

1 **The application of physiologically-based pharmacokinetic modelling to assess the impact of**  
2 **antiretroviral-mediated drug-drug interactions on piperazine antimalarial therapy during**  
3 **pregnancy**

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5

**6 ABSTRACT**

7 Antimalarial therapy during pregnancy poses important safety concerns due to potential  
8 teratogenicity and maternal physiological and biochemical changes during gestation. Piperaquine  
9 (PQ) has gained interest for use in pregnancy in response to increasing resistance towards  
10 sulfadoxine–pyrimethamine in sub-Saharan Africa. Co-infection with HIV is common in many  
11 developing countries, however, little is known about the impact of anti-retroviral (ARV) mediated  
12 drug-drug interaction (DDI) on PQ pharmacokinetics during pregnancy. This study applied  
13 mechanistic pharmacokinetic modelling to predict pharmacokinetics in non-pregnant and pregnant  
14 patients, which was validated in distinct customised population groups from Thailand, Sudan and  
15 Papua New Guinea. In each population group, no significant difference in day 7 concentrations  
16 were observed during different gestational weeks (GW) (weeks 10-40), supporting the notion that  
17 PQ is safe throughout pregnancy with consistent pharmacokinetics, although possible  
18 teratogenicity may limit this. Antiretroviral-mediated DDIs (efavirenz and ritonavir) had moderate  
19 effects on PQ during different gestational weeks with a predicted  $AUC_{ratio}$  ranging from 0.56-0.8  
20 and 1.64-1.79 for efavirenz and ritonavir respectively over GW 10-40, with a reduction in  
21 circulating human serum albumin significantly reducing the number of subjects attaining the day 7  
22 (post-dose) therapeutic efficacy concentrations under both efavirenz and ritonavir DDIs.

23 This present model successfully mechanistically predicted the pharmacokinetics of PQ in  
24 pregnancy to be unchanged with respect to non-pregnant women, in the light of factors such as  
25 malaria/HIV co-infection. However, ART-mediated DDIs could significantly alter PQ  
26 pharmacokinetics. Further model refinement will include collation of relevant physiological and  
27 biochemical alterations common to HIV/malaria patients.

28

29 **KEYWORDS**

30 Physiologically-based pharmacokinetics; malaria; anti-retroviral; drug-drug interaction;

31 pregnancy.

32

## 33 1. INTRODUCTION

34 The problem of malaria-induced maternal morbidity and mortality in endemic areas for the disease  
35 is far reaching, particularly with respect to the unborn child. Maternal death due to malaria was  
36 reported to account for up to 25% of maternal deaths due to all causes in malaria endemic regions  
37 while close to a million children born to malaria-infected mothers had low birth weights  
38 (Consortium, 2017).

39 Malarial infection in pregnancy triples the maternal risk of suffering from severe diseases compared  
40 with non-pregnant women (Murray & Bennett, 2009). This is further confounded by the added  
41 complication of coinfection with human immunodeficiency virus (HIV) as a result of the  
42 immunocompromised nature of pregnancy (Menendez et al., 2008; Ofori et al., 2009; Schantz-  
43 Dunn & Nour, 2009).

44 The treatment of malaria during pregnancy possess major challenges to healthcare systems. This is  
45 because antimalarial treatments (AMT) which yields satisfactory safety and efficacy profiles are  
46 often found to be unsafe during the early stages of pregnancy (Nosten et al., 2006). The WHO's  
47 current recommendations for AMT chemoprophylaxis are based on intermittent preventive  
48 treatment with sulfadoxine-pyrimethamine (IPTp-SP) (Organization., 2015). This recommendation  
49 was based on a review (Kayentao et al., 2013) of seven trials which assessed the use of monthly  
50 SP administration for malaria prevention in pregnant women across six African countries. The  
51 result of the review demonstrated that there was a significant reduction in both low birth weights  
52 and placental and maternal parasitaemia following administration of no less than two doses of SP  
53 monthly during pregnancy (Kayentao et al., 2013).

54 However, with the spread of SP resistance, new interventions have been sought. In high  
55 transmission settings where there may be widespread resistance to SP-IPTp, dihydroartemisinin-  
56 piperazine (DHA-PQ) has been demonstrated to result in a lower malarial burden (Kakuru et al.,  
57 2016). A recent study showed that, when compared to the use of SP in pregnant women, the

58 administration of DHA-PQ provided significantly higher protection against placental malaria;  
59 significantly lowered maternal parasitaemia and reduced prevalence of composite adverse birth  
60 consequences (Kakuru et al., 2016). More so, the safety of DHA-PQ in pregnancy is evident in  
61 numerous studies. In 2015, a randomised controlled superiority trial showed that in addition to the  
62 observed efficacy of DHA-PQ for the preventing malaria in pregnancy, DHA-PQ resulted in fewer  
63 detrimental maternal and infant side effects compared with SP-IPTp (Desai et al., 2015). Similarly,  
64 another study revealed that compared to quinine, DHA-PQ used for the treatment of multi-resistant  
65 malaria in 2<sup>nd</sup> and 3<sup>rd</sup> trimester of pregnancy resulted in less perinatal mortality, though in the 1<sup>st</sup>  
66 trimester, quinine appeared to be safer (Poespoprodjo et al., 2014).

67 Infectious diseases such as HIV are prevalent in malaria endemic regions (Benjamin et al., 2015;  
68 Tarning et al., 2012). Pregnant women with HIV and malaria coinfection are more vulnerable to  
69 all the complications of malaria in pregnancy such as anaemia, placental parasitaemia and low birth  
70 weights (Hayes et al., 2015). This can be further confounded by the potential for many antiretroviral  
71 (ART) drugs to elicit drug-drug interactions (DDIs) on common Cytochrome P450 isozymes, e.g.  
72 3A4 (Fichtenbaum & Gerber, 2002; Horita & Doi, 2014; Renjifo et al., 2015). Hence, these factors  
73 are significant causes for concern when treating this population. The reduced systemic  
74 concentration of DHA-PQ, due to co-administration with efavirenz in HIV infected pregnant  
75 women, has been demonstrated in a recent study which showed that in Ugandan pregnant women,  
76  $AUC_{0-8hr}$  and  $AUC_{0-21d}$  of piperazine was 50% and 40% lower respectively when DHA-PQ was  
77 co-administered with efavirenz compared to when DHA-PQ was taken alone (Kajubi et al., 2017a).  
78 A systemic review of data involving DDI between ARV drugs and AMT further accentuated the  
79 likelihood of a range of such DDIs (Seden et al., 2017).

80 Addressing the problem of AMT in malaria endemic areas requires consideration of physiological  
81 peculiarities in subjects that might impact upon the efficacy of the antimalarial treatment. Some  
82 example of factors that can impact upon the efficacy of AMT include, but not limited to,

83 geographical region differences in body weight (Hayes et al., 2015) and biochemistry (e.g serum  
84 albumin (Nanjul, 2007) and haematocrit (Newton et al., 2013; Othman, 2014)). Elucidating these  
85 factors individually in a clinical setting may be difficult due to the presence of other confounding  
86 factors and/or the ethical constraints of recruiting large number of pregnant women into clinical  
87 studies. With the aid of PBPK modelling techniques, these factors can be investigated separately  
88 to suggest the effect the impacts can make on the antimalarial therapy clinically.

89 In this study, through virtual clinical trials simulations, we investigate the impact changes in PQ  
90 plasma concentrations in the absence and presence of ARV-mediated DDIs in three malaria-  
91 specific geographical regions (Thailand, Papua New Guinea and Sudan) pregnant population  
92 groups, whereby changes in biochemical and haematology were incorporated into the design of the  
93 population groups.

94

95

## 96 2. METHODS

97 All population based PBPK modelling was conducted using the virtual clinical trials simulator  
98 Simcyp (Simcyp Ltd, a Certara company, Sheffield, UK, Version 16).

### 99 2.1 Model development

100 A four-stage stepwise approach was employed for model development (Figure 1) which is fully  
101 described in the supplementary materials and briefly summarised below.

#### 102 2.1.1 Base model development (Step 1)

103 The base model was developed from two reported studies of PQ dosed in fasted Caucasian healthy  
104 volunteers (Ahmed et al., 2008; Sim, Davis, & Ilett, 2005). Given the high lipophilicity and  
105 expected wide-spread tissue distribution of PQ, a full PBPK model was employed for all model  
106 simulations.

#### 107 2.1.2 Non-pregnant malaria population groups (Step 2)

108 To assess the predictive performance of the model in non-Caucasian non-complicated malaria  
109 population groups, we identified four studies where PQ was dosed to non-pregnant females in  
110 Thailand (Rijken et al., 2011; Tarning et al., 2012) [Tarning *et al* 2008 (Tarning et al., 2008) was  
111 excluded from this study due to the difficulty in obtaining individual data points for the study  
112 duration], Papua New Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012). The  
113 'Healthy Volunteer' (HV) population group within Simcyp was adapted. In order to address the  
114 differences in patient demographics (primarily body weight) and biochemistry (haematocrit/plasma  
115 proteins) between healthy-subjects and malaria-subjects (see supplementary materials Table S1).

### 116 **2.1.3 Pregnant malaria population groups (Step 3)**

117 The ‘Pregnancy’ population group within Simcyp was adapted (see supplementary materials) to  
118 create ‘Malaria-Pregnancy’ population groups based upon the three regional populations  
119 originating from the four clinical studies highlighted in section 2.1.2. These studies also detailed  
120 the pharmacokinetics of PQ in pregnant women and this was used as a basis to further validate the  
121 ‘Malaria-Pregnancy’ population groups. Final model parameters for PQ are detailed in  
122 supplementary materials Table S2.

### 123 **2.1.4. ‘What-If’ scenarios (Step 4)**

124 To assess ‘What-If’ scenarios (Figure 1), case studies were included, in order to demonstrate the  
125 impact of possible drug-drug interactions mediated by efavirenz (EFV) or ritonavir (RTV); the  
126 former was selected due to the potential for CYP3A4 induction and the latter for its CYP3A4  
127 inhibitory effects.

#### 128 **2.1.4.1 Efavirenz/Ritonavir-mediated drug-drug interactions**

129 Validation of DDIs mediated by ART was considered through the only published DDI study  
130 available (Kajubi et al., 2017b) with EFV and PQ in a Ugandan population group. A Ugandan  
131 population group was developed (see supplementary materials for details) and the trial design was  
132 replicated for the three arms of the study within the reported trial, namely non-pregnant women  
133 with no-DDI, pregnant women with no-DDI and pregnant women with a DDI scenarios, in order  
134 to compare the ability of the PQ PBKP model to capture the extent of the reported DDIs.

135 Subsequently, DDIs were simulated in a 10x10 virtual clinical trial with each population group  
136 described previously (see section 2.1.2). A standard daily dose approach was employed for EFV  
137 (600 mg once daily) and RTV (100 mg twice daily, in line with ritonavir/lopinavir combination  
138 dosing of 100mg/400mg twice daily) with EFV/RTV dosed for 14 days and PQ dosed on days 3,4  
139 and 5 (10 mg/kg PQ base). The malaria-pregnancy population groups were redefined during the



140 simulation duration on a daily basis to account for physiological/biochemical changes, and studies  
141 conducted across gestational weeks (GW) 10 to 40. EFV and RTV are pre-validated compounds  
142 developed by Simcyp and included into the Simcyp Simulator® compound library. The RTV  
143 compound file has been widely used as a CYP3A4 inhibitor in mechanistic modelling (Colbers,  
144 Greupink, Litjens, Burger, & Russel, 2016; Hyland, Dickins, Collins, Jones, & Jones, 2008;  
145 Kaspera et al., 2014; Marsousi et al., 2016; Wang, 2010), and compound-specific parameters and  
146 validation data for the use of EFZ as a CYP3A4 and 2B6 inducer within the Simcyp Simulator®,  
147 have recently been published (Ke, Barter, Rowland-Yeo, & Almond, 2016).

#### 148 **2.1.4.2. Human serum albumin**

149 Human serum albumin (HSA) concentrations was set at 20 g/L and 50 g/L within population  
150 groups, to mimic the reduction in serum albumin reported at different stages of malaria infection,  
151 with 20 g/L representing severe malaria (Sagaki et al., 2013) . The Simcyp ‘Pregnancy’ population  
152 includes a description for alterations in HSA during pregnancy and the baseline initial HSA  
153 concentration was fixed at the aforementioned concentrations.

#### 154 **2.1.4.3. Gestational week**

155 The impact of gestation week on PQ pharmacokinetic during EFV/RTV-mediated DDI was further  
156 assessed at weeks 10, 20 and 30 for all population groups.

### 157 **2.2 Predictive performance**

158 Although no uniform criterion has been accepted for defining an ‘optimal’ predictive performance  
159 range, a prediction to within 2-fold of the observed data is generally accepted in this context [40]  
160 and was employed as our criterion for  $C_{max}$  and AUC comparisons to those clinically reported. For  
161 EFV/RTV DDI simulation, as the clinical efficacy of PQ is determined by its day-7 concentration  
162 (post-first dose) of 30 ng/mL (Price et al., 2007), the impact of a DDI of PQ pharmacokinetics was  
163 assessed by direct analysis of the day-7 concentration.

**164 2.3 Data analysis**

165 Unless otherwise stated, all simulations of plasma concentration-time profiles were presented as  
166 arithmetic mean and 5-95<sup>th</sup> percentiles. Reported concentration-time profiles from clinical studies  
167 were digitally retrieved using the WebPlotDigitizer v3.10 [41] and superimposed onto simulated  
168 profiles for visual predictive checks.

### 169 3. RESULTS

#### 170 3.1. Healthy volunteer: base model development (Step 1)

171 The initial model development for health-volunteer (Caucasian) subjects focussed on addressing  
172 model validation to recover appropriate absorption kinetics coupled with an appropriate prediction  
173 of steady state volume of distribution ( $V_{ss}$ ) and CYP3A4 and CYP2C8-mediated metabolic  
174 clearance (see supplementary materials). The resultant model was found to be appropriate to  
175 capture  $C_{max}$  and  $t_{max}$  and resulted in a broadly consistent simulated  $C_{max}$  (21.5 ng/mL  $\pm$  9.2 ng/mL),  
176  $t_{max}$  (5.3 hours) and AUC (AUC<sub>0-24</sub>: 384.2 ng h/mL  $\pm$  145.9 ng h/mL; AUC<sub>0-last</sub>: 3207.3 ng h/mL  $\pm$   
177 1121 ng h/mL) when compared to Ahmed *et al* (Ahmed et al., 2008) ( $C_{max}$ : 41.6 ng/mL  $\pm$  29.5  
178  $\mu$ g/L;  $t_{max}$ : 4.0 hours; AUC<sub>0-24</sub>: 393 ng h/mL  $\pm$  149 ng h/mL; AUC<sub>0-last</sub>: 2312 ng h/mL  $\pm$  790 ng  
179 h/mL) (Figure 2A) and Sim *et al* (Sim et al., 2005) ( $C_{max}$  [range]: 21.0  $\mu$ g/L [14-31.4  $\mu$ g/L];  $t_{max}$ :  
180 6.8 hours [2.1-11.5 hours]; AUC<sub>0-last</sub>: 2818  $\mu$ g h/L [1566-5070  $\mu$ g h/L]) for a 500 mg PQP dose  
181 (Figure 2C). For a higher 1500 mg PQP dose, consistent simulated  $C_{max}$  (76.1 ng/mL  $\pm$  69 ng/mL),  
182  $t_{max}$  (5.1 hours) and AUC (AUC<sub>0-24</sub>: 1243 ng h/mL  $\pm$  193.8 ng h/mL; AUC<sub>0-last</sub>: 9065 ng h/mL  $\pm$   
183 1299 ng h/mL) were simulated when compared to Ahmed *et al* (Ahmed et al., 2008) ( $C_{max}$ : 147  
184 ng/mL  $\pm$  110  $\mu$ g/L;  $t_{max}$ : 2.5 hours; AUC<sub>0-24</sub>: 1418 ng h/mL  $\pm$  775 ng h/mL; AUC<sub>0-t</sub>: 6399 ng h/mL  
185  $\pm$  2067 ng h/mL) (Figure 2B).

186 These predictions supported the successful model development in healthy-volunteer population  
187 groups for fasted single dose studies only.

188

#### 189 3.2 Non-Caucasian, non-pregnant malaria population groups (Step 2)

190 In order to assess the predictive performance in multi-dose studies, three-population groups were  
191 developed for Thailand, Papua New Guinea and Sudan females based on published clinical studies

192 within these groups, under conditions of standard multi-dose regimens (10 mg/kg PQ base once  
193 daily for 3 days) (Figure 3).

194 For all population groups, the majority of estimated parameters (Table 1) fell within 2-3 fold of the  
195 reported metrics (Table 2). Notability however, for the Thailand population group, the predicted  
196 increase in median  $C_{max}$  following each dose was only moderately correlated with that reported by  
197 Rijken *et al* (Rijken et al., 2011) (Figure 3). However, the clinical end-point marker of successful  
198 antimalarial therapy (day 7 concentration) (Price et al., 2007) were all simulated (Table 1) to with  
199 2-fold of the reported clinical measures (Table 2), in addition to day 14 and day 28 concentrations.

200 Furthermore, the model predictions were also able to capture the differences in day 7 concentration  
201 across population groups, despite similar dosing strategies, e.g. Thai 24.74 ng/mL (4.42-64.93  
202 ng/mL) vs. Sudanese 34.0 ng/mL (6.8-86.7 ng/mL) population groups. A one-way ANOVA  
203 indicated statistical differences in the median day 7 concentrations, when comparing all 4 predicted  
204 population studies, with the Sudanese population group demonstrating a statistically higher median  
205  $C_{max}$  ( $p = 0.0415$ ) compared to the other population groups.

206 This highlighted the successful creation of sub-population group's validation in each population  
207 group.

208

### 209 **3.3 Non-Caucasian, pregnant malaria population groups (Step 3)**

210 The PBPK model was further adapted to evaluate PQ pharmacokinetics in non-Caucasian pregnant  
211 population groups (Figure 4). For all population groups, the majority of estimated parameters  
212 (Table 3) fell within 2-fold of the reported metrics (Table 4), with predictions of the median day 7,  
213 14 and 28 concentrations all simulated to with 2-fold of the reported clinical measures (Table 4).

214 These predicted point markers were not significantly different than those for non-pregnant subjects  
215 ( $p > 0.05$ ) (Tables 1 and 2)

216 A one-way ANOVA indicated statistical differences in the median day 7 concentrations, when  
217 comparing all 4 predicted population studies, with the Sudanese population group demonstrating a  
218 statistically higher median  $C_{\max}$  ( $p = 0.0392$ ) compared to the other population groups (Figure 4).  
219 However, when comparing non-pregnant to pregnant population groups, no significant difference  
220 in the median day 7 concentration was identified for each population. Further, predicted half-life  
221 in pregnancy population groups were significantly different ( $p < 0.01$  for all population groups [t-  
222 test]) from those in non-pregnancy population groups (Table 3).

223 This highlighted the successful creation of sub-population group's validation in each population  
224 group.

225

### 226 **3.4 'What-If' scenarios (Step 4)**

227 To evaluate and validate the impact of ART on PQ systemic exposure, the only known recent study  
228 investigating the impact of ART (efavirenz) on PQ systemic exposure in Ugandan pregnant women  
229 (Kajubi et al., 2017b) was replicated, following creation of a Uganda pregnancy-malaria population  
230 group (see supplementary materials) where EFV was orally dosed at 600 mg once daily (see  
231 supplementary materials Figure S1). The predicted day 7, 14 and 21 PQ concentrations were all  
232 within 2-fold of that reported by Kajubi *et al* (Kajubi *et al.*, 2017b), with a similar approximate 50  
233 % decrease in the predicted mean day 7 concentrations (No EFV: 20.5 ng/mL; EFV: 9.2 ng/mL)  
234 (see supplementary materials Table S3). Furthermore, our predicted  $AUC_{0-d21}$  was within 2-fold of  
235 that reported by Kajubi *et al* (this study: 0.51; Kajubi: 0.62).

236

#### 237 **3.4.1 The impact of change in HSA on the extent of ART-DDIs**

238 In all population groups (absence and presence of a DDI) an increase in HSA from 20 g/L to 50  
239 g/L significantly increased the median day 7 (total) plasma concentration of PQ (Figure 5). This

240 was associated with a significant increase in the number of subjects with a day 7 concentration >  
241 30 ng/mL in the absence of an ART (Thailand: 8 to 45,  $p = 0.007$ ; PNG: 11 to 53,  $p = 0.00009$ ;  
242 Sudan: 9 to 49,  $p = 0.0006$ ), and in the presence of EFV (Thailand: 7 to 48,  $p = 0.006$ ; PNG: 4 to  
243 24,  $p = 0.0003$ ; Sudan: 1 to 16,  $p = 0.0005$ ) or RTV (Thailand: 41 to 80,  $p = 0.0009$ ; PNG: 49 to  
244 85,  $p = 0.0008$ ; Sudan: 47 to 80,  $p = 0.00003$ ) (Figure 5).

245 Additionally, the presence of EFV or RTV significantly reduced or increased, respectively, the day  
246 7 PQ concentration across all population groups, however the overall impact of the DDI across  
247 population groups for both EFV and RTV were broadly similarly (Figure 5). This resulted in a  
248 similar number of subjects attaining a day 7 concentration  $\geq 30$  ng/mL except for the Sudanese  
249 population with a EFV-mediated DDI, where a statistically significant difference in the median day  
250 7 concentration across the three population groups was identified (One-Way ANOVA,  $p = 0.0023$ ).

251

### 252 **3.4.3 The impact of gestation on the extent of an ART-mediated DDI**

253 In the absence of an ART-mediated DDIs, the median predicted day 7 concentration was broadly  
254 consistent across all gestational weeks investigated (Thailand: 20.2-21.2 ng/mL; PNG: 22.5-23.5  
255 ng/mL; Sudan: 25.1-26.2 ng/mL) and demonstrated no significant difference across gestational  
256 weeks within the same population group (Figure 6).

257 In the presence of EFV, a significant decrease in PQ concentrations ( $p < 0.0001$ ) was simulated  
258 across all gestational weeks within each population group (Thailand: 9.8-11.3 ng/mL; PNG: 13.2-  
259 21.5 ng/mL; Sudan: 15.4-18.3 ng/mL), except for gestational week 40 with the PNG population  
260 group (Figure 6). In the presence of RTV, a significant increase in PQ concentrations ( $p < 0.0001$ )  
261 were simulated across all gestational weeks within each population group (Thailand: 34.2-37.9  
262 ng/mL; PNG: 40.7-42.2 ng/mL; Sudan: 41.3-46.1 ng/mL). Furthermore, a trend in increasing  
263 median concentration with increasing gestational week was observed for all population groups,  
264 although this was not statistically significant (Figure 6).

#### 265 4. DISCUSSION

266 The treatment of malaria in special populations, such as pregnant women and young children, is  
267 complicated by the ‘moving-target’ nature of such population groups and their associated reduced  
268 immune function with which to resist the individual impact and spread of malaria. Attempting to  
269 address the problem of AMT in malaria endemic areas requires careful consideration of the disease  
270 pathophysiology in those population groups which may in turn impact upon the efficacy endpoint  
271 of the antimalarial treatment. With the aid of PBPK modelling techniques, these factors can be  
272 investigated separately to suggest the effect specific impacts can make on antimalarial therapy  
273 clinically. Hence, the applied 4-stage workflow model (Figure 1) was aimed at developing and  
274 validating a PBPK model to assess the pharmacokinetics of PQ during pregnancy, as well as under  
275 conditions of altered serum albumin (mimicking severe malaria), and during potential DDIs; these  
276 were mediated by common ARTs available for use in pregnant women (efavirenz and ritonavir).

277 Despite the advantages of PBPK/mechanistic modelling, the application of modelling  
278 approaches to the prediction of plasma concentration profiles has largely been based around  
279 systems-parameters derived from Caucasian healthy subjects. The base model development in step  
280 1 followed this similar approach, but only to identify and optimise parameters for single dose PQ  
281 studies. A common feature of many antimalarials are the large variability in absorption kinetic  
282 processes, represented by a highly variable  $C_{max}$ , and it was important to capture this, where  
283 possible (Borrmann et al., 2010; Sim et al., 2005; Tarning et al., 2014; White, 2013). To this end,  
284 in the absence of appropriate *in-vitro* Caco-2 derived passive permeability ( $P_{app}$ ) measures for PQ,  
285 we applied a first-order absorption model with final estimates of 0.50 for  $f_a$  and  $0.45\text{ h}^{-1}$  for  $k_a$   
286 which were able to recover the  $C_{max}$  and  $t_{max}$  compared to the single-dose studies (Figure 2).  
287 However, to capture the range of reported values (e.g.  $C_{max}$ : 14-31.5  $\mu\text{g/L}$  and  $t_{max}$ : 2.1-11.7 h) a  
288 50 % CV was applied. It should be noted that an inclusion of a transit absorption model, such as  
289 the Simcyp ADAM module, may improve predictions (Tarning et al., 2012) but the lack of

290 appropriate *in-vitro* permeability measures makes this less attractive over a first order model. The  
291 development of the base model was successful for single dose studies.

292 Malaria is endemic in developing countries and this is reflecting by the availability of reported  
293 clinical studies we identified where PQ was administrated to pregnant and non-pregnant women  
294 from studies conducted with subjects from Thailand (Rijken et al., 2011; Tarning et al., 2012),  
295 Sudan (Hoglund et al., 2012) and Papua New Guinea (Benjamin et al., 2015). Application of the  
296 ‘Healthy-Volunteer’ population group to simulate PQ pharmacokinetic would not be appropriate  
297 given the difference in adult age across these geographic regions (Walpole et al., 2012) and  
298 therefore custom age-weight relationships (Hayes et al., 2015) were generated for each population  
299 group to develop non-pregnant and pregnant populations from these regions, which incorporated  
300 alterations in blood parameters (haematocrit, human serum albumin and alpha-1-acidic  
301 glycoprotein) (see supplementary materials Table S1). Change in haematological biochemistry are  
302 also common in malaria and it plays a major role in pathogenesis (Bakhubaira, 2013; Maina et al.,  
303 2010; van Wolfswinkel et al., 2013). From a pharmacokinetic perspective, such changes are likely  
304 impact upon the blood:plasma ratio, but more importantly the unbound fraction, a key driver for  
305 the prediction of clearance,  $V_{ss}$  and the DDIs.

306 The development of appropriate systems-based population groups, specific to the study design is  
307 important and highlighted by the stark differences in body weights compared to ‘Healthy  
308 Volunteer’ population. Using the customised age-weight relationships for malaria population (see  
309 supplementary material), body weight for Thai ( $49.65 \pm 7.13$  kg), PNG ( $58.32 \pm 12.2$  kg) and Sudan  
310 ( $53.2 \text{ kg} \pm 14.46$  kg) were generally consistent and significant difference ( $p < 0.01$ ) from a standard  
311 ‘Healthy Volunteer’ population group giving an average weight of  $66.7 \pm 13.1$  kg. As dosing for  
312 many AMT is based on body weight, this may have a direct effect on the dose administered and  
313 the resultant determination of endpoint concentrations (Terlouw, Courval, et al., 2003; Terlouw,



314 Nahlen, et al., 2003), particularly considered dosing in many developing countries is based on age  
315 as a surrogate for body weight, in situations where weight facilities are unavailable.

316 Further, the inclusion of potential alterations in biochemistry- based changes during pregnancy or  
317 because of a disease state are important if they are thought to directly impact upon the resultant  
318 pharmacokinetics of AMT. Haematological alterations are common in malaria and a marker of the  
319 severity of malaria is often determined from changes in serum albumin. Equally, albumin binding  
320 is a direct driver for the free fraction of drugs, and any alterations in this may directly impact upon  
321 drug distribution and metabolic pathways. Indeed, the stark difference in HSA in Sudanese (45.5  
322 g/L) and Thai (33.7 g/L) illustrates this difference across population groups (see supplementary  
323 materials Table S1).

324 For the three population groups developed, model predictions of key pharmacokinetics metrics  
325 were within 2-fold of those reported and illustrate the successful prediction of PQ in non-pregnancy  
326 (Table 1) (Figure 3) and pregnant women (Table 3; Figure 4). Notably, no significant differences  
327 in key pharmacokinetic parameters, including day 7 concentrations, were observed between non-  
328 pregnant or pregnant population groups, suggesting the systemic exposure of PQ is relatively  
329 unchanged between the two groups and concurs with other reports of unchanged PQ  
330 pharmacokinetics in non-pregnant and pregnant populations (Adam et al., 2012; Rijken et al., 2011;  
331 Tarning et al., 2012). However, model predictions were less successful at predicting the  
332 interindividual variability in the range of  $C_{max}$  for population groups for each dosing period (Figure  
333 3 and 4). This may be partially due to poor control of food intake during the trial study, e.g.  
334 Tarning *et al* (2012) (Tarning et al., 2012), but may also be a feature of the sparse collection points  
335 around the expected  $C_{max}$  for each dosing day compared to the much richer collection over the  
336 longer elimination phases (Tarning et al., 2012). Further, the larger predicted  $C_{max}$  for each dosing  
337 period could be a result of the splanchnic blood flow (as a result of increased cardiac out) seen in  
338 pregnancy (Dawes & Chowienczyk, 2001), and which is altered using ‘Pregnancy’ population

339 groups in Simcyp, and hence represent a slight increase in the bioavailability of PQ (Tarning et al.,  
340 2012).

341 During pregnancy, the activity of CYP3A4 is also known to increase (Little, 1999), directly  
342 impacting upon the metabolic clearance of any CYP3A4 substrates. In our simulations, increasing  
343 gestational week had a noticeable impact of the terminal elimination of the PQ (see supplementary  
344 materials Figure S2), as quantified by a decrease in the terminal elimination half-life of PQ during  
345 pregnancy populations (Table 3) compared to non-pregnancy populations (Table 1).

346 Having established a working PBPK for PQ in pregnant females, the importance of the risk of DDIs  
347 was next assessed. The only existing study assessing the risk of DDI with PQ and antiretroviral  
348 (efavirenz) was recently published by Kajubi *et al* (Kajubi et al., 2017b), and demonstrated a 40%  
349 reduction in  $AUC_{0-21d}$  along with a 50 % reduction in day-7 concentrations, highlighting the  
350 potential risk EFV-mediated DDIs pose, and our model was able to recapitulate these changes (see  
351 supplementary materials Figure S1). As EFV is known to induce CYP3A4, the reduction in AUC  
352 and day-7 concentration is likely to be a result of this effect (Hariparsad et al., 2004). Further, this  
353 effect would be augmented by the induction of CYP3A4 itself during pregnancy, as noted for other  
354 drugs (Costantine, 2014; Dawes & Chowienczyk, 2001), and therefore would likely reduce day-7  
355 concentrations below the clinical efficacy end-point of 30 ng/mL (Price et al., 2007). Indeed, our  
356 model simulation demonstrated the impact of this in non-pregnant, pregnant and pregnant +EFV  
357 populations, demonstrating the additive effect of EFV-mediated DDI and pregnancy-related  
358 induction of CYP3A4 expression (see supplementary materials Figure S1).

359 Although pregnancy has been associated with a reduction in haematological parameters, e.g. human  
360 serum albumin (decrease by 1% at week 8 and 12% at week 32 (Murphy, Scott, McPartlin, &  
361 Fernandez-Ballart, 2002)), the impact of such changes on the pharmacokinetics of highly bound  
362 drugs is not well characterised in malarial infected subjects. Further, as demonstrated by the  
363 development of specific populations, the overall haematological levels are often reduced in such

364 populations. It has also been speculated that *P. falciparum* plays a major role in proteolysis of  
365 albumin (El Tahir, Malhotra, & Chauhan, 2003; Kolakovich, Gluzman, Duffin, & Goldberg, 1997).  
366 Further, previous reports have demonstrated alteration in  $f_{u,plasma}$  for quinine (Mansor et al., 1991)  
367 and halofantrine (Cenni, Meyer, Brandt, & Betschart, 1995) during the progression of malaria. In  
368 attempting to assess the impact of potential changes in human serum, albumin on the overall extent  
369 of ART-mediated DDIs (via assessing change in PQ day 7 concentration), we set the HSA  
370 concentration to 20 g/L (severe malaria) and 50 g/L (healthy subjects). In all cases (absence and  
371 presence of an ART) and in all population groups, the change from 20 g/L to 50 g/L had a direct  
372 on day 7 concentrations, leading to a statistically significant increase (Figure 5). For all population  
373 groups developed, the reduction in HSA to 20 g/L, generally resulted in a statistically significant  
374 increase in PQ  $f_{u,plasma}$  ( $p < 0.001$ ) which subsequently propagated to an increase in the hepatic  
375 clearance ( $p < 0.001$ ), when compared to compared to healthy volunteer population groups. This  
376 trend was also demonstrated under conditions of efavirenz/ritonavir exposure when compared to  
377 non-DDI studies (see table 5 for representative illustration in the Thailand non-pregnant  
378 population). The impact of this combined reduction in baseline HSA concentration in malaria  
379 population coupled with the pregnancy-related reduction in HSA is important considering as it can  
380 directly impact upon the elimination of the drug.

381

382 As expected, the impact of EFV and RTV on day 7 concentration were in-line with their function  
383 as CYP3A4 inducer (EFV) and inhibitors (RTV) resulting in a direct effect on day-7  
384 concentration following the interaction (Figure 5). A reduction in AMT concentrations as a result  
385 of induction will lead to parasite recrudescence, as has been demonstrated for lumefantrine  
386 (Huang et al., 2012; WorldWide Antimalarial Resistance Network Lumefantrine, 2015),  
387 dihydroartemisinin (Lamorde et al., 2013) and piperazine (Kajubi et al., 2017b). Further,  
388 piperazine is known to prolong QTc in a concentration dependant manner (Darpo et al., 2015),

389 and increased concentration following inhibition of metabolic clearance may potentially lead to  
390 QTc prolongation, as demonstrated with an adapted 2-day treatment with DHA-PQ (Manning et  
391 al., 2014).

392 Physiological changes during gestation can result in significant changes in plasma volume, CYP  
393 expression and cardiac output (Costantine, 2014; Dawes & Chowienczyk, 2001), it would  
394 therefore be expected that significant changes in the pharmacokinetics would be expected during  
395 pregnancy. We explored the impact of gestation on the predicted day-7 concentrations in the  
396 three pregnancy population groups.

397 In all three populations, the baseline median day 7 concentration was consistent across all  
398 population groups and with increasing GW, approximately 20-26 ng/mL, with no significant  
399 differences when GW increased (Figure 6). **Given the long half-life of PQ, the impact of gestation  
400 on day 7 concentrations may not be significantly noticeable.** However, CYP3A4 activity is thought  
401 to increase during pregnancy, reaching a peak at approximately week 20-24 (Hebert et al., 2008;  
402 Hirt et al., 2006; Villani et al., 2006). When considering the Thai population as an example, at  
403 baseline, GW20 corresponded with the lowest median day-7 concentration and highest hepatic  
404 CL<sub>int</sub> (week 17-27) (see supplementary materials Figure S3). However the impact of this may be  
405 negligible given the long half-life and large volume of distribution of PQ (Adam et al., 2012;  
406 Benjamin et al., 2015; Rijken et al., 2011; Tarning et al., 2013).

407 When ARVs were concomitantly dosed with PQ, statistically significant decreases (efavirenz) or  
408 increases (ritonavir) in PQ median day-7 concentrations were predicted, in-line with the role of  
409 EFV and RTV as inducer/inhibitors of CYP3A4 expression. Surprisingly, there were no significant  
410 difference across GW for either ART, suggesting the magnitude of the DDI would be similar,  
411 irrespective of the gestation period of the mother. Further, when considering the Thai population  
412 as an example, although each ART resulted in a significant change ( $p < 0.0001$ ) in the hepatic  
413 CL<sub>int</sub> in the absence of ART (~290-320 L/h) and presence of ART (EFV: 1230-1271 L/h; RTV:

414 6.3-10.7 L/h), no significant differences across gestational weeks were simulated (see  
415 supplementary materials Figure S3). Similarly, although each ART resulted in a significant change  
416 ( $p < 0.0001$ ) in the oral CL in the absence of ART (~2000-2135 L/d) and presence of ART (EFV:  
417 4125-5142 L/d; RTV: 899-1239 L/d), no significant differences across gestational weeks were  
418 simulated (see supplementary materials Figure S3). The resultant  $AUC_{ratio}$  (last dose-to-end) in the  
419 presence of EFV was consistent across GW 7 to 27 (0.56-0.58) but increased to 0.72 at GW 37  
420 (One way ANOVA, Tukey post hoc analysis  $p < 0.01$ ) (see supplementary materials Figure S4).  
421 Similarly, inhibition results in an  $AUC_{ratio}$  across GW 7 to 27 (1.71-1.79) which decreased to 1.64  
422 at GW 37 (not significant) (see supplementary materials Figure S4).

423 Further, median day 7 concentration in the absence and presence of an ART-mediated DDI were  
424 consistently higher in Sudanese population compared to Thai and PNG populations, and this can  
425 be attributed to the lower body-weight corrected doses administered to Thai subjects (see  
426 supplementary materials) coupled with the higher HSA concentrations in Sudanese populations  
427 (see supplementary materials table S1).

428 Thus, although the impact of ARV on PQ pharmacokinetics in pregnancy may lead to treatment  
429 failure or an increase in the adverse effects, the overall effect and magnitude of the DDI during  
430 pregnancy is largely minimal, with little change in day-7 concentrations.

431 It should be noted that the population groups developed altered only the age-weight relationships  
432 and haematological parameters, and reflecting changes predominantly malaria-infected subjects.  
433 Genetic polymorphisms in CYP2B6 are common (Haas et al., 2009; Lang et al., 2001; To et al.,  
434 2009) and may impact upon the clearance of EFV and hence its ability to elicit a DDI, and CYP2B6  
435 population-based polymorphic changes were not incorporated into our customised population  
436 groups. Further, changes in the abundance of CYP-isozymes across population groups have also  
437 not been incorporated and this may enable better predictions of the terminal elimination phases for  
438 PQ across population groups (Bains, 2013). It should also be noted that only one previous study

439 reported PQ metabolic pathways (Lee et al., 2012) and our assumption of the fraction metabolised  
440 by CYP3A4 and CYP2B6 of 0.99 and 0.01, alongside parameter estimated CL<sub>int</sub>, may be  
441 optimised at a later date with *in-vitro* metabolic clearance data to enhance the application of the  
442 model, when such data becomes available.

443 Further, the complexity of diseases states which can present differently depending upon disease  
444 progress, as is common with malaria and HIV (Wanke et al., 2000), would dictate that the  
445 developed population groups should address these different stages of disease progression. Finally,  
446 the studies used for validation utilised two DHA-PQ combination formulation regimens,  
447 Eurartesim<sup>®</sup> (Sigma-Tau, Rome, Italy) or Artekin<sup>®</sup> (Holleykin Pharmaceutical Co., Guangzhou,  
448 China). However only Eurartesim<sup>®</sup> has gained Good Manufacturing Practice compliance, having  
449 been developed without the Medicines for Malaria Venture (MMV) (Ubben & Poll, 2013).  
450 Therefore, batch-to-batch variability in the manufacture of the Artekin<sup>®</sup> fixed-dose combination  
451 tablet, may lead to variability in disintegration/dissolution process resulting in altered absorption  
452 kinetics and this should be considered in the context of further validation of the absorption kinetics  
453 of the model development. Finally, estimates of PQ *in-vitro* Caco-2 permeability are currently  
454 lacking and therefore this precludes the application of the Simcyp ADAM model, to appropriately  
455 model the biopharmaceutics processes in greater mechanistic detail. The modelling of the  
456 absorption phase of PQ pharmacokinetics may therefore be improved when such information  
457 becomes available.

## 458 5. CONCLUSION

459 The present PBPK model provides the ability to mechanistically predict the pharmacokinetic of PQ  
460 in non-pregnancy and pregnant women, whilst also considering possible population differences in  
461 malaria-HIV co-infected subjects. The present model demonstrated that PQ pharmacokinetics in  
462 pregnancy is consistent and was relatively unchanged, compared to non-pregnant women and that  
463 the impact of ART-mediated DDIs can significantly alter the PQ pharmacokinetics, the magnitude

464 of which was generally consistent across GW. Further adaptations of the model presented is  
465 warranted and would require further detailed collation of relevant physiological and biochemical  
466 alterations common to HIV/malaria patients and which would further enhance the clinical  
467 application of the proposed model.

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695

696

697 **LIST OF FIGURES**

698

699 **Figure 1: A four-stage workflow approach for model development, validation and**  
700 **predictions**

701

702 **Figure 2: The simulated plasma fasted single-dose concentration-time profile of piperazine**  
703 **in healthy-volunteers**

704 (A) Simulation of PQ plasma concentration-time profile following a single oral dose of 500 mg  
705 PQP (left panel: open circles are observed mean points) and 1500 mg (right panel: open squares  
706 are observed mean points) to healthy volunteers (n=6). Observed data was obtained from Ahmed  
707 *et al* (2008) (Ahmed *et al.*, 2008). (B) Simulation of PQ plasma concentration-time profile  
708 following a single oral dose of 500 mg PQP to healthy volunteers (n=8). Observed data is  
709 represented by open circles and represents the 3 individual subject concentration-time points only  
710 that were reported by Sim *et al* (2005)(Sim *et al.*, 2005) out of a total study size of 8 subject. Insert  
711 graphs illustrate plasma concentration profiles in the first 24-hours post-dosing. Errors bars  
712 indicate either (A) lower SD at  $C_{max}$  for observed data or (B) reported range of  $C_{max}$  (vertical red  
713 line) or  $t_{max}$  (horizontal red line) values. Solid lines represent population mean prediction with  
714 dashed lines representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles of prediction.

715

716 **Figure 3: The simulated plasma fasted multi-dose concentration-time profile of piperazine**  
717 **in non-pregnant malaria-female subjects**

718 Multidose simulations of PQ (10 mg/kg base once daily for 3 days) were conducted on malaria-  
719 non-pregnant female population groups (Thailand (Rijken et al., 2011; Tarning et al., 2012), Papua  
720 New Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012), adapted from the ‘Healthy  
721 Volunteer’ population group with Simcyp with adaptations to the age-weight relationships and  
722 blood biochemistry and matching (where possible) the clinical trial design (subject numbers and  
723 age range) within Simcyp. Crosses indicate observed data obtained from reported individual subject  
724 plasma concentration-time profile lines, open circles indicated observed data sampling points  
725 obtained from individual plasma concentration points. Insert graphs illustrate plasma concentration  
726 profiles in the first 24-hours post-dosing. Solid lines represent population median predictions with  
727 dashed lines representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles of prediction.

728

729 **Figure 4: The simulated plasma fasted multi-dose concentration-time profile of piperazine**  
730 **in pregnant malaria subjects**

731 Multidose simulations of PQ (10 mg/kg base once daily for 3 days) were conducted on malaria-  
732 pregnant female population groups (Thailand (Rijken et al., 2011; Tarning et al., 2012), Papua New  
733 Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012), from, adapted from the  
734 ‘Pregnancy’ population group within Simcyp with adaptations to the age-weight relationships and  
735 blood biochemistry and matching (where possible) the clinical trial design (subject numbers and  
736 age range). Crosses indicate observed data obtained from reported individual subject plasma  
737 concentration-time profile lines, open circles indicated observed data sampling points obtained  
738 from individual plasma concentration points. Insert graphs illustrate plasma concentration profiles  
739 in the first 24-hours post-dosing. Solid lines (black: non-pregnant [for comparison]; red: pregnant)

740 represent population median prediction with dashed lines representing the 5<sup>th</sup> and 95<sup>th</sup> percentiles  
741 of prediction.

742

743 **Figure 5. The impact of changes in human serum albumin concentrations on the piperazine**  
744 **median day 7 concentration in the absence and presence of an EFZ or RTV-mediated DDI**

745 Multidose simulations of PQP (10 mg/kg base once daily for 3 days) were conducted on malaria-  
746 pregnant female population groups (Thailand, Papua New Guinea and Sudan). The human serum  
747 albumin concentration was fixed at 20 g/L or 50 g/L. EFV (600 mg once daily) (red bars) or RTV  
748 (100 mg twice daily) (green bars) were orally dosed throughout the simulation time period (30  
749 days) with piperazine dosed on days 10, 11 and 12. Box and whisker plots represent minimal, 25<sup>th</sup>  
750 percentile, median, 75<sup>th</sup> percentile and maximum values. Dashed lines indicate the 30 ng/mL  
751 clinical efficacy cut-off. Numbers above the box and whisker are median values and the number  
752 (n) of subjects with a predicted concentration of over 30 ng/mL is indicated. Vertical drop-lines  
753 indicated statistical comparisons between 20g/L or 50 g/L simulation. Asterisks above the  
754 maximum bar indicate statistical significance when compared to black (no DDI) simulations. \*\* p  
755  $\leq 0.01$ ; \*\*\* p  $\leq 0.001$ ; \*\*\*\* p  $\leq 0.0001$ .

756

757 **Figure 6. The impact of changes in gestational week on median day 7 PQ concentration in the**  
758 **absence and presence of a DDI mediated by EFZ (induction) or RTV (inhibition)**

759 Multidose simulations of PQP (10 mg/kg base once daily for 3 days) were conducted on malaria-  
760 pregnant female population groups (Thailand, Papua New Guinea and Sudan over gestational  
761 weeks (GW) 10, 20, 30 and 40. EFV (600 mg once daily) or RTV (100 mg twice daily) were orally  
762 dosed throughout the simulation time period (30 days) with PQ dosed on days 10, 11 and 12. Box  
763 and whisker plots represent minimal, 25<sup>th</sup> percentile, median, 75<sup>th</sup> percentile and maximum values.  
764 Dashed lines indicate the 30 ng/mL clinical efficacy cut-off. Numbers above the box and whisker

765 are median values and the number (n) of subjects with a predicted concentration of over 30 ng/mL  
766 is indicated. Horizontal drop-lines indicate statistical comparisons between each GW in the absence  
767 and presence of the ART. \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; \*\*\*\*  $p \leq 0.0001$ .



## 768 TABLES

769 **Table 1:** Simulated PQ pharmacokinetics in non-Caucasian non-pregnant females

	Thailand		PNG	Sudan
	<i>Rijken</i> <b>Median</b> <b>(Range)</b>	<i>Tarning</i> <b>Median</b> <b>(Range)</b>	<i>Benjamin</i> <b>Median</b> <b>(Range)</b>	<i>Hoglund</i> <b>Median</b> <b>(Range)</b>
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)	66.14 (17.2-182.1)	64.59 (18.7-189.5)	87.70 (20.76-216.39)	92.15 (25.11-261.7)
C <sub>max</sub> 2 <sup>nd</sup> (ng/mL)	85.25 (21.23-241.46)	88.17 (23.74-251.49)	116.15 (25.89-292.40)	116.8 (30.71-337.6)
C <sub>max</sub> 3 <sup>rd</sup> (ng/mL)	98.5 (23.9-280.62)	99.2 (24.2-284.45)	134.6 (29.25-342.48)	134.6 (34.51-388.5)
T <sub>max</sub> (h)				
T <sub>max</sub> 1 <sup>st</sup> (h)	4.80 (2.88-6.48)	4.81 (2.82-6.12)	4.67 (2.91-6.51)	3.84 (2.16-5.04)
T <sub>max</sub> 2 <sup>st</sup> (h)	4.56 (2.64-6.01)	4.61 (2.54-6.32)	4.46 (2.72-6.06)	3.6 (2.16-4.8)
T <sub>max</sub> 3 <sup>st</sup> (h)	4.56 (2.64-7.62)	4.62 (2.71-7.20)	4.39 (2.7-6.12)	3.6 (2.16-4.8)
AUC <sub>0-24</sub> (ng/mL.h)	998.64 (240-2830.8)	993.56 (248-2824.9)	1372.8 (296.83-3466.7)	1261.4 (318.24-3678.7)
AUC <sub>24-48</sub> (ng/mL.h)	1409.3 (324.5-4074.9)	1429.3 (331.5-4107.34)	1968.7 (404.8-5064.4)	1790.6 (436.08-5254.3)
AUC <sub>48-72</sub> (ng/mL.h)	1712.4 (384-4945.9)	1762.1 (381-4995.2)	2378.4 (479.4-6175.7)	2194 (520.6-6383.8)
AUC <sub>0-∞</sub> (µg/L.h)			21715 (4463-53645.8)	35201.3 (303.9-74180.6)
Day 7 Conc. (ng/mL)	24.74 (4.42-64.93)	25.11 (4.21-65.14)	29.17 (5.31-80.85)	34.0 (6.8-86.7)
Day 14 Conc. (ng/mL)	17.01 (3.17-41.63)	16.78 (3.22-42.99)	18.75 (3.67-49.9)	24.2 (5.3-54.9)
Day 28 Conc. (ng/mL)	12.03 (2.34-26.28)	11.63 (2.23-27.27)	11.70 (2.05-26.80)	17.5 (4.02-38.7)
Half-life (d)	27.3 (21.3-40.9)	28.5 (20.8-39.8)	18.3 (16.1-23.6)	32.1 (21.6-43.3)

770

771

772 **Table 2:** Literature reported PQ pharmacokinetics in non-Caucasian non-pregnant females

	Thailand		PNG	Sudan
	<i>Rijken</i> <b>Median</b> <b>(Range)</b>	<i>Tarning</i> <b>Median</b> <b>(IQR)</b>	<i>Benjamin</i> <b>Median</b> <b>(Range)</b>	<i>Hoglund</i> <b>Median</b> <b>(Range)</b>
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)		291 (194–362)		185 (109–363)
C <sub>max</sub> 2 <sup>nd</sup> (ng/mL)	138 (39.3–328)			
C <sub>max</sub> 3 <sup>rd</sup> (ng/mL)	201 (58.2–455)			
T <sub>max</sub> (h)	309 (138–575)			
T <sub>max</sub> 1 <sup>st</sup> (h)		3.14 (2.84–3.84)		3.07 (1.65-4.64)
T <sub>max</sub> 2 <sup>st</sup> (h)				
T <sub>max</sub> 3 <sup>st</sup> (h)				
AUC <sub>0-24</sub> (ng/mL.h)				
AUC <sub>24-48</sub> (ng/mL.h)	1480 (506–3,270)			
AUC <sub>48-72</sub> (ng/mL.h)	2400 (734–4,400)			
AUC <sub>0-∞</sub> (µg/L.h)	3660 (1,160–5,010)			
Day 7 Conc. (ng/mL)			23721 (21481–27951)	42700 (27100–68700)
Day 14 Conc. (ng/mL)	31.8 (13.3–80.2)	28.8 (23.6–34.6)		60.7 (40.1-103)
Day 28 Conc. (ng/mL)	19.5 (7.76–49.3)			
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)	10.7 (3.70–31.4)	10.3 (9.18–14.4)		16.1 (9.68-26.8)
Half-life (d) <sup>a</sup>	4.78-39.9	22-26.1	20.3	20.9-33.3

773

774 <sup>a</sup> Half-life is reported as a range or median

775 **Table 3:** Simulated piperquine pharmacokinetics in non-Caucasian pregnant females

	Thailand		PNG	Sudan
	<i>Rijken</i> <b>Median</b> <b>(Range)</b>	<i>Tarning</i> <b>Median</b> <b>(Range)</b>	<i>Benjamin</i> <b>Median</b> <b>(Range)</b>	<i>Hoglund</i> <b>Median</b> <b>(Range)</b>
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)	70.44 (33.56-153.08)	72.43 (31.92-167.89)	89.52 (38.20-175.42)	92.91 (41.04-202.38)
C <sub>max</sub> 2 <sup>nd</sup> (ng/mL)	90.47 (46.02-204.60)	86.23 (50.61-214.36)	118.35 (25.82-237.95)	116.6 (55.6-263.53)
C <sub>max</sub> 3 <sup>rd</sup> (ng/mL)	103.28 (53.95-237.19)	109.73 (53.54-264.38)	136.70 (61.99-276.93)	132.17 (65.25-303.4)
T <sub>max</sub> (h)				
T <sub>max</sub> 1 <sup>st</sup> (h)	4.80 (2.9-7.68)	4.62 (3.1-7.98)	5.04 (2.9-8.16)	4.32 (2.64-6.96)
T <sub>max</sub> 2 <sup>st</sup> (h)	4.56 (2.88-6.96)	4.86 (2.91-7.02)	4.8 (2.9-7.2)	4.08 (2.64-6.48)
T <sub>max</sub> 3 <sup>st</sup> (h)	4.56 (2.88-6.96)	4.79 (2.83-7.11)	4.56 (2.9-6.96)	4.08 (2.64-6.24)
AUC <sub>0-24</sub> (ng/mL.h)	1036.3 (564.96-2429)	1135.2 (536.9-2532)	1399.4 (681.1-2850)	1249 (112.72-2974.32)
AUC <sub>24-48</sub> (ng/mL.h)	1450.3 (770.4-3482.4)	1424.1 (779.3-3599.8)	2002.1 (938.9-4127.52)	1745.3 (931.92-4229.76)
AUC <sub>48-72</sub> (ng/mL.h)	1736.1 (912.5-4195.9)	1811.2 (964.9-4201.7)	2408.64 (1110.24-4977.1)	2424.96 (1114.1-5108.2)
AUC <sub>0-∞</sub> (μg/L.h)			21633.6 (8383.9-42237.8)	30067.4 (15267-84201.1)
Day 7 Conc. (ng/mL)	25.97 (10.87-52.59)	24.17 (11.03-53.13)	29.62 (12.2-57.7)	34.04 (15.13-70.60)
Day 14 Conc. (ng/mL)	19.11 (7.88-39.46)	19.92 (8.24-40.11)	20.19 (7.89-38.8)	26.14 (11.31-56.55)
Day 28 Conc. (ng/mL)	14.40 (5.63-31.20)	15.12 (5.91-39.97)	13.60 (4.82-27.2)	20.11 (7.94-45.62)
Half-life (d)	19.4 (18.7-35.4)	19.9 (18.64-35.7)	26.3 (16.7-39.25)	24.7 (14.9-27.2)

776

777

778

779 **Table 4:** Literature reported piperquine pharmacokinetics in non-Caucasian pregnant females

	Thailand		PNG	Sudan
	<i>Rijken</i> <b>Median</b> <b>(Range)</b>	<i>Tarning</i> <b>Median</b> <b>(IQR)</b>	<i>Benjamin</i> <b>Median</b> <b>(Range)</b>	<i>Hoglund</i> <b>Median</b> <b>(Range)</b>
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)		216 (139–276)		102 (40.6-235)
C <sub>max</sub> 2 <sup>nd</sup> (ng/mL)	71.6 (10.1–239)			
C <sub>max</sub> 3 <sup>rd</sup> (ng/mL)	136 (13.6–393)			
T <sub>max</sub> (h)	245 (53.4–798)			
T <sub>max</sub> 1 <sup>st</sup> (h)		3.04 (2.36–4.13)		1.48 (0.887-4.18)
T <sub>max</sub> 2 <sup>st</sup> (h)				
T <sub>max</sub> 3 <sup>st</sup> (h)				
AUC <sub>0-24</sub> (ng/mL.h)				
AUC <sub>24-48</sub> (ng/mL.h)	869 (157–2,940)			
AUC <sub>48-72</sub> (ng/mL.h)	1710 (167–4,740)			
AUC <sub>0-∞</sub> (µg/L.h)	2750 (500–8,280)			
Day 7 Conc. (ng/mL)			35644 (29546–39541)	38000 (12400–100000)
Day 14 Conc. (ng/mL)	25.9 (6.80–56.6)	22.7 (17.6–32.8)		55.4 (16.6-146)
Day 28 Conc. (ng/mL)	16.7 (2.24–59.2)			
C <sub>max</sub> 1 <sup>st</sup> (ng/mL)	9.17 (5.14–47.6)	10.3 (8.06–14.9)		15.4 (4.85-38.6)
Half-life (d) <sup>a</sup>	8.88-24.9	16.2-19.4	15.9	19.1-25.8

780

781 <sup>a</sup> Half-life is reported as a range or median

782

783 **Table 5:** Impact of changes in blood biochemistry on hepatic clearance in the absence and presence of a efavirenz or ritonavir mediated drug-drug  
 784 interaction for a representative population group (Thailand non-pregnant).

	<i>No DDI</i>			<i>Efavirenz</i>			<i>Ritonavir</i>		
	<b>Healthy</b>	<b>20 g/L<sup>a</sup></b>	<b>50 g/L<sup>a</sup></b>	<b>Healthy</b>	<b>20 g/L</b>	<b>50 g/L</b>	<b>Healthy</b>	<b>20 g/L</b>	<b>50 g/L</b>
<b>CL<sub>H</sub> (L/h)</b>	8.41 ± 4.48	16.24 ± 6.91	7.06 ± 3.75	33.86 ± 10.95	46.91 ± 10.26	29.94 ± 9.43	2.62 ± 1.31	5.1 ± 2.35	1.94 ± 0.87
<b>f<sub>u</sub>plasma</b>	0.0139 ± 0.0014	0.0368 ± 0.0037	0.0135 ± 0.0013	0.0138 ± 0.0016	0.0375 ± 0.0032	0.0136 ± 0.0014	0.0137 ± 0.0016	0.0381 ± 0.0031	0.0138 ± 0.0014
<b>HSA (g/L)<sup>b</sup></b>	45.28 ± 4.53	16.72 ± 1.73	46.81 ± 4.68	45.68 ± 4.66	16.62 ± 1.74	47.04 ± 4.63	45.16 ± 4.56	16.92 ± 1.72	47.21 ± 4.61

785

786 <sup>a</sup> Pre-defined fixed mean human serum albumin (HSA) concentration.

787 <sup>b</sup> Simcyp simulated population median HSA concentration

788 Data represented as median ± standard deviation

789 Healthy: Healthy Volunteer population group; CL<sub>H</sub>: hepatic clearance; f<sub>u</sub>plasma: unbound fraction in plasma; HSA: human serum albumin.