Population pharmacokinetics of artemether, lumefantrine and their respective metabolites in Papua New Guinean children with uncomplicated malaria

Sam Salman¹, Madhu Page-Sharp², Susan Griffin³, Kaye Kose³, Peter M. Siba³, Kenneth F. Ilett¹, Ivo Mueller³*, Timothy M. E. Davis¹

¹School of Medicine and Pharmacology, University of Western Australia, Fremantle Hospital, Fremantle, Western Australia, Australia; ²School of Pharmacy, Curtin University of Technology, Bentley, Australia; ³Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea

*Current addresses: Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Barcelona, Spain and Infection and Immunity Division, Walter and Eliza Hall Institute, Parkville, Victoria, Australia.

Correspondence and reprints: Professor T. M. E. Davis, University of Western Australia, School of Medicine and Pharmacology, Fremantle Hospital, PO Box 480, Fremantle, Western Australia 6959, Australia. Telephone: +618 9431 3229; Fax: +618 9431 2977; email: tdavis@cyllene.uwa.edu.au

Running head: Artemether-lumefantrine pharmacokinetics in children
Abstract

There are sparse published data relating to the pharmacokinetic properties of artemether, lumefantrine and their active metabolites in children, especially desbutyl-lumefantrine. We studied 13 Papua New Guinean children aged 5-10 years with uncomplicated malaria who received the six recommended doses of artemether (1.7 mg/kg) plus lumefantrine (10 mg/kg) given with fat over 3 days. Intensive blood sampling was carried out over 42 days. Plasma artemether, dihydroartemisinin, lumefantrine and desbutyl-lumefantrine were assayed using liquid chromatography-mass spectrometry. Multi-compartmental pharmacokinetic models for drug plus metabolite were developed using a population approach that included plasma artemether and dihydroartemisinin concentrations below the limit of quantitation. Although artemether bioavailability was variable and its clearance increased by 67.8% with each dose, the median areas under the plasma concentration-time curve (AUC$_{0-\infty}$) for artemether and dihydroartemisinin (3,063 and 2,839 µg.h/liter, respectively) were similar to those reported previously in adults with malaria. For lumefantrine, the median AUC$_{0-\infty}$ (459,980 µg.h/liter) was also similar to that in adults with malaria. These data support the 35% higher dose recommended for children 15-35 kg vs a 50 kg adult but question the recommendation for a lower dose in children weighing 12.5-15 kg. The median desbutyl-lumefantrine:lumefantrine ratio in our children was 1.13%, within the range reported for adults and higher at later time-points because of the longer desbutyl-lumefantrine terminal elimination half-life. A combined desbutyl-lumefantrine plus lumefantrine AUC$_{0-\infty}$ weighted on in vitro antimalarial activity was inversely associated with recurrent parasitemia, suggesting that both parent drug and metabolite contribute to artemether-lumefantrine treatment outcome.

Keywords: artemether, dihydroartemisinin, lumefantrine, desbutyl-lumefantrine, population pharmacokinetics, children, Papua New Guinea
Introduction

Artemether-lumefantrine (AL) is a fixed-dose combination therapy used widely for the treatment of malaria (35). Artemether (ARM) is a lipophilic artemisinin derivative that is converted in vivo to dihydroartemisinin (DHA), an active metabolite. Both ARM and DHA have short half-lives (14, 20-22, 25-26, 32) but a rapid effect on parasitemia. Lumefantrine (LUM) is a highly lipophilic drug with a longer half-life (11, 13-14, 20-21, 25, 31) which is combined with ARM primarily to prevent late recrudescence. Although the pharmacokinetic properties of ARM, DHA and LUM have been well documented in adults (4-5, 11, 13-15, 20-23, 25, 31), there are scant and inconsistent data relating to the disposition of desbutyl-lumefantrine (DBL), a potent LUM metabolite (27, 29-30, 33) that may influence AL treatment outcome (33). Reported plasma DBL:LUM concentration ratios after AL dosing in adults differ >10-fold (15, 25), while the pharmacokinetic properties of DBL in children are unknown. In addition, although several studies have attempted to characterize LUM disposition in children with malaria (1, 16, 26), methodologic issues complicate their comparison with adult data. One study involving a limited sampling schedule suggested that AL-treated children with malaria receive an inadequate dose of LUM relative to healthy adults (26), while the other studies either used pooled plasma concentrations (1) or used a truncated sampling schedule inadequate to characterize LUM pharmacokinetics (16).

In view of this situation, we have characterized the population pharmacokinetics of ARM, LUM and their metabolites in pediatric malaria using a rich sampling schedule to assess potential differences in disposition between children and adults, and to add to the limited data on DBL disposition and its role in AL treatment outcome.
Methods

Patients
We recruited children aged 5 to 10 years from Alexishafen Health Center, Madang Province on the north coast of Papua New Guinea (PNG). The clinic serves an area where \textit{P. falciparum} and \textit{P. vivax} are hyperendemic, and \textit{P. ovale} and \textit{P. malariae} are also transmitted.

Children with an axillary temperature $>$37.5°C or a history of fever in the previous 24 h were screened with a Giemsa-stained thick blood film read by an on-site trained microscopist.

Those with a mono-infection of \textit{P. falciparum} ($>$1,000 asexual parasites/microliter), or \textit{P. vivax}, \textit{ovale} or \textit{malariae} ($>$250/microliter) were eligible provided that the child’s parents gave informed consent, there were no features of severe malaria (34), they had not taken any antimalarial drug in the previous 14 days, there was no evidence of another cause of fever, and there were no features of malnutrition or other chronic co-morbidity. The study was approved by the Medical Research Advisory Committee of the Department of Health, PNG.

Clinical methods
After enrolment, a standardized history was taken and a clinical examination performed. A 3 milliliter blood sample was taken for blood film microscopy, a baseline haemoglobin and blood glucose, and subsequent drug assay of separated plasma. Urinalysis and audiometric assessment were performed. Each child was given artemether/lumefantrine (Coartem, Novartis Pharma Ltd, Switzerland) at a dose of 1.7 and 10 mg/kg, respectively to the nearest tablet. This dose was repeated at 8, 24, 36, 48 and 60 hours with the exact time of dosing recorded. All doses were given under direct observation with at least 50 milliliters of cow’s milk (equivalent to 2 g of fat). Further venous blood samples were taken from an indwelling intravenous catheter at 4, 8, 12, 24, 36, 40, 48, 60, 64, 68 and 72 h, and then by vesection.
on days 4, 5, 7, 14 and 28. All samples were centrifuged promptly and red cells and separated
plasma stored frozen at -80°C until assay. Detailed clinical assessment, including a symptom
questionnaire, blood film, hemoglobin and blood glucose, was repeated on days 1, 2, 3 and 7,
with additional clinical assessment and blood films on days 14, 28 and 42.

**Laboratory methods**

All blood smears taken at baseline and during follow-up were examined independently by
two skilled microscopists in a central laboratory. Each microscopist viewed 100 fields at
1,000x magnification before a slide was considered negative. Any slide discrepant for
positivity/negativity or speciation was referred to a third microscopist for adjudication.

For drug assays, HPLC-grade acetonitrile (Merck, Kilsyth, Australia), tert-butyl chloride,
ethyl acetate, glacial acetic acid and formic acid (Merck, Darmstadt, Germany), and
ammonium formate (Sigma-Aldrich, Gillingham, UK) were used. Other solvents and
chemicals were of analytical grade. Stock solutions (1 µg/liter in methanol) of ARM (AAPIN
Chemicals, Abingdon, UK), DHA (Sigma, St Louis, MO) and artemisinin (used as an internal
standard; Sigma) were stored protected from light at -80°C and used to prepare working
dilutions (0.1, 1, and 10 µg/milliliter). Calibration curves (2-200 µg/liter) were constructed
for DHA and ARM by spiking blank plasma. Quality control (QC) samples were prepared in
blank plasma at 10, 20, 50 and 200 µg/liter and also stored at -80 °C prior to use.

ARM and DHA were extracted as previously described (7) with the following modifications.
Briefly, solid phase extraction (SPE) Bond Elut® PH columns (Varian Inc, Palo Alto, CA)
were pre-conditioned with 1milliliter methanol followed by 1milliliter 1M acetic acid.
Plasma (0.5 milliliter) was spiked with internal standard (artemisinin 100 µg/liter) and loaded
onto the SPE column and drawn through with a medium vacuum. The column was then washed twice with 1M acetic acid (1 milliliter), followed by 20% v/v methanol in 1M acetic acid (1 milliliter). The column was dried under low vacuum for 30 min and the retained drugs eluted using 2 milliliter t-butyl chloride: ethyl acetate (80:20% v/v). The eluate was then evaporated under vacuum at 35°C and reconstituted in 50 microliter mobile phase and kept overnight to equilibrate the α and β anomers of DHA (7). Only the α-anomer was used for quantification. The injection volume was 10 microliter.

The liquid chromatography-mass spectrometry (LC-MS) system used was a single quad mass spectrometer (Shimadzu, Kyoto, Japan) with electrospray ionization (ESI) and atmospheric pressure ionization (APCI) systems. Assays were performed with 20 mM ammonium formate (pH 5):acetonitrile in 0.1% formic acid (40:60) at a flow rate of 0.2 milliliter/min, and chromatographic separation undertaken at ambient temperature on a Synergy fusion-RP C18 (150 mm x 2.0 mm i.d.) column coupled with a 4 mm x 3 mm i.d., 5 μm particle C18 guard column (Phenomenex, Lane Cove, Australia). Retention times were 4.5, 7.5 and 12.7 min for DHA, artemisinin and ARM, respectively. Optimized mass spectra were acquired with an interface voltage of 4.5 kV, a detector voltage of 1 kV, a heat block temperature of 400°C and a desolvation gas temperature of 250°C. Nitrogen was used as a nebulizer gas at a flow rate of 1.5 liter/min and dry gas flow of 10 liter/min. Quantitation was performed by selected ion monitoring using the dual ionization source mode. The predominant fragmented ions \(m/z\) 221 for ARM and \(m/z\) 221 for DHA were used. For artemisinin, \(m/z\) 283 was monitored.

Standard curves were linear \((r^2 \geq 0.999)\). Chromatographic data (peak area ratio of DHA:artemisinin and ARM:artemisinin) were processed using LAB Solution software (Version 5, Shimadzu, Japan). No matrix effect (ion suppression/enhancement) was observed.
under methodologies described elsewhere (24), and performance of both assays, assessed as
intra- and inter-day relative standard deviations across relevant concentration ranges, was
similar to that published previously (7, 18). Inter-day accuracies of QC assays were <15% of
nominal values on all occasions. The limits of quantification and detection for DHA and
ARM were 2 and 1 μg/liter, and 5 and 2 μg/liter, respectively.

LUM and DBL were quantified in plasma using a validated ultra-high-performance liquid
chromatography-tandem mass spectrometry assay as previously described (33). The linear
range for LUM was 20-20,000 ng/milliliter, and inter-day variability was 4.94%, 4.93%,
7.16% and 11.23% and intra-day variability 2.83%, 4.41%, 4.11%, 9.55% at 20,000, 2,000,
200 and 20 ng/milliliter, respectively. For DBL, the linear range was 0.5-100 ng/milliliter,
and inter-day variability was 3.36%, 3.47%, 9.98% and 6.74% and intra-day variability
2.47%, 3.46%, 8.16% and 3.48% at 50, 10, 1 and 0.5 ng/milliliter, respectively. As a LC-
MS/MS method was used for DBL, matrix effects were assessed where between subject
variability was 3.37%, 4.47% and 9.43% at 50, 10 and 1 ng/milliliter, respectively.

Pharmacokinetic modeling
Loge (natural log) plasma concentration-time datasets for LUM with DBL and ARM with
DHA were analyzed by nonlinear mixed effect modeling using NONMEM (v 6.2.0, ICON
Development Solutions, Ellicott City, MD) with an Intel Visual FORTRAN 10.0 compiler.
The first order conditional estimation with interaction (FOCE-I) estimation method was used
for the LUM/DBL model and the Laplacian with interaction method for ARM/DHA. The
minimum value of the objective function (OFV) and weighted residuals (WRES) plots were
used to choose suitable models during model-building. As FOCE-I estimation was used,
conditional weighted residuals were also considered in the initial stages of model building
(17). However, as they were similar to WRES, the latter was considered suitable for further
model-building. Concentrations were modeled as µg/milliliter with a conversion factor for all
metabolite parameters included into the model to account for the difference in molecular
weight between parent drug and metabolite. Allometric scaling was used a priori, with
volume terms multiplied by \( (WT/70)^{1.0} \) and clearance terms by \( (WT/70)^{0.75} \) (3). Residual
variability (RV) was estimated as additive error for the loge-transformed data. Models were
parameterized using \( k_a \) (absorption rate constant), \( V_C/F \) (central volume of distribution), \( CL/F \)
(clearance), \( V_P/F \) and \( Q/F \) (peripheral volumes of distribution(s) and their respective inter-
compartmental clearance(s)).

For the LUM/DBL model, plasma LUM concentrations were initially modeled using inbuilt
2- and 3- compartment model structures with first-order absorption and a fixed lag time of 2 h
(23) (ADVAN 4 and 12). Once a suitable (3-compartment) LUM model had been
determined, the DBL dataset was added and modeled simultaneously. User-defined linear
mammillary models (ADVAN 5) were constructed testing 1-, 2- and 3- compartments with
and without first-pass LUM metabolism. As no data exist regarding the degree of \textit{in vivo}
DBL conversion from LUM this was set to 100% to allow identifiability. Therefore, all
clearance and volume terms for DBL are relative to LUM bioavailability (\( F_{LUM} \)) as well as
the degree of metabolic conversion from LUM (\( F_{met-DBL} \)). The term \( F^*_{DBL} \) (representing \( F_{LUM} \)
\( x \) \( F_{met-DBL} \)) will be used for simplicity.

As 45% and 12% of plasma ARM and DHA concentrations, respectively, were below the
limit of quantification (BLQ), we used a published method known to produce reliable
pharmacokinetic parameters in this situation (9-10). The method (known as M3 (2)) models
continuous and categorical data simultaneously. Concentrations above the LOQ are included
as conventional continuous data while those BLQ are treated as categorical and the likelihood (probability) that they are BLQ maximized with respect to model parameters. This allows BLQ observations to contribute to the determination of the OFV and in finalizing model structure.

Initially plasma ARM concentrations were assessed using 1- and 2-compartment models with first order absorption (ADVAN2 and 4) to obtain a suitable structure. The $k_a$ for ARM was fixed to 1 h$^{-1}$ (32) as the data did not support its estimation. Once a suitable (2-compartment) ARM model had been determined, the DHA dataset was added and modeled simultaneously using a user-defined linear mammillary model (ADVAN 5). For DHA, 1- and 2-compartments were assessed and the conversion of ARM to DHA was considered complete for identifiability purposes. Therefore, all clearance and volume terms for DHA are relative to ART bioavailability ($F_{\text{ART}}$) as well as the degree of metabolic conversion from ART ($F_{\text{met-DHA}}$). The term $F^*_{\text{DHA}}$ (representing $F_{\text{ART}} \times F_{\text{met-DHA}}$) will be used for simplicity.

Once model structure was established, inter-individual variability (IIV), inter-occasion variability (IOV) and their correlations were estimated. Relationships between model parameters and the covariates age, sex, baseline parasitemia and baseline hemoglobin were identified using correlation plots and subsequently evaluated within NONMEM. Inclusion of the covariate relationship required a decrease in OFV $\geq 6.63$ ($\chi^2$ distribution with 1 d.f., $P<0.01$) accompanied by a decrease in the IIV of that parameter.

**Model evaluation**

A bootstrap using Perl speaks NONMEM (PSN) with 1,000 samples was performed and the parameters derived from this analysis summarized as median and 2.5th and 97.5th centiles.
(95% empirical CI) to facilitate evaluation of final model parameter estimates. Runs were included in the bootstrap analysis regardless of their minimization status. In addition, visual predictive checks (VPCs) were performed with 1,000 datasets simulated from the final models. The observed 10th, 50th and 90th percentiles were plotted with their respective simulated 95% CI to assess the predictive performance of the model. For the ARM/DHA model, the observed fraction of BLQ observations was compared with the median and 95% prediction intervals (PI) of BLQ observations from these simulated datasets (9).

The applicability of the final population models to younger patients from the present sample was assessed using a numerical predictive check. Day 7 plasma LUM concentrations from a previous study (18) from children aged 0.5 to 5 years were compared with simulated data from the final models. The actual and simulated number of data points above and below the 20%, 40%, 60%, 80%, 90% and 95% simulated prediction intervals (PI) were compared.

**Statistical analysis**

Changes in hemoglobin, glucose and audiometric data over time were assessed using the Wilcoxon signed-rank test. The AUC0–∞ of DBL and LUM were compared between subjects with or without recurrent parasitemia using the Mann–Whitney U test. A two-tailed level of significance of 0.05 was considered significant for all comparisons.

**Results**

**Clinical characteristics and course**

The baseline characteristics of the 13 recruited children are summarized in Table 1. Eleven
had a mono-infection (9 *P. falciparium*, 2 *P. malariae*) on confirmatory expert microscopy, while 2 had a mixed *P. falciparum/vivax* infection. AL treatment was well tolerated and reported symptoms were mild/moderate, short-lived (<3 days) and consistent with clinical features of uncomplicated malaria. Initial fever and parasite clearance were <48 h in all cases.

By 28 days of follow-up, three children had developed slide-positive *P. vivax* (two had *P. vivax* at enrolment) and two children had developed *P. falciparum* (one had *P. falciparum* at enrolment). By 42 days of follow-up, five children had been diagnosed with *P. vivax* (two had *P. vivax* at enrolment) and three with *P. falciparum* (two had *P. falciparum* at enrolment). These data are consistent with the PCR uncorrected results of a previous larger comparative treatment trial in younger children performed at the same location (18). The recurrent *P. vivax* parasitaemia could have resulted from i) recrudescent infection in those infected with this parasite before treatment, ii) acquisition of a new *P. vivax* infection after treatment or, since no primaquine therapy was administered, iii) appearance of *P. vivax* from hypnozoites present in the liver at study entry. For *P. falciparum* parasitemia detected during follow-up, this could have represented recrudescence or re-infection.

The mean hemoglobin concentration was significantly higher on day 28 compared to enrolment (10.7 vs 8.9 g/liter, *P*<0.01). There was no significant change in blood glucose over the first three days of enrolment or audiometric findings over 28 days (data not shown).

**Pharmacokinetic modeling**

LUM and DBL plasma concentration-time curves are shown in Figure 1. A 3-compartment model proved superior to a 2-compartment model for LUM with a lower OFV and reduced bias in the WRES plot. The addition of two compartments and the inclusion of first-pass
metabolism provided the best model once the DBL dataset had been added. Therefore, the final model comprised 3 compartments for LUM and 2 compartments for DBL. The structural model parameters were $k_a$, $V_C/F_{LUM}$, $V_{P1}/F_{LUM}$, $V_{P2}/F_{LUM}$, $CL/F_{LUM}$, $Q_1/F_{LUM}$, $Q_2/F_{LUM}$, $FP$ (percentage contribution of first-pass metabolism to DBL metabolic conversion), $V_C/F^*_{DBL}$, $V_P/F^*_{DBL}$, $CL/F^*_{DBL}$, $Q/F^*_{DBL}$. Inter-individual variability was able to be estimated for $k_a$, $CL/F_{LUM}$, $CL/F^*_{DBL}$, $VC/F^*_{DBL}$ and $F_{LUM}$ as well as inter-occasion variability for $F_{LUM}$ (the population value of $F_{LUM}$ remained fixed to 1). Variability in $F_{LUM}$ was smaller between individuals than it was between doses in the same individual (20 vs 67%). Once IIV and IOV terms were added, inspection of the WRES plot revealed a bias due to the absorption profile of the final dose. Estimation of a separate $k_a$ for the 6th (final dose) ($k_a_{D6}$) improved the bias and reduced the OFV (-7.519 p<0.01). None of the covariates tested improved the model. Residual variability (20.8% and 20.9% for LUM and DBL, respectively) was low.

The final model parameter estimates and the bootstrap results are summarized in Table 2. Bias was <10% for structural and random model parameters. Figures 2 and 3 show goodness-of-fit plots and VPCs, respectively. The half-lives and AUC of LUM and DBL are shown in Table 4. The first distribution, second distribution and terminal elimination half-lives for LUM had median values of 10.4, 46.6 and 126 h, while DBL had a median distribution half-life of 19.7 h and a median terminal elimination half-life of 141 h. Overall the metabolite to parent drug ratio was 1.13 % (obtained from AUC$_{0-\infty}$) but there was a higher ratio at later time-points. Day 7 LUM concentrations obtained from younger children were consistent with prediction based on the final model with an expected number of observations above and below the 20, 40, 60, 80, 90 and 95% simulated PIs. When the same data for DBL were compared, there was an excess of points above the 20, 40, 60 80 and 90% PIs and a lack of...
points below the 20 and 40% PI, especially at a younger age, demonstrating that the day 7 DBL levels in the younger children were higher than expected from the model.

Initial modeling of ARM/DHA datasets proved difficult given the large proportion of BLQ data (45% and 12% for ARM and DHA respectively). Once these data were incorporated into the model using the method ‘M3’ in Ahn et al. (2), more acceptable models were obtained. The dispositions of ARM and DHA were best described by a 2-compartment model for ARM and a 1-compartment model for DHA. The structural model parameters were $k_a$, $V_c/F_{ARM}$, $V_p/F_{ARM}$, $Q/F_{ARM}$, $V_c/F_{DHA}$ and $CL/F_{DHA}$. As with LUM the IIV and IOV of $F_{ARM}$ was estimated and variability between doses was larger than between individuals (84.1 vs 38.1 %). The IIV of $CL_{ARM}$ was also estimated. A relationship between $CL_{ARM}$ and dose number was included and demonstrated that for each subsequent dose of ARM $CL_{ARM}$ increased by 67.8% relative to its value after the first dose. This relationship was accompanied by a decrease in the OFV (-82.774, $P<0.001$) and a reduction in the RV of both ARM and DHA. No other covariate relationship improved the model. After the inclusion of IIV/IOV terms and the covariate relationship, RV was still high at 51.6% and 53.3% for ARM and DHA, respectively.

The final model parameter estimates and the bootstrap results are summarized in Table 3. As the covariance step was not successful, NONMEM-derived relative standard errors could not be obtained. Bias was <11% for structural and random parameters except IIV for $F_{ARM}$ which had a negative 48% bias. Figures 4 and 5 show goodness-of-fit plots and VPCs, respectively. The VPCs show all observed 10th, 50th and 90th percentiles within their simulated 95% CIs and the fraction of BLQ data at each time point within its 95% CI for both ARM and DHA.
Secondary parameters for study participants are shown in Table 4. The AUC$_{0-\infty}$ and half-lives of ARM decreased with each dose while the median DHA to ARM ratio increased.

**Relationship between drug exposure and treatment outcome**

The LUM AUC$_{0-\infty}$ tended to be lower in children with recurrent parasitemia on days 28 (n=5, $P=0.057$) and 42 (n=8, $P=0.086$), but this was not the case for DBL ($P=0.46$ and 0.89, respectively). However, a combined AUC$_{0-\infty}$ with DBL weighted four times more than LUM, consistent with its greater antimalarial potency *in vitro* (27, 29-30, 33), was significantly lower in children with recurrent parasitemia on day 28 (n=5, $P=0.028$) and of borderline significance on day 42 (n=8, $P=0.063$).

**Discussion**

In the present study of PNG children with uncomplicated malaria treated with a conventional AL regimen, rich datasets of plasma concentrations of LUM, ARM and their active metabolites measured during an extended follow-up period were successfully analyzed using population pharmacokinetic modeling that allowed for a high proportion of BLQ plasma ARM and DHA concentrations. Our analyses included the first compartmental PK analysis of plasma DBL levels. We found that current dose recommendations for AL in children result in a LUM AUC similar to that achieved in adults, despite children receiving a higher average mg/kg dose relative to a 50 kg adult. However, the subgroup of children weighing 12.5-15 kg receive the lowest mg/kg dose and may be at risk of under-dosing.
Three studies, all from Africa, have examined LUM pharmacokinetics after AL treatment in children. The first and simplest compared crushed tablets and a dispersible formulation using a pooled analysis of single blood sample taken at one of six time-points during a 14-day period from 726 children aged <12 years (1). The LUM AUC for both formulations was higher than in the present study (574,000 and 636,000 vs 459,980 µg h liter⁻¹). In the second study (26), six blood samples were taken from children aged 5-13 years starting when the last AL dose was given and the LUM AUC₆₀₋ₐ was calculated using non-compartmental analysis. When we used our final models to generate an AUC₆₀₋ₐ, this was higher (257,010 vs 210,000 µg h liter⁻¹). Based on their data, the authors reported that children have lower levels of exposure to LUM than adults using recommended AL dose schedules (26). A third study of children aged 1-10 years utilized a population approach (16) but there was no sampling beyond 72 h and no secondary pharmacokinetic parameters were provided. A comparison with LUM disposition in the present study was, therefore, not possible.

Comparisons of LUM AUC between studies in adults is also difficult as some report AUC from the first dose while others use AUC₆₀₋ₐ. Table 5 summarizes the available data for both measures of drug exposure. There is a difference between LUM exposure in healthy adults and subjects with malaria, but the AUCs for non-pregnant adults, pregnant adults and children with malaria are similar. Current AL dose recommendations for children ensure that those weighing 15-35 kg receive a 35% higher average mg/kg dose than a 50 kg adult, but those weighing 12.5-15 kg receive a lower mg/kg dose. The AUC data support the higher average mg/kg dose in children and suggest that those weighing 12.5 - 15 kg should receive 2 tablets rather than 1 to avoid under-dosing while not exceeding the highest recommended mg/kg dose (Figure 6). As LUM exposure, measured either as AUC or day 7 levels, has
previously been shown to be a prime determinate of efficacy (12, 28), it is important that under-dosing is avoided.

The three studies of AL in children also measured plasma ARM/DHA concentrations (1, 16, 26). The first was not able to calculate AUCs from pooled concentration data due to a sparse sampling schedule (1). The second employed a limited sampling schedule starting from the last AL dose (26), and the AUCs were therefore lower than those of the present study (168 vs 217 µg h liter\(^{-1}\) for ARM and 382 vs 402 µg h liter\(^{-1}\) for DHA). The population approach used in the third study (16) produced a similar model of the disposition of ARM (two compartments) and DHA (one compartment) to that of the present study. The authors reported a similar increase in CL/F\(_{ARM}\) with each dose (57% vs 67.8 % in our children) and a higher RV (61% vs 51.6% and 82 % vs 53.3% for ARM and DHA respectively), the latter observation likely a reflection of the fact that many plasma concentrations were close to or below the LOQ. As no secondary PK parameters were provided, a comparison of AUCs could not be performed. However the half-lives of ARM, estimated from the pharmacokinetic parameters provided, were longer than those in our children (0.89 vs 0.62 h and 32.0 vs 16.4 h for distribution and elimination, respectively, of the first dose), while the elimination half-life of DHA was shorter (0.38 vs 0.80 h).

The AUCs for ARM and DHA in the present study were similar to those reported previously in adults with malaria (22, 25) but higher than those in healthy adults (14, 20-21). Our terminal elimination half-life for ARM was longer than those reported in these studies (16.4 vs 1.5-3.9 h) while for DHA it was shorter (0.80 vs 1.2-2.1 h). The adult studies used non-compartmental methods to determine these half-lives and this may account for the
differences. Nevertheless, based on these comparisons, exposure to ARM and DHA in children is adequate with current AL dose recommendations.

Few studies have evaluated the disposition of DBL, an active metabolite of LUM. Our DBL:LUM ratio (1.13%) falls between values reported in previous treatment studies (0.33% and 5.2%) (15, 25). The lower value (0.33%) was from a study of non-immune Columbian adults with malaria that sampled to 168 h and reported AUC\(_{0-168}\). The higher value (5.2%) was from a study of pregnant Thai women with malaria in which sampling started after the last dose and AUC\(_{60-\infty}\) was reported. The difference between these values can, at least in part, be explained by the study designs as the metabolite-to-parent percentage calculated from AUC\(_{60-\infty}\) in the present study is more than double for AUC\(_{0-168}\) (1.96 vs 0.76 %). However it is likely that ethnicity and pregnancy contribute to the difference. Age may also influence metabolic conversion of LUM to DBL as our PK model was able to predict concentrations of LUM but not DBL in younger children effectively. It is uncertain as to whether malaria itself also influences the ratio since it was 0.45%, within the range of studies of malaria, after a single dose of AL in 22 healthy adults (19).

As reported previously (25) DBL had a longer terminal elimination half-life than LUM in the present study (141 vs 123 h) and therefore the DBL:LUM ratio will increase with time. Although the ratios found in available studies are low, the \textit{in vitro} potency of DBL is between 2.2 and 7.2 times that of LUM (27, 29-30, 33) and it may therefore contribute to therapeutic outcome. We found a combined weighted LUM/DBL AUC was more likely to be lower than the AUC of either LUM or DBL alone in subjects with recurrent parasitemia at days 28 and 42. This supports the suggestion that DBL may influence AL treatment outcome (33).
Although the variable bioavailability of ARM and LUM has been previously reported (13), it has not previously quantified in children. Given the significant increase in fed vs fasted healthy volunteers (23) it is recommended that AL is administered with fat in order to improve absorption. Based on a study in healthy adults who received a single dose of AL, 1.2 g of fat (equivalent to 35 mL of full cream milk) is required to achieve 90% of maximal LUM bioavailability (4). Although these results may not be directly applicable to the children with malaria in our study, they ingested 2 g of fat with each dose and there was still significant between-dose variability in the bioavailability of both LUM (67.0%) and ARM (84.1%). We were unable to identify factors that may be responsible for these observations.

In the analysis of the ARM/DHA dataset, there was a significant number of BLQ plasma concentrations. This is an issue encountered in pharmacokinetic analyses of a variety of other antimalarial drugs (6, 8, 16). Traditional approaches to this problem such as excluding BLQ data from the analysis or setting them to a specific value (such as zero or 50% of the LOQ) have been shown to bias the pharmacokinetic parameters even when only 10% of the data are BLQ (2, 9-10, 36). Our approach was to use a method within NONMEM shown to have little bias in situations with up to 40% BLQ data in population analysis (10). This method treats BLQ data points as categorical data and maximizes the likelihood that its value is truly below the LOQ (2). Although the implementation of this method has previously been difficult and time consuming, changes to NONMEM and more efficient data processing have increased its accessibility. The benefits of this method demonstrated in relatively simple models are likely to apply to more complex models with parent drug and metabolite. We were unable to obtain relative standard error (RSE) for our parameters in this model as the covariance step was unsuccessful, a common problem when this method is used (9-10). However this does not
Our novel data relating to DBL pharmacokinetics and its favorable pharmacodynamic effects suggest that future efficacy and pharmacokinetic studies of LUM should include DBL assay to further elucidate its role. We have also shown that analytical techniques that utilize BLQ data to refine pharmacokinetic parameter estimates can be applied in this situation. Extended sampling and a population pharmacokinetic approach allow flexibility in deriving secondary parameters, an important consideration when comparisons with published non-standard measures such as time-limited AUC are of interest. Our data confirm that current AL dose recommendations produce similar ARM, DHA and LUM exposure in children to that in adults with malaria. However, smaller children weighting 12.5-15 kg are at risk of under-dosing and AL doses could be doubled without exceeding the current weight-based maximum mg/kg dose in this patient group.

Acknowledgements
We thank the children and their parents/guardians for their participation. We are most grateful to Sr Valsi Kurian and the staff of Alexishafen Health Centre for their kind co-operation during the study. We also thank Jovitha Lammey, Christine Kalopo and Bernard (“Ben”) Maamu for clinical and/or logistic assistance, and Harin Karunajeewa for assistance with protocol design. The National Health and Medical Research Council (NHMRC) of Australia funded the study (grant #634343). TMED is supported by an NHMRC Practitioner Fellowship.
References:


Table 1. Baseline characteristics of study participants. Data are number (%), mean ± SD or median and [inter-quartile range].

<table>
<thead>
<tr>
<th></th>
<th>n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>8 (62%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>19.0 ± 3.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td>36.8 ± 1.0</td>
</tr>
<tr>
<td><em>P. falciparum</em> parasitemia</td>
<td>9 (69%)</td>
</tr>
<tr>
<td><em>P. falciparum/vivax</em> parasitemia</td>
<td>2 (15%)</td>
</tr>
<tr>
<td><em>P. malariae</em> parasitemia</td>
<td>2 (15%)</td>
</tr>
<tr>
<td>Respiratory rate (/min)</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Supine pulse rate (/min)</td>
<td>102 ± 16</td>
</tr>
<tr>
<td>Mean upper arm circumference (cm)</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>Hemoglobin (g/liter)</td>
<td>8.9 ± 1.6</td>
</tr>
</tbody>
</table>
Table 2 Final population pharmacokinetic estimates and bootstrap results for lumefantrine and desbutyl-lumefantrine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (relative standard error %)</th>
<th>Bootstrap median [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Function Value</td>
<td>-586.510</td>
<td>-601.901 [-668.687 - 559.564]</td>
</tr>
</tbody>
</table>

**Structural model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (relative standard error %)</th>
<th>Bootstrap median [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (/h)</td>
<td>0.461 (20)</td>
<td>0.442 [0.285-0.644]</td>
</tr>
<tr>
<td>$CL/F_{LUM}$ (liters/h/70kg)</td>
<td>7.29 (9)</td>
<td>7.21 [5.55-9.04]</td>
</tr>
<tr>
<td>$V_C/F_{LUM}$ (liters/70kg)</td>
<td>227 (12)</td>
<td>225 [147-284]</td>
</tr>
<tr>
<td>$Q_1/F_{LUM}$ (liters/h/70kg)</td>
<td>1.52 (16)</td>
<td>1.57 [0.96-2.32]</td>
</tr>
<tr>
<td>$V_{P1}/F_{LUM}$ (liters/70kg)</td>
<td>115 (19)</td>
<td>109 [57-214]</td>
</tr>
<tr>
<td>$Q_2/F_{LUM}$ (liters/h/70kg)</td>
<td>0.743 (13)</td>
<td>0.805 [0.208-1.27]</td>
</tr>
<tr>
<td>$V_{P2}/F_{LUM}$ (liters/70kg)</td>
<td>164 (8)</td>
<td>168 [97-240]</td>
</tr>
<tr>
<td>$ka_D6$ (/h)</td>
<td>1.20 (52)</td>
<td>1.14 [0.50-3.68]</td>
</tr>
<tr>
<td>FP (%)</td>
<td>6.29 (15)</td>
<td>6.45 [4.36-9.84]</td>
</tr>
<tr>
<td>$CL/F^*_{DBL}$ (liters/h/70kg)</td>
<td>701 (10)</td>
<td>694 [561-851]</td>
</tr>
<tr>
<td>$Q/F^*_{DBL}$ (liters/h/70kg)</td>
<td>51,100 (10)</td>
<td>51,200 [42,200-61,430]</td>
</tr>
<tr>
<td>$V_C/F^*_{DBL}$ (liters/70kg)</td>
<td>439 (19)</td>
<td>424 [305-632]</td>
</tr>
<tr>
<td>$V_{P1}/F^*_{DBL}$ (liters/70kg)</td>
<td>68,400 (14)</td>
<td>68,000 [51,800-88,600]</td>
</tr>
</tbody>
</table>

**Random model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (relative standard error %)</th>
<th>Bootstrap median [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIV in $F_{LUM}$ (%)</td>
<td>19.8 (42)</td>
<td>18.9 [2.5-29.3]</td>
</tr>
<tr>
<td>IIV in $ka$ (%)</td>
<td>55.4 (44)</td>
<td>55.8 [17.1-92.2]</td>
</tr>
<tr>
<td>IIV in $CL/F_{LUM}$ (%)</td>
<td>17.7 (20)</td>
<td>16.9 [6.8-23.7]</td>
</tr>
<tr>
<td>IIV in $CL/F^*_{DBL}$ (%)</td>
<td>26.2 (26)</td>
<td>26.0 [10.4-37.6]</td>
</tr>
<tr>
<td>IIV in $V_C/F^*_{DBL}$ (%)</td>
<td>34.1 (22)</td>
<td>33.3 [17.4-47.8]</td>
</tr>
<tr>
<td>IOV in $F_{LUM}$ (%)</td>
<td>67.0 (9)</td>
<td>66.4 [53.4-77.7]</td>
</tr>
<tr>
<td>RV for $LUM$ (%)</td>
<td>20.8 (7)</td>
<td>20.3 [17.4-22.5]</td>
</tr>
<tr>
<td>RV for $DBL$ (%)</td>
<td>20.9 (7)</td>
<td>20.6 [17.5-23.0]</td>
</tr>
</tbody>
</table>

a RSE% are the NONMEM produced values from the covariance step
Table 3 Final population pharmacokinetic estimates and bootstrap results for ARM and DHA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (relative standard error %)</th>
<th>Bootstrap Median [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Function Value</td>
<td>159.853</td>
<td>177.255</td>
</tr>
<tr>
<td></td>
<td>[77.606-249.014]</td>
<td></td>
</tr>
<tr>
<td>Structural model parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F&lt;sub&gt;ARM&lt;/sub&gt; (liters/h/70kg)</td>
<td>102 (27)</td>
<td>96.3 [57.0-167.0]</td>
</tr>
<tr>
<td>V&lt;sub&gt;C&lt;/sub&gt;/F&lt;sub&gt;ARM&lt;/sub&gt; (liters/70kg)</td>
<td>193 (62)</td>
<td>172 [40-506]</td>
</tr>
<tr>
<td>Q/F&lt;sub&gt;ARM&lt;/sub&gt; (liters/h/70kg)</td>
<td>49.6 (47)</td>
<td>45.8 [19.7-111.1]</td>
</tr>
<tr>
<td>V&lt;sub&gt;P&lt;/sub&gt;/F&lt;sub&gt;ARM&lt;/sub&gt; (liters/70kg)</td>
<td>1070 (59)</td>
<td>1220 [593-3011]</td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt; (/h)</td>
<td>1 [FIXED]</td>
<td>1 [1-1]</td>
</tr>
<tr>
<td>V&lt;sub&gt;C&lt;/sub&gt;/F*DHA (liters/70kg)</td>
<td>440 (40)</td>
<td>417 [69-826]</td>
</tr>
<tr>
<td>CL/F*DHA (liters/h/70kg)</td>
<td>277 (26)</td>
<td>275 [140-443]</td>
</tr>
<tr>
<td>% increase in CL/F&lt;sub&gt;ARM&lt;/sub&gt; for each subsequent dose (%)</td>
<td>67.8 (31)</td>
<td>73.3 [40.5-125]</td>
</tr>
<tr>
<td>Random model parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV in F&lt;sub&gt;ARM&lt;/sub&gt; (%)</td>
<td>38.1 (72)</td>
<td>19.7 [0.3-58.6]</td>
</tr>
<tr>
<td>IOV in F&lt;sub&gt;ARM&lt;/sub&gt; (%)</td>
<td>84.1 (38)</td>
<td>84.2 [52.2-113.6]</td>
</tr>
<tr>
<td>IIV in CL/F&lt;sub&gt;ARM&lt;/sub&gt; (%)</td>
<td>84.0 (33)</td>
<td>75.8 [49.1-108.2]</td>
</tr>
<tr>
<td>RV for ARM (%)</td>
<td>51.6 (12)</td>
<td>50 [37-61]</td>
</tr>
<tr>
<td>RV for DHA (%)</td>
<td>53.3 (20)</td>
<td>61 [42-83]</td>
</tr>
</tbody>
</table>

<sup>a</sup> RSE% are derived from the bootstrap.
Table 4: Secondary pharmacokinetic parameters derived from post hoc Bayesian estimates for study participants. Data are median [inter-quartile range].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LUM</th>
<th>DBL</th>
<th>ARM – Dose 1</th>
<th>ARM – Dose 6</th>
<th>ARM – All doses</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{\alpha}$ (h)</td>
<td>10.4 [10.3 – 11.8]</td>
<td>19.7 [18.4 – 22.5]</td>
<td>0.62 [0.60 – 0.64]</td>
<td>0.16 [0.12 – 0.33]</td>
<td>–</td>
<td>0.80 [0.76 – 0.82]</td>
</tr>
<tr>
<td>$t_{\gamma}$ (h)</td>
<td>123 [120 – 127]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AUC$<em>{METABOLITE}$/AUC$</em>{PARENT}$ (%)</td>
<td>1.13 [0.93 – 1.55]</td>
<td>–</td>
<td>36.8 [36.8 – 36.8]</td>
<td>186 [91.8 – 268]</td>
<td>92.7 [59.2 – 94.3]</td>
<td>–</td>
</tr>
</tbody>
</table>

$t_{\alpha}$, $t_{\beta}$ and $t_{\gamma}$ are the first distribution, second distribution and terminal elimination half-lives respectively for LUM, while for DBL and ARM $t_{\alpha}$ and $t_{\beta}$ represent the distribution and terminal elimination half-life respectively and for DHA $t_{\alpha}$ represents the terminal elimination half-life.

$^b$AUC$_{0-\infty}$ represents either the AUC$_{0-\infty}$ for all six doses together or the AUC$_{0-\infty}$ for individual doses as if they were given alone.
Table 5: Summary of studies reporting area under the plasma concentration-time curve (AUC) for lumefantrine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AUC_{60-∞} (µg.h/liter)</th>
<th>AUC_{0-∞} (µg.h/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults</td>
<td>383,000-456,000 (14, 20)</td>
<td>1,242,000-2,730,000 (11, 14)</td>
</tr>
<tr>
<td>Non-pregnant adults with malaria</td>
<td>-</td>
<td>335,000-758,000 (5, 13, 15, 23)</td>
</tr>
<tr>
<td>Pregnant women with malaria</td>
<td>252,000 (25)</td>
<td>472,000 (31)</td>
</tr>
<tr>
<td>Children with malaria</td>
<td>210,000 (16)</td>
<td>572,000-636,000 (1)</td>
</tr>
<tr>
<td>Present study</td>
<td>257,000</td>
<td>459,980</td>
</tr>
</tbody>
</table>

*a AUC was either a median or mean and was reported either to the last data point or to ∞

*b as subjects in Bindschedler et al (10) only received a single dose, the reported AUC has been multiplied by six

*c this study used a pooled approach from single observations in each subject to calculate AUC
**Figure 1.** Time-concentration plots showing LUM (○) and DBL (∇) in μg/liter on log\(_{10}\) scale. Curves of the median concentration for LUM (solid black line) and DBL (dashed black line) are also shown.

**Figure 2.** Population (○) and individual predicted (●) versus observed data for LUM (A) and DBL (B) concentrations (μg/liter) for the final model. The lines of identity are also shown.

**Figure 3.** Visual predictive check showing observed 50\(^{th}\) (●), 10\(^{th}\) (⊕) and 90\(^{th}\) (○) percentiles with the simulated 95\% CI for the 50\(^{th}\) (solid black line), 10\(^{th}\) (grey dotted lines) and 90\(^{th}\) (dashed grey lines) percentiles for LUM (A) and DBL (B) concentrations (μg/liter on log\(_{10}\) scale) from the final model.

**Figure 4.** Population (○) and individual predicted (●) versus observed data for ARM (A) and DHA (B) concentrations (μg/liter) for the final model. The lines of identity are also shown. The grey dashed line represents the LOQ of ARM in (A) and DHA in (B).

**Figure 5.** Visual predictive check showing observed 50\(^{th}\) (●), 10\(^{th}\) (⊕) and 90\(^{th}\) (○) percentiles with the simulated 95\% CI for the 50\(^{th}\) (solid black line), 10\(^{th}\) (grey dotted lines) and 90\(^{th}\) (dashed grey lines) percentiles for ARM (A) and DHA (B) concentrations (μg/liter on log\(_{10}\) scale) from the final model. The fraction of BLQ observations from the data (○ connected with a dotted black line) with the simulated 95\% prediction interval are also shown for both ARM and DHA.
Figure 6. The doses of lumefantrine and artemether in mg/kg given to children 5-35 kg under current (solid black line) and suggested (dashed grey line) dosing regimens. The horizontal dotted black line represents the dose in mg/kg recommended for a 50 kg adult.