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Multivariate Multi-Level Non-Linear  
Mixed-Effect Models and their Application to the  
Modeling of Drug-Concentration Time Curves

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## Abstract

The enormous burden and threat to global health posed by the spread of malaria is well documented and forms an extensive field of study. With over 250 million new cases per annum and close on a million deaths, mostly in Sub-Saharan Africa, [5], the role of pharmacokinetics in determining the contribution of drugs to the prevention and cure of malaria is of evident importance. During pregnancy, those stricken with the disease are at increased risk of severe morbidity and both maternal and foetal mortality.

As part of the current WHO recommendations, pregnant women in areas of high intensity malaria transmission are given intermittent preventative treatment (IPT), using Sulfadoxine-Pyrimethamine (SP). Despite this recommendation and widespread implementation of the policy, there is very limited information on the disposition of the compounds during pregnancy, information which is critical for the development of informed and justified dosing regimens.

Using a similar protocol, studies were conducted in Mozambique, Zambia, Mali and Sudan, where serially measured concentration data was collected for healthy self-matched pregnant subjects on SP-IPTp. The objective of the studies was to enable characterization of the processes underlying the concentration-time relationship, and to determine the impact of pregnancy and pregnancy-related factors on individual specific deviations from these processes. Data was collected for 98 pregnant women, 77 of whom returned postpartum to act as self-matched controls.

The mixed effect or hierarchical model, is an appropriate methodology in the analysis of longitudinal data, in that it copes with the repeated measures per subject through the addition of subject-specific random effects, [6]. It thus provides a flexible framework and structure for dealing with multiple sources of variation, [6, 3]. In addition, by pooling information from all sampled individuals, it effectively accommodates sparsely sampled data.

This thesis discusses the techniques involved in the fitting of nonlinear mixed effect (NLME) models. In particular, it looks at the application of these techniques to the analysis of concentration-time data for the aforementioned anti-malarial compounds, and details the necessary extensions to the basic modeling process that were required in order to accommodate multiple responses and multiple observation phases (pregnant and postpartum).

The existence of serial measurements for two phases on each individual necessitated the inclusion of an additional level of random effects, occasion-specific, nested within the individual-specific effects. This multi-level model was developed for each of the individual compounds, using a structural model based on additive poly-exponential expressions.

In addition to the quantification of the systematic impact of pregnancy, results from the multi-level models were contrasted with those achieved using single



level models with an explicitly specified correlation structure for the random effects. Various structures were explored for the modeling of heterogeneous residual variance (a complication not unusual in nonlinear repeated measurement data), and the impact of various different parameterizations on the convergence and stability of the models was also assessed.

The co-administration of the two drugs raises the possibility of an interaction or interdependence. Sequential and simultaneous modeling procedures traditionally used in the context of investigating the dose response relationship were adapted to the analysis of the joint absorption and distribution of these drugs.

The sequential modeling approach involved the use of the predicted values of one response in the covariate model of the other as a time-varying predictor. This approach was contrasted with two simultaneous model formulations, one in which different structural model forms were specified using a binary indicator for response type, and one wherein the same structural form was applied to both responses, and the response type was included as a covariate.

Bi- and triple- exponential models were deemed appropriate for Sulfadoxine and Pyrimethamine respectively, which are loosely analogous to traditionally defined one- and two-compartment pharmacokinetic model forms. Both single-level models with correlated random effects and multiply-nested models achieved the same results, and the parameterization of the random effects (fit in both an additive and multiplicative (or proportional) context, the latter using logged parameters), appeared to play a significant role in the ease and speed of convergence, and in the estimation of robust variance-covariance matrices for the random effects.

The Delta method was then employed in order to acquire estimated standard errors for clinically useful parameters, obtained via back transformation from the exponential specification. The pharmacokinetic parameters obtained via this approach were compared and contrasted with those previously obtained using a traditional two-stage approach to the analysis of this longitudinal data set, that is, those obtained by averaging results obtained from individual- and occasion-specific models. Additional comparisons were also made between the parameters obtained from the exponential specification, and those from a mechanistically specified NLME model.

Results from the individual models indicated that physiological changes in pregnancy play a differing role for the two compounds in the determination of both the range of concentrations reached following the standard dosage regimen, and in the elimination of the drug from the system.

Pregnancy appeared to increase the range of concentrations reached for Sulfadoxine, whilst simultaneously increasing the rate of decline over time. The overall clinical impact of this was a reduction in the total drug exposure, as measured by the area under the concentration-time curve (AUC), a reduction in the volume of distribution, and an increase in the clearance. Site also had an impact on the concentration-time profile of Sulfadoxine, with subjects in Mozambique and Zambia having the highest and lowest range of concentra-

tions, irrespective of pregnancy phase, and subjects in Mozambique having a slower rate of decline when compared to other sites, again irrespective of pregnancy phase.

The impact of pregnancy on Pyrimethamine concentrations was to similarly increase the range, although no direct effect could be ascertained for the various rate constants. Clinically, this translated to an increase in total drug exposure, again as measured by the AUC, and a decrease in clearance for pregnant subjects versus those postpartum. The impact of pregnancy was dependent on the study site of the subject: pregnant subjects had higher peak concentrations in all sites except Sudan, where pregnancy status appeared to have a limited impact. Additionally, there was a separate site effect, with subjects in Mozambique and Zambia having the highest and lowest concentrations respectively, regardless of pregnancy phase.

Pharmacokinetic parameters obtained from the exponential specification were similar to those obtained via the two-stage approach and the mechanistic NLME model.

In the sequential modeling, the impact seen was that of the predicted Pyrimethamine concentrations on the absorption properties of Sulfadoxine, rather than any effect vice versa. Both this sequential model and the simultaneous model in which different functional forms were accommodated for the different responses were of limited usefulness in determining the true nature of the interaction between the drugs.

The simultaneous model in which the same structural form was applied for both responses allowed for the formal statistical testing of the “correct” structural model form for the different responses, and bi- and triple-exponential models were again indicated for Sulfadoxine and Pyrimethamine respectively.

For this model specification, the parameter estimates for the response type indicator variable were effect modifiers, specifying changes to the parameters and thus the concentration-time curve for the baseline response (Pyrimethamine). The parameters defining the second compartment for Sulfadoxine were not significantly different from zero. Additionally, the impact of pregnancy could be directly determined only for parameters related to the range of concentrations reached.

Concerns arose with the determination of accurate starting estimates for the more general empirical specification of the model in terms of poly-exponential equations, which initially led to problems with convergence. These were later overcome using curve-stripping algorithms.

An additional problem area resulted from the multiply-nested structure of the data, in that the determination of the degrees of freedom was not possible, unless approximated using formulae appropriate in the *linear* mixed effect model context, which did not appear to be applicable in the non-linear context.

Covariate model building also presented several challenges, due in part to the

complexity of the base model specification, and also because of the loss of clinical information regarding the placement of covariates resulting from the exponential parameterization. Convergence and stability issues resulted in fewer covariates being considered for inclusion, despite indications that covariates not included might still play a role in determining the relationship between the two drug concentrations and the influence of pregnancy, such as anaemia.

This thesis demonstrates that the use of nonlinear mixed effect modeling techniques provides a flexible framework for the estimation of separate, sequential and simultaneous models of drug-concentration over time. We have been able to draw conclusions regarding the impact of pregnancy on the concentration-time profiles of the individual compounds, as well as examine the sequential and simultaneous approaches to the modeling of the interaction between the two drugs, although further work is required to obtain a specification for the simultaneous modeling approach that allows for different structural forms and also makes sense for drug-drug interactions.

University of Cape Town

# Chapter 1

## Problem Statement and Introduction to Data

### 1.1 Study Design

The data specific to the analyses presented in this thesis are from a prospective multi-center study, consisting of 98 self-matched pregnant women from four different countries, where self-matched refers to return of the same subjects postpartum for the purpose of providing a non-pregnant control group.

Data was initially collected for 31 pregnant women in Mozambique, with the same women returning postpartum to act as their own controls. Using a similar protocol, a study was undertaken in Sudan, with 25 self-matched pregnant women, and again in Mali and Zambia with 18 and 25 self-matched pregnant women respectively.

All subjects were healthy volunteers, with maternal age 18-45 and gestational age 15-36 weeks, receiving intermittent preventative treatment during pregnancy (IPTp) with Sulfadoxine-Pyrimethamine (SP), antimalarial compounds that are routinely administered as part of pre-natal care in areas of high malaria prevalence.

Three tablets of SP (1500 mg of Sulfadoxine and 75 mg of Pyrimethamine) were orally administered to each subject once during pregnancy and again postpartum.

The main purpose of the study was to determine the impact of pregnancy and pregnancy related factors on the disposition and characterization of the SP drug concentration-time profiles.

This is referred to as the study of Pharmacokinetics (PK), whereas the study of the impact of the drug on the disease is termed Pharmacodynamics (PD).

Of the original 98 subjects, 77 returned to complete the postpartum phase of the study, and hence the data is unbalanced.

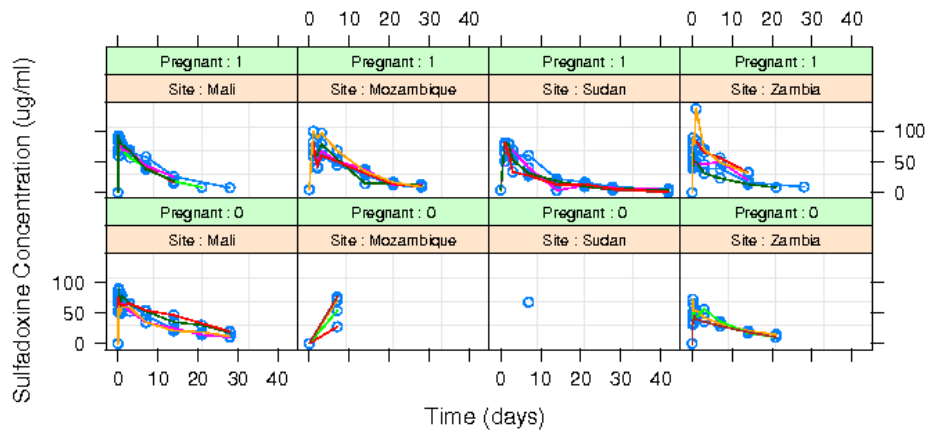


Figure 1.1: Sulfadoxine Concentration-Time Curves Grouped by Pregnancy Phase and Site

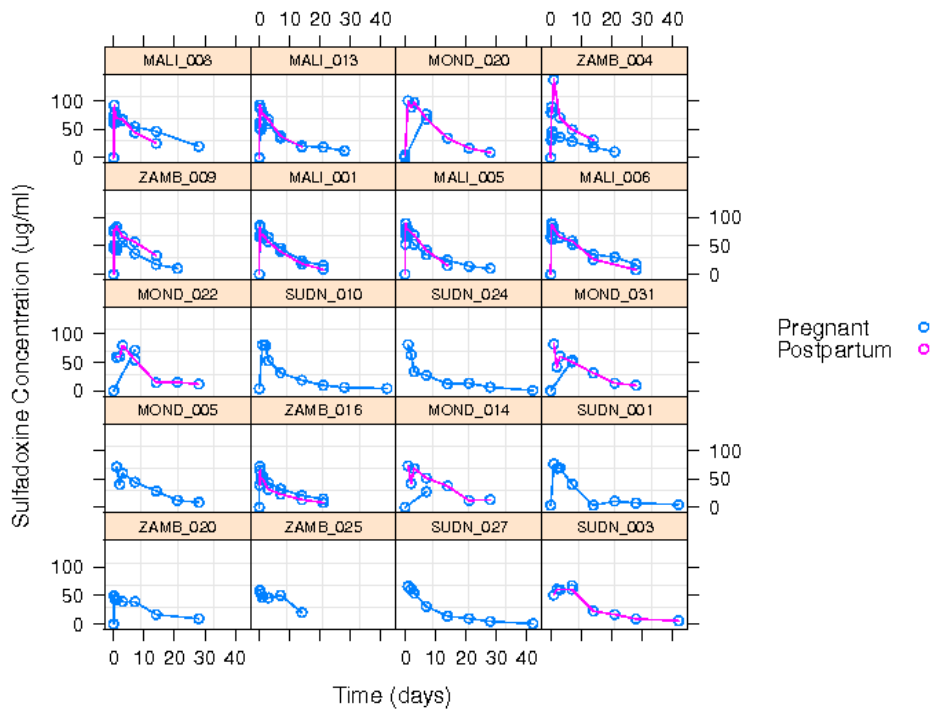


Figure 1.2: A Sample of Individual Sulfadoxine Concentration-Time Curves Grouped by Pregnancy Phase and Site

Blood samples were collected from women in Mali and Zambia at 3, 6, and 12 hours, and again at 1, 3, 7, 14, 21 and 28 days, and from women in Mozambique and Sudan on days 1, 3, 7, 14, 21 and 28 only. Postpartum samples in Mozambique and Sudan were taken on days 0 and 7 only, which was deemed adequate at the time owing to the reported high correlation between day 7 drug concentrations and overall drug exposure [5].

There are thus differing measurement occasions for different sites, and extremely sparse sampling for the postpartum phase in two of the sites where concentrations are only measured twice. There is also very limited information for the absorption phase, as data is only collected for the first 24 hours in two of the four sites.

This sparsity of information is demonstrated by Figure 1.1, which shows (multiple) Sulfadoxine concentration-time curves for each pregnancy phase grouped by site, and by Figure 1.2, which looks at a subset of the data, and allows for the examination of several *individual* subject profiles by pregnancy status and site. Large variation in the shape of the curves is observed both between individuals and within individuals between phases.

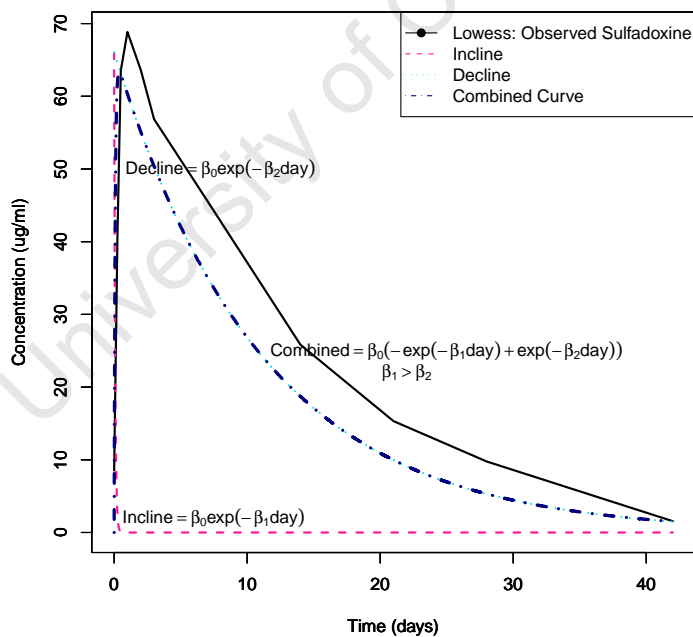


Figure 1.3: Decomposition of Bi-Exponential Curve

The methods involved in the evaluation of these curves must thus account for the sparseness of the data, the inherent correlation structure induced not only by the serial measurements per subject but by the multiple nested levels of group-

ing, (specifically: the measurements within observation phases within subject), and also the variable shape of the curves.

This latter aspect is addressed with the use of sums of exponentials, a routinely applied empirical methodology for curves such as these [7]. We chose to fit empirical rather than mechanistic models because we focused on the smoothing and interpolation of these incomplete observed concentration-time curves, approaching the analysis from a mathematical rather than pharmacological approach.

The suitability of the use of sums of exponentials is demonstrated graphically in Figure 1.3, which shows the decomposition of a particular bi-exponential equation into its requisite parts, superimposed over the lowest plot of the observed Sulfadoxine concentrations at the different time points.

In addition to accounting for all of the aforementioned aspects, the methodology must also accommodate the possible inter-dependent relationship of the two responses (the Sulfadoxine and Pyrimethamine concentrations over time), as the drugs are co-administered.

The correct quantification of this relationship is necessary in order to accommodate the simultaneous modeling of PK/PD data, although the focus of this thesis is on the PK component only. The concentration-effect relationship determined by the PK/PD analysis has yet to be accurately elucidated for SP in vivo, due in part to the lack of information regarding the interaction of the two drugs.

Table 1.1 is an excerpt of the data, highlighting the structure and the various factors under consideration as potential covariates.

Participant	Day	Sulfadoxine (ug/ml)	Pyrimethamine (ng/ml)	Pregnant	Gestational Age (wks)	Trimester	Site	Haemaglobin (g/dl)	Anaemic (HB <10d/l) (Yes/No)	Weight (kg)	Dosage (mg/kg)
MALL_002	0	0	0	1	24	2	Mali	9	1	60	1.25
MALL_002	0.125	83.2	354	1	24	2	Mali	9	1	60	1.25
MALL_002	0.25	83.9	310	1	24	2	Mali	9	1	60	1.25
MALL_002	0.5	86.8	311	1	24	2	Mali	9	1	60	1.25
MALL_002	1	80.7	266	1	24	2	Mali	9	1	60	1.25
MALL_002	3	69.4	178	1	24	2	Mali	9	1	60	1.25
MALL_002	7	49.7	71	1	24	2	Mali	9	1	60	1.25
MALL_002	14	25.3	11	1	24	2	Mali	9	1	60	1.25
MALL_002	0	0	0	0	0	0	Mali	14.2	0	56	1.34
MALL_002	0.125	55.3	350	0	0	0	Mali	14.2	0	56	1.34
MALL_002	0.25	55.9	277	0	0	0	Mali	14.2	0	56	1.34
MALL_002	0.5	66.2	245	0	0	0	Mali	14.2	0	56	1.34
MALL_002	1	63.4	201	0	0	0	Mali	14.2	0	56	1.34
MALL_002	3	59.9	112	0	0	0	Mali	14.2	0	56	1.34
MALL_002	7	39.2	24	0	0	0	Mali	14.2	0	56	1.34
MALL_002	14	34	12.3	0	0	0	Mali	14.2	0	56	1.34
MALL_002	21	19.7	0	0	0	0	Mali	14.2	0	56	1.34
MALL_002	28	11.3	0	0	0	0	Mali	14.2	0	56	1.34
SUDN_029	0	0.501	127	1	17	2	Sudan	10	1	78	0.96
SUDN_029	1	56.1	318	1	17	2	Sudan	10	1	78	0.96
SUDN_029	2	66.7	262	1	17	2	Sudan	10	1	78	0.96
SUDN_029	3	48.2	186	1	17	2	Sudan	10	1	78	0.96
SUDN_029	7	28.4	66.1	1	17	2	Sudan	10	1	78	0.96
SUDN_029	14	11.9	11.8	1	17	2	Sudan	10	1	78	0.96
SUDN_029	21	7.7	0.37	1	17	2	Sudan	10	1	78	0.96
SUDN_029	28	3.95	4.56	1	17	2	Sudan	10	1	78	0.96
SUDN_029	42	0.625	2.95	1	17	2	Sudan	10	1	78	0.96
SUDN_029	0	0	0	0	0	0	Sudan	7	1	53	1.42
SUDN_029	7	44.6	79	0	0	0	Sudan	7	1	53	1.42

Table 1.1: Subset of Data: Example



## Chapter 2

# Methodology

### 2.1 Introduction to Pharmacokinetics

#### 2.1.1 Background

In order to motivate the use of Non-Linear Mixed-Effect (NLME) modeling techniques, it is pertinent to define the broad goals behind the analysis of pharmacokinetic data, and to explore the characteristics of the data which render these techniques necessary.

Pharmacology may be defined as the science dealing with the fate and effects of the drugs or substances on the processes of the body and mind, where drugs are defined as a chemical agent, typically foreign or exogenous to the body, [8].

The branch of pharmacology dealing with the *fate* of drugs in the body, or the processes of absorption, distribution, metabolism and elimination (ADME), is known as pharmacokinetics (PK), and is more formally defined as the study of the time course of substances in the body, [2, 8, 9, 10, 11, 12, 13, 14].

Pharmacodynamics (PD) is defined as the study of the pharmacological response to a drug (the effect of the drug in the body), and it is typically studied together with pharmacokinetics in order to determine whether or not there is a concentration-effect relationship [8, 9, 10, 11, 12, 13, 14].

The goal behind the analysis of pharmacokinetic data is to provide a better understanding of how the concentrations of the drugs under evaluation (and the underlying processes which govern these concentrations) vary over time and across individuals. The quantification of systematic variation is critical for the development of sub-population specific dosing regimens, [6, 14, 15].

#### 2.1.2 General Data Characteristics

In general, pharmacokinetic data consists of serially measured drug concentrations on several individuals, that is, the data consists of repeated measurements over time which are grouped according to one or more classification factors, and is thus termed hierarchical, [6, 15].

The variation in the response (drug concentration) is non-linear over time, and from multiple sources: there is both within group variation (i.e. intra-individual), and between group variation (i.e. inter-individual), [3, 6, 15].

The usual ordinary least squares (OLS) assumptions of independent observations are thus no longer applicable because of the within-individual correlation between measurements, i.e. observations within a particular group are expected to be more similar than observations from different groups. Between group variation refers to the individual-specific deviation from the common or typical profile.

We expect the same basic nonlinear profile for drug concentrations over time, but the peak, rise and decay and the corresponding model parameters will differ slightly for each person, [3, 6, 15]. This deviation can be attributed to, and explicitly modeled as a function of both systematic and random components, where systematic refers to the impact of an individual or grouping level specific covariate pattern, [3, 6, 15].

## 2.2 Basic Pharmacokinetics

An in depth discussion of pharmacokinetics is beyond the scope of this thesis, but the rudimentary principles are introduced here in order to familiarize the reader with terms and concepts later referenced.

Since Sulfadoxine-Pyrimethamine is administered orally in a single dose, and the intention behind the collection of the data was to determine the impact of pregnancy induced changes on the disposition of SP, processes specific to oral administration, SP, and the impact of pregnancy have been highlighted. Calculations for parameters of interest are detailed in later sections.

In general, the aim of drug therapy is to achieve efficacy without toxicity, [16]. Figure 2.1 [1] represents the typical concentration profile for a single orally administered dose, where the minimum effective concentration (MEC) and toxic threshold are clearly demarcated.

The objective is thus to determine a regimen for dosing such that concentrations reach and exceed the MEC as quickly as possible, remaining in the therapeutic window for as long as required, without toxicity, [9, 10, 11, 12, 13, 14].

Pharmacokinetics allows us to determine the factors behind the variation of the concentration over time, and thus to create dosing regimens tailored to take into account changes in pertinent PK parameters brought about by physiological or environmental factors, (for example), [16].

From a dosing perspective, the most important parameters are the clearance, volume of distribution and elimination half-life of the drug, defined as [16]:

- Clearance (Cl): the volume of plasma cleared per unit time,

- Volume of Distribution ( $V_d$ ): the volume of space the drug would be distributed in at a concentration equal to that observed in the plasma (blood), given uniform and instantaneous distribution,
- Elimination half-life ( $t_{1/2}$ ): the time for the concentration of the drug to be halved.

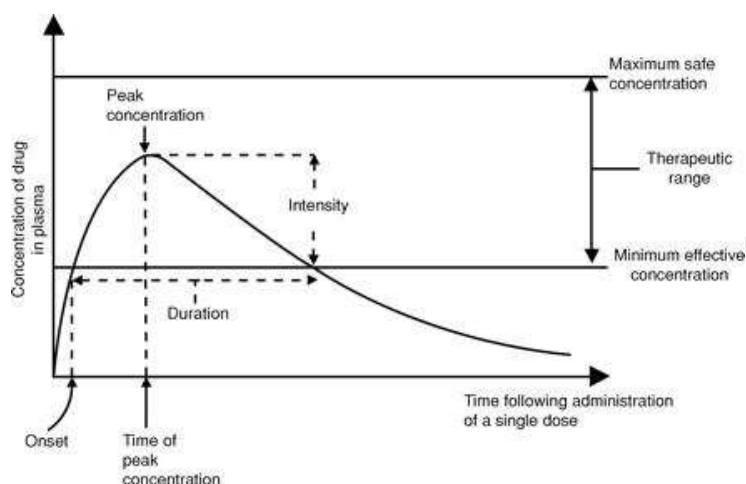
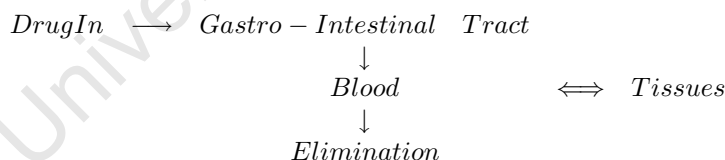


Figure 2.1: Typical Profile with Therapeutic Window Indicated [1]

The movement of drugs through the biological system is a complex process, and the models used to assess this movement are hence based on simplifying assumptions, [13, 14].

The schematic below is a much simplified version of the actual processes occurring in the body following the administration of a drug, [13]:



Essentially, a drug is administered for the purposes of producing a therapeutic effect, which is achieved through the absorption and subsequent distribution of drug molecules to what is known as the site of action, [14], which may broadly be described as a binding site for the drug, consisting of cells or tissues that interact with the drug to bring about the desired (or adverse) effects, [8, 17, 18, 19].

These target sites can be receptors or enzymes, where receptors are a kind of terminal on the cell surface which are able to detect stimuli, and through the transmission of signals (dependent on the type of stimulus), are able to bring about a type of reaction. Drugs can bind to these receptors, which will induce a certain effect, or to enzymes, which regulate the rate of chemical reactions, thereby altering this rate, either increasing (activators or inducers) or decreasing it (inhibitors), [8, 17, 18, 19].

Agonists are defined as drugs which stimulate the receptor in the same way that the body's natural substances would, and antagonists as drugs that block the action of the natural substance. Receptors may have several subtypes, and drugs may act on one or several, [8, 17, 18, 19].

Blood usually provides the method of transport from the site of administration to the surrounding tissues and organs (including the site of action), and is thus one of usual mediums of measurement of concentrations in the body (along with urine), [2, 11, 14].

For malaria, blood *is* the main site of action, and Sulfadoxine and Pyrimethamine are both folic acid antagonists, which in combination as SP, act as a two-fold interference in the synthesis of tetrahydrofolic acid in malaria parasites, which is essential for DNA synthesis and cell multiplication, i.e. they prevent the replication of the parasites at a crucial stage in their development cycle, [18, 20].

In intravenous administration, the administered drug is immediately and instantaneously absorbed into the systematic circulation, (i.e. no absorption is necessary) and 100% of the administered dose is available for distribution, [14, 16]. SP is, however, orally administered, and must therefore follow a process of absorption, (defined as the movement of the drug from the site of administration into the systematic circulation/site of measurement (in the case of SP, the blood or plasma), [2, 9, 10, 11, 12, 13, 14], or in biological terms, as the "uptake of materials from cells' external environment", [19]).

In general, following absorption into the system, i.e. once in the bloodstream, there is both bound and unbound (free) drug, which is circulated throughout the body, [9, 10, 11, 12, 13, 14]. Concentrations measured from plasma are assumed to be total drug concentrations (from both free and bound drug, which are assumed to be in equilibrium). Bound drug is that which is (e.g.) reversibly bound to either red cells or plasma proteins, such as albumin, and the protein-binding acts like a slow-release system from a reservoir, [9, 10, 11, 12, 13, 14].

The free drug is that which is allowed to travel across cell membranes, (semi-permeable barriers around cells, composed of phospholipids (fat molecules), proteins and aqueous pores), and distribute into peripheral tissues, [8, 9, 10, 11, 12, 13, 14, 19].

Tissue bound drug eventually leaves and re-enters the bloodstream. This reversible transfer of drug to and from the site of measurement (i.e. from the blood or plasma to the surrounding tissues and back again) is known as the process of distribution, [2, 9, 10, 11, 12, 13, 14].

From the bloodstream, the drug is able to perfuse the liver and kidneys, the main organs responsible for the elimination of drug from the system, where elimination is the irreversible transfer of drug from the site of body. this includes metabolism, where the parent drug or initial substance is converted into a slightly different chemical substance, and excretion, [2, 9, 10, 11, 12, 13, 14].

This is not a simple linear process: drug metabolized by the liver can be excreted in bile/faeces, but it can also be reabsorbed, (this re-absorption is referred to as the entero-hepatic cycle). Both the parent drug and any un-excreted metabolites are later filtered by the kidney, and again either reabsorbed or excreted from the body, [9, 10, 11, 12, 13, 14] and there are other routes of elimination e.g. sweat/tears, although these are generally minor.

In the subsections that follow, (which describe the individual processes of absorption, distribution, metabolism and elimination (ADME) in greater detail), the information presented is a para-phrasal and concatenation of work presented by Wagner [14] and Begg [16].

### 2.2.1 Input factors: Absorption

There are several possible routes of administration, which may loosely be categorized into intra- and extra-vascular routes.

In oral administration, (as is the case with most of the extra-vascular routes), following drug administration a small depository is formed, and some form of transport must occur from this site of administration to the systematic circulation. This transport usually involves the traversing of a cell membrane.

Oral absorption in particular usually occurs via passive diffusion of the drug through the small intestine, i.e. the process is one which requires no energy, and movement flows with the concentration gradient (from an area of high concentration to that of a lower concentration). Drug absorption may also occur via active transport (as opposed to passive diffusion) whereby transportation across the cell membrane is facilitated by an energy dependent membrane carrier mechanism such that transport can occur against the concentration gradient (transfer from low to high concentration).

The completeness (or extent) of the absorption of the drug reaching the systematic circulation (bioavailability) is ordinarily less than 100%. Most orally administered drugs must pass through the portal vein to the liver, which means that the drug is exposed to metabolizing enzymes; this is known as the first pass effect.

A number of factors, mechanical, physicochemical, and physiological, may impact on the absorption of a particular drug, including, (but not limited to), the surface area of the particles, or the size and shape of the granules, the pH of gastric secretions, the presence or absence of food in the gut, the blood flow rate to the site of administration, the individual's temperature etc.

### 2.2.2 Disposition factors

Disposition factors as a whole refer to all the processes of drug movement from the time that the drug appears in the systematic circulation until the time that it exits the body. They thus encompass the processes of distribution, metabolism and elimination.

## **Distribution**

Distribution in particular has already been defined as the reversible transfer of drug to and from the site of measurement (the systematic circulation) into different “volumes”: extra vascular, interstitial and intracellular spaces.

The systematic circulation carries the drug to all the tissues of the body, but the rate and extent of the distribution is determined by the ability of the drug to perfuse these tissues, which again depends on the blood flow rate to the tissue (the perfusion rate), and the pH of the drug.

Tissues fall into several groups: those that are highly perfused, less highly perfused tissues (muscle and skin), a negligible perfusion group which consists of ligaments and cartilage, and a fat group (adipose tissue and bone marrow).

Drugs distribute most easily into porous tissues with high blood flows, (such as the liver/kidney) and less easily into e.g. the brain, which is protected by the blood-brain barrier, a lipid membrane with very few aqueous pores.

Un-ionized, lipophilic (fat-loving) drugs are more widely distributed, and polar drugs (ionized, hydrophilic drugs i.e. dissolving more easily in water) less so because of the difficulty encountered in crossing non-porous lipid membranes. The polarity of a drug thus limits the rate of distribution and basic compounds tend to distribute more easily into tissues than acidic.

Distribution is also influenced by plasma-protein and tissue binding: at equilibrium (which is assumed to exist at all times because of the speed of rates of association and the disassociation of the reversible binding process) protein- and tissue-bound drugs are not available for distribution.

## **Metabolism and Elimination**

Metabolism, or biotransformation, typically forms part of elimination in that the parent substance is, by way of enzyme-catalyzed reactions, converted to metabolites, either in the gut wall during absorption, in the liver, or in certain tissues, such as the lung and kidney.

The rate and extent of metabolism is influenced by genetic, environmental and physiological factors. The form of metabolism that is of the most interest with oral absorption is that occurring in the gut wall, and the so called first pass effect already discussed, which reduces the overall bioavailability of the drug.

The route of elimination is largely dependent on the polarity and molecular weight of the drug. Sufficiently lipophilic components excreted in bile are reabsorbed in the GI Tract (entero-hepatic cycle), and cleared again by the liver, before finally being excreted. Some polar drugs are biotransformed by bacteria and the products reabsorbed, and they are usually eliminated via the kidneys.

Renal excretion, which occurs via the kidneys, and is the route of elimination for SP, is quantitatively the most important route, although excretion also occurs via bile/faeces and sweat and tears also play a minor role.

There are 3 processes of renal elimination: Glomerular filtration, passive tubular reabsorption, and active tubular secretion. Glomerular filtration refers to unbound drug only (and is thus dependent on the extent of plasma-protein binding). The rate of filtration (125ml/min) referred to as the GFR can be approximated by creatinine clearance. Passive diffusion in the kidney is dependent on the polarity and degree of ionization of the drug at the urinary pH: an increase in pH promotes the excretion of acids and inhibits that of bases. Total renal elimination is given by filtration plus secretion, minus reabsorption.

### 2.2.3 Pharmacokinetic Parameters of Interest

There are a number of pharmacokinetic parameters of interest, most particularly, the three previously mentioned: the volume of distribution, clearance and half-life.

Distribution is estimated by the volume of distribution (Vd), (the volume into which the drug appears to be distributed with a concentration equal to that measured in the plasma). Volume of distribution is most commonly expressed in litres, but may also be normalized for weight and measured in litres per kg.

The Vd is not a physiological volume, but may approximate to one of either plasma volume (3L), extracellular volume (13-16L), or total body water (40-46L). Values in excess of total body water indicate a high tissue uptake (increased uptake of drug by tissues leaves smaller concentrations in the circulation, and thus results in larger volumes of distribution).

It ranges from approximately 7 to 50000L.

Since we do not know the fraction (f) of the dose that was actually absorbed following extravascular administration, we express the volume as  $V/f$ , where f is a measure of bioavailability. We refer to  $V/f$  as the “apparent” volume of distribution.

The clearance (Cl) or apparent clearance ( $Cl/f$ ) of a drug refers to the amount of plasma that is cleared of drug per unit time, and both renal clearance and total clearance are defined, where total clearance is the sum of clearance from all routes. For calculations of the maintenance dose rate, the clearance of the drug is the most important parameter of interest, (while the loading dose is calculated based on the volume of distribution) [16].

The plasma elimination half-life provides an “index of the time-course of drug elimination”, [16]. It may be expressed in terms of  $k_e$ , the elimination rate constant, which may sometimes be determined from the log-linear plot of the concentration-time profile as the slope of the line following  $C_{max}$  (for a single compartment model, defined in section 2.5.)

There are several PK parameters relating to the absorption of the drug: the bioavailability, (the percentage of the dose available for distribution into the systematic circulation), is a measure of the extent of absorption. The C<sub>max</sub> is the maximum concentration reached, and the T<sub>max</sub> the time after dosing that this maximum is achieved. T<sub>max</sub> is related to the *rate* of absorption, along with the absorption constant (k<sub>a</sub>), [16].

The C<sub>max</sub> and T<sub>max</sub> may be obtained from the observed or predicted concentration versus time curves.

Additional parameters of interest are the AUC (the area under the plasma concentration-time curve), which is a measure of the extent of drug exposure. AUC is calculated using either the trapezoidal rule, should only the observed data be available (model-independent approach), or derived from parameters determined using appropriate models. Calculations for the AUC may be seen in section 2.8.3, along with those for the volume of distribution and clearance.

The mean residence time (MRT), interpreted as the average time taken for the drug to transition from administration to elimination, may also be determined, and is defined as the first statistical moment where the concentration-time curve is defined as a probability curve.

#### 2.2.4 SP and the Impact of Pregnancy

Changes in maternal physiology influence the processes of absorption, distribution, metabolism and elimination. In some instances, pregnant subjects may experience significantly higher or (more often) lower concentrations. For drugs with a narrow therapeutic window, there is thus an increased risk of toxicity for higher concentrations, or reduced efficacy for lower concentrations or total exposure, [21, 22, 23].

In general, information on the absorption and disposition of drugs during pregnancy is limited and largely based on theoretical principles and observational studies, as the ethical and practical constraints implicit in taking samples from such a vulnerable subpopulation prevents the collection of large amounts of data, [21, 22, 23].

During pregnancy, hormonal changes (such as elevated progesterone) may reduce gastric emptying and small intestine mobility, resulting in an increase in the time taken to reach maximum concentration, and a decrease in the actual maximum achieved. These effects are most pronounced during the third trimester, as it is during this time that the levels of progesterone are highest, [16, 21, 22, 23]. Changes in the gastric pH may also reduce the absorption of weak acidic drugs, but the most pronounced absorption related impact of pregnancy for orally administered drugs is that of nausea and vomiting, [22].

Pregnancy induces expansion of intra- and extra-vascular water content, and hence increases total body water by approximately 8 litres. It also increases body fat (approximately 4 kgs). This increase in body water and fat essentially



provides a larger space for drugs to distribute into, resulting in a decrease in the maximum concentrations reached and a larger volume of distribution. Decreases in protein binding, (specifically albumin), result in the displacement of protein-bound drug, which results in an increase in the amount of free drug available, [16, 21, 22, 23].

An increase in hepatic blood flow means that more drug passes through the liver and is thus available for metabolizing. Additionally, hormonal changes may induce or inhibit metabolizing enzymes, thus increasing or decreasing metabolism and hence elimination. The exact effect is dependent on the mechanism of change and the drug itself, [16, 21, 22, 23].

Elimination of drugs is expected to increase during pregnancy, due to an increase in renal blood flow of approximately 50 to 80%, and an increase in GFR of about 40 to 65%. This increase, together with the increase in the volume of distribution, indicates that there are lower concentrations during pregnancy, and thus that dosage adjustments may be required, [16, 21, 22, 23].

There may also be a potential build up of drug in the foetal-placental unit: drugs that may enter the foetal compartment with relative ease are occasionally unable to return, resulting in an accumulation of drug in the foetus. Additionally, because the umbilical cord feeds straight into the cardiac and systematic circulation of the foetus, bypassing the liver, and enzymatic activity is low in early development, elimination via liver-based metabolism is limited, (8).

In the case of SP, although the exact mechanism of effect as intermittent preventive treatment in pregnancy (IPTp) is not clearly understood, the general consensus indicates that efficacy is dependent on sustained effective concentrations, i.e. for the entire dosing interval, [5].

Despite the extensive use of SP in IPTp, the understanding of its performance and disposition during pregnancy is limited. As of July 2009, there were fewer than 150 enrolled participants in published pharmacokinetic studies, [23, 24, 25], data which is urgently required for the review and optimization of current IPTp strategies [26, 27].

In the non-pregnant population, both compounds are well absorbed, have greater than 90% bioavailability and are 85-90% protein bound. Both compounds are excreted primarily in urine, although Pyrimethamine is metabolized to several metabolic products, and only 15 to 30% of the drug is excreted unchanged, [5, 28, 29].

The average terminal elimination half-lives are 200 and 100 hours for Sulfadoxine and Pyrimethamine, respectively, in healthy non-pregnant adults, [5, 28, 29].

SP concentrations are expected to be lower during pregnancy, relative to after the postpartum period (6 to 8 weeks after delivery), as a result of pregnancy-associated changes in drug absorption, distribution, metabolism (notably hepatic metabolism), and renal elimination. This may result in a reduction in SP efficacy, particularly given the widespread resistance to this drug, [5, 23].

Table 2.1 summarizes the data currently available for SP pharmacokinetic parameters in the non-pregnant population, while Table 2.2 indicates the impact of covariates such as age and pregnancy on these parameters determined from several sources.

For Sulfadoxine, the overall drug exposure appears to decrease during pregnancy, (as indicated by the majority of sources), and the clearance appears to increase. Conflicting results are seen for the volume of distribution. For Pyrimethamine, there does not appear to be a common consensus on the impact of pregnancy for any of the three parameters.

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Antimalarial (metabolite)	Ref.	C <sub>max</sub>	T <sub>max</sub> (hr)	AUC (mg/mL.hr)	K <sub>a</sub> (/hr)	V/F	CL/F (L/hr.kg)	t <sub>1/2</sub> (days)
Pyrimethamine	[23, 30, 31]	193-591 (ng/mL)	12-19.8	25.2-72.7		3.8-7.2 (L/kg)	0.03-0.07	2.8-3.4
Sulfadoxine	[23, 30, 31]	57.92 (ug/mL)	5.7-13.5	11040-66192	0.3	372-660 (ml/kg)	1.4-3.0	4.1-8.9

Table 2.1: Summary of Available Data on Sulfadoxine and Pyrimethamine Pharmacokinetic Parameters

Antimalarial	Covariate	Clearance			Volume of distribution			Exposure (e.g. AUC, day 7)		
		↑	↔	↓	↑	↔	↓	↑	↔	↓
Sulfadoxine	Age (young children)	[30]			[30]				[30]	[30, 31]
	Pregnancy	[5, 23]			[23]		[5]			[5, 23, 24, 32]
Pyrimethamine	Age (young children)	[30]			[30]				[30]	[30]
	Pregnancy	[23]		[5]	[23]		[5]	[5]	[24]	[23]

Table 2.2: Summary of Available Data on the Effect of Demographic Factors on Sulfadoxine and Pyrimethamine Pharmacokinetic Parameters

## 2.3 Introduction to NLME Models

### 2.3.1 Role of NLME model: Background

In the traditional two stage or marginal approach to the analysis of PK data, separate models are fitted to each individual, and population parameters (mean, variance and covariance) may then be obtained through the averaging of parameters from several individuals.

The suitability of this method is reliant on the availability of rich sampling data, which is not always possible (e.g. in infants or similarly vulnerable sub-populations such as pregnant women), [3, 6, 12, 33].

The mean parameter estimates resulting from this approach are usually unbiased, but for sparsely sampled data, the estimates of the variance and covariance across subjects are not. Estimates of the inter-individual errors are overestimated as residual error is increased, [15, 34, 35].

The alternative population or mixed effect modeling approach, in which a model is simultaneously fitted to data from all subjects (thereby pooling information), was thus originally proposed as a solution to the aforementioned issues in the analysis of sparsely sampled data [3, 6, 15, 36].

The basic NLME model is expressed in terms of both fixed and random effects, where fixed effects are those parameters associated with the population as a whole: they express the basic shape of the concentration-time curve. Random effects are parameters associated with individual experimental units randomly drawn from population at large, (for the general case: individual subjects).

By associating common random effects to observations from the same level, (e.g. subject specific random effects), population averages and estimates of variation are obtained whilst simultaneously quantifying the between (inter)-individual variation separately to the within (intra)-individual variation, [3, 6].

This separation is important: deviations from the typical profile are usually expected to vary constantly within individuals, whereas elements of residual error, (deviations from the individual profile), change for every observation, [3, 6, 33, 37, 38, 39, 40, 41].

The different levels of variation (inter-individual and intra-individual) need to be independently dealt with in order to correctly account for the inherent covariance structure of the data, and to quantify the inter-individual variation attributable to systematic components.

In the pharmacokinetic context, we need to not only characterize the basic ADME processes which govern the concentrations, (the underlying features or mechanisms of individual profiles), but also explore how these processes vary in the population of subjects, [3, 6, 15], in order to elucidate the proportion of variance attributable to individual-specific characteristics (e.g. pregnancy, weight, age) and to thus develop appropriate and therapeutically beneficial dos-

ing strategies/regimens, tailored for specific population subgroups, [2, 9, 10].

General inference about the typical population profile is not adequate, because of the differential impact of the drugs on different individuals.

### 2.3.2 Relevance of Study Design

The details presented earlier in section 1.1 introduced the multiply-nested structure of the data: that of measurements taken within observation phase within subject, where the observation phases are determined by the pregnancy status.

These multiple levels of grouping result in a correlation structure that is inherently more complicated, as measurements within phases on the same individuals are more similar than those for different phases, and measurements on the same individual (irrespective of observation phase) are more similar than those for different individuals. The phases of observation are henceforth referred to as occasions. This leads to the following levels of random effects:

- Inter-individual variation (IIV), (level 1)
- Intra-individual, inter-occasion (IOV), (level 2)
- and Intra-individual, intra-occasion (WIV), (level 3: the innermost level or residual measurement error)

The non-linear mixed effect model allows for a flexible representation of this covariate structure.

In summary: we therefore have the basic deterministic model, consisting of fixed effect parameters only, which provides us with the average non-linear concentration-time profile for the population as a whole (the structural model), and that of strata within the population (the covariate model, which takes into account systematic deviations from the typical parameters). Non-systematic or random deviations are dealt with via the use of nested random effects- one for each level of grouping. These random effects are dealt with separately from the residual variation (level 3), and are quantified in terms of their deviation from the typical parameters through specific covariance matrices.

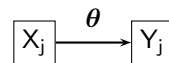
The development of the theoretical model may thus loosely be categorized into three sections: structural, covariate and stochastic, although these are not easily separated out.

### 2.3.3 Conceptual Framework

Overall, the application of the NLME model may be conceptually illustrated using the general structure of an ordinary least squares model (OLS), [39]. In this, and in the remainder of the thesis, the subscript  $i$  refers to subjects,  $j$  to specific measurements or observations, and  $k$  to pregnancy status and thus to observation phase or occasion.

### OLS Model:

For the usual ordinary least squares (OLS) model, the goal is invariably to model the effects of independent variables or predictors on a particular response. Assuming one predictor variable for the sake of simplicity, and thus the vector of intercept and slope parameters  $\theta$ , then for observations  $j = 1 \dots n$ , the vector of independent responses,  $\mathbf{Y} = [Y_1 \dots Y_n]'$ , is associated with the vector of predictor values,  $\mathbf{X} = [X_1 \dots X_n]'$ , through the vector of model parameters,  $\theta$ , which may be schematically represented as follows:

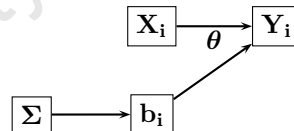


### 2-Level Random Effects Model:

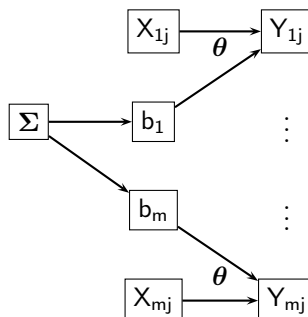
For the 2-level random effects model, i.e. for the situation in which there are multiple individuals and observations are grouped according to these individuals, the OLS assumptions of independent observations no longer apply.

We now have  $i = 1, \dots, m$  individuals, with  $j = 1, \dots, n_i$  observations on each individual.

Again assuming a single predictor variable, the vector of responses for the  $i$ th individual,  $\mathbf{Y}_i = [Y_{i1} \dots Y_{in_i}]'$  is now associated with the vector of predictor values,  $\mathbf{X}_i = [X_{i1} \dots X_{in_i}]'$  and a vector of individual specific random parameters,  $\mathbf{b}_i = [b_1 \dots b_m]'$ , through a vector of shared parameters  $\theta$ , and a parameter matrix  $\Sigma$  governing inter-individual variability:



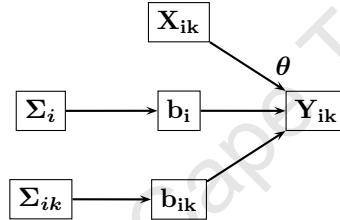
This relationship may be represented equivalently by the simple schematic above, and the more fully expanded version:



### 3 Level Random Effects Model:

Similarly, for the 3-level random effects model where we have multiple observations on several individuals for each of several possible occasions (given by pregnancy status), we have  $i = 1, \dots, m$  individuals,  $k = 1, \dots, n_i$  occasions on each individual, and  $j = 1, \dots, n_{ik}$  observations for each occasion on each individual.

For the  $i$ th individual on the  $k$ th occasion, the vector of  $j = 1 \dots n_{ik}$  responses,  $\mathbf{Y}_{ik} = [\mathbf{Y}_{ik1} \dots \mathbf{Y}_{ikn_{ik}}]'$ , is associated with the vector of predictor values (for a single predictor),  $\mathbf{X}_{ik} = [\mathbf{X}_{ik1} \dots \mathbf{X}_{ikn_{ik}}]'$ , the vector of individual specific random effects,  $\mathbf{b}_i = [b_1 \dots b_m]'$ , and the vector of occasion specific random effects,  $\mathbf{b}_{ik} = [b_{i1} \dots b_{in_i}]'$  through the vector of common parameters,  $\boldsymbol{\theta}$ , a parameter matrix governing inter-individual variability,  $\boldsymbol{\Sigma}_i$ , and a parameter matrix governing intra-individual inter-occasion variability,  $\boldsymbol{\Sigma}_{ik}$ .



## 2.4 Linear Mixed Effect Models

This section introduces mixed effect models in a purely linear fashion for a single level of grouping only, (observations  $j$  grouped by subjects  $i$ ). The purpose of this apparent digression is to actually demonstrate *how* the technique of mixed effect models accounts for the hierarchical structure of the data, in the simplest algebraic terms possible. Whilst this could be achieved for the non-linear case, the linear version is simpler, and contributes to the natural progression of understanding. The information presented in this section is based on lecture notes by Jack Weiss, [40, 41].

As was the case for the conceptual schematics, the mixed effect model is again contrast to the usual OLS model, where the model for the  $j$ th response is given by:

$$Y_j = X_j \beta + \epsilon_j$$

where  $\epsilon_j \sim N(0, \sigma^2)$ .

Now looking at  $p$  predictor-variables, and thus at the vector of model parameters  $\boldsymbol{\beta}$ , and expressing the above model in matrix notation:

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon}$$

where  $\boldsymbol{\epsilon} \sim N(0, \mathbf{I}\sigma_e^2)$ ,

or (now including an intercept term):

$$\begin{bmatrix} y_1 \\ \vdots \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & \dots & x_{1p} \\ \vdots & \vdots & \ddots & \vdots \\ 1 & x_{n1} & \dots & x_{np} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \vdots \\ \beta_p \end{bmatrix} + \begin{bmatrix} e_1 \\ \vdots \\ e_n \end{bmatrix}$$

The expectation and variance, and the distribution of the response  $\mathbf{Y}$ , are given by:

$$\begin{aligned} E(\mathbf{Y}) &= \mathbf{X}\boldsymbol{\beta} \\ \text{Var}(\mathbf{Y}) &= \text{Var}(\boldsymbol{\epsilon}) = \sigma_e^2 \mathbf{I} \\ \mathbf{Y} &\sim N(\mathbf{X}\boldsymbol{\beta}, \sigma_e^2 \mathbf{I}) \end{aligned}$$

The above OLS specification is that of a model with fixed effects only. We can now comfortably segue into the notation for a model in which  $j = 1, \dots, n_i$  observations are grouped according to  $i = 1, \dots, m$  individuals, (such that we have  $N = \sum_{i=1}^m n_i$  data points in total).

This new model may be expressed in two stages, the 1st encompassing the structural model form and the within-individual variation, and the second dealing with the inter-individual variation.

Thus stage I may be written as:

$$\mathbf{Y}_i = \mathbf{X}_i \boldsymbol{\beta} + \mathbf{Z}_i \mathbf{b}_i + \boldsymbol{\epsilon}_i$$

for the  $i$ th individual (suppressing subscript  $j$  for convenience), where  $\boldsymbol{\beta}$  is the  $p$ -dimensional vector of fixed effects,  $\mathbf{b}_i$  is the  $q$ -dimensional vector of random effects,  $\mathbf{X}_i$  and  $\mathbf{Z}_i$  are the fixed and random effects regressor matrices of dimension  $(n_i \times p)$  and  $(n_i \times q)$  respectively, and  $\boldsymbol{\epsilon}_i$  is the  $n_i$ -dimensional vector of residual (within-group) errors.

Or (again now including an intercept):

$$\begin{bmatrix} y_{i1} \\ \vdots \\ y_{in_i} \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & \dots & x_{1p} \\ \vdots & \vdots & \ddots & \vdots \\ 1 & x_{n_i1} & \dots & x_{n_i p} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \vdots \\ \beta_p \end{bmatrix} + \begin{bmatrix} 1 & z_{11} & \dots & z_{1q} \\ \vdots & \vdots & \ddots & \vdots \\ 1 & z_{n_i1} & \dots & z_{n_i q} \end{bmatrix} \begin{bmatrix} b_{0i} \\ \vdots \\ b_{qi} \end{bmatrix} + \begin{bmatrix} \epsilon_{i1} \\ \vdots \\ \epsilon_{in_i} \end{bmatrix}$$

Where the marginal expectation and covariance of  $\mathbf{Y}_i$  are given by:

$$\begin{aligned} E(\mathbf{Y}_i | \mathbf{b}_i) &= \mathbf{X}_i \boldsymbol{\beta} + \mathbf{Z}_i \mathbf{b}_i \\ \text{Cov}(\mathbf{Y}_i | \mathbf{b}_i) &= \mathbf{R}_i \end{aligned}$$

and thus  $\boldsymbol{\epsilon}_i \sim N(0, \mathbf{R}_i)$ .

The development of the inter-individual variability, (stage II), involves the addition of covariates and random effects. Focusing on the random effects, and defining  $q$  random effects where  $q \leq p$ , we can then define a covariance matrix  $\mathbf{D}_{q \times q}$ , such that  $\mathbf{b}_i \sim N(\mathbf{0}, \mathbf{D})$ , (assuming normality).



If the  $\mathbf{b}_i$  are independent of the  $\epsilon_i$  and of each other for different individuals, the unconditional expectation and covariance of  $\mathbf{Y}_i$  are as follows:

$$\begin{aligned} E(\mathbf{Y}_i) &= E\{E(\mathbf{Y}_i|\mathbf{b}_i)\} \\ &= E\{\mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i\} \\ &= \mathbf{X}_i\boldsymbol{\beta} \end{aligned}$$

$$\begin{aligned} Cov(\mathbf{Y}_i) &= E\{Cov(\mathbf{Y}_i|\mathbf{b}_i)\} + Cov\{E(\mathbf{Y}_i|\mathbf{b}_i)\} \\ &= \mathbf{R}_i + \mathbf{Z}_i\mathbf{D}\mathbf{Z}_i' \\ &= \mathbf{V}_i \end{aligned}$$

where  $\mathbf{V}_i$  is dependent on a vector  $\boldsymbol{\omega}$  of variance parameters.

If we look at an example where we have a model that includes an intercept term, and has random effects on both the intercept and slope parameters, stage I could be expressed as:

$$\begin{aligned} y_{ij} &= f(x_{ij}, \boldsymbol{\beta}_i) + \epsilon_{ij} \\ &= \beta_{0i} + \beta_{1i}x_{ij} + \epsilon_{ij} \end{aligned}$$

where  $\boldsymbol{\beta}_i = (\beta_{0i}, \beta_{1i})'$ ,  $f(x_{ij}, \boldsymbol{\beta}_i) = \beta_{0i} + \beta_{1i} \times t_{ij}$ , for a single predictor variable  $x_{ij} = t_{ij}$ , and  $i = 1, \dots, m$ , and  $j = 1, \dots, n_i$  where  $m$  is the number of individuals and  $n_i$  is the number of observations for individual  $i$ .

Assuming that the covariance matrix  $\mathbf{R}_i$  is given by  $\sigma_e^2 \times \mathbf{I}$ , the distribution of the residual errors may be written as  $\epsilon_{ij} \sim N(0, \sigma_e^2)$ .

Stage II could then be written as:

$$\begin{aligned} \beta_{0i} &= \beta_0 + b_{0i} \\ \beta_{1i} &= \beta_1 + b_{1i} \end{aligned}$$

Then assuming a compound symmetric correlation structure and using assumptions of independence for the elements of  $\mathbf{b}_i$  and  $\epsilon_{ij}$ , and for  $\mathbf{b}_i$  for different individuals:

$$Cov(b_{0i}, b_{0k}) = \begin{cases} \tau_0^2 & i = k \\ 0 & i \neq k \end{cases}$$

$$Cov(b_{1i}, b_{1k}) = \begin{cases} \tau_1^2 & i = k \\ 0 & i \neq k \end{cases}$$

$$Cov(b_{0i}, b_{1k}) = \begin{cases} \tau_{01} & i = k \\ 0 & i \neq k \end{cases}$$

$$Cov(b_{0i}, \epsilon_{ij}) = 0$$

$$Cov(b_{1i}, \epsilon_{ij}) = 0, \quad \forall i, j$$

Thus, covariance matrix  $\mathbf{D}$  and the distribution of the random effects  $\mathbf{b}_i$  is given by:

$$\begin{bmatrix} b_{0i} \\ b_{1i} \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_0^2 & \tau_{01} \\ \tau_{01} & \tau_1^2 \end{bmatrix}\right)$$

although the assumptions of normality may later be relaxed.

Simplifying the above for the purposes of illustration, and thus looking back at the OLS case where:

$$\begin{aligned}\beta_{0i} &= \beta_0 \\ \beta_{1i} &= \beta_1\end{aligned}$$

if we were to include random effects on the intercept parameter only for the mixed effect model:

$$\begin{aligned}\beta_{0i} &= \beta_0 + b_{0i} \\ \beta_{1i} &= \beta_1, \quad \text{for } b_{0i} \sim N(0, \tau_0^2)\end{aligned}$$

the form of the composite model would then be as follows:

$$\begin{aligned}y_{ij} &= \beta_0 + b_{0i} + \beta_1 t_{ij} + \epsilon_{ij} \\ &= \underbrace{\beta_0 + \beta_1 t_{ij}}_{\text{fixed effects}} + \underbrace{b_{0i} + \epsilon_{ij}}_{\text{random effects}}\end{aligned}$$

Then using the properties of covariance for the sum of random variables  $(X, Y, W, Z)$  with  $a$  and  $b$  depicting constants:

$$Cov(a + X, b + Y) = Cov(X, Y)$$

$$Cov(W + X, Y + Z) = Cov(W, Y) + Cov(W, Z) + Cov(X, Y) + Cov(X, Z)$$

and looking at observations from different groups  $(i, k)$  and at different times or for different observations  $(j, l)$ :

$$\begin{aligned}y_{ij} &= \beta_0 + \beta_1 X_{ij} + b_{0i} + \epsilon_{ij} \\ y_{kl} &= \beta_0 + \beta_1 X_{kl} + b_{0k} + \epsilon_{kl}\end{aligned}$$

$$\begin{aligned}Cov(y_{ij}, y_{kl}) &= Cov(\beta_0 + \beta_1 X_{ij} + b_{0i} + \epsilon_{ij}, \beta_0 + \beta_1 X_{kl} + b_{0k} + \epsilon_{kl}) \\ &= Cov(b_{0i} + \epsilon_{ij}, b_{0k} + \epsilon_{kl}) \\ &= Cov(b_{0i}, b_{0k}) + Cov(b_{0i}, \epsilon_{kl}) + Cov(b_{0k}, \epsilon_{ij}) + Cov(\epsilon_{ij}, \epsilon_{kl}) \\ &= Cov(b_{0i}, b_{0k}) + Cov(\epsilon_{ij}, \epsilon_{kl})\end{aligned}$$

where

$$\begin{aligned}cov(b_{0i}, b_{0k}) &= \begin{cases} \tau^2 & \text{if } i = k \\ 0 & \text{if otherwise} \end{cases} \\ cov(\epsilon_{ij}, \epsilon_{kl}) &= \begin{cases} \sigma^2 & \text{if } i = k \quad \& \quad j = l \\ 0 & \text{if otherwise} \end{cases}\end{aligned}$$

we can show that:

$$cov(y_{ij}, y_{kl}) = \begin{cases} 0 & \text{if } i \neq k \\ \tau^2 & \text{if } i = k \quad \& \quad j \neq l \\ \tau^2 + \sigma^2 & \text{if } i = k \quad \& \quad j = l \end{cases}$$

i.e. observations in the same group have a positive covariance (and are therefore more similar), and observations in different groups are uncorrelated.

In correlation terms, for distinct observations ( $j \neq l$ ):

$$\begin{aligned}\rho(y_{ij}, y_{kl}) &= \frac{\text{Cov}(y_{ij}, y_{kl})}{\sqrt{\text{Var}(y_{ij})}\sqrt{\text{Var}(y_{kl})}} \\ &= \frac{\text{Cov}(y_{ij}, y_{kl})}{\sqrt{\text{Cov}(y_{ij}, y_{ij})}\sqrt{\text{Cov}(y_{kl}, y_{kl})}}\end{aligned}$$

Thus

$$\rho(y_{ij}, y_{kl}) = \begin{cases} \frac{\tau^2}{\tau^2 + \sigma^2} & \text{if } i = k \\ 0 & \text{if otherwise} \end{cases}$$

If we specify (e.g.) 4 subjects, with  $N = 15$  observations, ( $n_1 = 4, n_2 = 4, n_3 = 3, n_4 = 4$ ), i.e. an unbalanced randomized block design; using the example from before with random effects on the intercept parameter only, we can show the form of the variance-covariance matrix more explicitly. Thus, (again suppressing subscript  $j$  for convenience):

$$\mathbf{Y}_i = \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\mathbf{b}_i + \boldsymbol{\epsilon}_i$$

$$\boldsymbol{\beta} = [\beta_0, \beta_1]'$$

$$\mathbf{b}_i = [b_{0i}].$$

Assuming  $\text{Var}(\boldsymbol{\epsilon}_i) \sim N(0, \mathbf{R})$  where

$$\mathbf{R} = \sigma_e^2 \mathbf{I}$$

$$\mathbf{R} = \begin{bmatrix} \sigma_e^2 & \dots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \dots & \sigma_e^2 \end{bmatrix}_{(15 \times 15)}$$

and

$$\mathbf{ZDZ}' =$$

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \end{bmatrix}_{15 \times 4} \begin{bmatrix} \sigma_s^2 & 0 & 0 & 0 \\ 0 & \sigma_s^2 & 0 & 0 \\ 0 & 0 & \sigma_s^2 & 0 \\ 0 & 0 & 0 & \sigma_s^2 \end{bmatrix}_{4 \times 4} \begin{bmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \end{bmatrix}'_{4 \times 15}$$

Thus

$$\mathbf{ZDZ}' + \mathbf{R} = \begin{bmatrix} \begin{bmatrix} \sigma_s^2 + \sigma_e^2 & \dots & \sigma_s^2 \\ \vdots & \ddots & \vdots \\ \sigma_s^2 & \dots & \sigma_s^2 + \sigma_e^2 \end{bmatrix}_{4 \times 4} & \dots & \begin{bmatrix} 0 & \dots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \dots & 0 \end{bmatrix} \\ \vdots & \ddots & \vdots \\ \begin{bmatrix} 0 & \dots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \dots & 0 \end{bmatrix} & \dots & \begin{bmatrix} \sigma_s^2 + \sigma_e^2 & \dots & \sigma_s^2 \\ \vdots & \ddots & \vdots \\ \sigma_s^2 & \dots & \sigma_s^2 + \sigma_e^2 \end{bmatrix}_{4 \times 4} \end{bmatrix}$$

We have thus shown the overall model formulation for this particular case, with the focus on the hierarchical covariance structure.

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## 2.5 Structural Model Formulation

Before continuing with the development of the theory behind nonlinear mixed effect models and the various extensions to the general techniques required for the analysis of our particular data set, we need to introduce the basic structural model formulation, and in so doing, the terminology used in the remainder of this chapter.

### 2.5.1 Background

In the traditional clinical approach to the modeling of pharmacokinetic data, linear compartment models form the basis for the structural model form.

In compartmental modeling, the input and disposition of “substances in the body are assumed to occur in a series of consecutive processes or steps” [14, 42], and the body is thus divided into a series of compartments (not representative of “real physical spaces” [7]), where the rates of transfer of the drug from one compartment to another are assumed to follow a first order process, (i.e. the rate is proportional to the amount of drug in the system), [2, 14].

These “compartmental” models are described by L.Aarons in the British Journal of Clinical Pharmacology [7] as semi-mechanistic, in that although the parameters are relatively easily related to physiological processes, and are thus easier to interpret, the assumption of compartments is a simplification of the biological system, and is in fact referred to as an “abstraction” by Wagner et al, [14], necessary for the development of practical mathematical relationships which can be used to describe the kinetics of the drug’s movement, [14].

The model itself is given by the equation or set of equations which describes the system, and the one and two compartment open models for oral absorption may be represented using schematic diagrams, from which the differential equations and resulting integrated solutions may be derived. The input, distribution and elimination rates are all assumed to be first order.

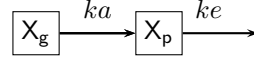
An alternative and more comprehensive approach would be to use the so-called physiologically based pharmacokinetic model (PBPK), wherein the compartments represent actual tissue and organ spaces with real physical volumes [7]. The uptake and elimination of substances may be linked to specific organs, and drivers of uptake and absorption such as the perfusion-rate limitation of different tissues may also be incorporated [7]. These models may be described by a series of differential equations. The use of these models is, however, limited by a lack of sufficiently complex quality data.

### 2.5.2 Semi-Mechanistic: Schematics and Derivation

The section below deals with the derivation of the equations used for the semi-mechanistic one- and two-compartmental models introduced above. Although these models are not those employed in the majority of the analyses presented in this thesis, they are described here for the purposes of contrast and comparison.

## One Compartment Model

The schematic for the one compartment model with oral absorption is given by, [2, 14]:



where  $X_g$  is the amount of drug in the gastro-intestinal tract immediately following administration, and  $X_p = V \times C_p$  is the amount of drug in the central compartment, where  $V$  is the volume of distribution (in Litres) and  $C_p$  (usually in  $ug/ml$  or  $ng/ml$ ) is the concentration of the drug. The rates of absorption and elimination are given by  $ka$  and  $ke$  respectively, both measured in units of reciprocal time (usually 1/hour).

Both  $ka$  and  $ke$  are assumed to be first order process, despite the oral absorption and resultant possible issues with dissolution and possible multiple absorption sites, [2, 43].

This model assumes that there is rapid equilibrium between the drug in the blood/plasma, and the surrounding extra vascular tissues, i.e. that the drug is immediately and evenly distributed, such that the body is made up of a single “well-mixed” (central) compartment.

Using this schematic, we can define differential equations for  $X_g$  and  $X_p$ , [2, 14] such that:

$$\begin{aligned} \frac{dX_g}{dt} &= -ka \times X_g \\ \frac{dX_p}{dt} &= ka \times X_g - ke \times X_p. \end{aligned}$$

Then taking the Laplace transformations we get:

$$\begin{aligned} L\left(\frac{dX_g}{dt}\right) &= s \times \bar{X}_g - X_g^0 = -ka \times \bar{X}_g \\ (s + ka)\bar{X}_g &= X_g^0. \end{aligned}$$

Thus

$$\bar{X}_g = \frac{X_g^0}{(s + ka)} \quad (2.1)$$

and:

$$\begin{aligned} L\left(\frac{dX_p}{dt}\right) &= s \times \bar{X}_p - X_p^0 = ka \times \bar{X}_g - ke \times \bar{X}_p \\ L\left(\frac{dX_p}{dt}\right) &= L\left(\frac{V \times dC_p}{dt}\right) = s \times V \times \bar{C}_p - V \times C_p^0 \\ &= ka \times \bar{X}_g - ke \times (V \times \bar{C}_p). \end{aligned}$$

We therefore have:

$$\begin{aligned} V \times \bar{C}_p \times (s + ke) &= ka \times \bar{X}_g \\ V \times \bar{C}_p &= \frac{ka \times \bar{X}_g}{(s + ke)} \end{aligned}$$

and substituting 2.1:

$$V \times \bar{C}_p = \frac{ka \times F \times D}{(s + ka) \times (s + ke)}$$

$$\bar{C}_p = \frac{ka \times F \times D}{V \times (s + ka) \times (s + ke)}$$

where  $X_g^0 = F \times D$  and  $F$  is the fraction of dose  $D$  absorbed,  $X_p = V \times C_p$  and  $C_p^0 = 0$  at time  $t = 0$ .

The general partial fraction (fingerprint) method is given by equation:

$$L^{-1}\left(\frac{N(s)}{D(s)}\right) = \sum_{i=1}^n \frac{N(\lambda_i)}{D(\lambda_i)} \times e^{\lambda_i \times t}$$

where the  $\lambda$  terms are the roots of the polynomial term in the denominator on the left, and with limitations that the degree of the denominator is higher than that of the numerator, with no repeating terms, we can backtransform in order to get the concentration of drug in the central compartment,  $C_p$ .

Thus, for:

$$\bar{C}_p = \frac{ka \times F \times D}{V \times (s + ka) \times (s + ke)},$$

the roots of the denominator are  $-ka$  and  $-ke$ , and:

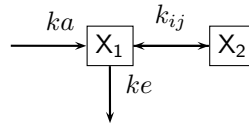
$$L^{-1}(\bar{C}_p) = C_p = \frac{ka \times F \times D}{V \times (ke - ka)} \times e^{-ka \times t} + \frac{ka \times F \times D}{V \times (ka - ke)} \times e^{-ke \times t}$$

Therefore:

$$C_p(t) = \frac{ka \times F \times D}{V \times (ka - ke)} \times [e^{-ke \times t} - e^{-ka \times t}]. \quad (2.2)$$

## Two Compartment Model

For the two compartment oral absorption model with  $i, j = 1, 2$ , the schematic is as follows:



Solution of the resulting differential equations makes use of both the partial fraction method outlined above, and convolution theory, [14].

The two compartment model allows for the specification of both a central and peripheral compartment, (the assumption of rapid and immediate distribution between the plasma and surrounding tissues is deemed to be overly simplistic), [14].

While exact anatomic assignment to these compartments is not possible, those tissues most rapidly perfused are assumed to be associated with the central compartment, [14].

Input, disposition and elimination rates are still assumed to be of first order, and further, terminal elimination is assumed to occur from the central compartment only, [14].

The anticipated number of phases of decline and thus the correct compartment model may be determined by examination of plots of the logged concentration over time, such as those in Figure 2.2: should the plot be linear, the decline is determined to be monophasic, whereas an additional deviation (i.e. a secondary altered rate of decline), would indicate the presence of an additional compartment, [2, 7, 14].

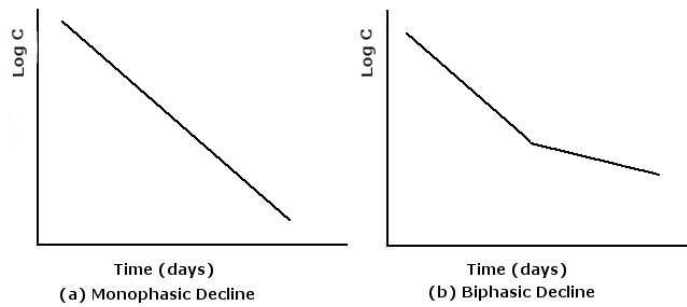
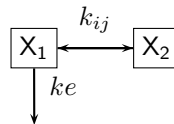


Figure 2.2: Mono- and Biphasic Decline: Log-Linear Scale

The derivation of the equation for the two compartment model is done in two stages, as described by Wagner [14].

Stage 1 determines the amount of drug in the central compartment ( $X_1$ ) following an *IV Bolus* dose, i.e. for the second schematic ( $i, j = 1, 2$ ):



The differential equations for the amount of drug in  $X_1$  and  $X_2$  are as follows:

$$\begin{aligned} \frac{dX_1}{dt} &= k_{21} \times X_2 - k_{12} \times X_1 - ke \times X_1 \\ \frac{dX_2}{dt} &= k_{12} \times X_1 - k_{21} \times X_2 \end{aligned}$$



Taking the Laplace transformation of the equations:

$$\begin{aligned} L\left(\frac{dX_1}{dt}\right) &= s \times \bar{X}_1 - X_1^0 = k_{21} \times \bar{X}_2 - k_{12} \times \bar{X}_1 - ke \times \bar{X}_1 \\ L\left(\frac{dX_2}{dt}\right) &= s \times \bar{X}_2 - X_2^0 = k_{12} \times \bar{X}_1 - k_{21} \times \bar{X}_2 \end{aligned}$$

and rearranging, given that  $X_1^0 = F \times D$  where  $F = 1$ , and  $X_2^0 = 0$ :

$$\begin{aligned} (s + k_{12} + ke) \times \bar{X}_1 - k_{21} \times \bar{X}_2 &= D \\ (s + k_{21}) \times \bar{X}_2 - k_{12} \times \bar{X}_1 &= 0 \end{aligned}$$

Now applying Cramer's Rule,  $\bar{X}_1$  is given by:

$$\begin{aligned} \bar{X}_1 &= \frac{|\text{determinant obtained by replacing 1st column by column of constants}|}{|\text{determinant of system}|} \\ &= \frac{\begin{vmatrix} D & -k_{21} \\ 0 & (s + k_{21}) \end{vmatrix}}{\begin{vmatrix} (s + k_{12} + ke) & -k_{21} \\ -k_{12} & (s + k_{21}) \end{vmatrix}} \end{aligned}$$

Thus:

$$\bar{X}_1 = \frac{D \times (s + k_{21})}{(s + \alpha) \times (s + \beta)}$$

where

$$\begin{aligned} \alpha &= 1/2 \times [(k_{12} + k_{21} + ke) + \sqrt{(k_{12} + k_{21} + ke)^2 - 4 \times k_{21} \times ke}] \\ \beta &= 1/2 \times [(k_{12} + k_{21} + ke) - \sqrt{(k_{12} + k_{21} + ke)^2 - 4 \times k_{21} \times ke}] \end{aligned}$$

Stage 2 uses the principle of convolution. Defining  $in_s$  as the Laplace transform of the input function for a given compartment, and  $d_s$  as the Laplace transform of the disposition function:

$$\bar{X}_1 = in_s \times d_s$$

For a two compartment model with oral absorption,

$$\begin{aligned} in_s &= \frac{F \times D \times ka}{(s + ka)}, \\ d_s &= \frac{(s + k_{21})}{(s + \alpha) \times (s + \beta)} \end{aligned}$$

Thus

$$\bar{X}_1 = \frac{F \times D \times ka \times (s + k_{21})}{(s + ka) \times (s + \alpha) \times (s + \beta)}$$

Taking the inverse, using the partial fraction method, we get:

$$\begin{aligned} C_1(t) &= \frac{ka \times F \times D}{V_1} \times \left[ \frac{k_{21} - \alpha}{(ka - \alpha) \times (\beta - \alpha)} \times \exp(-\alpha \times \text{time}) \right. \\ &\quad + \frac{k_{21} - \beta}{(ka - \beta) \times (\alpha - \beta)} \times \exp(-\beta \times \text{time}) \\ &\quad \left. + \frac{k_{21} - ka}{(\alpha - ka) \times (\beta - ka)} \times \exp(-ka \times \text{time}) \right] \end{aligned} \quad (2.3)$$

where  $\alpha$  and  $\beta$  are defined as before, for  $X_1 = V_1 \times C_1$ .

### 2.5.3 Empirical Approach

The clinical parameterization derived and outlined above, that of the semi-mechanistic compartmental model, is based on assumptions regarding the actual (albeit simplified) underlying processes of drug absorption and disposition.

This can be simplified by expressing the concentration-times curves as an additive series of exponential terms, such that the concentration at time  $t$  is given by:

$$C(t) = \sum_{i=1}^n C_i \times e^{(-\lambda_i t)}$$

where the  $\lambda_i$  are the rate constants,  $t = \text{time}$ , the number of terms ( $i = 1, \dots, n$ ) is determined by the number of differential phases of decline, and the coefficient of the exponential term related to the absorption phase is negative.

This model specification reasonably describes the “typical concentration-time [curves]”, resulting in profiles that are roughly equivalent to those obtained via the compartmental approach [7]. The parameters themselves however have no direct physiological interpretation, although pharmacokinetic parameters of interest and dosage regimens may easily be determined.

Thus for example, observing a monophasic decline on the log-linear scale, the response may be expressed as:

$$C(t) = \beta_0[-\exp(-\beta_1 \times \text{time}) + \exp(-\beta_2 \times \text{time})], \quad (2.4)$$

where  $\beta_0$  gives an indication of the range or level of concentrations reached, and  $\beta_1$  and  $\beta_2$  are the slopes of the incline and decline phases respectively.

Comparing this model to that previously defined by equation 2.2: the composite parameter  $\beta_0$  may be expressed as:

$$\beta_0 = \frac{F \times D \times ka}{V(ka - ke)}$$

and  $\beta_1$  and  $\beta_2$  as  $ka$  and  $ke$  respectively.

Similarly, for a curve displaying a biphasic log-linear profile, the concentration may be expressed as :

$$C(t) = A_1 \times \exp(-\alpha \times \text{time}) + A_2 \times \exp(-\beta \times \text{time}) + A_3 \times \exp(-k \times \text{time})$$

where (relating this model formulation back to the clinical specification in equation 2.3):

$$A_1 = \frac{k \times F \times D}{V_1} \times \left[ \frac{k_{21} - \alpha}{(k - \alpha) \times (\beta - \alpha)} \right]$$

$$A_2 = \frac{k \times F \times D}{V_1} \times \left[ \frac{k_{21} - \beta}{(k - \beta) \times (\alpha - \beta)} \right]$$

$$A_3 = \frac{k \times F \times D}{V_1} \times \left[ \frac{k_{21} - k}{(\alpha - k) \times (\beta - k)} \right]$$

Here,  $k = ka$ , the absorption rate constant, and  $\alpha$  and  $\beta$ , defined as before, represent the initial slope of the curve, corresponding to the distribution phase, and the slope for the final or terminal elimination phase respectively.

Further, in order to reduce collinearity and achieve a steeper slope for the incline, using  $A_1 \approx -(A_2 + A_3)$  and defining:

$$\begin{aligned}\beta_1 &= ka, \\ \beta_2 &= A_1 \\ \beta_3 &= \alpha, \\ \beta_4 &= A_2 \\ \beta_5 &= \beta\end{aligned}$$

we can define the model:

$$\begin{aligned}C(t) &= \beta_2 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_3 \times time)] \\ &+ \beta_4 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_5 \times time)]\end{aligned}\quad (2.5)$$

where  $\beta_3$  and  $\beta_5$  represent the initial and final slope of the decline,  $\beta_2$  and  $\beta_4$  combined represent the overall level of concentration reached, [3].  $\beta_1$  represents the slope for the incline.

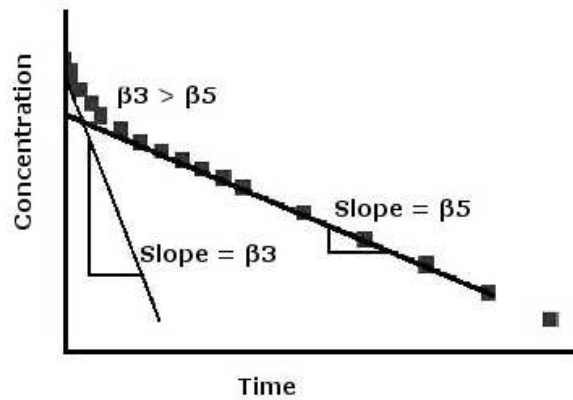


Figure 2.3: Triple-Exponential Curve (Decline only) [2]

Ignoring the absorption phase of the curve, Figures 2.3 and 2.4 illustrate the change in slope from the initial to the final phase of decline for the triple exponential model. Figure 2.4, [3], illustrates the final combined “elimination” curve (given by equation 2.6 below) and its constituent exponential parts, with  $\beta_2, \beta_4, \beta_3$  and  $\beta_5$  labeled.

$$C(t) = \beta_2 \times [\exp(-\beta_3 \times time)] + \beta_4 \times [\exp(-\beta_5 \times time)]\quad (2.6)$$

The two sets of parameters given by  $(\beta_2, \beta_3)$  and  $(\beta_4, \beta_5)$  are described as “exchangeable” by Pinheiro and Bates [3], in that the values of the pairs can be

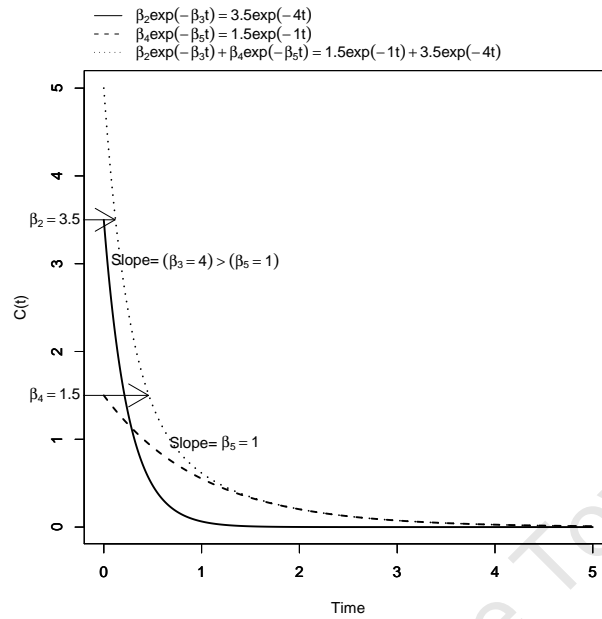


Figure 2.4: Bi-Exponential Decline of Triple-Exponential Curve (Absorption Phase Ignored) [3]

exchanged without altering the predicted value of  $C(t)$ . Requiring that  $\beta_3$  be greater than  $\beta_5$  creates an “identifiable” parameterization, [3].

The empirical modeling approach is limited in terms of extrapolation: it is difficult to determine the impact of underlying physiological changes on the parameters as specified, and thus the semi-mechanistic models are usually favoured from a pharmacokinetic perspective.

The more general parameterization is however preferred for use in this thesis, as the focus was on the smoothing and interpolation of the observed concentration-time curves, and the methodologies behind the use of non-linear mixed effect models for a multi-level design.

## 2.6 Nonlinear Mixed Effect Models

Now that the terminology for the structural model form has been introduced, we may proceed with the theoretical development of mixed effect modeling procedures, moving on to the nonlinear case for both single and multiple levels of nesting.

Similar to the development of the linear mixed effect model, the nonlinear model (with a single level of subject-specific random effects) may also be defined in two stages: the first stage again encompassing the structural model form, and the modeling of the intra-individual variance, and the second stage examining the inter-individual variability, and modeling it in terms of the impact of covariates on structural parameters. The extension to multiple levels and then to the case where we have multiple responses follows from there.

The methods outlined here are modifications of those presented in *Mixed Effect Models in S and S-Plus* by Pinheiro and Bates [3], and *Nonlinear Models for Repeated measurement Data*, by Davidian and Giltinan, [6].

### Hierarchical Model Development

#### Stage 1: Intra-individual (within-subject)

Ignoring the multiple levels of nesting (i.e. ignoring the occasion-specific random effect), and looking at  $j = 1, \dots, n_i$  observations on each of  $i = 1, \dots, m$  individuals, such that we have a total of  $N = \sum_{i=1}^m n_i$  available data values, we can then define a model for the  $j$ th response as:

$$y_{ij} = f(\mathbf{x}_{ij}, \boldsymbol{\beta}_i) + e_{ij} \quad (2.7)$$

where  $e_{ij}$  is a random error term, given individual  $i$ , with  $E(e_{ij}|\boldsymbol{\beta}_i) = 0$ .

The function  $f(\mathbf{x}_{ij}, \boldsymbol{\beta}_i)$  could be any linear/non-linear function, although for our purposes it follows one of the exponential forms previously identified (equations 2.4 and 2.5).

This function, which gives the structural form of the model, governs the within-individual behaviour through characterization of the systematic variation by a  $(p \times 1)$  vector of parameters  $\boldsymbol{\beta}_i$  which is specific to each individual  $i$ . It models the inter-subject variability in terms of the impact of covariates  $\mathbf{x}_{ij}$  on structural parameters.

The data for the  $i$ th individual may be summarized as:

$$\mathbf{y}_i = f(\boldsymbol{\beta}_i) + \mathbf{e}_i$$

where

$$\mathbf{y}_i = [y_{i1}, \dots, y_{in_i}]'$$

$$\mathbf{e}_i = [e_{i1}, \dots, e_{in_i}]'$$

and

$$f_i(\boldsymbol{\beta}_i) = [f(\mathbf{x}_{i1}, \boldsymbol{\beta}_i), \dots, f(\mathbf{x}_{in_i}, \boldsymbol{\beta}_i)]'$$

Then

$$E(\mathbf{e}_i|\beta_i) = 0,$$

$$Cov(\mathbf{e}_i|\beta_i) = \mathbf{R}(\beta, \xi)$$

where  $\mathbf{R}(\beta, \xi)$  is a covariance matrix which allows for the relaxation of the usual classical assumptions that the residual error terms  $e_{ij}$  are i.i.d normally distributed with mean zero and constant variance  $\sigma^2$ , ( $e_{ij} \sim i.i.d N(0, \sigma^2)$ ), where  $\xi$  is a function of  $\sigma$ , some vector of additional variance parameters  $\theta$  and a vector of correlation parameters  $\alpha$ . This is explained in more detail for the case where we have multiple levels of nesting.

### Stage 2: Inter-individual (between-subject)

Now specifying a model for the  $\beta_i$ , allowing for dependence on both systematic and random effects;

$$\beta_i = \mathbf{A}_i\beta + \mathbf{b}_i,$$

Where  $\mathbf{A}_i$  is an indicator matrix in the case of group effects but can also contain other types of covariate values, and  $\beta$  is a vector of fixed parameters common to all individuals.

More generally, allowing the random effects  $\mathbf{b}_i$  to be added to some parameters and not necessarily to others:

$$\beta_i = \mathbf{A}_i\beta + \mathbf{B}_i\mathbf{b}_i,$$

where  $\mathbf{B}_i$  is an indicator matrix, or;

$$\beta_i = \mathbf{d}(\mathbf{a}_i, \beta, \mathbf{b}_i),$$

The  $p$ -dimensional vector  $\mathbf{d}$  characterizes how the elements of  $\beta_i$  vary across individuals, allowing for both (i) systematic and (ii) random variation:

- (i) systematic variation is accounted for through systematic association with  $\mathbf{a}_i$ , an  $(a \times 1)$  vector of covariates for the  $i$ th subject, modeled through  $\beta$ , a vector of fixed parameters common to all individuals, and
- (ii) random (unexplained) variation in the population is modeled through the  $q$ -dimensional vector of random effects  $\mathbf{b}_i$ , where the  $\mathbf{b}_i$  are i.i.d and often,  $\mathbf{b}_i \sim (0, \psi)$ , where  $\psi$  is a  $(q \times q)$  covariance matrix.

The linear mixed effects model is a simplified version of the models presented, where  $\beta$  and  $\mathbf{b}_i$  are defined as before,  $\mathbf{X}_i$  is a design matrix for individual  $i$ , and  $\mathbf{Z}_i$  is a design matrix linking the random effects to the response.

Since  $\beta_i$  is specific to individuals through  $\mathbf{b}_i$  and the known individual characteristics  $\mathbf{a}_i$ ,

$$\mathbf{e}_i|\mathbf{b}_i \sim (0, \mathbf{R}_i(\beta, \xi))$$

A new vector of parameters,  $\varpi$ , may then be constructed, consisting of both the intra-individual parameter  $\xi$ , and the distinct elements of the inter-individual

covariate matrix  $\psi$ .

For the structural form dictated by equation 2.4, i.e. that of:

$$y_{ij} = \beta_{0i}(-e^{(-\beta_{1i} \times t_{ij})} + e^{(-\beta_{2i} \times t_{ij})}) + e_{ij},$$

$$\beta_i = \beta + \mathbf{b}_i$$

where  $\beta = [\beta_0, \beta_1, \beta_2]'$ , and  $\mathbf{b}_i = [b_{0i}, b_{1i}, b_{2i}]'$ .

For example, where we have subject-specific random effects on parameters  $\beta_0$  and  $\beta_1$  only, we could define:

$$A = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

and:

$$B = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

such that:

$$\beta_i = \mathbf{A}_i \beta + \mathbf{B}_i \mathbf{b}_i$$

can be written as:

$$\begin{bmatrix} \beta_{0i} \\ \beta_{1i} \\ \beta_{2i} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} b_{0i} \\ b_{1i} \\ b_{2i} \end{bmatrix}.$$

Alternatively, incorporating a subject-specific continuous covariate such as age (time-invariant) on parameters  $\beta_0$  and  $\beta_1$ , we could define:

$$A = \begin{bmatrix} 1 & 0 & 0 & age_i & 0 \\ 0 & 1 & 0 & 0 & age_i \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix}$$

and:

$$B = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

such that:

$$\beta_i = \mathbf{A}_i \beta + \mathbf{B}_i \mathbf{b}_i$$

can be written as:

$$\begin{bmatrix} \beta_{0i} \\ \beta_{1i} \\ \beta_{2i} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & age_i & 0 \\ 0 & 1 & 0 & 0 & age_i \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} b_{0i} \\ b_{1i} \\ b_{2i} \end{bmatrix}$$

where  $(\beta_0, \beta_3)$ ,  $(\beta_1, \beta_4)$  are the “intercept” and “slope” parameters respectively that determine the linear relationship between  $\beta_0$ ,  $\beta_1$  and age.  $\beta_2$  is defined as before. This example also assumes random (subject-specific) effects on  $\beta_0$  and  $\beta_1$  only.

### Extension to Multi-Level

Extension to the case where there are multiple levels of nested random effects (occasion within subject) is more easily achieved with slight changes to the notation used in the specification of a single level mixed effect model. Thus, redefining the model represented by equation 2.7 for the  $j$ th response on the  $i$ th individual:

$$y_{ij} = f(\mathbf{x}_{ij}, \boldsymbol{\beta}_{ij}) + e_{ij}$$

where  $i = 1, \dots, m$ ,  $j = 1, \dots, n_i$ ,  $f$  and  $x_{ij}$  are defined as before, and

$$\boldsymbol{\beta}_{ij} = \mathbf{A}_{ij}\boldsymbol{\beta} + \mathbf{B}_{ij}\mathbf{b}_i,$$

where the dependence of  $\mathbf{A}_{ij}$ ,  $\mathbf{B}_{ij}$  and thus  $\boldsymbol{\beta}_{ij}$  on  $j$  now allows for the incorporation of time-varying covariates.

Then for the  $i$ th individual:

$$\mathbf{y}_i = f(\mathbf{x}_i, \boldsymbol{\beta}_i) + \mathbf{e}_i$$

where

$$\begin{aligned}\mathbf{y}_i &= [y_{i1}, \dots, y_{in_i}]', \\ \mathbf{e}_i &= [e_{i1}, \dots, e_{in_i}]', \\ \boldsymbol{\beta}_i &= [\beta_{i1}, \dots, \beta_{in_i}]'\end{aligned}$$

and

$$f(\mathbf{x}_i, \boldsymbol{\beta}_i) = [f(\mathbf{x}_{i1}, \boldsymbol{\beta}_{i1}), \dots, f(\mathbf{x}_{in_i}, \boldsymbol{\beta}_{in_i})]'$$

Now defining the period (occasion determined by pregnancy status)  $k = 1, 2$ , we thus specify the model for the  $j$ th measurement in the  $k$ th period for the  $i$ th individual as:

$$\begin{aligned}y_{ikj} &= f(\mathbf{x}_{ikj}, \boldsymbol{\beta}_{ikj}) + e_{ikj} \\ i &= 1, \dots, m, k = 1, \dots, n_i, j = 1, \dots, n_{ik}\end{aligned}$$

where  $m$  is the number of individuals,  $n_i$  is the number of periods for the  $i$ th individual, and  $n_{ik}$  is the number of observations in the  $k$ th period for the  $i$ th individual.

The  $(p \times 1)$  vector of regression parameters  $\boldsymbol{\beta}_{ikj}$  is then defined as:

$$\boldsymbol{\beta}_{ikj} = \mathbf{A}_{ikj}\boldsymbol{\beta} + \mathbf{B}_{i,kj}\mathbf{b}_i + \mathbf{B}_{ik,j}\mathbf{b}_{ik},$$

$$\mathbf{b}_i \sim N(\mathbf{0}, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(\mathbf{0}, \boldsymbol{\psi}_2)$$

Where  $\boldsymbol{\beta}$  is a  $p$ -dimensional vector of fixed effects, with design matrix  $\mathbf{A}_{ikj}$  which may incorporate time-varying covariates. The first level (subject-specific) random effects  $\mathbf{b}_i$  are independently distributed  $q_1$ -dimensional vectors with variance-covariance matrix  $\boldsymbol{\psi}_1$ . The second level (occasion-specific) random effects  $\mathbf{b}_{ik}$  are independently distributed  $q_2$ -dimensional vectors with variance-covariance matrix  $\boldsymbol{\psi}_2$ , assumed independent of the first level random effects.

The random effects design matrices  $\mathbf{B}_{i,kj}$  and  $\mathbf{B}_{ik,j}$  depend on first and second level groups and possibly on the values of some covariates at the  $j$ th observation.



In the sequential modeling approach (which is explained in more detail later), the response or concentration of one drug is modeled as a function of the other, incorporating the predicted *time-varying* concentration of the other drug as a covariate. The dependence on  $j$  is thus required.

The within group errors  $e_{ikj}$  are assumed independently and identically normally distributed, with variance-covariance matrix  $\mathbf{R}_{\mathbf{ik}}$ .

As before, for the particular case represented by equation 2.4, looking at the model for the  $j$ th concentration in the  $k$ th period for the  $i$ th individual:

$$\begin{aligned} y_{ikj} &= f(\mathbf{x}_{\mathbf{ikj}}, \boldsymbol{\beta}_{\mathbf{ikj}}) + e_{ikj} \\ &= \beta_{0ikj}(-e^{-\beta_{1ikj} \times \mathbf{x}_{\mathbf{ikj}}} + e^{-\beta_{2ikj} \times \mathbf{x}_{\mathbf{ikj}}}) + e_{ikj} \end{aligned}$$

where the only covariate is time, i.e.  $\mathbf{x}_{\mathbf{ikj}} = time_{ikj}$ , we can expand the previously specified model (suppressing subscript  $j$  for convenience) as follows:

$$\begin{aligned} \beta_{0ik} &= \beta_0 + b_{0ik}, \\ \beta_{0i} &= \beta_0 + b_{0i} \end{aligned}$$

$$\begin{aligned} \beta_{1ik} &= \beta_1 + b_{1ik}, \\ \beta_{1i} &= \beta_1 + b_{1i} \end{aligned}$$

$$\begin{aligned} \beta_{2ik} &= \beta_2 + b_{2ik}, \\ \beta_{2i} &= \beta_2 + b_{2i}, \end{aligned}$$

i.e. in composite form

$$y_{ikj} = (\beta_0 + b_{0i} + b_{0ik})(-e^{-(\beta_1 + b_{1i} + b_{1ik}) \times time_{ikj}} + e^{-(\beta_2 + b_{2i} + b_{2ik}) \times time_{ikj}}) + e_{ikj}.$$

We can illustrate the incorporation of covariates as follows:

Fitting subject-specific covariate age (time and occasion (period)-invariant) to  $\beta_0$  would result in equations:

$$\begin{aligned} \beta_{0ik} &= \beta_0 + \beta_3 \times age_{i..} + b_{0i} + b_{0ik}, \\ \beta_{1ik} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ik} &= \beta_2 + b_{2i} + b_{2ik} \end{aligned}$$

Fitting time-invariant covariate weight (or equivalently pregnancy) which varies by occasion within subject, would result in equations:

$$\begin{aligned} \beta_{0ik} &= \beta_0 + \beta_{3k} \times weight_{ik.} + b_{0i} + b_{0ik}, \\ \beta_{1ik} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ik} &= \beta_2 + b_{2i} + b_{2ik} \end{aligned}$$

Re-introducing subscript  $j$ , and specifying both subject and occasion specific random effects on the intercept parameter only (in the linear relationship between  $\beta_0$  and the covariate), we could fit a time-varying covariate such as in the

sequential modeling approach using equations:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_{3kj} \times \text{concentration}_{ikj} + b_{0i} + b_{0ik}, \\ \beta_{1ikj} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ikj} &= \beta_2 + b_{2i} + b_{2ik}\end{aligned}$$

### General intra-individual, intra-occasion covariance structures

Individual non-linear data quite often exhibits a distinct departure from the classical OLS assumptions of independence and homoskedasticity imposed on the  $e_{ikj}$ , (previously the  $e_{ij}$ ), indicating a need for a generalization of this traditional approach.

This section deals with the description of the variance-covariance matrix  $\mathbf{R}_{ik}$  for the within group errors  $e_{ikj}$ .

- Variance heterogeneity

In PK data specifically, observations are often serially correlated over time, normality assumptions do not necessarily hold, and variance is often proportional to the square of the mean response, i.e. a log normal distribution is assumed, resulting in error terms which follow a gamma distribution with a mean of zero and variance:

$$\text{Var}(e'_{ikj}) = \sigma^2 \times (f(\mathbf{x}_{ikj}, \boldsymbol{\beta}))^2, \quad (2.8)$$

where  $\sigma$  is the coefficient of variation.

We could thus specify a model of the form:

$$\log(y_{ikj}) = \log(f(\mathbf{x}_{ikj}, \boldsymbol{\beta}_{ikj})) + e_{ikj}$$

which is alternatively expressed as:

$$y_{ikj} = f(\mathbf{x}_{ikj}, \boldsymbol{\beta}_{ikj}) \times e'_{ikj}$$

where  $e'_{ikj} = \exp(e_{ikj})$ , and thus  $e_{ikj} \sim N(0, \sigma^2)$ . This would also alter the linear relationship we saw before for the subject and occasion-specific random effects, such that for example (suppressing subscript j):

$$\beta_{0ik} = \beta_0 \times b_{0i} \times b_{0ik},$$

as well as changing the additive (linear) relationship between the parameters and the covariates.

The approach taken here is slightly different, in that a model is explicitly specified for the characterization of the systematic response variance and correlation patterns. It is referred to as a ‘‘band-aid’’ [6] approach, in that it only approximates the proportional error structure usually required for data of this kind.

This is detailed below for the general case, but because the same variance function is applied for both occasions (periods) in this thesis, only subscript  $j$  is

specified, which represents observations for the  $k$ th period for the  $i$ th individual.

Thus for a specific individual-occasion grouping, we can define a variance function  $g$ , which is dependent on the vector of regression parameters,  $\boldsymbol{\beta}$ , for that individual through the mean function  $f$ , on constants  $\mathbf{z}_j$ , which may include some or all of  $\mathbf{x}_j$  and on an additional  $q$ -dimensional parameter vector  $\boldsymbol{\theta}$ :

$$E(y_j) = \mu_j = f(\mathbf{x}_j, \boldsymbol{\beta}),$$

$$Var(y_j) = \sigma^2 g^2(\mu, \mathbf{z}_j, \boldsymbol{\theta})$$

In the above specification, the variance function  $g$  provides the general form of the variance model, with parameter  $\boldsymbol{\theta}$  fully specifying the functional form. The scale parameter  $\sigma$  governs the overall level of precision in the response.

An example of a variance function and in fact the function applied later on follows the general form of:

$$g^2(\mu, \mathbf{z}_j, \boldsymbol{\theta}) = (\theta_1 + \mu_j^{\theta_2})^2, \theta_1, \theta_2 > 0,$$

where  $\mu_j$  is the expected value of the  $j$ th response and  $\boldsymbol{\theta} = [\theta_1, \theta_2]$ , which allows for measurement error that appears constant at lower response values and exhibits increasing variance at higher levels of the response, i.e. approximately proportional errors. This form is preferred to the power function specified above (equation 2.8), because of the large number of fitted values at or close to zero in our data.

- Intra-individual correlation

An assumed correlation structure  $\boldsymbol{\Gamma}(\boldsymbol{\alpha})$  is also specified, where the correlation matrix  $\boldsymbol{\Gamma}(\boldsymbol{\alpha})$  is a function of a vector of correlation parameters  $\boldsymbol{\alpha}_{(s \times 1)}$ .

In some instances, both heterogeneous variance and a correlation pattern are evident.

For any individual-occasion grouping  $ik$ , we can then define the diagonal matrix  $G$ , where the elements are the same variance function previously defined, which depends on individually specific information ( $\mathbf{z}_j$ ) and on an individual mean response ( $\mu_j$ ), given  $\boldsymbol{\beta}_{ik}$ , i.e.,

$$\mathbf{G}(\boldsymbol{\beta}, \boldsymbol{\theta}) = \text{diag}[g^2(\mu_1, z_1, \boldsymbol{\theta}), \dots, g^2(\mu_{n_{ik}}, z_{n_{ik}}, \boldsymbol{\theta})]$$

If the correlation pattern is given by matrix  $\boldsymbol{\Gamma}(\boldsymbol{\alpha})$ , then assuming a common within-individual covariance pattern:

$$\text{Cov}(\mathbf{e}_{ij}) = \text{Cov}(\mathbf{e}),$$

we have:

$$\begin{aligned} \text{Cov}(\mathbf{e}) &= \sigma^2 \mathbf{G}^{(1/2)}(\boldsymbol{\beta}, \boldsymbol{\theta}) \boldsymbol{\Gamma}(\boldsymbol{\alpha}) \mathbf{G}^{(1/2)}(\boldsymbol{\beta}, \boldsymbol{\theta}) \\ &= \mathbf{R}(\boldsymbol{\beta}, \boldsymbol{\xi}) \end{aligned}$$

$$\boldsymbol{\xi} = [\sigma, \boldsymbol{\theta}', \boldsymbol{\alpha}'],$$

where  $\boldsymbol{\xi}$  is the  $(q_1 \times q_2 + s + 1) \times 1$  combined vector of all intra-individual parameters.

This implies that

$$\begin{aligned} \text{Var}(y_j) &= \sigma^2 g^2(\mu_j, \mathbf{z}_j, \boldsymbol{\theta}), \\ \text{Corr}(\mathbf{y}_1, \mathbf{y}_2) &= \boldsymbol{\Gamma}_{(j_1, j_2)}(\boldsymbol{\alpha}) \end{aligned}$$

Thus

$$e_{ik} | \boldsymbol{\beta}_{ik} \sim N(0, \mathbf{R}_{ik}(\boldsymbol{\beta}_{ik}, \boldsymbol{\xi}))$$

The above specification is general enough to accommodate both heterogeneous variance and within-subject correlations, where the functional form  $\mathbf{R}_{ik}$  is usually common to all subjects and is flexible enough to depend on covariates.

In matrix formulation: since we don't impose an additional correlation structure on our  $e_{ik}$ , (although we could), we essentially have an equal and exchangeable correlation structure such that:

$$\begin{aligned} \text{Cov}(\mathbf{e}_{ik})_{(n_{ik} \times n_{ik})} &= \\ &\left( \begin{array}{ccc} \frac{\sigma_1^2(\theta_1 + \mu_1^{\theta_2})^2}{\sigma_2 \sqrt{(\theta_1 + \mu_2^{\theta_2})^2} \times \sigma_1 \sqrt{(\theta_1 + \mu_1^{\theta_2})^2}} & \sigma_1 \sqrt{(\theta_1 + \mu_1^{\theta_2})^2} \times \sigma_2 \sqrt{(\theta_1 + \mu_2^{\theta_2})^2} & \dots \\ \sigma_2 \sqrt{(\theta_1 + \mu_2^{\theta_2})^2} \times \sigma_1 \sqrt{(\theta_1 + \mu_1^{\theta_2})^2} & \sigma_2^2(\theta_1 + \mu_2^{\theta_2})^2 & \dots \\ \vdots & \vdots & \ddots \end{array} \right). \end{aligned}$$

### Alternative approach to Nesting

Multilevel nesting can also be represented as a single level of grouping, using an explicitly specified correlation structure for the random effects that correctly accounts for the structure of the data. Computational algorithms used in the nlme package in R are however deemed more efficient for the nested approach.

The use of the alternative single-level representation of a multi-level model is best explained via an example:

Assuming that the model for the  $j$ th measurement in the  $k$ th period for the  $i$ th individual is given by equation:

$$y_{ikj} = \beta_{0ikj}(-e^{-\beta_{1ikj} \times \mathbf{x}_{ikj}} + e^{-\beta_{2ikj} \times \mathbf{x}_{ikj}}) + e_{ikj}$$

where (suppressing subscript  $j$  for convenience):

$$\begin{aligned} \beta_{0ik} &= \beta_{0i} + b_{0ik}, \\ \beta_{0i} &= \beta_0 + b_{0i} \\ \\ \beta_{1ik} &= \beta_{1i} + b_{1ik}, \\ \beta_{1i} &= \beta_1 + b_{1i} \end{aligned}$$

$$\begin{aligned}\beta_{2ik} &= \beta_{2i} + b_{2ik}, \\ \beta_{2i} &= \beta_2 + b_{2i}\end{aligned}$$

and:

$$\begin{aligned}e_{ikj} &\sim N(0, \mathbf{R}_{ik}) \\ b_i &\sim N\left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_0^2 & 0 & 0 \\ 0 & \tau_1^2 & 0 \\ 0 & 0 & \tau_2^2 \end{bmatrix}\right) \\ b_{ik} &\sim N\left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_{0k}^2 & 0 & 0 \\ 0 & \tau_{1k}^2 & 0 \\ 0 & 0 & \tau_{2k}^2 \end{bmatrix}\right)\end{aligned}$$

We can then define random effects  $b^*_i$ :

$$b^*_i = \begin{bmatrix} b_{0i} + b_{0i,0} \\ b_{0i} + b_{0i,1} \\ b_{1i} + b_{1i,0} \\ b_{1i} + b_{1i,1} \\ b_{2i} + b_{2i,0} \\ b_{2i} + b_{2i,1} \end{bmatrix}$$

where the variance-covariance matrix  $\boldsymbol{\psi}^*$  has a blocked diagonal structure, with a compound symmetry structure on the diagonals:

$$\begin{aligned}b^*_i &\sim N(\mathbf{0}, \boldsymbol{\psi}^*) \\ \boldsymbol{\psi}^* &= \begin{bmatrix} \begin{bmatrix} \tau_0^2 + \tau_{0k}^2 & \dots & \tau_0^2 \\ \tau_0^2 & \dots & \tau_0^2 + \tau_{0k}^2 \end{bmatrix} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \begin{bmatrix} \tau_1^2 + \tau_{1k}^2 & \dots & \tau_1^2 \\ \tau_1^2 & \dots & \tau_1^2 + \tau_{1k}^2 \end{bmatrix} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \begin{bmatrix} \tau_2^2 + \tau_{2k}^2 & \dots & \tau_2^2 \\ \tau_2^2 & \dots & \tau_2^2 + \tau_{2k}^2 \end{bmatrix} \end{bmatrix}\end{aligned}$$

The variance for the compound symmetry structure in the top-left block is then given by:

$$\tau_b^2 = \tau_0^2 + \tau_{0k}^2,$$

and the correlation is:

$$\rho = \frac{\tau_0^2}{\sqrt{\tau_0^2 + \tau_{0k}^2}}$$

Thus,

$$\tau_0^2 = \rho \times \tau_b^2,$$

and

$$\tau_{0k}^2 = \tau_b^2 - \tau_0^2.$$

### Extension to multiple responses:

Ordinarily in the context of pharmacokinetics, when we talk about the accommodation of multiple responses, we refer to the modeling of PK/PD data, undertaken in order to determine the concentration-effect relationship.

Sulfadoxine and Pyrimethamine are both folic acid antagonists which are simultaneously orally administered, acting in concert on different enzymes which are required for folate synthesis in the replication of the malaria parasites [4, 44].

Isobolograms, which look at the 50% inhibitory concentration ( $IC_{50}$ ) values for various SP concentrations have been developed in-vitro for different parasite genotypes, as mutations in the dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) genes result in resistance to the two drugs and therefore reduce their efficacy and increase the concentrations required to have an inhibitory effect, [4, 30].

The *in-vitro* relationship indicated by these isobolograms is an synergistic one: for a particular genotype, effective inhibitory concentrations can be achieved with low levels of one drug if combined with high levels of the other [4, 30], and the inhibition of parasite growth effected by using the two drugs in combination is significantly greater than what can be achieved by either drug alone, [44]. Isobolograms for Pyrimethamine and Sulfadoxine combinations are depicted in Figure 2.5 [4].

This synergistic concentration-effect relationship is more difficult to determine in-vivo however, due in part to the lack of information regarding the mechanisms of interaction between the two drugs.

This, therefore, was the motivation for exploring the drug-drug interaction for this dataset, from which the sequential and simultaneous modeling methods usually used in the PK-PD context were adapted to modeling two drug concentrations.

The most common PD model specification is that of the Emax model, given by:

$$y_{ikj} = E_{0i} + \frac{E_{maxi} - E_{0i}}{1 + \frac{EC_{50i}}{C_{ikj}}} + e_{ikj}$$

for the case in which we have multiply-nested random effects, and:

$$\beta_{ik} = \beta + \mathbf{b}_i + \mathbf{b}_{ik}$$

where

$$\beta = [\beta_0, \beta_1, \beta_2] = [E_0, E_{max}, EC_{50}]$$

$$\mathbf{b}_i = [b_{0i}, b_{1i}, b_{2i}]$$

$$\mathbf{b}_{ik} = [b_{0ik}, b_{1ik}, b_{2ik}]$$

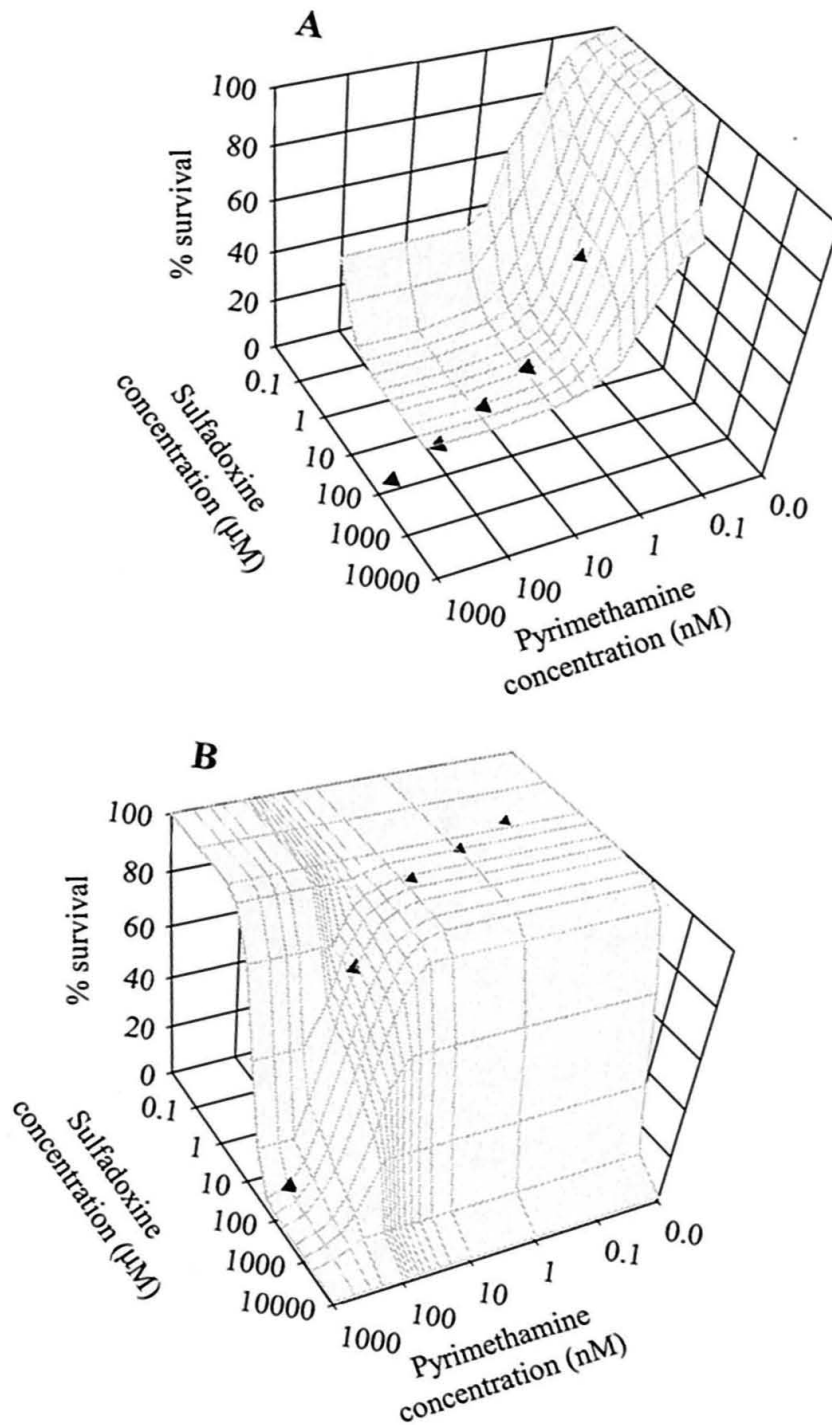


Figure 2.5: Isobologram [4]

and

$$\begin{aligned}\mathbf{b}_i &\sim (\mathbf{0}, \mathbf{D}_1) \\ \mathbf{b}_{ik} &\sim (\mathbf{0}, \mathbf{D}_2) \\ \mathbf{e}_{ikj} &\sim (\mathbf{0}, \mathbf{R}_{ik}(\boldsymbol{\beta}_{ik}, \boldsymbol{\xi}))\end{aligned}$$

where  $\mathbf{R}_{ik}(\boldsymbol{\beta}_{ik}, \boldsymbol{\xi})$  is some assumed intra-individual, intra-occasion covariance structure, and  $\mathbf{D}_1$  and  $\mathbf{D}_2$  are the covariance matrices associated with the different levels of random effects.

The parameters  $E_0$ ,  $E_{max}$  and  $EC_{50}$  are the effect at zero concentration, the maximum effect produced, and the concentration at which 50% of the effect occurs respectively, i.e. they measure the effect of drug concentration  $C_{ikj}$  on the PD outcome (some measurement of effect  $y_{ikj}$ ).

### Sequential:

In the sequential approach for PK-PD modeling, the predicted concentrations from the PK model are used in order to try and reduce the bias in the estimation of the PD model parameters, since the observed concentrations are measured with error and the predicted values provide a smooth interpolated curve, albeit with their own amount of estimation error.

We of course do not have PD data, and are in fact modeling the PK-PK relationship. The PK/PD relationship is mentioned merely as a precursor and introduction, hence: *adapting* this approach to model the drug-drug interaction, we replace the Emax model with the additive exponential models used before, and add the second drug's predicted concentration as a time-varying covariate.

Thus, for example, we could model the Sulfadoxine concentration at time  $j$  in period  $k$  for individual  $i$  as a function of the predicted Pyrimethamine concentration at the same point using:

$$\begin{aligned}y_{ikj} &= f(\mathbf{x}_{ikj}, \boldsymbol{\beta}_{ikj}) + e_{ikj} \\ &= \beta_{0ikj}(-e^{-\beta_{1ikj} \times time_{ikj}} + e^{-\beta_{2ikj} \times time_{ikj}}) + e_{ikj}\end{aligned}$$

where:

$$\boldsymbol{\beta}_{ikj} = \mathbf{A}_{ikj}\boldsymbol{\beta} + \mathbf{B}_{i,kj}\mathbf{b}_i + \mathbf{B}_{ik,j}\mathbf{b}_{ik},$$

may be written as:

$$\begin{bmatrix} \beta_{0ikj} \\ \beta_{1ikj} \\ \beta_{2ikj} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & C_{ikj} & 0 & 0 \\ 0 & 1 & 0 & 0 & C_{ikj} & 0 \\ 0 & 0 & 1 & 0 & 0 & C_{ikj} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \\ \beta_5 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} b_{0i} \\ b_{1i} \\ b_{2i} \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} b_{0ik} \\ b_{1ik} \\ b_{2ik} \end{bmatrix}$$



such that:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_3 \times C_{ikj} + b_{0i} + b_{0ik} \\ \beta_{1ikj} &= \beta_1 + \beta_4 \times C_{ikj} + b_{1i} + b_{1ik} \\ \beta_{2ikj} &= \beta_2 + \beta_5 \times C_{ikj} + b_{2i} + b_{2ik}\end{aligned}$$

where  $C_{ikj}$  is the predicted concentration of Pyrimethamine, and:

$$\begin{aligned}\mathbf{b}_i &\sim N(0, \boldsymbol{\psi}_1), \\ \mathbf{b}_{ik} &\sim N(0, \boldsymbol{\psi}_2), \\ e_{ikj} &\sim N(0, \mathbf{R}_{ik}).\end{aligned}$$

Of the two drugs, Pyrimethamine is the more quickly absorbed and eliminated, and the relationship presented above is thus in the hypothesized direction, i.e. where the Sulfadoxine concentration is impacted by that of Pyrimethamine rather than vice versa.

### Simultaneous:

The simultaneous approach is slightly more involved, as it specifies the two models at the same time, indicating which response type is appropriate with the use of a binary indicator variable.

The structure of the data is changed slightly, so that now the response is a stacked vector of concentrations for both drugs for each individual. The particular drug type being modeled is determined by a binary indicator variable in the initial structural model specification.

We thus have the same model specification as before, except that now it is additionally indexed by  $l$ , where  $l = 1, 2$  for the case where we have two responses.

$$y_{likj} = f_l(\mathbf{x}_{ikj}, \boldsymbol{\beta}_{ikj}) + e_{likj}$$

where  $y_{likj}$  is the stacked vector of responses  $y_{1ikj}$  and  $y_{2ikj}$ .

$$l = 1, 2, \quad i = 1, \dots, m_l, \quad k = 1, \dots, n_{li}, \quad j = 1, \dots, n_{lik}$$

$m_l$  is the number of individuals for response type  $l$ ,  $n_{li} = 2$  is the number of periods for the  $i$ th individual for response type  $l$ , and  $n_{lik}$  is the number of observations in the  $k$ th period for the  $i$ th individual for response type  $l$ .

The vector of  $\boldsymbol{\beta}_{ikj}$  is now a combined vector of regression parameters  $\boldsymbol{\beta}_{1ikj}$  and  $\boldsymbol{\beta}_{2ikj}$  defined as before, the components of which correspond to those in the separate models for each response type.

The covariance matrix  $\mathbf{R}_{ik}$  is now a  $(n_{ik} \times n_{ik})$  covariance matrix, where the upper left  $(n_{1ik} \times n_{1ik})$  submatrix corresponds to the covariance structure for  $y_{1ik}$ , the lower right  $(n_{2ik} \times n_{2ik})$  submatrix corresponds to the covariance structure for  $y_{2ik}$ , and the remaining elements may correspond to a model for within-individual correlations between the elements of  $y_{1ik}$  and  $y_{2ik}$ .

$$\mathbf{R}_{ik(n_{ik} \times n_{ik})} = \begin{bmatrix} R_{ik(11)} & R_{ik(12)} \\ R_{ik(21)} & R_{ik(22)} \end{bmatrix}$$

The covariance matrix for the residuals now accommodates the covariance structure for each individual response type, and allows for within-individual within-phase correlations between the different responses.

For combined vectors  $\mathbf{b}_i = [\mathbf{b}'_{1i}, \mathbf{b}'_{2i}]'$  and  $\mathbf{b}_{ik} = [\mathbf{b}'_{1ik}, \mathbf{b}'_{2ik}]'$ :

$$\mathbf{b}_i \sim N(0, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(0, \boldsymbol{\psi}_2)$$

where  $\boldsymbol{\psi}_1$  and  $\boldsymbol{\psi}_2$  are now joint covariance matrices, allowing correlations among group-specific regression parameters for the two different response types.

For the PK-PD model,  $\boldsymbol{\beta}_{ikj}$  is then a vector of both the PK parameters and the PD parameters. Thus for example, for a bi-exponential PK model and an Emax PD model similar to that previously specified:

$$\boldsymbol{\beta} = [\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5] = [\beta_0, \beta_1, \beta_2, E_0, E_{max}, EC_{50}]$$

$$\mathbf{b}_i = [b_{0i}, b_{1i}, b_{2i}, b_{3i}, b_{4i}, b_{5i}]$$

$$\mathbf{b}_{ik} = [b_{0ik}, b_{1ik}, b_{2ik}, b_{3ik}, b_{4ik}, b_{5ik}]$$

where the random effects at each level are assumed to be jointly distributed with an appropriate covariance matrix.

Now looking at the case in which the responses are the Sulfadoxine and Pyrimethamine concentrations, the approach outlined above is appropriate for the case in which we have different structural forms for the different responses, so for example bi- and triple-exponential models for Sulfadoxine and Pyrimethamine concentrations respectively.

Using these specifications, we would thus define a model such that:

$$f(\mathbf{x}_{likj}, \boldsymbol{\beta}_{likj}) = \underbrace{\left[ \beta_{0likj} (-e^{-\beta_{1likj} \times time_{likj}} + e^{-\beta_{2likj} \times time_{likj}}) \right]}_{\text{Bi-Exponential Model Form}} \times \delta_{l\dots}$$

$$+ \underbrace{\left[ \beta_{3likj} \times [-exp(-\beta_{5likj} \times time_{likj}) + exp(-\beta_{6likj} \times time_{likj})] \right.}_{\text{Triple-Exponential Model Form}}$$

$$\left. + \beta_{4likj} \times [-exp(-\beta_{5likj} \times time_{likj}) + exp(-\beta_{7likj} \times time_{likj})] \right]$$

$$\times (1 - \delta_{l\dots})$$

where  $\delta_{l\dots}$  is a binary variable indicating the response type as Pyrimethamine ( $\delta_{l\dots} = 0$ ) or Sulfadoxine ( $\delta_{l\dots} = 1$ ).

Since the triple-exponential model is merely an extension of the bi-exponential specification, a possible alternative approach would be to specify the larger and

more complicated of the two structural forms, and differentiate between the two responses by including the response type as a binary covariate.

Thus for example, for the triple-exponential model specification, we could fit:

$$\begin{aligned}
 y_{likj} &= f(\mathbf{x}_{likj}, \boldsymbol{\beta}_{likj}) + e_{likj} \\
 &= \beta_{2likj} \times [-\exp(-\beta_{1likj} \times time_{likj}) + \exp(-\beta_{3likj} \times time_{likj})] \\
 &\quad + \beta_{4likj} \times [-\exp(-\beta_{1likj} \times time_{likj}) + \exp(-\beta_{5likj} \times time_{likj})] \\
 &\quad + e_{likj}
 \end{aligned}$$

where:

$$\begin{aligned}
 \beta_{1likj} &= \beta_1 + \beta_6 \times \delta_{l...} + b_{1i} + b_{1ik} \\
 \beta_{2likj} &= \beta_2 + \beta_7 \times \delta_{l...} + b_{2i} + b_{2ik} \\
 \beta_{3likj} &= \beta_3 + \beta_8 \times \delta_{l...} + b_{3i} + b_{3ik} \\
 \beta_{4likj} &= \beta_4 + \beta_9 \times \delta_{l...} + b_{4i} + b_{4ik} \\
 \beta_{5likj} &= \beta_5 + \beta_{10} \times \delta_{l...} + b_{5i} + b_{5ik}
 \end{aligned}$$

where  $\delta_{l...}$  is a binary variable indicating the response type as Pyrimethamine ( $\delta_{l...} = 0$ ) or Sulfadoxine ( $\delta_{l...} = 1$ ), and:

$$\begin{aligned}
 \mathbf{b}_i &\sim N(0, \boldsymbol{\psi}_1), \\
 \mathbf{b}_{ik} &\sim N(0, \boldsymbol{\psi}_2), \\
 e_{ikj} &\sim N(0, \mathbf{R}_{ik})
 \end{aligned}$$

The regression parameters associated with the binary variable  $\delta_{l...}$  act as effect modifiers, describing the changes in the shape of the concentration-time curve, (as described by the number of exponential terms), for Sulfadoxine when compared to Pyrimethamine. For the above model specification, the conditional t-test for parameter  $\beta_4$  thus provides a formal statistical test for the suitability of the bi- or triple-exponential model for the two responses.

Sequential and/or simultaneous procedures allow for the accommodation of possible correlations between the different response types: they take into account the possible interdependence and relationship between the responses.

In the case of the simultaneous approach in particular, the combination of the two models accommodates the association among factors corresponding to the two responses, and allows for greater precision in the estimation of common elements (the mixed-effect model relies on the pooling of data, and with the simultaneous approach more information is available).

## 2.7 Estimation and Inference

Inferential procedures for the nonlinear regression model are based on the usual principles of least squares, and are analogous to the methods applied for the linear case, i.e. the iteratively re-weighted least squares approach, where the usual normality distributional assumptions are relaxed in order to account for heterogeneity and autocorrelation. The main difference lies in the use of numerical methods in the solving of the resulting estimating equations.

The main class of estimation techniques for nlme models is constituted by methods based on the linearization of the hierarchical model formulation presented earlier. Several methods of linearization are available, including first order linearization, which makes use of joint maximum likelihood and generalized least square methods and first order conditional linearization (FOCE), which is the estimation algorithm used in the nlme function in R, (R version 2.11.1 (2010-05-31) Copyright (C) 2010 The R Foundation for Statistical Computing), and thus the estimation procedure used in the attainment of all results presented in this thesis. The FOCE approach is a refinement of first order linearization, which takes the inter-individual variability into account, [6].

Inference is largely based on standard normal asymptotic theory: in the nonlinear case, since the solution of the estimating equations may not be explicitly obtained, exact distributional results for the OLS estimator are not available. Hence, approximations are developed using asymptotic theory, which hold even when the underlying normality assumptions are violated, and are easily adapted to take into account the existence of non-constant variance. Standard errors are obtained from the inverse of the information matrix, evaluated at the appropriate estimates. Confidence intervals and hypothesis tests are thus based on these approximations, and likelihood ratio tests and information criteria are used in model comparison, [6, 45].

A full description of these procedures may be found in *Nonlinear Models for Repeated Measurement Data* by Davidian and Giltinan [6] for both the linear and nonlinear mixed effect models, together with alternative inferential approaches for nlme models not described here. Extensions to the multi-level (nested) case may be found in *Mixed Effect Models in S and S-Plus* by Pinheiro and Bates [3].

The theoretical development of the multi-level NLME model is fundamental to the specification and understanding of the correct model structure for this data, and was thus presented in detail. Since the theoretical estimation and inferential procedures are widely available and would remain largely unchanged if detailed here, we chose to focus instead on the two aspects which impacted most on the analyses, namely, the starting estimates and degrees of freedom.

### 2.7.1 Starting Values

The estimation of parameters in the context of the nlme model and specifically in R requires solution of computationally intensive estimation algorithms. The ease of convergence of these algorithms and the reliability of the results is heavily dependent on the accurate specification of initial values.

Determining reasonable estimates is difficult, and depends largely on an understanding of the structure of the model. Techniques described by Bates and Watts [46] include the use of graphical or mechanistic interpretations of the parameters, and the refinement of particular estimates through an iterative procedure in which all other parameters are held fixed. Alternative options are to base the starting values on relevant previously published literature, or to obtain them from examination of the data on hand (via curve-stripping or method of residuals) or through simulation.

Another common strategy is to specify a simpler version of the model, and use the results to further refine the starting estimates for the intended analysis. Pinheiro and Bates [3] suggest specifying a diagonal variance-covariance matrix for the random effects, which, although not necessarily realistic, often speeds up the iteration process and allows for successful convergence of the algorithms.

Lack of appropriate initial values may cause the iteration procedure to loop, resulting in a lack of convergence, or should the model converge, results obtained may not be stable or robust. For the latter case, slight changes in starting values may result in different final parameter estimates.

Although different parameter estimates may result in the same predicted curve, particularly when dealing with sums of exponentials, unstable parameter values impact on the model building procedure, as they alter the potential positions of covariates, and hence the impact of these covariates on specific features of the curves.

Generally, the easiest way to ensure good starting estimates in the NLME model is to “update” from the individual model fits, [3], i.e. to use the aggregates of the predicted individual-specific parameter values as new starting values. Although this procedure is usually automated in the nlme package in R, for multi-level models it must be manually programmed.

For the separate Sulfadoxine and Pyrimethamine models, starting estimates for the least squares and individual-specific models were obtained from previously published literature (Nyunt et al., [5]) and from a curve-stripping procedure, although the latter methodology was only applied for the bi-exponential model specification. The estimates of the fixed effects parameters from the least squares models, and the averaged estimates from the individual-specific models were then used to inform the initial values used in the NLME model.

For the Pyrimethamine case, there were insufficient data points available to support the fitting of individual-specific triple-exponential models, and so for this specification, the literature-based starting values were used in a simpler single-level NLME model, and the final parameter estimates from this model were then used in the multi-level version.

Nyunt et al [5] provided starting estimates for PK parameters only, which were then transformed in order to get the values for the beta parameters in the exponential parameterization. The values for the PK parameters in their paper, [5],

were the averaged estimates from individual-specific one and two-compartment models run on the same dataset used here.

Curve stripping or peeling, (also known as the method of residuals), uses the observed data to provide starting estimates by essentially resolving the concentration time curve into a series of exponentials (much the same as the parameterization employed in the structural model forms used here). The curve-stripping procedure used for the bi-exponential model was that described in *Mixed Effect Models in S and S-Plus* [3] for a first order single compartment model, which again resulted in estimates for the clinical parameterization of the model in question, albeit a different version to that obtained from the literature (the model was specified in terms of clearance rather than volume of distribution). Transformation to our exponential parameterization was straightforward.

The method is described as follows by Pinheiro and Bates [3]:

Writing the equation for a first order open compartmental model as:

$$y(x) = \frac{D \times \exp(\phi_1) \times \exp(\phi_2)}{\exp(\phi_3) \times [\exp(\phi_2) - \exp(\phi_1)]} \times [\exp(-\exp(\phi_1) \times x) - \exp(-\exp(\phi_2) \times x)]$$

where  $D$  is the dose,  $\phi_1$  is the log of the elimination rate constant,  $\phi_2$  is the log of the absorption rate constant, and  $\phi_3$  is the log of the clearance, the starting estimates for  $\phi_1$ ,  $\phi_2$ , and  $\phi_3$  are obtained by following the following steps:

- The position time of the maximum response is determined, and the regression model  $\log(y) = a + bx$  is fit to the data with values of  $x$  that are greater than or equal to the position of the maximum response. Following this,  $\phi_1^0$  and  $\phi_2^0$  are defined as  $\phi_1^0 = \log|b|$  and  $\phi_2^0 = \phi_1^0 + 1$  respectively.
- An algorithm for partially linear models is used to fit the nonlinear regression model:

$$y(x) = k[\exp(-\exp(\phi_1) \times x) - \exp(-\exp(\phi_2) \times x)],$$

refining the estimates of  $\phi_1$  and  $\phi_2$

- The current estimates of  $\phi_1$  and  $\phi_2$  and an algorithm for partially linear models are used to fit

$$y(x) = kD \times \frac{\exp(-\exp(\phi_1) \times x) - \exp(-\exp(\phi_2) \times x)}{\exp(\phi_1) - \exp(\phi_2)},$$

and we define  $\phi_3 = \phi_1 + \phi_2 - \log(k)$ .

The curve-stripping procedure that would be used in order to obtain starting estimates for the two-compartment model is not described here.

## 2.7.2 Degrees of Freedom

In *Mixed Effect Models in S and S-Plus*, Pinheiro and Bates, [3], recommend that the conditional t- and F-tests provided in the model output be used to evaluate

the inclusion of a fixed effect term rather than the output from the likelihood-ratio tests, and show the likelihood ratio-tests to be slightly “anticonservative”, in that the “nominal p-values are smaller than the empirical p-values”. The likelihood-ratio tests may still be used however in the evaluation of the random effects structure.

The algorithm for the calculation of the denominator degrees of freedom for the conditional t- and F-tests used in the nlme package is described by Pinheiro and Bates [3] for the linear mixed effect model. The algorithm was designed to reproduce the results of the “BETWEENWITHIN” option in SAS PROC MIXED, [3, 47].

The degrees of freedom are determined by the level of grouping (subject or occasion-within-subject) at which the term is estimated. A term is classified as “inner” to a grouping level, if its value can change within the levels of the group. If not, it is classified as “outer”, [3].

Applying this to our dataset, pregnancy would be classified as “inner” to the individual level of grouping indexed by  $i$  (level 1), and “outer” to the occasion level of grouping indexed by  $k$ , (level 2).

Similarly, a factor such as site (constant for individuals and not dependent on the occasion/period of observation determined by pregnancy status), would be determined as “outer” to both levels of grouping, and would thus, like the intercept, be estimated at level 0, and its denominator calculated at level  $Q + 1$ , where  $Q$  is the total number of levels.

If we define  $m_f$  as the total number of groups in nesting level  $f$ , with  $m_0 = 0$ , (where  $f = 1, \dots, Q$ , and for the results presented here,  $Q = 2$ ), then the denominator degrees of freedom for the conditional t- and F-tests in the regression models should be given by:

$$denDF_f = m_f - (m_{f-1} + p_f),$$

where  $p_f$  is the sum of the degrees of freedom corresponding to the terms estimated at level  $f$ .

Defining level 1 as observations indexed by individual  $i$ , level 2 as observations indexed by occasion  $k$ , and the inner-most level  $(Q + 1) = 3$  as observations indexed by  $j$  respectively, the degrees of freedom for a parameter corresponding to a covariate “inner” to grouping level 1 and “outer” to grouping level 2 (such as pregnancy) should then be given by:

$$\begin{aligned} denDF_1 &= 97 - (0 + 1) \\ &= 96 \end{aligned}$$

where  $m_1 = 97$  is given by the number of individuals. The degrees of freedom calculated and reported in the model output for the various covariate models do not however appear to follow the above specification.

For the case of the linear mixed effect model, the degrees of freedom calculated by the algorithm are described as “approximate at best” [48] by Douglas Bates, who has also since debated whether this algorithm is “appropriate or not” [47].

For the nonlinear case, there does not as yet appear to be a reliable way to calculate the values required, although several approximations are suggested as potential solutions.

Paraphrasing from several responses to questions posed in the R mixed effects mailing list, primarily by Douglas Bates [47, 49, 50] and Bert Gunter (Genentech Nonclinical Biostatistics, [51]):

“Assuming that degrees of freedom (df) are defined as the “dimension of the null space when the data are projected on the linear subspace of the model matrix of a *linear model*”, there should thus be no df for the case in which there is no linear model.

Since nonlinear models are however fit by successive linear approximations, approximate df may be obtained. The problem appears to be that there is no “guarantee that the relevant residual distributions are sufficiently [chi-squared] with the approximate df to give reasonable answers”.

There does not appear to be a lot of consensus on the correct approach to take (with little mention of specifically nonlinear mixed effect models), with suggestions from (1) using the trace of the ‘hat’ matrix to (2) Satterthwaite and Kenward-Roger corrections.

For (1), the suggestion is based on the degrees of freedom calculated for an unpenalized least squares problem (n minus the trace of the hat matrix). This approximation is “frequently applied in penalized least squares problems, which may be viewed as the basic calculation in mixed effect models”. For (2), one of the biggest reasons cited for why these corrections are not feasible is that they rely on a normal distribution assumption, which is not necessarily correct, and the other is that the actual calculations are computationally intensive and difficult to institute.

Bootstrapping has also been suggested as a good way of overcoming the degrees of freedom problem, but this is also not ideal, as the non-diagonal covariance matrix makes re-sampling whilst maintaining the covariance structure difficult.

Douglas Bates has discussed the problem extensively, and for the lmer package, which is a “re-implementation” [49] of the nlme package, no degrees of freedom or p-values are printed in the model output, as he has yet to determine a reliable calculation for them.



While the text, *Mixed Effect Models in S and S-Plus*, [3], indicates that the linear algorithm described above is used in the nlme estimation procedures, Bates goes on to describe the denominator df as all being the same (as seen in the model output in this thesis) [50]:

“The denominator [of the F-test] is the penalized residual sum of squares divided by the REML degrees of freedom, which is  $n-p$  where  $n$  is the number of observations and  $p$  is the column rank of the model matrix for the fixed effects. All the F ratios use the same denominator . . . This is why I have a problem with the assumption that the reference distribution for these F statistics should be an F distribution with a known numerator degrees of freedom but a variable denominator degrees of freedom and we can answer the question of how to calculate a p-value by coming up with a formula to assign different denominator degrees of freedom for each test. The denominator doesn’t change.”

The current recommendation is to use a Markov Chain Monte Carlo sample to evaluate the properties of individual coefficients, or to calculate the F-ratio and assign a lower bound to the denominator df. ”

The above discussion illustrates the lack of consensus regarding the appropriate calculation for the degrees of freedom in NLME models. Hence, we decided not to rely on the degrees of freedom and associated p-values reported in the model output, instead basing our decisions regarding model evaluation and comparison on diagnostic assessments and initial hypotheses supported by graphical output.

## 2.8 Pharmacokinetics and the Delta Method

### 2.8.1 Introduction

The output from the models outlined in previous chapters enables us to achieve smooth predicted curves, both for individuals and for covariate profiles of interest (e.g. pregnant subjects vs. non-pregnant subjects).

It also provides us with estimates of the fixed effect parameters and their relevant standard errors. These fixed effect parameters are of limited use to the clinician however, as they are composite transformations of the pharmacokinetic parameters that are usually of interest, as discussed in section 2.5.3.

In the evaluation of the impact of pregnancy on the disposition of SP, certain pharmacokinetic parameters are more relevant than others, (e.g. Apparent Volume of Distribution and Clearance), because of previously formulated hypotheses regarding physiological changes during pregnancy and the resultant anticipated impact on the movement of drugs through the system. These parameters are also required for the purposes of dosage calculations.

Whilst back-transformation from the beta parameters used in these models to the mechanistic PK parameters is relatively straightforward, some measure of variability is also required.

The delta method, which was chosen to obtain these estimates of variability, is outlined and explained in this chapter. Additionally, calculations for the PK parameters of interest are provided.

### 2.8.2 The Delta Method

The delta method essentially enables us to derive an approximation for the mean value and variance of some nonlinear function, which is a transformation of one or more random variables, [52, 53].

It uses the first-order Taylor expansion of the function about its mean, ignoring higher order terms, following which the variance of the function can be calculated, [52, 53].

Thus, given a nonlinear function  $G(X)$ , where  $X$  is an asymptotically normal random variable with mean  $\mu$  and the function is differentiable (i.e. the derivative exists and is non-zero), the approximate Taylor expansion of  $G(X)$  is given by:

$$G(X) = G(\mu) + (X - \mu) \times G'(\mu)$$

The approximate variance of this function is thus given by:

$$\text{Var}(G(X)) = \text{Var}[G(\mu) + (X - \mu) \times G'(\mu)] = \text{Var}(X) \times [G'(\mu)]^2,$$

using:

$$\begin{aligned}\text{Var}(a) &= 0 \\ \text{Var}(a + b \times X) &= b^2 \times \text{Var}(X)\end{aligned}$$

where  $a$  and  $b$  are constants, [52, 53].

It should be noted that the accuracy of this approximation depends on the the variance of the random variable  $X$ : the larger this variance, the worse the approximation, [52, 53].

Extending this to the multivariate case, where  $\mathbf{X}$  is a  $(1 \times m)$  vector-valued function, [53]:

$$G(\mathbf{X}) = G(\mu) + (\mathbf{X} - \mu) \times G'(\mu)^T$$

And thus:

$$\begin{aligned} Var(G(\mathbf{X})) &= Var[G(\mu) + (\mathbf{X} - \mu) \times G'(\mu)^T] \\ &= Var[G'(\mu)^T \times \mathbf{X}] \\ &= G'(\mu)^T \times Var(\mathbf{X}) \times G'(\mu) \end{aligned}$$

### 2.8.3 PK Parameters

The PK parameters of interest, namely, the AUC, clearance (Cl), volume of distribution (Vd), elimination half-life ( $t_{1/2}$ ), Cmax, Tmax and the absorption and elimination rates  $k_a$  and  $k_e$ , have already been introduced and defined in the section on basic pharmacokinetics.

The exponential specification of the structural models necessitates that these parameters be calculated, and as such, the calculations required for these back-transformations are presented here. These calculations are based on those found in the user guide for the software *PK Solutions 2.0* [54].

The area under the concentration-time curve, or AUC, can be calculated using the trapezoidal rule below (for either observed or predicted concentrations):

$$AUC_{0-t} = \sum_{i=1}^n \frac{(C_{i+1} + C_i)}{2} \times (t_{i+1} - t_i)$$

where  $n$  is the total number of data points, and extrapolated to infinity using  $k_e$ :

$$\begin{aligned} AUC_{0-\infty} &= AUC_{0-t} + AUC_{t-\infty} \\ AUC_{t-\infty} &= \frac{C}{k_e} \end{aligned}$$

where  $C$  is the (observed or predicted) concentration at time  $t$ , the last observed data point.

Alternatively, based on the general model specification used in this thesis:

$$C(t) = \sum_{i=1}^n C_i \times \exp(-\lambda_i \times t)$$

where  $\lambda_i$  is the rate parameter as before,  $n$  is the number of differentiable phases and thus the number of exponential terms, and  $t$  is time, the AUC may be calculated as:

$$AUC = \sum_{i=1}^n \frac{C_i}{\lambda_i}$$

Thus for the bi-exponential model given by:

$$y_{ikj} = (\beta_{0ikj})[-e^{-(\beta_{1ikj}) \times time_{ikj}} + e^{-(\beta_{2ikj}) \times time_{ikj}}] + e_{ikj},$$

where(for example)

$$\beta_{0ikj} = \beta_0 + b_{0i} + b_{0ik}$$

the AUC is calculated as:

$$\begin{aligned} AUC &= \sum_{i=1}^2 \frac{C_i}{\lambda_i} \\ &= \frac{\beta_0}{\beta_1} + \frac{\beta_0}{\beta_2} \end{aligned}$$

The vector of the mean parameter estimates,  $\beta = [\beta_0, \beta_1, \beta_2]'$ , which are the estimates of the fixed effects from the model output, and the variance-covariance matrix of these parameter estimates would then be required in order to determine the standard error for the AUC using the Delta method.

Similarly, volume of distribution and clearance can be calculated as:

$$\begin{aligned} Vd &= \frac{F \times D}{\lambda_z \times \sum_{i=1}^n \frac{C_i}{\lambda_i}} \\ Cl &= \frac{F \times D}{\sum_{i=1}^n \frac{C_i}{\lambda_i}} \end{aligned}$$

where  $\lambda_z$  is the elimination rate constant, and  $F$  is the fraction of dose  $D$  absorbed.

Hence, assuming  $F = 1$ , for the bi-exponential model specification:

$$\begin{aligned} Vd &= \frac{Dose}{\beta_2 \times AUC} = \frac{Dose}{\beta_2 \times \left(\frac{\beta_0}{\beta_1} + \frac{\beta_0}{\beta_2}\right)} \\ &= \frac{Dose \times \beta_1 \times \beta_2}{\beta_2 \times \beta_0 \times (\beta_2 + \beta_1)} \\ &= \frac{Dose \times \beta_1}{\beta_0 \times (\beta_2 + \beta_1)} \end{aligned}$$

and:

$$\begin{aligned} Cl &= \frac{Dose}{AUC} \\ &= \frac{Dose \times \beta_1 \times \beta_2}{\beta_0 \times (\beta_2 + \beta_1)} \end{aligned}$$

Cmax and Tmax are calculated as:

$$Tmax = \frac{2.303}{\lambda_a - \lambda_z} \times \log\left(\frac{\lambda_a}{\lambda_z}\right)$$

$$Cmax = \frac{F \times D}{Vd} \times \exp(-\lambda_z \times Tmax)$$

where  $F$ ,  $D$ , and  $\lambda_z$  are defined as previously,  $\lambda_a$  is the absorption rate parameter, and  $Vd$  is the volume of distribution as calculated above. Thus:

$$Tmax = \frac{2.303}{\beta_1 - \beta_2} \times \log\left(\frac{\beta_1}{\beta_2}\right)$$

and:

$$\begin{aligned} Cmax &= (Dose \times 1/Vd) \times \exp(-\beta_2 \times Tmax) \\ &= (Dose \times \frac{\beta_2 \times AUC}{Dose}) \times \exp(-\beta_2 \times Tmax) \\ &= (\beta_2 \times AUC) \times \exp(-\beta_2 \times Tmax) \end{aligned}$$

Plasma half-life is calculated as:

$$t_{1/2} = \frac{0.693}{\lambda_n} = \frac{0.693}{\beta_2}$$

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## 2.9 Model Building

In general, several methods are available in the development of the covariate model, which may be loosely divided into “candidate covariate” and “regression based” procedures, [33].

In “regression-based” procedures, an initial NLME model is fitted ignoring covariates other than the primary time covariate, (which is already included in the relevant structural model specification). This basic model is used in order to obtain the individual- or occasion-within-individual-specific predicted parameter values, for each parameter, [33, 55].

For each parameter, this vector of predicted parameter values is then used as the vector of (independent) responses, and ordinary least squares regression of each estimated parameter against the potential covariates determines which covariates, and in what form, are most appropriate for that parameter. Model building procedures such as stepwise model selection may be used in order to obtain the “best” model for each parameter, [33, 55].

The methods used in this thesis are, however, what would be referred to as “candidate covariate” techniques. Unlike in the “regression-based” procedures, the selection of covariates is not determined by the (for example) stepwise regression of each individual parameter against prospective covariates.

The inclusion of covariates on a particular parameter, on several parameters, and in combination with other covariates or otherwise, is determined via comparison of the appropriate NLME models, i.e. comparison of the model fits for the appropriate non-linear models of the concentration over time.

For this thesis, prior to the development of the NLME models, least squares models (ignoring random effects) and individual-specific models were fitted, the latter being used in order to identify parameters which might require random effects, and to identify potentially influential individuals.

Various structural model forms were compared, and the models were fitted for the full dataset and for both pregnant and postpartum subsets. These models were used to inform starting estimates for use in the NLME models.

Starting from a basic NLME model which included all possible random effects, albeit with the most simplified variance-covariance structures, subsequent models were built in increasing and then decreasing levels of complexity, where the inclusion of random effects, covariates and variance structures was re-evaluated at each juncture.

This iterative re-assessment was required in order to accurately determine the effect of the inclusion or removal of a covariate, since the various components of the model all impact on one another. All possible combinations of model features that could feasibly be assessed were thus considered.

Model evaluation included the usual assessments of model improvement, such

as the stepwise addition and deletion of covariates based on the comparisons of log-likelihood ratios and the Akaike (AIC) and Bayesian Information Criteria (BIC) and the assessment of the appropriate model diagnostics.

Various other factors were also considered, including the successful convergence of model runs, the time taken for them to converge and the obtainment of robust variance-covariance matrices. Model fits were individually assessed in order to watch for the emergence of multi-collinearity and other signs of model instability.

While this seems straightforward, there were several difficulties in the identification of the appropriate covariate relationships, not unusual in the case of nonlinear mixed effect models.

In the stepwise approach outlined, the number of combinations of covariates and parameters was unfeasibly large. The number of possible covariate combinations, compounded by the required iterative model building procedure and finally, by the presence of correlated covariates, (the inclusion of which resulted in unstable models with nonsensical results), resulted in a reduction in the number of covariates assessed for inclusion in the models. The focus was deemed to be the impact of pregnancy/trimester, the inclusion of which followed the procedure outlined above.

An hypothesis-based model building procedure was required in order to assess the impact of the remaining covariates. However, the specification of the models in terms of multiple exponential terms ignores the usual compartmental breakdown and clinical parameterization, which in turn hampers the use of hypothesis-based covariate selection; since several beta parameters are composite parameters, despite the clearly defined relationships between the PK and beta parameters, the hypothesized clinical impact of a covariate on a specific parameter is no longer clear.

Acting without information regarding clinically feasible relationships for the beta parameters made the covariate selection more complicated, and alternative means of identifying potential parameter-covariate relationships were thus required. To this end, graphical techniques were employed, whereby plots of the random effects of each of the parameters against various covariates were assessed at every step of the model building procedure in order to determine possible associations. These plots were also used in the assessment of the pregnancy and trimester covariates.

The graphical approach to covariate selection may be affected by shrinkage: individual parameter estimates may tend to shrink towards the typical population or average value, in comparison with the true individual parameter values, particularly for sparse data, [33, 55]. This shrinkage may distort the actual parameter-covariate relationships, hiding real associations or even falsely inducing them (a correlation between structural model parameters may result in a false correlation between individual specific parameters, and thus, the relationship between a covariate and one parameter may falsely create the impression of a relationship between that covariate and several other parameters), [33, 55].

This is even more noticeable for individual parameter values that are not plausible or have little information (such as the absorption phase in our case), and this approach is not possible at all for parameters that have no random effects, [33].

The exponential parameterization makes this all the more likely: using the bi-exponential structural model as an example,  $\beta_0$  is seen to be a composite function of  $\beta_1$  and  $\beta_2$ . Thus, systematic patterns observed in the subject or occasion-specific variability about this parameter, which might ordinarily indicate the effect of some covariate on  $\beta_0$ , may in fact be more accurately captured through one of the other parameters.

The graphs were thus used as a guideline only.

University of Cape Town



## Chapter 3

# Analysis and Results

### 3.1 Data Preparation and Exploration

Data preparation and exploration was performed using Stata/MP 11.0 (Stata-Corp, College Station, Texas 2005) and R (R version 2.11.1 (2010-05-31) Copyright (C) 2010 The R Foundation for Statistical Computing).

Continuous covariates prospectively considered to be of interest included the subject's weight (kg), and gestational age (in weeks). Trimester, pregnancy and site were to be explored as categorical variables, with the cut off for 2nd and 3rd trimester determined as 14-26 weeks and 27-35 weeks respectively. Subjects with haemoglobin levels less than 10g/dl, were considered to be anaemic, [5], and the impact of anaemia was to be investigated as both a continuous and dichotomous variable with the above cut-off.

Variable	Postpartum		Pregnant		P-value (Kruskal-Wallis)
	N	Median (IQR)	N	Median (IQR)	
Weight (kg)	73	59.4 (53.3,67)	98	61 (56,67)	0.0016
Haemaglobin (g/dl)	74	11.75 (11,13.1)	98	10.7 (9.2,11.7)	<0.0001
	N	%	N	%	(Chi-Square)
% Haemaglobin < 10g/dl	74	9.46%	98	41.84%	<0.0001

Table 3.1: Baseline Characteristics by Pregnancy (I)

Baseline characteristics for the study participants were compared for different phases of pregnancy (Table 3.1) and across sites (Table 3.2 and Table 3.3) using appropriate tests, Kruskal-Wallis for continuous variables and Chi-squared tests of association for counts.

Although we do have paired samples, the design is unbalanced, since some of the participants did not return for the postpartum phase of the study. As such, the Kruskal-Wallis and Chi-squared tests appropriate for *independent* samples were used, albeit as a basic guideline only.

Parameter Median (IQR)	Total N=97	Mali N=18	Mozambique N=31	Sudan N=24	Zambia N=25	P-value
Age (years)	26 (23,32)	26 (22,32)	24 (22,27)	28 (26,32)	31 (25,36)	0.003
Weight (kg)	61 (56,67)	60 (56,65)	61 (56, 65)	66 (57,71)	60 (56,66)	0.37
Gestational age (wks)	27.5(24,29)	28 (25,29)	27 (24,29)	27 (21,33)	26.5 (24,28)	0.87
2nd Trimester (n (%))	44 (45%)	7 (39%)	13 (42%)	12 (50%)	12 (48%)	0.9
Haemoglobin (g/dl)	10.7 (9.2,11.7)	9.9 (9.4,11.4)	10.8 (9.6, 12)	9.2 (8.5, 10.9)	11.3 (10.5, 12.1)	0.0002
Anaemic (n (%))	41 (41.84%)	10 (55.6%)	11 (35%)	17 (71%)	3 (12%)	0.001
Dosage (mg/kg)	1.2 (1.1,1.3)	1.3 (1.2, 1.3)	1.2 (1.2,1.3)	1.2 (1.1, 1.3)	1.3 (1.1,1.3)	0.35

Table 3.2: Baseline Characteristics for Pregnant Subset

Parameter Median (IQR)	Total N=74	Mali N=18	Mozambique N=22	Sudan N=15	Zambia N=18	P-value
Weight (kg)	59.4 (53.3,67)	59 (52,64)	55 (51,63)	71 (61,82)	60.8 (55,66)	0.003
Haemoglobin (g/dl)	11.8 (11.0,13.1)	12.4 (11.6,13.2)	11.6 (11.2,12.9)	10.8 (10.2,11.5)	13.1 (11.6,14.9)	0.0003
Anaemic (n (%))	7 (9.5%)	1 (5.6%)	3 (13.6%)	3 (20%)	0	0.2
Dosage (mg/kg)	1.3 (1.1,1.4)	1.3 (1.2,1.4)	1.4 (1.2,1.5)	1.06 (0.9,1.2)	1.2 (1.1,1.4)	0.003

Table 3.3: Baseline Characteristics for Non-Pregnant Subset

Pregnant subjects appeared to weigh significantly more ( $p=0.0016$ ) and be significantly more anaemic ( $p<0.0001$ ) when compared to postpartum subjects for all sites, although the actual difference between the pregnant and postpartum median weights (59.4 versus 61) did not appear to be too large.

Within the pregnant and postpartum subsets, significant differences were seen across sites for age and anaemia (in the pregnant subset) and for anaemia and weight (and thus mg/kg dosage) in the postpartum subset.

Weight (kg), dosage (mg/kg) and gestational age (in weeks) were similar across sites for pregnant subjects, and moderate anaemia was observed for approximately 41.8% of pregnant subjects.

Subjects in Zambia appeared to be older and less anaemic than those in the remaining sites, with a median age of 31 and only 12% of subjects with anaemia in the pregnant subset, and no anaemic subjects in the postpartum subset. Subjects in Sudan were most often anaemic for both the pregnant (71%) and postpartum subsets (20%).

Individual concentration-times curves were examined for both Sulfadoxine and Pyrimethamine, in order to identify concentrations that might be deemed biologically implausible.

Subjects with all concentrations less than 10ug/ml for Sulfadoxine and 10ng/ml for Pyrimethamine could not be included in the analysis, as the limits of quantification for the assay methods were 10ug/ml and 10ng/ml for the two drugs respectively. Concentrations greater than 0ug/ml or 0ng/ml *prior* to dosing and those outside of the range of plausible values were also excluded. Table 3.4 details the subjects and/or concentrations that were not included in the analysis (for both Sulfadoxine and Pyrimethamine).

Subject	Site	Pregnancy Status	Concentration Measurements
11	Sudan	Pregnant/Postpartum	All
10	Mozambique	Pregnant	Days 2 and 21
26	Mozambique	Postpartum	Day 0
28	Mozambique	Pregnant	Days 1, 2 and 3
23	Mozambique	Pregnant	Day 1

Table 3.4: List of Subjects/Concentration Measurements Excluded from Analyses

Figures 3.1 and 3.2 show graphs of Pyrimethamine concentrations over time for subjects in Sudan and Mozambique, with the implausible points labeled by subject and by day of observation. Figures 3.3 and 3.4 show these same plots for the Sulfadoxine concentrations over time. These figures illustrate the general structural form of the observed drug concentration-time profiles, in addition to identifying those biologically implausible points removed prior to modeling. Similar curves were examined for subjects from Mali and Zambia, for which no implausible points were identified, (Appendix A, Figures A.1 to A.4).

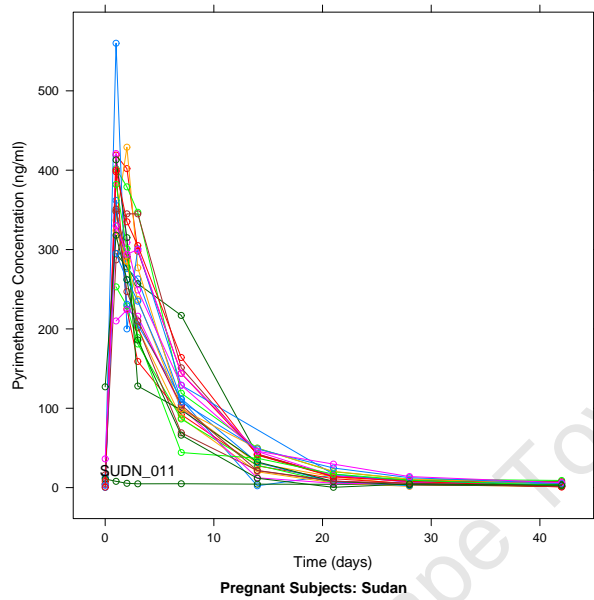


Figure 3.1: Identification of Biologically Implausible Pyrimethamine Concentrations: Sudan

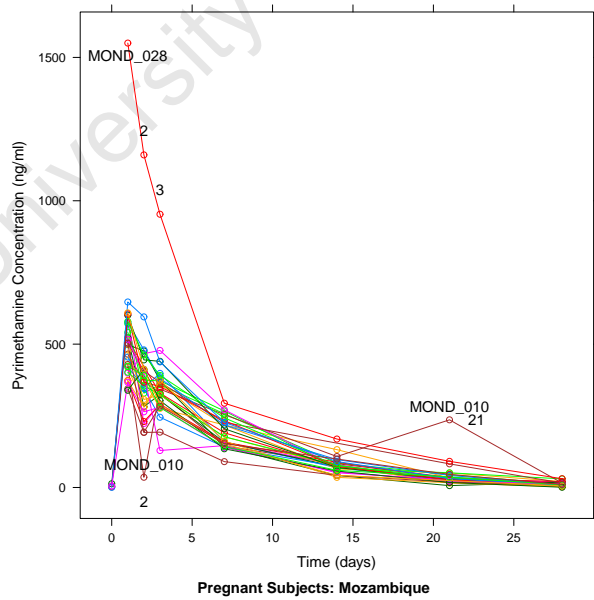


Figure 3.2: Identification of Biologically Implausible Pyrimethamine Concentrations: Mozambique

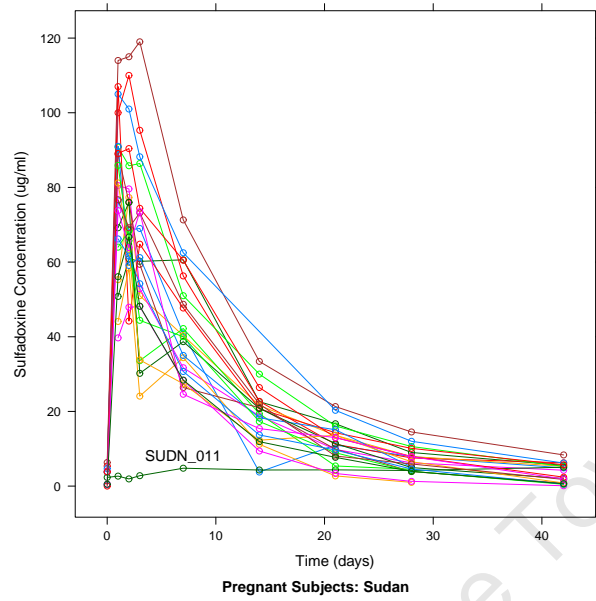


Figure 3.3: Identification of Biologically Implausible Sulfadoxine Concentrations: Sudan

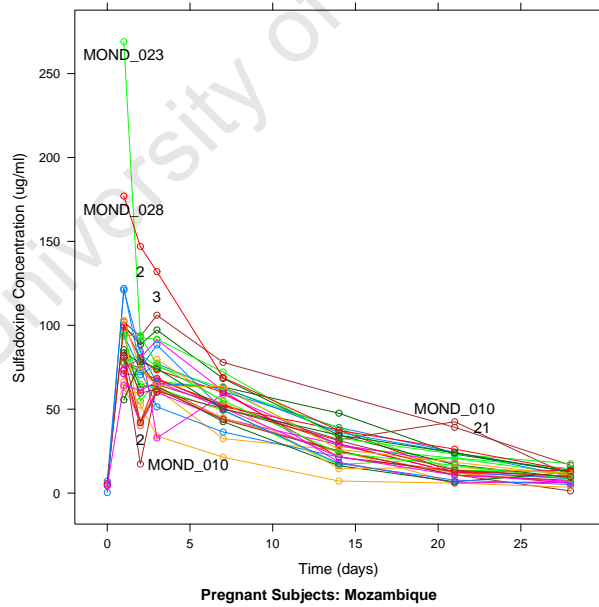


Figure 3.4: Identification of Biologically Implausible Sulfadoxine Concentrations: Mozambique

The distributions of individual covariates were examined using histograms (continuous covariates) and tables (categorical covariates). The relationships between continuous covariates were explored using pairs plots, and those between categorical covariates using appropriate tests of association. Box plots of the continuous variables by pregnancy status, site and anaemia were also used to indicate potential confounders (Appendix A, Figures A.5 and A.6).

As seen in Figure 3.5, relatively symmetrical distributions were indicated for weight (kg), haemoglobin measurement and dosage (mg/kg). The histogram for gestational age (gage) reflects zero's for the non-pregnant subjects and an approximately symmetrical distribution for pregnancy. Age appeared to be slightly skewed to the right. Weight and mg/kg dosage are directly correlated, as anticipated.

Day 7 concentrations were used to indicate relationships between concentrations and covariates; day 7 concentrations have previously been shown to be highly correlated with total drug exposure (as measured by the area under the concentration-time curve) for both Sulfadoxine and Pyrimethamine, and have also been shown to be associated with therapeutic efficacy, [5, 30].

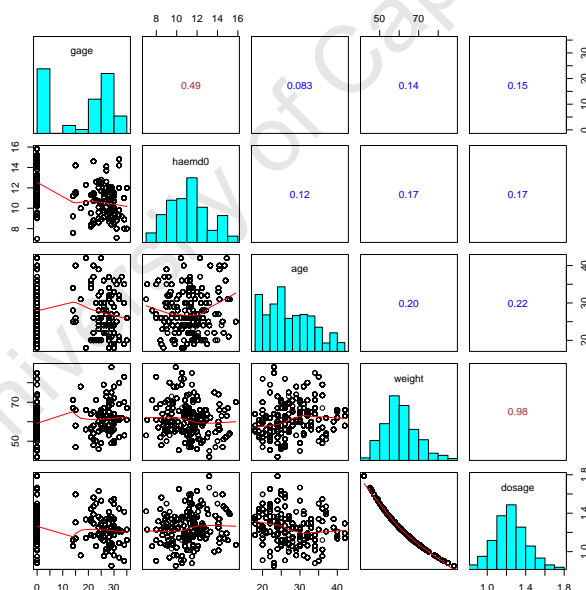


Figure 3.5: Pairs Plots

Box plots of day 7 concentrations by pregnancy status, site and anaemia indicated that the relationship observed between Pyrimethamine day 7 concentration levels and pregnancy phase appeared to differ dependent on whether or not subject was anaemic (Figure 3.6). For Sulfadoxine, the impact of pregnancy on the day 7 concentrations appears to be greater for anaemic subjects.

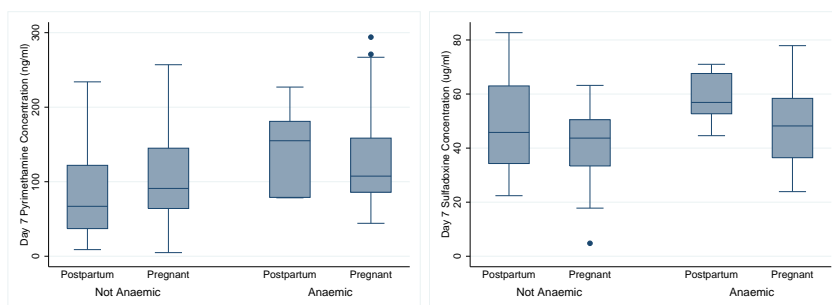


Figure 3.6: Boxplots of Day 7 Concentrations by Pregnancy and Anaemia

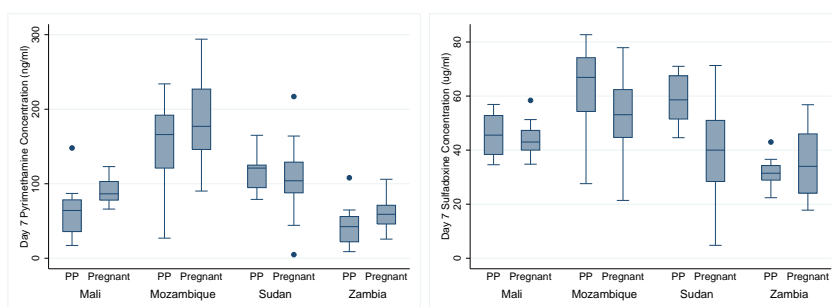


Figure 3.7: Boxplots of Day 7 Concentrations by Pregnancy and Site

The relationship between the day 7 concentrations and pregnancy did not appear to be altered by site for either of the two compounds (Figure 3.7), although for Pyrimethamine, more of an overlap is observed for subjects in Sudan than for those in the remaining sites, and for Sulfadoxine, the impact of pregnancy on the day 7 concentrations appears to be greater for subjects in Mozambique and Sudan than for those in Mali and Zambia.

Figure 3.8 demonstrates the different scales and units for Pyrimethamine and Sulfadoxine. Higher peak concentrations and a longer observation period were observed for pregnant subjects for both compounds, (Figure 3.9). For Sulfadoxine, a steeper elimination period was also seen in for pregnant subjects.

## 3.2 Sulfadoxine Models

Following the model building procedure outlined in section 2.9, least squares models (referred to as NLS models), that ignore the grouping structure of the data, and individual-specific models were fitted to the the Sulfadoxine concentration-time data prior to any nlme models.

This section presents some of the pertinent results from the NLS and individual-specific models, and compares the results from models using different starting values (those from Nyunt et. al [5] and from the curve-stripping procedure for the bi-exponential model).

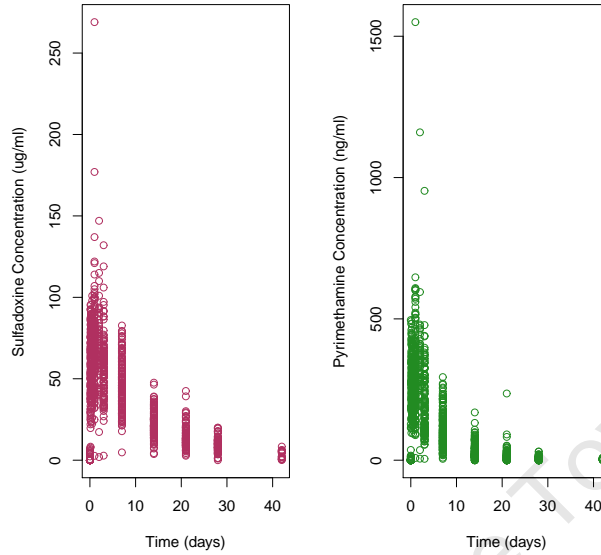


Figure 3.8: Scatter plots of Sulfadoxine and Pyrimethamine Concentrations over Time

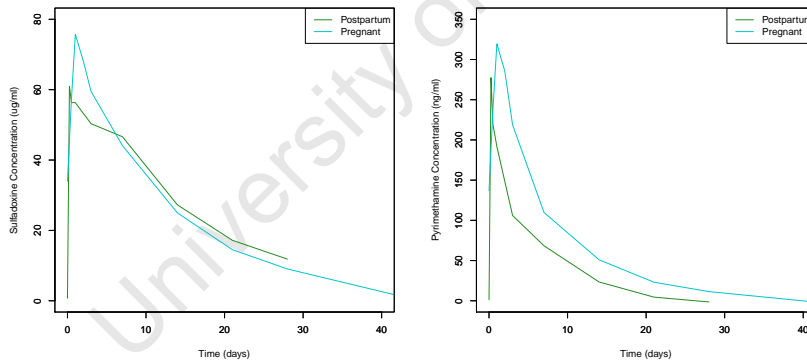


Figure 3.9: Lowess plots of Sulfadoxine and Pyrimethamine Concentrations over Time by Pregnancy Status

The covariate model-building procedure used in the development of the non-linear mixed effect model is also detailed, along with the results from the final NLME models. Predicted curves from the exponential model specification are shown, and clinical parameters are presented and contrasted with those obtained using the more traditional two stage approach to the analysis of PK data, for several models.

Results from the basic bi-exponential model, ignoring covariates, and the model



adjusted for pregnancy are also contrasted with those obtained from corresponding clinically (mechanistically) specified one-compartment models.

The results obtained from the NLME multi-level model adjusted for pregnancy only are compared to those achieved with a single-level representation of the grouping structure, and a model with proportional (multiplicative) random effects is also presented.

### 3.2.1 NLS Models

Nonlinear least squares models ignore the hierarchical grouping structure of the data, assuming independent observations. We are thus fitting a model of the form:

$$y_j = f(x_j, \boldsymbol{\beta}) + e_j \quad (3.1)$$

where  $y_j$  is the concentration at time  $j$ ,  $x_j = time_j$ , and  $\boldsymbol{\beta}$  is a  $p$ -dimensional vector of fixed effects.

Both the bi- and triple-exponential structural model forms were fitted, such that:

$$f(x_j, \boldsymbol{\beta}) = \beta_0 \times [-exp(-\beta_1 \times time_j) + exp(-\beta_2 \times time_j)] \quad (3.2)$$

or:

$$f(x_j, \boldsymbol{\beta}) = \beta_2 \times [-exp(-\beta_1 \times time_j) + exp(-\beta_3 \times time_j)] + \beta_4 \times [-exp(-\beta_1 \times time_j) + exp(-\beta_5 \times time_j)] \quad (3.3)$$

The models were fitted for the full dataset and for pregnant and postpartum subsets; in the case of the bi-exponential model, comparing the results obtained from starting values based on the article by Nyunt et. al [5] with those from the curve-stripping procedure.

Table 3.5 compares the starting values from the different sources for the full dataset and the various subsets. The units in table 3.5, and in the remainder of the results section, for the beta parameters corresponding to the range of concentrations (for example  $\beta_0$ ) and the rates of incline/decline (for example  $\beta_1$ ) are concentration, and concentration per hour respectively.

Parameter	Data	Curve Stripping	Nyunt et. al
$\beta_0$ :	Full	86.7	80.89 (60.88; 96.88)
	Pregnant	91.3	86.8 (68.6; 101.5)
	Postpartum	67.3	62 (50.2;70.7)
$\beta_1$ :	Full	5.92	14 (4.2; 39.8)
	Pregnant	6.58	15.7 (4; 45)
	Postpartum	8.76	11.5(5.3;20.7)
$\beta_2$ :	Full	0.1476	0.088 (0.069; 0.1)
	Pregnant	0.1476	0.094 (0.081,0.11)
	Postpartum	0.0978	0.07(0.062;0.079)

Table 3.5: Comparison of Starting Values for Bi-Exponential Model

The values obtained from the curve-stripping procedure appear to lie within the ranges calculated from the Nyunt et. al [5] results, with the exception of those for  $\beta_2$  (analogous to the elimination rate), which are consistently higher using curve-stripping for all subsets.

The suggested starting values for the pregnant subset indicate a higher range of concentrations than for the postpartum subset, together with a slower rate of incline and faster decline.

The large degree of uncertainty for the  $\beta_1$  parameter observed for all subsets (indicated by the wide interquartile ranges from the Nyunt et al. analysis) may be attributable to the limited amount of information available for the absorption phase of the data.

	Estimate	Std. Error	Pr(> t )
$\beta_0$	72.90	0.96	0.00
$\beta_1$	10.11	0.67	0.00
$\beta_2$	0.07	0.003	0.00

Table 3.6: Model Output for Sulfadoxine Bi-Exponential NLS model

	Estimate	Std. Error	Pr(> t )
$\beta_1$	17.95	5.23	0.00
$\beta_2$	-21.73	6.05	0.00
$\beta_3$	1.89	0.79	0.02
$\beta_4$	77.62	1.98	0.00
$\beta_5$	0.08	0.004	0.00

Table 3.7: Model Output for Sulfadoxine Triple-Exponential NLS model

Tables 3.6 and 3.7 are the results obtained for the full dataset from NLS models using the bi- and triple-exponential structural model forms respectively. The results were the same regardless of which set of starting values were applied. Comparisons of these models based on Aikake (9395.81 vs. 9377.96) and Bayesian Criteria (9415.97 vs. 9408.20) indicated that the triple-exponential model was preferred.

For the pregnant subset, comparisons of the Aikake criteria of the bi- and triple-exponential models indicated that the inclusion of the additional exponential term improved the general model fit, (6131.44 vs. 6123.79), although the Bayesian criteria favoured the simpler model (6149.9 vs. 6151.47). For the postpartum subset however, the triple-exponential model resulted in disproportionately large standard errors indicative of multi-collinearity, and so was discarded. This may in part be attributed to the very sparse sampling observed for postpartum subjects in Mozambique and Sudan (concentrations were measured on days 0 and 7 only for these subjects).

The validity of these triple-exponential models was brought into question by

a paper published in the Journal of Pharmacokinetics and Biopharmaceutics in 1998, entitled “Pitfalls in Pharmacokinetic Multicompartment Analysis”, by Liang and Derendorf [56]. Using simulation techniques, the authors explored the potential for erroneous model specification, and determined that for the model formulation below:

$$C = A_1 \times \exp(-\alpha \times \text{time}) + A_2 \times \exp(-\beta \times \text{time}) \\ + A_3 \times \exp(-k \times \text{time})$$

the values of parameters  $A_1$  and  $A_2$  played a more significant role in the accurate determination of a truly biphasic elimination profile than the rate constants  $\alpha$  and  $\beta$ .

They determined that a monophasic elimination profile was indicated for situations in which both parameters were negative, or if otherwise, for the case in which  $|A_2| > |A_1|$ . Biphasic elimination profiles were indicated for situations where  $|A_1| > |A_2|$ .

Using the triple-exponential model specification given by equation 3.3, parameters  $\beta_2$  and  $\beta_4$  are those corresponding to parameters  $A_1$  and  $A_2$  respectively. Examination of the parameter estimates presented in Table 3.7 shows that  $\beta_4 > |\beta_2|$ , which is thus indicative of a misspecification.

For the NLS models then, the bi-exponential models were deemed more appropriate for both the full dataset and the pregnant subset. Results from this model specification indicated that when compared to postpartum subjects, pregnant subjects exhibited higher values for  $\beta_0$  (78.4 vs. 61.5), a slower rate of incline (10.36 vs. 12.26), and faster rate of decline (0.084 vs. 0.054), as observed in the initial data exploration and indicated by the starting values obtained from the curve-stripping procedure, (Appendix B, Tables B.1 and B.2).

Plots of the standardized residuals against the subject ID (Figure 3.10), showed residuals for several subjects that were either strictly positive or negative, indicating the need for the subject-specific random effects.

### 3.2.2 Individual-Specific Models

Individual-specific model fits for both structural model specifications provided us with alternative starting values: parameter estimates from the individual fits were averaged across all individuals for later use in the nlme models.

Individual-specific fits could not be achieved for subjects in Mozambique or Sudan due to sparsely sampled data, since for pregnant subjects no samples were taken for the first 24 hours following dosing, and samples were taken for days 0 and 7 only in the postpartum subjects.

Plots of the parameter estimates and their 95% confidence intervals for each subject may usually be used in order to ascertain which of the parameters might require random effects. A basic range may also be established for each of the

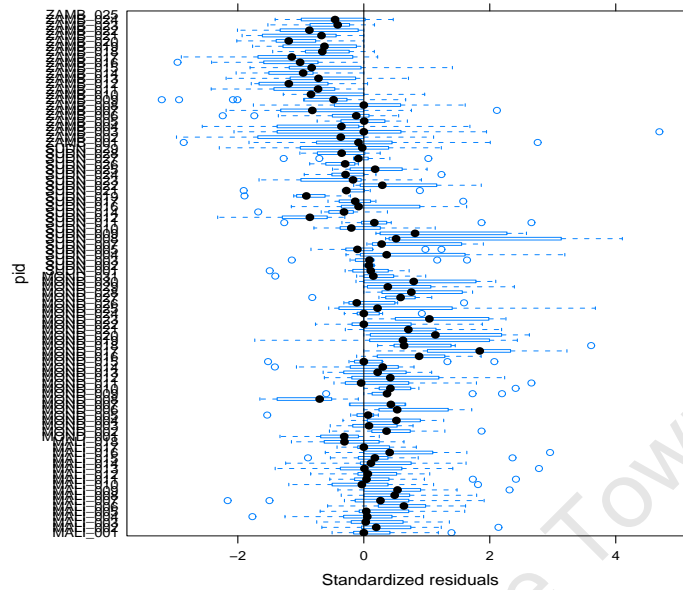


Figure 3.10: Residuals by Subject ID: Sulfadoxine Bi-Exponential NLS Model

parameters, and individual subjects with mean values and confidence intervals outside of this range may be identified for further examination as potential outliers. In this instance, because of the large number of individuals for whom these model fits and estimates could not be obtained (approximately half of the individuals in the dataset), the graphs are not overly informative.

Figure 3.11 shows the parameter estimates and 95% confidence intervals for the bi-exponential model specification. The lack of overlapping intervals seen for  $\beta_0$  indicated the potential need for random effects on this parameter. This was not so easily determined for  $\beta_1$  or  $\beta_2$ , as comparatively wide confidence intervals for some of the individuals obscured the scale and intervals for the remaining subjects. Removal of two of the individuals with overly large confidence intervals resulted in Figure 3.12, which indicated that random effects were required for  $\beta_2$ , but not necessarily for  $\beta_1$ .

The graphics for the triple-exponential model were not useful, as so few individual fits could be obtained for this specification.

### 3.2.3 Multi-Level NLME Models

The specification of starting values for the nlme models played a significant role in both the convergence and stability of the model.

Basic nlme models (ignoring the impact of covariates) were again fit for both the bi- and triple-exponential model specifications for the pregnant and postpartum

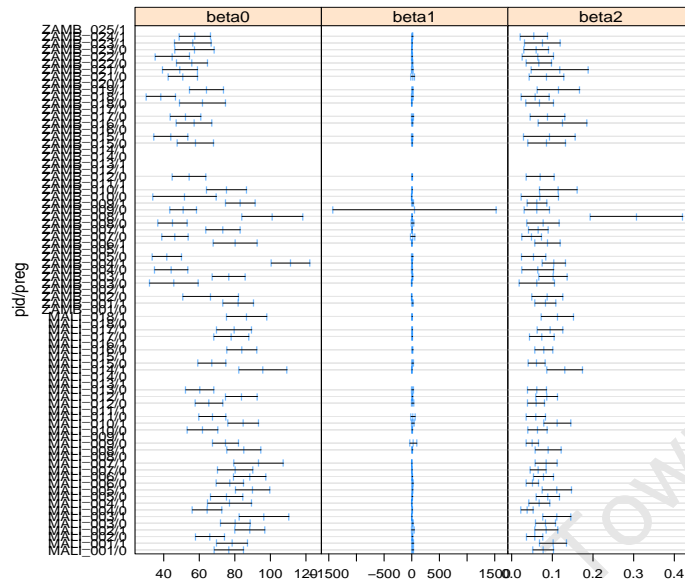


Figure 3.11: Parameter Estimates and 95% Confidence Intervals for Individual Model Fits (I)

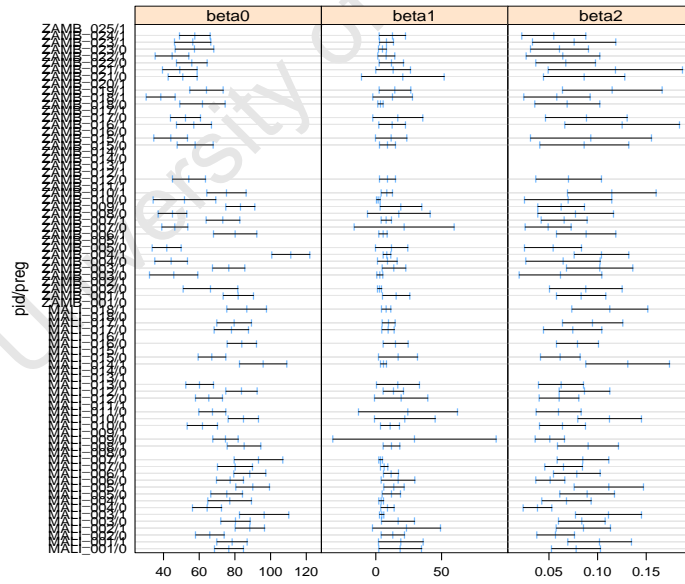


Figure 3.12: Parameter Estimates and 95% Confidence Intervals for Individual Model Fits (II)

subsets. These subset analyses were done in order to double check the impact of pregnancy on the beta parameters, and thus to better inform the starting values used later when incorporating pregnancy as a covariate in the model for the full dataset.

Stable versions of the models were achieved using starting values for the fixed effects based on the parameter estimates from the appropriate NLS models, and a positive-definite diagonal covariance matrix for both levels of random effects (subject and occasion specific).

We were unable to fit a triple-exponential model for the postpartum subset, and the results from this formulation for the pregnant subjects indicated misspecification of the structural model form, since the parameter estimates for  $\beta_2$  and  $\beta_4$  were such that  $|\beta_4| = 81.04 > |\beta_2| = 0.157$ .

Table 3.8 shows the Aikake and Bayesian information criteria for the comparison of the bi- and triple-exponential models for the pregnant subset: the model comparison supported the conclusion that the simpler model was preferred. The bi-exponential model form was thus selected to continue the model building process.

Model	df	AIC	BIC
Bi-Exponential	7	5599.207	5631.501
Triple-Exponential	11	5624.219	5674.966

Table 3.8: Model Comparison for Bi-Exponential NLME Models: Pregnant Subset

### Model 1:

The initial model fit to the Sulfadoxine concentration-time data was thus given by equation:

$$y_{ikj} = f(\text{time}_{ikj}, \boldsymbol{\beta}_{ikj}) + e_{ikj}$$

where  $y_{ikj}$  is the  $j$ th measurement in the  $k$ th period for the  $i$ th individual, and

$$\begin{aligned} f(\text{time}_{ikj}, \boldsymbol{\beta}_{ikj}) &= \beta_{0ikj} [-\exp(-\beta_{1ikj} \times \text{time}_{ikj}) + \exp(-\beta_{2ikj} \times \text{time}_{ikj})] \\ &= (\beta_0 + b_{0i} + b_{0ik}) \times [-\exp(-(\beta_1 + b_{1i} + b_{1ik}) \times \text{time}_{ikj}) + \exp(-(\beta_2 + b_{2i} + b_{2ik}) \times \text{time}_{ikj})] \end{aligned}$$

Thus, in the specification of the stage II equations:

$$\boldsymbol{\beta}_{ikj} = \mathbf{A}_{ikj}\boldsymbol{\beta} + \mathbf{B}_{i,kj}\mathbf{b}_i + \mathbf{B}_{ik,j}\mathbf{b}_{ik},$$

the  $p$ -dimensional vector of fixed effects  $\boldsymbol{\beta}$  is given by

$$\boldsymbol{\beta} = [\beta_0, \beta_1, \beta_2],$$

the  $q_1$  – and  $q_2$ -dimensional vectors of the subject and occasion-specific random effects are given by:

$$\begin{aligned} \mathbf{b}_i &= [b_{0i}, b_{1i}, b_{2i}] \\ \mathbf{b}_{ik} &= [b_{0ik}, b_{1ik}, b_{2ik}] \end{aligned}$$

Parameter	Value	Std.Error	DF	P-value
$\hat{\beta}_0$	79.09	1.99	977.00	<.0001
$\hat{\beta}_1$	13.95	1.07	977.00	<.0001
$\hat{\beta}_2$	0.08	0.002	977.00	<.0001

Table 3.9: Bi-Exponential Model Output: Sulfadoxine NLME Model 1

and the design matrices  $\mathbf{A}_{ikj}$ ,  $\mathbf{B}_{i,kj}$ , and  $\mathbf{B}_{ik,j}$  are  $(3 \times 3)$  identity matrices.

Table 3.9 summarizes the results from this model, showing the fixed effects parameter estimates together with their standard errors, the degrees of freedom and associated significance.

The within group errors  $e_{ikj}$  are assumed independently and identically normally distributed, with variance-covariance matrix  $\mathbf{R}_{ik}$  for every  $ik$  combination, where  $\mathbf{R}_{ik}$  is the positive-definite  $(n_{ik} \times n_{ik})$  matrix with diagonal elements  $\hat{\sigma}^2 = (7.36)^2$ .

The subject and occasion-specific random effects are also assumed to be normally distributed:

$$\mathbf{b}_i \sim N(\mathbf{0}, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(\mathbf{0}, \boldsymbol{\psi}_2)$$

with variance-covariance matrices,  $(\boldsymbol{\psi}_1$  and  $\boldsymbol{\psi}_2)$  given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} 14.85^2 & 0 & 0 \\ 0 & 3.06^2 & 0 \\ 0 & 0 & 7.77e-06^2 \end{bmatrix}, \hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} 13.72^2 & 0 & 0 \\ 0 & 5.85^2 & 0 \\ 0 & 0 & 0.018^2 \end{bmatrix}$$

The diagnostic plots for this model (Appendix B, figures B.1 to B.5) indicated that the normality assumptions for the random effects and residual errors appeared to be reasonable. QQ plots of the random effects for the respective grouping levels showed very little subject or occasion-specific variation for the  $\beta_1$  parameter. Pairs plots of the random effects for the different levels showed no distinct correlation patterns, (Appendix B, figures B.6 and B.7).

Figure 3.13 is a plot of the standardized residuals against the fitted values (with outliers identified by the labelled points), and indicated that the assumption of constant variance for the residual errors was invalid, as demonstrated by the outward fanning of the residuals which shows increasing variance for larger fitted values. The model thus appears to be over- or under-estimating the peak concentrations.

The overall fit of the model was examined using plots of the fitted values versus the observed, (Appendix B, figure B.8), and plots of the predicted concentration-time curves for the population, individual-specific and occasion-specific levels versus the observed values for each individual-occasion grouping.

Figure 3.14 shows the latter plots for a subset of the dataset; the blue lines represent the population curve obtained using the fixed effects parameter estimates,

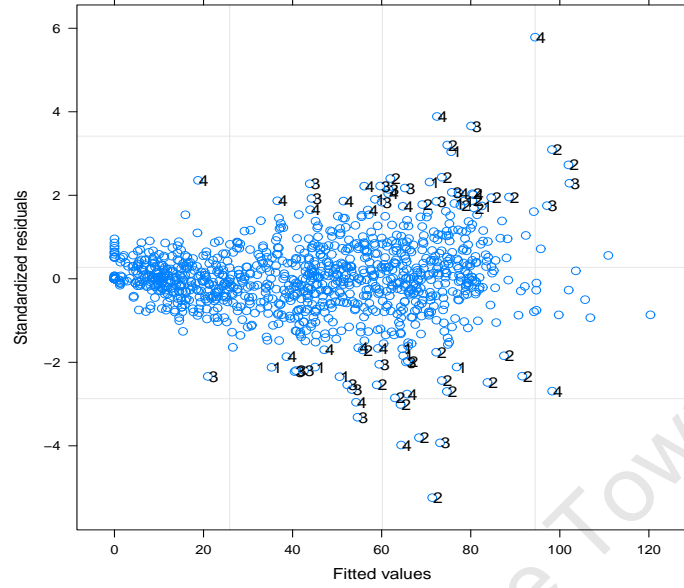


Figure 3.13: Standardized Residuals vs. Fitted Values: Sulfadoxine NLME Model 1

i.e. the predicted concentrations  $\hat{y}_{ikj}$  given by:

$$\begin{aligned}\hat{y}_{ikj} &= \hat{\beta}_0 \times [-\exp(-\hat{\beta}_1 \times time_{ikj}) + \exp(-\hat{\beta}_2 \times time_{ikj})], \\ &= 79.09 \times [-\exp(-13.95 \times time_{ikj}) + \exp(-0.08 \times time_{ikj})]\end{aligned}$$

The pink and green lines are the predicted curves for the subject and occasion-specific grouping levels respectively, i.e. the predicted concentrations given by:

$$\hat{y}_{ikj} = (\hat{\beta}_0 + \hat{b}_{0i}) \times [-\exp(-(\hat{\beta}_1 + \hat{b}_{1i}) \times time_{ikj}) + \exp(-(\hat{\beta}_2 + \hat{b}_{2i}) \times time_{ikj})]$$

and

$$\hat{y}_{ikj} = (\hat{\beta}_0 + \hat{b}_{0i} + \hat{b}_{0ik}) \times [-\exp(-(\hat{\beta}_1 + \hat{b}_{1i} + \hat{b}_{1ik}) \times time_{ikj}) + \exp(-(\hat{\beta}_2 + \hat{b}_{2i} + \hat{b}_{2ik}) \times time_{ikj})]$$

The peak concentration value is under-estimated for several individuals (e.g. subject SUDN\_012/1), and the overlapping of the blue, pink and green lines for the absorption phase of the curves demonstrates the lack of subject or occasion-specific variability for the parameter representing the slope of this line.

The small subject-specific variability for the  $\beta_2$  parameter ( $\hat{\tau}_2^2 = 7.77e - 06^2$ ) is also illustrated by the overlapping blue and pink lines in the elimination phase of the curves.

As described in the section on model building, for each grouping level and parameter, plots of the random effects versus covariates were used as a guideline



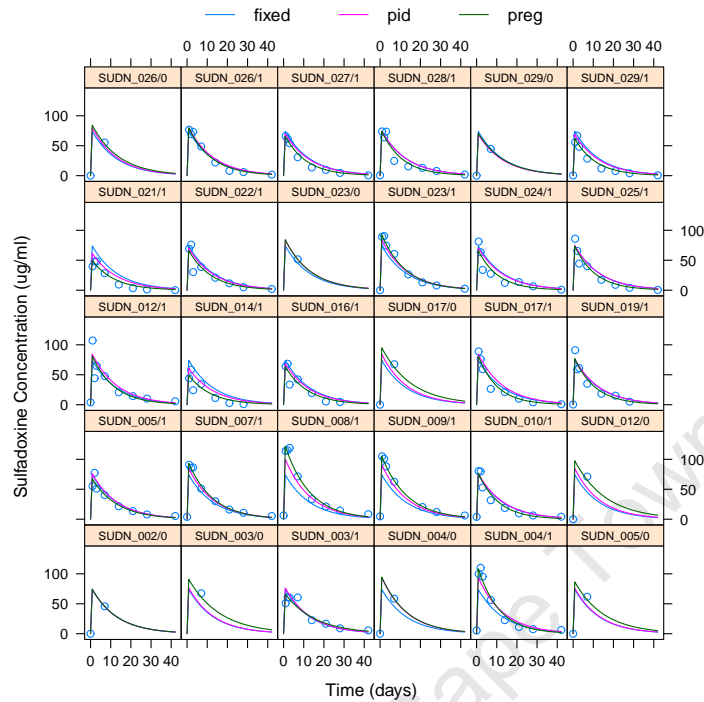


Figure 3.14: Population, Subject and Occasion-Specific Predicted Curves (Subset: Sudan): Sulfadoxine NLME Model

in deciding on the placement of covariates.

Figures 3.15, and 3.16 are plots of the subject-specific random effects for  $\beta_0$  and  $\beta_1$  vs. trimester, weight, site and anaemia. Figure 3.17 is the plot of the occasion-specific random effects vs. the same covariates for  $\beta_2$ . The plots for the parameters and grouping levels not shown here may be found in Appendix B, figures B.9 to B.11.

Using study site Mali as a reference category, subjects in study sites Mozambique and Zambia appear to have higher and lower median  $\beta_0$  values respectively. Anaemic subjects, and those in their 2nd or 3rd trimester appear to have higher median  $\beta_0$  values (when compared to non-anaemic and postpartum subjects respectively). There does not appear to be any weight effect for this parameter.

The systematic patterns detectable in the subject-specific variation for  $\beta_0$  are not as evident for the occasion-specific grouping level, with the exception of Anaemia, (Appendix B, figure B.10).

Figure 3.16 illustrates the limited amount of subject-specific variability for the  $\beta_1$  parameter, (representing the slope of the incline, and analogous to the absorption rate constant  $ka$ ), although this may not necessarily indicate a true lack of variability; it is more likely a consequence of the limited data available

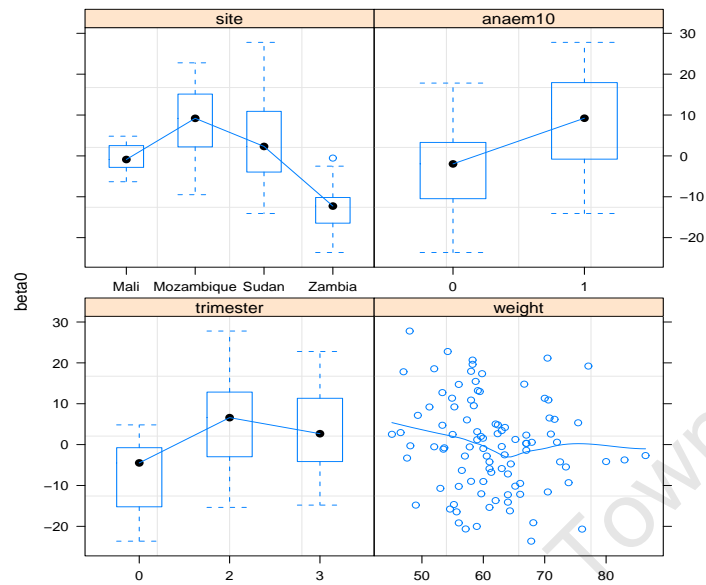


Figure 3.15: Subject-Specific Random Effects for  $\beta_0$  vs. Covariates: Sulfadoxine NLME Model

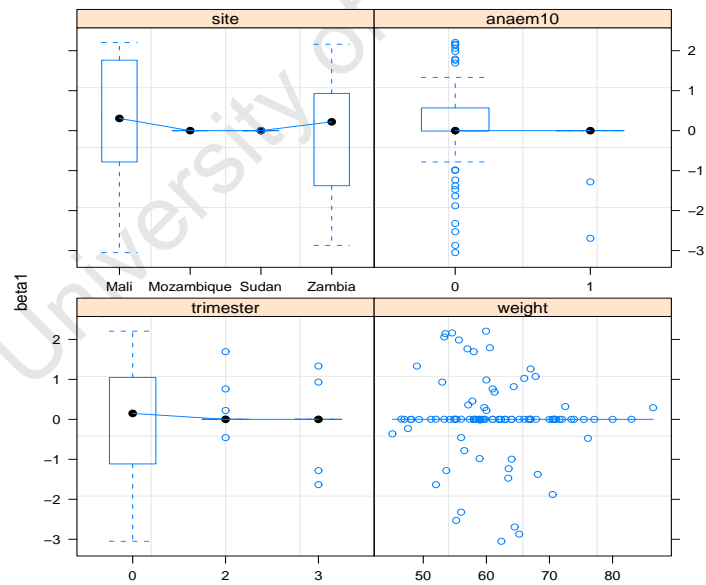


Figure 3.16: Subject-Specific Random Effects for  $\beta_1$  vs. Covariates: Sulfadoxine NLME Model

for this part of the curve. No systematic patterns are observed.

The same observations could be made for the plot of the occasion-specific random effects vs. covariates (Figure B.11 in Appendix B) for  $\beta_1$ .

There do not appear to be any large differences in the value of  $\beta_2$  attributable to study site, anaemia or weight, where  $\beta_2$  is the parameter giving the slope of the decline and is analogous to the elimination rate constant  $k_e$ , (Figure 3.17).

Subjects in their 2nd and 3rd trimester do appear to have higher median values, which would indicate a faster rate of decline. This systematic pattern in the occasion-specific variability is not observed in the plots of the subject-specific random effects for this parameter.

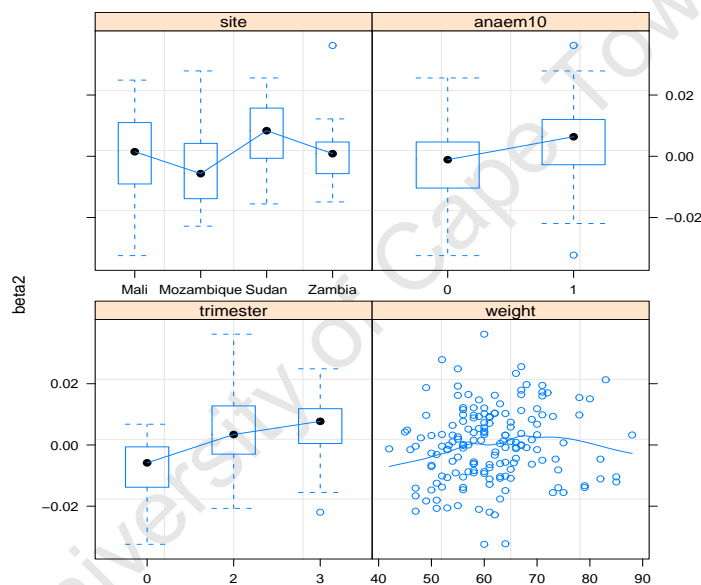


Figure 3.17: Occasion-Specific Random Effects for  $\beta_2$  vs. Covariates: Sulfadoxine NLME Model

Since the main objective of the study was to determine how the concentration-time relationship of the drugs was affected by pregnancy, we started the covariate model building procedure by looking at pregnancy and its proxy measurements, trimester and gestational age (plots not shown).

The graphs presented in figures 3.15, 3.16 and 3.17 and the initial data exploration indicated a possible systematic pregnancy effect for parameters  $\beta_0$  and  $\beta_2$ . These graphs were used as a guideline only however, and the inclusion of pregnancy as a covariate on the fixed effect parameters was examined through an iterative stepwise model building procedure, in which pregnancy was added to each parameter in turn and to various combinations of parameters in increas-

ing levels of complexity.

Model comparisons using the Aikaike and Bayesian information criteria and the (“anti-conservative”) likelihood ratio test (for nested models) indicated a preference for the initial model (excluding covariates) at every step.

**Model 2:**

The removal of the subject-specific random effect for parameter  $\beta_2$  ( $\hat{\tau}_2^2 = 7.77e - 06^2$ ) appeared to improve the model fit, (AIC: 8484.19 vs. 8482.19, BIC: 8534.59 vs. 8527.55), and we were then able to fit pregnancy to both  $\beta_0$  and  $\beta_2$ .

**Model 3:**

The resulting stage 2 equations are given by:

$$\beta_{ikj} = \mathbf{A}_{ikj}\beta + \mathbf{B}_{i,kj}\mathbf{b}_i + \mathbf{B}_{ik,j}\mathbf{b}_{ik},$$

where:

$$\begin{bmatrix} \beta_{0ikj} \\ \beta_{1ikj} \\ \beta_{2ikj} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & preg_{ik.} & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & preg_{ik.} \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \\ \beta_4 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} b_{0i} \\ b_{1i} \\ b_{2i} \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} b_{0ik} \\ b_{1ik} \\ b_{2ik} \end{bmatrix}$$

such that:

$$\begin{aligned} \beta_{0ikj} &= \beta_0 + \beta_3 \times preg_{ik.} + b_{0i} + b_{0ik}, \\ \beta_{1ikj} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ikj} &= \beta_2 + \beta_4 \times preg_{ik.} + b_{2ik}. \end{aligned}$$

The distributional assumptions for the  $e_{ikj}$  and the subject and occasion-specific random effects remain unchanged, and the results for the fixed effects are summarized in Table 3.10.

Parameter	Value	Std.Error	DF	P-value
$\hat{\beta}_0.(Intercept)$	71.91	2.58	975.00	<.0001
$\hat{\beta}_3.(Pregnancy)$	8.89	2.50	975.00	<.0001
$\hat{\beta}_1$	14.22	1.10	975.00	<.0001
$\hat{\beta}_2.(Intercept)$	0.06	0.003	975.00	<.0001
$\hat{\beta}_4.(Pregnancy)$	0.03	0.004	975.00	<.0001

Table 3.10: Model Output for Sulfadoxine NLME Model 3

The variance-covariance matrices for the random effects for each grouping level are given by:

$$\hat{\psi}_1 = \begin{bmatrix} 14.52^2 & 0 & 0 \\ 0 & 3.32^2 & 0 \\ 0 & 0 & 0 \end{bmatrix}, \hat{\psi}_2 = \begin{bmatrix} 11.98^2 & 0 & 0 \\ 0 & 5.80^2 & 0 \\ 0 & 0 & 0.015^2 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (7.32)^2$ .

This model was determined to be significantly better than model 2 (AIC: 8482.19 vs. 8427.35, BIC: 8527.55 vs. 8482.8 and lrtest p-value (anti-conservative):  $<0.0001$ ).

Examination of the diagnostic plots for this model (Appendix B, figures B.12 to B.18) indicate no departure from the assumptions aside from the assumption of constant variance, as demonstrated by Figure 3.18.

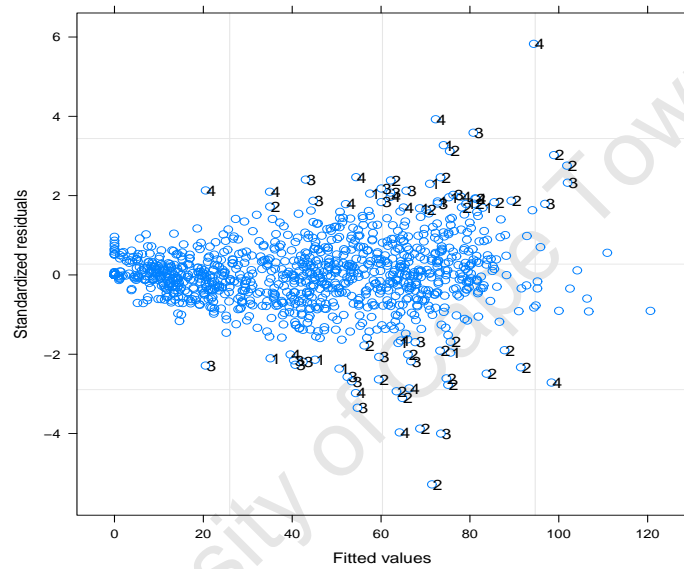


Figure 3.18: Standardized Residuals vs. Fitted Values: Sulfadoxine NLME Model 3

A variance model of the form:

$$g^2(\mu, \mathbf{z}_j, \boldsymbol{\theta}) = (\theta_1 + \mu_j^{\theta_2})^2, \theta_1, \theta_2 > 0,$$

was therefore specified for the residual errors ( $e_{ikj}$ ), where  $\mu_j$  is the expected value of the  $j$ th response (for each  $ik$  combination) and  $\boldsymbol{\theta} = [\theta_1, \theta_2]$ , which results in approximately proportional errors.

The inclusion of this variance model appeared to greatly improve the model fit, (AIC: 8427.35 vs. 7867.06, BIC: 8482.8 vs. 7932.59, and lrtest p-value:  $<0.0001$ ).

#### Model 4:

The results for the fixed effects from this model are summarized in Table 3.11, and the variance-covariance matrices for the subject and occasion-specific ran-

dom effects, ( $\psi_1$  and  $\psi_2$  respectively) are now given by:

$$\hat{\psi}_1 = \begin{bmatrix} 13.63^2 & 0 & 0 \\ 0 & 3.36^2 & 0 \\ 0 & 0 & 0 \end{bmatrix}, \hat{\psi}_2 = \begin{bmatrix} 11.22^2 & 0 & 0 \\ 0 & 5.02^2 & 0 \\ 0 & 0 & 0.014^2 \end{bmatrix}$$

The ( $n_{ik} \times n_{ik}$ ) variance-covariance matrix  $\mathbf{R}_{ik}$  for the residual errors is now:

$$\hat{\mathbf{R}}_{ik} = \begin{pmatrix} \frac{\sigma^2(\theta_1 + \mu_1^{\theta_2})^2}{\sigma_2\sqrt{(\theta_1 + \mu_2^{\theta_2})^2} \times \sigma_1\sqrt{(\theta_1 + \mu_1^{\theta_2})^2}} & \sigma_1\sqrt{(\theta_1 + \mu_1^{\theta_2})^2} \times \sigma_2\sqrt{(\theta_1 + \mu_2^{\theta_2})^2} & \dots \\ \sigma_2\sqrt{(\theta_1 + \mu_2^{\theta_2})^2} \times \sigma_1\sqrt{(\theta_1 + \mu_1^{\theta_2})^2} & \sigma^2(\theta_1 + \mu_2^{\theta_2})^2 & \dots \\ \vdots & \vdots & \ddots \end{pmatrix}$$

where  $\hat{\sigma}^2 = 0.077^2$ , and  $\hat{\boldsymbol{\theta}} = [20.37, 1.14]$ .

Parameter	Value	Std.Error	DF	P-value
$\hat{\beta}_0.(Intercept)$	70.69	2.47	975.00	<.0001
$\hat{\beta}_3.(Pregnancy)$	8.62	2.45	975.00	<.0001
$\hat{\beta}_1$	13.37	1.07	975.00	<.0001
$\hat{\beta}_2.(Intercept)$	0.06	0.003	975.00	<.0001
$\hat{\beta}_4.(Pregnancy)$	0.03	0.003	975.00	<.0001

Table 3.11: Model Output for Sulfadoxine NLME Model 4

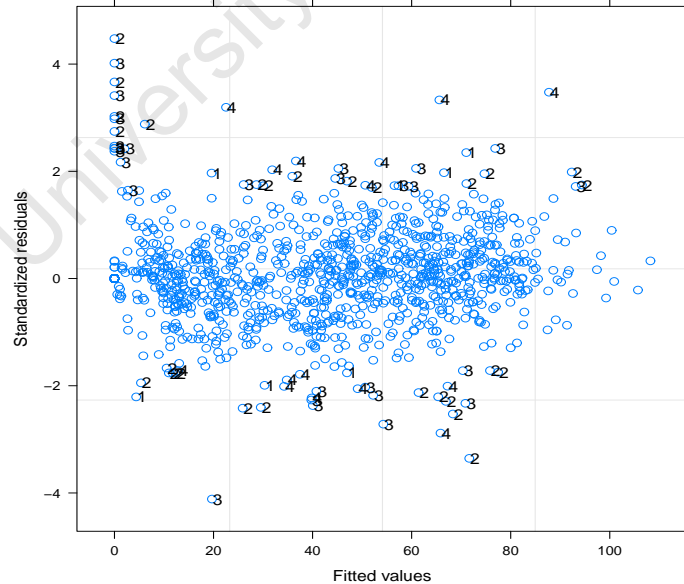


Figure 3.19: Standardized Residuals vs. Fitted Values: Sulfadoxine NLME Model 4

Figure 3.19 illustrates the reduction in the variance of the residuals for the larger fitted values for Model 4. As seen before, pregnant subjects in models 3 and 4 appeared to have systematically higher values for  $\beta_0$  and  $\beta_2$  compared to postpartum, illustrated by the positive effect modifiers  $\beta_3$  and  $\beta_4$  respectively. This indicates that pregnant subjects have a higher range of concentrations and a faster rate of decline than those postpartum.

This is illustrated in Figure 3.20 which is a plot of the mean predicted concentration-time curves for the pregnant and postpartum subjects.

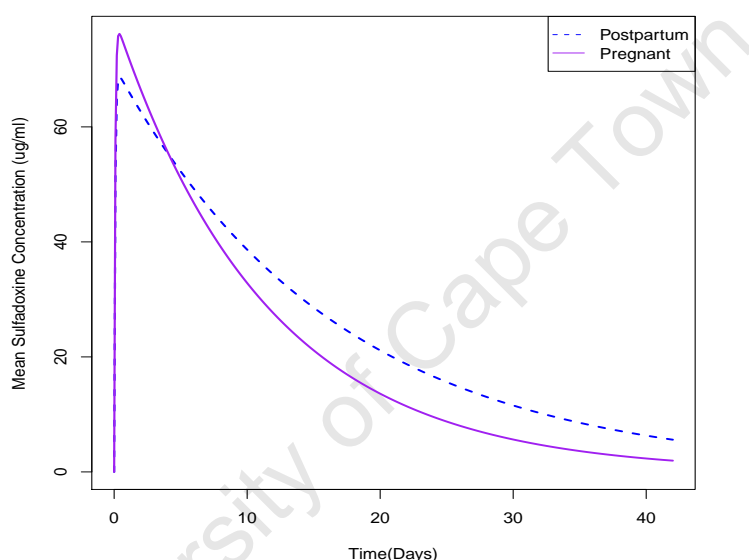


Figure 3.20: Predicted Mean Concentration-Time Curves by Pregnancy Phase: Sulfadoxine NLME Model 4

Examining the plot of the subject-specific random effects vs. covariates for the  $\beta_0$  parameter from Model 4, (Figure 3.21), we see that the trimester and anaemia effects previously noted have been somewhat reduced, although the site effect remains.

No patterns can be seen for the occasion-specific random effects for this parameter, (Appendix B, figure B.19).

Looking at the occasion-specific random effects for the  $\beta_2$  parameter, (Figure 3.22), both the trimester and anaemia effects are no longer in evidence.

No further adjustments to the random effects were deemed necessary, and the inclusion of trimester in place of pregnancy did not improve the model fit, (as anticipated since no differences were observed between the 2nd and 3rd trimesters for any of the parameters). Model 4 is therefore the final pregnancy model for

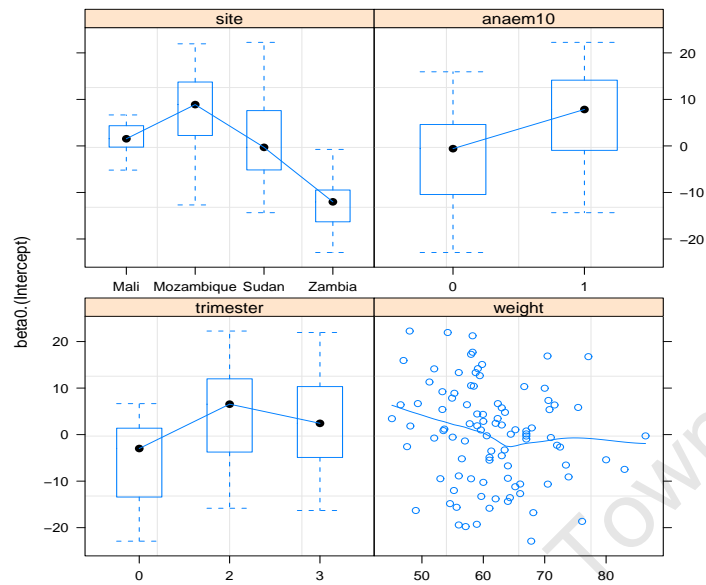


Figure 3.21: Subject-Specific Random Effects for  $\beta_0$  vs. Covariates: Sulfadoxine NLME Model 4

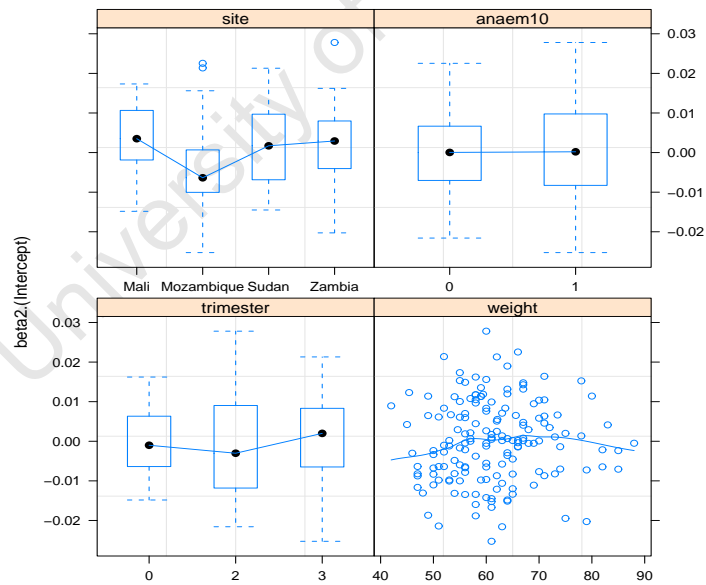


Figure 3.22: Occasion-Specific Random Effects for  $\beta_2$  vs. Covariates: Sulfadoxine NLME Model 4



Sulfadoxine.

Figure 3.23 is the plot of the fitted values vs. observed for this model, which indicates a relatively good fit.

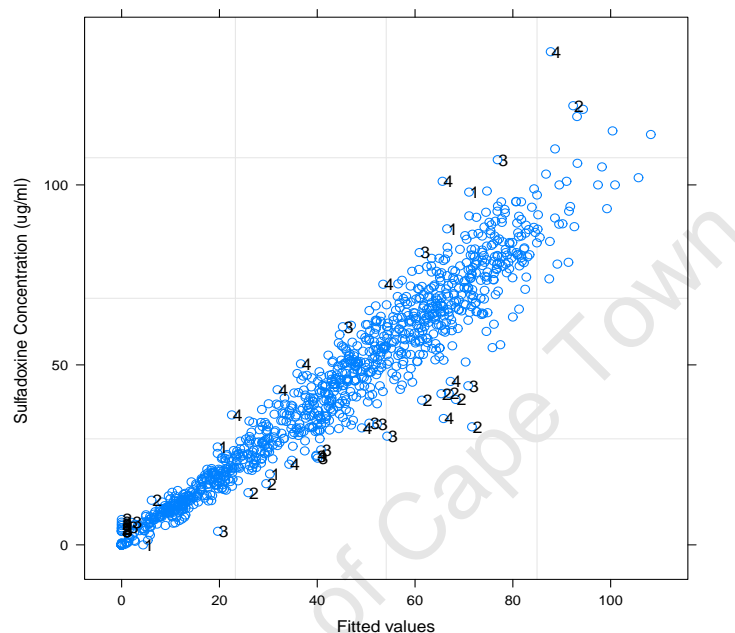


Figure 3.23: Fitted Values vs. Observed: Sulfadoxine NLME Model 4

The results for the fixed effects for model 4 presented in Table 3.10 have the same degrees of freedom for every parameter ( $df=975$ ), which seems intuitively incorrect if we assume that the algorithm for the calculation of these degrees of freedom described for the linear mixed effect model is applicable here.

Due to the ongoing discourse regarding the correct method of calculating the degrees of freedom, we have chosen not to interpret the statistical significance of the individual parameters. This impacts on the model building procedure, and as such, an hypothesized model building approach was adopted for the remaining covariates.

#### Model 5:

The visible site effect seen in Figure 3.21, the plot of the random effects vs. covariates for  $\beta_0$ , would suggest that site be incorporated as a categorical covariate on this parameter. However, models of this form would not converge.

Based on the supposition that effects seen for  $\beta_0$  might be incorporated through either  $\beta_1$  or  $\beta_2$ , (since  $\beta_0$  is a composite of these parameters), a model was fitted with site as a categorical covariate on  $\beta_2$ .

Since the inclusion of site on  $\beta_2$  alone did not reduce the effects seen in the graphs, site was then added to the  $\beta_0$  parameter. Following examination and adjustment of the random effects included in the model, the stage 2 equations for Sulfadoxine model 5 may be given by:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_3 \times \text{preg}_{ik.} + \beta_6 \times \text{mozambique}_{i..} + \beta_7 \times \text{zambia}_{i..} + b_{0i} + b_{0ik}, \\ \beta_{1ikj} &= \beta_1, \\ \beta_{2ikj} &= \beta_2 + \beta_4 \times \text{preg}_{ik.} + \beta_5 \times \text{mozambique}_{i..}\end{aligned}$$

The distributional assumptions for the  $e_{ikj}$  and the variance model form remain unchanged. The fixed effects are summarized in Table 3.12, where the degrees of freedom and significance have been excluded from the model output, (see Section 2.7.2).

Parameter	Value	Std.Error
$\hat{\beta}_0.(Intercept)$	73.43	2.49
$\hat{\beta}_3.(Pregnancy)$	8.05	2.62
$\hat{\beta}_6.(Mozambique)$	8.88	3.25
$\hat{\beta}_7.(Zambia)$	-21.61	2.79
$\hat{\beta}_1$	13.9	0.91
$\hat{\beta}_2.(Intercept)$	0.06	0.002
$\hat{\beta}_4.(Pregnancy)$	0.03	0.002
$\hat{\beta}_5.(Mozambique)$	-0.01	0.003

Table 3.12: Model Output for Sulfadoxine NLME Model 5

The distributions for the random effects for each grouping level are given by:

$$b_{0i} \sim N(0, 0.009^2), \quad b_{0ik} \sim N(0, 13.07^2)$$

and the parameters determining the elements of variance-covariance matrix  $\mathbf{R}_{ik}$  are given by:

$$\hat{\boldsymbol{\theta}} = [6.073, 0.871], \quad \hat{\sigma}^2 = 0.26^2$$

Models with subject- and occasion-specific random effects on parameters other than  $\beta_0$  resulted in non-positive definite variance-covariance matrices. The grouping of the site categories such that both Mali and Sudan form the baseline for the interpretation of the  $\beta_6$  and  $\beta_7$  parameters and Mali, Sudan and Zambia the baseline for the interpretation of the  $\beta_5$  parameter reduced the multi-collinearity observed in the model with site Mali as the baseline in all cases.

The inclusion of site as a covariate on  $\beta_0$  and  $\beta_2$  appears to have significantly reduced the effects previously visible in the plots of the random effects versus the covariates. This is illustrated in Figure 3.24. No further patterns of systematic variability appear to be unaccounted for, and as such, no further covariates were added to the model.

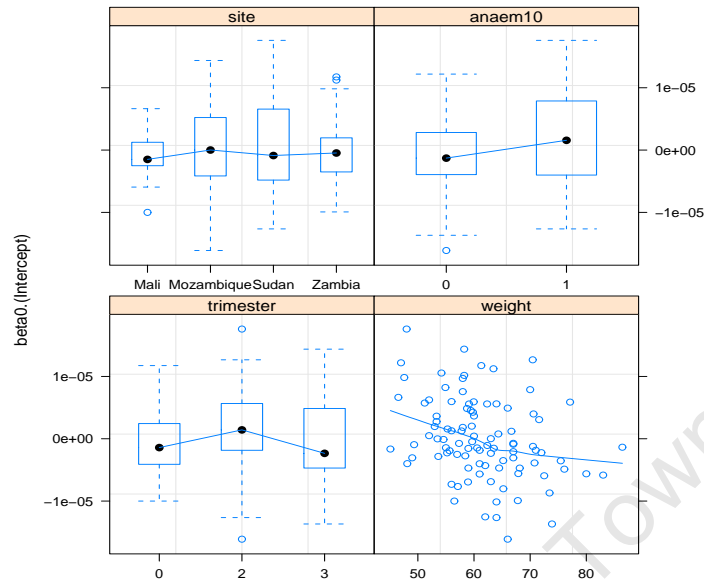


Figure 3.24: Subject-Specific Random Effects for  $\beta_0$  vs. Covariates: Sulfadoxine NLME Model 5

Examination of diagnostic plots (in Appendix B, figures B.20 to B.26) indicate that the assumptions of normality for the residuals and random effects are valid, as is the assumption of homoskedasticity. The model appears to fit reasonably well.

Figures 3.25 and 3.26 show the mean predicted curves by site and pregnancy phases generated from the final Sulfadoxine model, (model 5).

Faster rates of decline are observed for pregnant subjects in all sites, with higher peak concentrations for pregnant subjects in Mali and Zambia, (Figure 3.25). Subjects in Mozambique and Zambia had the highest and lowest concentrations respectively, regardless of pregnancy phase, (Figure 3.26).

### 3.2.4 The Delta Method

Using the mean parameter estimates for the fixed effects and the associated variance-covariance matrices for those fixed effects, the delta method was applied in order to calculate the standard errors for several PK parameters, obtained via back-transformation from the exponential specification using the calculations outlined in section 2.8.

Using the general exponential specification given by:

$$C = \sum_{i=1}^n C_i \times \exp(-\lambda_i \times t)$$

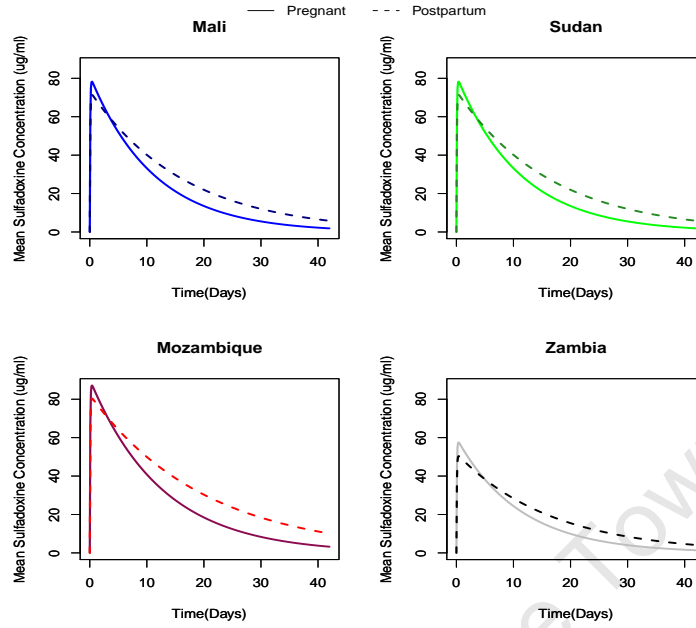


Figure 3.25: Predicted Mean Concentration-Time Curves by Site and Pregnancy Status: Sulfadoxine NLME Model 5

where  $\lambda_i$  is the rate parameter as before,  $n$  is the number of differentiated phases and thus the number of exponential terms, and  $t$  is time, the calculation for the AUC was given as:

$$AUC = \sum_{i=1}^n \frac{C_i}{\lambda_i}$$

Thus for the bi-exponential model used in Model 4:

$$\hat{y}_{ikj} = \hat{\beta}_{0ikj} \times [-\exp(-\hat{\beta}_{1ikj} \times \text{time}_{ikj}) + \exp(-\hat{\beta}_{2ikj} \times \text{time}_{ikj})],$$

where:

$$\begin{aligned} \hat{\beta}_{0ikj} &= \hat{\beta}_0 + \hat{\beta}_3 \times \text{preg}_{ik.} + \hat{b}_{0i} + \hat{b}_{0ik}, \\ &= 70.68906 + 8.61799 \times \text{preg}_{ik.} + \hat{b}_{0i} + \hat{b}_{0ik} \\ \hat{\beta}_{1ikj} &= \hat{\beta}_1 + \hat{b}_{1i} + \hat{b}_{1ik}, \\ &= 13.36970 + \hat{b}_{1i} + \hat{b}_{1ik} \\ \hat{\beta}_{2ikj} &= \hat{\beta}_2 + \hat{\beta}_4 \times \text{preg}_{ik.} + \hat{b}_{2ik} \\ &= 0.06037447 + 0.02777 \times \text{preg}_{ik.} + \hat{b}_{2ik} \end{aligned}$$

the AUC for the postpartum subjects may be calculated as:

$$\begin{aligned} AUC &= \frac{\hat{\beta}_0}{\hat{\beta}_1} + \frac{\hat{\beta}_0}{\hat{\beta}_2} \\ &= \frac{70.68906}{13.36970} + \frac{70.68906}{0.06037447} \\ &= 1176.13 \end{aligned}$$

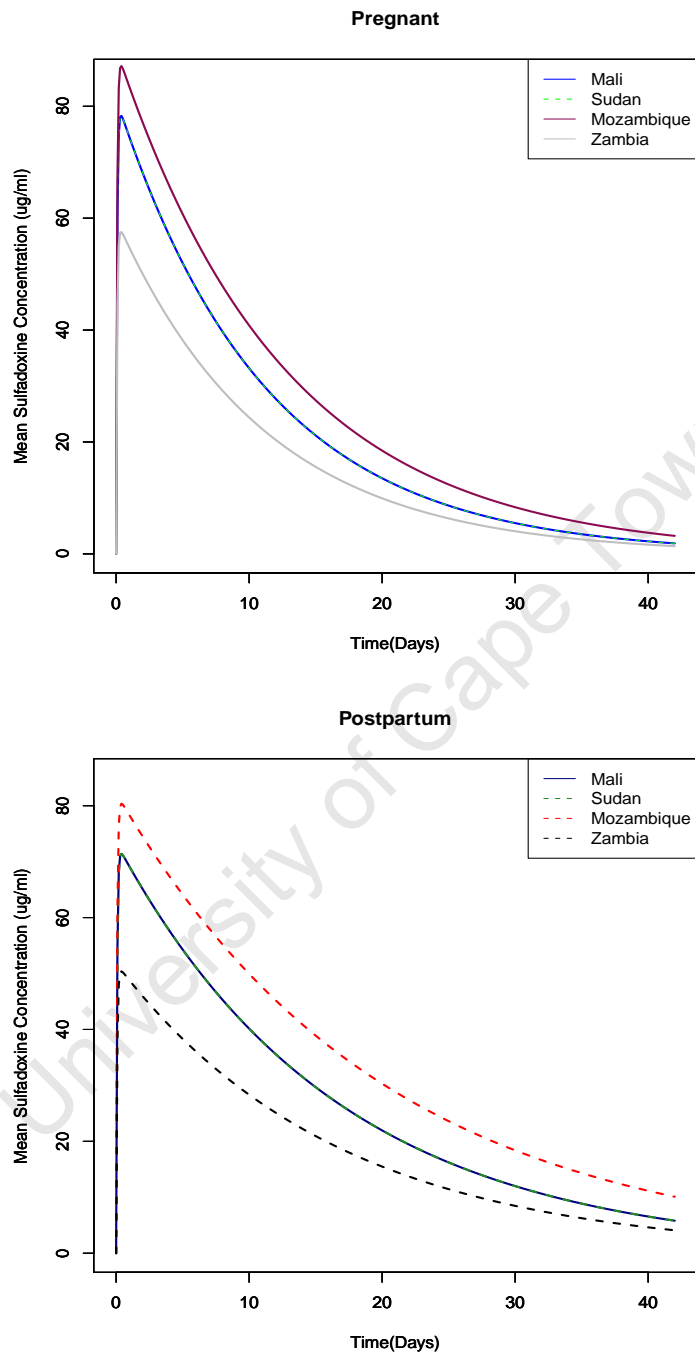


Figure 3.26: Predicted Mean Concentration-Time Curves by Pregnancy Status: Sulfadoxine NLME Model 5

The standard error for this AUC can be calculated using:

$$Var(F(\mathbf{X})) = F'(\mu)^T \times Var(\mathbf{X}) \times F'(\mu)$$

where  $\mathbf{X} = [70.68, 13.37, 0.06]'$ , and

$$Var(\mathbf{X}) = \begin{bmatrix} \hat{\beta}_0 & \hat{\beta}_1 & \hat{\beta}_2 \\ \hat{\beta}_0 & 6.088 & -1.524e-01 & 1.762e-03 \\ \hat{\beta}_1 & -0.152 & 1.135 & -2.65e-04 \\ \hat{\beta}_2 & 0.0018 & -2.138e-04 & 6.976e-06 \end{bmatrix}$$

For the pregnant subjects, the AUC is calculated as:

$$\begin{aligned} AUC &= \frac{\hat{\beta}_0 + \hat{\beta}_3}{\hat{\beta}_1} + \frac{\hat{\beta}_0 + \hat{\beta}_3}{\hat{\beta}_2 + \hat{\beta}_4} \\ &= \frac{70.68906 + 8.61799}{13.36970} + \frac{70.68906 + 8.61799}{0.06037447 + 0.02777} \\ &= 905.6712 \end{aligned}$$

The vector of mean parameter estimates for the fixed effects is then given by  $\hat{\mathbf{X}} = [70.69, 13.37, 0.06, 8.62, 0.03]'$ , and  $Cov(\mathbf{X})$  is the estimated  $(5 \times 5)$  variance-covariance matrix of the fixed effects  $\mathbf{X}$ .

Table 3.13 summarizes the results from this procedure for Model 2, the initial model excluding covariates (that displays increasing variance for the residuals), after the removal of the subject-specific random effect for  $\beta_2$ , and compares them to those obtained by Nyunt et al. [5] by averaging results from individual-specific one-compartment models.

Two sets of results are shown from Nyunt et al.: because of the skew distributions observed for most of the PK parameters, the summary statistics originally reported were restricted to medians and inter-quartile ranges. The results obtained here, however, are means and standard errors, and as such, corresponding results were obtained from the authors for the purposes of comparison. The values for the day 7 concentrations presented in this table are the average values of individual-specific day 7 concentrations, calculated using the predicted concentrations for each individual-phase grouping.

Parameter	Model 2		Nyunt et al.		Nyunt et al. (Reported)	
	Mean	Std. Error	Mean	Std. Error	Median	IQR
$AUC_{(0-\text{inf})}$ (ug.day/ml)	999.44	32.77	902.72	287.85	877.02	(731.93; 1069.47)
Cmax (ug/ml)	74.29	1.97	76.59	41.27	73.93	(59.85; 85.84)
Tmax (days)	0.86	0.05	0.59	0.63	0.38	(0.15; 0.88)
Vd/f (ml/kg)	314.30	7.97	338.95	116.55	305.83	(259.52; 402.48)
Cl/f (ml/kg/day)	25.01	0.86	30.32	11.13	28.20	(23.21; 34.07)
$t_{1/2}$ (days)	8.71	0.22	8.26	2.82	7.92	(6.67; 9.97)
$C_{day7}$ (ug/ml)	45.61	12.10	46.40	15.41	45.75	(34.7; 56.8)

Table 3.13: Comparison of PK Parameters: Sulfadoxine NLME Model 2

The same comparison is shown in Table 3.14, now for Model 4, the final *pregnancy* adjusted model, and thus for the pregnant and postpartum subjects.

The average values from the two methodologies are similar for all parameters.

	Model 4		Nyunt et al.		Nyunt et al. (Reported)	
	Pregnant					
Parameter	Mean	Std. Error	Mean	Std. Error	Median	IQR
$AUC_{(0-\text{inf})}$ (ug.day/ml)	905.63	26.54	903.27	309.06	877.02	(728.53 ; 1049.69)
Cmax (ug/ml)	73.93	1.94	82.74	46.22	80.62	(67.53 ;87.90)
Tmax (days)	0.87	0.056	0.61	0.70	0.34	(0.14 ;0.97)
Vd/f (ml/kg)	308.03	7.71	307.63	112.54	284.50	(248.61 ;358.63)
Cl/f (ml/kg/day)	27.15	0.81	30.56	12.31	28.20	(22.34 ;34.24)
$t_{1/2}$ (days)	7.86	0.16	7.58	2.87	7.38	(6.11 ;8.58)
	Postpartum					
$AUC_{(0-\text{inf})}$ (ug.day/ml)	1176.13	56.31	901.25	224.70	884.38	(732.49; 1079.57)
Cmax (ug/ml)	67.11	2.44	60.01	13.23	59.69	(50.11; 70.57)
Tmax (days)	0.93	0.42	0.55	0.41	0.46	(0.28; 0.82)
Vd/f (ml/kg)	355.63	12.5	423.33	80.28	410.17	(353.12 ;490.73)
Cl/f (ml/kg/day)	21.47	1.044	29.70	7.14	27.97	(24.48; 33.87)
$t_{1/2}$ (days)	11.48	0.50	10.09	1.66	9.89	(8.72; 11.11)

Table 3.14: Comparison of PK parameters during Pregnancy and after Postpartum

The standard errors are much larger, however, for the values obtained from Nyunt et al.

This is to be expected, as the authors followed the traditional two stage approach to the analysis of PK data, calculating population parameter estimates by averaging parameters from individual-specific models, which resulted in over-estimated values for the inter-individual errors as a consequence of the sparsely sampled data. For the postpartum subjects, complete PK parameters could in fact only be determined for subjects from Mali and Zambia using this approach, as the two measurements available for postpartum subjects in Mozambique and Sudan were not sufficient to fit the individual-specific models.

Tables B.3 and B.4 in Appendix B show the comparison between the Nyunt et. al parameters and the results obtained from Model 5. The mean parameters were again similar, with the exception of apparent volume of distribution and clearance, and smaller standard errors were obtained for the results from Model 5, (the nlme model).

### 3.2.5 Single-Level Models: Correlated Random Effects Structure

This section serves to illustrate the alternative method for dealing with the multiply-nested grouping structure of the data outlined in Section 2.6.

Model 4, the final *pregnancy* adjusted model presented earlier, was seen to have

stage 2 equations:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_3 \times preg_{ik.} + b_{0i} + b_{0ik}, \\ \beta_{1ikj} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ikj} &= \beta_2 + \beta_4 \times preg_{ik.} + b_{2ik}\end{aligned}$$

where the vector of the parameter estimates for the fixed effects was found to be  $\hat{\beta} = [70.69, 13.37, 0.06, 8.62, 0.03]$ ,

$$\mathbf{b}_i \sim N(\mathbf{0}, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(\mathbf{0}, \boldsymbol{\psi}_2)$$

$$\mathbf{e}_{ikj} \sim N(\mathbf{0}, \mathbf{R}_{ik})$$

and the elements of  $\mathbf{R}_{ik}$  depend on  $\hat{\sigma}^2 = 0.077^2$ , and  $\hat{\boldsymbol{\theta}} = [20.37, 1.14]$ .

The variance-covariance matrices for the random effects were given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} \tau_0^2 = 13.63^2 & 0 & 0 \\ 0 & \tau_1^2 = 3.36^2 & 0 \\ 0 & 0 & \tau_2^2 = 0 \end{bmatrix},$$

$$\hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} \tau_{0k}^2 = 11.22^2 & 0 & 0 \\ 0 & \tau_{1k}^2 = 5.02^2 & 0 \\ 0 & 0 & \tau_{2k}^2 = 0.014^2 \end{bmatrix}$$

A model was fitted using a single level of grouping, maintaining the same fixed effects structure and the same variance model for the residual errors.

### Model 6:

The stage 2 equations for this model would be given by:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_3 \times preg_{ik.} + b^*_{0i}, \\ \beta_{1ikj} &= \beta_1 + b^*_{1i}, \\ \beta_{2ikj} &= \beta_2 + \beta_4 \times preg_{ik.} + b^*_{2i}\end{aligned}$$

where, for example:

$$b^*_{0i} = \begin{bmatrix} b_{0i} + b_{0i,0} \\ b_{0i} + b_{0i,1} \end{bmatrix}$$

and

$$b^*_{i} \sim N(\mathbf{0}, \boldsymbol{\psi}^*)$$

The results for the fixed effects from this model are summarized in Table 3.15.

The variance-covariance matrix for the single level of random effects is given by:

$$\hat{\boldsymbol{\psi}}^* = \begin{bmatrix} \begin{bmatrix} 17.53^2 & 0.581 \\ 0.581 & 17.53^2 \end{bmatrix} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \begin{bmatrix} 5.72^2 & 0.303 \\ 0.303 & 5.72^2 \end{bmatrix} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \begin{bmatrix} 0.014^2 & 0 \\ 0 & 0.014^2 \end{bmatrix} \end{bmatrix}$$



	Value	Std.Error
$\hat{\beta}_0.(Intercept)$	71.09	2.47
$\hat{\beta}_3.(Pregnancy)$	8.60	2.47
$\hat{\beta}_1$	12.57	0.99
$\hat{\beta}_2.(Intercept)$	0.06	0.003
$\hat{\beta}_4.(Pregnancy)$	0.03	0.003

Table 3.15: Bi-Exponential Model Output: Sulfadoxine NLME Model 6

The variance for the compound symmetry structure in the top-left block is given by:

$$\tau_b^2 = \tau_0^2 + \tau_{0k}^2,$$

with correlation:

$$\rho = \frac{\tau_0^2}{\sqrt{\tau_0^2 + \tau_{0k}^2}}$$

Thus,

$$\begin{aligned}\hat{\tau}_0^2 &= 0.581 \times 17.53^2, \\ &= 13.35^2,\end{aligned}$$

and

$$\begin{aligned}\hat{\tau}_{0k}^2 &= 17.53^2 - \tau_0^2, \\ &= 17.53^2 - 13.35^2, \\ &= 11.36^2.\end{aligned}$$

Comparing the results from Models 6 and 4, we can then see that for both methods, the estimates of the fixed effects and the variance-covariance matrices for the random effects are almost identical. SAS software (Copyright, SAS Institute Inc., Cary, NC, USA) makes use of the single-level model specification, and in R, for this particular model, the single-level version was faster in terms of convergence. The computational algorithms used in the nlme package in R are, however, designed specifically for the nested model approach, and it is thus deemed more efficient.

### 3.2.6 Multi-Level Models with Proportional Random Effects:

The additive random effects specification used in the previous models is unusual for PK data: in the traditional clinical setting, the one and two compartment models are specified in terms of logged fixed effects parameters.

This logged parameterization ensures positivity in the parameters, and also results in a different random effects structure and a proportional covariate model specification.

Using the notation previously defined, for example:

$$\beta'_{0ik} = \log(\beta_0) + \mathbf{b}_{0i} + \mathbf{b}_{0ik}$$

when back-transformed, would be:

$$\beta_{0ik} = \beta_0 \times \exp(\mathbf{b}_{0i}) \times \exp(\mathbf{b}_{0ik})$$

The relationship between the fixed effects parameters and the random effects is thus no longer linear.

**Model 7:**

Maintaining the exponential parameterization of the structural model form, but now using logged parameters, the model building procedure outlined for the case in which there were additive random effects was repeated.

Parameter	Value	Std.Error
$\log(\hat{\beta}_0)$ .(Intercept)	4.25	0.037
$\hat{\beta}_3$ .(Pregnancy)	0.11	0.046
$\log(\hat{\beta}_1)$	2.62	0.05
$\log(\hat{\beta}_2)$ .(Intercept)	-2.83	0.048
$\hat{\beta}_4$ .(Pregnancy)	0.42	0.049

Table 3.16: Bi-Exponential Model Output: Sulfadoxine NLME Model 7

The resulting final model is summarized in Table 3.16, and corresponds to the model equation given by:

$$y_{ikj} = f(\text{time}_{ikj}, \beta'_{ikj}) + e_{ikj}$$

where  $y_{ikj}$  is the  $j$ th measurement in the  $k$ th period for the  $i$ th individual, and

$$f(\text{time}_{ikj}, \beta'_{ikj}) = \exp(\beta'_{0ikj})[-\exp(-\exp(\beta'_{1ikj}) \times \text{time}_{ikj}) + \exp(-\exp(\beta'_{2ikj}) \times \text{time}_{ikj})]$$

where:

$$\beta'_{0ikj} = \log(\beta_0) + \beta_3 \times \text{preg}_{ik} + b_{0ik},$$

$$\beta'_{1ikj} = \log(\beta_1),$$

$$\beta'_{2ikj} = \log(\beta_2) + \beta_4 \times \text{preg}_{ik} + b_{2i}$$

and hence:

$$\beta_{0ikj} = \beta_0 \times \exp(\beta_3) \times \text{preg}_{ik} \times \exp(b_{0i}),$$

$$\beta_{1ikj} = \beta_1,$$

$$\beta_{2ikj} = \beta_2 \times \exp(\beta_4) \times \text{preg}_{ik} \times \exp(b_{2ik})$$

This model does not differ too much from our previous Model 4. Despite obviously heterogeneous variance (Figure 3.27), attempts to account for this by specifying a variance model only worsened the model fit, as did the inclusion of additional covariates such as site.

The variance-covariance estimates for the random effects were greatly reduced, with  $\hat{\tau}_{0k}^2 = 0.27^2$  and  $\hat{\tau}_2^2 = 0.19^2$ . This change is not unexpected, since in logging the parameters we are altering the variance.

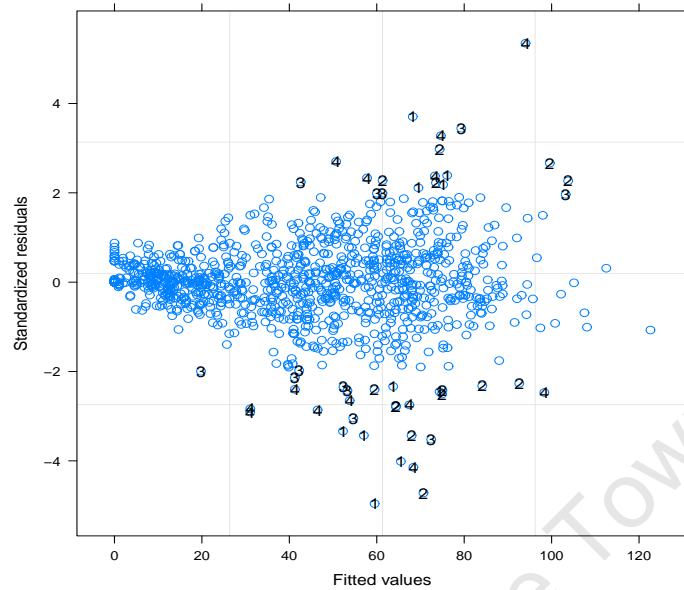


Figure 3.27: Std Residuals vs. Fitted Values: Sulfadoxine NLME Model 7

Parameter	Value	Std.Error
$\hat{\beta}_0.(Intercept)$	70.25	1.04
$exp(\hat{\beta}_3).(Pregnancy)$	1.12	1.0047
$\hat{\beta}_1$	13.77	1.051
$\hat{\beta}_2.(Intercept)$	0.06	1.049
$exp(\hat{\beta}_4).(Pregnancy)$	1.52	1.05

Table 3.17: Back-transformed Model Output for Bi-Exponential Model: Sulfadoxine NLME Model 7

The back-transformed results are presented in Table 3.17.

The covariate effects are interpreted as a proportional change, such that, for example, the fixed effect estimate of parameter  $\beta_0$  for pregnant subjects would be given by:

$$\begin{aligned}
 \hat{\beta}_{0preg} &= \hat{\beta}_0 \times exp(\hat{\beta}_3), \\
 &= 70.25 \times 1.12, \\
 &= 78.75.
 \end{aligned}$$

where previously, the same estimate obtained from model 4 is 79.31, which is approximately 1.12 times the postpartum estimate.

Examination of various diagnostic plots (Appendix B figures B.27 to B.31, and 'in-text' Figures 3.27 and 3.28) indicates that this model does not fit as well as

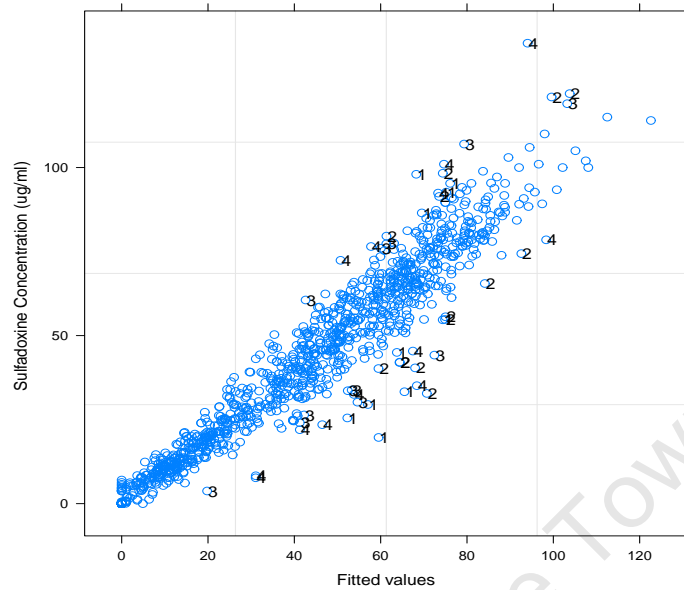


Figure 3.28: Fitted Values vs. Observed: Sulfadoxine NLME Model 7

that with additive random effects, despite much faster convergence.

This conclusion is supported by model comparison using the AIC and BIC (AIC: 7867.059 vs. 8538.254 , BIC: 7932.586 vs. 8578.578).

### 3.2.7 Mechanistic Model Specification: One-Compartment Model:

Using the specification outlined in Section 2.5 for the one-compartment model, models were fitted with the structural form given by:

$$C(t) = \frac{ka \times F \times D}{V/f \times (ka - ke)} \times [e^{-ke \times t} - e^{-ka \times t}].$$

where  $F = 1$ , and  $D = 1500$ ,  $ka$  is the absorption rate constant,  $ke$ , the elimination rate constant, and  $V/f$  is the apparent volume of distribution.

Although the same model building procedure was followed, the development of the covariate model was restricted, and the mechanistic models were adjusted for pregnancy only, since this section is purely for illustrative and comparative purposes.

#### Model 8:

Model 8 is the basic NLME model, ignoring covariates, and following adjustment to the random effects specification. The model may be summarized by stage 2

equations:

$$\begin{aligned} V_{ikj} &= V + b_{0i} + b_{0ik}, \\ ka_{ikj} &= ka + b_{1ik}, \\ ke_{ikj} &= ke. \end{aligned}$$

The results for the fixed effects are summarized in Table 3.18, and the variance-covariance matrices for the random effects  $\mathbf{b}_i = [b_{0i}]$  and  $\mathbf{b}_{ik} = [b_{0ik}, b_{1ik}]$  are given by:

$$\hat{\psi}_1 = \begin{bmatrix} 2.19^2 & 0 \\ 0 & 0 \end{bmatrix}, \hat{\psi}_2 = \begin{bmatrix} 0.02^2 & 0 \\ 0 & 1.31^2 \end{bmatrix}$$

The diagonal elements of  $\mathbf{R}_{ikj}$  are given by  $\hat{\sigma}^2 = 8.12^2$ .

Parameter	Value	Std.Error
$\hat{V}$	10.77	0.513
$\hat{ka}$	0.08	0.002
$\hat{ke}$	0.15	0.007

Table 3.18: Model Output for Sulfadoxine NLME Model 8

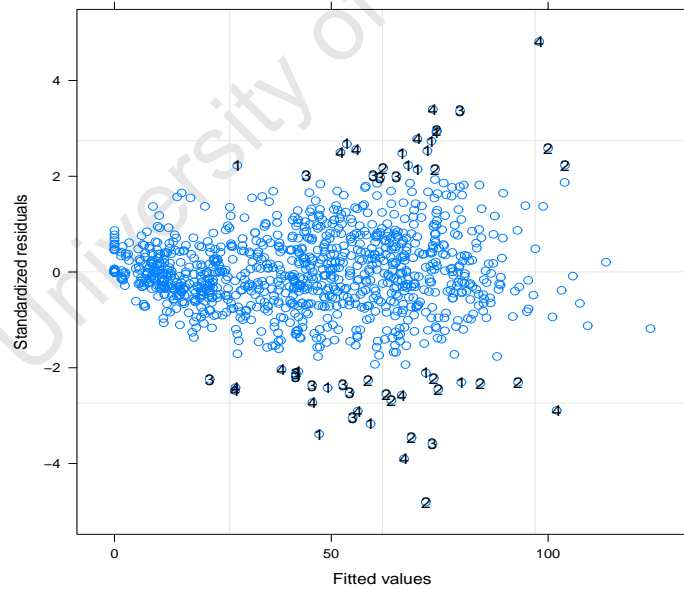


Figure 3.29: Std Residuals vs. Fitted Values: Sulfadoxine NLME Model 8

Examination of the diagnostic plots, (Appendix B, figures B.32 to B.36 and Figures 3.29 and 3.30 “in-text”) indicates that the assumptions of normality for the residuals and random effects are valid, although there does appear to be

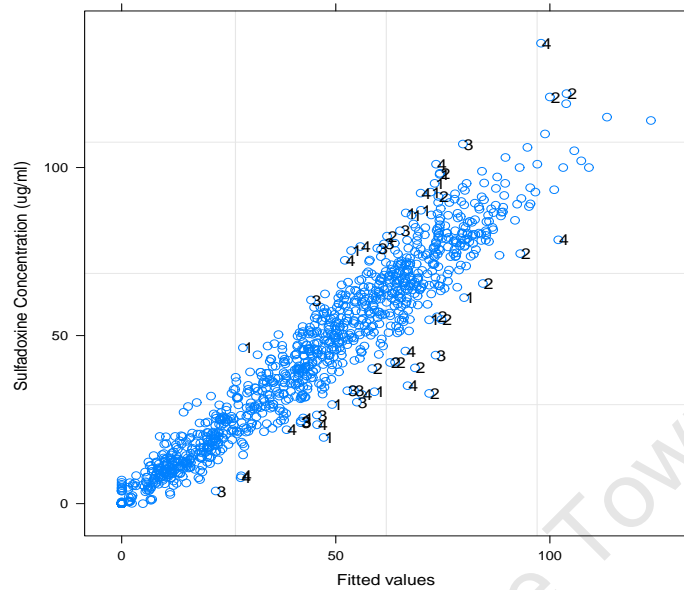


Figure 3.30: Fitted Values vs. Observed: Sulfadoxine NLME Model 8

non-constant variance, (Figure 3.29). The model appears to fit reasonably well, (Figure 3.30), although comparison to Model 2 (the most similar model using the exponential specification) indicates a better fit for the empirical parameterization (AIC: 8482.187 vs. 8584.446, BIC: 8527.552 vs. 8619.730).

#### Model 9:

Adjusting model 8 for pregnancy, and accounting for the heteroskedasticity observed by fitting a variance model of the same form as that previously specified, we have model 9.

The results for the fixed effects are summarized in Table 3.19, and the variance-covariance matrices for the random effects  $\mathbf{b}_i = [b_{0i}]$  and  $\mathbf{b}_{ik} = [b_{0ik}, b_{1ik}]$  are given by:

$$\hat{\psi}_1 = \begin{bmatrix} 2.28^2 & 0 \\ 0 & 0 \end{bmatrix}, \hat{\psi}_2 = \begin{bmatrix} 0.014^2 & 0 \\ 0 & 1.11^2 \end{bmatrix}$$

The diagonal elements of  $\mathbf{R}_{ikj}$  are now dependent on  $\hat{\sigma}^2 = 0.129^2$  and  $\hat{\boldsymbol{\theta}} = [12.089, 1.04]$ .

Figures 3.31 and 3.32 show the reduction in the non-constant variance of the residuals, and indicate a reasonably good fit.

Figures 3.33, 3.34 and 3.35 demonstrate the need for further covariate adjustment, in the clearly defined systematic patterns for site.

Parameter	Value	Std.Error
$\hat{V}$	9.49	0.49
$\hat{k}_a.(Intercept)$	0.06	0.002
$\hat{k}_a.(Pregnancy)$	0.03	0.003
$\hat{k}_e.(Intercept)$	0.15	0.007
$\hat{k}_e.(Pregnancy)$	0.04	0.005

Table 3.19: Model Output for Sulfadoxine NLME Model 9

Comparing Model 9 to Model 4 (the final pregnancy adjusted model for the exponential specification), we see that the empirical parameterization again provides the better fit, (AIC: 7867.059 vs. 8015.787, BIC: 7932.586 vs. 8071.233).

Tables 3.20 and 3.21 provide a comparison of the PK parameters obtained from the empirical and mechanistic specifications for both the basic (unadjusted) models, and the models adjusted for pregnancy.

For the basic models, the average values for the PK parameters appear to be reasonably similar, with comparable standard errors as well. For the models adjusted for pregnancy however, the standard errors for the AUC and  $Vd/f$  are lower for the mechanistic model specification (Model 9).

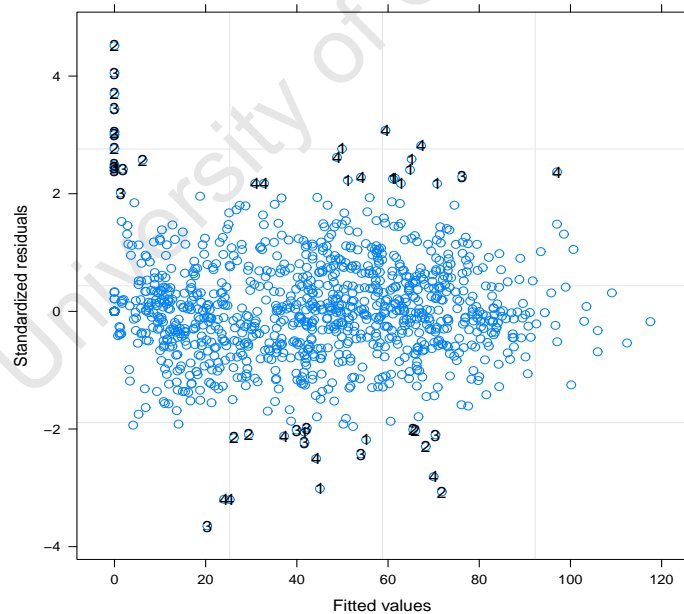


Figure 3.31: Std Residuals vs. Fitted Values: Sulfadoxine NLME Model 9

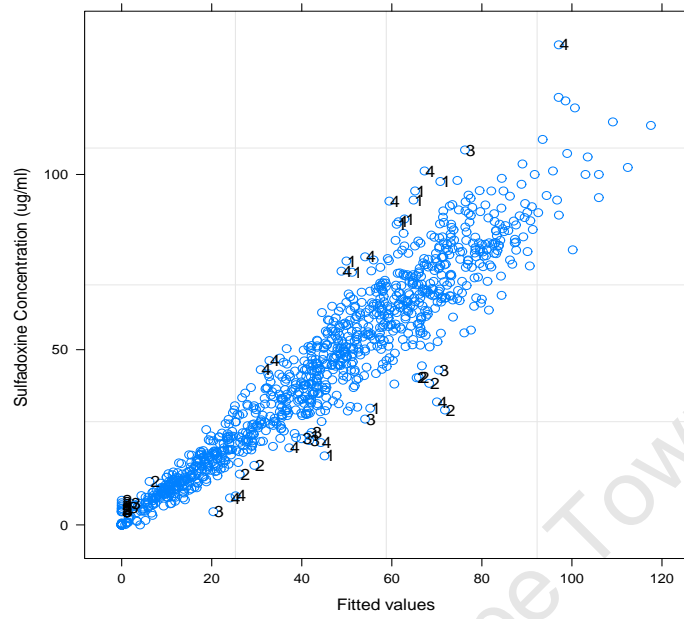


Figure 3.32: Fitted Values vs. Observed: Sulfadoxine NLME Model 9

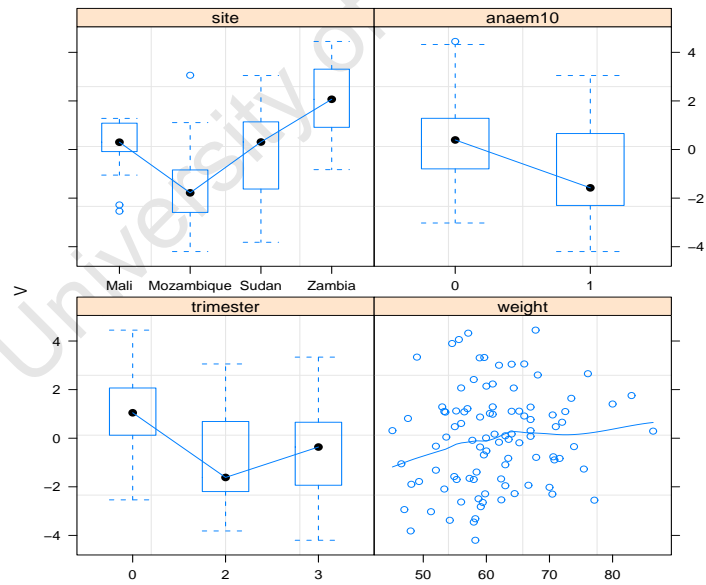


Figure 3.33: Subject-Specific Random Effects for V vs. Covariates: Sulfadoxine NLME Model 9



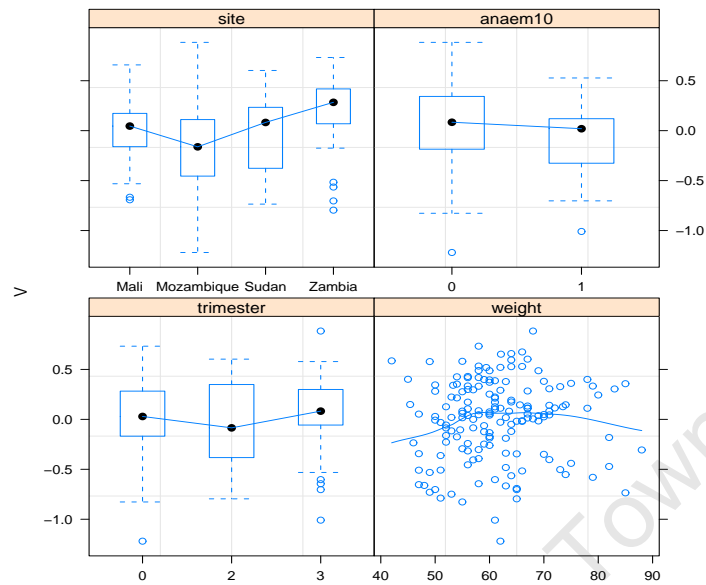


Figure 3.34: Occasion-Specific Random Effects for V vs. Covariates: Sulfadoxine NLME Model 9

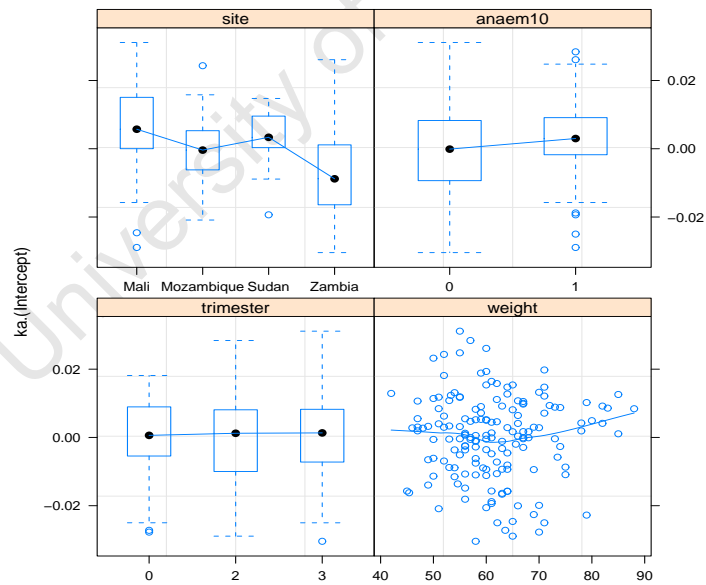


Figure 3.35: Occasion-Specific Random Effects for ka vs. Covariates: Sulfadoxine NLME Model 9

Parameter	Model 2		Model 8	
	Mean	Std. Error	Mean	Std. Error
$AUC_{(0-\text{inf})}$ (ug.day/ml)	905.63	26.54	952.63	26.90
Vd/f (ml/kg)	308.03	7.71	179.52	8.58
Cl/f (ml/kg/day)	27.15	0.81	26.21	0.74
$t_{1/2}$ (days)	7.86	0.16	4.74	0.22

Table 3.20: Comparison of PK Parameters: Sulfadoxine NLME Models 2 and 8

Parameter	Model 4		Model 9	
	Mean	Std. Error	Mean	Std. Error
Pregnant				
$AUC_{(0-\text{inf})}$ (ug.day/ml)	905.63	26.54	760.11	20.87
Vd/f (ml/kg)	308.03	7.71	176.58	0.79
Cl/f (ml/kg/day)	27.15	0.81	32.35	0.89
$t_{1/2}$ (days)	7.86	0.16	3.78	0.18
Postpartum				
$AUC_{(0-\text{inf})}$ (ug.day/ml)	1176.13	56.31	957.22	33.88
Vd/f (ml/kg)	355.63	12.5	181.33	8.15
Cl/f (ml/kg/day)	21.47	1.044	26.38	0.93
$t_{1/2}$ (days)	11.48	0.50	4.76	0.24

Table 3.21: Comparison of PK parameters during Pregnancy and after Postpartum: Sulfadoxine NLME Models 4 and 9

### 3.2.8 Discussion

Results from the exponential parameterization employed indicate that both pregnancy phase and study site have an impact on the concentration-time profile of Sulfadoxine.

Subjects in Mozambique and Zambia had the highest and lowest concentrations respectively, irrespective of pregnancy phase, and for Mali and Zambia, pregnant subjects showed higher peak concentrations than postpartum subjects. Faster rates of decline were observed for pregnant subjects in all sites, although the rate of decline for subjects in Mozambique was lower for both the pregnant and postpartum subjects when compared to those in other sites, (Figure 3.25 and Table 3.12).

The overall impact of this in terms of clinical parameters was a reduction in the total drug exposure, (as measured by the area under the concentration-time curve), for pregnant subjects in all sites except Zambia. The elimination half-life was shorter in all sites for pregnant subjects, and longest for subjects in Mozambique, (Appendix B, tables B.3 and B.4). Although the actual figures for the PK parameters differed slightly from results obtained by Nyunt et al. the overall conclusions remained the same.

The use of nlme models as opposed to the traditional two-stage approach previ-

ously taken with this dataset (Nyunt et al. [5]) resulted in a significant reduction in the standard errors of the estimates.

Even though the exponential parameterization is removed from the more mechanistic parameterization in terms of PK parameters, we were able to graphically illustrate the different concentration-time curves for the various strata, and through back-transformation, obtain sensible estimates of the PK parameters. The average values of the PK parameters obtained via the empirical and mechanistic NLME models were similar, (with the exception of  $Vd/f$ ), as were their standard errors.

Results obtained from a model specified with a single-level of grouping were very similar to those obtained with the multiply-nested design.

Comparisons of models fit with both additive and proportional random effects indicated that the additive structure was preferred, although this comparison may not be entirely valid, as the heteroskedasticity in the model with proportional random effects was unaccounted for. Parameter estimates and conclusions from the two models were similar.

### 3.3 Pyrimethamine Models:

As in the analysis of the Sulfadoxine concentration-time data, NLS (nonlinear least squares) models were fitted for Pyrimethamine, for both the bi- and triple-exponential structural model forms, on the full dataset and for the pregnant and postpartum subsets.

Results from these models and from individual-specific models are presented here, together with the results from various nlme models fitted during the course of model building.

The final pregnancy adjusted NLME model is detailed, together with an NLME model adjusted for site, and predicted curves are presented for both models.

#### 3.3.1 NLS Models

Nonlinear least squares models fitted to the Pyrimethamine data follow the model forms specified in equations 3.2 and 3.3 for the general model specified in equation 3.1., that is:

$$y_j = f(x_j, \boldsymbol{\beta}) + e_j$$

where  $y_j$  is the concentration at time  $j$ ,  $x_j = time_j$ , and  $\boldsymbol{\beta}$  is a  $p$ -dimensional vector of fixed effects.

Models following the bi-exponential specification were fitted to both the full dataset and the pregnant subset. Models for the postpartum subsets did not converge for this specification.

Although comparisons of results with different starting values were intended,

the starting values obtained from the analysis by Nyunt et al. for this structural model form were determined to be too inaccurate for use (estimated values for  $\beta_0$  ranging from 5 to 10 for the full dataset and from 6.1 to 10.7 for the pregnant subset were logically inconsistent with the concentration measurements observed).

The inaccuracy of these values is assumed to be a result of the two-stage approach taken in the analysis; despite expectations of a two-compartment model specification for Pyrimethamine, the sparseness of the data and the use of individual-specific models did not accommodate such a complex model form (since at least 6 data points are required to fit a two-compartment model).

For those subjects for whom a two-compartment model was fitted, the model fit was significantly better than that of the one-compartment model. Since the majority of subjects were unable to fit the two-compartment model, however, the results reported by Nyunt et al. were those obtained using a one-compartment model, and as such, the estimates of parameters such as the volume of distribution and clearance/elimination rate may be inaccurate. This in turn translates to ill-defined starting estimates for the beta parameters in the exponential specification.

The starting values determined from the curve-stripping procedure and the analysis performed by Nyunt et al. [5] are tabulated in Table 3.22.

Parameter	Data	Curve Stripping	Nyunt et. al
$\beta_0$ :	Full	399.58	7.43 (5.0; 10.05)
	Pregnant	497.26	8.59 (6.14; 10.72)
	Postpartum	N/D	5.31 (4.14; 6.67)
$\beta_1$ :	Full	7.63	54 (17.33; 292.79)
	Pregnant	4.47	53.9 (17.32; 243.86)
	Postpartum	N/D	102.3(18.48; 368.23)
$\beta_2$ :	Full	0.31	0.23 (0.18; 0.3)
	Pregnant	0.31	0.21 (0.17; 0.28)
	Postpartum	N/D	0.28(0.22; 0.35)

Table 3.22: Comparison of Starting Values for Bi-Exponential Model

Comparisons for different starting values for the triple-exponential structural model form were also not possible, as the curve-stripping procedure was not performed for this specification.

The values from Nyunt et al. for this specification (triple-exponential) were still contentious, since so few individual-specific model fits were obtained for the more complex two-compartment model in their analyses [5]. Estimates for  $\beta_2$  and  $\beta_4$  were therefore slightly adjusted before they were used in the models.

Tables 3.23 and 3.24 are the results obtained from NLS models using the bi- and triple-exponential structural model forms respectively, the latter for the postpartum subset, which was the only subset for which the triple-exponential model

	Estimate	Std. Error
$\hat{\beta}_0$	351.9	7.73
$\hat{\beta}_1$	14.35	1.96
$\hat{\beta}_2$	0.14	0.0008

Table 3.23: Model Output for Pyrimethamine Bi-Exponential NLS model

	Estimate	Std. Error
$\hat{\beta}_1$	18.51	5.58
$\hat{\beta}_2$	131.96	35.88
$\hat{\beta}_3$	1.88	0.97
$\hat{\beta}_4$	192.01	22.10
$\hat{\beta}_5$	0.13	0.02

Table 3.24: Model Output for Pyrimethamine Triple-Exponential NLS model (Postpartum subset)

specification converged. Comparisons between the bi- and triple-exponential models were therefore not possible.

Plots of the standardized residuals against the subject identification number (Figure 3.36) for the bi-exponential model on the full dataset, indicated the need for the subject-specific random effects.

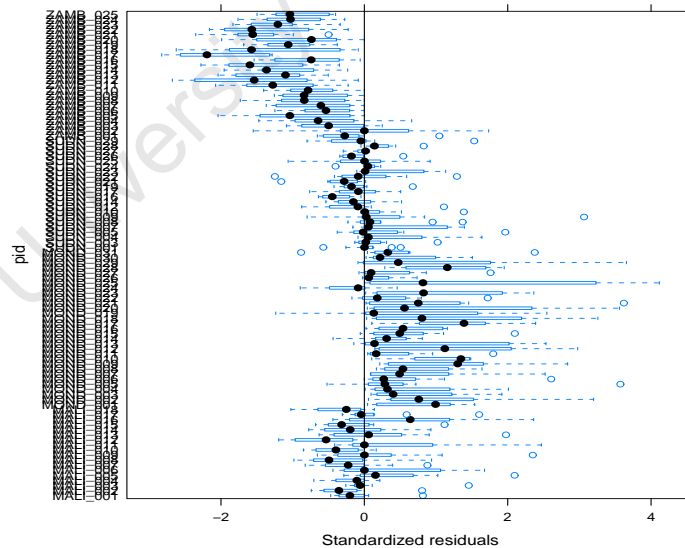


Figure 3.36: Residuals by Subject ID: Pyrimethamine Bi-Exponential NLS Model

### 3.3.2 Individual-Specific Models

Individual specific models were fitted for both structural model forms for the subjects from Mali and Zambia only (since there was insufficient data for the Mozambique and Sudan study sites).

Figure 3.37 shows the possible need for random effects on  $\beta_0$  and  $\beta_2$  for the bi-exponential model specification. For the triple-exponential model the placement of random effects was harder to determine, but  $\beta_2$  and  $\beta_4$  appeared to have intervals which did not overlap indicating that random effects were required for those parameters.

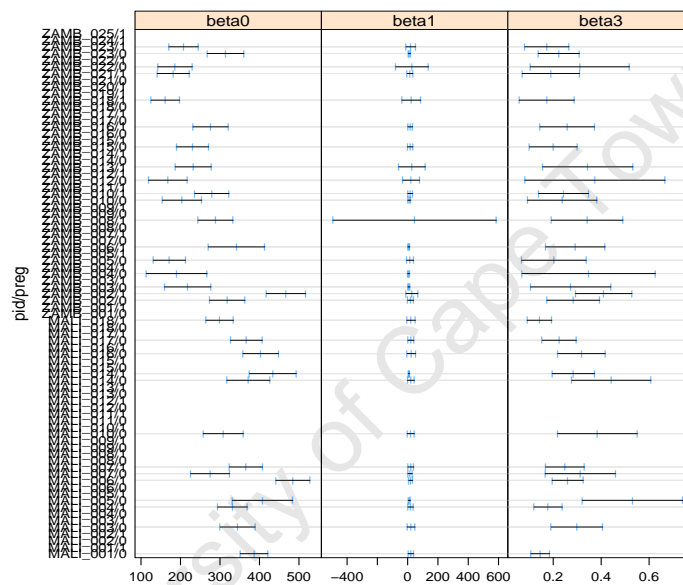


Figure 3.37: Parameter Estimates and 95% Confidence Intervals for Individual Model Fits (Please Note: label “beta3” for far right column should read “beta2”)

### 3.3.3 Multi-Level Models

Basic nlme models (ignoring covariates) were again fitted for both the bi- and triple-exponential model forms, but for the full dataset only. Models with the bi-exponential structural model form did not converge, regardless of simplification (reductions in the number of random effects and in the number of levels of random effects).

#### Model 1:

For the triple-exponential specification, stable models were achieved only once a simplified model structure, (ignoring the occasion-specific random effects),

was specified, and the parameter estimates from this model used as starting estimates in the multiple-nested version.

Parameter	Value	Std.Error
$\hat{\beta}_1$	36.65	11.23
$\hat{\beta}_2$	141.34	13.43
$\hat{\beta}_3$	0.77	0.09
$\hat{\beta}_4$	293.45	17.45
$\hat{\beta}_5$	0.14	0.005

Table 3.25: Triple-Exponential Model Output: Pyrimethamine NLME Model 1

The results from this model for the fixed effects are summarized in Table 3.25, and correspond to a model of the form:

$$y_{ikj} = f(\text{time}_{ikj}, \boldsymbol{\beta}_{ikj}) + e_{ikj}$$

where  $y_{ikj}$  is the  $j$ th measurement in the  $k$ th period for the  $i$ th individual, and

$$\begin{aligned} f(\text{time}_{ikj}, \boldsymbol{\beta}_{ikj}) &= \beta_{2ikj} \times [-\exp(-\beta_{1ikj} \times \text{time}) + \exp(-\beta_{3ikj} \times \text{time})] \\ &+ \beta_{4ikj} \times [-\exp(-\beta_{1ikj} \times \text{time}) + \exp(-\beta_{5ikj} \times \text{time})] \end{aligned}$$

Such that, in the specification of the stage II equations:

$$\boldsymbol{\beta}_{ikj} = \mathbf{A}_{ikj}\boldsymbol{\beta} + \mathbf{B}_{i,kj}\mathbf{b}_i + \mathbf{B}_{ik,j}\mathbf{b}_{ik},$$

the  $p$ -dimensional vector of fixed effects  $\boldsymbol{\beta}$  is given by

$$\boldsymbol{\beta} = [\beta_1, \beta_2, \beta_3, \beta_4, \beta_5],$$

the  $q_1$ - and  $q_2$ -dimensional vectors of the subject and occasion-specific random effects are given by:

$$\begin{aligned} \mathbf{b}_i &= [b_{1i}, b_{2i}, b_{3i}, b_{4i}, b_{5i}] \\ \mathbf{b}_{ik} &= [b_{1ik}, b_{2ik}, b_{3ik}, b_{4ik}, b_{5ik}] \end{aligned}$$

and the design matrices  $\mathbf{A}_{ikj}$ ,  $\mathbf{B}_{i,kj}$ , and  $\mathbf{B}_{ik,j}$  are  $(5 \times 5)$  matrices of the form:

$$\mathbf{A}_{ikj} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}, \quad \mathbf{B}_{i,kj} = \mathbf{B}_{ik,j} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

i.e., there are no random effects specified for  $\beta_1$ , as indicated by the first row of zeros in the matrix  $\mathbf{B}_{i,kj}$ , (which is equivalent to matrix  $\mathbf{B}_{ik,j}$ ).

The within group errors  $e_{ikj}$  are assumed independently and identically normally distributed, with variance-covariance matrix  $\mathbf{R}_{ik}$ , for every  $ik$  combination, where  $\mathbf{R}_{ik}$  is the positive-definite  $(n_{ik} \times n_{ik})$  matrix with diagonal elements  $\hat{\sigma}^2 = (26.93)^2$ .

The subject and occasion-specific random effects are also assumed to be normally distributed:

$$\mathbf{b}_i \sim N(\mathbf{0}, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(\mathbf{0}, \boldsymbol{\psi}_2)$$

with variance-covariance matrices, ( $\boldsymbol{\psi}_1$  and  $\boldsymbol{\psi}_2$ ) given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} 0.03^2 & 0 & 0 & 0 \\ 0 & 0.004^2 & 0 & 0 \\ 0 & 0 & 127.64^2 & 0 \\ 0 & 0 & 0 & 0.014^2 \end{bmatrix},$$

$$\hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} 103.02^2 & 0 & 0 & 0 \\ 0 & 0.0002^2 & 0 & 0 \\ 0 & 0 & 59.16^2 & 0 \\ 0 & 0 & 0 & 0.012^2 \end{bmatrix}$$

Based on the paper by Liang and Derendorf [56], the above model is misspecified ( $|\beta_4| = 293.45 > |\beta_2| = 141.34$ ).

However, since we were unable to fit a reasonable bi-exponential model, and since the semi-log profile clearly indicates a bi-phasic elimination phase for Pyrimethamine, (Figure 3.38), and the use of a two compartment model (analogous to the empirical triple-exponential specification used here) is not unusual for Pyrimethamine, [23], model building was continued using this structural model form.

The diagnostic plots for this model (Appendix C, figures C.1 to C.5) indicated that the normality assumptions for the residual effects and occasion-specific random effects were reasonable, although the QQplots of the subject-specific grouping level random effects indicated slightly skewed distributions for  $\hat{b}_{4i}$  and  $\hat{b}_{5i}$ .

No distinct correlation patterns were observed, (Appendix C, figures C.6 and C.7), and the assumption of constant variance for the residuals did not appear to be violated to any great extent, (Figure 3.39).

The overall fit of the model was examined using plots of the fitted values vs. the observed (Figure 3.40), and plots of the predicted concentration-time curves for the population, individual-specific and occasion-specific levels vs. the observed values for each individual-occasion grouping, (Figure 3.41).

As before, the blue lines represent the population curve obtained using the mean parameter estimates of the fixed effects, and the pink and green lines are the predicted curves for the subject and occasion-specific grouping levels respectively.

The overlapping of the blue, pink and green lines for the absorption phase of the curves demonstrates the lack of any subject or occasion-specific variability for the parameter representing the slope of this line (no random effects were



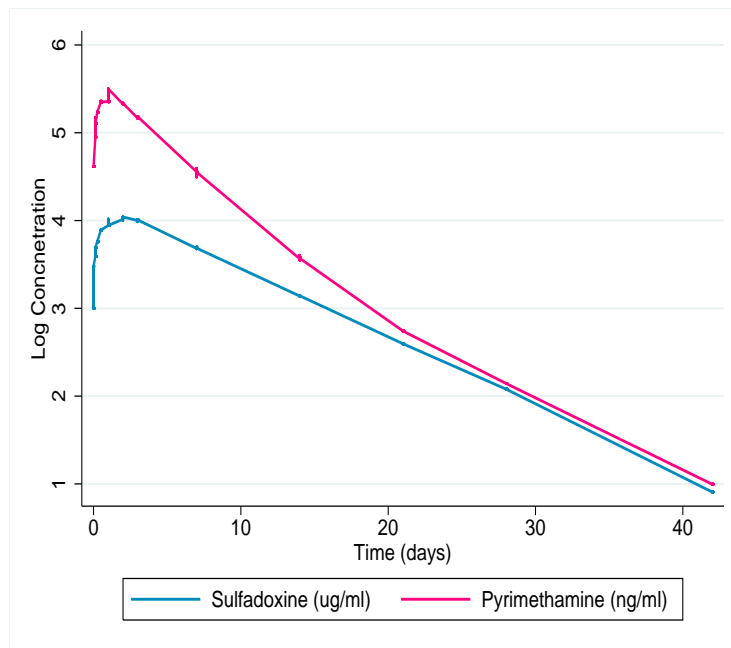


Figure 3.38: Semi-log Plot of Observed Concentration vs. Time

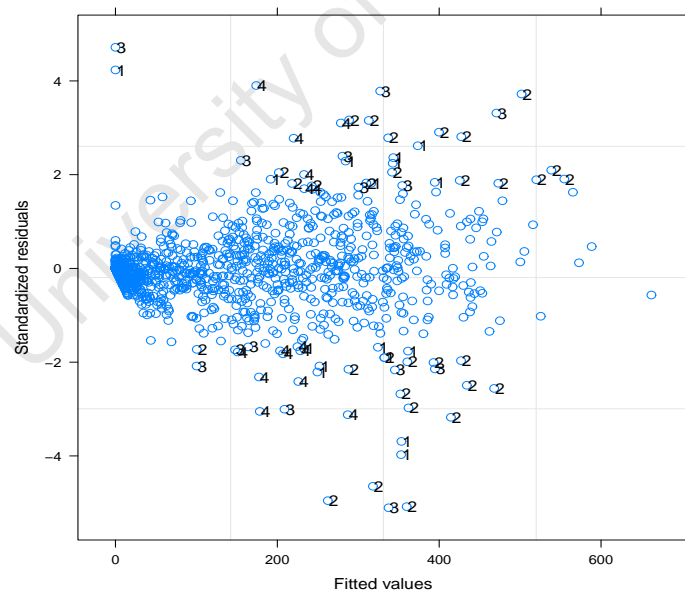


Figure 3.39: Standardized Residuals vs. Fitted Values: Pyrimethamine NLME Model 1

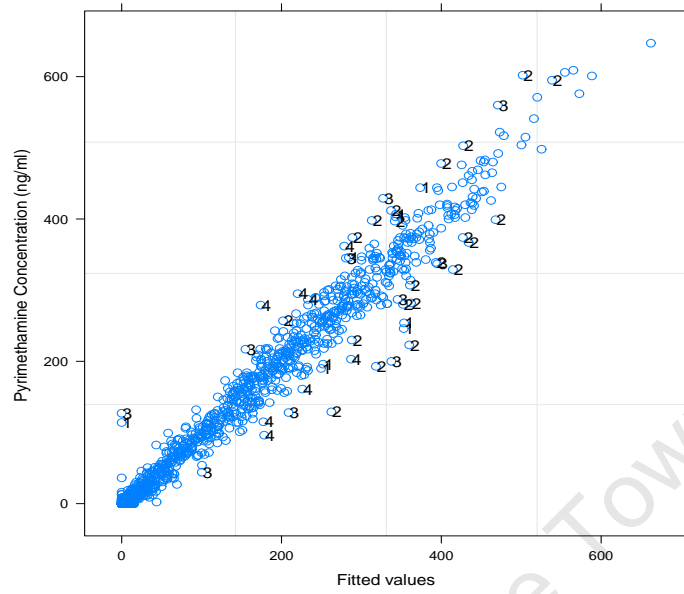


Figure 3.40: Fitted values vs. Observed: Pyrimethamine NLME Model 1

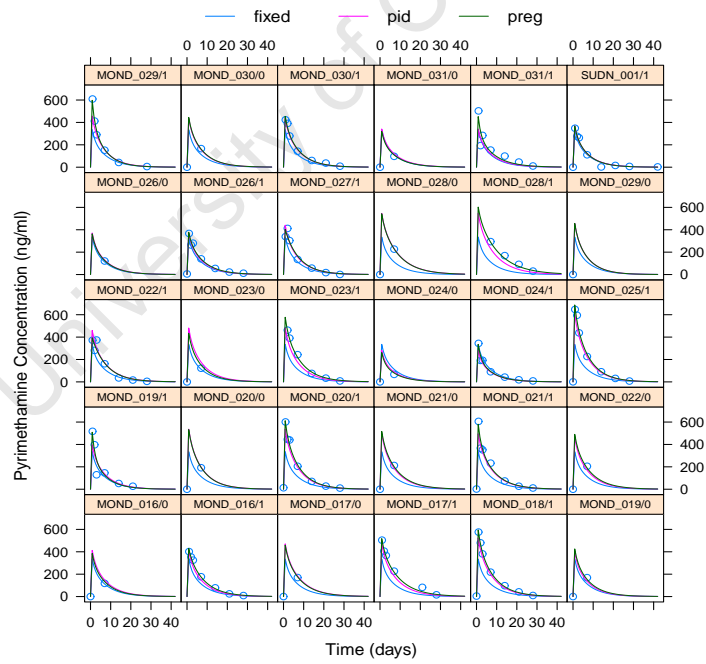


Figure 3.41: Population, Subject and Occasion-Specific Predicted Curves (Subset: Mozambique): Pyrimethamine NLME Model 1

included for this parameter). The model appears to fit reasonably well.

As previously shown, plots of the random effects vs. covariates were used to inform the placement of covariates.

Plots of the both the subject and occasion-specific random effects vs. trimester, weight, site and anaemia indicated clear systematic patterns for  $\beta_4$  only, although potential systematic differences in variation were observed for site on  $\beta_2$ , (Appendix C, figure C.8) and again on  $\beta_3$ , (Appendix C, figure C.10), for the subject-specific level of random effects.

Figures 3.42, and 3.43 are plots of the subject and occasion-specific random effects versus covariates for  $\beta_4$ . The plots for the parameters and grouping levels not shown here may be found in Appendix C, figures C.8 to C.13.

Using study site Mali as a reference category, subjects in study sites Sudan and Mozambique appear to have increasingly higher median  $\beta_4$  values. Subjects in Zambia appear to have lower median  $\beta_4$  values.

Subjects in their 2nd or 3rd trimester appear to have higher median  $\beta_4$  values (when compared to postpartum subjects). There does not appear to be any effect for weight or anaemia (overlapping IQR's).

For the occasion-specific grouping level the site effect is no longer in evidence. The trimester effect is still there however, albeit less pronounced.

The model building procedure followed for pregnancy and its proxy measurements was the same as that for the Sulfadoxine case; an iterative stepwise procedure, adding pregnancy to each parameter in turn and then in various combinations in increasing levels of complexity, with continuous assessment of the random effects and residual diagnostics.

For the models including random effects on all parameters except  $\beta_1$  for both levels of grouping (as for the basic Model 1), pregnancy could only reasonably be included on  $\beta_3$ , (Model 2), since adding it to any other parameters resulted in multi-collinearity.

High correlations were however observed between the intercept and slope parameters for the linear relationship between  $\beta_3$  and pregnancy, ( $\hat{\rho} = 0.9$ ).

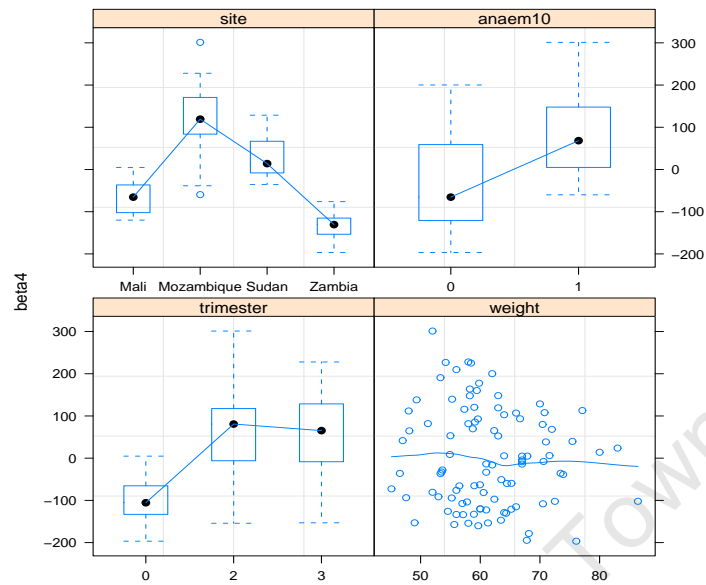


Figure 3.42: Subject-Specific Random Effects for  $\beta_4$  vs. Covariates: Pyrimethamine NLME Model

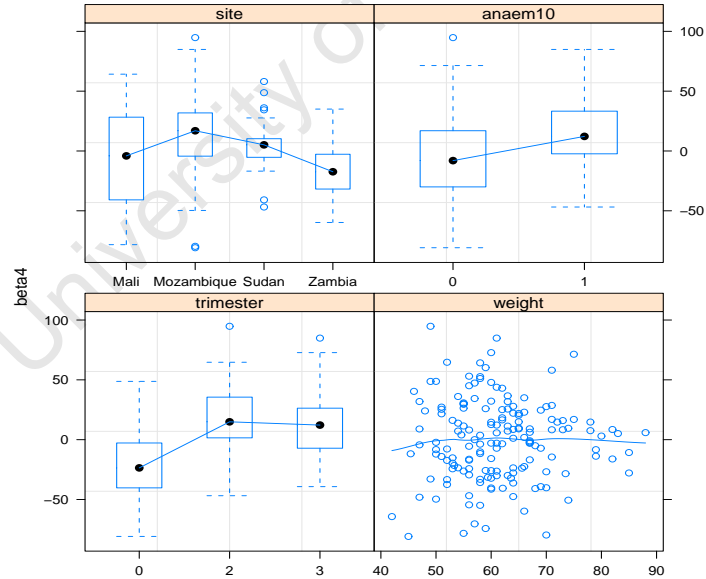


Figure 3.43: Occasion-Specific Random Effects for  $\beta_4$  vs. Covariates: Pyrimethamine NLME Model

**Model 3:**

Iterative reassessment of the random effects and fixed effects in turn resulted in a model with stage 2 equations given by:

$$\begin{aligned}\beta_{1ikj} &= \beta_1, \\ \beta_{2ikj} &= \beta_2 + b_{2ik}, \\ \beta_{3ikj} &= \beta_3 \\ \beta_{4ikj} &= \beta_4 + \beta_6 \times \text{preg}_{ik} + b_{4i}, \\ \beta_{5ikj} &= \beta_5 + b_{5i} + b_{5ik},\end{aligned}$$

The distributional assumptions for the  $e_{ikj}$  and the subject and occasion-specific random effects remained unchanged. No models including a variance function of any form would converge and the results for the fixed effects are summarized in Table 3.26.

Parameter	Value	Std.Error
$\hat{\beta}_1$	44.15	29.06
$\hat{\beta}_2$	134.7	13.14
$\hat{\beta}_3$	0.71	0.08
$\hat{\beta}_4$ .(Intercept)	253.72	18.56
$\hat{\beta}_6$ .(Pregnancy)	57.55	7.88
$\hat{\beta}_5$	0.14	0.005

Table 3.26: Triple-Exponential Model Output: Pyrimethamine NLME Model 3

The variance-covariance matrices for the random effects for each grouping level, ( $\hat{\mathbf{b}}_i = [\hat{b}_{4i}, \hat{b}_{5i}]'$  and  $\hat{\mathbf{b}}_{ik} = [\hat{b}_{2ik}, \hat{b}_{5ik}]'$ ), are given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} 132.64^2 & 0 \\ 0 & 0.005^2 \end{bmatrix}, \hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} 85.45^2 & 0 \\ 0 & 0.023^2 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (27.75)^2$ .

This model was determined to be significantly better than model 2 (AIC: 11540.66 vs. 11564.72, BIC: 11596.11 vs. 11640.33 and lrtest p-value: 0.0029).

Examination of the diagnostic plots for this model (Appendix C, figures C.14 to C.16) indicate no departure from the normality assumptions, for both the residual errors and both levels of random effects.

With the exception of one or two outliers, QQplots for the random effects were roughly centered around zero, (Figures 3.44 and 3.45), and no correlation patterns were observed for the random effects using pairs plots, (Appendix C, figures C.17 and C.18).

Examination of the plot of the random effects vs. covariates for  $\beta_4$ , (Figure 3.46) shows that the inclusion of pregnancy as a covariate on  $\beta_4$  has not lessened the systematic patterns of variation, i.e. that the model is not accurately capturing

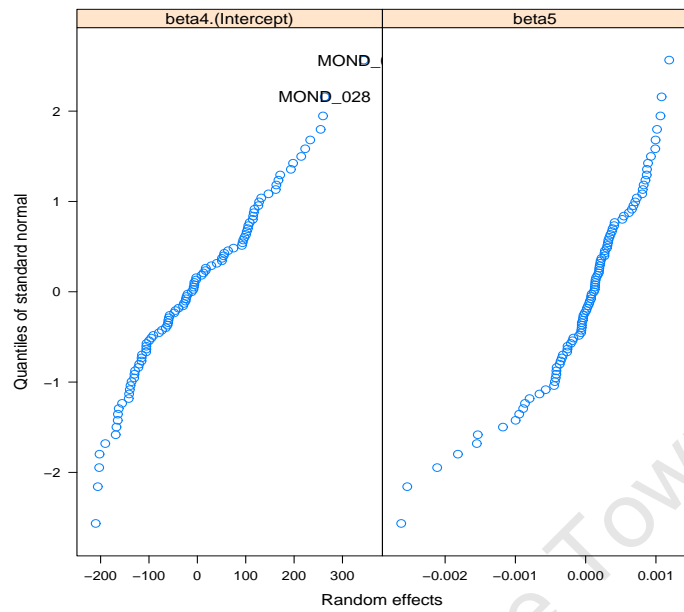


Figure 3.44: QQplots for Subject-Specific Random Effects: Pyrimethamine NLME Model 3

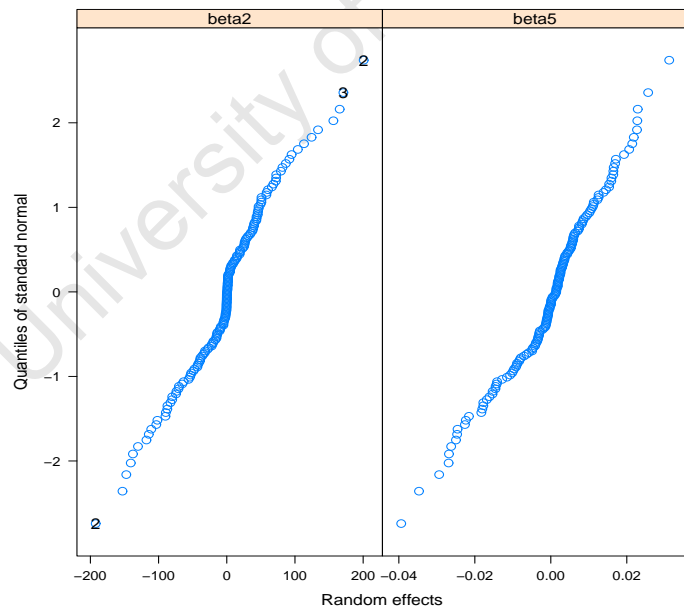


Figure 3.45: QQplots for Occasion-Specific Random Effects: Pyrimethamine NLME Model 3

the effects observed.

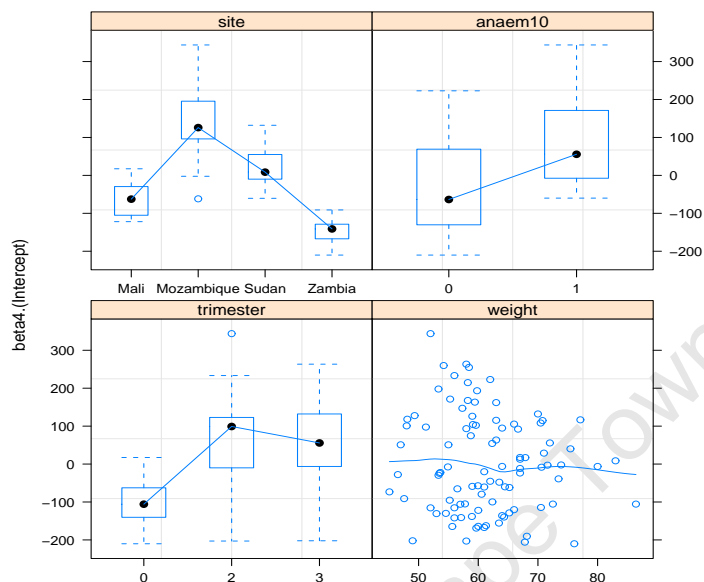


Figure 3.46: Subject-Specific Random Effects for  $\beta_4$  vs. Covariates: Pyrimethamine NLME Model 3

Further changes to the random effects and placement of the pregnancy covariate did not improve the model fit. Model 3 was therefore determined to be the “best” pregnancy adjusted model, not accounting for other covariates. Figure 3.47 is a plot of the mean predicted concentration-time curves by pregnancy phase.

The remaining model building followed an hypothesis driven approach, taking into account the degrees of freedom and the lack of physiological information regarding the placement of covariates together with the computational intensity involved in fitting all combinations of parameters and covariates.

#### Model 4:

The “final” pregnancy model (model 3) described above appears to be inadequate in terms of the quantification of the impact of covariates. Neither the pregnancy nor the site effect seen in Figure 3.46 have been reduced. Model building was therefore continued.

Despite the expected impact of site on  $\beta_4$ , models incorporating the categorical covariate on this parameter would not converge. Attempts were then made to capture this effect via the impact of site on the remaining parameters, in an approach similar to that undertaken during the Sulfadoxine model building procedure.

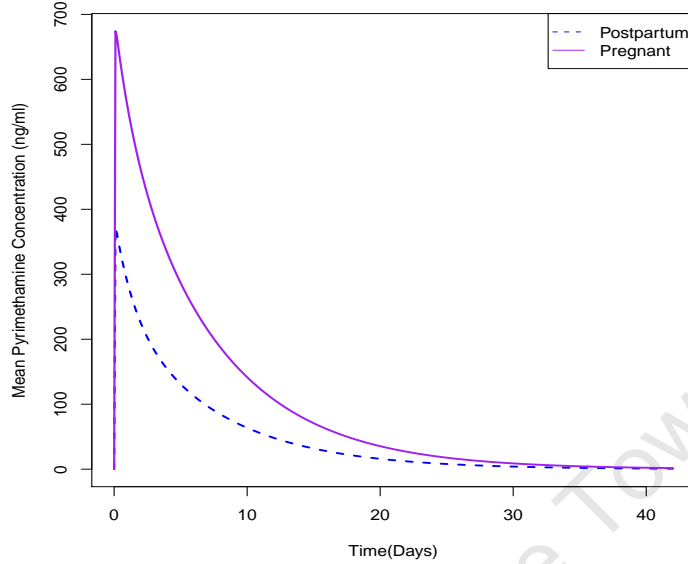


Figure 3.47: Predicted Mean Concentration-Time Curves by Pregnancy Phase: Pyrimethamine NLME Model 3

The final model for Pyrimethamine included site as a covariate on  $\beta_4$ , together with an interaction between site and pregnancy, such that:

$$\begin{aligned}
 \beta_{1ikj} &= \beta_1, \\
 \beta_{2ikj} &= \beta_2 + b_{2ik}, \\
 \beta_{3ikj} &= \beta_3 \\
 \beta_{4ikj} &= \beta_4 + \beta_6 \times \text{preg}_{ik.} + \beta_7 \times \text{mozambique}_{i..} + \beta_8 \times \text{sudan}_{i..} \\
 &\quad + \beta_9 \times \text{zambia}_{i..} + \beta_{10} \times \text{preg}_{ik.} \times \text{mozambique}_{i..} \\
 &\quad + \beta_{11} \times \text{preg}_{ik.} \times \text{sudan}_{i..} + \beta_{12} \times \text{preg}_{ik.} \times \text{zambia}_{i..} + b_{4i} \\
 \beta_{5ikj} &= \beta_5 + b_{5ik}.
 \end{aligned}$$

The results for the fixed effects from this model are summarized in Table 3.27.

The variance-covariance matrices for the random effects, ( $\hat{\mathbf{b}}_i = [\hat{b}_{4i}, \hat{b}_{5i}]'$  and  $\hat{\mathbf{b}}_{ik} = [\hat{b}_{2ik}, \hat{b}_{5ik}]'$ ), are given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} 48.93^2 & 0 \\ 0 & 0.012^2 \end{bmatrix}, \hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} 71.29^2 & 0 \\ 0 & 0.021^2 \end{bmatrix},$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (28.06)^2$ .

Both pregnancy and site appear to directly impact the range of concentrations only, in their effect on  $\beta_4$ . Close examination of the summarized results in Table 3.27 shows that  $\hat{\beta}_2 = 129.94 < \hat{\beta}_4 = 175.9$ .



Parameter	Value	Std.Error
$\hat{\beta}_1$	55.22	116.56
$\hat{\beta}_2$	129.94	12.96
$\hat{\beta}_3$	0.68	0.08
$\hat{\beta}_4$ .(Intercept)	175.94	18.69
$\hat{\beta}_6$ .(Pregnancy)	84.99	12.51
$\hat{\beta}_7$ .(Mozambique)	233.16	29.11
$\hat{\beta}_8$ .(Sudan)	141.71	37.32
$\hat{\beta}_9$ .(Zambia)	-60.58	19.99
$\hat{\beta}_{10}$ .(Pregnancy $\times$ Mozambique)	-31.00	27.35
$\hat{\beta}_{11}$ .(Pregnancy $\times$ Sudan)	-64.84	36.03
$\hat{\beta}_{12}$ .(Pregnancy $\times$ Zambia)	-58.46	16.92
$\hat{\beta}_5$	0.14	0.01

Table 3.27: Triple-Exponential Model Output: Pyrimethamine NLME Model 4

As previously demonstrated, this is indicative of a potential misspecification (as shown by Liang and Derendorf [56]). However, for the reasons previously cited in the case of Pyrimethamine, Model 4 is deemed satisfactory.

Figures 3.48 and 3.49 show the concentration-time curve for this model for the baseline site and pregnancy status (Mali, Postpartum), together with their constituent components.

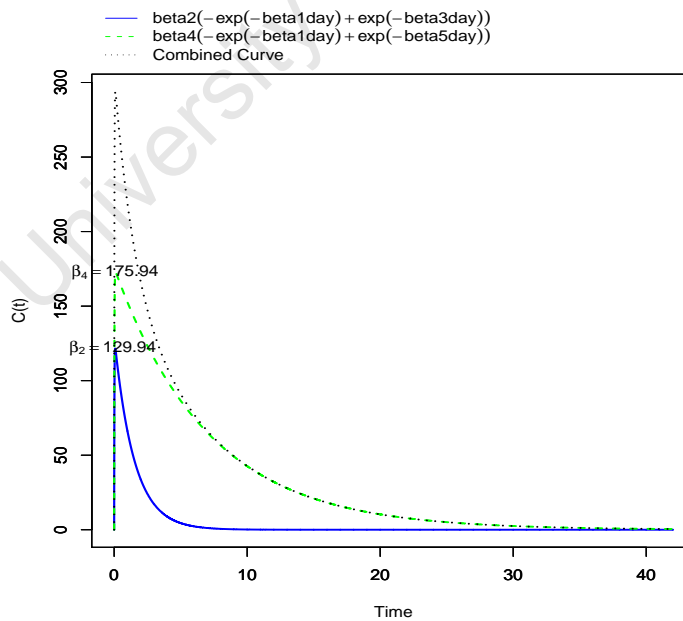


Figure 3.48: Concentration-Time Curve and Components: Pyrimethamine NLME Model 4

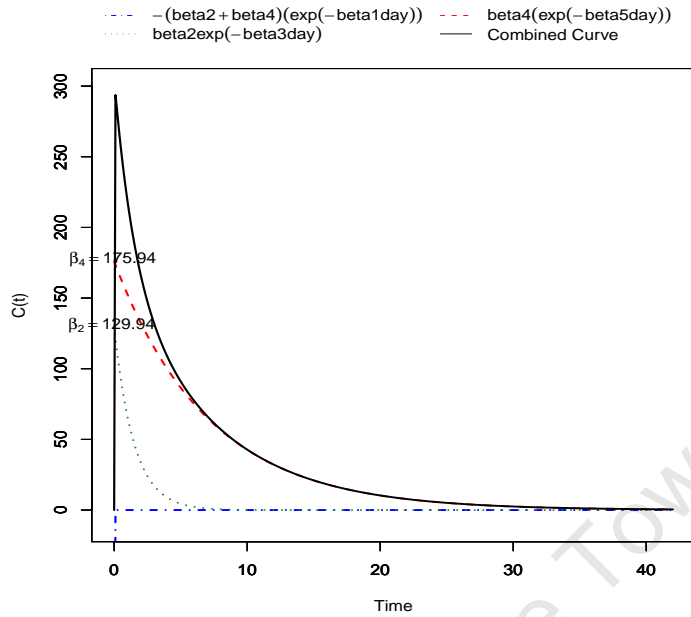


Figure 3.49: Concentration-Time Curve and Components: Original Triple-Exponential Form

Examination of Figures 3.48 and 3.49 demonstrate the different constituent curves for different structural parameterizations, that of

$$C(t) = \beta_2 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_3 \times time)] \\ + \beta_4 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_5 \times time)]$$

shown in Figure 3.48, and that of

$$C(t) = \beta_0 \times \exp(-\beta_1 \times time) + \beta_2 \times \exp(-\beta_3 \times time) \\ + \beta_4 \times \exp(-\beta_5 \times time)$$

which is the original exponential form, prior to any simplification, shown in Figure 3.49.

The constituent curves for the simplified model form are easily seen to be bi-exponential curves. For the original specification, the constituent parts of the combined curve are various single exponential curves.

It should be noted that the curves in Figure 3.49 were created using the parameter values for the simplified model structure: had the original (unsimplified) structural form been fitted, the parameter values would be slightly different, and the slope of the absorption phase in Figure 3.49 would be seen to be less steep.

The graphs here serve merely as an illustration of the break down of the combined exponential curve, and illustrate that in spite of the apparent misspecification, (due to  $\hat{\beta}_2 = 129.94 < \hat{\beta}_4 = 175.9$ ), the sum of the constituent parts

results in a combined curve that is biphasic in its decline.

Model 4 was determined to be significantly better than model 3 (AIC: 11382.50 vs. 11540.66, BIC: 11468.19 vs. 11596.11), despite the multi-collinearity noted for the  $\beta_1$  parameter.

The effects previously seen in Figure 3.46 are no longer in evidence, (Figure 3.50).

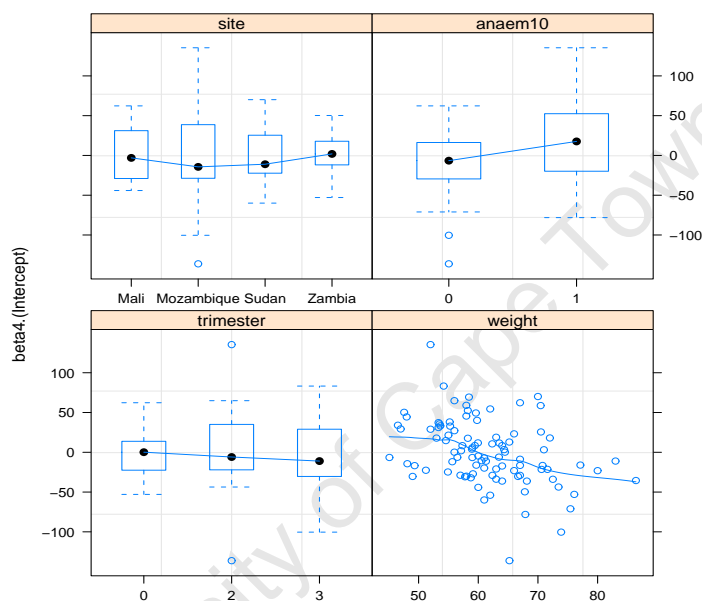


Figure 3.50: Subject-Specific Random Effects for  $\beta_4$  vs. Covariates: Primethamine NLME Model 4

Examination of the diagnostic plots for this model, (Appendix C, figures C.19 to C.26), showed no departure from the normality assumptions for either the residuals or the random effects.

The model fit, as determined by the plot of the fitted values versus the observed, (Figure 3.51), was also reasonably good.

Figures 3.52 and 3.53 show the mean predicted concentration-time profiles by site and pregnancy phase.

Irrespective of pregnancy phase, the predicted mean concentrations are highest in Mozambique and Sudan, and lowest in Zambia. Pregnant subjects are seen to have a consistently higher mean range of concentrations in all sites except for Sudan, where no pregnancy effect is observed.

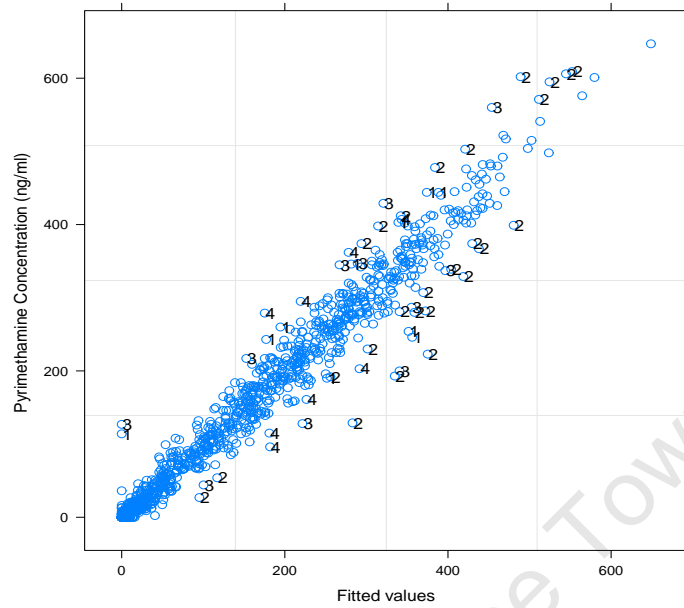


Figure 3.51: Fitted Values vs. Observed: Pyrimethamine NLME Model 4

### 3.3.4 The Delta Method

For the most part, the equations outlined in section 2.8.2 for the calculation of PK parameters via back-transformation apply only in the bi-exponential case.

The calculations for the area under the concentration-time curve, (AUC), apparent volume of distribution (Vd/f), and apparent clearance (Cl/f) may still be used however for the triple-exponential specification.

Recalling the original exponential specification for the triple exponential model:

$$C(t) = \beta_2 \times [-\exp(-\beta_1 \times \text{time}) + \exp(-\beta_3 \times \text{time})] \\ + \beta_4 \times [-\exp(-\beta_1 \times \text{time}) + \exp(-\beta_5 \times \text{time})]$$

using the general form for the calculation of the AUC given by:

$$AUC = \sum_{i=1}^n \frac{C_i}{\lambda_i}$$

we would specify:

$$AUC = \sum_{i=1}^3 \frac{C_i}{\lambda_i} = \frac{\hat{\beta}_0}{\hat{\beta}_1} + \frac{\hat{\beta}_2}{\hat{\beta}_3} + \frac{\hat{\beta}_4}{\hat{\beta}_5}$$

Since  $\beta_0 = -(\beta_2 + \beta_4)$ , however, we may express the above as:

$$AUC = \sum_{i=1}^3 \frac{C_i}{\lambda_i} = \frac{\hat{\beta}_2}{\hat{\beta}_1} + \frac{\hat{\beta}_2}{\hat{\beta}_3} + \frac{\hat{\beta}_4}{\hat{\beta}_1} + \frac{\hat{\beta}_4}{\hat{\beta}_5}$$

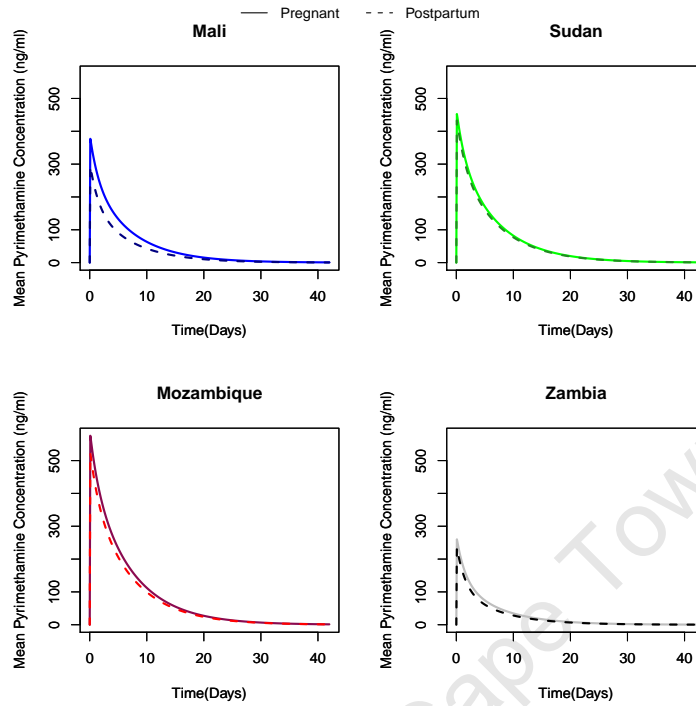


Figure 3.52: Predicted Mean Concentration-Time Curves by Site: Pyrimethamine NLME Model 4

Then, assuming  $F = 1$ , and defining:

$$ke = \frac{\hat{\beta}_3 \times \hat{\beta}_5}{k_{21}}$$

where

$$k_{21} = \frac{(\hat{\beta}_2 \times \hat{\beta}_5 \times \hat{\beta}_1 + \hat{\beta}_4 \times \hat{\beta}_3 \times \hat{\beta}_1 - (\hat{\beta}_2 + \hat{\beta}_4) \times \hat{\beta}_3 \times \hat{\beta}_5)}{(\hat{\beta}_2 \times (\hat{\beta}_1 - \hat{\beta}_3) + \hat{\beta}_4 \times (\hat{\beta}_1 - \hat{\beta}_5))}$$

we were able to calculate the  $Cl/f$  using the general form given by:

$$Cl/f = \frac{F \times D}{\sum_{i=1}^n \frac{C_i}{\lambda_i}} = \frac{F \times D}{AUC}$$

Individual-specific values for  $C_{max}$  were calculated using the predicted concentrations for each individual-phase grouping. These values were then averaged by site and pregnancy. The mean PK parameters and their standard errors resulting from this procedure and that using back transformation and the delta method are summarized in Table 3.28 for the various sites and pregnancy phases.

The clinical values obtained using nlme model 4 may not be explicitly compared with those from the analysis performed by Nyunt et al., since the structural model forms used in the different analyses are not compatible. The  $Vd/f$

was not calculated for this same reason.

However, as illustrated in Table 3.28, the overall conclusions are similar: Nyunt et al. [5] report the highest and lowest mean  $C_{max}$  and AUC values for Mozambique and Zambia respectively, and for those sites in which postpartum PK parameters could be obtained (Mali and Zambia), the mean  $C_{max}$  and AUC values corresponding to the pregnant subjects were consistently higher. The conclusions for apparent clearance and volume of distribution are also similar to those obtained by Nyunt et al., with postpartum subjects in both Mali and Zambia having higher mean values for these parameters. The medians and inter-quartile ranges actually reported for the analysis from Nyunt et al. may be found in Appendix C, Table C.1.

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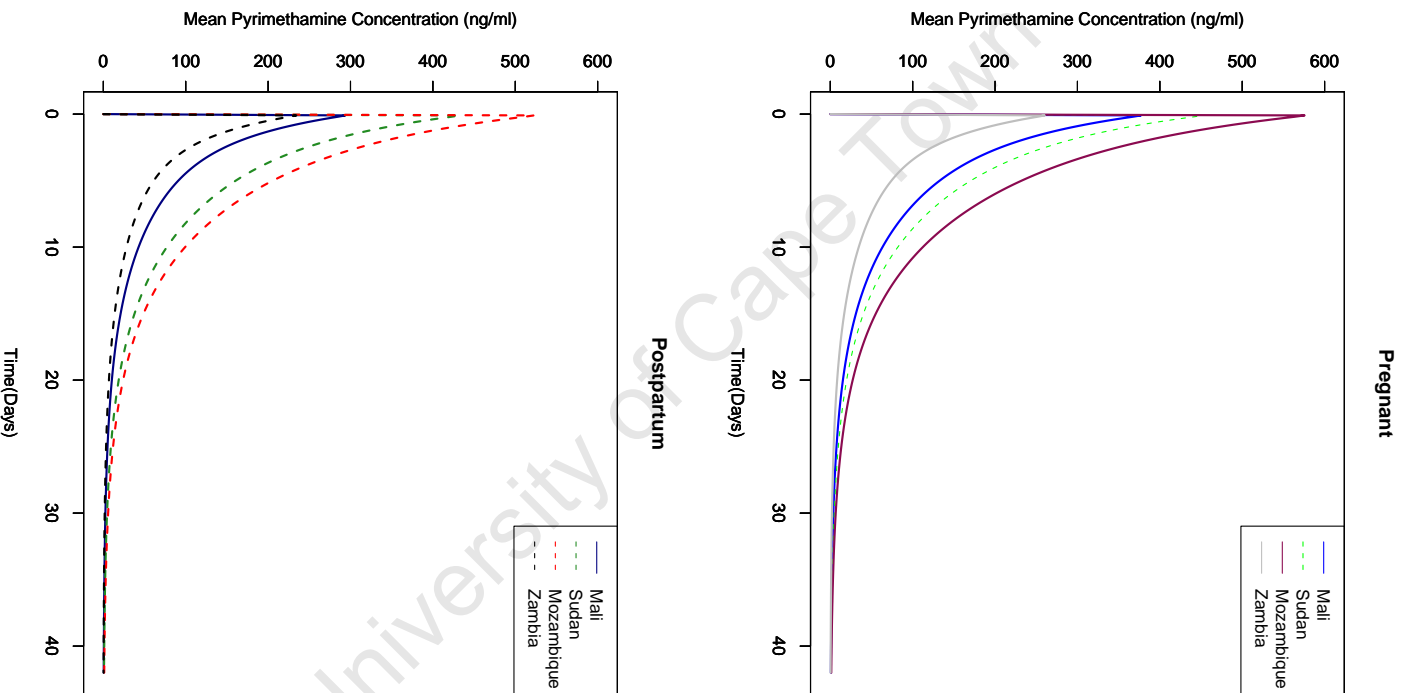


Figure 3.53: Predicted Mean Concentration-Time Curves by Pregnancy Phase: Pyrimethamine NLMPE Model 4

Country	Parameter	Model 4				Nyunt et al. [5]			
		Pregnant		Postpartum		Pregnant		Postpartum	
		Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
Mali	$AUC_{(0-inf)}$ (ng.day/ml)	2040.28	110.90	1439.23	107.13	1730.79	271.32	1135.15	466.35
	Cl/f (ml/kg/day)	602.62	32.75	877.30	65.30	738.2423	143.6674	1288.19	453.98
	Cmax (ng/ml)	304.07	40.70	250.90	35.96	373.7846	61.88207	325.66	49.71
Mozambique	$AUC_{(0-inf)}$ (ng.day/ml)	3469.98	120.95	3088.15	186.00	3599.25	1280.14	N/D	N/D
	Cl/f (ml/kg/day)	354.33	12.35	408.86	24.63	377.33	120.99	N/D	N/D
	Cmax (ng/ml)	489.77	73.18	431.75	51.23	561.487	274.93	N/D	N/D
Sudan	$AUC_{(0-inf)}$ (ng.day/ml)	2583.93	109.62	2441.41	243.73	2112.09	479.46	N/D	N/D
	Cl/f (ml/kg/day)	475.83	20.19	517.17	51.63	580.01	170.98	N/D	N/D
	Cmax (ng/ml)	372.92	50.75	359.52	30.66	405.50	106.02	N/D	N/D
Zambia	$AUC_{(0-inf)}$ (ng.day/ml)	1198.46	83.69	1010.82	NaN**	1039.53	323.96	832.38	249.46
	Cl/f (ml/kg/day)	1025.9	71.64	1249.11	NaN	1332.31	507.61	1613.75	430.43
	Cmax (ng/ml)	192.54	43.65	161.26	32.40	242.03	66.22	205.99	44.46

Table 3.28: Comparison of PK parameters by Site and Pregnancy Phase: Pyrimethamine NLME Model 4

N/D\*= Not determined, NaN\*\*= Computation not possible



### 3.3.5 Discussion

Both pregnancy status and study site appear to play a role in the determination of the Pyrimethamine concentration-time profiles, where the pregnancy effect is dependent on the study site.

For the exponential parameterization and the predicted curves, pregnant subjects appear to have a consistently higher range of concentrations in all sites except for Sudan, where no pregnancy effect is observed. Subjects in Mozambique and Zambia have the highest and lowest concentrations respectively, regardless of pregnancy phase. No direct impact of pregnancy or site could be determined for any of the rate parameters.

Calculation of the PK parameters using back-transformation of the parameters from the exponential specification is more complicated for the triple-exponential case, with only some of the relevant equations actually applicable. Although comparisons between the Nyunt et al. results and the results from the final model are not ideal, (since the Nyunt et al. analysis makes use of individual-specific *one-compartment* models, which are analogous to the bi-exponential model form and not the the triple exponential), the conclusions reached in both analyses are the same.

The final adjusted model for Pyrimethamine, determined via an hypothesis-driven model-building procedure appears to induce multi-collinearity for the  $\beta_1$  parameter, as illustrated by the large standard error. Simpler models with fewer covariates did not converge however, and the previous “best” model, adjusted only for pregnancy, did not appear to capture any of the covariate effects. In general, estimation of the Pyrimethamine models was more difficult than that of the Sulfadoxine models, most likely due to more complicated structural model form.

## 3.4 Sequential Models

The results presented in this section are those for the sequential model. NLME Models were run for both Sulfadoxine and Pyrimethamine, with the predicted concentrations of the drug not being modeled included as a time-varying covariate in the model of the partner drug.

### 3.4.1 Impact of Predicted Pyrimethamine Concentrations on Sulfadoxine

The models presented here illustrate the impact of the predicted Pyrimethamine concentrations on the concentration-time profile of Sulfadoxine. Model 1 is that previously presented in the section pertaining to the separate Sulfadoxine models (section 3.2), with results summarized by Table 3.9. The predicted Pyrimethamine concentrations were generated using the basic Pyrimethamine model (Model 1, section 3.3), which ignores the impact of covariates.

Figures 3.54 and 3.55 are plots of the subject-specific random effects vs. covari-

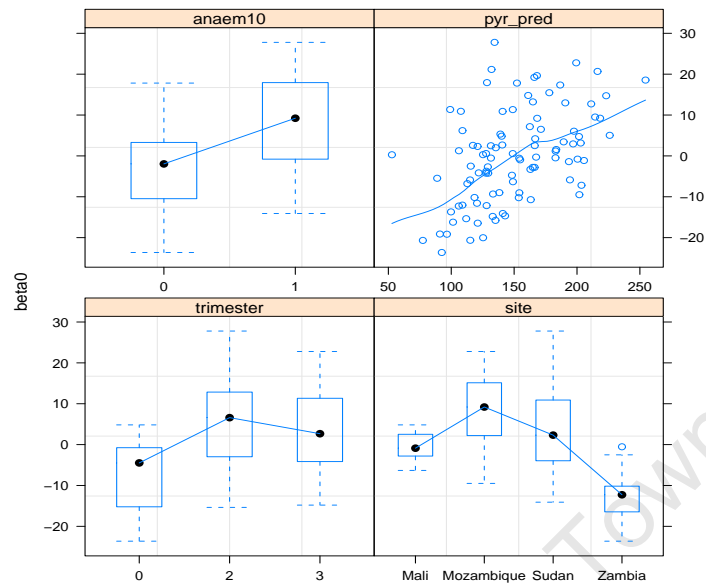


Figure 3.54: Subject-Specific Random Effects for  $\beta_0$  vs. Covariates: Sequential NLME Model 1

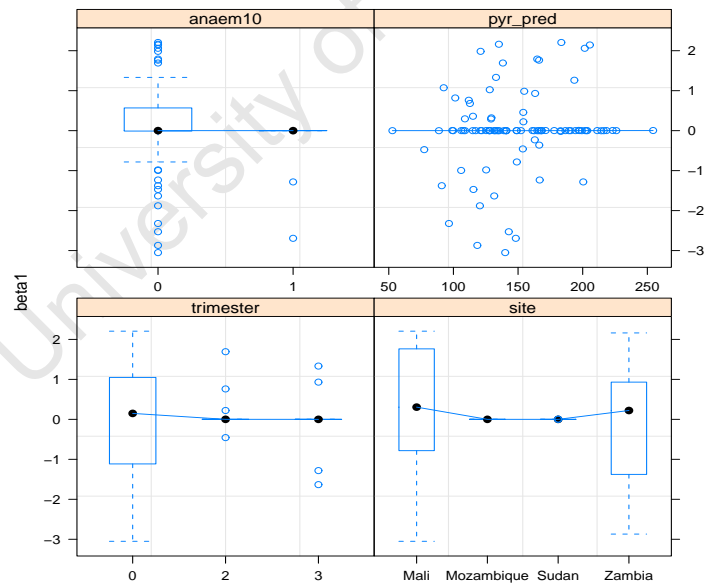


Figure 3.55: Subject-Specific Random Effects for  $\beta_1$  vs. Covariates: Sequential NLME Model 1

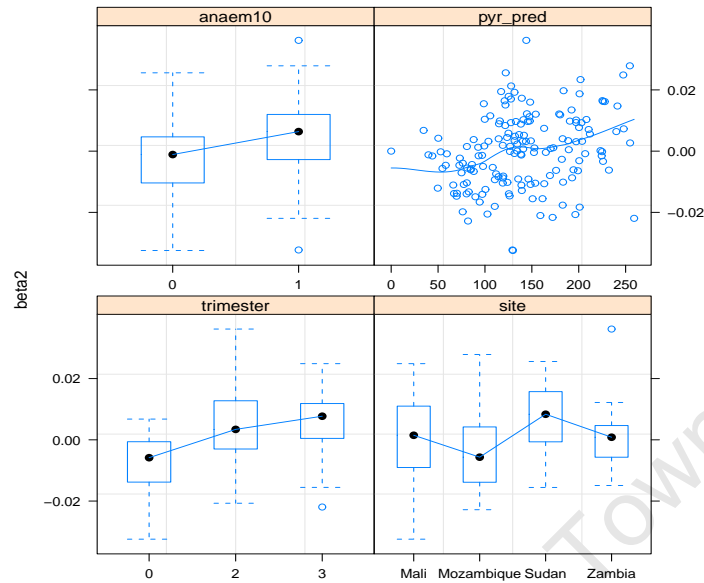


Figure 3.56: Occasion-Specific Random Effects for  $\beta_2$  vs. Covariates: Sequential NLME Model 1

ates for parameters  $\beta_0$  and  $\beta_1$ , and Figure 3.56 is the plot of the occasion-specific random effects vs. covariates for  $\beta_2$ . The plots for the random effects and grouping levels not presented here may be found in Appendix D, figures D.1 to D.3.

The site and pregnancy effects previously noted for this model are unchanged. A systematic pattern may be observed in the plot of the random effects versus the predicted Pyrimethamine concentrations for parameter  $\beta_0$ , (Figure 3.54).

## Model 2

The covariate plots were used as a guideline only, and the impact of the predicted Pyrimethamine concentrations on the fixed effects was determined through an iterative stepwise model building procedure.

The time-varying covariate was added to each parameter in turn, and then to various combinations of parameters in increasing levels of complexity, with the random effects and model diagnostics assessed at every turn.

The resulting “unadjusted” model is described by stage 2 equations:

$$\begin{aligned}\beta_{0ikj} &= \beta_0 + \beta_3 \times \text{pyrimethamine}_{ikj} + b_{0i} + b_{0ik}, \\ \beta_{1ikj} &= \beta_1 + b_{1i} + b_{1ik}, \\ \beta_{2ikj} &= \beta_2 + b_{2ik}.\end{aligned}$$

The subject and occasion-specific random effects are still assumed to be normally distributed:

$$\mathbf{b}_i \sim N(\mathbf{0}, \boldsymbol{\psi}_1), \mathbf{b}_{ik} \sim N(\mathbf{0}, \boldsymbol{\psi}_2)$$

with variance-covariance matrices, ( $\boldsymbol{\psi}_1$  and  $\boldsymbol{\psi}_2$ ) given by:

$$\hat{\boldsymbol{\psi}}_1 = \begin{bmatrix} 6.72^2 & 0 & 0 \\ 0 & 1.84^2 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$\hat{\boldsymbol{\psi}}_2 = \begin{bmatrix} 11.82^2 & 0 & 0 \\ 0 & 4.29^2 & 0 \\ 0 & 0 & 0.014^2 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (7.54)^2$ .

The results for the fixed effects are summarized in Table 3.29.

Parameter	Value	Std.Error
$\hat{\beta}_0$ .(Intercept)	52.81	2.29
$\hat{\beta}_3$ .(Pyrimethamine)	0.09	0.006
$\hat{\beta}_1$	10.91	0.72
$\hat{\beta}_2$	0.054	0.003

Table 3.29: Model Output for Sequential NLME Model 2

The plot of the subject-specific random effects versus covariates for parameter  $\beta_0$  for model 2, indicates a reduction in the systematic patterns previously observed for trimester and the predicted Pyrimethamine concentration.

The pattern observed for site remains, although it is much reduced, (Figure 3.57), with the only difference indicated for Zambia.

The same plot for parameter  $\beta_2$  indicates the possible impact of pregnancy (trimester), (Figure 3.58).

The diagnostics plots for this model, (Appendix D, figures D.4 to D.11) indicated roughly normal residuals and random effects for both grouping levels, but showed increasing residual variance, (Figure 3.59).

Following the incorporation of a variance model of the same form as previously shown, model building was continued using an hypothesis based approach, and the effects of pregnancy and site were examined.

The distributions and variance-covariance matrices for the random effects were iteratively examined for each prospective model.

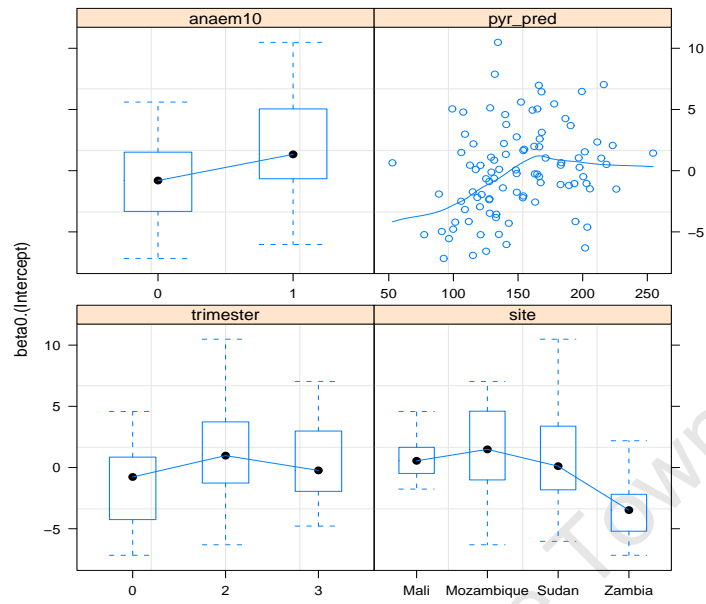


Figure 3.57: Subject-Specific Random Effects for  $\beta_0$  vs. Covariates: Sequential NLME Model 2

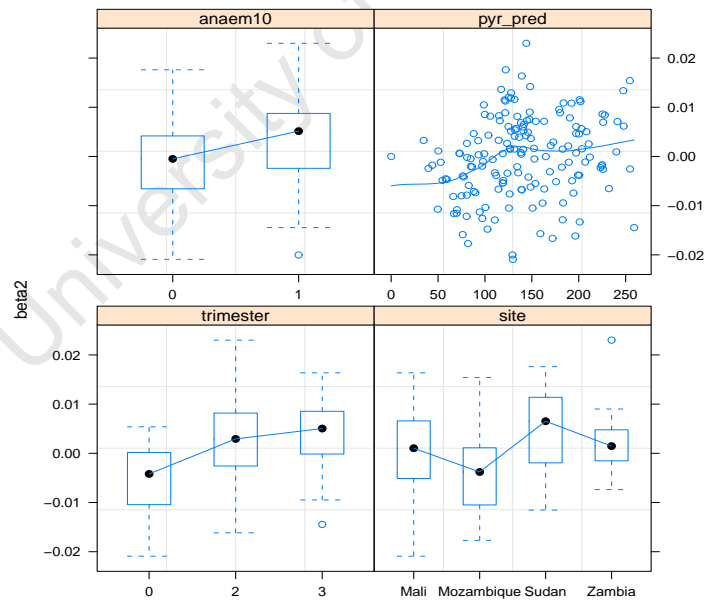


Figure 3.58: Occasion-Specific Random Effects for  $\beta_2$  vs. Covariates: Sequential NLME Model 2

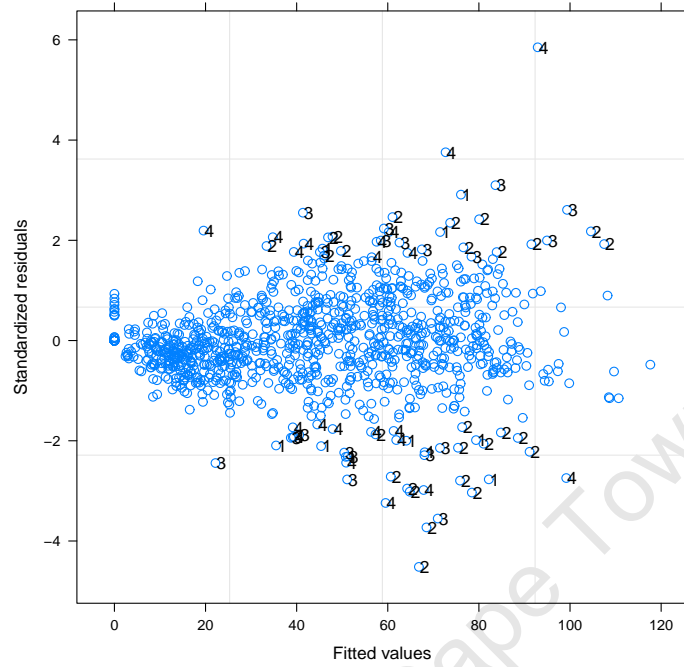


Figure 3.59: Standardized Residuals vs. Fitted Values: Sequential Model 2

### Model 3

The final adjusted sequential model is given by stage 2 equations:

$$\beta_{0ikj} = \beta_0 + \beta_3 \times \text{pyrimethamine}_{ikj} + \beta_5 \times \text{zambia}_{i..} + b_{0ik},$$

$$\beta_{1ikj} = \beta_1 + b_{1ik},$$

$$\beta_{2ikj} = \beta_2 + \beta_4 \times \text{preg}_{ik.} + b_{2i}.$$

The results for the fixed effects are summarized in Table 3.30.

Parameter	Value	Std.Error
$\hat{\beta}_0.(Intercept)$	61.07	2.38
$\hat{\beta}_3.(Pyrimethamine)$	0.08	0.007
$\hat{\beta}_5.(Zambia)$	-15.96	2.33
$\hat{\beta}_1$	10.09	0.65
$\hat{\beta}_2$	0.05	0.003
$\hat{\beta}_4.(Pregnancy)$	0.02	0.002

Table 3.30: Model Output for Sequential NLME Model 3

The random effects,  $\hat{b}_i = [\hat{b}_{2i}]'$  and  $\hat{b}_{ik} = [\hat{b}_{0ik}, \hat{b}_{1ik}]'$ , have variance  $\hat{\tau}_2^2 = 0.012^2$

and variance-covariance matrix:

$$\hat{\psi}_2 = \begin{bmatrix} 10.43^2 & 0 \\ 0 & 4.03^2 \end{bmatrix}$$

respectively, and the diagonal elements of  $\mathbf{R}_{ik}$  are dependent on  $\hat{\sigma}^2 = (0.097)^2$  and  $\hat{\theta} = [15.74, 1.09]$ .

This model was determined to be significantly better than both Model 1 and Model 2, as illustrated in Table 3.31, which details the Aikaike and Bayesian Information Criteria for each of the models.

Model	df	AIC	BIC
Model 1	9	8482.187	8527.552
Model 2	11	7881.795	7937.241
Model 3	12	7767.627	7828.114

Table 3.31: AIC and BIC for Sequential Models 1, 2 and 3

The assumptions of normality for both the residuals and random effects did not appear to be violated (Appendix D, figures D.12 to D.17), and Figure 3.60 illustrates the impact of the variance model on the heteroskedasticity of the residuals.

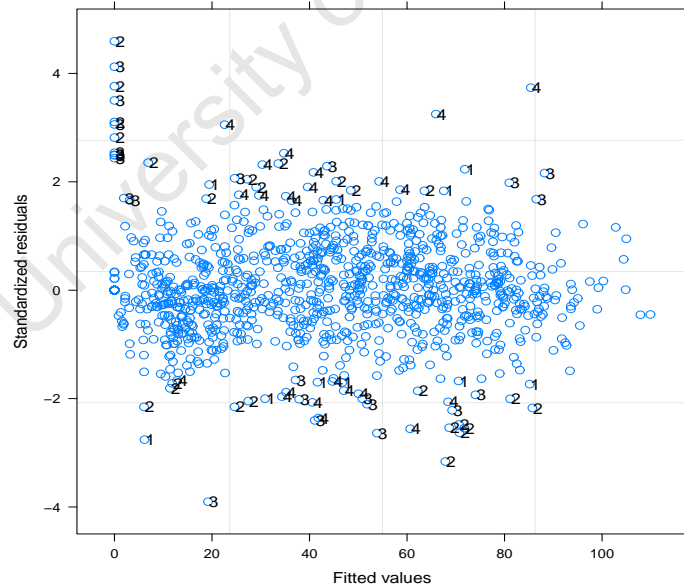


Figure 3.60: Standardized Residuals vs. Fitted Values: Sequential Model 3

Figures 3.62 and 3.63 demonstrate the lack of systematic patterns in the random variability about parameters  $\beta_0$  and  $\beta_2$ , indicating that Model 3 adequately

accounts for those covariate effects considered, and Figure 3.61, the plot of the fitted values versus the observed, indicates a relatively good fit, although the outward fanning towards the top right-hand side of the graph does suggest that the peak concentration values are being under- or over-estimated.

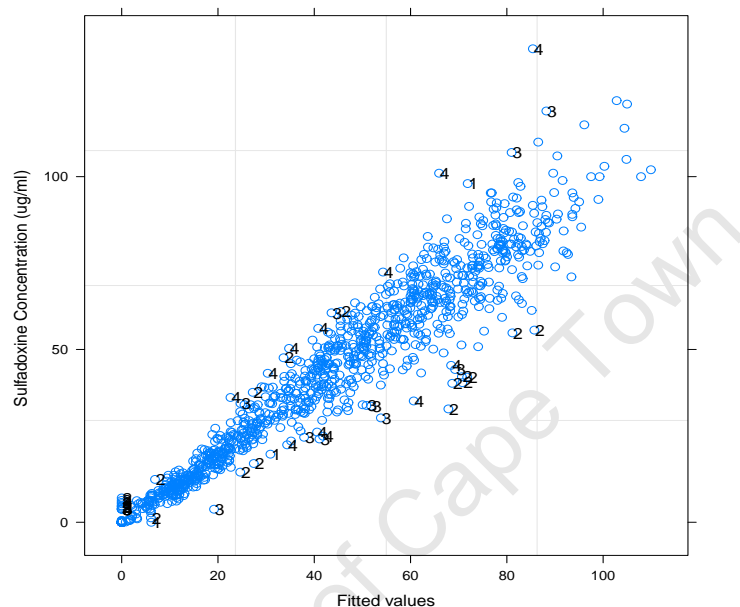


Figure 3.61: Fitted Values vs. Observed: Sequential NLME Model 3

This is further emphasized by Figure 3.64, which looks at the population, individual and phase-specific predicted concentration-time curves versus the observed values (for a subset of the data).

As shown by (for example) subject “ZAMB\_004/1”, whilst the phase-specific predicted concentration-time curve captures most of the observed information quite accurately, the peak concentration is under-estimated.

The mean predicted concentration-time profiles by site and pregnancy status, shown in Figures 3.65 and 3.66, illustrate the effects of pregnancy and site for the Model 3, for the case in which the predicted Pyrimethamine concentration is 0ng/ml, and the case in which the predicted Pyrimethamine concentration is 400ng/ml.

Pregnant subjects appear to have a faster rate of decline than those postpartum, regardless of study site, and higher peak concentrations in all sites except for Sudan.

Subjects in Zambia have the lowest range of concentrations compared to the other sites, irrespective of pregnancy phase.



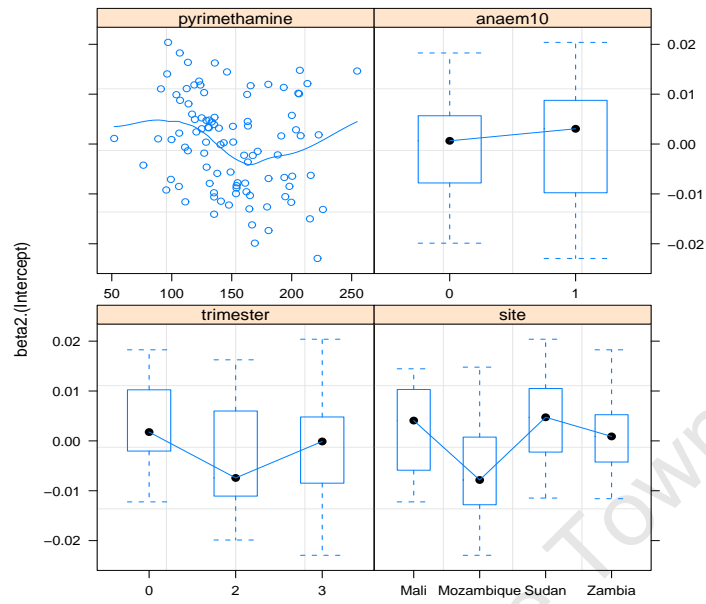


Figure 3.62: Subject-Specific Random Effects for  $\beta_2$  vs. Covariates: Sequential NLME Model 3

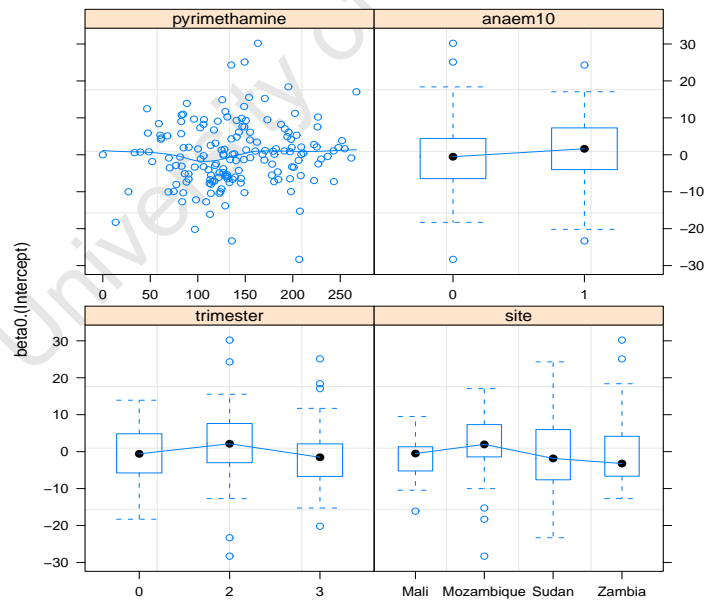


Figure 3.63: Occasion-Specific Random Effects for  $\beta_0$  vs. Covariates: Sequential NLME Model 3

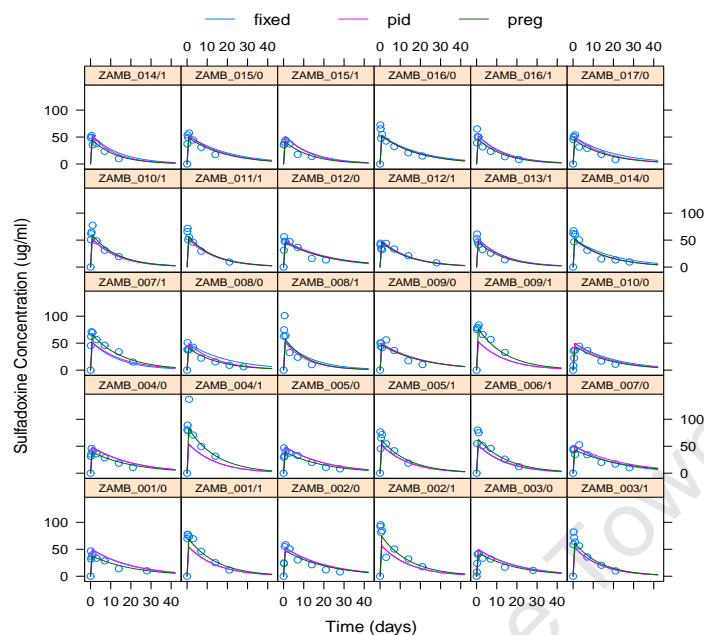


Figure 3.64: Population, Individual and Phase-Specific Predicted Concentration-Time Curves (Subset: Zambia): Sequential NLME Model 3

The curves in Figure 3.65 differ slightly from those in Figure 3.25: the overall range of concentrations reached is lower than that found with the separate Sulfadoxine Model, irrespective of study site or pregnancy phase, and the higher peak concentrations previously seen in pregnant subjects in Mali and Zambia are no longer observed.

As a result of the latter, in all sites, the slope of decline for pregnant subjects is consistently lower than that for postpartum subjects for the entire time period observed, where before it was lower for only the later portion of the curves in Mali and Zambia.

The approximate magnitude of the difference between the pregnant and postpartum slopes of decline is, however, roughly the same as that previously observed.

The curves in Figure 3.66, which account for an increase in Pyrimethamine predicted concentrations from 0 to 400ng/ml (amount arbitrarily chosen for illustrative purposes), show a much higher range of concentrations for all subjects in all sites, with the same basic relationship maintained between postpartum and pregnant subjects in Mali, Zambia and Sudan, albeit with a slightly greater magnitude of difference. For Mozambique, greater changes are observed.

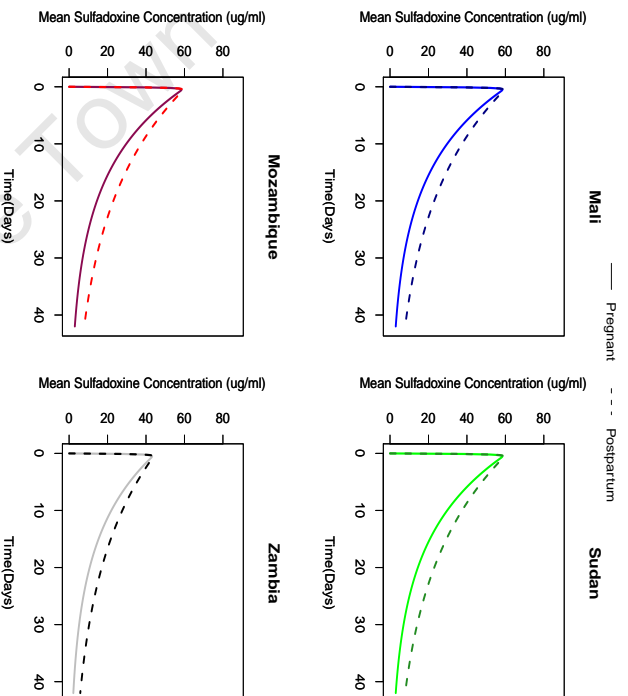


Figure 3.65: Mean Predicted Concentration-Time Curves by Site and Pregnancy Phase: Sequential NLME Model 3

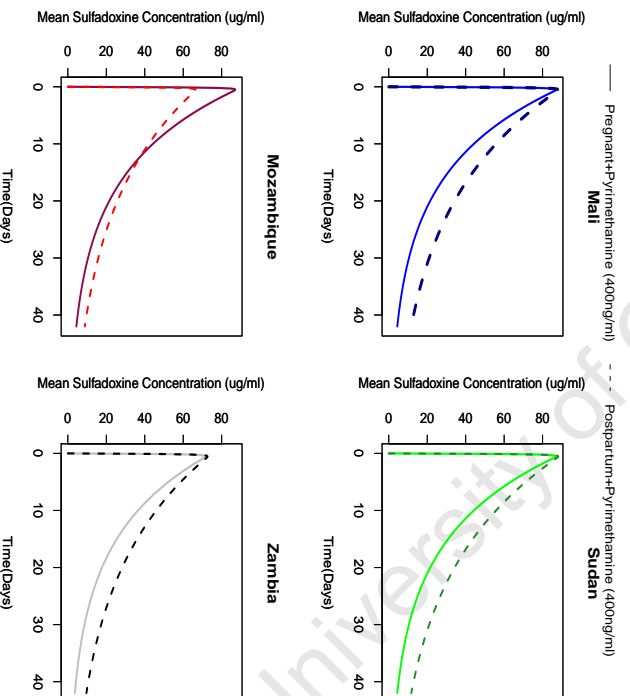


Figure 3.66: Mean Predicted Concentration-Time Curves by Site and Pregnancy Phase II: Sequential NLME Model 3

### 3.4.2 Impact of Predicted Sulfadoxine Concentrations on Pyrimeth-amine

Predicted Sulfadoxine concentrations were generated using the basic Sulfadoxine model ignoring covariates, given by Model 1 in section 3.2. the basic triple-exponential model previously used as a starting point for the Pyrimethamine concentration-time models (Model 1, section 3.3) was then re-fitted, with the predicted Sulfadoxine concentrations included in the covariate model. Despite the attempted inclusion of Sulfadoxine on each of the parameters in turn and in various combinations, none of the models reached convergence.

This was not entirely unexpected, as the original hypothesis was that the Pyrimethamine concentrations would impact on Sulfadoxine, rather than vice versa, due to the more rapid absorption and elimination of Pyrimethamine.

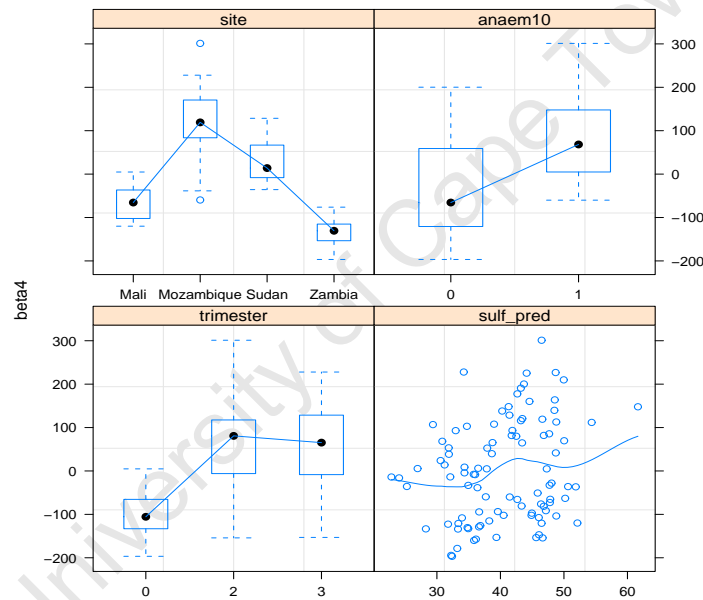


Figure 3.67: Subject-Specific Random Effects for  $\beta_4$  vs. Covariates: Sequential Pyrimethamine NLME Model 1

This hypothesis was further supported by the plots of the random effects versus the covariates for the basic Pyrimethamine model, (Figures 3.67 and 3.68), which showed no clear relationship for Sulfadoxine.

### 3.4.3 Discussion

In the sequential modeling, as hypothesized, the Pyrimethamine concentration appears to impact the concentration range of Sulfadoxine.

Including Pyrimethamine as a time-varying covariate also appears to influence

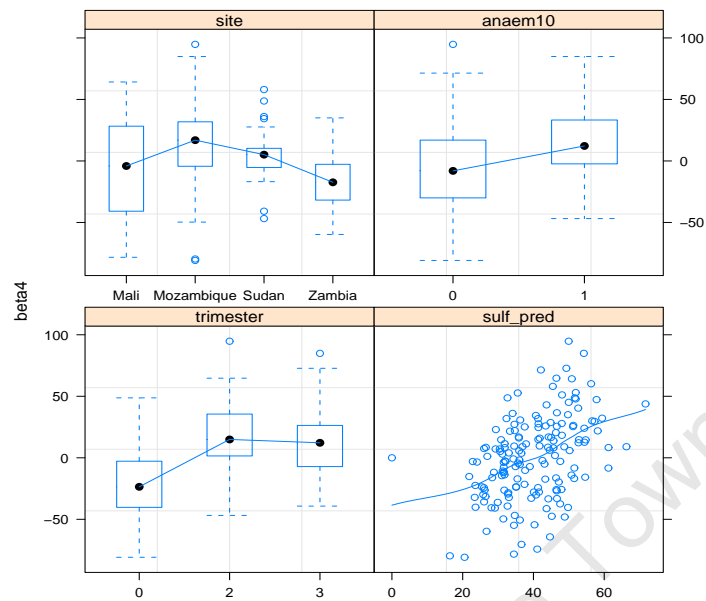


Figure 3.68: Occasion-Specific Random Effects for  $\beta_4$  vs. Covariates: Sequential Pyrimethamine NLME Model 1

the effects of pregnancy and site, removing pregnancy as a covariate on parameter  $\beta_0$ , and reducing the impact of site-based differences.

The Pyrimethamine concentrations may not necessarily influence those of Sulfadoxine directly. Since Pyrimethamine is more rapidly absorbed, it may act as a surrogate indicator of the individual/groups' ability to absorb drugs, rather than increasing or altering the Sulfadoxine absorption.

As such, the sequential model formulation appears to be of limited use in the PK-PK setting in which multiple drug concentrations are modelled. It would be more useful in the PK-PD setting, where the mechanism of interaction is more widely understood, and there is a direct impact of the predicted drug concentration on the PD response.

### 3.5 Simultaneous Models

This section details the results from the simultaneous models for both model specifications:

1. Response type fitted as a covariate to a single structural model form, and
2. Response type included as part of the original structural form, indicating which form is applicable for which response

In order for either model specification to be used, the data itself required restructuring.

Table 3.32 is an excerpt of the data demonstrating the “stacked” vector of responses.

Participant	Day	Concentration (ug/ml)/(ng/ml)	$\delta_{i...}$ (0/1)	Response	Pregnant
MALL001	0	0	0	Pyrimethamine	0
MALL001	0.125	343	0	Pyrimethamine	0
MALL001	0.25	444	0	Pyrimethamine	0
MALL001	0.5	328	0	Pyrimethamine	0
MALL001	1	286	0	Pyrimethamine	0
MALL001	3	260	0	Pyrimethamine	0
MALL001	7	148	0	Pyrimethamine	0
MALL001	14	48	0	Pyrimethamine	0
MALL001	21	18.7	0	Pyrimethamine	0
MALL001	0	0	0	Sulfadoxine	0
MALL001	0.125	66.4	1	Sulfadoxine	0
MALL001	0.25	86.3	1	Sulfadoxine	0
MALL001	0.5	66.2	1	Sulfadoxine	0
MALL001	1	65.3	1	Sulfadoxine	0
MALL001	3	64.5	1	Sulfadoxine	0
MALL001	7	45.8	1	Sulfadoxine	0
MALL001	14	23.6	1	Sulfadoxine	0
MALL001	21	15.4	1	Sulfadoxine	0
MALL001	28	9.9	1	Sulfadoxine	0

Table 3.32: Subset of Restructured Data: Example

The results from model specification 1 are presented first, including a detailed description of the model building procedure, the results from the final model and the predicted curves resulting from this model.

#### 3.5.1 Model Specification 1: Covariate Specification

Since the same structural model form is fitted for both response types in this model specification, the triple-exponential model was selected in order to account for the more complex Pyrimethamine concentration-time curves.

### Model 1

Fitting the response type as a binary indicator variable on all parameters, the stage 2 equations for Model 1 (the basic model, ignoring the impact of other covariates), may be given by:

$$\begin{aligned}\beta_{1likj} &= \beta_1 + \beta_6 \times \delta_{l\dots} \\ \beta_{2likj} &= \beta_2 + \beta_7 \times \delta_{l\dots} + b_{2i} + b_{2ik} \\ \beta_{3likj} &= \beta_3 + \beta_8 \times \delta_{l\dots} + b_{3i} + b_{3ik} \\ \beta_{4likj} &= \beta_4 + \beta_9 \times \delta_{l\dots} + b_{4i} + b_{4ik} \\ \beta_{5likj} &= \beta_5 + \beta_{10} \times \delta_{l\dots} + b_{5i} + b_{5ik}\end{aligned}$$

for a model of the form:

$$\begin{aligned}y_{likj} &= f(\mathbf{x}_{likj}, \boldsymbol{\beta}_{likj}) + e_{likj} \\ &= \beta_{2likj} \times [-\exp(-\beta_{1likj} \times time_{likj}) + \exp(-\beta_{3likj} \times time_{likj})] \\ &\quad + \beta_{4likj} \times [-\exp(-\beta_{1likj} \times time_{likj}) + \exp(-\beta_{5likj} \times time_{likj})] \\ &\quad + e_{likj}\end{aligned}$$

where the random effects and residual errors  $e_{likj}$  are defined as before, and where  $\delta_{l\dots}$  is a binary variable indicating the response type as Pyrimethamine ( $\delta_{l\dots} = 0$ ) or Sulfadoxine ( $\delta_{l\dots} = 1$ ).

The results for the fixed effects are summarized in Table 3.33.

Parameter	Value	Std.Error
$\hat{\beta}_1.(Baseline)$	37.47	10.90
$\hat{\beta}_6.(Sulfadoxine)$	-37.29	10.90
$\hat{\beta}_2.(Baseline)$	298.04	19.25
$\hat{\beta}_7.(Sulfadoxine)$	80.30	53.81
$\hat{\beta}_3.(Baseline)$	0.14	0.004
$\hat{\beta}_8.(Sulfadoxine)$	0.005	0.01
$\hat{\beta}_4.(Baseline)$	124.88	9.67
$\hat{\beta}_9.(Sulfadoxine)$	-191.99	9.91
$\hat{\beta}_5.(Baseline)$	0.65	0.09
$\hat{\beta}_{10}.(Sulfadoxine)$	12.35	1.96

Table 3.33: Triple-Exponential Model Output: Simultaneous NLME Model 1

The variance-covariance matrices for the random effects for each grouping level are given by:

$$\hat{\psi}_1 = \begin{bmatrix} 139.91^2 & 0 & 0 & 0 \\ 0 & 0.008^2 & 0 & 0 \\ 0 & 0 & 0.02^2 & 0 \\ 0 & 0 & 0 & 0.0002^2 \end{bmatrix},$$

$$\hat{\psi}_2 = \begin{bmatrix} 61.07^2 & 0 & 0 & 0 \\ 0 & 7.49e-07^2 & 0 & 0 \\ 0 & 0 & 0.001^2 & 0 \\ 0 & 0 & 0 & 1.88e-05^2 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (23.41)^2$ .

The diagnostic plots for this model indicated roughly normal residuals and random effects for both levels, although some skewness was observed in the QQplots of the random effects (Appendix E, figures E.1 to E.7).

Heteroskedasticity is however indicated for Sulfadoxine (Figure 3.69).

Although the fit for Pyrimethamine appears to be reasonable, for Sulfadoxine, the plot of the fitted values versus the observed shows an outward fanning towards to the top right-hand side of the graph, indicating a lack of fit for the higher concentrations, (Figure 3.70).

The incorporation of a variance function at this stage of the model building procedure was not possible, and the focus therefore turned to the number of random effects included in the basic model, and their specification.

## Model 2

The distributions of the random effects were restructured in order to take into account potential differences in the subject and occasion-specific variability due to different response types.

The resulting model had stage 2 equations:

$$\begin{aligned} \beta_{1likj} &= \beta_1 + \beta_6 \times \delta_{l...}, \\ \beta_{2likj} &= \beta_2 + \beta_7 \times \delta_{l...} + b_{2i} + b_{2ik} + b_{7i} + b_{7ik}, \\ \beta_{3likj} &= \beta_3 + \beta_8 \times \delta_{l...} + b_{3i}, \\ \beta_{4likj} &= \beta_4 + \beta_9 \times \delta_{l...} + b_{4i} + b_{4i}^*, \\ \beta_{5likj} &= \beta_5 + \beta_{10} \times \delta_{l...}, \end{aligned}$$

such that, in the linear relationship between (for example) parameter  $\beta_{2likj}$  and the response type indicator variable  $\delta_{l...}$ , random effects were specified for both  $\beta_2$  and  $\beta_7$  for both grouping levels.

The random effects for this model have been specified in two different ways.

The first method specifies random effects for the “intercept” term, and the term for the “slope” or effect modifier, i.e. in the linear relationship between parameter  $\beta_{2likj}$  and the indicator variable  $\delta_{l...}$ , we have random effects for the “intercept” parameter  $\beta_2$ , given by  $b_{2i}$  and  $b_{2ik}$ , and we have random effects for the “slope” parameter  $\beta_7$ , given by  $b_{7i}$  and  $b_{7ik}$ .

The second method specified random effects for the two different intercepts given by, a.) the original “intercept” parameter, and b.) the original “intercept”



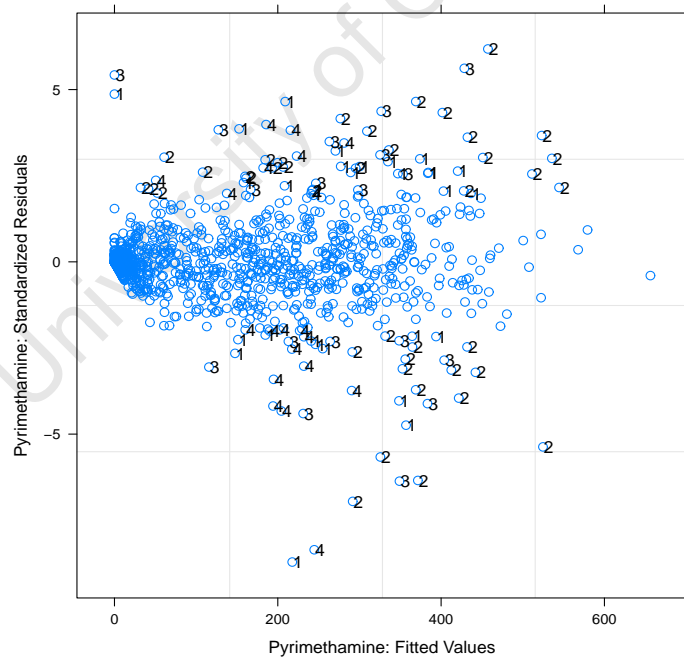
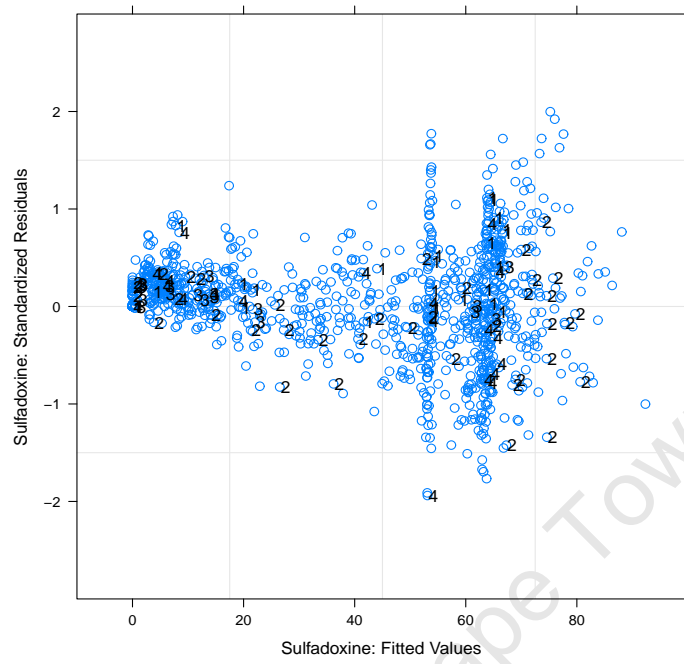


Figure 3.69: Standardized Residuals vs. Fitted Values: Simultaneous NLME Model 1

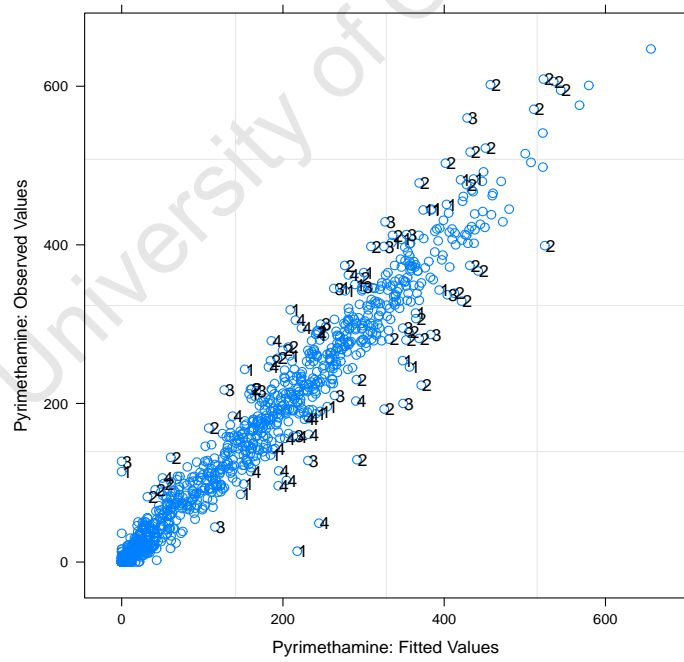
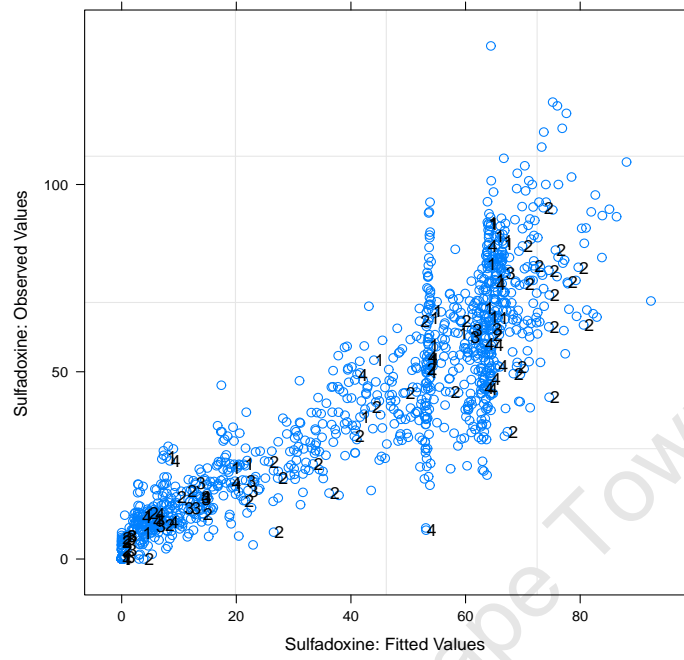


Figure 3.70: Fitted Values vs. Observed: Simultaneous NLME Model 1

parameter *plus* the parameter for the “slope”.

Thus, in the linear relationship between parameter  $\beta_{4likj}$  and the indicator variable  $\delta_{l...}$ , we have random effects for the original “intercept” parameter  $\beta_4$ , (the Pyrimethamine value) given by  $b_{4i}$ , and we have random effects for the second “intercept” parameter  $\beta_4 + \beta_9$ , (corresponding to the Sulfadoxine value), given by  $b_{4i}$ .

The different specifications came about during the model building procedure, with this particular combination providing the best fit.

The random effects for  $\beta_{2likj}$  were further constrained at this point so that  $b_{2i} = b_{7i}$  and  $b_{2ik} = b_{7ik}$ .

The results for the fixed effects from this model are summarized in Table 3.34.

Parameter	Value	Std.Error
$\hat{\beta}_1.(Baseline)$	26.32	2.30
$\hat{\beta}_6.(Sulfadoxine)$	-12.04	3.02
$\hat{\beta}_2.(Baseline)$	326.68	13.77
$\hat{\beta}_7.(Sulfadoxine)$	-227.93	27.37
$\hat{\beta}_3.(Baseline)$	0.15	0.004
$\hat{\beta}_8.(Sulfadoxine)$	-0.05	0.01
$\hat{\beta}_4.(Baseline)$	156.66	30.36
$\hat{\beta}_9.(Sulfadoxine)$	-178.74	38.28
$\hat{\beta}_5.(Baseline)$	1.30	0.09
$\hat{\beta}_{10}.(Sulfadoxine)$	-1.04	0.19

Table 3.34: Triple-Exponential Model Output: Simultaneous NLME Model 2

The variance-covariance matrices for the random effects  $\hat{\mathbf{b}}_i = [\hat{b}_{2i}, \hat{b}_{3i}, \hat{b}_{4i}, \hat{b}_{7i}, \hat{b}_{4i*}]'$  and  $\hat{\mathbf{b}}_k = [\hat{b}_{2ik}, \hat{b}_{7ik}]'$  are now given by:

$$\hat{\psi}_1 = \begin{bmatrix} 119.8298^2 & 0 & 0 & 0 & 0 \\ 0 & 119.8298^2 & 0 & 0 & 0 \\ 0 & 0 & 0.03^2 & 0 & 0 \\ 0 & 0 & 0 & 270.29^2 & 0 \\ 0 & 0 & 0 & 0 & 1.90e - 10^2 \end{bmatrix},$$

$$\hat{\psi}_2 = \begin{bmatrix} 53.12^2 & 0 \\ 0 & 53.12 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (18.95)^2$ .

The diagnostic plots for Model 2 indicate a much better model fit, (Figures 3.71 and 3.72), and a reduction in the heteroskedasticity previously seen for the residuals related to the Sulfadoxine response, (Figure 3.73).

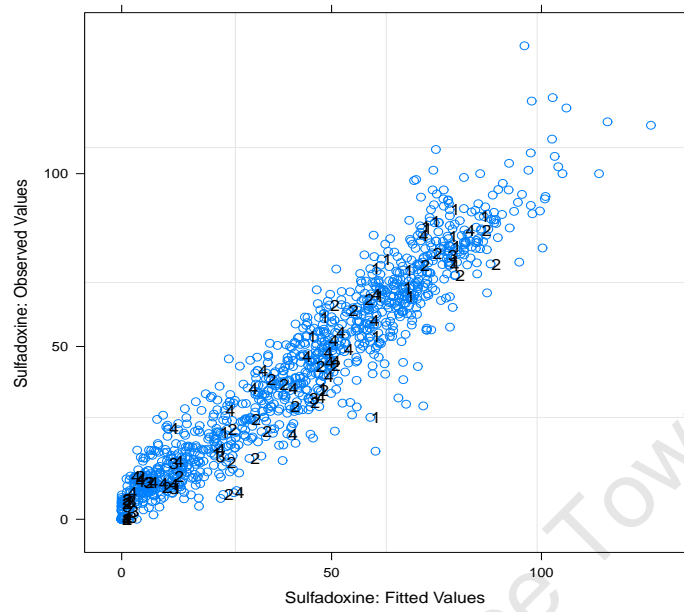


Figure 3.71: Sulfadoxine Fitted Values vs. Observed: Simultaneous NLME Model 2

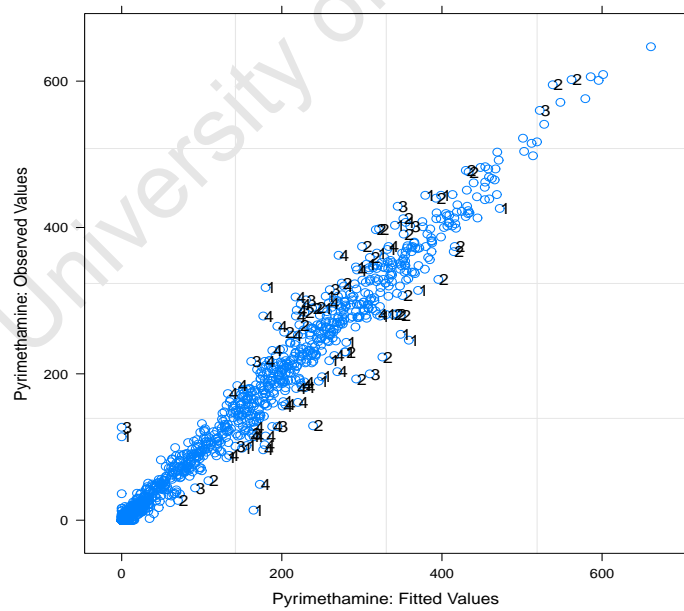


Figure 3.72: Pyrimethamine Fitted Values vs. Observed: Simultaneous NLME Model 2

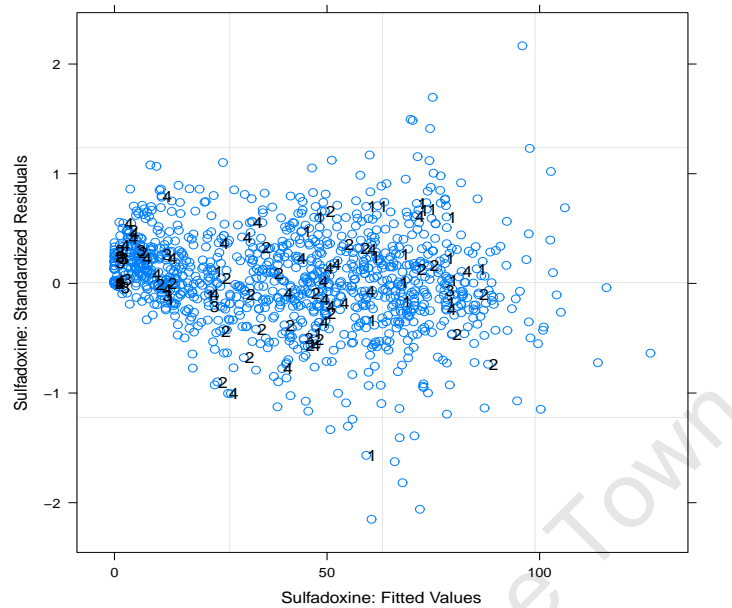


Figure 3.73: Standardized Residuals vs. Fitted Values: Simultaneous NLME Model 2

For parameter  $\beta_2$ , for the random effects  $b_{2i}$  and  $b_{2ik}$ , (labeled “beta2.(Intercept)”), the effects of pregnancy and site are indicated for both the subject and occasion-specific random effects.

For the random effects  $b_{7i}$  and  $b_{7ik}$  however, (labeled “beta2.pk\_factsulf”), the site and pregnancy effects for both the subject and occasion-specific random effects appear to be in the opposite direction to what they should be, (Figure 3.74 and Figure 3.75).

This is in part explained by the perfect negative correlation between the  $\beta_2$  and  $\beta_7$  random effects noted for both grouping levels, shown here for the phase within subject level (Figure 3.76).

The same phenomenon is *not* noted for the random effects  $b_{4i}$  and  $b_{*4i}$ , (labeled “beta4.pk\_factpyr” and “beta4.pk\_factsulf” respectively), which had the alternative specification previously described, (Figure 3.77).

A potential site effect is also noted for  $\beta_3$  in the plot of the subject-specific random effects versus covariates for this parameter, (Appendix E, figure E.8).

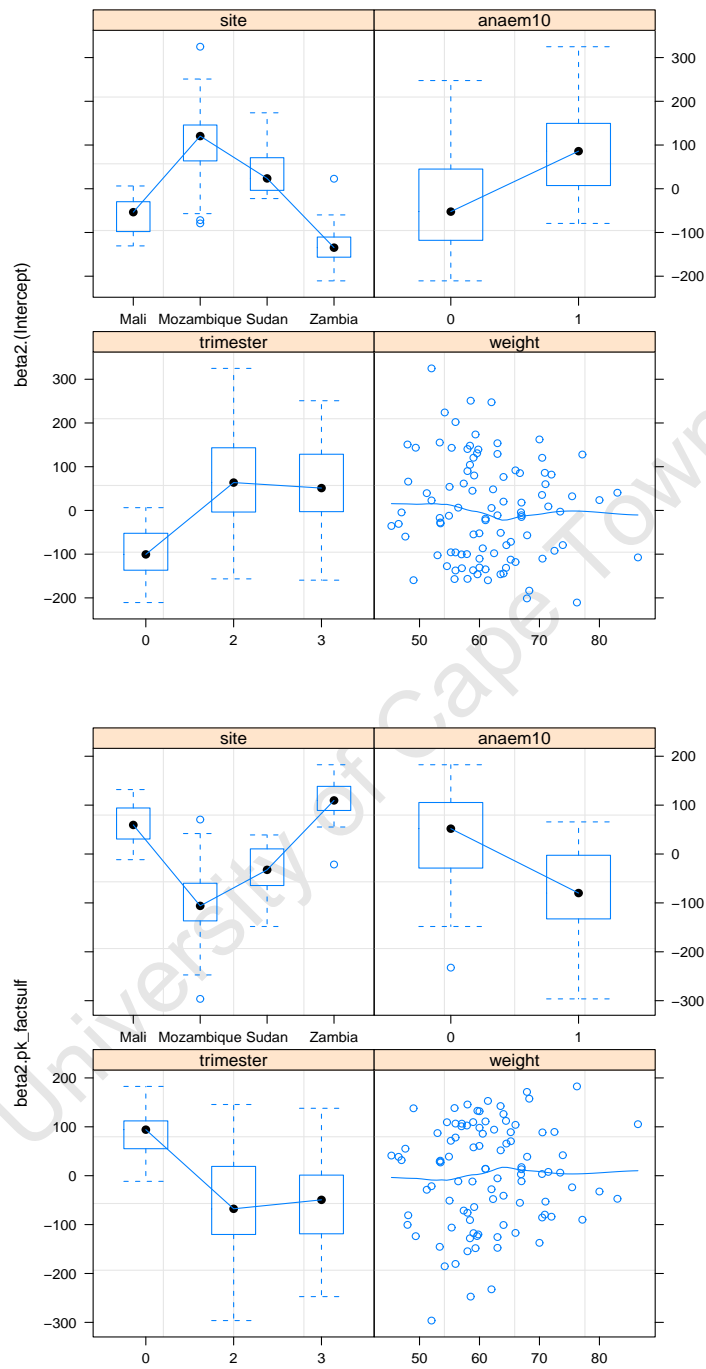


Figure 3.74: Subject-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 2

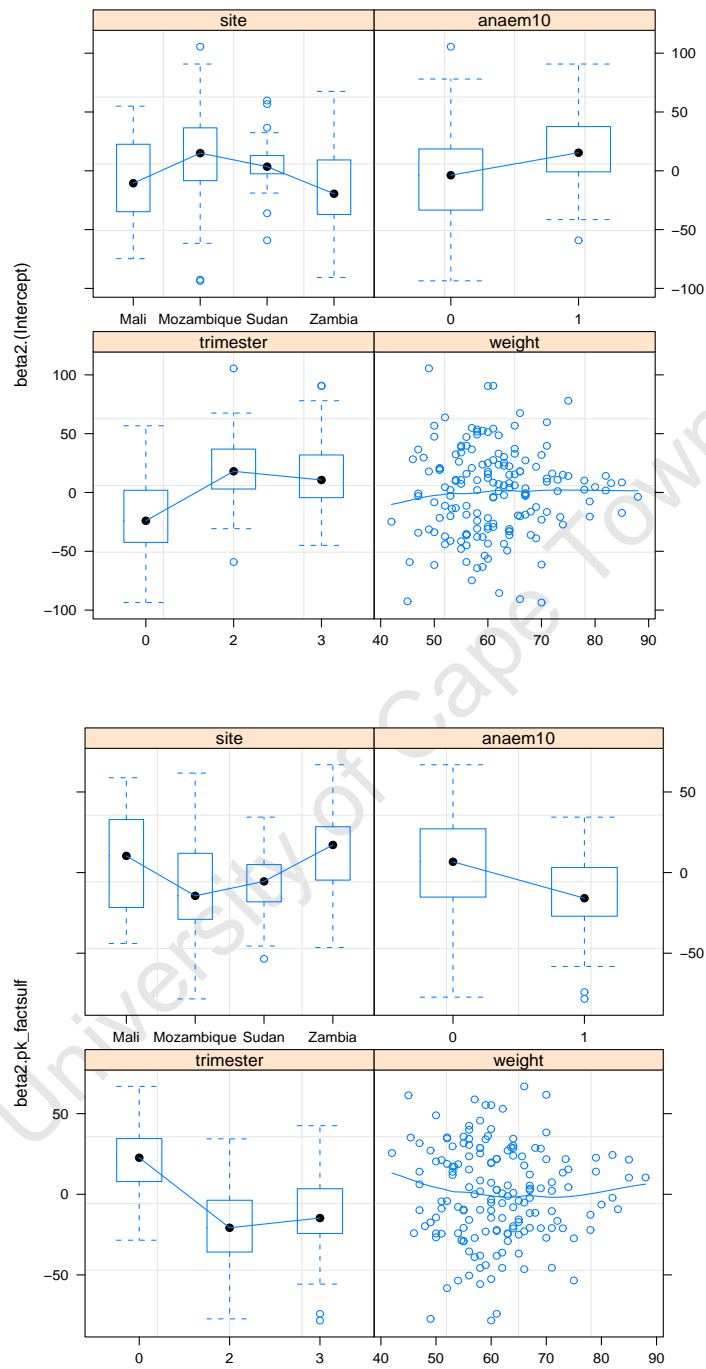


Figure 3.75: Occasion-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 2

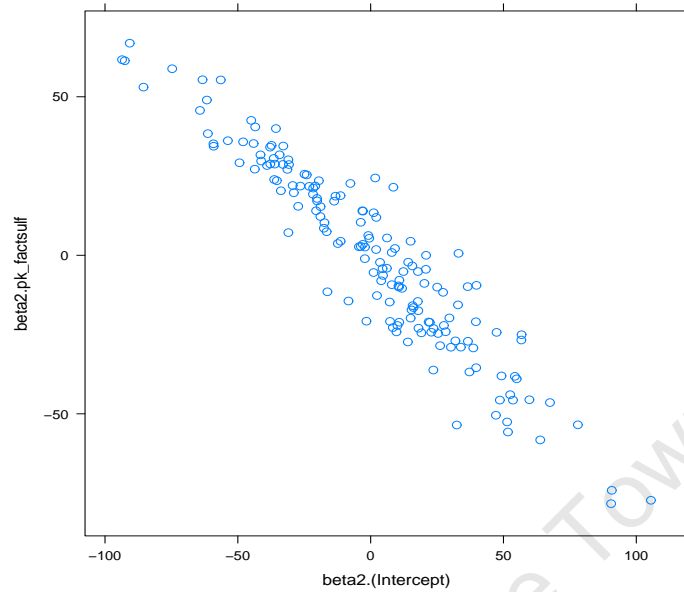


Figure 3.76: Pairs plots of Occasion-Specific Random Effects: Simultaneous NLME Model 2

### Model 3

This model adjusts for the impact of pregnancy and site, and corresponds to stage 2 equations:

$$\begin{aligned}
 \beta_{1likj} &= \beta_1 + \beta_6 \times \delta_{l...}, \\
 \beta_{2likj} &= \beta_2 + \beta_7 \times \delta_{l...} + \beta_{11} \times pregnancy_{lik.} + \beta_{12} \times mozambique_{li..} \\
 &\quad + \beta_{13} \times sudan_{li..} + \beta_{14} \times zambia_{li..} + b_{2i} + b_{2ik} + b_{7i} + b_{7ik}, \\
 \beta_{3likj} &= \beta_3 + \beta_8 \times \delta_{l...} + b_{3i}, \\
 \beta_{4likj} &= \beta_4 + \beta_9 \times \delta_{l...} + b_{4i} + b_{4i*}, \\
 \beta_{5likj} &= \beta_5 + \beta_{10} \times \delta_{l...},
 \end{aligned}$$

The constraint previously applied to the random effects for  $\beta_{2likj}$ , such that  $b_{2i} = b_{7i}$  and  $b_{2ik} = b_{7ik}$  has been removed, and the variance-covariance matrices for the random effects are thus given by:

$$\hat{\psi}_1 = \begin{bmatrix} 53.53^2 & 0 & 0 & 0 & 0 \\ 0 & 113.36^2 & 0 & 0 & 0 \\ 0 & 0 & 0.02^2 & 0 & 0 \\ 0 & 0 & 0 & 238.63^2 & 0 \\ 0 & 0 & 0 & 0 & 3.84e-5^2 \end{bmatrix},$$

$$\hat{\psi}_2 = \begin{bmatrix} 46.26^2 & 0 \\ 0 & 45.70 \end{bmatrix}$$



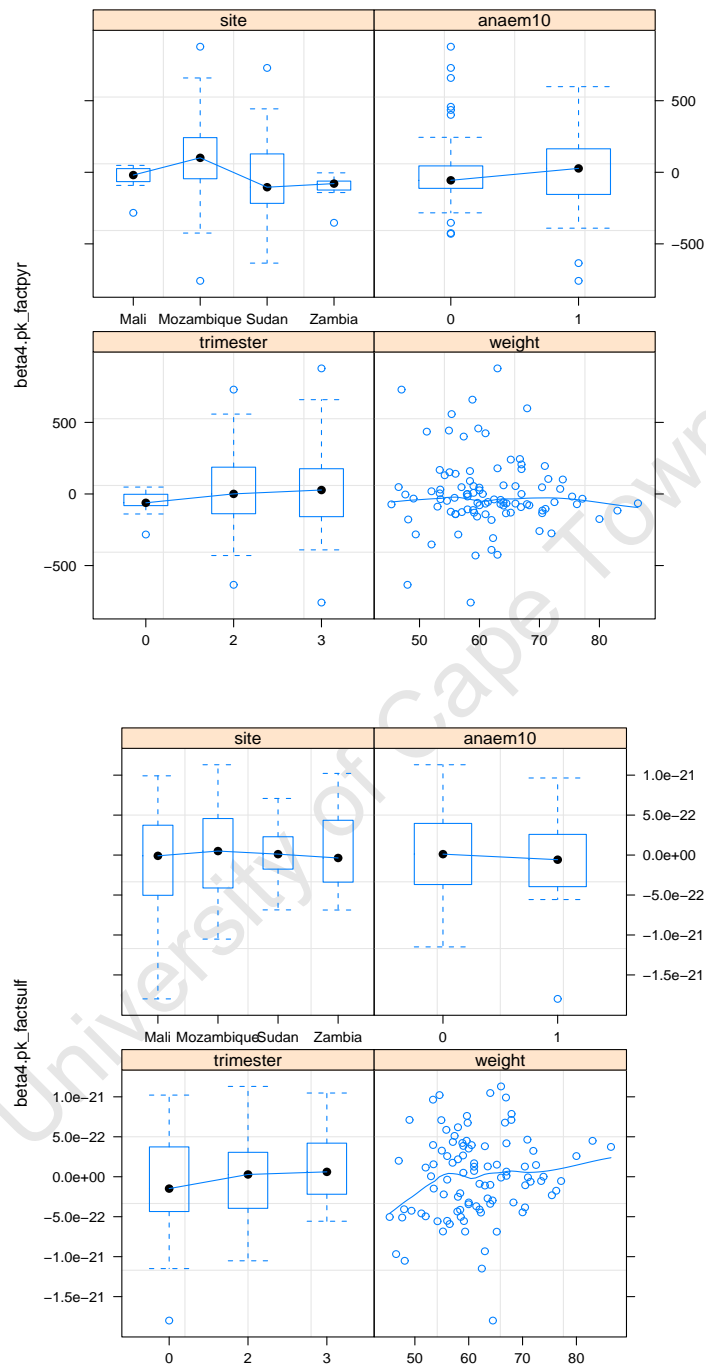


Figure 3.77: Subject-Specific Random Effects  $b_{4i}$  and  $b_{4i^*}$  vs. Covariates: Simultaneous NLME Model 2

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (19.23)^2$ .

The results for the fixed effects from Model 3 are summarized in Table 3.35.

Parameter	Value	Std.Error
$\hat{\beta}_1.(Baseline)$	25.55	2.12
$\hat{\beta}_6.(Sulfadoxine)$	-11.06	2.91
$\hat{\beta}_2.(Baseline)$	231.72	16.63
$\hat{\beta}_7.(Sulfadoxine)$	-220.33	33.71
$\hat{\beta}_{11}.(Pregnancy)$	45.78	8.73
$\hat{\beta}_{12}.(Mozambique)$	191.16	20.60
$\hat{\beta}_{13}.(Sudan)$	106.28	22.59
$\hat{\beta}_{14}.(Zambia)$	-86.63	20.70
$\hat{\beta}_3.(Baseline)$	0.15	0.004
$\hat{\beta}_8.(Sulfadoxine)$	-0.05	0.01
$\hat{\beta}_4.(Baseline)$	150.95	27.16
$\hat{\beta}_9.(Sulfadoxine)$	-179.06	41.10
$\hat{\beta}_5.(Baseline)$	1.26	0.08
$\hat{\beta}_{10}.(Sulfadoxine)$	-1.05	0.14

Table 3.35: Triple-Exponential Model Output: Simultaneous NLME Model 3

The plots of the subject-specific random effects for parameters  $\beta_2$  and  $\beta_7$ , ( $b_{2i}$  and  $b_{7i}$  respectively), shown in Figure 3.78, demonstrate that this model has captured the impact of pregnancy and site for Pyrimethamine only (plot labeled “beta2.(Intercept)”), since the patterns remain for  $\beta_7$ , (plot labeled “beta2.pk\_factsulf”).

A similar phenomenon is seen in Figure 3.79, for the occasion-specific random effects.

#### Model 4

Model 4, (output not shown) incorporates interaction terms between the  $\delta_{l...}$  variable and the variables for pregnancy and site.

The plots of the subject- and occasion-specific random effects versus the covariates for parameters  $\beta_2$  and  $\beta_7$ , (Figures 3.80, and 3.81), show the reduction in the patterns previously observed for  $\beta_7$ , (labeled “pk\_factsulf”).

#### Model 5

The final simultaneous model for the covariate specification is Model 5, which incorporates the interaction terms described, and no longer has the response type indicator variable for parameter  $\beta_5$  (since its inclusion induces multi-collinearity in this model). The results for this model are summarized in Table 3.36, corre-

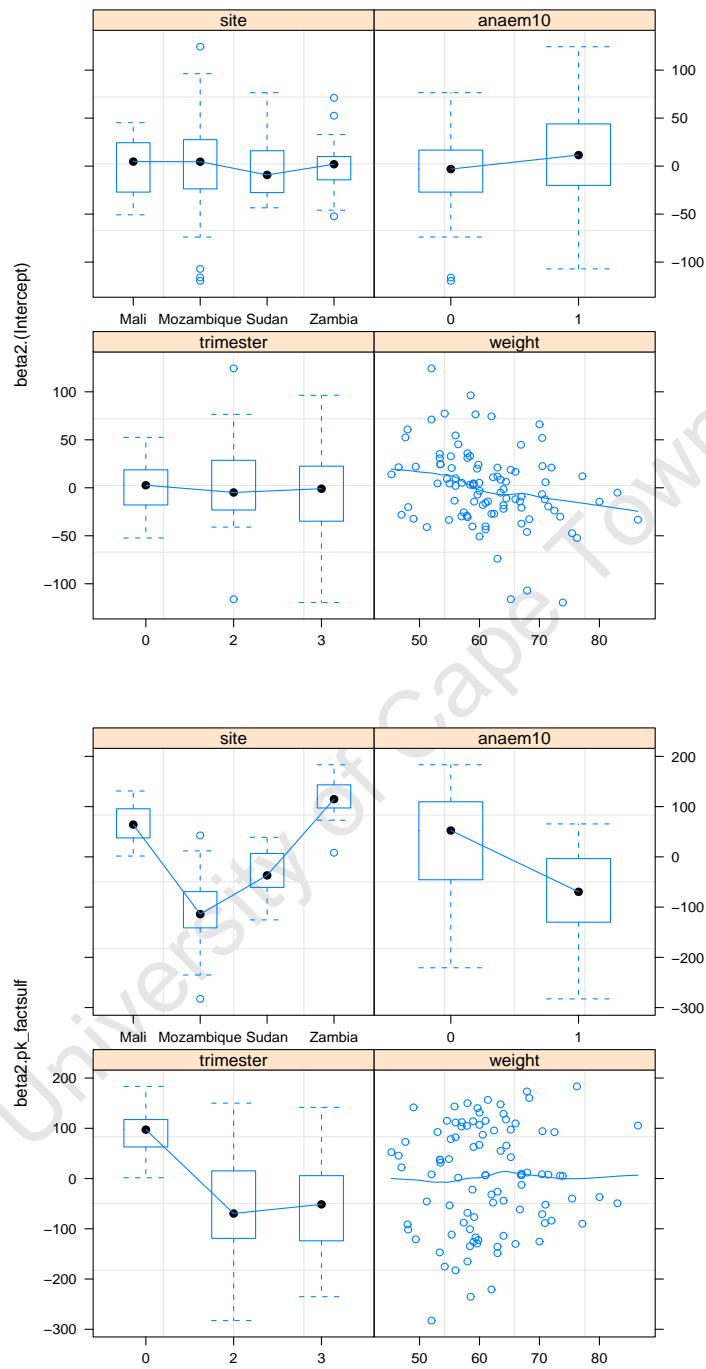


Figure 3.78: Subject-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 3

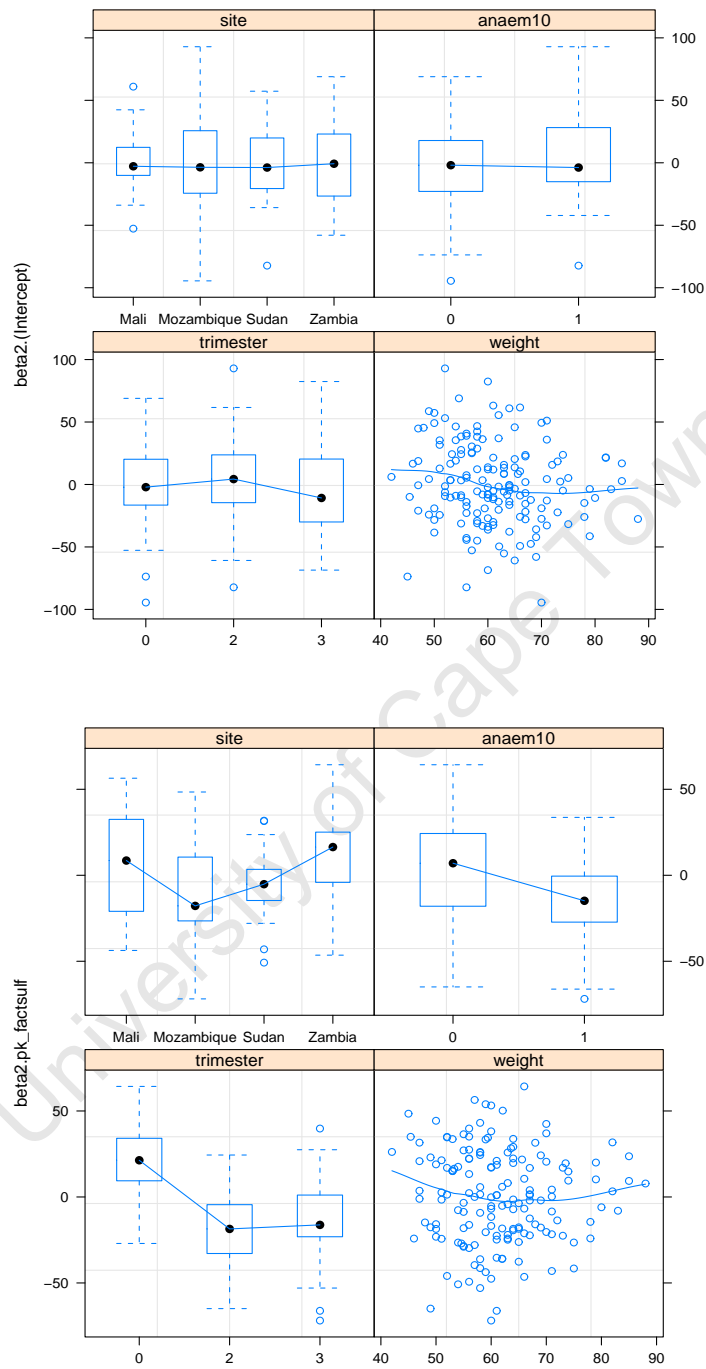


Figure 3.79: Occasion-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 3

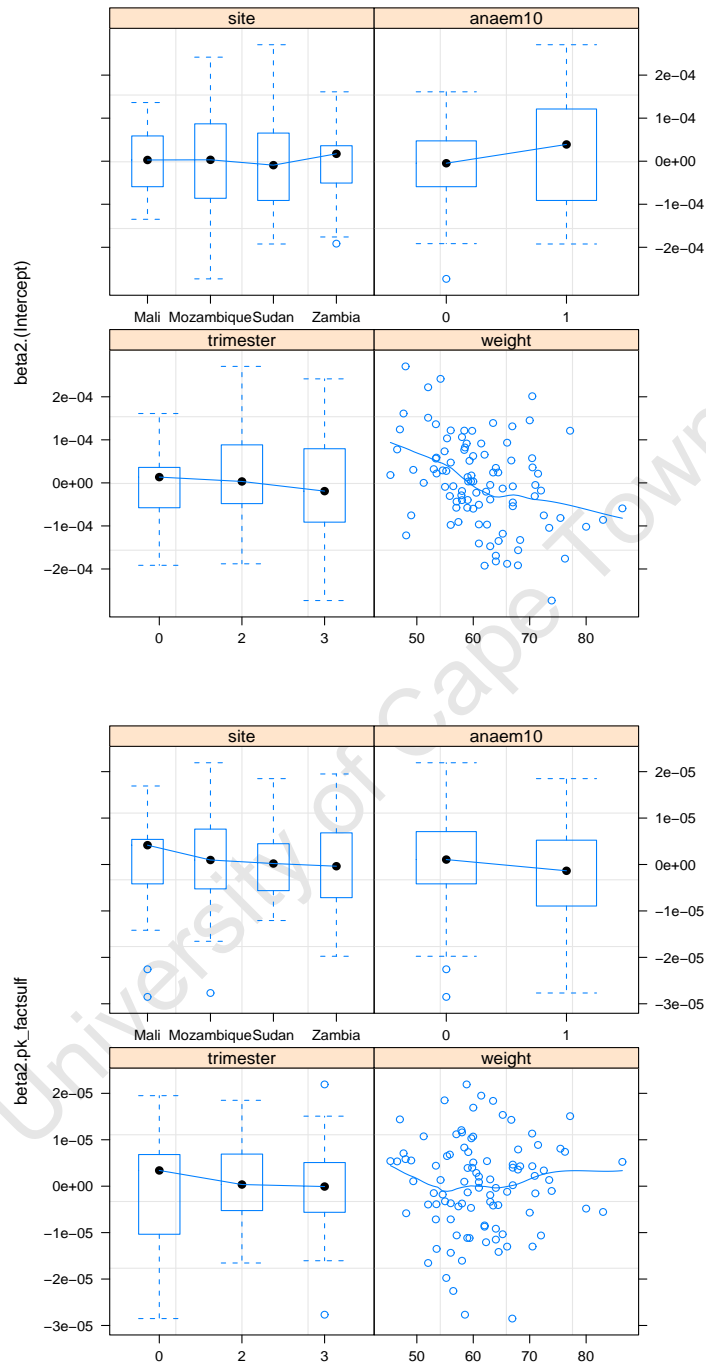


Figure 3.80: Subject-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 4

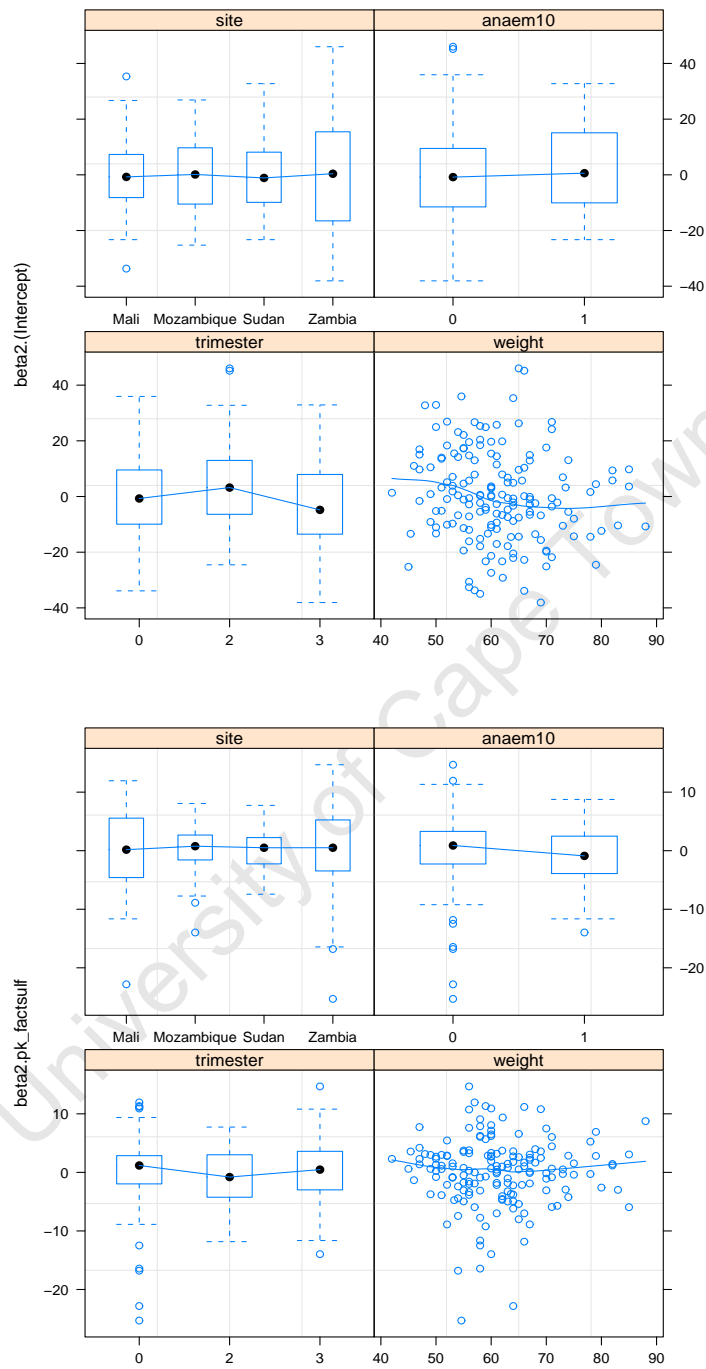


Figure 3.81: Occasion-Specific Random Effects for  $\beta_2$  and  $\beta_7$  vs. Covariates: Simultaneous NLME Model 4

sponding to stage 2 equations given by:

$$\begin{aligned}
\beta_{1likj} &= \beta_1 + \beta_6 \times \delta_{l...}, \\
\beta_{2likj} &= \beta_2 + \beta_7 \times \delta_{l...} + \beta_{10} \times \text{pregnancy}_{lik.} + \beta_{11} \times \text{mozambique}_{li..} \\
&\quad + \beta_{12} \times \text{sudan}_{li..} + \beta_{13} \times \text{zambia}_{li..} \\
&\quad + \beta_{14} \times \delta_{l...} \times \text{pregnancy}_{lik.} + \beta_{15} \times \delta_{l...} \times \text{mozambique}_{li..} \\
&\quad + \beta_{16} \times \delta_{l...} \times \text{sudan}_{li..} + \beta_{17} \times \delta_{l...} \times \text{zambia}_{li..} + b_{2i} + b_{2ik} + b_{7i} + b_{7ik}, \\
\beta_{3likj} &= \beta_3 + \beta_8 \times \delta_{l...} + b_{3i}, \\
\beta_{4likj} &= \beta_4 + \beta_9 \times \delta_{l...} + b_{4i} + b_{4i*}, \\
\beta_{5likj} &= \beta_5,
\end{aligned}$$

Parameter	Value	Std.Error
$\hat{\beta}_1.(Baseline)$	41.78	16.36
$\hat{\beta}_6.(Sulfadoxine)$	-27.74	16.52
$\hat{\beta}_2.(Baseline)$	172.62	13.23
$\hat{\beta}_7.(Sulfadoxine)$	-88.62	13.43
$\hat{\beta}_{10}.(Pregnancy)$	43.74	4.90
$\hat{\beta}_{11}.(Mozambique)$	234.65	12.33
$\hat{\beta}_{12}.(Sudan)$	119.52	13.41
$\hat{\beta}_{13}.(Zambia)$	-71.71	10.99
$\hat{\beta}_{14}.(Sulfadoxine \times Pregnancy)$	-42.23	4.30
$\hat{\beta}_{15}.(Sulfadoxine \times Mozambique)$	-223.19	11.46
$\hat{\beta}_{16}.(Sulfadoxine \times Sudan)$	-111.58	12.39
$\hat{\beta}_{17}.(Sulfadoxine \times Zambia)$	50.29	10.20
$\hat{\beta}_3.(Baseline)$	0.14	0.005
$\hat{\beta}_8.(Sulfadoxine)$	-0.05	0.0071
$\hat{\beta}_4.(Baseline)$	130.86	14.70
$\hat{\beta}_9.(Sulfadoxine)$	-138.79	15.79
$\hat{\beta}_5$	0.54	0.04

Table 3.36: Triple-Exponential Model Output: Simultaneous NLME Model 5

The variance-covariance matrices for the random effects for this model are given by:

$$\hat{\psi}_1 = \begin{bmatrix} 0.06^2 & 0 & 0 & 0 & 0 \\ 0 & 0.02^2 & 0 & 0 & 0 \\ 0 & 0 & 0.03^2 & 0 & 0 \\ 0 & 0 & 0 & 86.97^2 & 0 \\ 0 & 0 & 0 & 0 & 0.0001^2 \end{bmatrix},$$

$$\hat{\psi}_2 = \begin{bmatrix} 21.20^2 & 0 \\ 0 & 12.02 \end{bmatrix}$$

and the diagonal elements of  $\mathbf{R}_{ik}$  are given by  $\hat{\sigma}^2 = (22.1)^2$ .

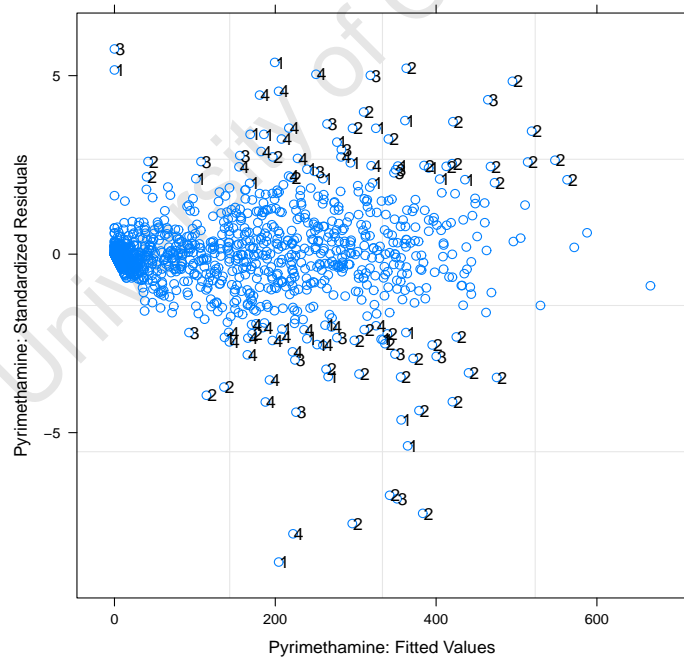
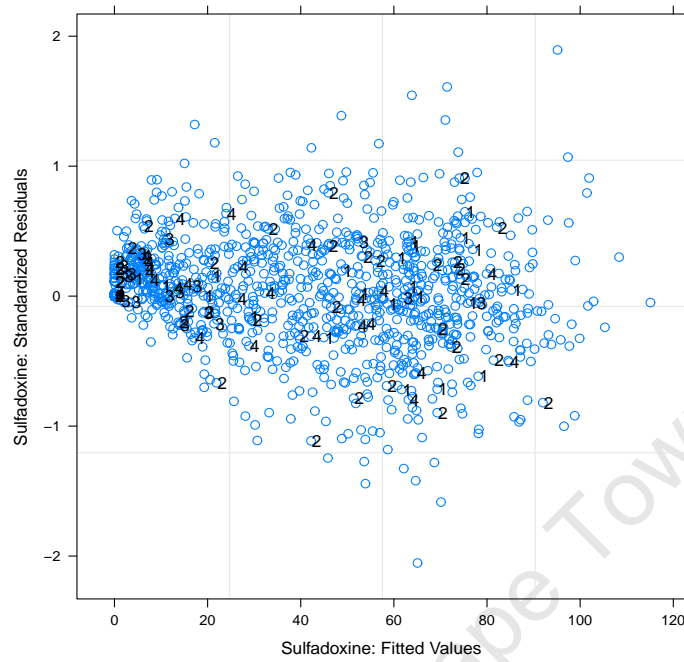


Figure 3.82: Standardized Residuals vs. Fitted Values: Simultaneous NLME Model 5



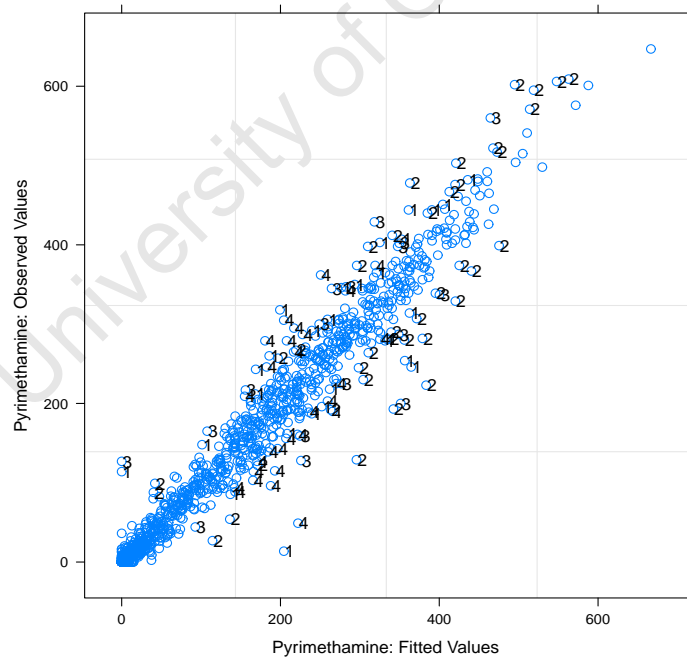
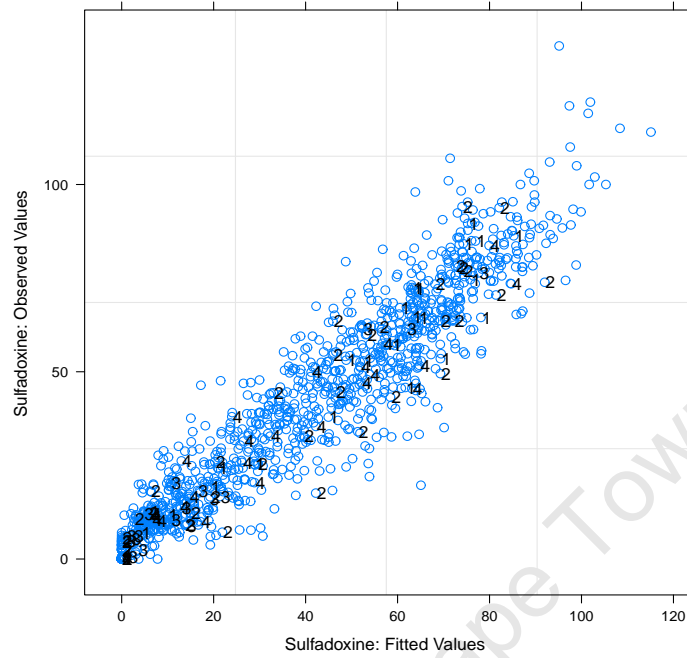


Figure 3.83: Fitted Values vs. Observed: Simultaneous NLME Model 5

Despite the slight heteroskedasticity still observed, (Figure 3.82), no variance models could be fitted. The diagnostic plots for this model (Appendix E, figures E.9 to E.14) indicate that the assumptions of normality are not violated for either the residuals or random effects, although the QQplots of the random effects do indicate slightly skewed distributions, led mainly by outliers, (Appendix E, figures E.11 and E.12).

The perfect correlation previously observed for some of the random effects is no longer apparent, (Appendix E, figures E.13 and E.14), and the model appears to give a reasonably good fit for both the Sulfadoxine and Pyrimethamine responses, (Figure 3.83).

Although the values of the variances for the random effects  $b_{2i}$  and  $b_{7i}$  are relatively small, models without these random effects could not be obtained.

This then is the “best” model that could be obtained for this specification, determined by an hypothesis-based model building procedure. Table 3.37 summarizes the fixed effects for each response and for the various categories of pregnancy and site, after taking the effect modification parameters and interaction terms into account.

Pyrimethamine								
Parameter	Postpartum				Pregnant			
	Mali	Mozambique	Sudan	Zambia	Mali	Mozambique	Sudan	Zambia
$\hat{\beta}_1$	41.77	41.77	41.77	41.77	41.77	41.77	41.77	41.77
$\hat{\beta}_2$	172.62	407.27	292.14	100.91	216.36	451.01	335.88	144.65
$\hat{\beta}_3$	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
$\hat{\beta}_4$	130.86	130.86	130.86	130.86	130.86	130.86	130.86	130.86
$\hat{\beta}_5$	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54

Sulfadoxine								
Parameter	Postpartum				Pregnant			
	Mali	Mozambique	Sudan	Zambia	Mali	Mozambique	Sudan	Zambia
$\hat{\beta}_1$	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03
$\hat{\beta}_2$	84.00	95.45	91.94	62.57	85.51	96.97	93.45	64.09
$\hat{\beta}_3$	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
$\hat{\beta}_4$	-7.93	-7.93	-7.93	-7.93	-7.93	-7.93	-7.93	-7.93
$\hat{\beta}_5$	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54

Table 3.37: Fixed Effects by Response Type, Pregnancy Phase and Site: Simultaneous NLME Model 5

This table, together with Figure 3.84, which shows the mean predicted concentration-time curves by response type, pregnancy phase and site (for Mali and Zambia only), illustrate the results from Model 5 more clearly.

Once again, the highest and lowest concentrations occur for subjects in Mozambique and Zambia respectively, irrespective of pregnancy phase, for both responses.

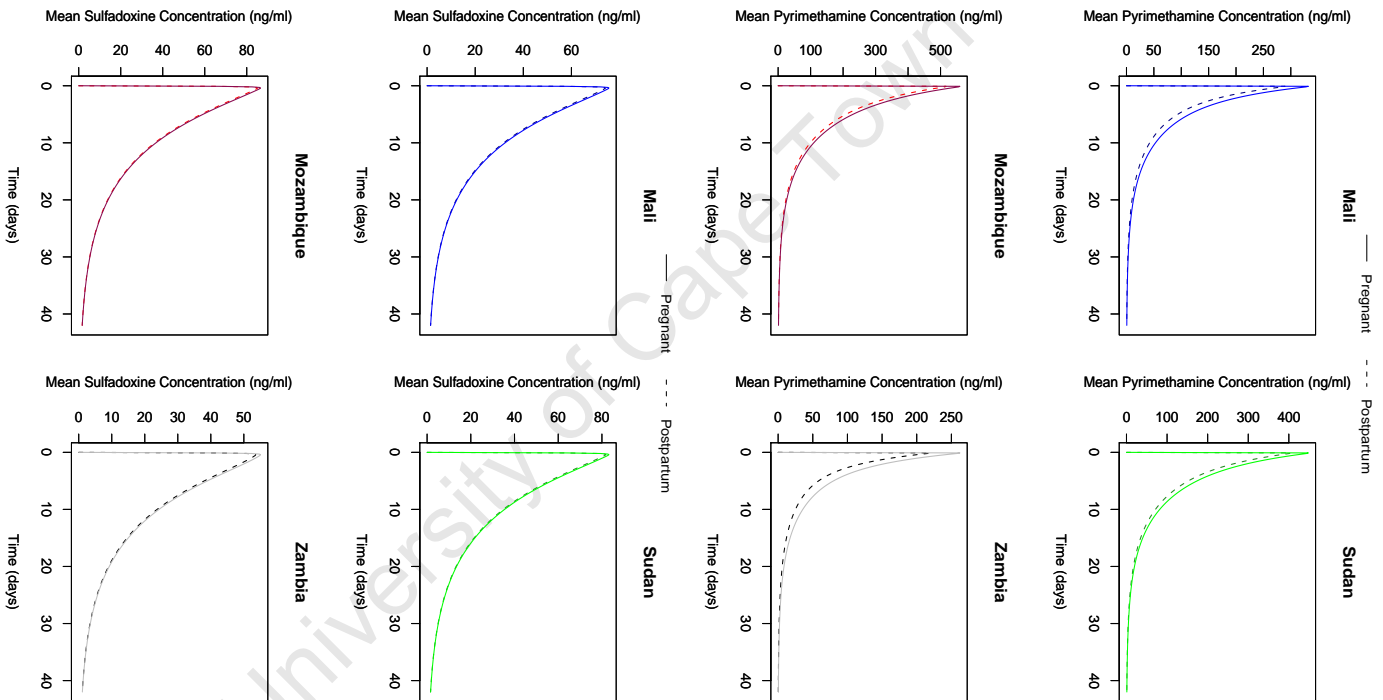


Figure 3.84: Mean Predicted Pyrimethamine and Sulfadoxine Concentration-time Curves by Response type, Pregnancy Phase and Site (Mali and Zambia): Simultaneous NLME Model 5

Pregnant subjects have higher concentrations in all sites, for both response types, although the difference in concentrations between postpartum and pregnant subjects is not as pronounced for Sulfadoxine. Although this model does not quite capture all the effects previously seen, the conclusions reached are similar to those found with the separate models.

It is worth noting that the greatest impact of the indicator variable are on parameters  $\beta_2$  and  $\beta_4$ , which indicate the overall range of concentrations reached, and would thus necessarily differ greatly for the two response types because of the different units used for the concentration measurements.

Pyrimethamine appears to have a much faster absorption rate, (shown by the larger  $\beta_1$  parameter value, which corresponds to the slope of the absorption phase), and a slightly faster initial rate of decline, (given by  $\beta_3$ ). The terminal rate of decline is unchanged.

The main benefit of this model lies in the comparison of the different structural forms for the different response types. Were the calculation of the degrees of freedom for the NLME models correct, the interpretation of the resultant p-values for the conditional t-tests for these parameters would provide a statistically appropriate test of the “correct” model form. Approximate p-values may, however, be obtained using Wald tests, since the diagnostic plots for this model (Appendix E, figures E.8) indicate that the assumptions of normality are not violated.

Table 3.38 summarizes the results for the fixed effects parameters as before, together with the values of the Wald test for each parameter, given by  $\hat{\beta}_i/s\hat{e}_{\beta_i}$  for  $i = 1, \dots, p$ , and the corresponding p-value.

Then testing the null hypothesis that the  $\beta_4$  parameter for Sulfadoxine, resulting from adding  $\hat{\beta}_9.(Sulfadoxine)$  to  $\hat{\beta}_4.(Baseline)$ , is actually zero, i.e. testing:

$$H_0 : \hat{\beta}_4.(Sulfadoxine) = 0$$

vs.

$$H_A : \hat{\beta}_4.(Sulfadoxine) \neq 0$$

using  $Var(A + B) = Var(A) + Var(B) + 2 \times Cov(A, B)$  for random variables  $A$  and  $B$ , the wald test would be given by:

$$\frac{-7.93111}{\sqrt{14.704917^2 + 15.794286^2 + 2 \times -0.915}}$$

and the corresponding p-value would be  $p = 0.713$ , indicating that the null hypothesis that the  $\beta_4$  parameter for Sulfadoxine is equal to zero may not be rejected.

Hence, we may conclude that the structural form for Sulfadoxine is that of

Parameter	Value	Std.Error	Z	P-value
$\hat{\beta}_1.(Baseline)$	41.78	16.36	2.55	0.011
$\hat{\beta}_6.(Sulfadoxine)$	-27.74	16.52	1.68	0.093
$\hat{\beta}_2.(Baseline)$	172.62	13.23	13.05	<.0001
$\hat{\beta}_7.(Sulfadoxine)$	-88.62	13.43	6.60	<.0001
$\hat{\beta}_{10}.(Pregnancy)$	43.74	4.90	8.93	<.0001
$\hat{\beta}_{11}.(Mozambique)$	234.65	12.33	19.03	<.0001
$\hat{\beta}_{12}.(Sudan)$	119.52	13.41	8.91	<.0001
$\hat{\beta}_{13}.(Zambia)$	-71.71	10.99	6.52	<.0001
$\hat{\beta}_{14}.(Sulfadoxine \times Pregnancy)$	-42.23	4.30	9.83	<.0001
$\hat{\beta}_{15}.(Sulfadoxine \times Mozambique)$	-223.19	11.46	19.47	<.0001
$\hat{\beta}_{16}.(Sulfadoxine \times Sudan)$	-111.58	12.39	9.01	<.0001
$\hat{\beta}_{17}.(Sulfadoxine \times Zambia)$	50.29	10.20	4.93	<.0001
$\hat{\beta}_3.(Baseline)$	0.14	0.005	27.43	<.0001
$\hat{\beta}_8.(Sulfadoxine)$	-0.05	0.0071	6.42	<.0001
$\hat{\beta}_4.(Baseline)$	130.86	14.70	8.90	<.0001
$\hat{\beta}_9.(Sulfadoxine)$	-138.79	15.79	8.79	<.0001
$\hat{\beta}_5$	0.54	0.04	13.27	<.0001

Table 3.38: Triple-Exponential Model Output (Wald Test): Simultaneous NLME Model 5

a bi-exponential model, since in the equation given by:

$$C(t) = \beta_2 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_3 \times time)] \\ + \beta_4 \times [-\exp(-\beta_1 \times time) + \exp(-\beta_5 \times time)]$$

the second bi-exponential function no longer applies.

This conclusion is further supported by an examination of Figure 3.85, which shows the combined curves (for the baseline study site and pregnancy status) and their constituent parts for Pyrimethamine and Sulfadoxine respectively.

In Figure 3.85, it is easily seen that the combined curve does not indicate a biphasic rate of decline, and that the constituent curve given by  $\beta_4 \times (-\exp(-\beta_1 \times time) + \exp(-\beta_5 \times time))$  serves merely to lower the Cmax value.

### 3.5.2 Model Specification 2: Different Functional Forms

The model specification in which different structural forms are accommodated is that directly adapted from the traditional simultaneous model in which the PK-PD relationship is investigated.

In this (adapted) model specification, as previously outlined, both a bi- and triple-exponential model are simultaneously fit to the full dataset, restructured so as to accommodate the multiple responses. The appropriate parameterization for the different responses is then indicated in the structural model, with the use of a binary indicator variable.

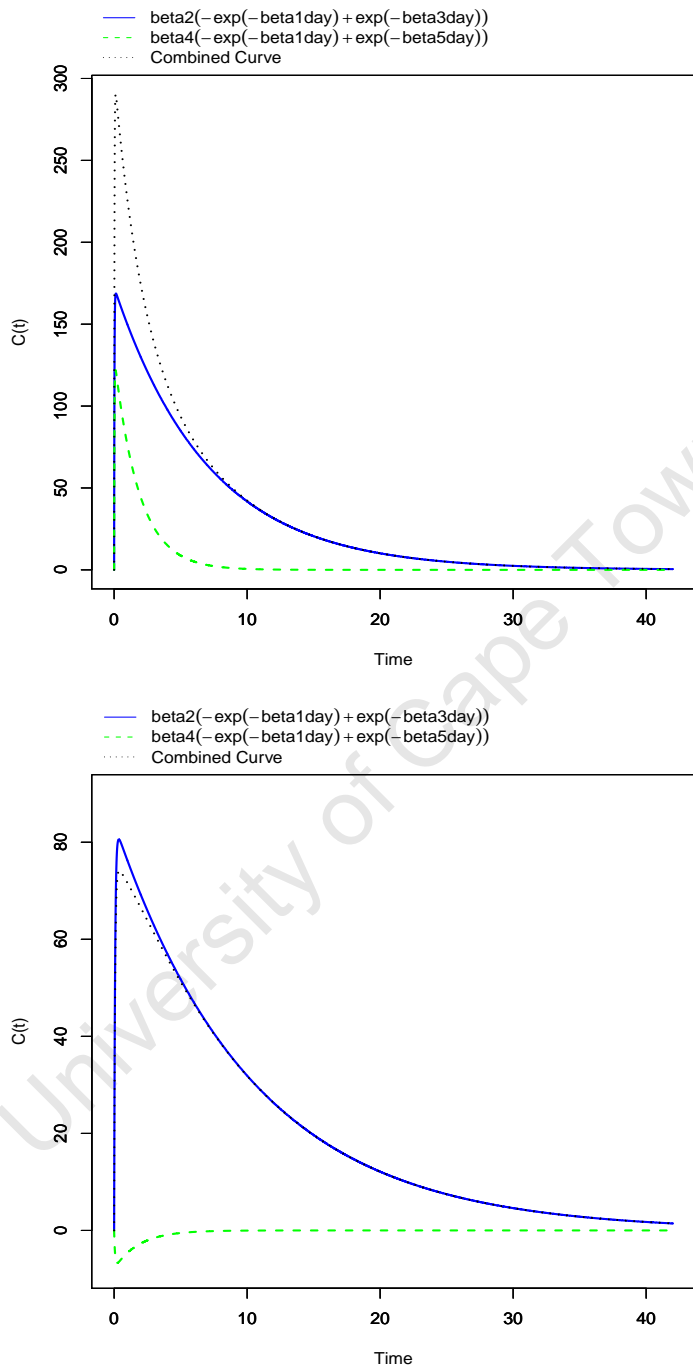


Figure 3.85: Mean Predicted Pyrimethamine and Sulfadoxine Concentration-time Curves by Response type, Pregnancy Phase and Site (Mali and Zambia): Simultaneous NLME Model 5

The model building procedure applied in all other instances in this thesis has not been used for the models presented here, owing to the computational intensity of such a procedure for a model of such complexity.

A single model is presented here, with the covariate and random effects structure most closely approximating those seen for the respective individual (separate) Sulfadoxine and Pyrimethamine models.

### Model 1

This model follows the basic structural form given by:

$$\begin{aligned}
 f(\mathbf{x}_{likj}, \boldsymbol{\beta}_{likj}) = & \underbrace{\left[ \beta_{0likj} (-e^{-\beta_{1likj} \times time_{likj}} + e^{-\beta_{2likj} \times time_{likj}}) \right]}_{\text{Bi-Exponential Model Form: Sulfadoxine}} \times \delta_{l\dots} \\
 & + \left[ \beta_{3likj} \times [-exp(-\beta_{5likj} \times time_{likj}) + exp(-\beta_{6likj} \times time_{likj})] \right. \\
 & \left. + \beta_{4likj} \times [-exp(-\beta_{5likj} \times time_{likj}) + exp(-\beta_{7likj} \times time_{likj})] \right] \\
 & \underbrace{\hspace{10em}}_{\text{Triple-Exponential Model Form: Pyrimethamine}} \\
 & \times (1 - \delta_{l\dots})
 \end{aligned}$$

where  $\delta_{l\dots}$  is a binary variable indicating the response type as Pyrimethamine ( $\delta_{l\dots} = 0$ ) or Sulfadoxine ( $\delta_{l\dots} = 1$ ).

The stage 2 equations, and hence the covariate model, are given by:

$$\begin{aligned}
 \beta_{0likj} &= \beta_0 + \beta_8 \times pregnancy_{lik.} + \beta_{11} \times mozambique_{li..} + \beta_{12} \times zambia_{li..} + b_{0i} + b_{0ik} \\
 \beta_{1likj} &= \beta_1 \\
 \beta_{2likj} &= \beta_2 + \beta_9 \times pregnancy_{lik.}
 \end{aligned}$$

$$\begin{aligned}
 \beta_{3likj} &= \beta_3 + b_{3i} + b_{3ik} \\
 \beta_{4likj} &= \beta_4 + \beta_{10} \times pregnancy_{lik.} \\
 & + \beta_{13} \times mozambique_{li..} + \beta_{14} \times sudan_{li..} + \beta_{15} \times zambia_{li..} + b_{4i} + b_{4ik} \\
 \beta_{5likj} &= \beta_5 \\
 \beta_{6likj} &= \beta_6 \\
 \beta_{7likj} &= \beta_7
 \end{aligned}$$

The results for the fixed effect parameters are summarized in Table 3.39.

Although the interaction effect previously seen between pregnancy and site for the Pyrimethamine model (Model 4) is not included here, together with the effect of Zambia on the  $\beta_2$  parameter in the previous Sulfadoxine model (Model

Parameter	Value	Std.Error
$\hat{\beta}_0.(Intercept)$	70.96	2.53
$\hat{\beta}_8.(Pregnancy)$	11.13	2.67
$\hat{\beta}_{11}.(Mozambique)$	10.35	3.09
$\hat{\beta}_{12}.(Zambia)$	-21.47	2.47
$\hat{\beta}_1$	14.96	1.98
$\hat{\beta}_2.(Intercept)$	0.06	0.005
$\hat{\beta}_9.(Pregnancy)$	0.03	0.006
$\hat{\beta}_3$	141.81	21.50
$\hat{\beta}_4.(Intercept)$	199.69	18.11
$\hat{\beta}_{10}.(Pregnancy)$	55.56	10.49
$\hat{\beta}_{13}.(Mozambique)$	228.78	22.10
$\hat{\beta}_{14}.(Sudan)$	94.06	23.92
$\hat{\beta}_{15}.(Zambia)$	-80.42	22.39
$\hat{\beta}_5$	25.85	2.19
$\hat{\beta}_6$	0.99	0.07
$\beta_7$	0.14	0.003

Table 3.39: Triple-Exponential Model Output: Simultaneous NLME Model 1 (Indicator Specification)

5), the values of the parameters that are included here are very similar to those obtained from the separate models (Tables 3.12 and 3.27), and the relationships indicated for pregnancy and site are of the same approximate magnitude and in the same directions.

The random effects for this model are restricted to subject and occasion-specific random effects on parameters  $\beta_2$ ,  $\beta_3$  and  $\beta_4$  only.

The variance-covariance matrices for these random effects are block-diagonal matrices, which do not yet take into account potential correlations between the random effects for the different model specifications.

The variance-covariance matrices for these random effects are given by:

$$\hat{\psi}_1 = \begin{bmatrix} [0.006] & \mathbf{0} \\ \mathbf{0} & \begin{bmatrix} 184.23 & 0 \\ 0.006 & 56.96 \end{bmatrix} \end{bmatrix}$$

$$\hat{\psi}_2 = \begin{bmatrix} [7.48] & \mathbf{0} \\ \mathbf{0} & \begin{bmatrix} 36.26 & 0 \\ 0.006 & 54.42 \end{bmatrix} \end{bmatrix}$$

This is an obvious limitation to the use and interpretation of the model, since for this specification, the joint distribution of the random effects and the accommodation of these potential correlations is the driver behind accounting for the possible interdependence and relationship between the responses.



Since the complexity of the indicator model specification forces the most simple approach, in this case, there is no obvious benefit to be had from using this version of the simultaneous model. Additionally, the “nested” structure of the two constituent structural model forms (the bi- and triple-exponential specifications) lends itself to the covariate version of the simultaneous model, which allows us to formally test which structural form is most appropriate for each response.

We were also unable to fit a variance function to this model, although the initial function specified was structured so as to allow for different  $\theta$  values for the different response types.

The diagonal elements of the matrix  $\mathbf{R}_{ik(n_{ik} \times n_{ik})}$  were thus given by  $\hat{\sigma}^2 = (19.76)^2$ .

Examination of the diagnostic plots for this model indicate a relatively good fit for Pyrimethamine, (Figures 3.86 and 3.87), with less heteroskedasticity observed for the residual variance, (Figure 3.86).

Figures 3.88 and 3.89 show the plot of the standardized residuals versus the fitted values, and the plot of the fitted values versus observed for Sulfadoxine respectively, which does not appear to have as accurate a fit.

The remaining diagnostic plots (Appendix F, figures F.1 to F.6) do not indicate any further departures from the assumptions of normality, although potential correlations are indicated between the subject-specific random effects for  $\beta_3$  and  $\beta_4$ , and between the occasion-specific random effects for  $\beta_0$  and  $\beta_4$ .

### 3.5.3 Discussion and Conclusions

The original undertaking theorized for this master’s thesis was to explore and in effect “master” the basic theoretical and computational constructs involved in the use of nonlinear mixed effect models.

The use of concentration-time data for the antimalarial compounds Sulfadoxine and Pyrimethamine further allowed for the extension of these NLME models to a “multivariate framework”.

Owing to the complexity of the available data, in particular, the self-matched study design, these single level NLME models (multivariate or otherwise) were no longer adequate, and hence, multilevel NLME models were required.

Ordinarily, the modeling of concentration-time data is achieved through the use of mechanistic pharmacokinetic models. In this thesis however, a more empirical approach was taken, and the structural model forms were specified as poly-exponential expressions, with the parameters having little direct mechanistic interpretation.

The use of the exponential parameterization was initially decided on as a means

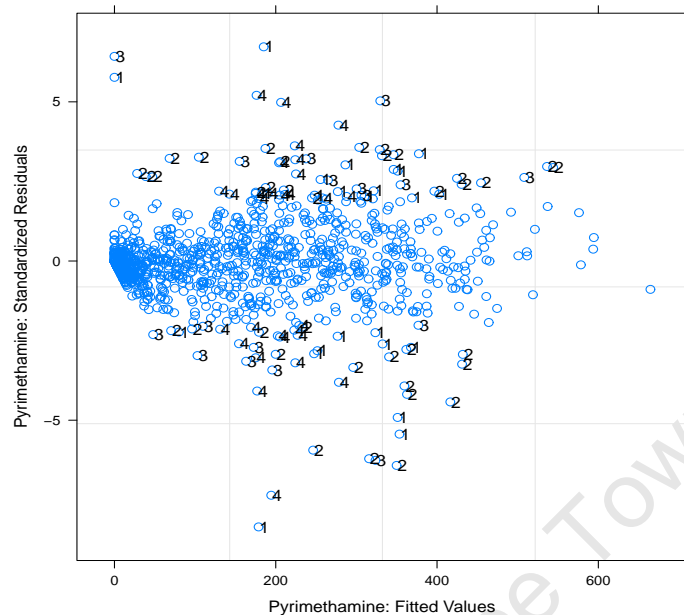


Figure 3.86: Standardized Residuals vs. Fitted Values: Simultaneous NLME Model 1 (Indicator)

of smoothing and interpolating the observed concentration-time curves, whilst simultaneously distancing the development of the methodology from a PK analysis.

The PK parameters obtained from the back transformation of these exponential parameters appeared to be consistent with those found using the mechanistic NLME models, and indeed with those found using the more traditional two-stage approach to the analysis of PK data, and despite the various complications arising from the use of the exponential specification (such as the loss of clinical information regarding the placement of covariates, and the issue of starting values), this parameterization allowed for the break down of the curves into their constituent components.

This thesis demonstrates that, in general, the use of NLME techniques provides a flexible and powerful tool for the analysis of longitudinal data, and in this particular case, for the estimation of separate, sequential and simultaneous models of drug-concentrations over time. The resulting models have enabled us to draw conclusions regarding the impact of covariates on the concentration-time profiles of the individual compounds, as well as examine the structural forms most appropriate to the different response types.

The sequential and simultaneous approaches used here are based on methods applicable in the traditional PK-PD context, and although clinically, the motivation behind the modeling of the drug-drug relationship was to develop a

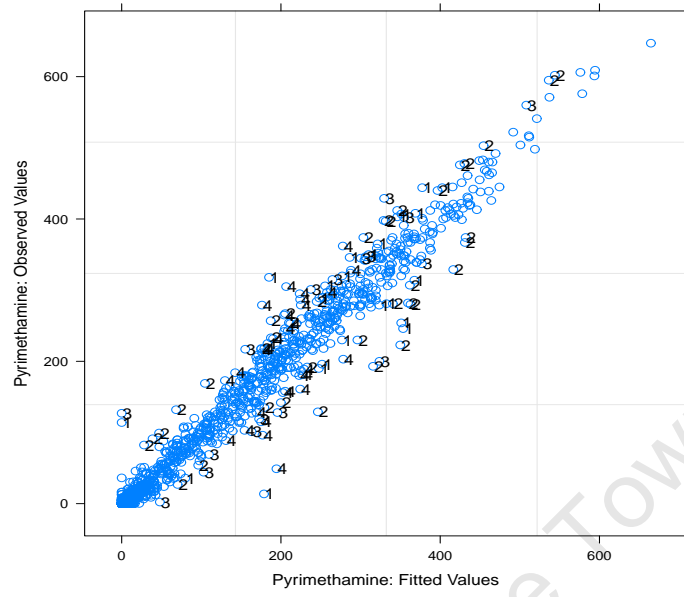


Figure 3.87: Fitted Values vs. Observed: Simultaneous NLME Model 1 (Indicator)

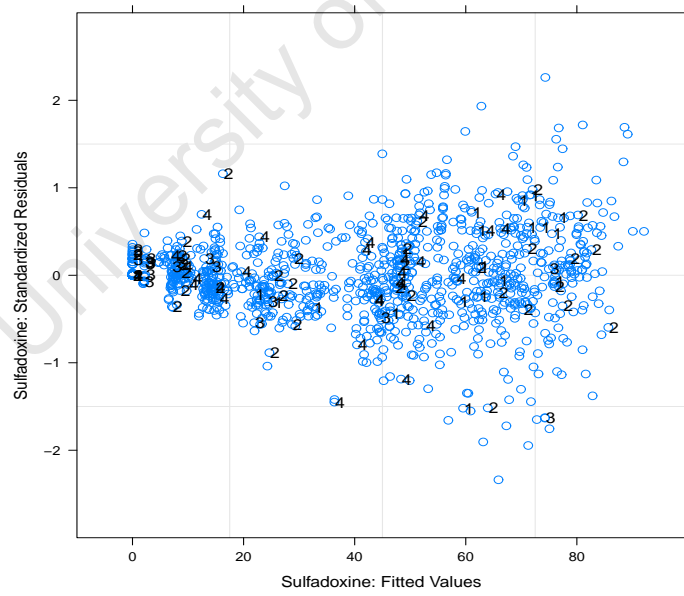


Figure 3.88: Standardized Residuals vs. Fitted Values: Simultaneous NLME Model 1 (Indicator)

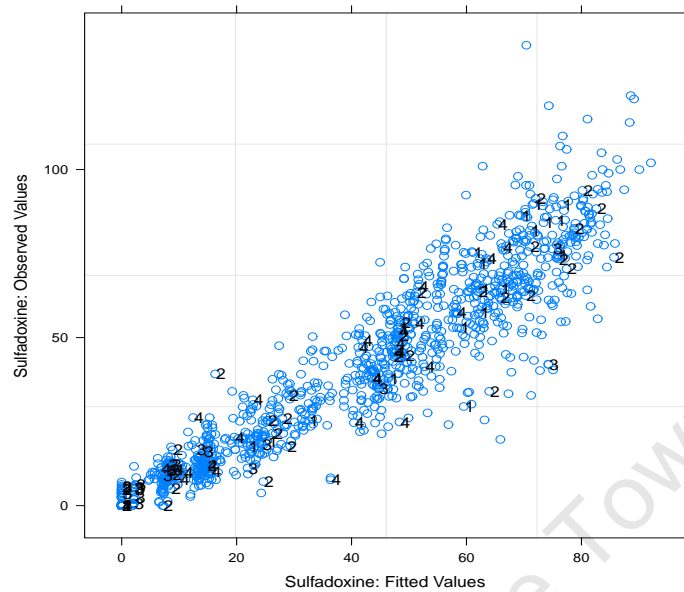


Figure 3.89: Fitted Values vs. Observed: Simultaneous NLME Model 1 (Indicator)

further understanding of the interaction between the two compounds and hence to understand the underlying synergistic mechanism of action on the malaria parasite, the models developed here do not appear to answer that question. Further work, undertaken for subjects in Mozambique for whom we have PD data, (not used in this analysis), might see the development of a PK-PD model in which the PD model accounts for the two predicted drug concentrations simultaneously.

From a mathematical perspective, the sequential and simultaneous models presented in this thesis provide an interesting approach to the determination of the correct underlying structural form (despite the use of approximate significance calculations necessitated by the incorrect calculation of degrees of freedom).

The flexibility of the NLME model is impressive: results from the multilevel specification may be effectively replicated using a single level model with a variance-covariance structure for the random effects that mimics the correlation induced by the nesting structure of the data, and the random effects may be specified in multiple ways (as demonstrated in the covariate specification of the simultaneous model). The use of explicit variance models appears to effectively handle the heterogeneous variance so often observed in models for data of this type, although the correct specification of this variance structure is not always straightforward.

The specification of accurate starting estimates appeared to play a significant role in the convergence of the models, and in the development of robust, stable

models. The multilevel nesting structure for the random effects exacerbated this problem, and in some instances (such as with the separate Pyrimethamine models), a simpler single-level model (which ignored the data structure) was required in order to obtain useable starting values. Curve-stripping procedures helped somewhat for the bi-exponential model specification, but were not applied for the more complicated triple-exponential model form. The exponential specification also contributed to the difficulty in the determination of robust model estimates, since with this specification, different parameter estimates often result in almost identical predicted curves.

The calculation of the degrees of freedom was a major obstacle, which has yet to be overcome, and will hopefully form part of further work on these models. There does not appear to be any common consensus on the correct approximation to use, which will only be further aggravated by the nested random effects structure. For the models presented here, with the exception of the simultaneous model in which the Wald test was used, the degrees of freedom, and hence the associated p-value and significance of the model parameters could not be determined, which made it difficult to interpret the impact of covariates, and also made model building more complicated.

As previously mentioned, the exponential parameterization further complicated the covariate model building procedure, in that clinical information on the placement of covariates was not as clearly applicable. Convergence and stability issues also resulted in fewer covariates being considered for inclusion, despite indications that covariates not included, such as anaemia, might still play a role in determining the relationship between the two drug concentrations and the impact of pregnancy induced changes. It should be noted that there are alternative methods available for the estimation of parameters of non-linear mixed effect models, which may not have the same convergence issues of the 'nlme' package in R. Markov Chain Monte Carlo (MCMC) methods are one such example, and are available in both WinBugs and Monolix.

The predicted population, individual- and occasion-specific curves generated by the models did, however, appear to capture the observed concentration-time relationships, and the determined site and pregnancy effects were consistent with previous results. The results obtained through the use of the delta method in the calculation of the standard errors for back transformed PK parameters appeared adequate, and the comparison of NLME results with those obtained from the two-stage approach used by Nyunt et al. [5] showed the reduction in the standard errors for the NLME model.

Results from the separate Sulfadoxine and Pyrimethamine models indicated that both pregnancy and site played a role in the determination of the concentration-time profiles for the different compounds. The overall range of concentrations was higher in pregnant subjects for both Sulfadoxine and Pyrimethamine, but the additional impact of pregnancy on the elimination rate and hence the clearance of Sulfadoxine resulted in a reduction in the overall drug exposure, as measured by the AUC, where the opposite was seen for Pyrimethamine. Study sites varied widely, and for Pyrimethamine, the pregnancy effect was dependent on the study site of the individual. The dosing implications of these results are

not clear, particularly as these models were developed from a purely empirical perspective.

The use of NLME modeling techniques is evidently of enormous potential benefit. This thesis manages an introduction to the large scope of possible uses and applications of these techniques, and provides a glimpse into the power, flexibility and elegance of NLME models, whilst demonstrating the computational and theoretical complexity involved in their development.

University of Cape Town

## Appendix A

# Data Exploration

---

Example Code for Pairs plot (Figure 3.5 in text) (58):

---

```
panel.cor <- function(x, y, digits=2, prefix=""){
  usr <- par("usr"); on.exit(par(usr))
  par(usr = c(0, 1, 0, 1))
  r <- abs(cor(x, y))
  txt <- format(c(r, 0.123456789), digits=digits)[1]
  txt <- paste(prefix, txt, sep="")
  #if(missing(cex.cor)) cex.cor <- 0.8/strwidth(txt)
  #text(0.5, 0.5, txt, cex = cex.cor * r)
text(0.5, 0.5, txt, col=ifelse(as.numeric(txt)>0.3,"brown","blue"))
}

panel.hist <- function(x, ...){
  usr <- par("usr"); on.exit(par(usr))
  par(usr = c(usr[1:2], 0, 1.5) )
  h <- hist(x, plot = FALSE)
  breaks <- h$breaks; nB <- length(breaks)
  y <- h$counts; y <- y/max(y)
  rect(breaks[-nB], 0, breaks[-1], y, col="cyan", ...)
}

pairs(iptsub[,-match(c("preg","trimester","site","anaem10"),
names(iptsub))],lower.panel=panel.smooth,diag.panel=panel.hist,
upper.panel=panel.cor,cex.labels=1)
```

---

Example Code for Figures A.1 to A.4 and Figures 3.1 to 3.4 in text: (Identification of Biologically Implausible Concentrations)

---

```
xyplot(sulfadoxine ~ day, initialsulf, groups = pid, type = 'b',
```

```

subset = (site=="Mali" & preg==1),
,sub="Pregnant Subjects: Mali",
xlab="Time (days)",ylab="Sulfadoxine Concentration (ug/ml)",
  panel = function( x, y, subscripts, ...) {
  panel.superpose( x,y,subscripts,...)
  panel.identify( x,y, subscripts = subscripts,
  labels = initialsulf$pid[subscripts],...)
  panel.identify( x,y, subscripts = subscripts,
  labels=initialsulf$day[subscripts],offset=1.25, ...)
})

```

-----

Identification of Biologically Implausible Concentrations, Mali and Zambia:

-----

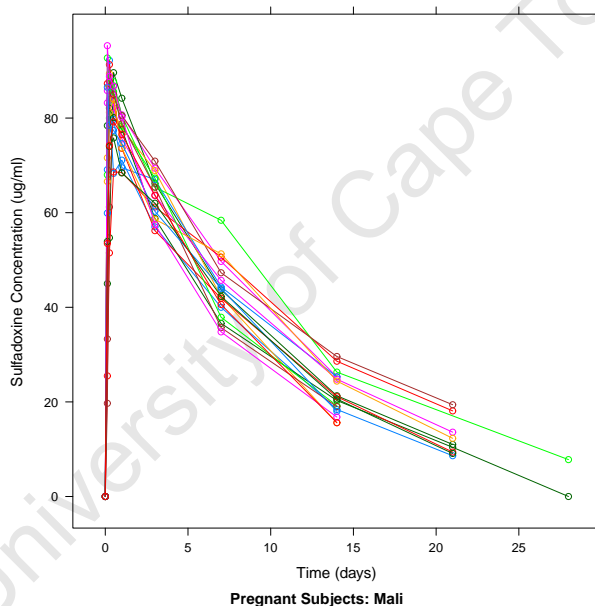


Figure A.1: Identification of Biologically Implausible Sulfadoxine Concentrations: Mali



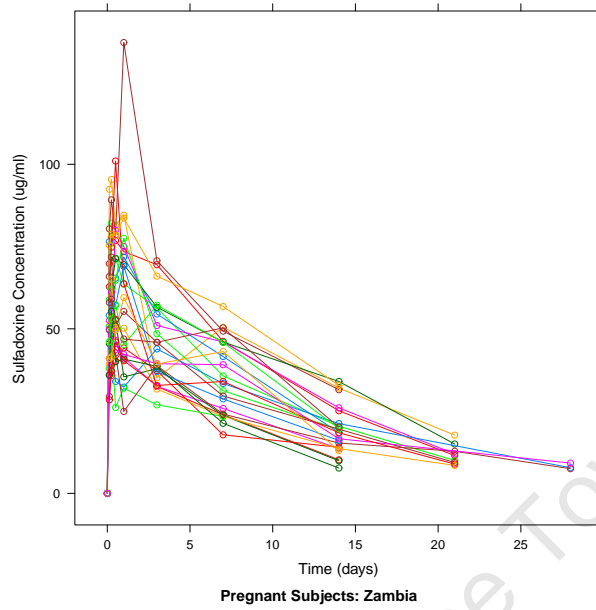


Figure A.2: Identification of Biologically Implausible Sulfadoxine Concentrations: Zambia

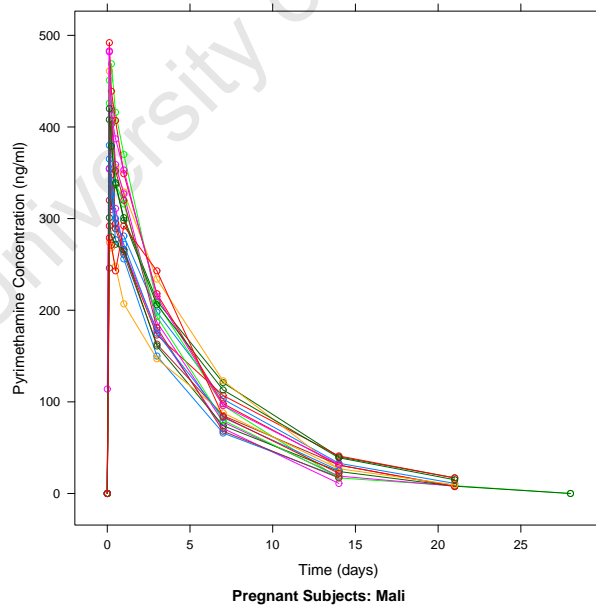


Figure A.3: Identification of Biologically Implausible Pyrimethamine Concentrations: Mali

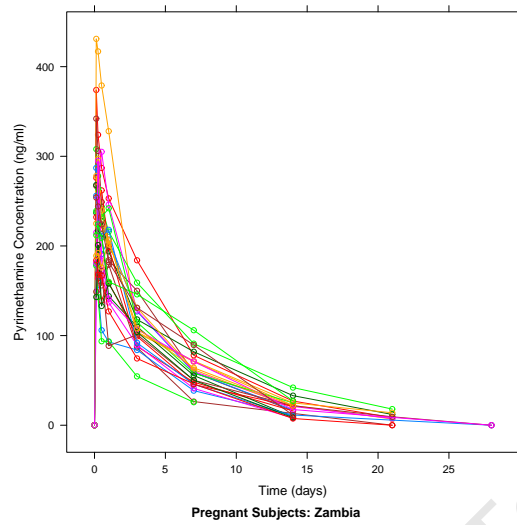


Figure A.4: Identification of Biologically Implausible Pyrimethamine Concentrations: Zambia

-----  
 Further Data Exploration: Boxplots of Continuous Covariates by Pregnancy Status and Site  
 -----

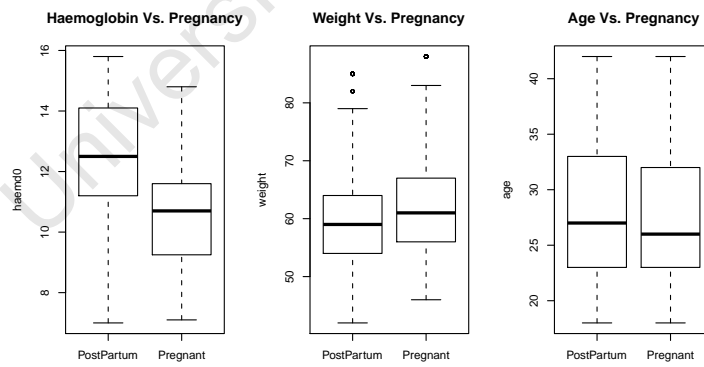


Figure A.5: Boxplots of Continuous Covariates vs. Pregnancy

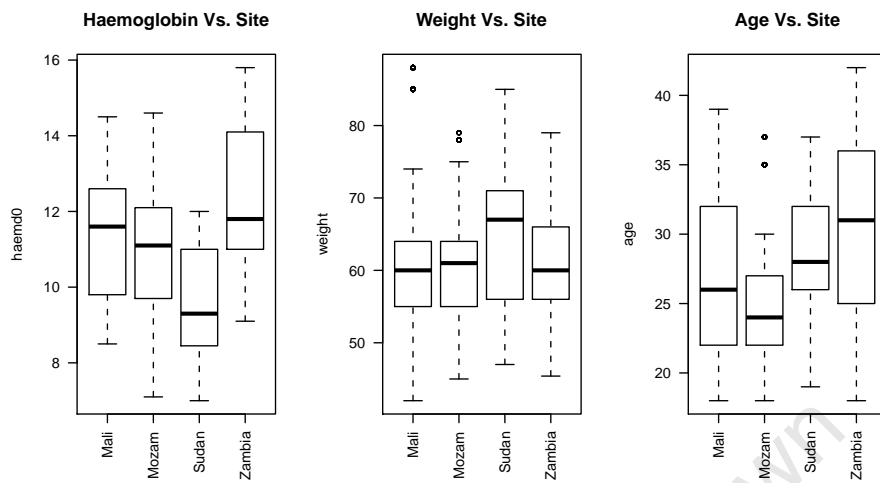


Figure A.6: Boxplots of Continuous Covariates vs. Site

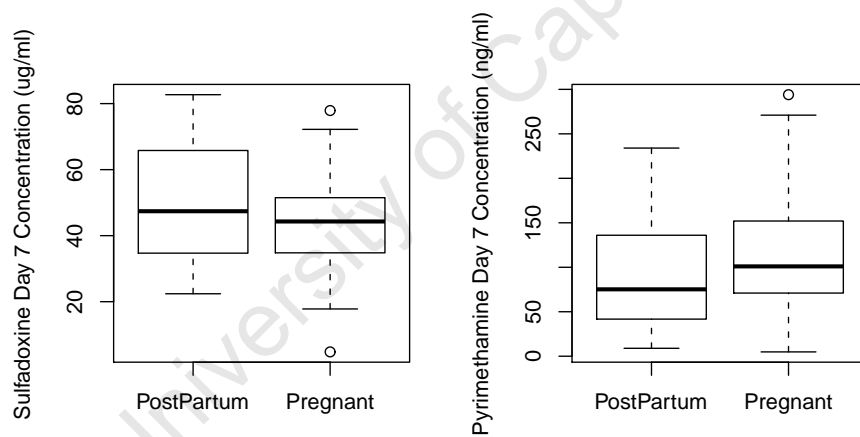


Figure A.7: Boxplots of Day 7 Concentrations by Pregnancy Phase

---

Data Exploration for Subset of Data

---

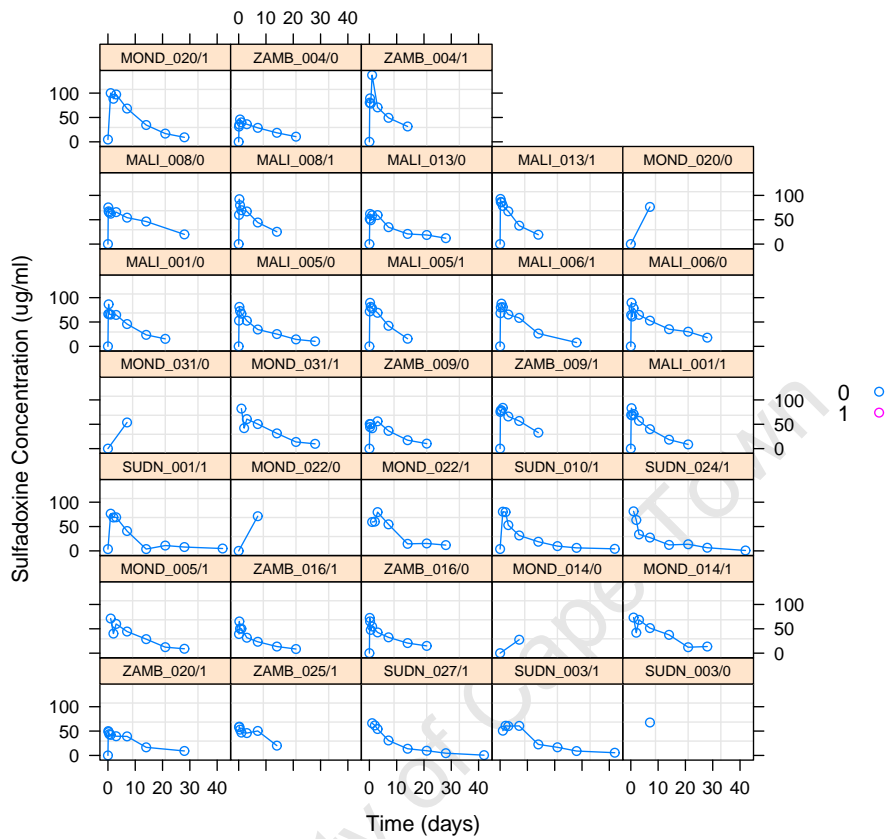


Figure A.8: Sulfadoxine Concentration vs. Time by Grouping (Multiple Levels)

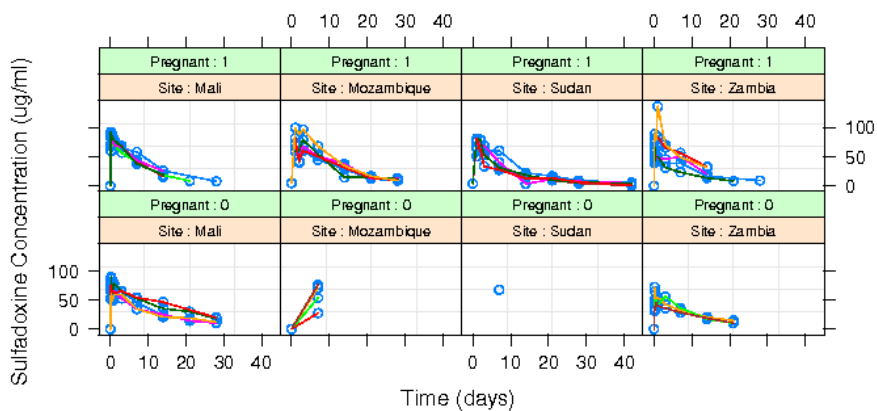


Figure A.9: Sulfadoxine Concentration vs. Time by Site and Pregnancy Status

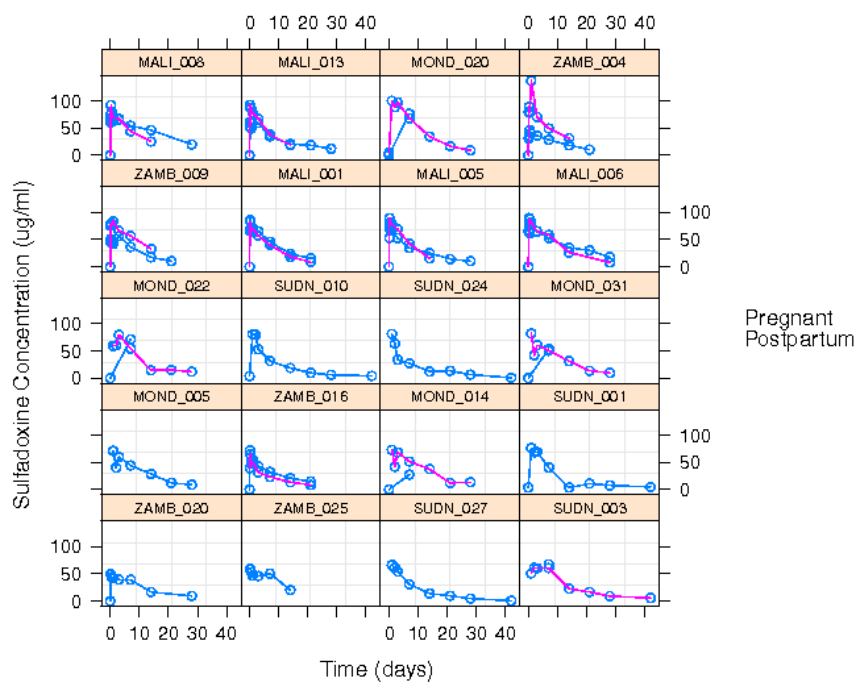


Figure A.10: Sulfadoxine Concentration vs. Time by Grouping (Subject Level)

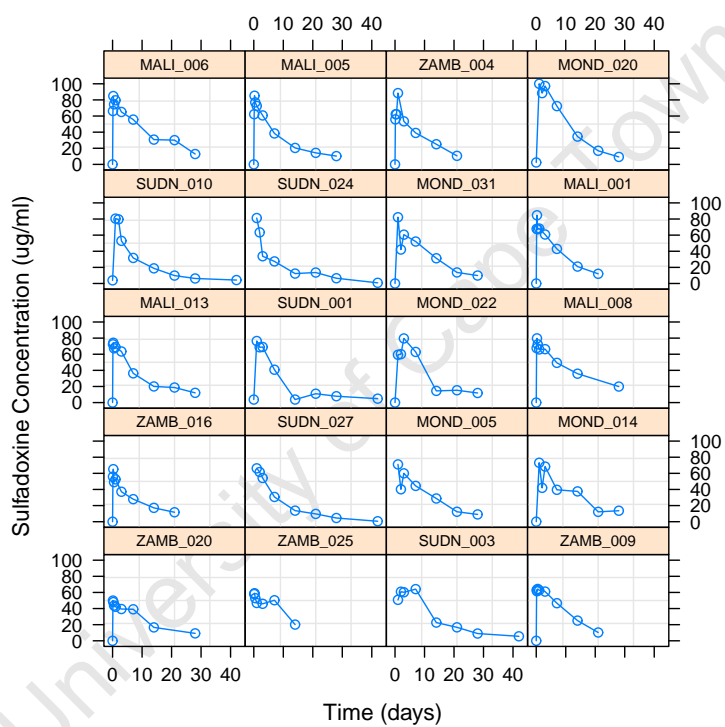


Figure A.11: Sulfadoxine Concentration vs. Time (Collapsed)

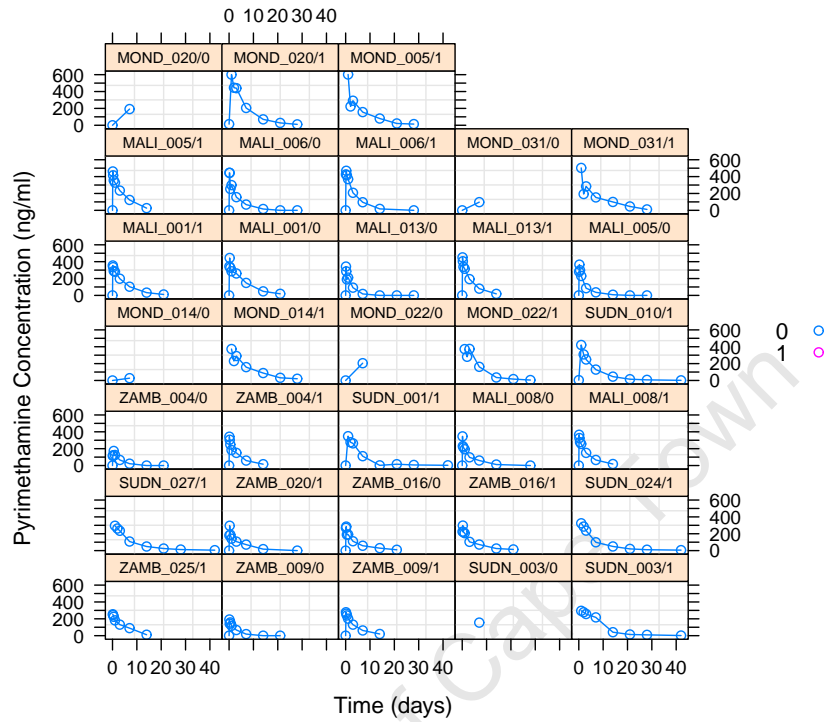


Figure A.12: Pyrimethamine Concentration vs. Time by Grouping (Multiple Levels)

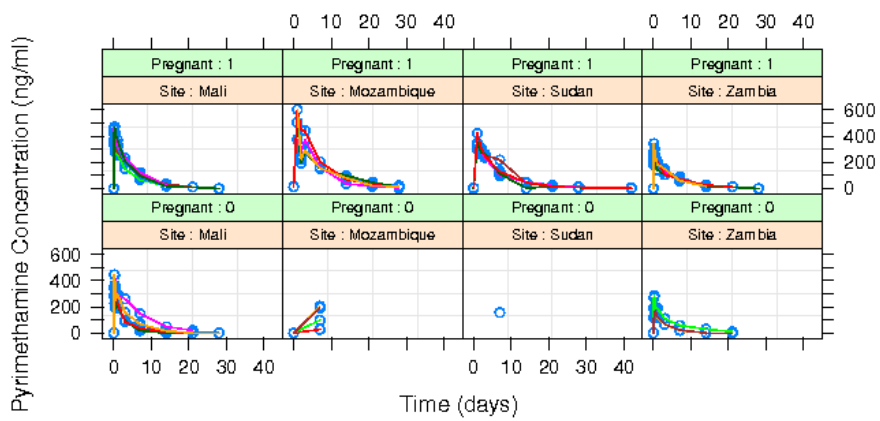


Figure A.13: Pyrimethamine Concentration vs. Time by Site and Pregnancy Status

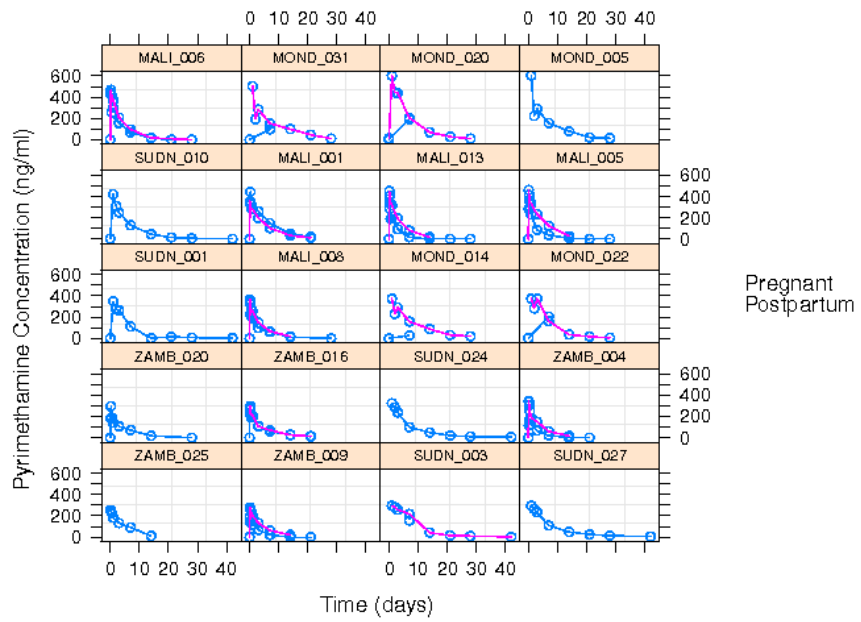


Figure A.14: Pyrimethamine Concentration vs. Time by Grouping (Subject Level)



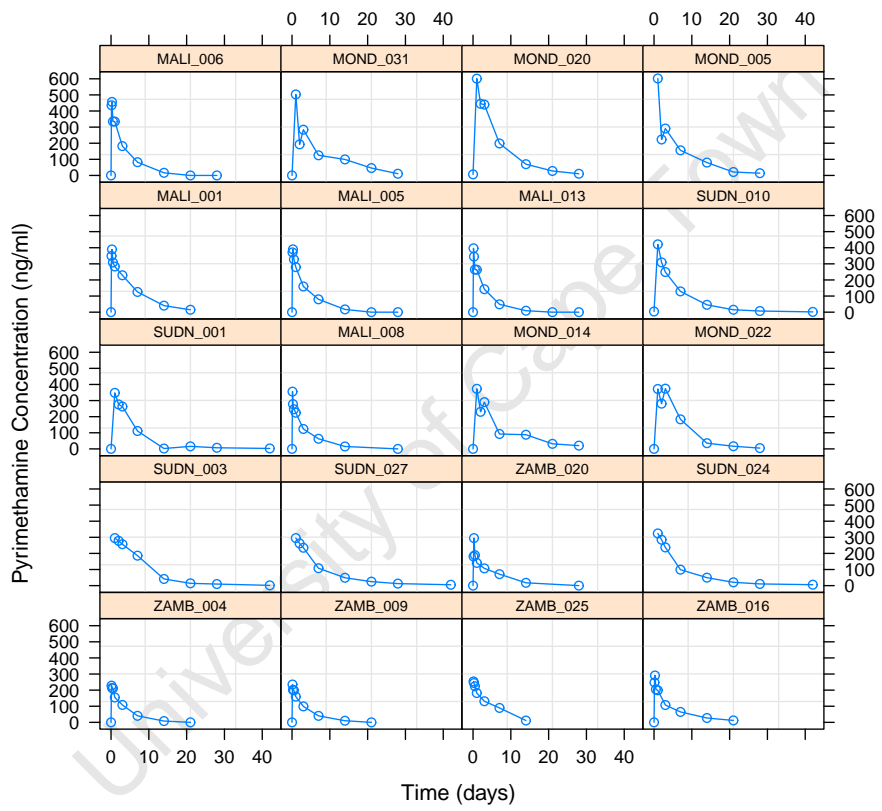


Figure A.15: Pyrimethamine Concentration vs. Time (Collapsed)

## Appendix B

# Sulfadoxine Models

-----  
Example Code for NLS Models:

```
-----  
sulf1comp_preg.nls=nls(sulfadoxine~fm1comp(beta0,beta1,beta2,day),  
  data=sulf,  
  start=c(beta0=91.2964786,beta1=6.5815878,  
  beta2=0.1476355),  
  control=controlS,,subset=preg==1)  
summary(sulf1comp_preg.nls)  
-----
```

Model Output: Sulfadoxine NLS Models (Pregnant and Postpartum Subsets)

	Estimate	Std. Error	t value	Pr(> t )
beta0	78.39	1.19	65.63	0.00
beta1	10.36	0.86	12.11	0.00
beta2	0.08	0.00	25.01	0.00

Table B.1: Model Output for Sulfadoxine Bi-Exponential NLS model: Pregnant Subset

	Estimate	Std. Error	t value	Pr(> t )
beta0	61.47	1.39	44.38	0.00
beta1	12.26	1.48	8.28	0.00
beta2	0.05	0.00	14.94	0.00

Table B.2: Model Output for Sulfadoxine Bi-Exponential NLS model: PostPartum Subset

-----  
Example Code for NLSLIST Models (Individual-Specific):  
-----

```
#NLSLIST MODELS: BI-EXPONENTIAL MODELS
#FULL DATASET
sulf1comp.lis=nlsList(sulfadoxine~fm1comp(beta0,beta1,beta2,day),
                    data=sulf,
                    start=c(beta0=86.7155320,beta1=5.9219280,
                             beta2=0.1476355),
                    control=controlS,,subset=!(site=="Mozambique")
                    &!(site=="Sudan"))

#INTERVALS PLOT: FIGURE 3.11 IN TEXT
plot(intervals(sulf1comp.lis),layout=c(3,1,1),devAskNewPage(ask=T))

#BETA ESTIMATES AVERAGED OVER INDIVIDUALS
summary(sulf1comp.lis)
coef_sulf1comp.lis=as.data.frame(coefficients(sulf1comp.lis))

avecoef=c(mean(as.numeric(coef_sulf1comp.lis[1:79,1]),na.rm=T),
           mean(as.numeric(coef_sulf1comp.lis[1:79,2]),na.rm=T),
           mean(as.numeric(coef_sulf1comp.lis[1:79,3]),na.rm=T))

#EXAMINING OUTLIERS
sulflis=coef(sulf1comp.lis)

subset(sulflis,sulflis$beta1==max(sulflis$beta1,na.rm=T))#ZAMB_009/0
subset(sulflis,sulflis$beta2==max(sulflis$beta2,na.rm=T))#ZAMB_008/1

sulf1comp2.lis=nlsList(sulfadoxine~fm1comp(beta0,beta1,beta2,day),
                    data=sulf,
                    start=c(beta0=86.7155320,beta1=5.9219280,
                             beta2=0.1476355),
                    control=controlS,,subset=!(site=="Mozambique")
                    &!(site=="Sudan")
                    &!(pid=="ZAMB_009" & preg==0)
                    &!(pid=="ZAMB_008"&preg==1))

#INTERVALS PLOT: FIGURE 3.12 IN TEXT
plot(intervals(sulf1comp2.lis),layout=c(3,1,1),devAskNewPage(ask=T))
```

-----  
Diagnostic plots: Sulfadoxine NLME Model 1  
-----

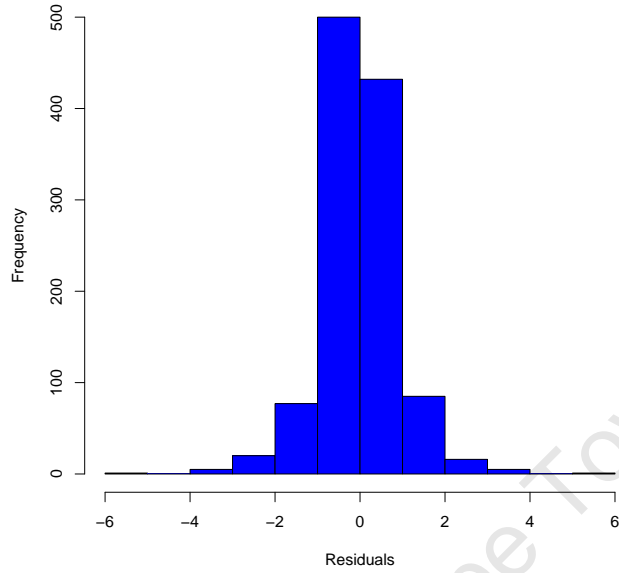


Figure B.1: Histogram of Residuals: Sulfadoxine NLME Model 1

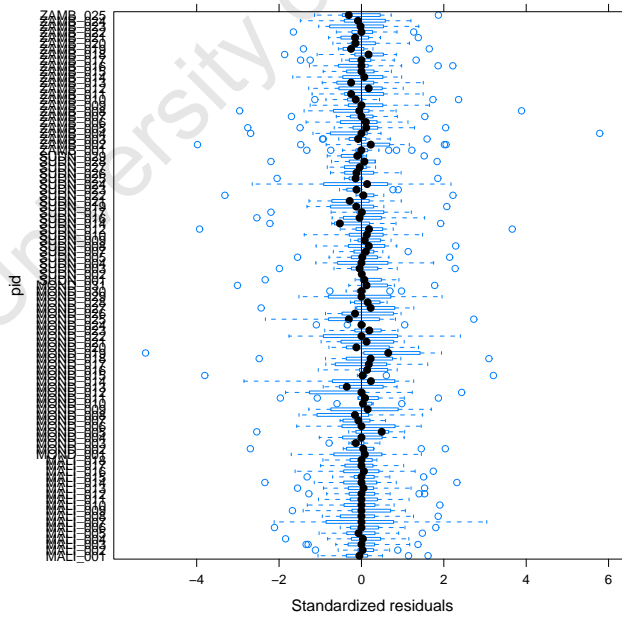


Figure B.2: Residuals by Subject ID: Sulfadoxine NLME Model 1

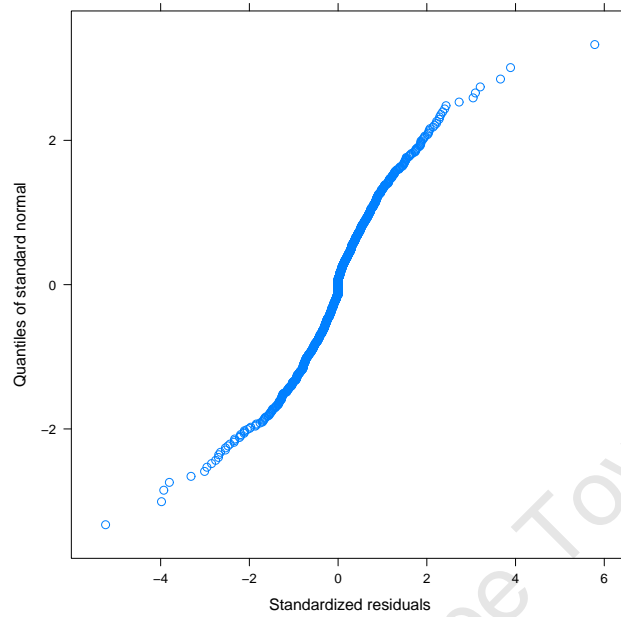


Figure B.3: QQplot of Residuals: Sulfadoxine NLME Model 1

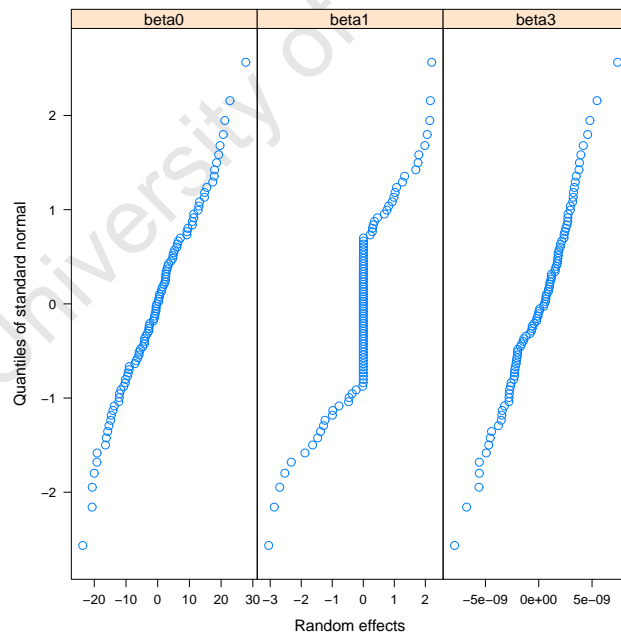


Figure B.4: QQplot of Random Effects (Subject Level): Sulfadoxine NLME Model 1

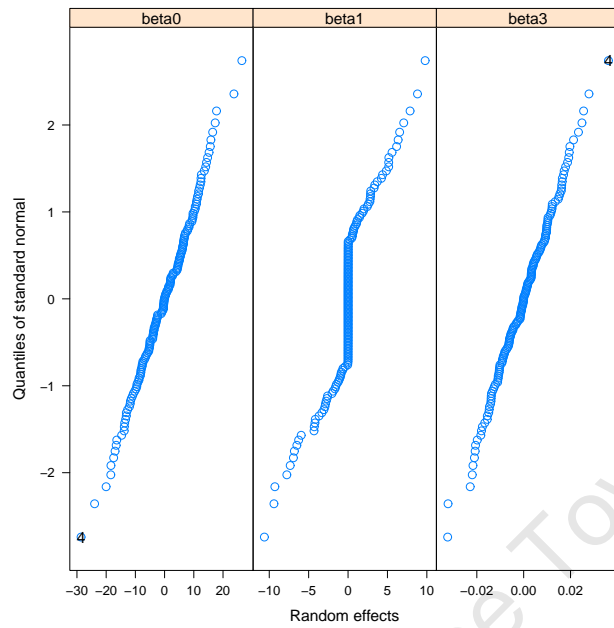


Figure B.5: QQplot of Random Effects (Phase-within-Subject Level) : Sulfa-doxine NLME Model 1

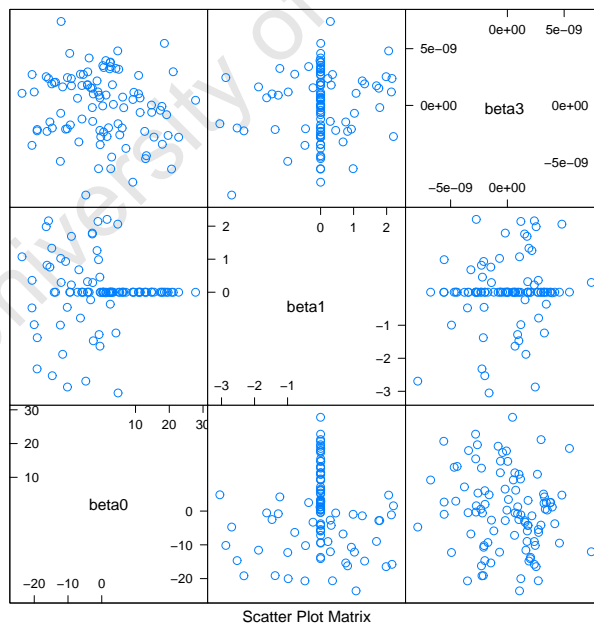


Figure B.6: Pairs Plot of Random Effects (Phase-within-Subject Level) : Sulfa-doxine NLME Model 1

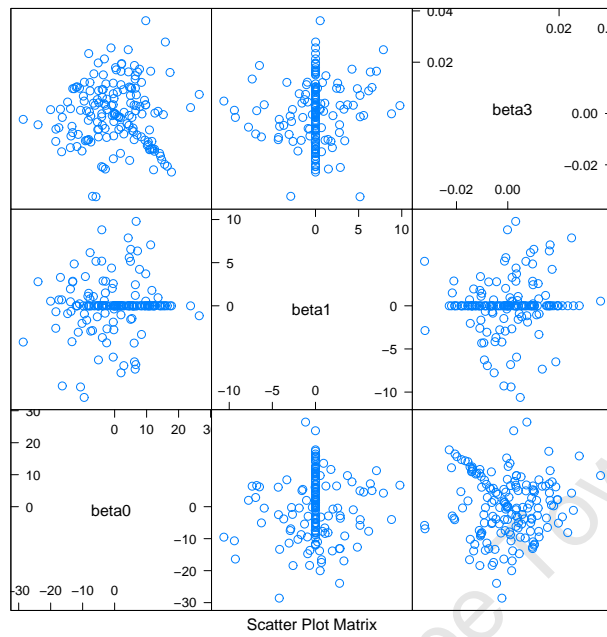


Figure B.7: Pairs Plot of Random Effects (Phase-within-Subject Level) : Sulfadoxine NLME Model 1

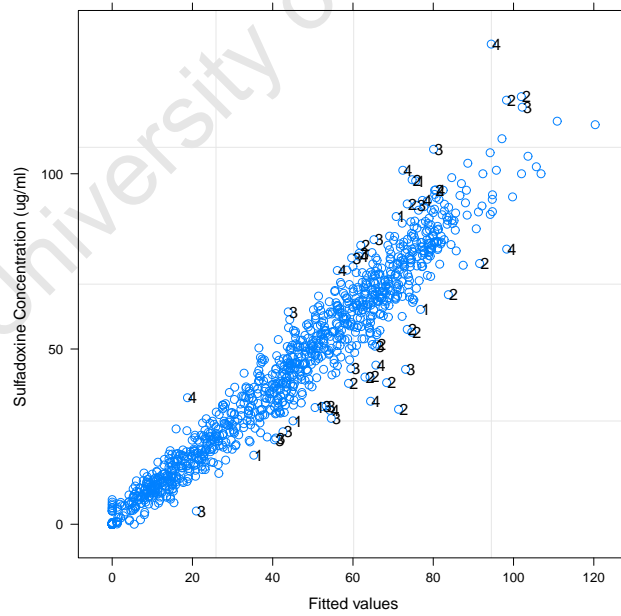


Figure B.8: Fitted Values vs. Observed: Sulfadoxine NLME Model 1

---

Random Effects versus Covariates: Sulfadoxine NLME Model 1

---

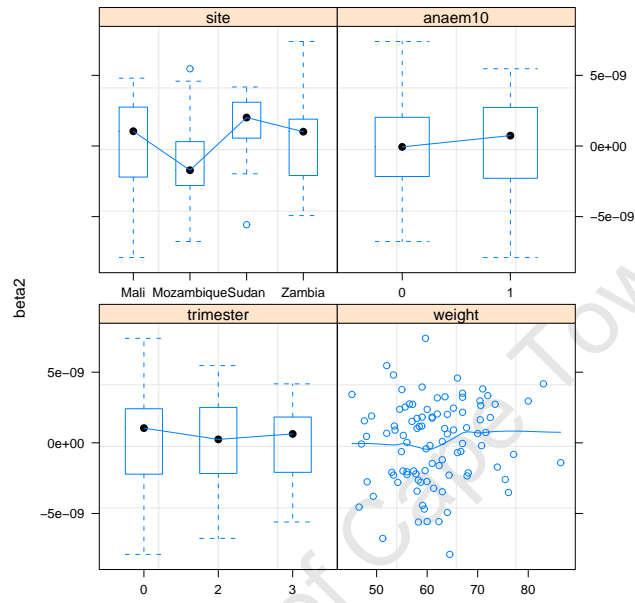


Figure B.9: Subject-Specific Random Effects for  $\beta_2$  vs. Covariates: Sulfadoxine NLME Model 1



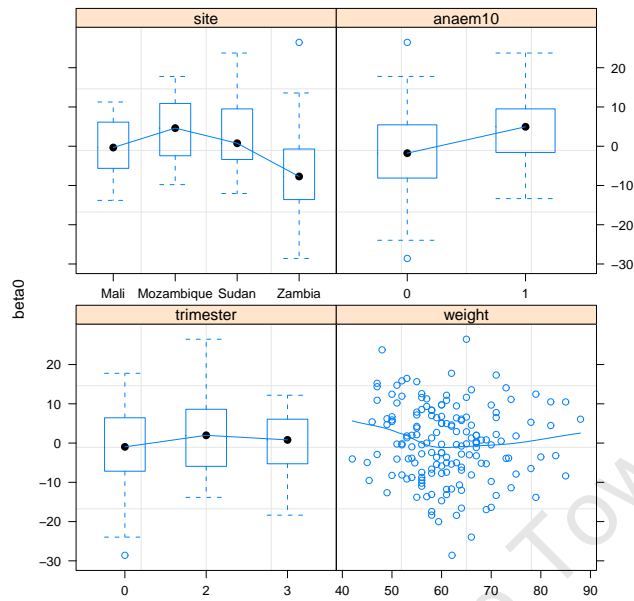


Figure B.10: Occasion-Specific Random Effects for  $\beta_0$  vs. Covariates: Sulfadoxine NLME Model 1

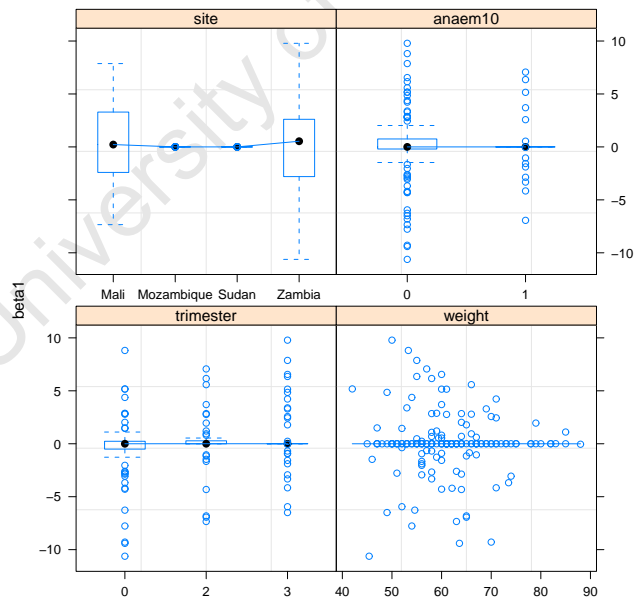


Figure B.11: Occasion-Specific Random Effects for  $\beta_1$  vs. Covariates: Sulfadoxine NLME Model 1

-----  
Example Code: Sulfadoxine NLME Model 3:  
-----

```
#MODEL
sulf1comp.13=update(sulf1comp.8,fixed=list(beta0~preg,beta1~1,
                                          beta2~preg),
                  random=list(pid=pdDiag(beta0+beta1~1),
                               preg=pdDiag(beta0+beta1+beta2~1)),
                  start=list(fixed=c(80,0,14,0.06,0)))
summary(sulf1comp.13)

#MODEL DIAGNOSTICS FOR MODEL 13
#RESIDUALS VS. FITTED
par(oma=c(0,0,0,0))
plot(sulf1comp.13,resid(.,type="p")~fitted(.,id=0.10,adj=-0.1,
idLabels=~as.numeric(site))

#HISTOGRAM, QQPLOT OF RESIDUALS, RESIDUALS BY SUBJECT ID
hist(resid(sulf1comp.13,type="p"),col="blue",main="")
plot(sulf1comp.13,pid~resid(.,type="p"),abline=0)
qqnorm(sulf1comp.13,~resid(.,type="p") )

#QQPLOTS OF RANDOM EFFECTS (BOTH LEVELS)
qqnorm(sulf1comp.13,~ranef(.,level=1),layout=c(2,1,1),
devAskNewPage(ask=T),id=0.05)
qqnorm(sulf1comp.13,~ranef(.,level=2),layout=c(3,1,1),
devAskNewPage(ask=T),id=0.05,adj=0.5,idLabel=~as.numeric(site))

#PAIRS PLOTS OF RANDOM EFFECTS
pairs(sulf1comp.13,~ranef(.,level=1),control=list(cex.axis=0.7),id=0.05)
pairs(sulf1comp.13,~ranef(.,level=2),control=list(cex.axis=0.7),id=0.05)

#PLOT OF PREDICTED CONCENTRATION-TIME CURVES BY INDIVIDUAL (MULTIPLE-LEVELS)
devAskNewPage(ask=TRUE)
plot(augPred(sulf1comp.13,level=0:2),layout=c(6,5,6),
strip=strip.custom(par.strip.text =list(cex=0.65)))

#FITTED VALUES VS. OBSERVED
plot(sulf1comp.13,sulfadoxine~fitted(.,id=0.05,adj=-0.1,
idLabel=~as.numeric(site))
```

-----  
Diagnostic Plots: Sulfadoxine NLME Model 3  
-----

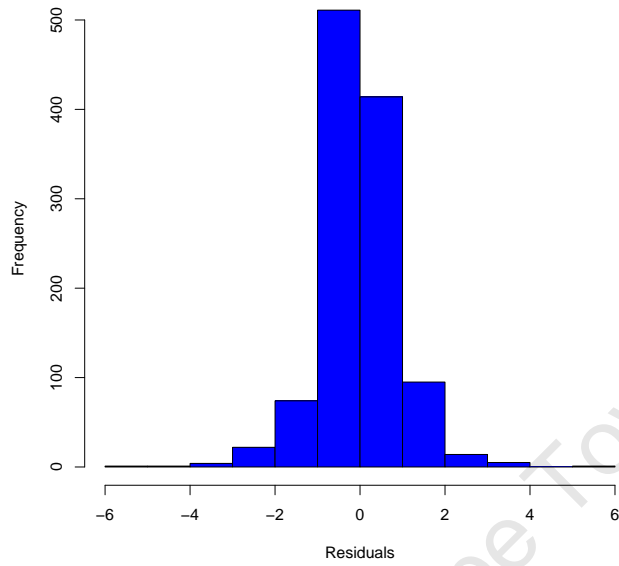


Figure B.12: Histogram of Residuals: Sulfadoxine NLME Model 3

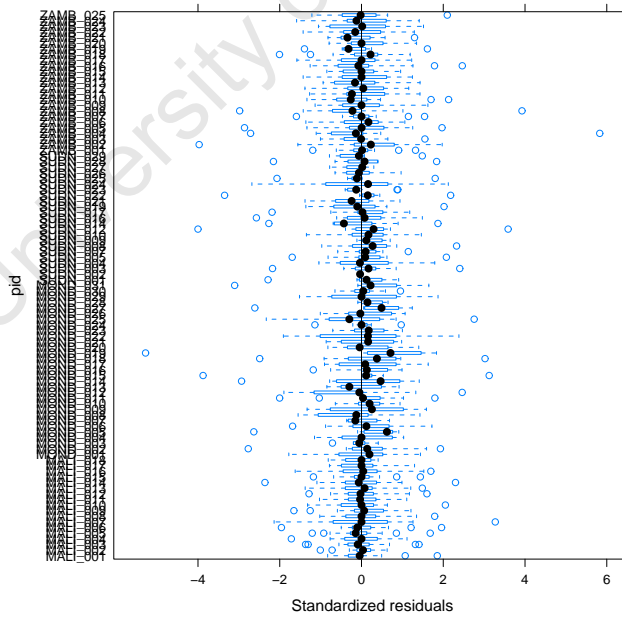


Figure B.13: Residuals by Subject ID: Sulfadoxine NLME Model 3

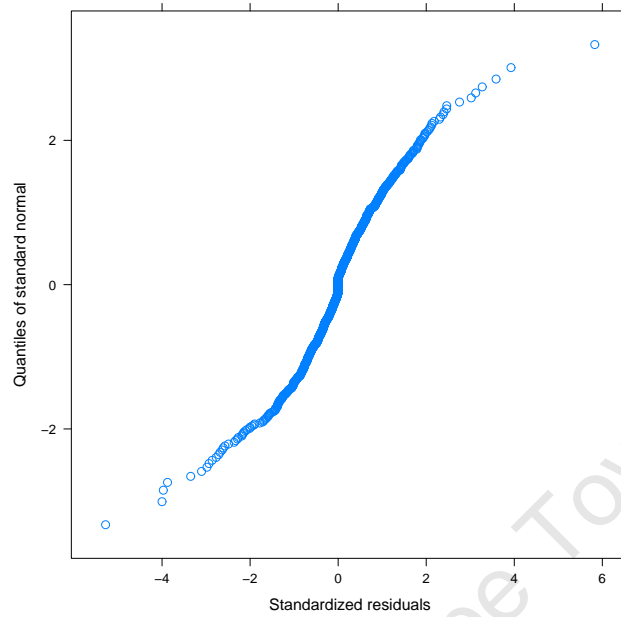


Figure B.14: QQplot of Residuals: Sulfadoxine NLME Model 3

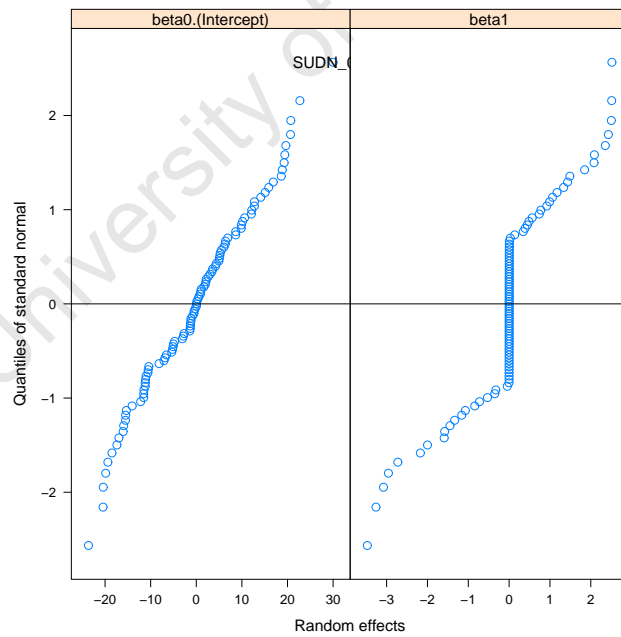


Figure B.15: QQplot of Random Effects (Subject Level): Sulfadoxine NLME Model 3

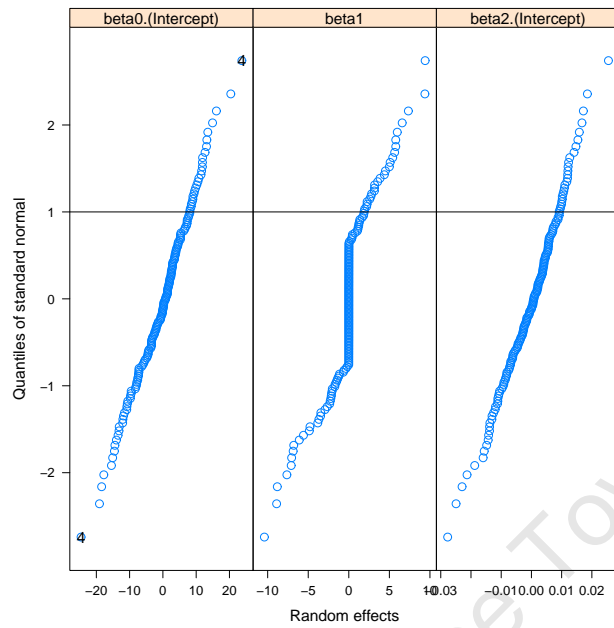


Figure B.16: QQplot of Random Effects (Phase-within-Subject Level): Sulfa-doxine NLME Model 3

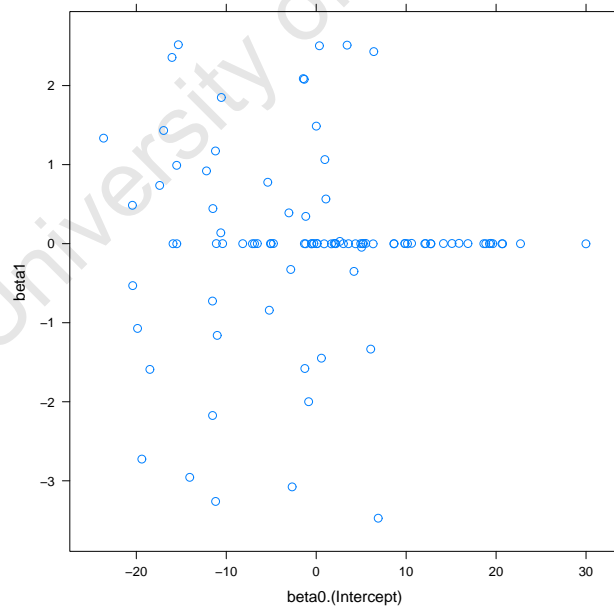


Figure B.17: Pairs Plot of Random Effects (Phase-within-Subject Level): Sulfa-doxine NLME Model 3

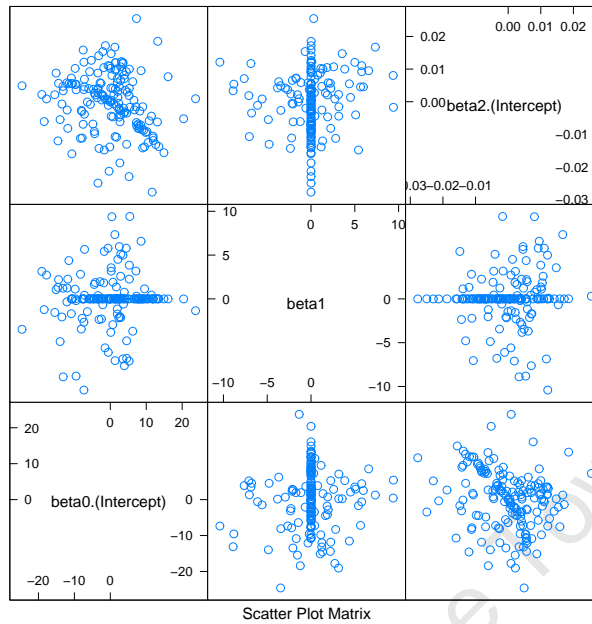


Figure B.18: Pairs Plot of Random Effects (Phase-within-Subject Level): Sul-fadoxine NLME Model 3

---

Example Code for Variance Model

---

```
var5=varConstPower(power=0.9,const=20)
sulf1comp.20=update(sulf1comp.13,weights=var5)

#RANDOM EFFECTS VS. COVARIATES
sulf1comp20.re=rانef(sulf1comp.20,level=1,aug=T)
sulf1comp20.re2=rانef(sulf1comp.20,level=2,aug=T)
sulf1comp20.fe=fixef(sulf1comp.20,level=1,aug=T)

plot(sulf1comp20.re,form=beta0.(Intercept)~trimester+weight+
site+anaem10,control=list(cex.axis=0.7))
plot(sulf1comp20.re,form=beta1~trimester+weight+site+anaem10
,control=list(cex.axis=0.7))

plot(sulf1comp20.re2,form=beta0.(Intercept)~trimester+weight
+site+anaem10,control=list(cex.axis=0.7))
plot(sulf1comp20.re2,form=beta1~trimester+weight+site+anaem10
,control=list(cex.axis=0.7))
plot(sulf1comp20.re2,form=beta2.(Intercept)~trimester+weight+
site+anaem10,control=list(cex.axis=0.7))
```

---

Random effects vs. Covariates for Model including Variance Function

---

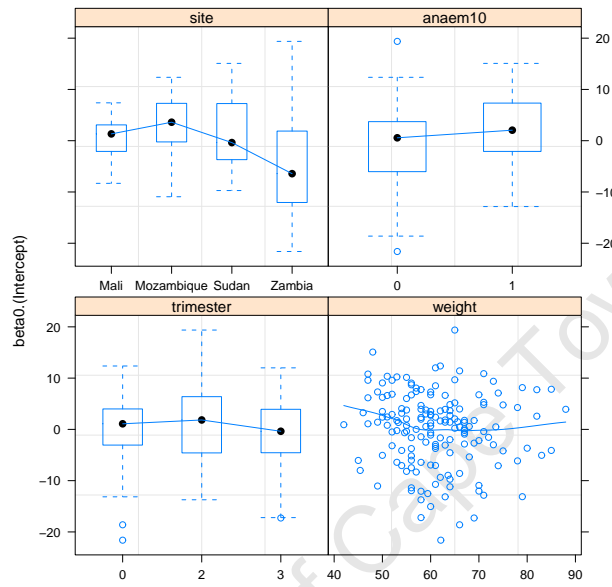


Figure B.19: Occasion-Specific Random Effects for  $\beta_0$  vs. Covariates: Sulfadoxine NLME Model 4

---

Diagnostic Plots: Sulfadoxine NLME Model 5

---

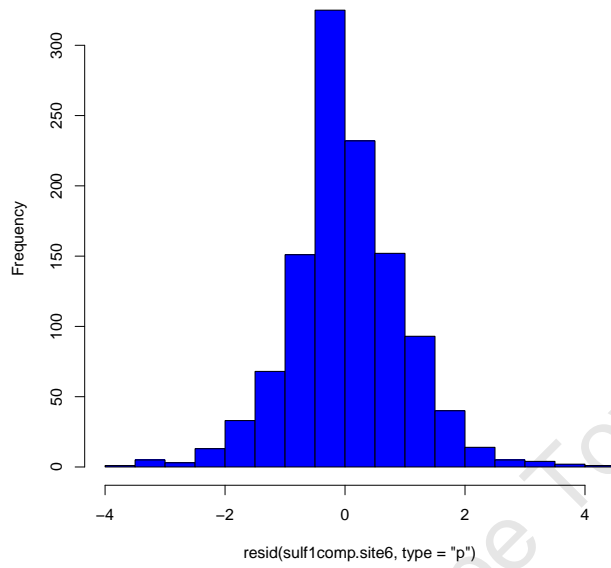


Figure B.20: Histogram of Residuals: Sulfadoxine NLME Model 5

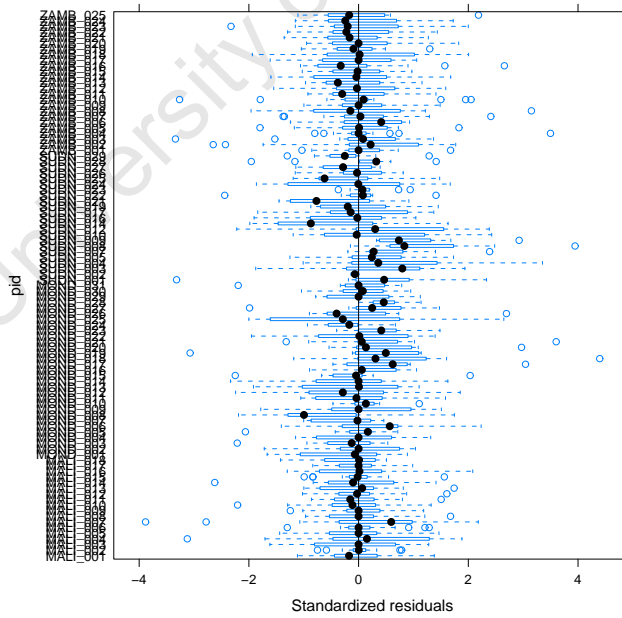


Figure B.21: Residuals by Subject ID: Sulfadoxine NLME Model 5



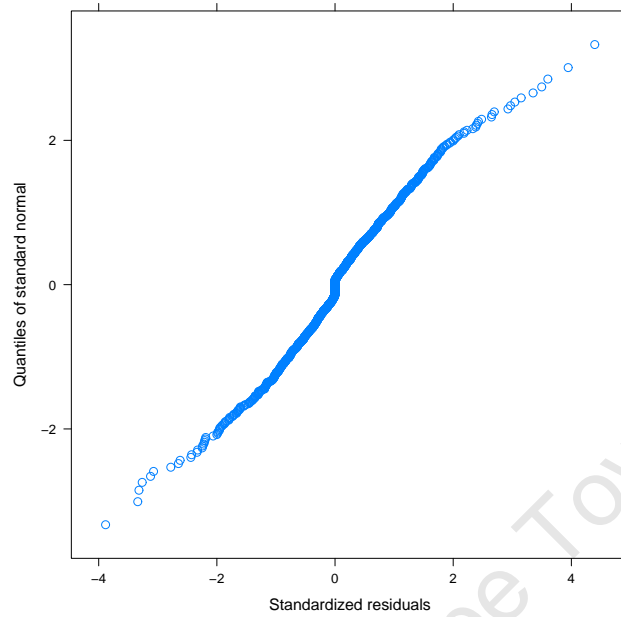


Figure B.22: QQplot of Residuals: Sulfadoxine NLME Model 5

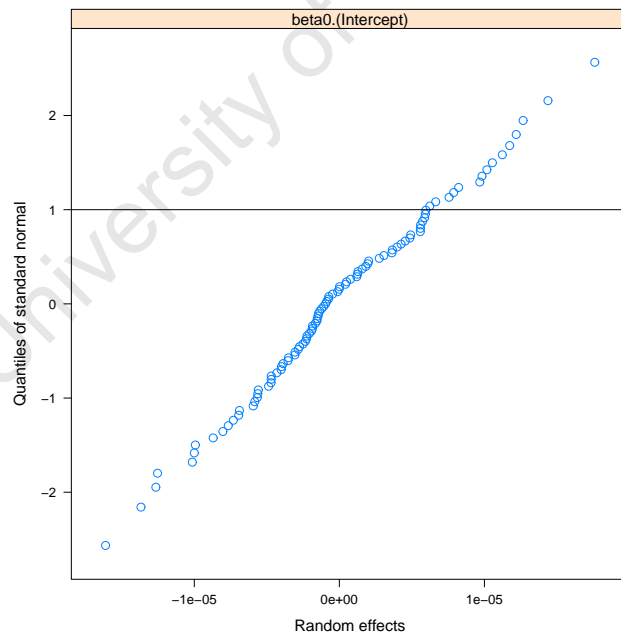


Figure B.23: QQplot of Random Effects (Subject Level): Sulfadoxine NLME Model 5

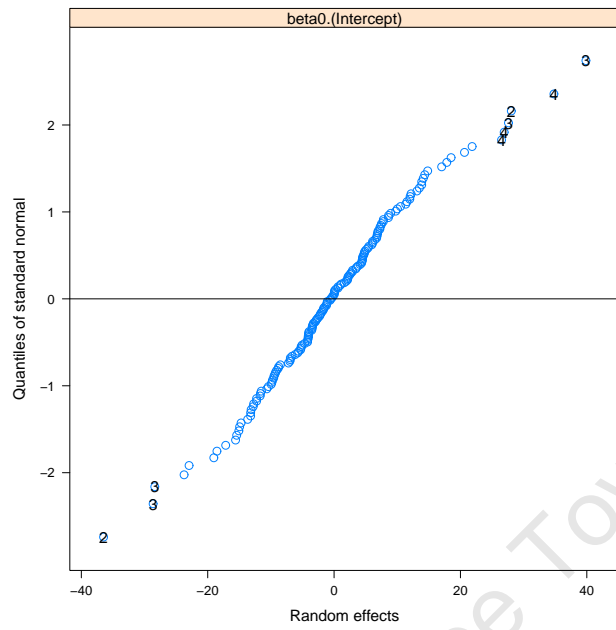


Figure B.24: Q-Qplot of Random Effects (Phase-within-Subject Level): Sulfadoxine NLME Model 5

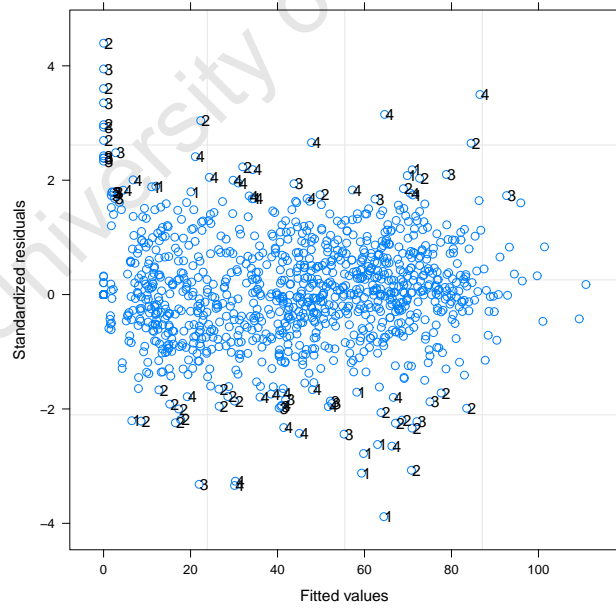


Figure B.25: Residuals vs. Fitted Values: Sulfadoxine NLME Model 5

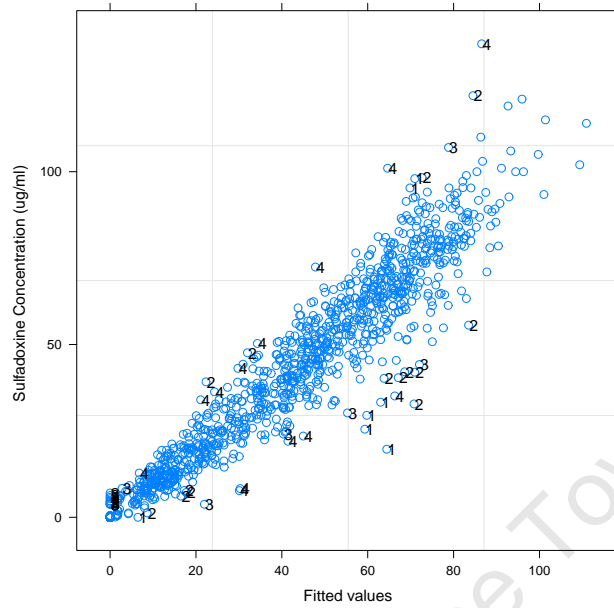


Figure B.26: Fitted Values vs. Observed: Sulfadoxine NLME Model 5

-----  
 Example Code for Figure 3.25  
 -----

```
#CREATING EMPTY DATASET
timenew2=seq(0,42,0.1)

conc_mali2=rep(0,421)
conc_mali_preg2=rep(0,421)

conc_moz2=rep(0,421)
conc_moz_preg2=rep(0,421)

conc_sud2=rep(0,421)
conc_sud_preg2=rep(0,421)

conc_zamb2=rep(0,421)
conc_zamb_preg2=rep(0,421)

newdata2=data.frame(timenew2, conc_mali2,conc_mali_preg2,
conc_moz2,conc_moz_preg2,
conc_sud2,conc_sud_preg2,
conc_zamb2,conc_zamb_preg2)

fixed=fixef(sulf1comp.site6)
```

```

is.vector(fixef(sulf1comp.site6))

#RENAMING VARIABLES OF INTEREST FOR EASE OF USE
beta0mali=fixed[1]
beta1mali=fixed[5]
beta2mali=fixed[6]

beta0malipreg=fixed[1]+fixed[2]
beta1malipreg=fixed[5]
beta2malipreg=fixed[6]+fixed[7]

beta0moz=fixed[1]+fixed[3]
beta1moz=fixed[5]
beta2moz=fixed[6]+fixed[8]

beta0mozpreg=fixed[1]+fixed[3]+fixed[2]
beta1mozpreg=fixed[5]
beta2mozpreg=fixed[6]+fixed[8]+fixed[7]

beta0zamb=fixed[1]+fixed[4]
beta1zamb=fixed[5]
beta2zamb=fixed[6]

beta0zambpreg=fixed[1]+fixed[4]+fixed[2]
beta1zambpreg=fixed[5]
beta2zambpreg=fixed[6]+fixed[7]

beta0sud=fixed[1]
beta1sud=fixed[5]
beta2sud=fixed[6]

beta0sudpreg=fixed[1]+fixed[2]
beta1sudpreg=fixed[5]
beta2sudpreg=fixed[6]+fixed[7]

#FILLING EMPTY DATASET
for (i in 1:nrow(newdata2)) {
newdata2[i,2]=beta0mali*(-exp(-beta1mali*newdata2[i,1])+
exp(-beta2mali*newdata2[i,1]))
newdata2[i,3]=beta0malipreg*(-exp(-beta1malipreg*newdata2[i,1])
+exp(-beta2malipreg*newdata2[i,1]))
newdata2[i,4]=beta0moz*(-exp(-beta1moz*newdata2[i,1])
+exp(-beta2moz*newdata2[i,1]))
newdata2[i,5]=beta0mozpreg*(-exp(-beta1mozpreg*newdata2[i,1])
+exp(-beta2mozpreg*newdata2[i,1]))
newdata2[i,6]=beta0sud*(-exp(-beta1sud*newdata2[i,1])
+exp(-beta2sud*newdata2[i,1]))
newdata2[i,7]=beta0sudpreg*(-exp(-beta1sudpreg*newdata2[i,1])
+exp(-beta2sudpreg*newdata2[i,1]))
newdata2[i,8]=beta0zamb*(-exp(-beta1zamb*newdata2[i,1])

```

```

+exp(-beta2zamb*newdata2[i,1]))
newdata2[i,9]=beta0zambpreg*(-exp(-beta1zambpreg*newdata2[i,1])
+exp(-beta2zambpreg*newdata2[i,1]))
}

#CURVES BY SITE (PREG AND PP)
par(mfrow=c(2,2))
plot(newdata2$conc_mali_preg2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lwd="2", col="blue",font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)", main="Mali")

par(new=TRUE)
plot(newdata2$conc_mali2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lty="44",lwd="2", col="navy",font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)")

plot(newdata2$conc_sud_preg2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lwd="2", col="green", font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)", main="Sudan")

par(new=TRUE)
plot(newdata2$conc_sud2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lty="44",lwd="2", col="forest green",font.sub="2",
xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)")

plot(newdata2$conc_moz_preg2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lwd="2", col="deeppink4",font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)", main="Mozambique")

par(new=TRUE)
plot(newdata2$conc_moz2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lty="44",lwd="2", col="red",font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)")

plot(newdata2$conc_zamb_preg2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lwd="2", col="gray",font.sub="2",xlab="Time(Days)",
ylab="Mean Sulfadoxine Concentration (ug/ml)", main="Zambia")

par(new=TRUE)
plot(newdata2$conc_zamb2~newdata2$timenew2,
ylim=range(newdata2$conc_moz_preg2),
type="l",lty="44",lwd="2", col="black",font.sub="2",xlab="Time(Days)",

```

```
ylab="Mean Sulfadoxine Concentration (ug/ml)")
```

```
par(xpd=NA)
```

```
legend(locator(1), legend=c("Pregnant", "Postpartum"), lty=c(1,44), bty="n", ncol=2)
```

-----  
Comparison of PK parameters: Sulfadoxine NLME Model 5

(N/D\*)= Not determined  
-----

University of Cape Town

Country	Parameter	Model 5				Nyunt et al.			
		Pregnant		Postpartum		Pregnant		Postpartum	
		Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
Mali	Tmax (days)	0.84	0.27	0.91	0.05	0.38	0.33	0.55	0.37
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	7.04	1.34	10.17	1.33
	$AUC_{(0\text{inf})}$ (ug.day/ml)	912.79	24.64	1221.72	45.78	873.07	142.52	1064.86	189.01
	Vd/f (ml/kg)	299.85	7.98	342.41	11.64	290.16	36.91	358.95	38.67
	Cl/f (ml/kg/day)	26.94	0.74	20.67	0.78	29.51	6.62	24.68	2.97
	Cmax (ug/ml)	76.04	2.12	69.83	2.46	83.35	5.44	70.01	9.87
Mozambique	Tmax (days)	0.86	0.29	0.94	0.39	0.72	0.86	(N/D*)	(N/D)
	$t_{1/2}$ (days)	8.72	0.21	13.87	0.84	8.12	3.55	(N/D)	(N/D)
	$AUC_{(0\text{inf})}$ (ug.day/ml)	1144.20	32.69	1653.88	90.90	1091.27	320	(N/D)	(N/D)
	Vd/f (ml/kg)	270.58	8.00	305.68	12.26	261.94	81.27	(N/D)	(N/D)
	Cl/f (ml/kg/day)	21.49	0.62	15.27	0.85	24.68	9.08	(N/D)	(N/D)
	Cmax (ug/ml)	84.88	2.62	78.84	3.25	100.78	74.92	(N/D)	(N/D)
Sudan	Tmax (days)	0.84	0.27	0.91	0.05	0.98	0.82	(N/D)	(N/D)
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	6.29	2.28	(N/D)	(N/D)
	$AUC_{(0\text{inf})}$ (ug.day/ml)	912.79	24.64	1221.72	45.78	844.64	322.5	(N/D)	(N/D)
	Vd/f (ml/kg)	299.85	7.98	342.41	11.64	253.99	79.34	(N/D)	(N/D)
	Cl/f (ml/kg/day)	26.94	0.74	20.67	0.78	30.67	9.75	(N/D)	(N/D)
	Cmax (ug/ml)	76.04	2.12	69.83	2.46	81.26	18.26	(N/D)	(N/D)
Zambia	Tmax (days)	0.84	0.27	0.91	0.34	0.3	0.24	0.54	0.45
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	8.5	2.83	10.02	1.97
	$AUC_{(0\text{inf})}$ (ug.day/ml)	996.20	27.39	862.22	44.85	745.84	262.48	737.63	107.39
	Vd/f (ml/kg)	274.74	16.62	485.17	24.54	426.23	123.85	487.72	54.73
	Cl/f (ml/kg/day)	24.68	1.52	29.29	1.54	38.49	16.55	34.72	6.53
	Cmax (ug/ml)	82.99	2.38	49.28	2.58	61.3	17.33	50.01	7.14

Table B.3: Comparison of PK parameters by Site and Pregnancy Phase: Sulfadoxine NLME Model 5

Country	Parameter	Model 5				Nyunt et al. (Reported)			
		Pregnant		Postpartum		Pregnant		Postpartum	
		Mean	Std. Error	Mean	Std. Error	Median	IQR	Median	IQR
Mali	Tmax (days)	0.84	0.27	0.91	0.05	0.36	(0.04,0.53)	0.46	(0.32,0.80)
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	6.83	(6.11,8.00)	9.89	(9.52,10.63)
	$AUC_{(0\text{ inf})}$ (ug.day/ml)	912.79	24.64	1221.72	45.78	831.98	(769.21,961.66)	1079.57	(958.38,1156.49)
	Vd/f (ml/kg)	299.85	7.98	342.41	11.64	291.05	(274.68,303.99)	353.12	(345.32,392.94)
	Cl/f (ml/kg/day)	26.94	0.74	20.67	0.78	28.86	(26.02,33.62)	24.48	(22.41,26.28)
	Cmax (ug/ml)	76.04	2.12	69.83	2.46	84.21	(80.78,86.09)	70.57	(63.46,77.07)
Mozambique	Tmax (days)	0.86	0.29	0.94	0.39	0.23	(0.15,1.32)	(N/D*)	(N/D)
	$t_{1/2}$ (days)	8.72	0.21	13.87	0.84	7.79	(6.15,9.79)	(N/D)	(N/D)
	$AUC_{(0\text{ inf})}$ (ug.day/ml)	1144.20	32.69	1653.88	90.90	1045.71	(886.15,1270.09)	(N/D)	(N/D)
	Vd/f (ml/kg)	270.58	8.00	305.68	12.26	270.21	(231.25,301.31)	(N/D)	(N/D)
	Cl/f (ml/kg/day)	21.49	0.62	15.27	0.85	23.14	(19.68,28.14)	(N/D)	(N/D)
	Cmax (ug/ml)	84.88	2.62	78.84	3.25	83.24	(73.70,96.90)	(N/D)	(N/D)
Sudan	Tmax (days)	0.84	0.27	0.91	0.05	1.09	(0.22,1.56)	(N/D)	(N/D)
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	6.72	(5.19,7.38)	(N/D)	(N/D)
	$AUC_{(0\text{ inf})}$ (ug.day/ml)	912.79	24.64	1221.72	45.78	758.75	(622.09,1009.25)	(N/D)	(N/D)
	Vd/f (ml/kg)	299.85	7.98	342.41	11.64	248.61	(217.63,295.44)	(N/D)	(N/D)
	Cl/f (ml/kg/day)	26.94	0.74	20.67	0.78	30.74	(23.87,35.99)	(N/D)	(N/D)
	Cmax (ug/ml)	76.04	2.12	69.83	2.46	83.96	(66.35,90.76)	(N/D)	(N/D)
Zambia	Tmax (days)	0.84	0.27	0.91	0.34	0.35	(0.03,0.43)	0.44	(0.27,0.86)
	$t_{1/2}$ (days)	7.71	0.14	11.48	0.33	7.89	(6.49,10.78)	10.07	(8.24,11.36)
	$AUC_{(0\text{ inf})}$ (ug.day/ml)	996.20	27.39	862.22	44.85	743.58	(468.67,911.07)	732.49	(679.03,817.37)
	Vd/f (ml/kg)	274.74	16.62	485.17	24.54	457.47	(321.05,518.36)	490.73	(452.06,508.75)
	Cl/f (ml/kg/day)	24.68	1.52	29.29	1.54	32.41	(27.60,47.45)	33.87	(29.53,38.45)
	Cmax (ug/ml)	82.99	2.38	49.28	2.58	56.94	(47.82,73.37)	50.62	(43.41,54.68)

Table B.4: Comparison of PK parameters by Site and Pregnancy Phase II: Sulfadoxine NLME Model 5



-----  
Example Code for Single-Level Model (Correlated Random Effects Structure)

```
-----  
sulf1comp.corr=update(sulf1comp.20,  
                      random=list(pid=pdBlocked(list(pdCompSymm(beta0~preg-1)  
                                                    ,pdCompSymm(beta1~preg-1),pdIdent(beta2~preg-1))))  
  
summary(sulf1comp.corr)  
VarCorr(sulf1comp.corr)  
-----
```

Example Code for Proportional Random Effects Structure

```
-----  
#SPECIFYING FUNCTIONS  
fm1comp_prop=function(lb0,lb1,lb2,day) {  
  b0=exp(lb0)  
  b1=exp(lb1)  
  b2=exp(lb2)  
  b0*(-exp(-b1*day)+exp(-b2*day))  
}  
  
fm2comp_prop=function(lb1,lb2,lb3,lb4,lb5,day) {  
  b1=exp(lb1)  
  b2=exp(lb2)  
  b3=exp(lb3)  
  b4=exp(lb4)  
  b5=exp(lb5)  
  b2*(-exp(-b1*day)+exp(-b3*day))+b4*(-exp(-b1*day)+exp(-b5*day))  
}  
  
#BASIC MODEL (BUILDING FROM SCRATCH)  
sulf1comp.propb_scr=nlme(sulfadoxine~fm1comp_propb(lb0,lb1,lb2,day),  
                          data=sulf,  
                          fixed=list(lb0~1,lb1~1,lb2~1),  
                          random=list(pid=pdDiag(lb0+lb1+lb2~1),  
                                       preg=pdDiag(lb0+lb1+lb2~1)),  
                          start=list(fixed=c(5,3,0.06)),control=controlS)  
summary(sulf1comp.propb_scr)  
-----
```

Diagnostic Plots for Sulfadoxine NLME Model 7 (Proportional Random Effects Structure)

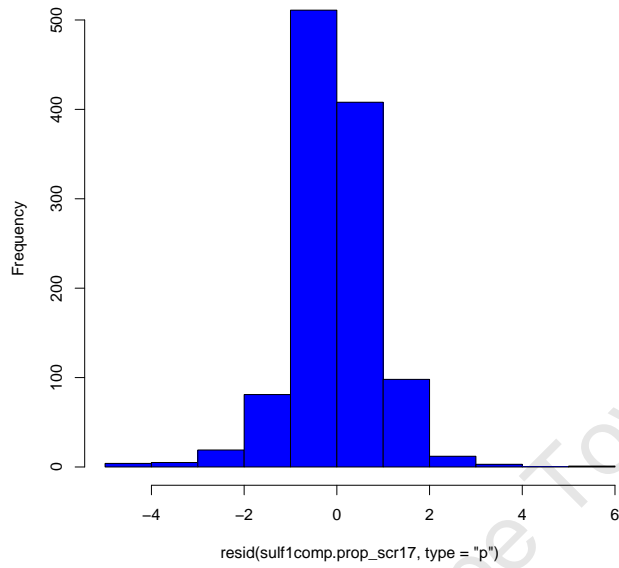


Figure B.27: Histogram of Residuals: Sulfadoxine NLME Model 7

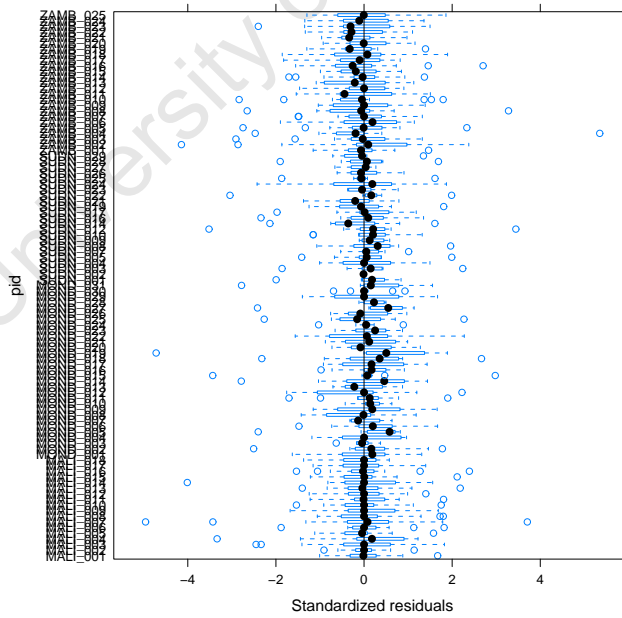


Figure B.28: Residuals by Subject ID: Sulfadoxine NLME Model 7

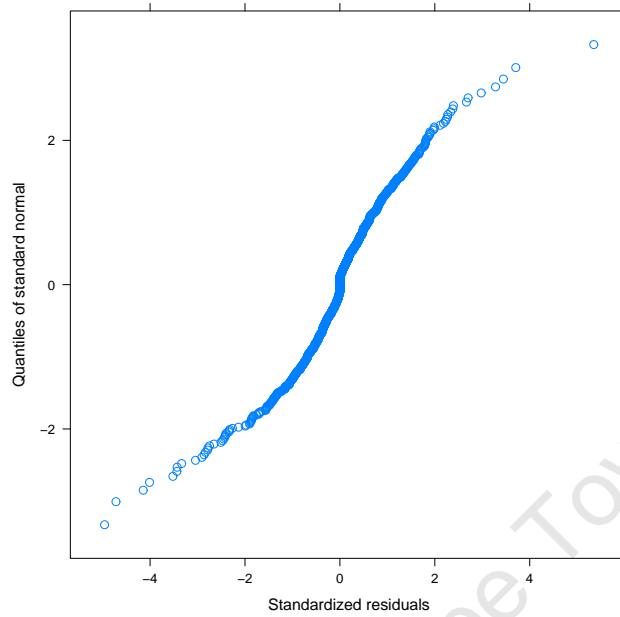


Figure B.29: QQplot of Residuals: Sulfadoxine NLME Model 7

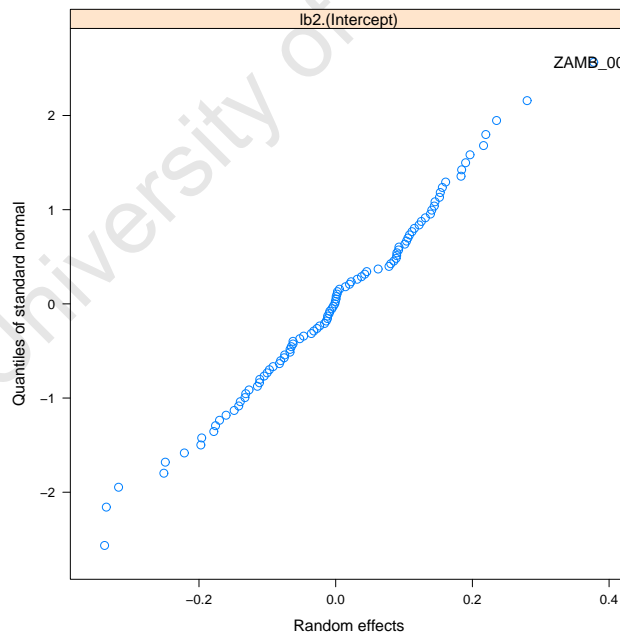


Figure B.30: QQplot of Random Effects (Subject Level): Sulfadoxine NLME Model 7

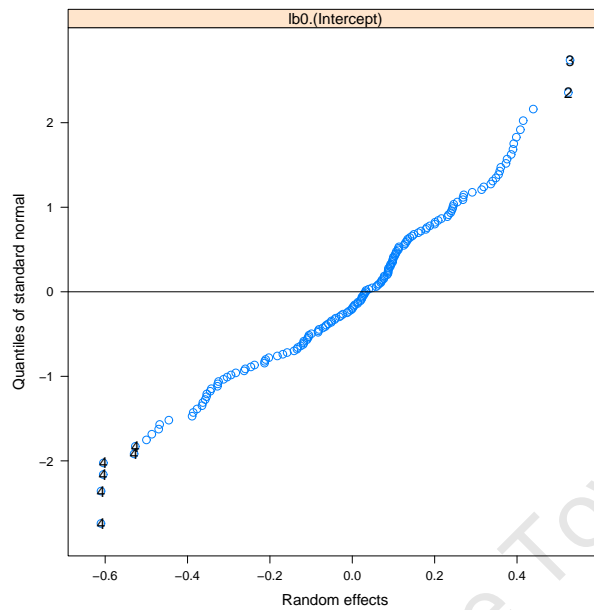


Figure B.31: QQplot of Random Effects (Phase-within-Subject Level): Sulfadoxine NLME Model 7

-----  
 Example Code for Mechanistic Models  
 -----

```
#MECHANISTIC MODEL SPECIFICATION
#ADDITIVE SPECIFICATION
#ka, ke, V formulation vs. ka, ke, Cl formulation
fn1=function(day,dose,ka,V,ke) {
  ((dose*20*ka)/(V*(ka-ke)))*(-exp(-ka*day)+exp(-ke*day))
}

#ka, Cl, V formulation
fn3=function(day,dose,ka,V,CL) {
  ((dose*20*ka)/(V*(ka-(CL/V))))*(-exp(-ka*day)+exp(-(CL/V)*day))
}

sulf.mech0=nlme(sulfadoxine~fn1(day,dose,ka,ke,V),
  data=sulf,
  fixed=list(ka~1,ke~1,V~1),
  random=list(pid=pdDiag(ke+ka+V~1),
  preg=pdDiag(ke+ka+V~1)),
  start=list(fixed=c(0.05,0.088,18)),
  control=controlS)
summary(sulf.mech0)
```

---

Diagnostic Plots for Sulfadoxine NLME Model 8 (Mechanistic Model Specification)

---

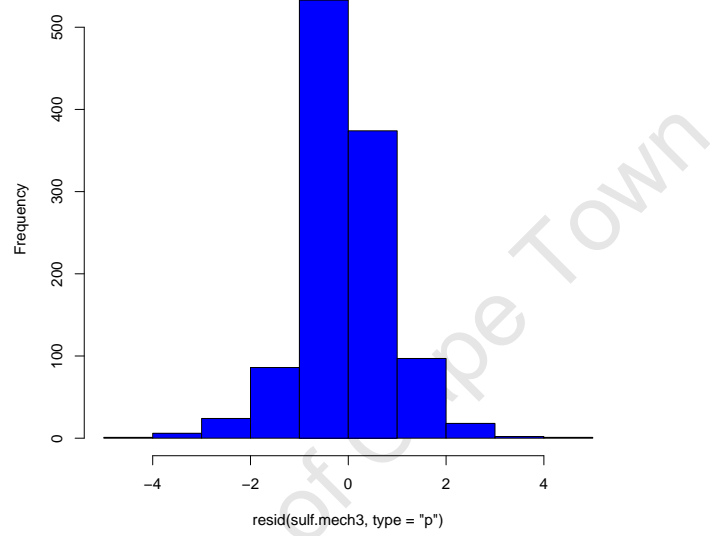


Figure B.32: Histogram of Residuals: Sulfadoxine NLME Model 8

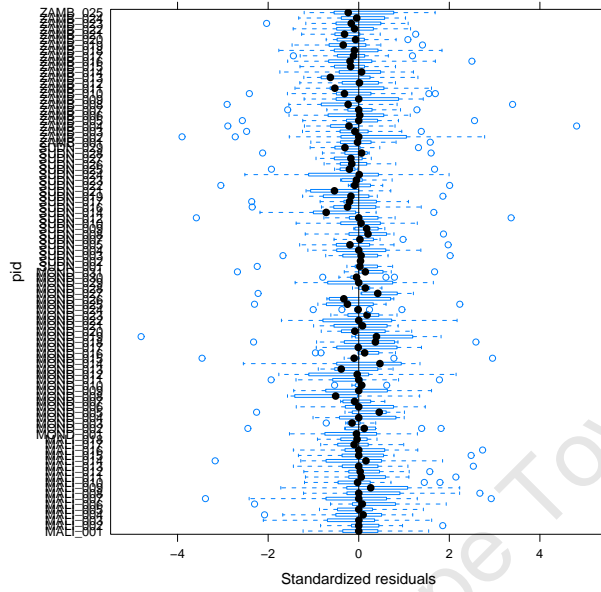


Figure B.33: Residuals by Subject ID: Sulfadoxine NLME Model 8

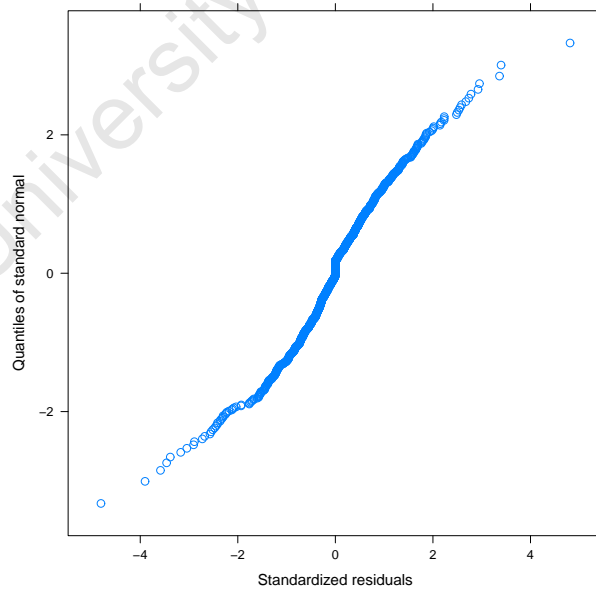


Figure B.34: QQplot of Residuals: Sulfadoxine NLME Model 8

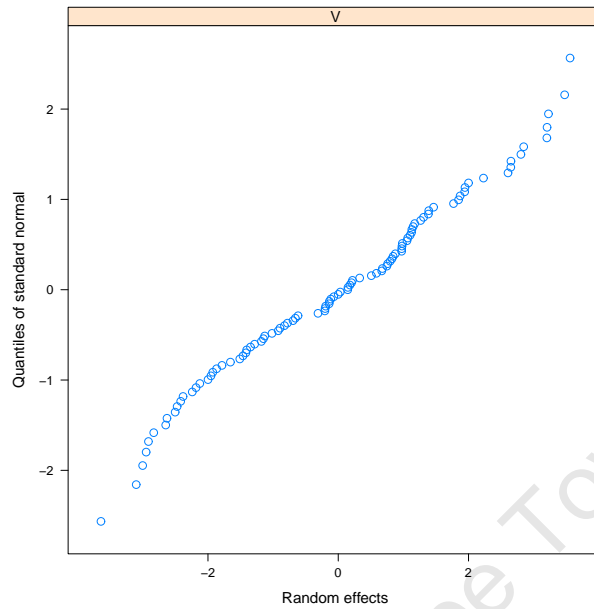


Figure B.35: Q-Qplot of Random Effects (Subject Level): Sulfadoxine NLME Model 8

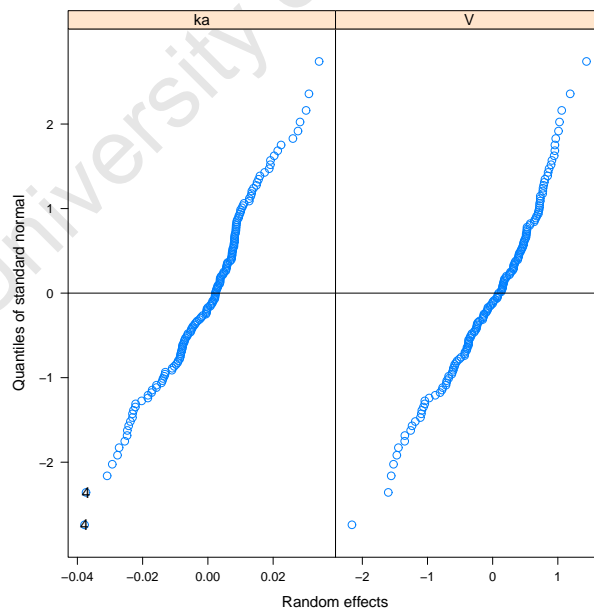


Figure B.36: Q-Qplot of Random Effects (Phase-within-Subject Level): Sulfadoxine NLME Model 8

## Appendix C

# Pyrimethamine Models

-----  
Example Code for Pyrimethamine NLME Model:  
-----

```
pyr2comp_simple.0=nlme(pyrimethamine~fm2comp(beta1,beta2,beta3,beta4,
      beta5,day),
      data=pyr,
      fixed=list(beta1~1,beta2~1,beta3~1,beta4~1,
      beta5~1),
      random=list(pid=pdDiag(beta1+beta2+beta3+beta4+
      beta5~1)
      #,preg=pdDiag(beta2+beta3+beta4+beta5~1)),
      start=list(fixed=c(41.16,417.067,1.22,448.43,0.16)),
      control=controlS)

fixef(pyr2comp_simple.0)

pyr2comp.0=nlme(pyrimethamine~fm2comp(beta1,beta2,beta3,beta4,
      beta5,day),
      data=pyr,
      fixed=list(beta1~1,beta2~1,beta3~1,beta4~1,
      beta5~1),
      random=list(pid=pdDiag(beta1+beta2+beta3+beta4+
      beta5~1),
      preg=pdDiag(beta2+beta3+beta4+beta5~1)),
      start=list(fixed=c(41,98,1.5,332,0.2)),
      control=controlS)

pyr2comp.2=update(pyr2comp.0,
      random=list(pid=pdDiag(beta2+beta3+beta4+
      beta5~1),
      preg=pdDiag(beta2+beta3+beta4+beta5~1)))

summary(pyr2comp.2)
ranef(pyr2comp.2)
-----
```



---

Diagnostic Plots for Pyrimethamine NLME Model 1:

---

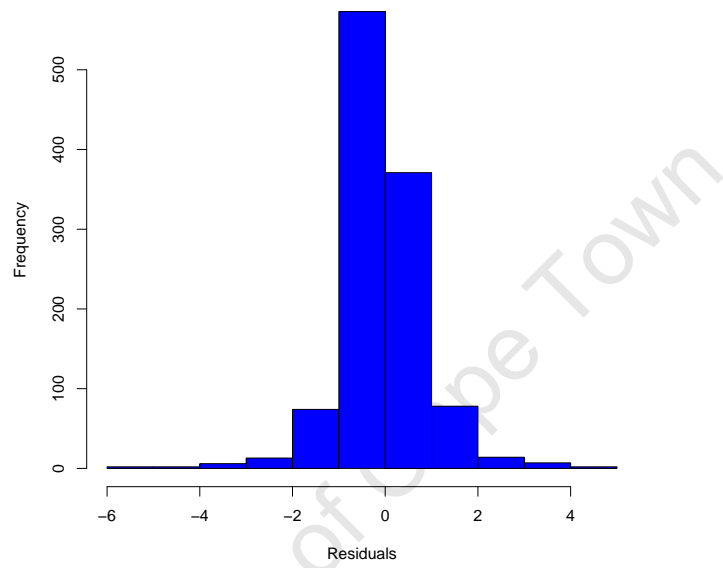


Figure C.1: Histogram of Residuals: Pyrimethamine NLME Model 1

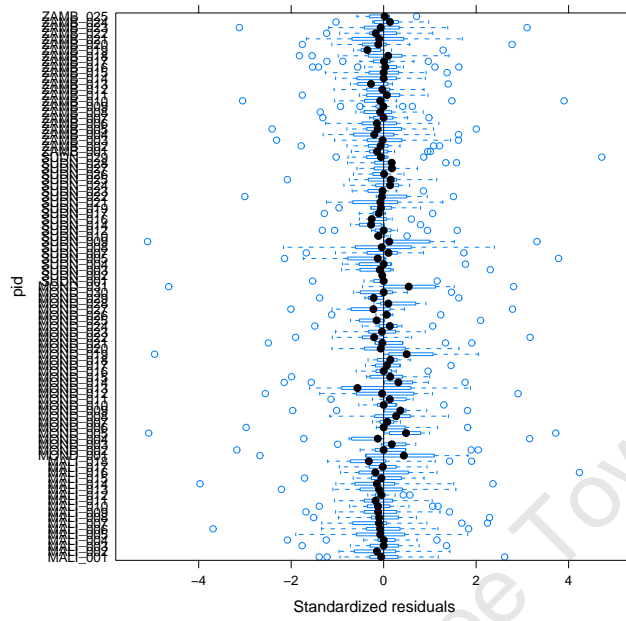


Figure C.2: Residuals by Subject ID: Pyrimethamine NLME Model 1

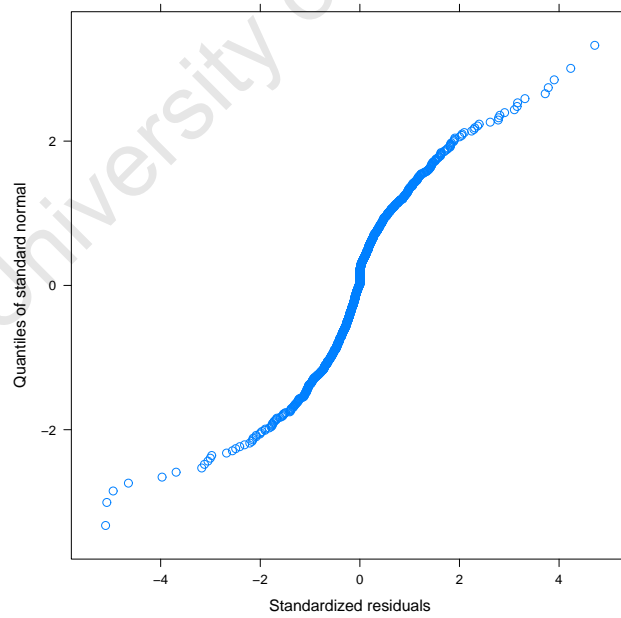


Figure C.3: QQplot of Residuals: Pyrimethamine NLME Model 1

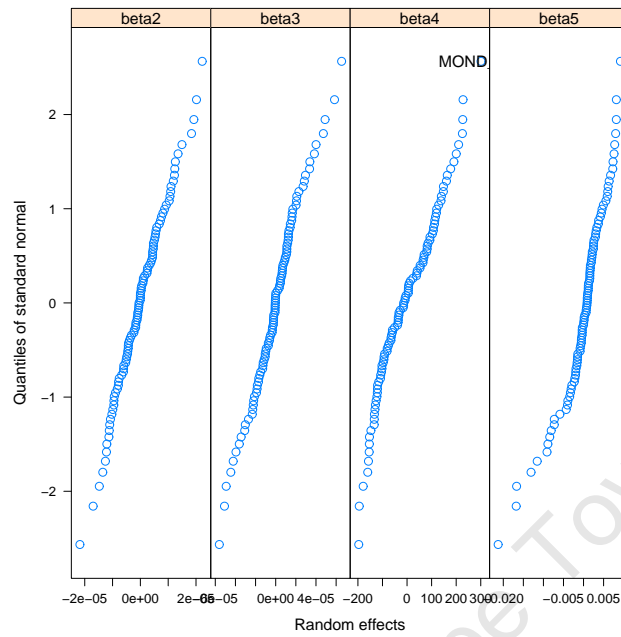


Figure C.4: QQplot of Random Effects (Subject Level): Pyrimethamine NLME Model 1

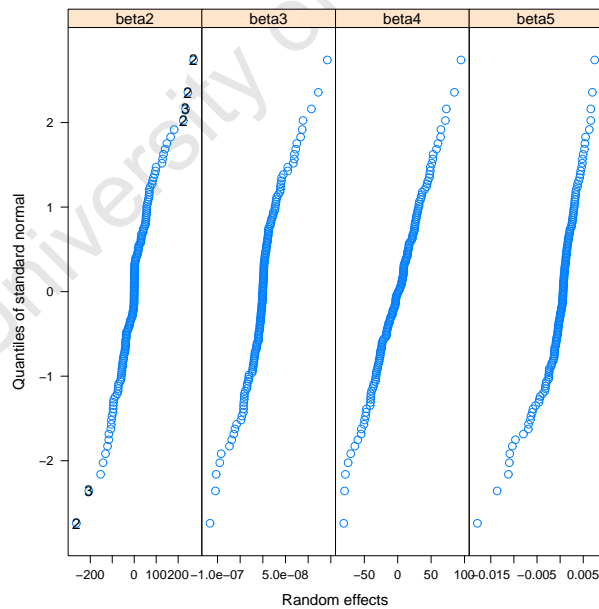


Figure C.5: QQplot of Random Effects (Phase-within-Subject Level): Pyrimethamine NLME Model 1

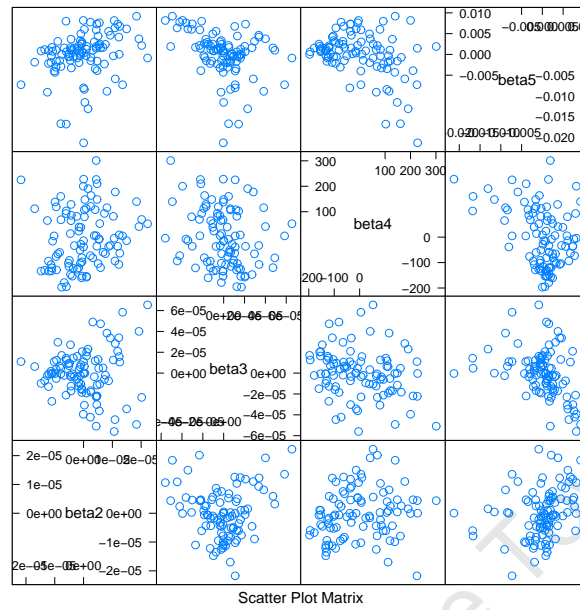


Figure C.6: Pairs Plot of Random Effects (Subject Level): Pyrimethamine NLME Model 1

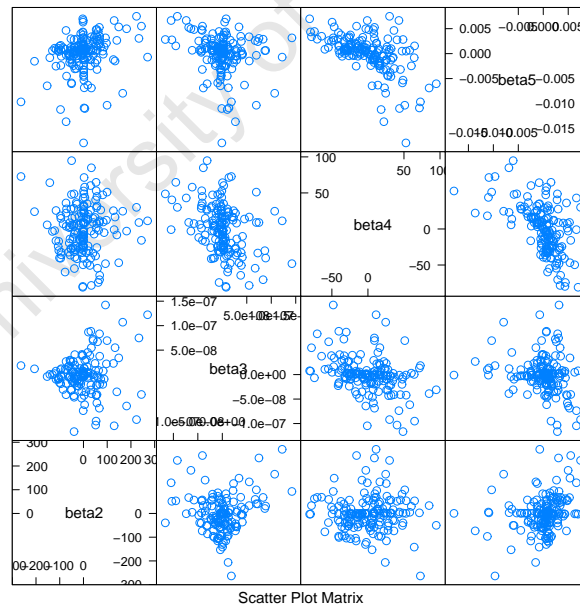


Figure C.7: Pairs Plot of Random Effects (Phase-within-Subject Level): Pyrimeth-amine NLME Model 1

-----  
Random Effects versus Covariates for Pyrimethamine NLME Model 1 (Subject-  
and Occasion-Specific):  
-----

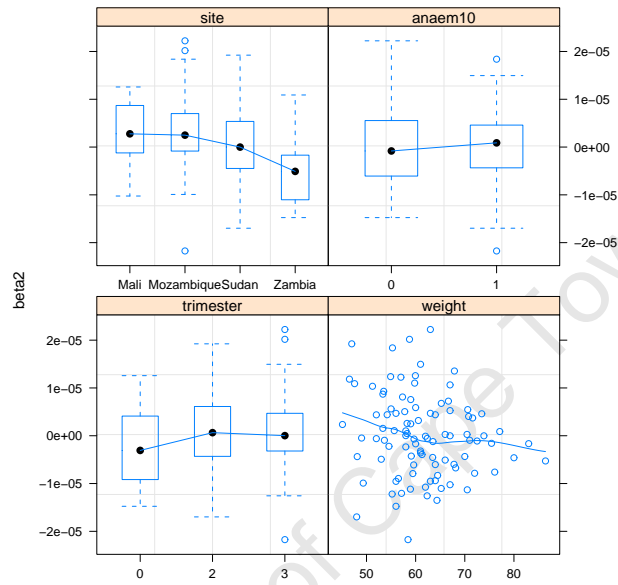


Figure C.8: Subject-Specific Random Effects for  $\beta_2$  vs. Covariates: Pyrimethamine NLME Model 1

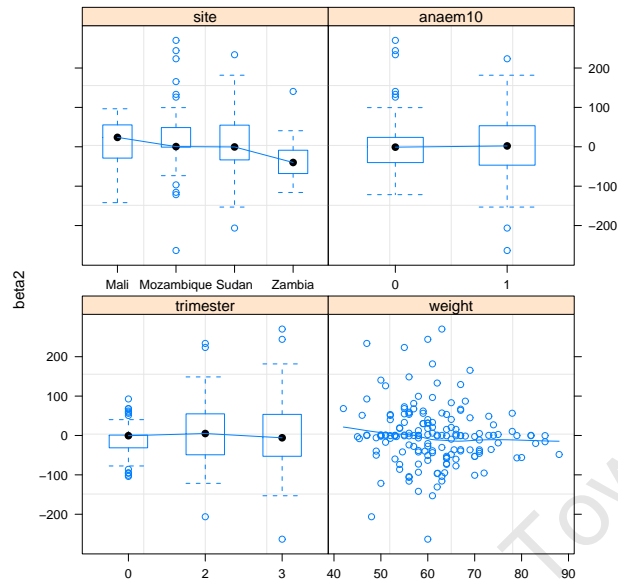


Figure C.9: Occasion-Specific Random Effects for  $\beta_2$  vs. Covariates: Pyrimethamine NLME Model 1

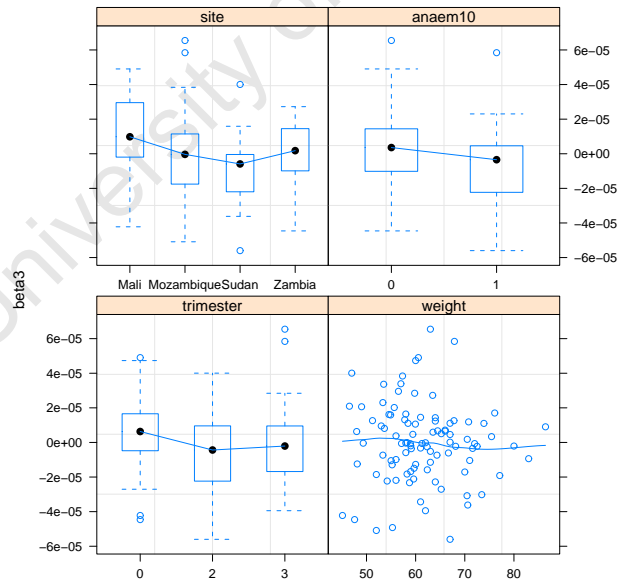


Figure C.10: Subject-Specific Random Effects for  $\beta_3$  vs. Covariates: Pyrimethamine NLME Model 1

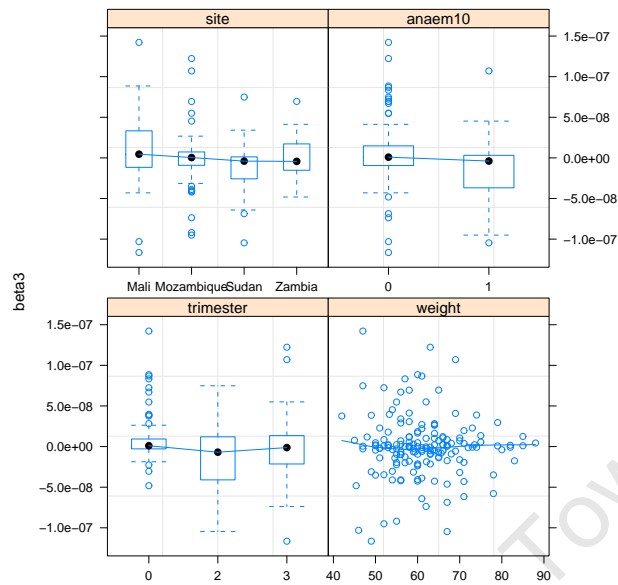


Figure C.11: Occasion-Specific Random Effects for  $\beta_3$  vs. Covariates: Pyrimethamine NLME Model 1

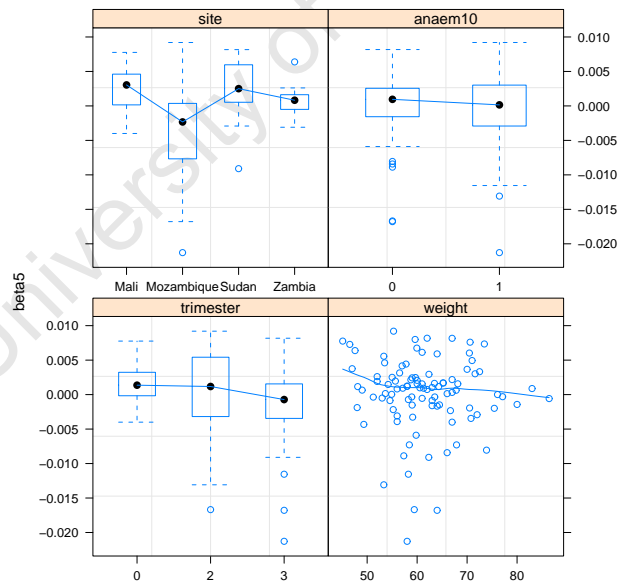


Figure C.12: Subject-Specific Random Effects for  $\beta_5$  vs. Covariates: Pyrimethamine NLME Model 1

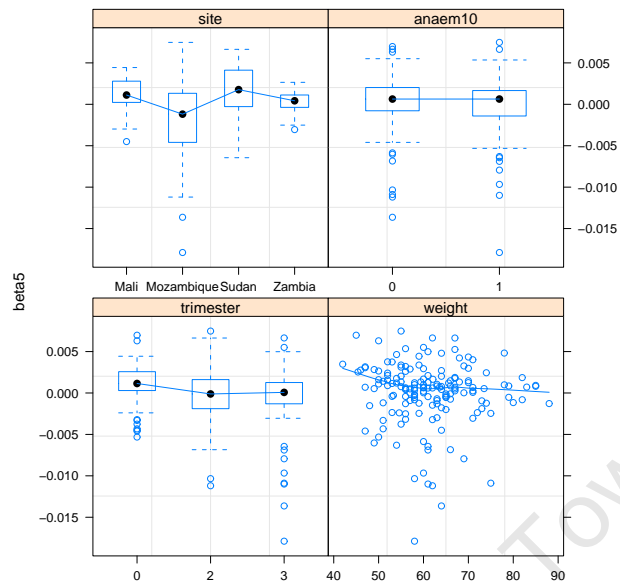


Figure C.13: Occasion-Specific Random Effects for  $\beta_5$  vs. Covariates: Pyrimethamine NLME Model 1

---

Diagnostic Plots for Pyrimethamine NLME Model 3:

---



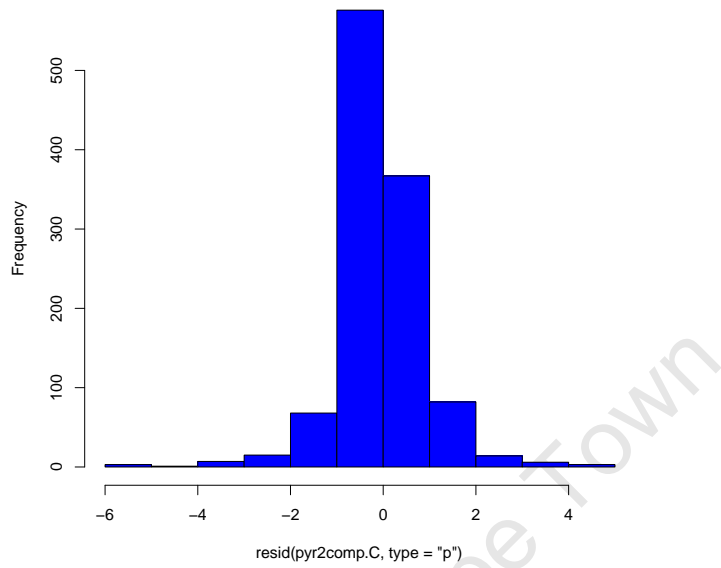


Figure C.14: Histogram of Residuals: Pyrimethamine NLME Model 3

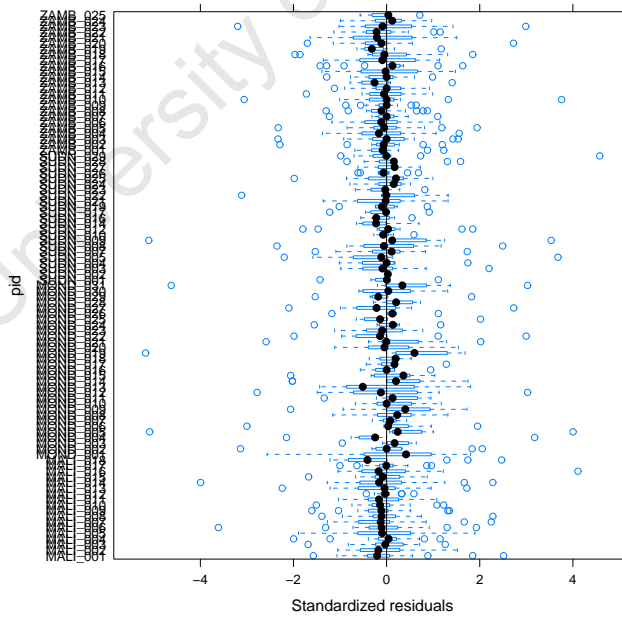


Figure C.15: Residuals by Subject ID: Pyrimethamine NLME Model 3

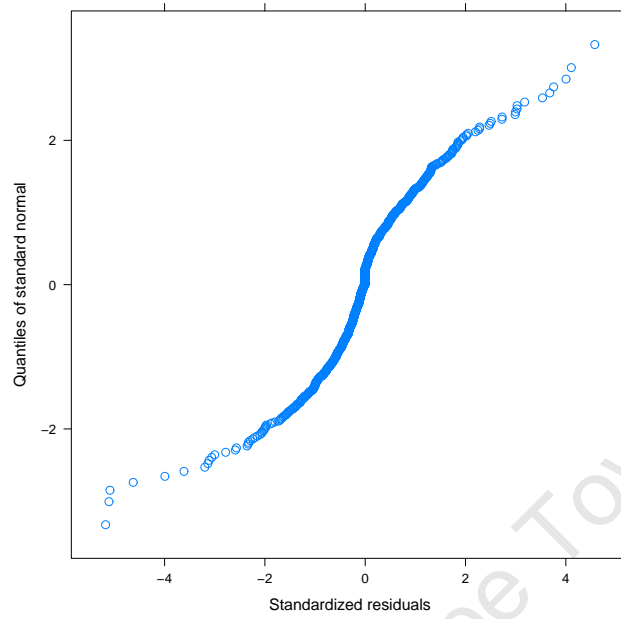


Figure C.16: Q-Qplot of Residuals: Pyrimethamine NLME Model 3

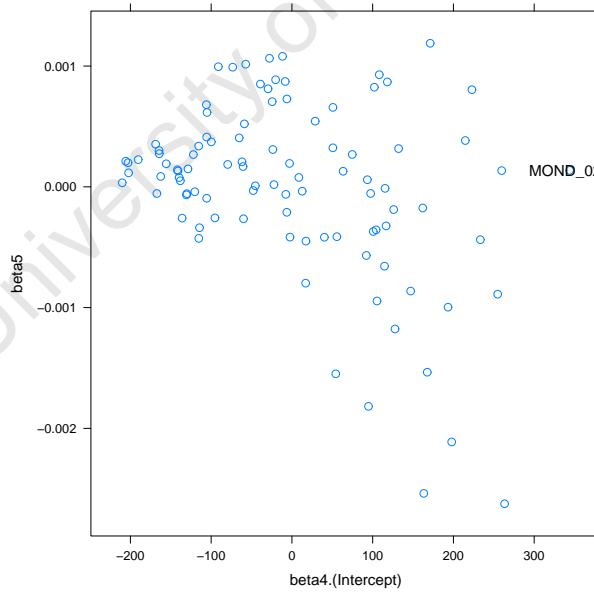


Figure C.17: Pairs Plot of Random Effects (Subject Level): Pyrimethamine NLME Model 3

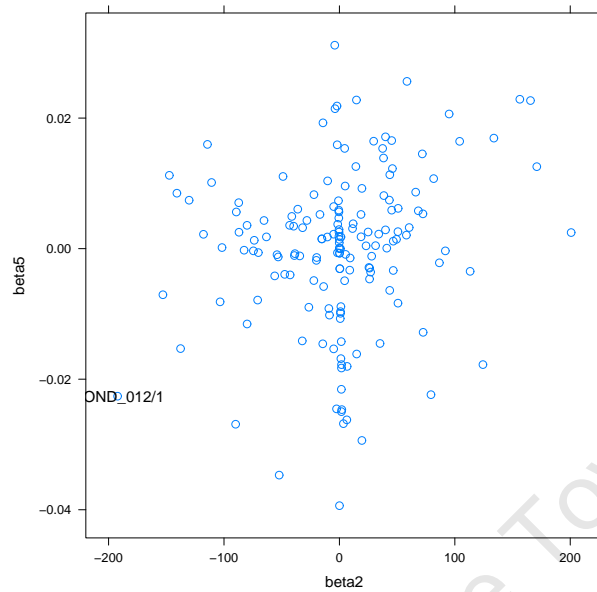


Figure C.18: Pairs Plot of Random Effects (Phase-within-Subject Level):  
Pyrimeth-amine NLME Model 3

-----  
Example Code for Pyrimethamine NLME Model 4:

```

pyr2comp.C=update(pyr2comp.2,
  fixed=list(beta1~1,beta2~1,beta3~1,beta4~preg,
    beta5~1),
  random=list(pid=pdDiag(beta4+beta5~1),
    preg=pdDiag(beta2+beta5~1)),
  start=list(fixed=c(40,100,2,400,70,0.2)),
  control=controlS)
pyr2comp.int=update(pyr2comp.2,
  fixed=list(beta1~1,beta2~1,beta3~1,beta4~preg*site,
    beta5~1),
  random=list(pid=pdDiag(beta4+beta5~1),
    preg=pdDiag(beta2+beta5~1)),
  start=
  list(fixed=c(40,100,2,400,70,500,300,10,5,5,5,0.2)),
  control=controlS)
anova(pyr2comp.C,pyr2comp.int)
summary(pyr2comp.int)

```

-----  
Diagnostic Plots for Pyrimethamine NLME Model 4:

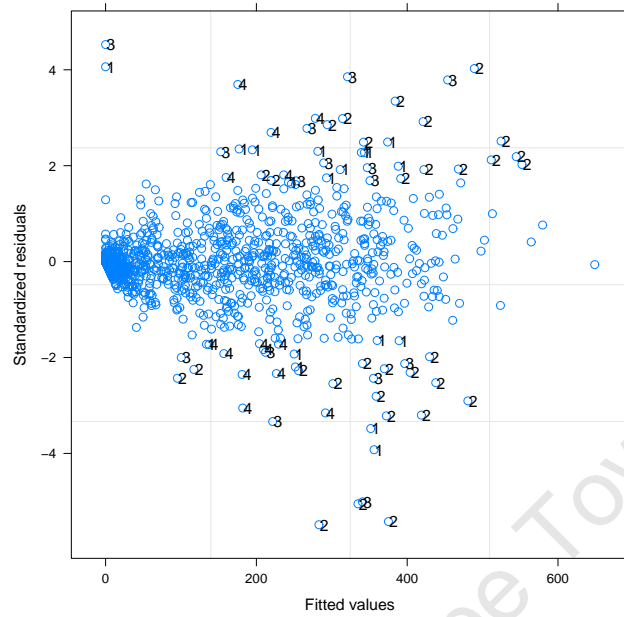


Figure C.19: Standardized Residuals vs. Fitted Values: Pyrimethamine NLME Model 4

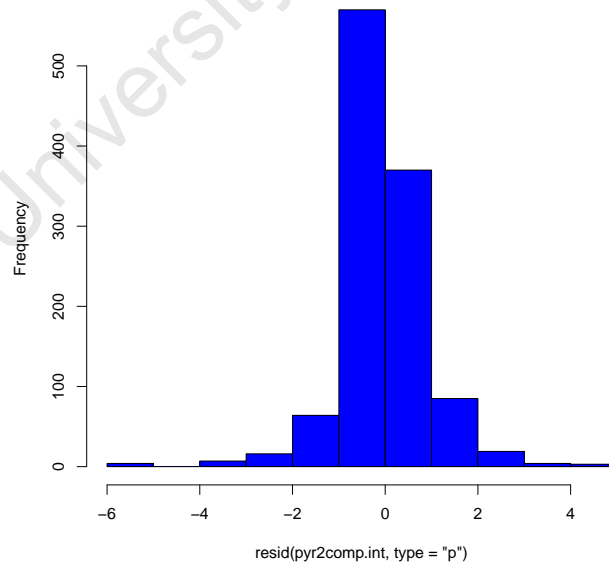


Figure C.20: Histogram of Residuals: Pyrimethamine NLME Model 4

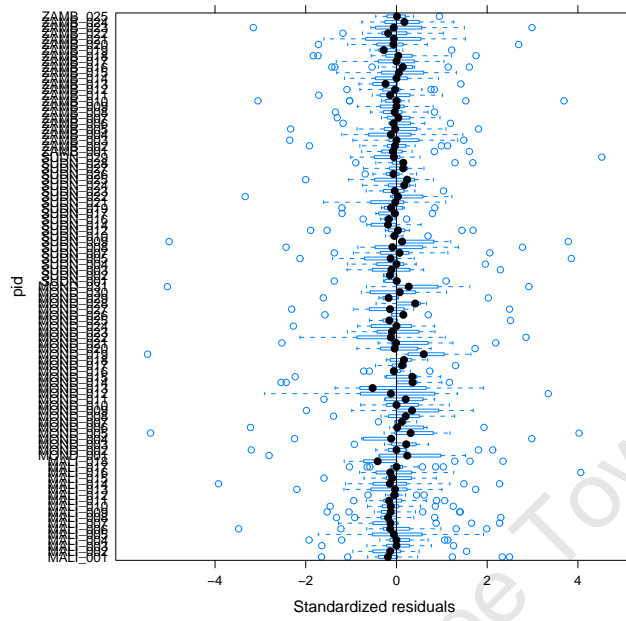


Figure C.21: Residuals by Subject ID: Pyrimethamine NLME Model 4

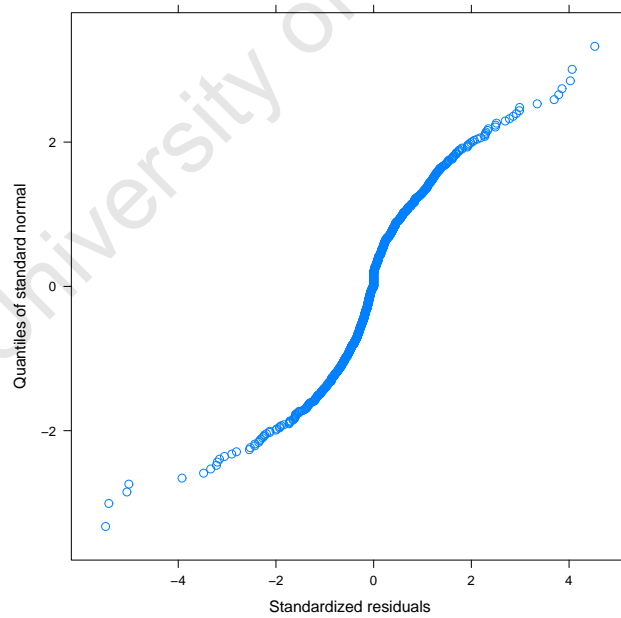


Figure C.22: QQplot of Residuals: Pyrimethamine NLME Model 4

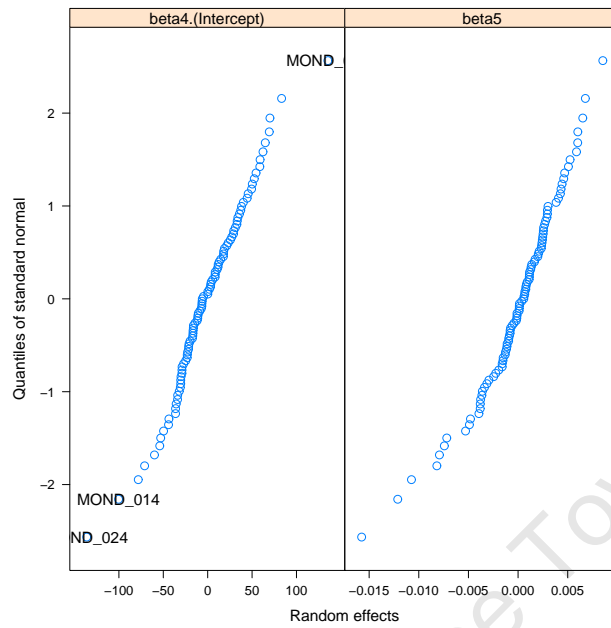


Figure C.23: QQplot of Random Effects (Subject Level): Pyrimethamine NLME Model 4

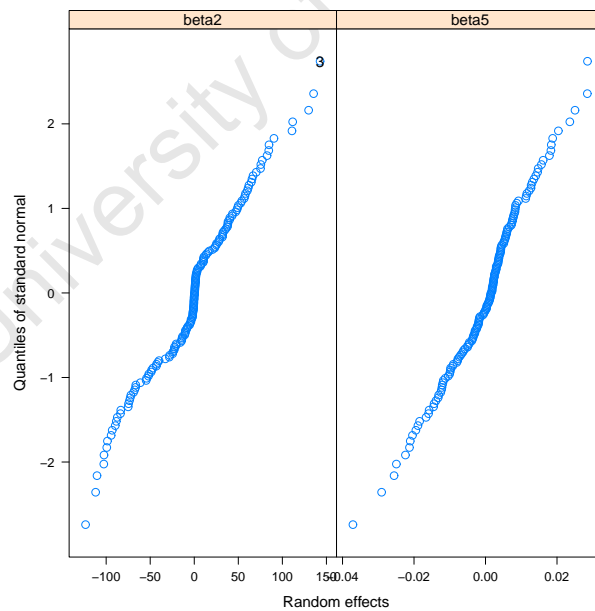


Figure C.24: QQplot of Random Effects (Phase-within-Subject Level): Pyrimethamine NLME Model 4

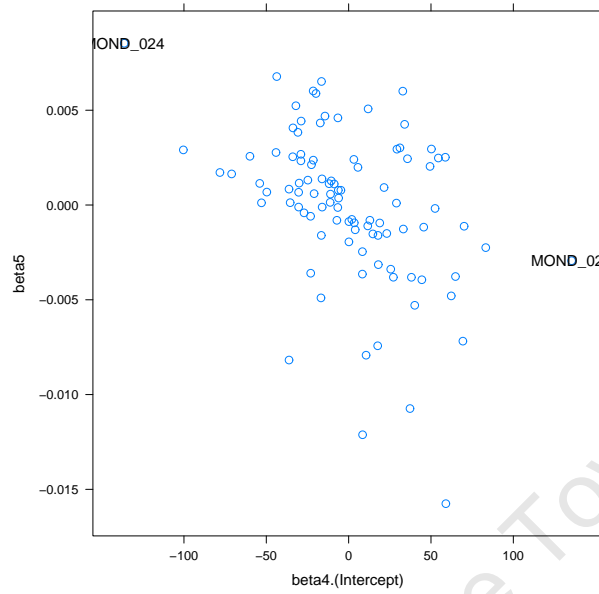


Figure C.25: Pairs Plot of Random Effects (Subject Level): Pyrimethamine NLME Model 4

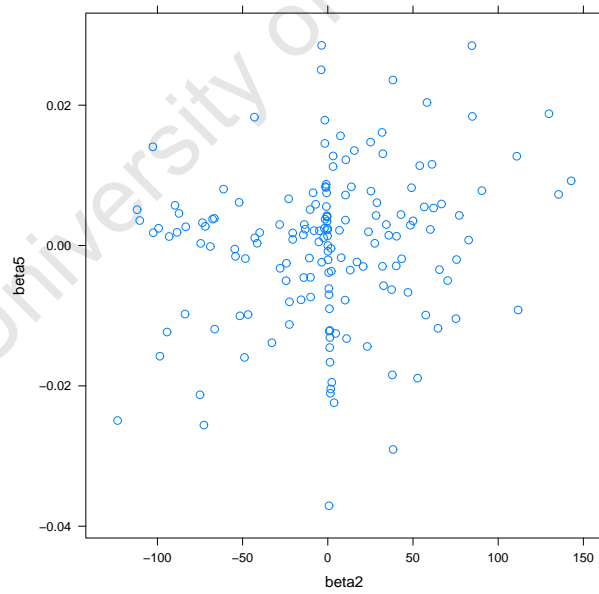


Figure C.26: Pairs Plot of Random Effects (Phase-within-Subject Level): Pyrimethamine NLME Model 4

Comparison of PK Parameters by Site and Pregnancy Status: Pyrimethamine NLME Model 4 (Nyunt et al. Reported Values [5])

Country	Parameter	Model 4				Nyunt et al. (Reported) [5]			
		Pregnant		Postpartum		Pregnant		Postpartum	
		Mean	Std. Error	Mean	Std. Error	Median	IQR	Median	IQR
Mali	$AUC_{(0-inf)}$ (ng.day/ml)	2040.28	110.9	1439.23	107.13	1,715	(1,528;1,920)	1,104	(791;1,251)
	Cl/f (ml/kg/day)	602.62	32.75	877.3	65.3	717	(620;827)	1,219	(1,019;1,695)
	Cmax (ng/ml)	304.07	40.7	250.9	35.96	362	(340;429)	323	(281;371)
Mozambique	$AUC_{(0-inf)}$ (ng.day/ml)	3469.98	120.95	3088.15	186	3,277	(2,810;4,237)	N/D	N/D
	Cl/f (ml/kg/day)	354.33	12.35	408.86	24.63	335	(309;466)	N/D	N/D
	Cmax (ng/ml)	489.77	73.18	431.75	51.23	508	(425;631)	N/D	N/D
Sudan	$AUC_{(0-inf)}$ (ng.day/ml)	2583.93	109.62	2441.41	243.73	2,014	(1,626;2,551)	N/D	N/D
	Cl/f (ml/kg/day)	475.83	20.19	517.17	51.63	600	(423;689)	N/D	N/D
	Cmax (ng/ml)	372.92	50.75	359.52	30.66	400	(325;473)	N/D	N/D
Zambia	$AUC_{(0-inf)}$ (ng.day/ml)	1198.46	83.69	1010.82	NaN**	1,047	(868;1,175)	804	(668;1,032)
	Cl/f (ml/kg/day)	1025.9	71.64	1249.11	NaN	1,305	(989;1,458)	1,466	(1,325;1,907)
	Cmax (ng/ml)	192.54	43.65	161.26	32.4	241	(198;271)	190	(174;231)

Table C.1: Comparison of PK parameters by Site and Pregnancy Phase: Pyrimethamine NLME Model 4

N/D\*= Not determined, NaN\*\*= Computation not possible



## Appendix D

# Sequential Models

---

Example Code for Sequential NLME Model (Impact of Pyrimethamine predicted concentrations on Sulfadoxine):

---

```
#BASIC MODEL
seq1comp.0=nlme(sulfadoxine~fm1comp(beta0,beta1,beta2,day),
               data=sulfnew,
               fixed=list(beta0~1,beta1~1,beta2~1),
               random=list(pid=pdDiag(beta0+beta1+beta2~1),
                             preg=pdDiag(beta0+beta1+beta2~1)),
               start=list(fixed=c(70,10,0.07)),
               control=controlS)

summary(seq1comp.0)

#COVARIATES
seq1comp.1=nlme(sulfadoxine~fm1comp(beta0,beta1,beta2,day),
               data=sulfnew,
               fixed=list(beta0~pyr_pred,beta1~1,beta2~1),
               random=list(pid=pdDiag(beta0+beta1~1),
                             preg=pdDiag(beta0+beta1+beta2~1)),
               start=list(fixed=c(70,0,10,0.06)),
               control=controlS)

summary(seq1comp.1)
```

---

Random Effects versus Covariates for Sequential NLME Model 1:

---

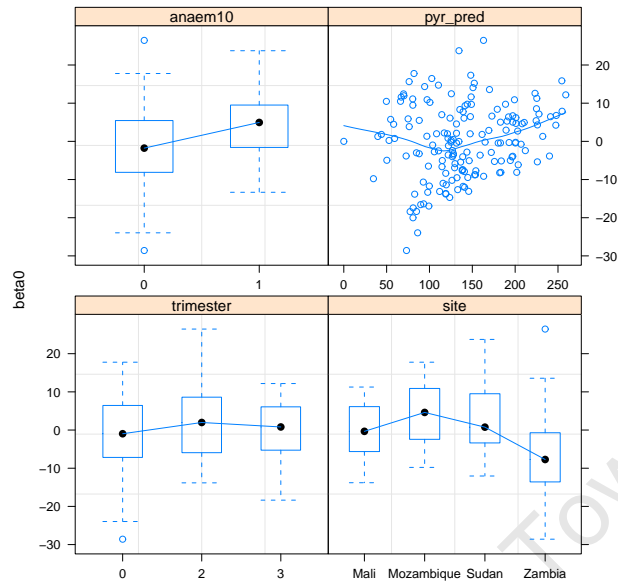


Figure D.1: Occasion-Specific Random Effects for  $\beta_0$  vs. Covariates: Sequential NLME Model 1

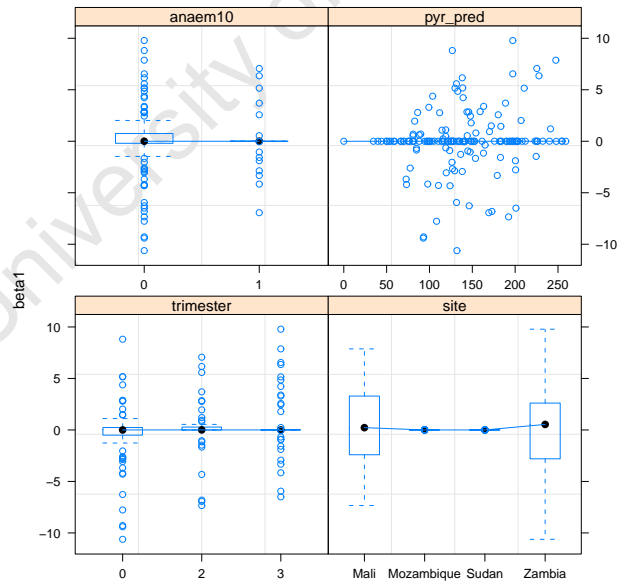


Figure D.2: Occasion-Specific Random Effects for  $\beta_1$  vs. Covariates: Sequential NLME Model 1

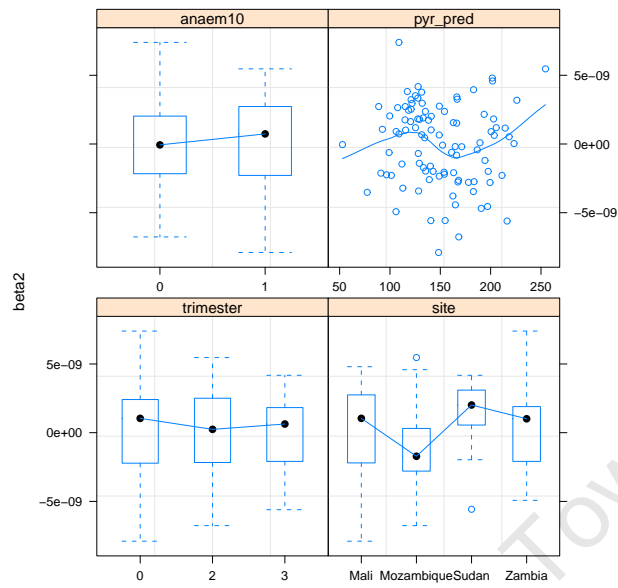


Figure D.3: Subject-Specific Random Effects for  $\beta_2$  vs. Covariates: Sequential NLME Model 1

---

Diagnostic Plots for Sequential NLME Model 2:

---

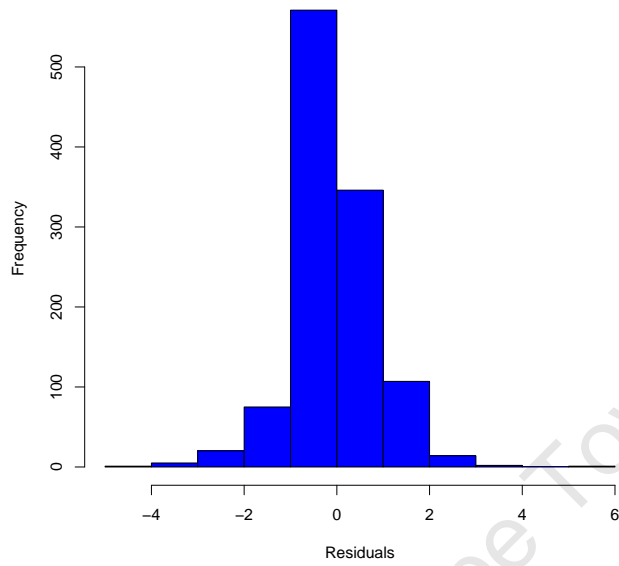


Figure D.4: Histogram of Residuals: Sequential NLME Model 2

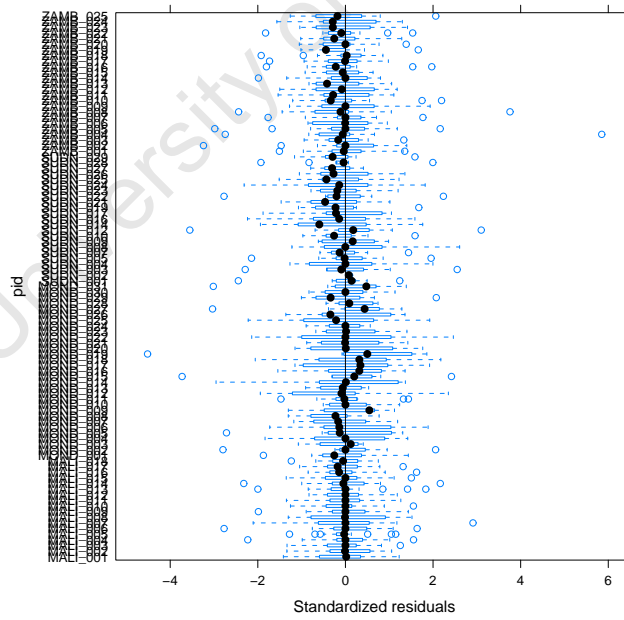


Figure D.5: Residuals by Subject ID: Sequential NLME Model 2

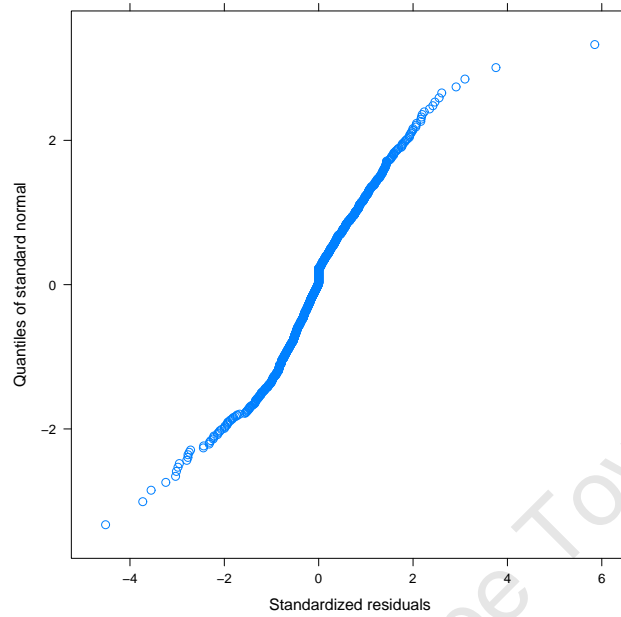


Figure D.6: QQplot of Residuals: Sequential NLME Model 2

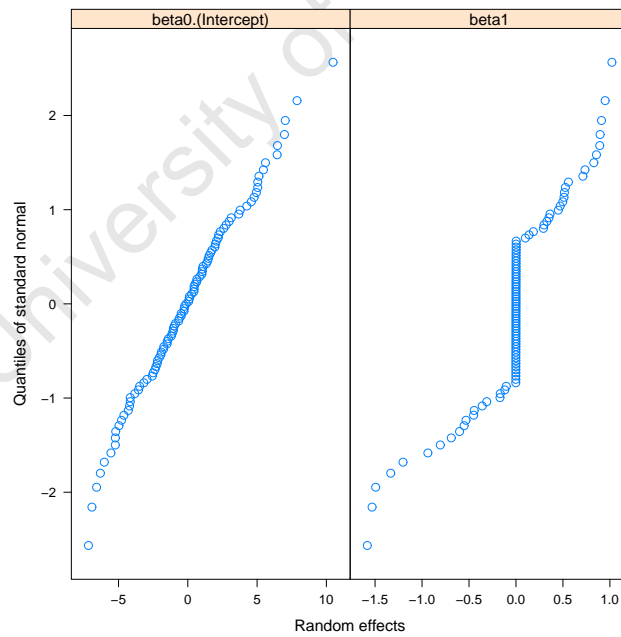


Figure D.7: QQplot of Random Effects (Subject Level): Sequential NLME Model 2

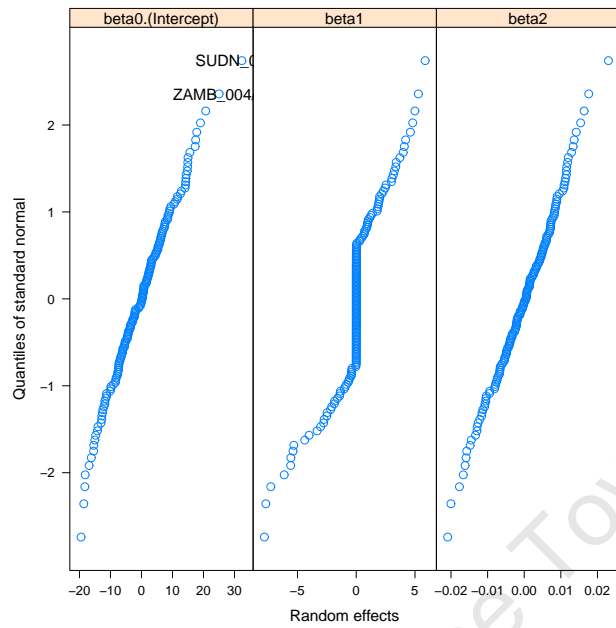


Figure D.8: QQplot of Random Effects (Phase-within-Subject Level): Sequential NLME Model 2

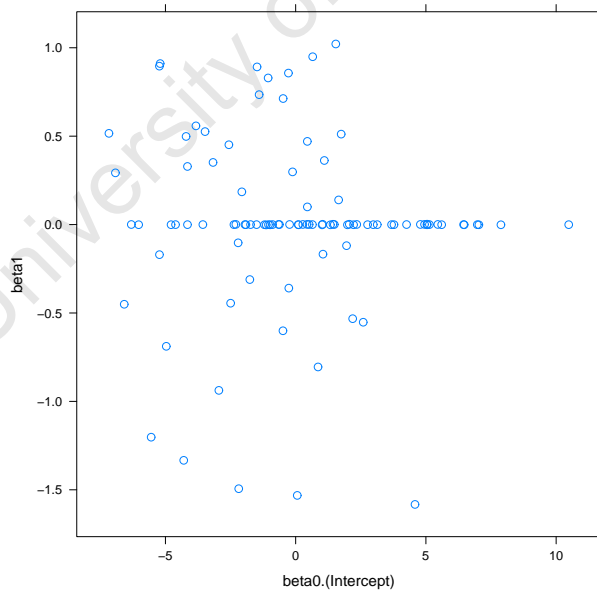


Figure D.9: Pairs Plot of Random Effects (Subject Level): Sequential NLME Model 2

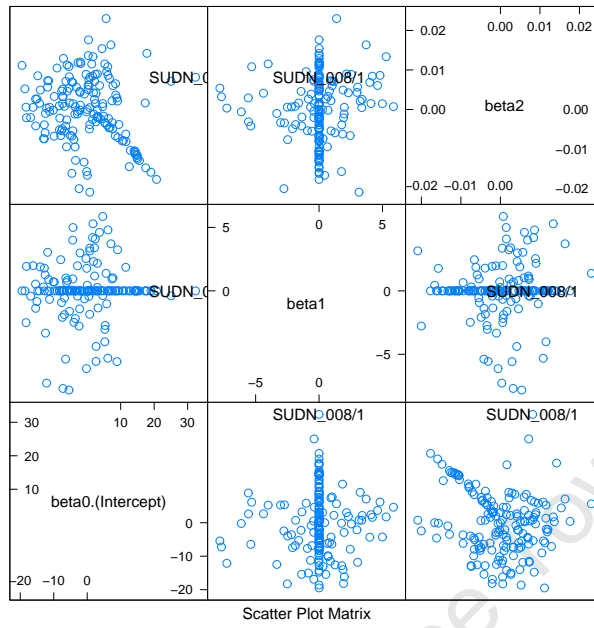


Figure D.10: Pairs Plot of Random Effects (Phase-within-Subject Level): Sequential NLME Model 2

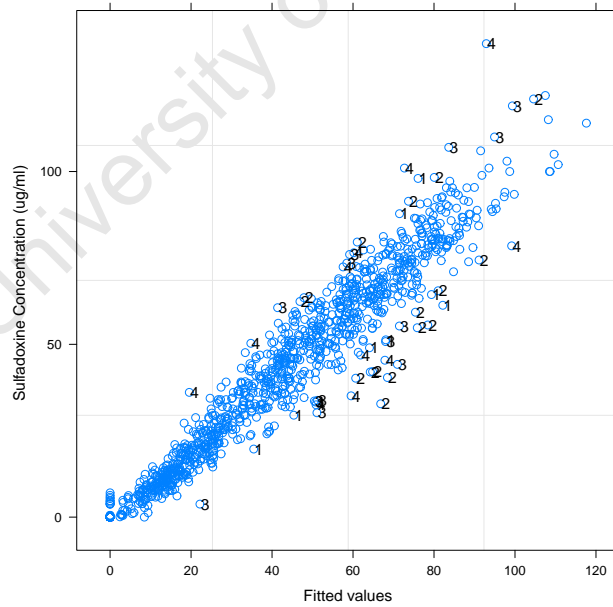


Figure D.11: Fitted Values vs. Observed: Sequential NLME Model 2

---

Diagnostic Plots for Sequential NLME Model 3:

---

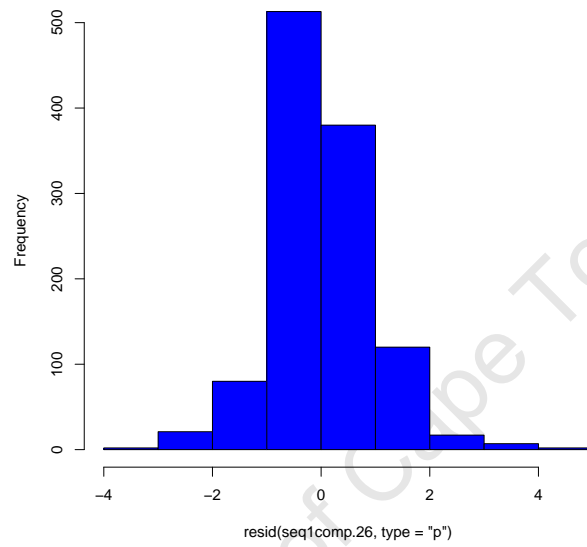


Figure D.12: Histogram of Residuals: Sequential NLME Model 3



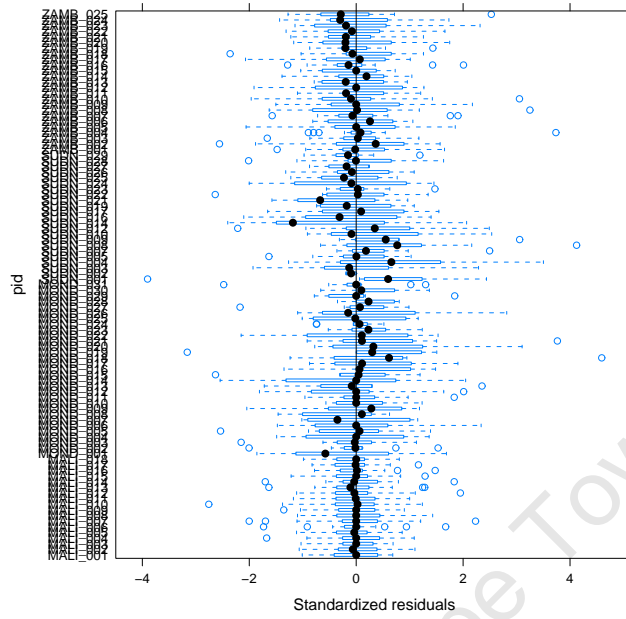


Figure D.13: Residuals by Subject ID: Sequential NLME Model 3

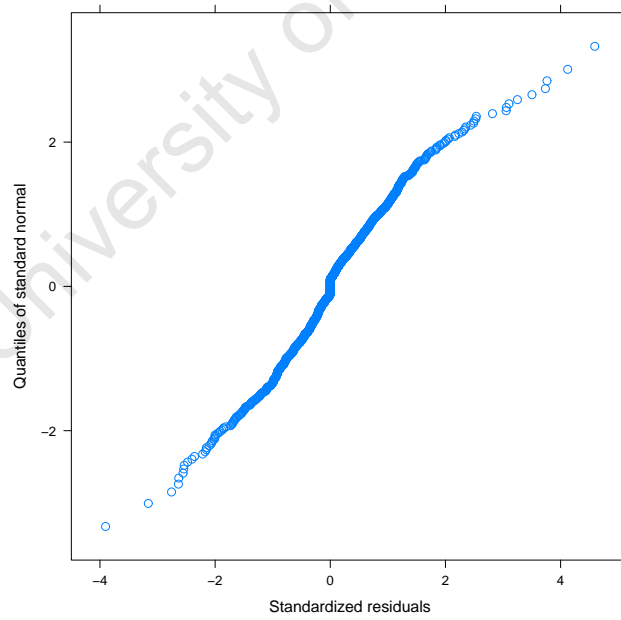


Figure D.14: QQplot of Residuals: Sequential NLME Model 3

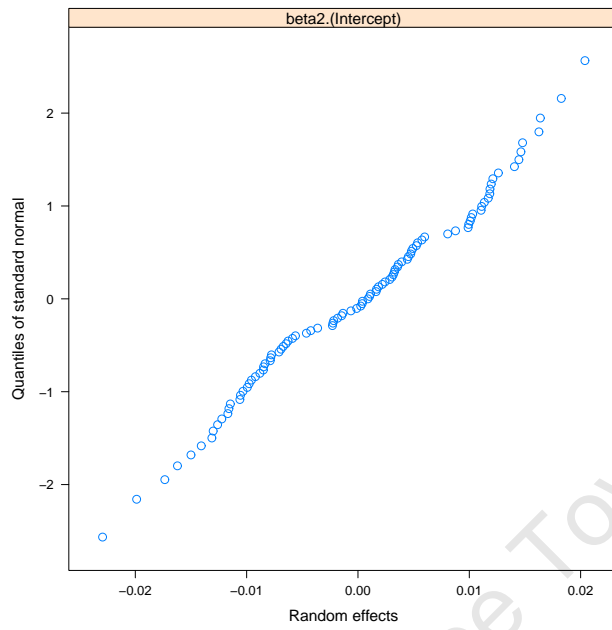


Figure D.15: QQplot of Random Effects (Subject Level): Sequential NLME Model 3

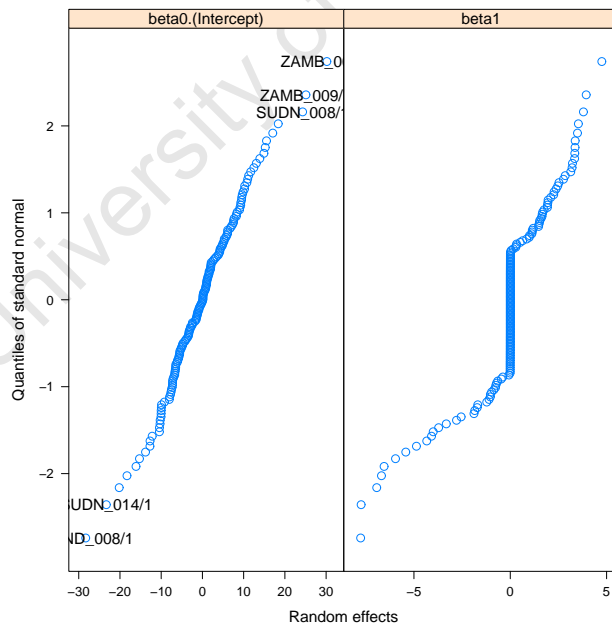


Figure D.16: QQplot of Random Effects (Phase-within-Subject Level): Sequential NLME Model 3

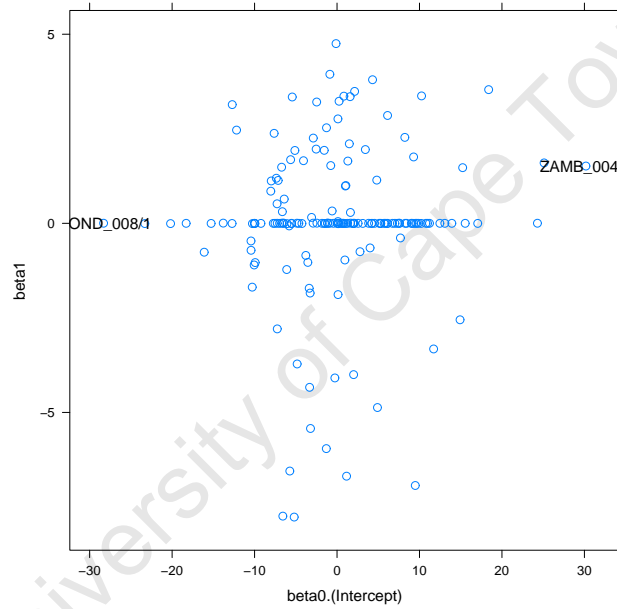


Figure D.17: Pairs Plot of Random Effects (Phase-within-Subject Level): Sequential NLME Model 3

## Appendix E

# Simultaneous Models (Covariate Specification)

---

Example Code for Simultaneous NLME Model (Covariate Specification):

---

```
#TRIPLE-EXPONENTIAL: BASIC MODEL
simcov2.basic=nlme(conc~fm2comp(beta1,beta2,beta3,beta4,beta5,day),
  data=dta,
  fixed=(beta1+beta2+beta3+beta4+beta5~1),
  random=list(pid=pdDiag(beta2+beta3+beta4+beta5~1),
  preg=pdDiag(beta2+beta3+beta4+beta5~1)),
  start=c(beta1=45,beta2=330,beta3=0.2,beta4=100,beta5=1),
  control=controls)
summary(simcov2.basic)

#FITTING DRUG TYPE
simcov2.1=update(simcov2.basic,
  fixed=list(beta1~pk_fact,beta2~pk_fact,beta3~pk_fact,
  beta4~pk_fact,beta5~pk_fact),
  random=list(pid=pdDiag(beta2+beta3+beta4+beta5~1),
  preg=pdDiag(beta2+beta3+beta4+beta5~1)),
  start=list(fixed=c(45,0,330,0,0.2,0,100,0,1,0)),
  control=controls)
anova(simcov2.basic,simcov2.1)
summary(simcov2.1)
```

---

Diagnostic Plots for Simultaneous NLME Model 1 (Covariate Specification)

---

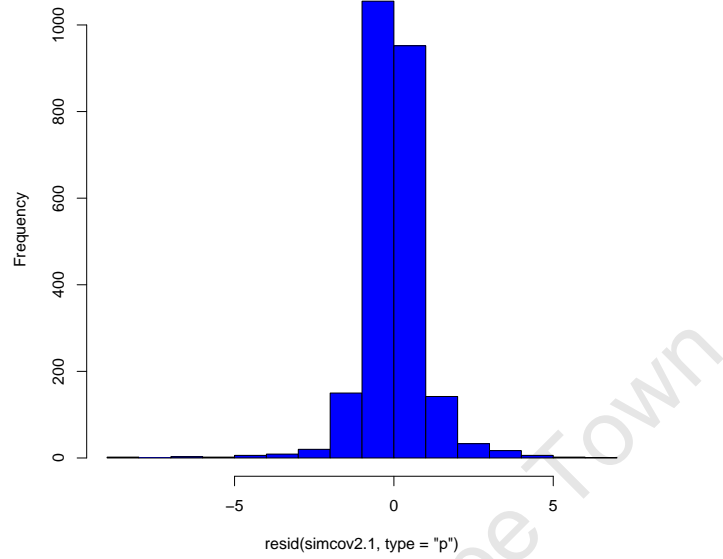


Figure E.1: Histogram of Residuals: Simultaneous NLME Model 1

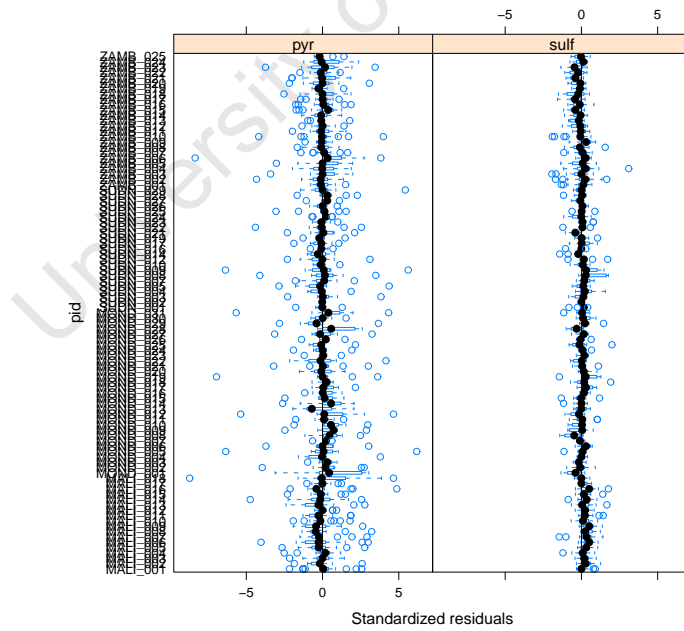


Figure E.2: Residuals by Subject ID: Sequential NLME Model 1

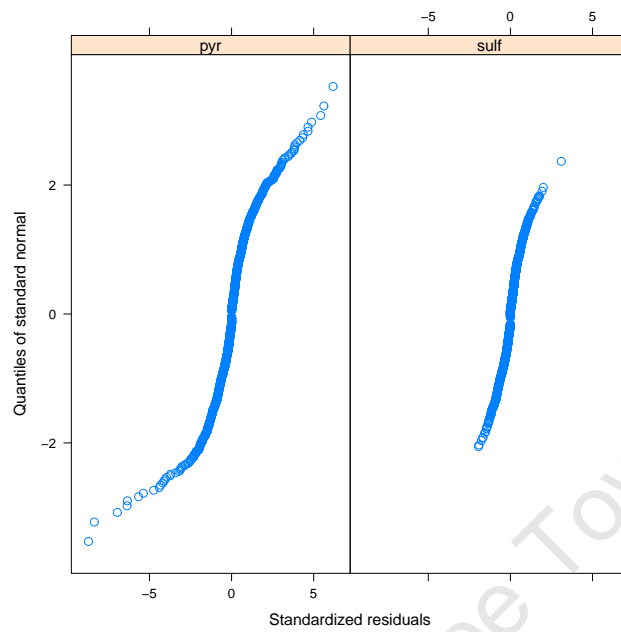


Figure E.3: QQplot of Residuals: Simultaneous NLME Model 1

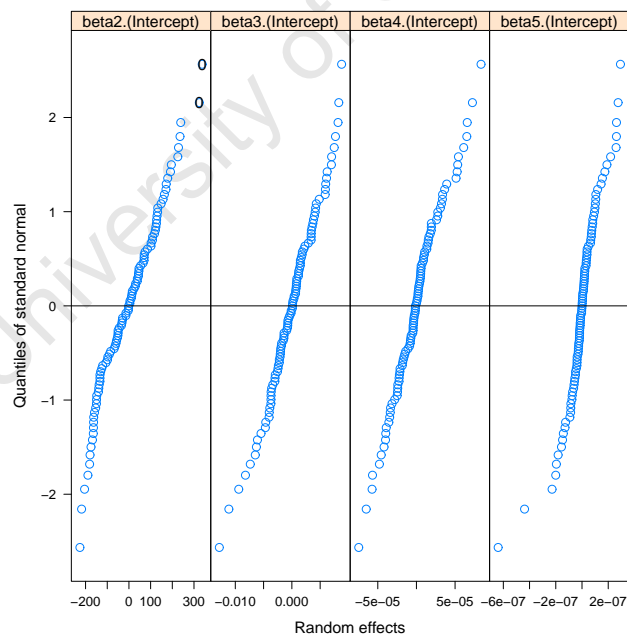


Figure E.4: QQplot of Random Effects (Subject Level): Simultaneous NLME Model 1

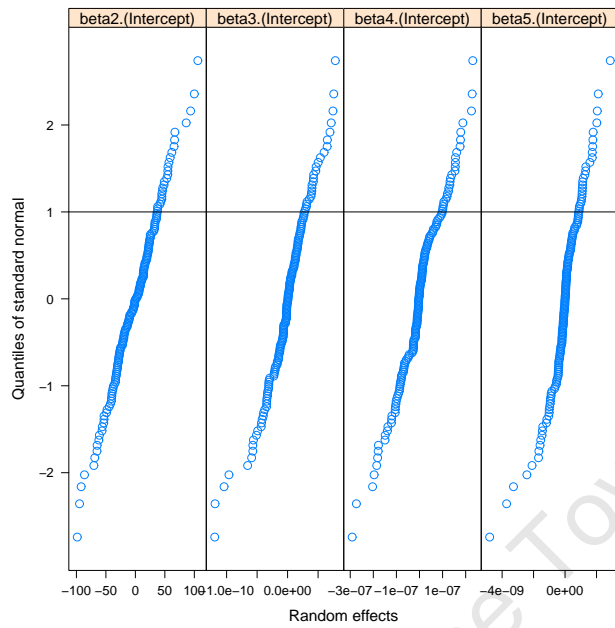


Figure E.5: QQplot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 1

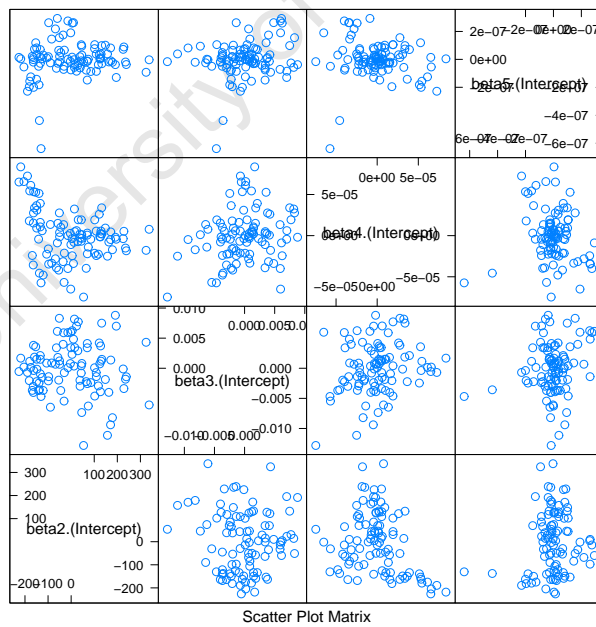


Figure E.6: Pairs Plot of Random Effects (Subject Level): Simultaneous NLME Model 1

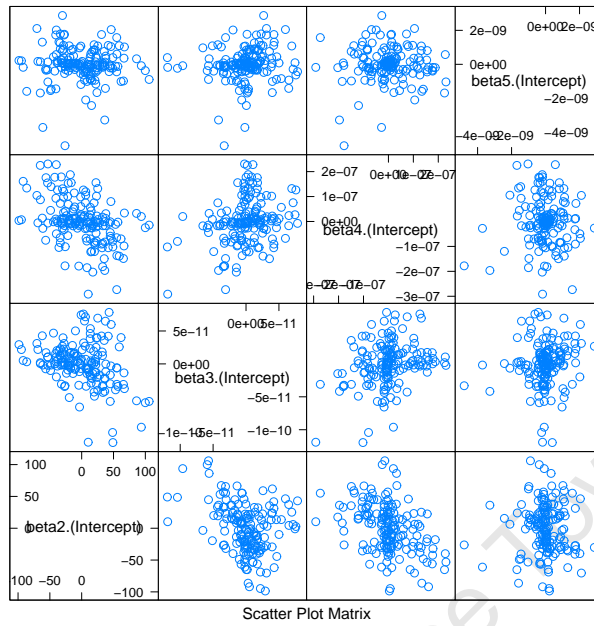


Figure E.7: Pairs Plot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 1

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Random Effects vs. Covariates for Simultaneous NLME Model 2 (Covariate Specification)

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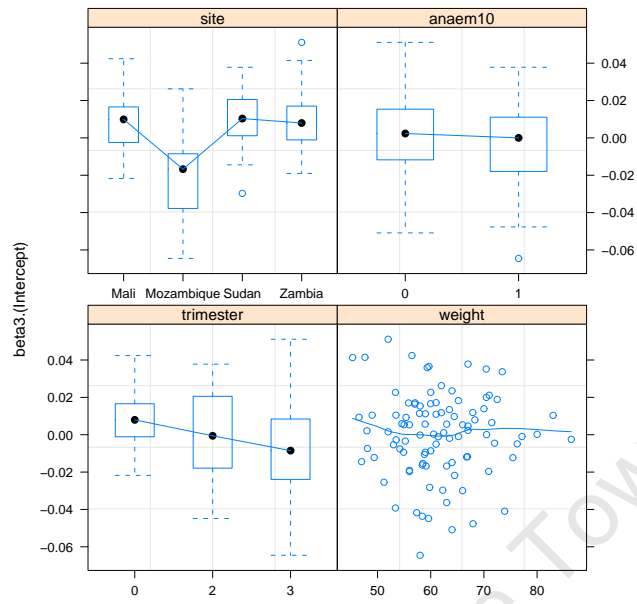


Figure E.8: Occasion-Specific Random Effects for  $\beta_3$  vs. Covariates: Simultaneous NLME Model 2

---

Diagnostic Plots for Simultaneous NLME Model 5 (Covariate Specification)

---

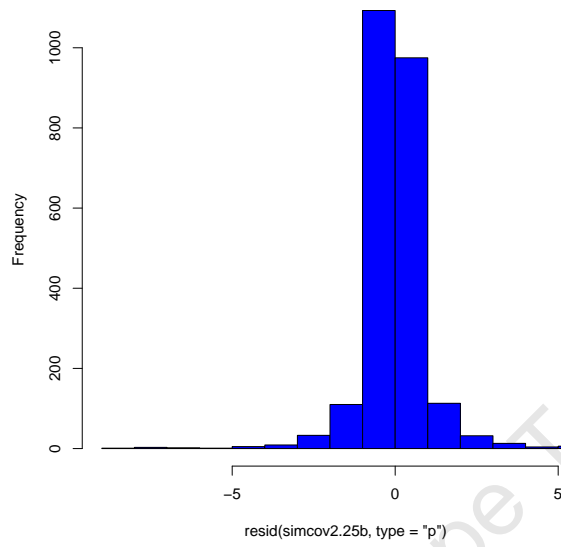


Figure E.9: Histogram of Residuals: Simultaneous NLME Model 5

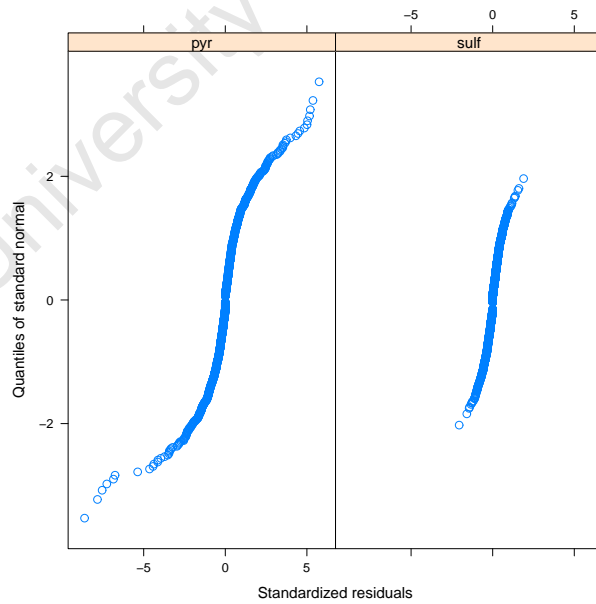


Figure E.10: QQplot of Residuals: Simultaneous NLME Model 5

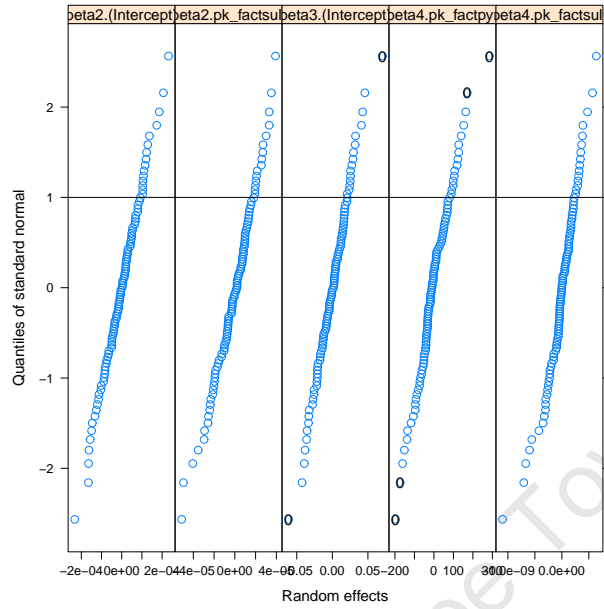


Figure E.11: QQplot of Random Effects (Subject Level): Simultaneous NLME Model 5

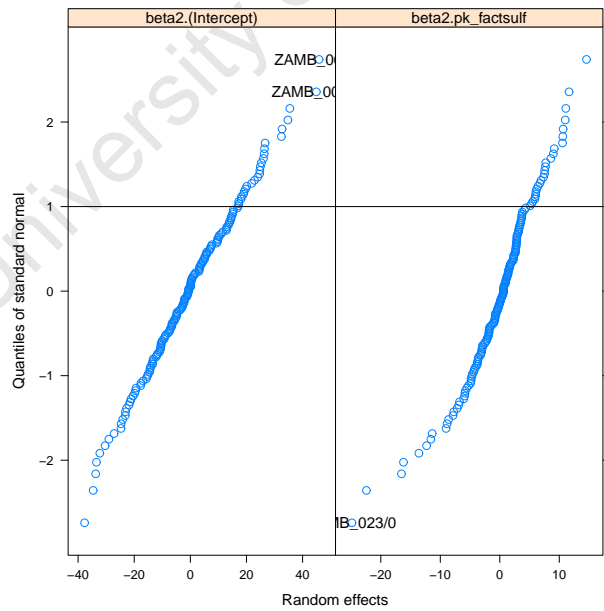


Figure E.12: QQplot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 5

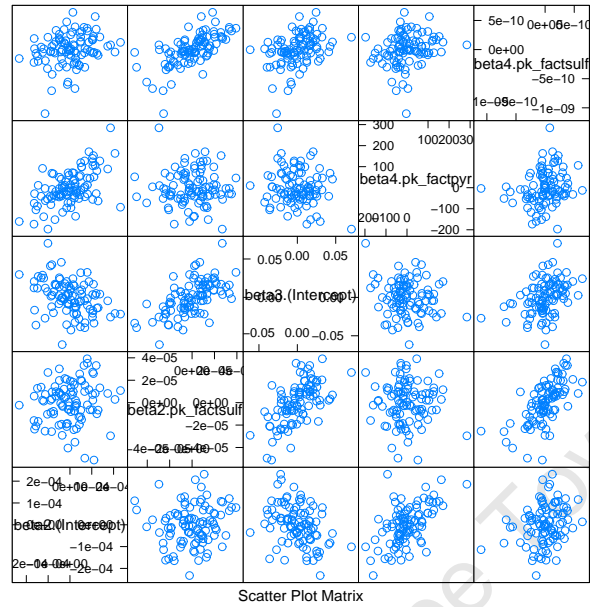


Figure E.13: Pairs Plot of Random Effects (Subject Level): Simultaneous NLME Model 5

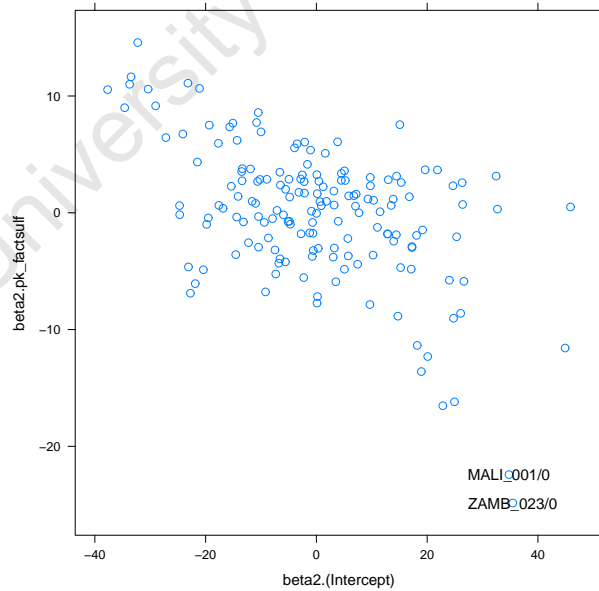


Figure E.14: Pairs Plot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 5

## Appendix F

# Simultaneous Models (Indicator Specification)

---

Example Code for Simultaneous NLME Model (Indicator Specification):

---

```
simcov_indic.16=update(simcov_indic.6,  
  fixed=list(beta0~preg+sitenew3,beta1~1,beta2~preg,  
  beta3~1,beta4~preg+site,beta5~1,beta6~1,beta7~1),  
  random=list(pid=pdBlocked(list(pdDiag(beta0~1),pdDiag(beta3+beta4~1))),  
  preg=pdBlocked(list(pdDiag(beta0~1),pdDiag(beta3+beta4~1))),  
  start=list(fixed=(c(70,0,0,0,10,0.06,0,150,400,70,500,300,50,40,2,0.2))),  
  control=controlS)  
summary(simcov_indic.16)  
anova(simcov_indic.14,simcov_indic.16)
```

---

Diagnostic Plots for Simultaneous NLME Model 1 (Indicator Specification)

---

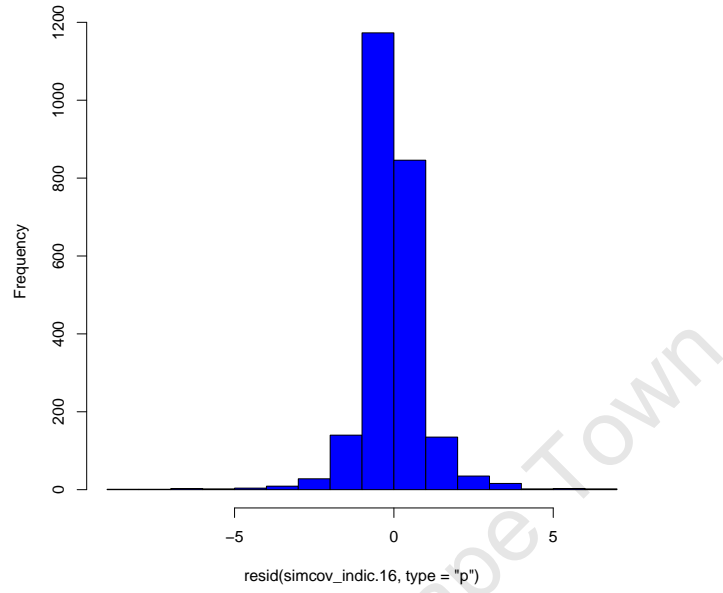


Figure F.1: Histogram of Residuals: Simultaneous NLME Model 1 (Indicator)

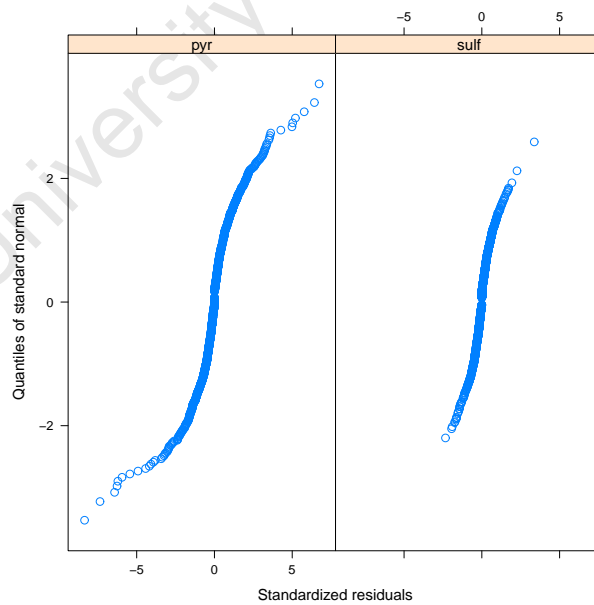


Figure F.2: QQplot of Residuals: Simultaneous NLME Model 1 (Indicator)

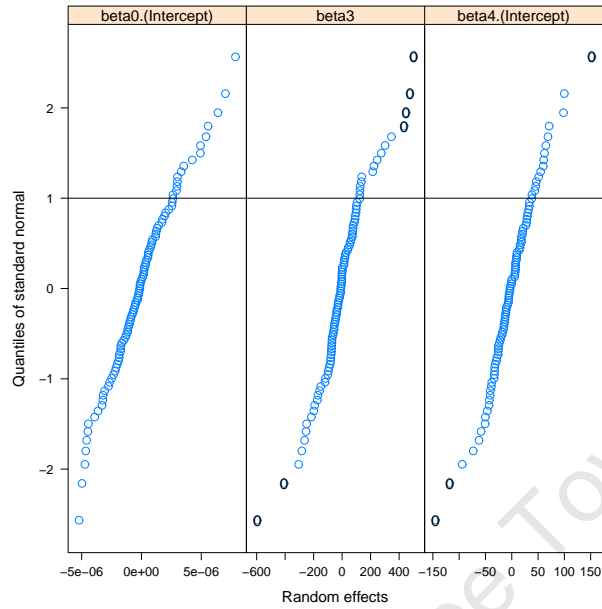


Figure F.3: QQplot of Random Effects (Subject Level): Simultaneous NLME Model 1 (Indicator)

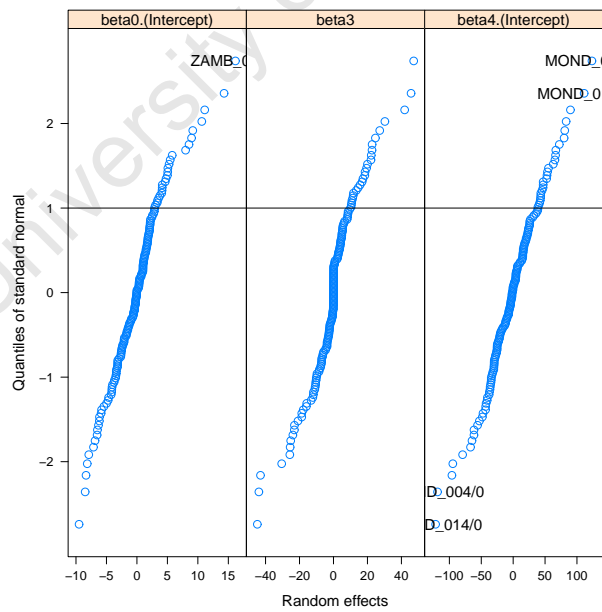


Figure F.4: QQplot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 1 (Indicator)

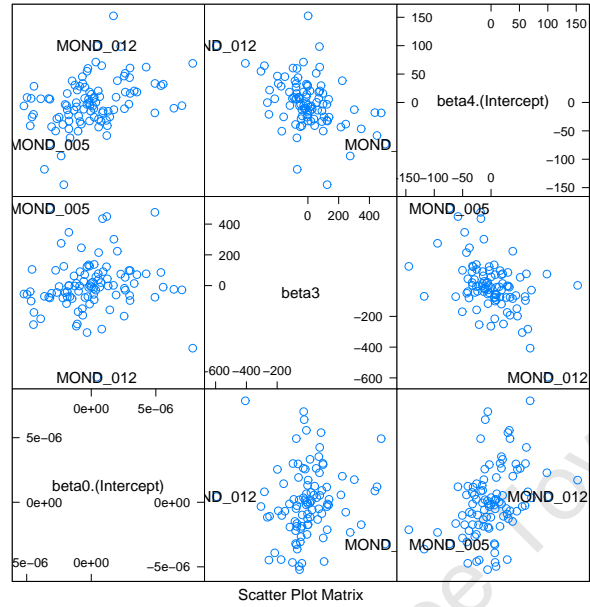


Figure F.5: Pairs Plot of Random Effects (Subject Level): Simultaneous NLME Model 1 (Indicator)

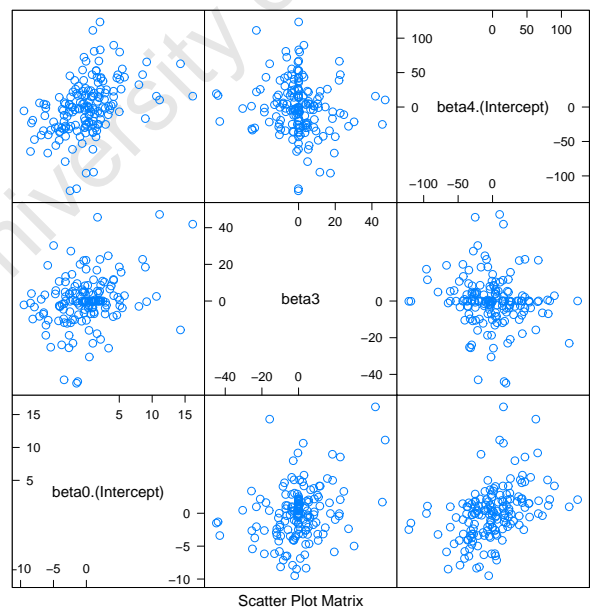


Figure F.6: Pairs Plot of Random Effects (Phase-within-Subject Level): Simultaneous NLME Model 1 (Indicator)



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