Identifying paediatric needs in cardiology and the prediction of sildenafil exposure in children with pulmonary arterial hypertension

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1 Introduction

1.1 Clinical studies in the paediatric population

Developmental changes influence the pharmacotherapy in children and make it different from that in adults. This may lead to overdosing and/or underdosing in children if drugs are given to children in doses extrapolated from adults.

Children were described as therapeutic orphans, since only little research on the paediatric pharmacotherapy had been carried out. Because of that, the European Parliament introduced a paediatric regulation (Regulation (EC) No 1901/2006, 2006) which came into force in January 2007. According to this regulation, pharmaceutical industries have to conduct clinical trials in children to receive approval for new drugs. Nevertheless, drugs, which are off-patent are frequently given to children without paediatric labelling or outside the terms of their label, i.e. off-label (Conroy, 2000). It is strongly recommended that such drugs be investigated in children to assure their efficacy and safety in the paediatric population. The priorities for paediatric clinical trials should be given to life-threatening diseases, especially those with a poor labelling state of their pharmacotherapy in children. In the present project, a drug prescription analysis was carried out to describe the off-label drug use on a paediatric cardiology ward in Germany, in order to identify paediatric needs especially for drugs given to children with cardiovascular diseases.

The age-related PK of a drug used off-label in children will be further investigated based on the results of this analysis. PK studies are essential in developing effective and safe dosing regimens in children, but they have to be carefully designed to provide the strongest evidence from a low number of subjects. An *a priori* simulation of drug exposure in children enables one to estimate the starting dose and an efficient sampling strategy, which should be helpful in the planning of clinical trials in paediatric patients. Physiologically-based pharmacokinetic (PBPK) modelling, which uses the knowledge on the age-related developmental changes of children to predict PK of a drug, is a suitable tool to simulate PK of drugs *a priori* in children. This simulation tool will be used in the present project to build a model for this drug and simulate its PK in virtual children to be used in the planning of future PK trials in children.

1.2 Off-label drug use in paediatrics

Many drugs given to children in Europe and the USA are used unlicensed or off-label (Dell'Aera, 2007; 't Jong, 2002; Shah, 2007; Conroy, 2002). The unlicensed use of drugs includes: The modification of a licensed drug, the use of chemicals as drugs, drugs used before a licence has been granted and the use of imported drugs (Turner, 1999). The off-label drug use means that a drug is prescribed at a dose, for an indication, by a route of administration or for an age group not covered by the product license (Conroy, 2000).

The major causes of concern about off-label drug use in children are safety and lack of efficacy (Neubert, 2008). Off-label prescriptions were associated with a higher rate of adverse drug reactions (Horen, 2002; Turner, 1999), which is probably due to the lack of evidence and a lack of information about the appropriate use of the off-label prescribed drugs in children. Inefficacy of the treatment with off-label prescriptions can also occur, if the age-related changes in PK and PD of drugs are not considered. This was the case of cidofovir, which had been used in an off-label manner to treat a life-threatening viral infection in a child after liver transplantation (Dohna-Schwake, 2010). Drug monitoring had shown that the child needed much higher doses of cidofovir than the dose used. The authors reported a dramatic improvement after increasing the dose considering the age-specific cidofovir PK. Unfortunately, the happy end reported by Dohna-Schwake, which was achieved in corporation with paediatric clinical pharmacologists, cannot be provided for all children treated with off-label prescribed drugs. Many drugs are still given to children with no sufficient scientific evidence, and may be ineffective. Data on the efficacy or inefficacy of the off-label prescribed drugs are sparse in the paediatric population because of the reduced number of clinical studies in children compared to adults. Considering the high cost of the extensive research required for the paediatric labelling, the reward of a paediatric drug labelling had not been attractive for the pharmaceutical industry. Furthermore, many ethical and technical aspects challenge the conducting of clinical trials in children. As a result of this situation, little information about the efficacy and safety of the off-label prescribed drugs in children is available.

Another important aspect concerning the off-label drug use among children is the lack of labelled alternative drugs. In contrast to adults, the off-label drug use among children cannot be switched to an alternative drug licensed for children simply because often there isn't any (Cras, 2007).

New drugs are still being introduced into the market offering better efficacy, higher selectivity, lower toxicity or better compliance than the conventional therapies. For many reasons, many of these new drugs may lack paediatric labelling. Such drugs may have

benefits for paediatric patients and may therefore be used in an off-label manner in children with the same diseases as adults. Thus, it is important to investigate the current state of the off-label drug use in children and to refer to drugs and therapeutic habits which are not evidence-based in paediatrics, thereby being able to determine paediatric needs and research priorities in children. The present project was carried out to analyse the off-label drug use in a paediatric ward in order to identify paediatric needs and focus on areas where information is lacking and where research is necessary.

1.3 Off-label use of cardiovascular drugs in children

The percentage of cardiovascular drugs in the published studies of off-label drug use in children was very low, especially among paediatric outpatients. Cardiovascular drugs made up only 0.1 to 0.4% of the total number of prescriptions in the studies of Horen and Bücheler, which analysed the off-label drug use in paediatric patients (Horen, 2002; Bücheler, 2002). Although the use of cardiovascular prescriptions is not common in children, the percentage of off-label prescribed drugs within this drug group seemed very high; for example, 100% of the antihypertensive drugs given to children on a paediatric ward in Germany were off-label (Neubert, 2004). The low percentage of cardiovascular drugs relative to other drug groups used in children demonstrates a limited experience of drug use in the paediatric cardiology, whereas the high rate of off-label use of cardiovascular drugs in paediatrics demonstrates limited evidence-based information on the appropriate use of this drug group in children. Adverse drug reactions are a considerable issue concerning the use of cardiovascular drugs in paediatric patients. Diuretics, antihypertensives, vasodilators and inotropic agents were associated with 29% of all observed adverse drug reactions in one study, which included prescriptions from five paediatric wards covering a variety of different paediatric specialities (Turner, 1999). In Turner's study, the percentage of unlicensed and off-label prescriptions including off-label prescribed cardiovascular drugs was significantly associated with the risk of adverse drug reactions. Turner's study showed that adverse drug reactions seen in 35% of the children in the cardiac intensive care were much higher than all other paediatric wards included in the study.

Until now, no study on the off-label drug use on any paediatric cardiology ward in Germany has been investigated, this being a main reason for the present trial. The off-label prescribed cardiovascular drugs observed on a German paediatric cardiology ward will be described and compared to the European Medicines Agency (EMEA) list of paediatric needs in field of cardiology and the EMEA priority list for studies into off-patent

paediatric drugs (EMEA, 2006; EMEA, 2007). This can show if the off-label prescribed cardiovascular drugs in Germany are comparable to those common in other European countries. The off-label use of cardiovascular drugs in children will be also discussed to objectively determine the prioritised paediatric needs in paediatric cardiology.

The use of cardiovascular drugs among children has to be investigated to assure their efficacy and safety in paediatric patients, especially since cardiovascular diseases include a spectrum of potentially life-threatening diseases such as heart failure, arrhythmias, thrombosis and pulmonary arterial hypertension (PAH). The analysis in the first part of the present project revealed many drugs which were given to children with cardiovascular diseases in an off-label manner. Sildenafil was one of these off-label prescribed drugs, which was given to paediatric patients although no sufficient data on its PK in children were available. Furthermore, this drug was given to children with PAH, which is a life-threatening disease with a poor prognosis. Sildenafil was therefore chosen to be further investigated in the second part of the present project.

1.4 Pulmonary arterial hypertension in children

Pulmonary arterial hypertension (PAH) is a progressive disease characterised by an increased pulmonary vascular resistance leading to right ventricular failure and occasionally to death. PAH has a bad prognosis with a very poor survival period of less than a year in untreated children (D'Alonzo, 1991). Even in the treated children with PAH the prognosis is still poor, where the 5-year survival rate was only 64% (Haworth, 2009).

Most of the information about the pharmacological treatments of paediatric PAH is based on research in adult patients. Children with PAH showed benefits from the current available treatment options (Karatza, 2005; Barst, 2003). This demonstrates the importance of research in the paediatric PAH to gain more information about the disease itself and about the paediatric pharmacotherapy of the disease in order to improve the quality of life and survival in children with PAH.

PAH is defined by a mean pulmonary artery pressure above 25 mm Hg at rest or above 30 mm Hg with exercise with a normal left atrial pressure (Barst, 2004). For children it is important to consider the hemodynamic abnormalities during exercise, since they sometimes have a normal mean pulmonary artery pressure at rest but they develop an extreme pulmonary vascular response to exercise compared to adults (Widlitz, 2003). There are different forms of PAH including idiopathic PAH, associated PAH and persistent pulmonary hypertension of the newborn (Simonneau, 2004). Persistent pulmonary hypertension of the newborn is usually transient; infants do not need treatment after

recovery. This is not the case for children with idiopathic PAH, as they require treatment indefinitely (Widlitz, 2003).

The pathophysiology of PAH consists of vasoconstriction and vascular hypertrophy within the pulmonary artery, leading to obstruction and increased pulmonary vascular resistance. A chronically elevated pulmonary arterial pressure results in right ventricular overload and consequently right ventricular failure. Patients with advanced disease suffer from a decreased cardiac output and abnormalities in oxygenation. Many factors contribute to the vasoconstriction and the obstructive remodelling of the pulmonary vascular bed in PAH such as the reduced synthesis of natural pulmonary vasodilators, e.g. nitric oxide and prostacycline; the overexpression of endothelial vasoconstrictors, e.g. endothelin-1; and the existence of mutations in genes responsible for the suppression of vascular cells (Boutet, 2008). The present therapies for PAH target three major pathological pathways: The prostacyclin-, nitric oxide- and endothelin-mediated pathways. The current approved therapies for adult PAH consist of synthetic prostacyclins and prostacyclin analogues, e.g. epoprostenol; endothelin receptor antagonists, e.g. bosentan; and phosphodiesterase type 5 inhibitors, e.g. sildenafil (Dimopoulos, 2008).

1.5 Sildenafil for the treatment of paediatric pulmonary arterial hypertension

Sildenafil (1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimdin-5-yl)phenylsulphonyl]-4-methylpiperazine) is a phosphodiesterase type 5 (PDE-5) inhibitor (Figure 1). Sildenafil is a potent and selective inhibitor of PDE-5, resulting in an increase of intracellular cGMP, which leads to smooth muscle relaxation (Ballard, 1998).

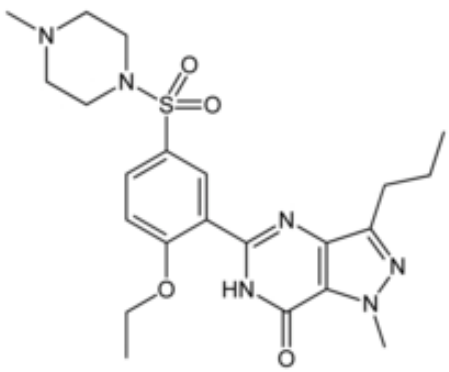

Figure 1: Structural formula of sildenafil

As PDE-5 is highly expressed in pulmonary vascular smooth muscle, sildenafil was successfully used to reduce pulmonary vascular resistance and to induce strong pulmonary vasodilatation (Pauvert, 2003). Sildenafil improved exercise capacity, functional status and haemodynamics in adult patients with symptomatic PAH in randomised controlled trials (Galiè, 2005; Sastry, 2004; Singh, 2006). The efficacy and safety of sildenafil in paediatric PAH were reported in many uncontrolled trials and case reports showing that children with PAH benefit from sildenafil (Karatza, 2005; Raja, 2007). However, children with PAH are treated with sildenafil without sufficient data on its PK/PD in paediatrics. The reported dosing patterns of sildenafil for the treatment of paediatric PAH in published studies are largely heterogeneous and no age-specific dosing recommendation for paediatric patients is available (Lee, 2008; Oliveira, 2005; Peiravian, 2007, Karatza, 2005). The lack of knowledge about the age-related developmental changes of sildenafil PK in children may lead to insufficient exposure or unsafe use of sildenafil in the paediatric population. Incompliance is also an important issue. Children with PAH took sildenafil up to six times a day compared to three times a day in adult patients (Raja, 2007). Thus, the optimal age-specific dosing of sildenafil has to be established in a well-designed PK/PD study considering the different paediatric age groups.

Based on the epidemiological data of PAH, only few paediatric patients would be included in a paediatric clinical trial of sildenafil. An *a priori* simulation for sildenafil PK in different paediatric age groups is therefore very helpful in the planning of a well-designed PK trial by the estimation of the effective age-specific dosing regimens and the efficient sampling points that appropriately describe the important PK parameters of oral sildenafil in children. For this purpose, the exposure of sildenafil in virtual children was simulated in the present project using PBPK modelling.

1.6 Pharmacokinetics of sildenafil

Sildenafil is rapidly absorbed with a time to maximum plasma concentration (t_{max}) of 0.25 to 3.5 h (Muirhead, 2002b; Jetter, 2002). The absolute bioavailability of oral sildenafil was limited to about 40% due to a first-pass metabolism (Muirhead, 2002b; Nichols, 2002; Walker, 1999). Its terminal half-life ($t_{1/2}$) after intravenous application was 2 to 4 h (Nichols, 2002; Muirhead, 2002b) with plasma clearance (CL) of 41 l/h (Nichols, 2002). Food affects sildenafil PK by delaying t_{max} and decreasing C_{max} and area under the plasma concentration-time curve (AUC), but this effect was considered clinically irrelevant

(Nichols,2002). Sildenafil is highly bound to plasma proteins (96%) and its protein binding is independent of its concentration (Walker, 1999).

Sildenafil undergoes an extensive hepatic metabolism, predominantly by cytochrome P450 CYP3A4 (79%) and for a less extent by CYP2C9 (20%) (Warrington, 2000). No unchanged drug was detected in either urine or faeces, demonstrating that metabolism is exclusively responsible for the elimination of sildenafil (Muirhead, 2002b).

Sildenafil exposure was much higher in Mexican subjects compared to Caucasians, demonstrating an inter-ethnic difference in sildenafil PK (Flores-Murrieta, 2000). No impact was observed in sildenafil PK in females compared to males (EMEA, 2005). However, due to the nature of its common indication, i.e. for erectile dysfunction, sildenafil was studied almost exclusively in men, no PK study of sildenafil in women has ever been published.

In contrast to adults, very few information on sildenafil PK in children is available (Karatza, 2005; Witjes 2010). In order to avoid overdosing or underdosing of sildenafil in children with PAH, its PK in paediatric patients should be investigated. An *a priori* simulation of sildenafil PK in children was carried out in the present project, in order to be used in the planning of PK studies in children with PAH.

1.7 Pharmacokinetic studies in paediatric patients

To establish the optimal age-specific dosing regimens for a drug in children, it is necessary to investigate its exposure, represented by the area under the plasma concentration-time curve or average steady state concentration in the different age groups. Information about the age-related PK is essential for a better interpretation and understanding of the efficacy and safety data of drugs in the paediatric population.

Many issues challenge the performance of PK studies in children, such as the invasive nature of the PK studies, which makes them unattractive for children, their parents, and doctors. Children, especially neonates and oncology patients, have small blood volumes; therefore it is some times impossible to obtain sufficient number of blood samples from such children. For diseases affecting a small number of children, it is difficult to include sufficient number of patients in a short period.

To date, three approaches have usually been used to estimate the PK of drugs in children: Population pharmacokinetics, allometric scaling and physiologically-based pharmacokinetic modelling (PBPK) (Alcorn, 2008). Population pharmacokinetic modelling enables the interpretation of drug PK with sparse plasma samples at random times from a

large group of children. It is an attractive method due to its ability to deal with sparse data, i.e. with a less number of blood samples required from each patient. However, this approach is *posteriori*, i.e. the drug has first to be applied to a large number of children before a model can be developed. Allometric scaling extrapolates a PK parameter to children depending on its value in adults using an empiric power exponent. Allometric scaling methods are very simple, but were shown to be unable to predict the clearance for all drugs or for all paediatric ages (Mahmood, 2006). PBPK modelling is very promising in the prediction of drug exposure, as it considers the maturation processes - especially of the eliminating organs. PBPK modelling was chosen to simulate sildenafil PK in children in the present project because it provides a science-based *a priori* simulation of the drug exposure in children, taking into consideration not only the weight of the child but also the ontogeny of different organs and eliminating processes.

1.8 Whole body physiologically-based pharmacokinetic (PBPK) modelling in paediatric patients

PBPK modelling is a simulation tool which mathematically describes the physical and physiological processes affecting the PK of drugs in mammalians. The basic approach underlying the PBPK modelling is the subdivision of the organism into many compartments; each represents a single organ or tissue relevant for the description of the absorption, distribution, metabolism and elimination. The physiology of each compartment is translated into mass-transport equations, which describe the distribution of a drug into the different organs and tissues via venous and arterial blood pools. Building a whole body PBPK model depends on two sets of parameters: the first set includes organism-specific anatomical and physiological parameters, such as organ volume, organ blood flow and organ composition; the second one includes compound-related physico-chemical parameters, such as lipophilicity, solubility and molecular weight. The concentration-time curve of a drug in blood and organs included in the model can be calculated by solving the different equations that establish the PBPK model (Schmitt, 2005).

The whole body PBPK modelling is a useful tool to predict the PK of drugs in children by utilizing the knowledge about the age-related physiological and anatomical changes such as tissue composition, organ weights, blood flow rates and the different eliminating processes. Age-dependent body weight, growth and organ volumes were gathered from the literature and used in the paediatric PBPK modelling. Age-dependent changes in body composition of adipose and muscles were considered (Edginton, 2006b). Neonates and infants have relatively larger extracellular and total body water spaces compared to adults,

which may affect the volume of distribution depending on the physico-chemical properties of the drug (Willmann, 2009). Age-dependent changes in blood flows for brain, kidney, muscle and skin are also considered in the paediatric model (Edginton, 2006b). To physiologically scale the clearance from adults to children, information about the processes that contribute to the elimination of a drug and the fraction of each process of the total clearance in adults have to be determined. The clearance of the simulated child can be then calculated depending on information about the developmental changes of every eliminating process (Edginton, 2006a). The expression of phase I enzymes, such as the P-450 cytochromes (CYPs), changes dramatically after birth. However, the development patterns of these enzymes are not the same. While CYP3A4 appears during the first week of life, CYP1A2 appears at one to three months of life (Kearns, 2003). The activity of hepatic CYP3A4 reaches the adult activity at 6 months of age, whereas the activity of CYP1A2 reachrs the adult activity between 8 and 10 years of ages (Edginton, 2006a). These age-related changes in enzyme activity in addition to age-dependent changes in plasma unbound fraction are considered in the PBPK modelling (Johnson, 2006). The available data on the ontogeny of phase II conjugating enzymes, such as glucuronosyltransferase UGT1A6 and UGT2B7, in addition to developmental changes in the glomerular filtration rate are also considered. Both UGT1A6 and UGT2B7 are detectable in neonates and reach adult activity at 1 and 10 years of age respectively (Edginton, 2006a). The glomerular filtration rate (ml/min/1.73 m^2) is very low in term neonates compared to adults, then increases rapidly through the first two weeks of life and reaches the adult value by approximately 8 to 12 months of age (Johnson, 2006; Kearns, 2003).

The whole body PBPK modelling is a suitable tool for suggesting the dosing strategy of a drug especially when applied for the first time in children (Johnson, 2006). Another benefit of PBPK modelling is its ability to predict the inter-individual variability in a paediatric population depending on information about the distribution of the demographic characteristics and about the variability of physiological factors such as metabolic clearance, gastric emptying time and intestinal transit time, which influence the variability of the population pharmacokinetics (Willmann, 2007).

PBPK modelling was successfully used to predict the PK of many drugs in paediatric ages, suggesting that PBPK can be a useful tool in decision-making regarding dosing, sample times and study design (Breddemann, 2008; Björkman, 2005; Johnson, 2006; Edginton, 2006b). This tool was used in the present project to predict the developmental changes in sildenafil PK in paediatric patients.

1.9 Aim of the project

As no epidemiological study of the off-label drug use on a paediatric cardiology ward in Germany was available, a prospective observational trial to describe the off-label drug use on a paediatric pneumology and cardiology ward in the University Hospital of Düsseldorf was conducted. The results of the trial should give a close picture of the off-label prescriptions in hospitalised children particularly among cardiovascular drugs. This drug group was already shown to be associated with a considerable rate of adverse drug reactions in children (Turner, 1999) and a poor paediatric labelling state. A comparison with the EMEA priority list for research into off-patent paediatric drugs and EMEA list of paediatric needs was made to compare the off-label prescriptions in Germany with those in Europe. The results of the present trial will be used to identify needs in paediatric pharmacotherapy, where information is lacking, and where research is necessary.

In the second part of this project, sildenafil pharmacokinetics (PK) were simulated in virtual children. Although very few data are available on sildenafil PK in paediatrics, children with PAH are frequently treated with sildenafil. As sildenafil PK were very well studied in adults, a PBPK simulation software which enables the prediction of PK of drugs in children based on data from adults was used. The aim was to simulate sildenafil PK in children *a priori*. The simulation should help in the planning of paediatric PK studies of sildenafil in the future.

The following issues were considered in the present project:

1. Presentation of a close analysis of the off-label drug use on a paediatric cardiology and pneumology ward in Germany.
2. Comparison of the off-label prescribed cardiovascular drugs and those included in the EMEA list for paediatric needs and priority list of off-patent drugs.
3. Development and evaluation of a PBPK model for sildenafil in adults.
4. Physiology-based scaling of the adult model to children to predict sildenafil PK in paediatrics. The predicted plasma concentrations will be compared with observed data from the literature to evaluate the performance of prediction.
5. Simulation of sildenafil exposure in different paediatric age groups to estimate age-specific starting doses and sampling points for future studies in children.

2 Methods

2.1 A prospective observational trial to analyse the off-label drug use in hospitalised children

2.1.1 Trial design

A prospective observational trial during a 6-month period was performed on the paediatric pneumology and cardiology ward at the University Hospital of Düsseldorf in Germany. Prescription records of all patients admitted to the ward between January and June 2006 were included and followed up from admission to discharge or transfer to another ward. Data collection occurred daily except for weekends and public holidays.

The following information was documented for each patient:

- Demographic data: Date of birth, age, gender, height and weight
- Prescription data: Dose, dose interval, form and route of administration of each drug
- Documented diagnosis, which was obtained from patient's discharge letter

Patients were excluded from the trial if there was:

- Lack of discharge letters
- No information about their age
- No information about their weight although weight was required to calculate the dose of the prescribed drugs

Prescriptions of intravenous nutrition, inhaled 0.9% sodium chloride solution and subcutaneously or intravenously administered heparin were excluded.

The electronic prescribing system Theriak™ from TM Software hf. Holtasmári 1, IS-201 Kópavogur, Iceland was used to record the patients' data and prescriptions.

2.1.2 Age classification

Patients in the trial were grouped according to the EMEA age classification system into:

- Neonates (0–27 days)
- Infants (28 days–23 months)

- Children (2–11 years)
- Adolescents (12–18 years)
- Adults (>18 years)

This classification was suggested by the EMEA as one possible categorisation system that provides a basis for study design in paediatrics. It was done taking into consideration the developmental biology and pharmacology of children, but a considerable overlap of developmental aspects exists across the age categories (EMEA, 2001).

Immature eliminating organs or the immature blood-brain barrier make neonates (0–27 days) especially susceptible for adverse drug reactions. High variability of absorption processes and rapid changes of eliminating organs make PK predictions very difficult for this age group.

According to EMEA, the infants' category (28 days–23 months) is mainly characterised by the rapid maturation of the central nervous system, the immune system, hepatic and renal clearance pathways and the total body growth. Nevertheless, inter-individual maturation variability has to be considered within this age group.

In children (2–11 years), most of the elimination pathways are mature. An important increase in mental and motor skills also occurs. This may affect the participation of children in efficacy studies for example with the endpoint improvement in walk-test. This test is applicable for children after four years of age (Lammers, 2008).

Adolescents (12–18 years) are characterised by a sexual maturation which may influence some diseases due to hormonal changes. Noncompliance is an important issue in this group, especially for drugs which may affect weight or appearance (EMEA, 2001).

2.1.3 Classification of diagnosis

The present trial was conducted on a ward specialised for paediatric cardiology and pneumology. However, the ward received many paediatric patients, transmitted from the paediatric ward for oncology, haematology and clinical immunology. The main diagnosis or the reason for admission to hospital for patients was classified into five main groups, three of them (cardiovascular diseases; Onco-, haemato- and immunologic diseases; and diseases of respiratory system and cystic fibrosis) represent the main three specialities on the ward, in addition to the category infections, which included infections of respiratory tract and infections after surgical or medical care and those as complications of chemotherapy. The remaining diagnoses were classified under the category "others". The

diagnoses were classified by means of the International Classification of Diseases (ICD-10 German modification; Internationale Statistische Klassifikation der Krankheiten und verwandter Gesundheitsprobleme. 10. Revision. Version 2006). The ICD-10 coding classified the diseases into categories each is coded with a capital letter followed by an Arabic numeral indicating a subcategory, which can be in turn subdivided into particular diseases or health problems. For example: I27.0 refers to the primary pulmonary hypertension, which is included in the subcategory “diseases of the pulmonary heart diseases and diseases of pulmonary circulation (I26-I28)”, which makes a part of the category “disease of the circulatory system (I00-I99)”.

The main diagnoses or the reasons for admission to hospital for patients in the present trial were classified as follows:

- Cardiovascular diseases: This category includes diseases of the circulatory system (I00-I99) in addition to the congenital malformations of the circulatory system (Q20-Q28).
- Onco-, haemato- and immunologic diseases: This category includes malignant neoplasm (C00-C97) and diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism (D50-D90).
- Respiratory system and cystic fibrosis: This category includes cystic fibrosis (E84.0-E84.9) and diseases of the respiratory system which are not caused by infections (J30-J99).
- Infections: This category includes certain infectious and parasitic diseases (A00-B99), infectious diseases of the respiratory system (J00-J22) and infections which occur as complications of surgical or medical care (T82.7, T85.7 and T87.4).
- Others: This category includes diseases which are not frequently seen on the ward, e.g. drug poisoning (T36-T50) or drug withdrawal syndrome in infants of dependent mothers (P96.1).

2.1.4 Classification of drug groups

The prescriptions were coded using the German ATC classification, i.e. Anatomical Therapeutic Chemical Classification (Anatomisch-therapeutisch-chemische Klassifikation mit Tagesdosen, 2007). Drugs in the ATC system are classified in groups at five different levels. The first level of the code indicates the anatomical main group and consists of one letter. The second level indicates the therapeutic main group and consists of two digits. The 3rd and 4th levels are chemical/pharmacological/therapeutic subgroups, each consists

of a letter. The 5th level is the chemical substance and consists of two digits. For example, Propranolol has the ATC code: C07AA05, which belongs to the beta blocking agents, non-selective: C07AA, a subgroup of the beta blocking agent C07A. The latest is one of the subcategories of the therapeutic main group beta blocking agents C07, which is included in the anatomical main group: Cardiovascular system C. This classification was taken to classify the prescriptions in the present trial with some modifications. For example, the platelet aggregation inhibitor acetylsalicylic acid (ATC code: B01AC06), which belongs to the anatomical main group: Blood and blood forming organs in the ATC classification, was classified in the main group "cardiovascular drugs" in the present trial. This is because this drug was chosen from the cardiologists to treat paediatric patients with cardiovascular diseases. The existence of such an agent in the cardiovascular drugs in the present project is more helpful to identify paediatric needs in the field of cardiology and to compare the off-label prescribed cardiovascular drugs in the present trial with those included in the EMEA list of paediatric needs – cardiovascular products, in which acetylsalicylic acid and other "antithrombotics" were considered as cardiovascular products (EMEA, 2006).

The prescriptions in the present trial were classified into the following groups:

- Anti-infectives: In addition to anti-infectives for systemic use (J) this class includes intestinal anti-infectives, anti-infectives for dermatological use and for sensory organs (A07A, D01, D06 and S01A).
- Respiratory system: Drugs with the ATC code: R.
- Analgesics and antipyretics: Drugs with the ATC codes: N02 and M01.
- Alimentary tract and metabolism: Drugs with the ATC code A without A07A.
- Cardiovascular system: Drugs with the ATC code: C, in addition to antithrombotic agents (B01).
- Systemic hormone preparations: Drugs with the ATC code: H.
- Blood and blood forming organs: Drugs with the ATC codes: B without B01.
- Dermatologicals: Drugs with the ATC code: D without D01 and D06.
- Nervous system: Drugs with the ATC code: N without N02.
- Antineoplastic and immunomodulating agents: Drugs with the ATC code: L.

2.1.5 Reference sources for drug information

Drug information was obtained from the German Summary of Product Characteristics (SPCs) as a primary source for drug information and from the Pharmaceutical Index for Germany, i.e. Rote Liste® as an alternative source.

According to German Drug Law (Arzeimittelgesetz, AMG), information about drugs which are approved in Germany and Europe have to be available for physicians, dentists and pharmacists. For this purpose, about 8000 SPCs are documented and can be accessed online for medical health professionals under http://www.fachinfo.de. Information about the pharmaceutical form, strength, route of administration, indication with corresponding dosing recommendation and the approved age groups is included.

Rote Liste®, available under http://www.rote-liste.de, is a compendium with information on approved drugs in Germany and Europe. Drug information is presented as a short abstract and was used as an alternative information source when no SPC for the medical preparation was available online.

2.1.6 Classification of off-label prescriptions

The identification of off-label drug use was carried out by the analysing of the license information for drugs with reference sources mentioned in the section 2.1.5. Off-label prescribed drugs were hierarchically categorised, i.e. with respect to age, indication, route of administration and dose, respectively. First, the prescriptions were analysed with respect to age; a drug was considered to be off-label due to age if it was given to a patient younger than the age group for which the drug was recommended. If the drug was labelled with respect to age, then the drug was analysed with respect to indication. If the prescribed drug was labelled with respect to age and indication, it was then analysed with respect to the route of administration, and finally with respect to dose. According to this classification system, an off-label prescribed drug can not be classified in more than one group. For example, propranolol for the treatment of portal hypertension in children was considered off-label due to age and not due to indication although the drug was not labelled for this indication.

When determining if a prescribed dose was above or below that recommended, a 10% margin of error was allowed, i.e. a prescription was considered off-label due to dose when the prescribed dose was ±10% of the recommended dose. Dosing intervals had to be identical with the recommendations. This was in accordance to previous trials that analysed the prescribing compliance with dosing recommendations or trials that analysed prescribing errors in paediatrics (Cheng, 2009; Condren, 2010). Pro re nata (PRN) analgesics and antipyretics, i.e. those to take as needed, were considered off-label due to dose if the prescribed single dose was 10% less than the recommended single dose or when the prescribed daily dose was 10% more than the maximum recommended daily dose.

A prescription was considered labelled for all paediatric age groups when a dose recommendation was generally given to children, even if no age specific dosing was mentioned. In this case the recommended dose for children in the SPC was adopted for all paediatric age groups. If a dose recommendation was given in the SPC for children up to 12 years of age without specific dose recommendation for adolescents, the drug was also considered labelled for adolescents. In this case, the dose for adolescents was regarded the same as for adults.

Similar to the studies of Dell'Aera and Bücheler, drugs with no paediatric information were classified in the current trial as off-label prescriptions due to age (Dell'Aera, 2007; Bücheler, 2002). This differs from the classification of other studies, which had classified such prescriptions as unlicensed drugs ('t Jong, 2002). The present trial was not designed to analyse the unlicensed drug use including extemporaneous preparation.

2.1.7 Result comparison with the EMEA list of paediatric needs and the priority list

The cardiovascular drugs classified as off-label prescriptions due to age or indication in the current trial were compared with the list of paediatric needs and the priority list for research into off-patent paediatric medicinal products established by the EMEA (EMEA, 2006; EMEA, 2007). This comparison was made to see if the off-label prescriptions and their indications were recognized by the EMEA, which can indicate if the off-label drug use identified in the present trial is also widespread in other European countries.

The EMEA list for paediatric needs in cardiovascular drugs is established to identify the paediatric needs in cardiology where there should be research and development of medicinal products for children, either old (i.e. off-patent) or new ones (including those under development) (EMEA, 2006).

The EMEA priority list for studies into off-patent paediatric medicinal products includes only products which are not covered by a basic patent or a supplementary protection certificate (EMEA, 2007). This list was established as a consequence of the paediatric regulation (Regulation (EC) No 1901/2006, 2006), which aimed to increase the availability of drugs authorised for children as well as to increase the information available on the use of drugs in paediatrics. The regulation includes provisions for funding studies into off-patent medicinal products. The EMEA priority list for studies into off-patent paediatric medicinal products aimed to direct the funds into research of drugs with the highest need in the paediatric population (EMEA, 2007).

2.1.8 Data analysis

Frequencies and percentages were used to summarise the categorical variables of the data: Patient' genders, patients age groups, diagnosis categories, prescriptions in every drug group and the off-label prescriptions within every drug group.

The number of prescribed drugs for every patient, the age of patients, and the average applied dose of off-label prescriptions among the cardiovascular drugs were summarised as medians and presented with their ranges.

Statistical analysis was done with Microsoft Excel 2003, Microsoft Office XP version (Microsoft Corporation, Redmond, USA).

2.2 Development of a PBPK model for sildenafil in adults and children

2.2.1 Pharmacokinetic simulation with PK-Sim®

2.2.1.1 Structure of PK-Sim®

The simulations were run using PK-Sim® Version 4.1.3 (Bayer Technologies Services, Leverkusen, Germany). PK-Sim® is a software package for PBPK modelling software which mathematically represents the different biophysical and biochemical processes that affect PK of a drug in mammalians. The concept of PBPK modelling was presented in the introduction in section 1.8.

PK-Sim® allows the analysis of concentration-time curves of a compound in blood as well as in different organs; relevant PK parameters can also be automatically calculated by PK-Sim®. PK-Sim® consists of seventeen organs and tissues represented as different compartments. The structure of PK-Sim® is presented in Figure 2, showing the different organs connected with their arteries and veins to the arterial and venous blood pool. The transport of a compound among the different organs occurs via organ-specific blood flow rates. Each organ or tissue consists of three sub-compartments: vascular, interstitial and cellular spaces. The vascular space contains the plasma and the blood cells. Intracellular mass transfer occurs via passive diffusion or via user-defined active transport processes. The distribution rate of a drug depends on organ-specific blood flow and the surface area product. The amount of drug partitioning into tissue is determined by the organ plasma partition coefficient, which is a compound-related parameter internally calculated by PK-Sim® from the lipophilicity of the compound.

To simulate PK of a drug using PK-Sim®, two input data sets are required:

1. Compound-related data: Lipophilicity, molecular weight, solubility and plasma unbound fraction, and clearance.
2. Anthropometric data: Age, weight, height and race of the simulated individual.

The compound-related data have to be entered into PK-Sim® by the user to calculate parameters that affect the uptake, distribution and elimination of the drug, such as fraction absorbed from the gastric intestinal tract, partition coefficients and permeability. Hepatic, renal and biliary clearances can be described as user-defined parameters to be saturable processes or first-order intrinsic clearances.

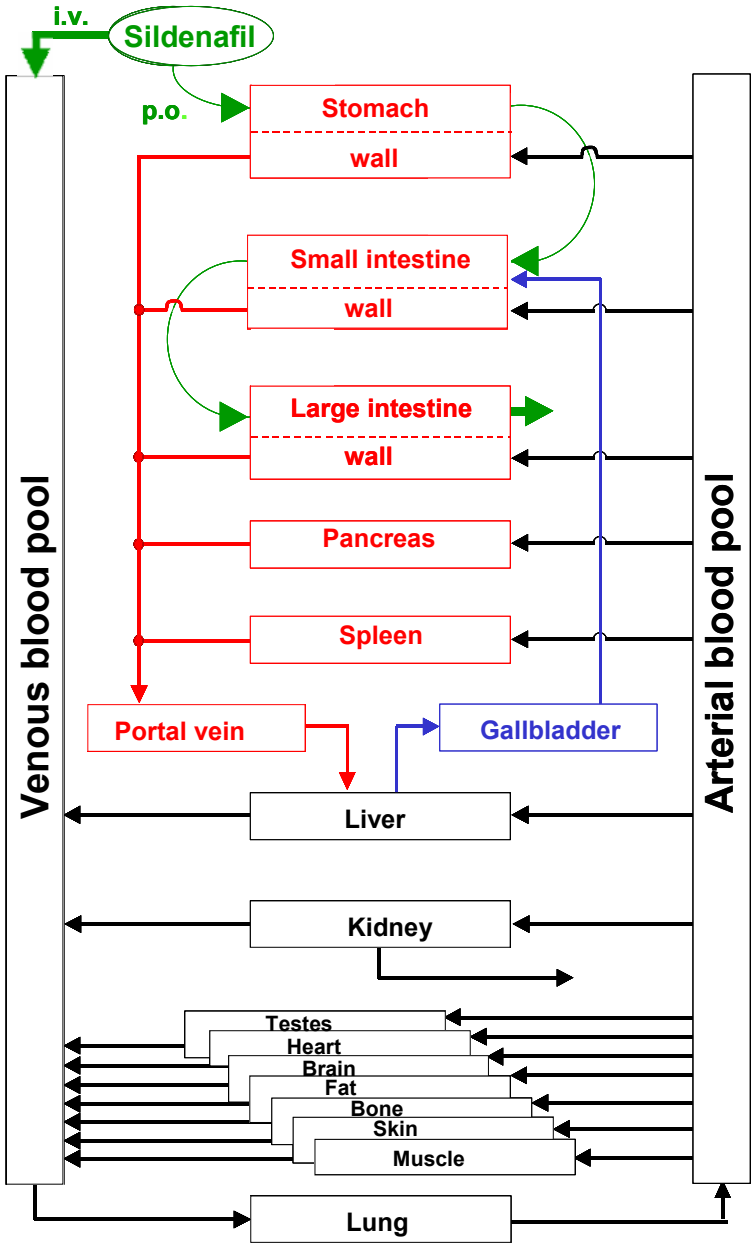

Figure 2: Structure of the PK-Sim® software (adopted from Willmann, 2003)

Different organs and tissues are represented and considered as different compartments, which are connected with their arteries and veins to the arterial and venous blood pool.

The absorbed amount and uptake rate of a drug from gastric intestinal tract is simulated by PK-Sim® considering the gastric and intestinal pH, active surface area across the small intestine, gastric emptying time and intestinal transit time.

For drugs undergoing intestinal first-pass metabolism, such as sildenafil, PK-Sim® enables the user to define different intestinal cytochrome P450 enzymes with their corresponding activities across the different intestinal segments.

2.2.1.2 Creating a single virtual individual with PK-Sim®

A single virtual individual can be created by PK-Sim® using the demographic characteristics of the simulated subject. Depending on the age of the simulated subject, PK-Sim® calculates the corresponding mean body weight, height and body composition considering the gender and race of the simulated subject. Consequently, PK-Sim® allometrically scales the body organ weights from the mean individual for the corresponding gender and age to the simulated subject using the entered information about the height of the simulated subject (Willmann, 2007). Height rather than body weight or BMI was seen to be correlated to the organ weights in humans (La Grandmaison, 2001). The skin weight is calculated by PK-Sim® using allometric scaling depending on the subject weight. This is based on published results, which demonstrated that skin weight is proportional to body weight with an exponent around 0.5 (Willmann, 2007). The allometric scaling is applied internally by PK-Sim® to calculate all organ weights of a simulated subject except for brain, and fat tissue. The brain weight is kept constant independent of the subject weight or height according to published results (ICRP, 2002). The weight of fat tissue is estimated by PK-Sim® to be the difference between the subject weight and the sum of the estimated weights of all other organs is estimated to be (Willmann, 2007).

The organ blood flows for the simulated subject are calculated based on the constant relative fractions from the scaled cardiac output. Cardiac output in the simulated subject is scaled from the mean cardiac output, i.e. that of the mean subject for the corresponding age and gender. Cardiac output is scaled from the mean subject using allometric scaling function with height (Willmann, 2007).

2.2.1.3 Creating a virtual population with PK-Sim®

PK-Sim® also enables the user to create a virtual population and to estimate the PK variability in this population. This is based on the knowledge of the variability of physiological parameters that affect the variability of PK in a population. For this purpose, PK-Sim® has a “population module” which uses the information about the age-dependent distribution of weight, height and BMI to build a virtual population with the corresponding organ volume, blood flow rates, gastric intestinal tract dimensions and tissues composition (Willmann, 2007). This information was obtained from the NHANES III study and the ICRP report (National Center for Health Statistics, 1997; ICRP, 2002). The NHANES III study, which included approximately 30,000 individuals aged 2 months and older from both gender and of different races, provided statistically distribution curves for body weight,

height and BMI over the whole age range for Caucasian, Afro-American and white Mexican American populations. Information on mean organ weights and blood flows were gathered from the ICRP report (ICRP, 2002).

In order to create a target population, the user first has to enter the number of individuals, race, age range, gender distribution and the minimum and maximum values for two parameters out of: Body weight, height, and BMI of the simulated individuals in the Population module window in PK-Sim®. First, virtual individuals are randomly given ages and genders according to the user-defined ranges and distributions. A “mean individual” is then created for every age and gender as explained in section 2.2.2. Consequently, a new height is randomly chosen within a normal distribution with a mean value being equal to the height of the mean individual. The organ weights are then calculated for this new height keeping the same BMI as the mean individual. After that, each organ weight is randomly varied using normal distributions for the weight of all organs except muscle, fat tissue, lung and spleen, which are log-normally distributed (Willmann, 2007).

2.2.1.4 Clearance scaling from adults to children with PK-Sim®

PK-Sim® implements a clearance scaling module to scale the clearance (biliary, renal or hepatic) from adults to a child depending on the clearance value, plasma unbound fraction and the main plasma binding protein in adults. In addition, the fraction of contribution of every hepatic eliminating enzyme has to be given. The estimation of the paediatric clearance in the simulated age depends on the maturation of the eliminating organ or the activity of the eliminating enzyme in the simulated age relative to adults. PK-Sim® individually scales each pathway to the desired age, inclusive of protein-binding prediction. The scaled pathways are then summed to generate a total plasma clearance for the simulated child (Edginton, 2006a). In the present project, the hepatic clearance of sildenafil was scaled to paediatric age groups using the clearance scaling module in PK-Sim®. The concept of this physiology-based scaling is presented in detail in section 2.2.11.1.

2.2.2 Work flow for the development of a PBPK model for sildenafil in children

Figure 3 illustrates the workflow for building the final PBPK model for sildenafil in children.

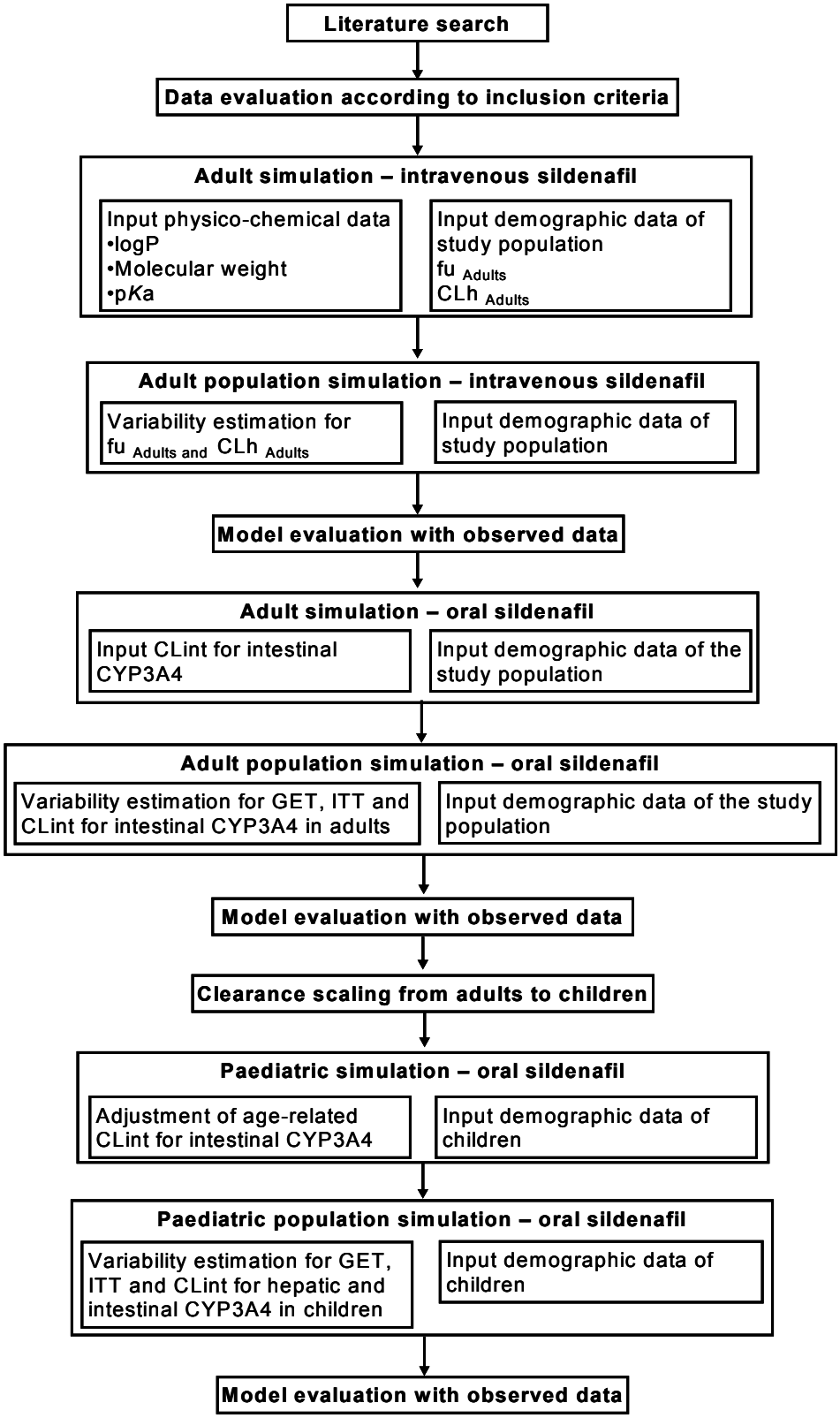

Figure 3: Workflow for the development of a PBPK model for sildenafil in children modified from (Edginton, 2006b)

PK: Pharmacokinetic. logP: Lipophilicity. p*K*a: Ionization constant. Fu_{Adults}: Unbound plasma fraction in adults. CLh_{Adults}: Hepatic clearance in adults. CLint: Intrinsic clearance. ®: Cytochrome P450 enzymes. GET: Gastric emptying time. ITT: Intestinal transit time.

A literature search was carried out to summarise compound-related parameters of sildenafil such as clearance, unbound fraction and physico-chemical parameters. A literature search was also done to obtain PK data for sildenafil in adults and children. Data on the inter-individual variability of particular physiological parameters that affect the inter-individual variability of sildenafil PK were obtained from the published literature. The results of the literature search were evaluated according to inclusion criteria (listed in section 2.2.3). A PBPK model for intravenous sildenafil in adults was developed and evaluated by a comparison to observed mean data. A simulation for intravenous sildenafil in a virtual adult population was run considering the variability of the physiological parameters. The population simulation was evaluated by a comparison with observed individual data. Depending on the model for intravenous sildenafil, a PBPK model for oral sildenafil in adults was developed and evaluated by a comparison to observed mean data. A simulation for oral sildenafil in a virtual adult population was performed in a similar way to that for intravenous sildenafil. Consequently, the clearance of sildenafil was scaled from adults to children to predict the age-related exposure of sildenafil in the different paediatric age groups. The PBPK simulation of oral sildenafil in children was compared to observed data if available

2.2.3 Literature search

2.2.3.1 Literature search for compound-related parameters

A literature search using biomedical databases (MEDLINE via PubMed and Google Scolar) was conducted using the following keywords: “sildenafil”, “lipophilicity”, “solubility”, “ionization constant”, “plasma protein binding” and “clearance”. The reference lists of the identified articles were also screened to find any pertinent articles. Product monograph and additional literature, which was found by browsing literature about sildenafil, were also considered. The results were listed in a spreadsheet. Only parameters for which determination methods were mentioned and those extracted from the product monograph were considered for the estimation of a reference range for model input. Input parameters were manually adjusted to fit the sildenafil plasma concentration-time curves in adults. The selected values for model input had to be taken from the reference ranges, which were estimated from the literature search.

2.2.3.2 Literature search for pharmacokinetic studies of sildenafil

The same process mentioned in section 2.2.3.1 was carried out to search PK data of sildenafil in adults and children. The following two terms were used: “sildenafil” and “pharmacokinetics”. The search was limited to “human”. The articles were screened and all PK studies were listed in a spreadsheet with information about the following criteria:

- Number of subjects
- Demographic data: Age, race, gender, weight, height and body mass index (BMI)
- Assay: Calibration range, accuracy and precision
- Protocol: Fasted or fed state
- Dose

PK studies were chosen for the development and evaluation of the adult model if they fulfilled the following inclusion criteria:

- Healthy subjects
- Caucasian population
- Validated assay
- Age: 18 – 65 years
- Fasted state for the simulation of orally administered sildenafil

The authors of the studies were contacted via email to obtain the mean plasma concentration-time data or individual data if possible in numeric form. In the case that this data could not be received, ScanData® (Bayer Technology Services GmbH, Leverkusen, Germany) was used to scan the mean plasma concentration values from the PK profiles presented in the figures of the articles. The numeric values of the plasma concentration-time profiles could be approximately obtained in this way,.

2.2.3.3 Literature search for inter-individual variability for physiological parameters

A literature search using biomedical databases (MEDLINE via PubMed and Google Scolar) was carried out to find studies investigating the inter-individual variability for particular physiological parameters that affect the inter-individual variability of the sildenafil PK. These physiological parameters were: Hepatic clearance, unbound plasma fraction, gastric emptying time, intestinal transit time and intestinal clearance. The inter-individual

variability of hepatic clearance and unbound plasma fraction affects the inter-individual variability of PK of both intravenous and oral sildenafil. The inter-individual variability of gastric emptying time, intestinal transit time and intestinal clearance contributes to the variability of PK of oral sildenafil. If the variability of a parameter was already reviewed and analysed in previous studies, the results of the review articles were preferred. Otherwise, relevant studies in healthy subjects were selected. Data on the distribution of these parameters in adults and paediatric populations were obtained. If a high variability between studies existed, an average value for the identified parameters was then calculated. The estimated variability for the physiological parameters was given to simulate the sildenafil PK in virtual populations.

2.2.4 Development of a PBPK model for intravenous sildenafil in adults

As mentioned in section 2.2.3.1, a reference range for the physico-chemical and other compound-related parameters were estimated. Values from these ranges were manually adjusted to fit the plasma concentration-time profiles of intravenous sildenafil. Plasma concentration-time curves of the intravenous and not of the oral sildenafil were fitted with values for logP and pK_a. This was necessary to exclude other factors, such as absorption and first-pass effect, which influence the plasma concentration-time curves after the oral administration of sildenafil. Values which were selected in this step were not allowed to be modified in the next steps of the model development.

The following input parameters were used at this stage:

- Lipophilicity (logP)
- Unbound plasma fraction (fu)
- Molecular weight [g/mol]
- Ionization constant for the acidic and basic functions (pK_a)
- Solubility for different pH values [mg/ml]
- Plasma clearance [ml/min/kg]

Lipophilicity in the simulation software refers to the membrane affinity which is the partition coefficient of the compound between water and lipid bilayer (Willmann, 2004). This value may differ from the experimental logP values which are defined as the octanol/water partition coefficient.

The molecular weight and lipophilicity of the compound are essential to calculate the permeation into each organ (Willmann, 2003).

The ionization constants for both acidic and basic functions, unbound fraction and lipophilicity are relevant parameters to predict the distribution of the compound into different tissues (Rodgers, 2007).

Clearance values, given either as intrinsic clearance from *in vitro* experiments or as plasma clearance from *in vivo* experiments, are essential to calculate the fraction of dose eliminated by the eliminating organs.

2.2.5 Simulation of the pharmacokinetics of intravenous sildenafil in a virtual adult population

First, the population module of PK-Sim® was used to create a virtual population of 1000 subjects with demographic characteristics matched to those of the subjects in the reference PK study. The range of age, weight and height in addition to the race, gender and the applied dose were given. Before the population simulation was run, the variability of the hepatic clearance and the unbound plasma fraction of sildenafil was given to the module.

2.2.6 Development of a PBPK model for oral sildenafil in adults

A PBPK model for oral sildenafil was developed depending on the PBPK model for intravenous sildenafil. The intrinsic clearance of the intestinal cytochrome P450 enzymes involved in the first-pass effect of sildenafil was fitted to achieve a bioavailability of approximately 40% and to achieve an adequate visual check for the simulation. Data on the distribution of the cytochrome P450 enzymes across the human intestine were obtained from the published literature (Zhang, 1999) and used to estimate the intrinsic clearances in the different segments of the small intestine. Zhang and colleagues measured the amount of cytochrome P450 enzymes in enterocytes taken from different sites along the human small intestine. As the lengths of the duodenum (0.25 m), the jejunum and the ileum (each 3 m) in humans were known (Kararli, 1995), and considering the distance of the sample sites from the proximal end of the small intestine in Zhang's study, the mean value for the amount of cytochrome P450 enzymes in the duodenum, the jejunum and the ileum was calculated in the present project. The relative distribution of the cytochrome P450 enzymes along the small intestine was considered to be correlated with the relative distribution of the intrinsic clearance (CLint) of these enzymes. The estimated distribution of the CLint of intestinal cytochrome P450 enzymes was taken into consideration when the intestinal CLint was fitted to the PK porfile of oral sildenafil.

2.2.7 Simulation of the pharmacokinetics of oral sildenafil in a virtual adult population

A virtual population of 1000 subjects was created as explained in section 2.2.5. The variability which was estimated in the development of the population pharmacokinetic model for intravenous sildenafil was not changed; however, the variability of additional physiological parameters that affect the PK variability of oral sildenafil in adults was given. These parameters were: Gastric emptying time, intestinal transit time and intrinsic clearance of intestinal CYP3A4 which are involved in the first-pass effect of sildenafil.

2.2.8 Development of a PBPK model for oral sildenafil in children

2.2.8.1 Hepatic clearance scaling from adults to children

Using the clearance scaling module in PK-Sim®, the hepatic clearance of sildenafil was scaled from adults to children. PK-Sim® scales clearances to children depending on the following input parameters in adults: Clearance, unbound plasma fraction, and the contribution of every eliminating pathway to the total clearance.

To scale the drug clearance to children, PK-Sim® first calculates the intrinsic hepatic clearance in adult using equation 1 (Edginton, 2006a).

$$\mathrm{CLint} = \mathrm{CL_H} \frac{\mathrm{Q_H}}{\left(\mathrm{Q_H} - \mathrm{CL_H}\right)} \times \frac{1}{\mathrm{fu}} \qquad (1)$$

where CLint is the intrinsic hepatic clearance, CL_H is the hepatic clearance, Q_H is the hepatic blood flow and fu is the unbound plasma fraction. Intrinsic hepatic clearance is then normalised to the liver weight [ml/min/g] and the proportion of every metabolic enzyme is calculated. Based on the age-related enzyme activity, the intrinsic clearance is scaled to the age of the simulated child. The scaled intrinsic clearance is then calculated back to the hepatic clearance based on age-specific Q_H, age-specific fu and liver weight (Edginton, 2006a).

2.2.8.2 Intestinal clearance scaling from adults to children

The intrinsic clearance (CLint) of the small intestine was scaled from adults to children depending on published data on the ontogeny of intestinal CYP3A4. For this purpose, the fraction of intestinal CYP3A4 activity in the different paediatric age groups relative to their activity in adults was used to predict the age-related CLint of intestinal CYP3A4 as a

fraction of adult value. For the calculation of CLint [ml/min] of the intestinal CYP enzymes, the adult value was multiplied by age-related intestinal surface area and age-related intestinal CYP3A4 activity as a fraction of adult value using equation 2:

$$CLint_{Paed} = CLint_{Adult} \times F_{CYP3A4\ activity} \times F_{IntestinalSA} \quad (2)$$

Where $CLint_{paed}$ is the age-related intrinsic intestinal clearance of the simulated age, $CLint_{Adult}$ is the intrinsic intestinal clearance in adults, $F_{CYP3A4\ activity}$ is the age-related intestinal CYP3A4 activity as a fraction of the adult activity and $F_{Intestinal\ SA}$ is the age-related intestinal surface area as a fraction of the adult value.

$F_{CYPs\ activity}$ was calculated for every simulated paediatric age based on published literature on the ontogeny of intestinal CYP3A4, whereas $F_{Intestinal\ SA}$ was calculated using PK-Sim® data on the surface areas of different intestinal segments.

2.2.9 Simulation of the pharmacokinetics of oral sildenafil in virtual paediatric populations

Similar to the development of the population pharmacokinetic model for oral sildenafil in adults, the variability for physiological parameters that affect the PK variability of oral sildenafil in children was given to the module. These parameters were: The intrinsic hepatic clearance, the gastric emptying time, the intestinal transit time and the intrinsic intestinal clearances.

2.2.10 Calculation of the pharmacokinetic parameters

The PK parameters were calculated using non-compartmental methods. Area under the curve to infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal rule for data from time 0 to the last data point plus the area under the curve from the last data point to infinity ($AUC_{last-\infty}$), where $AUC_{last-\infty}$ was calculated using equation 3:

$$AUC_{last-\infty} = \frac{C_{last}}{k_e} \quad (3)$$

where k_e is the elimination rate constant and was determined from the slope of the natural logarithm of the last 20% of the data points from the concentrations versus time plot.

Terminal half-life ($t_{1/2}$) was calculated using equation 4:

$$t_{1/2} = \frac{\ln 2}{k_e} \quad (4)$$

Clearance (CL) was calculated using equation 5:

$$CL = \frac{Dose}{AUC_{0-\infty}} \quad (5)$$

Volume of distribution at steady state (V_{ss}) was calculated using equation 6:

$$V_{ss} = MRT \times CL \quad (6)$$

where MRT is mean residence time and was calculated using equation 7:

$$MRT = \frac{AUMC}{AUC} - \frac{\tau}{2} \quad (7)$$

where τ is the infusion time and AUMC is the area under the moment curve and was calculated using the trapezoidal rule for data up to the last data point plus the area under the moment curve from last point data to infinity ($AUMC_{last-\infty}$), where $AUMC_{last-\infty}$ was calculated using equation 8:

$$AUMC_{last-\infty} = \frac{t_{last} \times C_{last}}{k_e} + \frac{C_{last}}{k_e^2} \quad (8)$$

2.2.11 Evaluation of the PBPK model of sildenafil

The performance of the simulations was evaluated by:

- Visual predicted check
- Goodness-of-fit plots
- Numerical evaluation

To visually evaluate the simulations for intravenous or oral sildenafil, simulated versus observed mean plasma concentration-time plots for all simulated studies were presented. For the population pharmacokinetic simulations, plots for median, 5th and 95th percentiles, minimum and maximum of the simulated versus individual plasma concentrations were presented.

To evaluate the population pharmacokinetic simulations of both intravenous and oral doses, diagnostic goodness-of-fit plots were presented showing:

- Population predicted versus observed plasma concentrations
- Weighted residuals versus population predicted plasma concentrations
- Individual predicted versus observed plasma concentrations

- Weighted residuals versus time after dose

Population predicted values were presented based on the predicted population median. Weighted residuals were calculated using equation 9.

$$\text{Weighted residual} = \frac{C_{pre} - C_{obs}}{C_{obs}} \quad (9)$$

where C_{pre} and C_{obs} is the predicted and the observed plasma concentration respectively.

To numerically evaluate the performance of the intravenous and oral model, the median percentage error (MPE) as a measure for the bias and the median absolute percentage error (MAPE) as a measure for the precision of the simulation of plasma concentrations and the prediction of PK parameters (V_{ss}, $AUC_{0-\infty}$ and $t_{1/2}$ for intravenous doses and C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ for oral doses) in adults were used. The percentage error (PE) was estimated using equation 10.

$$PE = \frac{y_{pre} - y_{obs}}{y_{obs}} \times 100 \quad (10)$$

where y_{pre} and y_{obs} is the predicted and the observed value respectively.

The absolute percentage error (APE) was estimated using equation 11.

$$APE = \frac{\left|y_{pre} - y_{obs}\right|}{y_{obs}} \times 100 \quad (11)$$

To numerically evaluate the predictive performance of the model in children, the mean relative deviation (MRD) was used in addition to MPE and MAPE. MRD is estimated by the arithmetic average of distance of the observed plasma concentration values from the predicted values on a logarithmic scale using equation 12.

$$MRD = 10^{x};\ x = \sqrt{\frac{\sum(\log C_{obs} - \log C_{pre})^2}{n}} \quad (12)$$

where n is the number of observed data points, log C_{obs} is the logarithm of the observed concentration and log C_{obs} is the logarithm of the predicted concentration.

An MRD value of ≤ 2 was considered an acceptable prediction meaning that the average of the predicted values is equal or less than a factor of 2 from the observed values.

Statistical analysis was done with Microsoft Excel 2003, Microsoft Office XP version (Microsoft Corporation, Redmond, USA).

3 Results

3.1 A prospective observational trial to analyse the off-label use of drugs in hospitalised children

3.1.1 Patients' characteristics

The trial consisted of 417 patients. Their age ranged from few hours after birth to 40 years. The number of males was larger than females with male:female ratio of 1.3:1. The characteristics of the patients are summarised in Table 1.

Patients' characteristics	Number (%) or median (range)
All patients	417
Gender	
Male	237 (56.8)
Female	180 (43.2)
Age (year)	3.6 (0.0 – 40.3)
Age group	
Neonate (0 – 27 days)	30 (7.2)
Infants (28 day – 23 months)	142 (34.1)
Children (2 – 11 years)	153 (36.7)
Adolescents (12 – 18 years)	69 (16.5)
Adults (> 18 years)	23 (5.5)
Diagnosis*	
Infection	184 (43.8)
Cardiovascular diseases	68 (16.2)
Onco-, haemato- and immunologic diseases	51 (12.1)
Respiratory system and cystic fibrosis	30 (7.1)
Other (for example: epilepsy)	87 (20.7)
Drug prescriptions per patient	4 (0 – 23)

Table 1: Patients' characteristics

* Some patients had more than one main diagnosis; therefore, the total number of diagnoses is more than number of patients.

The diagnoses presented in Table 1 were classified by means of ICD-10 codes as follows: Infections (A00-B99; J00-J22; T82.7, T85.7 and T87.4), cardiovascular diseases (I00-I99; Q20-Q28), oncologic, haematologic and immunologic diseases (C00-C97; D50-D90) and respiratory system and cystic fibrosis (J30-J99; E84.0-E84.9). Infections were the main reason for the admission to hospital in 184 patients (44%), followed by cardiovascular diseases in 68 patients (16%), oncologic, haematologic and immunologic diseases in 51 patients (12%) and diseases of respiratory system and cystic fibrosis in 30 patients (7%).

Children (2 to 11 years) and infants (1 to 23 months) constitute the majority of the patient collective with 37% and 34% of the total patients respectively.

3.1.2 Most frequently prescribed drugs

The number of drug prescriptions given to every patient ranged from 0 to 23 prescriptions with an average of 4 drug prescriptions per patient. The trial included 1,812 prescriptions representing 211 different active agents. The ten most frequently prescribed drugs with their frequencies are presented in Figure 4. This list consists of three anti-infectives, two analgesics and antipyretics, two drugs for respiratory system, one cardiovascular drug, one drug for alimentary tract and metabolism and one systemic hormone preparation.

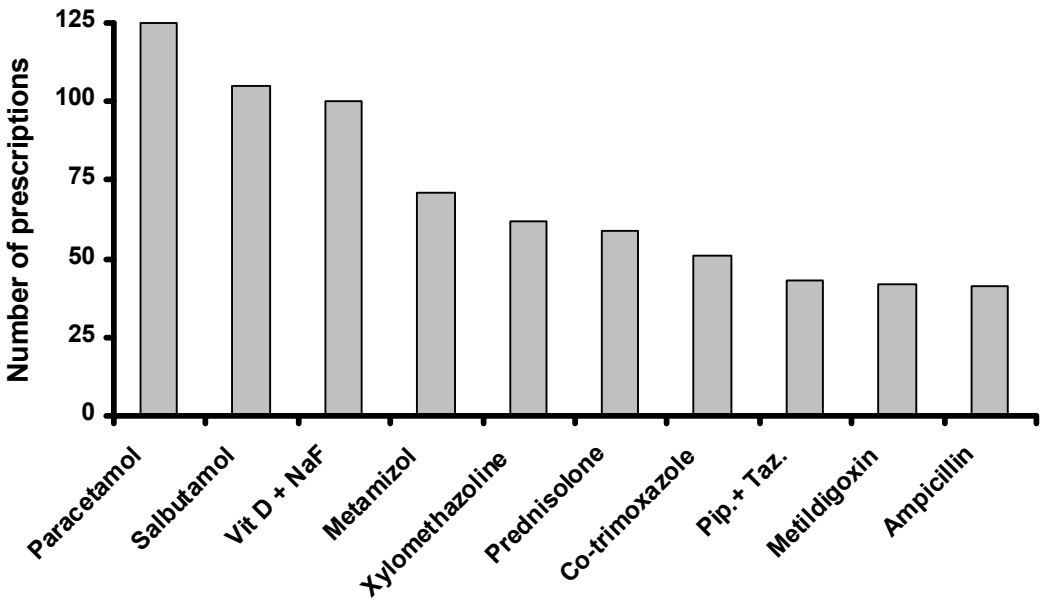

Figure 4: The ten most frequently prescribed drugs in the trial

Vit D + NaF: the combination of cholecalciferol and sodium fluoride; Pip.+Taz: the combination of piperacillin and tazobactam.

Paracetamol was the most frequently prescribed drug with 125 prescriptions including oral, rectal and intravenous formulations, followed by salbutamol with 105 prescriptions either as aerosol or as a solution to inhale. The combination preparation cholecalciferol and sodium fluoride was the third most frequently prescribed drug with 100 prescriptions.

3.1.3 Drug prescriptions in the different drug groups

The most frequently prescribed drug group was anti-infectives with 449 prescriptions (25%), followed by drugs for the respiratory system with 335 prescriptions (18%), drugs for the alimentary tract and metabolism with 269 prescriptions (15%), analgesics and antipyretics with 264 prescriptions (15%), cardiovascular drugs with 216 prescriptions (12%) and systemic hormone preparations with 100 prescriptions (6%). The remaining drug groups were less frequently prescribed for the patients in the trial with percentages less than 5% of the whole analysed prescriptions for every separate drug group. The number and percentage of prescriptions for all drug groups are shown in Table 2.

Drug group*	Number of drug prescriptions [%]
Anti-infectives	449 (24.8)
Respiratory system	335 (18.5)
Alimentary tract and metabolism	269 (14.8)
Analgesics and antipyretics	264 (14.6)
Cardiovascular drugs	216 (11.9)
Systemic hormone preparations	100 (5.5)
Blood and blood forming organs	77 (4.2)
Nervous system	36 (2.0)
Other	30 (1.7)
Antineoplastic and immunomodulating agents	24 (1.3)
Dermatologicals	12 (0.7)

Table 2: Number of drug prescriptions in each drug group (total number is 1,812)

In Table 2, the drug groups are classified by means of ATC codes as follows: Anti-infectives (J, A07A, D01, D06 and S01A), respiratory system (R), analgesics and antipyretics (N02 and M01), alimentary tract and metabolism (A without A07), cardiovascular drugs (C and B01), systemic hormone preparations (H), blood and blood forming organs (B without B01), nervous system (N without N02), antineoplastic and immunomodulating agents (L) and dermatologicals (D without D01 and D06).

3.1.4 Most frequently prescribed off-label drugs

There were 253 out of 417 patients (61%) who received at least one off-label prescription. Of the 1,812 drugs 553 (31%) were used off-label; out of these, 216 (39%), 170 (31%) and 167 (30%) were used off-label due to dose, indication and age respectively.

The ten most frequently prescribed off-label drugs with their frequencies are presented in Figure 5. The list consists of three cardiovascular drugs, three anti-infectives, two drugs for respiratory system, one drug for alimentary tract and metabolism and one systemic hormone preparation.

Salbutamol was the most frequently prescribed off-label drug, followed by co-trimoxazole and oxacillin with 55, 44 and 30 prescriptions respectively. Salbutamol was often prescribed in an off-label manner due to dose, as its prescribed dose was up to 200% of the approved dose. The second and third most frequently prescribed off-label drugs were co-trimoxazole for the prophylaxis for Pneumosystis-carinii-Pneumonia in children and oxacillin given in a dose interval of 8 h.

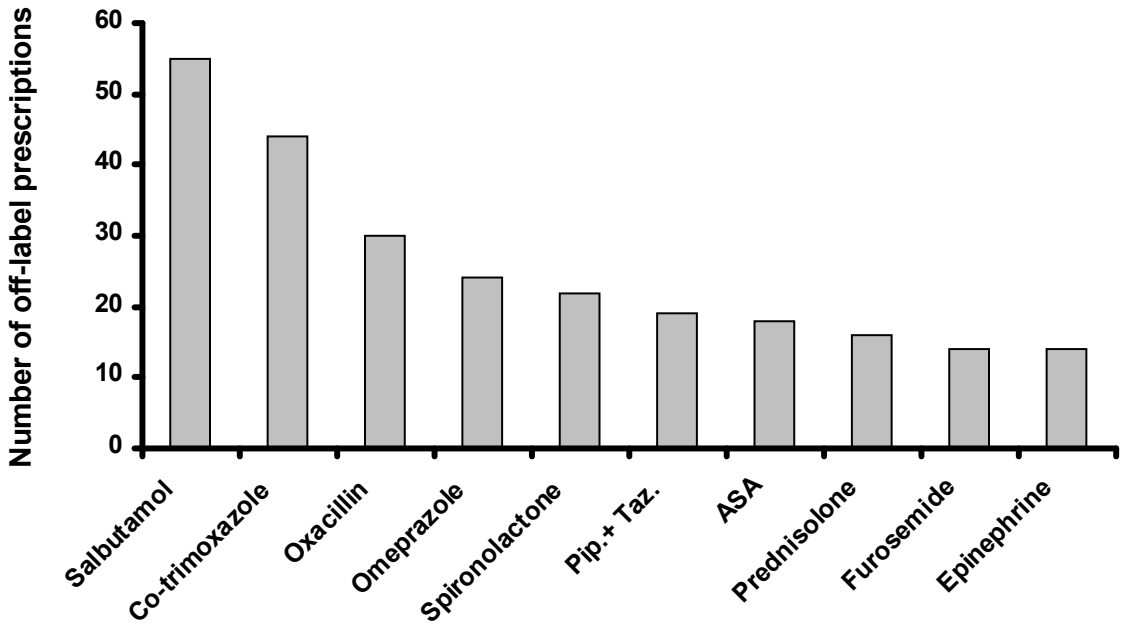

Figure 5: The ten most frequently prescribed off-label drugs in the trial

Pip.+Taz: The combination of piperacillin and tazobactam. ASA: Acetylsalicylic acid

3.1.5 Off-label drug use among the different drug groups

Cardiovascular drugs had the highest percentage of off-label prescriptions, where 129 out of 216 cardiovascular drugs (60%) were used off-label. The off-label use in cardiovascular

drugs was mainly due to age with 72 prescriptions (33%), whereas 38 (18%) and 19 (9%) cardiovascular drugs were used off-label due to indication and dose respectively. The status of off-label drug use among the remaining drug groups is illustrated in Table 3.

	Off-label prescriptions due to:			Total number of
Drug group* (number of prescriptions)	**Dose (%)**	**Indication (%)**	**Age (%)**	**off-label prescriptions (%)**
Anti-infectives (449)	86 (19.2)	72 (16.0)	32 (7.1)	190 (42.3)
Respiratory system (335)	75 (22.4)	16 (4.8)	9 (2.7)	100 (29.9)
Alimentary tract and metabolism (269)	11 (4.1)	27 (10.0)	29 (10.8)	67 (24.9)
Analgesics and antipyretics (264)	5 (1.9)	0 (0)	3 (1.1)	8 (3.0)
Cardiovascular drugs (216)	19 (8.8)	38 (17.6)	72 (33.3)	129 (59.7)
Systemic hormonal preparations (100)	6 (6.0)	13 (13.0)	0 (0.0)	19 (19.0)
Blood and blood forming organs (77)	8 (10.4)	1 (1.3)	7 (9.1)	16 (2.8)
Nervous system (36)	2 (5.6)	0 (0.0)	3 (8.3)	5 (13.9)
Other (30)	2 (6.7)	1 (3.3)	3 (10.0)	6 (20.0)
Antineoplastic & immunomodulating agents (24)	1 (4.2)	2 (8.3)	6 (25.0)	9 (37.5)
Dermatologicals (12)	0 (0.0)	0 (0.0)	4 (33.3)	4 (33.3)

Table 3: Status of off-label drug use among the different drug groups

In Table 3, the drug groups are classified by means of ATC-Codes as follows: Anti-infectives (J, A07A, D01, D06 and S01A), respiratory system (R), analgesics and antipyretics (N02 and M01), alimentary tract and metabolism (A without A07), cardiovascular drugs (C and B01), systemic hormone preparations (H), blood and blood forming organs (B without B01), nervous system (N without N02), antineoplastic and immunomodulating agents (L) and dermatologicals (D without D01 and D06).

Table 3 shows three categories of off-label prescriptions: Due to dose, due to indication and due to age, but no off-label prescription due to route of administration is presented. Epinephrine was the only drug that was applied via a route of administration that was not included in the SPC. Epinephrine was given to inhale in asthma patients, whereas it is licensed for intravenous application as a treatment for anaphylaxis and in cardiac resuscitation. Nevertheless, according to the classification system mentioned in the method

section 2.1.6, these prescriptions were considered off-label due to indication and not due to route of administration.

The number of different ATC codes and the number different off-label prescribed ATC codes within each drug group in the current trial are presented in Table 4. This analysis reflects the paediatric needs and the availability of different therapeutic alternatives that cover these needs in every drug group in the present trial.

The highest number of different prescribed ATC codes were among anti-infectives (55 different ATC codes) followed by drugs for alimentary tract and metabolism (34) and cardiovascular drugs (28). However, cardiovascular drugs were associated with the highest number of different off-label prescribed ATC codes due to age with 17 different drugs.

Drug group*	**Number of different ATC codes**	**Number of different ATC codes used off-label due to age**
Cardiovascular drugs	28	17
Anti-infectives	55	14
Alimentary tract and metabolism	34	11
Blood and blood forming organs	9	6
Respiratory system	20	5
Nervous system	19	3
Dermatologicals	7	3
Antineoplastic und immundulatinagents	11	3
Analgesics and antipyretics	7	1
Systemic hormonal preparations	10	0

Table 4: Comparison between the number of different ATC codes and the number of different ATC codes used off-label due to age within every drug group

In Table 4, the drug groups are classified by means of ATC codes as follows: Anti-infectives (J, A07A, D01, D06 and S01A), respiratory system ®, analgesics and antipyretics (N02 and M01), alimentary tract and metabolism (A without A07), cardiovascular drugs (C and B01), systemic hormone preparations (H), blood and blood forming organs (B without B01), nervous system (N without N02), antineoplastic and immunomodulating agents (L) and dermatologicals (D without D01 and D06).

3.1.6 Off-label prescribed cardiovascular drugs in comparison with EMEA lists

As seen in section 3.1.5, cardiovascular drugs had the highest percentage of off-label prescriptions and the highest number of different off-label prescribed ATC codes compared to the other drug groups in the trial.

A list of 21 different cardiovascular drugs, which were prescribed in an off-label manner due to indication or age, was established. Drugs in this list were compared with the drugs in the EMEA list of paediatric needs and the priority list and are presented in Table 5 (EMEA, 2006; EMEA, 2007). The aim of this comparison was to see if the off-label prescribed drugs in the present trial were also widespread in other European countries.

As seen in Table 5, 18 out of 21 off-label prescribed cardiovascular drugs in the present trial (86%) were included in one or both of the EMEA lists. However, some of the identified indications for which the cardiovascular drugs were prescribed in children in the present trial were not considered by the EMEA. For example, the indication for which ß-blockers were considered by the EMEA was arterial hypertension in children, whereas ß-blockers were prescribed among the children in the current trial for the treatment of heart failure, Arrhythmias or portal hypertension.

Off-label prescriptions University Düsseldorf	Indication	EMEA lists	
		Priority off-patent	Paediatric needs
Bisoprolol	Heart failure	+ [1]	-
Carvedilol	Heart failure	+	+
Metoprolol	Angina pectoris	+ [1]	+ [4]
Nebivolol	Heart failure	+ [1]	-
Propranolol	Arrhythmias, heart failure, portal hypertension	+ [1]	+ [4]
Spironolactone	Heart failure	+ [1]	+
Potasium canrenoat	Heart failure	-	-
Furosemide	Forced diuresis	+ [2]	+ [3]
Hydrochlorthiazide	Heart failure	+	+
Nifedipine	Hypertension	+	+
Amlodipine	Hypertension	+	+
Amiodarone	Arrhythmias	+	+
Sotalol	Arrhythmias	+	+
Isosorbide dinitrate	Hypertension	-	-
Glyceryl trinitrate	Angina pectoris	+ [1]	-
Sildenafil	Pulmonary hypertension	- [a]	+
Phenoxybenzamine	Hypertension	- [a]	-
Acetylsalicylic acid	Prevention of thrombosis, Kawasaki syndrome	-	+
Phenprocoumon	Prevention and treatment of thrombosis	-	+
Enoxaparin	Prevention and treatment of thrombosis	-	+
Urokinase	Thrombotic events	-	+

Table 5: Off-label prescribed cardiovascular drugs in comparison with EMEA lists

+ Listed by EMEA: - Not listed by EMEA. [a] Products are under patent.
[1]: Only for the treatment of hypertension. [2]: Only for the treatment of hypertension and heart failure. [3]: Only for the treatment of hypertension, heart failure and oedema. [4]: Only for the treatment of hypertension and Arrhythmias.

3.1.7 Average prescribed dose for the off-label prescribed cardiovascular drugs

The median (range) of the prescribed single doses of the off-label prescribed cardiovascular drugs compared to the lowest commercially available strength is presented in Table 6. This analysis reflects the paediatric needs and the availability of the appropriate formulations that cover these needs within the cardiovascular drugs.

In the list of the off-label prescribed cardiovascular drugs, eight drugs were prescribed to at least three different patients. For every one of these eight drugs, the median single dose which was prescribed for children was calculated and listed in Table 6. The average single dose as seen in Table 6 was much less than the lowest commercially available strength for some drugs. That means that the commercially available formulation had to be modified to obtain the suitable dose for children. For example, the average single dose of spironolactone was 7.5 mg, whereas the lowest commercially available strength is 25 mg. For sildenafil, all of its applied single doses, which ranged from 2.8 to 8 mg, were lower than the lowest commercially available strength, i.e. 20 mg.

Drug	Number of prescriptions	Route of administration	Single dose [mg] median (range)	The lowest commercially available strength [mg]
Spironolactone	20	Oral	7.5 (2.5-100.0)	25
Acetylsalicylic acid	16	Oral	29.0 (8.0-100.0)	50
Furosemide	9	Intravenous	20.0 (10.0-60.0)	20*
Phenprocoumon	8	Oral	1.5 (0.5-3.8)	3
Propranolol	8	Oral	11.3 (1.0-40.0)	10
Enoxaparin	7	Subcutaneous	30.0 (5.0-50.0)	20
Amiodaron	5	Oral	150.0 (15.0-200.0)	100
Sildenafil	4	Oral	3.5 (2.8-8.0)	20

Table 6: Median single dose for off-label prescribed cardiovascular drugs

* 2 ml ampule contains 20 mg furosemide

3.2 Development of a PBPK model for sildenafil in adults and children

Sildenafil was selected from the list of off-label prescribed cardiovascular drugs in the present trial. It is used to treat children with PAH, which is a life-threatening disease with a poor prognosis in children. It has also been recognised in the EMEA list for paediatric needs. Very few PK data on sildenafil in children exist, which highlights the need for investigation of its PK in children. In the present project, PBPK modelling was used to simulate sildenafil PK in children *a priori* based on published data on the physico-chemical properties and PK of sildenafil in adults.

3.2.1 Results of literature search

3.2.1.1 Literature search for compound-related parameters

3.2.1.1.1 Ionization constants

A summary of values for ionization constant pK_a of both basic and acidic function published in the literature is shown in Table 7. In 2 out of 6 sources, the pK_a determination method had been mentioned. Both of these sources in addition to the product monograph were considered to demonstrate a reference range for pK_a of sildenafil. A reference range of 6.53 to 7.10 for basicity and 9.12 to 9.84 for acidity pK_a was estimated.

Ionization constants pK_a	Value	Reference
Protonation of tertiary amine	6.53*	Pfizer Canada Inc., 2008
Deprotenation of pyrimidine moiety	9.17*	Pfizer Canada Inc., 2008
Basicity pK_a	7.6*	Rodgers, 2007
Basicity pK_a	6.5*	Walker, 1999
Basicity pK_a	8.7*	Cooper, 1997
Basicity pK_a	7.10	Al Omari, 2006
Acidity pK_a	9.84	Al Omari, 2006
Basicity pK_a	6.78	Gobry, 2000
Acidity pK_a	9.12	Gobry, 2000

Table 7: Summary of values published in literature for ionization constants of sildenafil

*The parameter determination method was not mentioned.

3.2.1.1.2 Aqueous solubility and lipophilicity

Published values for sildenafil solubility at different pHs are summarised in Table 8. One out of 3 different sources mentioned the determination method and was therefore included (Wang, 2008). In Wang's study, the solubility of sildenafil was measured at pHs from 3 to 11 at body temperature (37˚C), whereas the solubility in the product monograph was measured in 1 M HCl, water and 1 M NaOH at 23˚C. In the human gastrointestinal tract pH of 1 to 7.5 were measured (Evans, 1988). Therefore, the solubility values in Wang's study were able to better reflect the solubility of sildenafil in the physiological condition in the gastrointestinal tract than those in the product monograph and were therefore considered as input parameters.

Solubility	**Value [mg/ml]**	**Reference**
In water (23˚C)	3.5*	Pfizer Canada Inc., 2008
In 1M HCl (23˚C)	5.8*	Pfizer Canada Inc., 2008
In 1M NaOH (23˚C)	42.3*	Pfizer Canada Inc., 2008
In water	0.285*	de Souza, 2009
In pH 3 (37˚C)	6.965	Wang, 2008
In pH 7 (37˚C)	0.025	Wang, 2008
In pH 11 (37˚C)	0.322	Wang, 2008
Lipophilicity	**Value**	**Reference**
XlogP	1.5*	NCBI**, 2009
$LogD_{7.4}$	2.7*	Walker, 1999
Partition Coefficient (Oct/Water)	2.7*	Pfizer Canada Inc., 2008
LogP	2.75*	Rodgers, 2007
LogP neutral	3.18	Gobry, 2000
LogP cation	0.32	Gobry, 2000
LogD cation	-0.52	Wang, 2008
LogD neutral	1.59	Wang, 2008
LogD anion	1.13	Wang, 2008

Table 8: Summary of values published in literature for aqueous solubility and ionization constants of sildenafil

*The method of the parameter determination was not mentioned. **National Center for Biotechnology Information.

The lipophilicity of sildenafil was found in 6 sources. Its published values are summarised in Table 8. Only two sources mentioned the parameter determination method (Wang, 2008; Gobry, 2000). The lipophilicity in Wang's study was represented by logD whereas it was

represented by logP in Gobry's study. The lipophilicity in PK-Sim® refers to the membrane affinity or the partition coefficient between lipid membranes and water at physiological pH. If the membrane affinity is not available, it is recommended to use logP. Wang's study was therefore excluded, whereas Gobry's study and the product monograph were considered to establish a reference range for the lipophilicity, which was estimated to be 2.7 to 3.18.

3.2.1.1.3 Unbound plasma fraction

Literature search for the unbound plasma fraction (fu) of sildenafil in adults revealed three different sources (Table 9). Sildenafil unbound fraction was independent of its concentration, but age seemed to affect its value. A reference value of 2.7 to 5.71% for fu in adults was suggested.

Unbound fraction mean (SD or range)	Age of subjects [year] mean (range)	Reference
4 (3 – 4)	Not available	Walker, 1999
5.71 (2.25)*	24 (19 – 34)	Purvis, 2002
4.71 (1.64)**	24 (19 – 34)	Purvis, 2002
4.3 (1.1)	30 (19 – 45)	Muirhead, 2002
3.4 (1.1)	70 (65 – 81)	Muirhead, 2002
3.46 (0.61)	(32 – 63)	Muirhead, 2002
2.7 (0.8)	(22 – 72)	Muirhead, 2002

Table 9: Summary of values published in literature for unbound plasma fraction of sildenafil

* Unbound fraction 1 hour after dose, **unbound fraction 4 hours after dose.

3.2.1.1.4 Plasma clearance

Mean clearances were calculated from individual PK data of intravenous sildenafil from five studies. These values are shown in Table 10. Plasma clearance after 80 mg intravenous sildenafil was excluded. This dose is equal to the oral dose of 200 mg, which is out of the therapeutic range where a non-linearity in sildenafil PK was seen (Milligan, 2002). Sildenafil mean clearance after 20 and 40 mg intravenous sildenafil was 6.3 and 5.6 ml/min/kg respectively (Jackson, 1999). This was similar to the value of 6.0 ml/min/kg reported in Walker's study but much less than the value of 9.4 ml/min/kg observed in Nichols' study. Depending on the values summarised in Table 10, a reference range of 5.6 to 9.4 ml/min/kg for plasma clearance of sildenafil was suggested.

Mean clearance [ml/min/kg]	Dose [mg]	Number of subjects	Mean age [year]	Reference
6.0	25	3	51.33	Walker, 1999
6.3	20	8	24.75	Jackson, 1999
5.6	40	8	24.75	Jackson, 1999
4.9	80	8	24.75	Jackson, 1999
9.4	50	12	25.75	Nichols, 2002

Table 10: Summary of plasma clearance values of sildenafil from pharmacokinetic studies of intravenous sildenafil

3.2.1.2 Pharmacokinetic studies of sildenafil

3.2.1.2.1 Pharmacokinetic studies of intravenous sildenafil in adults

A literature search revealed three publications that investigated PK of five different intravenous doses of sildenafil (Nichols, 2002; Walker, 1999; Jackson, 1999). The characteristics of these studies are listed in Table 11. Information about the analytical method was available only in two papers. The majority of the volunteers in all studies were white. Mean age was available for all studies and was around 25 years in four studies and 51 years in one.

Five different doses in a range of 20 to 80 mg sildenafil were studied in the papers. The study which investigated PK of 80 mg intravenous sildenafil was excluded. This dose is equal to the oral dose 200 mg, which is out of the therapeutic range where a non-linearity in sildenafil PK was seen (Milligan, 2002).

According to the FDA guideline for the bioanalytical methods validation, the precision of an analytical method should not exceed 15% of the coefficient of variation (CV) , except for the lower limit of quantification, where it should not exceed 20% of CV (FDA, 2001). As shown in Table 11, the precision of the assay in study 1 was -35 to 8.6% and was, therefore, outside of the FDA criteria. The characteristics of the analytical methods in study 3a and 3b were not mentioned. Although the assay of study 2 was a validated method, there were only three included subjects in this study and were aged more than 45 years. Therefore, the PBPK model for intravenous doses was developed considering all four intravenous studies: 1, 2, 3a and 3b irrespective of their limitations.

Study	Dose [mg]	Mean age [year]	Race of subjects	Assay	Calibration range [ng/ml]	Precision [%]	Accuracy [%]
1	50	25.75	12/12 white	LC-MS	0.1 – 2.0	-35 - 8.6	NA
2	25	51.33	3/3 white	LC-UV	1 – 250	<5.1	-2.3 to 3.5
3a	20	24.75	7/8 white	HPLC	NA	NA	NA
3b	40	24.75	7/8 white	HPLC	NA	NA	NA
3c	80	24.75	7/8 white	HPLC	NA	NA	NA

Table 11: Characteristics of the pharmacokinetic studies of intravenous sildenafil in adults

NA: not available. LC-MS: Liquid chromatography mass spectrometry. LC-UV: Liquid chromatography with ultra violet detection. HPLC: High performance liquid chromatography. Study 1: (Nichols, 2002), Study 2: (Walker, 1999), Studies 3a, 3b and 3c: (Jackson, 1999).

3.2.1.2.2 Pharmacokinetic studies of oral sildenafil in adults

A literature search revealed 26 PK studies found in 19 publications. In these studies, 44 different mean plasma-concentration time profiles of oral sildenafil in healthy adults were presented. These studies with information about the demographic data and protocol are listed in Table 26, Appendix and Table 27, Appendix. There was only one PK profile for the dose of 200 mg. The highest number of profiles was for the dose of 100 mg with 22 profiles. For the dose of 50 mg, 17 profiles were published, whereas for the dose of 25 mg, only one profile was found. For the dose of 20 mg, the approved dose for PAH, only two profiles were found.

The number of volunteers ranged from 3 to 34 subjects in every collective for which a mean plasma concentration-time profile of sildenafil was presented. Information about average age or age range of the volunteers was available for 37 out of 44 profiles and was missing in 7. The race of subjects was mentioned only for 16 profiles. White subjects consist more than 90% of the whole collective in 13 out of 16 profiles. Information about the race or the ethnicity of the volunteers was missing in 28 investigations. Subjects were fasted in 24 investigations and fed in 10 investigations, whereas information about fed/fasted state of the volunteers was missing in 10 investigations.

Information about sildenafil assay in the PK studies are summarised in Table 28, Appendix and Table 29, Appendix. In 23 out of 26 PK studies, information about the precision of the analytical methods was mentioned, whereas in 3 studies, the authors mentioned that the assay was validated but no descriptions of the precision or accuracy was given. In one study, the precision of sildenafil assay was outside of the FDA criteria with a range of -35 to 8.6%.

Only 3 out of 44 mean plasma concentration-time profiles of sildenafil from three different studies met the inclusion criteria for the evaluation and development of the PBPK model for oral sildenafil. Demographic data of subjects in the included studies are summarised in Table 12.

Study	Number of subjects	Dose [mg]	Age mean [years]	Weight mean [kg]	Height mean [cm]
Walker 1999	3	50	51	73.3	175.3
Muirhead 2002c	15	50	30	NA	NA
Jetter 2002	24	50	29	81.8	184

Table 12: Demographic data of the volunteers in the included pharmacokinetic studies of oral sildenafil in adults

3.2.1.2.3 Pharmacokinetic studies of oral sildenafil in children

A literature search revealed only two papers where plasma concentrations of oral sildenafil were investigated in children (Karatza, 2005; Witjes, 2010). Karatza and colleagues measured plasma concentrations 1 h after 0.5, 1 and 2 mg/kg, as well as 3 h after 2 mg/kg oral sildenafil in three children in an age range of 6.6 to 13.9 years. Witjes and colleagues measured plasma concentrations 0, 2, 4 and 6 h after 1.5 mg/kg dose in a 4-month-old infant. Data on the calibration range, precision and accuracy of sildenafil assay in both publications are presented in Table 13. The children in Karatza's study were fasted. No information concerning the fed state of the child in Witjes' study was mentioned. Although no information about the body weight of children in Karatza's study and no data on the fed state of the child in Witjes' study were mentioned, both studies were considered in the evaluation of the paediatric model.

Study	Dose [mg]	Age mean [year]	Number of children	Meal	Assay	Calibration range [ng/ml]	Precision [%]	Accuracy [%]
1	0.5, 1 and 2	10.4	3	Fasted	LC-UV	1 - 250	≤5.1	-2.3 – 3.5
2	1.5	0.3	1	NA	LC-MS	1 - 1000	≤13.4	-4 – 2

Table 13: Characteristics of the published pharmacokinetic studies of oral sildenafil in children

NA: not available. LC-MS: Liquid chromatography mass spectrometry. LC-UV: Liquid chromatography with ultra violet detection. Study 1: (Karatza, 2005), Study 2: (Witjes, 2010).

3.2.1.3 Variability estimation for the physiological parameters

3.2.1.3.1 Variability estimation for the hepatic clearance in adults

The inter-individual variability of sildenafil plasma clearance was not presented in the published studies of intravenous sildenafil (Nichols, 2002; Walker, 1999; Jackson, 1999). Inter-individual variability of sildenafil clearance after oral doses was estimated to be 29% (Milligan, 2002). But this estimation was made for an adult population where the ages (18 – 87 years) and weights (49 – 159 kg) of subjects were very heterogeneous. In addition, this estimation described the variability distribution of clearance after oral doses where additional factors may influence the variability of clearance such as the first-pass effect or absorption process. The inter-individual variability of the clearance of different drugs whose metabolism is mediated by hepatic CYP3A4 was analysed in a previous publication based on pooled results from many *in vivo* studies (Dorne, 2003). Dorne and colleagues estimated a lognormal distribution for the clearance of drugs metabolised by hepatic CYP3A4 with a geometric standard deviation of 1.4. As CYP3A4 is responsible for about 80% of sildenafil metabolism, it was accepted that the variability for the metabolism by hepatic CYP3A4 represents the variability of total hepatic clearance of sildenafil in the present project.

3.2.1.3.2 Variability estimation for the hepatic clearance in children

The published data on sildenafil PK in children are not sufficient to be used in the estimation of the inter-individual variability of sildenafil clearance in children. Dorne and colleagues pooled data on the inter-individual variability of clearance for different drugs metabolised mainly by hepatic CYP3A4 from different paediatric PK studies (Dorne, 2003). According to the results of Dorne and colleagues, a lognormal distribution for the hepatic CLint of sildenafil was estimated with a geometric standard deviation of 1.4 for infants and children (Dorne, 2003).

3.2.1.3.3 Variability estimation for the unbound plasma fraction in adults

Values for the unbound plasma fraction (fu) in adult subjects are summarised in Table 9. The mean value for fu of sildenafil ranged from 2.7 to 5.71% with a coefficient of variation ranging from 18 to 39%. Although the variability of fu of sildenafil was presented as mean ± SD, the authors did not mention that fu values normally distributed. Therefore, a uniform variability for fu in adults was assumed in the present project. The range was 3 to 4% for subjects older than 45 years of age, and 4 to 5% for subjects younger than 45 years of age.

This estimation was based on experimental data which showed that the mean fu was higher in younger ages (4.3%) relative to older ages (3.4%) (Muirhead, 2002).

3.2.1.3.4 Variability estimation for the unbound plasma fraction in children

The unbound plasma fraction (mean ± SD) in neonates was 6.1 ± 2.46 %. Data in other age groups were not found in the literature. As this value was scaled by PK-Sim® from adults to the simulated age and because no data on the variability distribution of fu in children were available, a physiological variability for fu was accepted. It means that all simulated children at a particular age have the same value for fu.

3.2.1.3.5 Variability estimation for the gastric emptying time in adults

Values for the gastric emptying time (GET) with the existence of liquid, solid or mixed meal in healthy adults were pooled from 11 studies. A summary of the results is shown in Table 14. The distribution of the gastric half emptying time (T50) was skewed in one study, which was conducted in nine subjects (Maes, 1995), while T50 in two other studies with higher numbers of volunteers (90 and 28 subjects) normally distributed (Hellmig, 2006; Lartigue, 1994). Therefore, a normal distribution for GET was estimated in the present project.

Sildenafil, as citrate salt, is manufactured as immediate release film-coated tablets. Sildenafil citrate has a relatively high aqueous solubility (3.5 mg/ml) and is expected to rapidly dissolve in the gastrointestinal medium. Therefore, GET for sildenafil was accepted to be an average value for GET for tablets taken with water or for liquid markers with the existence of liquid meal. In the simulated PK studies of oral sildenafil, sildenafil was taken with water in a fasted condition. Thus only the results of studies that investigated GET with existence of liquid meals with low energy content were considered. As seen in Table 14, 6 values for T50 for liquid markers or for tablets with the existence of liquid meals with low energy were found. Average T50 values were: 15, 23, 24, 24, 28 and 81 min with coefficient of variation of: 19, 48, 54, 63, 54 and 27% respectively (Gentilcore, 2006; Hveem, 1996; Hellmig, 2006; Perkins, 2001). Median T50 was calculated to be 24 min and the median coefficient of variation was 51%.

As PK-Sim® defines GET as the time in which 63% of the dose is released from the stomach into the intestine in a fasted state, emptying time for 63% of the dose (T63) was calculated according to equation 13.

$$T63 = T50 \times \frac{63}{50} \qquad (13)$$

The coefficient of variation of T63 was accepted to be the same for T50, i.e. 51%. T63 (mean ± SD) of 30 ± 15 min was estimated in the present project to describe the inter-individual variability of gastric emptying time in adults.

Parameter	Mean ± SD or median (range) [min]	Meal	Study
For liquid markers			
GET	77 (32 - 118)	Mixed liquid-solid	Graff, 2001
T50	35 ± 13	Mixed liquid-solid	Lartigue, 1994
T50	24 ± 8	Mixed liquid-solid	Brophy, 1986
T50	29 ± 30	Mixed liquid-solid	Waller, 1991
T50	28 ± 15	Liquid 12 kcal	Gentilcore, 2006
T50	24 ± 15	Liquid 12 kcal	Gentilcore, 2006
T50	23 ± 11	Liquid 20 kcal	Hveem, 1996
T50	24 ± 13	Liquid 20 kcal	Hveem, 1996
T50	81 ± 22	Liquid	Hellmig, 2006
T50	35 (31)*	Milk	Maes, 1995
T50	122 ± 42	Liquid 314 kcal	Gentilcore, 2006
T50	132 ± 32	Liquid 314 kcal	Gentilcore, 2006
T50	107 ± 42	Liquid 300 kcal	Hveem, 1996
T50	108 ± 48	Liquid 300 kcal	Hveem, 1996
For solid markers			
GET	112 (49 - 164)	Mixed liquid-solid	Graff, 2001
GET	215 (199 - 225)	Mixed liquid-solid	Kuo, 2008
T50	75 ± 15	Mixed liquid-solid	Lartigue, 1994
T50	58 ± 17	Mixed liquid-solid	Brophy, 1986
T50	55 (21)*	Solid 150 kcal	Maes, 1995
T50	95 (29)*	Solid 250 kcal	Maes, 1995
T50	144 ± 55	Solid	Hellmig, 2006
Film-coated tablets			
T50	15 ± 2.9	Water	Perkins, 2001
GET	62 ± 17	Water	Ofori-Kwakye, 2004

Table 14: Gastric emptying time in healthy adults from different publications

Data are presented as median (interquartile range). **Intended for colonic or biphasic release. GET: Gastric emptying time. T50: Half emptying time.

3.2.1.3.6 Variability estimation for the gastric emptying time in children

Data on the distribution behaviour of GET in children were not found in the published literature. However, a higher variability was shown for GET in children than in adults (Maes, 1995). Van Den Driessche reviewed all published studies of GET in different paediatric age groups and concluded that it was impossible to establish reference values based on the published data, since different techniques, different outcomes and only few studies with a small number of subjects were available (Van Den Driessche, 2003). Nevertheless, a range of 7 to 87 min for T50 for liquids in children was pooled from all published papers (Van Den Driessche, 2003). Therefore, a range of 10 to 110 min for T63 with uniform distribution was calculated according to equation 13. This range was used to describe the variability of GET for children in the current project.

3.2.1.3.7 Variability estimation for the intestinal transit time in adults

Intestinal transit time (ITT) was not significantly affected by the digestive state (Davis, 1986) and no significant difference between male and female subjects was observed (Graff, 2001). ITT values from 4 published studies are summarised in Table 15. Triantafyllou and colleagues estimated a normal distribution for ITT (Triantafyllou, 2007). Although the results of other studies were presented as mean ± SD, the authors did not mention that the data normally distributed. However, in the present project a normal distribution for ITT in adults was estimated based on the results of Triantafyllou (Triantafyllou, 2007). As shown in Table 15, ITT ranged from 3 to 5 h with SD of about 1 h. Therefore, ITT (mean ± SD) of 4 ± 1 h was used for the present project to describe the variability of ITT in adult virtual populations.

Intestinal transit time for:	**Mean ± SD or median (range) [min]**	**Meal**	**Study**
Liquid markers	4 (1.5 - 6.6)	Mixed liquid-solid	Graff, 2001
Solid markers	3.63 (2.4 - 5.9)	Mixed liquid-solid	Graff, 2001
Solutions	4.2 ± 1	Variable	Davis, 1986
Pellets	3.2 ± 1.6	Variable	Davis, 1986
Single unites	3.2 ± 1.2	Variable	Davis, 1986
Video-capsule endoscopy	5 ± 1	Clear liquid diet	Triantafyllou, 2007
Non-disintegrating tablets	3.4*	Fasted	Fadda, 2009
Non-disintegrating tablets	3.5*	Fed	Fadda, 2009
Non-disintegrating tablets	2.4*	45 min before food	Fadda, 2009

Table 15: Intestinal transit time in healthy adults from different publications

* Data are presented as median.

3.2.1.3.8 Variability estimation for the intestinal transit time in children

Orocaecal transit time in 3- to 17-year-old children ranged from 165 to 390 min with average value of 255 min, which is similar to adult values (Van Den Driessche, 2000). Myo-Khin and colleagues reported a shorter ITT being about 90 min in 1- to 5-year-old children (Khin, 1999). However, two other publications support the results of Van Den Driessche, where Orocaecal transit time was shown to remain constant from the second month of age (Vreugdenhil, 1986) and ITT values in children were shown to be identical to adult values measured with the same methods (Fallingborg, 1990). Thus, the same variability estimation for ITT of adults was also estimated for children in the present project, i.e., a normal distribution with mean ± SD of 4 ± 1 h.

3.2.1.3.9 Variability estimation for the intrinsic intestinal clearance in adults

A uniform distribution from 0 to a value equal to twice the intestinal CLint was assumed to describe the variability of CLint of intestinal CYP enzymes in the current project. Few data on the inter-individual variability of the activities of intestinal CYP3A4 were published. However, CLint of intestinal CYP3A4, which was presented by Paine and colleagues, showed no normal distribution in the data (Paine, 1997). The activity of intestinal CYP3A4 was investigated by another author by measuring the fentanyl metabolism in duodenal microsomes (Labroo, 1997). However, the six samples were too little to identify the distribution pattern in the mentioned study.

3.2.1.3.10 Variability estimation for the intrinsic intestinal clearance in children

The activity of intestinal CYP3A4 in children was investigated in one published study (Johnson, 2001). Although the results in the mentioned publication were presented as mean ± SD, the authors did not mention that the data normally distributed. As data on the distribution behaviour of the intestinal intrinsic clearance in children were not found in other published literature, the estimation of the variability of intestinal intrinsic clearance for children was the same for adults in the present project, i.e. a uniform distribution with a range of 0 to 2*CLint, where CLint is the intrinsic intestinal clearance in the simulated age group.

3.2.2 Development and evaluation of a PBPK model for intravenous sildenafil in adults

Considering the reference ranges which were estimated from the literature search for the physico-chemical parameters and other compound-related parameters of sildenafil, input parameters were adjusted to fit the mean plasma concentration-time profiles in the included PK studies. The input parameters are shown in Table 16.

Parameter	Value
Lipophilicity	2.70
Unbound plasma fraction [%]	3.4 or 4.5*
Molecular weight [g/mol]	474.58
Ionization constant (pK_a) for:	
acidic function	9.12
basic function	6.99
Solubility at pH 7 [mg/ml]	0.025
Hepatic plasma clearance [ml/min/kg]	7

Table 16: Input parameters which were given in the development of the physiologically-based pharmacokinetic of sildenafil

* Unbound plasma fraction was estimated to be 3.4 and 4.5% for studies conducted in subjects aged > 45 and < 45 years respectively.

A PBPK model for intravenous sildenafil was developed and run to simulate the doses: 20, 25, 40 and 50 mg in healthy adult male volunteers. The simulated plasma concentration-time profiles compared to mean observed data are presented in Figure 6.

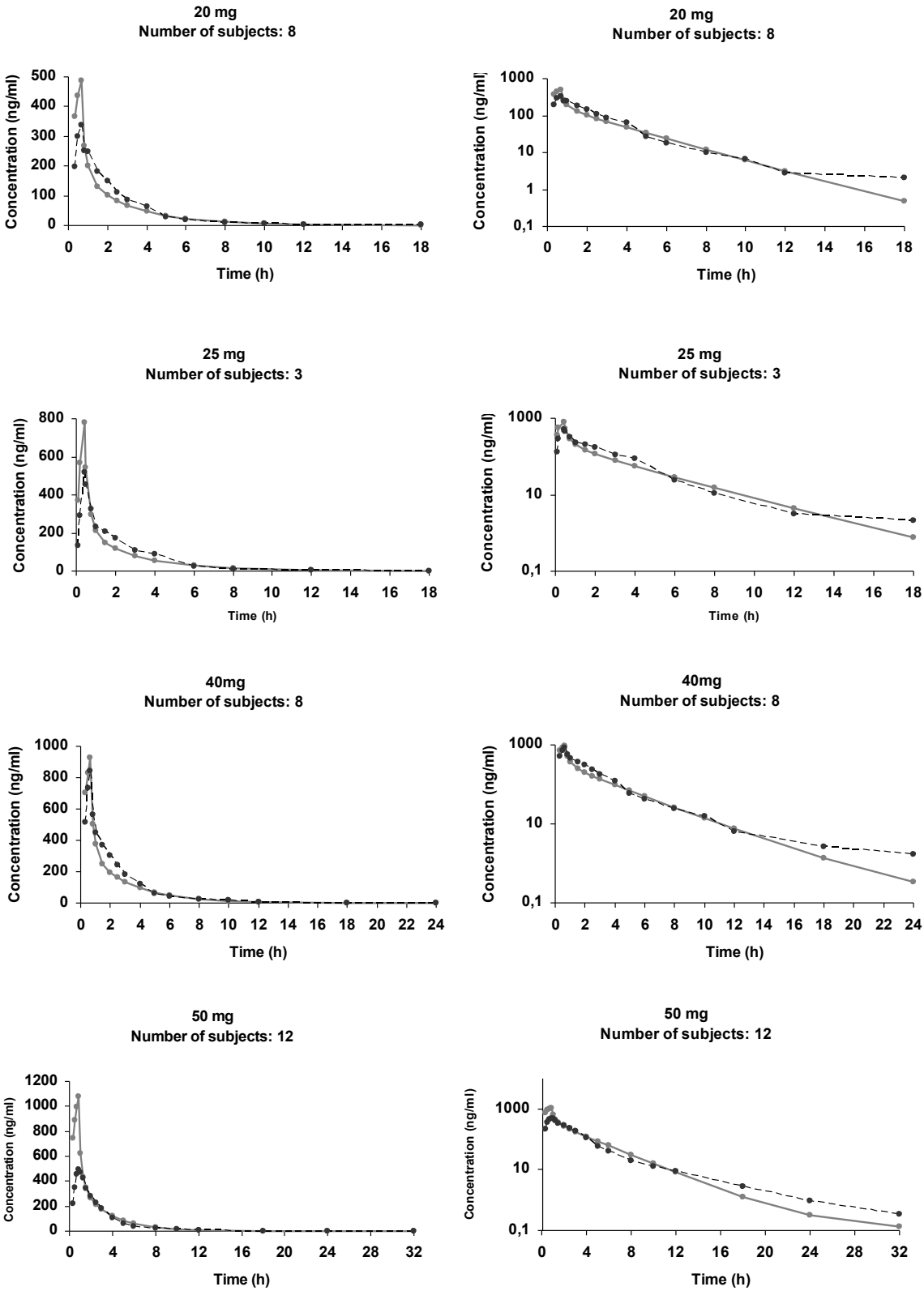

Figure 6: Simulated plasma concentrations of intravenous sildenafil compared to observed mean data

Linear (left) and logarithmic (right) plot of simulated (grey) and mean observed (black) concentration-time profiles for 20 and 40 mg (Jackson, 1999), 25 mg (Walker, 1999) and 50mg (Nichols, 2002) given intravenously as 40, 25 and 50 minute infusions respectively.

The bias of the prediction of all mean plasma concentrations after four intravenous doses was low with an MPE of 1.3% (25th to 75th percentile, -26.7 to 30.8%). The precision was also acceptable with an MAPE of 27.5% (25th to 75th percentile, 13.4 to 47.4%).

The simulated parameters: V_{ss}, $t_{1/2}$ and $AUC_{0-\infty}$ were compared with mean observed data from the four reference studies. The simulated versus observed PK parameters and the percentage error for their estimation are summarised in Table 17. With exceptions of Nichols' study, all predicted PK parameters in all studies were within 19% of the observed parameters. The estimation of the PK parameters in Nichols' study was more biased with predicted V_{ss}, $AUC_{0-\infty}$ and $t_{1/2}$ being within 41, 42 and 22% of the observed data.

Pharmacokinetic parameter	Simulated value	Observed value*	Percentage error [%]	Study
V_{ss} [l]	62.59	105.33	- 40.6	Nichols, 2002
	54.48	56.72	- 3.9	Walker, 1999
	58.98	70.95	- 16.9	Jackson, 1999
	63.08	58.85	+ 7.2	Jackson, 1999
$AUC_{0-\infty}$ [ng·h/ml]	1831.70	1290.86	+ 41.9	Nichols, 2002
	958.12	970.56	- 1.3	Walker, 1999
	720.75	730.90	- 1.4	Jackson, 1999
	1443.06	1573.92	- 8.3	Jackson, 1999
$t_{1/2}$ [h]	3.30	4.22	- 21.8	Nichols, 2002
	2.32	2.21	+ 5.0	Walker, 1999
	2.39	2.88	- 17.0	Jackson, 1999
	2.67	3.30	- 19.1	Jackson, 1999

Table 17: Simulated versus observed pharmacokinetic parameters for intravenous sildenafil

V_{ss}: Volume of distribution at steady state; $AUC_{0-\infty}$: Area under the curve to infinity; $t_{1/2}$: Terminal half-life. * The observed data are presented as arithmetic, geometric and harmonic means for V_{ss}, $AUC_{0-\infty}$ and $t_{1/2}$ respectively.

MPE for prediction of V_{ss}, $AUC_{0-\infty}$ and $t_{1/2}$ in all studies was: -10.4% (25th to 75th percentile, -1.2 to -22.8%), -1.3% (25th to 75th percentile, -3.1 to 9.5%) and -18.1% (25th to 75th percentile, -19.8 to -11.5%) respectively. Although the bias values referred to underprediction for both V_{ss}, and $t_{1/2}$, the precision of the prediction was acceptable with the MAPE for V_{ss}, $AUC_{0-\infty}$ and $t_{1/2}$ being: 12% (25th to 75th percentile, 6.4 to 22.8%), 4.9% (25th to 75th percentile, 1.4 to 16.7%) and 18.1% (25th to 75th percentile, 14 to 19.8%) respectively.

3.2.3 Simulation of the pharmacokinetics of intravenous sildenafil in a virtual adult population

Using the population module in PK-Sim®, a virtual population of 1000 subjects with the characteristics listed in Table 16 was created. These demographic characteristics are the same for the volunteers in the reference study with the following exception: 1 out of 8 subjects (12.5%) was black (Jackson, 1999) whereas all subjects in the virtual population were white. Variability for the hepatic clearance and the unbound plasma fraction (Table 18) were estimated as explained in section 3.2.1.3.

Parameter		**Value**
Gender		Male
Race		White
Age [year]		19 – 30
Weight [kg]		64.6 – 81.4
Height [cm]		165 – 194
Dose [mg]		40
Parameter	**Distribution category**	**Deviation**
Hepatic clearance [ml/min/kg]	Lognormal distribution	1.4*
Unbound fraction [%]	Uniform	4 – 5**

Table 18: Demographic characteristics of the virtual population and the estimated variability for physiological parameters used to develop the population pharmacokinetic model of intravenous sildenafil

Demographic characteristics were given according to (Jackson, 1999). * Geometric standard deviation. ** Range.

A PBPK simulation of 40 mg intravenous sildenafil for a population of 1000 male adults was run. The simulated plasma concentrations are presented in Figure 7 with comparison to 130 observed plasma concentrations from 8 subjects, where 110 observed concentrations (84.6%) were included between the 5th and 95th percentiles of the simulation and 15 observed plasma concentrations (11.5%) were under the 5th percentile of the simulation; most of them (13 points) were measured through the infusion period. Five observed concentrations (3.8%) were above the 95th percentile of the simulation.

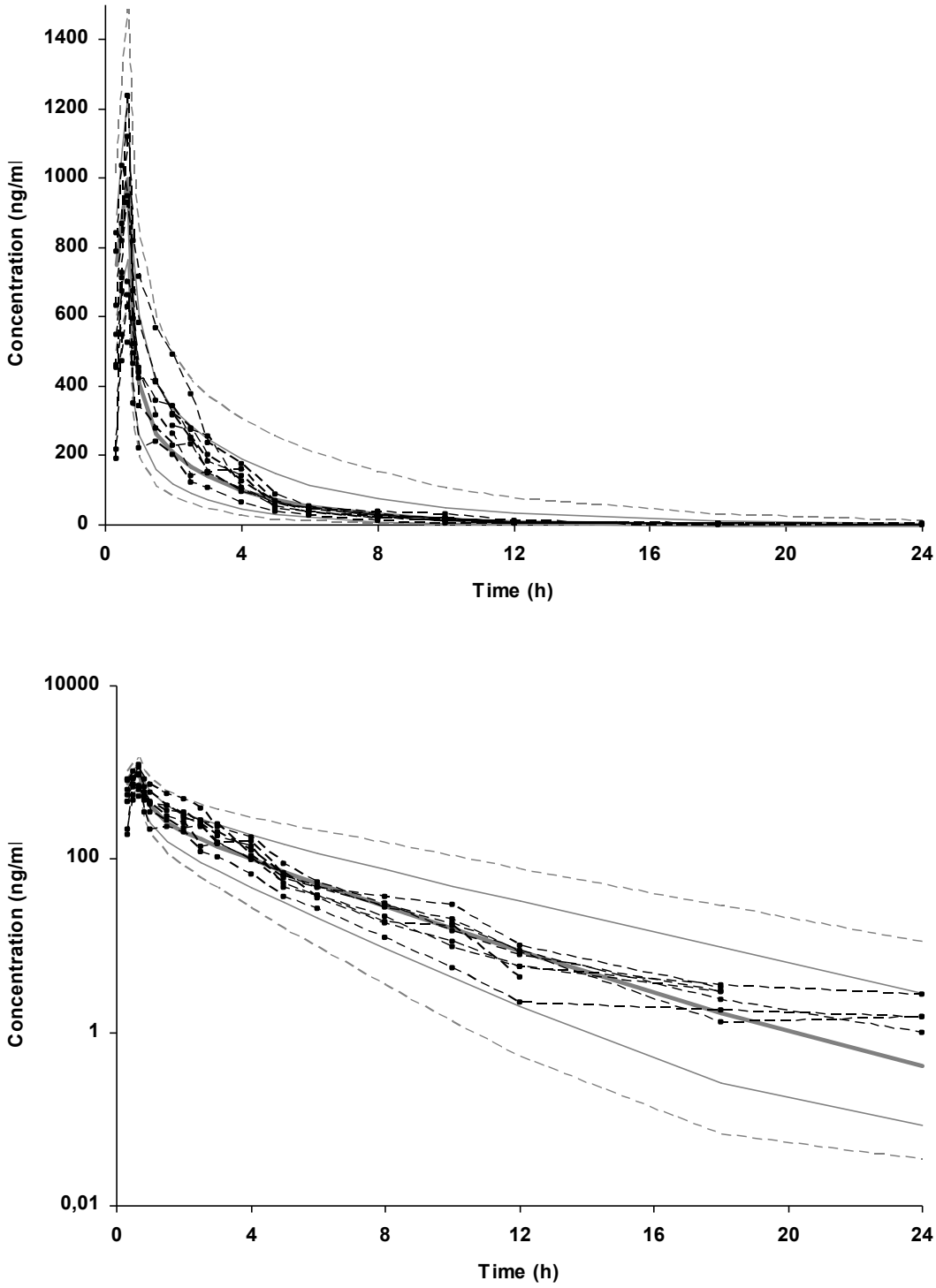

Figure 7: Comparison between simulated and observed individual sildenafil plasma concentrations after intravenous administration

Linear (top) and logarithmic (bottom) plot of the simulated plasma concentrations after the intravenous application of 40 mg sildenafil in 1000 virtual subjects compared to observed data from 8 subjects. ---: maximum and minimum simulation; ▬: simulation median; —: simulation 5th and 95th percentile; -•-: observed data (Jackson, 1999).

Goodness-of-fit plots for the population model for intravenous sildenafil are presented in Figure 8 showing the population and individual predicted versus observed plasma concentrations in eight subjects and the weighted residuals versus population predicted plasma concentrations and time after the beginning of the infusion. The goodness-of-fit plots indicate that the population predicted and individual predicted plasma concentrations are closely correlated with the observed data, with weighted residuals ranged from 0.8 to 3.

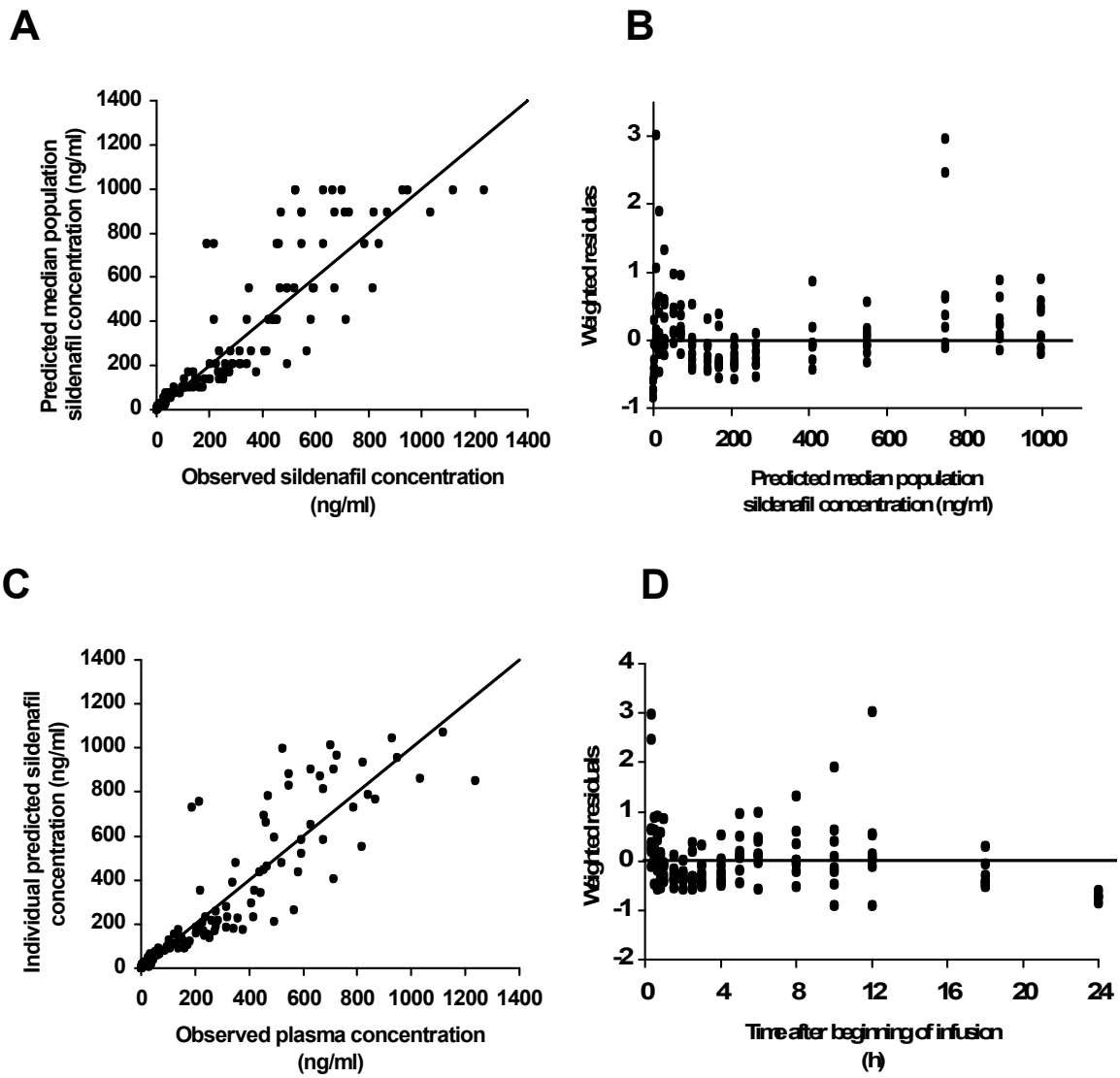

Figure 8: Diagnostic goodness-of-fit plots for the population pharmacokinetic model of intravenous sildenafil

(A) Predicted median population versus observed individual plasma concentrations; (B) weighted residuals versus population predicted plasma concentrations; (C) individual predicted versus observed plasma concentrations; and (D) weighted residuals versus time after beginning of infusion. Lines represent the line of identity (left) and the value zero (right). Observed data are obtained from 8 subjects (Jackson, 1999).

The prediction of plasma concentrations in adult subjects based on the population simulated median was performed with an MPE (bias) of -7.5% (25^{th} to 75^{th} percentile, -38.4 to +26.1%). The MAPE (precision) of the prediction of plasma concentrations based on the population simulated median was 34.6% (25^{th} to 75^{th} percentile, 12.9 to 52.5%). The prediction based on individual simulated plasma concentrations had better precision with an MAPE of 26.9% (25^{th} to 75^{th} percentile, 13.4 to 43.7%).

Individual simulations for intravenous sildenafil were carried out for every volunteer in the reference study using information about age, weight and height of every individual subject. The simulated individual plasma concentration-time profiles of sildenafil compared with observed individual data are presented in Figure 20, Appendix.

3.2.4 Development and evaluation of a PBPK model for oral sildenafil in adults

3.2.4.1 Estimation of the intestinal intrinsic clearances in the different intestinal segments

Cytochrome P450 contents in the different intestinal segments were adjusted according to *in vitro* studies of the human intestinal Cytochrome P450 (Zhang, 1999). Zhang and colleagues measured the Cytochrome P450 amount in different areas in the human intestine. The results were used to estimate the intestinal clearances along the small intestine (Table 19).

	Duodenum	Upper jejunum	Lower jejunum	Upper ileum	Lower ileum
P450 amount [nmol/mg]	0.12	0.13	0.12	0.06	0.03
Fraction of total intestinal CLint	0.26	0.28	0.26	0.13	0.07

Table 19: Cytochrome P450 content and the predicted fraction of total intestinal intrinsic clearance in the different intestinal segments in humans

Values for cytochrome P450 amount in every intestinal segment were calculated depending on the results from (Zhang, 1999) considering the length of every intestinal segment (Kararly, 1995). CLint: intrinsic clearance.

The intestinal intrinsic clearances were adjusted to fit the plasma concentration-time curves in the PK studies of oral sildenafil considering the relative contribution of CYP3A4 in every

intestinal segment. The final values for the intrinsic clearance of CYP3A4 are shown in Table 20.

Intestinal segment	Intrinsic clearance [ml/min]
Duodenum	0.42
Upper jejunum	0.46
Lower jejunum	0.42
Upper ileum	0.21
Lower ileum	0.11

Table 20: Estimated intrinsic clearance for intestinal CYP3A4 involved in the first-pass effect of sildenafil in the different segments of small intestine

3.2.4.2 Simulation of oral doses of sildenafil in adults

The mean age, weight and height of subjects in every study were considered. As the mean value for weight and height of the volunteers in Muirhead's study were not known, sildenafil plasma concentrations were simulated using the standard weight and height for the corresponding average age in this study.

Simulations of the three included PK studies compared to observed data are shown in Figure 9. The simulated plasma concentration-time profiles have a close correlation to the observed data in the three studies except for the plasma concentrations measured before t_{max} in Jetter's study, where these concentrations, as seen in figure 9, were underpredicted.

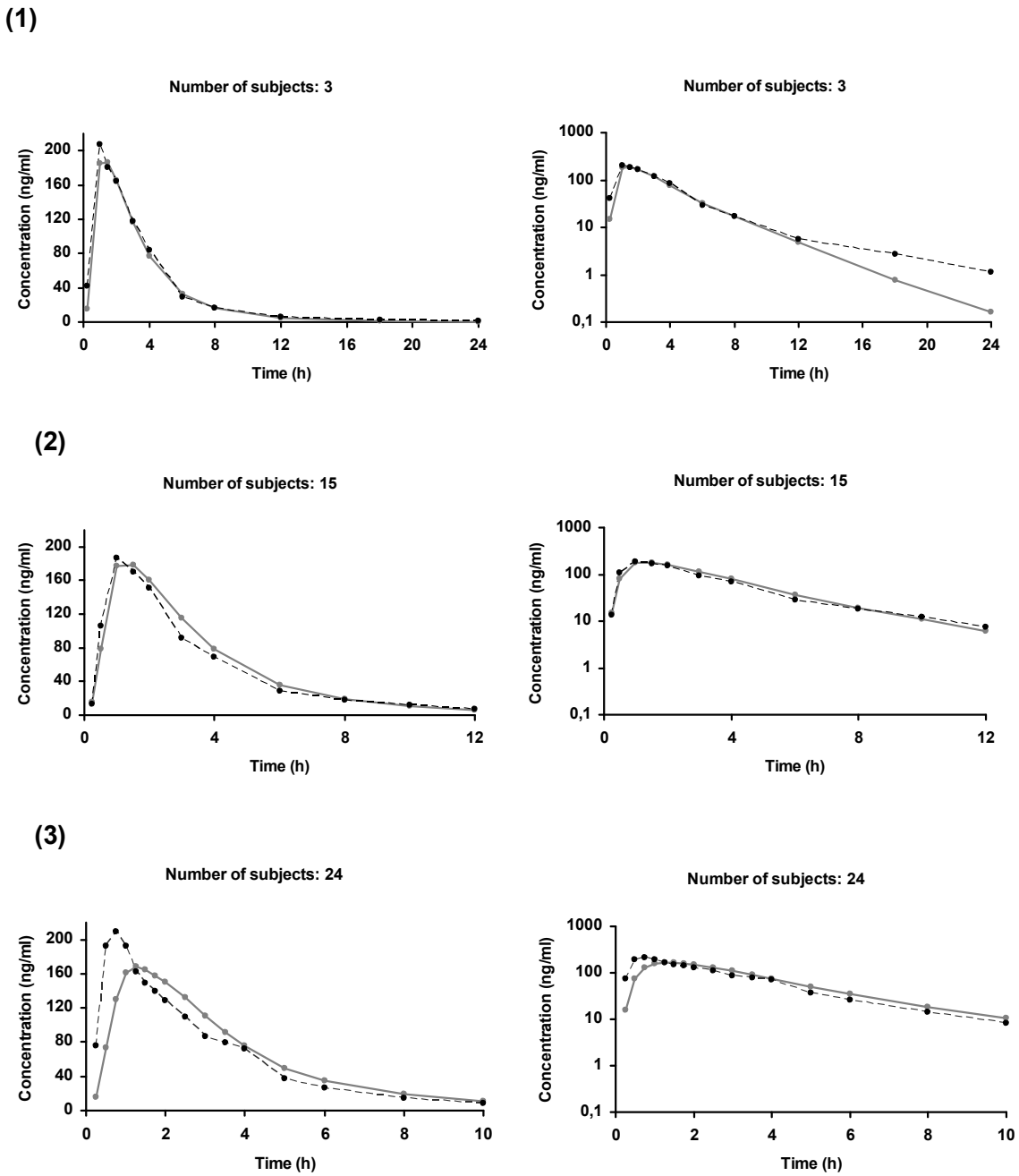

Figure 9: Simulated plasma concentrations of oral sildenafil compared to mean observed data

Linear (left) and logarithmic (right) plot of simulated (grey) and observed mean (black) concentration-time profiles for 50 mg sildenafil given orally in studies: 1 (Walker, 1999), 2 (Muirhead, 2002) and 3 (Jetter, 2002).

The prediction of mean plasma concentrations in the three PK studies had a good performance with an MPE (bias) of 4.5% (25^{th} to 75^{th} percentile, -14.2 to +14.3%) and an MAPE (precision) of 14.9% (25^{th} to 75^{th} percentile, 7.6 to 29.4%).

The simulated PK parameters: C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ in the three PK studies compared to their mean observed values are presented in Table 21.

Pharmacokinetic parameter	Simulated value	Observed value	Percentage error [%]	Study
C_{max} [ng/ml]	192.12	207.16	-7.26	Walker, 1999
	183.88	178	+3.30	Muirhead, 2002
	168.06	254.89	-34.07	Jetter, 2002
$AUC_{0-\infty}$ [ng·h/ml]	700	708.94	-1.26	Walker, 1999
	720	586	+22.87	Muirhead, 2002
	680	619	+9.85	Jetter, 2002
$t_{1/2}$ [h]	2.85	2.63	+8.37	Walker, 1999
	3.03	2.6	+16.54	Muirhead, 2002
	2.97	3.79	-21.64	Jetter, 2002

Table 21: Simulated versus observed pharmacokinetic parameters for oral sildenafil

C_{max}: maximum plasma concentration; $AUC_{0-\infty}$: Area under the curve from time 0 to infinity; $t_{1/2:}$ Terminal half-life.

The MPE (bias) for the prediction of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ was: -7.3% (25^{th} to 75^{th} percentile, -20.7 to -2%), +9.9% (25^{th} to 75^{th} percentile, +4.3 to +16.4%), and +8.3% (25^{th} to 75^{th} percentile, -6.7 to +12.4%) respectively. Although the values of the MPE of the prediction of the PK parameters referred to an underprediction of C_{max} and overprediction of $AUC_{0-\infty}$**,** the prediction of the PK parameters was performed with a good precision estimated by an MAPE of 7.3% (25^{th} to 75^{th} percentile, 5.3 to 20.66%), 9.9% (25^{th} to 75^{th} percentile, 5.6 to 16.4%), and 16.5% (25^{th} to 75^{th} percentile, 12.4 to 19.1%) for the prediction of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ respectively.

3.2.5 Simulation of the pharmacokinetics of oral sildenafil in a virtual adult population

Depending on the PBPK model for oral sildenafil, a population pharmacokinetic model for 1000 virtual adult subjects was developed. First, a virtual population of 1000 subjects with the same demographic characteristics in Walker's study was created by the population module in PK-Sim®. The characteristics of the virtual population are shown in Table 22.

To develop the population pharmacokinetic model for oral sildenafil, variability for the physiological parameters which contribute to the variability of PK of drugs administered orally was estimated as shown in Table 22.

Parameter		**Value**
Gender		Male
Race		White
Age [year]		50 – 53
Body mass index [kg/m^2]		21 – 26
Parameter	**Distribution category**	**Deviation**
Hepatic clearance [ml/min/kg]	Lognormal distribution	7 (1.4)*
Unbound fraction [%]	Uniform	3 – 4**
Gastric emptying time [h]	Normal distribution	0.5 (0.25)***
Intestinal transit time [h]	Normal distribution	4 (1)***
Intrinsic clearance for intestinal cytochrome P450 in:		
• Duodenum [ml/min]	Uniform	0 – 0.84**
• Upper jejunum [ml/min]	Uniform	0 – 0.96**
• Lower jejunum [ml/min]	Uniform	0 – 0.84**
• Upper ileum [ml/min]	Uniform	0 – 0.42**
• Lower ileum [ml/min]	Uniform	0 – 0.22**

Table 22: Demographic characteristics of the virtual population and the estimated variability for physiological parameters used to develop the population pharmacokinetic model of oral sildenafil

The demographic characteristics were given according to (Walker, 1999). * Geometric standard deviation. ** Range. *** Standard deviation.

The PBPK simulation of 50 mg oral sildenafil in a virtual adult population compared to 31 observed concentrations from three subjects (Walker, 1999) is presented in Figure 10, where 29 observed concentrations (93.5%) were included between the 5th and 95th percentiles of the simulation and 2 observed plasma concentrations (6.4%) were under the 5th percentile of the simulation; these concentrations were, however, measured 15 minutes post dose. No observed concentration was above the 95th percentile of the population.

Individual simulations for 50 mg oral sildenafil were carried out for every volunteer in Walker's study using information about age, weight and height. The simulated individual plasma concentration-time profiles of sildenafil compared with individual observed data are presented in Figure 21, Appendix.

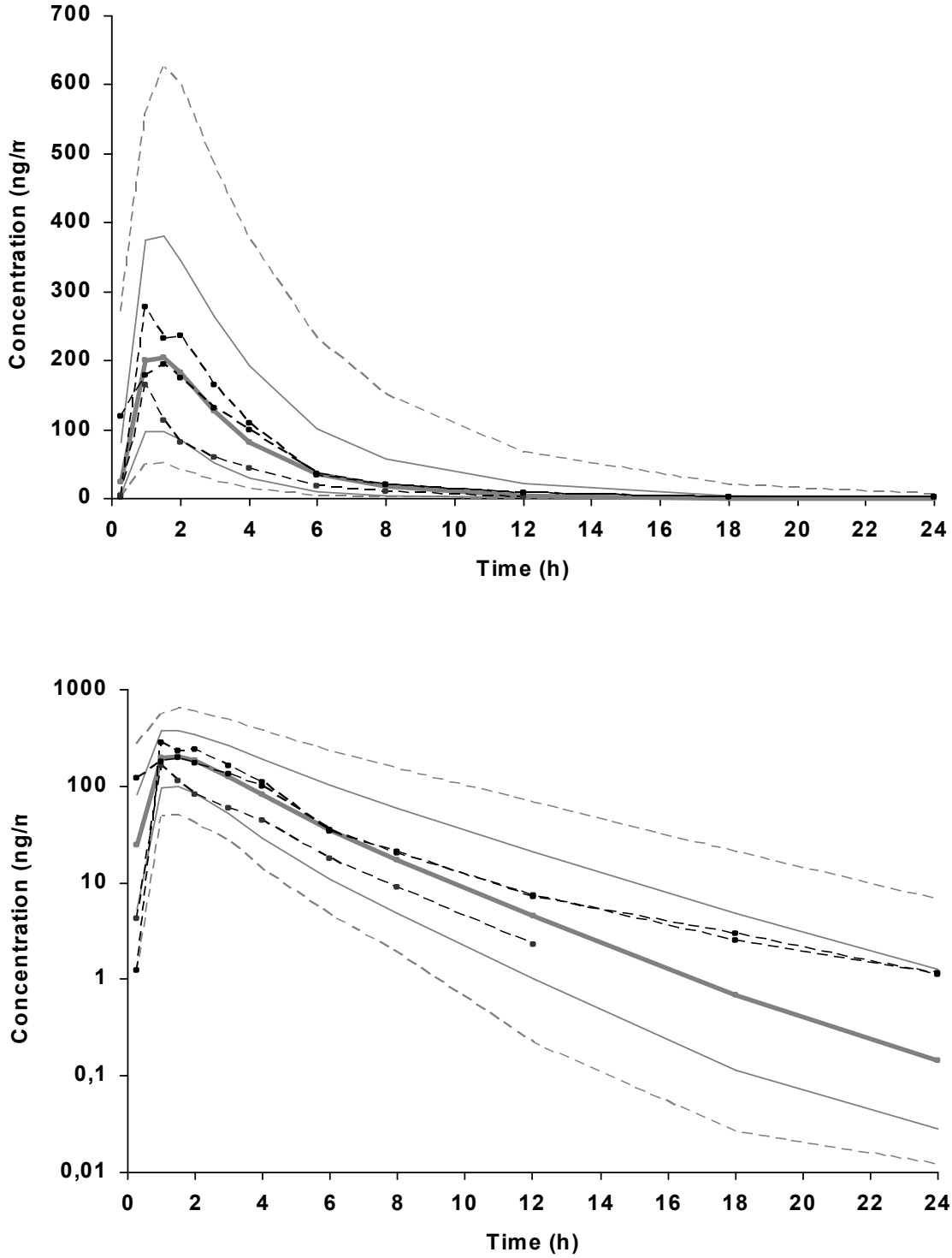

Figure 10: Comparison between simulated and observed individual sildenafil plasma concentrations after oral application

Linear (top) and logarithmic (bottom) plot of the simulated plasma concentrations after the oral application of 50 mg sildenafil in 1000 virtual subjects compared to observed data. ---: maximum and minimum simulation; ▬: simulation median; —: simulation 5th and 95th percentile; -•-: observed data (Walker, 1999).

Goodness-of-fit plots for the population model of oral sildenafil are presented in Figure 11, which shows the population and individual predicted versus observed plasma concentrations in three subjects and the weighted residuals versus population predicted plasma concentrations and time after dose. The goodness-of-fit plots indicate that the population predicted and individual predicted plasma concentrations are closely correlated with the observed data. With exceptions of two values, which were measured 15 min after dose, all observed plasma concentrations could be predicted with weighted residuals ranged from -0.9 to +1.2.

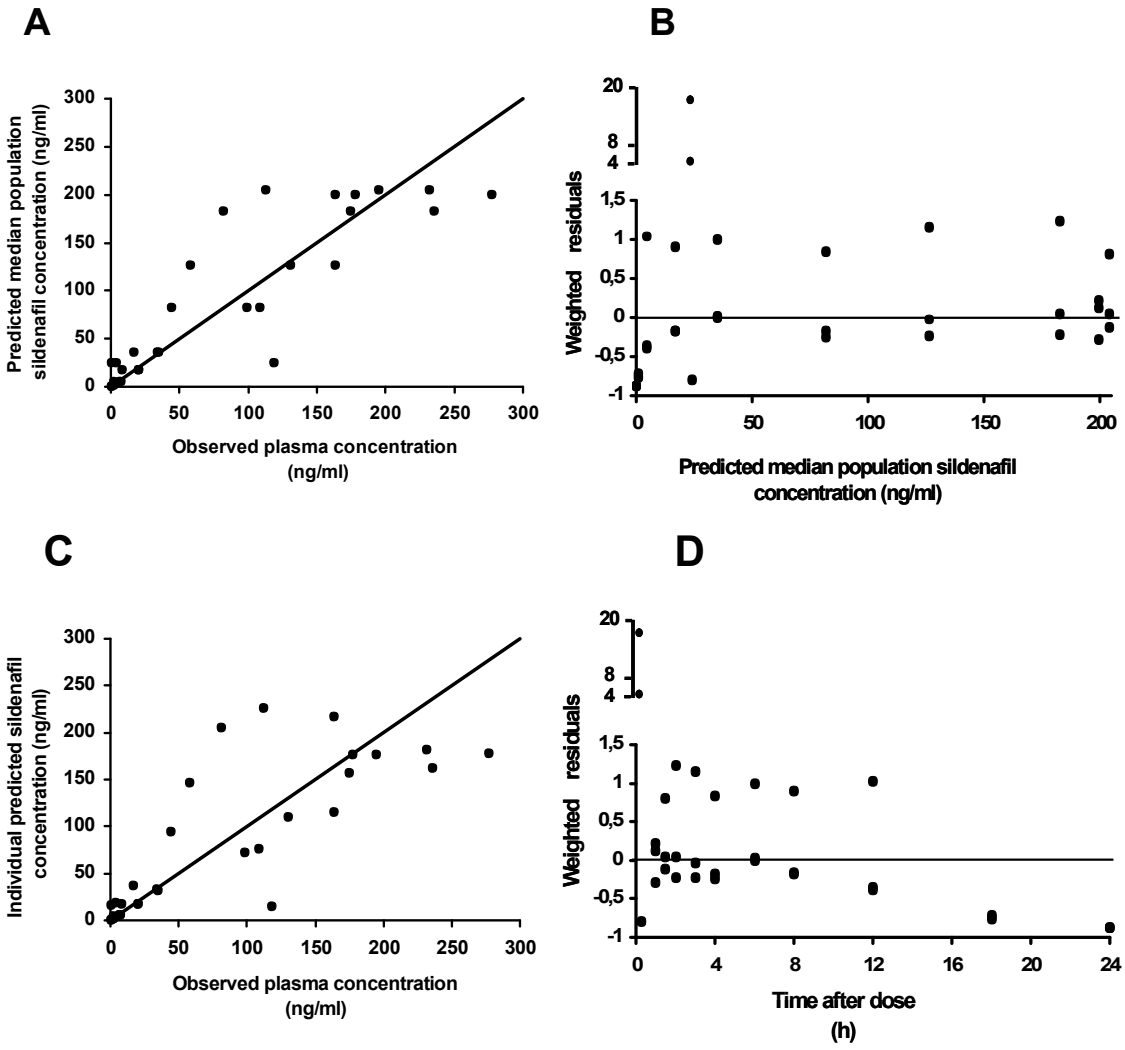

Figure 11: Diagnostic goodness-of-fit plots for the population pharmacokinetic model of oral sildenafil

(A): Population predicted versus observed plasma concentrations; (B): weighted residuals versus population predicted plasma concentrations; (C): Predicted individual versus observed individual plasma concentrations; (D): Weighted residuals versus time after beginning of infusion. Lines represent the line of identity (left) and the value zero (right). Observed data in three subjects (Walker, 1999)

The prediction of plasma concentrations in adult subjects based on the population simulated median was performed with an MPE (bias) of -3.6% (25th to 75th percentile, -26.5 to +82.2%) and MAPE (precision) of 35.6% (25th to 75th percentile, 16.9 to 87.3%).

3.2.6 Development and evaluation of a PBPK model for oral sildenafil in children

3.2.6.1 Age-related hepatic clearance

The clearance scaling module in PK-Sim® was used to scale sildenafil hepatic clearance from adults to the simulated age in children. The predicted age-related plasma clearance of sildenafil in children is shown in Figure 12. After the first month of age, the body weight-normalised plasma clearance of sildenafil is predicted to reach adult values. Sildenafil clearance is predicted to increase continually up to four years of age; subsequently, it decreases progressively to reach adult values at 18 years of age.

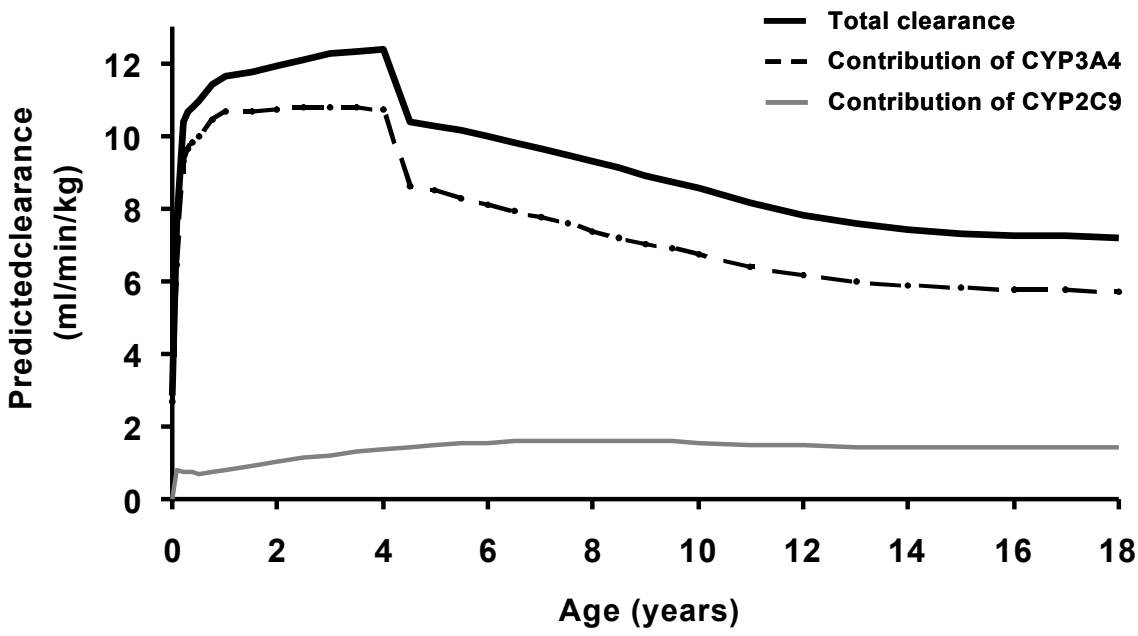

Figure 12: Predicted age-dependent sildenafil hepatic clearance across the different paediatric ages based on the clearance scaling module in PK-Sim®

3.2.6.2 Age-related intestinal clearance

The intrinsic intestinal clearance was scaled from adults to children using published data on the ontogeny of intestinal CYP3A4 as mentioned in method section 2.2.8.2. For this purpose, the fraction of intestinal CYP3A4 activity in the different paediatric age groups relative to adult activity (Johnson, 2001) was used to predict the age-related intrinsic clearance of intestinal CYP3A4 as a fraction of the adult value. The development of intestinal CYP3A4 activity across the paediatric ages is shown in Figure 13.

The intestinal CYP3A4 activity for neonates is predicted to be 0.36 of the adult value. Its value increases as the age of the child increases and is expected to reach an activity twice that of the neonate value at 1.5 years of age. Considering the intestinal surface area in children as a fraction of the adult value, the predicted intestinal CLint [ml/min/kg] is higher in all paediatric age groups than in adults.

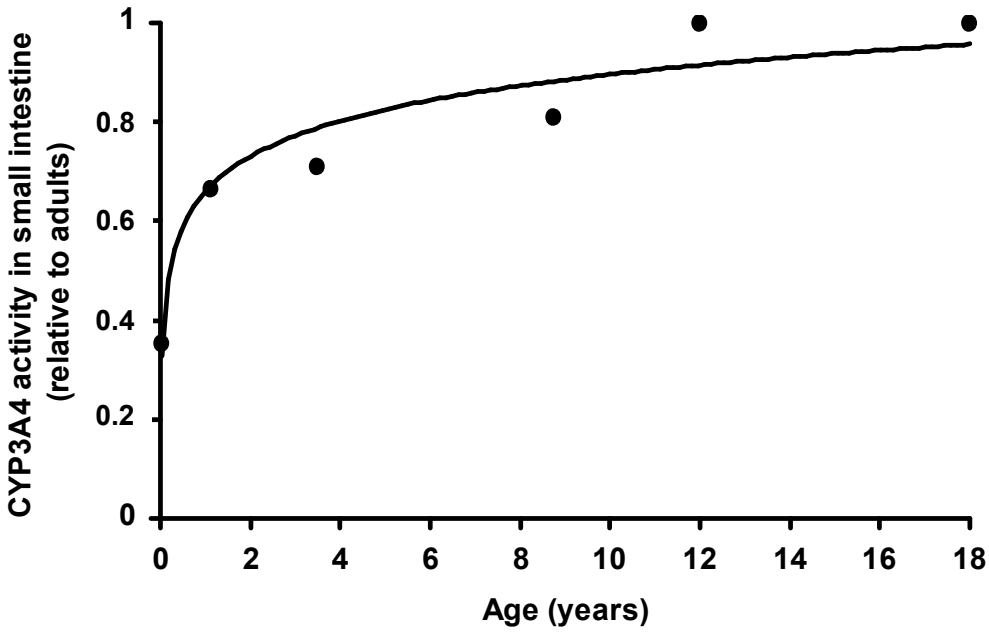

Figure 13: Predicted age-related intestinal CYP3A4 activities as a fraction of the adult value

The presented progression was estimated depending on ontogeny data of intestinal CYP3A4 from (Johnson, 2001)

3.2.6.3 Simulation of oral doses of sildenafil in children

PK of three different oral doses of sildenafil: 0.5, 1 and 2 mg/kg in three individual children were simulated. The children were 6.58, 10.58 and 13.91 years old. The dosage regimens were simulated according to those described in Karatza's study (Karatza, 2005). A dose of 0.5 mg/kg was given to the children every 4 h with omitting the overnight dose for one month. Consequently, 1 mg/kg sildenafil was given by the same regimen for another one month. At the third month, the dose was increased to 2 mg/kg. The simulated mean plasma concentrations of oral sildenafil in the three children compared to observed mean data are illustrated in Figure 14.

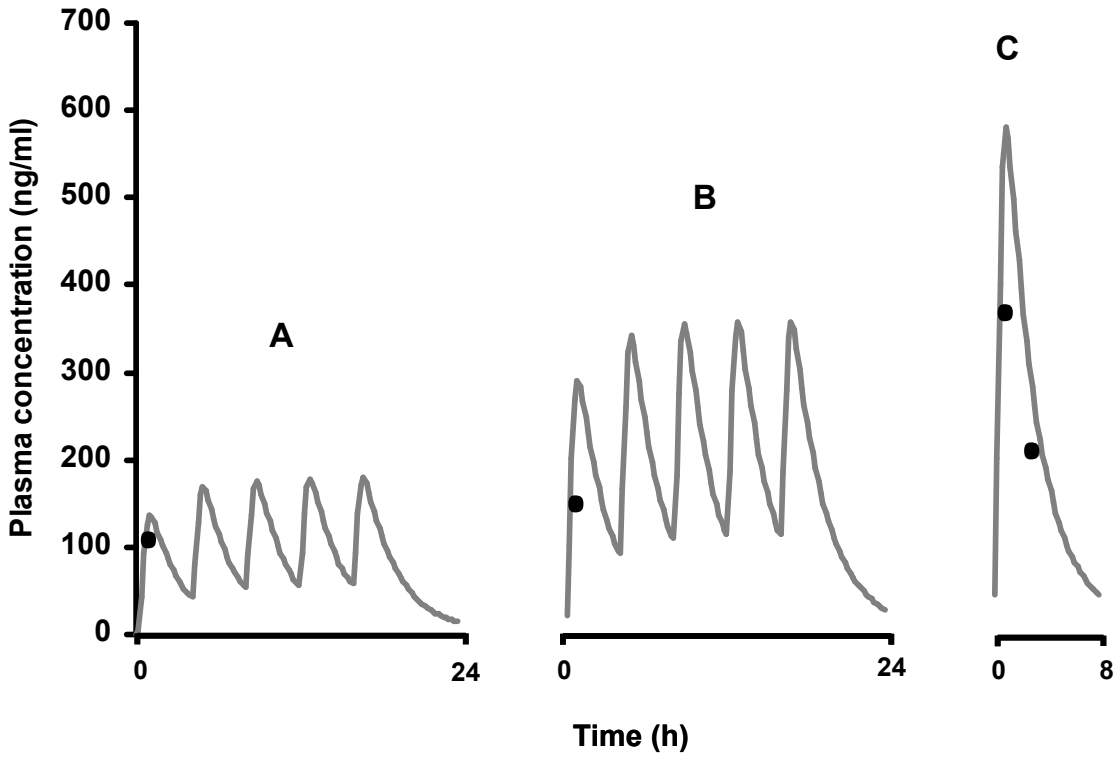

Figure 14: Predicted versus observed mean plasma concentrations of sildenafil in three children

Predicted (lines) versus observed (plots) mean plasma concentrations after 0.5 mg/kg (A), 1 mg/kg (B) and 2 mg/kg (C) oral sildenafil in three children in the first, second and third month of therapy respectively. The observed data are from (Karatza, 2005).

The predicted versus observed mean plasma concentrations of the three different doses of oral sildenafil with the percentage error of their prediction are presented in Table 23.

Time after dose [h]	Dose [mg/kg]	Predicted mean value [ng/ml]	Observed mean value [ng/ml]	Percentage error* [%]
1	0.5	137	109	+25.7
1	1	290	150	+93.3
1	2	580	368	+57.6
3	2	285	211	+35.1

Table 23: Predicted and observed mean plasma concentrations of oral sildenafil in three children

Children were 6.58, 10.58 and 13.91 years old. The observed data are from (Karatza, 2005)

The prediction for all mean plasma concentrations was performed with an MPE (bias) of +46.3% (25^{th} to 75^{th} percentile, 32.7 to 66.5%) and an MAPE (precision) of 46.3% (25^{th} to 75^{th} percentile, 32.7 to 66.5%).

The MRD for the prediction of mean plasma concentrations in Karatza's study was 1.6, which means that the predicted plasma concentrations were within a factor of 1.6 of the observed values.

3.2.6.4 Simulation of oral sildenafil in a 4-months-old infant

Plasma concentrations of sildenafil after 7 mg oral sildenafil given every 8 h to a 4-month-old girl were simulated. The simulation compared to observed data from Witjes' study is shown in Figure 15 (Witjes, 2010). Sildenafil was predicted to reach its maximum plasma concentration 40 min after dose, with predicted C_{max} of 363 ng/ml. The simulation shows a rapid elimination of sildenafil, which is expected to reach a plasma concentration of 1.7 ng/ml 8 h after dose, i.e. trough level.

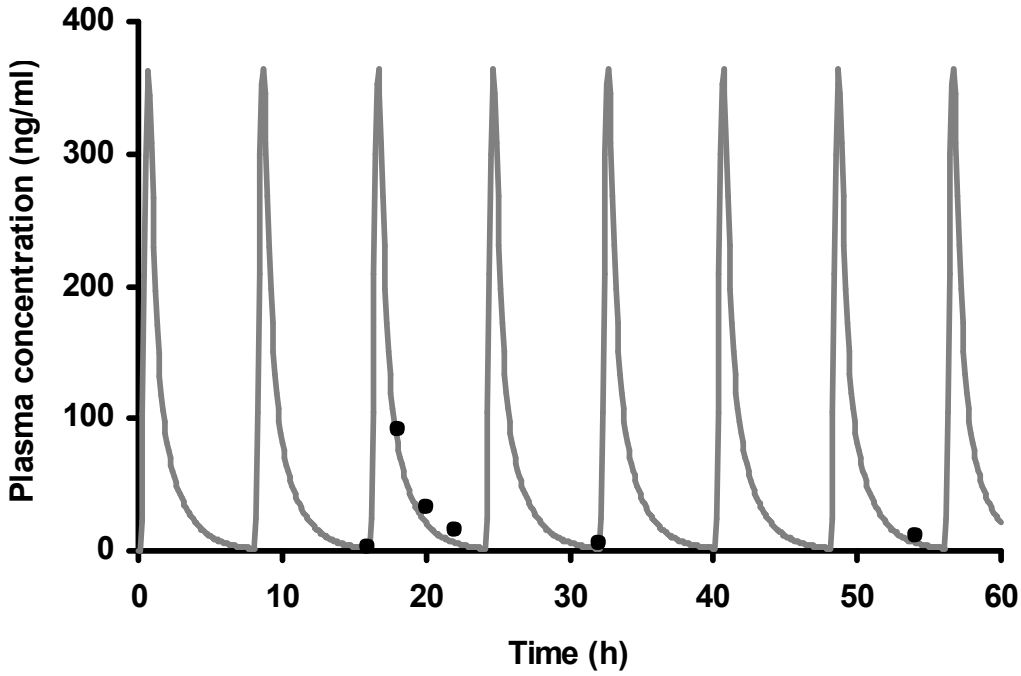

Figure 15: Predicted versus observed plasma concentrations of sildenafil in a 4-month-old infant

Predicted (lines) versus observed (plots) plasma concentration-time profiles after 7 mg oral sildenafil given 8-hourly in a 4-month-old girl. The observed data are from (Witjes, 2010)

Predicted versus observed plasma concentrations of 7 mg sildenafil given orally 3 times a day in a 4-month-old girl and the percentage error of their estimation are presented in Table 24.

Time after dose [h]	Predicted value [ng/ml]	Observed value [ng/ml]	Percentage error [%]
8	1.84	3.57	-48.46
2	82.27	92.41	-10.97
4	21.14	33.04	-36.02
6	6.07	15.63	-61.16
8	1.92	5.36	-64.18
6	6.14	11.16	-44.98

Table 24: Predicted versus observed plasma concentrations of oral sildenafil in a 4-month-old infant

The applied dose was 1.5 mg/kg given every 8 h. The observed data are from (Witjes, 2010)

The prediction bias, estimated by MPE was -46.7% (25^{th} to 75^{th} percentile, -58% to -38.3%) and the precision estimated by MAPE was 46.7% (25^{th} to 75^{th} percentile, 38.3 to 58%). MRD for the prediction of plasma concentrations in Witjes' study was 2.0, which means that the predicted plasma concentrations were within a factor of 2 of the observed values.

3.2.7 Simulation of the pharmacokinetics of oral sildenafil in a virtual paediatric population

A virtual population of 200 children in the ages of 6 to 13 years was created. This is the same age range of the children in Karatza's study (Karatza, 2005). Female:Male ratio of children was 1:1. The estimated variability for the physiological parameters that affect the PK variability of sildenafil in children is presented in Table 25.

Parameter	Distribution category	Mean (deviation) or range
Hepatic clearance [ml/min]	Lognormal distribution	CL_{scaled} (1.4)*
Gastric emptying time [h]	Uniform	0.17 – 1.83
Intestinal transit time [h]	Normal distribution	4 (1)**
Intrinsic clearance for intestinal cytochrome P450 in:		
• Duodenum [ml/min]	Uniform	0 to 2*CL_{scaled}
• Upper jejunum [ml/min]	Uniform	0 to 2*CL_{scaled}
• Lower jejunum [ml/min]	Uniform	0 to 2*CL_{scaled}
• Upper ileum [ml/min]	Uniform	0 to 2*CL_{scaled}
• Lower ileum [ml/min]	Uniform	0 to 2*CL_{scaled}

Table 25: Estimated variability for physiological parameters relevant to access the pharmacokinetic variability of sildenafil in the paediatric virtual population

$CL_{scaled:}$ scaled intrinsic clearance from adults to the paediatric age; * geometric standard deviation, ** range; ***: standard deviation.

The predicted plasma concentrations of 0.5 mg/kg in the created paediatric population compared to observed data are presented in Figure 16. According to the population simulation, sildenafil is expected to reach a maximum plasma concentration 1.4 h post dose. Predicted C_{max} is 126 ng/ml and predicted $t_{1/2}$ is 2 h. Plasma concentration of sildenafil is expected to decrease rapidly to reach undetectable level 14 h post dose.

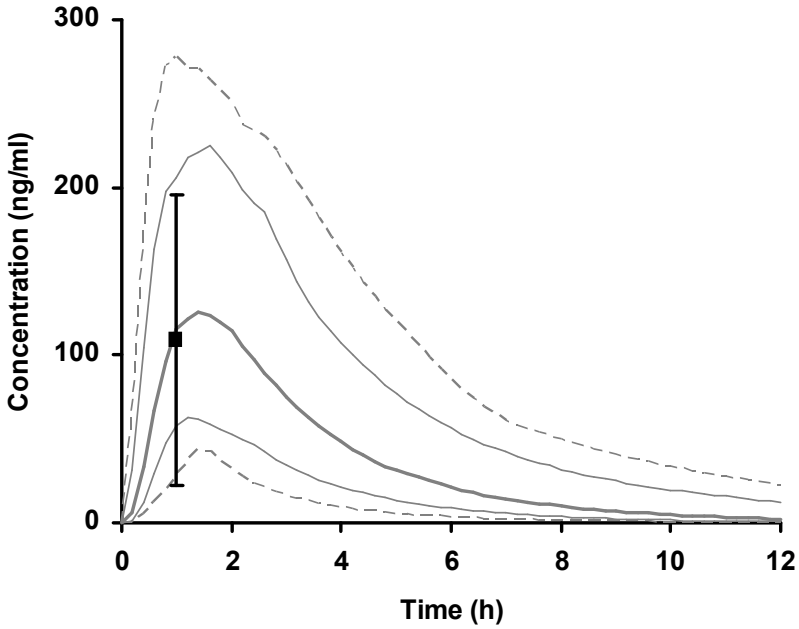

Figure 16: Comparison between simulated and observed plasma concentrations after oral doses of sildenafil in children

The population pharmacokinetic model was developed in 200 virtual children aged 6 to 13 years and compared to observed data in three children (Karatza, 2005). ---: maximum and minimum population; ▬: population median; —: population 5th and 95th percentile; ■: observed data, and error bars are standard deviation (Karatza, 2005).

The predicted mean plasma concentration 1 h after dose in 6- to 13-year-old children was within 14% of the observed mean plasma concentration and the predicted coefficient of variation was within 50% of the observed one.

3.2.8 Age-related exposure of oral sildenafil

The exposure of 0.5 mg/kg oral sildenafil was simulated in paediatric populations in an age range of 3 months to 18 years. This dose is widely used for the treatment of children with PAH, and was therefore chosen for the population simulations. The exposure of 20 mg oral sildenafil in adults was also simulated to be compared with sildenafil exposure of 0.5 mg/kg in children. Twenty milligrams of sildenafil is the approved dose for the treatment of PAH in adults, and was therefore selected.

Simulations for 1000 individual subjects were run in every age,. The calculated $AUC_{0-\infty}$ with the distribution of its value in each age is presented in Figure 17. As shown in Figure 17, the exposure of the same body weight-normalised dose across the paediatric age groups increases as the age of children increases. The progression of sildenafil exposure over the

paediatric ages can be divided into three subgroups. The first one includes 0.25 to 4-year-old children, in which the simulated exposure of 0.5 mg/kg sildenafil for many children is under the therapeutic levels estimated by the median of the simulated exposure of 20 mg oral sildenafil in adults. The second group includes children between 5 and 8 years of age, in which the simulated exposure is equal to the therapeutic levels in adults. The third group includes children ≥ 9 years of age, in which the simulated exposure of 0.5 mg/kg sildenafil is higher than the therapeutic levels of sildenafil in adults.

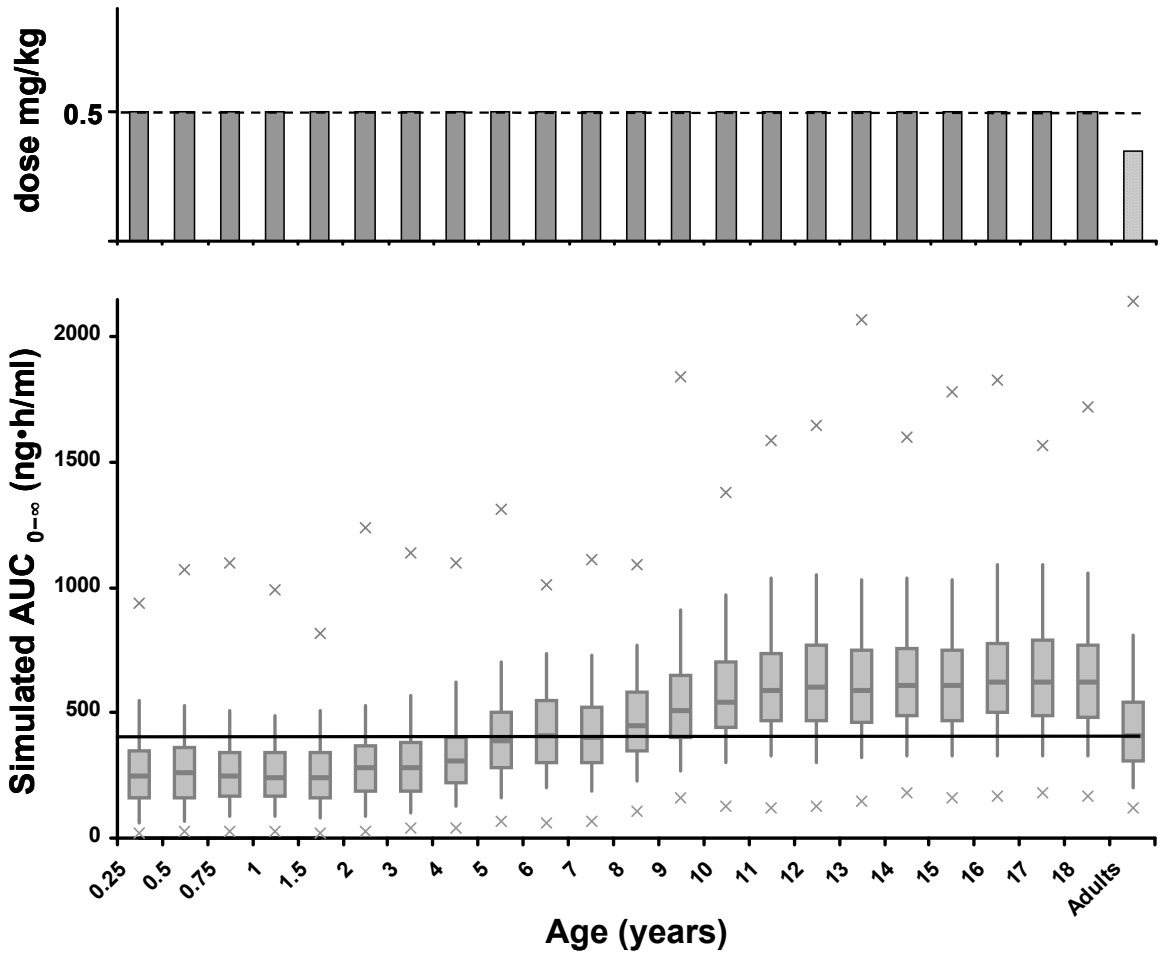

Figure 17: Simulated age-related exposure of sildenafil in children compared to adults

The figure shows the predicted area under the curve from time 0 to infinity ($AUC_{0-\infty}$) after 0.5 mg/kg and 20 mg oral sildenafil in children and adults respectively. Box plots represent median, 25th and 75th percentiles (box), 5th and 95th percentiles (error bar) and maximum and minimum values (x) of $AUC_{0-\infty}$ from 1000 simulations in each age.

3.2.9 Estimation of age-related doses of sildenafil and efficient sampling points for pharmacokinetic studies in children

In order to achieve the median exposure of the therapeutic dose of sildenafil in adults, the sildenafil dose was calculated in all paediatric age groups. The exposure of the estimated doses of sildenafil was simulated in children between 0.25 and 18 years of age. Simulations for 1000 individual subjects were runIn every age. The calculated $AUC_{0-\infty}$ with the distribution of its value in each age is presented in Figure 18.

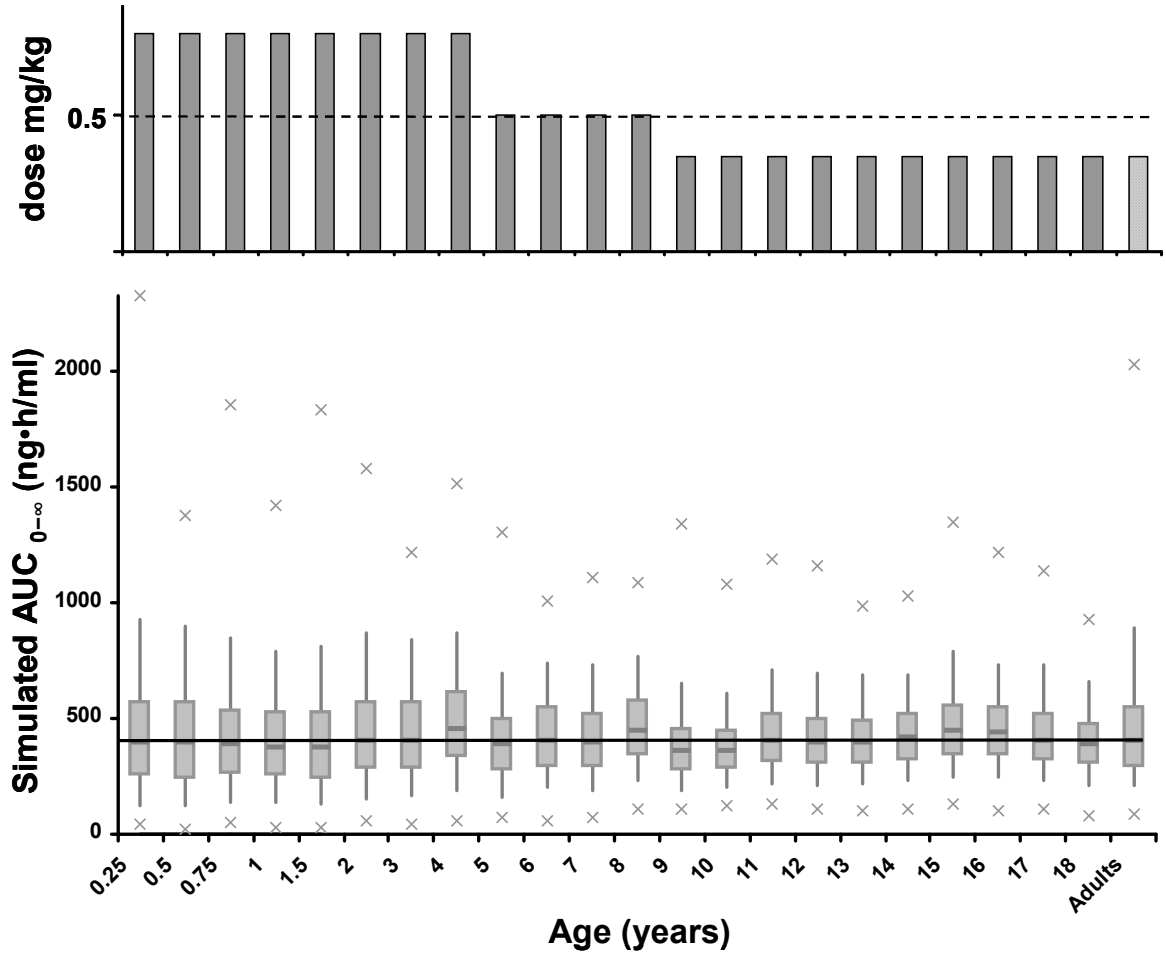

Figure 18: Simulated age-related exposure of the recommended doses of sildenafil in children compared to adults

The figure shows the predicted area under the curve from time 0 to infinity ($AUC_{0-\infty}$) after the estimated age-related doses of sildenafil in children and adults respectively. Box plots represent median, 25th and 75th percentiles (box), 5th and 95th percentiles (error bar) and maximum and minimum values (x) of $AUC_{0-\infty}$ from 1000 simulations in each age.

All children younger than 9 years of age need higher body weight-normalised doses than older children or adults in order to achieve therapeutic levels of sildenafil. The body weight-normalised dose of oral sildenafil for children older than 8 years of age is the same for adults, i.e. 0.35 mg/kg.

Infants and younger children need higher doses [mg/kg] relative to older children. For example the recommended dose for a 1-year-old infant is 0.8 mg/kg, whereas it is 0.5 and 0.35 mg/kg for a 7- and 15-year-old child respectively.

The PBPK model for sildenafil in children was used to estimate sampling times that appropriately describe the PK parameters: C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$. According to the simulation, time to reach maximum plasma concentration (t_{max}) of sildenafil is expected to be different among different paediatric ages. For example t_{max} is predicted to be 0.8, 1.1 and 1.3 h after oral application of 0.8, 0.5 and 0.35 mg/kg sildenafil in children of 0.25, 7 and 17 years of age respectively. Therefore, samples taken 0.8, 1.1 and 1.3 h after dose in children of 0.25, 7 and 17 years of age respectively should help to estimate the C_{max}. Additional sampling times 3, 6 and 10 h after dose in a 3-month-old infant should help to appropriately calculate the AUC, and to get points in the elimination phase to accurately investigate k_e, hence $t_{1/2}$. As shown in Figure 19, the simulated plasma concentrations of sildenafil 11 h after dose will be lower than the limit of quantification. Therefore, additional samples after this time in a 3-month-old infant are not necessary according to the simulation.

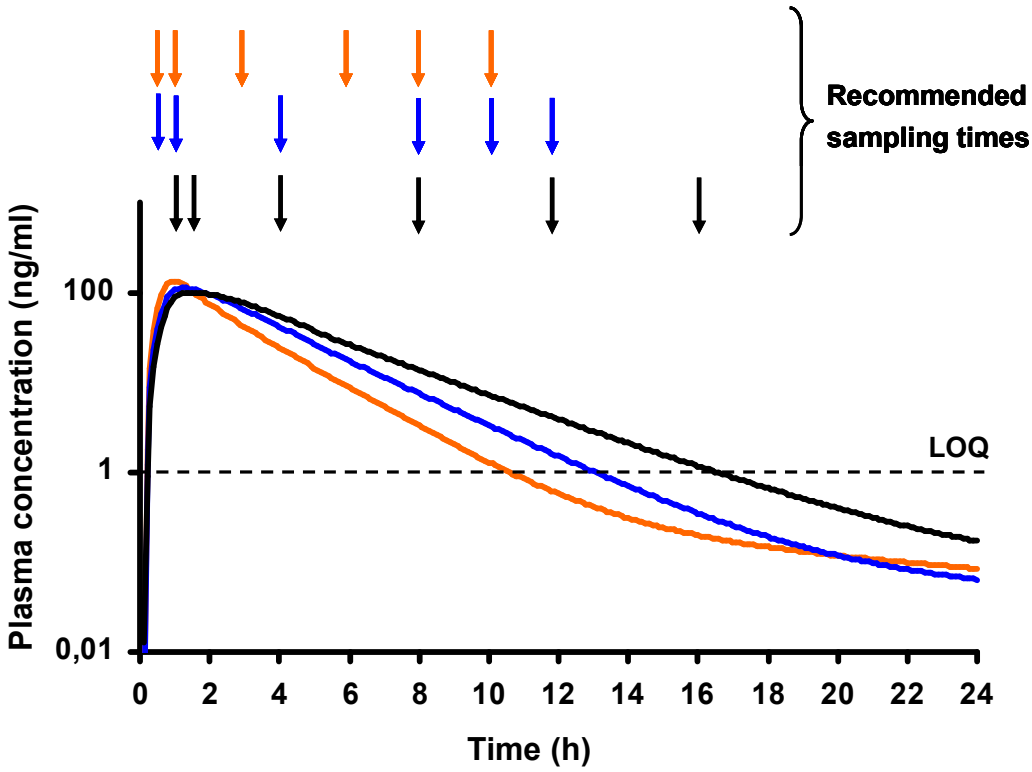

Figure 19: Estimated sampling times which appropriately describe the relevant pharmacokinetic parameters of oral sildenafil in children

The figure presents the logarithmic plot of the simulated plasma concentration-time curves for 0.8, 0.5 and 0.35 mg/kg oral sildenafil in children of 0.25 (—), 7 (—) and 17 (—) years of age respectively. The arrows represent the sampling times suggested to achieve an accurate description of the pharmacokinetic characteristics of sildenafil in children from different paediatric ages. LOQ: Limit of quantification.

4 Discussion

4.1 Most important results to be discussed

Off-label drug use on the paediatric pneumology and cardiology ward of the University Hospital of Düsseldorf was analysed. Sildenafil was used off-label in children with PAH although few PK data in children were available. This may lead to an overdosing or underdosing of sildenafil in children and demonstrates an insufficient scientific basis for the use of sildenafil in a serious and life-threatening disease in children. The paediatric PK of sildenafil have to be investigated to assure the efficacy and safety of sildenafil in children and to develop age-specific dosing regimens. This has to be done in well-designed PK studies to get the strongest evidence from few patients. Therefore, sildenafil PK were simulated in the current project using PBPK modelling to estimate the age-specific effective doses and the appropriate sampling time points *a priori*.

The main results of the present project were as follows:

- Sixty-one percent of the patients in the present trial received at least one off-label prescription, and 31% of the prescribed drugs were used off-label. The ten most frequently prescribed off-label drugs were: Salbutamol, co-trimoxazole, oxacillin, omeprazole, spironolactone, piperacillin/tazobactam, acetylsalicylic acid, prednisolone, furosemide and epinephrine.
- Cardiovascular drugs had the highest percentage of off-label prescriptions with a considerable overlap with drugs listed by the EMEA to be prioritized for urgent research. This highlights the need for paediatric studies on the presented cardiovascular drugs in the current trial including sildenafil for children with PAH.
- A PBPK model for sildenafil was developed for adults. The simulated plasma concentrations of sildenafil were closely correlated with the observed ones, with acceptable performance of the prediction of the major PK parameters in adults.
- The PBPK model for sildenafil was scaled from adults to children. The predicted plasma concentrations were within an acceptable range of the observed values.
- The simulated exposure of sildenafil is lower in children 0.25 to 8 years of age than older children or adults. Children ≤ 8 years of age are expected to need higher doses [mg/kg] than older children or adults whereas children ≥ 9 years of age need the same dose [mg/kg] of sildenafil for adults.

4.2 A prospective observational trial to analyse the off-label drug use in hospitalised children

Off-label drug use was defined in the present trial due to dose, age, indication and route of administration. The drug use despite a contraindication in children was not considered in this categorization because data essential to define a contraindication in a child, e.g. data on the liver or kidney function, were not always available in the prescription records of the patients. In previous studies, the use of contraindicated drugs in children was also not considered in the classification of off-label use (Conroy, 2000) or was considered unlicensed use (‘t Jong, 2004). In Neubert’s study, the contraindicated drugs were included in the classification system of off-label drug use but no off-label prescription under this category was presented (Neubert, 2004).

Drug prescriptions whose SPCs did not include dosing recommendation for children were classified in the current trial as off-label prescriptions due to age. This was similar to the studies of Dell’Aera, 2007 and Bücheler, 2002 but differs from the classification in the study of ‘t Jong, 2004, in which such prescriptions were considered unlicensed drugs (Dell’Aera, 2007; Bücheler, 2002; 't Jong, 2002). Eevery comparison with results from previous studies should therefore consider the differences in the off-label categorisation system.

The present trial showed that 253 out of 417 patients (61%) on the paediatric pneumology and cardiology ward of the University Hospital of Düsseldorf were given at least one off-label prescription. This means that more than half of the children had the risk of receiving a drug whose paediatric use is not adequately analysed, which may affect its efficacy and safety in this population. This rate of children who received off-label prescriptions (61%) is similar to the rate reported recently among hospitalized children in Finland (58%) (Lindell-Osuagwu, 2009). The mean rate of children who received at least one off-label or unlicensed prescription on paediatric wards from five European countries: United Kingdom, Sweden, Italy, Netherlands and Germany was 67% (Conroy, 2000) which is similar to that reported in the present trial (61%).

In the present trial, 553 out of 1,812 prescriptions (31%) were used off-label. A similar rate of off-label use among hospitalised children in Germany was also shown by Neubert and colleagues in 2004, where 198 out of 740 prescriptions (26.4 %) were off-label (Neubert, 2004). The results of Conroy and colleagues in 2000 showed also a similar rate of off-label prescriptions, where 37% of the prescriptions on a paediatric ward in Germany were off-label (Conroy, 2000). These similar rates of off-label use among hospitalised children

recorded since 2000 reflect an on-going use of off-label prescriptions among paediatric inpatients in Germany.

Rates of off-label prescriptions similar to the rate reported in the present trial (31%) were also seen in hospitalised children in other European counrtries. Conroy and colleagues reported a mean rate of 39% of off-label prescriptions of all prescriptions analysed from five European countries (Conroy, 2000), and 36% of the drugs given to children on a paediatric ward in Finland were prescribed off-label (Lindell-Osuagwu, 2009).

The main reasons for concern of off-label drug use in children are the safety and efficacy. Off-label prescribed drugs in children are associated with a higher rate of adverse drug reactions (Horen, 2002; Turner, 1999), probably due to the lack of evidence and a lack of information about the appropriate use such drugs. The off-label drug use in children may also be associated with an insufficient treatment of the children. The ß-receptor blocker carvedilol, which was used off-label in children with congestive heart failure in the present project, was supposed in a previous trial to be ineffective in the treatment of heart failure in children (Shaddy, 2007). The doses of carvedilol given to children in Shaddy's study, which was extrapolated from adults, seemed to be too low to achieve effective plasma levels, partially due to the very rapid elimination of carvedilol in children compared to adults. This explanation was supported with later results from a PK study of carvedilol in children, which showed that infants, children and adolescents up to 15 years of age needed higher doses [mg/kg] of carvedilol than those for adults to achieve effective plasma levels (Albers, 2008).

4.2.1 Most frequently prescribed off-label drugs

4.2.1.1 Salbutamol

Salbutamol was the most frequently prescribed off-label drug in the current trial and was also one of the most frequently prescribed off-label drugs in previous studies from different European countries (Turner, 1998; Conroy, 2000; 't Jong, 2004). More than half of salbutamol prescriptions in the present trial were overdosed. The overdosing of salbutamol was up to 200% of the recommended dose. For children under the age of 12 years, the maximum daily dose of salbutamol according to the SPC was 6 puffs, where every puff contains 100 µg salbutamol. High doses of salbutamol may worsen the cardiovascular safety in children. However, higher doses of nebulised salbutamol showed a significantly greater improvement in lung function than standard doses in children with

acute asthma; children who received higher doses of salbutamol showed a rate of adverse drug reactions similar to those who received standard doses (Schuh, 1990).

The physicians prescribed high doses of salbutamol for young children in the present trial to get a better efficacy than that which resulted from the standard doses. A possible explanation for this off-label use is that children do not use the delivery system adequately which can lead to a less exposure of the drug, hence less efficacy. However, clinical studies on the use of high doses of inhaled salbutamol in children younger than 12 years are very necessary to investigate the efficacy and safety of this frequently off-label prescribed drug.

4.2.1.2 Co-trimoxazole

Co-trimoxazole was the second most frequently prescribed off-label drug in the present trial. Co-trimoxazole was given as a prophylaxis for Pneumosystis-carinii-Pneumonia in children, an indication for which the drug was not labelled. Co-trimoxazole was labelled for the therapy but not for the prophylaxis for Pneumosystis-carinii-Pneumonia in children. However, evidence exists for the efficacy of co-trimoxazole as Pneumosystis-carinii-Pneumonia prophylaxis in oncology paediatric patients (Ohata, 2009; Lindemulder, 2007) and co-trimoxazole was recommended in the guidelines for the prevention of Pneumosystis-carinii-Pneumonia in children undergoing chemotherapy (Groll, 2001). This specific indication in children has to be considered in the SPC to ensure the availability of adequate dosing information for paediatricians.

4.2.1.3 Oxacillin

The daily dose of oxacillin has to be divided into four single doses according to the SPC. However, oxacillin was given three times per day in the present trial and was therefore considered an off-label prescription due to dose. A highly significant correlation was observed between the therapeutic efficacy of ß-lactams and cephalosporins and the percentage of time that the serum levels exceeded the minimum inhibitory concentration (MIC) (Craig, 1998). The same correlation is expected in the case of oxacillin. The division of the daily dose of oxacillin into three single doses instead of four can decrease the time in which the plasma concentrations of oxacillin exceed the MIC, which may decrease its efficacy. On the other hand the administration of intravenous drugs less often would save the nurses time and would be preferred by the patients. However, the benefit and risk of the administration of different dose regimen of oxacillin has to be evaluated in clinical studies of the efficacy of the same doses of oxacillin with different dosing intervals.

4.2.1.4 Omeprazole

Omeprazole was often given to children in the present trial for the prophylaxis for drug-induced ulcer, which was not included in the SPC. The efficacy of omeprazole for this indication in children was not investigated in clinical trials. Nonsteroidal anti-inflammatory drugs are associated with significant gastrointestinal abnormalities in children; therapy with ranitidine or misoprostol led to clinical improvement (Mulberg, 1993). Data on the benefit of omeprazole in the prevention of drug-induced ulcer in children treated with high doses of corticosteroid for short time, which was seen frequently in the present trial, were not found in the literature. Thus, the use of omeprazole for this indication has to be evaluated through clinical trials.

4.2.1.5 Spironolactone

Spironolactone was used off-label in the present trial due to indication, as it was given as potassium-sparing diuretic during treatment with loop diuretics. This indication was not mentioned in the SPC neither for adults nor for children. Spironolactone was licensed for the management of primary hyperaldosteronism and the oedematous conditions for patients with secondary hyperaldosteronism in children but not for the prophylaxis of hypokalaemia in patients taking loop diuretics. The benefit and appropriate use of spironolactone as a potassium-sparing diuretic during treatment with loop diuretics in children was not investigated. Therefore, paediatric randomized controlled trials are necessary to investigate this indication of spironolactone in children.

4.2.1.6 Piperacillin/Tazobactam

The combination of piperacillin and tazobactam was often given as empirical therapy for infections in immunocompromised patients or sickle cell anaemia patients in ages less than 12 years. For children under this age, the combination of piperacillin and tazobactam was recommended only for intraabdominal infections. Nevertheless, the update version of the SPC now gives dose recommendation for the treatment of infections in 2- to 12-year-old children with neutropenia, which had not been done at the time when the analysis of the presented results was performed (Hsien, 2008).

4.2.1.7 Acetylsalicylic acid

Acetylsalicylic acid prescriptions for the treatment of Kawasaki syndrome or for the prevention of platelets aggregation in paediatrics were considered off-label due to

indication. Acetylsalicylic acid was given to children in the current trial for its anti-platelet effect but the evidence for its appropriate dosing and efficacy in the paediatric population is very limited (Israels, 2006). Studies of the appropriate use of acetylsalicylic acid in the prevention of thromboses in children as well as studies that evaluate the benefit of acetylsalicylic acid relative to other anti-platelet agents and anticoagulant are required.

4.2.1.8 Prednisolone

Prednisolone given intravenously for patients with acute bronchitis or bronchiolitis was considered off-label due to indication. Intravenous prednisolone was approved for children but for other indications such as anaphylactic shock, severe acute asthma exacerbation and rejection after kidney transplantation. A very frequent condition of the use of intravenous prednisolone in the present trial was for acute viral bronchiolitis. However, according to a Cochrane review, glucocorticoids for acute viral bronchiolitis in infants and young children have no benefit in either length of hospital stay or clinical score (Patel, 2004). Despite no evidence of its benefit and despite a potential risk of acute adverse effects of corticosteroids, children with acute viral bronchiolitis in the present trial were frequently given prednisolone.

4.2.1.9 Furosemide

The intravenous furosemide prescriptions for the postoperative management of patients undergoing cardiac surgical procedures or for forced diuresis with hyperhydration after chemotherapy were off-label due to indication. The appropriate dose regimen of intravenous furosemide after cardiac surgery was investigated in previous clinical studies and observational reports (van der Vorst, 2001; Klinge, 1997; Singh, 1992). A systematic review and analysis of the pooled results from these studies is necessary to establish a rational dosing recommendation or to determine paediatric needs for further clinical studies of intravenous furosemide in children after cardiac surgery. The appropriate use of intravenous furosemide with hyperhydration in children treated with nephrotoxic chemotherapy has not been yet studied and therefore should be investigated in clinical trials.

4.2.1.10 Epinephrine

Epinephrine used via inhalation for the treatment of children with acute inflammatory airway obstruction was considered off-label due to indication. The use of epinephrine by

inhalation was not included in the SPC. Epinephrine was often used in the present trial as additional treatment to manage acute inflammatory airway obstruction episodes in children. Epinephrine resulted in a clinical improvement in children with asthma or bronchiolitis but was not found to be more efficacious than salbutamol (Mull, 2004; Plint, 2000). Evidence exists for the safety of nebulised epinephrine in children (Zhang, 2005). Therefore, children with dyspnoea may benefit from the nebulised epinephrine, especially if the treatment with nebulised salbutamol does not cause a clinical improvement. However, the appropriate use of epinephrine by inhalation and the corresponding safety data in paediatric patients should be included in the SPC in order to assure the availability of dosing information and safety data for the paediatricians.

4.2.2 Off-label drug use among cardiovascular drugs

Cardiovascular drugs had the highest percentage of off-label prescriptions and the highest number of different ATC codes which were off-label due to age compared to the other drug groups in the present trial. This reflects an imbalance between the paediatric needs in the field of cardiovascular diseases and the low number of drugs labelled for paediatric use in this field. Sixty percent of the cardiovascular drugs were used off-label in the present trial mainly due to age, with 33% of the cardiovascular prescriptions being off-label due to age. Three cardiovascular drugs – Spironolactone, acetylsalicylic acid and furosemide – were included in the ten most frequently prescribed off-label drugs in the present trial. Their off-label use was discussed in section 4.2.1.

Only one trial conducted by Bajcetic and colleagues in Serbia, has so far investigated the off-label drug use among children on a paediatric cardiovascular ward in Europe (Bajcetic, 2005). The rate of off-label drug use among cardiovascular drugs in Bajcetic's study (44%) was lower than the rate observed in the present trial (60%). The off-label drug use due to age was the main reason for the off-label use of cardiovascular drugs in the present trial with 33% of the cardiovascular prescriptions being off-label due to age; whereas the off-label drug use due to dose was the main reason for the off-label use of cardiovascular drugs in Bajcetic's study with 39% of the cardiovascular prescriptions being off-label due to dose. These differences in results between both studies can be partially due to the differences in the classification of off-label drug use between both studies. While the present trial did not analyse the unlicensed use of drugs, 9% of the cardiovascular drugs in Bajcetic's study were unlicensed prescriptions; many of them might have been classified under off-label prescriptions if the unlicensed use had not been analysed. The differences in the results between both studies might also reflect

differences in the national approval status and prescribing habits as well as differences in the definition of the off-label use due to dose between both studies. In the current trial a drug prescribed in a dose < 90% or >110% of the recommended dose was considered off-label prescription due to dose. The definition of the off-label use due to dose was not mentioned in Bajcetic's study.

The rate of off-label drug use among cardiovascular drugs in paediatric outpatients in Germany (55%) was similar to the rate observed in the present trial (60%) although the rate of off-label drug use in paediatric outpatients is generally lower than in hospitalised children (Bücheler, 2002). The unlicensed and off-label use of cardiovascular drugs among paediatric outpatients in the Netherlands reached 74.7% (Schirm, 2003). This means that high rates of off-label use of cardiovascular drugs exist not only among hospitalised children but also in paediatric outpatients and that the off-label use of cardiovascular drugs in children is widespread not only in Germany but also in other European countries as well. This highlights the need for studies on the safety and efficacy of cardiovascular drugs in children.

Twenty one different off-label prescribed cardiovascular drugs including beta blockers, diuretics, calcium-channel blockers, antiarrhythmics, vasodilators and antithrombotic agents were determined in the present trial (Table 5). Most of them were also recognised by the EMEA, either in the list of paediatric needs or in the priority list of off-patent drugs. The list of paediatric needs and priority list established by the EMEA are a result of a review process by European experts in the field of paediatric pharmacotherapy. Thus, the overlap of the off-label prescribed cardiovascular drugs observed in the present trial and those included in the EMEA list reaffirm that these off-label prescriptions are not only common in Germany but also in other European countries. Furthermore, most off-label prescribed cardiovascular drugs presented in the current trial were also recognized in a recent study from the USA (Pasquali, 2008). Pasquali and colleagues presented a list of cardiovascular drugs used off-label in hospitalised children with heart diseases, demonstrating a lack of safety and efficacy data of these prescriptions in both Europe and the USA. This further substantiates the necessity of performing clinical trials on the cardiovascular drugs in the paediatric population in Europe.

Regarding the list of off-label cardiovascular prescriptions, it is obvious that off-label drug use among children differs markedly from adults concerning the number of labelled drugs for cardiovascular diseases. This reflects a very poor situation concerning the data on the safety and efficacy of drugs given for the treatment of cardiovascular diseases in children and demonstrates the need to concentrate the clinical research on the appropriate use of these drugs in children. Until now, there is no ß-receptor blocker approved for paediatric

use on the German market despite their common administration in children with cardiovascular diseases. A recent Cochrane review showed that the pooled results from published studies were insufficient to recommend or discourage the use of ß-receptor blockers in children with congestive heart failure (Frobel, 2009). Further well designed clinical studies on the use ß-receptor blockers in paediatric patients were therefore strongly recommended.

There is no dihydropyridine calcium channel blocker (CCB), e.g. nifedipine and amlodipine, with a paediatric marketing authorisation in Germany. Nifedipine and amlodipine were used in the management of paediatric hypertension in the present trial. In contrast to adults, data regarding safety and efficacy of CCBs in children have mostly been obtained from retrospective analyses (Sahney, 2006). Paediatric hypertension became a considerable problem as its prevalence was 7.4% and 4.2% in paediatric populations from Canada and Italy respectively (Genovesi, 2005; Salvadori, 2008). As long-acting CCBs are considered an appropriate first-line therapy in the pharmacological management of hypertension in adults, children with hypertension will probably benefit from drugs from this therapeutic class. Thus, prospective clinical studies on the safety, efficacy and the relative benefit of CCBs in children with hypertension are necessary.

Anticoagulants for the treatment and prevention of thrombosis and embolism in children, e.g. enoxaparin and phenprocoumon, were used off-label due to age in the present trial. Enoxaparin and phenprocoumon belong to the standard therapy and prophylaxis of thrombotic events in adults. Thrombotic events do not occur frequently in children compared to adults, but their management in children is essential in order to prevent consequent mortality and morbidity. In a review published recently, differences in the dosing regimens of enoxaparin and other low molecular weight heparins (LMWHs) administered in children were observed (Nowak-Göttl, 2008). Further future studies in paediatric patients with thrombosis are therefore recommended to evaluate the optimum use of LMWHs in children. Compared to other oral anticoagulants, fewer data on the use of phenprocoumon in paediatric patients are available (Bonduel, 2006). Data on the paediatric use of phenprocoumon were reported in only a few children (Engelhardt, 1989) and PK studies of phenprocoumon in children are not available in the literature. Therefore, studies of PK, efficacy and safety of phenprocoumon in children are necessary.

PK of some drugs in the list of off-label cardiovascular prescriptions in the present trial were already investigated in previous paediatric studies. However, no dose recommendation for children was given in the SPCs, which may result in inadequate dosing of these drugs in children despite the existence of relevant data on the paediatric use of these drugs in the literature. Age-related changes in clearance and exposure of

sotalol, carvedilol and amlodipine in children were observed demonstrating age-specific dose regimens differerent from those for adults (Läer, 2005; Albers, 2008; Flynn, 2006). For example, the daily dose of carvedilol for congestive heart failure in infants, children, adolescents and adults were 3, 2, 1 and 0.7 mg/kg respectively (Albers, 2008). In contrast to sotalol, carvedilol and amlodipine, PK of other drugs in the list of off-label prescribed cardiovascular drugs, e.g. sildenafil, were not yet studied in paediatric trials. Age-dependent PK are also expected for these drugs. Thus, the extrapolation of their doses from adult population to children, which occurs normally in the absence of paediatric data, can either be unsafe or ineffective depending on the maturation of eliminating organs and pharmacodynamic differences in the different paediatric age groups. This demonstrates the need for paediatric studies on the off-label prescribed cardiovascular drugs, for which very few data on PK/PD in children are available. Sildenafil was one of these off-label prescribed drugs whose PK/PD in children were not yet sufficiently investigated. Therefore, sildenafil was selected for the second part of the present project in order to simulate its PK characteristics in paediatric patients.

4.2.3 Off-label use of sildenafil in children with pulmonary arterial hypertension

Sildenafil prescriptions given to children with PAH in the present trial were off-label due to age. Sildenafil was also included in the EMEA list of paediatric needs which demonstrates that its off-label use is common not only in Germany but also in other European countries. Although very few data on sildenafil PK were available, children with PAH were frequently given sildenafil. PAH is a rare disease with an annual incidence of 2 to 8 cases per million (Humbert, 2006; Peacock, 2007), but it was considered as a fatal disease with a bad prognosis (Jing, 2007; D'Alonzo, 1991). However, the prognosis seems at present better in the western countries (Humbert, 2006), probably due to the availability of the new treatment options and a better understanding of the disease resulting from intensive research and clinical trials. Nevertheless, most of the available information about the pharmacotherapy of PAH is obtained from studies in adults. The efficacy and safety of sildenafil in the treatment of PAH in adults were investigated in randomised controlled trials (Galie, 2005; Sastry, 2004; Singh, 2006). In children with PAH, the efficacy and safety of sildenafil were reported in uncontrolled trials and case reports (Karatza, 2005; Raja, 2007). The reported dosing patterns of sildenafil for the treatment of paediatric PAH in the different published studies are largely heterogeneous. A daily dose of 0.88 to 10 mg/kg of oral sildenafil with dose interval of 3 to 8 hours was reported in children aged

from a few days to 18 years (Lee, 2008; Oliveira, 2005; Peiravian, 2007, Karatza, 2005). In addition, the use of sildenafil was described in different types of PAH and in some trials in a combination with Bosentan, which may affect its PK and thereby its dosing adjustment (Lunze, 2006). Therefore, the optimal dosing of sildenafil cannot be extrapolated from the published data and has to be established in a well-designed PK/PD study considering the different paediatric age groups. Based on the epidemiological data of PAH, only a low number of paediatric patients would be included in a paediatric clinical trial of sildenafil. An *a priori* simulation for sildenafil PK in different paediatric age groups is therefore very helpful in the planning of a well-designed PK trial by the estimation of the effective age-specific dosing regimens and the efficient sampling points that appropriately describe the important PK parameters of oral sildenafil in children.

4.3 Development of a PBPK model for sildenafil in adults and children

The simulation of sildenafil PK was carried out using the PBPK software PK-Sim®. Additionally to the whole-body model underlying PK-Sim®, three steps had been carried out in order to develop a PBPK model for sildenafil in adults and children:

1. Integrating data on the CYP3A4 activities in the different intestinal segments in adult subjects based on published *in vitro* studies on the expression and activities of CYP3A4 in the human duodenum, jejunum and ileum.
2. Incorporating information on the ontogeny of the intestinal CYP3A4 in order to scale the PBPK model of sildenafil from adults to children. This step was based on published *in vitro* studies of the development of CYP3A4 expression and activity in the paediatric intestine.
3. The inter-individual variability of the physiological parameters that affect the variability distribution of sildenafil PK in adults and children was estimated based on a literature search in order be used in the simulation of sildenafil PK in virtual populations.

4.3.1 Pharmacokinetic studies of sildenafil in the literature

4.3.1.1 Intravenous studies

Four different doses of intravenous sildenafil – 20, 25, 40 and 50 mg – in adults were included from the literature review. The PK after these doses were investigated in three

different publications (Walker, 1999; Jackson, 1999; Nichols, 2002). The numeric values for the plasma concentration-time profiles after the doses were obtained per email from the research group of the studies. The four doses included were used to develop the PBPK model for intravenous sildenafil in adults.

The literature search revealed three PK studies which investigated five different doses of intravenous sildenafil in healthy male adults: 20, 25, 40, 50 and 80 mg (Walker, 1999; Jackson, 1999; Nichols, 2002). The intravenous dose of 80 mg sildenafil, which is equal to the oral dose of 200 mg, was excluded because a non-linearity in sildenafil exposure was seen at the 200 mg dose relative to the lower doses and was therefore considered out of the therapeutic range (Milligan, 2002).

The studies of the remaining doses met all inclusion criteria except of the one relating to the sildenafil assay. The precision of the assay in the PK study of 50 mg sildenafil (Nichols, 2002) was -35 to 8.6 %, which is outside of the FDA criteria for the bioanalytical methods validation, i.e. a precision within 15%, and within 20% for the lower limit of quantification (FDA, 2001). The high imprecision of the assay in Nichols' study was not the only disadvantage of the assay; the calibration range was 0.1 – 2.0 ng/ml which was too low relative to the measured plasma concentrations of sildenafil, where C_{max} in the mentioned study was 531 ng/ml. This means that additional dilution steps for the plasma samples had to be carried out to get the concentrations in the calibration range. This may increase the inaccuracy of the determination of plasma concentrations observed in Nichols' study, hence a high bias of the determination of the PK parameters.

The characteristics of the analytical method in the PK study of 20 and 40 mg intravenous sildenafil (Jackson, 1999) were not mentioned; it was therefore not possible to evaluate it. The assay of the PK study of 25 mg was within the FDA criteria, but the study included only three subjects which were older than 45 years of age (Walker, 1999). Therefore, the studies that investigated the 20, 25, 40 and 50 mg doses were included irrespective of their limitations and the PBPK of intravenous sildenafil was developed considering the results of all these studies.

4.3.1.2 Oral sildenafil

Three different studies which investigate the PK of 50 mg oral sildenafil were included from the results of the literaturue review (Muirhead, 2002c; Walker, 1999; Jetter, 2002). These studies met all inclusion criteria: the subjects had to be healthy, 18 – 65 years old, Caucasian and fasted, and sildenafil assay had to be validated. The plasma

concentration-time profiles in the included studies were used to develop the PBPK model for oral sildenafil in adults.

A literature search revealed 44 mean plasma concentration-time profiles of oral sildenafil included in 19 different publications. However, only three studies met all inclusion criteria and were therefore considered in the development and evaluation of the PBPK model for oral sildenafil in adults (Muirhead, 2002c; Walker, 1999; Jetter, 2002).

The main reason for the exclusion of the remaining studies was missing information about race or about the fed state of volunteers. The PBPK model for sildenafil was developed for a Caucasian population; therefore, the simulations were evaluated with observed data from Caucasian volunteers. Studies that included subjects from other ethnic groups were excluded in order to eliminate any potential effect of the inter-ethnic differences on the PK of sildenafil. Inter-ethnic variability in the PK of CYP3A4 substrates were observed in previous studies (Chávez-Teyes, 1999). Furthermore, sildenafil exposure was for example higher in Mexicans than in Caucasians probably due to a reduced CYP3A4 activity in Mexican population (Flores-Murrieta, 2000). The regional activities of intestinal cytochromes P450 were also preferred to be fitted in a fasted state to exclude the effect of food on C_{max} and t_{max}. Evidence exists for a delayed gastric emptying for single unit dosage forms with the existence of food (Davis, 1986). This leads to a delayed delivery of the drug to the small intestine, where the absorption takes place, which in turn leads to a delayed t_{max} and reduced C_{max}.

4.3.2 PBPK model for intravenous sildenafil in adults

Plasma concentrations after intravenous doses of sildenafil were simulated and compared to observed data to ensure that the distribution was well described by the PBPK model. The prediction of mean plasma concentrations after the four simulated intravenous doses was performed with a low bias represented by a median percentage error (MPE) of 1.3% (25^{th} to 75^{th} percentile, -26.7 to 30.8%) and acceptable precision represented by a median absolute percentage error (MAPE) of 27.5% (25^{th} to 75^{th} percentile, 13.4 to 47.4%).

The PBPK model for sildenafil could simulate the mean PK parameters: Volume of distribution at steady state (V_{ss}), terminal half-life ($t_{1/2}$) and area under the curve to infinity ($AUC_{0-\infty}$) with an MAPE (precision) of ≤18%.

Based on the PBPK model for intravenous sildenafil, the PK of sildenafil in a population of 1000 virtual subjects were simulated. The population simulation was able to describe the inter-individual variability in the plasma concentration-time profiles, where 84% of the

observed values were within the simulated 5th to 95th percentiles. The goodness-of-fit plots indicate that the population predicted and individual predicted plasma concentrations are closely correlated with the observed data, with weighted residuals ranged from 0.8 to 3.

However, some limitations of the model of intravenous sildenafil have to be mentioned. The plot of the simulated mean plasma concentrations against observed ones showed a trend toward overestimation of the plasma concentrations through the infusion period. This overestimation is considerable in the case of Nichols' study. One possible explanation is the effect of the sample dilution on the accuracy of the measurement of sildenafil concentrations in all simulated studies. A calibration range of 1 - 250 ng/ml and 0.1 – 2 ng/ml was mentioned in Walker's and Nichols' study respectively. Plasma concentrations measured through the infusion period were above the mentioned calibration ranges, which necessitated a dilution of the plasma samples to get the concentrations in the calibration range. No information was available on the accuracy and precision of the measurement of sildenafil concentrations in the diluted samples. Moreover, the precision estimated by CV was -35 to 8.6% in Nichols' study, which means that the assay may underestimate the concentrations of sildenafil by 35% of the observed value. This demonstrates that the overprediction of sildenafil concentrations may be partially due to a positive bias of the simulation but also due to a negative bias in the measurement of sildenafil concentrations through the infusion period.

An underestimation of the observed data within the first two hours after the end of infusion was also seen. Secondary plasma peaks in the observed individual plasma concentration-time profiles were seen after the end of infusion in most volunteers. These secondary peaks were relevant especially in the next two hours after the end of infusion. Usually, multiple secondary peaks result from either enterohepatic recirculation or discontinuous absorption of oral doses. The second reason was excluded because the secondary peaks were seen after both oral and intravenous administration. No unchanged sildenafil could be detected in faeces or urine by Muirhead and colleagues, which made the authors conclude that the metabolism of sildenafil is the main mechanism of its elimination (Muirhead, 2002b). However, unchanged sildenafil could be found in the bile in post-mortem analysis (Dumestre-Toulet, 2002; Lewis, 2006; Pagani, 2005), which refers to a biliary elimination of sildenafil. The absorption of sildenafil is more than 90% of its dose (Muirhead, 2002b). Therefore, secreted sildenafil from the gallbladder into the intestine might be completely reabsorbed, so that no unchanged sildenafil could be detected in the gastrointestinal excreta by Muirhead and colleagues. One point of criticism with the assumption of an enterohepatic recirculation of sildenafil is that the secondary peaks in the plasma concentration-time profiles appear relative early, whereas the emptying of

gallbladder usually occurs a few hours after dose approximately at the time of eating (Roberts, 2002). However, the emptying of the gallbladder is regulated by the sphincter of Oddi (SO), which regulates the flow of bile into the duodenum, and diverts hepatic bile into the gallbladder reservoir (Bosch, 2007). It was shown that the PDE-5 inhibitor, Vardenafil, reduces the motility and the pressure of SO in patients with SO dysfunction (Cheon, 2009). Glyceryl trinitrate, which has a similar effect on the smooth muscle, reduces the resistance to the bile flow through a reduction in the contraction amplitude and SO baseline pressure (Staritz, 1985). Regarding the results of Cheon and Startz, a similar effect of sildenafil is expected in normal subjects, hence, the resistance of SO could be reduced and the flow of bile may take place even in fasted situation. This might explain the early secondary peak seen in the plasma concentration-time profiles of sildenafil in fasted subjects. However one has to interpret this conclusion cautiously because the sildenafil bile/blood ratio ranged between 11 and 121 in post-mortem analysis (Dumestre-Toulet, 2002; Lewis, 2006; Pagani, 2005). This ratio is relatively small compared to what was observed in the case of Buprenorphine, where a bile/blood ratio of about 9638 was seen in post-mortem analysis (Tracqui, 1998). Buprenorphine showed a secondary peak in its plasma concentration-time profile 90 min after infusion due to enterohepatic recirculation but its biliary secretion is probably much higher than sildenafil.

The predicted PK parameters were within 19% of those observed in all studies with the exception of Nichols' study. The PBPK model overestimated $AUC_{0-\infty}$ in Nichols' study by 42%, whereas V_{ss} and $t_{1/2}$ were underestimated by 41 and 22% respectively. However, as discussed above, the imprecision of the assay in Nichols' study was relatively high and the calibration range was too low relative to the measured data, demonstrating a possible bias in the measurement of plasma concentrations in Nichols' study. The difference between the results of Nichols' and other studies confirms this point. For example V_{ss} in Nichols' study was 105 l, which was much higher than those observed in the other studies where V_{ss} was 57, 71 and 59 l. The dose normalised $AUC_{0-\infty}$ (516 ng.h/ml) was lower in Nichols' study than in other studies (787, 731 and 776 ng.h/ml). The inter-individual variability can partially explain the difference in the results between studies, but the sildenafil assay which was used in Nichols' study probably had an important effect on the results.

4.3.3 PBPK model for oral sildenafil in adults

4.3.3.1 The intrinsic clearance of CYP3A4 in the different intestinal segments in adult subjects

The intestinal intrinsic clearance values were fitted to achieve a bioavailability of about 41% and to result in an adequate visual check for the simulation of oral doses. The relative contribution of the intestinal intrinsic clearance [ml/min] in the duodenum, jejunum and ileum to the total intestinal intrinsic clearance was assumed to be correlated with the relative amounts of CYP3A4 in these segments.

The regional intrinsic clearances as a fraction of the total intestinal intrinsic clearance in the present project were calculated based on *in vitro* experiments on the human intestinal P450 (Zhang, 1999) and was estimated in the current project to be 0.26, 0.54 and 0.2 in the duodenum, jejunum and ileum respectively. This was similar to the values estimated in a previous study, where the fraction of the intrinsic clearance of CYP3A4 was 0.24, 0.66 and 0.1 in the duodenum, jejunum and ileum respectively (Paine, 1997).

The bioavailability of sildenafil is 41% due to an effective first-pass effect (Walker, 1999; Nichols, 2002). Probably both hepatic and intestinal metabolisms are responsible for the first-pass effect of sildenafil. Data about the involvement of human intestinal CYP enzymes in the metabolism of sildenafil are not available. However, data from animal experiments showed that AUC after intragastric administration was significantly lower than those after intraportal administration of sildenafil, demonstrating an intestinal first-pass effect of sildenafil of about 71% of an oral dose in rats (Shin, 2006). The hepatic metabolism of sildenafil in human is mediated mainly by CYP3A4 (79%) and CYP2C9 (20%) (Warrington 2000). CYP3A4 and CYP2C9 consist of about 80% and 15% of the total immunoquantified cytochrome P450 enzymes in the proximal small intestine respectively (Paine, 2006). Therefore, both CYP3A4 and CYP2C9 in the human small intestine may contribute to the total intestinal first-pass metabolism of sildenafil. Therefore, the activity of the intestinal CYPs involved in the first-pass metabolism of sildenafil were integrated into the different intestinal segments in the development of the PBPK model of oral sildenafil.

4.3.3.2 Evaluation of the PBPK model for oral sildenafil in adults

The simulated plasma concentrations after oral doses had a close correlation to the observed data and the prediction of mean plasma concentrations in the three PK studies

of oral doses of sildenafil had a good performance with an MPE (bias) of 4.5% (25^{th} to 75^{th} percentile, -14.2 to +14.3%) and an MAPE (precision) of 14.9% (25^{th} to 75^{th} percentile, 7.6 to 29.4%).

The MPE (bias) for the prediction of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ in the three studies was -7.3% (25^{th} to 75^{th} percentile, -20.7 to -2%), +9.9% (25^{th} to 75^{th} percentile, +4.3 to +16.4%), and +8.3% (25^{th} to 75^{th} percentile, -6.7 to +12.4%) respectively. Although the values of the MPE of the PK parameters prediction referred to an underprediction of C_{max} and overprediction of $AUC_{0-\infty}$**;** the prediction of the PK parameters was performed with a good precision estimated by an MAPE of 7.3% (25^{th} to 75^{th} percentile, 5.3 to 20.7), 9.9% (25^{th} to 75^{th} percentile, 5.6 to 16.4%), and 16.5% (25^{th} to 75^{th} percentile, 12.4 to +19.1%) for the prediction of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ respectively.

The PBPK model could simulate the PK parameters: $C_{max,}$ $AUC_{0-\infty}$ and $t_{1/2}$ in Walker's study with a relative error within 9%. The relative error for the estimation of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ in Muirhead's study was +3.30, +22.87 and +16.54% respectively. The overestimation of both $AUC_{0-\infty}$ and $t_{1/2}$ might be due to an underestimation of the mean body weight which was given to the simulation software. The mean body weight of the volunteers in Muirhead's study was not mentioned in the paper, so a virtual individual was created with data provided by PK-Sim® using mean values for the body weight of a European male subject at the mean age of the subjects in Muirhead's study. This estimated value for body weight may, however, differ from the real value in Muirhead's study and may therefore affect the simulation results. Body weight affects the simulated exposure of sildenafil, so that the simulated $AUC_{0-\infty}$ will be higher in the virtual subjects with low body weights.

The relative error for the simulation of C_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ in Jetter's study was -34.07, +9.85 and -21.64. The measured C_{max} in Jetter's study was very variable with geometric mean (geometric standard deviation) of 254.89 (1.65). In addition, the measured C_{max} in Jetter's study (255 ng/ml) was higher than those measured in Walker's and Muirhead's studies: 207 and 178 ng/ml respectively. The prediction of such a variable parameter is difficult, since many factors such as body position, gastric emptying time and intestinal cytochromes activities may affect C_{max} for a drug with an extensive first-pass effect (Renwick, 1992). The negative bias in the simulation of $t_{1/2}$ in Jettre's study can be due to an inter-individual variability in the hepatic CYP3A4 activity which in turn affects the $t_{1/2.}$

Depending on the PBPK model for oral sildenafil, a population pharmacokinetic simulation for 1000 virtual adults was performed. Individual data could be obtained for only three

subjects in Walker's study, whereas it was not possible to get access to the individual data of the other two PK studies which met the inclusion criteria

The population simulation was able to describe the inter-individual variability in the plasma concentration-time profiles, where most of the observed values (94%) were within the simulated 5th to 95th percentiles. The goodness-of-fit plots indicate that the model is able to describe the observed data with weighted residual range of -0.9 to +1.2 for all observed points with the exception of two values, which were measured 15 min after dose and were overestimated with a weighted residual of 4.7 and 18.6.

4.3.4 PBPK model for oral sildenafil in children

4.3.4.1 Age-related hepatic and intestinal clearance of sildenafil in children

Prior to the simulation in children, the hepatic clearance was scaled from adults to the simulated paediatric age. The model for the development of hepatic clearance of sildenafil in children is an additive function of two models describing the development of CYP3A4 and CYP2C9. The hepatic clearance of sildenafil [ml/min/kg] in all paediatric ages after the first month of life is expected to be greater than in adults. The largest values of hepatic clearance arise in the first four years of age, thereafter the clearance decreases gradually to reach the adult value at 18 years of age. This progression was mainly affected by the CYP3A4 ontogeny, as its contribution to the hepatic clearance of sildenafil in adult is about 80% (Warrington, 2000).

In contrast to hepatic CYPs enzymes, PK-Sim® does not scale the clearance of intestinal CYPs from adults to children. An intestinal clearance scaling from adults to children was therefore manually carried out by incorporating information on the ontogeny of the intestinal CYP3A4. The ontogeny of the intestinal CYP3A4 enzymes was investigated in only one publication, which showed significant increases in CYP3A4 expression and activity with age (Johnson, 2001). The results of Johnson's study were used in the present project to develop a model for the age-related changes in the intestinal CLint as a fraction of the adult value. The model for age-related changes of the CLint of the intestinal CYP3A4 was developed considering that the intestinal CLint in children as a fraction of the adult value is correlated with the CYP3A4 activity and intestinal surface area in children as a fraction of the adult values. The intestinal CLint by CYP3A4 was scaled from adults to children in one published study (Johnson, 2006). Johnson used his already published results about the ontogeny of intestinal CYP3A4 to predict the disposition and

bioavailability of midazolam in different paediatric ages (Johnson, 2006; Johnson, 2001). Johnson calculated the paediatric CLint of intestinal CYP3A4 by multiplying the age-dependent gut CYP3A abundance by the CLint derived using human liver microsomes. In the present project, the adult intestinal CLint values, which were fitted to the mean plasma concentration-time profiles of oral sildenafil, were scaled to children. Although no data on the relative distribution of CYP3A4 in the paediatric human gut exist, it was assumed in the present project that this distribution pattern does not differ from adults. In addition, it was assumed that the development of CYP3A4 activity in paediatric duodenum is the same in both the ileum and jejunum. This assumption was also made by Johnson and colleagues (Johnson, 2006).

4.3.4.2 Evaluation of the PBPK model for oral sildenafil in children

This is the first time that a PBPK model for oral sildenafil in children is presented. As the published PK studies of oral sildenafil were investigated in very few children, the performance of the PBPK model of sildenafil in children was evaluated by comparison with few observed data. However the MRD for the prediction of plasma concentrations of sildenafil was 1.6 and 2.0 in Karatza's and Witjes' studies respectively, indicating an acceptable performance for the PBPK model for sildenafil in children, and that the simulation of sildenafil exposure can be used in the planning of PK studies of sildenafil in children, especially in the absence of sufficient age-specific observed data.

The mean plasma concentration 1 h after 0.5 mg/kg sildenafil in children aged 6.6, 10.6 and 13.9 years was predicted with a relative error of 26%. The prediction of mean plasma concentration 1 h after 1 and 2 mg/kg in the same children in the second and third month of the treatment with sildenafil was more biased with a relative error of 93 and 58% respectively. It is to be noted that an unexpected reduction in the measured C_{max} after 1 and 2 mg/kg sildenafil in the second and third month of therapy respectively occurred. The observed mean C_{max} after 1 mg/kg was only 1.4 of C_{max} after 0.5 mg/kg (Karatza, 2005), whereas C_{max} was expected to be linear relative to dose. This could be partially due to intra-individual variability in C_{max} caused by many factors including the intra-individual variability of gastric emptying time and hepatic or intestinal CYP3A4 activities. Another explanation is a potential delaying in the gastric emptying after a long-term therapy with sildenafil. The inhibitory effect of sildenafil on the gastrointestinal tract was reported in animals and humans. Sildenafil has an antispasmodic effect on rat duodenal segments (Araújo, 2005) and an inhibitory effect on the contractile activity of oesophageal musculature both in normal subjects and in patients with oesophageal disorders (Bortolotti, 2000; Bortolotti 2001a; Eherer, 2002). Sildenafil was also shown to inhibit the

gastroduodenal motility and to delay the gastric liquid emptying in humans and the gastric emptying and intestinal emptying of a liquid meal in rats (Bortolotti, 2001b; Sarnelli, 2004; Rosalmeida, 2003). Therefore, a prolonged intake of sildenafil may delay the gastric emptying in children and lead to a slow absorption of oral sildenafil. According to this scenario, a simulation for oral doses of sildenafil in the steady state phase without considering the altered gastric emptying time will lead to an overestimation of C_{max} and all concentrations measured in the first hour after administration.

An MRD of 2.0 for the prediction of plasma concentrations of oral sildenafil in a 4-month-old infant in Witjes' study was acceptable. In contrast to the simulation of Karatza's study, the model underestimated the plasma concentrations of sildenafil observed in Witjes' study with an MPE of -47%. This negative bias can be due to an inter-individual variability in hepatic or intestinal CYP3A4 activities or inter-individual variability in unbound plasma fraction which may affect the clearance and hence the plasma concentrations. Another possible explanation for the underestimation of the plasma concentrations of sildenafil is an overestimation of the hepatic clearance or the first-pass effect. The infant in Witjes' study had a severe form of PAH which might lead to a delayed development of eliminating organs due to a chronic hypoxemia. Nevertheless, the performance of the prediction of sildenafil concentration is still in an acceptable range.

4.3.5 Age-related changes in sildenafil exposure and dosing in children

The aim of the simulation of the age-related sildenafil exposure in children was to determine the age-related dosing that ensures levels of drug exposure in children similar to those of therapeutic doses in adults. For this purpose, the simulation of 20 mg of sildenafil, which is the approved dose for PAH in adults, was simulated in a virtual adult population of 1000 subjects. A simulation of 0.5 mg/kg oral sildenafil in paediatric populations was performed and the results were compared to adult exposure. The exposure of 0.5 mg/kg oral sildenafil differs among the paediatric age groups. $AUC_{0-\infty}$ of the same dose [mg/kg] is predicted to be less in infants and children up to 4 years of age compared to older children or adults. For example, the simulated median $AUC_{0-\infty}$ after 0.5 mg/kg sildenafil in 1-, 7- and 15-year-old children is 240, 400 and 610 ng·h/ml. The simulated median $AUC_{0-\infty}$ after 20 mg oral sildenafil in adults is 410 ng·h/ml. This means that a dose of 0.5 mg/kg of sildenafil given every 8 h to infants and young children will be insufficient to achieve an effective exposure for all simulated children; therefore, the dose has to be increased or the dose-interval has to be reduced in order to ensure a sufficient

exposure of sildenafil for all children in this age group. In contrast, children older than 8 years of age and adolescents are predicted to have a higher exposure after 0.5 mg/kg sildenafil than those after 20 mg in adults. Hence lower doses should be given to this age group to avoid overdosing of sildenafil.

The recommended doses of sildenafil based on the PBPK model in children range from 0.35 to 0.8 mg/kg three times a day. However age-specific doses should be considered. Children ≤ 8 years of age seem to need higher doses of oral sildenafil [mg/kg] compared to older children or adults. The recommended body weight-normalised dose in children ≥9 years of age is the same for adults; children in this age group with body weight similar to that of adults can be given the adult dose of sildenafil, i.e. 20 mg. All children younger than 9 years of age need higher doses [mg/kg] than adults.

Age-related changes for paediatric doses were also seen in the treatment with other drugs metabolised mainly by CYP3A4. In a population pharmacokinetic study of amlodipine in children, the body weight-normalised clearance for 1- to < 6-year-old children was about 1.6 of its value in 6- to < 13-year-old children (Flynn, 2006). In the same study, children 13 years of age or older have body a weight-normalised clearance of amlodipine similar to those of adults; therefore, no adjustment of amlodipine dose was recommended for adolescents with body weight comparable to adults.

The body weight-normalised clearance of ciclosporin decreases with increasing age after the second year of life, and children ≤ 8 years of age including infants (0.36 to 1 years of age) have a higher weight normalised-clearance of ciclosporin compared to older children (Fanta, 2007). This resulted in a higher dose requirement in young relative to older children, so that children aged 0 to 8 years need about 25% higher doses in mg/kg of ciclosporin than those of older children (Fanta, 2007).

5 Conclusion

The present project provides a close analysis of the off-label drug use on the paediatric pneumology and cardiology ward of the University Hospital of Düsseldorf. A high rate of off-label use of cardiovascular drugs was observed. The results of the present trial clearly reveal the need for prioritizing clinical trials in the paediatric area, especially in cardiovascular diseases. A list of 21 off-label prescribed cardiovascular drugs, most of which have no other authorized alternatives currently available for the indications in children, was presented in the current trial. A later trial from the USA included many of the presented cardiovascular drugs in the current project to be also used in an off-label manner in children (Pasquali, 2008), indicating a poor labelling state for cardiovascular drugs in children in both Europe and the USA. Furthermore, these drugs cover a spectrum of potentially life-threatening diseases in children, such as heart failure, arrhythmias, thrombosis and PAH. Therefore, these drugs should have the priority of intensive research in the near future.

Sildenafil was included in the list of off-label cardiovascular drugs in the present trial. It was also considered in the EMEA list of paediatric needs for the treatment of PAH in children. Sildenafil was very promising in the treatment of PAH in adults and is at present very essential in the modern therapy of this disease. In contrast to adults, there were very few data on sildenafil PK in children. Data available on sildenafil PK in adults were used in the present project to develop a PBPK model for oral sildenafil in children. The simulated PK parameters and plasma concentrations were closely correlated with the observed data in adults. The predicted plasma concentrations in children were within two fold of the of the observed values, demonstrating an acceptable performance of the PBPK model in children. The simulation of sildenafil PK in paediatric populations referred to an age-related exposure of oral sildenafil indicating the need for age-specific dose recommendation for sildenafil in order to avoid overdosing or underdosing of sildenafil. Children younger than 9 years of age are expected to need higher doses [mg/kg] than older children or adults whereas children 9 years of age and older need the same dose of sildenafil [mg/kg] as for adults. Although the simulations were compared with only small amounts of data from paediatric patients, its performance in both adults and children was acceptable and demonstrated that the PBPK model for sildenafil is able to be used to design PK studies of sildenafil in children. The *a priori* age-specific dose estimation, in addition to the estimated sampling time points presented in the current project should help in the planning of future PK studies of sildenafil in paediatric patients.

6 Summary

6.1 Zusammenfassung

Epidemiologische Studien über den Off-Label-Gebrauch von Arzneimitteln bei Kindern sind wichtig, um Forschungsbedarf in pädiatrischer Pharmakotherapie zu identifizieren. Dadurch kann der Fokus auf Arzneimittel gesetzt werden, die ohne wissenschaftliche Erkenntnisse bei kranken Kindern eingesetzt werden.

Eine Off-Label-Verordnungsanalyse wurde von Januar bis Juni 2006 auf der Station für Kardiologie und Pneumologie der Kinderklinik an der Universität Düsseldorf durchgeführt. Daten der Patienten, deren Diagnosen und Arzneimittel wurden aufgenommen und mit der deutschen Fachinformation verglichen. Der Off-Label-Gebrauch wurde nach Altersgruppe, Dosis, Indikation und Applikationsweg des Arzneimittels ermittelt. Die Ergebnisse zeigten, dass 253 von 417 Patienten (61%) mindestens eine Off-Label-Verordnung erhielten. Insgesamt waren 553 von 1812 Verordnungen (31%) off-label. Die Herz-Kreislaufarzneistoffe besaßen mit 129 von 216 Verordnungen (60%) den höchsten Off-Label-Anteil. Eine hohe Übereinstimmung dieser Off-Label-Herz-Kreislaufarzneistoffe fand sich mit den Arzneistoffen, die von der European Medicines Agency (EMEA) gelistet wurden, um auf dringend erforderliche pädiatrische Forschung aufmerksam zu machen. Ein beispielhafter Arzneistoff aus der Off-label-Liste der Herz-Kreislaufsubstanzen ist Sildenafil, bei dem völlig unzureichende Daten zur Pharmakokinetik bei Kindern vorliegen. Es war daher das Ziel im zweiten teil der Forschungsarbeit, ein Physiologie-basiertes Model zu entwickeln, um die Sildenafil Pharmakokinetik *a priori* in Kindern vorherzusagen als Grundlage für zukünftige kindgerechte klinische Studien. Zunächst wurde dazu ein Erwachsenenmodel erstellt, welches dann auf Kinder skaliert wurde. Die simulierten Plasmakonzentrationen und PK-Parameter konnten in beiden Schritten die gemessenen Datenwerte adäquat darstellen. Die Ergebnisse der Simulation zeigten eine altersabhängige Exposition von Sildenafil: Kinder, die jünger als 9 Jahre sind, benötigen höhere körpergewichtsnormierte Dosierungen von Sildenafil, um eine den älteren Kindern und Erwachsenen vergleichbare Exposition zu erzielen. Mit den Simulationen lassen sich auch altersspezifische Zeitpunkte für die pharmakokinetischen Untersuchungen planen. Auf der Grundlage der Simulationen können demnach nun kinderspezifische Besonderheiten von Sildenafil in den klinischen Studien von vornherein berücksichtigt werden. Damit sollten die Studien einen höheren Aussagewert erzielen können.

6.2 Summary

Epidemiological studies of off-label drug use in children are important to identify domains of pharmacotherapy with the highest need for research in paediatrics. They refer to drugs, which are frequently given to children without adequate information on their safety and efficacy for paediatric use.

An analysis of drug prescriptions was conducted on the paediatric ward of cardiology and pneumology of the University Hospital of Düsseldorf in Germany between January and June 2006 to describe the off-label drug use among hospitalised children. Data about patients, their diagnoses and prescribed drugs were collected. The off-label drug use was defined due to patient age, indication, route of administration and dose of the prescribed drug. In total, 253 out of 417 (61%) patients received at least one off-label prescription. Of all analysed prescriptions, 553 out of 1812 (31%) were off-label. Cardiovascular drugs had the highest percentage of off-label use: 129 out of 216 (60%) prescriptions were off-label. There was a considerable overlap between the off-label prescribed cardiovascular drugs in the current trial and those listed by the European Medicines Agency (EMEA) to be prioritised for urgent research in Europe.

Sildenafil was included in the list of off-label prescribed cardiovascular drugs in the present trial, and was also mentioned in the list of paediatric needs of EMEA. Very few pharmacokinetic data were available on sildenafil in children. A physiologically-based pharmacokinetic (PBPK) model for sildenafil in children was therefore developed to better interpret the efficacy and safety of sildenafil in the paediatric population. Firstly, a PBPK model for sildenafil was developed in adults and was then scaled to children. The simulated plasma concentrations and the pharmacokinetic parameters were closely correlated with observed data from adults. The predicted plasma concentrations of sildenafil in children were in an acceptable range of those observed. The paediatric model showed an age-related exposure of sildenafil: Children less than 9 years of age are expected to require higher doses [mg/kg] than older children and adults in order to achieve effective exposure of sildenafil.

The present project demonstrated a high need for research on the paediatric use of cardiovascular drugs, e.g. sildenafil. It was shown that PBPK modelling is an adequate method to predict the plasma concentrations of sildenafil in children. The PBPK model for sildenafil in children can help design future pharmacokinetic studies by providing the optimal sampling times and age-specific doses of oral sildenafil.

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8 Appendix

8.1 List of abbreviations

AUC	Area under the curve
AUMC	Area under the moment curve
BMI	Body maß index
CCB	Calcium channel blocker
cGMP	Cyclic guanosine monophosphate
CL	Plasma clearance
CLh	Hepatic clearance
CLint	Intrinsic clearance
C_{max}	Maximum concentration
CV	Coefficient of variation
CYP	Cytochrome P450
EHC	Enterohepatic circulation
EMEA	European Medicines Agency
fu	Unbond fraction
g	Gramme
GET	Gastric emptying time
h	Hour
HPLC	High performance liquid chromatography
ITT	Intestinal transit time
kcal	Kilocalorie
k_e	Elimination rate constant
kg	Kilogramme
l	Litre
LC-MS	Liquid chromatography mass spectrometry
LC-UV	Liquid chromatography with ultraviolet detection
m^2	Square metre
mg	milligramme
min	Minute
ml	Millilitre
MAPE	Median absolute percentage error
MPE	Median percentage error
MRD	Mean relative deviation

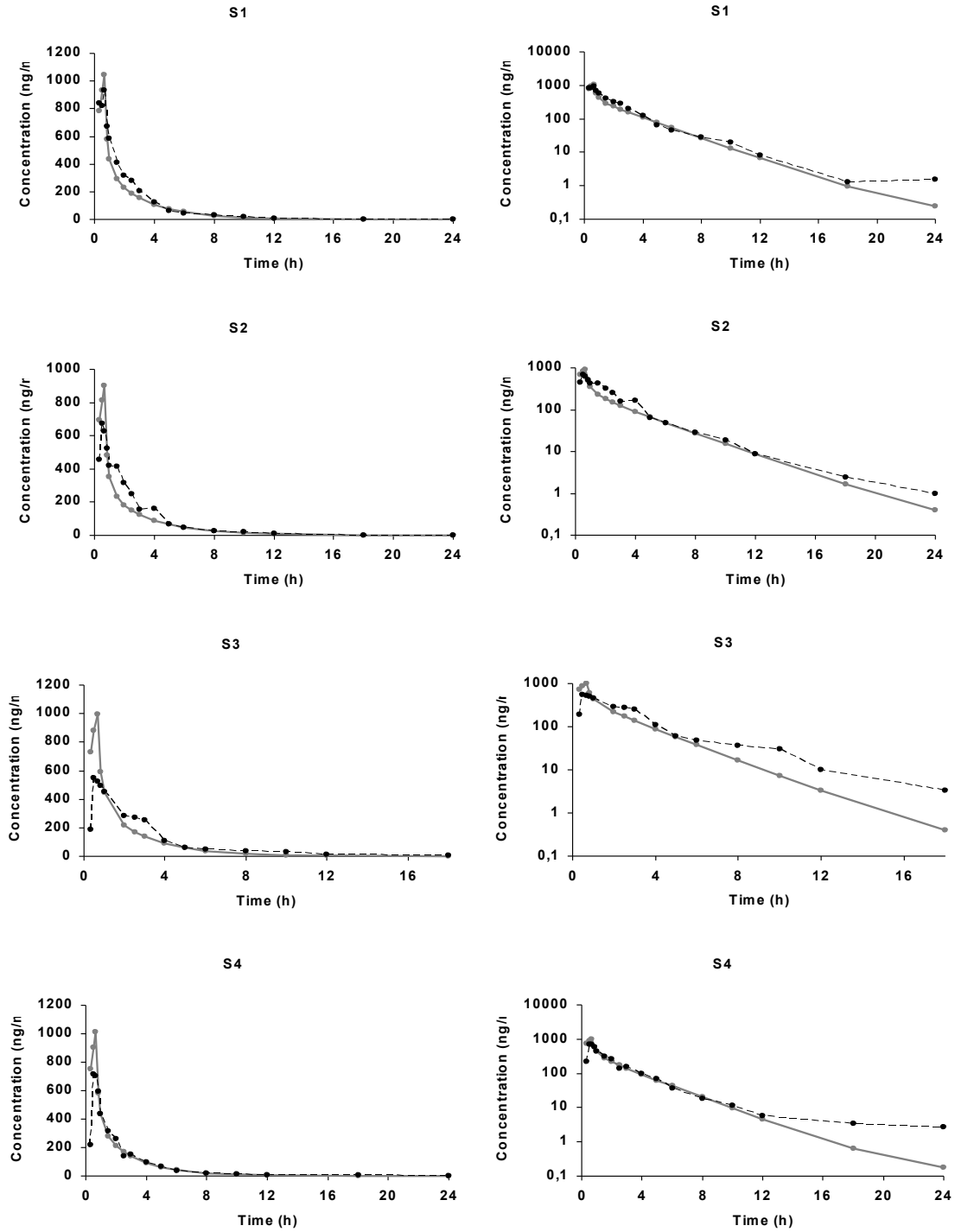
MRT	Mean residence time
NCBI	National Center for Biotechnology Information
PBPK	Pysiologically-based pharmacokinetic
PD	Pharmacodynamic
PDE-5	Phosphodiesterase type 5
PK	Pharmacokinetic
Q_H	Hepatic blood flow
RE	Relative error
SD	Standard deviation
SPCs	Summary of Product Characteristics
$t_{1/2}$	Terminal half-life
t_{max}	Time to maximum plasma concentration
V_{ss}	Volume of distribution at steady state

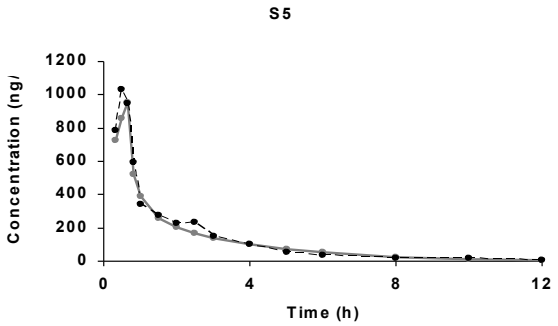
8.2 List of figures

8.3 List of tables

Figure 20: Comparison between simulated individual and observed individual plasma concentrations after the intravenous administration of 40 mg sildenafil

Linear (left) and logarithmic (right) plot of simulated individual (grey) and individual observed (black) concentration-time profiles for 40 mg given intravenously (Jackson, 1999).

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Concentration (ng/
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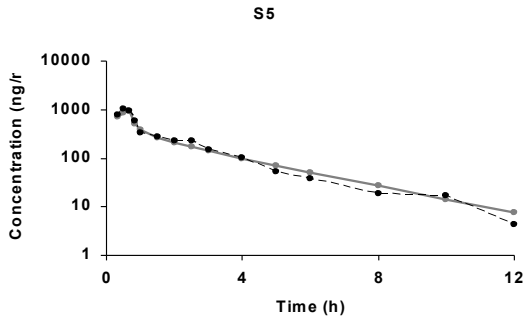
800
600
400
200
0
0
4
8
12
Time (h)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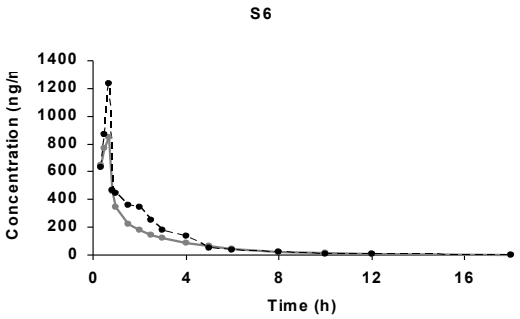
100
10
1
0
4
8
12
Time (h)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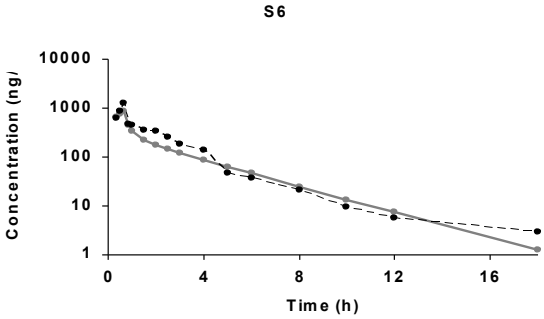
400
200
0
0
4
8
12
16
Time (h)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Concentration (ng/
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1000
100
10
1
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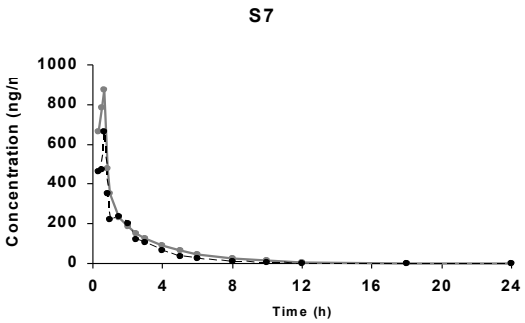
8
12
16
Time (h)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Concentration (ng/m
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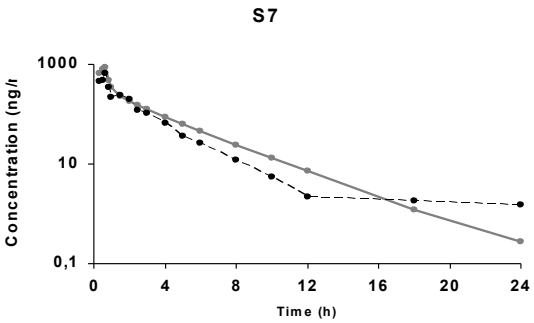
800
600
400
200
0
0
4
8
12
16
20
24
Time (h)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Concentration (ng/l
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0
4
8
12
16
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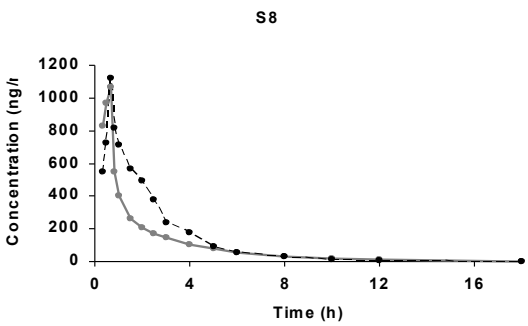
Time (h)


S8
Concentration (ng/l
1200
1000
800
600
400
200
0
0
4
8
12
16
Time (h)

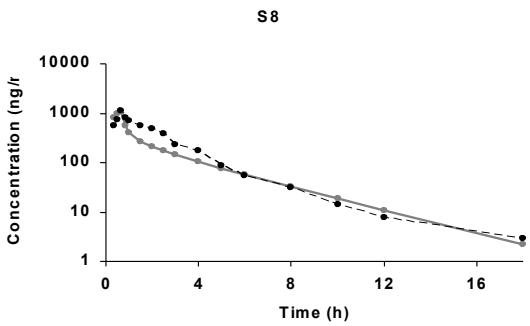

S8
Concentration (ng/r
10000
1000
100
10
1
0
4
8
12
16
Time (h)

Figure 21: Comparison between simulated individual and observed individual plasma concentrations after the oral administration of 50 mg sildenafil

Linear (left) and logarithmic (right) plot of simulated (grey) and individual observed (black) concentration-time profiles for 50 mg given orally (Walker, 1999).

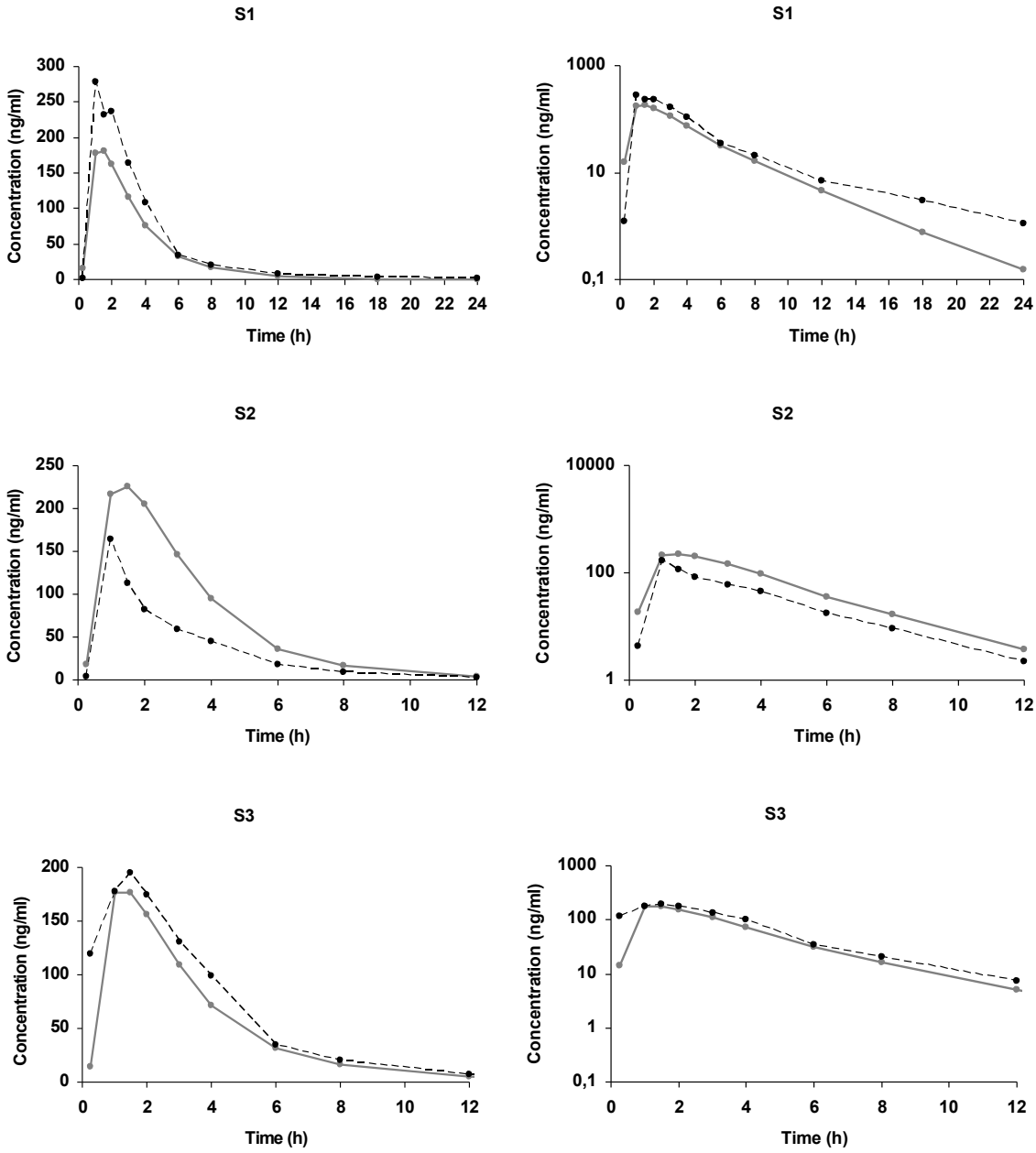


Table 26: Demographic characteristics and fed/fasted state of the volunteers in the published pharmacokinetic studies of the doses 200 and 100 mg oral sildenafil

Study	Number of subjects	Race	Meals	Age years mean (range)
Dose 200mg				
Nichols 2002	32	NA	Fasted	(18 - 45)
Dose 100mg				
Sekar 2008	16	75% white	Fed	27 (18 - 54)
Purvis 2002	17	White	After breakfast	24 (19 – 34)
Nichols 2002	32	NA	Fasted	(18 - 45)
Nichols 2002	34	NA	Fasted	(18 - 45)
Nichols 2002	34	NA	Fed	(18 - 45)
Muirhead 2002a	13	NA	After breakfast	28 (19 - 45)
Muirhead 2002a	13	NA	After breakfast	28 (19 - 45)
Muirhead 2002a	13	NA	After breakfast	29 (18 - 43)
Muirhead 2002a	12	NA	After breakfast	22 (19 - 25)
Muirhead 2002a	12	NA	After breakfast	22 (19 - 25)
Muirhead 2002a	12	NA	After breakfast	24 (19 - 33)
Muirhead 2000	12 or 13	96% white	NA	29 (18 - 44)
Muirhead 2000	12 or 13	96% white	NA	29 (18 - 44)
Muirhead 2000	12 or 13	96% white	NA	29 (18 - 44)
Muirhead 2000	14	96% white	NA	29 (18 - 44)
Muirhead 2000	14	96% white	NA	29 (18 - 44)
Muirhead 2000	14	96% white	NA	29 (18 - 44)
Spinola 2008	26	NA	Fasted	33 (18 - 52)
Spinola 2008	26	NA	Fasted	33 (18 - 52)
Flores-Murrieta 2000	24	Mexican	NA	NA
Kanjanawart 2008	15	NA	Fasted	29 (19 - 43)
Kanjanawart 2008	15	NA	Fasted	29 (19 - 43)
Kim 2003	4	NA	Fasted	NA

Every row presents a particular mean plasma concentration-time profile of sildenafil. NA: Not available.

Table 27: Demographic characteristics and fed/fasted state of the volunteers in the published pharmacokinetic studies of the doses 50, 25 and 20 mg oral sildenafil

Study	Number of subjects	Race	Meals	Age years mean (range)
Dose 50mg				
Walker 1999	3	White	Fasted	51 (50 -53)
Nichols 2002	12	White	Fasted	26 (18 - 42)
Nichols 2002	32	NA	Fasted	(18 - 45)
Muirhead 2002c	15	93.9% white	Fasted	30 (19 - 45)
Muirhead 2002c	15	White	Fasted	70 (65 - 81)
Muirhead 2002c	12	NA	After breakfast	(32 - 63)
Muirhead 2002c	8	White	Fasted	(22 - 72)
Jetter 2002	24	White	Fasted	29
Wilner 2002	10	NA	Fasted	(18 - 39)
Wilner 2002	10	NA	Fasted	(18 - 39)
Wilner 2002	10	NA	Fasted	(18 - 39)
Wilner 2002	12	NA	Fasted	NA
Hedaya 2006	12	NA	Fasted	32
Mandal 2004	24	NA	NA	NA
Mandal 2004	24	NA	NA	NA
Al-Ghazawi 2007	26	Jordanien	Fasted	NA
Jeong 2001	4	NA	NA	(40 - 45)
Dose 25mg				
Nichols 2002	32	NA	Fasted	(18 - 45)
Dose 20mg				
Wang 2005	10	NA	Fasted	NA
Spence 2008	19	NA	Fasted	36

Every row presents a particular mean plasma concentration-time profile of sildenafil. NA: Not available.

Table 28: Characteristics of the analytical methods, by which sildenafil plasma concentrations were measured in the published pharmacokinetic studies of the doses 200 and 100 mg oral sildenafil

Study	Detector	Calibration range [ng/ml]	Precision [%]	Inaccuracy [%]
Dose 200mg				
Nichols 2002	UV	0.1 – 2.0	CV ≤5.1	-2.3 - 3.5
Dose 100mg				
Sekar 2008	MS/MS	2 - ?	Validated method	Validated method
Purvis 2002	UV	1 - 250	CV ≤6	-5 - 3
Nichols 2002	UV	0.1 – 2.0	CV ≤5.1	-2.3 - 3.5
Nichols 2002	UV	0.1 – 2.0	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002a	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2000	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Spinola 2008	MS/MS	2.01 - 501.60	FDA guidance	
Spinola 2008	MS/MS	2.01 - 501.60	FDA guidance	
Flores-Murrieta	Spectrophotometry	35 - 1053	CV <14.9	-0.4 - 14
Kanjanawart 2008	UV	10 - 1500	FDA guidance	-
Kanjanawart 2008	UV	10 - 1500	FDA guidance	-
Kim 2003	MS/MS	2 - 1000	Intra CV ≤7.6	Intra CV -3.6 - -1
			Inter CV ≤ 8.1	Inter CV -0.6 - 1.2

Every row presents a particular mean plasma concentration-time profile of sildenafil. CV: Coefficient of variation. UV: Ultraviolet. MS: Mass spectrometry. MS/MS: Tandem mass spectrometry.

Table 29: Characteristics of the analytical methods, by which sildenafil plasma concentration was measured in the published pharmacokinetic studies of the doses: 50 , 25 and 20 mg oral sildenafil

Study	Detector	Calibration range [ng/ml]	Precision [%]	Inaccuracy [%]
Dose 50mg				
Walker 1999	UV	1 – 250	CV ≤5.1	-2.3 - 3.5
Nichols 2002	MS	0.1 – 2.0	Inter CV -35 to 8.6	-
Nichols 2002	UV	0.1 – 2.0	CV ≤5.1	-2.3 - 3.5
Muirhead 2002c	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002c	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002c	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Muirhead 2002c	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Jetter 2002	MS/MS	0.987 - 395	Intra CV ≤ 8.1	-7.5 - 4
Wilner 2002	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Wilner 2002	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Wilner 2002	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Wilner 2002	UV	1 - 250	CV ≤5.1	-2.3 - 3.5
Hedaya2006	UV	50 - 1000	Intra CV 1.1 - 7.8	
			Inter CV 3.2 - 9.4	
Mandal 2004	UV	10 - 1000	Intra CV 1.4 - 2.1	Intra -3.77 - 19.23
			Inter CV 1.7 - 12.5	Inter -3.97 - 7.69
Mandal 2004	UV	10 - 1000	Intra 1.37 - 2.05	intra -3.77 - 19.23
			Inter 1.65 - 2.50	inter -3.97 - 7.69
Al-Ghazawi 2006	Electrochemical	7.858 - 449.032	CV <11	<6
Kyun Jeong 2001	UV	10 - 1000	CV <2.3	-2.0 - 2.0
Dose 25mg				
Nichols 2002	UV	0.1 - 2.0	CV ≤5.1	-2.3 - 3.5
Dose 20mg				
Wang 2005	MS/MS	0.125 - 40.0	CV <5	<10
Spence 2008	MS/MS	1 - 1000	CV ≤15	±15

Every row presents a particular mean plasma concentration-time profile of sildenafil. CV: Coefficient of variation. UV: Ultraviolet. MS: Mass spectrometry. MS/MS: Tandem mass spectrometry.

Publications

I. Research papers

Hsien L, Breddemann A, Frobel A, Heusch A, Schmidt KG, Läer S. Off-label drug use among hospitalised children: identifying areas with the highest need for research. Pharmacy world & science: PWS 2008; 30(5):497–502.

Breddemann A, Hsien L, Tot E, Läer S. Quantification of cidofovir in human serum by LC-MS/MS for children. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2008; 861(1):1–9.

II. Poster Presentations

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Hsien L, Breddemann A, Frobel A-K, Heusch A, Schmidt K G, Läer S. Off-label drug use among hospitalised children: Identifying areas with the highest need for research. In Proceedings of the 11th ESDP Congress, Rotterdam, Netherlands, pp.115 (2008).

Hsien L, Breddemann A, Frobel AK, Heusch A, Schmidt K, Läer S. High rate of off-label use in cardiovascular paediatric pharmacotherapy requires new focus in research. Naunyn Schmiedebergs Arch Pharmacol, 375 Supplement, R 564 (2007).

III. Oral presentations

Hsien L, Breddemann A, Frobel AK, Heusch A, Schmidt K, Läer S. High rate of off-label use in cardiovascular paediatric pharmacotherapy requires new focus in research. PSWC Congress, Amsterdam, Netherlands (2007).

IV. Book chapters and reviews

Frobel AK, Hsien L, Breddemann A, Läer S. Arzneimitteltherapie von Kindern soll sicherer werden. Deutsche Apothekerzeitung 5, 50-60 (2007).

9 Curriculum Vitae

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09/1998 – 08/2003	Degree in Pharmacy at Tishreen University in Latakia, Syria
09/2003 – 08/2004	Postgraduate Diploma in Microbiology, Haematology and Immunology at Damascus University, Syria

Working experiences

11/2003 – 06/2004	Laboratory assistant in the Clinical Laboratory Department of Al-Assad University Hospital, Damascus, Syria
01/2005 – 06/2005	Pharmaceutical sales representative at the pharmaceutical company Rim in Damascus, Syria
01/2006 – 10/2006	Research project in preparation for a Ph.D. at Dept. Clinical Pharmacy and Pharmacotherapy at Heinrich-Heine-University Düsseldorf under the supervision of Prof. Dr. med. S. Läer
10/2006- 12/2009	Ph.D. student at Dept. Clinical Pharmacy and Pharmacotherapy, at Heinrich-Heine-University Düsseldorf under the supervision of Prof. Dr. med. S. Läer

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Erklärung zur Dissertation

Die hier vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfe angefertigt. Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf, den

Unterschrift