

3 Running title: **Primaquine exposures in vivax malaria**

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20 Keywords: *Plasmodium vivax*, primaquine, pharmacokinetics, relapse, chloroquine,  
21 dihydroartemisinin-piperaquine

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## 24 Abstract

### 25 Background

26 Primaquine is the only widely available drug for radical cure of *Plasmodium vivax* malaria. There  
27 is uncertainty whether the pharmacokinetic properties of primaquine are altered significantly in  
28 childhood or not.

### 29 Methods

30 Glucose-6-phosphate dehydrogenase normal patients with uncomplicated *P. vivax* malaria were  
31 randomized to receive either chloroquine (25mg base/kg) or dihydroartemisinin-piperaquine  
32 (dihydroartemisinin 7mg/kg and piperaquine 55mg/kg) plus primaquine; given either as 0.5 mg  
33 base/kg/day for 14 days or 1 mg/kg/day for 7 days. Pre-dose day 7 venous plasma  
34 concentrations of chloroquine, desethylchloroquine, piperaquine, primaquine and  
35 carboxyprimaquine were measured. Methemoglobin levels were measured on day 7.

### 36 Results

37 Day 7 primaquine and carboxyprimaquine concentrations were available for 641 patients. After  
38 adjustment for the primaquine mg/kg daily dose, day of sampling, partner drug, and fever  
39 clearance, there was a significant non-linear relationship between age and trough primaquine  
40 and carboxyprimaquine concentrations, and day methemoglobin levels. Compared to adults 30  
41 years of age, children 5 years of age had trough primaquine concentrations 0.53 (95% CI: 0.39-  
42 0.73) fold lower, trough carboxyprimaquine concentrations 0.45 (95% CI: 0.35- 0.55) fold lower,  
43 and day 7 methemoglobin levels 0.87 (95% CI: 0.58-1.27) fold lower. Increasing concentrations  
44 of piperaquine and chloroquine and poor metabolizer *CYP 2D6* alleles were associated with

45 higher day 7 primaquine and carboxyprimaquine concentrations. Higher blood methemoglobin  
46 concentrations were associated with a lower risk of recurrence.

47 Conclusion

48 Young children have lower primaquine and carboxyprimaquine exposures, and lower levels of  
49 methemoglobinemia, than adults. Young children may need higher weight adjusted primaquine  
50 doses than adults.

## 51 Introduction

52 Primaquine is used for radical cure of *Plasmodium vivax* and *Plasmodium ovale* malaria, for  
53 antimalarial chemoprophylaxis and as a single dose gametocytocide in *Plasmodium falciparum*  
54 malaria (1). Although the slowly eliminated 8-aminoquinoline tafenoquine has been registered  
55 in a few countries for radical cure or chemoprophylaxis in adults, and pamaquine and quinocide  
56 were once used, primaquine is by far the most widely deployed drug in this class. It is generally  
57 agreed that the dosing of antimalarial chemotherapy should be designed to provide equivalent  
58 exposures to the active antimalarial across the age range (1). There is uncertainty whether or not  
59 children have lower exposures to primaquine and its bioactive metabolites than adults for the  
60 same weight adjusted doses, and thus whether they should receive higher doses than currently  
61 recommended (2–5). Primaquine is combined with either an artemisinin combination treatment  
62 (ACT) or chloroquine to prevent relapse of vivax or ovale malaria (radical cure) (1). Primaquine is  
63 a pro-drug requiring metabolism to bioactive metabolites mainly via the liver cytochrome P450  
64 isoenzyme *CYP 2D6* (6). The prevalence of intermediate and non-functioning *CYP 2D6* alleles is  
65 over 50% in some populations, particularly in Asians (7–9). Decreased *CYP 2D6* function, resulting  
66 from intermediate or poor metabolizer genotypes, potentially lowers radical cure efficacy (10,  
67 11).

68 Chloroquine has been the first-line treatment for the blood stages of *P. vivax* malaria for over 70  
69 years (1). High grade resistance in *P. vivax* remains confined to Oceania and Indonesia (12) but,  
70 in the last decade, low grade chloroquine resistance, manifest by earlier appearance of relapses,  
71 has been reported increasingly in other locations. Piperaquine (a slowly eliminated bisquinoline),  
72 combined with dihydroartemisinin, is a well-tolerated and highly efficacious ACT which provides

an excellent alternative blood stage treatment for *P. vivax* malaria (13). An open label two-way randomized controlled trial, conducted on the Thailand-Myanmar border, showed previously that a 7-day high dose primaquine regimen (1 mg/kg/day) , combined either with chloroquine or dihydroartemisinin-piperaquine, was non-inferior to the standard 14-day (0.5 mg/kg/day) regimen (14). This large study allowed investigation of the pharmacokinetic properties of primaquine in adults and children, characterization of the determinants of drug exposures, and correlation with therapeutic outcomes.

## Methods

### Clinical trial

This study was conducted by the Shoklo Malaria Research Unit (SMRU), which is located on the northwest Thailand-Myanmar border. Full details of the study have been reported previously (14). In brief, patients who presented to an outpatient clinic with microscopy confirmed uncomplicated *P. vivax* mono-infections were enrolled if they were  $\geq 6$  months old, and if they were glucose-6-phosphate dehydrogenase (G6PD) normal by the fluorescent spot test. Patients were excluded if they were pregnant or breastfeeding an infant  $\leq 6$  months, had a hematocrit  $\leq 25\%$ , or had received a blood transfusion within 3 months. Written informed consent was obtained from patients or from parents or carers of children below 18 years old.

Patients were randomized 1:1:1:1 to each of the following groups:

1. Chloroquine (10 mg base/kg on the first two days of treatment, then 5 mg base/kg on day 3; Remedica, Ltd., Cyprus) and primaquine (1 mg base/kg/day for 7 days; Government Pharmaceutical Organization, Thailand)

94 2. Chloroquine as above and primaquine (0.5 mg base/kg/day for 14 days)

95 3. Dihydroartemisinin-piperaquine (dihydroartemisinin 7 mg/kg and piperaquine 55 mg/kg

96 for 3 days; Guilin Pharmaceutical company, China) and primaquine (1 mg base/kg/day for

97 7 days)

98 4. Dihydroartemisinin-piperaquine as above plus primaquine (0.5 mg base/kg/day for 14

99 days)

100 Food and drink were given before drug administration. Primaquine was usually started on the

101 same day as the schizonticide (i.e. the first day of treatment) but in a subset of patients it was

102 started on the third day of treatment. Patients were assessed daily while on treatment.

103 Methemoglobin was measured either daily or on days 1, 3, 6 and 13, and additionally on day 10

104 in the primaquine 14-day groups, using a transcutaneous pulse oximeter (Masimo Radical-7®).

105 One recruitment clinic did not have access to a transcutaneous oximeter, so 80 study patients

106 did not have methemoglobin measurements. All drug doses were supervised.

107 A venous blood sample for antimalarial drug levels was taken before drug administration on day

108 6 (i.e. the 7<sup>th</sup> day of treatment) +/- 2 days, and plasma was separated and stored at -70°C. Patients

109 were followed at weeks 2 and 4, and then every 4 weeks until 52 weeks. If there was a *P. vivax*

110 recurrence then a venous plasma blood sample for drug levels was taken again. Standard high

111 dose primaquine (0.5 mg base/kg/day for 14 days) and chloroquine were given for the treatment

112 of recurrences. Follow-up was re-started as if newly recruited, and day 6 sampling for drug levels

113 was collected again. The total study duration was 52 weeks from enrolment.

## 114 Drug measurements

115 Chloroquine/desethylchloroquine, piperaquine, and primaquine/carboxyprimaquine plasma  
116 concentrations were measured using three validated liquid chromatography (LC) – tandem mass  
117 spectrometry (MS/MS) methods (15–17). The lower limits of quantification (LLOQ) were set to  
118 1.4, 1.5, 1.14 and 4.88 ng/mL for chloroquine/desethylchloroquine, piperaquine, primaquine and  
119 carboxyprimaquine, respectively. All three quantification methods were validated according to  
120 regulatory standards and three levels of quality control samples were analyzed in triplicate within  
121 each batch of clinical samples. Total imprecision (i.e. relative standard deviation) for all quality  
122 control samples was below 10% during drug quantification.

123 *CYP 2D6* genotyping

124 We carried out a nested case-control genotyping study to assess the effect of *CYP 2D6*  
125 polymorphisms on recurrent vivax malaria. Cases were all patients with a recorded recurrent  
126 episode of vivax malaria during follow-up and controls were patients without observed  
127 recurrences matched by age and sex (in total n=158 patients). The *CYP 2D6* gene  
128 duplications/multiplications and gene deletions were determined by extra-long range  
129 polymerase chain reaction (XL-PCR) using a previously published protocol (18). The functional  
130 *CYP 2D6* and nonfunctional *CYP2D8* and *CYP2D7* genes were then differentiated by intron 2  
131 sequencing (INT2). The variants \*1, \*2, \*4, \*5, \*10, \*39, and \*36 were identified with rapid  
132 identification of four polymorphic loci by allele-specific oligonucleotide probes using real-time  
133 SNPs genotyping. From the *CYP 2D6* genotyping result the *CYP 2D6* “activity score” was obtained  
134 as described previously (15, 19–21).

## 135 Ethics approval

136 This study was approved by both the Mahidol University Faculty of Tropical Medicine Ethics  
137 Committee (MUTM 2011-043, TMEC 11-008) and the Oxford Tropical Research Ethics  
138 Committee (OXTREC 17-11) and was registered at ClinicalTrials.gov (NCT01640574).

## 139 Statistical analysis

140 All drug concentration data were analyzed on the log base 10 scale. Three main generalized  
141 additive linear regression models were fitted to the  $\log_{10}$  primaquine day 7 concentrations; the  
142  $\log_{10}$  carboxyprimaquine concentrations; and the  $\log_{10}$  carboxyprimaquine to primaquine ratios.  
143 We used the following predictors age (years), mg/kg daily dose of primaquine (the administered  
144 mg/kg dose not the target dose), study arm (DHA-piperaquine versus chloroquine), fever  
145 clearance time (days), the number of days since start of primaquine dosing (6 versus 4), and the  
146 time since the previous primaquine dose (in hours). We excluded drug measurements taken  
147 outside of a 18-30 hour window after the previous dose (only trough levels approximately one  
148 day after dosing), except for those for which the previous dose was given less than 30 minutes  
149 before the drug measurement. More than 90% of samples were taken in this window  
150 (Supplementary Figure 1). A smooth spline term with 3 degrees of freedom was used to estimate  
151 non-linear effects of age. Individual random intercept terms were specified to account for  
152 multiple day 7 measurements in the same patient. To assess any residual effect of *CYP 2D6*  
153 diplotypes, we fitted linear regression models to the model residual concentrations with the *CYP*  
154 *2D6* activity score as a linear predictor. To estimate the effect of the partner drug concentrations  
155 (not available for every day 7 measurement), we fitted separate generalized additive regression  
156 models to the primaquine and carboxyprimaquine  $\log_{10}$  concentrations with either the  $\log_{10}$



157 chloroquine+desethychloroquine (assuming equal antimalarial activities of parent drug and  
158 metabolite and designated as 'chloroquine' in the remainder of the paper) concentration or the  
159  $\log_{10}$  piperaquine concentration as additional predictors.

160 We fitted a generalized additive regression model to the day 7 blood methemoglobin  
161 concentrations (expressed as a percentage of the hemoglobin concentration) with age, mg/kg  
162 daily dose of primaquine, study arm (DHA-piperaquine versus chloroquine), fever clearance time  
163 (days), and days since start of primaquine dosing as predictors. A spline term with 3 degrees of  
164 freedom was fit to age. Secondary models included the *CYP 2D6* activity score (available only for  
165 the subset of patients in the nested case control investigation) as an additional covariate, both  
166 on the linear scale and as a binary covariate ( $\leq 0.5$  versus  $> 0.5$ ). The activity score has an arbitrary  
167 scale so it is unclear how best to encode it in regression models (i.e. an activity score of 2 versus  
168 1 is not the same as 1 versus 0).

169 We fitted Cox proportional hazard models to the time to first recurrence (right censored event  
170 for patients who did not have a recurrence during follow-up), with continuous predictors: age,  
171 partner schizonticidal drug (chloroquine versus piperaquine), and either the  $\log_{10}$  primaquine or  
172 the  $\log_{10}$  carboxyprimaquine day 7 concentration (they are highly co-linear so we fitted two  
173 separate models). Additional models included the day 7 methemoglobin levels as an additional  
174 predictor (measured in all sites except the one where a transcutaneous pulse oximeter was not  
175 available).

176 The generalized additive models were fitted in R version 4.0.2 using the package *mgcv* version  
177 1.8. The Cox proportional hazard model used the *survival* package version 3.2. Code in the form

178 of an RMarkdown script along with all analyzed data are available on an accompanying github  
179 repository for full reproducibility ([https://github.com/jwatowatson/Primaquine\\_day7](https://github.com/jwatowatson/Primaquine_day7)).

## 180 Results

181 Between February 2012 and July 2014, 680 patients with uncomplicated acute *P. vivax* malaria  
182 were enrolled. In total 641 patients had at least one day 7 plasma primaquine and  
183 carboxyprimaquine concentration measured, either during the enrollment episode or during a  
184 recurrent *P. vivax* episode (there were a total of 720 episodes with day 7 concentrations including  
185 patients with multiple episodes). For 692 of these day 7 concentrations, the corresponding  
186 plasma chloroquine or piperaquine concentration was also available (measured from the same  
187 blood sample). We excluded 41 drug measurements considered not to be trough levels (see  
188 Methods) resulting in a total of 679 day 7 measurements included in the analysis.

189 In total, 71 of the patients with measured primaquine and carboxyprimaquine concentrations  
190 were 6 to  $\leq 10$  years of age (median weight 20kg, range 13-30kg), and 34 were  $\leq 5$  years of age  
191 (median weight 14kg, range 8-20kg) (Table 1). Three patients were less than 3 years old, and the  
192 youngest was 1 year 6 months old. Fever and parasite clearance times were similar across all age  
193 groups. The proportion of patients with microscopy detected gametocytemia was higher in the  
194 older age groups  $>10$  years.

## 195 Effect of age

196 After adjustment for the daily mg/kg primaquine dose, the number of days of dosing, the fever  
197 clearance times, and the partner drug (piperaquine versus chloroquine), there was a significant  
198 non-linear association between age and day 7 primaquine trough concentration ( $p < 10^{-16}$  for the

199 smooth spline term fit to age) and day 7 carboxyprimaquine trough concentration ( $p < 10^{-16}$  for  
200 the smooth spline term fit to age) (Figure 1). For both primaquine and carboxyprimaquine,  
201 increasing age correlated with higher trough levels. The strongest effect was seen for the  
202 carboxyprimaquine trough levels. Thus the ratio of carboxyprimaquine to primaquine was also  
203 associated with age, with an approximate doubling when comparing patients aged  $\leq 5$  years  
204 versus patients 30 years or older (Figure 1).

#### 205 Effect of partner drug

206 Our previously reported healthy volunteer studies showed that co-administration of piperaquine  
207 and chloroquine both increased exposures to primaquine and carboxyprimaquine (15, 16). In this  
208 study of patients with vivax malaria, a ten-fold increase in the chloroquine day 7 concentration  
209 was associated with a 2.5 fold increase in day 7 primaquine concentrations (95% CI: 1.9 to 3.1;  
210  $p = 0.0003$ ), and a 2.0 fold increase in day 7 carboxyprimaquine concentrations (95% CI: 1.7 to 2.4;  
211  $p = 0.0002$ ). A ten-fold increase in the piperaquine day 7 concentrations was associated with a 2.5  
212 fold increase in primaquine concentrations (95% CI: 1.9 to 3.2;  $p = 0.0005$ ), and a 2.5 fold increase  
213 in carboxyprimaquine concentrations on day 7 (95% CI: 2.1 to 3.0;  $p = 10^{-7}$ ). Scatterplots showing  
214 the univariate relationships between the partner drug concentrations and  
215 primaquine/carboxyprimaquine concentrations are shown in Supplementary Figure 2. Treatment  
216 with chloroquine or piperaquine had no statistically significant effect on the  
217 carboxyprimaquine/primaquine ratios.

218 Effect of *CYP 2D6* genotypes

219 *CYP 2D6* genotypes were determined in 154 patients. The estimated *CYP 2D6* allele frequencies  
220 in this population were as follows: 25% for \*1; 23% for \*2; 2% for \*4; 10% for \*5; 38% for \*10;  
221 0.5% for \*39; and 1% for \*36. Alleles \*4, \*5, and \*36 are considered null-metabolizer alleles  
222 (enzyme activity score of 0); \*10 is a poor metabolizer allele (activity score of 0.25); and \*1 and  
223 \*39 are normal metabolizer alleles (activity score of 1). In total, there were 3 patients with an  
224 activity score of 0 (non-metabolizers) and 36 patients with activity scores of either 0.25 or 0.5  
225 (poor metabolizers). For primaquine day 7 plasma concentrations, model residuals (defined as  
226 the observed concentration minus the predicted concentration, both on the log scale, where the  
227 model is based on age, mg/kg dose, fever clearance, time since last dose, number of days of  
228 dosing, and partner drug), were correlated significantly with the genotype-defined *CYP 2D6*  
229 enzyme activity scores (Figure 2;  $p = -0.2$ ,  $p = 0.004$ ). Patients with low *CYP 2D6* activity scores had,  
230 on average, higher observed concentrations of primaquine than predicted (i.e. positive  
231 residuals), whereas patients with normal activity scores had lower concentrations than expected  
232 (i.e. negative residuals). For plasma carboxyprimaquine concentrations, a trend in the same  
233 direction was observed, but the correlation was substantially smaller, and it was not significant  
234 ( $p = 0.1$ ).

## 235 Methemoglobinemia and hemolysis

236 Primaquine causes predictable methemoglobinemia. As reported previously methemoglobin  
237 concentrations rose over the first week of primaquine treatment, and then plateaued in the 14-  
238 day primaquine groups. The day 7 values were usually the peak recorded values  
239 (Supplementary Figure 3). Methemoglobin (expressed as a percentage of the hemoglobin

240 concentration) was lower in children than in young adults ( $p < 10^{-16}$  for a spline term fit to age in  
241 a generalized additive regression model, Figure 3A), and was lower in patients with loss of  
242 function *CYP 2D6* polymorphisms (Figure 2C; absolute decrease of 1.2 percentage points in the  
243 mean day 7 methemoglobin level in patients with an activity score  $\leq 0.5$  compared to patients  
244 with an activity score  $> 0.5$ ; 95% CI: 0.1-2.3%,  $p=0.04$ ). Day 7 methemoglobin levels were  
245 associated with both primaquine and carboxyprimaquine day 7 plasma concentrations. A ten-  
246 fold increase in primaquine day 7 concentrations was associated with an absolute increase of  
247 0.7 percentage points in day 7 methemoglobin levels (95% CI: 0.14-1.23), and a ten-fold  
248 increase in carboxyprimaquine concentrations was associated with an absolute increase of 1.1  
249 percentage points in day 7 methemoglobin levels (95% CI: 0.5 – 2.0). Four patients stopped  
250 taking primaquine because of elevated methemoglobin levels and associated symptoms. Two of  
251 these patients stopped on day 5 and, as the day 6 primaquine dose was not administered, they  
252 had low day 7 primaquine and carboxyprimaquine levels. One of these patients had a recorded  
253 methemoglobin level of 20.9% on day 6 followed by 18.1% on day 7. The other three patients  
254 had peri-oral cyanosis (blue lips). Their peak recorded methemoglobin levels were between 10  
255 and 12%.

256 There was no relationship between age and hemolysis defined as the percentage drop in  
257 hematocrit between baseline and day 7 (Figure 3B).

#### 258 Primaquine exposure and *P. vivax* recurrence

259 We did an exploratory analysis of the relationship between primaquine exposures and times to  
260 the first *P. vivax* recurrence. A total of 564 patients had recorded day 7 drug levels and  
261 methemoglobin levels, and 70 had at least one recurrence during follow-up. After adjusting for

age and partner drug, day 7 concentrations of primaquine and carboxyprimaquine were not associated with the risk of recurrence, but a 1% absolute increase in day 7 methemoglobin was associated with a hazard ratio for recurrence of 0.9 (95% CI: 0.85-0.99,  $p=0.02$ ). Two of the three patients with a *CYP 2D6* activity score of 0 had a *P. vivax* recurrence, and one of them had 2 recurrences (the interval between recurrences was >65 days). Studies are ongoing to characterize the relationship between *CYP 2D6* genotypes and risk of *P. vivax* relapse in this population.

## Discussion

Radical cure of *P. vivax* malaria is necessary to prevent relapses. Relapses of vivax malaria are a major cause of morbidity and developmental delay in children living in endemic areas, so optimization of primaquine dosing is critical in this age group. The higher *P. vivax* relapse rates in children compared with adults living in endemic areas observed in some studies (22) are attributed usually to lower levels of immunity, but lower drug exposures could also be a contributory factor. For many antimalarial drugs, exposures in children are lower than in adults and an increase in the weight adjusted dosing is needed (1). The limited pharmacokinetic evidence to date for primaquine does not provide a clear picture, with a study from Papua New Guinea suggesting little difference between adults and older children (2), whereas a study of single dose primaquine (used as a *P. falciparum* gametocytocide) in Tanzania (3) and a study of primaquine for *P. vivax* radical cure in Brazil (4) both suggesting that younger children had lower exposures. The relationship between plasma primaquine concentrations and antimalarial effect is complex. Primaquine is metabolized rapidly (elimination half-life ~5 hours) via monoamine oxidase to a biologically inert metabolite carboxyprimaquine and, through a separate pathway,

284 mainly via *CYP 2D6*, to several active hydroxylated metabolites (6, 23). The exact mechanism of  
285 antimalarial action remains uncertain. Recent laboratory studies suggest that these unstable  
286 hydroxylated metabolites are oxidized to quinoneimines generating local hydrogen peroxide -  
287 which is parasiticidal. The quinoneimines in turn are substrates for cytochrome P450  
288 NADPH:oxidoreductase (POR or CPR) resulting in hydrogen peroxide accumulation and  
289 augmenting the parasiticidal effect (24). These reactions may mediate both the antimalarial  
290 therapeutic effects, and also the oxidant hemolytic adverse effects. Alternatively the  
291 quinoneimine itself may cause the hemolytic and possibly parasiticidal effects by forming  
292 irreversible ligands with heme. There is also evidence for synergy between the 8-aminoquinolines  
293 and other quinoline antimalarials in liver stage and blood stage activities (25, 26). The 8-  
294 aminoquinolines have innate schizonticidal activity as well as radical curative activity and 4-  
295 aminoquinoline antimalarials increase primaquine concentrations (15, 16, 26). Unfortunately,  
296 although the metabolic pathway to the production of the active primaquine metabolites has  
297 been characterized ex-vivo, no validated measure of their concentrations, or of their more stable  
298 metabolites, in whole blood, plasma, or urine has yet been correlated with antimalarial  
299 therapeutic responses in-vivo. Thus, measurement of primaquine and carboxyprimaquine  
300 concentrations does not provide a direct correlate of antimalarial activity. Furthermore, because  
301 the apparent volume of distribution of carboxyprimaquine is not known, the proportion of parent  
302 drug that passes through this inactive metabolic pathway cannot be estimated.

303

304 This large study of day 7 “trough” primaquine and carboxyprimaquine plasma concentrations  
305 shows clearly that exposures to both parent compound and inactive metabolite are lower in

306 children than in adults. This is unlikely to be explained by lower oral bioavailability as primaquine  
307 is generally well absorbed, the children in the study were clinically well by seven days after  
308 starting treatment (14), all doses were observed, and only 7 children  $\leq 10$  years old ( $\leq 27$ kg)  
309 vomited. It presumably reflects either larger primaquine (and carboxyprimaquine) distribution  
310 volumes, or increased clearance, or both. The lower ratio of carboxyprimaquine to primaquine  
311 in children may also be explained by these age-related pharmacokinetic changes. Although  
312 greater diversion down the bioactivating CYP pathway cannot be excluded as an explanation for  
313 these findings- there is no convincing evidence for augmented drug efficacy (e.g. no relationship  
314 with fever or parasite clearance times) or increased hemolytic toxicity in children to support this.  
315 Furthermore, in this study, methemoglobinemia was lower in children than in young adults,  
316 indicating that formation of oxidant metabolites was reduced (27). Lower methemoglobin  
317 concentrations were associated with an increased risk of *P. vivax* recurrence. The importance of  
318 CYP 2D6 primaquine bioactivation in the generation of oxidant metabolites was evidenced by the  
319 lower levels of methemoglobinemia in patients with loss of function CYP 2D6 polymorphisms  
320 (Figure 2C). Taken together this evidence strongly suggests that children have lower exposures  
321 to the biologically active metabolites of primaquine than do adults receiving the same weight  
322 adjusted doses.

323

324 This study was sufficiently large that the influence of other covariates affecting primaquine  
325 exposures could be examined. We have reported previously that co-administration of quinoline  
326 or structurally related antimalarials increases primaquine and carboxyprimaquine concentrations  
327 (15, 16). This was confirmed in this study. The effects on the parent compound of chloroquine



328 and piperazine were similar whereas piperazine had a larger effect on carboxypiperazine. In  
329 Asia loss of function *CYP 2D6* genetic polymorphisms (mainly the \*10 allele) are common. In this  
330 study low *CYP 2D6* activity scores (derived from the genotype) were associated with higher  
331 piperazine levels and lower levels of methemoglobinemia as would be expected from reduced  
332 metabolic conversion through this bioactivation pathway.

333 There are several limitations to this study. This analysis is limited to drug concentrations at one  
334 time point during radical cure treatment providing a limited description of drug exposures. Very  
335 young children are under-represented, and the analysis does not include any infants < 1 year old.  
336 In addition, *CYP 2D6* genotyping was not performed in all patients.

337 There is no evidence that the adverse effects of piperazine are significantly worse in children  
338 than adults. The main therapeutic implication of this large pharmacometric evaluation is that  
339 children may require larger weight adjusted doses of piperazine than adults.

#### 340 Funding

341 This work was supported by the Wellcome Trust [grant number 089179/Z/09/Z] to NJW. CSC, JW,  
342 APP, JT, CL, FHN and NJW are supported by the Wellcome Trust [Programme grant number  
343 089179]. CSC, JW, APP, JT, CL, FHN, and NJW are part of the Wellcome Trust Mahidol University  
344 Oxford Tropical Medicine Research Programme funded by the Wellcome Trust.

345

346 **Acknowledgements**

347 This research was funded by The Wellcome Trust. A CC BY or equivalent license is applied to the  
348 author accepted manuscript arising from this submission, in accordance with the grant's open  
349 access conditions.

350 The authors would like to thank the SMRU staff for their hard work in running this study and  
351 the MORU Clinical Pharmacology Laboratory in Bangkok for their diligent work in sample  
352 processing and drug measurements. Special appreciation goes to the contributions of Htet Htet  
353 Chaw, Nwe Wah Lin, Thida Zin, Say Paw, Naw Eh Shee, Thu Lay Paw, Rattanporn Raksapraidee,  
354 Moo Koo Paw, the data entry team headed by Jacher Wiladphaingern, the logistics team, and  
355 the laboratory teams headed by Kanlaya Sriprawat and Germana Bancone.

356 We would also like to thank the Data Safety and Monitoring Board: Bob Taylor, Charles Woodrow,  
357 and Professor Nicholas Day for their time and commitment.

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450 **Tables and Figures**

451 Table 1. Patient characteristics at enrolment by age group

	Age group			
	≤5 years	6-10 years	11-15 years	≥16 years
	n=34	n=71	n=118	n=418
Chloroquine+Primaquine-7	8 (5%)	22 (13%)	29 (18%)	104 (64%)
Chloroquine+Primaquine-14	10 (6%)	18 (11%)	29 (18%)	103 (65%)
DHAP+Primaquine-7	7 (4%)	15 (10%)	37 (24%)	98 (62%)
DHAP+Primaquine-14	9 (6%)	16 (10%)	23 (14%)	113 (70%)
Median age years (IQR, range)	4 (3.2-5, 1.5-5)	8 (6-10, 5.1-10)	13 (12-14, 11-15)	28 (20-39, 16-63)
Median weight, kg (IQR, range)	14 (12-16, 8-20)	20 (16-23, 13-30)	35 (29-43, 21-57)	52 (47-56, 31-88)
Median Body Mass Index, kg/m <sup>2</sup> (IQR, range)	15 (14-16, 13-18)	14 (14-15, 11-18)	17 (15-18, 12-23) <sup>1</sup>	20 (19-22, 13-36)
History of fever, n (%)	34 (100%)	71 (100%)	118 (100%)	411 (98%)
Median temperature >37.5°C, (IQR, range)	37.5 (36.7-38.2, 36-40)	37.4 (36.8-38.2, 36-40.1)	37.6 (37-38.5, 36-40.9) <sup>1</sup>	37.3 (36.8-38.1, 36-40.9)
Fever clearance; days (range)	0 (0-4)	0 (0-3)	1 (0-2)	0 (0-4)
Geometric mean parasitemia, parasites/μL (range)	3096 (16-40,192)	3633 (16-221,056)	4886 (16-75,109)	3804 (16-816,400)
Parasite clearance, days (range)	2 (1-4)	2 (1-3)	2 (1-4)	2 (1-5)
Gametocytemia, n (%)	23 (68%)	56 (79%)	104 (88%)	352 (84%)
Median chloroquine level <sup>a</sup> , ng/mL (range)	54.3 (6.4-115)	50.1 (27.3-138)	56.1 (27.6-161)	77.9 (26.9-248)
Median piperaquine level <sup>a</sup> , ng/mL (range)	37.5 (15.3-109)	29.4 (10-7-78.2)	35.3 (7.7-131)	37 (7.3-181)
Median carboxyprimaquine level <sup>a</sup> , ng/mL (range)	261 (29-1410)	406 (9-1590)	563 (40-2740)	793 (59-4710)
Median primaquine level <sup>a</sup> , ng/mL (range)	3.5 (1.1-43.8)	3.8 (0.9-349)	5.2 (0.9-106)	6.9 (1-293)
Median carboxyprimaquine: primaquine ratio <sup>a</sup> , (range)	86 (12-207)	97 (4-256)	98 (23-516)	106 (2-564)

452

453 Primaquine-7: primaquine dosing 1 mg/kg/day for 7 days

454 Primaquine-14: primaquine dosing 0.5 mg/kg/day for 14 days

455 Numeric superscript denotes the number of missing values

456 <sup>a</sup> Only data from the first episode are included



457 **Legends to Figures**

458 Figure 1: The effect of age on primaquine and carboxyprimaquine exposures in patients with acute vivax  
459 malaria.

460 Panel A: age versus mg/kg primaquine daily dose;

461 Panel B: age versus the primaquine concentration on day 7;

462 Panel C: age versus the carboxyprimaquine concentration on day 7;

463 Panel D: age versus the ratio of the day 7 carboxyprimaquine to primaquine concentration.

464 The black lines show model fits using a generalized additive model with a smooth spline term for age  
465 and adjusting for daily mg/kg dose; number of days of primaquine received, time since last dose, partner  
466 drug and fever clearance time (dashed: mean predicted concentration for 1mg/kg daily; solid: mean  
467 predicted concentration for 0.5 mg/kg daily).

468

469 Figure 2: The relationship between *CYP 2D6* genotypes and primaquine and carboxyprimaquine  
470 exposure, and day 7 methemoglobin levels. In panels A and B, the y-axis shows the model residuals  
471 (observed minus predicted) for the main model of exposure (adjusting for age, daily mg/kg primaquine  
472 dose, partner drug, and fever clearance time). In panel C, the y-axis shows the observed day 7  
473 methemoglobin %. In all three panels the x-axis is the activity score predicted from the *CYP 2D6*  
474 diplotype (20). Each box-and-whisker plot shows the median value, the interquartile range and the  
475 range. The box widths are proportional to the square-roots of the number of observations per activity  
476 score.

477

478 Figure 3: Age and drug exposure relationships with hemolysis and methemoglobinemia.

479 Panel A: Age and day 7 methemoglobin

480 Panel B: Age and the day 7 change in hematocrit from baseline.

481 Panel C: Day 7 methemoglobin and pre-dose primaquine concentrations.

482 Panel D: Day 7 methemoglobin and pre-dose carboxyprimaquine concentrations. In panels A&B the

483 black lines show model fits under a generalized additive model with a smooth spline term for age and

484 adjusting for daily mg/kg dose; number of days of primaquine received, the time since last dose, and

485 G6PD deficiency (dashed: mean predicted concentration for 1mg/kg daily; solid: mean predicted

486 concentration for 0.5 mg/kg daily).





