# Preclinical and pharmacokinetic studies of praziquantel, the cornerstone of schistosomiasis treatment

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# Summary

Schistosomiasis remains the most important helminthic disease, infecting over 240 millions of people in tropical and subtropical areas of the world, while close to 800 millions of people live at risk of contracting it. Unfortunately, children are among the most affected and the disease often results in stunting, malnutrition and cognitive and physical retardation. Praziquantel (PZQ), being effective, cheap and safe, remains the cornerstone of schistosomiasis treatment and is distributed on a wide scale within drug administration programs. Until recently it was believed schistosomiasis among young children, below the age of six years, is not very common and consequently, they were not regularly treated. However, in 2011 WHO acknowledged these children are a risk group and could be included in the administration programs in the future, but pharmacokinetic data (PK), crucial to establish effective and safe dose of PZQ for pre-schoolers, is not readily available. Furthermore, *in vitro* and *in vivo* data on antischistosomal activity of PZQ for *S. haematobium,* responsible for the highest number of infections, is lacking. Moreover, PK studies in this sensitive population are tedious in conduct and call for a more patient friendly sampling approach, while the quality of sampling remains uncompromised.

The aim of the present thesis was to gain more information about activity of both enantiomers of PZQ, R- and SPZQ, as well as the racemic drug and the main human metabolite (R-*trans*-4-hydroxy-PZQ) *in vitro*, *in vivo* and in humans.

*S. haematobium* was studied *in vitro* and *in vivo* to evaluate and confirm its greater susceptibility to PZQ, compared to *S. mansoni* and *S. japonicum*. This species of schistosomes is characterised with a life-cycle, tedious to maintain in laboratory conditions and consequently, understudied compared to other species of the parasites. We determined $IC_{50}$ values for racemic PZQ, both enantiomers and the main human metabolite on adult worms *in vitro*. Moreover, $ED_{50}$ values for both enantiomers and the racemic drugs in hamster model were reported *in vivo*. In light of the development of paediatric formulation for PZQ, it would be important to evaluate how these findings translate to humans.

Two PK studies were conducted within dose finding studies to investigate PK of both PZQ enantiomers and the main human metabolite. For the first time, PK parameters, such as area under the curve, maximal blood concentration and half-life of these analytes were revealed and compared. Influences, e.g. age and infection species on the PK processes were investigated. Moreover, a PK model for in depth study of influence on metabolic processes of RPZQ is currently under development.

As a sub-study within SAC infected with *S. haematobium*, a novel micro-sampling device, called Mitra™, was evaluated in comparison to established dried blood spots technique, in the laboratory and under field conditions. A sample preparation method for PZQ with Mitra™ was established, optimised and validated in compliance with Food and Drug Administration guidelines. Owing to practicality and simplicity during both sampling and extraction process, Mitra™ showed great potential; however, overestimation of concentrations compared to dried blood spots in incurred, but not in spiked samples, is yet to be clarified.

To conclude, we revealed PK parameters of the main entities, contributing to antischistosomal activity of PZQ. The PK model for RPZQ will reveal influences on metabolic processes of the proposed eutomer of PZQ. These findings will contribute to establishment and tailoring of guidelines for treating paediatric populations, infected with schistosomiasis, using PZQ. Validation of Mitra™ as a potential micro-sampling tool for PK studies will pave the way towards higher quality of sampling while maintaining high patient adherence. Last but not least, the study of *S. haematobium in vitro* and *in vivo* will compliment the existing data on activity of PZQ towards different species of schistosomes. Since SPZQ and the main human metabolite showed non-negligible activity towards *S. haematobium*, the decision whether to develop an enantio-pure paediatric formulation, consisting of RPZQ only, should be carefully evaluated.

# List of Abbreviations

| | |
|---|---|
| AUC | Area under the curve |
| $C_{max}$ | Maximal concentration |
| CR | Cure rate |
| CV | Coefficient of variation |
| DBS | Dried blood spot |
| DMSO | Dimethylsulfoxide |
| $ED_{50/90}$ | Effective dose (to reduce parasitic load by 50/90%) |
| EPG | Number of eggs per gram of faeces |
| ERR | Egg reduction rate |
| FDA | Food and Drug Administration |
| HPLC | High pressure liquid chromatography |
| $IC_{50/90}$ | Inhibitory concentration (dose to kill 50/90% of the parasites) |
| iFCS | Inactivated foetal calf serum |
| IS | Internal standard |
| ISR | Incured sample reanalysis |
| LC-MS/MS | Liquid chromatography coupled to tandem mass spectrometry |
| LLOQ | Lower limit of quantification |
| ME | Matrix effects |
| MRM | Multiple reaction monitoring |
| MS | Mass spectrometry |
| NTD | Neglected tropical disease |
| NTS | Newly transformed schistosomulae |
| PK | Pharmacokinetics |
| PSAC | Preschool-aged children (<6 yrs) |
| PZQ | Praziquantel |
| QC | Quality control |
| Rac | Racemic/racemate |
| RRE | Relative recovery |
| SAC | School-aged children (6-16 yrs) |
| SD | Standard deviation |
| $T_0$ | Time of treatment |
| $t_{1/2}$ | Half-life |
| $T_{max}$ | Time needed to reach maximal concentration |
| ULOQ | Upper limit of quantification |
| WB | Worm burden |
| WBR | Worm burden reduction |

# Chapter 1

General Introduction

# 1 Schistosomiasis

## 1.1 Epidemiology

Schistosomiasis, caused by blood-dwelling flukes of the genus *Schistosoma*, belongs to the group of so-called neglected tropical diseases (NTD) (WHO, 2013). As a subgroup of infectious diseases, NTDs affect over a billion people worldwide, predominantly in rural tropical and sub-tropical areas, where poverty is concentrated (Adenowo *et al.*, 2015; WHO, 2015). Despite their high social, economic and clinical impact, these diseases have been neglected to a great extent in the recent past by funders, researchers and decision-makers (King and Dangerfield-Cha, 2008; Feasey *et al.*, 2010). One of the main causes behind NTDs is attributed to inadequate access to safe water, proper sanitation, health services and infrastructure (Chitsulo *et al.*, 2000).

The most vulnerable group, affected by schistosomiasis, are children, where the prevalence of the disease is increasing with age (Gryseels *et al.,* 2006; Verani *et al.,* 2011). The latest numbers show over 250 million people are infected with schistosomiasis, while a staggering 779 million people, accounting for 10% of world's population, are at risk of contracting a schistosome infection (GBD 2015 DALYs and HALE Collaborators, 2016). The burden of schistosomiasis is estimated to be 2.6 million of disability adjusted life years (DALYs), which account for the years lost due to premature mortality (YLL) and years lived with disability (YLD) (GBD 2015 DALYs and HALE Collaborators, 2016). Nonetheless, the estimations can vary greatly depending on how the burden was calculated and can peak as high as 56 million DALYs (King and Dangerfield-Cha, 2008).

There are three principle species of schistosomiasis infecting humans that are of public health importance: *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*. Together they cause two main forms of the disease: hepatosplenic and urogenital schistosomiasis (Gryseels *et al*., 2006; Knopp *et al*., 2013). *S. haematobium* is responsible for the highest number of infections (approximately 64%), followed by *S. mansoni* (approx. 34%) (Hotez *et al*., 2014). *S.*

*japonicum* causes urinary schistosomiasis in South-East Asia, while *S. mekongi* and *S. intercalatum* are of a local importance only (Gryseels *et al.*, 2006).

Schistosomiasis is a water-borne disease, endemic in 78 countries in tropical regions of Africa, Asia and South America (Chitsulo *et al.*, 2000; Steinmann *et al.*, 2006) (see Figure 1). *Biomphalaria* snails are intermediate host of *S. mansoni*, which causes intestinal and hepatic schistosomiasis in Africa, the Arabian peninsula and South America. *S. haematobium*, the infectious agent behing urinary schistosomiasis, is transmitted by *Bulinus* snails and common in Africa and the Arabian peninsula. *S. japonicum*, parasitizing *Oncomelania* snails, results in intestinal and hepatosplenic schistosomiasis in Indonesia, China and the Philippines (Lockyer *et al.*, 2003; Colley *et al.*, 2014). The expansion of water infrastructure to meet the power and agricultural requirements raising with increasing development resulted in growing transmission, especially of *S. mansoni*, while population growth and migration have added up to the introduction of the disease to new areas (Chitsulo *et al.*, 2000; Ross *et al.*, 2002).
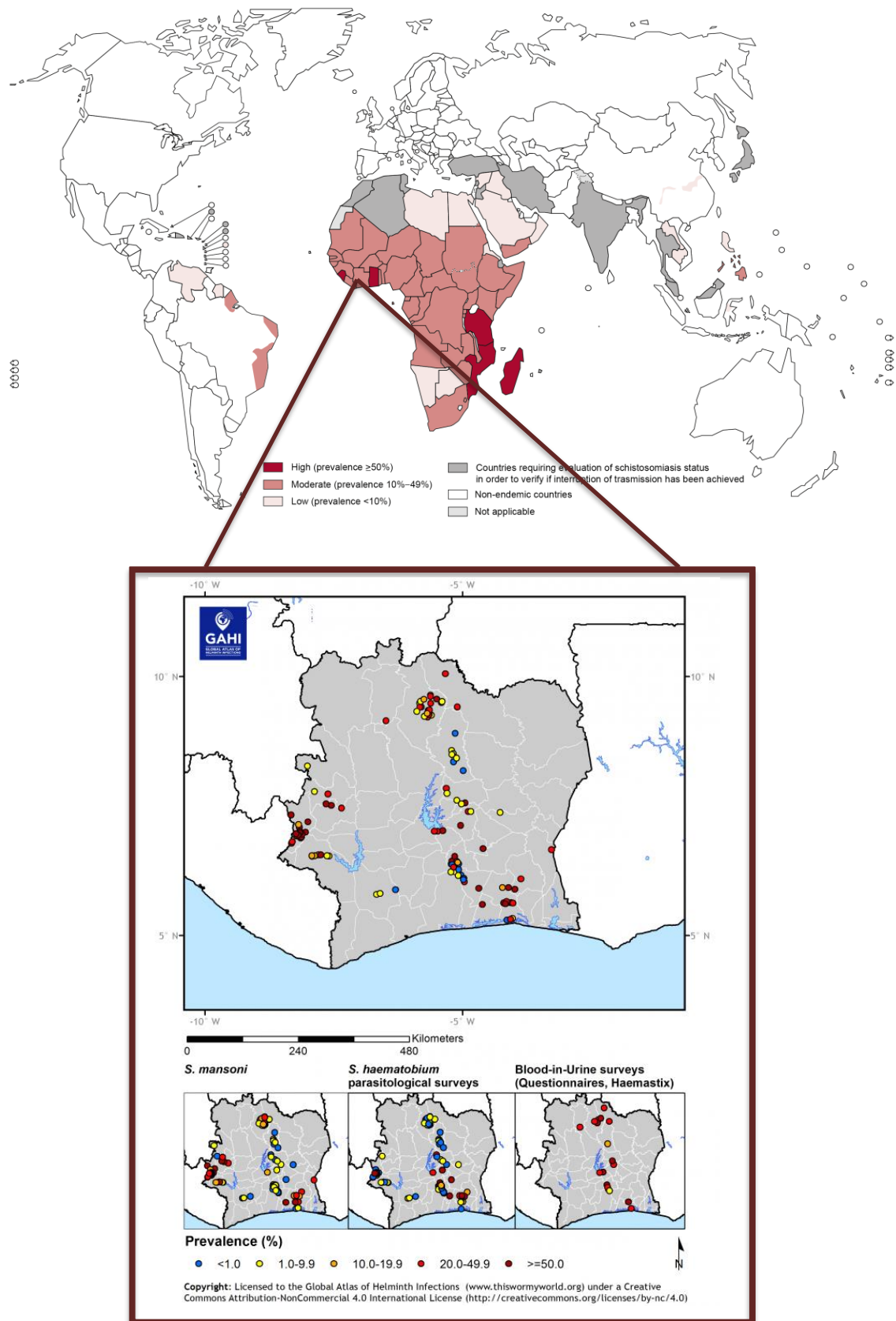
**Figure 1:** Distribution of schistosomiasis in the world (WHO 2012) and in Côte d'Ivoire

(GAHI), where the field component of this thesis took place

## 1.2 Biology and life-cycle

Schistosomes belong to the Trematoda class, forming the Platyhelminthes phylum together with the Cestoda and Monogenea classes. Trematodes are further divided into two subclasses, namely Aspidogastrea, obligate parasites of molluscs, and Digenea, obligate parasites of both molluscs and vertebrates. Most trematodes are hermaphrodites and posses both male and female organs, except schistosomes, which are dioecious (Farley, 1971; Cribb *et al.*, 2003; Olson *et al.*, 2003).

All *Schistosoma* species that infect humans are characterised by a very similar life cycle (Figure 2.1), which includes a fresh-water mollusc as an intermediate host, where asexual reproduction takes place and a human as a definitive host, where schistosomes reproduce sexually. Fresh-water sources (typically natural streams, ponds and lakes), where gastropods reside, become contaminated when an infected individual defecates (*S. mansoni* and *S. japonicum*) or urinates (*S. haematobium*) in or near the water, excreting parasitic eggs, which can remain viable up to 7 days (Gryseels *et al*., 2006). Eggs hatch upon contact with fresh water and miracidiae penetrate a mollusc of a certain species (Lockyer *et al.*, 2003; Gryseels *et al.*, 2006). After two generations of sporocysts over a course of 4-6 weeks, free-swimming cercaria (Figure 2.2) are released, recognised by their characteristic bifurcated tail (Gryseels *et al.,* 2006). Transmission of the infection occurs when an individual comes into contact with a fresh-water source, carrying infectious cercariae, which are able to survive in water for up to 72 h (Gryseels *et al.,* 2006). Those will penetrate human skin, shedding their tails and becoming schistosomula (Figure 2.3) (Gryseels *et al*., 2006; McKerrow & Salter, 2002). Following skin penetration, schistosomula migrate to the lungs, where they mature to the juvenile stage. They then move through the circulatory system to the liver and finally, to the portal venous system (*S. mansoni)* or veins draining pelvic organs (*S. haematobium*), where they reach maturation within 5-7 weeks post infection (Colley *et al*., 2014).

Adult schistosomes have separate sexes and are 7-20 mm long, characterised by a syncytial tegument and two terminal suckers. Females, which are typically thinner and longer, will reside inside the so-called gynaecophoric channel of the thicker male (Steinauer, 2009). The

parasitic pairs will move to their final destination- mesenteric veins of the intestine (*S. mansoni* and *S. japonicum*) or pelvic venous plexus (*S. haematobium*), where they can live on average 3-5 years, but can reach also as high as 20 years (Warren *et al.*, 1974). In the veins, they feed on blood via anaerobic glycolysis and produce and release up to thousands of eggs, containing miracidium, per day (Cheever *et al.*, 1994). The eggs migrate to the lumen of the intestine or bladder with the aid of proteolytic enzymes and are expelled with stool (*S.mansoni* and *S. japonicum*) or urine (*S. haematobium*). Some eggs will get trapped in the surrounding tissues, eliciting host immune responses resulting in eosinophilic inflammatory and granulomatous reactions, which are progressively replaced by fibrotic deposition, typical for chronic infection (Colley *et al.*, 2014).
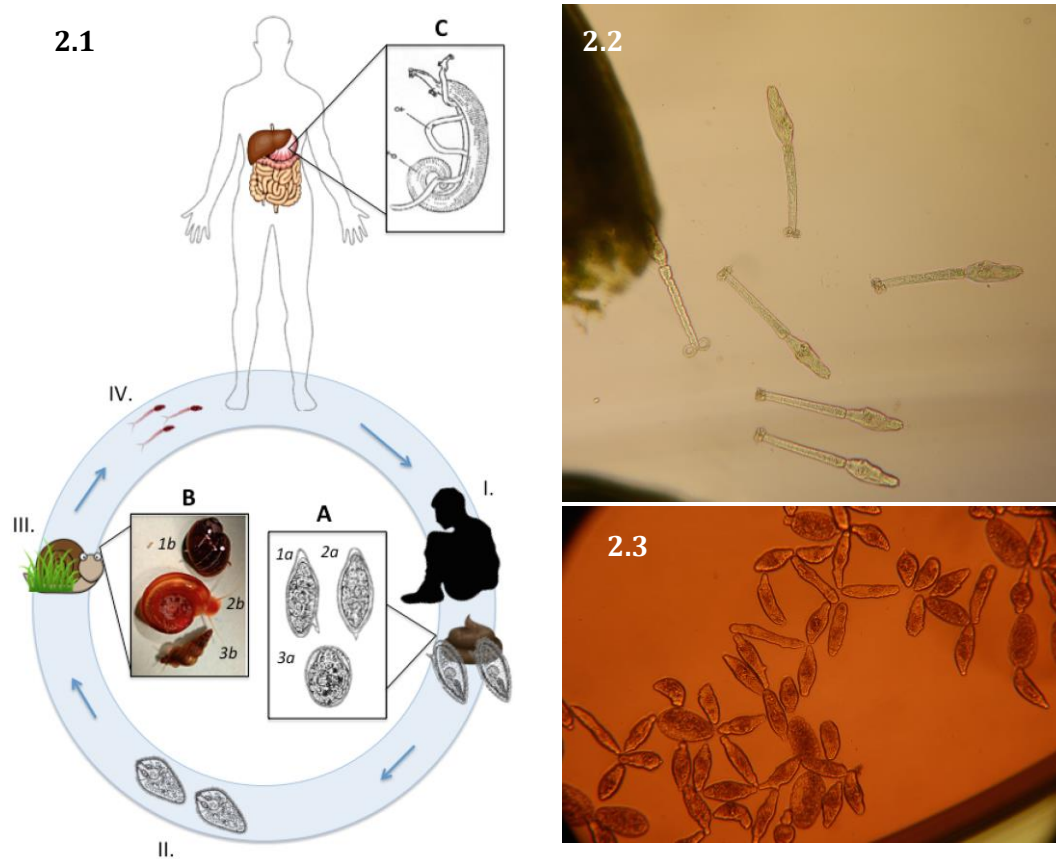
**Figure 2:** Life cycle of *S. mansoni* (**2.1**): infected individual defecates in fresh water sources (*I*), excreting parasitic eggs (A: *1a S. mansoni*, *2a S. haematobium*, *3a S. japonicum*), which hatch to free swimming miracidiae (*II*), penetrate a mollusc (*III*) (B: *1b Bulinus spp.*; *2b Biomphalaria spp.*; *2c Oncomelania spp.*) and after asexual reproduction, infectious cercariae (*IV*) are excreted, penetrating human skin and moving through the body to reach their final destination in mesenteric veins (*S. mansoni* and *S. japonicum*) or veins draining pelvic organs (*S. haematobium*), where they live as mature adult worm pairs, shedding eggs; (**2.2**) cercariae shedded from a snail; (**2.3**) schistosomulae after transformation

### 1.3 Pathological and clinical aspects

Schistosomiasis manifests in two stages. Acute infection is characterised by flu-like symptoms but can often remain asymptomatic in endemic populations. However, if not treated, it can progress to the chronic phase, which can carry severe health consequences.

#### 1.3.1 Acute schistosomiasis

The percutaneous penetration of cercariae can cause a temporary urticarial skin rash on the location of the entry (so called swimmer's itch), especially after first-time infections, more common in tourists and migrants (Colley *et al.*, 2014). Acute schistosomiasis, also known as Katayama fever, is a systemic hypersensitivity reaction caused by migrating schistosomula in the body and is species-independent. It can appear up to a few weeks or months after the primary infection and is characterised by very unspecific symptoms, such as fever, fatigue, malaise, myalgia, cough and possibly abdominal symptoms in the later phase (Lambertucci, 1993). Katayama fever is common for people without a history of previous infection, for example tourists, travellers and other people accidentally exposed to transmission (Ross *et al.*, 2007). People living in endemic areas, which are chronically infected, rarely observe acute symptoms due to *S. mansoni* or *S. haematobium* infections *(*Bottieau *et al.,* 2006; Gryseels *et al.*, 2006; Ross *et al.*, 2007). In contrast, Katayama fever due to *S. japonicum* has been observed also in patients with a history of chronic infections (Gryseels *et al.,* 2006; Ross *et al.*, 2007).

#### 1.3.2 Chronic schistosomiasis

Chronic schistosomiasis is a result of host immune responses, due to parasitic eggs trapped in tissues during perivesical (*S. haematobium*) or peri-intestinal (*S. mansoni*) migration (Gryseels *et al.*, 2006; Pearce & MacDonald, 2002). Antigens excreted by trapped eggs will evoke development of granulomatous lesions around the eggs, which will be gradually replaced by fibrotic deposition, as the eggs die (Pearce and MacDonald, 2002; Colley *et al.*, 2014). Hence, the severity of chronic clinical manifestations depends on individual immune response and severity of the infection (Gryseels *et al.,* 2006). Especially in children, schistosomiasis is connected to malnutrition, growth-stunting and hindered cognitive

development (Stephenson, 1993; Jukes *et al.*, 2002; Colley *et al.*, 2014). Organs affected in this process are species-dependent and since the focus of this PhD thesis was on *S. haematobium* and *S. mansoni,* chronic infection caused by these two species only are described below.

- **Hepatosplenic schistosomiasis**

This type of schistosomiasis is caused by *S. mansoni* and results in hepatic pathology. A very typical symptom, expressed commonly by children and adolescents, is hepatomegaly, following inflammation, granulomatous lesions and finally calcifications (Ross *et al.*, 2002; Gryseels *et al.*, 2006; Barsoum *et al.*, 2013). Liver cirrhosis, portal hypertension and premature death all stem from enlarged liver (Danso-Appiah *et al.*, 2013). Since the liver is one of the main organs involved in metabolic processes of drugs, changes in its functionality might influence deposition of praziquantel enantiomers and with it, pharmacokinetics (Prescott *et al.*, 1975). For this reason, we were interested to study the impact of schistosomiasis, among other factors, on the pharmacokinetics of praziquantel, in a paediatric population (See Chapter 3). Additionally to the hepatic morbidity, *S. mansoni* infection results in intestinal pathology as well. During the peri-intestinal migration, eggs trapped mainly in colon and rectum provoke inflammation, leading to symptoms such as abdominal pain and discomfort, loss of appetite and blood in stool, the latter being important indicator and a common first sign of the infection (Gryseels *et al.*, 2006). Extensive fibrosis, leading to hepatosplenic disease with periportal fibrosis, also known as Symmer's pipe-stem fibrosis, is a common manifestation in the immune-compromised patients (Colley *et al*., 2014).

- **Urogenital schistosomiasis**

Urogenital schistosomiasis is caused by *S. haematobium* and rarely by the other two main species of the parasite. Early symptoms, apparent two to three months post infection, occur due to poor immunoregulation of parasitic antigens excreted by parasitic eggs, trapped in vesical and ureteral wall, leading to haematuria and dysuria (King and Bertsch, 2013; Colley e*t al*., 2014). A firm connection between haematuria and schistosomiasis has been established and consequently, haematuria is recognised by the World Health Organisation (WHO) as one

of the markers for mapping prevalence of urinary schistosomiasis, additionally to standard egg count based criteria (WHO, 2006). Following early symptoms, chronic lesions result in fibrosis and calcifications of the bladder and lower ureters, advancing to hydronephrosis and hydroureter (Gryseels *et al.,* 2006). Kidney failure, which is the ultimate manifestation after parenchymal damage caused by the disease, is recognised as one of the risk factors of squamous bladder cancer (Johansson and Cohen, 1997; Brindley *et al*., 2015). Female genital schistosomiasis results in inflammatory lesions of ovaries, cervix and vulva, affecting female reproductive health (Colley *et al.*, 2014). A connection with increased risk of abortion and infertility has been confirmed and furthermore, this type of infection is recognised as one of the risk factors for HIV infection (Kjetland *et al.*, 2006). For men, the consequences of urogenital schistosomiasis are typically haematospermia and prostatitis, among others (Colley *et al.*, 2014). However, treatment with PZQ can reverse the schistosomiasis caused morbidity to a certain extent, more readily for men than women but confirmed also for young children, emphasising the importance of regular chemotherapy (Barda *et al.*, 2017).

### 1.4 Diagnostics

While tourists and travellers presenting symptoms are usually examined for and diagnosed with schistosomiasis upon return to their home country, inhabitants of endemic areas are commonly treated within large-scale mass drug administration programs, without prior diagnosis (WHO, 2006) . Diagnostic tools used for diagnosing travellers without previous history of infection differ from those used at different stages of disease control and monitoring programs with regards to sensitivity and detection limits (Utzinger *et al.*, 2015). While tools for rapid confirmation of infection in endemic areas for assessing prevalence and intensity of infection during morbidity control programs are available, highly sensitive and specific assays for monitoring and surveillance for post-transmission control and elimination phases are lacking (Montresor *et al.*, 1998; Utzinger *et al.*, 2015).

The current methods can roughly be grouped as urine/stool microscopy and serological methods (Utzinger *et al.*, 2015), briefly summarised below.

**Microscopic examination of excreta** (faeces for *S. mansoni* and *S. japonicum*, urine for *S. haematobium*) remains the diagnostic gold standard. Due to their typical size and shape, with a lateral (*S. mansoni*) or terminal (*S. haematobium*) spine, the eggs are easily detected, identified and distinguished using light microscopy (Gray *et al.*, 2011). The Kato-Katz method (*S. mansoni*) and urine filtration (*S. haematobium*) method are commonly used for quantification of infection. The result is expressed as eggs per g of faeces or eggs per 10 ml of urine (Utzinger *et al.*, 2015). Kato-Katz is a thick smear stool examination and is known to be simple, inexpensive and rapid (Katz *et al.*, 1972). It is recommended by the WHO for diagnosing intestinal schistosomiasis with high infection intensity and often used in field studies (Montresor *et al.*, 1998). Urine filtration is a technique used to diagnose urinary schistosomiasis and consists of filtration of 10 ml urine through a nitrocellulose filter (or other poly-carbonate filter with pores of 8-30 μm) (Peters *et al.*, 1976). However, there are several drawbacks when it comes to these techniques: the parasitic eggs are excreted in irregular intervals (e.g. mid-day urine should be used for diagnosing *S. haematobium* infection) and samples need to be taken on several days in duplicates (or even triplicates) to enhance the accuracy (Utzinger *et al.*, 2001). Additionally, light intensity infections are difficult to detect without concentrating the sample using methods, such as centrifugation or filtration followed by examination of the sediment (Utzinger *et al.*, 2015). This can result in underestimating the prevalence of the infection on one hand and overestimating the cure rates after treatment on the other (Utzinger *et al.*, 2001; Stete *et al.*, 2012; Knopp *et al.*, 2013).

Simple point-of-care (POC) diagnostic tools in the form of microhaematuria-detecting dipsticks for *S. haematobium* and circulating cathodic antigen (CCA) to diagnose *S. mansoni* and *S. japonicum* are very simple and have shown superiority over microscopic methods in terms of sensitivity (Coulibaly *et al.*, 2011; Danso-Appiah *et al.*, 2016). They can be successfully used for mapping and subsequent monitoring of the treatment distribution programs (Utzinger *et al.*, 2015).

Other methods, such as FLOTAC, can be used for *S. mansoni* detection. Originating from veterinary medicine, FLOTAC has been validated for diagnosing human nematodes. Faeces

are homogenised, filtered and the flotation solution is added, bringing helminthic eggs to float which can then be counted under the microscope (Utzinger *et al.*, 2015). Mini-FLOTAC is a simpler version of FLOTAC, more suitable for rural settings since it does not require centrifugation, but is still reasonably sensitive (limit of 10 epg) (Barda *et al.*, 2013a). Studies evaluating performance of mini-FLOTAC in the field showed higher accuracy compared to other techniques (direct faecal smear, formalin ether concentration technique and Kato-Katz) (Barda *et al.*, 2013a; Barda *et al.*, 2013b).

There are several options among **serological tests**, however most of them are based on the same principle- detection of anti-schistosome antibodies in blood, which develop within 6-8 weeks post-infection (Utzinger *et al.*, 2015). Usually antibodies can be detected before eggs are excreted in stool or urine; nonetheless antibodies are not schistosome-species specific and very early infections might be missed using this approach (Nausch *et al.*, 2014; Utzinger *et al.*, 2015). They are commonly used to determine if a person has been exposed to schistosomiasis infection, although they cannot differentiate between past and current infection since high antibodies titres can persist after successfully treating the patient; therefore, their usefulness in endemic areas is limited (Nausch *et al.*, 2014; Utzinger *et al.*, 2015). Serological tests are useful for diagnosing schistosomiasis in travellers returning from endemic areas, before the onset of clinical symptoms, often absent or unspecific in light infections (Utzinger *et al.*, 2015). Tests able to detect and quantify parasitic DNA in clinical samples have been emerging in high number, offering great specificity and sensitivity, comparable to or higher than conventional microscopic methods. The great advantage of these techniques, one of them being polymerase chain reaction (PCR), is the ability to use other specimens additionally to stool and urine, including semen and vaginal lavages. However, regardless of advantages, PCR requires highly skilled personnel and expensive equipment, limiting its use in resource-scarce settings (Utzinger *et al.*, 2015).

### 1.5 Chemotherapy against schistosomiasis

Praziquantel (PZQ), a pyrazino-isoquinoline derivative, has remained a cornerstone of schistosomiasis treatment for decades (Utzinger and Keiser, 2004; Cioli *et al.*, 2014; Olliaro *et al.*, 2014). Characterised with mild adverse events (e.g. abdominal symptoms) and no long-term toxicity, PZQ has been successfully used in mass drug administration (MDA) programs for years, with hundreds of millions of people treated (Raso *et al.*, 2004; Doenhoff *et al.*, 2008; Geary *et al.*, 2010; Olliaro *et al.*, 2014). However, PZQ is only effective against adult worms and to successfully treat the infection, the drug needs to be re-administered in the weeks following the first treatment (Pica-Mattoccia and Cioli, 2004). Furthermore, dependence on a single drug is not ideal and lack of knowledge regarding the mechanism of action of PZQ represents one of the main obstacles on the way to develop effective analogues (Doenhoff *et al.*, 2008; Pica-Mattoccia *et al.*, 2008; Wu *et al.*, 2011).

PZQ is a racemic compound and is currently marketed as a mixture of both enantiomers, R- and S-praziquantel (R-/SPZQ), in equal parts (Cioli *et al.*, 2014; Olliaro *et al.*, 2014). Although there are still some disagreements, several researchers have confirmed RPZQ bears the main antischistosomal activity against *S. mansoni* (Shua-Hua and Catto, 1989; Meister *et al.*, 2014) and *S. japonicum* (Tanaka *et al.*, 1989), while SPZQ is believed to be responsible for side effects, including the awfully bitter taste (Meyer *et al.*, 2009). *S. haematobium*, however, remains largely unstudied, although it is responsible for the largest part of schistosomes infections (Botros *et al.*, 2005). Halving the dose and maintaining the level of activity while reducing adverse events is a very attractive solution, especially for paediatric populations, which have difficulties swallowing the tablet due to its size and very bitter taste (Meyer *et al.*, 2009). To compliment existing data on *S. mansoni* and *S. japonicum* and contribute to a better understanding of antischistosomal activity of PZQ, we conducted *in vitro* and *in vivo* studies with *S. haematobium* (see Chapter 2).

With no available alternative, resistance development to PZQ has been a hot topic in recent years due to increasing drug pressure originating from MDA programs. Oxamniquine, effective solely against *S. mansoni*, was used in Brazil successfully for many years and is no

longer available (Gryseels *et al.*, 2006; Cioli *et al.*, 2014). Metrifonate, used to treat schistosomiasis caused by *S. haematobium*, has shown little efficacy as a single dose but could potentially be used as a multiple dose treatment (Kramer *et al.*, 2014). No evidence of resistance to PZQ, except in the laboratory, has been documented so far (Vale *et al.*, 2017). However with a wide coverage, planned by the London declaration (*London Declaration On Negelected Tropical Diseases*, 2012) and high frequency of treatment, essential for control and eventually elimination of schistosomiasis, resistance development is only a matter of time (Fallon and Doenhoff 1994; WHO, 2006). New effective drugs for treating schistosomiasis are very much needed.

### 1.6 Intervention and control

The main strategy of schistosomiasis control remains preventive chemotherapy (PC) with PZQ (Hotez *et al.*, 2007). These programs aim to lessen the extent, severity and duration of the infection morbidity by distributing PZQ to people living in endemic areas, without prior positive diagnosis (Shuford *et al*., 2016). The priority is to achieve high coverage of risk groups: school-aged children (SAC) are one of the groups considered to be at the highest risk of contracting the infection, apart from adults in frequent contact with infected water due to occupation (e.g. fishermen, farmers…) and pregnant or lactating women (WHO, 2006). The frequency of treatment depends on the risk magnitude- high risk communities, where the prevalence of schistosomiasis is 50% detected by parasitology methods (or at least 30% of urinary schistosomiasis prevalence based on history of haematuria) are treated annually while moderate risk communities with a prevalence of the disease at least 10%, are given treatment every 2 years (Inobaya *et al.*, 2014). Within low-risk communities, only SAC are treated twice during their primary schooling (WHO, 2006). Treating pregnant women is of great importance, since heavy helminthic infection can exacerbate maternal anemia, leading to increased danger of labour complications and mortality, especially in the regions where malaria is co-endemic (Ajanga *et al.*, 2006). Additionally, connection between women with genital schistosomiasis and higher risk of HIV establishment and AIDS acceleration has been confirmed (Kjetland *et al.*, 2006). Due to the lack of safety data for this age group, preschool-

aged children (PSAC) are excluded from PC programs and can only be treated on an individual basis (WHO, 2006).

To monitor the impact of drug administration program on morbidity, disease specific indicators are used. For schistosomiasis, these are prevalence and intensity of infection (using parasitological methods), prevalence of micro- and macro-haematuria, prevalence of anemia and prevalence of lesions in urinary tract and liver (using ultrasound) (WHO, 2006). Usually, PC programs lean on the existing health systems or other established community based approaches for drug distribution (WHO, 2006). PC has been implemented in numerous countries as a part of national schistosomiasis control programs; however, regardless the low costs of PZQ, these countries are struggling to sustain it and the coverage is still below optimal- in 2016, 52 % of SAC were globally reached, distant from the aim set to minimum 75% by World Health Assembly in 2001 (WHO, 2006, 2017).
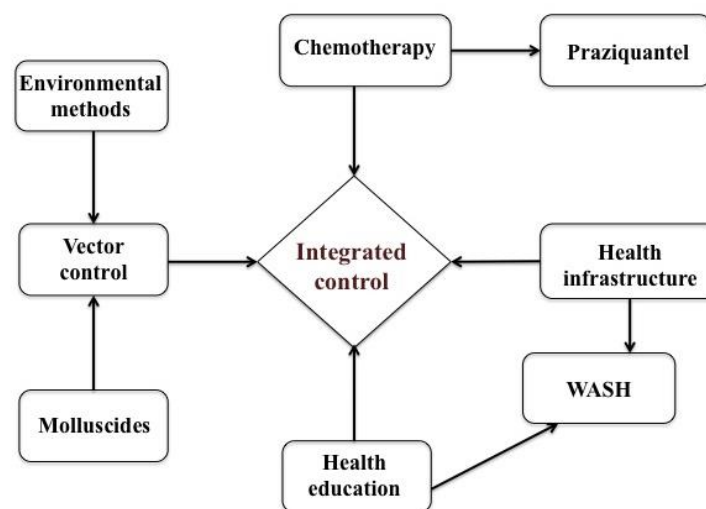


**Figure 3:** Integrated schistosomiasis control (adapted from Ross *et al.,* 2017)

The WHO goal was to control morbidity of schistosomiasis by 2016 and eliminate it by 2020. Bearing the parasitic life-cycle and transmission pathways in mind, there are two possible targets: influencing the transmission from human to the vectors or the other way around, from snails to humans. Nonetheless, treatment administration alone affects solely the human to snails pathway and only for as long as the treatment is given and consequently, prevalence

levels can quickly reach the baseline values within 18-24 months - therefore, it is not enough to successfully interrupt the transmission of the disease (Gray *et al.*, 2010). A multicomponent approach of the treatment, complimented with health education and promotion, improved water infrastructure and sanitation together with vector control (e.g. molluscicides) could aid to approach schistosomiasis elimination (Inobaya *et al.*, 2014; Ross *et al.*, 2017) (see Figure 3).

### 1.7 Treatment gap

While SAC are recognised as one of the main groups at risk for contracting schistosomiasis and have been included in PC programs (Figure 4B), their younger peers, PSAC, have been left out and need to wait up to the age of six to receive their first treatment or they are treated off label with the WHO recommended dose for adults (WHO, 2006; Bustinduy *et al.*, 2017). One of the reasons for this exclusion is the assumption that young children (< 6 years) are not as affected by schistosomiasis as they don't come into contact with infected water as actively (e.g. swimming) as older children (Odogwu *et al.*, 2006). Additionally, they are also harder to diagnose using standard parasitological methods, since they often harbour a lower burden of the parasites (Ross *et al.*, 2002; Bosompem *et al.*, 2004; Geary *et al.*, 2010). Nonetheless, researchers showed PSAC are regularly bathed by their mothers and guardians, using water from infected water sources, unaware of the risks or simply without sources of clean water for bathing (Figure 4A) (Mafiana *et al.*, 2003; Sousa-Figueiredo *et al.*, 2010). Furthermore, epidemiological surveys have confirmed schistosomiasis among young children (< 6 years) is indeed very common and the prevalence can peak as high as 86% (Ekpo *et al.*, 2012).

Overlooking schistosomiasis in PSAC is of great concern, since this age group might have a role in maintaining local transmission of the disease within communities integrated in PC programs (Stothard and Gabrielli, 2007). Although the intensity of infection is usually milder compared to older children, they are in contact with water many times a day, adding to the contamination of water sources (Odogwu *et al.*, 2006). Furthermore, it is not yet clear whether early parasitic infection can exacerbate or attenuate the clinical impact of schistosomiasis and its subsequent morbidity (Stothard and Gabrielli, 2007).

Since PSAC have been recognised by the WHO in 2010 as one of the risk populations for contracting schistosomiasis infection, the incentive to include them in PC programs is getting stronger (WHO, 2010). However, metabolic processes of PZQ have never been studied in children and pharmacokinetic (PK) data crucial to establish a safe and effective dose for this age group is lacking (Keiser *et al*., 2011). Since differences in PK processes between children and adults, a consequence of maturation processes, have been described in details, a simple extrapolation of doses used for adults to children is very uncertain (Anderson, 2002; Hattis *et al.*, 2003). Furthermore, the use of standard PZQ formulation, characterised with bitter taste and big tablets, is only aggravating integration of young children into drug administration programs. All these facts underline a compelling need for a child-friendly paediatric formulation (Meyer *et al.*, 2009; Stothard *et al.*, 2011).



**Figure 4:** Mother bathing her preschool-aged child (**A**); SAC waiting to receive treatment (**B**)

### 1.8 Pharmacokinetic methods

PK studies are used to investigate and quantify the effect the human body has on an active substance after administration (Batchelor and Marriott, 2015). The aim is to assess absorption, distribution, metabolism and elimination (ADME) of the active substance and the influences on these processes, affecting the concentration of the drug in body fluids (Toomula *et al.*, 2011). Typically, an intensive sampling scheme is applied to determine concentration of the drug in blood at several points in time after treatment, resulting in concentration over time curve (Patel *et al.*, 2010). These studies are important to determine a suitable dose of drug for patients and are especially important for specific populations of patients, such as those with liver failure or other diseases, where impairment of organs involved in ADME processes could have an impact on the PK of the active ingredient. Use of a drug lacking evidence-supported tailored dosing can result in sub-optimal efficacy and unexpected adverse events (Yewale and Dharmapalan, 2012).

Although PZQ has been successfully used to treat schistosomiasis for decades, not much is known about its PK. The only available PK data originates from studies performed with healthy adult volunteers and in patients with different conditions (such as liver impairment) (El Guiniady *et al.*, 1994; Lima *et al.*, 2009). To this point, only a single study, reporting PK parameters of R- and SPZQ, is available for paediatric populations (Bustinduy *et al.*, 2016). Since the demand to include PSAC in PC programs is growing and with it, a need for a paediatric PZQ formulation, PK data to establish a safe and effective dose for this age-group of children is crucial (Keiser *et al*., 2011) and therefore, the aim of our PK studies was to fill this gap in knowledge and aid to the development of the paediatric formulation of PZQ (See Chapter 3).

Mass spectrometry (MS) is an analytic tool frequently applied to measure concentration of a drug in PK samples. Coupling of liquid chromatography with mass spectrometry joins the abilities of physical separation of liquid chromatography with the sensitive detection capabilities of MS. Generally, the analytes of interest are first separated from the remaining matrix by partitioning between the stationary phase, packed in a column of suitable

characteristics, and a liquid mobile phase, which elutes them further into the MS part. In the first part of the MS, the source, the analytes are ionised, then separated based on different principles and finally detected and quantified by a detector. Triple quadrupole is a type of tandem mass spectrometer, used commonly for quantification in the bio-chemical field due to ease of use and good quality of results. Multiple reaction monitoring, one of the popular operation modes of triple quadrupole, enabling detection of targeted analytes with high sensitivity, was used also in our experiments and described in more details in Figure 5 and 6 (Pitt, 2009).

To be able to measure and quantify the concentration of the analytes in a sample using liquid chromatography tandem mass spectrometry (LC-MS/MS), the samples need to be in a liquid state, in contrast to gas chromatography, where analyte is in gaseous state. The sample preparation method for each analyte from different sampling matrices needs to be developed, optimised and validated, assuring constant recovery of analytes and accounting for possible matrix effects. The sample preparation method for PZQ from dried blood spots, DBS, (discussed in more details below) had already been developed in our group (Meister *et al.*, 2016), while the extraction method for Mitra™ was a part of this PhD project, as described in Chapter 4.

All the PK analysis used to obtain results for this thesis were performed on an 6460 Series triple quadrupole LC-MS/MS machine using a method for detection of PZQ, validated in compliance with FDA regulations for analytical method validation (Food and Drug Administration, 2015). The equipment used is described in detail in Chapter 3.



| Analyte | Parent ion (m/z) | | Product ion (m/z) | |
|---|---|---|---|---|
| PZQ | 313 | | 203 | |
| PZQd11 | 324 | | 204 | |
| R-*trans*-4-OH-PZQ | 329 | | 203 | |

**Figure 5:** Fragments of interest for multiple reaction monitoring
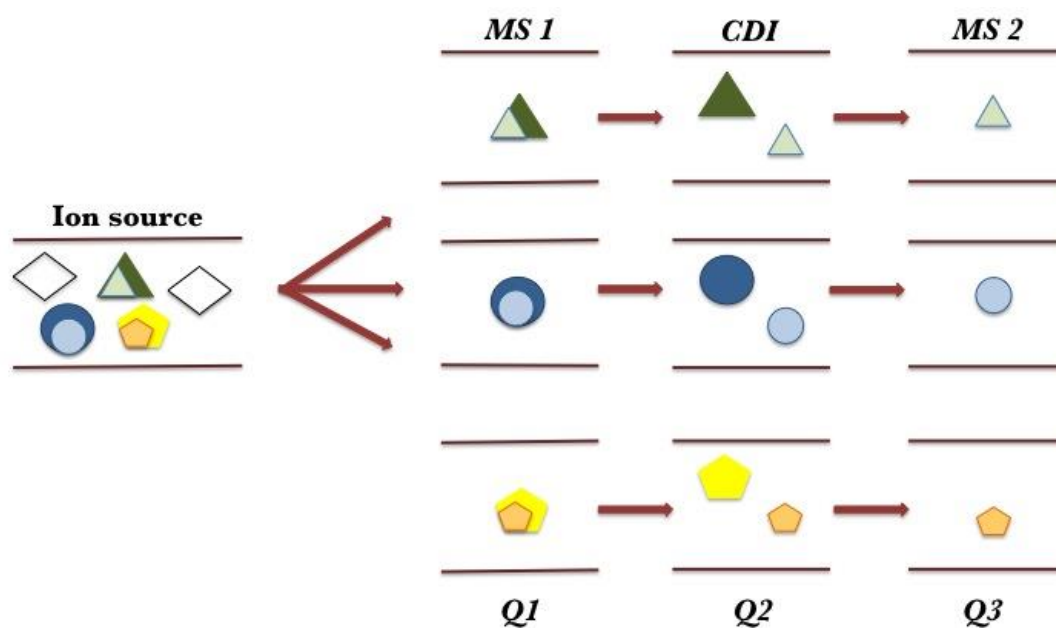
**Figure 6: Multiple Reaction Monitoring**. Only targeted parent ions are selected in Q1 (MS level 1) and allowed to pass to the second quadrupole (Q2) for collision-induced dissociation (CID). Third quadrupole, Q3 (MS level 2), allows only selected fragment ions to pass to the detector.

### 1.9 Micro-sampling in pharmacokinetic studies

As a consequence of recent updates in legislation, PK trials in paediatric population are becoming an essential part of the drug development process (Zisowsky *et al.*, 2010). When conducting clinical trials with sensitive populations, such as children, the focus of clinical research is to reduce the invasiveness of the sampling process and increase the patient compliance (Goodenough *et al.*, 1997; Batchelor and Marriott, 2015; Altamimi *et al.*, 2016). PK trials in particular typically have an intensive sampling scheme; therefore assuring the sampling process is as comfortable for the patient as possible is of utmost importance (Altamimi *et al.*, 2016). One of the most commonly used sampling techniques is venepuncture, known to be very uncomfortable and invasive, therefore not appropriate for sensitive populations (Patel *et al.*, 2013; Altamimi *et al.*, 2016).

In search of alternatives, one technique has stood out- DBS have been successfully used in new-born screening for genetic disorders for decades and have been gaining popularity as a PK sampling tool in the recent past (Guthrie and Susi, 1963; Meesters and Hooff, 2013). DBS are droplets of capillary blood, collected from a finger after a finger prick, disposed on filter paper cards and dried at room temperature (Mei *et al.*, 2001). They offer several advantages over venepuncture, in addition to increased compliance (Edelbroek *et al.*, 2009). Since they do not require a hospital environment and cold chain storage or transport, they are very suitable for use in low resource settings, such as tropical areas of Africa (Denniff and Spooner, 2014). However, DBS sampling requires additional equipment to transfer blood droplets from a finger to the card, such as coagulant-coated capillaries (Figure 7B), to assure a spot of a sufficient size and acceptable shape for quantification (De Kesel *et al.*, 2015). Within an intensive sampling scheme under a time pressure and with short blood coagulation times, often seen in young children, preparing spots acceptable for quantification can be challenging. Another issue, emerging from the DBS technique, is the haematocrit bias, which has been extensively studied and described in the literature (Capiau *et al.*, 2014; Denniff and Spooner, 2014). These factors together can result in unreliable and variable measurements.

Due to all of the above-mentioned issues stemming from the use of DBS, efforts have been aimed towards developing a better alternative. Phenomenex has recently introduced a device called Mitra™, a volumetric absorptive micro-sampling tool (Denniff and Spooner, 2014). The sample collection is based on wicking- using capillary forces to draw the liquid inside the pores of the substrate (De Kesel *et al*., 2015; Kok and Fillet, 2017). The volume absorbed is controlled by the porosity and quantity of the polymeric material from which the tip is made of. The substrate is directly in contact with the liquid sample and as such, it avoids the need for additional equipment, e.g. capillaries, to transfer the sample. Moreover, it assures consistent and repeatable volume of samples, regardless of blood haematocrit (Figure 7A) (De Kesel *et al.*, 2015; Kip *et al.*, 2017; Kok and Fillet, 2017). The whole substrate is subsequently extracted, eliminating the need for punching and simplifying the workflow as compared to DBS (Denniff *et al.*, 2015; Spooner *et al.*, 2015). Moreover, it can be used for sampling different biological liquids, such as blood, plasma, urine or saliva (Denniff and Spooner, 2014; Mercolini *et al.*, 2016). Mitra™ has been studied and tested in laboratory conditions, using both animal and human blood, but never in field conditions (Kok and Fillet, 2017). Thus, our aim was to evaluate its performance in the field compared to DBS not only from the quantification point of view, but also user-friendliness and practicality (see Chapter 4).
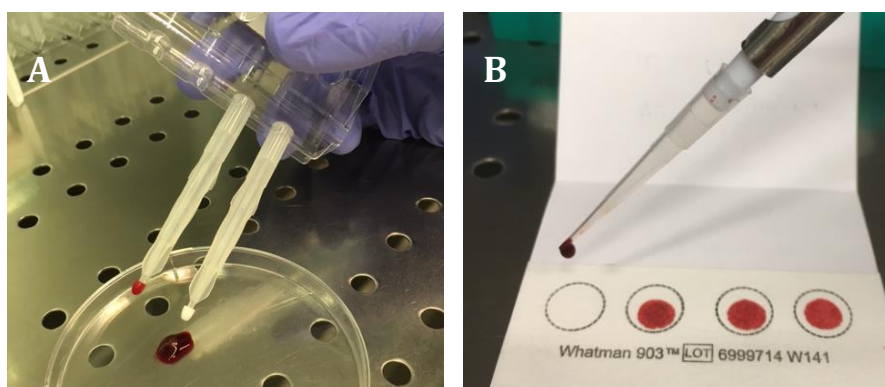


**Figure 7:** Preparation of Mitra™ (**A**) and DBS (**B**) samples in laboratory conditions

## 2 Aim and objectives

Schistosomiasis is one of the most prevalent NTDs. Children are typically the most affected population and while SAC are regularly treated, PSAC are left out of drug distribution programs or treated off-label with the WHO recommended dose for adults of 40 mg/kg. Since PSAC have been recognised as commonly affected by schistosomiasis, there is a growing consensus to include them in PC programs with PZQ as well, directed by WHO in 2010 (WHO, 2010). However, PK data, crucial to establishing safe and effective doses to treat this age group, are sparse.

The main **aim** of this thesis was to elucidate the pharmacokinetics of PZQ and its enantiomers and its relation to efficacy in PSAC and SAC infected with *S. mansoni* and *S.haematobium.* This was pursued with the following specific **objectives**:

**1.** To evaluate the *in vitro* and *in vivo* activity of PZQ enantiomers and its main metabolite against *S. haematobium* (Chapter 2)

**2.** To conduct PK studies with PZQ in SAC and PSAC, infected with *S. mansoni* and *S. haematobium* and obtain PK parameters using non-compartmental analysis (Chapter 3)

**3.** To further deepen the understanding of factors influencing PK of PZQ and with it the dose-response relationship by constructing a model for R-PZQ (in collaboration with Dr. C. Falcoz) and using it to model the data collected during PK studies, resulting in evidence-supported guidelines for dosing regimen in PSAC (see Chapter 7.2)

**5.** To develop, optimise and validate an extraction method for a new conventional micro-sampling device, Mitra™, a potential substitute for DBS and compare its performance to the DBS method by sampling a subset of *S. haematobium* infected patients with both methods after treatment with PZQ (Chapter 4)

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# Chapter 2

# *In vitro* and *in vivo* activity of R- and S- praziquantel enantiomers and the main human metabolite *trans*- 4-hydroxy-praziquantel against *Schistosoma haematobium*

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# Activity of R- and S- Praziquantel enantiomers and the main human metabolite against *Schistosoma haematobium*

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## Abstract

**Background:** Praziquantel (PZQ) is the mainstay of schistosomiasis control and has been successfully used for decades. Despite, its mechanism of action is not fully understood. While the majority of studies have been conducted on *Schistosoma mansoni*, it is not known which enantiomer, R- or S-Praziquantel (R-/S-PZQ), is responsible for the activity on *S. haematobium*.

**Methods:** I*n vitro* and *in vivo* studies were conducted to evaluate the activity of R- and S-PZQ, racemic PZQ and the main human metabolite, namely *trans*-4-OH-PZQ, on *S. haematobium*. $IC_{50}$ values on adult *S. haematobium* were determined in vitro. Dose response relationship studies were performed in golden Syrian hamsters, harbouring a chronic *S. haematobium* infection.

**Findings:** R-PZQ displayed the highest activity against adult worms *in vitro*, revealing an $IC_{50}$ of 0.007 μg/ml at 4 and 0.01 μg/ml at 72 h. S-PZQ on the other hand was 501x less active (eudysmic ratio at 4 h), with an $IC_{50}$ of 3.51 and 2.46 μg/ml (4 and 72 h, respectively). Racemic PZQ resulted in an $IC_{50}$ of 0.03 μg/ml at 72 h. *Trans*-4-OH-PZQ had an $IC_{50}$ of 1.47 μg/ml at 4 and 72 h, respectively, 2.3-2.4-fold lower than S-PZQ. *In vivo*, R-PZQ was the most potent drug with worm burden reductions (WBRs) of 98.5, 75.6 and 73.3% at 125, 62.5 and 31 mg/kg, respectively. A single oral dose of 250 mg/kg PZQ resulted in a WBR of 99.3%. S-PZQ was highly active at 250 and 500 mg/kg with WBRs of 83.0 and 94.1%, respectively. The lowest tested dose of S-PZQ, 125 mg/kg, showed moderate activity (WBR of 46.7%). The calculated $ED_{50}$ for R- and S-PZQ were 24.7 and 127.6 mg/kg, respectively, with a corresponding eudysmic ratio of 5.17.

**Conclusion:** Our data supports the theory of R-PZQ driving the antischistosomal activity. Interestingly, also S-PZQ proved to possess a significant activity towards *S. haematobium*, particularly *in vivo*.


**Key words:** praziquantel, *Schistosoma haematobium*, *in vivo*, *in vitro*

## Introduction

Schistosomiasis is one of the prominent Neglected Tropical Diseases (NTDs), caused by blood-dwelling flukes of the genus *Schistosoma*. It is affecting over 230 millions of people around the world, mostly concentrated in poor, tropical and subtropical areas (1–3).

Intestinal schistosomiasis, caused by *S. mansoni* and *S. japonicum*, manifests with abdominal symptoms (e.g., blood in stool, abdominal discomfort, diarrhoea) and can lead to liver failure (4, 5). *S. haematobium* on the other hand causes urinary schistosomiasis, triggering bladder pathology and often resulting in bladder cancer (4, 6). In addition, schistosomiasis influences the course and outcome of pregnancy and affects child's intellectual and physiological development (7, 8).

Praziquantel (PZQ) is the only effective drug available against schistosomiasis and has been successfully in use for decades (4, 6, 9–13). Originating from veterinary medicine and repurposed for human use, it has been thoroughly studied; however knowledge regarding the mechanism of action is scarce (9, 11). PZQ is a racemic compound consisting of two enantiomers, R- and S-PZQ (9, 11). While there have been some *in vitro* and *in vivo* studies on the activity of PZQ, they mostly studied the racemic drug (14–20). In the few studies, which explored the activity of either R- or S-PZQ, the reported findings vary. Nevertheless, most studies reported greater activity of R-PZQ (21–24) over S-PZQ. Staudt *et al*. (24) suggested that the main metabolite, *trans*- 4-hydroxy-praziquantel (*trans*-4-OH-PZQ), also possesses a high antischistosomal activity on *S. mansoni*. A similar finding was reported by Xiao *et al.* (25) for *S. japonicum*.

It is worth highlighting that the above-mentioned studies, testing the enantiomeric activity of R- and S-PZQ, were conducted using exclusively *S. mansoni* and *S. japonicum*. *S. haematobium* remains largely unexplored, regardless of the fact that it is responsible for the largest number of infections (26). One of the many reasons of negligence might be the life cycle of the parasite, which is difficult to maintain in the laboratory conditions (27–29). However, drug activity shoul be tested on *S. haematobium* as well, since there is evidence that the activity of drugs, e.g. PZQ, oxamniquine or metrifonate differs between species of the parasite (18, 30).

In this study, the activity of both PZQ enantiomers, R- and S-PZQ, as well as the racemic drug and the main metabolite (*trans*-4-OH-PZQ) on *S. haematobium* was assessed. The activity of all entities was tested *in vitro* on adult worms and the results were reported as $IC_{50}$ values. The *S. haematobium* hamster model was used for testing different dosages of R-PZQ and S-PZQ compared with racemic PZQ *in vivo*. $ED_{50}$ values were reported and worm burden reductions (WBRs) were compared between different treatment groups of R- and S-PZQ and the control group.

## Methods and materials

### Drugs, media and animals

### Drugs

Pure analytes, R-, S- and *trans*-4-OH- PZQ were kindly supplied by Merck (Darmstadt, Germany). Racemic PZQ was purchased from Sigma Aldrich (Buchs, Switzerland). Drugs for *in vitro* studies were dissolved in dimethyl sulfoxide (DMSO; Fluka, Buchs, Switzerland). A mixture of 7% (vol/vol) Tween 80 and 3% ethanol (vol/vol) was used to suspend the drugs for *in vivo* treatment.

### Media

For cultivating adult schistosomes, standard RPMI 1640 medium (Life Technologies, Carlsbad, CA) with addition of 5% heat-inactivated foetal calf serum (iFCS), 100 U/ml of penicillin (Life Technologies) and 100 μg/ml of streptomycin (Life Technologies) was used.

### Animals

30 LVG Golden Syrian Hamsters (male, weight approximately 150 g) infected with *S. haematobium* were obtained from the Biomedical Research Institute (NR-21966, Rockville, MD). The animals were kept under controlled conditions (22 °C, 50% humidity, 12/24 h of light and free access to water and rodent diet) to allow development of chronic infection for 3 months post infection date.

### In vitro and in vivo studies

### *In vitro studies*

Adult worms were tested at a range of 0.01-3 μg/ml for R-PZQ and PZQ, at 0.1-30 μg/ml for S-PZQ and 0.1-3 μg/ml for *trans*-4-OH-PZQ. Drugs were prepared in medium using serial dilutions in flat bottom 24-well plates (BD, Falcon). Control wells consisted of 0.3% DMSO, which was the highest concentration of DMSO used to dissolve the drugs. 3 months post infection, *S. haematobium* infected hamsters were euthanized with $CO_2$ and dissected. Adult worms were collected from hepatic portal and mesenteric veins. 2-3 worms, sexes equally represented, were placed per well and each concentration of the drug was tested in duplicates. Worms were incubated at 37 °C and 5% $CO_2$ and the phenotypic changes were evaluated 1 h, 4 h, 24 h, 48 h and 72 h post incubation using a motility scale ranging from 3 (normal activity) to 0 (no activity, granularity present).

$IC_{50}$ values were calculated with CompuSyn® software (version 1.0) from motility values at different concentrations of each drug. The eudysmic ratio was calculated using the following formula:

$IC_{50}$ distomer/ $IC_{50}$ eutomer,

where R-PZQ is the eutomer and S-PZQ the distomer.

## *In vivo studies*

 Infected *S. haematobium* hamsters in groups of 3-4 were treated 3 months post infection with a single oral dose of 250 mg/kg PZQ, 125 mg/kg R-PZQ, 62.5 mg/kg R-PZQ, 31 mg/kg R-PZQ, 500 mg/kg S-PZQ, 250 mg/kg S-PZQ or 125 mg/kg S-PZQ. 10 days post treatment hamsters were euthanized with $CO_2$ and dissected. Adult worms from intestinal veins were counted and sexed and the liver was inspected for live/dead worms and eggs. The control group (untreated) was dissected at the same time and the mean worm burden of treated hamsters was compared with untreated hamsters to determine the worm burden reduction (WBR). $ED_{50}$ and eudysmic ratios were calculated as described above.

## Statistics

Statistical tests were performed using Prism software (version 7.03, GraphPad, CA, USA). Unpaired t-test allowing for unequal variances was used to determine differences in worm burden between the control group and the treatment groups. $p< 0.05$ was considered to be significant.

## Results

## *In vitro* **studies**

*In vitro* $IC_{50}$ and $IC_{90}$ values (4 and 72 h of incubation) of racemic PZQ, pure enantiomers and *trans*-4-OH-PZQ obtained against adult worms of *S. haematobium* are summarised in Table 1. The $IC_{50}$ of R-PZQ was 0.007 µg/ml at 4 and 0.01 µg/ml at 72 h, while S-PZQ was 501x less active (eudysmic ratio at 4 h) yielding $IC_{50}$ values of 3.51 and 3.40 µg/ml (4 and 72 h, respectively). The $IC_{50}$ of PZQ was 0.03 µg/ml, which is 4.3x higher compared to R-PZQ. *Trans*-4-OH-PZQ revealed an $IC_{50}$ of 1.47 µg/ml at 4 and 72 h, respectively.

## *In vivo* **studies**

In Table 2, total WBRs and female WBRs following different single oral doses of R-, S-PZQ and PZQ are presented. For all drugs and dosages tested, a higher activity on the female worms was observed. PZQ reduced the total worm burden by 99.3% at a single dose of 250 mg/kg. R-PZQ showed the highest total WBR at 125 mg/kg (98.5%) while with a half of the dose (62.5 mg/kg) the worm burden reduction was lower (75.6%). The lowest dose of R-PZQ, 31 mg/kg, yielded still a high total WBR of 73.3 %.  S-PZQ revealed a high activity at 500 and 250 mg/kg with total WBRs of 94.1% and 83.0%. A moderate total WBR of 46.7% was observed when the hamsters were treated with 125 mg/kg of S-PZQ. The calculated $ED_{50}$s for R- and S-PZQ were 24.7 and 127.6 mg/kg, respectively, with a corresponding eudysmic ratio of 5.17. All WBRs of the different treatment groups were significantly different from the control group ($p < 0.05$), except for the lowest doses of R- and S-PZQ (31.5 mg/kg and 125 mg/kg, respectively), which showed not to be significantly better compared to the control group.

**Discussion**

With no available alternative drug, PZQ is the mainstay of schistosomiasis control (9–12). Apart from reliance on a single drug, an additional drawback is the large dose required resulting in huge size of the tablet, containing a racemic mixture of PZQ (31–33). The discussion about the activity of each enantiomer of the drug, namely R- and S-PZQ, has been on-going and therefore it is time to reach a conclusion which enantiomer is responsible for the antischistosomal activity (9, 32–34). Moreover, development of a paediatric PZQ formulation is currently undergoing and thorough examination of *in vitro* and *in vivo* activity of PZQ and its enantiomers will not only contribute to a better understanding of the drug but also aid to select the optimal entity for the final formulation (R-PZQ or racemic PZQ) (35). While *S. mansoni* has been thoroughly researched, *S. haematobium* remains neglected in the laboratory, despite of being responsible for a large share of the burden of schistosomiasis (26, 29). This holds true also for drug sensitivity testing including studies on PZQ. While a few studies reported the activity of PZQ towards *S. haematobium*, all of them only evaluated the activity of racemic PZQ (18, 19, 29). Studies on *S. haematobium* are pivotal as many antischistosomals, oxamniquine, metrifonate and PZQ have very distinct profiles on the different schistosome species (30). Our study is the first to report the activity of both enantiomers of PZQ, at three different doses, compared to a single dose of PZQ, *in vivo*. Additionally, the activity of both enantiomers was compared also to the main human metabolite, *trans*-4-OH-PZQ, and the racemic drug, *in vitro*.

Our results show that R-PZQ is driving the antischistosomal activity of PZQ, both *in vitro* and *in vivo*. The $IC_{50}$ value of racemic PZQ was 4.3x higher compared to the enantiopure R-PZQ *in vitro*.

*In vivo* results followed a similar pattern: R-PZQ at 125 mg/kg resulted in WBRs above 98%, as did a twice higher dose of PZQ, 250 mg/kg. The latter result is in the line with findings from the dose response relationship study with PZQ in *S. haematobium* infected hamsters conducted by Webbe and James, yielding an $ED_{50}$ of 118 mg/kg (18).

Strikingly, it seems that in case of *S. haematobium* in contrast to *S. mansoni* (36), also S-PZQ possesses non-negligible activity. An $ED_{50}$ of 127.6 mg/kg was calculated for S-PZQ, which is close to the value of the racemic drug. For comparison, *S. mansoni* infected mice treated with 800 mg/kg S-PZQ showed only a low WBR of 19.6%. Hence, the eudysmic ratio is 64-fold lower for *S. haematobium* compared to *S. mansoni* (36). However, it is worth highlighting that differences in the drug sensitivity between the two species might also be due to differences in the model, hamster versus mouse model (27). Finally, also *trans*-4-OH-PZQ revealed a 2.3-2.4-fold higher activity (72 and 4 h, respectively) against *S. haematobium in vitro* when compared to *S. mansoni*. A contribution of S-PZQ and *trans*-4-OH-PZQ to PZQ's activity could explain the higher sensitivity of PZQ to *S. haematobium* when compared to *S. mansoni* in humans (30). In humans *S. haematobium* are residing in the venus

plexus of the bladder, getting exposed mostly to high concentrations of S-PZQ and the metabolite, as a consequence of first pass metabolism. This is in contrast with *S. mansoni*, where the adult worms are exposed to un-metabolised drug in the mesenteric veins, prior reaching the liver.

Last but not least, we observed increased sensitivity of female worms compared to the male in vivo- the female WBRs were higher compared to total WBRs in all cases. The lowest dose of S-PZQ reached the female WBR of 95.6%, while total WBR was only 46.7%. The increased sensitivity of female worms to PZQ has been reported previously (20).

## Conclusion

To sum up, we observed that R-PZQ possesses the highest activity among the PZQ enantiomers and main human metabolite tested against *S. haematobium.* Surprisingly, S-PZQ- showed a high activity *in vivo*. Additionally, the main human metabolite displayed an activity higher than S-PZQ *in vitro*.

In the line with the current efforts to develop a paediatric formulation, an enantioselective R-PZQ formulation might bear some risk; however, clinical trials, including pharmacokinetic/pharmacodynamics relationship studies, would be required to confirm our findings.

**Declarations**

**List of abbreviations**

PZQ: praziquantel; WBR: worm burden reduction; $IC_{50}$: concentration of the drug, needed to kill 50% of the parasites; $ED_{50}$: dose of the drug needed to reduce the worm burden by 50%

**Consent for publication**

Not applicable.

**Ethics approval**

The current study was approved by the local veterinary agency based on Swiss cantonal and national regulations (permission no. 2070).

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The datasets supporting the conclusion of this article are included within the article.

**Authors' contributions**

JK and JKo designed the experiment. MV and JKo performed the experiments. The statistics and the manuscript were prepared by JK. All authors read and approved the final manuscript.

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**Table 1:** $IC_{50}$ and $IC_{90}$ values of PZQ, R-PZQ, S-PZQ enantiomers and *trans*-4-OH-PZQ against adult worms of *S. haematobium*

| | $IC_{50}$ at 4 h (µg/ml) | r-value | $IC_{50}$ at 72 h (µg/ml) | r-value | $IC_{90}$ at 72 h (µg/ml) | r-value | Eudysmic ratio |
|---|---|---|---|---|---|---|---|
| **PZQ** | 0.03 | 0.978 | 0.03 | 0.965 | 0.09 | 0.978 | 501 |
| **R-PZQ** | 0.007 | 0.803 | 0.01 | 0.940 | 0.03 | 0.940 | |
| **S-PZQ** | 3.51 | 0.925 | 3.40 | 0.923 | 5.98 | 0.923 | |
| ***Trans*-4-OH-PZQ** | 1.47 | 0.891 | 1.47 | 0.891 | 3.31 | 0.891 | |

**Table 2:** Worm burden reductions (WBRs) following different single oral doses of R, S-PZQ and PZQ.

| | Number of hamsters cured/treated | Mean number of alive worms (SD) | | | | WBR (%) | Female WBR (%) | ED$_{50}$ (mg/kg)[a] |
|---|---|---|---|---|---|---|---|---|
| | | Liver | Mesenteric veins | Total | Females | | | |
| **Control** | 0/4 | 2.75 (3.77) | 31.0 (9.49) | 33.8 (16.8) | 15.3 (5.2) | -- | -- | -- |
| **PZQ** | | | | | | | | |
| 250 mg/kg | 3/4 | 0.25 (0.5) | 0 | 0.3 (0.5) | 0 | 99.3 | 100.0 | |
| 200 mg/kg* | | | | | | 77.2 | | |
| 150 mg/kg* | | | | | | 66.1 | | 118.1 |
| 100 mg/kg* | | | | | | 39.2 | | |
| **R-PZQ** | | | | | | | | |
| 125 mg/kg | 2/4 | 0.25 (0.5) | 0.25 (0.5) | 0.5 (0.6) | 0 | 98.5 | 100.0 | |
| 62.5 mg/kg | 1/4 | 5.00 (4.69) | 3.25 (3.59) | 8.3 (8.1) | 2.3 (3.3) | 75.6 | 85.2 | 24.7 |
| 31 mg/kg | 0/3 | 6.67 (11.55) | 2.33 (2.31) | 9.0 (13.9) | 4.0 (6.9) | 73.3 | 73.8 | |
| **S-PZQ** | | | | | | | | Eudysmic ratio |
| 500 mg/kg | 3/4 | 1.25 (2.5) | 0.75 (1.5) | 2.0 (4.0) | 0 | 94.1 | 100.0 | |
| 250 mg/kg | 2/4 | 4.0 (4.90) | 1.75 (2.06) | 5.8 (6.9) | 0 | 83.0 | 100.0 | 127.6 | 5.17 |
| 125 mg/kg | 0/3 | 7.0 (6.24) | 11.0 (6.0) | 18.0 (12.1) | 0.7 (0.6) | 46.7 | 95.6 | |

# Chapter 3

# Pharmacokinetics of praziquantel in *Schistosoma mansoni* and *Schistosoma haematobium* infected school- and preschool- aged children

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**Pharmacokinetics of praziquantel in *Schistosoma mansoni* and *Schistosoma haematobium* infected school- and preschool- aged children**

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**Abstract**

There is a growing consensus to include preschool-aged children in preventive chemotherapy programs with praziquantel to improve schistosomiasis control. However, pharmacokinetic data, crucial to establish safe and effective dose for this age group, are sparse. The objective of this study was to establish and compare pharmacokinetic parameters of praziquantel in preschool- and school-aged children with schistosomiasis. Two pharmacokinetic trials in school- and preschool-aged children infected with *Schistosoma mansoni* or *S. haematobium*, were conducted in Côte d'Ivoire. Dried blood spot samples were taken from 492 children at 10 time points following a single oral dose of 20, 40 or 60 mg/kg of bofy weight of praziquantel and analysed using liquid chromatography mass spectrometry. Non-compartmental analysis (NCA) was performed to obtain the pharmacokinetic parameters of R-praziquantel (RPZQ), S-praziquantel (SPZQ) and R-*trans*-4-hydroxy-praziquantel. No significant differences in pharmacokinetic parameters between species-specific infections were observed. While pharmacokinetic parameters differed significantly between age groups for *S. mansoni*, this trend was not observed with *S. haematobium*. Neither the area under the curve (AUC) nor the maximal blood concentration ($C_{max}$) presented clear dose proportionality for R- and SPZQ. Logistic regression indicated a relationship between the RPZQ AUC and $C_{max}$ and the probability of cure. Praziquantel is subject to complex metabolic processes following erratic absorption. While the results of NCA are a very informative base for a better understanding of the drug, a more targeted approach in the form of population modelling is needed to quantify the factors influencing metabolic processes and draw conclusions.

**Keywords:** pharmacokinetics, praziquantel, schistosoma, schistosomiasis, preschool-aged children

**Introduction**

Schistosomiasis, first described in 1851 by Theodor Bilharz (1), represents a major public health problem in rural tropical and subtropical areas of the world (2, 3). Caused by blood–dwelling flukes of the genus *Schistosoma*, with *S. haematobium*, *S. japonicum* and *S. mansoni* being the principle species infecting humans, it affects over 250 million people (4). Acute schistosomiasis manifests with flu–like symptoms however, if it is not treated, it can result in severe chronic consequences, e.g. hepatic fibrosis, kidney failure or bladder cancer (5, 6).

Despite years of research, praziquantel (PZQ) remains the only available drug effective against schistosome infections (7, 8). As a drug with relatively good efficacy and tolerability, PZQ has been successfully used in large scale drug administration programs (i.e. preventive chemotherapy) for the last decade (8, 9).

One of the most vulnerable groups, affected by schistosomiasis, is children (10, 11). Preventive chemotherapy programs target school-aged children (SAC), while their younger peers, preschool-aged children (PSAC; age <6 years), are excluded from official treatment programs and treated only on individual basis, creating the so called "treatment gap" (12, 11). Nonetheless, early parasitic infection could exacerbate the clinical impact of schistosomiasis and its subsequent morbidity (13). Additionally, PSAC might have a role in maintaining the local transmission of the disease; therefore it is crucial to include them in treatment programs (13).

One of the reasons behind targeting only SAC in preventive chemotherapy programs is the lack of the pharmacokinetic (PK) data, which would guide the establishment of safe and effective dose of PZQ for PSAC (11, 14, 15). Available data on the drug's absorption, distribution, metabolism and elimination processes (ADME) is mostly derived from studies in healthy adult volunteers, carried out years ago, while basic PK information for the target population is lacking (8, 9, 16). Since physiological and enzyme differences in drug

metabolism between adults and children have been described in detail, the extrapolation of adult dosages to children is dubious (17–19). Furthermore, the only commercially available formulation of PZQ is a racemic mixture of both enantiomers, R-praziquantel (RPZQ) and S-praziquantel (SPZQ), resulting in a big tablet difficult for small children to swallow, while the discussion regarding which entity is responsible for antischistosomal activity of the drug is ongoing (8, 16). The metabolism of both enantiomers has been studied and there is some evidence that the main human metabolite, R-*trans*-4-hydroxy-praziquantel (R-*trans*-4-OH-PZQ), mainly originating from enantio-selective metabolism of RPZQ, might contribute to antischistosomal activity of PZQ (20–22).

We conducted two PK studies using dried blood spot sampling (DBS) with the aim to elucidate the PK parameters of PZQ in PSAC and SAC, infected with *S. mansoni* or *S. haematobium*, in which each child was embedded in a single-blind, randomised, placebo controlled dose-finding study (23, 24). For the first time, the concentrations over time course of all three entities, namely, RPZQ, SPZQ and the main human metabolite, R-*trans*-4-OH-PZQ, were analysed. Non-compartmental analysis (NCA) was conducted in order to derive PK parameters of the analytes, and relationships between drug exposure and efficacy were explored. Our study contributes to a better understanding of PZQ and possibly to the development of a paediatric formulation.

## Results

### *Method revalidation*

The linearity range of the calibration lines was 0.009–2.232 μg/ml for R- and SPZQ and 0.179 to 44.600 μg/ml for R-*trans*-4-OH-PZQ, with a coefficient of correlation ($R^2$) above 0.99. The results of the partial revalidation are summarized in Table S1 to S3 of the supplemental material.

### *S. mansoni study*

A total of 2,540 DBS were analysed from 229 children (94 PSAC and 135 SAC) (median, 11 DBS per participant, range, 9 to 11 DBS per participant.) Of these, 398 (16%) measurements for RPZQ were under the lowest level of quantification (LLOQ), while 311 (12 %) and 439

(17 %) were under the LLOQ for SPZQ and *trans*-4-OH-PZQ, respectively. Between 29 (40 mg/kg of body weight) and 33 (60 mg/kg) PSAC and 42 (60 mg/kg) and 47 (20 mg/kg) SAC per treatment arm participated in the PK study. Both sexes were uniformly represented in each group and treatment arm (55% females for PSAC, 53% for SAC). Actual doses administered ranged from 17.0–23.3 (median, 20.2 mg/kg) for the 20 mg/kg treatment arm, 35.4–42.7 (median, 39.2 mg/kg) for the 40 mg/kg treatment arm and 56.3–64.3 (median, 60.0 mg/kg) for the 60 mg/kg treatment arm.

**Table 1:** Patient characteristics. (Presented by Coulibaly et al.(23, 24)

|  | Pre-school aged children | | | School-aged children[a] | | |
|---|---|---|---|---|---|---|
| ***Schistosoma mansoni*** | | | | | | |
| Treatment arm | **20 mg/kg** | **40 mg/kg** | **60 mg/kg** | **20 mg/kg** | **40 mg/kg** | **60 mg/kg** |
| No. of children | 32 | 29 | 33 | 47 | 46 | 42 |
| No. of girls | 18 | 18 | 16 | 25 | 21 | 25 |
| Mean (minimum – maximum age) (years) | 3.8 (2 - 6) | 3.8 (2 - 5) | 3.9 (2 - 6) | 9.1 (6 - 15) | 9.0 (6 - 14) | 9.0 (6 - 14) |
| Mean ± SD Wt (kg) | 13.8 (±2.5) | 14.0 (±2.4) | 14.0 (±2.5) | 25.2 (±7.6) | 24.3 (±7.8) | 25.9 (±7.6) |
| No. of children absent at follow-up | 3 | 2 | 1 | 1 | 1 | 1 |
| **Geometric mean no. of eggs/g of stool** | | | | | | |
| Before treatment | 19.6 (6 - 168) | 21.1 (6 - 330) | 37.5 (6 – 15) | 76.5 (6 – 2034) | 84.1 (6 – 2196) | 80.2 (6 – 2286) |
| After treatment | 1.8 (0 – 48) | 1.0 (0 – 36) | 0.8 (0 – 30) | 12.1 (0 – 768) | 1.4 (0 – 72) | 0.7 (0 – 1116) |
| Egg reduction rate - % | 90.8 | 95.3 | 97.9 | 84.2 | 98.3 | 99.1 |
| **No. of children positive for infection** | | | | | | |
| Before treatment | 29 | 27 | 32 | 46 | 45 | 41 |
| After treatment | 10 | 7 | 7 | 32 | 14 | 7 |
| Cure rate - % | 65.5 | 74.1 | 78.1 | 30.4 | 68.9 | 82.9 |
| ***Schistosoma haematobium*** | | | | | | |
| Treatment arm | **20 mg/kg** | **40 mg/kg** | **60 mg/kg** | **20 mg/kg** | **40 mg/kg** | **60 mg/kg** |
| No. of children | 39 | 43 | 41 | 45 | 46 | 44 |
| No. of girls | 21 | 20 | 26 | 25 | 25 | 25 |
| Mean (minimum – maximum age) (years) | 3.7 (2 - 5) | 3.9 (2 - 5) | 4.0 (2 - 5) | 8.8 (6 - 15) | 9.0 (6- 14) | 9.2 (6 - 15) |
| Mean ± SD Wt (kg) | 14.9 (±2.8) | 15.0 (±2.6) | 15.2 (±2.1) | 24.3 (±5.2) | 25.0 (±6.2) | 25.3 (±8.0) |
| No. of children absent at follow-up | 5 | 3 | 3 | 1 | 5 | 1 |
| **Geometric mean no. of eggs/ml urine** | | | | | | |
| Before treatment | 6.9 (1 – 63) | 6.8 (1 – 145) | 8.0 (0.3 – 72) | 20.4 (1 – 2317) | 16.6 (1 – 237) | 15.7 (1 – 223) |

| After treatment | 0.1 (0 – 7) | 0.2 (0 – 5) | 0.3 (0 – 27) | 0.5 (0 – 14) | 0.2 (0 – 5) | 0.4 (0 – 20) |
|---|---|---|---|---|---|---|
| Egg reduction rate - % | 98.6 | 97.1 | 96.3 | 97.5 | 98.8 | 97.5 |
| **No. of children positive for infection** | | | | | | |
| Before treatment | 34 | 40 | 37 | 44 | 40 | 41 |
| After treatment | 4 | 9 | 12 | 20 | 11 | 15 |
| Cure rate - % | 88.2 | 77.5 | 67.6 | 54.6 | 72.5 | 63.4 |

Egg reduction rates (ERRs; based on the geometric mean) for PZQ doses of 20, 40 and 60 mg/kg were 90.8%, 95.3% and 97.9% , respectively, for PSAC and 83.9%, 98.3% and 99.1%, respectively, for SAC. Cure rates (CRs) ranged from 65.5% for 20 mg/kg to 78.1% for 60 mg/kg (74.1% for the standard dose of 40 mg/kg), for PSAC. Among SAC, CRs were 30.4%, 68.9% and 82.9% for PZQ doses of 20, 40 and 60 mg/kg respectively. All the patients' characteristics are summarised in Table 1.

### *S. haematobium study*

Altogether, 2808 DBS were analysed, (median, 11 DBS per participant; range, 9–11 DBS per participant. Four hundred two measurements (14 %) were under the LLOQ for RPZQ, 345 (12 %) were under the LLOQ for SPZQ and 474 (17 %) were under the LLOQ for R-*trans*-4-OH-PZQ. Thirty-nine PSAC received 20 mg/kg, 43 PSAC receivws40 mg/kg and 41 PSAC received 60 mg/kg (the numbers for SAC were 45, 47 and 45, respectively). The number of boys and girls was balanced between the treatment groups. The actual doses were 18.8–22.2 mg/kg (median, 20.0 mg/kg), 37.5–41.4 mg/kg (median, 40.0 mg/kg) and 58.3–61.4 mg/kg (median, 60.0 mg/kg). CRs for PSAC were 88.2%, 77.5% and 67.6% for doses of 20, 40 and 60 mg/kg, respectively, with corresponding ERRs of 98.6%, 97.1% and 96.3%. SAC exhibited CRs of 54.6%, 72.5% and 63.4%, respectively and ERRs of 97.5%, 98.8% and 97.5%, respectively. The children's demographics and parasitological characteristics are presented in more detail in Table 1.

*Non–compartmental analysis*

PK parameters are summarised in Table 2 for *S. mansoni* and in Table 3 for S. *haematobium*, with the results presented as medians with interquartile ranges (IQR). The concentration-over-time profiles of R- and SPZQ and the metabolite for *S. mansoni-* and S. *haematobium*-infected SAC and PSAC are presented in Figures 1 and 2, respectively.

Overall, great inter-patient variability was observed. The half-life ($t_{1/2}$) could be estimated in 75 % (IQR, 70 to 81%) of subjects and was similar for all analytes across different dosages and the two age groups and parasite species, at approximately 3–8 h for R- and SPZQ and 2–5 h for the metabolite. The time to the maximal blood concentration ($T_{max}$) was 1.5 to 6 h, for the metabolite, while the enantiomers attained their highest concentration in blood at 0.5– 3.0 h in the four populations of children studied.

In *S. mansoni*–infected SAC, the highest maximal blood concentration ($C_{max}$) values were observed at 60 mg/kg for R-*trans*-4-OH-PZQ (median, 13.57 μg/ml; IQR 9.81 to 16.43 μg/ml), followed by SPZQ (median, 1.00 μg/ml; IQR 0.62 to 1.37 μg/ml) and RPZQ (median, 0.29 μg/ml; IQR 0.22–0.53 μg/ml). PSACs revealed considerablz higher $C_{max}$s than SAC for all three analytes as summarized in Table 2. In the *S. haematobium* study (Table 3), in SAC, the highest values for $C_{max}$ at 60 mg/kg PZQ were observed for R-*trans*-4-OH-PZQ (median 11.49 μg/ml; IQR 9.05–13.78 μg/ml), followed by SPZQ (median 1.25 μg/ml; IQR 0.78–1.86 μg/ml) and RPZQ (median 0.44 μg/ml; IQR 0.25–0.81 μg/ml). PSAC revealed similar $C_{max}$s and no significant differences from SAC were observed for any dose or analyte.

Area-under-the-curve (AUC) values showed considerable and significant differences between SAC and PSAC infected with *S. mansoni* for the metabolite and RPZQ at all three dosages administered and for SPZQ at 20 and 60 mg/kg. No sdifference in AUC values between SAC and PSAC with urinary schistosomiasis was observed for any of the analytes at any dose. Logistic regression confirmed this finding: a significant effect of dose on AUC was found for children infected with *S. mansoni* but not for children infected with *S. haematobium.*

When comparing the AUC of cured and uncured PSAC using Mann-Whitney analysis, no differences were observed for any of the analytes and both species of the parasite, while cured

SAC infected with *S. mansoni* reached AUCs significantly higher than those for uncured SAC. In *S. haematobium* infected SAC significantly higher AUC values of RPZQ and SPZQ were observed in cured compared to uncured children, while there was no significant difference in metabolite exposure (data not shown). Logistic regression indicated a positive relationship between RPZQ exposure (for both AUC and $C_{max}$) and the probability of cure, in which a higher exposure led to a higher probability of cure for all children analysed together, as shown in Figure 3 and 4.

**Discussion**

Schistosomiasis remains a considerable public health problem, despite the years of efforts to control it (2, 3). PZQ is the treatment of choice and has been successfully used for decades (7, 8). While SAC are treated regularly, PSAC have been, up to this day, either excluded from preventive chemotherapy programs or treated off-label with the WHO recommended dose of 40 mg/kg PZQ, used for adults and SAC (11, 12, 25).

To date, several studies describing efficacy and safety of PZQ in young children have been conducted (13, 26, 27). The PK of PZQ have been studied mostly in healthy adults (28–31), while ADME processes of PZQ in children remain largely unexplored, except for the recent study by Bustinduy *et al.* (16).

We have, for the first time, quantified all three main analytes of PZQ (RPZQ, SPZQ and R-*trans*-4-OH-PZQ) using an intensive sampling scheme in blood. Our PK studies were embedded in two randomised controlled clinical trials allowing us to explore the PK of a large cohort of children in both age groups, PSAC and SAC, infected with either *S. mansoni* or *S. haematobium* and treated with three different dosages of PZQ (23, 24). The sampling technique used was novel DBS technology, which proved to be an excellent tool. It does not require medical staff or a hospital environment, which is crucial for rural settings. Furthermore, collection of blood is comparably less invasive than venepuncture, which is a benefit for sensitive populations, such as children. Adding up to these advantages are also the transport conditions: DBS do not require a cold chain and can be easily transported from the field to the laboratory (32, 33).

**Table 2:** PK parameters of SAC and PSAC infected with *S. mansoni*

| *S. mansoni* | | *R-trans-4-OH-PZQ* | | *RPZQ* | | *SPZQ* | |
|---|---|---|---|---|---|---|---|
| | | SAC | PSAC | SAC | PSAC | SAC | PSAC |
| **20 mg/kg** | $T_{1/2}$ (h)$^□$ | 2.73 (2.24–3.46) | 2.88 (2.24–3.81) | 3.12 (2.15–5.97) | 5.02 (3.96–6.70) | 3.59 (1.82–6.44) | 4.32 (3.31–6.63) |
| | $T_{max}$ (h) | 2 (2.0–2.5) | 2.50 (1.50–3.00) | 1.0 (0.5–1.5) | 1.50 (1.00–2.50) | 1.0 (1.0–1.5) | 1.50 (1.00–2.50) |
| | $C_{max}$ (µg/ml) | 6.43 (3.94–9.53)*,a,b | 3.24 (2.26–7.34)*,a,b | 0.07 (0.05–0.14)*,+,#,a,b | 0.33 (0.15–0.60)*,b | 0.19 (0.14–0.38)*,+,#,a,b | 0.50 (0.32–1.04)*,b |
| | $AUC_{last}$ (h*µg/ml) | 29.87 (19.60–43.11)*,a,b | 16.84 (11.52–33.28)*,a,b | 0.27 (0.16–0.46)*,#,+,a,b | 1.35 (0.74–2.86)*,b | 0.60 (0.36–1.13)*,#,+,a,b | 1.82 (1.04–3.33)*,a |
| **40 mg/kg** | $T_{1/2}$ (h)$^□$ | 3.09 (2.66–3.82) | 3.42 (2.68–3.80) | 3.91 (1.86–6.57) | 5.65 (3.70–7.80) | 2.25 (1.46–4.61) | 4.64 (3.12–6.36) |
| | $T_{max}$ (h) | 2.5 (2.5–3.0) | 2.00 (1.50–3.00) | 1.50 (1.00–2.00) | 1.00 (0.50–2.00) | 1.50 (1.00–2.00) | 1.00 (0.50–2.00) |
| | $C_{max}$ (µg/ml) | 10.32 (8.21–14.17)*,+,a | 6.92 (4.08–10.16)*,a | 0.25 (0.15–0.37)*,#,a | 0.49 (0.36–0.93)*,# | 0.77 (0.52–1.02)#,a | 0.90 (0.59–1.44)# |
| | $AUC_{last}$ (h*µg/ml)+ | 56.59 (46.18–81.85)*,a,c | 32.74 (21.76–50.42)*,a | 0.78 (0.57–1.08)*,#,+,a | 1.71 (1.15–2.73)* | 2.19 (1.61–3.20)#,a,c | 2.20 (1.64–4.42)c |
| **60 mg/kg** | $T_{1/2}$ (h)$^□$ | 3.19 (2.96–4.40) | 3.54 (3.00–4.41) | 4.49 (2.00–7.82) | 5.95 (4.42–8.75) | 4.05 (1.60–6.04) | 4.58 (4.04–5.96) |
| | $T_{max}$ (h) | 3.00 (2.50–6.00) | 2.50 (1.50–3.00) | 1.50 (1.00–2.00) | 1.00 (0.50–2.00) | 2.00 (1.50–2.75) | 1.00 (1.00–2.00) |
| | $C_{max}$ (µg/ml) | 13.57 (9.81–16.43)*,b | 8.29 (5.69–11.85)*,+,b | 0.29 (0.22–0.53)*,#,b | 0.69 (0.43–1.25)*,#,b | 1.00 (0.62–1.37)#,b | 1.33 (0.80–1.78)#,b |
| | $AUC_{last}$ (h*µg/ml) | 89.22 (61.98–141.97)*,c | 49.94 (28.57–75.12)*,+,b | 1.00 (0.83–1.93)*,#,+,b | 2.74 (1.71–4.03)*,#,b | 3.50 (2.54–4.71)*,#,+,b,c | 4.99 (3.15–6.03)*,#,a,c |

*Significant difference between SAC and PSAC of the same dose and analyte; +Significant difference between *S. haematobium* and *S. mansoni* infections of same dose, age group and analyte
#Significant difference between R and SPZQ of same dose, age group and species; Significant difference between the doses of 20 and 40 mg/kg (a), 20 and 60 mg/kg (b) or 40 and 60 mg/kg (c)
for same age group, analyte and species of the parasite; $^□$ $T_{1/2}$ could be estimated in 75 (70–81)% (median (IQR)) of the subjects

**Table 3:** PK parameters of SAC and PSAC infected with *S. haematobium*

| *S. haematobium* | | R-trans-4-OH-PZQ | | RPZQ | | SPZQ | |
|---|---|---|---|---|---|---|---|
| | | SAC | PSAC | SAC | PSAC | SAC | PSAC |
| **20 mg/kg** | $T_{1/2}$ (h)[◻] | 3.28 (2.54–4.78) | 3.49 (2.79–5.21) | 4.41 (3.52–9.36) | 7.39 (4.03–9.42) | 4.92 (2.15–7.75) | 5.58 (2.08–8.57) |
| | $T_{max}$ (h) | 2.50 (1.50–3.00) | 2.00 (1.50–3.00) | 2.50 (1.50–6.00) | 1.00 (0.50–2.00) | 1.50 (1.00–3.00) | 1.00 (0.50–1.50) |
| | $C_{max}$ (µg/ml) | 4.64 (4.08–5.84)[a,b] | 4.92 (3.92–7.73)[a,b] | 0.18 (0.09–0.35)[+,#,b] | 0.32 (0.19–0.74)[#,b] | 0.34 (0.21–0.77)[+,#,a,b] | 0.60 (0.41–0.90)[#,b] |
| | $AUC_{last}$(h*µg/ml) | 22.20 (20.17–22.84)[a,b] | 23.08 (16.49–41.36)[a,b] | 1.01 (0.49–1.80)[#,+,b] | 1.16 (0.50–2.03)[#,b] | 1.67 (0.87–2.39)[#,+,a,b] | 1.80 (1.25–2.59)[#,b] |
| **40 mg/kg** | $T_{1/2}$ (h)[◻] | 3.70 (3.18–4.52) | 3.71 (2.92–4.71) | 4.33 (2.37–8.20) | 5.57 (2.40–8.61) | 4.61 (1.86–7.44) | 4.94 (2.13–7.78) |
| | $T_{max}$ (h) | 3.00 (2.25–6.00) | 3.00 (1.75–3.00) | 2.00 (1.50–3.00) | 1.50 (0.75–2.50) | 2.00 (1.00–3.00) | 1.50 (0.50–2.00) |
| | $C_{max}$ (µg/ml) | 8.16 (6.44–10.16)[+,a,c] | 8.14 (6.18–11.47)[a,c] | 0.32 (0.16–0.62)[#] | 0.41 (0.21–0.62)[#,c] | 0.74 (0.46–1.38)[#,a] | 0.91 (0.54–1.14)[#,c] |
| | $AUC_{last}$ (h*µg/ml) | 47.79 (39.14–60.79)[+,a,c] | 42.59 (29.78–76.56)[a,c] | 1.15 (0.75–2.30)[#,+] | 1.17 (0.84–2.13)[#,c] | 2.73 (1.73–4.37)[#,a,c] | 2.24 (1.65–3.60)[#,c] |
| **60 mg/kg** | $T_{1/2}$ (h)[◻] | 3.86 (3.39–4.55) | 3.64 (3.03–4.09) | 5.34 (3.30–7.90) | 5.09 (3.03–7.32) | 5.58 (3.02–7.73) | 4.80 (3.06–6.83) |
| | $T_{max}$ (h) | 4.50 (2.88–6.00) | 3.00 (2.00–3.00) | 2.50 (1.00–3.00) | 1.50 (0.50–2.00) | 2.50 (1.50–3.00) | 2.00 (1.00–2.50) |
| | $C_{max}$ (µg/ml) | 11.49 (9.05–13.78)[b,c] | 13.49 (10.65–15.09)[+,b,c] | 0.44 (0.25–0.81)[#,b] | 0.62 (0.37–1.18)[#,b,c] | 1.25 (0.78–1.86)[#,b] | 1.22 (0.96–1.78)[#,b,c] |
| | $AUC_{last}$(h*µg/ml) | 88.32 (68.10–136.91)[b,c] | 107.44 (58.31–148.66)[+,b,c] | 1.84 (1.07–2.67)[#,+,b] | 2.25 (1.58–3.79)[#,b,c] | 5.09 (3.37–7.06)[#,+,b,c] | 4.87 (3.85–7.59)[#,b,c] |

*Significant difference between SAC and PSAC of the same dose and analyte; [+]Significant difference between *S. haematobium* and *S. mansoni* infections of same dose, age group and analyte [#]Significant difference between R and SPZQ of same dose, age group and species; Significant difference between the doses of 20 and 40 mg/kg ([a]), 20 and 60 mg/kg ([b]) or 40 and 60 mg/kg ([c]) for same age group, analyte and species of the parasite; [◻] $T_{1/2}$ could be estimated in 75 (70–81)% (median (IQR)) of the subjects

**Figure 1:** Concentration over time profiles (mean with standard deviation as a shaded area) for increasing doses of all analytes in *S. mansoni* infected children. The blue lines indicate 20 mg/kg, the red lines 40 mg/kg and the yellow lines 60 mg/kg.
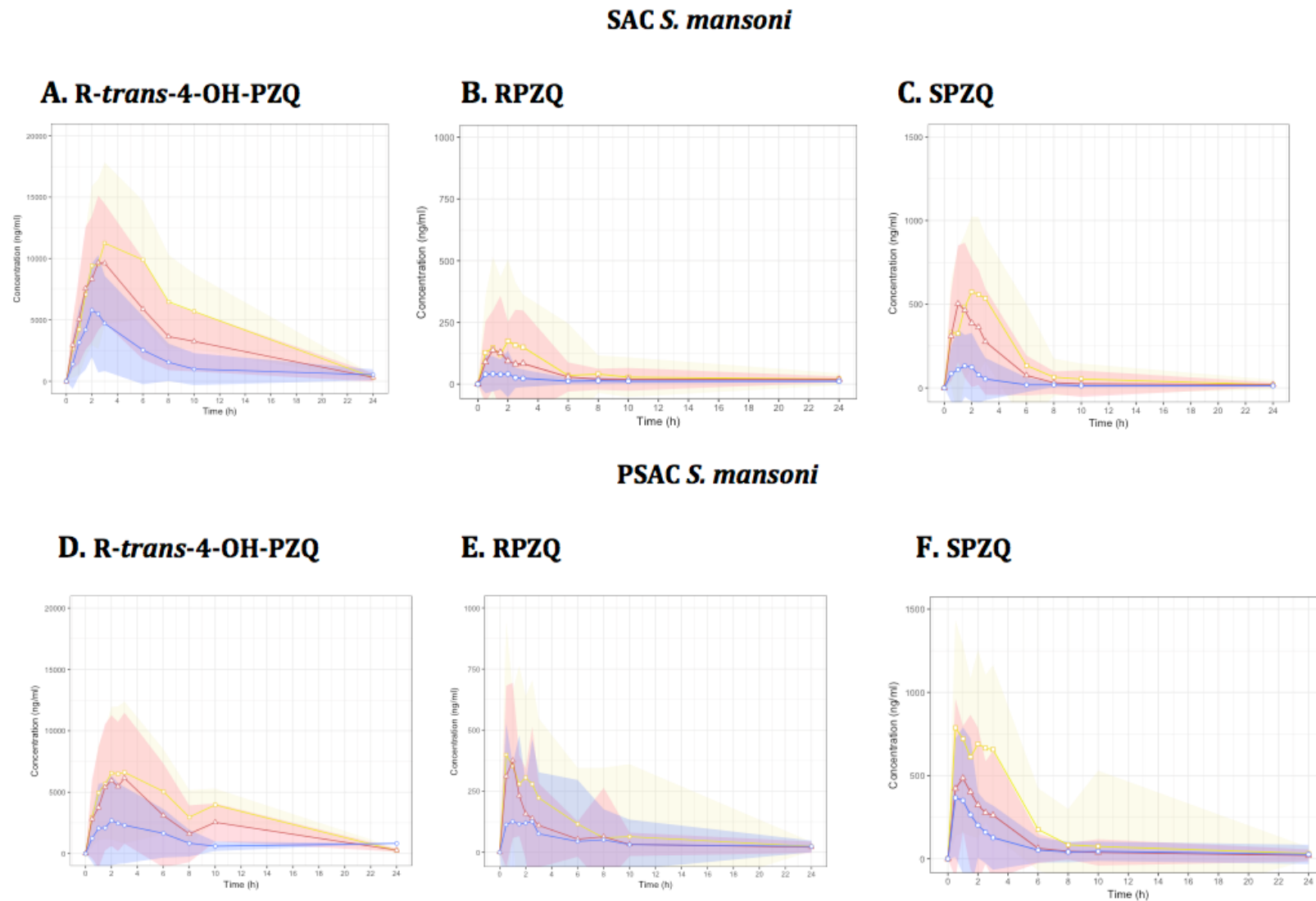
**Figure 2:** Concentration over time profiles (mean with standard deviation as the shaded area) for increasing doses of all analytes in *S. haematobium* infected children. The blue lines indicate 20 mg/kg, the red lines 40 mg/kg and the yellow lines 60 mg/kg.
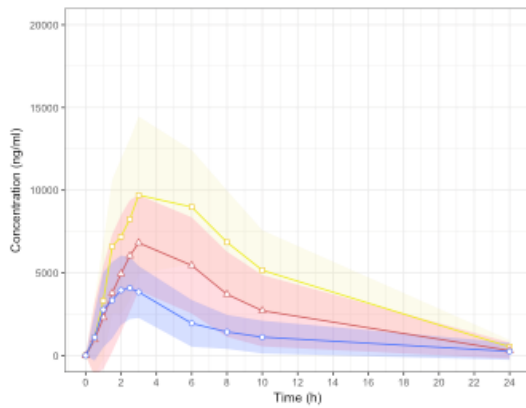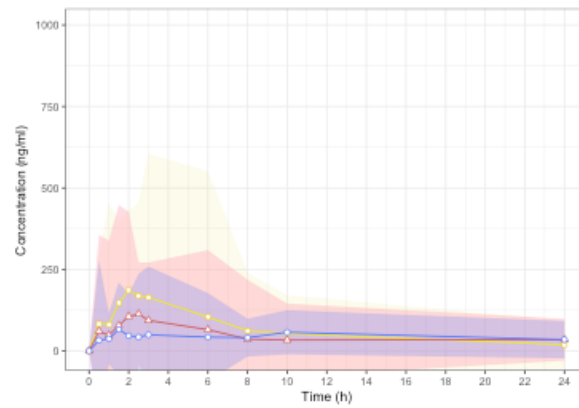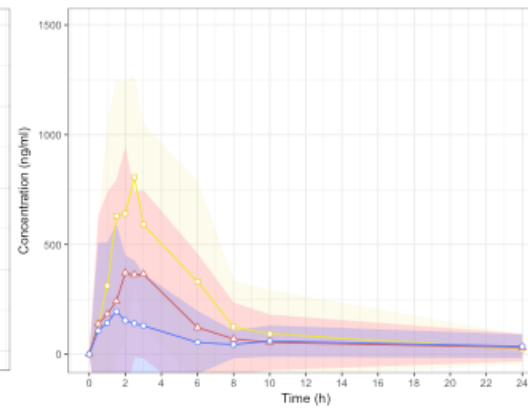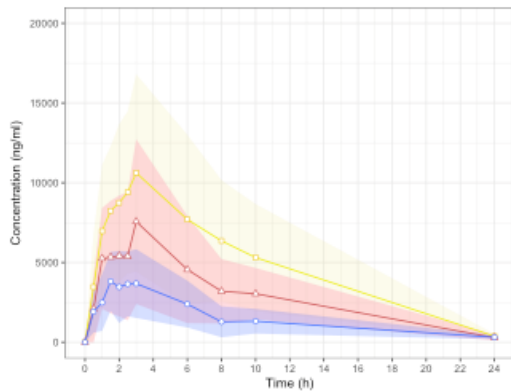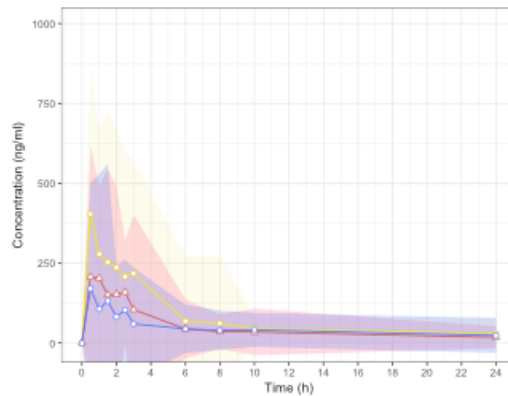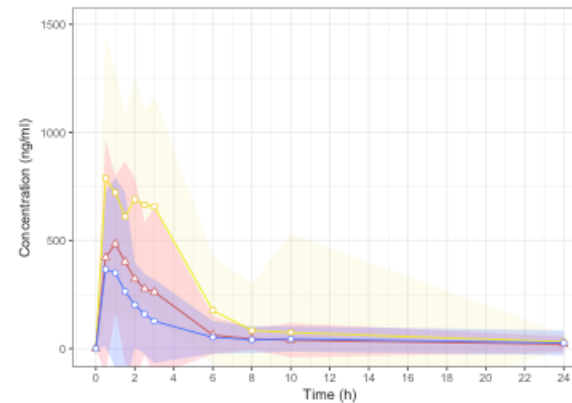
We did not observe pronounced differences in half-life and $T_{max}$ parameters between SAC and PSAC and between species-specific infections (Tables 2 and 3). While we observed significantly higher exposure for the enantiomers (R- and SPZQ) in PSAC than in SAC, infected with *S. mansoni*, the exposure for the metabolite significantly lower. This finding could indicate slower metabolism or lower clearance in young children than in their older peers. Interestingly, these differences were not significant in children harbouring urinary schistosomiasis, where liver pathology is less pronounced.

**Figure 3:** AUC versus the probability of cure for RPZQ and all groups of children.
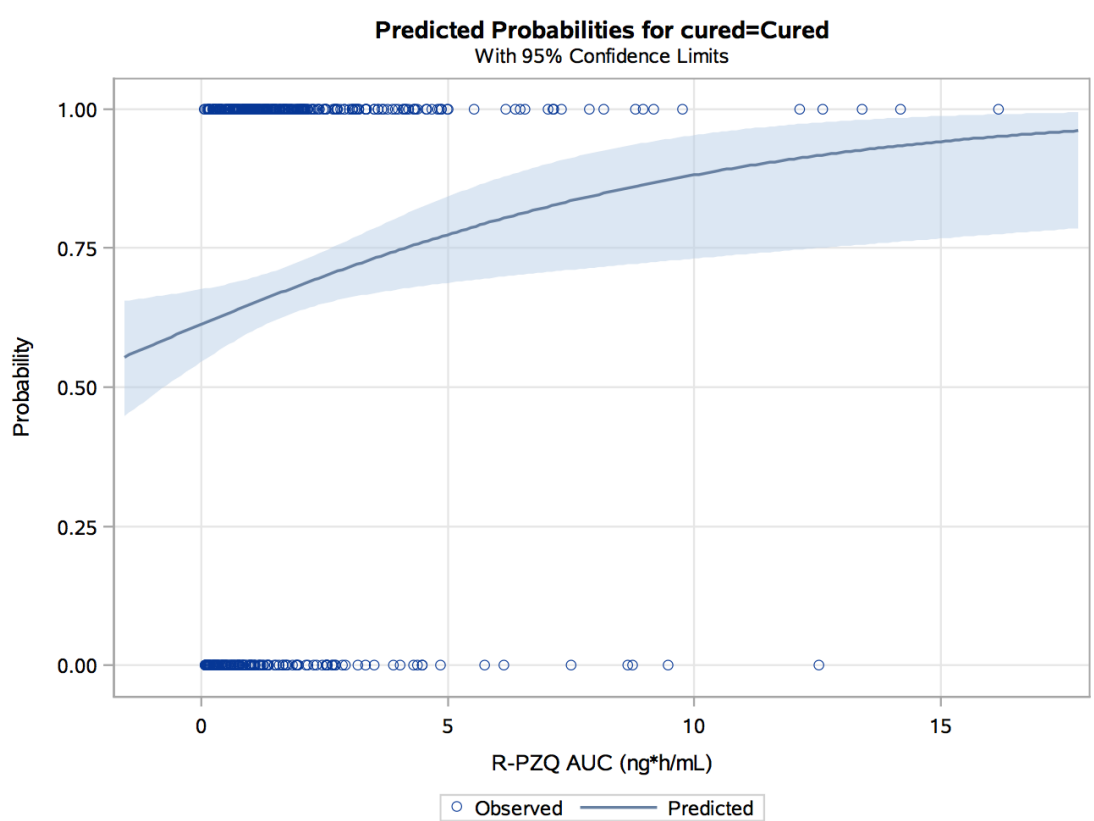


The debate about which analyte drives the antischistosomal activity of PZQ is still ongoing. In a recent PK study in *S. mansoni*-infected children, Bustinduy *et al* proposed SPZQ to be the eutomer, since it exhibited a higher and longer exposure (AUC and half–life) compared to RPZQ(16). They also suggested a correlation between AUC of PZQ, in particular that of SPZQ, and CR. In our study, we observed higher AUCs for SPZQ compared to RPZQ as well, however logistic regression analysis points to a positive relationship between the AUC and $C_{max}$ of RPZQ and the probability of cure for both infecting species and age groups,

indicating that there might be a relationship between the exposure of RPZQ and the CR. However, one should bear in mind that the sample size on which our assumptions are based on is quite small. As CRs increased with increasing dosages, we had a few uncured children with which to sufficiently represent the exposure of this population.

**Figure 4:** $C_{max}$ versus the probability of cure for RPZQ and all groups of children.
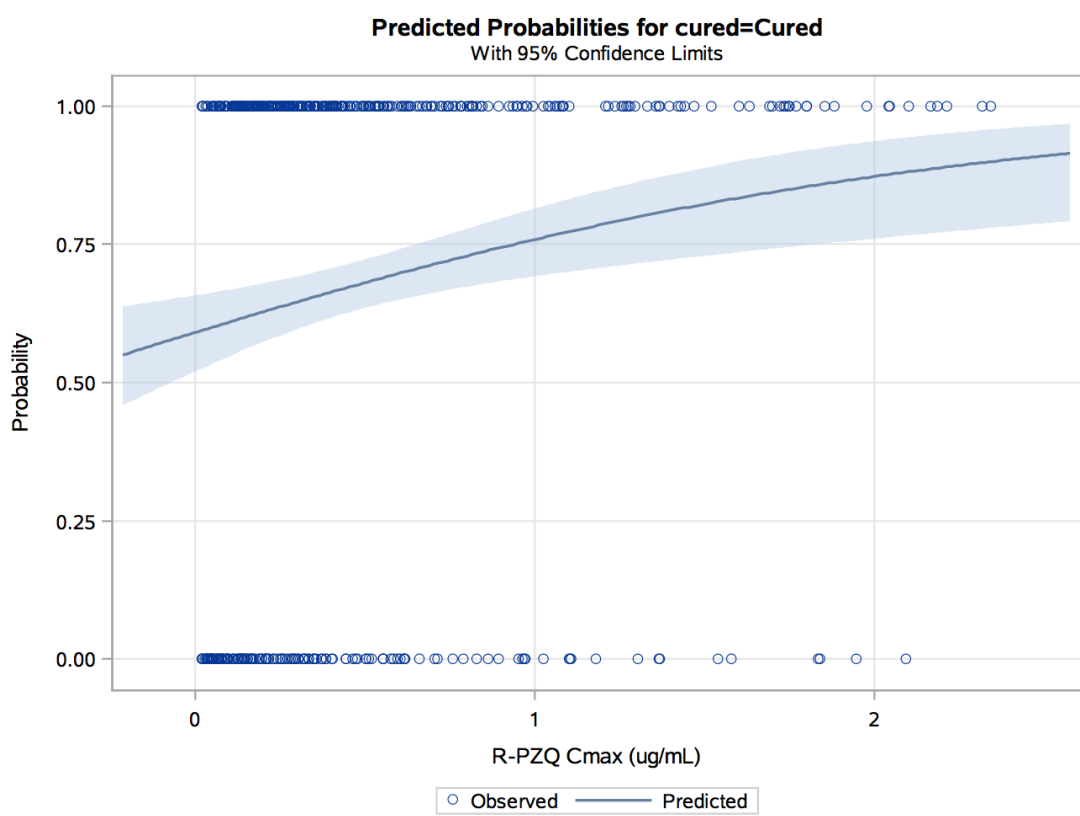


The observed interspecies differences in susceptibility to PZQ enantiomers and their impact on efficacy of the drug in humans are important issues. We recently showed that SPZQ had a higher and significant efficacy against *S. haematobium* compared to that of *S. mansoni* both *in vitro* and *in vivo* (21). It might therefore contribute to the overall activity of PZQ on *S. haematobium*. R-*trans*-4-OH-PZQ also showed signs of antischistosomal activity *in vitro* towards both species of the parasite, and this was more pronounced in the case of *S. haematobium* (21). Its contribution to the activity of RPZQ could explain a higher sensitivity of *S. haematobium* to PZQ within *in vitro*/*in vivo* frame; however in our studies we did not observe any correlation between exposure of the metabolite and the probability of cure.

In conclusion, we have described the PK processes of PZQ in a paediatric population by using three different dosages and comparing the two most prevalent species of *Schistosoma spp.* Based on our findings, the schistosome species causing infection (*S. mansoni* versus *S. haematobium*) does not play a role in influencing the PK parameters of PZQ. Age seems to have an effect though mostly with respect to $C_{max}$ and AUC in *S.mansoni* infection. While SPZQ is present in higher quantities than RPZQ, it did not show a relationship with CRs, while logistic regression indicated a positive relationship between RPZQ exposure and cure. Population pharmacokinetics studies with RPZQ are currently on going to study the patterns and eventual relationships of patient characteristics (e.g. weight, co-infections) with PK parameters in more detail.

## Methods and materials

### *Chemicals and reagents*

R- and SPZQ and R-*trans*-4-OH-PZQ were kindly provided by Merck KgaA (Darmstadt, Germany). Eleven-fold deuterated PZQ (PZQd11), acquired from Toronto Research Chemicals (Ontario, Canada), was used as an internal standard (IS). Acetonitrile, ammonium formate, ammonium acetate and formic acid of mass spectrometry (MS) grade were purchased from Sigma-Aldrich (Buchs, Switzerland). Ultrapure water was obtained using a Millipore Milli-Q water purification system (Merck Millipore, MA, USA). Human blood was supplied by the local blood donation centre (Basel, Switzerland). PZQ tablets (Cesol™, 600 mg) were donated by Merck KgaA (Darmstadt, Germany).

### *LC–MS/MS equipment*

A 6460 Series triple quadrupole liquid chromatography- mass spectrometry, LC-MS/MS, (Agilent Technologies, Basel, Switzerland) was used to perform all the measurements. The LC module consisted of a 1290 series binary pump (G1312B), followed by 1200 Series Micro Vacuum (G1379B) degasser, Agilent 1260 Infinity High Performance autosampler (G1367E), equipped with a 12900 Infinity series Thermostat (G1330B) and electrospray ionization source (G1958–65138). MS/MS analyses were performed in positive ionization mode. Mass

Hunter Workstation software B.06.00 (Agilent Technologies, Basel, Switzerland) served to operate the instrument and analyse the data.

### *LC–MS/MS method and partial validation*

The LC-MS/MS method was adapted from a recently validated method by Meister *et al* (34). Briefly, the compounds of interest were primarily separated from remaining matrix by a column trapping system (HALO C–18, 4.6 x 5 mm, Optimize Technologies, OR, USA), to minimize instrument contamination. 10 mM ammonium acetate with 0.015% formic acid in water (mobile phase A) at a flow rate of 0.3 ml/min served as a loading solution. After 1.00 minute of loading, a mixture of 20 mM ammonium acetate and acetonitrile (1:4, mobile phase B) was used to elute the analytes from the trapping to the chiral column (Lux Cellulose–2 (150x4.6 mm, 3 μm, Phenomenex, CA, USA)), with a flow rate ascending to reach 0.4 ml/min at 3 minutes and remaining steady until 9.49 min. In the last minute (9.50–10.50), mobile phase A was used again to re-equilibrate the trapping column at a flow rate of 1.0 ml/min. LC-MS/MS parameters are summarised in Table S4 in the supplemental materials and chromatograms are depicted in Figures S1 to S2.

Partial revalidation was performed to ensure compliance with Food and Drug administration guidelines, since the LC-MS/MS method was transferred to another system (34).

In brief, selectivity was assured by analysing 18 blank DBS extracts compared to double blank (pure extraction solvent without IS) DBS samples to exclude potential endogenous substances interference. Calibration lines (CL) were plotted as analyte peak area (normalised to IS) vs. concentration and fitted using linear regression. The suitable weighting factor ($1/x^2$) was chosen to result in the minimal total error. Accuracy and precision were determined using quality control (QC) samples with known analyte concentration, 6 replicates of four concentration levels across the linearity range (lower level of quantification (LLOQ), low, middle and high concentrations). By comparing measured to nominal concentration (in percentage), accuracy was calculated. Inter- and intra-batch precision was analysed by measuring 3 batches of samples per day on 3 different days. Precision of +/− 15% (+/− 20% at LLOQ) was considered adequate, while the acceptable accuracy ranged from 85–115 %

(80–120% at LLOQ). Possible enhancing or suppressive matrix effects were evaluated by comparing the signal of biological matrix (DBS extract) and organic solvent, both spiked with analytes. The stability of all entities has been reported elsewhere (28, 34).

*Quality control and standard preparation*

The preparation of the solutions was adapted from Meister *et al* (34). Briefly, stock solutions of the analytes were prepared freshly in acetonitrile to obtain 1.0 mg/ml concentrations for R- and SPZQ, 5.0 mg/ml for R-*trans*-4-OH–PZQ and 1.25 mg/ml for PZQd11. A working solution of 53.0 μg/ml PZQ and 1071.0 μg/ml R-*trans*-4-OH-PZQ was used to prepare fresh CL and QC samples and diluted with acetonitrile to reach a range of 0.2–53.0 for PZQ and 4.0–1071.0 μg/ml for R-*trans*-4-OH-PZQ. The IS solution was diluted 4:1 (v/v) with water to produce extraction solvent.

CL and QC samples were prepared freshly for every analytical run. For the CL, blank human blood was spiked with the mixture of analytes to reach a final concentration range of 2.232 to 0.009 (LLOQ) μg/ml for R- and SPZQ, and of 44.6 to 0.179 (LLOQ) μg/ml for R-*trans*-4-OH-PZQ. For the QC, 6 samples of high, medium, low and LLOQ concentrations were prepared similarly and spotted on the DBS cards (903 Protein Saver Snap Apart Cards®, Whatman, UK). Disks of 5 mm in diameter were punched from DBS samples and extracted with 200 μl of extraction solvent.

*Study design and ethical considerations*

Ethical clearance was obtained by the Ethics Committee of North-western and Central Switzerland (EKNZ 162/2014) and the Ministère de la Santé et de l'Hygiène Publique in Côte d'Ivoire (CNER, 037/MSLS/CNER-dnk). The trial was registered as International Standard Randomised Controlled Trial (ISRCTN15280205). The *S. mansoni* study was carried out in the Azaguié region of Côte d'Ivoire between November 2014 and February 2015 (23, 24). *S. haematobium* PK study was implemented in Azaguié, Côte d'Ivoire between November 2015 and January 2016 (24).

94 PSAC (age 2–5 years) and 135 SAC (age 6–15 years) with confirmed *S. mansoni* infection using duplicate Kato-Katz method were included in the PK study. 122 PSAC and 137 SAC

infected with *S. haematobium* (diagnosed using the urine filtration method) participated in the second PK trial. All children were stratified according to infection intensity and randomised to receive 20, 40 or 60 mg/kg PZQ or placebo (data not shown). Randomisation, masking, field and laboratory procedures have been presented elsewhere (Coulibaly *et al*, Coulibaly *et al* submitted) (23). PZQ tablets (600 mg Cesol™) were administered according to the calculated dose per kilogram of body weight in half (*S. mansoni*) and quarter (*S. haematobium*) tablet increments. Since the bioavailability of PZQ is known to be influenced by food (8, 35), the treatment was administered after a standardised breakfast. For PSAC, the tablets were crushed and the powder was suspended in a mixture of sugar syrup and water to mask the taste.

### *DBS samples collection*

Capillary blood (+/− 0.1 ml) was obtained using a finger pricker (e.g. Accu-check Softclix Pro®; Roche, Switzerland) at 0:00, 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 6:00, 8:00, 10:00 and 24:00 hours after treatment with PZQ. Four drops of blood at each time point were transferred on the DBS cards, dried for approximately 1 h and stored afterwards in plastic bags with desiccant. The cards were transferred to Basel and kept at −80°C.

### *Non–compartmental analysis*

The following PK parameters of RPZQ, SPZQ and *trans*-4-OH-PZQ were obtained using the Winonlin software (version 5.2; Certara, Princeton, NY, USA):

$C_{max}$       maximal blood concentration (μg/ml)

$T_{max}$       time needed to reach $C_{max}$  (h)

$AUC_{last}$   area under the curve between 0 and the last positive concentration (h*μg/ml)

$T_{1/2}$        terminal half-life; time in which half of the absorbed drug is eliminated (h)

$C_{max}$ and $T_{max}$ are observed parameters, while $T_{1/2}$ was calculated as $T_{1/2} = \ln2/\lambda$. Constant of elimination ($\lambda$) was determined by the program using non-linear regression of the natural logarithm of concentration values in the elimination phase. Since the absorption of PZQ is erratic, half-life was only estimated for those patients who had a single peak in concentration and where the elimination phase was well estimated by the algorithm. $AUC_{last}$ was calculated

from 0 to the last quantifiable positive concentration, using linear trapezoidal rule. Volume of distribution and renal clearance were not reported, since bioavailability is needed to estimate them adequately. PK parameters were estimated for each study participant and the median and interquartile range (IQR) were calculated for patients of each treatment arm. AUC and $C_{max}$ values of both R- and SPZQ were compared between cured and uncured patients. Cure rate (CR) was defined as the percentage of patients who were positive for infection at the baseline but were not excreting eggs at the follow up. Egg reduction rate (ERR) was expressed as the geometric mean egg output after treatment divided by the geometric mean egg output before treatment (36).

To compare the PK parameters, statistical analysis was conducted using Prism (version 7.03, GraphPad, CA, USA). Mann-Whitney or Kruskal-Wallis multiple comparison statistical test, depending on the number of groups being compared, were used to study parameters of children in different treatment arms, age groups and parasite species. A *P* value of $<0.05$ was considered as statistically significant.

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**Transparency declarations**

None to declare.

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## Supplemental material

**Table 1:** Optimized MS/MS parameters for detection of PZQ enantiomers, main human metabolite and PZQd11 on 6460 Series Triple Quadrupole LC-MS/MS

| Parameters | PZQ | *Trans*-4-OH-PZQ | PZQd11 |
|---|---|---|---|
| | | **COMPOUNDS** | |
| ESI polarity | | positive | |
| m/z | 313/203 | 324/204 | 324/204 |
| Fragmentor | 110 | 110 | 140 |
| Cell accelerator voltage | | 7 | |
| Collision energy (CE) | 10 | 15 | 15 |
| Gas temperature (°C) | | 350 | |
| Gas flow (l/min) | | 12 | |
| Ion source temperature (°C) | | 400 | |
| Nebulizer (psi) | | 40 | |
| Sheath gas temperature (°C) | | 400 | |
| Sheath gas flow (l/min) | | 12 | |
| Chamber current (nÅ) | | 32 | |

**Figure S1:** Chromatogram of a pure internal standard PZQd11.
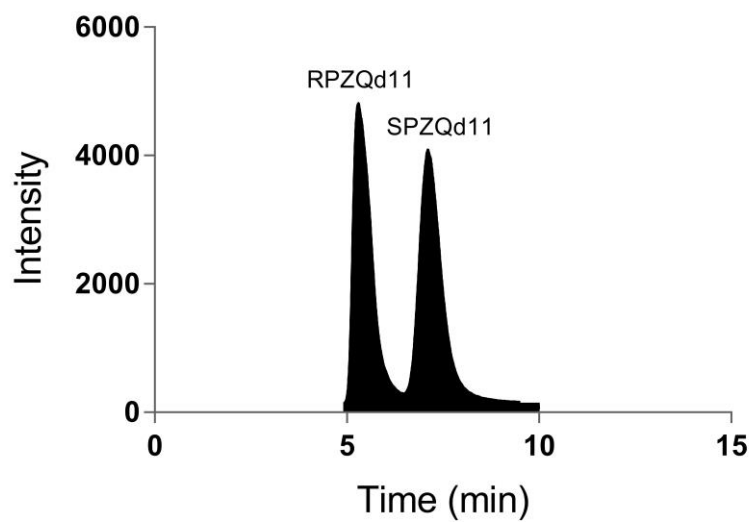


**Figure S2:** Chromatogram of *trans*-4-OH-PZQ, RPZQ and SPZQ at the upper level of quantification
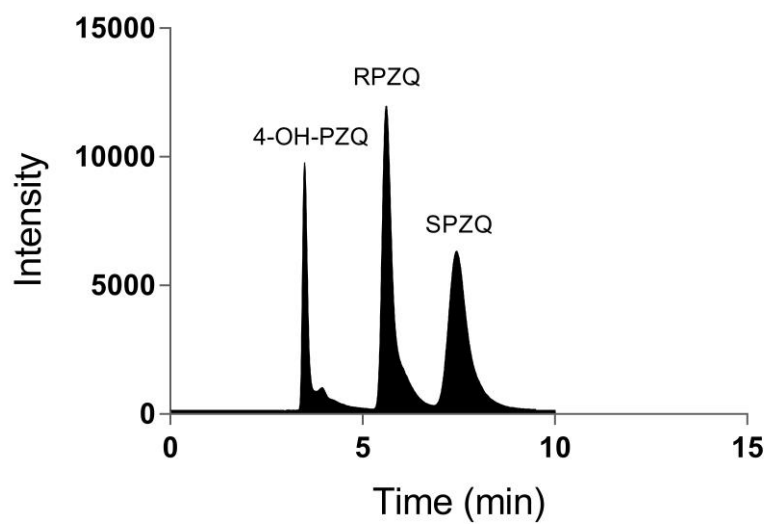
**Table S2:** Intra- and inter-assay accuracy and precision for RPZQ, SPZQ and *trans*-4-OH-PZQ

| RPZQ | Intra-assay [a] ($n_{min}$=12)[a] | | | Inter-assay ($n_{min}$=12) [a] | | |
|---|---|---|---|---|---|---|
| Nominal concentration (µg/mL) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) |
| **0.009** | 0.009 | 99 | 6 | 0.009 | 102 | 9 |
| **0.0161** | 0.016 | 105 | 6 | 0.016 | 102 | 8 |
| **0.161** | 0.166 | 107 | 6 | 0.158 | 101 | 9 |
| **1.61** | 1.58 | 102 | 5 | 1.59 | 102 | 7 |
| **SPZQ** | Intra-assay [a] ($n_{min}$=12)[a] | | | Inter-assay ($n_{min}$=12) [a] | | |
| Nominal concentration (µg/mL) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) |
| **0.009** | 0.009 | 98 | 6 | 0.009 | 98 | 10 |
| **0.0161** | 0.016 | 103 | 4 | 0.016 | 102 | 8 |
| **0.161** | 0.158 | 102 | 9 | 0.58 | 101 | 9 |
| **1.61** | 1.58 | 102 | 5 | 1.60 | 103 | 8 |
| **Trans-4-OH-PZQ** | Intra-assay [a] ($n_{min}$=12)[a] | | | Inter-assay ($n_{min}$=12) [a] | | |
| Nominal concentration (µg/mL) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) | Mean concentration (µg/mL) | Accuracy (%) | RSD (%) |
| 0.179 | 0.178 | 98 | 8 | 0.178 | 99 | 10 |
| 0.321 | 0.32 | 101 | 6 | 0.33 | 102 | 7 |
| 3.21 | 3.24 | 104 | 7 | 3.12 | 100 | 9 |
| 32.0 | 30.9 | 99 | 5 | 31.87 | 102 | 6 |

[a]$n_{min}$ is the minimal amount of replicate measurements used for each mean concentration calculation.

**Table S3:** Matrix effects for all analytes

| Analyte | **RPZQ** | **SPZQ** | | *Trans*-**4-OH-PZQ** |
|---|---|---|---|---|
| Nominal concentration (μg/mL) | **Matrix effect ± RSD (%)** | **Matrix effect ± RSD (%)** | Nominal concentration (μg/mL) | **Matrix effect ± RSD (%)** |
| **0.0161** | 106 ± 4 | 103 ± 5 | **0.321** | 103± 5 |
| **0.161** | 103 ± 2 | 94 ± 4 | **3.21** | 93 ± 3 |
| **1.61** | 103 ± 2 | 97 ± 2 | **32.0** | 90 ± 2 |

**Table S4:** Selectivity and carryover

| **Analyte** | **Average peak area (n = 18)** | | | **Signal to noise (LLOQ to blank)** | **Carryover %** |
|---|---|---|---|---|---|
| | Solvent | Double blank | Blank DBS extract | | |
| RPZQ | 1.7 ± 1.6 | 2.7 ± 3.8 | 96 ± 12 | 6 ± 1 | 0.6 ± 0.6 |
| SPZQ | | | | | |
| *Trans*-4-OH-PZQ | 1.3 ± 1.2 | 1.1 ± 1.0 | 90 ± 8 | 6 ± 1 | 0.3 ± 0.5 |
| | 3.0 ± 3.6 | 4.7 ± 3.9 | 7 ± 6.1 | 100 ± 80 | 0 |

# Chapter 4

# Evaluation of a novel micro-sampling device, Mitra™, in comparison to dried blood spots, for analysis of praziquantel in *Schistosoma haematobium*-infected children in rural Côte d'Ivoire

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**Evaluation of a novel micro-sampling device, Mitra™, in comparison to dried blood spots, for analysis of praziquantel in *Schistosoma haematobium*-infected children in rural Côte d'Ivoire**

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**Abbreviations**

$C_{max}$, maximal blood concentration; $T_{max}$, time needed to reach maximal blood concentration; $T_{1/2}$, half-life of the drug; AUC, area under the concentration-time curve; DBS, dried blood spots; PZQ, praziquantel, PK, pharmacokinetics; SAC, school-aged children, LC-MS/MS, liquid chromatography tandem mass spectrometry; CL, calibration line; QC, quality control; LLOQ, lower level of quantification

**Key words:** praziquantel, schistosomiasis, pharmacokinetics, dried blood spots, Mitra™, micro-sampling

**Abstract**

Pharmacokinetic (PK) studies with paediatric populations are increasing in importance for drug development. However, conventional PK sampling methods are characterised by invasiveness and low patient adherence, unsuitable for use with sensitive population, such as children. Mitra™ is a novel volumetric absorptive micro-sampling device, which offers an alternative to the dried blood spotting (DBS) technique, a current popular sampling technique within PK studies. We tested Mitra™ for the first time in the framework of a randomized controlled trial in rural Côte d'Ivoire. Thirty-five school-aged children, infected with *Schistosoma haematobium*, were sampled with both DBS and Mitra™, at 10 time points after treatment with praziquantel (PZQ). A extraction method for PZQ from Mitra™ was developed, optimised and validated. Analytes, namely R- and S-praziquantel (R-/SPZQ) and the main human metabolite, R-*trans*-4-OH-praziquantel, were measured using liquid chromatography-tandem mass spectrometry and the results were compared with Bland-Altman analysis to determine

agreement between matrices. PK parameters, such as maximal plasma concentration and area under the concentration-time curve, were estimated using non-compartmental analysis.

While we observed strong positive correlation ($R^2 > 0.98$) and agreement between both matrices within the calibration line and quality control samples, Mitra™ revealed higher concentrations of all the analytes in the majority of patients' samples compared to DBS sampling, namely 63% samples for RPZQ, 49% for SPZQ and 78% for the metabolite were overestimated. While $T_{1/2}$ and $T_{max}$ were in agreement between both matrices, area under the curve and maximal blood concentration were up to 2x higher for Mitra™ samples, with $P < 0.005$ for all parameters except $C_{max}$ of SPZQ, which was not significantly different between the two matrices. The reasons for the higher PZQ concentrations, more pronounced in incurred Mitra™ samples compared to spiked samples, are yet to be fully explored. Mitra™ appears superior to DBS in terms of simplicity and practicality however labelling issues and the high price of Mitra™ are difficult to overlook.

## 1. Introduction

Clinical pharmacokinetic (PK) studies are crucial to determine the relationship between administered drug dosage and the resulting concentration of active substance in body fluids, particularly in patients with impaired renal or liver function or paediatric populations (1, 2). Physiological differences between children and adults, affecting absorption, distribution, metabolism and elimination of drugs have been well described and therefore PK processes and drug dosages cannot be simply extrapolated from adults to children (1, 3, 4). Use of a drug without supporting evidence for a tailored dose can result in sub-optimal efficacy and unpredictable adverse events (2). The number of paediatric clinical trials has been increasing in the recent past, due to new legislation enforced by both European Medicines Agency and American Food and Drug Administration (FDA) (5). Nonetheless, clinical studies involving PK analysis are especially difficult to conduct in children, given that intensive sampling schemes and invasive sampling techniques, (i.e. venepuncture drawing large volumes of blood) are often applied (6). While bruising is a common consequence of frequent sampling, venepuncture has also been associated with infection of the sampling site (3). Therefore, micro-sampling techniques have been evaluated in the recent past in order to carry out paediatric PK studies in a more ethical manner(2, 7).

Lately, the dried blood spot (DBS) technique, commonly used in new-born genetic screening, has been gaining in popularity for PK studies given its low-invasiveness and hence increased patient adherence (8, 9). DBS are droplets of capillary blood, obtained after a finger prick and deposited on a filter paper (10, 11). However, DBS require either an exact volume of blood spotted or a sub-punch of a certain diameter for quantification (12–14). Unfortunately, in non-controlled settings, such as rural areas of the tropics and especially when working with young children, inhomogeneous spots of irregular shape or insufficient size can be common, giving rise to unreliable results (15, 16). Furthermore, the haematocrit is well known to have an effect on the accurate quantification of analytes from DBS(11, 13, 17–19)

To overcome these obstacles, a new device, Mitra™, based on absorptive volumetric micro-sampling, was launched recently (17). The volume of blood it absorbs is controlled by the porosity and quantity of polymeric material, eliminating a need for sampling aids and assuring a precise and repeatable sampled volume, regardless of haematocrit (12, 13, 17). Mitra™ has been tested in the laboratory using both human and animal blood, however it has not been evaluated in human clinical PK studies yet (12, 20, 21).

The aim of the present work was to compare the quantification of the anthelmintic drug praziquantel (PZQ) extracted from DBS and Mitra™, in the framework of a PK trial. 35 school-aged children (SAC) with a confirmed *Schistosoma haematobium* infection were sampled with both sampling techniques at 10 time points post treatment with 20, 40 or 60 mg/kg PZQ (Kovac et al, *submitted*, Coulibaly et al, *submitted*). A method for extracting both enantiomers of PZQ, namely R- and S-praziquantel (R-/SPZQ) and the main human metabolite (R-*trans*-4-OH-PZQ) from Mitra™ was developed, optimised and validated in compliance with FDA guidelines (22). Liquid chromatography- tandem mass spectrometry (LC-MS/MS) analysis was performed. DBS and Mitra™ results were compared using Bland-Altman statistics and PK parameters were calculated using non-compartmental analysis. Our findings will compliment the laboratory evaluations of Mitra™ and contribute to knowledge crucial for decision-making when choosing a suitable sampling technique for PK trials.

## 2. Methods and materials

### *2.1 Chemicals and reagents*

Merck KgaA (Darmstadt, Germany) provided enantio-pure R- and SPZQ and the main human metabolite, R-*trans*-4-hydroxy-PZQ (*trans*-4-OH-PZQ). As internal standard (IS), deuterated PZQ (PZQd11), purchased from Toronto Research Chemicals (Ontario, Canada), was used. Solvents (methanol, acetonitrile, isopropanol) and reagents (ammonium formate, ammonium acetate and formic acid) of mass spectrometry grade were acquired from Sigma-Aldrich (Buchs, Switzerland). Ultrapure water was filtered using a Millipore Milli-Q water purification system (Merck Millipore, MA, USA). Human blood was supplied in lithium heparin-coated vacutainer tubes (BD, Allschwil, Switzerland) by the local blood donation centre (Basel, Switzerland). DBS 903 Protein Saver Snap Apart cards were purchased from Whatman (GE Healthcare Life Sciences, Cardiff, UK) and Mitra™ (10 µl) was ordered from Neoteryx® (Torrance, CA, USA). PZQ tablets (Cesol™, 600 mg) were donated by Merck KgaA (Darmstadt, Germany).

### *2.2 LC-MS/MS equipment and DBS sample preparation method*

The LC-MS/MS equipment and DBS method used were described in details by Kovac *et al*. (submitted). Briefly, DBS (5 mm diameter) were punched out of the filter cards. Samples were extracted with 200 µl acetonitrile:water (4:1, v/v) containing 400 ng/ml IS, thermo-mixed, ultra-sonicated and filtered through 2 µm PVDF membrane filter plates (Corning Life Sciences, Tewksbury, MA, USA) into 96-well plates by centrifugation (10 min at 2250 ×g and 25°C).

A column-trapping system (HALO C–18, 4.6 x 5 mm, Optimize Technologies, OR, USA) was used to remove the analytes from the remaining matrix before eluting to the main chiral column (Lux Cellulose–2 (150x4.6 mm, 3 µm, Phenomenex, CA, USA)), for separation. Ammonium acetate (aqueous, 10 mM) with 0.015% formic acid served as mobile phase A, while mobile phase B consisted of a mixture of ammonium formate (20 mM) and acetonitrile (1:4, v/v).

All measurements were performed on a 6460 Series triple quadrupole LC-MS/MS (Agilent Technologies, Basel, Switzerland). Mass Hunter Workstation software B.06.00 (Agilent Technologies, Basel, Switzerland) was used to operate the instrument and analyse the data.

### 2.3 Mitra™ sample preparation method optimisation and validation

A method for extracting R-, SPZQ and R-*trans*-4-OH-PZQ from Mitra™ was developed and validated in compliance with the FDA guidelines for bioanalytical method validation (22). A volume of 200µl of a mixture of acetonitrile and water (4:1, v/v) was used as an extraction solvent (400 ng/ml IS). Samples were then thermo-mixed 5 min at 1400 rpm at room temperature followed by ultra-sonication during 40 min.

Matrix effects and relative and total recovery were estimated at three different concentration levels (low, medium and high calibration range). Possible enhancing or suppressive matrix effects were evaluated by comparing the signal of biological matrix (blank blood Mitra™ extract) and blank extraction solvent, both spiked with analytes. Relative recovery was evaluated by comparing Mitra™ extracts of blood spiked with analytes to blank blood Mitra™ extracts spiked after extraction with corresponding concentrations, to estimate the uniformity of the extraction procedure. Mitra™ spiked blood samples compared to blank extraction solvent spiked with analytes, yielded total recovery. The limits were set to 85-115% for all three parameters.

Linearity of the selected method was evaluated in the calibration line (CL) range chosen for analysis. Analyte peak areas were normalised to those of the IS, plotted versus concentration and fitted with linear regression. Quality control (QC) samples were prepared in six replicates at four concentrations across the linearity range (lower limit of quantification (LLOQ), low, middle and high concentrations), to determine accuracy and precision. Inter- batch precision was analysed by comparing three batches of samples, extracted and measured on three different days, while intra-batch precision was determined by comparing three batches, extracted and measured on the same day. A precision of $+/-$ 15% ($+/-$ 20% at LLOQ) was considered adequate, while the acceptable accuracy ranged from 85–115 % (80–120% at

LLOQ). ). The haematocrit (HTC) effect was evaluated by including different HTC values in the QC sample preparation (25, 30, 35, 40, 45 and 50% HTC), as described below.

4 h bench-top stability, 72 h auto-sampler/fridge stability, short-term (72 h) freezer stability and long-term (2 months) freezer stability at -80 °C of both PZQ enantiomers and the main metabolite in Mitra™ were evaluated and linearity, accuracy and precision calculated.

## *2.4 Calibration line and quality control sample preparation*

Mitra™ and DBS CL samples were prepared freshly before every analytical run by spiking the blood of average HTC (35%) with a mixture of analytes to reach a concentration of 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 (LLOQ) µg/ml for R- and SPZQ, and of 50, 20, 10, 5, 2, 1, 0.5 and 0.2 (LLOQ) µg/ml for R-*trans*-4-OH-PZQ. QC samples were similarly obtained by spiking blood in six replicates, using different HTC values (25–50%), to reach final concentrations of 1.75, 0.175, 0.0175 and 0.01 µg/ml (high, medium, low and LLOQ concentrations) for R- and SPZQ, and to 35.0, 3.5, 0.35 and 0.2 µg/ml for the metabolite. DBS samples were prepared by depositing droplets of blood (20 µl) on filter paper cards, dried overnight and stored at room temperature in plastic bags, containing silica gel desiccants. Mitra™ samples were prepared similarly, by dipping the tip of Mitra™ into the blood and allowed to dry overnight in the respective container. DBS and Mitra™ samples were extracted and measured with LC-MS/MS as described in details above.

## *2.5 Ethical considerations and field sample collection*

The *S. haematobium* PK study was performed in Azaguié, Côte d'Ivoire between November 2015 and January 2016(23). Ethical clearance was obtained by the Ethics Committee of Northwestern and Central Switzerland (EKNZ 162/2014) and the Ministère de la Santé et de l'Hygiène Publique in Côte d'Ivoire (CNER, 037/MSLS/CNER-dnk). The trial was registered as International Standard Randomised Controlled Trial (ISRCTN15280205).

35 SAC with a confirmed *S. haematobium* infection (using urine filtration) were treated with 20, 40 or 60 mg/kg of PZQ, following a standardised meal. The exact dose of PZQ (600 mg Cesol™ tablets) was determined based on the body weight and the drug administered in quarter tablet increments. Capillary blood (+/− 0.1 ml) was obtained using a finger pricker

(e.g. Accu-check Softclix Pro®; Roche, Switzerland) at 0:30, 1:00, 1:30, 2:00, 2:30, 3:00, 6:00, 8:00, 10:00 and 24:00 hours post treatment with PZQ. Four drops of 20 µl blood at each time point were transferred on the DBS cards using 75 µl glass capillaries coated with heparin, allowed to dry and subsequently stored in plastic bags with desiccant. At the same time, one Mitra™ sample of 10 µl was taken at every time point from each patient. Mitra™ samples were stored in pre-designed racks and allowed to dry for several hours. The samples were transferred to Basel and kept at −80 °C until assayed.

### 2.6 Statistical analysis

To assess the agreement between both matrices, Pearson's correlation coefficient and Bland-Altman statistics were used, plotting the percentage difference between the matrices (DBS/Mitra™) against the average concentrations (24). All statistical analyses were performed using Prism (version 7.03, GraphPad, CA, USA). PK parameters of RPZQ, SPZQ and *trans*-4-OH-PZQ were obtained using the Winonlin software (version 5.2; Certara, Princeton, NY, USA). The following parameters were estimated:

$C_{max}$         maximal blood concentration (µg/ml)

$T_{max}$         time needed to reach $C_{max}$   (h)

$AUC_{last}$     area under the curve between 0 and the last positive concentration (h*µg/ml)

$T_{1/2}$          terminal half-life; time in which half of the absorbed drug is eliminated (h)

$T_{1/2}$ was calculated as $T_{1/2} = \ln2/\lambda$. Constant of elimination ($\lambda$) was determined with non-linear regression of the natural logarithm of concentration values in the elimination phase. Trapezoidal rule was applied to calculate $AUC_{last}$ (AUC) from 0 to the last quantifiable concentration. PK parameters were estimated for all children and the median, interquartile range (IQR), minimum and maximum were reported. Cure rate (CR) was calculated as the percentage of patients, which were positive for *S. haematobium* infection at the baseline and not excreting eggs at the follow up. Egg reduction rate (ERR) was defined as the geometric mean egg output after treatment divided by the geometric mean egg output before treatment (25). Mann-Whitney test was used to evaluate differences in PK parameters between Mitra™ and DBS. A P-value of <0.05 was considered to be statistically significant.

## 3. Results

### 3.1 Patients' characteristics

Thirty-five SAC were sampled with both Mitra™ and DBS. Eleven children received 20 mg/kg of PZQ, while 12 children were treated with 40 mg/kg and 11 with 60 mg/kg. Median age was 8 years, with interquartile range of 7–10 years and the median weight was 22 (20–29) (IQR) kg. 20 girls and 15 boys were enrolled in the study. Infection intensity was 8 (3–22) eggs/10 ml of urine. All children, except one child in the 60 mg/kg treatment group, were cured after treatment. Characteristics of study participants are summarised in Table 1.

**Table 1:** Patient characteristics

| Patient characteristics | | | |
|---|---|---|---|
| **Treatment arm** | 20 mg/kg | 40 mg/kg | 60 mg/kg |
| **Median actual dose (IQR) in mg/kg** | 20.7 (19.4–21.4) | 40.0 (38.9–41.4) | 61.1 (60.0–61.4) |
| **No. of children** | 11 | 13 | 11 |
| **No. of girls (%)** | 8 (73) | 7 (54) | 5 (45) |
| **Median age (IQR) (yrs)** | 8 (7–9.5) | 8 (8–10) | 8 (7.5–9) |
| **Median weight (IQR) (kg)** | 22 (19–29.5) | 26 (20–29) | 22 (20.5–26) |
| **Geometric mean before treatment (eggs/ml urine)** | 6.6 | 8.2 | 7.9 |
| **Cure rate (%)** | 100 (11/11) | 100 (13/13) | 90.9 (10/11) |
| **Egg reduction rate (%)** | 100 | 100 | 99.4 |

### 3.2 Mitra™ sample preparation method validation

The correlation coefficient ($r^2$) of CLs were > 0.998 for all analytes. QCs' accuracy and precision of all analytes were in line with FDA guidelines (Table 3) and results were independent of HTC values. Consistent matrix effects were obtained within the range of 99–107%, with a RSD of less than 10%. Both relative and total recovery were in line with set requirements. Matrix effects, relative and total recovery results are summarised in the Table 2. Results of intra- and inter-day precision and accuracy evaluation are presented in the Table 3.

**Table 2:** Matrix effects, total and partial recovery of all analytes

| Nominal concentration (µg/mL) | Matrix effect ± RSD (%) | Relative recovery ± RSD (%) | Total recovery ± RSD (%) |
|---|---|---|---|
| **RPZQ** | | | |
| **1750** | 103.5 ± 2.4 | 101.9 ± 7.1 | 105.2 ± 8.9 |
| **175** | 103.2 ± 2.6 | 96.2 ± 5.5 | 98.1 ± 4.3 |
| **17.5** | 99.7 ± 5.5 | 93.1 ± 7.8 | 97.6 ± 6.5 |
| **SPZQ** | | | |
| **1750** | 102.2 ± 1.8 | 104.5 ± 4.2 | 102.2 ± 8.3 |
| **175** | 105.9 ± 2.0 | 90.1 ± 3.7 | 99.3 ± 9.7 |
| **17.5** | 100.9 ± 1.6 | 93.6 ± 4.9 | 93.6 ± 1.7 |
| **R-*trans*-4-OH-PZQ** | | | |
| **35000** | 101.3 ± 3.1 | 105.8 ± 7.2 | 106.7 ± 8.1 |
| **3500** | 99.9 ± 2.1 | 100.9 ± 6.0 | 94.5 ± 7.8 |
| **350** | 100.0 ± 7.3 | 97.1 ± 5.6 | 101.6 ± 2.8 |

Stability of the analytes was evaluated for Mitra in different conditions. Stability for DBS was previously evaluated and reported elsewhere. All three analytes, namely R-, SPZQ and R-*trans*-4-OH-PZQ proved to be stable after 4 h at room temperature, with the average accuracy and precision ranging from 91-101%. The stability of all analytes at 4 °C for 72 h (autosampler/fridge stability) was confirmed to be sufficient, with an average accuracy and precision in the lines of 93-105%. Both long- and short-term freezer stability of all analytes did not deviate from the average more than tolerated +/- 15% (+/- 20% at LLOQ). Long-term (4 months) room temperature stability was confirmed as well. Stability of freeze-thaw cycles was not evaluated since we did not freeze and thaw samples more than a single time during our procedures. Stability results are further detailed in Table 4.

**Table 3:** Inter- and intra-day precision and accuracy

| RPZQ | Intra-assay [a] (n$_{min}$=12)[a] | | | Inter-assay (n$_{min}$=12) [a] | | |
|---|---|---|---|---|---|---|
| Nominal concentration (μg/mL) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) |
| **0.010** | 0.010 | 105 | 13 | 0.011 | 109 | 7 |
| **0.0175** | 0.017 | 96 | 7 | 0.017 | 94 | 4 |
| **0.175** | 0.161 | 92 | 7 | 0.166 | 95 | 9 |
| **1.750** | 1.810 | 103 | 5 | 1.862 | 106 | 6 |
| **SPZQ** | Intra-assay [a] (n$_{min}$=12)[a] | | | Inter-assay (n$_{min}$=12) [a] | | |
| Nominal concentration (μg/mL) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) |
| **0.010** | 0.010 | 102 | 14 | 0.010 | 98 | 10 |
| **0.0175** | 0.016 | 90 | 7 | 0.016 | 90 | 3 |
| **0.175** | 0.158 | 90 | 6 | 0.167 | 95 | 8 |
| **1.750** | 1.841 | 105 | 6 | 1.874 | 108 | 5 |
| **R-*trans*-4-OH-PZQ** | Intra-assay [a] (n$_{min}$=12)[a] | | | Inter-assay (n$_{min}$=12) [a] | | |
| Nominal concentration (μg/mL) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) | Mean concentration (μg/mL) | Accuracy (%) | SD (%) |
| 0.200 | 0.214 | 107 | 6 | 0.212 | 106 | 7 |
| 0.350 | 0.302 | 86 | 1 | 0.306 | 87 | 2 |
| 3.500 | 3.170 | 91 | 6 | 3.301 | 94 | 7 |
| 35.00 | 35.59 | 102 | 6 | 36.46 | 104 | 6 |

### 3.3 DBS and Mitra™ comparison

For CL and QC samples, the data showed strong positive correlation based on Pearson's coefficient, with $R^2$ of 0.994 for RPZQ, 0.993 for SPZQ and 0.986 for the main human metabolite (P value of <0.0001 for all analytes). Both sampling methods were comparable when applying the Bland-Altman test within the full range of concentrations and HTC, with lower concentrations showing higher conformity and the differences growing with increasing

concentration. The same trend was noticed also within the correlation test. The graphs of correlation and Bland-Altman are depicted in Figure 1.

**Table 4:** Stability results

| Nominal concentration (µg/mL) | 4 h bench-top stability (average ± SD (%)) | 72 h at 4 °C stability (average ± SD (%)) | Short-term freezer stability (average ± SD (%)) | Long-term freezer stability (average ± SD (%)) |
|---|---|---|---|---|
| **RPZQ** | | | | |
| 1750 | 95.22 ± 10.79 | 100.50 ± 1.93 | 102.76 ± 10.43 | 91.62 ± 5.02 |
| 175 | 94.75 ± 5.06 | 96.78 ± 8.21 | 88.76 ± 3.08 | 90.01 ± 3.34 |
| 17.5 | 97.91 ± 7.95 | 101.10 ± 9.39 | 101.93 ± 3.77 | 94.99 ± 4.05 |
| 10 | 91.07 ± 4.69 | 99.98 ± 7.07 | 104.28 ± 8.36 | 96.21 ± 7.20 |
| **SPZQ** | | | | |
| 1750 | 100.38 ± 11.64 | 100.50 ± 2.07 | 101.07 ± 7.17 | 93.30 ± 5.85 |
| 175 | 98.25 ± 6.43 | 95.55 ± 6.24 | 92.43 ± 5.06 | 88.75 ± 2.46 |
| 17.5 | 91.47 ± 4.25 | 97.99 ± 9.47 | 99.10 ± 11.32 | 95.13 ± 6.63 |
| 10 | 101.39 ± 10.04 | 99.89 ± 10.09 | 9.50 ± 5.22 | 93.96 ± 9.61 |
| **R-*trans*-4-OH-PZQ** | | | | |
| 35000 | 100.63 ± 10.81 | 104.60 ± 1.75 | 99.41 ± 1.23 | 98.80 ± 6.11 |
| 3500 | 91.72 ± 4.10 | 96.46 ± 5.63 | 94.53 ± 6.85 | 89.57 ± 3.02 |
| 350 | 92.33 ± 2.63 | 94.79 ± 6.89 | 95.83 ± 4.97 | 87.76 ± 2.65 |
| 200 | 96.35 ± 7.86 | 93.11 ± 3.41 | 95.30 ± 4.39 | 91.49 ± 3.68 |

Patients' samples were tested for the agreement between the two matrices in the same manner. Compared to R- and SPZQ, the metabolite was measured in higher quantities. Mitra™ samples reached higher values of analyte concentration compared to DBS samples for all three analytes for most of the patients. 63% (192/305) samples had higher values measured from Mitra™ for RPZQ and 78% (240/308) for the metabolite while for SPZQ the percentage was slightly lower, namely 49% (148/300). Only small percentages of samples was comparable between Mitra™ and DBS for the metabolite (13.6%), RPZQ (22%) and SPZQ (35%). Graphs in Figure 2 illustrate mean concentrations over time with 95% confidence intervals (dashed lines) and Bland-Altman graphs (dashed lines indicating upper and lower 95% limit of agreement and red dashed line the mean difference) for both matrices and all analytes.

PK parameters are summarised in Table 5 for all three analytes and both matrices. While half-life and $T_{max}$ did not differ significantly in value between DBS and Mitra™, $C_{max}$ and AUC were up to 2x higher based on concentrations measured in Mitra™, for all analytes and were significantly different (P<0.005) for RPZQ and the metabolite. For SPZQ, only $C_{max}$ did not differ significantly between both matrices.

**Figure 1:** Graphs of correlation (A, B, C) and Bland-Altman (a, b, c) for CL and QC samples (dashed lines indicating upper and lower 95% limit of agreement and red dashed line the mean difference).
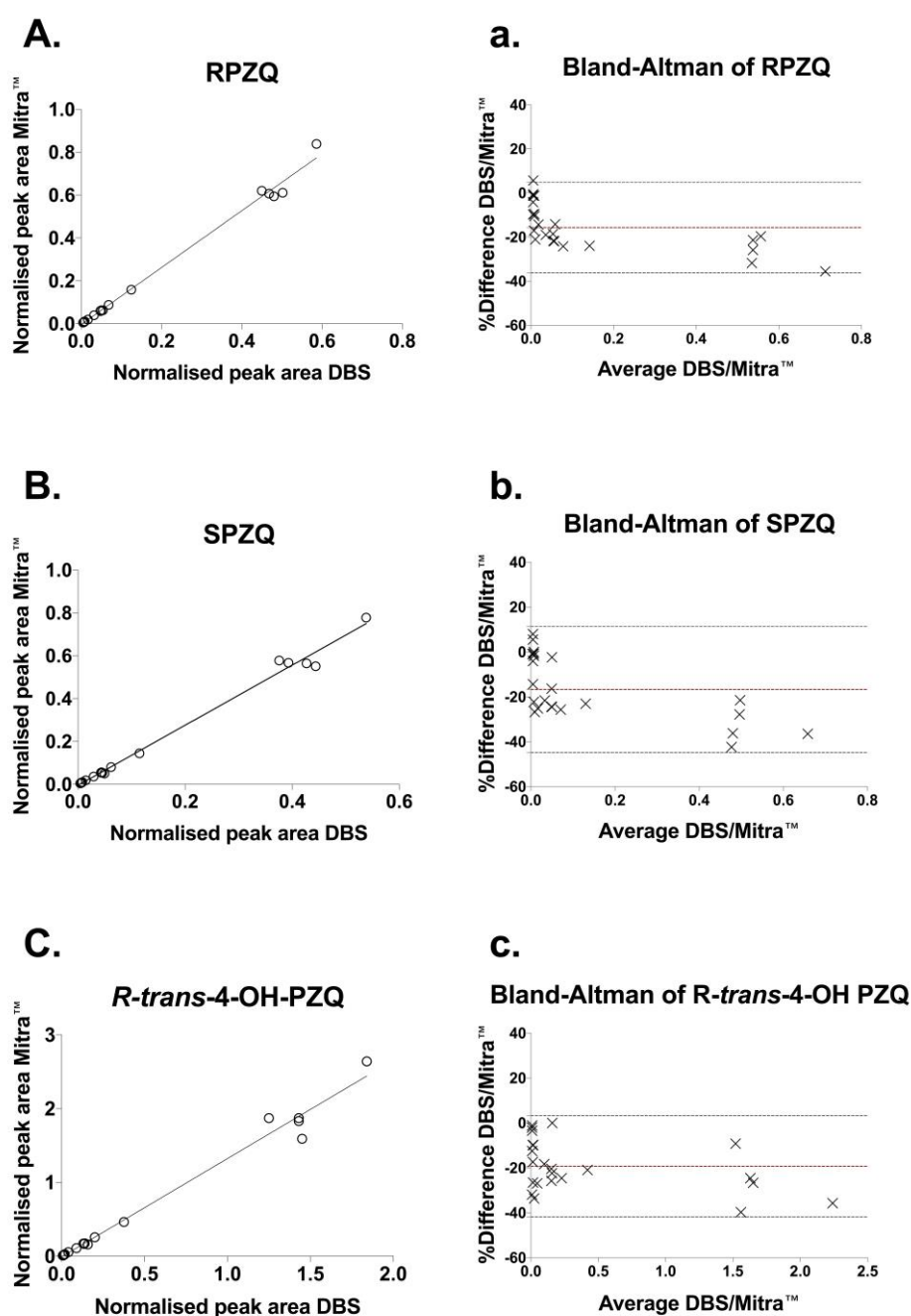
**Figure 2:** Graphs showing mean concentrations over time (A, B, C) with 95% confidence intervals (dashed lines) and Bland-Altman graphs (a, b, c) for patient samples (dashed lines indicating upper and lower 95% limit of agreement and red dashed line the mean difference).
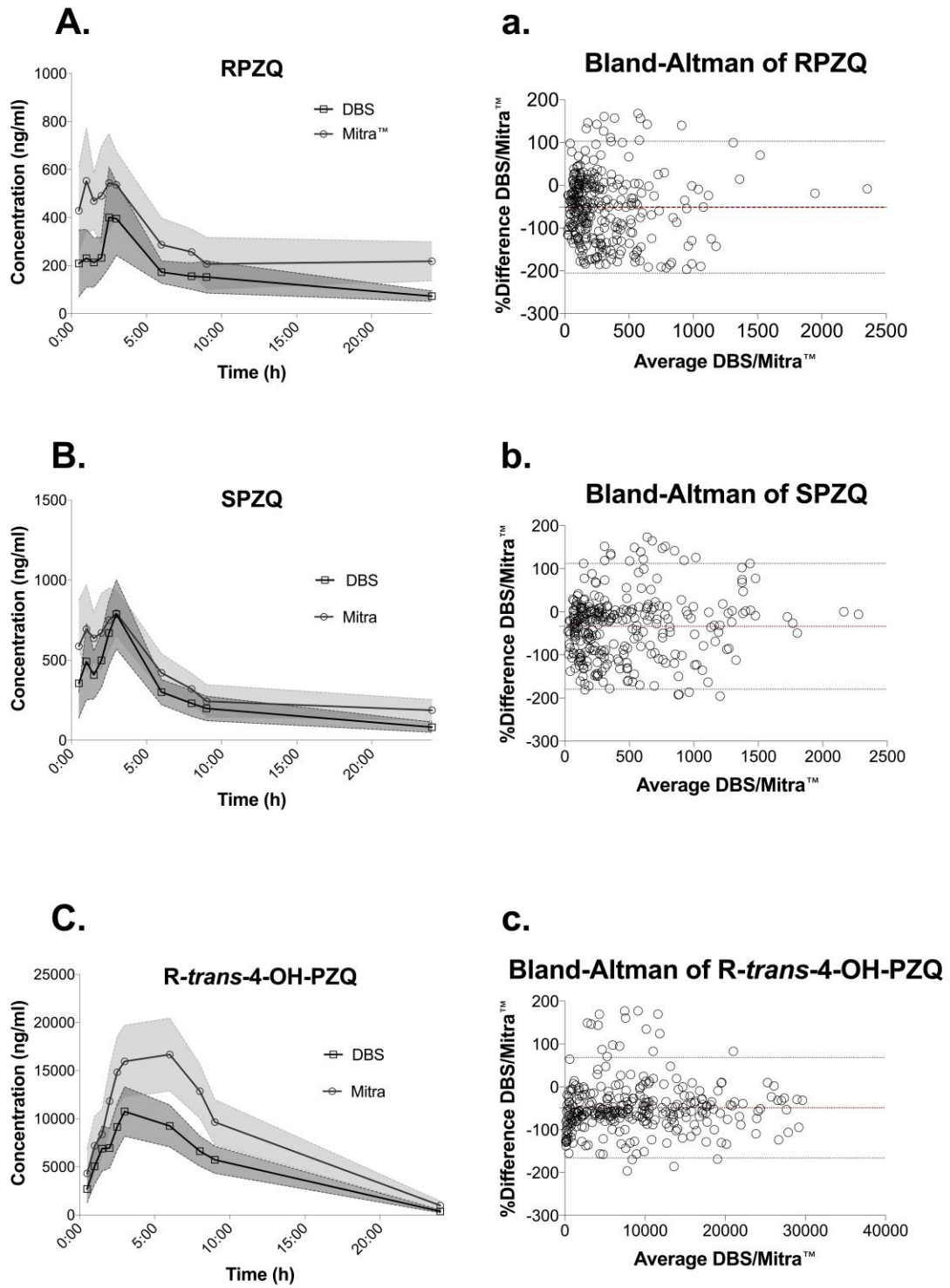
**Table 5:** PK parameters of patients for all 3 analytes and both matrices

| | DBS | Mitra™ | DBS | Mitra™ | DBS | Mitra™ | DBS | Mitra™ |
|---|---|---|---|---|---|---|---|---|
| **R-*trans*-4-OH-PZQ** | | | | | | | | |
| | $T_{1/2}$ (h) | $T_{1/2}$ (h) | $T_{max}$ (h) | $T_{max}$ (h) | $C_{max}$ (µg/ml) | $C_{max}$ (µg/ml) | AUC (µg*h/ml) | AUC (µg*h/ml) |
| MEDIAN (IQR) | 3.53 (2.87-4.19) | 3.84 (3.25-5.06) | 3.0 (2.5-6.0) | 3.0 (2.5-6.0) | 11.09 (6.75-16.15) | 18.09 (10.34-25.01) | 88.57 (42.43-150.14) | 146.05 (85.27-238.72) |
| MIN | 2.15 | 1.99 | 1.00 | 1.00 | 1.44 | 2.85 | 10.82 | 26.13 |
| MAX | 6.56 | 8.15 | 9.00 | 9.00 | 29.69 | 43.17 | 322.75 | 379.51 |
| **RPZQ** | | | | | | | | |
| MEDIAN (IQR) | 9.12 (6.30-12.55) | 8.69 (5.89-14.02) | 2.5 (2.0-3.0) | 2.0 (1.0-3.0) | 0.44 (0.25-0.88) | 0.77 (0.61-1.26) | 2.56 (1.70-3.57) | 4.13 (3.34-5.60) |
| MIN | 2.01 | 1.72 | 0.50 | 0.50 | 0.12 | 0.28 | 0.62 | 2.03 |
| MAX | 17.37 | 21.07 | 9.00 | 9.00 | 2.25 | 2.45 | 14.43 | 27.84 |
| **SPZQ** | | | | | | | | |
| MEDIAN (IQR) | 6.61 (4.34-10.26) | 7.82 (5.51-12.84) | 2.5 (2.0-3.0) | 2.5 (1.0-3.0) | 0.84 (0.43-1.49) | 1.03 (0.72-1.76) | 4.39 (2.62-7.45) | 5.52 (4.18-9.64) |
| MIN | 1.37 | 1.60 | 0.50 | 0.50 | 0.25 | 0.44 | 1.22 | 2.53 |
| MAX | 19.47 | 21.20 | 8.00 | 9.00 | 2.24 | 2.38 | 22.19 | 24.97 |

## 4. Discussion

Pharmacokinetic studies in paediatric populations are gaining in importance for the drug development process and are increasing in number (1, 5). However, conducting PK studies in children is a complex undertaking due to ethical requirements. DBS technology is an attractive tool for PK studies, since painful venepuncture is substituted with a simple and comparably less invasive finger-prick (26, 27). In addition to boosted patient adherence, convenient shipping and storage at room temperature are especially important features when studies are conducted in remote, rural settings (26). Disadvantages of the method generally include the haematocrit bias (17, 18) and tedious workflow (12, 13, 19, 20). The Mitra™ sampling method, based on volumetric absorptive micro-sampling, was thus introduced to mitigate some of the drawbacks (17).

To date, Mitra™ has been studied under laboratory conditions, but not yet in the framework of a clinical PK study (12, 20, 21). Our study aimed to fill this gap by evaluating the performance of Mitra™, as compared to the current micro-sampling device, DBS, within a PK study in rural Côte d'Ivoire with *S. haematobium* infected children treated with PZQ (Kovac et al, *submitted*, (23).

Both matrices showed a good agreement and a strong positive correlation when comparing CL and QCs samples using both correlation test based on Paerson's coefficient and Bland-Altman. However, when analysing patients' samples, Mitra™ exhibited higher concentrations compared to DBS for all three analytes, as demonstrated by Bland-Altman graphs in Fig.2. The reasons behind this phenomenon are not yet clear. One explanation might be difference in the matrices, influencing the partition and recovery of PZQ. While the filter paper of DBS is of a more hydrophobic nature, Mitra™ consists of a hydrophilic polymer (17). Since PZQ is rather non-polar, extraction could result in the higher partition of analytes into more preferred mixture of acetonitrile and water. However, relative and total recovery were evaluated during method development and found within requirements at the full concentration range. Moreover, the difference in concentrations between DBS and Mitra™ was noticed solely in the incurred samples collected from the patients. Therefore, it is unlikely that the

recovery is the main reason for apparent higher extraction yield from Mitra™. Additionally, the overestimation of analytes concentration from incurred samples of Mitra™ has previously been reported, while the reasons for a different behaviour between spiked and incurred samples have not been elucidated yet (7, 12, 13). A bridging study with PZQ between whole blood, plasma and DBS in *O.viverrini* infected participants has been carried out by Meister et al., which showed that plasma concentrations tend to be higher for both R- and SPZQ, compared to whole blood and DBS, while the opposite trend was observed for the main human metabolite (28). Notably, a hypothesis that Mitra™ preferentially binds the plasma component of the blood and therefore exhibiting concentrations closer to the plasma concentrations than that in whole blood has been proposed (13). This could be a likely explanation for a drug such as PZQ, which binds to plasma proteins in a great extent, up to 80%, however this phenomenon should be explored further (29, 28). Last but not least, the reason for differences in the concentrations of incurred versus spiked samples could be the preparation itself- while the spiked samples for CL and QC were prepared fresh, incurred samples from the field were stored frozen at -80 °C prior analysis. Although DBS did not exhibit this phenomenon regardless the same preparation and storage procedure, the differences in matrices could be the reason for the noticed discrepancy.

Both Mitra™ and DBS have proven to be an excellent tool for sampling within the PK study in rural sub-Saharan Africa. While neither of the two methods requires cold chain for shipping or storage, Mitra™ was characterised with a higher practicality, since it does not require aids (i.e. capillaries) to transfer the blood. However, the problem of insufficient soaking and filling the tip, the equivalent of poorly soaked DBS, remains with Mitra™. The extraction procedure developed for Mitra™ was shorter and simpler, since no punching is necessary. Another practical advantage of Mitra™ is the drying process; while DBS need to be dried on separate drying racks, Mitra™ can be simply returned into the provided box and kept closed, enabling immediate transport and reducing contamination from the environment. This is in line with the literature, where the simplicity of extraction of Mitra™ has been discussed (12–14, 20). Nonetheless, Mitra™ is much pricier compared to DBS. Furthermore,

DBS filter cards are easier to label and the chances of sample mix up are lower compared to

Mitra™, which does not offer a suitable labelling surface on the plastic holder (Table 6).

**Table 6:** Comparison of advantages between Mitra™ and DBS

|  | DBS | Mitra™ |
|---|---|---|
| **Minimal sample volume needed (µL)** | 20 | 10 |
| **Storage and shipping temperature (°C)** | 21 | 21 |
| **Need for sampling aids (i.e. glass capillaries)** | Yes | No |
| **Need for punching** | Yes | No |
| **Need for additional drying racks** | Yes | No |
| **Haematocrit bias[+]** | Yes | No |
| **Haematocrit bias[#]** | Limited | No |
| **Labelling surface** | Yes | No |
| **Stability** | Long-term | Long-term |
| **Price (CHF/sample)\*** | 0.36 | 2.57 |

[+] Based on the literature
[#]Based on our analytes
\* Calculated based on price for 96-samples box of Mitra**™** and the price of a 100 cards package of DBS

One of the limitations of our work is the reliance on a single sample- due to a high price of

Mitra™, only one sample was taken per time point and per patient, therefore re-analysis of

samples which would strengthen the reliability of our results, was not possible.

To conclude, Mitra™ showed great potential as a tool to be used in PK trials, including the

low-resource settings. The method validation was successful and resulted in consistently good

results for all parameters evaluated. However, there is still room for improvement, with

emphasis on labelling issues and affordability. Finally, the reasons for discrepancy in

quantification of PZQ between incurred and spiked Mitra™ samples should be further

explored.

**Authors' contributions**

**Transparency declarations**

None to declare.

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# Chapter 5

# General discussion

# 5 General discussion

## 5.1 Rationale and objectives

The rationale of this PhD thesis was to uncover more information and gain valuable insight into praziquantel (PZQ), a drug successfully used for decades to treat schistosomiasis but still remaining a mystery on so many different levels (see Figure 1 for the flowchart of this work). Firstly, to compare activity of PZQ on different species of *Schistosoma spp*, by conducting studies with *S. haematobium*, *in vitro* and *in vivo*, in order to reveal potential inter-species differences in antischistosomal activity of PZQ. The results of these studies fill the gap created by the lack of studies with *S. haematobium* and compliment the existing knowledge of antischistosomal activity of PZQ towards other species of schistosomiasis (see Chapter 2).

Secondly, we wanted to explore the pharmacokinetics (PK) of PZQ in children of different age and infected with one of the main two species of the schistosomes, *S. haematobium* or *S. mansoni*, to explore the influences of covariates (e.g. age, parasite species) on PK processes of PZQ. Non-compartmental analysis (NCA) was a building base for initial insight into PK parameters of all three important analytes, namely R-praziquantel, S-praziquantel (R-/SPZQ) and the main human metabolite, R-*trans*-4-OH-PZQ (see Chapter 3). Following NCA, we were focusing on the analyte of interest, RPZQ, the proposed eutomer, aiming to construct a PK model, able to account for variability and effects of different covariates on processes of PZQ in the body and with it, efficacy. This model, currently under development in collaboration with Dr. Christine Falcoz (Certara, NJ, USA) would be able to predict with higher accuracy the concentration over time course of PZQ in patients and based on individual characteristics, a suitable treatment dose. Drawing a connection between dose-response relationship and PK would let us adapt the current treatment guidelines for treating young children and achieve the most optimal treatment efficacy while reducing adverse events. The need to perform additional clinical studies in sensitive populations, such as young children, would be reduced as well (see Appendix, Chapter 7.2).

Clinical studies we conducted in paediatric population brought into discussion also a question of improving the sampling quality, while maintaining the patient adherence. While dried blood spots (DBS) method for collecting PK samples has been well established in the recent years, there are still some drawbacks arising from this technique, which have been extensively described in the literature, underlining a need for an improved sampling tool (Spooner *et al.*, 2009). Since a new product, called Mitra™, has been recently launched, we were interested to evaluate it in the frame of fieldwork and compare its performance to DBS, which in the lack of alternatives remain the current gold standard (Denniff and Spooner, 2014) (see Chapter 4).
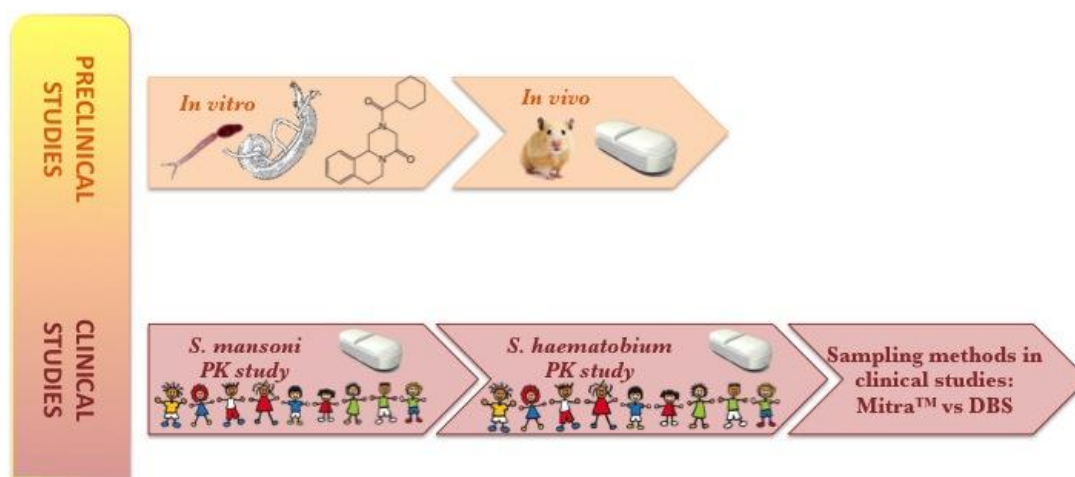


**Figure 1:** Flowchart of the projects involved in this PhD thesis: *in vitro* and *in vivo* studies of antischistosomal activity of PZQ on *S. haematobium* (Chapter 2), PK studies embedded in dose-finding studies with *S. mansoni* and *S. haematobium* infected children (Chapter 3) and comparison of DBS and Mitra™ within the frame of *S. haematobium* PK study (Chapter 4)

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**Table 1:** Contribution of different chapters of this PhD thesis to the nexus of Swiss TPH- innovation, validation and application

| Chapter | Title | Innovation | Validation | Application |
|---|---|---|---|---|
| 2 | *In vitro* **and** *in vivo* **activity of R- and S- praziquantel enantiomers and the main human metabolite** *trans-* **4-hydroxy-praziquantel against** *Schistosoma haematobium* | First time both enantiomers of PZQ were tested within a range of doses separately on *S. haematobium in vivo* and the main human metabolite was tested *in vitro*. | | Contributing to the knowledge about activity of PZQ on different species of *Schistosoma spp.* and the mechanism of action. Data might aid decision of whether to select the racemate or enantiomer for the development of a paediatric formulation. |
| 3 | **Pharmacokinetics of praziquantel in** *Schistosoma mansoni* **and** *Schistosoma haematobium* **infected school- and preschool- aged children** | . | Pharmacokinetic study with PZQ was carried out for the first time in schistosomiasis infected school- and preschool-aged children. Pharmacokinetic parameters were estimated and compared, influences on pharmacokinetics were studied. | Influencing guidelines for treating preschool-aged children by understanding the dose-response relationship and the covariates affecting the treatment efficacy. |
| 4 | **Evaluation of a novel micro-sampling device, Mitra™, in comparison to dried blood spots, for analysis of praziquantel in schistosomiasis patients in rural Côte d'Ivoire** | Development and optimisation of sample preparation method for analysis of Mitra™. | A novel micro-sampling device, Mitra™, is used for the first time in the field settings and evaluated not only from quantitation aspect, but also from the usefulness and practicality point of view. | Leading the way for new sampling techniques to enter the field of rural clinical trials and increase the quality and simplicity of sampling. |

### 5.2 *In vitro/ in vivo* **studies with** *S. haematobium*

The objective of *in vitro/in vivo* studies of antischistosomal activity of PZQ on *S. haematobium* was to evaluate whether this species of the parasites exhibits a different sensitivity to main analytes of PZQ, namely the racemate itself, both enantiomers (R- and SPZQ) and the main human metabolite, R-*trans*-4-OH-PZQ, compared to *S. mansoni*. There have been some suggestions in the literature that this might be the case and furthermore, there are several examples of antiparasitic drugs, such as oxamniquine and metrifonate, differing in activity between parasitic species (Zwang and Olliaro, 2014). While *S. mansoni* has been extensively studied, followed closely by *S. japonicum*, *S. haematobium* on the other hand remains in the background (Botros *et al.*, 2005).

We evaluated the antischistosomal activity of both PZQ enantiomers, R- and SPZQ, and the racemate, *in vitro* and *in vivo*. Additionally, we assessed the activity of main human metabolite, R-*trans*-4-OH-PZQ, which is believed to contribute to antischistosomal activity as well, *in vitro*. This knowledge is contributing to the understanding of PZQ and on the higher level, underlining the importance of studying inter-species differences in susceptibility to PZQ, in humans.

As expected based on *S. mansoni* data by Meister *et al.*, our results showed that RPZQ is driving the antischistosomal activity of PZQ against *S. haematobium*, both *in vitro* and *in vivo* (Meister *et al.*, 2014). The $IC_{50}$ value of racemic PZQ was 4.3× higher compared to the enantiopure RPZQ *in vitro*. Similar pattern was observed also *in vivo*, with comparable worm burden reductions (WBRs) for RPZQ and a twice higher dose of PZQ. Strikingly, SPZQ also proved to possess a non-negligible antischistosomal activity towards *S. haematobium*, in contrast to *S mansoni* (Meister *et al.*, 2014). An $ED_{50}$ value estimated for SPZQ was very close to that of the racemic PZQ. We also observed increased sensitivity of female worms compared to the males *in vivo* for all entities, which has previously been reported (Pica-Mattoccia and Cioli, 2004). Last but not least, also the metabolite, R-*trans*-4-OH-PZQ, revealed a higher effect on *S. haematobium*.

Our findings are in the line with literature (Webbe and James, 1977), suggesting *S. haematobium* is more sensitive to PZQ, possibly due to its residence in venus plexus of the bladder, where it gets exposed to the drug following the first pass metabolism, mainly to SPZQ and the metabolised form. *S. mansoni*, in contrast, resides in mesenteric veins (Figure 2B), coming in contact with PZQ prior reaching the liver, meaning mostly un-metabolised drug. Stemming from our findings is the question how does the increased sensitivity of *S. haematobium* to PZQ *in vitro* and *in vivo* translate to humans.

To conclude, our study contributed to the knowledge about PZQ and its antischistosomal activity on *S. haematobium*; however, there were some limitations that should be kept in mind. First, the differences stemming from different animal models used (hamster for *S. haematobium*, mice for *S. mansoni*) could not have been accounted for. Secondly, a low yield of adult worms for *in vitro* studies limited the number of replicates per experiment and with it, the strength of our findings. Lastly, due to inability of infected snails to sufficiently adapt to the artificial environment*, in vitro* studies with larval stages could not have been conducted. These are the factors that should be addressed in the future experiments.
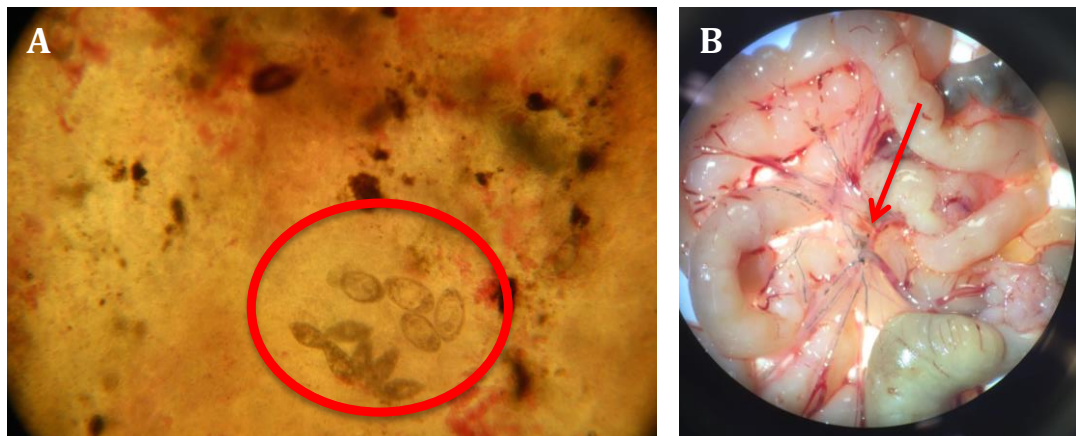


**Figure 2:** *S. haematobium* eggs (circled) found in the liver of the infected rodent (A) and adult worms of *S. mansoni* in the veins surrounding the intestine (B)

### 5.2.1 Praziquantel and schistosomes

There are three principal species of *Schistosoma spp.* of importance in human parasitology, namely *S. haematobium*, *S. mansoni* and *S. japonicum*, additionally to *S. intercalatum* and *S. mekongi*, which are of local importance (Colley *et al.*, 2014). The main difference between them is the final destination in human body where adult worms reside and with it, the organs where pathology is expressed (Gryseels *et al.*, 2006). Connected to the residence in the veins is also the path by which parasitic eggs leave the human body, via either stool (*S. mansoni, S. japonicum*) or urine (*S. haematobium*). In the part of the parasitic life cycle taking place outside the main host, these species differ in the intermediate host species, the water snails (Gryseels *et al.*, 2006). Since all three species of the parasite are characterised with obvious similarities, the question of the debate in the recent years has been differences in sensitivity to drugs, mainly PZQ, which is the standard treatment against all species of the parasite. However, *S. haematobium* is characterised with a life cycle, challenging to maintain in laboratory conditions (Moore and Meleney, 1954; Botros *et al.*, 2005; Doenhoff *et al.*, 2009). Additionally, time to develop a mature infection is much longer compared to *S. mansoni* and the infection intensities are often low, resulting in a low amount of adult worms yielded from animals (Loker, 1983; Rheinberg *et al.*, 1998). Trying to comply with the 3R guidelines for animal work, it becomes difficult to perform experiments in a reasonable framework (Fenwick *et al.*, 2009). Therefore, *S. haematobium* has remained largely unexplored, compared to *S. mansoni* and *S. japonicum*, although it is responsible for the highest share of the schistosomes infections (Keiser, 2010; Hotez *et al.*, 2014).

Reported results from studies evaluating antischistosomal activity of PZQ on different species of schistosomes generally support RPZQ as the carrier of antischistosomal activity towards *S. japonicum* (Irie *et al.*, 1989; Tanaka *et al.*, 1989; Wu *et al.*, 1991), while there have been some controversial results with *S. mansoni*; some studies claim SPZQ as the active enantiomer (Irie *et al.*, 1989; Tanaka *et al.*, 1989), which is in disagreement with other reports (Xiao and Catto, 1989; Staudt *et al.*, 1992; Meister *et al.*, 2014) (see Table 2). Nonetheless, inconsistencies in the design of experiments could explain these discrepancies. Additionally,

there are examples of antiparasitic drugs where the metabolites originating from the main entity have been proven to possess activity, indispensable in addition to that of the parent compound (Staudt *et al.*, 1992; Barrera *et al.*, 2012). Activity of the metabolites could add to the overall activity of the drug, if not carry the main effect, bringing into the light a question of antischistosomal activity, contributed by the main human metabolite in case of PZQ. Except on *S. mansoni* studied by Meister *et al.* from our group and our *S. haematobium* study (Chapter 2), metabolites of PZQ remain largely unstudied, leaving room for further explorations (Meister *et al.*, 2014; Kovač *et al.*, 2017).

One possible explanation for differences in activities reported could be that there are inter-species variations in presence of chiral receptors, only binding the respective enantiomer of PZQ; one chiral form only binding RPZQ and the other only SPZQ. This could be a reason for contradictory results reported between species and it underlines the need for a further exploration of both mechanism of action of PZQ and the activity in different species and strains. In addition, an interesting aspect to study would be also to assess differences in susceptibility to enantiomers of PZQ on other parasites- other intestinal worms, such as the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis*, lung worms of *Paragonimus spp.* and the other intestinal worms, such as *Fasciolopsis buski* and *Echinostoma spp.*, besides cestodes, are sensitive to PZQ as well. Interestingly, *Fasciola hepatica* is the only trematode, insubmissive to PZQ and comparison of these parasites could contribute to the knowledge regarding the mechanism of action of PZQ (Andrews, 1985; Cioli and Pica-Mattoccia, 2003).

**Table 2:** Summary of *in vitro* and *in vivo* studies with PZQ from literature

| | Analyte studied | Stage of parasite | $IC_{50}$ (µg/ml) at 4 h | Animal model | DOSE | WBR (%) | $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|
| | | | | *S. mansoni* | | | |
| Xiao and Catto, 1989 | PZQ | N/A | N/A | Mouse | 50 / 100 / 200 | 3.1 / 24.5 / 53.5 | N/A |
| | RPZQ | N/A | N/A | Mouse | 50 / 100 / 200 | 19.6 / 47.5 / 73.2 | N/A |
| Tanaka *et al.*, 1989 | PZQ | N/A | N/A | Mouse | 50 / 500 | 12.6 / 38.9 | N/A |
| | RPZQ | N/A | N/A | Mouse | 50 / 500 | 6.8 / 32.1 | N/A |
| | SPZQ | N/A | N/A | Mouse | 50 / 500 | 25.5 / 50.9 | N/A |
| Meister *et al.*, 2014 | RPZQ | Adult worms | 0.04 | Mouse | 100 / 200 / 400 | 52.0 (30.8) / 98.1 (2.3) / 100.0 (0) | 95.4 |
| | SPZQ | Adult worms | 5.7 | Mouse | 400 / 800 | 18.0 (21.4) / 19.6 (22.2) | 3066777 |
| | PZQ | Adult worms | 0.1 | Mouse | 400 | 94.1 (8.6) | 246.5 |
| | R-*trans*-4-OH-PZQ | Adult worms | 16.7 | N/A | N/A | N/A | N/A |
| | | | | *S. japonicum* | | | |
| Tanaka *et al.*, 1989 | PZQ | N/A | N/A | Mouse | 50 / 500 / 2x50 / 2x250 | 10.9 / 76.8 / 34.5 / 83.5 | N/A |
| | RPZQ | N/A | N/A | Mouse | 50 / 500 / 2x50 / 2x250 | -1.8 / 62.2 / 67.9 / 83.6 | N/A |
| | SPZQ | N/A | N/A | Mouse | 50 / 500 / 2x50 / 2x250 | -0.5 / 1.9 / 13.8 / -13.9 | N/A |
| Webbe and James, 1977 | PZQ | N/A | N/A | Mouse | 50 / 100 | 73.2 / 96.2 | N/A |
| | | | | *S. haematobium* | | | |
| Webbe and James, 1977 | PZQ | N/A | N/A | Hamster | 100 / 150 / 200 | 39.2 / 66.1 / 77.2 | 118 |
| Kovač *et al.*, 2017 | RPZQ | Adult worms | 0.007 | Hamster | 31 / 62.5 / 125 | 73.3 / 75.6 / 98.5 | 24.7 |
| | SPZQ | Adult worms | 3.51 | Hamster | 125 / 250 / 500 | 46.7 / 83.0 / 94.1 | 127.6 |
| | PZQ | Adult worms | 0.03 | Hamster | 250 | 99.3 | 118.1 |
| | R-*trans*-4-OH-PZQ | Adult worms | 1.47 | N/A | N/A | N/A | N/A |

### 5.2.2 Drug discovery for schistosomiasis- the bottleneck of anthelminthic development

Drug development for schistosomiasis has been on the back burner compared to other parasites, such as nematodes, where the importance in the animal health sector is driving the research (Geary *et al.*, 2009). There is also apparent lack of initiatives for anthelmintic drug development, common for other diseases, such as "The Big Three" (e.g. Medicines for Malaria Venture) (Utzinger *et al.*, 2011). One of the reasons for this is PZQ, which is readily available, effective and cheap, resulting in health policy makers and philanthropic organisations to focus their efforts on the distribution of PZQ rather than to support and finance the search of alternative drugs (Caffrey and Secor, 2011).

One of the crucial obstacles hindering development of new antischistosomal drugs is the lack of simple screening system and processes. Target-based approach is not feasible when it comes to schistosomiasis and cell lines or transgenic parasites do not exist either (Caffrey and Secor, 2011). Therefore, the screening relies on parasitic life cycle, which, additionally to being complex and requiring both a vertebrate and a molluscan host, is also difficult to maintain in the laboratory conditions. There is a growing need for robust and simple *in vitro* systems with objective and straight-forward, possibly automatized read-out, to move antitrematodicidal drug discovery to the higher gear (Keiser, 2010).

The significant breakthrough of the antischistosomal drug development was in 1960s with the ability to maintain the life cycle in artificial conditions. With the greater demand for parasites, newer and better methods for cultivation and artificial transformation of cercariae were developed (Ramirez *et al.*, 2007). *In vitro* methods are based on microscopic evaluation of phenotypic changes to the parasite after incubation with an investigational compound. A scale to evaluate phenotypic activity is standardised however the readouts themselves can be very subjective, not to mention time consuming. Partly automated screenings have emerged in the recent past, although the manual technique is still very common (Ramirez *et al.*, 2007). The development of different methods, able to quantitatively estimate the phenotypic changes and based on it, predict the efficacy of a drug, is a current hot topic. Methods under development range from measuring the change in electrical impedance or heath flow (Smout *et al.*, 2010),

to fluorescence based staining (Panic *et al.*, 2015). One of the methodologies under development in our laboratory is based on measuring the difference in electrical impedance, caused by the movement or the lack of there of, of the parasites after adding the drug.

There are two stages of the parasite used for <u>*in vitro*</u> testing, namely the larval stage (newly transformed schistosomula, NTS) and adult worms. NTS are obtained by forcing the cercaria, shed by infected snails, to loose the tail using different mechanic or chemical methods (Keiser, 2010). The process itself is cost effective and reasonably fast, nonetheless the infection rate of the snails can vary greatly and the shedding of cercariae is unreliable and variable as well, making it difficult to efficiently plan the experiments. The advantage of using larval stages is avoiding the need for animal use however the differences, which exist between mechanically produced schistosomula and the schistosomula, which have penetrated human skin, cannot be accounted for (Brink *et al.*, 1977).

Adult worms, on the other hand, are picked from the veins manually or using venous perfusion, surrounding the intestine or bladder of experimental animals, infected with cercariae and allowed to develop a chronic infection (Keiser, 2010). While it can take up to 3 months to develop a mature chronic infection (*S. haematobium*), there are also other issues when it comes to animal models (Botros *et al.*, 2005). The mouse model, for example, is not suitable for *S. haematobium* and rats as a semi-permitive hosts are not a good model for any of the schistosome species. *S. haematobium* in particular is very difficult to maintain under laboratory conditions and the infection rates are often very low, resulting in insufficient yield of adult parasites (Moore and Meleney, 1954; Botros *et al.*, 2005; Doenhoff *et al.*, 2009). As a consequence, the throughput for testing drug on adult worms is highly limited.

Different animals can be used as schistosoma model for <u>*in vivo*</u> testing of the drugs. Each species of parasites has a preferred animal model (hamsters for *S. haematobium*, mice or hamsters for *S. mansoni*) in which it's growth is substantial and possible differences, originating from inter-species variability of these animals, remain unaccounted for, leaving a zone of uncertainty (Keiser, 2010). While most mice strains can be used as *S. mansoni* model, also other animals have been used, e.g. primates (Keiser, 2010). Similarly to *in vitro*

procedure, the animals are first exposed to the infectious cercariae using different techniques (subcutaneous or intraperitoneal injections are common) and allowed to develop a chronic infection. Subsequently, the animals are treated with the drugs of interest and later sacrificed. The worms from the mesenteric veins are collected, sexed and counted. The liver is removed, compressed and examined for worms, alive or dead, and eggs (Xiao *et al.*, 2007; Keiser, 2010). Treatment outcome can be recorded in different ways, depending of the laboratory; most common parameters are adult worm count, liver egg count and oogram pattern (Ramirez *et al.*, 2007). New approaches of investigating the activity and effect of drugs using non-invasive determination of worm burden by *in vivo* imaging of schistosomes with positron emission tomography, with perspective to be used also in humans, are emerging (Salem *et al.*, 2010).

To conclude, PZQ remains the only available antischistosomal drug, while the funding directed to the search of the alternatives is small compared to the burden the disease is causing. There is a great need to develop molecular target-based approach for drug screening, which would speed up the drug discovery process. Additionally, there are still challenges to overcome in the cultivation techniques and last but not least, automatized and objective readouts for the *in vitro* screenings are a necessity (Keiser, 2010).
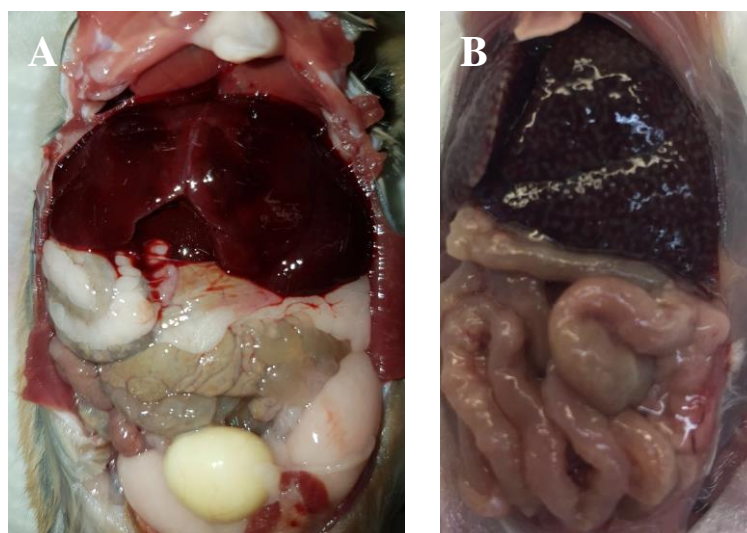


**Figure 3:** Liver of healthy (**3A**) versus infected (**3B**) experimental animal (courtesy of Valérian Pasche)

## 5.3 Pharmacokinetic studies with praziquantel in paediatric population

This project represents the main part of this PhD thesis. The aim was to conduct PK sampling within the dose-finding trial with PZQ, in school-aged (SAC) and preschool-aged (PSAC) children, infected with *S. mansoni* or *S. haematobium* (see Chapter 3) (Figure 4). By following the concentration of the drug in blood over the time span of 24 hours, we were able to understand more about absorption, distribution, metabolism and elimination (ADME) processes of PZQ. NCA analysis was conducted with all of the collected data and secondary PK parameters (namely AUC, $C_{max}$, $T_{1/2}$, $T_{max}$) obtained were compared within children of different age, parasite-specific infections and treatment dosages. We were interested in investigating the dose-response relationship and to elucidate the factors influencing PK of PZQ. This knowledge would be invaluable in guiding the clinicians on the way to tailor the dose for younger children. However, the strength of information obtained using NCA is only limited. Therefore, as an upgrade, we connected with Dr. Christine Falcoz to construct a population PK model for RPZQ, able to explore in depths and better explain influences and covariates on the metabolic processes of PZQ. This work is currently still on-going (see Appendix).

PK parameters of all three analytes in children of different age and infected with either species of the parasite, are presented and discussed in details in Chapter 3. To summarise, we did not observe significant differences in PK parameters between parasite-specific infections. Both AUC and $C_{max}$ were lacking the signs of dose proportionality, since the exposure increased less than proportional with the dose. No correlation between probability of cure and AUC or $C_{max}$ was found, therefore, our findings do not support the use of neither AUC nor $C_{max}$ as an indicator of cure.

Nonetheless, as mentioned above, NCA is not an ideal technique to describe metabolic processes for a drug with erratic absorption, such as PZQ. A population model, fitting all the concentration data from children of different ages and accounting for maturation processes and allometry, is a necessity in order to adequately describe ADME of PZQ. We hope this model will aid on the way to determine a suitable dose of PZQ for PSAC (see Chapter 7.2).
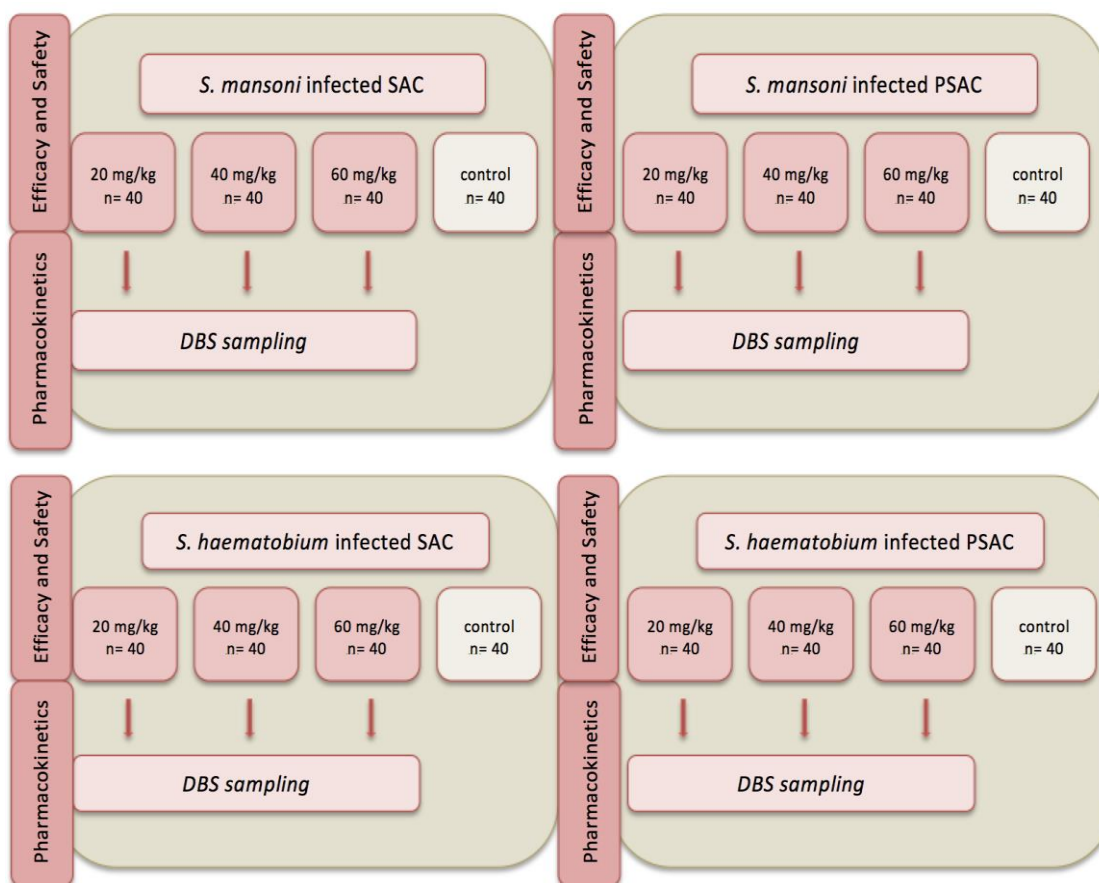
**Figure 4:** Flowchart of two clinical trials with infected SAC and PSAC

The metabolism of PZQ has been studied and it is well known that the drug undergoes extensive first pass metabolism in the liver with CYP P450 enzyme family. For this reason, PK processes of PZQ can be influenced to a smaller or greater extent by the following factors: (i) inter-individual variability due to polymorphism of CYP isoenzymes, (ii) other substances influencing CYP system, taken concomitantly and (iii) the status of liver function (Olliaro *et al.*, 2014). Until this date, the influence of these variables on the metabolism of PZQ has not been thoroughly explored. Additionally, the degree of polymorphism of the CYP system isoenzymes in the target populations, where PZQ is distributed on a large scale, has only recently been documented (Rajman *et al.*, 2017). Moreover, the extent of people being treated with medicaments, affecting PK processes of PZQ, has not been recorded (Mutapi *et al.*, 2017). For example, rifampicin, a drug used to treat tuberculosis infection, can significantly reduce levels of PZQ in blood when taken concomitantly and since these two infections might

coincide geographically, this factor should be taken into consideration (Ridtitid *et al.*, 2002). Studies of pharmacogenomics would be a very interesting addition to PK studies and could explain, at least to a certain extent, inter-patient variability in PZQ exposure and down the line, the treatment efficacy and the treatment failure (Mduluza and Mutapi, 2017).

### *5.3.1 Young children and preventive chemotherapy with PZQ*

For many years, it was believed young children (<6 years) are not as affected by schistosomiasis as their older peers, since they do not come into contact with infected water as actively (Odogwu *et al.*, 2006). Consequently, they have been, until recently, excluded from PC programs or are treated with a standard WHO recommended dose of 40 mg/kg, used for adults (Bustinduy *et al.*, 2017). However, researches closely looking at the transmission and water contact patterns have realised young children are frequently exposed to infected water when bathed by their guardians several times a day, using water from infected sources (Mafiana *et al*., 2003; Odogwu *et al.*, 2006). Furthermore, studies have confirmed a correlation between infection intensity and frequency of water exposure (Ekpo *et al.*, 2012). Adding up to this is the fact younger children (< 6years), when left out of the treatment program, could be the overlooked source of infection, maintaining the transmission cycle in villages regularly treated with PZQ (Stothard and Gabrielli, 2007). Early infection with schistosomiasis is also believed to exacerbate the later morbidity (Bosompem *et al.*, 2004; Odogwu *et al.*, 2006; Stothard and Gabrielli, 2007). Thus, there is a growing consensus to tailor the dose of PZQ for young children and furthermore, WHO has recognised that PSAC living in endemic areas should be considered in the future as a target group for administration of PZQ (WHO, 2010).

That being said, most of the information on PK of PZQ available originates from studies with healthy adults or studies in patients of a certain condition (e.g. liver failure) (Mandour *et al.*, 1990; El Guiniady *et al.*, 1994; Olliaro *et al*., 2014). Furthermore, the differences in metabolic and enzymatic systems between children and adults, influencing PK of active substances, have been well described (Hattis *et al.*, 2003; Kearns *et al.*, 2003). Therefore, one cannot simply extrapolate the dose of a drug from adults to children. PK studies to closely observe

the differences in ADME processes are essential in order to adapt the dosing of children (Yewale and Dharmapalan, 2012; Batchelor and Marriott, 2015). In depth study of response exposure relationship is crucial to understand whether children are under-dosed when receiving the standard adult dose of 40 mg/kg and to tailor the WHO guidelines, with the aim of highest efficacy and lowest adverse events.

Including children in treatment programs calls not only for an adapted dose, but also for suitable drug formulation. One of the well described issues when treating children with the standard commercially available formulation of PZQ stems from the formulation itself. The tablet consists of 600 mg racemic mixture of both enantiomers, R- and SPZQ, in 1:1 ratio (Olliaro *et al.*, 2014). As a result, the size of the tablet is big and combined with an awfully bitter taste, presumably originating from SPZQ, it is very hard for young children to swallow, which we observed within our clinical studies as well (Meyer *et al.*, 2009). Together with a risk of choking, these facts underline a need for a paediatric formulation. Ideally, it would consist only of RPZQ, which is the proposed protagonist of antischistosomal activity (Webbe and James, 1977; Meister *et al.*, 2014b; Olliaro *et al.*, 2014). SPZQ is presumably responsible for the adverse events (e.g. nausea, abdominal symptoms) originating from the drug and by removing it from the new formulation, perhaps these could be circumvented as well (Meyer *et al.*, 2009). However, studies collecting evidence to firmly establish connection of SPZQ and undesirable adverse events are yet to be conducted. Moreover, in the light of our results from *in vitro* and *in vivo* studies of antischistosomal activity of PZQ on *S. haematobium*, indicating SPZQ might be contributing to the overall activity for this species, the answer to the question of whether the paediatric formulation should be racemic or enantio-pure, might not be that straight forward and requires further studies with different species of the parasites in humans.

A public-private partnership was established in 2012 between Merck, Astellas and Swiss TPH, aiming to develop a paediatric formulation for PSAC. The efforts of this consortium are focused mainly on adapting the treatment dose to obtain safe and effective dose for young children, mitigating the bitter taste of PZQ and reduce the size of the tablet. Currently in the phase 2 clinical trial is an orodispersible tablet, much smaller compared to the current

formulation and with additional advantages, such as no need for water when administrating (except for babies and infants for which the tablet would need to be dissolved on a teaspoon), important in low-resource areas where PZQ is typically used (Mduluza and Mutapi, 2017).

A very important reason why young children were for so long believed not to be infected by schistosomes and consequently not regularly treated, lies in the lack of suitable diagnostic tools (Knopp *et al.*, 2013). Schistosomiasis prevalence and the burden of rises from the early age to reach the peak in SAC, therefore standard diagnostic tools are often not sensitive enough to use with young children, leaving them under-diagnosed (Gryseels *et al.*, 2006). Additionally, the consistence of stool originating from young children might be unsuitable for methods such as Kato-Katz (Coulibaly *et al.*, 2012). Furthermore, the worms themselves are maturing and the eggs can be excreted at very irregular intervals, resulting in a significant time-lag in the ability of the infection being discovered using the direct egg detection methods (such as stool smear or urine filtration), which are otherwise considered a gold standard (Poole *et al.*, 2014). The consequence of underestimating the prevalence of schistosomiasis among young children and overestimating the efficacy of treatment are the misguided recommendations in helminth control programs. This underlines the need to develop new diagnostic tools and methods, able to meet predefined target product profile (Knopp *et al.*, 2013).

### 5.3.2 Single drug dependence- PZQ forever?

With PZQ being the only effective drug against schistosomiasis and immense drug pressure due to massive drug administration, the fear of resistance is non-negligible. The need to uncover new antischistosomal leads is now greater than ever, although the first case of resistance to PZQ is yet to occur (Vale *et al.*, 2017). While researchers are working around the clock to find an alternative candidate, the antischistosomal pipeline remains rather empty (Caffrey and Secor, 2011).

One of the many challenges when trying to develop novel candidates is the lack of information on mechanism of PZQ (Vale *et al.*, 2017). There have been some theories about

the mechanism of action, in connection with $Ca^{2+}$ ion influx to the worm, resulting in intense muscular paralysis and blebbing and structural alterations of tegument (Cioli *et al.*, 2014). As a consequence of tegument disruption, parasitic surface antigens are exposed and recognised by host immune system, resulting in clearance of the parasite. Nonetheless, it is yet to be explained how PZQ disrupts the homeostasis of the parasite (Vale *et al.*, 2017). Furthermore, subsequent studies failed to confirm the connection between calcium accumulation itself and antischistosomal activity of PZQ; e.g. cytochalasin D, antagonist of PZQ, blocks antischistosomal effect of PZQ while it does not prevent influx of calcium (Pica-Mattoccia *et al.*, 2008). Additionally, it appears the genes coding for calcium channels are the same for both juvenile schistosomes, insensitive to PZQ and the susceptible adult worms (Aragon *et al.*, 2009). Transcriptomic studies found genes possibly involved in aerobic metabolism and regulation of cytosolic calcium, which are differently regulated by PZQ exposure, suggesting that schistosomes undergo a transcriptomic response, similar to that of oxidative stress (Aragon *et al.*, 2009). Last but not least, also calcium/calmodulin- dependent protein kinase type II might have a role in the antischistosomal activity of PZQ, representing a possible drug target (Vale *et al.*, 2017). That being said, there are still plenty of questions waiting to be answered regarding the mechanism of PZQ and the molecular target remains in the dark, hindering the drug development for schistosomiasis (Vale *et al.*, 2017).

Similar to the mechanism of action, also a possible resistance mechanism remains to be proven. While there have been cases of reduced susceptibility to treatment, both in the laboratory and in the field, it is not yet clear whether these were cases of actual resistance or rather misinterpreted (Cupit and Cunningham, 2015). Attempts to induced resistance under laboratory conditions, first *in vitro* and followed by *in vivo*, go as far back as to 1970s, although the focus was on *S. mansoni* (Vale *et al.*, 2017). In 1993, Couto and colleagues presented a simple and cost effective method of inducing resistance to PZQ, by treating infected snails with 100 mg/kg PZQ for 5 consecutive days and then using released cercaria to infect mice (Couto *et al.*, 2011). So far, no resistance to PZQ in the field has been confirmed; however, there have been studies reporting reduced susceptibility to PZQ or treatment failure

(Herwaldt *et al.*, 1995; Ismail *et al.*, 1996). That being said, it is not yet clear whether these findings were confounded by other factors (e.g. possible mal-absorption of PZQ due to *Giardia lamblia* co-infection) (Vale *et al.*, 2017).

While PZQ is effectively treating schistosomiasis, it does not prevent reinfection and furthermore, since it is not able to clear the juvenile stages of the parasites, it requires re-treatment few weeks post the initial treatment (Cioli and Pica-Mattoccia, 2003). One of the exciting aspects, which would have ameliorated many of the difficulties of PZQ treatment, is a possible antischistosomal vaccine, the search of which is currently undergoing. Ideally, antischistosomal vaccine would offer rather a reduced morbidity than sterile immunity (Siddiqui and Siddiqui, 2017). 75% worm burden reduction and significant reduction of egg-induced pathology in animal models, preferably baboons, due to similarities in immune response to humans among other reasons, was suggested as a target value for a good vaccine (Mo *et al.*, 2014). Furthermore, the possibility of multivalent vaccine, targeting not only schistosomes, but also other parasitic worms, would be of great benefit since these infections tend to coincide (Hotez *et al.*, 2010). While schistosomes offer plethora of distinct antigens due to their advanced life cycle, out of 100 identified vaccine antigens, only three compounds have shown potential to enter human clinical trials (Merrifield *et al.*, 2016). A single candidate against *S. haematobium* (Sh28GST), a glutathione S-transferase, demonstrated promising activity and acceptable safety profile during early clinical trials. Two vaccine candidates against intestinal schistosomiasis, a membrane protein domain (Sm-TSP-2) and a fatty acid binding protein (Sm-14) are currently in early stage clinical trials (Merrifield *et al.*, 2016). Smp80 (calpain) showed promising results in primates for both *S. mansoni* and *S. haematobium* and is moving towards the phase 1 and 2 clinical trials (Tebeje *et al.*, 2016; Siddiqui and Siddiqui, 2017). However, no vaccine against any of the schistosome species infecting humans is currently commercially available (Tebeje *et al.*, 2016).

### *5.3.3 From control to elimination of schistosomiasis: status quo*

Schistosomiasis remains one of the most prevalent neglected tropical diseases, regardless the efforts of control put in place by WHO in 2001 and endorsed by the member states (WHO, 2013). Several countries implemented schistosomiasis control programs and progress has been made in the recent years (Inobaya *et al.*, 2014). However, there are still significant issues hindering the effective control of schistosomiasis and trying to move to the next stage and towards the elimination, these will need to be taken into careful consideration.

Access to PZQ itself used to be one of the issues but since Merck KGaA committed to donate 250 millions of tablets annually, until the elimination of schistosomiasis is achieved, this is no longer the main problem (Tchuem Tchuenté *et al.*, 2017). However, the target coverage of 75% has not been achieved- furthermore, the global coverage lies as low as 20.74% of the population in need of preventive chemotherapy in 2014 (WHO, 2016). One must bear in mind the donation of PZQ itself is not enough- the countries where it is distributed, are struggling with weak and poorly structured and resource-lacking health systems, failing to act as effective delivery channel (Tchuem Tchuenté *et al.*, 2017). Furthermore, assuring safe and efficient consumption of PZQ, connected to patient's adherence, represent a whole new dimension of issues- having trained health professionals to distribute and supervise the treatment is rather an exception than a rule (Ross *et al.*, 2017). The reasons for poor patient adherence are interconnected and complex- schistosomiasis can be asymptomatic or only manifest with mild symptoms for years and many people are not aware they are infected; connected to it is also the fact that patients will not necessarily notice a great improvement in their health and not feel the need to be treated. What the patients might notice, however, are the adverse events of treatment with PZQ, although usually mild and transient- this can add to the reduced patient adherence as well (Ross *et al.*, 2017). Distribution of PZQ is largely bound to the education programs, distributing the drug to SAC; while this approach certainly has advantages, rural communities, non-school attending children and other risk groups are challenging to reach (Rollinson *et al.*, 2013). Sustainability of control programs will be difficult to achieve without engaging the local community.

Elimination of schistosomiasis will not be possible without an integrated approach, as acknowledged by World Health Assembly resolution 65.21- while treatment with PZQ is the core of schistosomiasis control, it has only a temporary effect on transmission interruption and requires maintenance of the treatment for success (Rollinson *et al.*, 2013). Intervention programs, such as WASH (Water, Sanitation and Hygiene), typically integrate access to safe water, proper sanitation (e.g. improved latrines and sludge management) and hygiene related education (e.g. safe defecation, hand washing, soap use, water storage practices), can have a significant effect on reducing transmission of schistosomiasis (Esrey *et al.*, 1991; Bieri *et al.*, 2013; Campbell *et al.*, 2014). However, these measures are unfortunately not prioritized by national governments in terms of funding and often left out. Additionally, not only establishment but also maintenance of the infrastructure is important to encourage people to use them. Targeting the snails population and with it, the pathway of transmission from the animal vector to humans, is one of the important measures for the disease control as well (Tchuem Tchuenté *et al.*, 2017). Nonetheless, due to negative experience with niclosamide, known pollutant and widely distributed in the past, it brings to the spotlight the impact molluscides have on the environment. One of the solutions could be targeting only the so-called focal hotspots- approach, which had proven to be successful in China (Rollinson *et al.*, 2013). Furthermore, this approach would reduce also the high costs, otherwise associated with mollusciding (Ross *et al.*, 2017). To conclude, schistosomiasis will not be eliminated as a public health problem by 2025, as planned by the WHO; nonetheless, learning from the experience and carefully considering and planning the next steps, is the move in the right direction.

## 5.4 Mitra™: new gold standard for microsampling?

Pharmacokinetic studies with paediatric population have become a standard part of drug development process in the recent years. Regulatory agencies have recognised a need for a tailored dose of medicaments, resulting in optimal efficacy and low adverse events for children and have released legislations, obliging to include studies of ADME processes in children, in registration dossier (Zisowsky *et al.*, 2010). PK studies with children are not amongst popular and there are several reasons for it- they are typically characterised with invasive and painful phlebotomy, considering intensive sampling scheme and large blood volumes it requires, this technique is inappropriate for sampling in children (Goodenough *et al.*, 1997; Patel *et al.*, 2010; Batchelor and Marriott, 2015). Consequently, efforts have been aimed to find an alternative for collecting blood samples from young patients, allowing for lower invasiveness and still resulting in reliable and standardised quantification (Patel *et al.*, 2010; Altamimi *et al.*, 2016).

However, disadvantages stemming from this technique, such as haematocrit bias as one of the most important, quickly forced researchers to keep on looking for other options (De Kesel *et al.*, 2015). Mitra™ is a new volumetric absorptive micro-sampling device, launched recently and promising to overcome the issues of DBS (Denniff and Spooner, 2014).

We compared the performance of both techniques in the field, with regards not only to both practicality and work-flow, but also PZQ extraction yield (see Chapter 4). 35 SAC infected with *S. haematobium* were sampled using both DBS and Mitra™. A sample preparation method for PZQ with Mitra™ was developed and validated in compliance with FDA guidelines (FDA, 2013). It is essentially the same method as used for DBS, except with a shorter thermo-mixing time, reduced from 20min to 5min. Both samples were extracted and the concentration of PZQ enantiomers and the main human metabolite was measured using a validated LC-MS/MS method. The concentrations were compared for each patient using Bland-Altman statistics to determine if the matrices were comparable. Furthermore, PK parameters, namely $T_{max}$, $C_{max}$, $T_{1/2}$ and AUC were calculated for both matrices and compared

as well. Additionally, we evaluated both tools regarding the practicality and usefulness in the field and in the laboratory conditions.

The results are presented and discussed in details in Chapter 4. To summarise our findings, calibration line and quality control samples showed a strong positive correlation between both matrices for all three analytes, but moving to the patients' samples, Mitra™ seemed to be over-estimating the concentrations. This phenomenon has previously been described, but not fully explained. Meister *et al*. described a similar situation for DBS and whole blood, where the concentrations of R- and SPZQ were lower compared to plasma (Meister *et al.*, 2016). This could be due to the binding to plasma proteins, which is typical for PZQ (Olliaro *et al*., 2014). That would mean concentrations obtained with using Mitra™ are closely related to plasma concentration values. Regarding usefulness and practicality of Mitra™, we confirmed it does carry additional advantages to those of DBS. Since no aids to transfer blood from a finger to the filter paper are needed, the workflow is simpler and faster (see Figure 5 and 6). The same goes for the laboratory work as well, where no punching is necessary and a whole tip can be extracted. However, the issue of incomplete soaking of the tip, an equivalent of insufficient spot size in DBS, remains with Mitra™ as well (see Figure 7). In addition, Mitra™ does not offer much of a labelling surface and consequently, the possibility of a mix-up or sample misplacement is more likely. To overcome this issue, one can order Mitra™ pre-labelled with barcode, allowing to have all information in an electronic database- however, it comes at a higher costs not only for the Mitra™ itself, but also to establish such database. To conclude, Mitra™ is about 4x more expensive per sample compared to DBS and since reliance on a single sample in PK analysis is not ideal, the overall costs of taking duplicates or triplicates of samples would result in much higher expenses of the sampling process. Therefore, cost-effectiveness must be carefully considered when selecting a suitable sampling method.
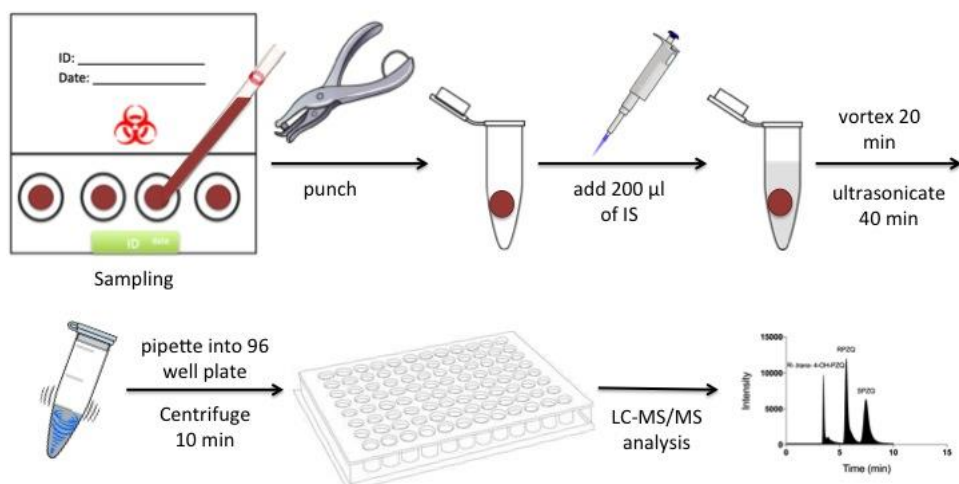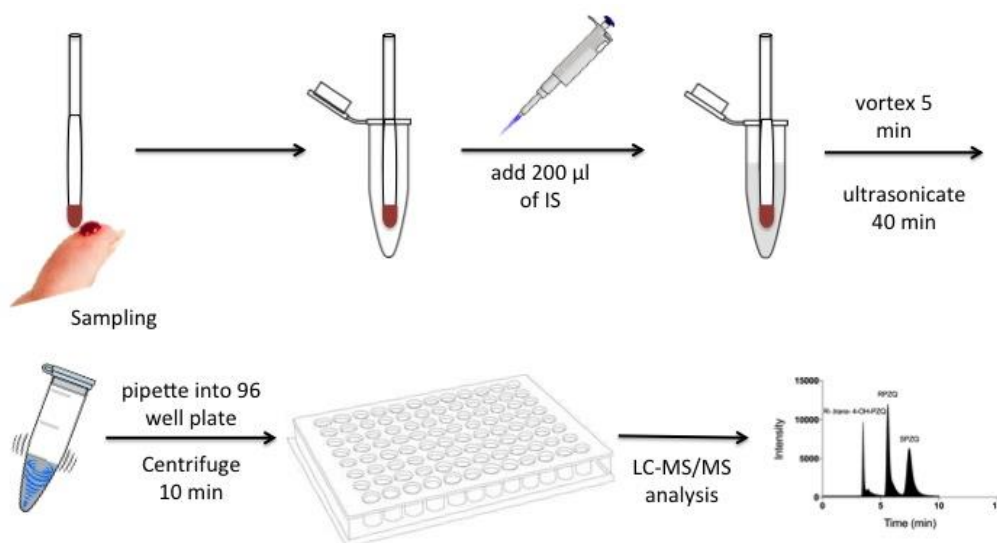
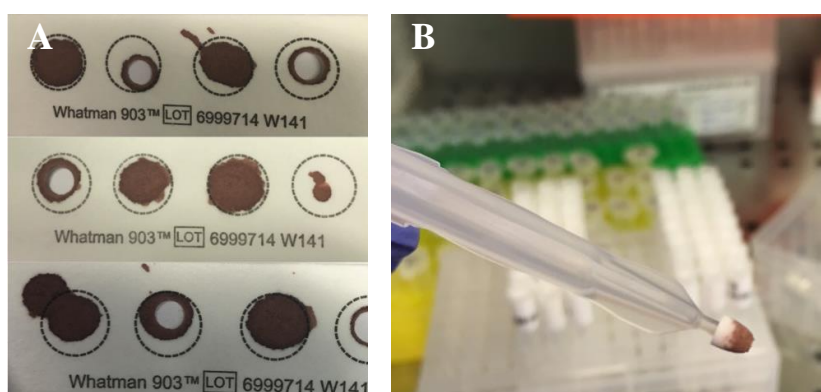**Figure 5:** DBS workflow



**Figure 6:** Mitra™ workflow



**Figure 7:** Examples of DBS (**A**) and Mitra™ (**B**) of a bad quality from our field studies

# Chapter 6

# General conclusion

This thesis aimed to study antischistosomal activity of praziquantel enantiomers (R-/SPZQ) and its main metabolite, R-*trans*-4-OH-PZQ, firstly in laboratory, including both *in vitro* and *in vivo* experiments with *S. haematobium*, the neglected species of *Schistosoma spp.* when it comes to research. We confirmed a suspected greater sensitivity of *S. haematobium* to PZQ compared to *S. mansoni*, with both SPZQ and the main metabolite having a non-negligible effect. Secondly, our work strived to translate results of the animal studies to humans- evaluation of PK behaviour of PZQ in paediatric population not only elucidated parameters, valuable in tailoring a treatment regimen for young children but also revealed influences on drug efficacy, critical to understand dose-response relationship. While NCA itself might not be sufficient to draw conclusions, it is a good building base for a PK model of RPZQ, the proposed eutomer of PZQ, which will aid to predict behaviour of the drug in the body using only simulations, reducing the need for invasive sampling. Thirdly, DBS, commonly used in PK sampling, are well known for their disadvantages originating mostly from haematocrit bias- thus, the aim of this thesis was to evaluate a promising novel micro-sampling device, Mitra™, in comparison to the current gold standard, DBS. The great practicality of Mitra™ was confirmed both in the laboratory and in the field; nonetheless, unexplained overestimation of concentrations in incurred samples compared to spiked samples, calls for further exploration.

PZQ remains cornerstone of schistosomiasis treatment, although several aspects of its mechanism of action and dose-response relationship are not well understood. Reliance on a single drug when treating an infection, affecting over 200 millions of people in the tropics, is far from ideal. While PZQ is being widely distributed in endemic areas, not enough attention is paid to other pillars of successful disease control, such as sanitation and education. Last but not least, with prevalence rates descending and countries announcing the disease is no longer a major public health problem, the attention of funders and policy makers, needed now more than ever to take the next steps towards elimination, is fading. An integrated and persisting approach of control hand in hand with a greater understanding of both the target as well as the weapon, is needed to fight and eliminate this debilitating disease once and for all.

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**Chapter 7**

**Appendix**

**7.1**

# Extrapolation of Praziquantel Pharmacokinetics to a Pediatric Population – A Cautionary Tale

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# Extrapolation of Praziquantel Pharmacokinetics to a Pediatric Population – A Cautionary Tale

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## ABSTRACT

L-praziquantel (PZQ) pharmacokinetic data were analyzed from two relative bioavailability Phase 1 studies in adult, healthy subjects with two new oral dispersion tablet (ODT) formulations of L-PZQ administered under various combinations of co-administration with food, water, and/or crushing. Linear mixed effects models adequately characterized the noncompartmental estimates of the pharmacokinetic profiles in both studies. Dose, food, and formulation were found to significantly affect L-PZQ exposure in both studies. The model for AUC was then extrapolated to children 2 to 5 years old accounting for enzyme maturation and weight. The predicted exposures were compared to an external Phase 1 study conducted by the Swiss Tropical and Public Health Institute using a currently marketed formulation (Cesol 600 mg immediate-release tablets) and found to be substantially lower than observed. A root cause analysis was completed to identify the reason for failure of the models. Various scenarios were proposed and tested. Two possible reasons for the failure were identified. One reason was that the model did not account for the reduced hepatic clearance seen in patients compared to the healthy volunteer population used to build the model. The second possible reason was that PZQ absorption appears sensitive to meal composition and the model did not account for differences in meals between a standardized Phase 1 unit and clinical sites in Africa. Further studies are needed to confirm our hypotheses.

## INTRODUCTION

Praziquantel (PZQ) is the current gold standard treatment for schistosomiasis, one of the most neglected tropical diseases that remains one of the most prevalent parasitic diseases in developing countries. Treatment and control of schistosomiasis is caused primarily by three main schistosome species, *Schistosoma haematobium*, *S. japonicum* and *S. mansoni*, and relies exclusively on PZQ [1]. PZQ was co-developed by Bayer and Merck in the 1970s and commercialized under the name of Biltricide® 600 mg (Bayer), Cisticid® 600 mg and Cisticid® 500 mg (Merck KGaA) for human use. Other generic PZQ products are also marketed worldwide. Both products exist as a 1:1 racemic mixture with L-PZQ (or R-(-)-Praziquantel) being the biologically active enantiomer and the D-isomer (or (S-(+)-Praziquantel) being the inactive enantiomer mostly responsible for its bitter taste [2-4]. The absorption of PZQ from the gastrointestinal tract is nearly complete, with a peak concentration reached within 1 to 2 hours. Due to extensive first-pass metabolism, as little of the drug is excreted unchanged, almost exclusively via the renal path, PZQ has a short half-life of 1 to 3 hours in both healthy normal volunteers and infected adults [4].

The prevalence of schistosomiasis among Sub-Saharan children is very high. In 2015, 53.2 million of 118 million school-aged children in need for treatment received preventive chemotherapy for schistosomiasis [5]. Current treatment is a single dose of 40 mg/kg using 500 mg or 600 mg PZQ tablets. The large size of the commercially available PZQ tablets makes it difficult, especially for young children, to swallow. Hence, PZQ in this population is mostly administered after crushing the tablet. While school-aged children are recognized as one of the most affected populations and regularly treated, pre-school children were until recently not considered. Nonetheless, it was shown that schistosomiasis among young children is very common and there is consensus that they should be included in treatment programs [6]. To address the gap of non-treatment of pre-school aged children,

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the Pediatric Praziquantel Consortium (http://www.pediatricpraziquantelconsortium.org) was established under the umbrella of Lygature (Utrecht, the Netherlands) with partners from the pharmaceutical industry (Merck KGaA, Germany, Astellas Pharma, Japan and SimCyp, United Kingdom), the academic sector (Swiss Tropical and Public Health Institute, Swiss TPH), as well as Fiocruz foundation attached to the Brazilian Ministry of Health. The Schistosomiasis Control Initiative (SCI), part of Imperial College London, joined the Consortium in 2016. The consortium aspires to develop a new pediatric formulation of PZQ and register its use in the pediatric schistosomiasis indication. In the framework of the development of the pediatric formulation, two Phase 1 pharmacokinetic studies were conducted in healthy adult volunteers. The objectives of this current analysis were to characterize L-PZQ pharmacokinetics in adult, healthy subjects enrolled in these Phase 1 studies and to extrapolate these results to those obtained from a Phase 2 study in an African pediatric population infected with *S. mansoni* in order to determine the equivalent pediatric dose for use in a Phase 2 study in the target populations of children 2 to 6 years old to be conducted under the auspices of the PZQ Pediatric Consortium development program.

## METHODS

### *Overview of Studies 200585-001 and 200661-001*

Study 200585-001 (https://clinicaltrials.gov/ct2/show/NCT02325713?term=200585-001&rank=1) was a Phase 1, open-label, randomized, four-period, crossover, single center trial to assess the relative bioavailability of a single oral dose of the new 150 mg Oral Dispersible Tablet (ODT) formulation of PZQ at different dose levels vs. the current commercial 500 mg tablet formulation of PZQ in healthy male subjects. The primary objective of the trial was to assess the relative bioavailability of the newly developed racemic ODT-PZQ tablet of 150 mg dispersed in water versus the current racemate Cysticide® tablet of 500 mg after single oral administration at a dose of 40 mg/kg in healthy subjects under fed conditions.

Subjects were dosed in a 4-period crossover in different cohorts for logistic reasons with a 7 day washout between each administration of study drug. Treatments were:

A. Racemic ODT-PZQ formulation at 40 mg/kg dispersed in water after a meal uncrushed (Period 1 and 2) (n=30).

B. Current PZQ formulation (Cysticide) at 40 mg/kg given with water after a meal uncrushed (Period 1 and 2) (n=30).

C. Racemic ODT-PZQ formulation at 20 mg/kg dispersed in water after a meal (C1) or at 60 mg/kg dispersed in water after a meal (C2) uncrushed (Period 3 and 4) (C1 n=14, C2 n=15).

D. Racemic ODT-PZQ formulation at 40 mg/kg dispersed in water without a meal (D1) or current PZQ formulation at 40 mg/kg given as crushed tablets (using a mortar and pestle) with water after a meal (D2) (Period 3 and 4) (D1 n=14, D2 n=14).

Study 200661-001 (https://clinicaltrials.gov/ct2/show/NCT02271984?term=200661-001&rank=1) was a Phase 1, open-label, randomized, single dose, five period, crossover, single center trial to assess the relative bioavailability of the 150 mg ODT formulation of L-PZQ vs. the current 500 mg PZQ commercial racemate tablet formulation in healthy male subjects. The ODT formulation in Study 200661-001 was different than Study 200585-001 in that the latter was a racemic ODT and the former was a pure enantiomeric L-PZQ ODT. The primary objective of the trial was to assess the relative bioavailability of the recently developed L-PZQ 150 mg ODT tablet versus the current 500 mg racemate PZQ tablet (Cysticide) after single oral administration at a dose of 20 mg/kg of L-PZQ in healthy subjects under fed conditions.

Subjects were dosed in a 5-period crossover in different cohorts for logistical reasons with a 7 day washout between each administration of study drug. Treatments were:

A) L-PZQ ODT formulation at 20 mg/kg dispersed in water, after a meal (Period 1 and 2) (n = 36).

B) Current PZQ formulation (Cysticide) at 40 mg/kg given with water, after a meal uncrushed (Period 1 and 2) (n = 36).

C) L-PZQ ODT formulation at 10 (C1) or 30 (C2) mg/kg (randomized 1 to 1) given dispersed in water, after a meal (Period 3, 4, and 5) (C1 n = 17, C2 n=17).

D) L-PZQ ODT formulation at 20 mg/kg given dispersed in water without a meal (Period 3, 4, and 5) (n = 35).

E) L-PZQ ODT formulation at 20 mg/kg directly disintegrated in the mouth without water after a meal (Period 3, 4, and 5) (n = 36).

Doses in both studies were rounded to the nearest integer tablet size. So, for example, a 70 kg adult scheduled to receive 40 mg/kg of Treatment B, 2800 mg, would be rounded to 3000 mg (6 tablets).

The population to be included in each study consisted of male subjects aged 18 to 55 years inclusive, and a body weight (BW) of 55 to 95 kg, who were certified as healthy by a comprehensive clinical assessment and fulfilled the inclusion and exclusion criteria. When a meal was to be administered with the dose in both studies, a standard high-carbohydrate meal was given consisting of a 100 g Breakfast cereal (All-Bran Flakes); 40 g Bread (Health Loaf/Granary); 250 g Milk (Low Fat/2% Fat, Fresh); 5 g Marmite, Yeast Extract; 10 g Sugar, White, Granulated. The meal contained ~75% carbohydrates of ~650 Calories. Serial plasma samples for pharmacokinetic analysis were collected from each subject in each period in each study. The primary endpoint for each study was the pharmacokinetic parameter $AUC_{0-\infty}$ of L-PZQ assessed in plasma.

All studies were conducted in accordance with the Declaration of Helsinki. Approval of the studies was done by the Medicine Control Council of South Africa and the Ethics Committee of the Faculty of Health Sciences, the University of the Free State, Bloemfontein.

## Population Pharmacokinetic Analysis Using NONMEM

Standard population pharmacokinetic (PopPK) methods and models using NONMEM (version 7.3, ICON Development Solutions, Ellicott City, MD) were used to analyze data from Study 200661-001[7]. However, it became apparent during the analysis that the concentration-time profiles were not well-behaved and had multiple, irregular peaks (Figure 1 and Figure 2). Over a hundred models were examined with different absorption functions (split inputs, recycling, mixed first- and zero-order, etc.). Different estimation methods were tried (FOCEI, SAEM, etc.). These peaks caused severe problems and after weeks of intense effort, a suitable model could not be found. Hence, further model development using population methods was terminated.

## Population Pharmacokinetics Using Linear Mixed Effects Modeling

Since PopPK analysis using NONMEM failed, an alternative plan was devised. The concentration-time data comprised a single dose and the proposed Phase 2 pediatric study also was a single dose study. Therefore, it was concluded that a linear mixed effect model approach using the noncompartmental pharmacokinetic estimates would be a viable alternative to nonlinear mixed effects modeling of the concentration-time profiles and would lead to the same conclusions regarding proposed AUC in the pediatric population.

Noncompartmental analysis was done using Phoenix version 6.3 (Certara, St. Louis, MO). AUC(0-∞) was estimated using the linear up-log down trapezoidal method extrapolated to infinity. Cmax were estimated by direct examination of the data. Log-transformed noncompartmental estimates of AUC(0-∞), denoted AUC hereafter, were the dependent variables in the analysis. Linear mixed effects models were used to analyze the dependent variable as a function of the covariates [8]. This approach can be considered to be an extension of the power model for dose proportionality with the addition of covariates to the model

$$Ln(AUC) = Ln(Dose) + \text{covariates}. \tag{1}$$

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All models were developed using the Mixed procedure in SAS for Windows (Version 9.3, SAS Institute, Cary NC). All models were fit using restricted maximum likelihood (REML). Fisher scoring was done if the initial model could not estimate the parameters. Each study was analyzed separately.

First, a full model with all covariates was fit to the data. For Study 200585-0001, the covariate list included: log-transformed L-PZQ dose administered (DOSE), period (PERIOD), log-transformed weight (WEIGHT), formulation (ODT, 0=current, 1=ODT), whether the tablet was crushed (CRUSH, 0=no, 1=yes), whether drug was administered with food (FOOD, 0=no, 1=yes), age of subject (AGE), and number of tablets administered (TABLETS). For Study 200661-0001 the covariate list included log-transformed DOSE, PERIOD, WEIGHT, ODT, drug taken with water (WATER, 0=no, 1=yes), FOOD, AGE, and TABLETS. Both intercept and log-transformed DOSE were treated as uncorrelated random effects. A simple residual covariance was assumed. Nonsignificant terms ($p > 0.05$ based on the Kenward-Rogers T-test of the parameter estimate) were removed from the model until a parsimonious model with only statistically significant terms ($p<0.01$) remaining in the model.

### Simulations and Extrapolation to a Pediatric Population

The strategy for simulating exposures in African children (2 years to 18 years old) was as follows:

1. Model L-PZQ AUC data from Studies 200661-001 and 200585-001 separately using a linear mixed effects model based on noncompartmental estimates (Eq. (1)).

2. Establish Target AUC in Adults: Simulate L-PZQ exposure from 40, 50, and 60 mg/kg of current racemic PZQ formulation using model from Study 200661-001 using 1000 random resamples from 18 to 55 year olds weighing between 55 and 95 kg in the National Health and Nutritional Examination Status (NHANES) database [9]. These exposures are the reference exposures for comparison to children. Study 200585-001 would then be used as an external validation dataset in adults.

3. External Validation to Swiss TPH Study in *S. mansoni* infected Children (study details are given later in the section): Using the model developed for Study 200661-001, simulate L-PZQ AUC exposures following doses of 20, 40, and 60 mg/kg of the current formulation with food in an African pediatric population (2 to 5 years old) and compare the results to the Swiss TPH study.

4. Predict Doses in African Children to Match Adult Exposures: If Step 3 is successful then simulate and optimize the pediatric equivalent dose in African children for racemic ODT equivalent to current racemic formulation at 60 mg/kg in adults.

5. Predict Doses in African Children to Match Adult Exposures: If Step 3 is successful then simulate and optimize the pediatric equivalent dose for African children L-PZQ ODT equivalent to current racemic formulation at 40, 50, and 60 mg/kg in adults.

In Step 3, clearance in children ($CL_{children}$) is expressed as

$$CL_{children} = CL_{adults} \left( \frac{Weight}{70 \text{ kg}} \right)^{0.75} \times MFA. \tag{2}$$

where MFA is the degree of enzyme maturation. Hence, using the relationship AUC = F×Dose/CL and substituting Eq. (2) for CL, AUC in children can be modeled as:

$$AUC_{children} = \frac{\left[ \text{Predicted AUC from Adult Model in Eq. 1} \right]}{MFA \times \left( \frac{Weight}{70 \text{ kg}} \right)^{0.75}}. \tag{3}$$

Modeling Cmax is problematic because no such equation exists. Hence, simulation of PZQ exposures in children was limited to AUC.

In order to simulate the AUC in African children, the weight of the children and the degree of enzyme maturation (MFA) relative to adults must be accounted for[10]. To account for weight, age was varied in the simulations and the weight of the African child was imputed using growth charts reported by

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the Liverpool School of Tropical Medicine [11], hereafter called the Liverpool dataset. MFA, which was provided by Simcyp within the Pediatric Praziquantel Consortium, was used to correct for enzyme maturation in very young children and was calculated on the basis of the ontogenies of each CYP isoform involved in the metabolism of L-PZQ and the fraction of L-PZQ clearance mediated by each CYP isoform determined from scaling *in vitro* metabolism data provided by Merck KGaA (data on file). Eq. (4) to (8) describe the maturation of CYP isoforms involved in the metabolism of L-PZQ as a fraction of the adult abundance based on AGE:

$$CYP1A2 = \begin{cases} 0.24 + \dfrac{1.47 \times AGE^{1.73}}{0.36^{1.73} + AGE^{1.73}} & \text{if age} < 2 \\ 0.83 + 0.79 \exp\left(-0.06\left(AGE - 1.8\right)\right) & \text{if age} \geq 2 \end{cases} \quad (4)$$

$$CYP2C9 = 0.17 + \dfrac{0.81 \times AGE^{0.53}}{0.0157^{0.53} + AGE^{0.573}} \quad (5)$$

$$CYP2C19 = 0.3 + \dfrac{0.68 \times AGE^{2.44}}{0.29^{2.44} + AGE^{2.44}} \quad (6)$$

$$CYP3A4 = \begin{cases} 0.11 + \dfrac{0.95 \times AGE^{1.91}}{0.64^{1.91} + AGE^{1.91}} & \text{if AGE} < 2.3 \\ 1.1 - 0.123 \exp\left(-0.05\left(AGE - 2.2\right)\right) & \text{if AGE} \geq 2.3 \end{cases} \quad (7)$$

$$CYP3A5 = \begin{cases} 0.11 + \dfrac{0.95 \times AGE^{1.91}}{0.64^{1.91} + AGE^{1.91}} & \text{if AGE} < 2.3 \\ 1.1 - 0.123 \exp\left(-0.05\left(AGE - 2.2\right)\right) & \text{if AGE} \geq 2.3 \end{cases} \quad (8)$$

$$MFA = \begin{cases} \begin{array}{c} 0.21 \times CYP1A2 + 0.23 \times CYP2C9 + 0.29 \times CYP2C19 + \\ 0.19 \times CYP3A4 + 0.08 \times CYP3A5 \end{array} & \text{if AGE} < 25 \\ 1 & \text{if AGE} \geq 25 \end{cases} . \quad (9)$$

*External Comparison to Swiss TPH Study*

After completion of the analysis and all simulations of Steps 1 and 2, the results were compared to the observations from a randomized, single blind, parallel group Phase 2 study in African preschool (2 to 5 years old) and school-aged (6 to 11 years old) *S. mansoni* patients [12]. In this study, children were randomized to receive a single-dose of placebo, 20, 40, or 60 mg/kg racemic praziquantel (Cesol 600 mg tablets, Merck KgA). Praziquantel was administered based on weight (to the nearest half tablet, respectively), which was measured during the physical examination before treatment. For the preschool children the tablets were crushed and mixed with 20-50 mL syrup-flavored water to mask the taste. A standardized food item (sandwich with butter or fishpaste) was provided before treatment.

Dried blood spots were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, 8, 12, and 24 h after dosing and analyzed for L- and D-praziquantel concentrations (linear calibration range of 0.01 to 2.5 µg/mL), as well as its active metabolite trans-4-hydroxypraziquantel, using a validated, enantioselective LC-MS/MS method [13]. L-PZQ AUC results are presented here. These were compared to the predictions based on the linear mixed effect models developed in Steps 1 and 2. To make the comparisons valid, the raw noncompartmental results from that study were corrected for crushing of tablets (+20% multiplier, value taken from Treatment Arm D2 in Study EMR200585-001) and for use of dried blood spots as the bioanalytical matrix (+10% multiplier, value from [14]).

# Results

*Description of Observed Data*

*Study 200585-001*: A total of 119 noncompartmental estimates of AUC from 32 subjects were available for analysis. Subjects ranged in age from 21 to 44 years with a mean of 29.0 years and from 53.5 to 91.6 kg in weight with a mean of 71.7 kg. The number of tablets administered (for both the current formulation and ODT formulation) ranged from 4 to 25 with a mean of 12.6. The number of subjects per treatment is shown in Table 1. Of all 32 (100%) healthy male subjects included in the

trial 19 subjects (59.4%) were Black or African American, 11 subjects (34.4%) were White and 2 subjects (6.3%) were of other races. *Study 200661-001*: A total of 177 noncompartmental exposure parameters of AUC from 36 subjects were available for analysis. Subjects ranged in age from 19 to 47 years with a mean of 26.3 years and ranged from 55.2 to 87.8 kg in weight with a mean of 69.4 kg. The number of tablets administered ranged from 8 to 12 with a mean of 9.5. The number of subjects per treatment is shown in Table 1. Of all 36 healthy male subjects included in the trial, 27 subjects (75.0%) were Black or African American, 6 subjects (16.7%) were White and 3 subjects (8.3%) were of other races. *Swiss Tropical and Public Health Institute Study:* A total of 28 to 33 AUC measurements per treatment group (95 children in total) ranging in age from 2 to 5 years old, treated with 20, 40 and 60 mg/kg, and ranging in weight from 8.0 to 22.3 kg were used for our analyses.

## *Model Development using Nonlinear Mixed Effect Modeling with NONMEM in Study 200661-001*

A total of 977 observations were available from 36 individuals from Study 200661-001. The concentration-time profiles for L-PZQ were erratic within and across subjects. Dozens of models using population pharmacokinetic methods were tested with a variety of absorption models. No acceptable base model could be found. The reasons for failure were myriad, but were predominantly due to unacceptable goodness of fit or convergence failure. As such, no model development was attempted for Study 200585-001.

## *Modeling of AUC Estimates Using Linear Mixed Effect Models in SAS*

*Study 200585-001:* The Akaike Information Criterion (AIC) for the full model with all covariates was 154.5. The best model, which had an AIC of 139.2, was one where only log-transformed DOSE and FOOD were included in the model. All other terms were not statistically significant ($p > 0.01$). The parameter estimates for the AUC model are shown in Table 2. Goodness of fit plots for the model are shown in Figure 3. The best fit AUC model was:

Model $\text{Ln}(\text{AUC}) = -9.99 + 2.3 \times \text{Ln}(\text{L-PZQ Dose in mg}) + 0.81 \times \text{FOOD}$ (10)

which in the original domain can be written as:

$$Ln(AUC \text{ in ng*h/mL}) = -9.99 + 2.3 \times Ln(\text{L-PZQ Dose in mg}) + 0.81 \times FOOD$$ (11)

$$AUC = \frac{DOSE^{2.3} \exp(0.81)^{FOOD}}{\exp(9.99)}$$ .

$$AUC = \frac{(DOSE)^{2.3} \times 2.25^{FOOD}}{22026}$$

Eq. (11) can also be expressed in terms of apparent oral clearance. It was assumed that the effect of Food would be manifest through its effect on F. Eq. (11) was then rewritten as

$$AUC = \frac{(DOSE)^{2.3}}{\dfrac{CL}{F}} = \frac{(DOSE)^{2.3}}{\dfrac{22026}{2.25^{FOOD}}} .$$ (12)

These results showed that the presence of food increased AUC by 125% and that the dose-AUC relationship was supraproportional. They further showed that none of the other available covariates (age, weight, number of tablets, etc.) significantly influenced AUC.

***Study 200661-001:*** The AIC for the full model with all covariates was 288.2. The best model, which had an AIC of 269.0, was one where only log-transformed DOSE, ODT formulation, and FOOD were included in the model. All other terms were not statistically significant (p > 0.01). The parameter estimates in the AUC model are shown in *Table 2*. Goodness of fit plots for the model are shown in Supplemental Figure 1. The best fit AUC model was:

Model $\text{Ln}(\text{AUC}) = -7.52 + 2.0 \times \text{Ln}(\text{L-PZQ Dose}) - 0.81 \times \text{ODT} + 0.62 \times \text{FOOD}$ (13)

Which in the original domain can be written as:

$$Ln(AUC) = -7.52 + 2.0 \times Ln(\text{L-PZQ Dose in mg}) - 0.81 \times ODT + 0.62 \times FOOD \qquad (14)$$

$$AUC = \frac{DOSE^{2.0} \exp(0.62)^{FOOD}}{\exp(7.52)\exp(0.81)^{ODT}} \qquad .$$

$$AUC = \frac{(DOSE)^2 \times 1.86^{FOOD}}{1845 \times 2.25^{ODT}}$$

These results showed that the presence of food increased AUC by 86%, that there was a 55% decrease in AUC with the L-PZQ ODT formulation, and that the dose-AUC relationship was supraproportional. They further showed that none of the other available covariates (age, weight, number of tablets, etc.) influenced AUC.

### External Comparison to Swiss TPH Study

Aggregate noncompartmental pharmacokinetic data from the Swiss TPH study were used from 95 patients. The results for total AUC stratified by total dose are shown in *Table 3*.

### Extrapolation and Simulation of Pediatric Exposures

L-PZQ exposures in adults were simulated at doses of 40, 50, and 60 mg/kg with the current racemic formulation when administered with food and water using the NHANES adult database and the model from Study 200661-001 (Step 2 of the Simulation). Because of high variability of the tails of the distribution, the upper and lower tails were trimmed by 10% prior to data summarization. The observed and trimmed simulated results are presented in *Table 3*. Note that the observed target AUC in adults from 200661-001 study was 2066 ng*h/mL. The model did a reasonable job of predicting total AUC and was within 20% relative error for the median prediction.

The next step (Step 4) was to simulate doses of 20, 40, and 60 mg/kg of the current formulation with food using an African pediatric population and compare the results to the Swiss TPH study. Supplemental Figure 2 presents a band plot of weight as a function of age in Africans and its comparison to Western subjects in the US NHANES database. Although the growth trajectories are similar, the weight of Africans is smaller than Western population. This importance of this difference

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will manifest itself later in the dose requirements for Africans requiring smaller doses than their Western counterparts. Supplemental Figure 3 presents a scatter plot of the observed doses used in the clinical studies compared to the simulated doses based on the Liverpool weight data. The simulated subject weights using the Liverpool dataset were similar to the observed subject weights in the Swiss TPH study confirming the validity of the Liverpool dataset to estimate age-specific weights in African children.

Figure 4 presents a series plot of the effect of MFA on clearance in children (using the MFA equation used in this analysis compared to a published CYP3A4 MFA function published by Anderson and Larsson [15] as a function of age. Although the effect of MFA tends to plateau around 2 years of age, the overall effect on clearance, as reflected by the product of MFA and weight, doesn't asymptote until well into adulthood and is predominantly linear in nature until then. Based on the results of the modeling of noncompartmental parameters from Study 200661-001, Eq. (14) was modified to incorporate the allometric effect of weight and effect of MFA in young children:

$$AUC_{children} = \frac{\left(\text{L-PZQ DOSE}\right)^2 \times 1.86^{FOOD}}{1845 \times 2.25^{ODT}\left(\dfrac{\text{Weight}}{70 \text{ kg}}\right)^{0.75} \times MFA}. \qquad (15)$$

Figure 5 presents the observed AUC in Study 200585-001, 200661-001, and the Swiss TPH study compared to simulated values under the model developed using Study 200661-001. The model predicted values were significantly less than observed values from the Swiss TPH study. Simulated median AUC was underpredicted by ~10-fold compared to observed values from the Swiss TPH study. At this point further modeling and simulation efforts ceased because, despite our best efforts, a suitable model that could reliably extrapolate to a pediatric population could not be developed.

## Discussion

### *Failure Analysis – Post-Mortem*

It was clear from the results of the Swiss TPH study that the predicted exposures in African children extrapolated from the adult model were significantly lower than the observed results of the Swiss TPH study and that a dose of 40 mg/kg of the current formulation in African children would provide equivalent exposure as 40 mg/kg of the same formulation in adult Western healthy volunteers. The question is why? Either the model was "wrong" and the predictions from the model were too low or the model was "right" and the observed data were higher than expected. Were there clues that might have told us to be wary of the simulation results? Maybe. In hindsight.

After it was realized that the modeling results were not successful, a root cause failure analysis (RCFA) was undertaken to look for possible reasons for the failure. RCFA has its origin in the NASA program to understand why rockets failed in their launches [16]. One definition of root cause analysis is [16]:

> *"The primary aim of root cause analysis is: to identify the factors that resulted in the nature, the magnitude, the location, and the timing of the harmful outcomes (consequences) of one or more past events; to determine what behaviors, actions, inactions, or conditions need to be changed; to prevent recurrence of similar harmful outcomes; and to identify lessons that may promote the achievement of better consequences. ("Success" is defined as the near-certain prevention of recurrence)".*

A brainstorming session was held and a number of factors were identified as possible causes:

1. The model was "wrong" and predicted exposures were lower than expected, or

2. The model was "right" but the observed Swiss TPH exposure data were higher than expected because of:

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a. Differences in the study population (healthy volunteers vs patients);

b. Differences between crushed tablets and intact tablets, number of tablets administered, or solubility saturation;

c. Differences in the meal composition;

d. Differences in bioavailability between adults and children; and/or

e. Differences in biological matrix (plasma vs dried blood spot).

Each factor was examined and either accepted or rejected as a possible factor:

1.) Could the model have been "wrong"? Could the discrepancy between simulated and observed exposures be explained by an inadequate model? Without going into the semantics of all models being wrong, could the extrapolation of adults to children resulted in predicted exposures that were too low? It should be pointed out that this was the first and immediate reason team members used to explain the discrepancy because the modeling approach that was used was not a standard approach and because "it's a model." The typical pediatric extrapolation approach is to first model the concentration-time profiles using PopPK and then extrapolate to children using an allometric scaling factor on clearance to account for differences in weight and, in really young children, to use a maturation factor to account for differences in enzyme immaturity. The approach herein modeled the AUC directly, and by extension, clearance indirectly since an adequate PopPK model could not be developed due to the erratic nature of the concentration-time profiles in adults. Since only a single dose of PZQ is given therapeutically, these approaches should have produced equivalent results. Indeed, the LMEM was very good at predicting the observed data in adults across all treatment arms. It may be that the traditional allometric scaling correction and maturation function used in this analysis do not apply to African children. There is only 1 report in the literature that we could find related to pediatric extrapolation in an African population. Zvada SP et al. [17] reported on the successful extrapolation of rifampicin,

pyrazinamide, and isoniazid exposures in African children with tuberculosis. For those 3 drugs the authors used the standard allometric equations to scale down clearance from adults to children and used a maturation function to account for age-related differences in children less than a year old. The maturation function was defined by a Hill model that starts from zero and asymptotes to an adult value of 1, and in which the values of $TM_{50}$ (the age at which maturation is 50% of the adult value) and Hill (the slope coefficient) were fitted to the available data [18]. Our analysis used similar extrapolation methods as Zvada et al., although our maturation function was different than theirs, so the likelihood that extrapolation functional differences explain the discrepancy is low.

We also questioned whether the population-extrapolation approach is universally successful. It could be that the PopPK approach, which works often in practice, has led to an unreasonable expectation that, due to publication bias, this approach will work uniformly for all drugs in every case. Certainly there is this impression in the literature as any example of extrapolation failure could not be found on PubMed and it does seem unlikely that any single method would work universally in every case for every drug. Bonate and Howard [19] stated that the predictability of human pharmacokinetic parameters from allometric scaling of animals to man was overly optimistic because of a positive publication bias in the literature and yet here we are again, almost 20 years later, suggesting that allometric scaling of adult data to pediatrics might have the same bias, but may still be correct.

Another suggestion was that the simulations were based only on the model developed from the 200585-001 data and that perhaps a model using all the data from both studies would result in better predictions. A secondary analysis was completed after this suggestion was made and the results are shown in Supplemental Model 1. Using a combined model did not improve predictions – there was still a wide discrepancy between observed and predicted exposures. In the end, it's

impossible to say with certainty if the discrepancy was due to model failure without another external dataset to validate against.

2a.) The other reason for the discrepancy could be broadly categorized as the model was "correct" but the observed data in African children was higher than expected. There were many differences in the study design and populations used to develop the model and the target population the model would be extrapolated to. The model was built using data from a well-controlled, Phase 1 adult, Western South African population and was being extrapolated to a pediatric infected African population. Could the differences be explained by race? It seems unlikely. In Study 200585-001, 59% of the subjects were Black, while in Study 200661-001 the number was 75% Black. While the Phase 1 results were largely based on a Black study population, it seems reasonable to generalize the results to all Black Africans on face-value. A second factor considered were differences in subject weight. The simulations used a dataset developed by the University of Liverpool to simulate a given weight based on a given age range. If the weight ranges in the Liverpool dataset were not representative of the weight ranges used in the Swiss TPH study then different exposures could have been obtained. But examination of the simulated weights and observed weights in the Swiss TPH study revealed them to be similar (Supplemental Figure 3), ruling this out as a possibility.

A third factor considered was that the Western adult studies were in healthy volunteers while the Swiss TPH study was in infected children. Mandour et al. [20] studied the differences in PZQ pharmacokinetics in healthy volunteers and Sudanese schistosomiasis patients with different grades of liver impairment using the Distocide®  and Biltricide® formulations. Large differences in exposure were seen related to the degree of liver impairment. For example, AUC was 4- to 5-times higher in severely impaired patients (Child Pugh C) than healthy volunteers after administration of 40 mg/kg Biltricide (15928 vs. 3823 ng*h/mL, respectively). Watt et al. [21] reported similar finding in Filipino patients with a disease-dependent increase in exposure with severe ($AUC_{24}$ = 37.8 μg*h/mL) > moderate ($AUC_{24}$ = 22.9 μg*h/mL) > unapparent ($AUC_{24}$ = 8.9 μg*h/mL) hepatic disease. Since

schistosomiasis causes periportal fibrosis and liver cirrhosis due to deposition of eggs in the small portal veins [22], an increase in AUC in the disease state seems entirely reasonable, although the disease in young children is generally not accompanied by liver abnormalities. In addition, 80% of preschool children analyzed in the Swiss TPH study harbored light infections [12], hence liver abnormalities are not expected.

And lastly, it was considered was that PZQ absorption is sensitive to gastrointestinal (GI) pH. Sammon et al. [23] showed that in rural South African adults the mean 24 h stomach pH was 2.84 and at night was as high as 3.7, which was considerably higher than historical data in Western subjects. Reaching pH levels seen after cimetidine administration (pH 3.1 to 6, cimetidine package insert), higher basal stomach pH in African children may have contributed to higher than expected absorption and higher than expected exposure in the Swiss TPH study. Therefore, there were differences in the patient population that could explain part of the differences in the predicted exposures simulations.


2b.) Another possible reason for the difference could have been differences in absorption and bioavailabilty due to crushing, the number of administered tablets, or solubility saturation. In the Swiss TPH study, the PZQ tablets were crushed prior to administration in preschool aged children.  In Study 200585-001, crushing decreased the L-PZQ AUC by 18% (90% CI: 31% to 2%) for the marketed formulation Cysticide. Hence, crushing decreased absorption, it did not increase it. Therefore, the effect of crushing could not account for the larger than expected exposures seen in the Swiss TPH study.

Maybe the difference could have been due to the number of tablets administered? In Study 200585-001, a large number of tablets were administered, 7 to 35 for the ODT formulation and 4 to 8 with Cysticide. In Study 200662-001, 5 to 7 Cysticide tablets and 4 to 17 ODT tablets were administered. For the Swiss study, 1 to 3 tablets were administered. Administration of a large number of tablets could result in a different dissolution profile and change the oral absorption of the drug. This

hypothesis was put forth early as a reasonable explanation. However, statistical analysis of the 200585-001 and 200662-001 data did not detect any effect of number of tablets administered, i.e., the number of administered tablets did not affect exposure. Therefore, this seems an unlikely reason for the difference in exposures.

Related to this was the hypothesis that PZQ saturation in GI fluids and its effect on absorption was the reason for the difference. PZQ is a Biopharmaceutics Class System (BCS) II drug meaning it has high permeability, low solubility and dissolution is the rate-limiting step in the absorption of PZQ. The therapeutic dose of 40 mg/kg in a 70 kg adult is around 3000 mg, which is quite high. With a water solubility of 0.38 mg/mL (https://www.drugbank.ca/drugs/DB01058) and a fasted state simulated intestinal fluid (FaSSIF) solubility of 0.26 mg/mL (Fagerberg JH et al., 2015), this means that in 70 kg adults receiving a dose of 2800 mg, drinking a water volume of 250 mL, only 95 mg of drug is in solution at any given time with more than 97% of the dose initially not in solution (Figure 6). In a 5 yr. old child weighing 13 kg who received a dose of 500 mg drinking a water volume of 50 mL (Shawahna R, 2016), 19 mg PZQ is in solution (~3% of the dose). One hypothesis assumed that with the bulk of drug being undissolved in the stomach and since water in the GI tract is not uniform but found in pockets [24], the erratic concentration-time profiles with multiple peaks could be explained by drug solubilization in water pockets and subsequent absorption, or by non-documental additional water intake by the subjects. But again, since the 80% of the drug is absorbed, despite a large amount of initially undissolved drug, sink conditions on the basolateral side of the enterocytes during intestinal absorption would provide an explanation for the amount of drug-related material absorbed, and thus it seems unlikely that an increase in F in children would result in the kind of exposure discrepancy that was observed

2c.) There were also differences in the meal composition between the Western adult studies and Swiss TPH study that may have led to a difference in predicted exposures. The Western adult studies used a

standard high-carbohydrate meal, while the Swiss TPH study used a sandwich with butter or fishpaste, which is more towards a high fat meal. Castro et al. [25] showed that PZQ pharmacokinetics were dependent on the composition of the meal. A standard high fat meal increased AUC by 172% and Cmax by 212% compared to the fasting state, but a high carbohydrate meal increased AUC and Cmax even more, 298% and 484%, respectively. It is possible that differences in meal type between studies could account for some of the differences between the observed Swiss TPH results and predicted exposures.

2d.) Another reason for the discrepancy could be related to differences between adults and children in oral absorption and first pass metabolism. It is assumed in any pediatric extrapolation that oral bioavailability (F) is the same in adults and children such that apparent oral clearance (CL/F) scales with weight. This works only if F is a constant between adults and children. Often this assumption is left unsaid in manuscripts and in presentations but it is a critical assumption. The correction using weight and maturation factors during the extrapolation process are for changes in total systemic clearance (CL) with age. There are no corrections for F with age, despite there being known differences in young children, particularly very young children less than a year of age [26]. Is F a constant in adults and children for PZQ? Patzschke et al. [27] showed that following a standard breakfast (200 mL water, 1 roll with margarine, boiled ham, and a cup of coffee), renal excretion after a radioactive dose of 46 mg/kg PZQ was $80 \pm 6\%$. Therefore, the fraction of dose absorbed, either as parent drug or metabolites, must be at least 80%. Hence, while there might be some role for differences in F, it seems unlikely to explain the discrepancy. Related to this explanation is another possibility that adults may have a clearance pathway that school-aged children do not. Although CYP pathways mature by 2 years of age, it's possible there is an extra-hepatic pathway that hasn't fully matured yet. There is no experimental evidence for this nor are there examples such pathways exist, so this possibility cannot be confirmed. In total, differences in first pass metabolism and oral absorption seem an unlikely cause to explain the simulation differences.

2e.) Another explanation could have been that the Swiss study used dried blood spots as the sample matrix and the Western studies used plasma. This was quickly ruled out because the analytical methods were both validated and were largely interchangeable with only a 10% difference in measured concentrations (plasma was slightly higher than DBS)[14].

## Conclusions

Because of the erratic nature of the concentration – time profiles, a suitable population pharmacokinetic model could not be developed using standard nonlinear mixed effect models. Using linear mixed effect modeling of the noncompartmental estimates for AUC a suitable predictive model could be developed, which produced parameter estimates consistent with the statistical analysis of the noncompartmental estimates. However, when this model was used to extrapolate to a pediatric population, the simulated exposures were ~10-fold lower compared to results obtained from a clinical study in the population of interest (the Swiss TPH study). A post-mortem afterwards suggested possible reasons for this difference, with differences in the meal composition and study populations being of sufficient magnitude to explain the discrepancy.

Root cause analysis highlighted a number of important considerations that are not often made or reported in the literature. First, pediatric extrapolation likely has a publication bias – negative studies where the extrapolation has failed are not reported. Journals need to encourage publication of failed pediatric extrapolations so that modelers can learn from them and not make the same mistakes next time. Second, a very important assumption made in pediatric extrapolation is constant oral bioavailability from adults to children. The allometric scaling equation and maturation function were designed to work for scaling total systemic clearance assuming absorption is a constant in adults and children. This may or may not be the case for every drug. Shawahna [28] showed that the Biopharmaceutics Classification System Class can change from adults to children due to differences

in gastric volume and, if this is the case, it seems likely that bioavailability may change as well. This factor should be at least considered in any pediatric extrapolation.

Study- and population-related differences seem the most likely cause to explain the difference between the observed and simulated data (Table 4). Two of them, patients vs. healthy volunteers and differences in meal composition, were of sufficient magnitude to suggest that these differences seen in published accounts could explain the discrepancy. Could all or some of these factors have played a role? Yes. But it is impossible to identify with any certainty the reasons for the discrepancy. The reasons do lead to possible future studies and hypotheses to be tested.

While the idea of a root cause analysis to identify the reasons for model failure is useful, one must be careful not to put too much weight in their results because of confirmation bias. We know that the simulated and observed exposures were off by about 10-fold. So going back and looking for factors that might lead to a 10-fold increase in exposure and bring the exposure predictions in agreement with the observed Swiss TPH results is self-confirmatory. In  other words, we look for reasons that are in agreement with our 10-fold discrepancy and rule out those that don't increase agreement between observations and predictions. Nevertheless, the root cause analysis did identify some factors and assumptions that might be useful to test and control in future pediatric extrapolations. These include a careful examination of differences in pharmacokinetics between healthy volunteer and patients, differences in drug administration and possible changes in BCS Class between adults children, and an examination that oral bioavailability is a constant between adults and children.

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**Tables**

| Study | Treatment | Formulation | Dose (mg/kg) | With Water | Fast or Fed | Crush Tablets | Number of Subjects |
|-------|-----------|-------------|--------------|------------|-------------|---------------|---------------------|
| 200585-001 | A | Racemic ODT | 40 | Water | Food | No | 31 |
| | B | Current Formulation | 40 | Water | Food | No | 31 |
| | C1 | Racemic ODT | 20 | Water | Food | No | 14 |
| | C2 | Racemic ODT | 60 | Water | Food | No | 15 |
| | D1 | Racemic ODT | 40 | Water | Fasting | No | 14 |
| | D2 | Current ODT | 40 | Water | Food | Yes | 14 |
| 200661-001 | A | L-PZQ ODT | 20 | Water | Food | No | 36 |
| | B | Current Formulation | 40 | Water | Food | No | 36 |
| | C1 | L-PZQ ODT | 10 | Water | Food | No | 17 |
| | C2 | L-PZQ ODT | 30 | Water | Food | No | 17 |
| | D | L-PZQ ODT | 20 | Water | Fast | No | 35 |
| | E | L-PZQ ODT | 20 | ---- | Food | No | 36 |

Table *1:* Number of Subjects for Each Treatment Group by Study

**Table 2**: **Parameter Estimates from Best Linear Mixed Effect Models For L-PZQ AUC in Healthy Volunteers Enrolled in Studies 200585-001 and 200661-001**

| Study | Parameter | Estimate | Standard Error | T-test | p-value |
|---|---|---|---|---|---|
| 200585-001 | Intercept | -9.99 | 0.850 | -11.76 | < 0.0001 |
| | Ln-DOSE | 2.30 | 0.116 | 19.9 | < 0.0001 |
| | Food | 0.811 | 0.0956 | 8.49 | < 0.0001 |
| | Var(Intercept) | 0.209 | | | |
| | Residual Variance | 0.0972 | | | |
| 200661-001 | Intercept | -7.52 | 0.930 | -8.09 | <0.0001 |
| | Ln-DOSE | 2.01 | 0.126 | 15.98 | <0.0001 |
| | ODT formulation | -0.81 | 0.0746 | -10.85 | <0.0001 |
| | Food | 0.62 | 0.0754 | 8.17 | <0.0001 |
| | Var(Intercept) | 0.372 | | | |
| | Residual Variance | 0.148 | | | |

**Table 3: Comparison of Observed and Simulated L-PZQ AUC Estimates**

| Dose (mg/kg) | Observed Study 200661-001 | Simulated Using Model 200661-001 | STPHI Study |
|---|---|---|---|
| 20 | | | 2730 (489-17871) |
| 40 | 2066 (660-6746) | 2164 (599 – 7893) | 3256 (726-7987) |
| 50 | | 3474 (1011-11666) | |
| 60 | | 5101 (1427-17235) | 5567 (855-22822) |

Data are reported as mean (range).

**Table 4: Root Cause Analysis**

| Reason | Possible Magnitude of Effect | Likelihood to Explain Discrepancy |
|---|---|---|
| Model was "wrong" | | |
| • Use of LMEM was not appropriate | Small | Unlikely (but unknown for sure) |
| • Allometric scaling was inappropriate | Unlikely | Unlikely |
| • Wrong maturation factor used | Unlikely | Unlikely |
| • Did not use all the data | Small | Unlikely |
| • Differences in infected patients and healthy volunteers not accounted for in model | Small to Large | Possible to Likely |
| Study results were higher than normal | | |
| • Racial differences (Western vs. African, Caucasians vs Blacks) | Small | Unlikely |
| • Weight differences | Small | Unlikely |
| • Healthy volunteers vs infected patients | High | Likely |
| • Differences in meal types | High | Likely |
| • Differences in stomach pH between Western and African patients | Small | Possible |
| • Extrahepatic metabolic pathway in adults not seen in children | Small to moderate | Unlikely |
| • Crushing of tablets in Swiss TPH study | Small | Possible |
| • Differences in oral bioavailability between adults and children | Small | Possible |
| • Differences in analytical methods | Small | Unlikely |
| • Number of tablets administered | Small | Unlikely |
| • Differences in PZQ saturation in GI tract between adults and children | Small | Unlikely |

**Index to Figures**

Figure 1.  Spaghetti plot of L-PZQ concentration-time profiles after oral administration in Study 200585-001 stratified by treatment.  Black line is the median concentration.  Gray lines are individual subjects.

Figure 2.  Spaghetti plot of L-PZQ concentration-time profiles after oral administration in Study 200661-001 stratified by treatment.  Black line is the median concentration.  Gray lines are individual subjects.

Figure 3.  Goodness of fit plot for L-PZQ AUC model for Study 200585-001.  Upper left: red line is the LOESS smooth to the data.  Symbols are by treatment.  Upper right: blue line is standard normal distribution, red line is kernel smooth to the empirical data.  Lower left: black line is line of unity, red line is LOESS smooth.  Symbols are by treatment.  Lower right: QQ plot of residuals; blue line is theoretical normal distribution line.

Figure 4.  Effect of allometric weight and MFA, as calculated by two different methods (SimCYP method used in this analysis and CYP3A4 function reported by Anderson and Larrson [15], on clearance in Africans.

Figure 5.  Forest plot comparing the observed L-PZQ AUC in Study 200585-001, Study 200661-001, and the Swiss Tropical and Public Health Institute Study to simulated AUCs based on the model developed using Study 200661-00.  Each box is the 1$^{st}$ and 3$^{rd}$ quartile.  The middle line in the box is the median (2$^{nd}$ quartile).  The diamons are the mean. The whiskers are 1.5 times the inter-quartile range.

Figure 6:  Cartoon of drug dissolution in the stomach of adults and children.  It should be noted that dissolution can also take place in the intestine.

**Index to Supplemental Figures**

Supplemental Figure 1. Goodness of fit plot for L-PZQ AUC model for Study 200661-001

Supplemental Figure 2. Weight as a function of age in Africans as reported by Hayes et al. (2015) and comparison the US NHANES database. Band is the 5rd and 95th percentile for Africans. Solid blue line is the median for Africans. NHANES data are reported as red symbols. Red line is the loess smooth to the NHANES data.

Supplemental Figure 3. Total dose administered in Study 200585-001, Study 200661-001, and the Swiss Tropical and Public Health Institute Study compared to simulated doses using the current formulation

## Figures

## Figure 1.
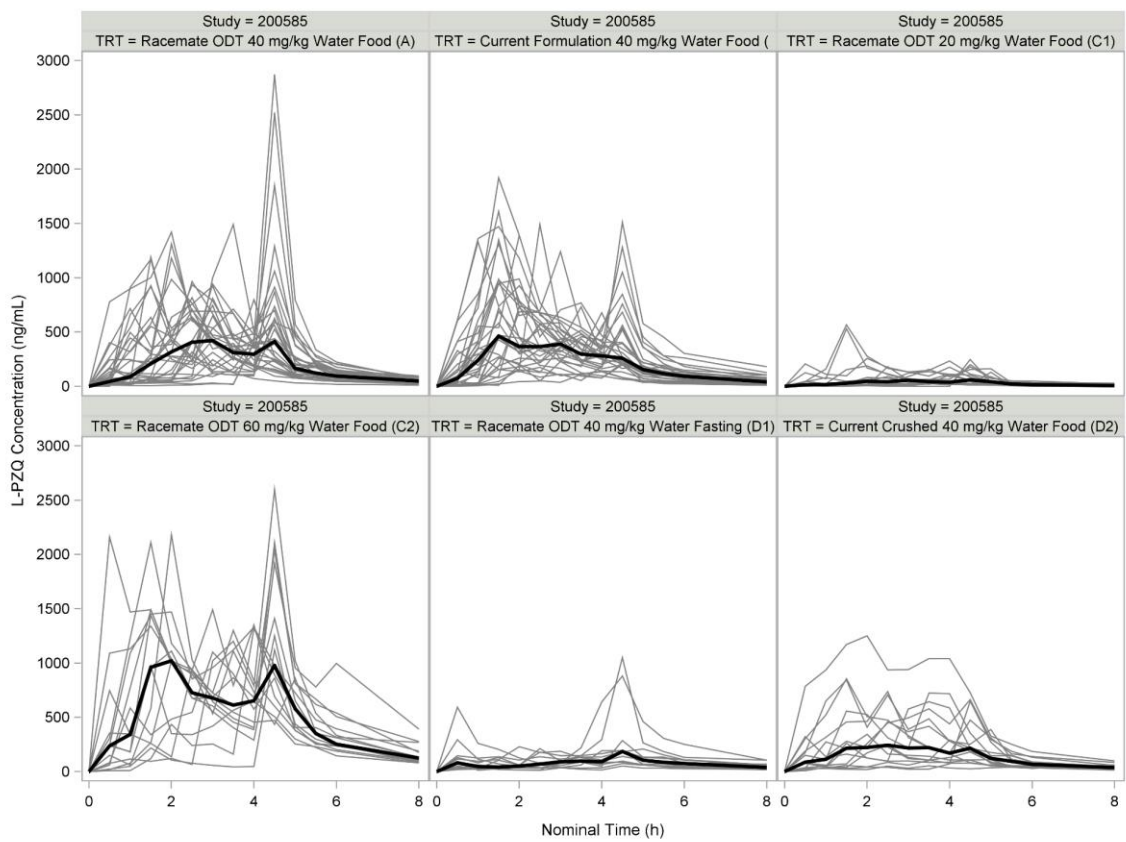
**Figure 2.**
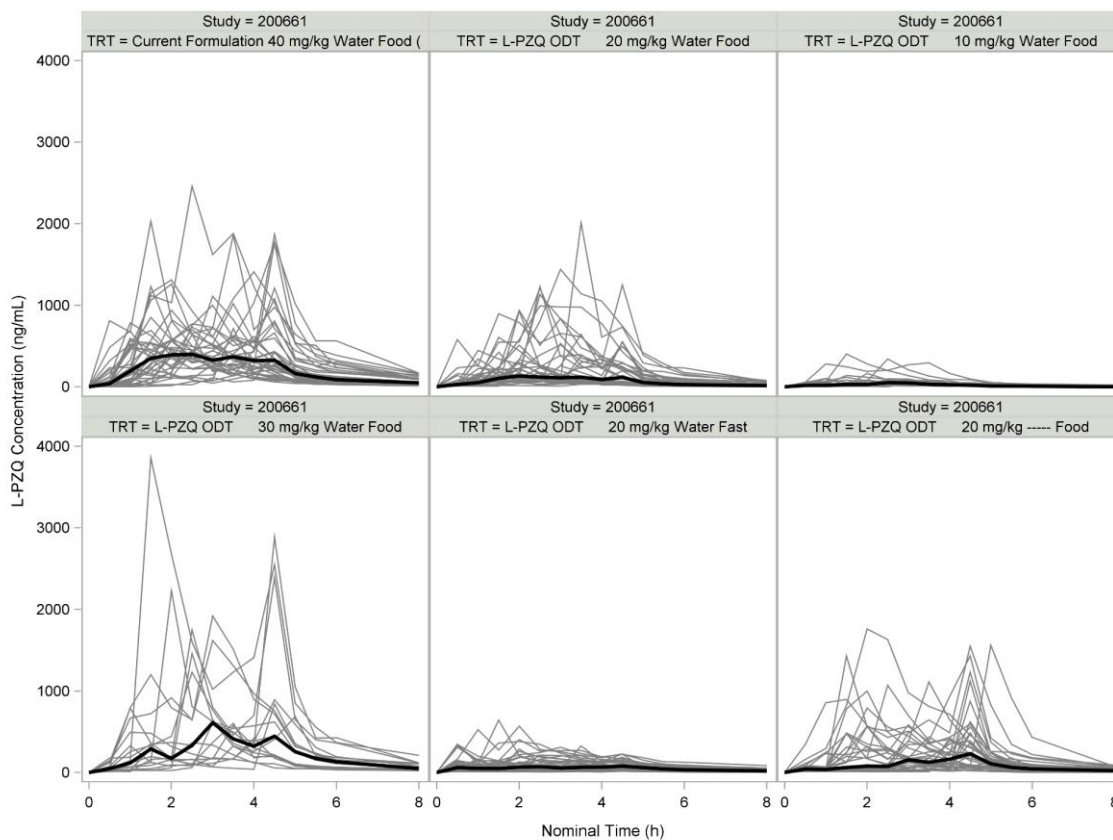
**Figure 3.**
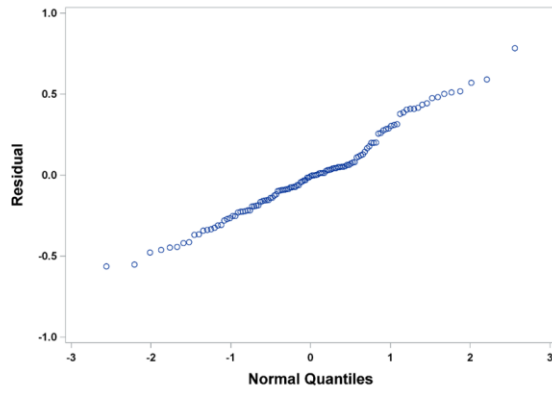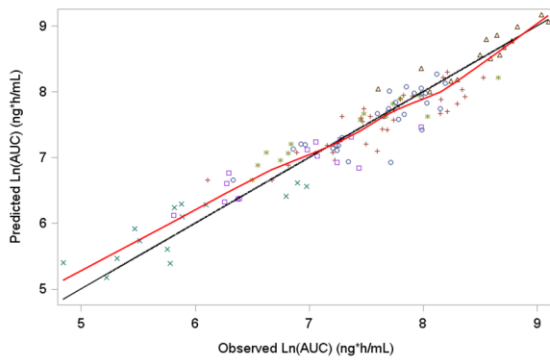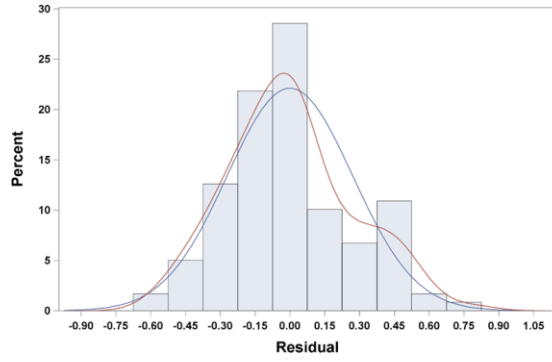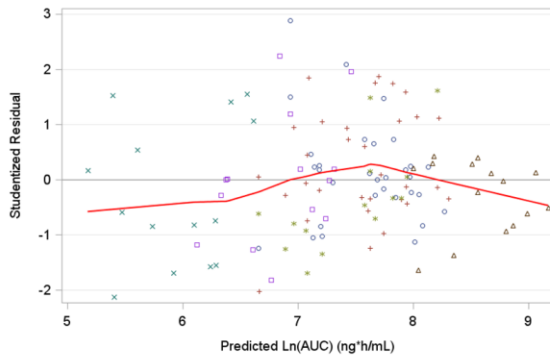
**Figure 4.**

**Figure 5.**

**Figure 6.**



ADULTS

40 mg/kg @ 70 kg
2800 mg total

Dose Number = 29

2705 mg
undissolved

95 mg in 250 mL
0.38 mg/mL

0.38 mg/mL

CHILDREN

40 mg/kg @ 13 kg
500 mg total

Dose Number = 34

481 mg
undissolved

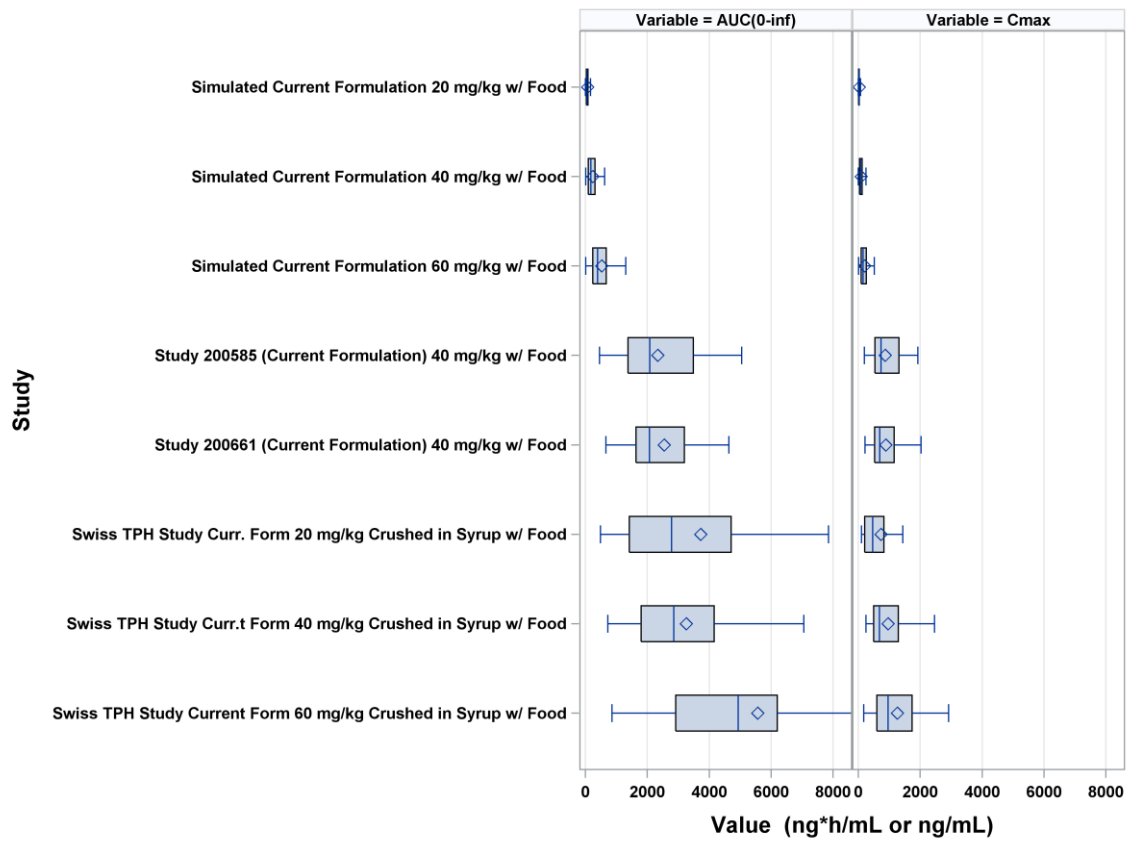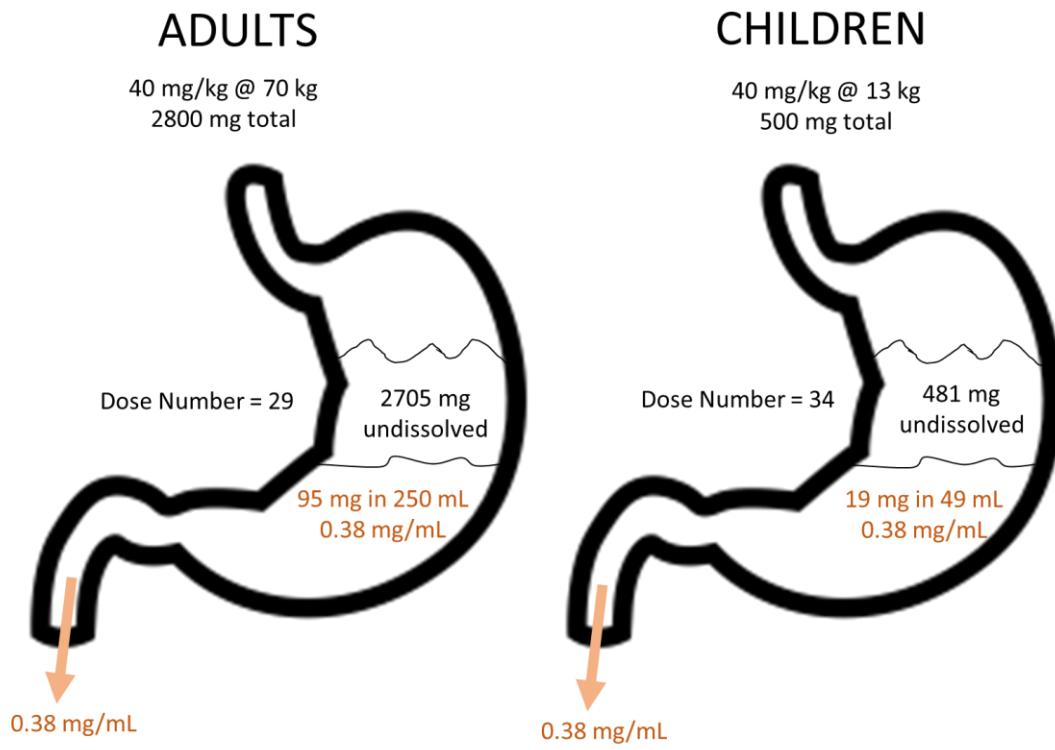19 mg in 49 mL
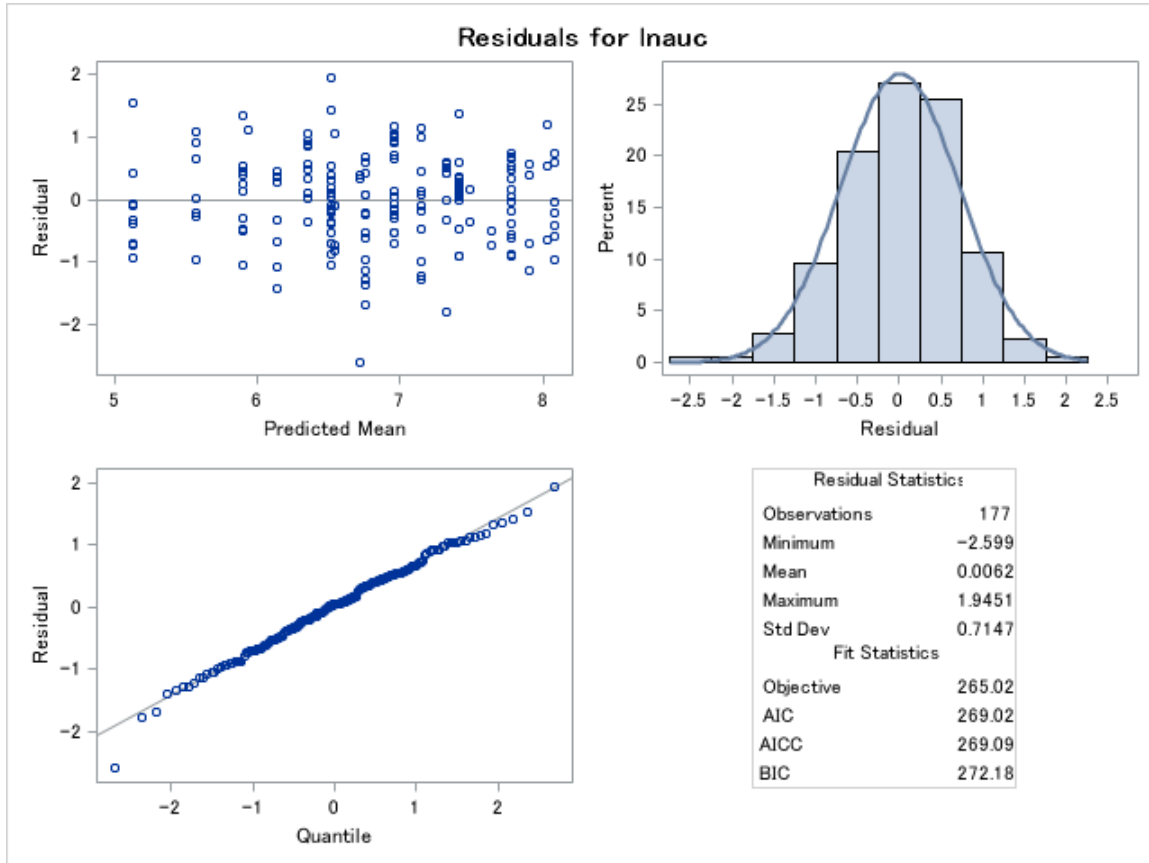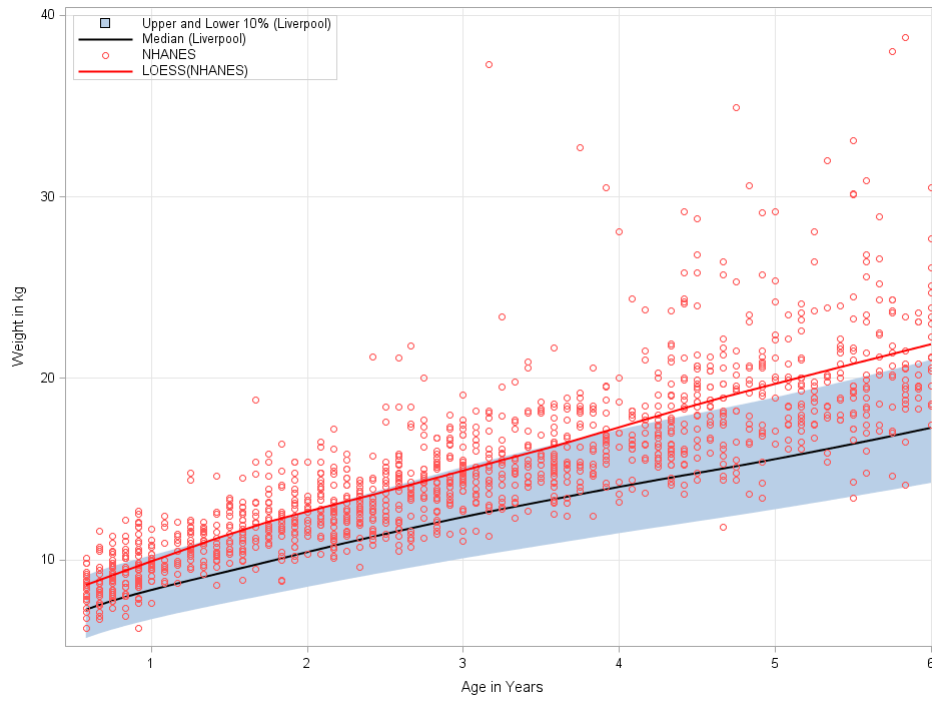0.38 mg/mL

0.38 mg/mL
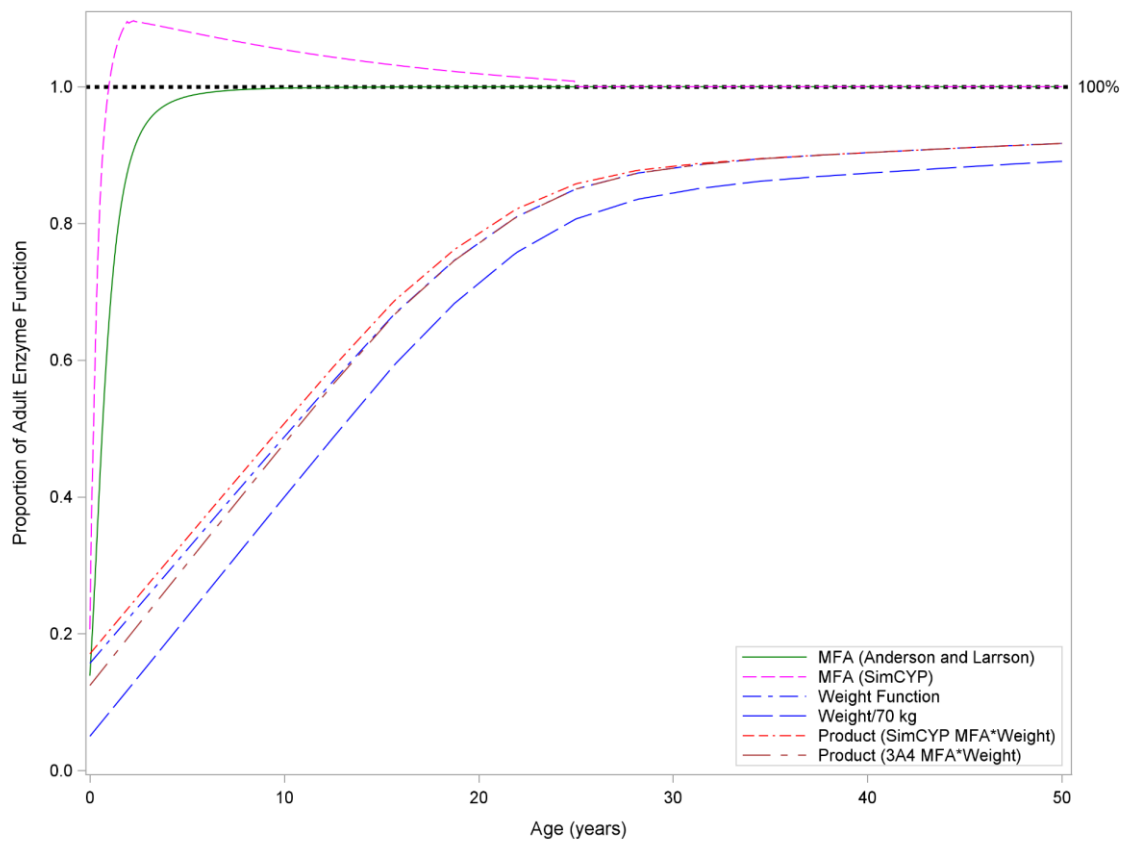
## Supplemental Figures

Supplemental Figure 1.

Supplemental Figure 2.

Supplemental Figure 3.

## SUPPLEMENTAL MODEL 1

A criticism of the analysis was the initial decision to model the 200585-001 and 200660-001 data separately and then base the pediatric extrapolation solely on Study 200585-001. A later analysis was conducted wherein a combined data model was developed using the same methods (with one exception) and procedures as before and then base the simulation on the joint model. In order to account for the differences in ODT formulations between studies, ODT was added as categorical factor in the linear mixed effects model (0=current formulation, 1=Racemic ODT, 2=L-PZQ ODT), as was STUDY (0=200585, 1=200660). Under the joint model, a total of 296 observations were available from 32 subjects in Study 200585-001 and 36 subjects from Study 200660-001.

*AUC:* The AIC for the full model with all covariates was 416.4. The best model, which had an AIC of 409.2, was one where only log-transformed DOSE, ODT, and FOOD were included in the model. All other terms were not statistically significant (p > 0.01), including STUDY. The parameter estimates for the AUC model are shown in Supplemental Model Table 1. Goodness of fit plots for the model are shown in Supplemental Model Figure 1. The best fit AUC model was:

$$\text{Model Ln}(\text{AUC}) = -8.54 + 2.13 \times \text{Ln}(\text{Dose}) + 0.667 \times \text{FOOD} + \begin{cases} 0 & \text{Current formulation} \quad (1) \\ -0.0794 & \text{L-PZQ ODT} \\ -0.776 & \text{Racemic ODT} \end{cases}$$

In a comparison to the fixed effects of the two individual study-specific models, the fixed effect estimates were quite comparable. What wasn't comparable was the intercept, which in the combined data model was a weighted mean of the intercepts from the study-specific values.

***Extrapolation and Simulation of Pediatric Exposures:*** A repeat of the simulations using the combined models showed similar results to results obtained using the study-specific model (Supplemental Model Figure 2). These results showed that a combined model did not improve the accuracy of the predictions and that there was still a discrepancy between the observed Swiss TPH data and the predicted data.

**Supplemental Model Table 1***: Parameter Estimates from Best Linear Mixed Effect Models Under the Combined Data Model for L-PZQ AUC*

| Parameter | ODT | Estimate | Standard Error | T-test | p-value |
|---|---|---|---|---|---|
| Intercept | | -8.54 | 0.653 | -13.08 | < 0.0001 |
| Ln-DOSE | | 2.13 | 0.0884 | 24.14 | < 0.0001 |
| ODT | Current | 0 | --- | | |
| | Racemic | -0.079 | 0.0690 | -1.15 | 0.2504 |
| | L-PZQ | -0.776 | 0.0657 | 11.87 | < 0.0001 |
| Food | | 0.668 | 0.0601 | 11.13 | < 0.0001 |
| Var(Intercept) | | 0.290 | | | |
| Residual Variance | | 0.130 | | | |

**Figure 1 For Supplemental Model**
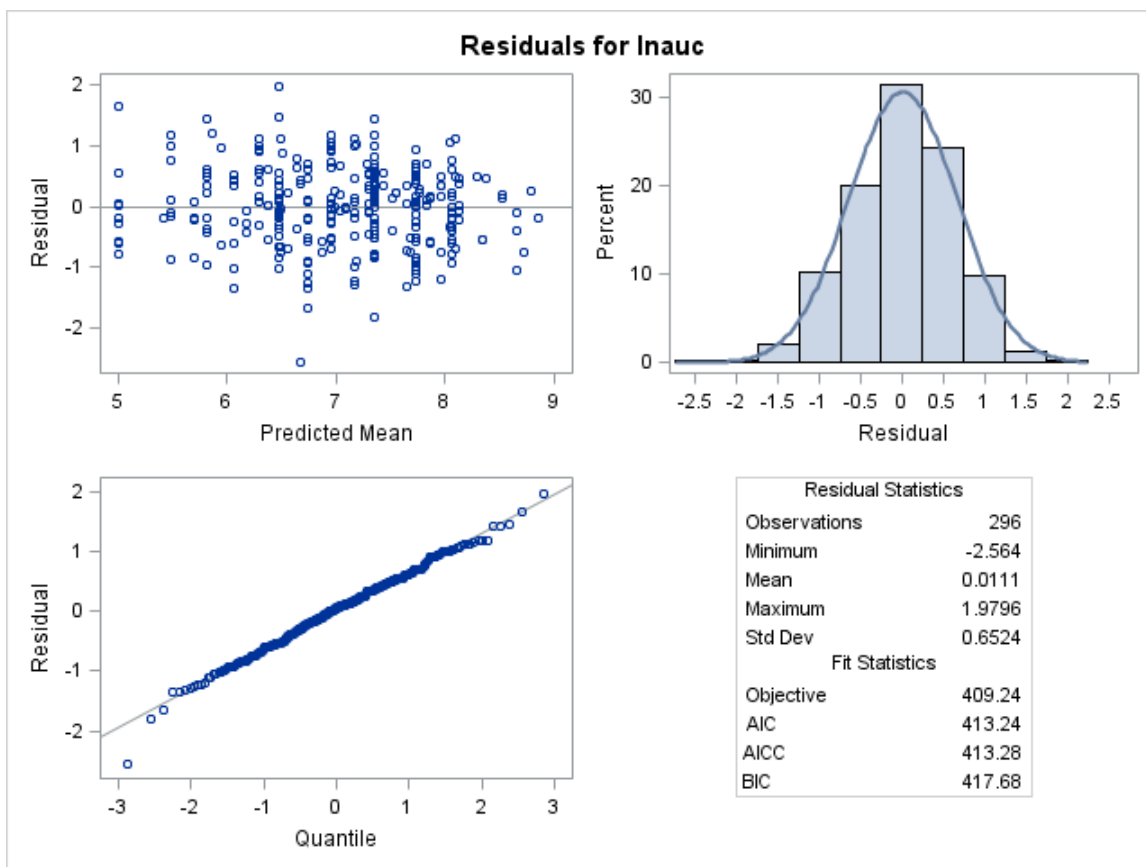


Goodness of fit plots for L-PZQ AUC under the combined data model.

**Figure 2 For Supplemental Model**
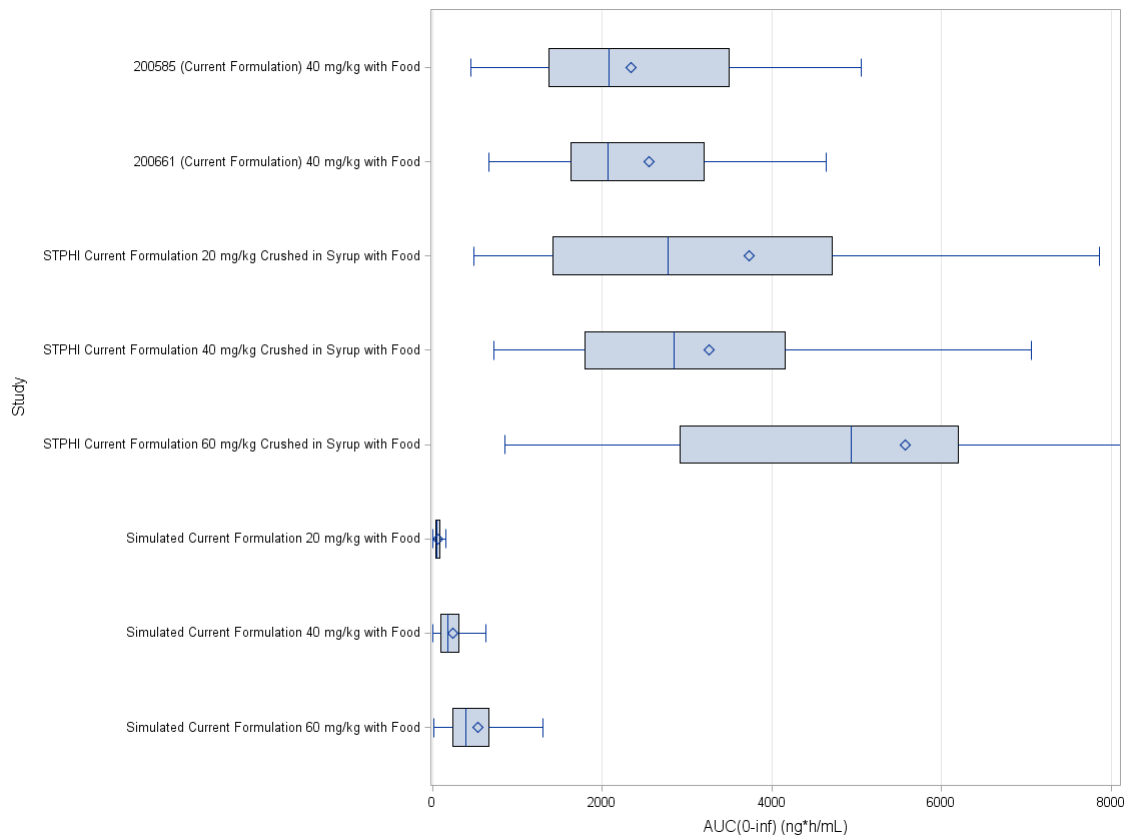


Comparison of observed L-PZQ AUC in Study 200585-001, Study 200661-001, and the Swiss Tropical and Public Health Institute Study to simulated L-PZQ AUC based on the combined data model.

**7.2**

**Curriculum vitae**

## Curriculum Vitae

Name: **Jana KOVAČ**

Address: Oetlingerstrasse 10, 4057 Basel, Switzerland

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      jana2kovac@gmail.com

Date and place of birth: 09.05.1989 (Maribor, Slovenia)

Citizenship: Slovenian

## EDUCATION

**2004-2008**    Gymnasium Ptuj, Slovenia, Major in Biology and Chemistry

        **Certificate: Federal Matura**

**2008-2014**    Faculty of Pharmacy, University of Ljubljana, Slovenia

        **Certificate: Masters of Pharmacy and License to practice pharmacy**

**2014-2017**  PhD student at Swiss Tropical and Public Health Institute, Basel, Switzerland

         (Helminth Drug Development Unit, supervision: Prof. Dr. Jennifer Keiser)

**PhD Thesis: Pharmacokinetic and pharmacological studies of praziquantel, cornerstone of schistosomiasis treatment**

## WORK EXPERIENCE

**2013**        <u>Research internship at UCL School of Pharmacy, London, United Kingdom</u>

        (Pharmaceutical and Biological Chemistry Unit, supervision: Prof. Dr. Ijeoma Uchegbu and Prof. Dr. Andreas Schätzlein)

        **-** Synthesis, characterisation and degratation of chitosan-based polymers

        **-** Formulation and characterisation of polymeric nanoparticles

        - analytical techniques used: MALDI TOF, GPC MALLS, LC-MS, DLS

**2014-2017**    <u>PhD student at Swiss Tropical and Public Health Institute</u>

        **-** involved in organisation, planning and execution of large scale pharmacokinetic studies in paediatric population in rural settings (Ivory Coast, Africa)

        **-** preparation and organisation of study material

        **-** sampling, analytics and data management

        **-** method development, optimisation and validation for LC-MS/MS

        **-** in vitro/in vivo research on different stages of helminths (*Schistosoma spp.*)

**2014-2017**         Global Program Regulatory Manager at Novartis Pharma AG, Basel, Switzerland

**OTHER SKILLS**

*Languages:* **Slovenian** (native)

**English** (IELTS certificate: Listening 8.5, Reading 8.5, Writing 8.0, Speaking: 7.0; overall: 8.0 out of 9.0)

**German** (certificate B2, ECAP)

*Certifications:*  Understanding clinical research: Behind the statistics (Coursera)

Learning how to lead and to build a successful working environment

Good Clinical Practice certificate

*Honors and awards:*  participant of Antelope@Novartis 2017

First class honors for Master thesis

**PRESENTATIONS**

7-10. September 2015: European Congress for Tropical Medicine and International Health (Basel, CH), **poster** (Praziquantel dose-finding and pharmacokinetic studies in preschool-aged children infected with *S.mansoni*)

24-25. October 2016: Annual meeting of Swiss Society for Parasitology and Tropical Medicine, students' section (Grindelwald, CH), **oral presentation** (Towards greater understanding of Praziquantel: pharmacological, preclinical and clinical investigations)

13. February 2017: Annual Research Meeting (Basel, CH), **poster** (Population pharmacokinetic modeling of praziquantel in *Schistosoma spp.* infected school- and preschool-aged children)

11. May 2017: Medical Parasitology Infection Biology Research Seminar (Basel, CH), **oral presentation** (Praziquantel: old drug, new discoveries)

26-29. June 2017: Annual conference of Australian Society for Parasitology (Leura, Blue Mountains, NSW, Sydney, Australia), **oral presentation** (Population pharmacokinetics of Praziquantel in *Schistosoma spp.* infected school- and preschool-aged children)

02. October 2017: Advances in Infection Biology, Epidemiology and Public Health (Basel, CH), **oral presentation** (Praziquantel: old drug, new discoveries)

03. November 2017: Annual meeting of Swiss Society for Parasitology and Tropical Medicine, students' section (Luzern, CH), **oral presentation** (Praziquantel: in the spotlight)

## PUBLICATIONS

I. Meister, A. Leonidova, **J. Kovac**, U. Duthaler, J. Keiser, J. Huwyler. Development and validation of an enantioselective LC-MS/MS method for the analysis of anthelmintic drug praziquantel and its main metabolite in human plasma, blood and dried blood spots. *J. Pharm. Biomed. Anal*.; **2015**: *118,* 81-88

I. Meister, **J. Kovac**, U. Duthaler, P. Odermatt, J. Huwyler, S. Sayasone,  J. Keiser. Pharmacokinetic study of praziquantel enantiomers and its main metabolite measured in blood, plasma and dried blood spots in *Opisthorchis*-infected patients, *PLoS Neglected Tropical Diseases;* **2016:** *10*(5)

I.I. Bogoch, J. T. Coulibaly, J. Rajchgot, J. R. Andrews, **J. Kovac**, J. Utzinger, G. Panic, J. Keiser. Poor validity of non-invasive hemoglobin measurements using the Masimo Pronto-7® system compared to conventional absorptiometry in *Schistosoma mansoni*-infected children, *Am J Trop Med Hyg*; **2017:** *96*(1)

J. T. Coulibaly, G. Panic, D. K. Silué, **J. Kovac**, J. Hattendorf, J. Keiser. Efficacy and safety of praziquantel in school-aged and preschool-aged children infected with *Schistosoma mansoni:* a randomized controlled dose-finding trial, *Lancet Glob Health*; **2017:** *5*(e688-98)

**J. Kovac**, M. Vargas and J. Keiser. *In vitro* and *in vivo* activity of R- and S- praziquantel enantiomers and the main human metabolite *trans-* 4-hydroxy-praziquantel against *Schistosoma haematobium; Parasit Vectors;* **2017;** 10: 365

**J.Kovac**, I.Meister, A.Neodo, G.Panic, J.T.Coulibaly, C.Falcoz, J.Keiser. Pharmacokinetics of praziquantel in *Schistosoma mansoni* and *Schistosoma haematobium* infected school- and preschool- aged children. **2017**. *Antimicrobial Agents and Chemotherapy,* **2018**

P.Bonate, T.Wang, P.Passier, P.Bagchus, H.Burt, C.Lupfert, N.Abla, **J.Kovac**, J.Keiser. Extrapolation of Praziquantel Pharmacokinetics to a Pediatric Population - A Cautionary Tale. *Journal of Pharmacokinetics and Pharmacodynamics*, 45: 747 – 726; **2018**

**J.Kovac**, G.Panic, A.Neodo, I.Meister, J.T.Coulibaly, J.D.Schulz, J.Keiser. Evaluation of a novel micro-sampling device, Mitra™, in comparison to dried blood spots, for analysis of praziquantel in schistosomiasis patients in rural Côte d'Ivoire. Journal of Pharmaceutical and Biomedical Analysis, 151: 339 – 346; **2018**