	**Adults**	**Neonates**	**Infants**	**Children**	**Adults**	**Neonates**	**Infants**	**Children**	**Adults**
Caffeine	0.004	NA	0.11	0.10	0.06	NA	NA	0.30	NA	NA	3.20	9.47
Theophylline	0.005	0.17	0.04	0.01	0.33	0.14	0.27	0.06	NA	3.45	2.39	5.20
Ibuprofen	0.005	0.1	0.03	0.05	0.11	0.14	0.11	0.03	25.89	5.42	8.56	4.47
Itraconazole	NA	0.55	0.31	0.70	NA	15.20	5.64	2.6	NA	0.52	1.71	1.84
Midazolam	0.10	0.20	0.24	0.70	NA	0.02	0.24	2.4	6.00	0.56	0.51	0.67

NA: No literature data for these paediatric age group were available.

6.4.1.2 PBPK simulation of SD for PK parameters

The $\bar{\mu}$ and δ of simulated PK parameters after normalisation for body weight and dose (mg/kg) are calculated for the same compounds as in NCA section. The simulated parameters are presented in Table 6.4.2 for neonates, Table 6.4.3 for infants and Table 6.4.4 for children.

Table 6.4.2 Parameter estimates obtained from analysis of simulations using PBPK model in neonates

Compound	CL ($L.h^{-1}.kg^{-1}$)		V_d ($L.kg^{-1}$)		AUC [(mg.h/L)/(mg/kg)]	
	Weighted Mean	Overall SD	Weighted Mean	Overall SD	Weighted Mean	Overall SD
Caffeine	0.01	0.007	0.45	0.14	2.12	3.09
Theophylline	0.02	0.01	0.47	0.14	7.34	17.17
Ibuprofen	0.001	0.0005	0.19	0.02	2.01	2.39
Itraonazole	0.19	0.16	10.68	3.22	0.04	0.10
Midazolam	0.15	0.17	0.99	0.30	5.50	5.57

Table 6.4.3 Parameter estimates obtained from analysis of simulations using PBPK model in infants

Compound	CL ($L.h^{-1}.kg^{-1}$)		V_d ($L.kg^{-1}$)		AUC [(mg.h/L)/(mg/kg)]	
	Weighted Mean	Overall SD	Weighted Mean	Overall SD	Weighted Mean	Overall SD
Caffeine	0.04	0.03	0.45	0.14	2.12	3.09
Theophylline	0.07	0.04	0.47	0.14	7.34	17.17
Ibuprofen	0.06	0.04	0.19	0.02	2.01	2.39
Itraonazole	0.51	0.23	329.98	116.71	0.04	0.10
Midazolam	0.31	0.20	1	0.3	5.50	5.57

Table 6.4.4 Parameter estimates obtained from analysis of simulations using PBPK model in children

Compound	CL ($L.h^{-1}.kg^{-1}$)		V_d ($L.kg^{-1}$)		AUC [(mg.h/L)/(mg/kg)]	
	Weighted Mean	Overall SD	Weighted Mean	Overall SD	Weighted Mean	Overall SD
Caffeine	0.07	0.06	0.45	0.14	9.40	15.89
Theophylline	0.13	0.08	0.47	0.14	5.34	10.43
Ibuprofen	0.07	0.04	0.19	0.02	12.28	17.92
Itraonazole	0.67	0.27	400.55	130.75	0.17	0.28
Midazolam	0.42	0.25	1	0.3	4.12	4.03

6.4.1.3 Impact of variability source on calculated sample size

Calculations for classical PK studies

Sample size estimates from the NCA approach are presented in Figure 6.4-2 to Figure 6.4-4. The results indicate that an overall trend could not be established with regard to whether

paediatric or adult clinical PK studies or PBPK simulations will lead to consistently higher or lower variability and hence higher or lower sample size. There were variations in the estimated variability between age groups. For example the order of highest to lowest estimates of variability for midazolam CL in “neonates” follows the order of PBPK> neonates> adults but for the same parameter in “infants” and “children” the order is adults>PBPK>infants/children. Using precision limits (0.8 and 1.25 folds) as the measure for the level of significance, SD_{Paed} from theophylline CL in “infants” returned significantly higher sample size than SD_{Adult} and SD_{PBPK}. There were discrepancies in the estimated variability between compounds. For example, SD_{Paed} from V_d in “children” returned over 2 fold higher sample size than SD_{Adult} for “theophylline” whereas both sources returned similar number of subjects in “itraconazole”. The order of estimated variability was consistently different between PK parameters. For example, for ibuprofen SD_{Paed} from “V_d” in neonates follows the order of neonates>adults>PBPK but “AUC” for the same compound in the same age groups follows PBPK> neonates= adults.

Even for PK parameters of a compound in the same age category an overall trend could not be established. For example for children, SD_{Paed} of V_d from ibuprofen, sample size is over threefold higher than estimated by SD_{PBPK} (10 versus 3). Assuming precision limits as a measure of significance, SD_{Adult} for ibuprofen AUC retuned significantly lower sample size than SD_{Paed} and SD_{PBPK} (5 versus 9 and 11). The CL and V_d SD_{Adult} for ibuprofen, shows four-fold difference in sample size.

Considering this lack of information in neonates and infants, in around 60% of the cases SDs from paediatric groups estimated significantly different sample size with PBPK simulations and in over 70% of the occasions estimated a significantly different sample size from adult. Table 6.4.5 and Figure 6.4-1 provide all the calculated sample sizes for these compounds.

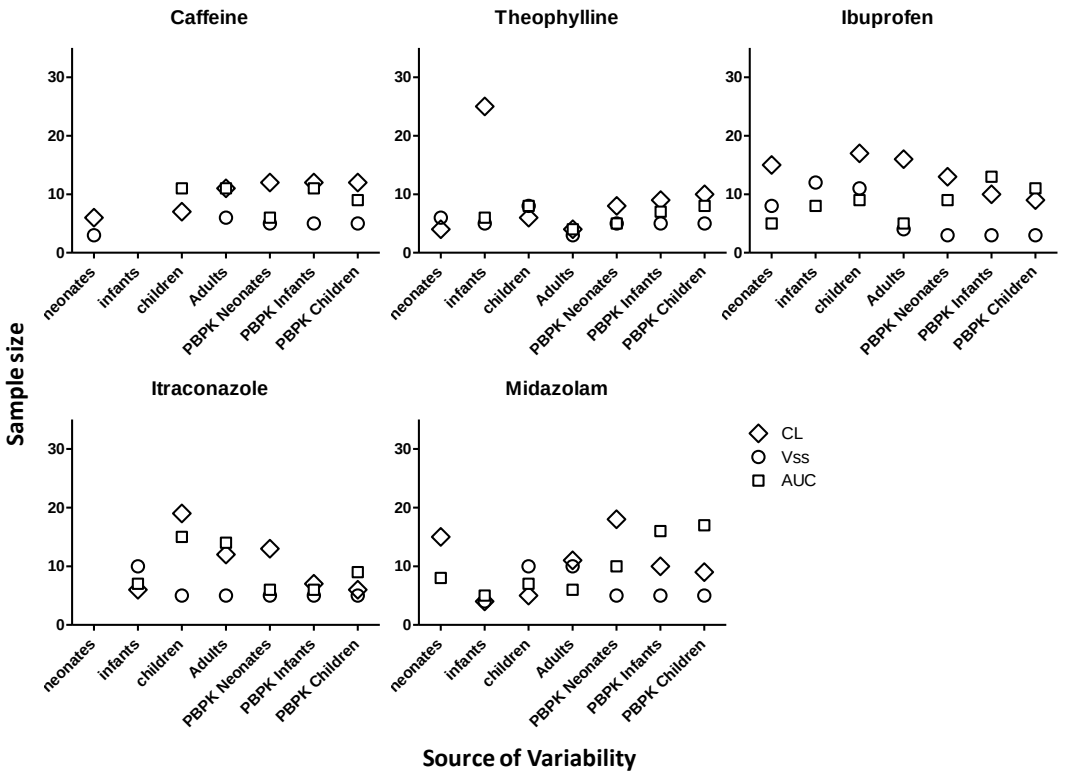

Figure 6.4-1 Comparing sample size estimates for three PK parameters for caffeine, theophylline, ibuprofen, itraconazole and midazolam with regard to age groups as the source of variability in estimation of sample size.

Table 6.4.5 Sample size obtained using Wang's method for NCA from different sources of variability

Compound	Parameter	Sources of variability						
		Neonates	Infants	Children	Adults	PBPK Neonates	PBPK Infants	PBPK Children
Caffeine	CL	6	NA	7.00	11	12	12.00	12
	V_d	3	NA	NA	6	5	5.00	5
	AUC	NA	NA	11	11	6	11	9
Theophylline	CL	4.00	25	6	4	8	9	10
	V_d	6	5	8	3	5	5	5
	AUC	NA	6	8	4	5	7	8
Ibuprofen	CL	13	13	6	16	13	10	9
	V_d	13	12	10	4	3	3	3
	AUC	5	8	9	5	9	13	11
Itraconazole	CL	NA	6	19	12	13	7	6
	V_d	NA	10	5	5	5	5	5
	AUC	NA	7	15	14	6	6	9
Midazolam	CL	15	4	5	11	18	10	9
	V_d	NA	11	10	10	5	5	5
	AUC	8	5	7	6	10	16	17

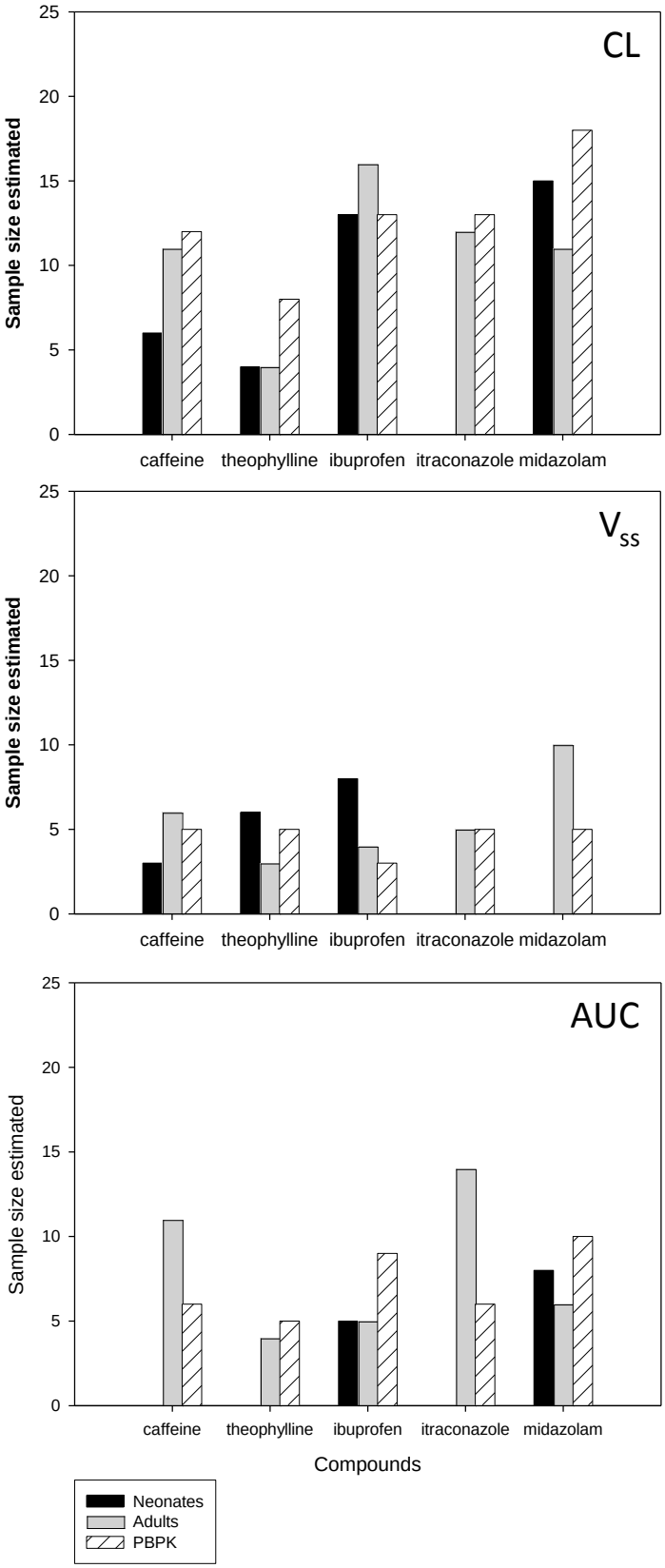

Figure 6.4-2 Calculated Sample size for a prospective PK clinical trial in neonates using the variability around CL, V_d and AUC from conventional PK studies in neonates or adults and the variability from simulation of the relevant PK parameter in neonates using Simcyp paediatric simulator.

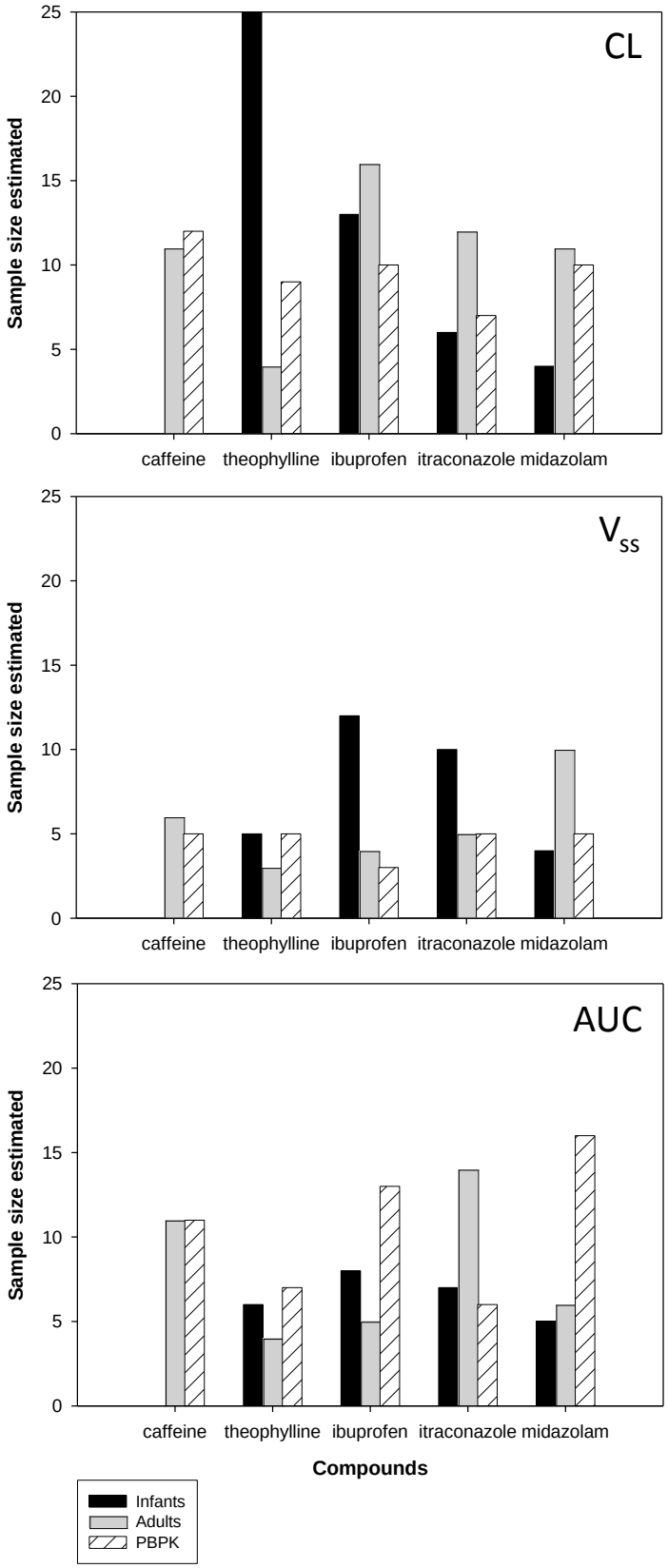

Figure 6.4-3 Calculated Sample size for a prospective PK clinical trial in infants using the variability around CL, V_d and AUC from conventional PK studies in infants or adults and the variability from simulation of the relevant PK parameter in infants using Simcyp paediatric simulator.

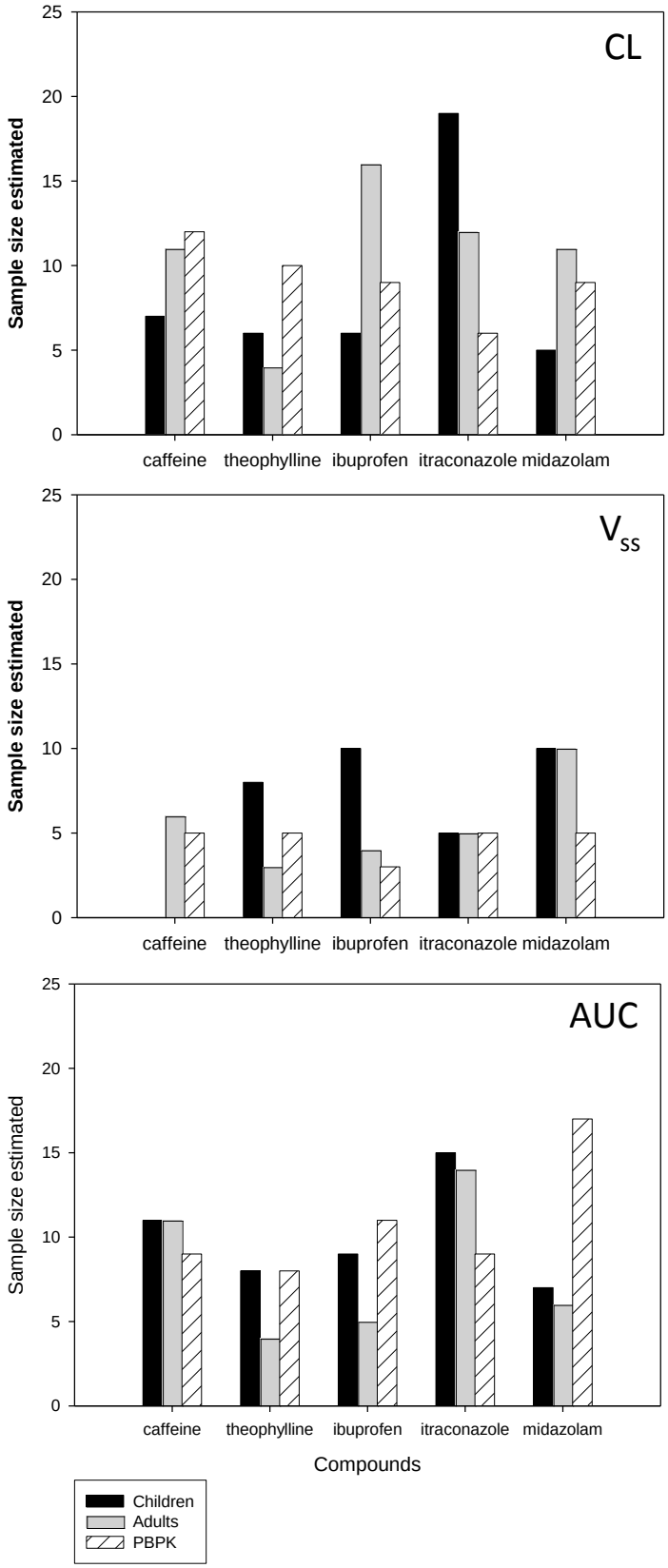

Figure 6.4-4 Calculated Sample size for a prospective PK clinical trial in children using the variability around CL, V_d and AUC from conventional PK studies in children or adults and the variability from simulation of the relevant PK parameter in children using Simcyp paediatric simulator.

6.5 Discussion

FDA and European Medicine Agency (EMA) support conducting PK studies in children of all age groups especially where the drugs are used for a specific indication in paediatric patients or when a drug has non-linear PK in adults (FDA, 1998; Conroy and McIntyre, 2005). In designing any experiment it is important to include sample size calculation at the design stage, this allows inference from the experiment to be drawn with adequate statistical power. Sample size calculation is often based on the hypothesis to be tested and the method for analysis of the data from the experiment. However, in cases such as PK studies there is no clear hypothesis to be tested, and the sample size can be related to some degree of precision for estimating the confidence interval on parameters of interest (Armitage et al., 2002; Ogungbenro and Aarons, 2008). In these cases the precision on the PK parameters especially CL will be applied on appropriate dosing decisions.

Although, adults and paediatric clinical PK studies have been conducted for many years, there is lack of a defined strategy to calculate the sample size for a potential PK study. The FDA 1998 draft guidance advocates the use of 6 to 12 paediatric subjects in clinical trials, irrespective of the PK of the compound (FDA, 1998). Wang *et al*., (2012) described methodologies for sample size calculations for NCA and population PK study design based on estimating confidence interval on parameters of interest with certain precision limits. For NCA analysis, expressions and scripts implemented in the R programming language were provided. These expressions cannot be applied directly to population PK experiments but the principle can be applied. Sample size for population PK experiment is determined by using simulation to obtain the power to estimate the confidence interval of a parameter of choice within pre-specified precision limit by making stepwise increases in the sample size. The method uses prior information on the model and parameter estimates in the simulation. The approach is described in details by Ogungbenro et al (2008). The prior information could be obtained from any related clinical population PK study in adult or paediatric or simulation using PBPK models.

We compared the variability from a number of sources and used them to estimate the samples size. As for any other experiment, higher variability leads to larger sample size. The results obtained from this analysis show that the magnitude of variability between sources

and age groups are not consistent therefore there is no consistency in the estimated sample sizes for different age groups, PK parameters and drugs.

The physiological and biochemical changes in paediatric subjects from birth onwards influence PK parameters and their variability and therefore, variability in adult PK parameters may not reflect the variability in children. A recent study compared the variability for CL in a number of drugs between children over 6 years and adults and suggests that there is no significant statistical difference between the CV% from these groups (Edginton et al., 2013). However the study did not consider variability in neonates and infants where most of the changes in anatomy and physiology are expected to be more pronounced. In the present study, children from birth to 12 years have been considered and the results showed there are significant differences in the calculated sample sizes when the SD was obtained from meta-analysis of observed paediatric studies compared to SD from adult studies or paediatric PBPK simulations.

Wang's approach could also be applied in calculation of sample size to study a new drug for which adult PK data is not readily available. In this case variability for a drug in that category with a similar physiochemical and elimination properties can be used for the sample size calculation. For example, the knowledge of PK variability from omeprazole or pantoprazole to estimate the sample size for a potential PK study on a new proton pump inhibitor with similar physiochemical and metabolic characteristics can be used in the sample size calculation. The fact that caffeine and theophylline sample sizes from PBPK simulations based on the results obtained in the present analysis are not significantly different may suggest potential validity of this assumption. However, if the physiochemical characteristics were different to a degree that they affect the PK component (e.g. protein binding, affinity to certain enzymes or transporters, passive permeability through membranes), some differences in variability of the PK parameters (CL, F, V_d) should be expected and variability should be handled more carefully.

The review of the literature on classic paediatric clinical studies revealed that methods for calculation of CL can result in a different variability estimates for this parameter. The paediatric studies used to derive sample size in the current study calculated CL from (i) dose and AUC, (ii) rate of drug delivery and established steady state concentration, or (iii) V_d and

elimination rate constant in one or two compartmental models. We also found the paucity of data on the investigated parameters (CL, V_d and AUC) in neonates (especially for theophylline and itraconazole) and in infants (especially for caffeine) in the literature. In addition, the number of samples taken from subjects at different ages may impact the variability around the PK parameters of interest. However, analysis of the impact of number of sampling and sampling times on calculated sample size is beyond the scope of this report.

There are a number of limitations of the analysis presented in this study. For instance not all the PK parameters were investigated in all clinical studies and thus some were not available in selected paediatric studies. Many studies did not provide individual doses or body weights and average values were used or assumptions were made in the normalisation. This can affect the estimate of the variability. It should also be noted that these shortcomings will equally apply to any exercise involving samples size calculations for paediatric studies and are a reflection of the reality regarding available sources to give initial estimates of variability and not specific to this study only.

6.6 Conclusion

In conclusion, the estimation of the number of subjects for the design of a paediatric study with adequate power is essential. However, such calculations rely on estimates of variability for the parameter of interest. The outcome of the current study demonstrates the significant variations in estimates of variability depending on the “source” of the data to derive the values. There was no consistent discrepancy in the sample size calculated according to the source of variability used in the sample size calculations. Moreover, it is not known which of the sources investigated in this study is a better reflection of the true PK parameter variability for a given drug in a given age range. Variability around simulated PK parameters from PBPK models could be used to calculate the sample size although in our study it led to significantly different sample sizes from paediatric observed SDs. Well-established large studies in paediatric population for various types of drugs (using different administration routes) will be necessary to better understand the reliability of the sources of initial estimates for PK parameter variability.

Chapter 7. Summary, concluding remarks and perspective

7.1 Background

This chapter briefly summarises the work described in this thesis and makes suggestions for future expansion of the work. An overview of how the chapters of this thesis fit together is shown in Figure 7.1-1.

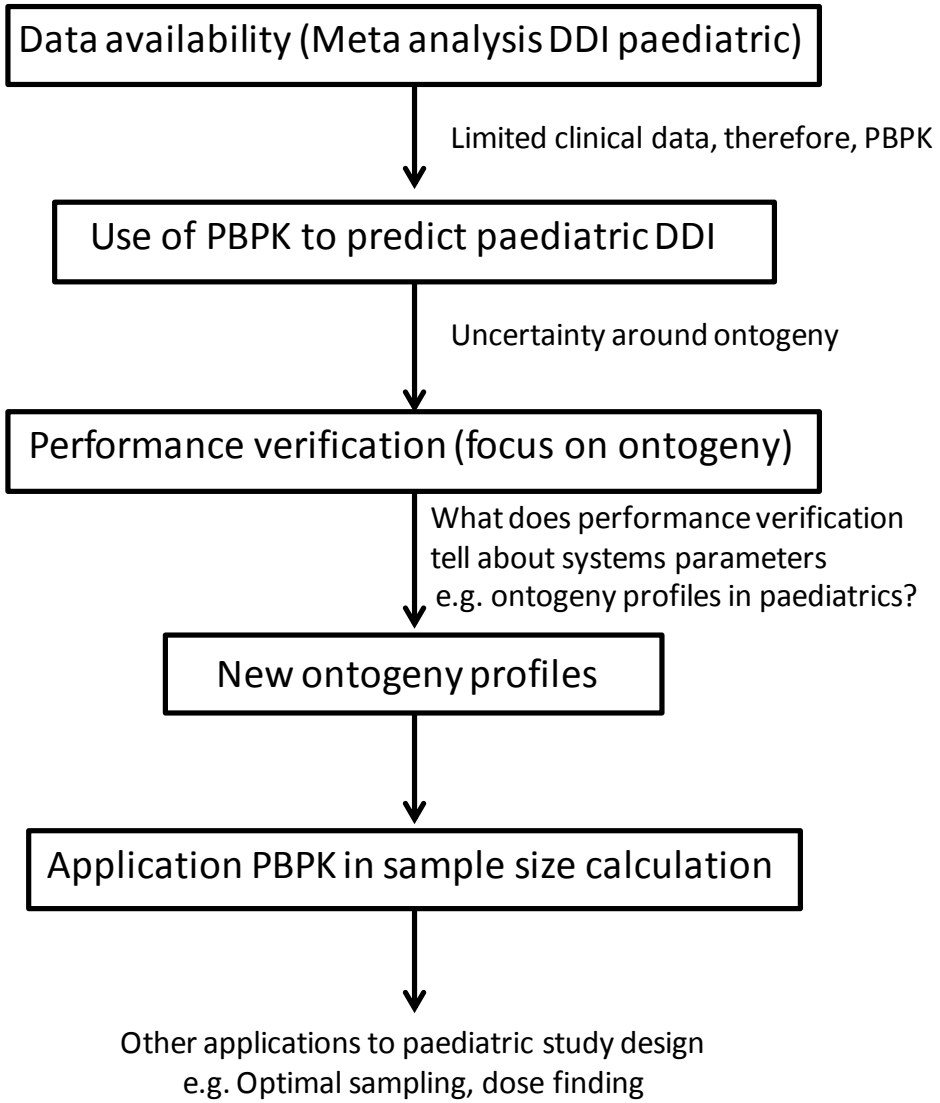

Figure 7.1-1 summary of sequence of chapters in this thesis

As outlined in the Introduction (chapter one), DDI is a clinical problem and is an important cause of suboptimal therapeutic effect or drug toxicities manifested by adverse drug effects. While paediatric patients especially neonates are the most vulnerable group of patients and

clinicians take extra care when dealing with these patients, there is little information on PK of many drugs in this age group. This knowledge gap causes decision making in these situations to rely mainly on adult observation/data and guesswork. We hypothesised that the level of DDI might change with age due to growth and maturation of metabolic pathways involved in metabolism and elimination of drugs; however, there is little evidence about DDI differences between paediatrics and adults. In this thesis, we have attempted to highlight the knowledge gap on DDIs in paediatric clinical pharmacology and apply modelling and simulation techniques to investigate the effect of age on DDIs. Since several parameters are involved in quantification of DDI, we had to investigate the determining factors that indirectly contribute to drug metabolism and DDI.

7.2 Summary

Critical evaluation of DDIs in the literature: An evaluation of the published literature on paediatric metabolic DDI studies and corresponding pairs in adults has been performed in this thesis to highlight the DDI differences between adult and paediatric populations and the lack of a formal guideline for dealing with DDIs in paediatric patients. Clinical data collection from two UK children hospitals was conducted to provide an overview of paediatric DDIs in current clinical practice and a few DDI cases were identified. The results of critical evaluation of the literature showed that although DDIs in adults are well-understood through numerous well-conducted clinical studies, paediatric data in this context is still sparse. Even, when the data is available, the analysis of data is not simple because of diversity in the reported parameters and their variability. However, we identified several cases of DDIs that made the comparison possible and also we identified several cases of DDIs in children that are not reported in adults. The lack of DDI in adults could be associated with clinical use of drug restricted to paediatric patients only or there is also a chance that DDI exists in adults but not reported. The age related changes in CL of drugs combined with the evidence on DDI differences in some paediatric and adults clinical PK studies suggests that DDI could change with age but the amount of data available in the literature is not sufficient to establish a trend. Perhaps if adequate data on plasma concentration-time profile n presence and absence of perpetrator were available from patients, a POPPK analysis identifies a trend by accounting for different covariates. However, Duan et al (2010) have shown that most POPPK models

give biased estimates due to structural models not accounting for links between the inhibition of the first-pass metabolism alongside the changes in clearance (Duan et al., 2011). Due to the dearth of DDI data in different paediatric age groups in the literature and difficulties in conducting paediatric clinical DDI studies, modelling and simulation approach was adopted to hypothesise the DDI changes using a paediatric PBPK simulator (Simcyp paediatric). The model was used to try and quantify changes in the magnitude of DDIs with age for some theoretical drugs eliminated by different pathways.

Age related changes in DDI: The principal behind the level of DDI relies on the ratio of exposure to the victim drug in presence and absence of a perpetrator and a major element in this approach is the fraction of drug metabolised by an inhibited pathway (fm). The activity (CL_{int}) and protein abundance of enzymes are the most important factors deriving fm and consequently, the metabolism and elimination of drugs from the body. Age related changes in activity and protein abundance that are shown in the form of ontogeny of metabolic pathways combined with size factors determine the CL changes with age. Therefore, inhibition of a metabolic pathway at each age leads to a different DDI level that may or may not significantly be different from adults. As a consequence, to investigate any age related changes in DDI, it is crucial to step back and investigate the ontogeny of major metabolic pathways.

Hepatic metabolic pathways mature at different rates as shown in the ontogeny profile of CYPs. These ontogeny profiles are created on the basis of *in vitro* enzyme activity or enzyme abundance and are reported in the literature by different groups (Bjorkman, 2005; Edginton et al., 2006a; Johnson et al., 2006). We have shown the differences in the rate of ontogeny for different CYPs relative to each other and associated it with the ages at which these discrepancies will lead to a more significant DDI in presence of a potent inhibitor. We used modelling approach and bootstrapping techniques to build confidence intervals around these age ranges. The reason for adopting this methodology was the variable amount of *in vitro* data on human liver microsomes from subjects of various ages without reporting the variability around the data in an appreciable number of reports.

The age varying fm suggests that based on the rate of differential ontogeny, one pathway might be absent at birth and develop later in neonatal and infancy periods. Therefore,

another pathway may have a more dominant role in metabolism of the drug. Introducing a potent inhibitor of the dominant pathway at a specific age leads to a more significant DDI at that age. These types of data are sparse in the literature for real compounds but can be simulated with hypothetical compounds to investigate the validity of this hypothesis. The results of simulations for our hypothetical compounds show that DDI could be higher or lower than adults in neonates and change with age. We had a critical look at p-PBPK models and especially Simcyp paediatric simulator. We assessed Simcyp performance with the view of potentially applying PBPK models in drug development and clinical application in children. Because the accurate prediction of changes in DDI with age will depend on how accurately the ontogeny profiles for the different elimination pathways are described in the model the next stage was to undertake a performance review on how well the simulator describes the age related changes in CL for substrates predominantly metabolised by one enzyme.

Performance verification of Simcyp paediatric simulator: Prior to utilising p-PBPK models for DDI predictions, the performance of the model should be tested using a variety of compounds in absence of an inhibitor. It is not expected that the paediatric model performs well if that PBPK model does not adequately simulate the adult PK parameters. For this reason performance verification for four compounds was conducted to simulate adults and paediatric PK clinical data. There are several PK parameters and options in Simcyp PBPK model but in this thesis we focused on prediction of AUC from ''enzyme kinetic'' and ''retrograde *in vivo*'' CL options. The outcome of the simulations suggested that there are some discrepancies in simulated *vs.* observed data especially for CYP1A2 and CYP3A and further refinement of the p-PBPK model is required through examining ontogeny models and model assumptions. Following the results of the performance verification and independent simulations performed by Leong et al., (Leong et al., 2012), we decided to investigate further the ontogeny of CYP1A2 and CYP3A4 and to try to develop *in vivo*-based ontogeny functions based on CL of probe substrates and compare against the existing *in vitro* derived functions in the model.

Novel ontogeny functions for CYP1A2 and CYP3A4: In order to address the discrepancies between predicted and observed CL values, a critical comparison of CYP1A2 and CYP3A4 from *in vitro* with corresponding *in vivo* data was performed. The reason why

CYP1A2 and CYP3A4 were selected for this investigation was that the probes for these enzymes are commonly used in paediatrics of all age groups and PK data is available at different age ranges.

Ontogeny models are incorporated into p-PBPK models and considered as system data in p-PBPK models. The accuracy of ontogeny models is highly important because any problem in these ontogeny models is directly reflected by over- or under-prediction of CL in simulations. There are a number of p-PBPK models and in this thesis we concentrated on the Simcyp p-PBPK model. Ontogeny profiles from Johnson et al, (Johnson et al., 2006) were re-visited and updated based on the most recent available data (Hines, 2007; Stevens et al., 2008). Novel ontogeny profiles were created by deconvolution of *in vivo* CL data using a retrograde well-stirred model approach to remove the effect of size from CL_{int}. New ontogeny profiles were derived on the basis of *in vivo* paediatric CL_{int} relative to adult CL_{int} data and appeared to describe clinical data more precisely. A few studies applied a similar approach and modelled the CL of probe compounds with age (Anderson et al., 2000; Anderson and Holford, 2011; Anderson and Larsson, 2011; Ince et al., 2013). The difference in the two approaches is that the latter profile is specific to certain compounds but since we used stripped data, the models can be applied to any CYP3A4 or CYP1A2 substrate. New ontogeny CYP1A2 showed significant improvement in prediction of CL for theophylline and ropivacaine but CYP3A4 showed little improvement when alfentanil CL was predicted. Very recently, it was suggested CYP3A4 activity is affected by clinical condition of patients. Critical illness, prematurity, inflammation and infection are the factors that reduce CYP3A4 activity (Ince et al., 2013; Machavaram et al., 2013) and these factors were considered in the new models as much as possible. Application of p-PBPK models is becoming more popular especially with the surge of interest from regulatory authorities and scientists in paediatric drug development. These models are still evolving and the on-going refinement of these models when the data becomes available is required. However, to avoid unnecessary clinical trials and in the absence of adequate clinical data on DDI, PBPK models are a good alternative. Regulatory authorities advocate conducting paediatric clinical PK studies and as well as use of PBPK models where there is confidence in performance of these models. PBPK models allow design of a rational clinical trial and take guess work out of clinical

practice. It is expected that the outcome of simulations by PBPK models be confirmed by clinical studies (Johnson and Rostami-Hodjegan, 2011). Existing PBPK models have some limitations and inevitably make some assumptions. A recent regulatory report highlighted some benefits of using p-PBPK models. In this report, some paediatric clinical PK data were simulated and compared with corresponding observed values (Leong et al., 2012). Another important aspect of this paper is how to compare the predicted *vs.* observed data.

PBPK models not going to replace clinical studies but they can feed into study design. Despite the current limitations, potential application of p-PBPK models is wide ranging from dose finding, optimal study design, sample size calculation, DDI prediction, individualisation of drug therapy and new drug development. Validated PBPK models have the potential to save drug developers from conducting unnecessary clinical studies and spending millions of pounds. Further refinement of p-PBPK models by continually updating the algorithms for different elements such as CYP ontogeny will potentially facilitate their application in new areas such as sample size estimation for paediatric clinical studies.

Calculation of sample size for conduct of paediatric clinical studies: As supported by the FDA and EMA, paediatric clinical PK studies are conducted under special conditions. As a by-product of p-PBPK models we utilised a methodology by Wang et al. 2012 (Wang et al., 2012) and applied the predicted variability around the PK parameters generated by the Simcyp paediatric simulator to determine the sample size for conducting potential paediatric clinical PK studies. Clearly, higher variability (SD here) will lead to a larger sample size for a potential study; however, none of the PK parameters or age groups consistently returned higher or lower samples size.

7.3 Conclusion

The work described in this thesis has highlighted some limitations and problems associated with prediction of DDI in paediatric patients. A critical evaluation of literature indicates that there is lack of (qualitative) data and analysis of these data is not simple. DDIs might be different between paediatric and adult groups and despite limitations of PBPK models, this difference can be predicted by p-PBPK models when there is a disparity in the rate of differential ontogeny. *In vitro* based ontogeny models sometimes do not adequately predict PK parameters. It was shown in this thesis that *in vivo*-based ontogeny models for CYP1A2

and CYP3A4 will perform more efficiently in prediction of CL. Modelling and simulation tools such as p-PBPK models facilitate the prediction of DDIs. These models require validation in adults and then in children for prediction of PK parameters. The application of p-PBPK models is becoming increasingly popular but these models still require further refinements which will ideally involve close collaboration between academia, commercial p-PBPK model developers, the pharmaceutical industry and the regulators.

7.4 Future work

Considering the novelty of p-PBPK models and their potentials and limitations in conducting clinical PK studies, there are several directions to take and help development of existing models. The quality of the predictions directly relates to the quality of input data.

- In the future more quality *in vitro* and *in vivo* data is required to refine the currently used models and especially CYP3A4. These models require constant evaluation with a wide range of drugs and at different age groups. To refine p-PBPK models through adding more complicated features such as absorption procedure, entrohepatic cycle, transporter abundance and additional organs or compartments.
- It is beneficial if clinical PK studies report adequate and detailed demographic information for individuals rather than mean parameters of the population. This information will facilitate the comparisons between observed and predicted data and reasoning why certain differences are expected.
- Considering the dearth of data on ontogeny of CYPs, UGTs and transporters more data is required to further develop the current ontogeny models. It is an interesting practise to investigate top-down *vs*. bottom up approach ontogeny for other CYPs such as CYP2C9.
- The newly developed *in vivo* based CYP1A2 and CYP3A4 models need to be tested for other several probes of CYP3A4 and CYP1A2 or with a new data set for the same probes when the data becomes available.
- Hypothetical DDIs should be tested using *in vivo*-based ontogeny models to investigate whether the pattern of hypothetical DDIs with age are consistent with those of *in vitro* models.

- The focus of this thesis was on inhibition and it is interesting to see how age related changes will apply to induction of metabolic pathways.
- To further evaluate and explore novel applications of p-PBPK in sample size calculation and in optimal PK sampling.
- To look at the pharmacogenetic aspect and investigate how genetic differences at early ages will affect the DDI.

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Appendix 1

Supplemental Table S 1 Comparison between paediatric and adult DDI studies for the drug pairs reported in the literature.

Paediatric DDI							**DDI outcome**	**Adult DDI**			**Measuring fold changes in exposure**		
Disease/indication	**N**	**Age range (y)**	**Victim Drug**	**Interacting Drug**	**Study Type**	**Paediatrics References**	**Impact of perpetrator on subjects or victim drug**	**N**	**Age range (y)**	**Adult Refs**	**PK Parameter used in adults (fold change in exposure)**	**PK Parameter used in paediatric (fold change in exposure)**	**Ratio of fold change in exposure Paed: Adult**
Acute lymphoblastic leukaemia	14	3 to 14	6-mercaptopurine	Methotrexate	Prospective study	(Balis et al., 1987)	increased exposure	**NO DDI reported in adults for 6-mrcaptopurine and Methotrexate**					
	10	4 to 16			Prospective study	(Innocenti et al., 1996)	increased exposure						
	2	4 to 6.5			Case report	(Andersen et al., 1998)	ADR						
bronchopulmonary dysplasia & Apnea of prematurity	1	28 w GSA	Adenosine	Theophylline	Case report	(Berul, 1993)	suboptimal effect	5		143			
Attention-deficit/hyperactivity disorder (ADHD)	127	7 to 17	Atomoxetine	Fluoxetine	Prospective study	(Kratochvil et al., 2005)	no DDI in paediatrics	**NO DDI reported in adults for Atomoxetine and Fluoxetine**					
HIV	10	4 to 12	Azithromycin	Atovaquone	Prospective study	(Ngo et al., 1999)	lower exposure	**NO DDI reported in adults for Azithromycin and Atovaquone**					
Perigastrointestinal disease	11	2.5 to 15	Caffeine	Cimetidine	Prospective study	(Parker et al., 1997)	no DDI in paediatrics	5	20-21	144			

	1	17		Clarithromycin	Case report	(Stafstrom et al., 1995)	ADR			145				
Epilepsy/ Attention-deficit/hyperactivity disorder (ADHD)	2	NA	Carbamazepine	Erythromycin/Pediazole	Case report	(Straughan, 1982)	ADR	7	24-27	146				
	1	8				(Mota et al., 1996)	ADR	8	24-36	147				
	1	9				(Kessler, 1985)	ADR	1	41	148	trough concentration (2.14)	Concentration (3.17)	2.41	
	2	3 & 9				(Stafstrom et al., 1995)	ADR	4	20-25	149				
	1	6				(Zitelli et al., 1987)	ADR				AUC (1.4)	Concentration (2.17)	1.64	
	4	1 to 10			Retrospective study	(Hedrick et al., 1983)	ADR				CL (1.32)	Concentration (2.00)	1.52	
	1	13		Methylphenidate	Prospective study	(Schaller and Behar, 1999)	suboptimal effect	**NO DDI reported in adults for Carbamazepine and Methylphenidate**						
	13	9.38[a]		Phenobarbitone	Prospective study	(Liu and Delgado, 1995)	lower CBZ level to dose ratio but not statistically significant	41	19-51	150				
	17	10.88[a]		Phenytoin	Prospective study	(Liu and Delgado, 1995)	lower CBZ level to dose ratio							
	1	15		Primidone	Case report	(Benetello and Furlanut, 1987)	Suboptimal effect	135		151				
	31	8.98[a]		Valproate	Prospective study	(Liu and Delgado, 1995)	higher exposure to CBZ-E	17	20 to 62	152	Concentration (1.08)	Concentration (0.82)	0.76	
	90	2 to 19			Prospective study	(Schoeman et al., 1984)	lower plasma concentration	24		153	Concentration (1.08)	Concentration (0.92)	0.86	

	55	6.3 to 18.2		Other AED		(Steinborn, 2005)	no difference in unbound PK between bitherapy groups						
Meningitis, pneumonia, brain abscess, rocky mountain spotted fever and Staphylococcus epidermidis shunt infectionventriculojugular	34	19 m	Chloramphenicol	Phenytoin/ Phenobarbitone	Prospective study	(Krasinski et al., 1982)	lower plasma concentration with phenobarbital and higher olasma concentration with phenytoin	**NO DDI reported in adults for chloramphenicol and phenytoine/phenobarbitone**					
	1	7			Case report	(Powell et al., 1981)	subtherapeutic peak concentrations						
Malaria	17	6 to 12	Chloroquine	Chlorpheniramine	Prospective study	(Okonkwo et al., 1999)	higher exposure	15	20-28	154	AUC (0.79)	AUMC (1.73)	2.2
Renal transplant	6	3 to 14	CsA	Carbamazepine	Prospective study	(Cooney et al., 1995)	subtherapeutic concentrations			155			
Renal transplant/ idiopathic nephritic syndrome	1	3		Clonidine	Case report	(Gilbert et al., 1995)	increased exposure	8	39[a]	156			
	78	0.51[a]		ketoconazole	Retrospective study	(El-Husseini et al., 2006)	increase therapeutic efficiency	36	20-59	157	trough concentration (1.15)	trough concentration (0.88)	1.01
	15	13		Mycofenolate mofetil	Prospective study	(Pape et al., 2003)	subtherapeutic concentrations	60	21-66	158			
	12	9.8[a]			Retrospective study	(Filler, 2004)	subtherapeutic concentrations						
	6	3 to 18		Nifedipine/verapamil	Prospective study	(Crocker et al., 1994) *	decreased CL	1	30	159			
	2	4 to 11			Case report	(Ogborn et al., 1989)	decreased CL by verapamil						
	5	8[a]		Norfloxacin	Retrospective study	(McLellan et al., 1995)	decreased CL	6	NA	160	trough concentration	Dose (1.64)	1.41

							1	57	161	(1.16)			
Severe aplastic anaemia	1	4		Phenobarbitone	Case report	(Carstensen et al., 1986)	subtherapeutic concentrations	1	43	162			
Kidney transplant	1	NA			Prospective study	(Burckart, 1984)	CL is influenced						
Cancer	22	2m to 18y	Cyclophosphamide	Fluconazole	Retrospective study	(Yule et al., 1999)	decreased CL	56	NA	163	AUC (1.79)	AUC (1.29)	0.72
HIV	60	2m to 12y	Dapsone	Rifabutin	POPPK study	(Mirochnick et al., 2001)	increased CL	12	24-63	164			
Congestive cardiomyopathy	9	6m to 18y	Digoxin	Amiodarone	Prospective study	(Koren et al., 1984)	higher exposure	34	43-84	165	Clearance (1.40)	Concentration (3.07)	2.19
								10	21-36	166			
								7	NA	167			
								8	23-34	168			
								22	18-75	169			
Ventricular failure	8	2w to 7.8		Carvedilol	Prospective study	(Ratnapalan et al., 2003)	decreased CL	8	23-27	170	AUC (1.20)	Clearance (1.9)	1.69
Congenital heart disease	1	7 d		Josamycine	Case report	(Cambonie et al., 2006)	higher exposure	**NO DDI for digoxin and josamycin**			-		
HIV & tuberculosis	15	3 to 15	Efavirenz	Rifampicin	Prospective study	(Ren et al., 2009)	no significant DDI in paediatrics	24	22-58	171	AUC (0.78)	trough concentration (0.97)	1.23
Solid tumours	18	4.4 to 20.7	Etoposide	CsA	Prospective study	(Bisogno et al., 1998)	increased exposure	16	NA	172	AUC (1.25)	AUC (1.47)	1.52
Acute myeloid leukaemia	38	8m to 17y				(Lacayo et al., 2002)	decreased CL				AUC (1.25)	AUC (1.9)	1.18
Acute lymphoblastic leukaemia	109	0.4 to 18.7		Prednisone		(Kishi et al., 2004)	increased CL	**NO DDI for Etoposide and Prednisone**					

NA	40	<216m	Felbamate	Phenytoin	POPPK study	(Kelley et al., 1997)	increased CL	700	<6 to >66	173			
HIV	16	0 to 24	Fluticasone	Ritanovir/lopinavir	Retrospective study	(Arrington-Sanders et al., 2006)	ADR			174			
	1	9			Case report	(Bhumbra et al., 2007)	ADR	1	45	175			
	1	13			Case report	(Pessanha et al., 2007)	ADR	1	44	176			
								1	27	177			
Tinea capitis	2	7 & 8	Griseofulvin	phenobarbitone	Case report	(Beurey et al., 1982)	suboptimal therapeutic effect	16	NA	178	AUC (0.69)	AUC (0.24)	0.35
Elective procedure	83	3m to 17y	Halothane	Epinephrine	Prospective study	(Karl et al., 1983)	ADR	100	13-72	179	-		
								9	39.9[a]	180			
Minor elective surgery	60	2 to 9		Vecuronium/ atracorium	Prospective study	(Sloan et al., 1998)	no significant DDI in paediatrics	20	46[a]	181			
								25	35 & 40[a]	181			
Acute lymphoblastic Leukemia	1	8	Ifosphamide	Phenytoin	Case report	(Ducharme et al., 1997)	increase therapeutic efficiency	**NO DDI reported in adults for ifosphamide phenytoine**					
Attention-deficit/ ADHD/ agression	36	7 to 16	Imipramine	Carbamazepine	Prospective study	(Brown et al., 1990)	subtherapeutic concentrations	13	48[a]	182	Concentration (0.58)	Concentration (1.06)	1.83
	2	9		propranolol	Case report	(Gillette and Tannery, 1994)	increased exposure	**NO DDI reported in adults for imipramine and propranolol**					
	1	3	Ketoconazole	Rifampicin	Case report	(Engelhard et al., 1984)	decreased exposure	6	24[a]	183			
Epilepsy	19	8m to 30y	Lamotrigine	Sodium valproate/ AED	Prospective	(Battino et al., 2001)	increased/decreased clearanceconcentration	20	33.4[a]	184	AUC (1.29)	Clearance	0.76

					study							(0.99)	
	1	13			Case report	(Ferrie and Panayiotopoulos, 1994)	increase therapeutic efficiency	6	20-32	185			
	45	3 to 38			Prospective study	(Bartoli et al., 1997)	increased/decreased concentration	16	16-60	186			
	296	0 to 19.9			Retrospective study[b]	(Reimers et al., 2007)	increased/decreased concentration						
	2	7 & 14			Case report	(Panayiotopoulos et al., 1993)	increased exposure						
	2	NA			Case report	(Pisani et al., 1993)	increased exposure						
Hernia repair or orchidopexy.	21	2 to10	Local anaesthetics Lidocaine/Bupivacaine	Diazepam	Prospective study	(Giaufre et al., 1988)	increased exposure to bupivacaine	19	50[a]	187	AUC (1.25)	AUC (1.7)	1.36
Elective cystourethroplasty	10	1 to 9		Clonidine	Prospective study	(Inomata et al., 2001)	decreased concentration	6	37[a]	188	AUC (0.92)	AUC (0.64)	0.7
Ureteroneocystostomy	35	1 to 10			Prospective study	(Tripi et al., 2005)	increase therapeutic efficiency	46	20-68	189			
Juvenile idiopathic Arthritis	16	12 to 25	Methotrexate	Chloroquine	Prospective study	(Kimura et al., 2007)	no significant DDI in paediatrics	11	41 to 75	190			
Acute lymphoblastic leukaemia	9	2 to 11		Co-trimoxazole	Prospective study	(Ferrazzini et al., 1990)	increased exposure	1	82	191			
	7	5 to 13				(Beach et al., 1981)	no significant DDI in paediatrics						
Chronic arthritis	7	8 to 18		NSAID	Prospective study	(Dupuis et al., 1990)	no significant DDI in paediatrics	2	56 & 72	192			
Attention-deficit/hyperactivity	1	7	Methylphenidate	Carbamazepine	Case report	(Behar et al., 1998)	Suboptimal effect	**NO DDI reported in adults**					

disorder (ADHD)/ mental retardation	1	13			Case report	(Schaller and Behar, 1999)	subtherapeutic concentrations						
	1	6		Ketamine	Case report	(Ririe et al., 1997)	suboptimal effect & subtherapeutic concentrations	NA	NA	193			
Asthmatic	5	6 to 18	Methyl prednisolone	Phenobarbitone/carbamazepine/phenytoin	Prospective study	(Bartoszek et al., 1987)	increased CL	**NO DDI reported in adults**					
Giardiasis/amebiasis	NA /36	NA	Metronidazole	phenobarbitone	Retrospective study	(Gupte, 1983)	suboptimal effect	6	25-62	194			
Respiratory distress	6	12 to 36 h	Midazolam	Fentanyl	Case report	(Burtin et al., 1994)	ADR	180	20-50	195			
Malignant astrocytoma	1	14m			Case report	(Yaster et al., 1990)	ADR						
Liver transplant	21	2 to 16	Mycophenolate mofetil	CsA/tacrolimus	Prospective study	(Brown et al., 2002)	subtherapeutic concentrations	110	>18	196			
Kidney transplant	23	2.2 to 18.6			Retrospective study	(Filler, 2004)	higher exposure	18	NA	197			
Epilepsy	109	3 to 17	Oxcarbazepine	AED	POPPK	(Sallas et al., 2003)	increased CL	**NO DDI for oxcarbazepine and AED**					
Epileptsy	349	0.4 to 33	Phenobarbitone	Carbamazepine	POPPk	(Yukawa et al., 1998)	decreased CL	349	0.4-33	75			
	29	NA		Sodium valproate	Prospective study	(Fernandez de Gatta et al., 1986)	higher exposure	20	19-54	198	Level/Dose ratio (1.51)	Level/Dose ratio (2.13)	1.41
								10	NA	76			
Asthmatic	6	6 to 18	Prednisolone	Carbamazepine	Prospective study	(Bartoszek et al., 1987)	increased CL	5	21-36	199			
								1	70	200			
	6	6 to 18		Phenobarbitone	Prospective study	(Bartoszek et al., 1987)	increased CL	6	16-38	201			
LeshNyhan	1	13	Phenytoin	Allopurinol	Case	(Yokochi et	increased exposure	84	6 to	202			

Syndrom					report	al., 1982)			54				
Epilepsy	1	4		Co-trimoxazole	Case report	(Gillman and Sandyk, 1985)	ADR	8	NA	203	Clearance (1.52)	Concentration (1.27)	0.84
	1	10		Halothane	Case report	(Karlin and Kutt, 1970)	ADR	**NO DDI for Halothane and Phenytoin**			-		
Malaria	1	9m to 13y		Chloramphenicol	Prospective study	(Ogutu et al., 2002)	no significant DDI in paediatrics	3	NA	204	AUC (1.98)	AUC (0.94)	0.47
Hydrocephalus	1	7			Case report	(Powell et al., 1981)	increased CL	1	64	205			
Convulsion	2	6 w & 15 y		Diazoxide	Case report	(Roe et al., 1975)	subtherapeutic concentrations	**NO DDI for Phenytoin and diazoxide**					
Epilepsy	1	5		Methylphenidate	Case report	(Garrettson et al., 1969)	ADR	1	58	206			
	1	10		Methlphenidate	Case report	(Ghofrani, 1988)	ADR	4	20 to 23	207			
Petit mal	5	NA		Dipropylacetate	Prospective study	(Windorfer et al., 1975)	increased exposure	**NO DDI for Phenytoin and Dipropylacetate**					
Petit mal	9	NA	Primidone	Dipropylacetate	Prospective study	(Windorfer et al., 1975)	increased exposure	**NO DDI reported in adults for Primidone and Dipropylacetate**					
Esophagogastroduodenoscopy	32	3 to 10	Propofol	Remifentanyl	Prospective study	(Drover et al., 2004)	positive PD DDI	20	19-72	208			
Ambulatory adenoidectomy	60	1 to 3		Midazolam	Prospective study	(Viitanen et al., 1999)	delayed recovery from aneasthesia	140	18-60	209			
Seizure	1	38m	Quinidine	AED	Case report	(Rodgers and Blackman, 1983)	suboptimal effect	4	23-37	210			
HIV	15	5.7 to 16.3	Ritanovir /lopinavir	Efavirenz	Prospective	(Bergshoeff et al., 2005)	decreased exposure	182	16-63	211	AUC (1.09)	AUC (1.27)	1.16

					study								
Juvenile rheumatoid arthritis	1	11	Salicylate	prednisone	Case report	(Koren et al., 1987)	subtherapeutic concentrations	14	20-66	212			
	5	5 to 22			Case report	(Klinenberg and Miller, 1965)	increased CL						
	42	1.3 to 13.9			Prospective study	(Bardare et al., 1978)	increased CL						
Rheumatic fever	1	8		Griseofulvin	Case report	(Phillips et al., 1993)	subtherapeutic concentrations	**NO DDI reported in adults for Salicylate and Griseofulvin**					
Epilepsy	4	13-17	Sodium valproate	Carbamazepine/oxcarbazepine	Prospective study	(Battino et al., 2001)	increased exposure	5	21-48	213			
	1	29m		Erythromycin	Case report	(Sanchez Romero and Onsurbe Ramirez, 1990)	increased exposure	1	38	214			
	1	10			Prospective study	(Gopaul SV et al., 1996)	no significant DDI in paediatrics						
	13	2.1 to 16.8		Ethosuximide	Prospective study	(Salke-Kellermann et al., 1997)	increased exposure				AUC (0.7)	Concentration (0.72)	1.02
				Felbamate	Prospective study	(Liu and Delgado, 1995)	suboptimal effect	10	20 to 39	215			
	10	2 to 14				(Delgado, 1994)	increased exposure						
	1	5		Isoniazid	Case report	(Jonville et al., 1991)	ADR	**NO DDI reported in adults**					
	1	13			Case report	(Dockweiler, 1987)	ADR						
			Tacrolimus	Amlodipine		(Zhao et al., 2012a)	increased exposure	1	19	216			

Refractory solid tumours	32	3 to 21.3	Temazolomide	O6-benzylguanine	Prospective study	(Meany et al., 2009)	no significant DDI in paediatrics	**NO DDI reported in adults**					
Asthma/ apnea of prematurity/ seizures	2		Theophylline	Cefaclor	Case report	(Hammond and Abate, 1989)	ADR	11	21-30	217			
	2	18m & 2y		Cimetidine	Case report	(Fenje et al., 1982)	ADR	4	56-77	218			
								1	71	219			
	1	11		Fluvoxamine	Case report	(Sperber, 1991)	ADR	12	22-30	220			
								10	42	221			
	1	6		Halothane	Case report	(Naito et al., 1986)		2	54 & 57	222			
	1	10			Case report	(Richards et al., 1988)	ADR						
	6	4 to 13		Isoproterenol	Prospective study	(Hemstreet et al., 1982)	increased clearance	**NO DDI reported in adults**					
	1	14			Case report	(Griffith and Kozloski, 1990)	ADR						
	12	2 to 18			Prospective study	(O'Rourke and Crone, 1984)	decreased exposure						
	6	8 to 14		Ketotifen	Prospective study	(Garty et al., 1987)	no significant DDI in paediatrics	12	20-30	223			
	10	2 to 6		Methylprednisolone	Prospective study	(de la Morena et al., 1982)	increased exposure	6	27-38	224			
	1	newborn		Phenobarbitone	Case report	(Delgado, 1996)	decreased exposure	6	23-32	225	Clearance (0.75)	Clearance (0.7)	0.93
	7	6 to 12			Prospective study	(Saccar et al., 1985)	subtherapeutic concentrations	6	23-35	226	Clearance (0.75)	Clearance (0.94)	1.26
	24	1w[c]			Prospe	(Kandrotas	no significant DDI in						

					ctive study	et al., 1990)	paediatrics							
	7	6 to 12			Prospective study	(Goldstein, 1977)	no significant DDI in paediatrics							
	NA	NA			Prospective study	(Greene, 1977)	no significant DDI in paediatrics							
	9	Premature Infants [d]			Retrospective study	(Yazdani et al., 1987)	suboptimal effect							
	1	8		Pyrantel	Case report	(Hecht and Murray, 1989)	increased exposure	**NO DDI reported in adults**						
	1	19m		Salbutamol	Case report	(Amirav et al., 1988)	suboptimal effect	10	23-30	227	Concentration (0.82)	Clearance (0.42)	0.51	
	30	5 to 13			Prospective study	(Dawson and Fergusson, 1982)	ADR				Concentration (0.82)	AUC (0.54)	0.66	
	1	3 w		Secobarbitone	Case report	(Paladino et al., 1983)	increased CL	**NO DDI reported in adults**						
	12	7 to 11		Terbutaline	Prospective study	(Danziger et al., 1985)	decreased exposure	6	28-54	228	AUC/ Clearance (0.91)	AUC (0.79)	0.87	
								9	25-42	229				
								12	19-23	230				
Minor elective surgical procedures	60	7 to 12	Thiamylal	Clonidine	Prospective study	(Nishina et al., 1994)	increase therapeutic efficiency	**NO DDI reported in adults**						
Status asthmaticus	1	2	Tolazoline	H_2-blockers	Case report	(Huang and Huang, 1996)	ADR	**NO DDI reported in adults**						
Epilepsy	94	1 to	Topiramate	AED	Retros	(Adin et al.,	decreased exposure	11	28 &	231				

		66			pective study	2004)		6	33				
Patent ductus aortus	11	35 w	Vancomycin	Indomethacin	Prospective study	(Spivey and Gal, 1986)	decreased CL	**NO DDI reported in adults**					
Gram-positive infection	23	26 to 46 w			Retrospective study	(Asbury et al., 1993)	decreased CL						
General surgery	30	3 to 10	Vecuronium	Bupivacaine	Prospective study	(Taivainen et al., 1994)	increase therapeutic efficiency	15	18 to 50	232			
Epilepsy/neurological/orthopaedic procedure/Elective surgery	10	4 to 22		Carbamazepine	Prospective study	(Soriano et al., 2001)	increased CL	10	32.2[a]	233			
	74	4 to 12		Vecuronium	Prospective study	(Lynas et al., 1988)	lower dose required						
Acute lymphoblastic leukaemia/ T-cell non-Hodgkin's lymphoma	1	9	Vincristine	Posaconcazole	Case report	(Eiden et al., 2009)	ADR	1	21	234			
	1	4				(Jain and Kapoor, 2010)	ADR						
	1	5		Itraconazole		(Jeng and Feusner, 2001)	ADR	4	16-24	235			
	8	3 to 14				(Kamaluddin et al., 2001)	ADR						
	5	2 to 16				(Murphy et al., 1995)	ADR						
	1	5				(Sathiapalan and El-Solh, 2001)	ADR						
	1	5		Voriconazole		(Porter et al., 2009)	ADR	16	23-77	236			
Cancer	29	1 to 14	Warfarin	Steroids	Prospective study	(Ruud et al., 2008)	increase therapeutic efficiency	32	40-91	237			

HIV	8	3 to 14	Zidovudine	Didanosine	Prospective study	(Gibb et al., 1995)	no significant DDI in paediatrics	8	32-48	238	AUC (1.28)	AUC (1.11)	0.87
	54	3m to 21				(Mueller et al., 1994)	no significant DDI in paediatrics	69	34[a]	239			
Epileptic	12	5 to 16	Zonisamide	Carbamazepine	Prospective study	(Miura, 2004)	decreased exposure	18	18-55	240			
	12	5 to 16			Prospective study	(Abo et al., 1995)	decreased exposure						

Appendix 2

Data collection form for Paediatric drug interaction study
Patient details
Age

Weight

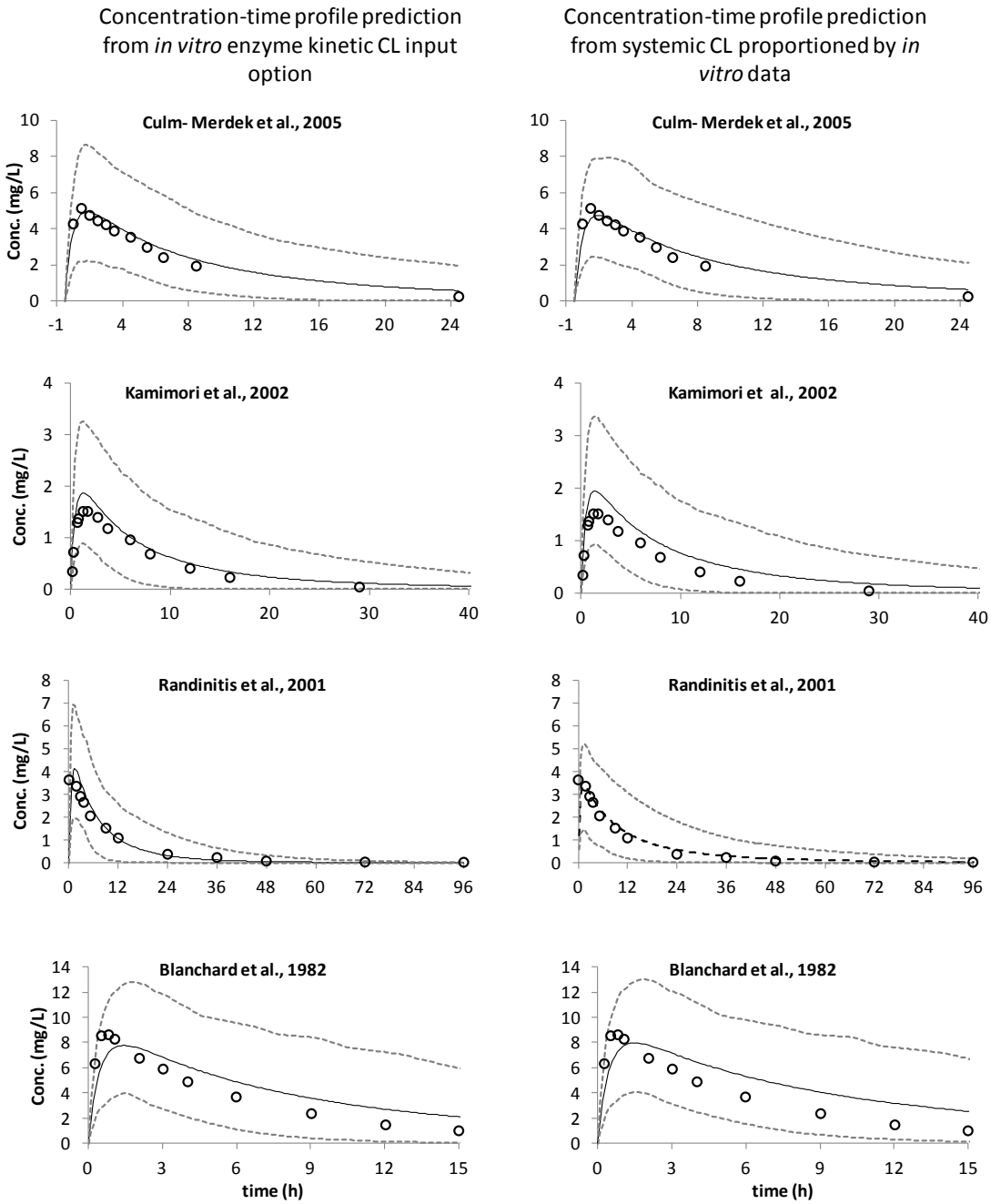
Sex
Drug Interaction Details (Specifically Pharmacokinetic interactions where one drug potentially affects the plasma concentration of another, underlying mechanism not important)
Drug 1
Start date:
Dose and frequency:
Rout of administration:
Stop date:

Drug 2 (interacting):
Start date:
Dose and frequency:
Rout of administration:
Stop date:

Drug 2 (interacting):
Start date:
Dose and frequency:
Rout of administration:
Stop date:

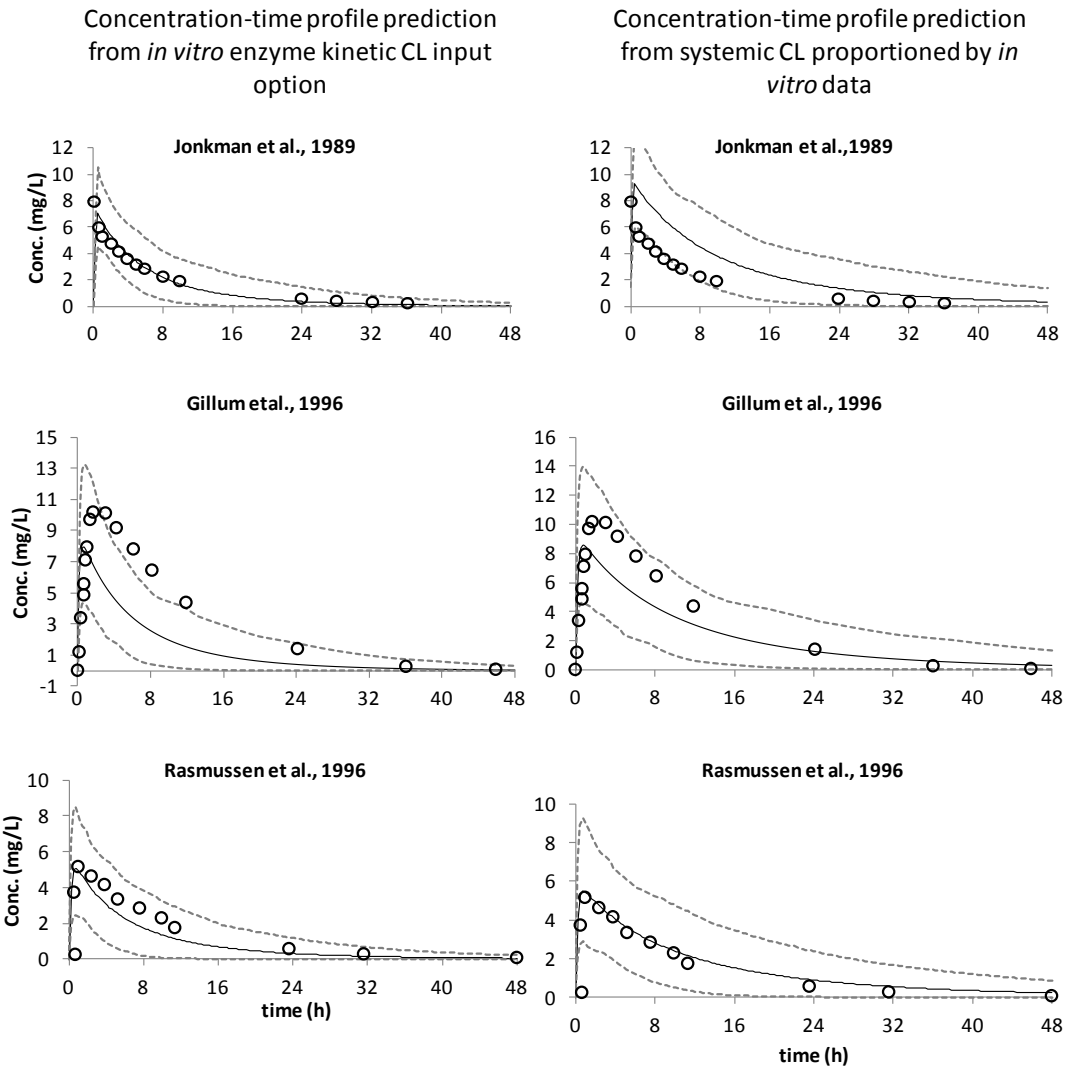
Drug 2 (interacting):
Start date:
Dose and frequency:
Rout of administration:
Stop date:

Appendix 3



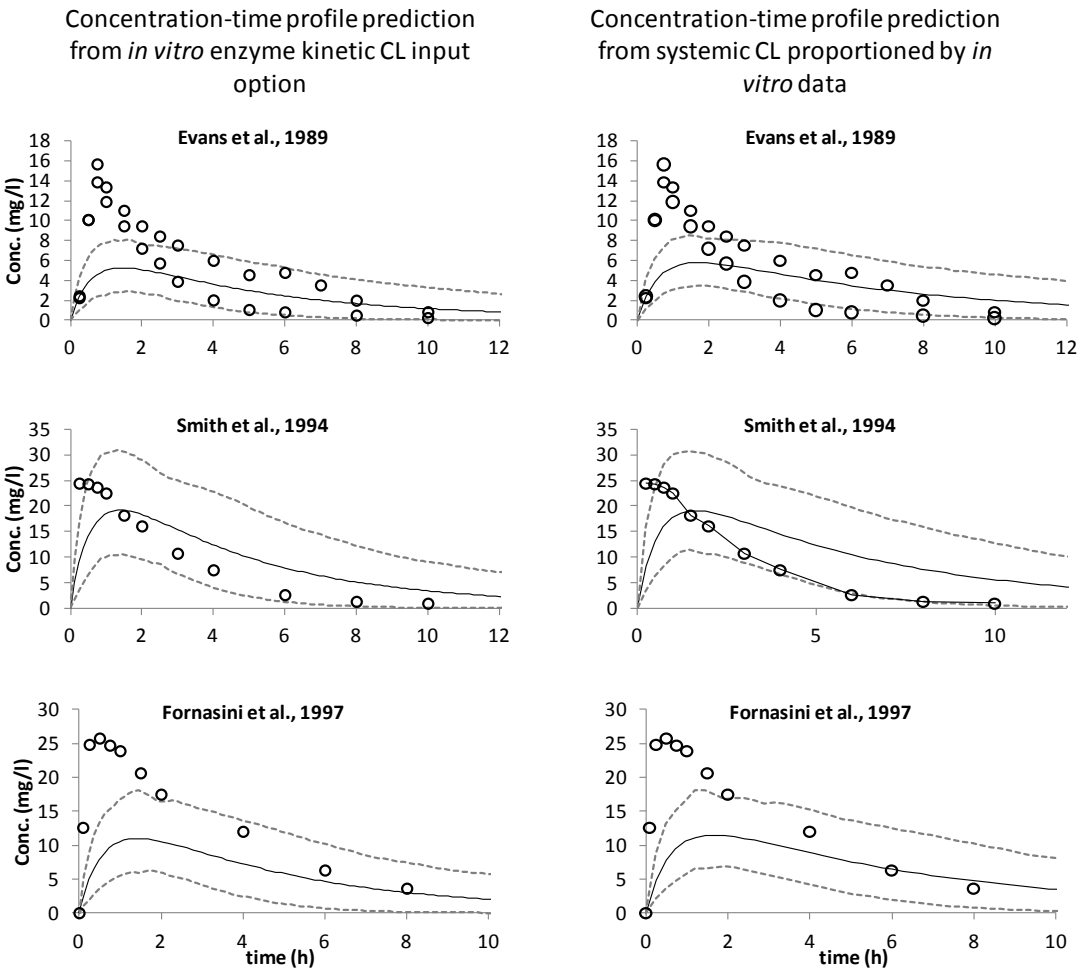
Supplemental Figure S 1 Comparison of predicted and observed plasma concentration-time profile for caffeine in adults.

Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.



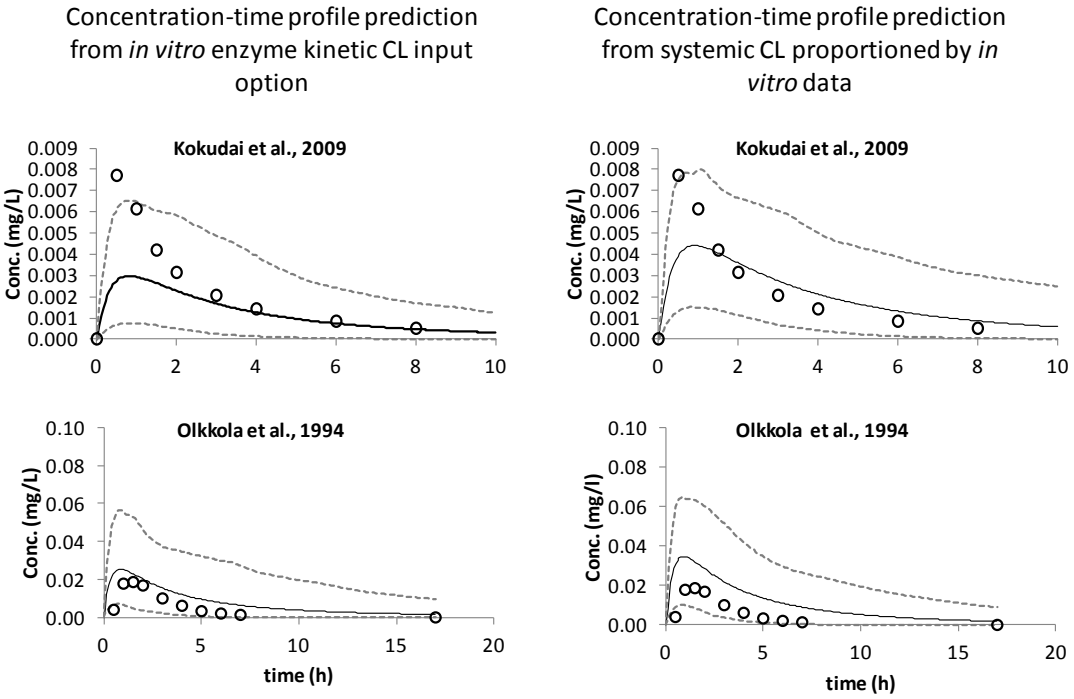
Supplemental Figure S 2 Comparison of predicted and observed plasma concentration-time profile for theophylline in adults.

Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.



Supplemental Figure S 3 Comparison of predicted and observed plasma concentration-time profiles for ibuprofen in adults.

Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.



Supplemental Figure S 4 Comparison of predicted and observed plasma concentration-time profiles for midazolam in adults.

Solid black line is the mean predicted concentration and the dotted lines are the 5 and 95th percentiles. Open circles are the observed data.

Appendix 4

Supplemental Table S 2 Studies reporting the clearance of caffeine in paediatric subjects (oral administration)

Study	n	GSA (weeks)	Age (Years)	CL (ml/min)
	2		1 to 2.5 months	3.2
(Aranda et al., 1979a)	3		3 to 4.5 months	11.67
	2		5 to 6 months	43.4
(Charles et al., 2008)	110	24 to 29		0.12
(Lee et al., 1997)	89	28		0.10
(Thomson et al., 1996)	60	33		0.13
	1	30	5 days	0.24
	1	28	18 days	0.18
(Gorodischer and Karplus, 1982)	1	25	21 days	0.13
	1	31	6 days	0.15
	1	33	3.5 days	0.22
(Aranda et al., 1979b)	12	29	3 to 32 days	0.17
(De Carolis et al., 1991)	5	29 to 31		0.02

Supplemental Table S 3 Studies reporting the clearance of theophylline in paediatric subjects (iv and oral administration)

Study	n	GSA (weeks)	Age (Years)	CL (L/h)	Route
	1	28	0.03	0.01	
	1	28	0.1	0.02	
	1	28	0.1	0.02	
	1	28	0.1	0.02	
(Dothey et al., 1989)	1	29	0.1	0.03	IV
	1	30	0.1	0.03	
	1	26	0.2	0.04	
	1	28	0.2	0.06	
	1	25	0.5	0.09	
	1		9.3	3.56	
	1		7.3	2.82	
	1		8.3	2.53	
	1		12.3	1.77	
(Arnold et al., 1981)	1		8.5	1.99	IV
	1		10	2.25	
	1		9.4	1.59	
	1		12.3	4.19	
	6		1 to 5	2.24	
(Kumar et al., 1989)	7		6 to 10	4.21	po
	8		11 to 16	7.75	
(Nassif et al., 1981)	4		6 to 48 weeks	0.22	po

(Aranda et al., 1976)	6	25 to 32	3 to 15 days	0.01	IV
(Jones et al., 1993)	1	28	0.01	0.03	IV
	1	27	0.01	0.02	
	1	25	0.01	0.02	
	1	29	0.09	0.02	
	1	27	0.003	0.01	
	1	27	0.1	0.01	
	1	28	0.1	0.03	
	1	29	0.03	0.02	
	1	29	0.03	0.03	
	1	30	0.1	0.02	
	1	27	0.1	0.02	
(Giacoia et al., 1976)	1	28	0.08	0.08	po
	1	29	0.08	0.04	
	1	28	0.16	0.09	
	1	28	0.15	0.09	
	1	26	0.13	0.07	
	1	26	0.13	0.03	
	1	32	0.10	0.06	
	1	29	0.07	0.05	
(Brazier et al., 1979)	20	34	2.9 days	0.03	po
(Rosen et al., 1979)	1		0.33	0.32	po
	1		0.4	0.34	
	1		0.4	0.44	
	1		0.5	0.86	
	1		0.6	1.15	
	1		0. 7	0.79	
	1		1	1.61	
	1		1.1	0.81	
	1		1.3	1.09	
	1		1.3	0.86	
	1		1.4	0.86	
	1		1.5	1.22	
	1		1.5	1.00	
(Loughnan et al., 1977)	1		1.3	0.64	IV
	1		1.7	1.22	
	1		2	0.76	
	1		2	1.10	
	1		2	1.83	
	1		2.5	1.43	
	1		2.7	1.20	
	1		3	2.15	
	1		3.2	0.95	
	1		4.4	1.10	
(Bolme et al., 1979)	1		0.2	0.77	IV
	1		0.8	0.19	

	1		0.3	0.66	
	1		4.3	1.12	
	1		0.5	1.30	
	1		1.2	0.96	
(Franko et al., 1982)	1		0.1	0.12	IV
	1		0.1	0.11	
	1		0.2	0.05	
	1		0.2	0.13	
	1		0.2	0.28	
	1		0.2	0.12	
	1		0.3	0.20	
	1		0.4	0.12	
	1		0.4	0.25	
	1		0.4	0.26	
	1		0.4	0.27	
	1		0.5	0.30	
(Kjellman et al., 1988)	8		4 to 10	4.18	po
(Yano et al., 1993)	66		0.25 to 18.3	1.52	IV
(Lonnerholm et al., 1983)	3	28 to 34	6-11 days	0.02	po
(Latini et al., 1978)	1	33	0.01	0.04	IV
	1	33	0.02	0.01	
	1	32	0.02	0.02	
	1	30	0.02	0.01	
	1	32	0.02	0.01	
	1	29	0.02	0.04	
	1	30	0.02	0.01	
(Simons and Simons, 1978)	1		0.3	0.25	IV
	1		0.3	0.17	
	1		0.3	0.27	
	1		0.3	0.38	
	1		0.4	0.59	
	1		0.7	0.84	
	1		0.7	0.29	
	1		0.8	0.78	
	1		0.8	0.66	
	1		1.4	0.66	
	1		1.5	0.95	
	1		1.7	1.70	
	1		1.9	0.37	
(Ginchansky and Weinberger, 1977)	23		4 to 15	2.50	IV
(Ellis et al., 1976)	30		6 to 16	3.00	IV
(Walson et al., 1989)	1		15.8	9.81	IV
	1		9.1	3.42	
	1		7.6	2.76	
	1		11.7	3.51	
	1		11.1	2.10	

	1	12.4	5.18	
	1	10.4	2.94	
(Leung et al., 1977)	1	6.2	1.72	IV
	1	10.1	3.52	
	1	10.3	2.16	
	1	11.3	4.04	
	1	11.3	4.45	
	1	11.3	1.97	
	1	11.5	1.97	
	1	11.8	2.41	
	1	12.3	3.14	
	1	12.5	1.98	
	1	12.5	4.36	
	1	12.5	3.92	
	1	12.5	2.32	
	1	12.8	2.78	
	1	12.8	2.11	
	1	13.7	3.68	
	1	13.8	2.04	
	1	13.8	4.21	
	1	13.8	2.58	
	1	13.8	2.88	
	1	13.9	2.78	
	1	14.3	2.97	
	1	14.4	1.85	
	1	14.5	3.69	
	1	14.8	1.99	
	1	14.9	5.59	
	1	15	2.07	
	1	15.5	3.51	
	1	16.3	3.76	
	1	16.5	3.84	
(Ginchansky and Weinberger, 1977)	1	4.3	1.17	IV
	1	4.1	1.53	
	1	5.8	1.86	
	1	8.3	2.59	
	1	8.9	3.60	
	1	9.1	3.82	
	1	9.8	3.50	
	1	10.2	2.02	
	1	10.3	3.39	
	1	10.3	3.57	
	1	10.8	2.51	
	1	11.3	2.18	
	1	11.2	3.44	
	1	11.7	4.09	

	1	11.9	4.36	
	1	12	4.29	
	1	12.3	3.32	
	1	12.6	2.02	
	1	12.6	1.53	
	1	13.9	3.04	
	1	14.1	2.56	
	1	14.3	2.89	
	1	15.2	2.41	
	20	0.1	0.05	
(Kraus et al., 1993)	15	0.3	0.12	
	17	0.6	0.27	

Supplemental Table S 4 Studies reporting the clearance of midazolam in paediatric subjects (IV administration)

Study	n	GSA (weeks)	Age (Years)	Critically ill	CL (L/h)
(Lee et al., 1999)	27	27	2 to 15 days	Yes	0.05
	33	27	2 to 15 days		0.07
Harte et al, 1997	10	28	2 to 4 days	Yes	0.10
(Jacqz-Aigrain et al., 1992)	15	33	1 to 5 days	Yes	0.23
(Jacqz-Aigrain et al., 1990)	1	35	2 to 5 days	Yes	0.19
	1	34			0.23
	1	35			0.17
	1	37			0.12
	1	39			0.16
	1	41			0.52
	1	38			0.22
	1	37			0.76
	1	38			0.86
	1	40			0.87
(de Wildt et al., 2001)	24	29	3 to 11 days	Yes	0.11
(Rey et al., 1991)	6		1.75 to 4 days	No	11.7
(Mathews et al., 1988)	6		0.9 to 7.7 days	No	13.2
	6				8.1
	4				4.8
(Hughes et al., 1996)	16		<1 days	Yes	2.9
	12		1 to 2 days		3.4
	10		> 3 days		23.7
(Reed et al., 2001)	5		0.5 to 2	No	5.9
	14		2 to 12		11.7
	2		12 to 16		34.7
	3		0.5 to 2		3.0
	3		2 to 12		12.7
(Salonen et al., 1987)	3		7	No	13.1

	6	7		8.1
	6	6		11.2
	6	6		15.0
(de Wildt et al., 2000)	20	8	No	16.2
(Wells et al., 1991)	13.00	13	No	25.6
(Tolia et al., 1991)	20	8 to 17	No	29.0
(de Wildt et al., 2003)	1	2 days to 17	Yes	0.80
	1			0.87
	1			0.82
	1			0.64
	1			0.84
	1			0.98
	1			1.09
	1			0.82
	1			1.7
	1			7.4
	1			4.8
	1			5.6
	1			7.1
	1			8.9
	1			8.2
	1			9.3
	1			18.6
	1			22.3