THE DETERMINATION AND VALIDATION OF POPULATION PHARMACOKINETIC PARAMETERS OF PHENYTOIN IN ADULT EPILEPTIC PATIENTS IN THE WESTERN CAPE USING NONLINEAR MIXED-EFFECTS MODELLING

BY

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To my wife, Indira, for her understanding, sacrifice, support and constant encouragement.

To my children, Sandeep and Rushika for the sacrifice they had to endure.

DECLARATION

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GLOSSARY OF SYMBOLS AND ABBREVIATIONS

θ	Fixed effect population pharmacokinetic parameter
σ^2_{Vm}	Inter-individual variance for Vm
σ^2_{Km}	Inter-individual variance for Km
σ^2_{Cl}	Inter-individual variance for Cl
σ^2_{ϵ}	Residual intra-individual variance
η	Random error term for inter-individual variability with a mean equal to zero and variance matrix σ^2
ε	Random error term for intra-residual variability with a mean equal to zero and variance matrix σ^2_{ϵ}
ALC	Alcohol
BSA	Body surface area
CI	Confidence interval
Cl	Linear clearance of phenytoin
Cp	Serum or plasma phenytoin concentration
Cp_{ss}	Cp at steady-state
CP	Cp at steady-state
Cp_{ss1}	Cp_{ss} obtained after first dose
Cp_{ss2}	Cp_{ss} obtained after second dose
CV	Coefficient of variation
DOBF	Difference between the values of 2 objective functions

DV	Dependent variable
EVID	Event identification
EXP	Exponential
F	Bioavailability factor
FE	Fixed effect
FO	First-order
FOCE	first-order conditional estimation
HT	Height
IBM	Ideal body mass
ID	Identification
Km	Michaelis-Menten constant
LD	Loading dose
MOF	Minimum objective function
MPE	Mean prediction error
MSE	Mean squared error
NONMEM	Nonlinear Mixed-Effects Modelling computer programme
P1, P2	Parameters models P1 and P2, respectively
PE	Prediction error
R	Dosing rate
R_1	First dosing rate
R_2	Second dosing rate
RES	Residuals
RMSE	Root mean squared error
S	Salt form factor
S1, S4	Structural models S1 and S4, respectively
SD	Standard deviation

SE	Standard error of the fixed effect parameter
SMK	Smoking
$t_{90\%}$	Time to reach 90% of steady-state
τ	Time interval between doses (hours)
Var	Variance
Vd	Volume of distribution
Vm	Maximum metabolic rate
WRES	Weighted residuals
WT	Body weight

CONVERSION FORMULA

Readers who wish to convert phenytoin serum concentrations to or from SI units may use the conversion factor of 3.96 :

To convert to SI units : (µg/ml or mg/l) x 3.96 = (µmol/l)

To convert from SI units : (µmol/l) / 3.96 = (µg/ml or mg/l)

ABSTRACT

The pharmacokinetics of phenytoin is complicated by the nonlinearity of the dose-concentration relationship which is a consequence of capacity-limited metabolism. Individualized therapy with phenytoin is therefore optimally required. As no data are available on the population pharmacokinetics of phenytoin in the Western Cape, this study was undertaken to address this issue. This study was conducted prospectively primarily to: (1) investigate the influence of various patient variables on the population pharmacokinetic parameters of phenytoin, (2) assess whether the parallel Michaelis-Menten and first-order elimination model provides a better fit to the data than the Michaelis-Menten model, (3) determine population pharmacokinetic parameter estimates of phenytoin representative of the patient population, and (4) validate and compare the clinical applicability of the parameter estimates and the models.

The study population comprised 332 black and coloured, adult, male and female epileptic patients residing in the Western Cape, South Africa. All patients were on phenytoin monotherapy for the management of their epilepsy and no drugs known to interfere with phenytoin pharmacokinetics were taken concurrently. Clinical pharmacokinetic dosing services were initiated at 9 clinics from which patients were selected for this study. The service entailed a patient interview, a chart review, drug analysis and provision of either a written or verbal consultation report.

The data were analyzed using NONMEM (nonlinear mixed-effects modelling), a computer programme designed for population pharmacokinetic analysis that allows pooling of data from many individuals. The Michaelis-Menten and the parallel Michaelis-Menten and first-order elimination models were fitted to 853 steady-state dose : serum concentration pairs.

The results indicate that patient variables such as body weight, smoking, ethnicity and age ($\geq$65 years), in descending order of importance, significantly influenced Vm (the maximum metabolic rate)($p < 0.05$). Although a significant difference ($p = 0.03$) in Km (the Michaelis - Menten constant, a constant equal to the serum concentration at which the rate of metabolism is one-half the maximum) between black and coloured patients was found, incorporating the influence of ethnicity in Km in the final regression model did not improve the fit of the model to the data. This indicated that the variability in Km was accounted for

by Vm. The scaling factors for smoking, coloured patients and 65 years of age or older in Vm were 1.12, 1.06 and 0.968, respectively.

The parallel Michaelis-Menten and first-order elimination model fitted the data better than when the Michaelis-Menten model was considered alone. With the former model either dose or steady-state serum concentration was used as the dependent variable whereas with the latter model only dose was used as the dependent variable. Three structural models were therefore tested in this study. The results indicated that linear clearance (Cl) of phenytoin should be taken into account, especially when predicting steady-state concentrations. When using the parallel Michaelis-Menten and first-order elimination model with steady-state serum concentrations as the dependent variable, the mean population parameter estimates (standard error in brackets) for Vm, Km and Cl were 5.27 (0.59) mg/kg/day, 6.81 (1.30) mg/l and 2.68 (0.78) l/day, respectively. The inter-individual variability in these parameters was 12.5, 67.9 and 46.3% respectively, and the intra-individual variability was 20.1%. Using this model, Vm values for subpopulation groups were reported. The mean population estimates for Vm and Km obtained with the Michaelis-Menten model were 5.71 (0.10) mg/kg/day and 3.45 (0.24) mg/l, respectively. The inter-individual variability in these parameters was 13.2 and 49.1%, respectively, and the intra-individual variability was 5%. The Vm and Km estimates obtained in this study were generally lower than those reported in the literature.

Each model was evaluated for relative predictive performance using the measures of bias and precision, i.e. mean prediction error and the root mean squared error respectively. Three sets of population parameter estimates, i.e. one set for each model, were tested. The methods of cross-validation and naive feedback were employed. Predictions obtained with the first-order and first-order conditional estimates were compared. The results indicated that the parallel Michaelis-Menten and first-order elimination model was not much better than the Michaelis-Menten model when predicting dose. However, when predicting steady-state serum concentration, the former model was clearly superior to the latter. This study has also confirmed that when using representative parameter estimates (taking into account factors shown to influence these parameters) for a population, predictive performance improved. All predictions using the estimates obtained with the first-order method were biased. When using the estimates obtained with the first-order conditional estimation method, the bias improved without an improvement in the precision of the predictions.

Although the Bayesian feedback technique has proved superior to all other currently available techniques for phenytoin dosage design, prerequisites for obtaining accurate results with this method are good initial estimates of the pharmacokinetic parameters and their variances. The greater the confidence in the prior information, the better will be the performance of the associated Bayesian system. This study has provided estimates of Vm, Km and linear clearance, and their inter- and intra-individual variability, for use in a pharmacokinetic programme with Bayesian forecasting.

CHAPTER 1

INTRODUCTION

Phenytoin is one of the most effective drugs against partial and generalized tonic-clonic seizures. It is also used for the control of status epilepticus.

Few thorough evaluations of the influence of demographic factors on phenytoin kinetics have been performed. A number of investigators have studied the pharmacokinetics of phenytoin in different population groups and it has been well documented that this may differ in the different population groups. To prescribe phenytoin optimally it is necessary to investigate its pharmacokinetics in the respective population groups (Breimer, 1983). Most often, these studies have involved a small number of patients, a limited number of serum phenytoin concentrations, and they have been retrospective in nature. The pharmacokinetic methods of analysis are also often suboptimal.

In the individualization of phenytoin dosage, it is important to use estimates of the pharmacokinetic parameters (Vm - the maximum metabolic rate and Km - the Michaelis-Menten constant, a constant which reflects the serum concentration at which the rate of metabolism is one-half the maximum) that are representative of the population concerned or, even better, the individual for whom the drug is prescribed (Miller *et al.*, 1987). The determination of representative Vm and Km values, their inter- and intra-individual variability, and knowledge of which factors influence these values in a population will allow rapid establishment of a patient's parameter values (Sheiner and Beal, 1982). This, in turn, will allow optimal maintenance dosage to be determined early in the course of therapy (Vozeh *et al,* 1981). Proper characterization of pharmacokinetic behaviour is also a prerequisite for the rational interpretation of dose-response relationships. Studies of this nature should preferably be performed prospectively (Vesell and Penno, 1983).

The influence of various factors (ethnicity, gender, age, mass and body surface area) on Vm and Km has been previously investigated, but not in South African patients. The influence of these factors needed investigation in a carefully controlled and prospective manner in the South African population. An understanding of how patient characteristics may influence dosage requirements is particularly important for drugs with a narrow

therapeutic range e.g. phenytoin. Miller *et al.* (1987), in a retrospective study, demonstrated that the Vm and Km values representative of the black population attending Baragwanath Hospital, Johannesburg, South Africa, were lower than those reported in the literature. Whether this difference also applies to the coloured population of South Africa was one of the aspects investigated in this study.

The inter-individual variability in Vm and Km is large and using average Vm and Km values, as is commonly done despite the wide inter-patient variability in these parameters, may lead to large prediction errors. To prevent this, the determination of Vm and Km values for subpopulation groups will enable the clinical pharmacist to choose Vm and Km values most appropriate for a selected patient.

Knowledge of representative Vm and Km values for the South African population should: (a) allow calculation of an accurate initial dosage regimen; (b) provide a good indication of when the serum phenytoin concentration will be at steady state, and when blood samples should ideally be drawn; (c) improve the accuracy of the prediction of steady-state serum concentrations; (d) allow estimation of the maximum dosage that should initially not be exceeded in patient subgroups; (e) facilitate construction of applicable nomograms for the South African population; (f) allow identification of patients who are likely to be more sensitive to phenytoin dosage adjustment; and (g) in the case of toxicity, allow more accurate calculation of how long a patient should be withdrawn from therapy so that the serum level might fall to a targeted level, and when therapy should be reinstituted.

All the methods that have been used clinically to predict phenytoin dose or serum concentration have traditionally taken into account Vm and Km values only. The influence of parallel Michaelis-Menten metabolism and first-order kinetics (i.e. linear clearance, Cl, of phenytoin) on prediction outcome has not been tested. In other words, the incorporation of linear clearance of phenytoin in a model with Vm and Km has not been validated in patients routinely taking phenytoin. The estimates of Vm, Km and Cl in such a model are also not available.

The population parameter estimates and their inter- and intra-subject variances obtained from this analysis will be used in a Bayesian forecasting computer programme for the optimization of individual dosage regimens for the population under investigation. The

accuracy and precision of Bayesian regression based on limited feedback is dependent on accurate initial population parameter estimates (Killilea *et al.*, 1989; Nonaka *et al.*, 1993). Since the Bayesian method relies on previous estimates of population parameters (means and standard deviations), it may be necessary to adjust these population values for different groups of patients (Yuen *et al.*, 1983a).

The objectives of this study were:

(a) to evaluate the influence of ethnicity, gender, age, mass, body surface area, smoking status and mild to moderate alcohol consumption on the population pharmacokinetic parameters (Vm and Km) of phenytoin using the NONMEM approach;

(b) to determine the mean population pharmacokinetic parameter estimates of phenytoin (Vm, Km and Cl) and their inter- and intra-individual variability in representative patient populations;

(c) to determine whether the population pharmacokinetic parameters of phenytoin applicable to South African patients are different from those reported in the literature for patients elsewhere;

(d) to assess whether the parallel Michaelis-Menten and first-order elimination model fits the data better than the Michaelis-Menten model;

(e) to establish a set of valid Vm and Km value combinations for subpopulation groups;

(f) to validate the applicability of the estimated Vm, Km and Cl values;

(g) to assess and compare the reliability of 3 steady-state pharmacokinetic models in predicting phenytoin doses or serum concentrations in epileptic patients.

CHAPTER 2

LITERATURE REVIEW

Numerous investigators have shown the benefit of monitoring phenytoin (formerly called diphenylhydantoin) serum concentrations to maximize efficacy and minimize drug-related toxicity. The optimal therapy with this drug requires that its administration be tailored to the needs of each individual (Klein *et al.*, 1987; Miller *et al.*, 1982; Lund, 1974). This is necessary as the therapeutic concentration of phenytoin is close to that which causes toxicity; i.e., it has a narrow therapeutic index, and its elimination is saturable within the therapeutic range. Phenytoin is one of the few drugs that, at clinical doses, can saturate hydroxylation by the hepatic microsomal enzymatic system that metabolizes the drug. As a result, serum phenytoin concentrations do not change in proportion to dose but, instead, a relatively small increase in phenytoin dose can cause a disproportionately large increase in the serum phenytoin concentration (Bochner *et al.*, 1972; Richens and Dunlop, 1975; Martin *et al.*, 1977; Mawer *et al.*, 1974; Schiller and Buchthal, 1958). Even a small change in the bioavailability of phenytoin may dramatically affect steady-state serum concentrations and clinical response (Gugler *et al.*, 1976; Sawchuk *et al.*, 1982). The half-life of phenytoin changes with dose and serum concentration. The higher the dose and serum concentration of phenytoin, the longer the half-life (Arnold and Gerber, 1969; Dodson, 1980; Houghton and Richens, 1974). Thus, the time required to reach a new steady state is difficult to predict as it depends on the dose and the patient's pharmacokinetic parameters (Allen *et al.*, 1979). Phenytoin is, therefore, said to exhibit nonlinear pharmacokinetics. The saturation of the microsomal enzymes varies widely among individuals. Wide inter-patient variations in serum phenytoin concentrations also occur (Buchthal and Svensmark, 1959; Lascelles *et al.*, 1970; Ludden *et al.*, 1978). The serum concentration of phenytoin is for this reason undoubtedly difficult to interpret pharmacokinetically (Ludden *et al.*, 1977). This justifies therapeutic drug monitoring of phenytoin (Morrow and Richens, 1989).

2.1 POPULATION PHARMACOKINETICS

Population pharmacokinetics is defined as the study of the variability in serum drug concentrations between individuals in a target population when conventional dosage regimens are administered. This variability within the population is measured and accounted for by patient variables such as ethnicity, age, sex, mass or disease states (Aarons, 1991; Whiting *et al.*, 1986). Good therapeutic practice should ideally be based on an understanding of pharmacokinetic variability. This ensures that dosage adjustments can be made to accommodate differences in pharmacokinetics (Whiting *et al.*, 1986). Population pharmacokinetic methods focus on the central tendency of a response across an entire population and account for deviations from the central tendency in subpopulations or among individual patients (Colburn, 1989). A nomogram designed from data obtained from a particular population may not be suitable for another patient population group. More recently, population pharmacokinetics has also been defined as the design, execution and analysis of sparsely collected data (Aarons, 1991).

Population pharmacokinetics is especially useful in patients where only one or two blood samples for drug assay can be obtained. In this case, population pharmacokinetic parameters can be used together with therapeutic drug monitoring programmes that apply Bayesian feedback techniques (Sheiner and Beal, 1982; Vozeh *et al.*, 1981, 1985) for the adjustment of dosage regimens. A prerequisite for the satisfactory performance of this technique of drug dosage optimization is good estimates of the population parameter distributions. If these are accurate, then the performance of the Bayesian system is improved (Whiting *et al.*, 1986).

Population pharmacokinetic parameters quantify population mean kinetics, inter- and intra-individual kinetic variability, and measurement error (Sheiner and Beal, 1983). A knowledge of the magnitude of the inter- and intra-individual variability should enable one to place a certain level of confidence on the predicted dose or concentration.

2.1.1. POPULATION PARAMETER ESTIMATION IN PHARMACOKINETIC STUDIES

The most commonly used methods of analysing pharmacokinetic data are the Naive Pooled Data (Sheiner, 1984; Sheiner and Beal, 1981), Standard Two Stage (Sheiner and Beal, 1981) and the Nonlinear Mixed-Effects Modelling (NONMEM) methods.

The NONMEM method has been studied in detail and is applied throughout this study. The NONMEM approach is currently considered to be the most satisfactory method for population pharmacokinetic data analysis (Sheiner *et al.*, 1972; 1977). With the Naive Pooled Data method, the averaging procedure may mask the most appropriate model and allow a different model to be justified. No estimate of inter- or intra-individual variability is possible with this method (Sheiner, 1984; Sheiner and Beal, 1981). NONMEM estimates are as good as those of the Standard Two Stage method for mean parameters and for residual variability and they are considerably better for inter-individual variability (Sheiner and Beal, 1981). Other approaches include a nonparametric maximum likelihood method (Mallet, 1986), Bayesian methods (Racine-Poon and Smith, 1990), and variants of NONMEM (Amisaki, and Tatsuhara, 1988; Lindstrom and Bates, 1990).

2.1.2. DESCRIPTION OF NONMEM

NONMEM is a sophisticated computer programme and complex data analysis technique that concentrates on the central tendency of the pharmacokinetic information, and it is designed for the calculation of population pharmacokinetic parameters from sparsely collected data (Aarons, 1991). This programme uses the extended least squares method to estimate population pharmacokinetic parameters. Nonlinear mixed-effects regression recognizes two sources of deviation from a predicted response, i.e. inter- and intra-individual variation.

The introduction of the NONMEM computer programme has facilitated the identification of factors which influence pharmacokinetics, and it has enabled the quantification of pharmacokinetic variability using routine clinical data (Sheiner *et al.*, 1972). The ability of NONMEM to use routine clinical data is a distinct advantage compared with other methods of population pharmacokinetic data analysis.

NONMEM describes the available data in terms of a number of fixed and random effect parameters. Fixed effects are population average values of parameters such as clearance, volume of distribution, maximum metabolic rate and Michaelis-Menten constants. Random effects quantify the amount of pharmacokinetic variability which is not explained by the fixed effects i.e. inter- and intra-subject variability. The NONMEM programme simultaneously derives fixed effects and random effects - hence the name 'mixed-effects modelling'. The fixed effect parameters measure central tendency in the values of the parameters and/or a number of parameters which describe these mean values as functions of various patient characteristics or pathophysiology, e.g. age, sex, height, body mass, underlying pathology, and other influences on drug disposition such as concomitant drug therapy, smoking habits and alcohol intake. The inter-individual random effects parameter describes a probability distribution about a particular mean value for a fixed effect parameter. The difference between an individual's parameter and population mean values is referred to as the inter-individual variability. On the other hand, the intra-individual random effects parameter explains all the sources of variability that cannot be accounted for on the basis of the fixed effects and the inter-individual variability. The intra-individual pharmacokinetic variability involves the change in response of an individual to drug treatment with time. Examples include inhibition or induction of metabolic elimination (changes in clearance), variable absorption due to intestinal flora or gut wall metabolism, diurnal variation due to circadian rhythms, assay error and model misspecification error. (Sheiner, 1984; Sheiner and Grasela, 1984; Vozeh *et al.*, 1984).

Data analyzed by NONMEM need not be from a homogeneous population since NONMEM can be used to determine the pharmacokinetic characteristics of specific patient subpopulations such as smokers, the elderly, patients with renal or liver impairment, and others. NONMEM's estimation of population parameters do not require the data to be restricted to special types of routine data such as those obtained only at steady state, or only at peak or trough, nor do estimates improve with such data. NONMEM's estimates do improve, however, when a data set is enhanced by the addition of many observations from many individuals. In addition, the number of samples per individual need not be the same. NONMEM is particularly useful when the data collection design varies considerably between individuals (Boeckmann *et al.*, 1991).

Besides being used to develop a pharmacokinetic model, NONMEM is also used to develop a statistical model. The NONMEM approach uses a statistical model in which the individual patient's pharmacokinetic parameters are assumed to arise from a distribution which has a population mean and a particular quantifiable deviation from this mean. The emphasis is, therefore, shifted away from the individual patient to a system in which the population is treated as a unit of analysis, thus allowing the simultaneous analysis of data from all individuals in the study. Observations are pooled to characterise a central tendency for the population rather than the individual subject. Fewer samples are needed from each individual because the individual is no longer of central interest (Sheiner and Beal, 1983).

Minimization of the NONMEM objective function is equal to twice the negative log-likelihood of the data (-2logLmax) which is equivalent to maximizing the probability (likelihood) of the data. Thus, the monitoring of changes in the minimum objective function (MOF) can serve as a statistical test to show which parameter values render the data most probable. The MOF is a goodness-of-fit statistic. Hypothesis testing is done by noting the changes in the MOF when 1 (reduced) or 2 (full) compartment pharmacokinetic models are fitted to the data. The difference in the MOF values obtained for the full versus the restricted models is approximately chi-square distributed, with degrees of freedom equal to the number of parameters set equal to a fixed value in the restricted model. This approach is used to estimate the significance of various patient characteristics, usually referred to as fixed effects or covariates, as predictors of pharmacokinetic parameters (Sheiner *et al.*, 1977).

NONMEM provides estimates of the standard errors for all parameters estimated and these can be used to construct confidence intervals for the parameter values, thereby allowing hypothesis testing. Under the assumptions of normality for distributions of random variables, NONMEM provides another method of testing hypotheses, the likelihood ratio test which is used in comparing models. The NONMEM computer package is supplied with a powerful scattergram subroutine which enables the user, for example, to plot variables against residuals and weighted residuals. One can detect trends which can subsequently be formally tested in an additional run (Vozeh *et al.*, 1984).

Three input files are required to run NONMEM: (a) the data file which contains the concentration - time data, (b) the PRED file, a FORTRAN subroutine which defines the structural and variance models, and (c) the control file which details information on the organization of data in the data file, initial estimates of parameters with upper and lower limits, and instruction for presentation of results, tables and graphs.

The NONMEM approach has proved itself in the human clinical setting (Vozeh *et al.*, 1982; Grasela *et al.*, 1983; Grasela and Sheiner, 1984; Grevel *et al.*, 1988) and has accordingly been chosen for this study.

2.2. CLINICAL PHARMACOKINETIC CONSIDERATIONS FOR PHENYTOIN

The clinical pharmacokinetics of phenytoin has been reviewed in the literature (Aronson *et al.*, 1992; Levine and Chang, 1990; Winter and Tozer, 1986; Richens, 1979), and therefore only the basic concepts and essential information which have a bearing on this study are presented here.

2.2.1. CHARACTERISTICS OF CAPACITY-LIMITED METABOLISM

Detailed investigation has demonstrated the complex nature of phenytoin elimination, which can be explained in terms of the Michaelis-Menten equation (Gerber and Wagner, 1972; Glazko *et al.*, 1969).

The Michaelis-Menten model was developed to individualize phenytoin therapy based on serum concentrations obtained at steady state. The Michaelis-Menten equation for the rate of phenytoin elimination (dAe/dt) is represented as follows:

$$dAe/dt = \frac{Vm \times Cp_{ss}}{Km + Cp_{ss}} \qquad (1)$$

where Vm is the maximum metabolic rate (mg/day), Km is the Michaelis-Menten constant (mg/l) and Cp_{ss} is the steady-state phenytoin concentration in serum or plasma (mg/l). By analogy with enzyme kinetics, the reaction velocity is the phenytoin elimination rate which, at steady state, is equal to the daily dose (Dodson, 1982).

From the steady-state serum concentration values, phenytoin pharmacokinetic parameters (Vm and Km) are determined in each patient to allow the calculation of dose (Ludden *et al.*, 1977). Vm is calculated using the following equation:

$$Vm = \frac{S.F.R}{Cp_{ss}} Km + S.F.R \qquad (2)$$

where R is the dosing rate (mg/day), S - the salt form factor, and F the bioavailability factor. The other variables have been defined above. This method fixes Km to the population mean value and uses one Cp_{ss} - dosage pair to solve for Vm. This Vm value, the population mean Km, and the target serum concentration can be used to solve equation 3 for the corresponding dose:

$$DOSE = \frac{Vm \times Cp_{ss} \times \tau}{(Km + Cp_{ss})(S)(F)} \qquad (3)$$

where τ is the dosing interval.

Estimates of Vm and Km can also be obtained graphically if at least 2 reliable steady-state serum phenytoin levels at different daily doses are available (Ludden *et al.*, 1977). Briefly, R (mg/day phenytoin sodium) is plotted on the y-axis against R/Cp_{ss} on the x-axis. The y-intercept is the value of Vm and the slope is the negative value of Km. The graphic method is necessary only if at least 3 reliable steady-state serum phenytoin concentrations at different daily doses are available. When steady-state phenytoin concentrations are known for only 2 dosage regimens, there is no need to use the graphic method. Km could be calculated from the following equation:

$$Km = \frac{R_2 - R_1}{\frac{R_1}{Cp_{ss1}} - \frac{R_2}{Cp_{ss2}}} \qquad (4)$$

where Cp_{ss1} and Cp_{ss2} are the steady-state serum phenytoin concentrations obtained after doses 1 and 2 respectively, and R_1 and R_2 are the first and second dosing rates respectively. The Km value is substituted in equation 2 and Vm is calculated. At this stage, the Vm and Km values specifically pertaining to the individual have been calculated. Now that estimates of the individual's Vm and Km are known, the appropriate dosing rate

required to produce a desired steady-state serum concentration can be predicted more accurately using equation 3 (Driessen *et al.*, 1980; Ludden *et al.*, 1976).

If no estimates of Vm and Km are available for a particular patient as a result of having no Cp_{ss}-dosage pairs, then the population values of Vm and Km obtained from the literature are used.

The serum phenytoin concentration that will be obtained at steady state (Cp_{ss}) on a particular dose can be calculated by rearranging equations 2 or 3 (Ludden *et al.*, 1976):

$$Cp_{ss} = \frac{Km \times (S)\,(F)\,(R)}{Vm - (S)\,(F)\,(R)} \qquad (5)$$

Phenytoin dosage regimens derived from individually calculated pharmacokinetic parameters (Vm and Km) have been shown to be superior to conventional regimens, more accurate and effective clinically without increasing the risk of toxicity. It has been shown that there is no significant difference between predicted and observed mean steady-state phenytoin concentrations using individual values of these apparent Michaelis-Menten parameters (Blain *et al.*, 1981).

The Michaelis-Menten model predicts that, when the serum phenytoin concentration is greater than the Km value and the dose of phenytoin is increased, the serum phenytoin concentration will increase disproportionately. This causes a nonlinear relationship between the maintenance dose administered and the resulting serum concentration (Richens and Dunlop, 1975a). The most important clinical consequence of this is that increments in dose need to be progressively smaller as the upper limit of the therapeutic range is approached. If this is not so, phenytoin intoxication is likely to develop. As a result, there is a tendency among clinicians who have knowledge of Michaelis-Menten pharmacokinetics or the nonlinear pharmacokinetics of phenytoin to administer small and therapeutically inadequate doses (Rambeck *et al.*, 1979). Conversely, a small decrease in phenytoin dose will result in a disproportionately large decrease in the serum phenytoin level.

A large change in serum level after a small change in dose may reflect a low Km value. Careful adjustment of phenytoin dosage is necessary for all patients with low Km values. A low Km value indicates a high percentage of enzyme saturation operating at low serum

concentrations of phenytoin, and the risk of phenytoin accumulation on dosage adjustment increases as the body's maximum capacity to metabolize phenytoin is approached (Taylor *et al.*, 1983). When serum phenytoin concentrations are well below the apparent Km, a linear relationship between dose and serum concentration exists in the lower part of the therapeutic range.

When the rate of phenytoin administration exceeds the individual's Vm, accumulation of the drug is rapid and this further contributes to the sudden appearance of toxicity after a small increase in dose. In this case, the drug accumulates continuously and the serum concentration never reaches steady state (Chiba *et al.*, 1980; Garrettson and Jusko, 1974). Appreciation of the variability in the rate of phenytoin accumulation is extremely important for the pharmacokinetic interpretation of serum phenytoin concentrations (Ludden *et al.*, 1978). The time of onset of phenytoin toxicity is related to the magnitude by which the rate of phenytoin administration exceeds the Vm values (Garrettson and Jusko, 1974). A high Vm value should reduce the risk of drug accumulation.

Km values may vary from 1.5 to 24 mg/l (Mawer *et al.*, 1974; Richens and Dunlop, 1975a). Mean values have been found to range from 2.3 to 9.4 mg/l. Rambeck *et al.* (1979) reported that Vm ranged from 155 to 818 mg/day, the mean being 355 ± 106 mg/day. A drawback of this study was that all patients received one or more other antiepileptic drugs in addition to chronic phenytoin therapy. Ludden *et al.* (1977) estimated individual Michaelis-Menten pharmacokinetic parameters for phenytoin from two reliable steady-state serum concentrations resulting from different daily doses in 12 patients. They reported Vm and Km values of 8.53 (range 5.25 to 12.4) mg/kg/day and 8.42 (range 1.03 to 16.2) mg/l, respectively. Estimates of Vm and Km values obtained by other investigators are reported in Table 1 (page 13).

Km and Vm vary substantially from person to person, but remain constant in the individual provided the pathological and physiological status of the patient remains stable (Rambeck *et al.*, 1979). Saturation kinetics tend to amplify the inter-subject variability in Vm and bioavailability.

TABLE 1: LITERATURE REVIEW OF Vm AND Km VALUES IN ADULTS AND CHILDREN

REFERENCE	Vm		Km (mg/l)	AGE (yr)	NO. OF PATIENTS	NO. OF R: Cp_{ss} PAIRS	ETHNIC GROUP	NON-MEM
	mg/day	mg/kg/ day						
Bauer & Blouin, 1982		7.5 ± 2.2	5.7 ± 2.2	20-39	26	-	-	No
		6.6 ± 1.8	5.4 ± 2.4	40-59	32			
		6.0 ± 1.9	5.8 ± 2.3	60-79	34			
Bauer *et al.*, 1983		13.5	6.59	0.5-3.0	42	432	Caucasian	No
		10.93	6.82	4-6	28			
		10.05	6.51	7-9	33			
		8.25	5.69	10-16	32			
Blain *et al.*, 1981	238 ± 25.5	20.4 ± 2.1	7.5 ± 1.2	0.67-2.75	40	159	English	No
	542.2 ± 37	8.7 ± 0.7	9.4 ± 2.3	18-66	21			
Chiba *et al.*, 1980		13.8 ± 4.3	4.1 ± 5.6	0.5-3.0	34	236	Japanese	No
		11.2 ± 3.0	4.1 ± 3.6	4-6	24			
		9.5 ± 1.5	3.6 ± 4.11	7-9	24			
		8.0 ± 1.7	3.0 ± 2.51	10-16	22			
Chan & Lee, 1990	499		2.307	2-76	66	174	Singapore Chinese	Yes
		10.9		<15	23	64		
		8.05		>15	43	110		
Dodson, 1982		17.9 ± 5.67	4.54 ± 2.19	0≤1	9	-	-	No
		12.0 ± 2.33	5.23 ± 2.09	1≤4	8			
		10.4 ± 3.68	3.85 ± 1.63	4≤8	9			
		11.1 ± 2.97	5.69 ± 4.51	8≤12	14			
		7.85 ± 2.31	5.14 ± 4.08	12≤16	11			
		9.57 ± 5.04	7.15 ± 4.02	16≤22	7			

TABLE 1: Continued

REFERENCE	Vm		Km (mg/l)	AGE (yr)	NO. OF PATIENTS	NO. OF R: Cp_{ss} PAIRS	ETHNIC GROUP	NON-MEM
	mg/day	mg/kg/ day						
Eadie *et al.*, 1976		8.1 12.5	5.8 5.3	Adults Children	21 15	-	-	No
Grasela *et al.*, 1983	415	5.93	5.7	Adults			European	Yes
	355	6.53	5.7	29.5 ± 15.2	178	385	German	
	208	9.08	2.2	6.05 ± 3.94	104	236	Japanese	
	138	11.69	3.2	1.33 ± 0.52	40	159	English	
Mawer *et al.*, 1974	435	0.8 - 13.2	3.8		15			No
Miller *et al.*, 1987	455	6.5	3.43	11-60	37	100	Black South Africans	Yes
Rambeck *et al.*, 1979	355 ± 106		6.01 ± 3.71	6-72	127		German	No
Taylor *et al.*, 1983	550 ± 168	7.1 ± 2.2	7.14 ± 4.6	43.2 ± 18.9	30	-	American	No
Vozeh *et al.*, 1981		7.22	4.44	22-78	49	124	Swiss	Yes
Yukawa *et al.*, 1990	325	5.42	2.41	0.6-71.1	101	-	Japanese	Yes

At lower serum concentrations of phenytoin the decline in serum concentration is a first-order process, but at higher serum concentrations, the decline in serum concentration approximates more closely a zero-order process, suggesting saturation of phenytoin metabolizing mechanisms (Arnold and Gerber, 1969). The decline from a toxic level is at first slow, but it becomes more rapid as the concentration falls. Thus, a gradual change from zero- to first-order kinetics occurs as the concentration decreases. The time of decline of the serum concentration to control values varies from 1 to at least 6 days (Loeser, 1960).

2.2.2. PARALLEL MICHAELIS-MENTEN AND FIRST-ORDER ELIMINATION

Since virtually all drugs are likely to have at least a small parallel first-order pathway, an appropriate expression for the rate of phenytoin elimination would be:

$$dAe/dt = \frac{Vm \times Cp_{ss}}{Km + Cp_{ss}} + (Cl \times Cp_{ss}) \qquad (6)$$

where Cl is the clearance for the linear pathway(s).

At low concentrations relative to Km, the elimination of the drug is first-order with constant clearance, i.e.

$$dAe/dt = \left(\frac{Vm}{Km} + CL\right) \times Cp \qquad (7)$$

Nonlinear pharmacokinetic behaviour will be most evident when concentrations are close to or above the Km value. At high concentrations relative to Km, the rate of elimination approaches

$$dAe/dt \sim Vm + Cl \times Cp \qquad (8)$$

At very high concentrations, the rate of elimination may again be first-order when the rate of elimination via the linear pathway greatly exceeds the Vm value for the saturable pathway:

$$dAe/dt \sim Cl \times Cp \qquad (9)$$

Even a minimal linear pathway can have a marked effect on predicted steady-state concentrations at high dosing rates (Ludden, 1991). Unchanged phenytoin is excreted in the urine in amounts ranging from 1 to 5% of the dose (Butler, 1957; Glazko *et al.*, 1982). Bochner *et al.* (1973) reported that the renal clearance of phenytoin ranged from 0.18 to 1.38 l/h. This would involve a total urinary loss of approximately 15 mg/day of phenytoin. This is small compared with an average total clearance of approximately 25 l/day at a

concentration of 15 mg/l. However, it is possible that the total of all parallel linear pathways, including the minor metabolic ones, could be much greater. At rates of administration approaching Vm, renal elimination of unchanged phenytoin can significantly influence the steady-state serum phenytoin concentration and the time required to achieve steady state. An equation which takes parallel Michaelis-Menten metabolism and first-order kinetics of phenytoin into account has been described (Ludden *et al.*, 1978; Ludden, 1991). This equation predicts steady-state phenytoin concentration as follows:

$$Cp_{ss} = -\frac{1}{2}\left[\left(\frac{Vm}{Cl} + Km - \frac{R}{Cl}\right) - \sqrt{\left(\frac{Vm}{Cl} + Km - \frac{R}{Cl}\right)^2 + \frac{4.R.Km}{Cl}}\right] \quad (10)$$

where Cl is the first-order (linear) clearance value for phenytoin in l/day. The population pharmacokinetic parameters used in this model, i.e. Vm, Km and Cl, have not been quantified. This model, as far as can be determined, has also not been clinically validated.

Computer simulations have shown that the time required to reach a particular fraction of the steady state is a function of the individual pharmacokinetic parameters, the rate of administration (the higher the dose, the longer the time required to achieve a percentage of steady state), and the magnitude of the parallel first-order elimination pathway (Ludden *et al.*, 1978; Wagner, 1978). Even a first-order pathway that is only 1 to 2% of the maximum clearance (which occurs at low concentrations) can be an important determinant of steady-state concentration and cumulation rate when serum concentrations are high (Ludden, 1991).

2.2.3. ALTERED PHARMACOKINETICS

A wide variation in the disposition of phenytoin occurs. This may be due to differences in liver drug-metabolizing activity, in the plasma concentration of phenytoin at which enzyme induction may occur or in the level at which rate-limiting enzyme reactions become saturated (Arnold and Gerber, 1969).

Demographic factors such as age, height (Houghton *et al.*, 1975), weight (Eadie *et al.*, 1973; Hooper *et al.*, 1974; Houghton *et al.*, 1975; Mawer *et al.*, 1974), body surface area

(Barot *et al.*, 1978; Sherwin, 1974) and sex (Eadie *et al.*, 1973; Hooper *et al.*, 1974; Houghton *et al.*, 1975) have been studied with regard to the serum phenytoin concentration. A number of factors and conditions that have been reported to alter the pharmacokinetics of phenytoin are discussed below:

2.2.3.1. AUTO-INDUCTION

This phenomenon occurs with phenytoin and has been reported in mice (Gerber and Arnold, 1969) and humans (Miller *et al.*, 1989a; Yuen *et al.*, 1983). It may result in therapeutic failure, and an upward adjustment in dose may be required. Reynolds *et al.* (1981) reported that declining phenytoin levels on a constant dose occurred in 39% of patients during the first few months of therapy. Although there was no evidence of poor compliance in this study, they could not entirely rule out this possibility.

2.2.3.2. HEPATIC DISEASE

In hepatic disease there is an increase in the unbound fraction of phenytoin. This may be due to a decrease in the serum albumin concentration or a decrease in the protein binding of phenytoin. The latter may be due to elevated levels of bilirubin, which competitively displaces phenytoin from its protein binding sites. In severe hepatic failure, a total serum phenytoin concentration that is apparently either subtherapeutic or therapeutic may actually be associated with phenytoin toxicity. In this context, effective antiepileptic therapy may be achieved with total phenytoin concentrations that are lower than the normal therapeutic range (Hooper *et al.*, 1973).

2.2.3.3. RENAL DISEASE

In the presence of uraemia the percentage of bound phenytoin is reduced (Hooper *et al.*, 1973). This is most probably due to a change in the conformation of the albumin molecule, rather than a decrease in albumin concentration. An altered metabolic disposition of phenytoin in uraemia has also been suggested (Letteri, 1971). The higher free serum levels of phenytoin result in a shorter half-life (Odar-Cederlof and Borga, 1974) and this may necessitate more frequent dosing to prevent wide fluctuations in serum phenytoin concentrations. The therapeutic range may decrease in uraemia to 4-10 mg/l.

No change in the daily dose of phenytoin may be necessary in uraemic patients despite the lower total serum concentrations. In nephrotic syndrome the reduced protein binding of phenytoin should be taken into account.

2.2.3.4. FEBRILE ILLNESS

During febrile illness (body temperature > 38.3^0 C for more than 24 hours), the phenytoin concentration in blood decreases by more than 40% from the pre-illness baseline. Neither phenytoin binding nor absorption is altered by the febrile illness, so the most probable cause of the fall in phenytoin levels was considered to be induction of hepatic oxidative enzymes (Leppik *et al.*, 1986).

2.2.3.5. INFECTIOUS MONONUCLEOSIS

One case report on a hospitalized patient indicated a marked decrease in phenytoin levels in a 22-year old woman. It was suggested that an increase in the capacity of the phenytoin hepatic oxidation pathway might have occurred during infectious mononucleosis (Leppik *et al.*, 1979).

2.2.3.6. DIABETES MELLITUS

Steady-state phenytoin concentrations were shown to be significantly lower in type 1 and type II diabetic patients compared with controls. The Vm and Vm/Km of phenytoin were significantly increased in type 1 diabetics. Vm was unaltered in type II diabetics, but the Vm/Km was higher. Protein binding of phenytoin decreased in both groups (Adithan *et al.*, 1991).

2.2.3.7. CRITICALLY ILL AND HEAD TRAUMA PATIENTS

Preliminary data have suggested that phenytoin systemic clearance increases during initial therapy in critically ill (Boucher *et al.*, 1988) and head trauma (Bauer *et al.*, 1983) patients. This may be a consequence of changes in protein binding, induction of metabolism, or influence of stress on hepatic metabolic capacity (Boucher *et al.*, 1988).

2.2.3.8. AGE

It has been suggested that age plays an important role in determining the phenytoin dosage required, particularly in paediatric patients (Dawson and Jamieson, 1975), who have a higher rapid metabolic rate.

2.2.3.8.1. PRETERM NEONATES, NEWBORNS, INFANTS AND CHILDREN

Preterm neonates younger than 2 weeks have a prolonged half-life because of a larger volume of distribution (1.2 l/kg) and incomplete maturation of the hepatic enzymes. Children 6 months to 6 years of age have a higher body clearance and a shorter elimination half-life than adults. The daily dose in mg/kg of phenytoin from 2 weeks to 6 years of age is generally higher than in neonates, older children and adults because of a higher basal metabolic rate. In this age group, a shorter dosing interval is required to minimize large fluctuations in serum phenytoin concentrations (Dodson, 1982; Sherwin *et al.*, 1974; Svensmark and Buchthal, 1964).

2.2.3.8.2. THE ELDERLY

The elderly have a lower body clearance of phenytoin compared with adolescents and younger adults (Bach *et al.*, 1981; Hayes *et al.*, 1975; Houghton *et al.*, 1975; Sherwin *et al.*, 1974). The influence of age on the dose and plasma concentrations of phenytoin was studied in 223 adult patients (Sherwin *et al.*, 1974). There was a marked increase in plasma phenytoin concentration in female patients over 65 years of age (when doses were normalized to 5.1 mg/kg/day) compared with women under 45 years of age receiving equivalent doses.

2.2.3.9. GENETIC FACTORS

Genetic variation is considered to be responsible for individual differences in the rate of phenytoin metabolism (Andreasen *et al.*, 1973; Kutt, 1971). The inter-individual variation in the saturability of the hydroxylase enzyme is genetically determined. The actual proportion of the drug which is metabolized by liver enzymes is determined by the prevailing steady-state serum phenytoin concentration (Houghton and Richens, 1974). It

seems likely that genetic differences are more important in determining a patient's steady-state plasma phenytoin concentration than age, weight and height (Houghton *et al.*, 1975).

The treatment of patients of different ethnic groups may create problems if clinicians are unaware of kinetic differences. From studies of negroes and Ghanaians compared with Caucasians (Andoh *et al.*, 1979; Arnold and Gerber, 1969), and black South Africans (Buchanan *et al.*, 1977), it is recognized that phenytoin may cause such problems. Inter-ethnic and inter-phenotype differences among Ghanaians and Caucasians in the metabolic hydroxylation of phenytoin have been found (Andoh *et al.*, 1979).

A pronounced difference in the mean plasma half-life of phenytoin in Caucasian (18.5, range 7 - 42 hours) and negro (26.5, range 11.5 - 38.5 hours) males was found, although the ranges did overlap (Arnold and Gerber, 1969). Haerer and Grace (1969) reported that the mean serum phenytoin concentration in white patients (8.6 mg/l) was lower than that in negro patients (10.3 mg/l). While there was a small difference in the mean weights between these groups, this did not sufficiently account for the differences in the mean drug levels.

Phenytoin disposition was studied in Greenland Eskimos and in Caucasian Danes in order to discover possible ethnic differences in pharmacokinetics. The total body clearance of phenytoin per kg body weight was nearly twice as high in Eskimos, irrespective of the place of residence, diet or sex, compared with the Danes. The volume of distribution was larger in the Eskimo group but protein binding did not differ. The reason for these differences is not clear, but the results emphasize that ethnic differences in pharmacokinetics can be of significance (Kromann *et al.*, 1981). In another study of Eskimo patients from Greenland (Dam *et al.*, 1977), the dose/plasma concentration ratio for phenytoin was higher in this ethnic group than in Danes treated with the same dosage. Furthermore, the 24-hour urine samples of the Greenlanders contained more 5-(*p*-hydroxyphenyl)-5-phenylhydantoin than that of the Danes, indicating more rapid metabolism of phenytoin in Greenlanders.

Kutt *et al.* (1964) were the first to describe a group of individuals who apparently had an inherited defective ability to oxidise phenytoin. Subsequently, a genetically determined

polymorphism of drug oxidation was described which influences the ability to oxidise a number of drugs, including phenytoin (Sloan *et al.*, 1978). A family with an inheritance of phenytoin hypometabolism has been described by Vasko *et al.* (1980).

2.2.3.10. GENDER

Women receiving the same daily dose of phenytoin as men had lower mean serum phenytoin concentrations (Gallagher and Baumel, 1971; Houghton *et al.*, 1975; Sherwin *et al.*, 1974; Travers *et al.*, 1972). In the study by Houghton *et al.* (1975) the difference was not statistically significant. Other investigators have suggested that gender itself does not alter the relationship between plasma phenytoin concentration and dose in adults or children (Eadie *et al.*, 1973; Galdames *et al.*, 1980; Hooper *et al.*, 1974). Richens (1975) found that after allowing for weight (women were lighter than men), women had slightly, but not significantly, lower serum concentrations. No marked differences in plasma protein binding of phenytoin between the sexes were found (Lunde *et al.*, 1970)

2.2.3.11. PREGNANCY

Serum phenytoin levels decrease during pregnancy and increase in the postpartum period (Lander *et al.*, 1977). Pregnancy is associated with altered phenytoin absorption or metabolism or both (Lander *et al.*, 1984), and periodic measurement of serum phenytoin concentration is necessary when managing a pregnant epileptic patient (Kochenour *et al.*, 1980; Levy and Yerby, 1985). Ezquer *et al.* (1992) reported that Vm during pregnancy was barely modified while the Km decreased.

2.2.3.12. POSTURE

An average increase of 10% in total plasma phenytoin concentration caused by standing for 45 minutes has been demonstrated (Albalan *et al.*, 1990).

2.2.3.13. BODY SURFACE AREA

Barot *et al.* (1978) found that dosage requirements correlated most strongly ($r = 0.57$, $p < 0.001$) with body surface area. This relationship (approximately 200 mg/day per m^2) accounted for about one-third of the total dosage variance. A limitation of this study was that only 3 of the 90 patients studied were receiving phenytoin monotherapy.

2.2.3.14. MISCELLANEOUS FACTORS

Other conditions in which altered pharmacokinetics of phenytoin have been reported are extensive burns, cystic fibrosis, AIDS and malnutrition. All influence the pharmacokinetics of phenytoin as a result of reduced protein binding of phenytoin (Bauer *et al.*, 1983; Boucher *et al.*, 1988; Ehrnebo *et al.,* 1971; Ehrnebo and Odar-Cederlof, 1975; Perrucca, 1980; Toler *et al.*, 1990).

2.2.4. FACTORS INFLUENCING Vm AND Km VALUES

Blain *et al.* (1981) estimated the Vm and Km from 159 dose-serum concentration pairs in English children (40 patients, aged 8-33 months) and adults (21 patients, aged 18-66 years) and found that Vm (mg/day) was significantly greater in adults than in children, but the apparent Km was similar in children and adults. In relation to body weight (mg/kg/day), however, the Vm was significantly greater in children than adults. The Vm and Km values were estimated using the method of Mullen (1978) and Ludden *et al.* (1977). Limitations of this study were that it did not investigate differences in Vm and Km within the wide range of ages in the adult patients. This study also did not control for gender. The merit of this study was that patients were receiving phenytoin monotherapy. The plasma concentrations of phenytoin reported were actually adjusted saliva concentrations and this created uncertainty about the mean value of Km for the data set.

Chiba *et al.* (1980), in a retrospective study, estimated Vm and Km values from 385 dose-serum concentration pairs in 104 Japanese paediatric patients aged 0.5 to 16 years. Their results indicated that Vm is inversely related to age but that there is no relationship between age and Km. The Vm in mg/kg/day decreased from a mean value of 13.8 to 8.0 as age increased from 3 years to more than 10 years. The method of Ludden *et al.* (1977)

was used to estimate the values of Vm and Km. A drawback of this study was that only 17 of the 104 patients were taking phenytoin as the only anticonvulsant. Subsequently, the same authors (Chiba *et al.*, 1980a) published a paper on the same patients in which they stated that Km tended to increase and become more variable as the number of co-administered anticonvulsants increased. This made the previous results questionable.

Rambeck *et al.* (1979) studied retrospectively 127 German patients aged 6-72 years who were receiving long-term phenytoin therapy. They found that Km was independent of age and body surface area, but Vm correlated positively with both. This study, however, did not control for gender. A further limitation of the study was that in addition to phenytoin, one or more anticonvulsant drugs (phenobarbitone, primidone, carbamazepine, sodium valproate, succinimides or benzodiazepines) were prescribed.

Grasela *et al* (1983) re-analysed retrospectively the phenytoin data of Blain *et al.* (1981), Chiba *et al.* (1980) and Rambeck *et al.* (1979) using NONMEM. The influence of age, gender, ethnicity, height and weight on Vm and Km were investigated. Their results, contrary to some of the initial conclusions, showed that Vm was not influenced by ethnicity, gender, height or age, but was influenced by weight; a nonlinear function of weight, $(weight)^{0.6}$, was used to reflect this relationship. Km, on the other hand, was 43% lower for patients under 15 years of age than for older patients. Ethnicity was shown to influence the Km value, as the Km for Japanese patients was 23% lower than for European patients. The apparent effect of age on Km, especially in the Europeans, may be in part due to the uneven distribution in age between the age groups in the patients from the different countries. In the Japanese group the majority of patients were under 15 years of age, but in the Europeans, those under 15 years were mainly from England and those older than 15 years mainly from Germany. The estimates of Vm and Km values, as reported by Grasela *et al.* (1983), were lower than those reported in the original studies. The assumptions made regarding compliance and the absence of any effect of other co-medication limit the confidence that can be placed in the Vm and Km estimates.

Bauer *et al.*, (1983) demonstrated in 135 Caucasian paediatric patients that Vm values were significantly different for different age groups (0.5 - 3; 4 - 6; 7 - 9 and 10 - 16 years), but not the Km values. Linear regression analysis of Vm against age revealed a significant decline in Vm with increasing age. The plotting of Km against age showed a poor

correlation and large variability. No differences were found in either parameters between boys and girls in each age group. Patients received phenytoin as their only anticonvulsant therapy. The results of Bauer *et al.* (1983), in comparison with those of Chiba *et al.* (1980), showed that for each age group, the Km values (mg/ml) were higher in Caucasian children (5.69 - 6.59) than in Japanese children (3.0 - 4.1). The Vm values did not differ substantially between the two ethnic groups when like age groups were compared. Bauer and Blouin (1982), in a similar study, investigated the influence of age on phenytoin kinetics in adult epileptics. They reported Vm values for 3 age groups: 7.5 ± 2.2; 6.6 ± 1.8; and 6.0 ± 1.9 mg/kg/day for the 20 to 39, 40 to 59 and 60 to 79-year-old subjects, respectively. Values of Vm for the geriatric patients were substantially lower than those for the younger adults (20 to 39 year olds, $p < 0.05$). As a result, it was calculated that the geriatric group would require an average of 21% less phenytoin daily than the younger adults to maintain a steady-state concentration of 15 mg/l. However, the Km values did not appear to be influenced by age. The mean values for Km ranged from 5.4 to 5.8 mg/l. No gender difference was noted for Vm and Km.

Dodson (1982) reported Vm and Km values for phenytoin in 54 children, of whom 39 received other drug therapy in addition to phenytoin. The ethnic background was not reported. The mean Vm values declined with increasing age, while mean Km values remained nearly constant. The Km was influenced primarily by drug interactions. These results of the relationship of Vm and Km with age were similar to those of Bauer *et al.* (1983). Both studies used linear regression analysis to study these relationships.

Chan and Lee (1990) studied retrospectively the population pharmacokinetics of phenytoin in 66 (43 > 15 years and 23 < 15 years) epileptic Singapore Chinese children and adults. Using NONMEM, they found that there were no age- or gender-related effects on Vm or Km. Vm was estimated to be 30.72 $mg.kg^{-0.656}$ and Km 2.307 mg/l. They reported that the Km value obtained was much lower than those reported in studies of European patients. Vm was influenced by mass ($p < 0.001$) and the relationship between the two variables was nonlinear, as had previously been reported by Grasela *et al.* (1983). The estimate of the power parameter of mass for Chinese patients (0.656) was similar to that of 0.625 obtained by Grasela *et al.* (1983). In another study, Lee and Chan (1981) reported that the low serum phenytoin versus daily dose ratio obtained in Chinese paediatric patients

compared with that obtained in other population groups could be due to ethnic differences e.g. a fast rate of hydroxylation of phenytoin.

The retrospective study of Taylor *et al.* (1983) yielded no significant correlation between patient physical data (age, sex, weight, body surface area) and pharmacokinetic values of Vm and Km. Twenty of the 69 patients received other anticonvulsants. The study was limited by the small number of patients. The method of Ludden *et al.* (1977), with the Bayesian forecasting technique, was used.

From this literature review, it is clear that most of the Vm and Km values reported in the literature were derived from retrospective studies and routine data and are not precise (Whiting *et al.*, 1986). The information in the literature is also conflicting. The method of analysis also has a significant influence on the results (Grasela *et al.*, 1983). Whether, and to what extent, ethnicity, gender, age, mass, body surface area and smoking influence the Vm and Km values of South Africans requires investigation in a controlled and prospective manner.

Table 1 (page 13) presents a literature review of studies reporting Vm and Km values in adults and children.

CHAPTER 3

EXPERIMENTAL PROCEDURES

As this study was the first pharmacokinetic service of its kind to be established in the Western Cape, a detailed description of the procedures involved in this service is given.

3.1. SELECTION OF HOSPITALS

The hospitals were selected on the basis of the following criteria: the size of the hospital (i.e. number of patients attending the hospital for all ailments), the existence of an epilepsy clinic (club), and day of the week on which the epilepsy clinic was usually held. The hospitals were chosen so that a pharmacokinetic consulting service could be offered by the investigator every day with minimal interference with the regular epilepsy clinics held at some of the hospitals. The following hospitals and institutions were chosen for this study: the Heideveld, Guguletu, Khayelitsha, Dr. Abdurahman, Lotus River and Elsies River Day Hospitals, the Neurology Outpatient Clinic at Groote Schuur Hospital, the South African National Epileptic League Protective Workshop (SANEL) and Betel School for Epilepsy. All of the clinics were situated in the Western Cape. It was arranged that the smaller clinics, i.e. Dr. Abdurahman Day Hospital, SANEL and Betel School for Epilepsy, would take place on the same day as a larger clinic. The Day Hospital Organization serving the greater Cape Town area consists of health care centres for outpatients, providing primary health care to lower-income groups not covered by medical aid (health insurance). Patients were registered after confirmation of the diagnosis by and referral from a neurology department. Although this was a multi-site study, only one clinic per week was initially offered to identify and minimize problems in the clinical pharmacokinetic consulting service.

3.2. INITIATION OF CLINICAL PHARMACOKINETIC CONSULTING SERVICE

Before commencing the service at some of the hospitals, the availability of patients for enrolment in this study was determined. In order to assess patient numbers, the

pharmacists working at the dispensary were requested to list all patients taking phenytoin as the only anticonvulsant. They were also requested to list respective ages and folder numbers so that the patients could be traced, if necessary. This was usually done two to three months before the planned commencement date of the pharmacokinetic service.

Before the initiation of a clinical pharmacokinetic consulting service for phenytoin at the various hospitals, a briefing on phenytoin pharmacokinetics and the proposal of this study was presented to the doctors, nurses and pharmacists at the respective hospitals. An information brochure written by the investigator and entitled 'Pharmacokinetic guidelines for oral phenytoin use' (Annexure 1) was provided at this lecture. Monotherapy for the treatment of epilepsy was encouraged. A continual, informal in-service education programme was provided throughout this study. The investigator requested that only one doctor be responsible for the clinic at each hospital so that better communication might exist between the doctor and the investigator. A starting date for the service was usually arranged a month after the presentation of the project to the hospital staff. This was to allow the doctors and primary health care nurses sufficient time to make appointments and to inform the patients of the service.

Before the initiation of the pharmacokinetic services, the two nurses who were employed at separate clinics to assist the investigator, were trained in phenytoin usage and encouragement of patient compliance. Special emphasis was placed on explanation to the patient of how to fill in the patient diary.

Certain of the guidelines published by Robinson *et al.* (1984) on how to establish a pharmacokinetic consulting service for ambulatory patients were followed.

3.3. PATIENT SELECTION

The patients were chosen consecutively with regard to the time of presentation at the hospital, and it was assumed that they represented typical patients receiving phenytoin. All ambulatory patients routinely taking phenytoin as the only anticonvulsant were screened for entry into the study. Of these patients those who satisfied the entry criteria were selected for this study. All patients were seen personally by the investigator and treated on an outpatient basis.

3.3.1. ENTRY CRITERIA

All patients satisfied the following entry criteria:

(a) compliant with drug therapy and able to understand the instructions

(b) above 15 years of age

(c) no hepatic or renal disease

(d) no evidence or history of alcohol or drug abuse

(e) no major depressive or psychiatric diseases

(f) no intake of medicines that might interfere with phenytoin serum levels within two weeks of entering the study

(g) taking phenytoin routinely as the only anticonvulsant

(h) taking phenytoin by oral administration only

(i) not pregnant or breastfeeding

(j) subjective judgement of the likelihood that the patient would comply with the treatment protocol, for example, by assessment of the patient's attitude to treatment, and

(k) a constant weight during the treatment period under consideration.

3.3.2. EXCLUSION CRITERIA

It was decided in the study plan that patients would be withdrawn from the study if they:

(a) were non-compliant with the prescribed dosage regimen

(b) developed renal, hepatic, major depressive or other psychiatric diseases subsequent to entering the study

(c) showed evidence of alcohol or drug abuse subsequent to entering the study. Consumption of alcohol more than thrice weekly and of more the 750 ml of beer or 125 ml of spirits was regarded as alcohol abuse. Any habit-forming drug not taken for therapeutic reasons was regarded as drug abuse.

(d) became pregnant

(e) developed significant adverse effects to the medication during the course of the study

(f) developed abnormalities of laboratory examinations which were judged to be clinically significant; namely, liver and renal function tests, and full blood count

(g) failed to respond to treatment

(h) took other medications that might interfere with phenytoin pharmacokinetics subsequent to entering the study, and

(i) failed to adhere to the protocol and reduced patient co-operation.

A full account was made of each withdrawal from the study.

3.4. LABORATORY TESTS

Laboratory tests were performed with special attention to liver (alkaline phosphatase, alanine transaminase, aspartate transaminase, glutamyltransferase, total bilirubin) and renal (serum creatinine and urea) function tests. Plasma albumin concentration determinations were also done. These tests were performed for all patients at SANEL and Groote Schuur Hospital as they were required by the neurologist. At the other clinics these laboratory tests were especially performed for geriatric patients and those who previously abused or were suspected of abusing alcohol. Laboratory tests were also done when there was difficulty in interpreting the serum phenytoin concentrations.

3.5. THE CLINICAL PHARMACOKINETIC CONSULTING SERVICE

3.5.1. DOCTOR-PATIENT CONSULTATION

It was requested that the doctor screen the patient initially and that if he/she satisfied the inclusion criteria that the patient be referred to the investigator for a pharmacokinetic consultation. For a new patient, the doctor prescribed the 'standard' dose of phenytoin, if indicated, and referred the patient to the investigator for counselling.

3.5.2. NURSE - PATIENT INTERVIEW

At Guguletu and Elsies River Day Hospitals the patients were usually screened initially by the primary health care nurses before being referred for a pharmacokinetic consultation. The primary health care nurses were asked to refer all patients who were taking phenytoin as the only anticonvulsant. If the patient attended the hospital on a day other than the fixed day of the week designated for the epilepsy clinic, an appointment was made for the patient by the doctor or primary health care nurse to see the investigator.

explained. The hospital folder was carefully perused for information such as the seizure type, the cause of the seizures, present dosage regimen and adverse effects. Medical records were, however, usually incomplete. All information collected was confirmed, where possible, by the hospital records. If the seizure type was not recorded in the hospital folder, an attempt was made by the investigator to classify the seizures into generalized tonic-clonic, tonic, myoclonic, simple or complex partial, or partial seizures with secondary generalization. If the seizures were due to an identifiable reason then the date of such cause (e.g. head injury due to a motor vehicle accident or assault) was noted. The date of the first seizure (if known) and/or the approximate duration of the epilepsy were also recorded. The patient was asked to describe the seizure, and if a family member or friend was present he/she was asked to describe the seizure/s. Much time was spent acquiring information regarding the frequency of the seizures. The number of seizures, if any, experienced in the past week, month, 3 months and year was recorded. The patient's statement on seizure frequency was compared with the doctor's or with the primary health care nurse's notes in the hospital folder. An attempt was made to count the number of seizures experienced during the past year, as reported in the folder. The prescription chart was checked to determine if any other anticonvulsant besides phenytoin had previously been used and whether the dose of phenytoin had been different from the present dose. Previous results of serum phenytoin concentrations reported in the folder were not used in this study as compliance in the taking of phenytoin was not known. Drug compliance was carefully evaluated by questioning the patient. The compliance status was documented as follows: 'claims compliant', 'poor compliance' or 'tendency to run out of medication'. If the patient had run out of phenytoin, the length of time without medication was documented. Questions such as 'when last did you forget to take your tablets?', 'do you sometimes forget to take your tablets?', 'do you still have tablets left?' or 'when did you finish your tablets?' were asked. A careful inquiry into adverse effects was made and, if applicable, the frequency and severity of the adverse effects were noted.

The patient was given a diary designed by the investigator (Annexure 3) to record when phenytoin was taken, the occurrence of seizures and the date of the next appointment. The cover of the diary contained the following information: name and telephone numbers of the investigator; name, address and folder number of the patient, and the name of hospital attended by the patient. The diary was valid for 3 months and those particular months were written in the diary by the investigator. The patient was asked to make a cross for every 100 mg tablet, a filled circle for every 50 mg tablet (both manufactured by

Lennon Pty Ltd), and an empty circle for half an Epanutin Infatab^{R} (manufactured by Warner-Lambert Pty Ltd) taken on a daily basis in the appropriate column (morning, afternoon or night) corresponding with the date when the tablets were taken. The patient was also asked to make a cross for every seizure that occurred and to record any side-effects or comments corresponding with the date when the events occurred. The next appointment, which was usually a month later, was recorded by circling the date in the diary and by writing 'appointment' in the appropriate column. This date was also filled in the hospital's appointment card. Patients were usually consulted once a month, except for selected patients who attended more frequently. Poorly controlled patients were reviewed more frequently, either to obtain a duplicate blood sample for phenytoin concentration or to adjust the dose. If a possibility existed that the patient might not be able to fill in the diary correctly, then the patient was requested to return a week or two later to check on how the diary was being filled in. The importance of filling in the diary accurately was stressed. It was requested that the diary and the phenytoin tablets be brought to every visit at the hospital. This was written prominently on the cover of the diary as a reminder. The patient was counselled about phenytoin use and this advice was reinforced by written instructions in the language (English, Afrikaans, Xhosa) of his/her choice. A copy of these instructions in English is given in Annexure 4.

The initial dose was decided empirically by the attendant doctor. In most cases, the dose was prescribed by a doctor in a neurology department at a tertiary hospital and repeated by the doctor at the day hospital. No attempt was made by the investigator to influence the initial dose prescribed.

It was stressed to the patient that if phenytoin was being taken once a day, then the missed dose ought to be taken as soon as remembered, as long as it was taken at least 12 hours before the next dose. If the missed dose was remembered within 12 hours of the next dose, then the missed dose was to be omitted altogether. It was also stressed that double the daily dose was never to be taken as a single dose. If phenytoin was taken twice a day and the morning dose was missed, then the missed dose was to be taken as soon as remembered or with the evening dose. The patient was advised that this might lead to side-effects; if this occurred the investigator was to be informed at the next visit and the daily dose never to be taken as a single dose again. If a dose was missed at night, then it was to be taken with the morning dose the following day. If phenytoin was taken thrice daily and the morning dose was missed, then it was to be taken in the

afternoon; if the afternoon dose was also missed, then the missed doses were to be taken with the evening dose. If the evening dose was missed, then it was to be taken with the morning dose the following day. If any signs or symptoms of transient toxicity were observed with the dosage regimens, the patient was requested not to take the missed dose within four hours of the next dose. In order to clarify the patient's understanding of the dosage regimen, he/she was asked to explain what was to be done in the event of a missed dose. The patient was also asked to show how he/she would fill in the diary every day, especially in the case of a missed dose. The patient was also briefly advised about the other information contained in the brochure (Annexure 4).

Patients and their escorts were informed that a few dosage adjustments might be necessary during the course of therapy to establish an effective dose with minimal side-effects. In this way, the patient and relatives were prepared for later changes without doubting the competence of the investigator, if change became necessary. The patient was also informed that the full therapeutic response of phenytoin might only be obtained four to six weeks after the initiation of therapy or following a change in dose. Every effort was made to secure the understanding and confidence of the patient and the family and thus improve the chance of obtaining successful seizure control. Adequate time was spent with the patient to ensure the best understanding of the therapy. The idea that medication controls rather than cures seizures was strongly reinforced. Patients and their families were educated about epilepsy and the signs and symptoms of phenytoin toxicity, and were requested to contact the investigator if toxicity occurred. The importance of drug compliance was stressed, and patients were encouraged to have a relative or close friend accompany them, supervise medication and assist in keeping a record of seizures. It was also preferred that such a person should personally observe the patient having a seizure. They were encouraged to visit any time they wished, and were informed that it was not always necessary to draw the hospital folder and pay the hospital fees. The only time they needed to draw the hospital folder was when more medication was required. The patient was also encouraged to telephone the investigator at any time for advice or with any query that he/she may have. A copy of the informed consent (Annexure 5) was also given to the patient in the language of his/her choice.

The first consultation usually took 45 to 60 minutes. At the end of the interview, a formal consultation note was written in the patient's hospital folder.

3.5.3. FIRST INVESTIGATOR-PATIENT INTERVIEW

The first interview usually took place immediately after the doctor or primary health care nurse had consulted with the patient. The main purpose of the first interview was to screen the patient for inclusion in the study, to gather information about the patient, and to establish a baseline regarding seizure frequency and adverse effects.

Once the investigator had ensured that the patient satisfied all the inclusion criteria, the patient was entered into the study. A red identification sticker was then placed on the cover of the patient's folder and the word 'phenytoin' was written on the sticker. If the inclusion criteria were not satisfied, the patient was counselled regarding phenytoin usage and then asked to consult the doctor or primary health care nurse at the following visit. A note was written in the hospital folder stating the reason for the patient not being included in the study. Written informed consent was obtained before entry into the study.

During the first interview, demographic and medical data were collected which included the patient's name, address, folder number, telephone number, occupation, age, date of birth, gender, race, mass, height, frame size, social class, concurrent medication, duration of therapy and dosages, smoking history, alcohol intake, previous history of other diseases, history of seizures and frequency, description of seizures, present clinical status, previous admissions to hospital and drug history with emphasis on the history of anticonvulsant therapy. The patient was questioned about previous experiences with phenytoin to discover any known problems with the drug. A data collection form (Annexure 2) was used to facilitate the interview. Patients were interviewed according to a standardised detailed format.

The patient's height and mass were recorded. Body mass was measured to the nearest 0.1 kg using a calibrated scale with the patient in light clothing. Height was measured to the nearest 0.5 cm with a vertical ruler.

During the interview the following information was also carefully acquired and documented: if the patient consumed alcohol, the type, frequency and amount of alcohol was recorded. At this point, the relationship between alcohol intake and the severity and frequency of seizures, and the influence that alcohol intake has on phenytoin pharmacokinetics, were

A folder for the investigator's personal use was opened for each patient. Each folder contained the data collection form (Annexure 2), signed copy of the informed consent (Annexure 5), blank 'request for taking of blood sample' forms (Annexure 6) and 'phenytoin assay results and recommendations' forms (Annexure 7). The completed diaries that the patients were requested to return were also placed in this folder.

3.5.4. SECOND INVESTIGATOR-PATIENT CONSULTATION

The investigator's diary was examined to determine the reason for the patient's visit. The patient's diary was checked to determine if it had been filled in correctly. The patient was questioned about compliance and occurrence of seizures since the preceding visit and this information was compared with that in the patient's diary. The diary was evaluated for completeness, and this was recorded on the data collection form. The patient was again questioned about the correct completion of the diary. The events recorded in the diary were then discussed.

Whether any phenytoin tablets were left over was assessed. If the answer was affirmative, the number of tablets left over was assessed. If the tablets were brought to the hospital, they were counted, and the following details noted on the data collection form: the number of tablets remaining, date of dispensing as indicated on the tablet packet or container, and the date of the tablet count. It was requested that the old tablets be placed in a safe place and that the new lot of phenytoin tablets be started on the day dispensed. The tablet counts were performed as far as possible without the knowledge of the patients.

If the patient was compliant and at steady state, a request form (Annexure 6) for blood collection was filled out by the investigator. A month was usually considered sufficient time for steady state to be reached (Barot *et al.*, 1978; Ludden *et al.*, 1978; Rambeck, 1979; Welty *et al.*, 1986; Yukawa *et al.*, 1991). Steady state was defined as the same dosage being maintained for at least 30 days without a trend toward an increasing or decreasing serum concentration after that time (Bourgeois and Wad, 1985; Yuen *et al.*, 1983a).

Good compliance was again encouraged and reinforced. The patient was then sent to the nurse for blood collection. If it was suspected that compliance was less than optimal,

blood sampling was delayed until compliance could be assumed and steady-state serum concentrations were reached.

3.5.5. BLOOD SAMPLE COLLECTION

The time of the last dose and the time when the blood sample was collected were documented on the request form for blood collection. This information was filled in by the investigator or the nurse. The time the samples were taken did not bear a fixed relationship to the time of the previous dose of phenytoin (Aronson *et al.*, 1992; Bryson *et al.*, 1988; Miller at al., 1987). Variation of serum concentration from this source was found to be negligible by comparison with variation due to progressive accumulation or elimination extending over several weeks (Haerer and Buchanan, 1972; Mawer *et al.*, 1974). The blood samples were collected by the nurse by direct venipuncture in plain red-top tubes without anticoagulant (Mauro and Mauro, 1991; Parish and Alexander, 1990). These samples were fetched by the investigator at the end of the clinical session.

All the blood samples were then stored in a cool place and delivered by the investigator to the analytical laboratory of the Department of Pharmacology, University of Cape Town. All the blood samples for serum phenytoin assay were accompanied by the 'request for taking of blood sample' form (Annexure 6). The assay procedure is discussed in section 3.6.

3.5.6. PHENYTOIN ASSAY RESULTS

The phenytoin assay results were carefully checked by the investigator the day after the collection of blood. The results were reported on the 'request for taking of blood sample' forms (Annexure 6). A copy of the result was filed in the patient's folder kept by the investigator and a second copy was placed in the patient's hospital folder.

If a phenytoin level confirmed toxicity, the doctor was informed immediately. The patient was informed telephonically of the result, where possible, and was asked either to stop the medication for a certain period or to return to the hospital on the next clinic day. In some instances, the patient was telephonically advised how to reduce the daily dose until the next visit. For example, if a dose of 350 mg/day phenytoin sodium was being taken

and it was decided that the dose should be reduced to 325 mg/day, then 300 and 350 mg/day phenytoin on alternate days were prescribed until the next epilepsy clinic.

3.5.7. DOSE CALCULATION

If the serum phenytoin level was subtherapeutic, as indicated by inadequate seizure control, then there was a need to adjust the dose. It was preferred not to use the first serum phenytoin concentration obtained to adjust to a new dose without the concentration being confirmed with a second measurement. If a new dose was to be calculated at that stage, equations 2 and 3 in Chapter 2 (page 10) were used. A Km value of 4.4 mg/l was assumed, and Vm was calculated using equation 2. The Vm and Km values were then substituted in equation 3.

Serum phenytoin concentrations were initially targeted to either 15 mg/l or 20 mg/l, as these values represented the midpoint and uppermost point of the usual therapeutic range. When necessary, the time for the phenytoin level to reach steady state on the new dose was calculated using equation 11 (page 41) described later in this chapter. If the serum phenytoin level was below the therapeutic range and the patient was seizure free, no adjustment of dose was made. Phenytoin doses were modified according to clinical response.

Throughout this thesis, doses are expressed as phenytoin sodium and complete bioavailability has been assumed. When Epanutin Infatabs^{R} (phenytoin acid) were used for small increments or decrements of dose, the equivalent dose expressed as phenytoin sodium was calculated. This was done by dividing the dose of phenytoin acid by 0.92. The latter figure represents the fraction of phenytoin sodium that is free acid. The Epanutin Infatabs^{R} contain 50 mg phenytoin acid per tablet, and the phenytoin sodium tablets are available in 50 mg and 100 mg strengths.

In order to interpret the serum concentrations and to make recommendations on dosage design for individual patients, the Bayesian technique of drug dose optimization was used. OPT^{R}, version 4.1b (Clydesoft Statistical and Scientific Software), is an interactive, user-friendly, personal computer software package used for this purpose (Kelman *et al.*, 1982). A laptop computer was used at the clinics to run OPT^{R}. The patient's mass, height,

gender, ideal body mass and steady-state phenytoin concentrations were entered into the programme and the most likely estimates of Vm, Km and dose were derived. When no serum phenytoin concentration feedback was available, the assigned constants in the programme for phenytoin were taken as 7.22 mg/kg/day and 4.44 mg/l for Vm and Km, respectively. Doses were often calculated for two target serum phenytoin concentrations. The nearest practical dose attainable with 100 mg, 50 mg and 27 mg (i.e. half an Epanutin Infatab^{R}) phenytoin sodium was entered and the corresponding target phenytoin concentration was calculated and reported. Dosing recommendations based on the pharmacokinetic calculations were decided on by the clinical judgement of the investigator. The recommended dose and the corresponding target serum concentration were then entered on the data collection form. The daily dose was normally prescribed as a single dose (Strandjord and Johannessen, 1974). The dose was prescribed twice daily only if there was a special reason to do so, or in selected cases where the daily dose exceeded 400 to 450 mg daily. The patient's data were recorded in an OPT^{R} file which could be updated by adding details of subsequent doses and concentration measurements. OPT^{R} allowed only steady-state serum phenytoin concentrations to be used, and only steady-state concentrations could be predicted. When OPT^{R} was not available at the clinic, the equations described in Chapter 2 were employed.

3.5.8. REPORT TO DOCTOR

A verbal and/or written report (Annexure 7) of the measured serum phenytoin concentration and recommendation of the dose was given to the doctor at the patient's next clinic visit. This report also stated the targeted serum concentration, estimates of Vm and Km values, and an estimate of when the serum level would be at steady state. Each written report was discussed personally with the doctor to ensure that the contents of the report were fully understood. A copy of the written report, if one had been prepared, was then placed in the patient's folder. The doctor made the final decision about the necessity of a dosage adjustment. A prescription for the new dosage regimen was always written by the doctor.

The pharmacokinetic service also evaluated the serum phenytoin concentration, patient response (e.g. seizure frequency and adverse effects), compliance with dosage regimen

and concomitant drug administration. The report included, where possible, any explanation for a large discrepancy between predicted and measured drug concentrations.

3.5.9. SUBSEQUENT INVESTIGATOR-PATIENT INTERVIEWS

A routine consultation was conducted with emphasis on the number and description of seizures since the last visit, the date when the last seizure was experienced, and the presence of side-effects to phenytoin. The phenytoin assay result was explained to the patient and the result recorded on the data collection form.

If there was no urgency to adjust the dose, a second assay while the patient was on the same dose was performed to confirm the result. Initial serum phenytoin concentrations below 10 mg/l were seldom used to adjust the dose before confirming the result. This served as a test for compliance. If two serum phenytoin levels were available on the same dose then the average of the two levels was used in the calculation of the new dose. If at least two reliable steady-state phenytoin serum levels on different daily doses were available, the patient's individual Vm and Km could be calculated using equations 2 and 4 in Chapter 2 (page 10), respectively. These parameter estimates were used to calculate a new dose by means of equation 3 in Chapter 2.

If more than one phenytoin dosage form was prescribed, the dosage forms were always shown to the patient. For example, if a dose of 377 mg phenytoin sodium was prescribed, three 100 mg phenytoin tablets, one 50 mg phenytoin tablet and half an Epanutin Infatab^{R} were shown to the patient. This was done to ensure that no mistakes in the dosage regimen occurred, given that many patients were illiterate. If a new dose was prescribed, the patient was counselled regarding the new dosage regimen. Decision to change therapy was a co-operative effort between the patient, investigator and the doctor.

The next appointment was scheduled by recording the date in the patient's diary provided by the investigator. The appointment was, as far as possible, scheduled to coincide with the patient's return to the hospital to collect his/her medication. The patient was then shown how to fill in the diary, with the new dose, if a new dose had been prescribed. If a complex dosage regimen had been prescribed the patient was often requested to return to our clinic after obtaining the phenytoin from the dispensary. This was to ensure that the

correct dosage forms had been dispensed and that the directions on the tablet packets or containers were correct. All the important principles of phenytoin use and compliance were reinforced at every visit. The patient was advised of drug-related precautions. Letters were written to patients who defaulted on clinic attendance to offer them another appointment date.

The patient was followed up for as long as possible to obtain more blood samples or to check on progress. The more dose-concentration pairs available, the more accurate the estimation of Vm and Km would be. The patient was continually reassessed to ensure optimal therapy. Towards the end of the study, many patients were discharged from the pharmacokinetic service if they had been seizure free for at least six months.

3.5.10. SANEL, GROOTE SCHUUR HOSPITAL AND BETEL SCHOOL FOR EPILEPSY

The clinics held at these institutions are discussed separately because the procedure followed differed slightly from that described previously.

The clinic at SANEL was managed by a nurse. The patient was initially consulted jointly by the neurologist and investigator once a week. An electro-encephalogram, full blood count and a SMAC (electrolytes, renal and liver function tests as well as albumin concentrations) were performed on all the patients. Besides the patients recording their seizures in the patient diary, a record of seizures was kept on a daily basis by a supervisor at the protective workshop. The phenytoin medication was obtained from Heideveld Day Hospital and distributed at work by the nurse. The investigator was promptly kept informed by the nurse of any problems the patients might have experienced.

At the outpatient clinic at Groote Schuur Hospital the blood samples were assayed for serum phenytoin concentration by a technician at the clinic and the results of the assays were available within an hour. This allowed a written report to be generated for the doctor during the same clinical session. This also reduced the number of patient visits to the hospital relative to all the other clinics where the prompt assay service was not available. This clinic was discontinued after 30 clinic sessions because of an insufficient number of patients on phenytoin monotherapy. Patients already entered in the study were still

followed up at this hospital on an appointment basis for a period of two months after officially discontinuing the service.

The clinic at Betel School for Epilepsy was managed by three full-time nurses and a medical officer who visited the clinic once a week. The phenytoin dose was placed in labelled bottles daily by the nurses and dispensed to the hostel students.

3.5.11. GENERAL COMMENTS REGARDING THE CLINICAL PHARMACO-KINETIC SERVICE

If there was a large discrepancy between two measurements of serum phenytoin concentrations on the same dose, then a third measurement was taken to assess which was most likely to have been correct. The first blood sample for the measurement of serum phenytoin concentration was taken after a minimum of 4 weeks on a constant maintenance dose. Subsequent samples were taken after intervals of 2 to 4 weeks provided that the dosage regimen was the same and compliance was optimal (Barot *et al.*, 1978).

A dosage increase was only considered when seizures occurred after at least 3 to 4 weeks following the last dosage increment or when the seizures occurred after the length of time required to reach steady state in a compliant patient.

In cases of suspected phenytoin toxicity, the treatment was stopped for 1 to 7 days before the new maintenance dose was started. The size of the dosage reduction was usually determined empirically. This depended on the degree of toxicity, seizure control and the serum phenytoin level, if available. The patient was telephoned, where possible, the next day to confirm toxicity and the dose was re-evaluated in the light of the available serum phenytoin level.

Doses were often adjusted empirically without any pharmacokinetic calculations. This was usually done in compliant patients with serum phenytoin levels of less than 15 mg/l. If the steady-state serum level was lower than 6 mg/l, an increase of 100 mg in the daily dose was recommended. If the serum phenytoin level ranged from 6 to 10 mg/l - a 50 mg/day increase was recommended, and if the level was between 10 and 15 mg/l, an increase

of 27 to 50 mg phenytoin sodium per day was recommended. A modification of the recommendations of Mawer *et al.* (1974) and Aronson *et al.* (1992) was used. Even an increase of 27 mg/day phenytoin sodium in a few instances was too large and it was necessary to prescribe e.g. 400 mg and 427 mg on alternate days. An extra 50 mg phenytoin tablet twice per week was also occasionally recommended. Given the lack of knowledge of the population pharmacokinetic parameters of phenytoin in the South African population, conservative increases in dosage were recommended. The number of doses prescribed daily were kept to a minimum to improve compliance. If the seizures were observed to occur at a time corresponding to the trough serum concentration or if adverse effects were experienced at a peak concentration, then it was advised that phenytoin be taken twice daily without changing the size of the daily dose. The only changes that were made were alterations in the phenytoin dosage regimen, substitution of drugs interfering with phenytoin or discontinuation of drugs not required by the patient.

The time taken for the serum concentration of phenytoin to reach 90% of steady state was often calculated using the following equation:

$$t_{90\%} = \frac{Km \times Vd}{(Vm - S.F.R)^2} \times (2.303\ Vm - 0.9\ S.F.R) \quad \textbf{(11)}$$

where $t_{90\%}$ is the time (days) taken to reach 90% of steady state, Vd (l) is the volume of distribution. The other parameters have previously been explained in Chapter 2 (Levine *et al.*, 1987). A few days to a week were allowed between the time taken to reach steady state as calculated above and the time of blood collection to ensure that a steady state had indeed been achieved. Vm was calculated using equation 2 in Chapter 2 (page 10) and Km was assumed to be 4.4 mg/l (Vozeh *et al.*, 1981). Vd was assumed to be 0.65 l/kg (Winter and Tozer, 1986). If individualized parameters were available then these values were used in the calculation (Allen *et al.*, 1979; Vozeh and Follath, 1980).

In the case of frequent seizures caused by non-compliance, an oral loading dose (LD) was calculated using the following equation:

$$LD = Vd \times Cp_{ss} \quad \textbf{(12)}$$

The Vd of phenytoin was assumed to be 0.65 l/kg and the targeted steady-state serum

concentration (Cp_{ss}) was usually 20 mg/l. If a reliable feedback serum level was available, then the following equation was used:

$$LD = Vd\ (Cp_{desired} - Cp_{achieved}) \qquad (13)$$

The oral loading dose was administered by giving an immediate dose of 400 mg followed by 200 mg every 2 hours over a period of 4 to 6 hours. The usual or new maintenance dose was also prescribed on that day.

A nurse or interpreter was always present for Xhosa-speaking patients. Nurses were employed at Guguletu, Khayelitsha, Lotus River and Elsies River Day Hospitals to assist with obtaining routine information concerning the patient. Other functions of the nurse included the drawing of the patient's hospital folder (if required for inserting information or prescribing of a new dose), escorting the patient to the utility room for blood collection, taking the folder to the doctor for a repeat prescription of phenytoin and requesting the doctor to examine the patient for other complaints.

The individual patient's clinical response in terms of seizure frequency and severity was used as the major monitoring parameter for the efficacy of the phenytoin therapy.

3.5.12. COMPLIANCE CHECKS

Compliance was assessed by an extensive patient interview, tablet counts (Blain *et al.*, 1981), correct filling in of the patient's diary and variation between two measured serial serum phenytoin concentrations taken at different times on the same dose (Ludden *et al.*, 1977; Wilson and Wilkinson, 1974). After steady state was attained and if the variation between two serum phenytoin concentrations on the same dose was less than 20%, then the patient was considered to be compliant (Yuen *et al.*, 1983a; 1989). This method allows direct measurement of compliance.

A large variation between predicted and measured serum phenytoin concentrations was sometimes used as a measure of non-compliance. The accuracy of this method depends on the accuracy of the estimates of Vm and Km used in the calculation (Miller *et al.*, 1982). The accuracy of the method improved when more feedback serum phenytoin

concentrations were available. The accuracy of the prediction was categorized as good, average or poor. A good prediction was considered to be within 20% of the measured concentration, an average prediction between 20 and 40%, and poor prediction greater than 40% of the measured concentration. A good and average accuracy of prediction were considered acceptable.

Compliance was also assessed by checking the hospital prescription cards. Patients collecting medication more than 28 days after the date of dispensing were suspected of being non-compliant as they were never supplied with more than 28 days medication unless otherwise arranged with the investigator. The number of tablets left over at the previous visit was taken into account in the assessment of compliance. All possible reasons for default were also sought. Where possible, the patient was telephoned to check on status and to encourage compliance.

A good investigator-patient relationship was sought in order to improve compliance. The patient was not made to feel guilty about poor compliance and a feedback on all laboratory results was provided to all patients. The patient's spouse, family and/or support group were involved as active participants in the planning of the treatment programme. As far as possible, phenytoin was prescribed as a single daily dose at night to improve compliance. Some patients were informed that their compliance in taking phenytoin could be verified by serum concentration determinations. The latter two procedures have been reported by Miller *et al.* (1982) to improve patient compliance.

3.5.13. ADVERSE EFFECTS

At every visit, the adverse effects to phenytoin were assessed by direct enquiry or by clinical examination. The main adverse effects investigated were dizziness, double vision, blurred vision, nystagmus, tremor, slurred speech and ataxia. Nystagmus was classified as horizontal and/or vertical; on straightforward gaze, at 45^0 or 180^0 deviation and unilateral or bilateral. Lower limb ataxia was assessed at every visit but upper limb ataxia was only assessed if deemed necessary by the investigator. The patient and family or close friends were questioned about whether the patient staggered or had an ataxic gait. The time of the day when the adverse effects occurred was noted. Whether the adverse effects occurred simultaneously was also taken into account. Other less frequent and

serious adverse effects of phenytoin were also noted. The relationship between the adverse effect/s and the serum phenytoin concentration was recorded (Buchthal *et al.*, 1960; Gerber *et al.*, 1972; Kutt *et al.*, 1964).

3.6. ASSAY

3.6.1. CALIBRATION OF MACHINE

The TDx and TDxFLx analyzers (Abbott Laboratories, Diagnostic Division, North Chicago) were calibrated by the technical staff of the Department of Pharmacology, University of Cape Town every four to six months. This was done in accordance with the manufacturer's specifications. Six phenytoin calibrator vials supplied by Abbott Laboratories were used in the calibration procedure. The first vial contained no phenytoin while the remaining five vials contained accurately measured amounts of phenytoin (2.5, 5.0, 10.0, 20.0 and 40.0 µg/ml) in human serum. This was done to establish linearity over the specified range of phenytoin concentrations.

3.6.2. QUALITY CONTROL

3.6.2.1. EXTERNAL QUALITY CONTROL

External quality control was performed once a month by Cardiff Bioanalytical Services Ltd., Cardiff Business Technology Centre, Senghenyold Road, Cardiff, Wales, CF2 4A7, United Kingdom.

3.6.2.2. INTERNAL QUALITY CONTROL

Internal quality control procedures were performed daily with the phenytoin controls supplied by Abbott Laboratories. Each vial labelled L, M or H read within the following ranges 6.75 - 8.25, 13.5 - 16.5, and 27.0 - 33.0 mg/l, respectively. Only one of these phenytoin controls was used daily for the batch analysis of the phenytoin samples.

3.6.2.3. INTER-ASSAY COEFFICIENT OF VARIATION

Six phenytoin duplicate blood samples obtained routinely from patients were used to determine the inter-assay coefficient of variation. During the quality control procedure the duplicate samples were labelled with fictitious patient names.

3.6.2.4. ADSORPTION OF PHENYTOIN TO RUBBER STOPPERS

Six duplicate phenytoin blood samples obtained routinely from patients were used to determine whether adsorption of phenytoin to the rubber stoppers of the blood collection tubes occurred. Six phenytoin blood samples were stored upright and the other six duplicate samples were stored in an inverted position. Samples were stored as described above for 6 hours before assay. A paired *t* test[1] was used to determine whether a significant difference between the two groups existed. A significant difference was indicated when a *p* value was ≤ 0.05. The coefficient of variation in the serum phenytoin concentration was calculated.

3.6.2.5. STABILITY OF PHENYTOIN IN WHOLE BLOOD SAMPLES

The effect of storage on the phenytoin concentration in the blood samples at room temperature was investigated. Six triplicate samples obtained routinely from patients were used. The first, second and third batch were assayed 2, 6 and 26 hours after blood collection. All samples were stored as whole blood samples. A paired *t* test[1] was used to determine whether a significant difference between the samples stored for 2 and 6, and 6 and 26 hours existed. A significant difference was indicated when a *p* value was ≤ 0.05. The coefficient of variation between the serum phenytoin concentrations was also calculated.

3.6.3. ASSAY PROCEDURE

The assays were performed on the same day when the blood was collected from the patient. On rare occasions, the serum was stored overnight at -20° C. The interval

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[1]the Wilcoxon signed rank test was also performed

between the time when the blood was collected from the patient and the time of centrifugation was kept as short as possible. This time seldom exceeded 6 to 8 hours, although phenytoin has been shown to be stable in serum for at least 24 hours at temperatures up to 32° C (Parish and Alexander, 1990). The blood samples were centrifuged at 3000 rpm for 15 minutes and 80 µl of the serum were transferred to a TDx^R cuvette using a micropipette. Although 50 µl of serum were required, an excess was pipetted to allow for the presence of any air bubbles. The TDx^R cuvettes were then placed in a carousel which had the capacity to accommodate 20 such TDx^R cuvettes. The carousel and a phenytoin reagent pack were placed in the TDx^R Analyzer and the batch assay process was started. Total serum phenytoin concentrations were thus measured with a fluorescent polarization immunoassay. The assays were performed with an automated TDx^R or TDxFLx^R system. All assays were performed in accordance with the manufacturer's specifications and quality control procedures. The drug assays were performed by the technical staff at the Department of Pharmacology, University of Cape Town.

3.7. DATA ANALYSIS

3.7.1. DATA ORGANIZATION

All information on the patients was recorded on 4 spreadsheets by means of the LOTUS 123 computer programme:

spreadsheet 1 : coloured patients excluded from the study
spreadsheet 2 : black patients excluded from study
spreadsheet 3 : coloured patients included in study
spreadsheet 4 : black patients included in study

Spreadsheets 3 and 4 were used for the NONMEM analysis.

3.7.2. NONMEM DATA ANALYSIS

The unnecessary information contained in spreadsheets 3 and 4 was deleted and the remaining information was reorganised into a format suitable for NONMEM. The

information was then coded according to NONMEM's specifications. The 2 spreadsheets were then combined and converted into an ASCII format to form a NONMEM data input file. Inputs to NONMEM consisted of a data file, control files and user-coded subroutines.

Double precision NONMEM version III level 1.2 and version IV level 1.0 (NONMEM Project Group, University of California, San Francisco) were used in this study (Beal *et al.*, 1988 - 1992; Sheiner *et al.*, 1977, 1979). The NONMEM version III and IV programmes were run on a 486 computer at the University of Durban-Westville, South Africa. Version IV was also run on a Sparc Station^{R} and UNIX was used as the operating system at the Food and Drug Administration, United States of America.

The posthoc option allows the estimation of individual parameters and associated individual predictions of the dependent variable given the population parameters. It does this through a Bayesian regression and uses all the data in the data set for each individual. The usual purpose of the posthoc option is to obtain individual parameter estimates. The conditional estimation method, an option of NONMEM version IV level 1.0, uses an E-M algorithm to derive improved estimates of the population pharmacokinetic parameters. This is an iterative procedure that involves multiple, sequential estimation of individual parameters, then population parameters, then individual parameters etc. until convergence is achieved for the population values. By combining data on population pharmacokinetic parameters (prior distributions of the parameters) with information from sampled concentrations in an individual patient, an optimal estimate of this individual's pharmacokinetic parameters (posterior distribution of the parameters) was obtained. The procedure therefore makes use of the Bayesian principle (Frakes *et al.*, 1986; Peck *et al.*, 1980; Sheiner and Beal, 1982; Sheiner *et al.*, 1979; Vozeh *et al.*, 1981). The main purpose of the conditional estimation method is to obtain population parameter estimates. The estimates obtained with the conditional estimation method were given greater credence (Beal *et al.*,1988-1992).

3.7.3. PHARMACOKINETIC MODELS

A one compartment model with either Michaelis-Menten or parallel Michaelis-Menten and first-order elimination was used.

3.7.3.1. STRUCTURAL MODELS

Three pharmacokinetic models, termed structural models S1, S2 and S3, were used.

3.7.3.1.1. STRUCTURAL MODEL S1 (MICHAELIS-MENTEN MODEL)

In this model, dose rather than Cp_{ss} as the dependent variable was chosen to ensure stability during data fitting (Vozeh *et al.*, 1981). This model was represented as follows:

$$R_{ij} = \frac{Vm_j \times Cp_{ssij}}{Km_j + Cp_{ssij}} \qquad (14)$$

where R*ij* is the *i*th dosing rate of phenytoin in mg/day predicted to achieve the *i*th Cp_{ss} in the *j*th patient; Vm*j* and Km*j* are the maximum metabolic rate (mg/day) and Michaelis-Menten constant (mg/l) of phenytoin respectively in the *j*th patient and Cp_{ssij} is the steady-state serum phenytoin concentration measured in the *j*th patient receiving dosage R*ij*. Vm*j* and Km*j* are assumed to be constant over time within a patient, but may differ between patients (Grasela *et al.*, 1983; Sheiner and Beal, 1980; Yukawa *et al.*, 1990).

3.7.3.1.2. STRUCTURAL MODEL S2 (PARALLEL MICHAELIS-MENTEN AND FIRST-ORDER ELIMINATION MODEL WITH DOSE AS THE DEPENDENT VARIABLE)

Since a small amount of phenytoin is eliminated unchanged in the urine, the contribution of a parallel first-order elimination pathway was considered. In this model, dose was used as the dependent variable and was represented as follows (Ludden *et al.*, 1991) :

$$R_{ij} = \frac{Vm_j \times Cp_{ssij}}{Km_j + Cp_{ssij}} + (Cl_j \times Cp_{ssij}) \qquad (15)$$

where Cl_j is the linear clearance of phenytoin for the *j*th patient in l/day.

3.7.3.1.3. STRUCTURAL MODEL S3 (PARALLEL MICHAELIS-MENTEN AND FIRST-ORDER ELIMINATION MODEL WITH STEADY- STATE SERUM CONCENTRATION AS THE DEPENDENT VARIABLE)

When using equation 15 and solving for Cp_{ss}, the following equation was derived :

$$Cp_{ssij} = -\frac{1}{2}\left[\left(\frac{Vm_j}{Cl_j} + Km_j - \frac{R_{ij}}{Cl_j}\right) - \sqrt{\left(\frac{Vm_j}{Cl_j} + Km_j - \frac{R_{ij}}{Cl_j}\right)^2 + \frac{4 . R_{ij} . Km_j}{Cl_j}}\right] \quad (16)$$

The derivation of this equation has been described by Ludden *et al.* (1978). Our equation differed from that of Ludden *et al.* (1978) in that linear clearance (Cl) was substituted for the product of Vd (volume of distribution) and K_{el} (first-order elimination rate constant).

3.7.3.2. PARAMETER MODELS

With NONMEM, the influence of various demographic or patient factors (fixed effects, FE) was tested by relating them to the pharmacokinetic parameters (P) using parameter models of the type:

$$P = \theta(FE) \quad (17)$$

where P is the expected value of the pharmacokinetic parameter (e.g. Vm or Km) in any individual, FE is an identifiable patient factor (e.g. mass), and θ is a regression coefficient. When quantifying the influence of a discontinuous variable such as smoking status, the model was of the type:

$$P = [\theta_1(FE) + \theta_2] * SMK \quad (18)$$

where SMK equals 1 for non-smokers and θ_{SMK} for smokers.

3.7.4. STATISTICAL MODELS

The statistical model takes into account the inter-individual variation and intra-individual error. The inter-subject variability in Vm, Km and Cl, as well as the intra-subject error, were modelled with additive and proportional error models. With the conditional estimation

method, the proportional error model was used to avoid obtaining negative values for the parameters. In the first set of analysis, only the additive and multiplicative error models were tested. The intra-individual variability in the dose : serum concentration relationship can be modelled as follows:

$$R_{ij} = R_{mij} + \epsilon_{ij} \quad \text{(additive error)} \qquad \textbf{(19)}$$

$$R_{ij} = R_{mij} \exp \epsilon_{ij} \quad \text{(proportional error)} \qquad \textbf{(20)}$$

$$C_{ij} = C_{mij} + \epsilon_{ij} \quad \text{(additive error)} \qquad \textbf{(21)}$$

$$C_{ij} = C_{mij} \exp \epsilon_{ij} \quad \text{(proportional error)} \qquad \textbf{(22)}$$

where

R_{ij} is the actual dosage rate for the *i*th R-Cp_{ss} pair in the *j*th individual (mg/kg). R_{mij} is the predicted dosage rate to obtain the *i*th Cp_{ss} in the *j*th patient. This value includes the contribution of the η_j^{Vm}, η_j^{Km} and η_j^{Cl} terms;

C_{ij} is the *i*th actual concentration for the *j*th individual, C_{mij} is the *i*th concentration predicted by the model for the *j*th individual. This value includes the contribution of the η_j^{Vm}, η_j^{Km} and η_j^{Cl} terms;

ε_{ij} is assumed to represent independent normally distributed statistical errors (intra-subject or residual error) with mean zero and variance σ^2 (Yukawa *et al.*, 1990). The variable ε_{ij} models all sources of error or deviation of the actual dosage or steady-state phenytoin concentrations from those that are predicted from the model. The models given above by equations 19-22 should be regarded as only an approximation of what are undoubtedly more complex error models, since ε_{ij} must represent all uncertainty caused by, e.g. pharmacokinetic model misspecification, analytical error in measuring serum phenytoin concentrations, sampling error and non-compliance.

It is, however, more convenient for the numerical aspects of fitting data to the Michaelis-Menten model to cast the statistical model in the form of the equation above (i.e. $R_{ij} = R_{mij} + \varepsilon_{ij}$) where dose is predicted. Otherwise, infinitely large residuals occur when Vm (or Vm_j) values are less than some values of R. In this model, the dosage rate is

regressed on Cp_{ss}, and it is therefore regarded as a random variable whose distribution is conditional on Cp_{ss}. This is contrary to usual pharmacokinetic practice, although it is commonplace when fitting phenytoin data (Sheiner and Beal., 1980).

The statistical models for individual pharmacokinetic parameters were as follows (Driscoll, 1989; Grevel *et al.*, 1988; Miller and Ludden, 1993; Mungall *et al.*, 1985; Sheiner and Beal, 1980; 1981):

$$Vm_j = Vm + \eta_j^{Vm} \quad \text{(additive error)} \qquad \textbf{(23)}$$

$$Vm_j = Vm \exp \eta_j^{Vm} \quad \text{(proportional error)} \qquad \textbf{(24)}$$

$$Km_j = Km + \eta_j^{Km} \quad \text{(additive error)} \qquad \textbf{(25)}$$

$$Km_j = Km \exp \eta_j^{Km} \quad \text{(proportional error)} \qquad \textbf{(26)}$$

$$Cl_j = Cl + \eta_j^{Cl} \quad \text{(additive error)} \qquad \textbf{(27)}$$

$$Cl_j = Cl \exp \eta_j^{Cl} \quad \text{(proportional error)} \qquad \textbf{(28)}$$

where

Vm_j, Km_j and Cl_j are the true pharmacokinetic parameters for the individual as predicted by the regression model;

Vm, Km and Cl are the population means of the maximum metabolic rate, Michaelis-Menten constant and linear clearance, respectively;

η_j^{Vm}, η_j^{Km} and η_j^{Cl} are assumed to represent random normally distributed terms with zero means and variances;

σ^2_{Vm}, σ^2_{Km}, σ^2_{Cl}, respectively distinguish the *j*th individual's Vm, Km and Cl from those predicted by the regression model;

σ_{Vm}, σ_{Km}, σ_{Cl} are approximately the coefficients of variation of Vm, Km and Cl, respectively.

3.7.5. VALIDATION OF POPULATION PHARMACOKINETIC PARAMETERS AND STRUCTURAL MODELS

3.7.5.1. THE NAIVE FEEDBACK METHOD USING ESTIMATES OBTAINED WITH THE FIRST-ORDER AND THE FIRST-ORDER CONDITIONAL ESTIMATION METHODS

The validity of the estimated population parameter values and pharmacokinetic models was tested using the naive prediction method (i.e. a method that uses the mean population estimates of Vm, Km and Cl but no patient-specific phenytoin concentration data). All the steady-state phenytoin concentrations obtained during treatment with the initial and adjusted dosage regimens were used. For models S1 and S2, the estimated population parameters (obtained from the NONMEM outputs and calculated from the parameter models) were used to predict the dosage of phenytoin that would achieve the measured concentration. For model S3, the estimated population parameters were used to predict the steady-state serum concentration that would be achieved when a particular dosing rate was administered. The predictions obtained with the mean population estimates derived with the first-order (FO) and the first-order conditional estimation (FOCE) methods were compared.

The same patient population used in the NONMEM analysis was used in this validation procedure. To minimize possible bias in favour of the model by predicting dose or serum concentrations for patients whose data were used for the estimation of the population parameters, the estimation and validation of the pharmacokinetic parameters were undertaken in separate patient groups. A method of cross-validation was used. All patients producing only one steady-state concentration on a single dose of phenytoin were excluded from this analysis. The reason for this was to exclude the possibility of non-compliance influencing the predictive performances of the models. The remaining patients were then randomly allocated to two equally matched groups (1 and 2) stratified according to the factors (ethnicity; smoking - non-smoker, smoking less than 10 cigarettes per day, 10 to 20 cigarettes per day, or more than 20 cigarettes per day; and age - less than 65 or 65 years or older) demonstrated to influence the pharmacokinetics of phenytoin.

The NONMEM fixed-effects parameter estimates obtained in Group 1 (θ_1, θ_2 etc.) were validated or constrained to their estimated values in Group 2. Similarly, the NONMEM estimates obtained in Group 2 were validated in Group 1. A similar validation procedure was used by Vozeh *et al.* (1982) when studying population pharmacokinetics of oral mexiletine. The predictive performances of the 3 final models were compared. The fixed effects obtained by a NONMEM first-order method in Group 1 was validated in Group 2 by fixing these values in the PRED file and the MAXEVAL option of the $ESTIMATION command omitted by typing '$EST OMITTED'. This step calculated the Vm using the final parameter models determined in Set 1 and Set 2 analysis for each patient. A fixed FO or FOCE Km and Cl values were used for each of the 3 structural models evaluated. The output tables (ID, VM, KM, CL, RI (or CP), PRED, RES and WRES were imported into LOTUS 123 spreadsheets. The DV - PRED which is equal to RES was used in the prediction error analysis as discussed in section 3.7.6.6 was performed. Thereafter the fixed effects obtained by the NONMEM first-order method in Group 2 were validated in Group 1 and the same procedure as described above was followed. The fixed effects obtained by a NONMEM first-orderconditional estimation method were validated as described for the FO method.

A method of cross-validation was used to validate the results in preference to the method where a separate validation population is used, for the following reasons:

(1) The NONMEM estimates were determined in a larger patient population;
(2) The stability of the NONMEM predictions was determined, e.g. if the bias obtained in the two matched groups for each of the structural models was significantly different, then either the NONMEM parameter estimates were unstable or the models used were inadequate;
(3) The validation of the results was based on a larger population;
(4) As more bias values were available, this method provided a better comparative method. This method of cross-validation also ascertained the variability in the bias between the two groups;
(5) The validation population was representative of the patient population routinely attending the epilepsy clinics.

3.7.5.2. PREDICTIONS OF STEADY-STATE SERUM CONCENTRATIONS USING THE MICHAELIS-MENTEN MODEL (S4)

The purpose of this part of the study was to compare the predictive performance of model S4 with that of model S3, and indicate the importance of Cl in the latter model. The estimates of Vm and Km obtained with model S1 were used in model S4. The latter model, which has been discussed previously (section 2.2.1) is represented as follows:

$$Cp_{ss} = \frac{Km \times (S)\ (F)\ (R)}{Vm - (S)\ (F)\ (R)} \qquad (5)$$

3.7.5.3. PREDICTIONS OF DOSE AND STEADY-STATE SERUM CONCENTRATION USING ESTIMATES OBTAINED FROM THE STUDY OF VOZEH *et al.* (1981) IN MODELS S1 and S4

The purpose of this part of the study was to compare the predictive performance of our estimates of Vm and Km with those obtained by Vozeh *et al.* (1981) using the Michaelis-Menten models S1 and S4. The values of 7.22 mg/kg/day and 4.44 mg/l for Vm

and Km respectively obtained from the study of Vozeh *et al.* (1981) were also programme-assigned values used in the OPT^R pharmacokinetic computer programme.

The correlation between the predicted dose or serum concentration and the actual dose or serum concentration was determined by calculating the mean prediction error (MPE) and the mean squared prediction error (RMSE) (Miller and Rheeders, 1989).

3.7.6. STATISTICAL ANALYSIS

3.7.6.1. SUMMARY AND FREQUENCY ANALYSIS

The spreadsheets were imported into the Statgraphics^R computer package (Statistical Graphics Corporation) for summary and frequency statistical analysis. The level of significance was set at $p = 0.05$.

3.7.6.2. NONMEM ANALYSIS

To test the hypothesis of whether the fit of the model to the data was significantly different, the value of the MOF determined in the NONMEM fitting routine was used. Minimizing the objective function is equivalent to maximizing the probability (likelihood) of the data, and monitoring changes in the MOF can thus provide a statistical test as to which parameter values render the data most probable. Assuming that inter- and intra-individual variances were normally distributed, a difference in the minimum objective function (DOBF) of more than 3.8 indicated statistical significance ($p < 0.05$, assuming chi-square distribution) in the improvement or worsening of the fit of the model to the data when the restricted model had one regression parameter less than the full model, i.e. one degree of freedom (Sheiner *et al.*, 1977). In order to identify potentially significant factors, a DOBF of > 7.9 for one degree of freedom associated with a p value of 0.005 was required. The validity of the p - value rests in the validity of the underlying model. This approach was only used when comparing nested models. When building a final model from a simple model in a forward procedure, a decrease in the MOF indicated an improvement in the fit of the model to the data. Conversely, when fixed-effect parameters were removed from a model, an increase in the MOF indicated an improvement in the fit of the model to the data.

When two models that were not restricted in terms of each other (i.e. zero degree of freedom) were compared, the likelihood ratio (Lmax ratio) was used to estimate the relative probability of the two models (Mungall *et al.*, 1985; Sheiner and Grasela, 1984). The following equation was used to calculate the Lmax ratio:

$$\frac{L_1}{L_2} \propto e^{-[MOF(1) - MOF(2)]0.5} \qquad (29)$$

An estimate of 10 or more was regarded to indicate a difference between two models.

In this study, either the DOBF or Lmax ratio was regarded as a sufficient criterion on the basis of which to select the most appropriate model to describe the data. If the DOBF was not sufficiently decisive, then the following criteria were considered in addition to the DOBF (Fattinger *et al.*, 1991; Grevel *et al.*, 1988):

(1) inspection of the correlation matrix of the parameter estimates to indicate whether a minimum correlation between the parameters existed;

(2) whether the scatterplot of weighted residuals versus predicted concentration was randomly scattered around zero. All other residual plots were also examined;

(3) whether smaller standard errors for the estimates were obtained.

(4) whether a decrease in the size of the inter-individual variance of the pharmacokinetic parameters and residual error were obtained.

3.7.6.3. CORRELATION ANALYSIS

Model parameters were not estimated independently but as an interrelated set giving rise to the generation of a correlation matrix for parameters. In the course of this thesis, the incidence of 'high' correlation between parameter estimates was used to examine the reliability of the parameter estimates. Two parameters were judged to be highly correlated if the pairwise correlation was ≥ 0.75[2], otherwise it was termed low. Inspection of the correlation matrix should indicate a minimum correlation between the parameters.

[2]this value has been used by the FDA, no reference is available

3.7.6.4. CONFIDENCE INTERVALS

The reliability of the fixed effect parameter (θ) estimates is important in parameter estimation. This statistical problem was approached through the construction of 'confidence intervals' for the parameter estimates. The standard error of the parameter estimates (SEE) produced by NONMEM can be used to calculate confidence intervals for the true parameter values, thereby allowing hypothesis tests for these (Sheiner and Beal, 1980). The approximate 95% confidence interval for (θ) was given by $\theta \pm 1.96$(SEE) (Miller and Ludden, 1993). Efficient parameter estimation required low standard errors for the parameters.

The width of the confidence interval depends on the precision of the current parameter estimates. To a certain extent, it indicates the degree of caution the clinician should exercise in adjusting the dose. The reliability of the reported confidence interval thus determines the safety and the efficacy of the algorithm (Vozeh and Steimer, 1985).

3.7.6.4.1. CALCULATION OF THE 95% CONFIDENCE INTERVAL FOR RMSE

Because of the large number of observations used in this study (i.e. a large number of degrees of freedom - 768), the χ^2 distribution tables presented in the standard textbooks on statistics could not be used. The χ^2 percentage points in terms of the standard normal percentage points were calculated using the following formula:

$$\chi^2_{\nu;p} = \nu \left[1 - \frac{2}{9\nu} + Zp\sqrt{\frac{2}{9\nu}}\right] \qquad (30)$$

where ν = the degrees of freedom, p = the tail area and Zp = 1.645 - the ordinate of the standard normal which gives the tail area p. The upper percentage point was then calculated by symmetry (Abrahamowitz and Stegun, 1972). The upper and lower limits of the 95% confidence interval range were calculated as follows:

$$\left[\sqrt{\frac{(\nu)\,MSE}{\chi^2_{0.975,\,\nu}}} \;,\; \sqrt{\frac{(\nu)\,MSE}{\chi^2_{0.025,\,\nu}}} \right] \qquad (31)$$

where $\chi^2_{0.975}$ and $\chi^2_{0.025}$ represent the lower and upper percentage points respectively, determined using equation 30 (Larsen and Marx, 1990).

3.7.6.5. CALCULATION OF THE STANDARD ERROR FOR THE ESTIMATED AVERAGE Vm VALUE

The parameter model used in this calculation was $Vm = \theta_1\ wt + \theta_3$ where wt was considered the mean weight value for the population. The mean weight of 64 kg was used in this calculation. Although the variance of Vm would have to take the variance of weight into account, for this study where the interest was in the precision with which Vm was estimated at average weight, this was not needed.

The following standard theorem was used to calculate the stardard error for Vm:

$$var(aX + bY) = a^2\ varX + 2ab\ cov(X,Y) + b^2\ var(Y) \qquad (32)$$

where a = wt

$X = \theta_1$

b = 1

$Y = \theta_3$

(Chatfield and Collins, 1980; Johnson and Wichern, 1988)

Therefore,

$$var(Vm) = wt^2\ var(\theta_1) + 2wt\ cov(\theta_1, \theta_3) + var(\theta_3) \qquad (33)$$

Therefore,

$$SE(Vm) = \sqrt{var(Vm)} \qquad (34)$$

The values for var (θ_1), cov (θ_1, θ_3) and var (θ_3) were obtained from the NONMEM output for each structural model.

3.7.6.6. PREDICTION ERROR ANALYSIS

Given that the actual dose or the measured concentration was known, the efficiency with which each model predicted dose or concentration could be judged. Prediction error analysis involved the calculation of the MPE and the RMSE (Rhoney *et al.*, 1992; Vozeh *et al.*, 1981).

The MPE described the bias that may be present, a positive bias indicated that the prediction method overpredicted the actual dose or concentration and a negative bias indicated that the prediction method underpredicted the actual dose or concentration. The MPE was determined by calculating the prediction error, which is the predicted dose or concentration minus the actual dose or concentration for each patient. The result was then applied to the following equations (Beck *et al.*, 1987; Privitera *et al.*, 1989; Sheiner and Beal, 1981) :

$$MPE = \frac{1}{n}\sum_{i=1}^{n}(PE) \qquad (35)$$

where n is the number of dose and serum concentration pairs and PE the prediction error. In order to express the predictions on the same scale, percentage error was computed for dosage prediction:

$$\%\ MPE = \frac{MPE\ for\ dose}{mean\ actual\ dose} \times 100 \qquad (36)$$

and for concentration prediction :

$$\%\ MPE = \frac{MPE\ for\ concentration}{mean\ measured\ concentration} \times 100 \qquad (37)$$

The percentage MPE obtained with each of the matched groups for each of the 3 models were compared. The average percentage MPE for each of the 3 sets of matched groups was then calculated and compared. Since the MPE has a sign associated with it, it was not a measure of the absolute magnitude of error. To remove the sign, the prediction error was therefore squared and the mean taken; this gave the mean squared error (MSE) (Graves *et al.*, 1986):

$$MSE = \frac{1}{n}\sum_{i=1}^{n} (PE)^2 \qquad (38)$$

The precision of the prediction method was evaluated by calculating the RMSE:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n} (PE)^2} \qquad (39)$$

The RMSE was expressed as a percentage of the actual dose or serum phenytoin concentration (Yuen *et al.*, 1983a; 1989). The smaller the RMSE, the greater the precision of the model with its associated population estimates in predicting the actual dose or serum phenytoin concentration. Bias and precision were further evaluated by constructing the 95% confidence intervals about the MPE and RMSE for each method (Flint *et al.*, 1985).

In summary, the following factors were taken into account when making comparisons of predictive performance:

(1) magnitude of MPE (bias)
(2) magnitude of RMSE (precision)
(3) 95% confidence interval of MPE
(4) standard deviation of MPE
(5) 95% confidence interval of RMSE
(6) comparison of mean predicted dose or serum concentration with the mean actual dose or measured concentration
(7) comparison of the standard deviation of the predicted dose or serum concentration with that of the actual dose or measured serum concentration.
(8) statistical difference between the actual and predicted dose or serum concentration using Student's *t*-test. The level of significance was set at $p < 0.05$
(9) percentage of under- or overpredictions
(10) percentage of prediction errors of more than 10% for dose and of more than 20% for serum concentrations
(11) number of dose : serum concentrations pairs excluded due to the dosage rate being greater than Vm
(12) number of highly unrealistic serum phenytoin predictions greater than 75 mg/l.

3.8. ETHICS

Informed written consent was obtained from each patient included in this study. The study was carried out with prior approval of the Ethics Committee of the Medical School, University of Cape Town and it was conducted strictly in accordance with the Helsinki Declaration. The choice of phenytoin for the patient was based on clinical grounds by the doctor attending to the patient. A dose was changed for therapeutic reasons only, and never for the purpose of this study.

CHAPTER 4

RESULTS

The data for this study were collected between August 1990 and July 1992 during routine clinical consultations at the various epilepsy clinics.

4.1. PATIENT CHARACTERISTICS

A total of 538 (233 black and 305 coloured) adult epileptic patients residing in the Western Cape were entered in the study. Of these patients, 332 were included in the data analysis as they were considered to have produced reliable phenytoin levels. Of the 332 patients included in the data analysis, 226 were male and 106 were female, 149 were black and 183 coloured (Table 2, page 62). Of the patients classified as coloured, 67 were of Cape malay origin.

The demographic characteristics of patients included in the NONMEM analysis are recorded in Table 3 (page 63). Details of the latter are given separately for black and coloured patients and the two ethnic groups combined. The age distribution of patients is shown in Table 4 (page 63). Only 24 patients in the age group 60 - 79 were available for inclusion in the study.

Of the 206 patients not included in the data analysis, 78.2% produced no phenytoin levels. The remainder (21.8%) produced unreliable levels, i.e. a large variation between two levels on the same dose. As a result of this, 89 phenytoin levels were excluded from the study. The details of the demographic characteristics of patients who defaulted or were excluded from the study are shown in Table 5 (page 64). These patients experienced an average of 1.08 ± 1.22 seizures per month, ranging from 0 to 8 seizures per month. Some patients spent as much as 14 months in the study without producing any reliable serum phenytoin level for inclusion in the data analysis. The demographic characteristics of the patients excluded from the study differed little from those of patients included in the study.

TABLE 2: DETAILS OF THE TOTAL NUMBER OF PATIENTS ENTERED IN THE STUDY

	Patient Numbers
Total patients entered in study	538
Black patients entered in study	233
Coloured patients entered in study	305
Black patients included in NONMEM analysis	149
Coloured patients included in NONMEM analysis	183
Malay patients included in NONMEM analysis	67
Total number of patients included in NONMEM analysis	332
Black male patients included in NONMEM analysis	100
Black female patients included in NONMEM analysis	49
Coloured male patients included in NONMEM analysis	126
Coloured female patients included in NONMEM analysis	57
Black patients who were excluded or defaulted from clinic	84
Coloured patients who were excluded or defaulted from clinic	122

A further description of patients who produced no phenytoin levels, either because of defaulting or being excluded from the study, is given in Table 6 (page 64). The majority of these patients were unemployed (88.7%) and only 23.8% had their epilepsy controlled. The reasons for excluding patients from the study are outlined in Table 7 (page 65). The most common reason was non-compliance as determined by comparison of at least two serum phenytoin levels obtained on the same dose. Most patients who were excluded from the study were excluded for more than one reason.

TABLE 3: DEMOGRAPHIC CHARACTERISTICS OF PATIENTS INCLUDED IN THE NONMEM ANALYSIS

	BLACK		COLOURED		COMBINED	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Age (yr)	33.5 (12.3)	15.0 - 82.0	38.7 (15.0)	15.0 - 78.0	36.4 (14.1)	15.0 - 82.0
Height (cm)	167.2 (9.2)	147.0 - 190.0	165.2 (9.6)	142.5 - 197.0	166.2 (9.4)	142.5 - 197.0
Mass (kg)	66.4 (13.2)	44.0 - 135.0	61.9 (12.0)	36.9 - 113.1	64.0 (12.7)	36.9 - 135.0
Body surface area (m^2)	1.7 (0.2)	1.3 - 2.6	1.7 (0.8)	1.2 - 2.5	1.7 (0.2)	1.2 - 2.6
Duration in study (months)	9.0 (4.7)	1.0 - 19.0	6.6 (5.5)	1.0 - 22.0	7.7 (5.3)	1.0 - 22.0
Duration of epilepsy (yr)	10.4 (8.5)	1.0 - 48.0	12.2 (9.0)	1.0 - 34.0	11.3 (8.8)	1.0 - 48.0
Phenytoin dosage at start of study (mg/day)	295.3 (38.3)	200.0 - 450.0	302.1 (63.1)	100.0 - 500.0	299.1 (53.4)	100.0 - 500.0
Dosage at end of study (mg/day)	307.1 (57.0)	150.0 - 500.0	313.1 (70.8)	100.0 - 500.0	310.4 (64.9)	100.0 - 500.0
Optimized phenytoin dose (mg/day)	308.8 (56.6)	150.0 - 500.0	302.4 (66.4)	100.0 - 500.0	305.8 (60.7)	100.0 - 500.0
Optimized phenytoin level (µmol/l)	65.3 (26.4)	15.0 - 133.0	58.3 (20.9)	22.0 - 115.0	62.7 (23.9)	15.0 - 133.0

TABLE 4: NUMBER OF PATIENTS IN SELECTED AGE CATEGORIES

	NUMBER OF PATIENTS			
	AGE GROUPS			
	15 - 19	20 - 39	40 - 59	60 - 79
BLACK	13	90	44	2
COLOURED	11	95	55	22
TOTAL	24	185	99	24

TABLE 5: DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WHO DEFAULTED OR WERE SUBSEQUENTLY EXCLUDED FROM THE STUDY

	BLACK			COLOURED			COMBINED		
	Mean (SD)	Range	n	Mean (SD)	Range	n	Mean (SD)	Range	n
Age (yr)	32.91 (11.46)	15-73	79	41.35 (14.86)	16-74	119	37.98 (14.19)	15-74	198
Height (cm)	168.26 (11.01)	144-189	36	166.78 (8.76)	149.5 ± 179.5	43	167.44 (9.8)	144-189	79
Mass (kg)	64.74 (14.04)	41.6-113	37	60.73 (12.60)	34.3-85.0	51	62.41 (13.3)	34.3-113	88
Duration of epilepsy (yr)	11.34 (9.71)	1-50	56	11.77 (11.36)	1-50	112	11.58 (10.64)	1-50	168
Phenytoin dose (mg/day)	291.78 (47.68)	200-400	76	295.09 (47.91)	100-500	112	293.75 (47.72)	100-500	188
No. of seizures experienced during the 3 months before entering the study	2.49 (3.15)	0-14	78	3.35 (3.80)	0-24	116	3.24 (3.65)	0-24	194
Duration in study (months)	1.81 (2.64)	0-13	81	2.64 (3.52)	0-14	107	2.28 (3.20)	0-14	188

TABLE 6: DESCRIPTION OF PATIENTS WHO DEFAULTED OR WERE EXCLUDED FROM THE STUDY

	BLACK	COLOURED	COMBINED
DESCRIPTION	%	%	%
Total patients who defaulted or were excluded	36.1	40.0	38.3
Excluded from study	3.9	12.5	20.9
Defaulted from study	32.2	27.5	79.1
Males	67.5	84.5	76.0
Females	32.5	15.5	24.0
Employed	7.4	12.4	9.9
Unemployed	91.4	86.0	88.7
Scholars	1.2	1.6	1.4
Seizures apparently controlled	28.6	18.9	23.8

TABLE 7: REASONS FOR EXCLUSION FROM THE STUDY

REASON	PERCENTAGE OF PATIENTS	
	BLACK	COLOURED
	n = 9	n = 38
Alcohol abuse (suspected or confirmed)	44.4	15.8
Irregular attendance at clinic	22.2	15.8
Abnormal laboratory finding	11.1	7.9
Non-compliance (a) Determined by phenytoin levels and, (b) Tablet counts/missing phenytoin doses	33.3 1.1	63.2 -
Adverse/hypersensitivity reactions	-	2.6
Wrong anticonvulsant prescribed	-	2.6
Anticonvulsant changed or added	11.1	10.5
Interfering drugs prescribed	11.1	5.3
Patient or family members unable to fill in diary	-	5.3
Patient or family members uncooperative	22.2	15.8
Unable to obtain blood for phenytoin levels	-	2.6
Phenytoin stopped	-	5.3
Became pregnant subsequent to entering study	22.2	7.9
Deceased	-	2.6

Of the patients, 49.7% smoked cigarettes; 40.7% of black patients smoked whereas a significantly greater proportion (58.7%) of coloured patients smoked. Black patients smoked 9.1 ± 6.6 (range 1 - 30) cigarettes per day whereas coloured patients smoked 10.6 ± 7.6 (range 1 - 40) cigarettes per day. Of black and coloured patients, 27.4% and 27.5% respectively reported mild-to-moderate alcohol consumption. Alcohol abusers were not included in the study. A large proportion of patients (67.8%) were unemployed and this contributed to patients defaulting on clinic attendance, given the lack of finances for transport costs and hospital fees.

Most of the patients were from Heideveld (HDH), Elsies River (ERD), Lotus River (LRD), Khayelitsha (KDH) and Guguletu Day Hospitals (GDH) and fewer patients were from the Outpatients Neurology Clinic at Groote Schuur Hospital (GSH), Dr Abdurahman Day (DAD) Hospital, Betel School for Epilepsy and the South African National Epileptic League (SANEL) (Table 8, page 66). Details of the clinical pharmacokinetic services offered at the

various hospitals and institutions are given in Table 8. The duration of the study at each clinic, the number of clinic sessions held, the number of patients producing results, and the percentage of defaulting patients at each of the hospitals and institutions are shown in the table.

TABLE 8: SUMMARY OF CLINICAL PHARMACOKINETIC SERVICES OFFERED AT THE VARIOUS HOSPITALS AND INSTITUTIONS IN THE WESTERN CAPE

HOSPITAL / INSTITUTION *	DURATION OF STUDY (months)	NO. OF CLINIC SESSIONS	NO. OF PATIENTS	NO. OF PATIENTS PRODUCING RESULTS	DEFAULTERS (%)
HDH	21.0	91	110	66	40.0
SANEL	22.0	46	24	16	33.3
GSH	5.0	30	12	10	16.7
GDH	19.8	83	129	81	37.2
ERD	18.0	74	70	42	40.0
KDH	15.0	67	94	68	27.7
BETEL	8.3	6	7	7	0.0
LRD	6.7	30	60	26	56.7
DAD	7.6	36	32	16	50.0
TOTALS		463	538	332	
%				61.7	

* the full names of the hospitals and institutions are reported in the text

Altogether 3341 consultations were conducted by the investigator. Further details of these are given in Table 9 (page 67). The number of repeat consultations depended on the severity of the epilepsy, the presence of adverse reactions and the need for dose adjustments.

TABLE 9: NUMBER OF CONSULTATIONS CONDUCTED BY THE INVESTIGATOR

	NUMBER OF CONSULTATIONS		
	BLACK	COLOURED	COMBINED
Patients included in study	1459	1289	2748
Patients who defaulted or were excluded from study	198	395	593
Total number of consultations			3341

A total of 1095 serum phenytoin levels were taken during this study. As serum phenytoin concentrations are not routinely measured at the hospitals concerned, the number of measured serum phenytoin concentrations per individual is greater than would routinely be available. The 332 patients included in the study produced a total of 1006 serum phenytoin levels. Only 853 of these levels were judged reliable for inclusion in the study. A minimum of two serum phenytoin concentrations at steady state on one dosage regimen was preferred for each patient to permit adequate analysis and assessment of reliability of the data. The characterization of serum phenytoin levels to dose relationship is shown in Table 10 (page 68). In 81 (24.4%) patients, only one serum phenytoin level was obtained. Even one data point per individual obtained during routine treatment can be used by NONMEM and can contribute useful information (Sheiner and Beal, 1983). In 48 (14.5%) patients, one level on each of 2 to 4 doses of phenytoin was obtained. Because of the prospective nature of the study, patients who produced a single dose : serum concentration were included in the analysis provided they claimed good compliance, the tablet counts were correct and the diaries correctly filled in. If a single level was lower than 30 µmol/l such a level was not included in the analysis. In the balance of 203 (61.1%) patients at least 2 serum phenytoin levels on one or more doses of phenytoin was obtained. The average number of serum phenytoin concentration measurements obtained per patient was 2.6, range 1 to 8. These samples were obtained as part of the therapeutic drug monitoring programme.

On admission, 235 patients were taking phenytoin alone, while 140 (40 black and 100 coloured) patients were taking other medication concurrently with phenytoin. The concurrent medications are reported in the literature not to interfere with phenytoin pharmacokinetics. In this study, paracetamol (Panado^{R}) was the most frequently prescribed drug used concurrently with phenytoin (Table 11, page 69). However, all patients were taking phenytoin as the only drug for the treatment of their epilepsy. In 52 (15.7%) patients, other disease states besides epilepsy were present. A list and the

occurrence of these diseases are reported in Table 12 (page 71). Hypertension was the most frequent disease (4.8% of patients) occurring concurrently with the epilepsy.

TABLE 10: CHARACTERIZATION OF SERUM PHENYTOIN CONCENTRATION : DOSE RELATIONSHIPS

	NUMBER OF PATIENTS		
	BLACK	COLOURED	TOTAL
1 CONC : 1 DOSE	31	50	81
2 CONCS : 1 DOSE	46	61	107
3 CONCS : 1 DOSE	5	4	9
4 CONCS : 1 DOSE	-	2	2
2 CONCS : 2 DOSES(2)	4	13	17
2 CONCS : 2 DOSES (3)	21	19	40
2 CONCS : 2 DOSES (4)	19	21	40
2 CONCS : 2 DOSES (5)	4	1	5
3 CONCS : 3 DOSES	14	8	22
4 CONCS : 4 DOSES	5	4	9

CODES:

1 CONC : 1 DOSE	= 1 serum phenytoin concentration obtained on a single dose of phenytoin
2 CONCS : 1 DOSE	= 2 concentrations obtained on a single dose of phenytoin.
2 CONCS : 2 DOSES (2)	= total of 2 concentrations, 1 concentration obtained on each of 2 doses.
2 CONCS : 2 DOSES (3)	= total of 3 or more concentrations obtained on 2 doses of phenytoin; 2 or more concentrations obtained on one dose and 1 concentration on another dose.
2 CONCS : 2 DOSES (4)	= total of 4 or more concentrations obtained on 2 doses; 2 levels on 1 dose and at least 2 levels on another dose, etc.

Phenytoin was initially prescribed in 91.0, 7.1 and 1.9% of the patients once, twice or thrice daily, respectively. Two hundred and sixty-five dosage adjustments were made during this study in 156 patients. The phenytoin dose was increased in 31.9% and reduced in 15.1% of patients taking phenytoin. Altogether, dose adjustments were made in 47.0% of patients. No change in dose therefore represented the most common therapeutic decision in patients on phenytoin (Table 13, page 72). When considering only the patients in whom dosage adjustments were made - one, two or three dosage adjustments were made in 69.8, 24.4 and 5.8% of patients, respectively. Similarly, if only patients optimized on phenytoin were considered, the results differ little.

TABLE 11: FREQUENCY OF DRUGS ADMINISTERED CONCURRENTLY WITH PHENYTOIN AT THE TIME OF BLOOD COLLECTION FOR PHENYTOIN LEVELS

GENERIC NAME	TRADE NAME	NUMBER OF PATIENTS	
		BLACK	COLOURED
		n = 40	n = 100
Amitriptyline	Tryptanol	-	2
Aspirin	Disprin	-	1
Atenolol	Tenormin	-	1
Astemizole	Hismanal	-	1
Clonidine	Dixarit	-	1
Diclofenac	Voltaren	-	1
Digoxin		-	1
Ferrous gluconate		-	1
Folic acid		2	-
Furosemide		-	1
Glibenclamide		2	-
Glicazide	Diamicron	-	1
Ibuprofen	Brufen	3	6
Indomethacin	Indocid	-	4
Isosorbide dinitrate	Isordil	-	2
Ispaghula	Fybogel	1	-
Medroxy-progesterone	Provera, Depo-Provera	1	1
Methyldopa	Aldomet	3	3
Naproxen	Naprosyn	-	1
Norethisterone	Nur-Isterate	2	-
Oxazepam	Serepax	-	1
Paracetamol	Panado	13	40
Potassium chloride	Slow-K	-	1

TABLE 11: continued

GENERIC NAME	TRADE NAME	NUMBER OF PATIENTS	
		BLACK	COLOURED
Prazosin	Minipress	-	1
Promethazine	Phenergan	-	1
Propranolol	Inderal	-	1
Pyridoxine		-	1
Salbutamol	Ventolin	-	2
Sodium bicarbonate		-	1
Vitamin B. Co.		-	6
Vitamin B1		-	1
Aspirin, codeine	Codis	1	-
Paracetamol, codeine	Panadeine	2	-
Diphenhydramine, ammonium chloride, sodium citrate	Benylin	1	-
Aluminium hydroxide	Amphojel	-	2
Aluminium hydroxide, magnesium oxide, dicyclomine, sodium lauryl sulphate, methyl-cellulose, methylpoly-siloxane	Propan Gel-S	1	2
Triamterene, hydrochloro-thiazide	Dyazide	-	1
Amiloride, hydrochloro-thiazide	Moduretic	8	8
Estradiol, estriol, norethisterone acetate	Trisequens	-	3

TABLE 12: FREQUENCY OF THE OCCURRENCE OF OTHER DISEASE STATES BESIDES EPILEPSY

DISEASE	NUMBER OF PATIENTS	
	BLACK	COLOURED
	n = 20	n = 32
Alcoholic cardiomyopathy	-	1
Anaemia	-	1
Arthritis	1	1
Asthma	-	3
Bronchiectasis	-	1
Bronchitis	-	1
Cerebral palsy	2	1
Diabetes	1	1
Endometriosis	-	1
Cerebral granuloma	1	-
Hemiplegia	2	1
Hydrocoele	1	-
Hypertension	6	10
Lung abscess	1	-
Mental retardation	1	1
Neurocysticercosis	2	-
Peripheral neuropathy	-	1
Pleurisy	-	1
Poliomyelitis	-	1
Psoriasis	-	1
Rickets	1	-
Schizophrenia	-	1
Sinusitis	-	1
Stroke	1	1
Venereal disease	-	2

TABLE 13: PERCENTAGE OF PATIENTS IN WHOM THE PHENYTOIN DOSE WAS EITHER INCREASED, DECREASED OR UNCHANGED

	PERCENTAGE OF PATIENTS		
	BLACK	COLOURED	COMBINED
	n = 149	n = 183	n = 332
Dose increased	34.2	30.1	31.9
Dose decreased	17.5	13.1	15.1
No change in dose	48.3	56.8	53.0
Dose adjusted	51.7	43.2	47.0

Patients were considered to be optimized on phenytoin if they experienced one seizure in 3 months or were seizure free for this period. One hundred and ninety-four (58.4%) patients were optimized on phenytoin at the end of the study. In 13.34% of patients, dosage adjustments were made without obtaining a subsequent serum phenytoin level at steady state at that dose because of defaulting. One hundred and ninety-nine patients were studied on 1 dose, 102 patients on 2 different doses, 22 patients on 3 different doses and 9 patients on 4 different doses. The average optimized dose of phenytoin was 305.8 mg/day, range 100 - 500 mg/day. This value may be biased as the calculation thereof did not take into account the lowest dose necessary to control the seizure. Many of the patients were started on 300 mg phenytoin and the small increments and decrements in doses made by the investigator were probably overshadowed by the large number of patients on 300 mg phenytoin. The number of prescriptions for 50 mg and 27 mg phenytoin sodium (half an Epanutin Infatab^{R}) increased from 20 to 116 and from zero to 51, respectively. The following doses of phenytoin sodium in mg/day were prescribed during this study : 100, 150, 171, 200, 227, 250, 277, 286, 300, 307, 314, 327, 334, 341, 350, 364, 377, 384, 400, 414, 427, 450 and 500.

In 55.6% of patients no cause for their epilepsy could be determined by questioning the patient or perusing the folder. Head trauma due to motor vehicle accidents or assault was the cause of the epilepsy in 34.7% of patients. The other causes of epilepsy are available, but not reported here.

Forty-four percent of patients experienced adverse effects resembling those of phenytoin at the first visit, of whom 23.5% had experienced adverse effects not related to phenytoin. At the first visit, 20.5% of patients had experienced adverse effects probably related to phenytoin. At the last visit, 3.2% of patients experienced adverse effects to phenytoin.

The decision whether the adverse effects were related or unrelated to phenytoin was based on serum phenytoin concentrations and the clinical judgement of the investigator. The correlation of serum concentration with the adverse effects of dizziness, diplopia, nystagmus and ataxia are outlined in Table 14. These adverse effects were experienced at the time when the blood samples were taken for the determination of serum phenytoin concentrations.

TABLE 14: SERUM PHENYTOIN CONCENTRATION VERSUS ADVERSE EFFECT RELATIONSHIPS

ADVERSE REACTION	SERUM PHENYTOIN CONCENTRATIONS (µmol/l)								
	BLACK			COLOURED			COMBINED		
	Mean (SD)	Range	n	Mean (SD)	Range	n	Mean (SD)	Range	n
Dizziness	119.5 (34.8)	66-205	22	121.9 (40.4)	59-208	31	120.7 (37.9)	59-208	53
Diplopia	121.0 (38.0)	73-205	19	129.8 (56.8)	65-208	16	125.4 (47.0)	65-208	35
Nystagmus	110.7 (36.7)	61-205	18	118.6 (48.5)	52-208	23	114.7 (43.4)	52-208	41
Ataxia	130.9 (31.6)	80-205	19	137.3 (36.0)	79-206	21	134.1 (33.7)	79-206	40

One hundred and fourteen (53 black and 61 coloured) patients defaulted on clinic attendance after producing levels that were used in the NONMEM analysis. Of these, 47 (27 black and 20 coloured) were optimized on phenytoin and the balance of the patients defaulted on attendance, even though they were not optimally controlled. Sixty-three (42 black and 21 coloured) patients were discharged from the pharmacokinetic consulting service prior to termination of the study as they had been optimally controlled on phenytoin.

4.2. THERAPEUTIC CONCENTRATIONS OF PHENYTOIN

The relationship between the therapeutic concentration ranges of phenytoin and the number of patients optimized in the specific range is given in Table 15 (page 74). Most patients (61.9%) were optimized in the therapeutic range of 40 to 79 µmol/l. A significant number of patients (21.1%) were optimized on phenytoin at serum concentrations above

that range and 1.6% of patients were optimized at serum concentrations above 120 µmol/l. Conversely, 17% of patients were optimized at serum levels below 40 µmol/l. This serves to emphasize the need to view the therapeutic range as a statistical concept with regard to group means, within which individuals differ both in efficacy and side-effects.

TABLE 15: RELATIONSHIP BETWEEN THERAPEUTIC CONCENTRATION OF PHENYTOIN AND THE PERCENTAGE OF PATIENTS OPTIMIZED AT THAT CONCENTRATION RANGE

THERAPEUTIC CONCENTRATION (µmol/l)	PERCENTAGE OF PATIENTS		
	BLACK	COLOURED	COMBINED
	n = 93	n = 78	n = 171
0.0 - 19	0.0	0.0	0.0
20 - 39	16.1	18.0	17.0
40 - 59	22.6	39.7	31.2
60 - 79	34.4	26.9	30.7
80 - 99	12.9	9.0	10.9
100 - 119	10.8	6.4	8.6
120 - 139	3.2	0.0	1.6

4.3. SEIZURE FREQUENCY REDUCTION

The response to treatment was based on an assessment over a minimum period of 3 months (Callaghan *et al.*, 1985). A total of 1202 seizures during the 3 months before entering the study (1st baseline period) were reported by the 332 patients. A total of 1070 seizures were reported by the same patients during the 3 months after entering the study (2nd baseline period). The same patients reported 643 seizures 3 months before termination of the study (test period). These results were not used to determine the benefit of the clinical pharmacokinetic service (i.e. a reduction in seizure frequency) as many of these patients were in the study for less than 3 months. This makes comparison of the number of seizures occurring during the 1st baseline with those occurring during the test period difficult as an overlap between these periods existed. In this case, a second baseline period was also not available.

To determine the benefit of our service, the following criteria were set: (a) the patient had to participate in the study for at least 6 months so that the second baseline and the test

periods did not overlap, and (b) the patient had to attend the clinic for at least 6 consultations. One hundred and ninety-five (113 black and 82 coloured) patients satisfied these criteria. The total number of seizures experienced by these patients during the 1st and 2nd baseline periods and the test period are reported in Table 16. The percentage reduction in seizure frequency was determined by comparing the number of seizures during the 1st and 2nd baseline periods with those occurring during the test period. The average of the number of seizures occurring during the 1st and 2nd baseline periods was considered as the initial frequency. The results of this analysis are reported in Table 17 (page 76). A 60.1 and 69.5% reduction in seizure frequency were obtained in black and coloured patients, respectively. In 29 black and 50 coloured patients, the dose of phenytoin was adjusted within 3 months of their entering the study. As the seizure frequency during this period was used as the 2nd baseline period, the percentage reduction in seizure frequency stated above as a result of the clinical pharmacokinetic service is most probably an underestimate.

TABLE 16: TOTAL NUMBER OF SEIZURES EXPERIENCED DURING THE FIRST AND SECOND BASELINE PERIODS AND THE TEST PERIOD[1]

PERIOD	NUMBER OF SEIZURES		
	BLACK	COLOURED	COMBINED
	n = 113	n = 82	n = 195
First baseline period	273	394	667
Second baseline period	284	304	588
Test period	111	105	216

[1] Only patients who participated in the study for at least six months and attended the clinic for at least 6 consultations were included in this analysis.

TABLE 17: PERCENTAGE REDUCTION IN SEIZURE FREQUENCY

PERIOD	PERCENTAGE IMPROVEMENT IN SEIZURES		
	BLACK	COLOURED	COMBINED
	n = 113	n = 82	n = 195
Based on the first baseline period	59.3	73.4	66.4
Based on the second baseline period	60.9	65.5	63.2
Based on the average of the first and second baseline periods	60.1	69.5	64.8

The percentage reduction in seizure frequency was grouped into 5 categories as indicated in Table 18. Of the 195 patients who satisfied the inclusion criteria in this analysis, 127 experienced a reduction in seizure frequency. This represented 64.9% of patients. The seizure frequencies per month (mean and range) for specific time periods before and after entering the study are reported in Table 19 (page 77). The patients in this analysis were evaluated according to the same criteria as above.

TABLE 18: PERCENTAGE REDUCTION IN SEIZURE FREQUENCY GROUPED INTO VARIOUS CATEGORIES[1]

REDUCTION IN SEIZURE FREQUENCY (%)	PERCENTAGE OF PATIENTS		
	BLACK	COLOURED	COMBINED
	n = 113	n = 82	n = 195
0	37.2	32.9	35.1
1 - 24	0.9	0.0	0.5
25 - 49	5.3	6.1	5.7
50 - 74	12.4	12.2	12.3
75 - 99	3.5	12.0	7.2
100	40.7	37.8	39.2

[1]Patients who were already optimized on phenytoin are included in this analysis.

TABLE 19: MEAN SEIZURE FREQUENCY PER MONTH FOR SPECIFIC PERIODS BEFORE AND AFTER THE INITIATION OF THE STUDY

PERIOD	SEIZURE FREQUENCY PER MONTH					
	BLACK		COLOURED		COMBINED	
	Mean	Range	Mean	Range	Mean	Range
1st baseline period	2.44	0-15	4.80	0-48	3.62	0-48
2nd baseline period	2.54	0-29	3.75	0-22	3.15	0-29
Test period	1.0	0-16	1.36	0-14	1.18	0-16

Patients whose seizure frequency had fallen from baseline level by 50% or more in the test period were considered to have a 'good improvement' in seizure control. A reduction of less than 50% was categorized as 'poor to intermediate improvement' in seizure control (Travers *et al.*, 1972). After this categorization, 58.7% of patients exhibited a good improvement in their seizure control. This analysis included patients who were well-controlled or seizure-free on phenytoin.

The decrease in seizure frequency reported in this study may be greater than that indicated by the results. The seizure frequency for the first baseline period was in most cases obtained from the patient and/or the family, and often from the scant information in the hospital folder. Epileptic patients tend to be forgetful or unaware that they had a seizure and this may result in the number of seizures occurring being under-reported. Also the compliance during this time was in question. The second baseline period was taken into consideration to minimize the influence of non-compliance on the interpretation of the results.

4.4. CLINICAL PHARMACOKINETIC SERVICE

There was a high degree of acceptance of the service judged by the requests for advice from the doctors on the management of epilepsy, even for patients not included in this study. The extent to which the advice was followed was a further indicator of this. For the 332 patients included in this study, only 7 dosage recommendations were not accepted,

for the following reasons: (a) a dose increment of 27 mg instead 100 mg of phenytoin sodium was considered too conservative. The doctor wanted to follow the standard practice of increasing the dose of phenytoin by 100 mg. The patient subsequently became toxic, and the recommendation of a 27 mg increase in dose was then accepted; (b) resistance to increasing the dose so that the serum concentration would not exceed the therapeutic range: this occurred in 2 patients although they were still experiencing seizures and showed no signs of toxicity; (c) the regimen recommended was too complex, and it was feared the patient would not comply with the proposed regimen. This happened in 4 cases. All of the above rejections of the recommendations happened at the start of the pharmacokinetic consultation service.

As this study was conducted among outpatients, compliance was critical. Many patients were compliant as long as they possessed phenytoin, but they defaulted when they ran out of medication. Other patients defaulted in keeping their clinic appointments. These patients were also regarded as non-compliant. In the majority of patients, more than one phenytoin level was obtained and this greatly assisted the assessment of patient compliance. In most patients, at least two levels on a particular dose were taken before further adjustment. It was found in numerous instances that 2 blood samples obtained on the same dose in the same subject showed markedly differing values. If non-compliance was suspected, a third level was taken on the same dose. Thereafter, the two levels that differed the least from each other were used. For these reasons, non-compliance was not considered to be a major problem in this study. As phenytoin is used prophylactically in the management of epilepsy, one could expect occasional under-compliance in a patient who had good epileptic control. A number of patients remained obstinately non-compliant despite the best efforts of the investigator. Frequent visits, good relationships with the patients and their families, and the regular checking of the serum phenytoin levels contributed greatly to improved compliance (Gibberd *et al.*, 1970). Patient morale also appeared to improve.

4.5. LIMITATIONS OF THE STUDY

The following limitations and drawbacks were experienced in offering a clinical pharmacokinetic service:

(1) Non-compliance posed a problem, especially during the initial period after the patient entered the study. As a result, 242 serum phenytoin samples were not

used in this analysis. A number of patients who claimed to be taking their phenytoin every day had a zero or near zero serum phenytoin level.

(2) As most of the patients involved in this study were from the lower socio-economic group, they were often not able to keep an appointment because of lack of finance for transport or hospital fees.

(3) The frequent change of doctors at the Outpatient Neurology Clinic at Groote Schuur Hospital often led to doctors being unaware that the service was being offered and resulted in confusion as to the follow-up treatment of the patient. The short duration (approximately two and a half hours) of the clinic posed a problem. Some patients were sent for X-rays and other investigations and only returned at the end of the clinic session. By the time the necessary calculations had been completed and a report written, the doctor had often left and the recommendations were not acted on. If the patient was seen by the doctor close to the end of the clinic session there was often no time for the pharmacokinetic consultation. As this was the last clinic of the day, the clinic was closed at the end of the clinic session and the investigator was unable to consult with the patient.

(4) Patients were taken off phenytoin or another anticonvulsant which had been added before the dose of phenytoin was optimized. However, it must be stated that the latter seldom occurred once the doctors were fully aware of the service that was being offered. When this happened, it was most often when the patient attended the hospital on a day other than that on which the epilepsy clinic was held.

(5) The frequent change of doctors at some of the hospitals presented a problem. This required regular discussions of the project and phenytoin pharmacokinetics throughout the study.

(6) Patients were being treated for epilepsy at more than one hospital, for example, a neurology clinic at a major hospital and a day hospital. This often resulted in the patient obtaining an extra supply of phenytoin. This led to patients missing the appointment with the investigator if they had enough medication. It made the assessment of compliance based on tablet counts difficult. Doses were also changed without a follow-up report.

(7) Patients may have falsely reported seizures in order to obtain a disability grant. As far as could be determined this seldom occurred in our clinic, as most patients reported an improvement.

(8) Pharmacists often supplied phenytoin acid instead of phenytoin sodium. This occurred especially when the dispensary had no stock of phenytoin sodium 50 mg tablets. In such cases, the serum phenytoin concentration measurements were usually delayed for a month.

(9) On numerous occasions a drug which interfered with the pharmacokinetics of phenytoin was concurrently prescribed by the doctor or primary health care nurse. The most common interfering drugs prescribed were theophylline and co-trimoxazole. The patients affected were temporarily or permanently withdrawn from the study. Otherwise, the doctor or primary health care nurse was advised to consider prescribing an alternative drug, if possible.

(10) The possibility of the patients taking extra doses of phenytoin before attending the clinic, after experiencing a seizure or when having a feeling that a seizure was imminent, was always borne in mind. If this happened, blood collection for assessment of serum phenytoin concentration was deferred, usually for a month.

(11) The initial reluctance of a doctor to prescribe complex dosage regimens of phenytoin, e.g. 3 different dosage forms of phenytoin at the same time, presented a problem.

(12) A policy of the hospital authorities that entailed rotation of the nurses to different hospitals every three months presented a problem in that some of the incoming nurses were unaware of the pharmacokinetic service and therefore did not refer the patients to our clinic, but instead requested the patients to collect their medication at the dispensary. If the repeat prescription of phenytoin was written up by the nurse, the patients often defaulted on attendance at the pharmacokinetic service as they thought it unnecessary to consult the investigator, or were reluctant to wait in a queue. The incoming nurses often erroneously sent the phenytoin blood sample to the state laboratory instead of keeping the blood samples aside for collection by the investigator at the end of the clinic session.

(13) Women of child-bearing age were taken off phenytoin by the doctor and prescribed carbamazepine in the erroneous belief that carbamazepine is safer in pregnancy.

(14) The high number of defaulters was partly due to lack of interest on the part of the patients. Epilepsy is such a common condition in the communities in which this study was performed that having a seizure was acceptable to the patient and his/her family. A typical initial response from the patient was "I have **only** had two or three fits the past month." Some patients mistakenly felt that nothing could be done to improve their epilepsy as they had previously consulted many doctors.

(15) It was often difficult to obtain a phenytoin level after the first dose adjustment as many patients defaulted on attendance at the pharmacokinetic service once better seizure control was achieved.

(16) At two of the hospitals, most of the patients were Xhosa-speaking and this required the assistance of an interpreter. Consultations with these patients tended to be prolonged.

(17) The old age home organisation which manages almost all the old age homes in Cape Town refused to allow access to their epileptic residents taking phenytoin. No reason was given, and this was a major limiting factor in the study as few geriatric patients were seen at the hospitals. In Cape Town, there are two old age homes which care for approximately 40 black geriatrics. Of these residents, 3 were on phenytoin therapy of whom 2 were suitable for the study.

(18) Widespread alcohol abuse excluded many patients from the study.

(19) Illiteracy was high among the target patient population, and some patients were unable to fill in the diary or understand the instructions. If family assistance could not be found, these patients were excluded from the study. It should be stressed that although many patients were illiterate they nevertheless were capable of filling in the diary by making crosses when taking phenytoin and when seizures occurred. This required the instructions to be repeated many times.

(20) Hunger and stress due to financial problems were common trigger factors for seizures. The patients' perception that they should not take phenytoin if they had not eaten added to the problem of non-compliance.

(21) While the information gained in this study has been most useful for understanding population pharmacokinetics, the clinical pharmacokinetic consulting services which had to be offered to obtain data were labour intensive, and required the cooperation of many patients over a prolonged period of time.

4.6. INTER-ASSAY COEFFICIENT OF VARIATION

An inter-assay coefficient of variation of 2.21% was obtained in this study (n = 12). The level of acceptance was set at 5% (Abbott Laboratories).

4.7. ADSORPTION OF PHENYTOIN TO RUBBER STOPPERS

In the samples (n = 6) used to determined whether adsorption of phenytoin to the rubber stoppers occurred, no significant difference[2] (t = 0.20, $p > 0.05$) between the samples was found. The estimate of the mean difference (95% CI in brackets) was found to 0.17 (-1.95 to 2.3) µmol/l. The median difference was 1,5 (range 4,0) µmol/l. The coefficient of variation was 0.5 %.

4.8. STABILITY OF PHENYTOIN IN WHOLE BLOOD SAMPLES

In the samples (n = 6) used to determine the effect of storage on the phenytoin serum concentration, no significant difference[2] (t = 0.273, $p > 0.05$) was found between the samples stored for 2 hours and 6 hours. The estimate of the mean difference (95% CI in brackets) was 0.2 (-1.83 to 2.23) µmol/l. The median difference was 1 (range 5) µmol/l. However, a significant difference[2] (t = 7.07, $p \leq 0.05$) was found between the samples (n = 6) stored for 6 and 26 hours. The estimate of the mean difference (95% CI in brackets) was 5.8 (1.61 to 9.99) µmol/l. The median difference was 5 (range 4) µmol/l. The coefficient of variation was 2.4 % between the samples assayed two hours after blood collection and the samples stored as whole blood for six hours. The coefficient of variation between the samples assayed 6 and 26 hours after blood collection was 7.6 %.

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[2]parametric and nonparametric tests produced the same results

4.9. NONMEM ANALYSIS

A total of 74 NONMEM runs were performed in this study, 20 runs on NONMEM version III level 1.2 and 54 runs on version IV level 1.0. Only 61 of the important runs are reported in this study. The results of this study are reported as 3 sets of analysis. Set 1 analysis was performed at the University of Durban-Westville, South Africa, under the supervision of Prof. R. Miller. In this analysis, the influence of various patient factors on Vm and Km values was investigated using the Michaelis-Menten model. Population estimates of Vm and Km and their inter- and intra-individual variability were also obtained. Set 2 analysis was performed at the Food and Drug Administration of the United States of America under the supervision of Dr T. Ludden, and investigated primarily with regard to whether the parallel Michaelis-Menten and first-order elimination model fitted the data better than the Michaelis-Menten model. Population parameter estimates of Vm, Km and Cl and their inter- and intra-individual variability were obtained using the parallel Michaelis-Menten and first-order elimination model with dose or steady-state serum concentration as the dependent variables. Set 3 analysis was performed at the University of Durban-Westville and validated the applicability of the determined Vm, Km and Cl values, and assessed and compared the reliability of the 3 steady-state pharmacokinetic models in predicting phenytoin doses or serum concentrations.

The abbreviations used in the NONMEM data file (Annexure 8) and the control files (Annexures 9 - 11) are reported in the Glossary of Symbols and Abbreviations on pages XV - XVII.

The information was coded as follows (Annexures 8 - 11):

Gender:	Female	= 0
	Male	= 1
Race:	Black	= 0
	Coloured	= 1
	Malay	= 2
Smoking:	Non-smokers	= 0
	Smokers :	
	less than 10 cigarettes/day	= 1
	10 to 20 cigarettes/day	= 2
	more than 20 cigarettes/ day	= 3
Alcohol:	Non-drinkers	= 0
	Drinkers	= 1

4.9.1. NONMEM ESTIMATES

The following estimates were obtained from the NONMEM output (Driscoll, 1989):

(a) typical (mean) population pharmacokinetic parameter values of Vm, Km and Cl,

(b) variances of the population values i.e . σ^2_{Vm} , σ^2_{Km}, σ^2_{Cl} and inter-individual random effects of Vm, Km and Cl i.e. η^{Vm}, η^{Km} and η^{Cl},

(c) variance of the residual random error, σ^2_ϵ i.e. intra-individual differences ε,

(d) standard errors of the parameter estimates,

(e) correlation matrix of the estimates, and

(f) value of the MOF

Estimates of the variance components (σ^2, σ^2_ϵ) from NONMEM were converted to standard deviation by derivation of their square root.

4.9.2. SET 1 ANALYSIS

In this set of analysis the structural model S1 was evaluated. The results are presented in Tables 20 (page 85) and 21 (page 88).

After the first NONMEM run, dose : serum concentration pairs with weighted residuals of more than three standard deviations from the population mean were identified from the scatterplots of identification versus weighted residuals. This indicated that these data points were considerably different from the others. Four dose : serum concentration pairs were identified to be outliers in four different patients. In three patients, a dose : serum concentration pair was omitted for the following reasons: 2 of the dose : serum concentration pairs were excluded as the phenytoin samples were possibly taken before steady state was reached and the third dose : serum concentration pair was excluded because of possible poor compliance. One outlier on the scatterplot was included in the NONMEM data set as the reason for it was attributed to significant enzyme induction of phenytoin, given that non-compliance had been ruled out and no error could be found. The revised NONMEM data set is presented in Annexure 8. The goodness of fit was judged by comparing the scatterplots of the actual dose versus the predicted dose obtained with the original and the revised data sets. The scatterplots of the weighted residuals versus predicted dose, weight, age, sex, body surface area, smoking or patient's identification number were also compared. Less scatter of the data points were obtained for the revised data set.

TABLE 20: DETAILS OF NONMEM PARAMETER MODELS USING THE MICHAELIS-MENTEN STRUCTURAL MODEL (S1) - (SET 1 ANALYSIS)

MODEL NO.	PARAMETER MODEL	MINIMUM OBJECTIVE FUNCTION
P1	$Vm = \theta_1 + \eta_1$ $Km = \theta_2 + \eta_2$	6792.103
P2 [3]	$Vm = \theta_1 + \eta_1$ $Km = \theta_2 + \eta_2$	6714.30
P3	$Vm = (\theta_1 * WT + \theta_3) + \eta_1$ $Km = \theta_2 + \eta_2$	6656.0
P4	$Vm = (\theta_1 * WT + \theta_3)(1 + \eta_1)$ $Km = \theta_2 + \eta_2)$	6693.575
P5	$Vm = (\theta_1 * BSA + \theta_3) + \eta_1$ $Km = \theta_2 + \eta_2$	6658.213
P6	$Vm = (\theta_1 * WT + \theta_3) + \eta_1$ $Km = \theta_2 * RACE + \eta_2$ where RACE = θ_4 if black, otherwise = 1	6651.213
P7	$Vm = (\theta_1 * WT + \theta_3) + \eta_1$ $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured $= \theta_5 + \eta_2$ if malay	6647.681
P8	$Vm = (\theta_1 * WT + \theta_3)$ RACE $+ \eta_1$ where RACE = θ_4 if malay and coloured, otherwise = 1 $Km = \theta_2 + \eta_2$	6646.514
P9	$Vm = (\theta_1 * WT + \theta_3)$ RACE $+ \eta_1$ where RACE = θ_4 if coloured, otherwise = 1 RACE = θ_5 if malay, otherwise = 1 $Km = \theta_2 + \eta_2$	6644.701
P10 [4]	$Vm = (\theta_1 * WT + \theta_3) + \eta_1$ $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if malay and coloured	6651.213
P11	$Vm = \theta_1 * WT^{\theta_3} + \eta_1$ $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6651.975

[3] the MOF and the parameter estimates obtained with model P2 were for the revised NONMEM data set.
[4] for models subsequent to P10, coloured and malay patients will be grouped together as 'coloured'

TABLE 20: continued

MODEL NO.	PARAMETER MODEL	MINIMUM OBJECTIVE FUNCTION
P12	$Vm = (\theta_1 * WT + \theta_3)\ SEX + \eta_1$ where SEX = θ_5 if male, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6649.728
P13	$Vm = (\theta_1 * WT + \theta_3)\ SMK + \eta_1$ where SMK = θ_5 if smoker, otherwise =1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6639.44
P14	$Vm = (\theta_1 * WT + \theta_3)\ ALC + \eta_1$ where ALC = θ_5 if drinker, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6648.290
P15	$Vm = (\theta_1 * WT + \theta_3)\ SMK + \eta_1$ where SMK = θ_5 if smoking $\geq$ 10 cigarettes per day, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6640.915
P16	$Vm = (\theta_1 * WT + \theta_3)\ SMK + \eta_1$ where SMK = θ_5 if smoking $\geq$ 20 cigarettes per day, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6650.632
P17	$Vm = (\theta_1 * WT + \theta_3)\ SMK * AGE + \eta_1$ where SMK = θ_5 if smoker, otherwise = 1 AGE = θ_6 if $\geq$ 60 years, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_4 + \eta_2$ if coloured	6638.596
P18	$Vm = (\theta_1 * WT + \theta_3)\ RACE + \eta_1$ where RACE = θ_4 if coloured, otherwise = 1 $Km = \theta_2 + \eta_2$ if black $= \theta_5 + \eta_2$ if coloured	6646.462
P19	$Vm = (\theta_1 * WT + \theta_3)\ RACE * SMK + \eta_1$ where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 $Km = \theta_2 + \eta_2$	6635.893
P20	$Vm = (\theta_1 * WT + \theta_3)\ RACE * SMK + \eta_1$ where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 $Km = \theta_2 + \eta_2$ if < 60 years $= \theta_6 + \eta_2$ if $\geq$ 60 years	6634.882

4.9.2.1. EVALUATION OF THE MODEL

To assess which model was the most suitable, the simplest model describing the data was analyzed initially (model P1, Table 20, page 85). Thereafter, a stepwise procedure was used to find the model that fitted the data best. The influence of various patient variables, such as ethnicity, mass, gender, age, body surface area, smoking and mild-to-moderate alcohol intake, on Vm was evaluated by including them in the model. The influence of ethnicity and age on Km was evaluated. The building of the final regression model from the simplest model is outlined in Table 20. In the development of the most suitable model to describe the data, only factors that proved to be significant were included in the model. A detailed description of each model and the resulting MOF are presented in Table 20. The models that were compared, the resulting DOBF and the associated *p* values obtained are presented in Table 21 (page 88). The *p* values were obtained by interpolation, where possible. The NONMEM parameter estimates obtained in Set 1 analysis are presented in Table 22 (page 90).

4.9.2.2. SIMPLEST MODEL

In the simplest model (model P1, Table 20), it was assumed that all patients had the same mean Vm and Km values with differences arising from the random inter- and intra-subject variation. A stepwise forward procedure was used to build up an adequate regression model from the simplest model. In the screening of fixed effects for a significant influence on pharmacokinetic parameters, we chose to begin with a minimum number of parameters and then to incorporate additional parameters into the model as necessary to account for influential factors.

4.9.2.3. ERROR MODEL

The application of a multiplicative error model (P4) for inter-subject variability in Vm and Km did not improve the fit of the model to the data when compared with the additive error model (P3). No improvement in the goodness of fit was observed when comparing the scatterplots of the actual dose versus the predicted dose obtained with the models P3 and P4. The scatterplots of weighted residuals versus predicted dose, age, sex, body surface area, smoking or patient's identification number obtained with these models were also compared and no improvement in the goodness of fit was observed.

TABLE 21: MODELS TESTED FOR FACTORS INFLUENCING Vm AND Km VALUES OF PHENYTOIN - (SET 1 ANALYSIS)

TEST	RUN	MODEL	MODEL FOR COMPARISON	DOBF	*p*-Value	COMMENT
Initial Vm and Km values	1	P1	-	-	-	Full data set
Revised Vm and Km estimates	2	P2	P1			Improvement in fit was obtained with the edited data set. Refer to section 4.9.2.
Weight in Vm	3	P3	P2	58.3	<0.0005*	Vm is related to weight
Multiplicative model for inter-subject variation	4	P4	P3			No improvement in fit to data. Refer to section 4.9.2.3
BSA in Vm	5	P5	P3	2.213	0.146	BSA provides no advantage over weight
Race in Km (Race separated into 2 groups)	6	P6	P3	4.787	0.03*	Km is related to race
Race in Km (Race separated into 3 groups)	7	P7	P6	3.532	0.064	No improvement in fit
Race in Vm (Race separated into 2 groups)	8	P8	P3	9.486	0.003*	Vm is related to race
Race in Vm (Race separated into 3 groups)	9	P9	P8	1.813	0.184	No improvement in fit
Race in Km	10	P10	P3	4.787	0.03*	Same as model P6-2 flags used
Weight raised to a power	11	P11	P10	0.762	0.385	No improvement in fit
Gender in Vm	12	P12	P10	1.485	0.228	Vm is not related to gender
Smoking in Vm	13	P13	P10	11.773	<0.0007*	Vm is influenced by smoking
Mild-to-moderate alcohol consumption in Vm	14	P14	P10	2.923	0.09	Vm is not influenced by mild-to-moderate alcohol consumption

TABLE 21: continued

TEST	RUN	MODEL	MODEL FOR COMPARISON	DOBF	*p*-Value	COMMENT
Smoking in Vm (<10 versus ≥10 cigarettes/day)	15	P15	P13			No improvement in fit. Refer to section 4.9.2.4.8
Smoking in Vm (<20 versus ≥20 cigarettes/day)	16	P16	P13			No improvement in fit. Refer to section 4.9.2.4.8.
Age in Vm (<60 versus ≥60 years)	17	P17	P13	0.844	0.363	Vm is not influenced by a discontinuity at 60 years of age
Race in Vm and Km	18	P18	P6	4.751	0.031*	Incorporating the influence of race in Vm significantly improved fit
	18	P18	P8	0.052	0.825	Incorporating the influence of race in Km did not significantly improve the fit. Therefore only the influence of race in Vm is incorporated
Race and smoking in Vm	19	P19	P8	10.621	0.001*	Best model. Influence of race and smoking combined in Vm provided a better fit than race alone in Vm
Age in Km (<60 versus ≥60 years)	20	P20	P19	1.011	0.317	Km is not influenced by a discontinuity at 60 years of age

* indicates statistical significance ($p \leq 0.05$) and improvement in fit of the model to the data

TABLE 22: PARAMETER ESTIMATES OBTAINED FOR SET 1 ANALYSIS

MODEL NO.	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	σ_{Vm}^2	σ_{Km}^2	σ_ε^2
P1	382	3.24					3290	3.35	252
P2	384	3.37					3320	3.33	230
P3	2.26	3.40	241				2560	3.04	236
P4	1.95	3.01	253				0.0394	0.296	0.003
P5	156	3.39	118				2620	2.96	237
P6	2.30	3.13	237	1.17			2480	3.03	237
P7	2.24	3.64	241	3.25	2.41		2480	2.96	237
P8	2.33	3.42	225	1.06			2460	3.0	238
P9	2.29	3.42	228	1.05	1.10		2450	2.95	238
P10	2.30	3.66	237	3.13			2480	3.03	237
P11	74	3.67	0.397	3.13			2500	3.0	237
P12	2.27	3.66	233	3.13	1.02		2470	3.01	237
P13	2.39	3.67	222	3.19	1.06		2370	2.89	240
P14	2.30	3.68	234	3.13	1.04		2450	2.99	238
P15	2.26	3.64	235	3.16	1.07		2350	3.01	238
P16	2.30	3.67	237	3.13	1.08		2470	3.03	237
P17	2.35	3.67	225	3.16	1.06	0.965	2350	2.88	240
P18	2.33	3.46	226	1.05	3.38		2450	3.01	237
P19	2.41	3.45	212	1.05	1.06		2350	2.87	241
P20	2.38	3.42	214	1.05	1.06	3.90	2360	2.85	241

4.9.2.4. INFLUENCE OF COVARIATES ON Vm AND Km VALUES

These results are summarized in Table 21 (pages 88 and 89).

4.9.2.4.1. Weight in Vm

Incorporation of Vm as a function of weight (model P3) resulted in a decrease of the MOF and a DOBF of 58.3 ($p < 0.0005$) when compared with model P2. This indicated that weight significantly influenced Vm. Thus, Vm expressed in proportion to weight is more appropriate than to assume the same Vm for all patients. As weight had a significant effect, it was left in the model. In the building of the full model, weight raised to a power was tested alone as a scaling function for Vm. No other factors were included in the Vm model (model P11). This resulted in an increase in the MOF and a DOBF of 0.762 ($p = 0.385$), indicating that this worsened the fit of the model to the data. The exponent was estimated as 0.397, and therefore was not expected to make a significant difference.

4.9.2.4.2. Body surface area in Vm

When body surface area was incorporated in Vm (model P5), the MOF increased and a DOBF of 2.213 ($p = 0.146$) was obtained when compared with model P3. This indicated that body surface area as a function of Vm provided no additional information over weight when estimating Vm. Therefore, only the influence of weight in Vm was included in the model.

4.9.2.4.3. Ethnicity in Km (2 ethnic groups)

Incorporating ethnicity in Km when the patients were classified as either black or coloured (model P6) resulted in a decrease in the MOF and a DOBF of 4.787 ($p = 0.03$) compared with model P3, which indicated that ethnicity significantly influenced Km and improved the fit of the model to the data. Km values of 3.13 and 3.66 mg/l were obtained for coloured and black patients, respectively. The Km value of coloured patients was 14.5% lower than that of black patients. The influence of ethnicity on Km was therefore included in subsequent models.

4.9.2.4.4. Ethnicity in Km (3 ethnic groups)

The inclusion of ethnicity in Km when the patients were classified as either black, coloured or malay (model P7) resulted in a decrease in the MOF and a DOBF of 3.532 ($p = 0.064$) compared with model P6. This indicated that separating the patients into malay and coloured groups did not significantly improve the fit of the model to the data.

4.9.2.4.5. Ethnicity in Vm (2 ethnic groups)

Incorporating ethnicity in Vm when the patients were classified as either black or coloured (model P8) resulted in a decrease in the MOF and a DOBF of 9.486 ($p = 0.003$) was obtained compared with model P3, which indicated that ethnicity significantly influenced Vm and improved the fit of the model to the data.

4.9.2.4.6. Ethnicity in Vm (3 ethnic groups)

The inclusion of ethnicity in Vm when the patients were classified as either black, coloured or malay (model P9) resulted in a decrease in the MOF and a DOBF of 1.813 ($p = 0.184$), compared with model P8. This, as with the investigation of the influence of ethnicity on Km, indicated that separating the patients into malay and coloured groups made no significant difference. Therefore, for the purpose of this study, the patients were classified either as black or coloured. As coloureds and malays did not significantly differ from each other in Vm and Km values, they were grouped together as coloureds. It was decided to do this as most of the indigenous malay population are now mixed with the coloured population and ethnically 'pure' or 'almost pure' malays are not found in South Africa.

4.9.2.4.7. Gender in Vm

When gender was incorporated in Vm (model P12), a decrease in the MOF and a DOBF of 1.485 ($p = 0.228$) resulted, compared with model P10. This showed that Vm was not significantly related to gender.

4.9.2.4.8. Smoking in Vm

The inclusion of smoking in Vm (model P13) resulted in a decrease in the MOF and a DOBF of 11.773 ($p < 0.0007$) compared with model P10. This indicated that smoking significantly influenced Vm. When the smokers were separated into those who smoked

less than 10 cigarettes and those who smoked 10 or more cigarettes per day (model P15), no improvement in the fit of the model to the data was obtained compared with model P13. When the smokers were separated into those who smoked less than 20 cigarettes and those who smoked 20 or more cigarettes per day (model P16), no improvement in the fit of the model to the data was obtained compared with model P13. No improvement in the goodness of fit was observed when comparing the scatterplots of the actual dose versus the predicted dose obtained with models P13, P15 and P16. The scatterplots of weighted residuals versus predicted dose, age, sex, body surface area, smoking or patient's identification number obtained with these models were also compared and no improvement in the goodness of fit was observed. No large decrease in the size of the interindividual variance of the pharmacokinetic parameters and residual error were also observed (Table 22, page 90). Thus, separating smokers into the groups referred to above did not improve the fit of the model to the data. Accordingly, the patients were categorized as smokers or non-smokers.

4.9.2.4.9. Alcohol consumption in Vm

The incorporation of mild-to-moderate alcohol consumption in Vm (model P14) resulted in a decrease in the MOF and a DOBF of 2.923 (p = 0.09), compared with model P10. This indicated that mild-to-moderate alcohol consumption did not influence Vm significantly. Because of the homogeneity of the patient population in respect of alcohol consumption (abusers excluded), no effect of alcohol consumption on Vm was expected. It was found that moderate consumption of alcohol, i.e. week-ends only or once or twice per week, did not influence Vm significantly.

4.9.2.4.10. Age in Vm

The analysis could not detect a continuous effect of age, and therefore discontinuity at 60 years of age was tested. A cut-off point of 60 years was arbitrarily chosen. When patients were separated into 2 groups, i.e. less than 60 years and 60 years of age or older (model P17), a decrease in the MOF and a DOBF of 0.844 (p = 0.363) were obtained compared with model P13. This indicated that age, in the categories referred to above, did not influence Vm significantly.

4.9.2.4.11. Age in Km

When patients were separated into 2 groups (less than 60 years and 60 years of age or older (model P20), a decrease in the MOF and a DOBF of 1.011 (p = 0.317) were obtained compared with model P19. This indicated that discontinuity at 60 years of age did not influence Km significantly.

4.9.2.5. FINAL REGRESSION MODEL FOR SET 1 ANALYSIS

A stepwise procedure was used to find the model that fitted the data best. On investigating the influence of ethnicity alone on the variation in Km (model P6), a DOBF of 4.787 (p = 0.03) was obtained compared with model P3, indicating that ethnicity produced a statistically significant influence on the variation in Km. On investigating the influence of ethnicity alone on Vm (model P8), a DOBF of 9.486 (p = 0.003) was obtained compared with model P3, indicating that ethnicity produced a statistically significant influence on the variation in Vm (Table 21, page 88). This indicated that the influence of ethnicity is greater on the variation in Vm than in Km. Incorporating the influence of ethnicity in Vm and Km combined (model P18) produced a DOBF of 0.052 (p = 0.825), compared with model P8. This indicated virtually no improvement in the fit of the model to the data when Km was incorporated in model P8, confirming that ethnicity has a greater influence on the variation in Vm. However, when Vm was incorporated in model P6 which only included the influence of ethnicity in Km, a DOBF of 4.751 (p = 0.031) was obtained. It can be concluded that the final model only needed to account for the influence of ethnicity on the variation in Vm. The incorporation of the influence of ethnicity on the variation in Km did not significantly improve description of the data. The above is explained schematically in Figure 1 (page 95).

Model P19 (Table 20, page 86) was found to be the model which best described the data in this study. In this model, Vm was expressed as a function of ethnicity, weight and smoking. No influence of covariates on Km was taken into account. A DOBF of 10.621 (p = 0.001), compared with model P8, indicated that the combined influence of ethnicity and smoking on the variation in Vm was more significant than ethnicity in Vm alone (Table 21, page 89).

The final population estimates (with the standard error of the estimate in parentheses) are presented in Table 27 (page 111). The NONMEM control file for the final model is presented in Annexure 9.

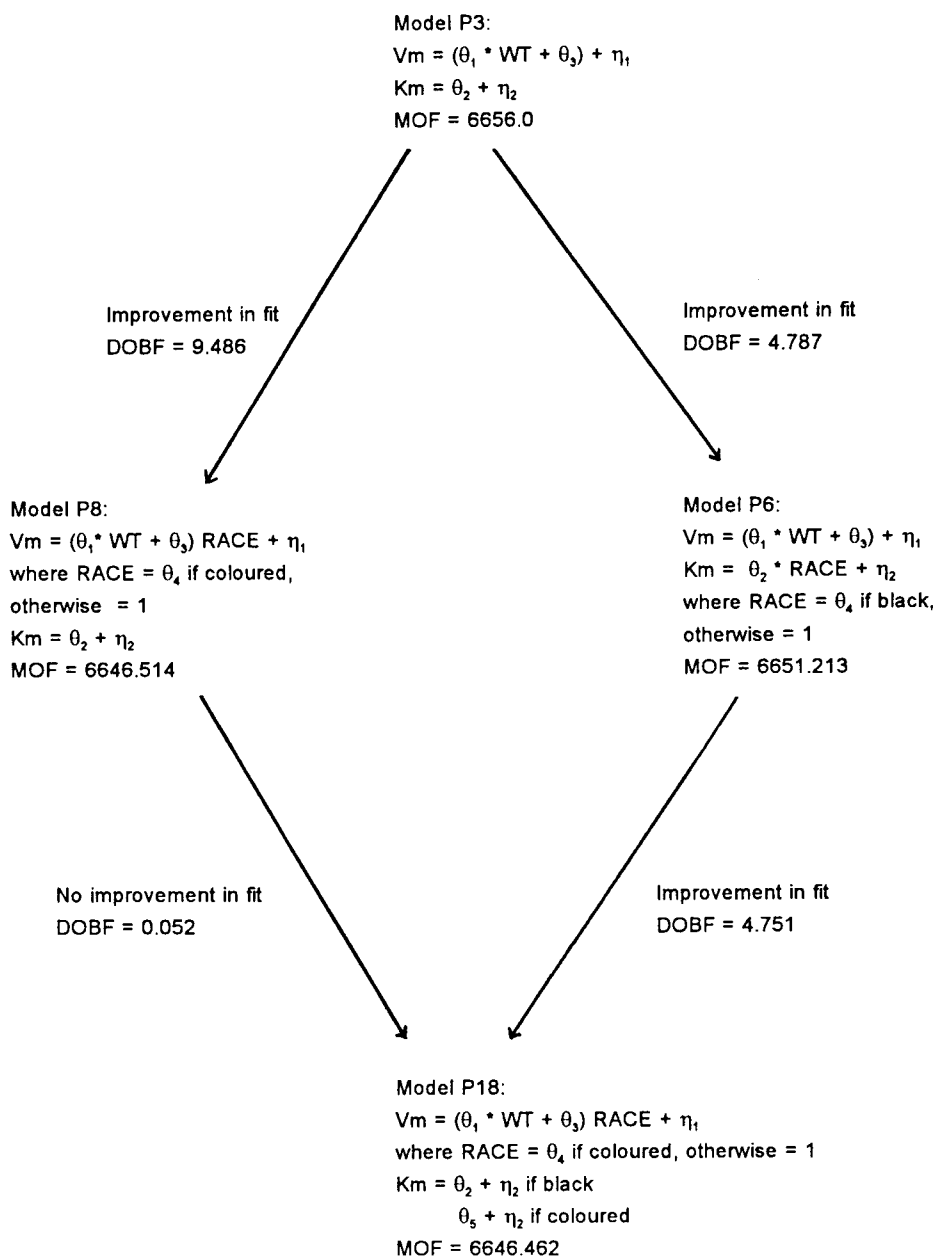

FIGURE 1: SCHEMATIC PRESENTATION OF THE INFLUENCE OF ETHNICITY ON Vm AND Km ALONE, AND ON Vm AND Km COMBINED

4.9.3. SET 2 ANALYSIS

In Set 2 analysis, the structural models S2 and S3 were evaluated. These models have been presented in sections 3.7.3.1.2 and 3.7.3.1.3 in Chapter 3. The results are presented in Tables 23 - 28. The same data used in Set 1 analysis was used in Set 2 analysis. The parameter models (P21 to P32) are depicted in Table 23 (page 97).

The MOF values obtained with the Michaelis-Menten model (S1) were compared with those obtained with the parallel Michaelis-Menten and first-order elimination model (S2). Given the nonlinear pharmacokinetics of phenytoin, unrealistic phenytoin concentrations, such as infinite concentrations, can be predicted. Both models, therefore, used dose as the dependent variable. Model S2 (Run 22) fitted the data significantly better, as indicated by a decrease in the MOF and a DOBF of 2922.524 ($p < 0.0005$), compared with model S1 (Run 21, Table 24, page 99). The results indicate that linear clearance is important in model S2.

As the same parameter and structural models are used in many of the NONMEM runs (e.g. when a run was performed with or without the conditional estimation method), the results are often reported by comparing runs and not parameter models as was previously the case. Model S3 predicted steady-state serum concentration (Cp_{ss}) and used Cp_{ss} as the dependent variable. For this reason, the MOF obtained with models S1 and S2 which predicted dose could not be compared with that obtained with model S3. The importance of Cl in model S3 was evaluated by using a variation of a method described by Sheiner *et al.* (1973). To illustrate the importance of Cl in model S3, the MOF was used to construct the 90% confidence interval for the Cl estimate by evaluating the MOF associated with a change in the Cl value. This entailed the Cl value being fixed at different values ranging from 0.9 to 4.5 l/day, and the resulting MOF generated by each run was observed (Table 26, page 103). The value of Cl was fixed at values greater than or less than the Cl value of 2.68 l/h (Run 23, Table 25, page 101) which was derived from model P21, and which fitted the data best. Cl values were first fixed to values decreasing from 2.68 l/h and the MOF of each consecutive run was observed until the DOBF exceeded the critical value of 3.8 compared with the MOF obtained in Run 23. Similarly, Cl values were fixed at values increasing from 2.68 l/h and the same procedure was followed. A plot of the Cl values (x-axis) and the corresponding MOF values (y-axis) is shown in Figure 2 (page 105). The dashed horizontal line in Figure 2 indicates a DOBF of 3.8 (measured from the lowest MOF value on the graph), and where this line intersects the graph the

TABLE 23: DETAILS OF NONMEM PARAMETER MODELS FOR SET 2 ANALYSIS

RUN	MODEL NO.	PARAMETER MODEL	MOF
21	P19	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK + η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 $Km = \theta_2 + \eta_2$	6720.3
22	P21	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK + η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 $Km = \theta_2 + \eta_2$ $Cl = \theta_6 + \eta_3$	3797.776
23	P22	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK * EXP η_1 where RACE = θ_4 if coloured, otherwise 1 SMK = θ_5 if smoker, otherwise = 1 $Km = \theta_2 * EXP\ \eta_2$ $Cl = \theta_6 * EXP\ \eta_3$	3798.902
39	P23	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK * ALC * SEX * AGE * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 ALC = θ_8 if drinker, otherwise = 1 SEX = θ_9 if male, otherwise = 1 AGE = θ_{10} if $\geq$ 65 years, otherwise = 1 $Km = \theta_2 *$ RACE * AGE * EXP η_2 where RACE = θ_7 if coloured, otherwise = 1 AGE = θ_{11} if $\geq$ 65 years, otherwise = 1 $Cl = \theta_6 * EXP\ \eta_3$	3791.125
40	P24	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK * ALC * SEX * AGE * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 ALC = θ_8 if drinker, otherwise = 1 SEX = θ_9 if male, otherwise = 1 AGE = θ_{10} if $\geq$ 65 years, otherwise = 1 $Km = \theta_2 *$ RACE * EXP η_2 where RACE = θ_7 if coloured, otherwise = 1 $Cl = \theta_6 * EXP\ \eta_3$	3792.412
41	P25	$Vm = (\theta_1 * WT + \theta_3)$ RACE * SMK * ALC * SEX * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 ALC = θ_8 if drinker, otherwise = 1 SEX = θ_9 if male, otherwise = 1 $Km = \theta_2 *$ RACE * EXP η_2 where RACE = θ_7 if coloured, otherwise = 1 $Cl = \theta_6 + \eta_3$	3797.908

TABLE 23: continued

RUN	MODEL NO.	PARAMETER MODEL	MOF
42	P26	Vm = (θ_1 * WT + θ_3) RACE * SMK * ALC * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 ALC = θ_8 if drinker, otherwise = 1 Km = θ_2 * RACE * EXP η_2 where RACE = θ_7 if coloured, otherwise = 1 Cl = θ_6 * EXP η_3	3798.375
43	P27	Vm = (θ_1 * WT + θ_3) RACE * SMK * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 Km = θ_2 * RACE * EXP η_2 where RACE = θ_7 if coloured, otherwise = 1 Cl = θ_6 * EXP η_3	3798.846
44	P28	Vm = (θ_1 * WT + θ_3) RACE * SMK * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 Km = θ_2 * EXP η_2 Cl = θ_6 * EXP η_3	3798.861
45	P29	Vm = (θ_1 * WT + θ_3) RACE * EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 Km = θ_2 + η_2 Cl = θ_5 + η_3	3833.681
46	P30	Vm = (θ_1 * WT + θ_3) * EXP η_1 Km = θ_2 * EXP η_2 Cl = θ_4 * EXP η_3	3846.102
47	P31	Vm = (θ_1 * WT + θ_3) RACE * SMK * AGE *EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 AGE = θ_7 if $\geq$ 60 years, otherwise = 1 Km = θ_2 * EXP η_2 Cl = θ_6 * EXP η_3	3796.889
48	P32	Vm = (θ_1 * WT + θ_3) RACE * SMK * AGE *EXP η_1 where RACE = θ_4 if coloured, otherwise = 1 SMK = θ_5 if smoker, otherwise = 1 AGE = θ_7 if $\geq$ 65 years, otherwise = 1 Km = θ_2 * EXP η_2 Cl = θ_6 * EXP η_3	3793.587

TABLE 24: COMPARISON OF MODELS FOR SET 2 ANALYSIS

RUN	PARA-METER MODEL	STRUCTURAL MODEL	MODEL USED FOR COMPARISON RUN NO.	DOBF	*p*-VALUE	COMMENT
21	P19	S1	-	-	-	Same as Run 19
22	P21	S2	21 (P19)	2922.524	<0.0005*	Linear clearance (Cl) improved fit of model to data. Dose was used as dependent variable
23	P21	S3	-	-	-	Cannot be compared with Run 22 as serum steady-state concentration was used as dependent variable
24 to 35						See Tables 25 and 26
36	P22	S3	23 (P21)	1.126		No significant difference between additive and proportional error models
37	P22	S3	-	-	-	Posthoc analysis
38	P22	S3	36 (P22)	145.22		Conditional estimation method
39	P23	S3	-	-	-	All patient characteristics included in the model
40	P24	S3	39 (P23)	1.29	0.262	Similar to Run 39 except that the influence of age in Km has been removed
41	P25	S3	40 (P24)	5.496	0.021*	Similar to Run 40 except that the influence of age in Vm has been removed
42	P26	S3	41 (P25)	0.467	0.495	Similar to Run 41 except that the influence of gender in Vm has been removed
43	P27	S3	42 (P26)	0.471	0.494	SImilar to Run 42 except that the influence of mild to moderate alcohol consumption in Vm has been removed
44	P28	S3	43 (P27)	0.014	0.908	Similar to Run 43 except that the influence of race in Km has been removed

TABLE 24: continued

RUN	PARA-METER MODEL	STRUCTURAL MODEL	MODEL USED FOR COMPARISON RUN NO.	DOBF	*p*-VALUE	COMMENT
45	P29	S3	44 (P28)	34.821	<0.0005*	Similar to Run 44 except that the influence of smoking in Vm has been removed
46	P30	S3	45 (P29)	12.421	<0.0005*	Similar to Run 45 except that the influence of race in Vm has been removed
47	P31	S3	36 (P30)	2.013	0.165	Vm is not influenced by a discontinuity at 60 years of age
48	P32	S3	36 (P22)	5.315	0.022*	Vm is influenced by a discontinuity at 65 years of age
49	P32	S3	48 (P32)	140.159		Similar to Run 48, conditional estimation procedure performed

* indicates statistical significance ($p \leq 0.05$) and improvement in fit of the model to the data

corresponding Cl values were read off. The Cl values of 1.49 and 3.89 l/day represent the approximate lower and upper limits of the 90% confidence interval of the Cl estimate, respectively. As the lower limit of the confidence interval was observed well before the Cl value approached zero, the importance of Cl in the model is confirmed.

4.9.3.1. ERROR MODEL

The additive error models for inter-individual variability in Vm, Km and Cl were compared with proportional error models. Using structural model S3, model P22 (Run 36, Table 24, page 99) was compared with model P21 (Run 23) to assess whether the additive or proportional error model resulted in a better data fit. A Lmax ratio of 0.569 indicated that little difference existed between the two error models.

TABLE 25: PARAMETER ESTIMATES OBTAINED FOR SET 2 ANALYSIS

RUN	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	θ_7	θ_8	θ_9	θ_{10}	θ_{11}	σ_{Vm}^2	σ_{Km}^2	σ_{Cl}^2	σ_{ε}^2
21	2.29	3.30	217	1.05	1.06	-	-	-	-	-	-	2430	2.73	-	266
22	2.28	1.60	140	1.06	1.09	2.09	-	-	-	-	-	2410	1.13	0.354	228
23	3.93	6.81	86	1.10	1.17	2.68	-	-	-	-	-	1780	21.4	1.54	10.1
24	4.03	9.98	171	1.09	1.15	0.9	-	-	-	-	-	511	30.5	0.46	10.3
25	4.02	9.50	160	1.09	1.15	1.1	-	-	-	-	-	731	29.9	0.52	10.3
26	4.03	9.14	148	1.09	1.15	1.3	-	-	-	-	-	917	28.6	0.613	10.3
27	4.03	8.78	138	1.09	1.16	1.5	-	-	-	-	-	1090	27.5	0.716	10.2
28	4.03	8.43	127	1.09	1.16	1.7	-	-	-	-	-	1240	26.3	0.827	10.2
29	4.02	7.91	113	1.09	1.16	2.0	-	-	-	-	-	1430	24.7	1.02	10.1
30	3.95	7.09	93.1	1.10	1.17	2.5	-	-	-	-	-	1700	22.2	1.39	10.1
31	3.9	6.63	82.4	1.10	1.17	2.8	-	-	-	-	-	1810	20.8	1.65	10.0
32	3.81	5.89	67.5	1.10	1.17	3.3	-	-	-	-	-	1950	18.7	2.16	10.0
33	3.74	5.47	60.0	1.10	1.18	3.6	-	-	-	-	-	1990	17.6	2.52	10.0
34	3.67	5.01	49.5	1.10	1.18	4.0	-	-	-	-	-	2001	16.1	3.05	10.0
35	3.53	4.32	41.5	1.09	1.18	4.5	-	-	-	-	-	2020	14.6	3.82	10.2
36	4.0	7.15	91.5	1.09	1.17	2.49	-	-	-	-	-	0.008	0.464	0.265	10.1

TABLE 25: continued

RUN	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	θ_7	θ_8	θ_9	θ_{10}	θ_{11}	σ_{Vm}^2	σ_{Km}^2	σ_{Cl}^2	σ_e^2
37	3.93	6.81	85.8	1.10	1.17	2.68	-	-	-	-	-	1770	21.3	1.54	10.0
38	3.58	6.48	141	1.06	1.12	2.16	-	-	-	-	-	0.009	0.291	0.919	7.66
39	3.95	7.63	119	1.13	1.14	2.10	8.31	1.03	0.972	0.805	0.728	0.0009	0.460	0.335	10.1
40	3.94	7.56	118	1.12	1.15	2.14	7.94	1.03	0.976	0.870	-	0.0006	0.466	0.345	10.1
41	4.08	7.24	93.3	1.09	1.16	2.43	7.23	1.03	0.979	-	-	0.0074	0.468	0.262	10.1
42	3.98	7.09	89.9	1.09	1.16	2.54	7.04	1.02	-	-	-	0.0068	0.484	0.271	10.1
43	4.00	7.09	89.1	1.09	1.17	2.57	6.97	-	-	-	-	0.0066	0.486	0.279	10.1
44	3.99	7.05	89.5	1.09	1.17	2.55	-	-	-	-	-	0.0066	0.485	0.278	10.1
45	3.93	6.22	89.2	1.12	3.02	-	-	-	-	-	-	0.0102	0.58	0.270	10.2
46	3.55	4.06	71.0	1.12	-	-	-	-	-	-	-	2.51^{-8}	1.17	0.294	9.99
47	3.90	7.21	101	1.10	1.16	2.45	0.929	-	-	-	-	0.0035	0.491	0.306	10.1
48	3.86	7.45	112	1.10	1.16	2.31	0.875	-	-	-	-	0.0017	0.478	0.336	10.1
49	3.55	6.48	143	1.06	1.12	2.15	0.968	-	-	-	-	0.009	0.29	0.927	7.67

TABLE 26: SUMMARY OF NONMEM RUNS PERFORMED TO DEMONSTRATE THE SIGNIFICANCE OF Cl IN STRUCTURAL MODEL S3[1]

RUN	FIXED VALUE OF Cl	MOF
24	0.9	3806.293
25	1.1	3804.345
26	1.3	3802.712
27	1.5	3801.341
28	1.7	3800.25
29	2.0	3798.917
30	2.5	3797.838
23	2.68	3797.776
31	2.8	3797.829
32	3.3	3798.870
33	3.6	3800.118
34	4.0	3802.483
35	4.5	3806.505

[1] parameter model P21 was used throughout this analysis

4.9.3.2. POSTHOC AND CONDITIONAL ESTIMATION METHODS

The posthoc estimates of the population parameters are presented in Table 25 (Run 37, page 102). Often a FOCE method is not needed as this is an optional NONMEM run. The goodness-of-fit plots between the conventional and the conditional estimation methods were similar, this provided additional assurance that the model could not be substantially improved. Little difference was found between the two sets of scatterplots, and further analysis using the conditional estimation method was therefore not required.

The FOCE method was used to obtain improved estimates of the parameters (Run 49, Table 25, page 102). A decrease in the MOF of 145.22 (Run 38 compared with Run 36) was expected, as this procedure allowed for better fitting of the model to the data (Table 24, page 99). The standard errors of the estimates were found to be smaller, indicating a better fit of the model to the data (Yukawa *et al.*, 1991).

4.9.3.3. INFLUENCE OF COVARIATES ON Vm AND Km VALUES

All patient variables showing possible evidence of influence on the pharmacokinetic parameters were re-evaluated using model S3. The influence of the following patient characteristics was re-evaluated: ethnicity, smoking, mild-to-moderate alcohol intake, gender, age less than 65 and 65 years or older in Vm; and ethnicity, and age less than 65 and 65 years or older in Km. This was done by comparing the full model with all the patient factors included (P23) with a regression model from which one of the covariates was independently removed stepwise (P24 - P30, Table 23, page 97) (Fattinger *et al.*, 1991). The DOBF compared the difference in the goodness-of-fit between the reduced model (which does not include the parameter in question) and the full model (Sambol and Sheiner, 1991). The same factors shown to influence Vm in Set 1 analysis using structural model S1 were found to influence Vm in this set of analysis. Removal of the influence of covariates on Km did not significantly influence the fit of the model to the data. These results are presented in Table 24 (page 99). In this case, an increase in the MOF when comparing two models represented an improvement in the fit of the model to the data.

In Set 1 analysis, age was categorized into 2 groups: less than 60 and 60 years or older. When this was tested in Vm, no improvement in the fit of the model to the data was observed ($p = 0.363$, Table 21, page 89). In this set of analysis, age was categorized into 2 groups, less than 65 and 65 years or older. As pharmacokinetic changes are expected to occur in patients at about 65 years of age, a cut-off point at 65 years was chosen. When model P32 was compared with model P22, a DOBF of 5.315 ($p = 0.022$) was obtained (Run 48, Table 24, page 100). This difference was statistically significant at $p < 0.05$ but not at $p < 0.005$. Discontinuity at 65 years, therefore, provided a better fit than at 60 years. A comparatively small, yet significant, effect of age on Vm was observed. This model implies that Vm remains constant up to 65 years of age and then decreases. Only 2 black and 15 coloured patients were 65 years of age or over. More patients in this age group are required to make a sound statistical conclusion regarding the effect of age on Vm.

RACE, SMK, ALC, SEX and AGE were indicator variables which had a value of unity for the jth patient if black, non-smoker, teetotaller, female and less than 65 years of age and θ_4, θ_5, θ_8, θ_9 and θ_{10} if coloured, smoker, mild-to-moderate alcohol drinker, male and 65 years of age or older, respectively. θ_4, θ_5, θ_8, θ_9 and θ_{10} represent the fractional increase or decrease in Vm with the presence of the corresponding indicator variable. The inter-individual differences in serum concentration profile of phenytoin could be described by assigning inter-individual variability to Vm and Km.

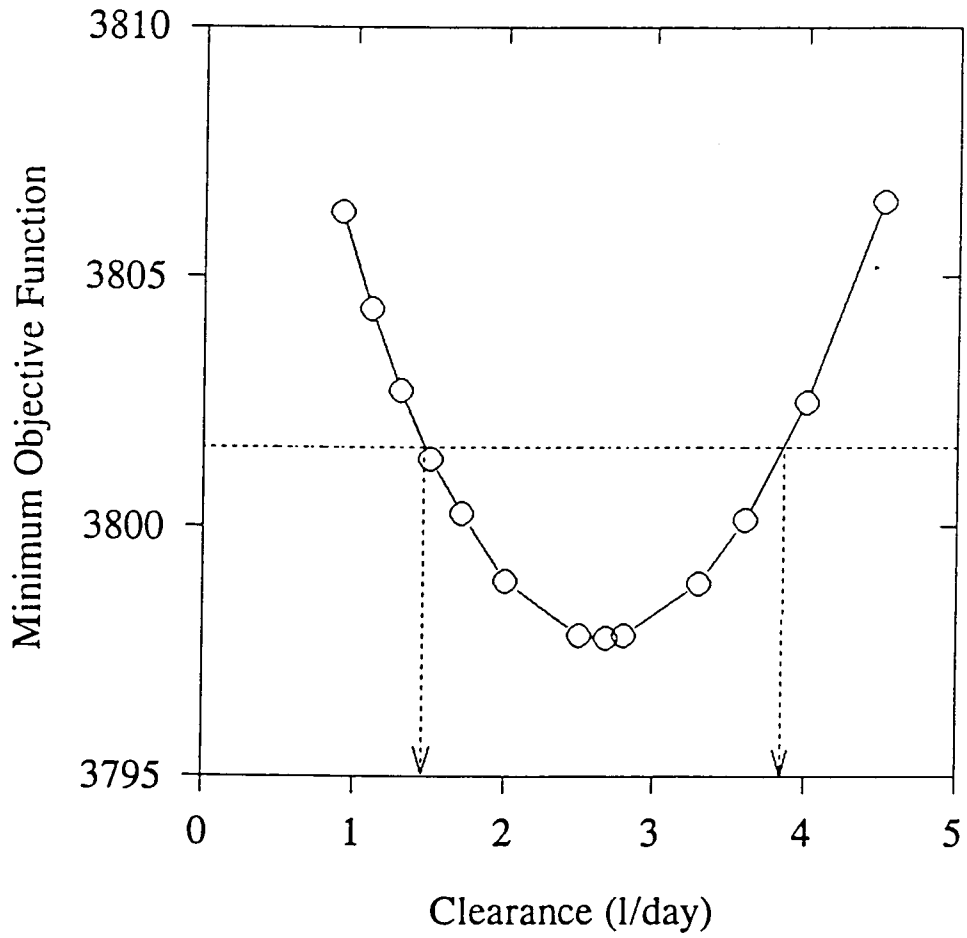

FIGURE 2: CONSTRUCTION OF THE 90% CONFIDENCE INTERVAL FOR THE CLEARANCE ESTIMATE OF 2.68 l/day. THE MINIMUM OBJECTIVE FUNCTION WAS OBTAINED BY FIXING CLEARANCE AT VARIOUS VALUES USING MODEL S3. THE POINTS AT WHICH THE DASHED HORIZONTAL LINE INTERSECTS THE TWO ARMS OF THE CURVE DELIMIT THE 90% CONFIDENCE INTERVAL FOR THE CLEARANCE ESTIMATE OF 2.68 l/day.

4.9.3.4. SCATTERPLOTS OF FINAL MODEL (S3)

A method of assessing the 'goodness-of-fit' was to examine the scatterplots of weighted residuals generated by NONMEM. Besides monitoring changes in the MOF, the model-building steps were guided by examination of the scatterplots of weighted residuals versus the various fixed effects. Predicted concentration more closely equalled observed concentration as the accuracy improved and weighted residuals approached zero. The scatterplots of weighted residuals versus the predicted concentrations for black (Figure 3, page 107) and coloured (Figure 4, page 108) patients were found to be randomly scattered about zero. The scatterplots of the measured serum phenytoin concentration versus the predicted serum concentration for black (Figure 5, page 109) and coloured (Figure 6, page 110) patients are also presented. The scatterplots seem to indicate a proportional model as the residuals' variance increases with the mean.

4.9.4. FINAL MEAN PARAMETER ESTIMATES

The values of the mean parameter estimates of Vm, Km and Cl and their inter-individual and intra-individual variability (expressed as the percentage coefficient of variation - %CV) obtained when using structural models S1, S2 and S3 are given in Table 27 (page 111). The standard errors of the estimates are given in parentheses. The 95% CI for the fixed effects are also reported. The Vm estimate of 286.4 mg/day (12.3 mg/kg/day) obtained with model S2 was the lowest and appeared to be inconsistent with that obtained with models S1 and S3. This indicated that different Vm values should be used with different models. Thus, when predicting dose by means of model S2 (which includes Cl) a lower Vm value should be used. When using this model, the general concept that dose should not exceed Vm, is not applicable. The latter concept applies to the Michaelis-Menten model (S1). The % CV in Vm obtained with all 3 models was considered acceptable (Table 27, page 111) . The square root of the variance of the additive error terms divided by θ (i.e. fixed effect) multiplied by 100% is a reasonable approximation of the CV of the fixed effect.

For all the structural models used, the Vm obtained for black patients was consistently lower than for coloured patients. The estimates of Km obtained with all 3 models differed markedly. Km values of 3.45, 1.60 and 6.81 mg/l were obtained with model S1, S2 and S3, respectively. The high % CV obtained in Km was expected. Although ethnicity was found to influence Km, this factor was not included in the final model as ethnicity accounted more for the variability in Vm. This has been discussed in section 4.9.2.5. The % CV in Km obtained with models S2 and S3 compared well, whereas that obtained with

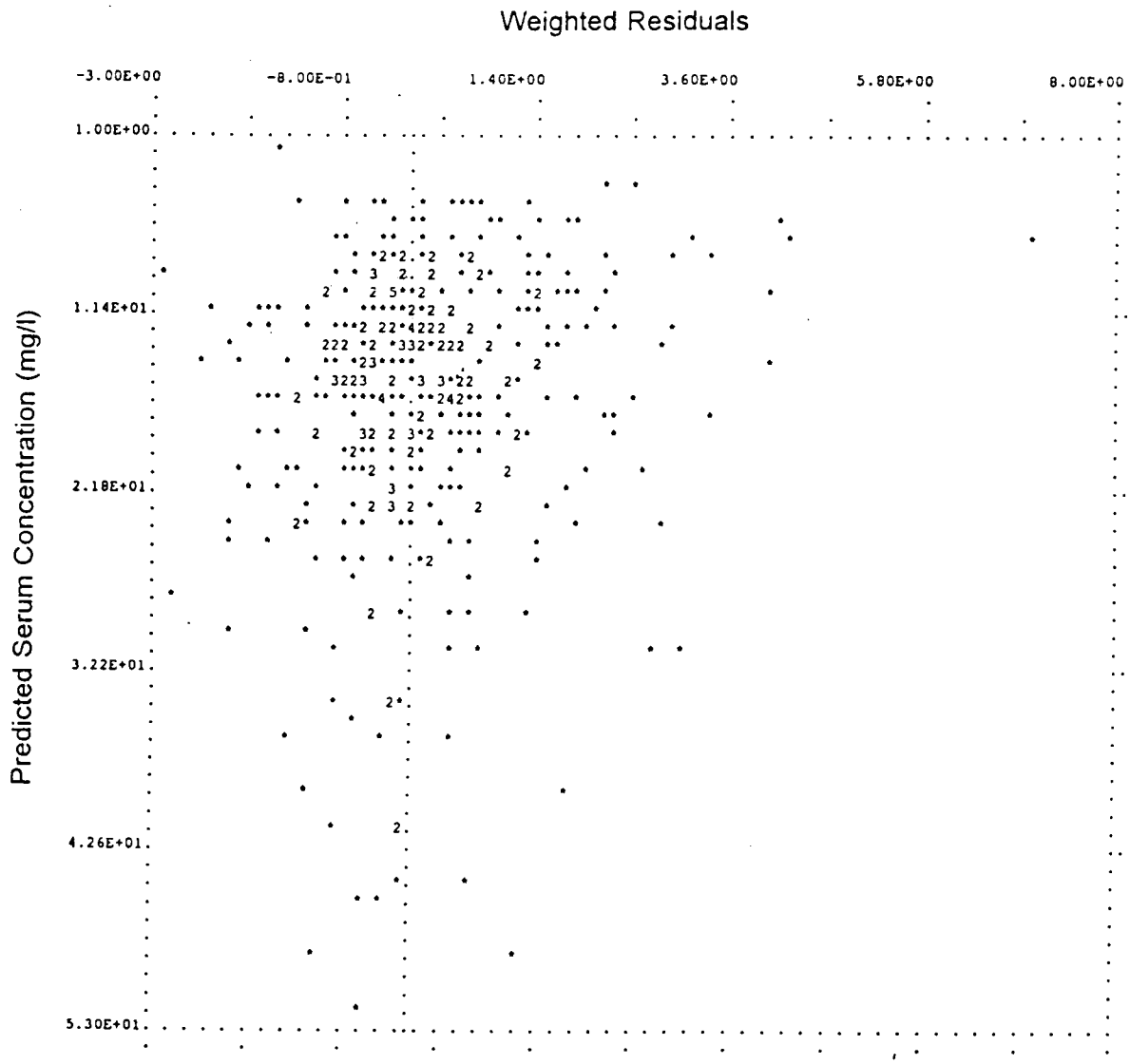

FIGURE 3: SCATTERPLOT OF WEIGHTED RESIDUALS (UPPER AXIS) VERSUS PREDICTED SERUM CONCENTRATION (VERTICAL AXIS) OBTAINED FOR BLACK PATIENTS WITH THE FINAL MODEL

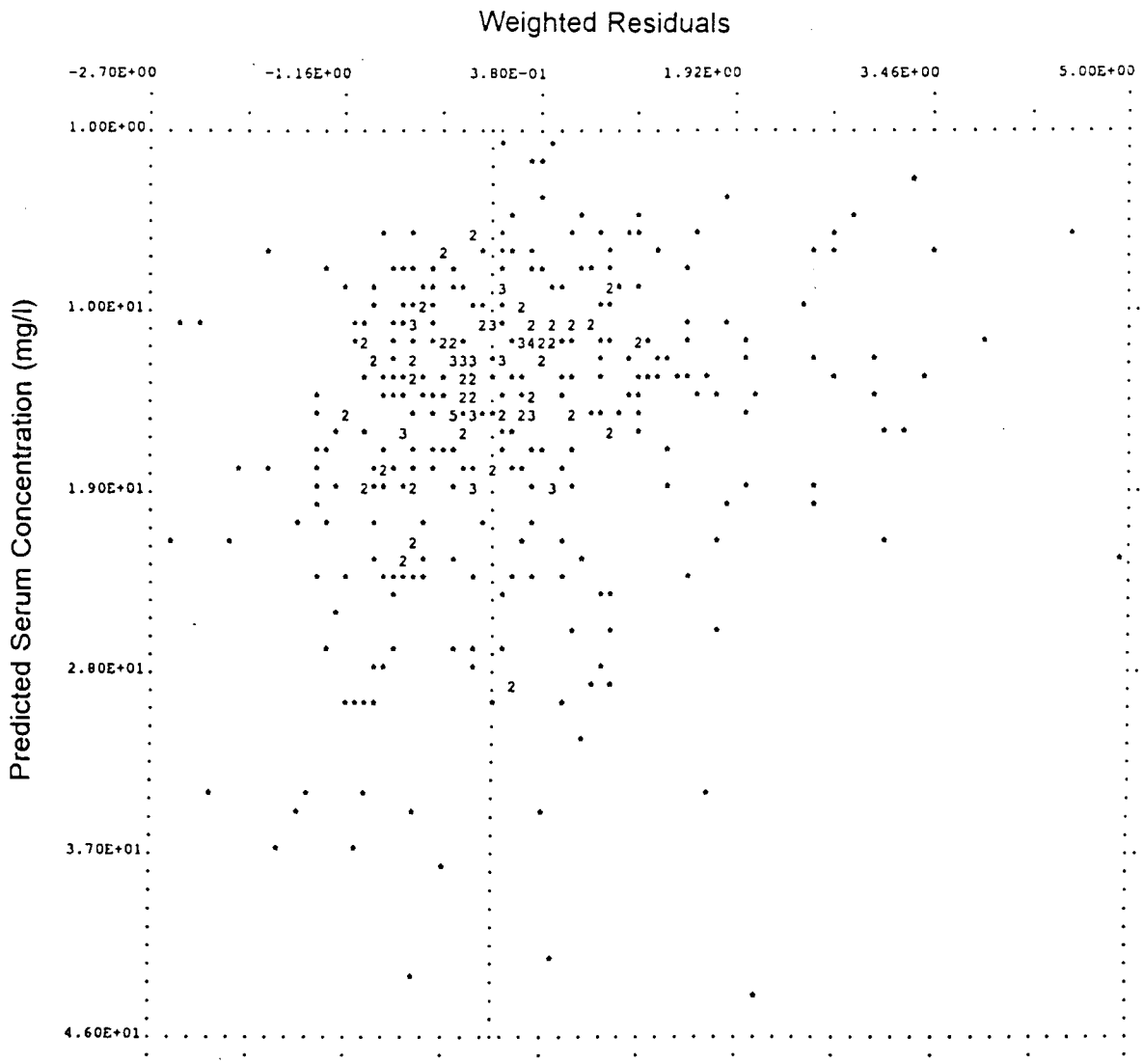

FIGURE 4: SCATTERPLOT OF WEIGHTED RESIDUALS (UPPER AXIS) VERSUS PREDICTED SERUM CONCENTRATION (VERTICAL AXIS) OBTAINED FOR COLOURED PATIENTS WITH THE FINAL MODEL

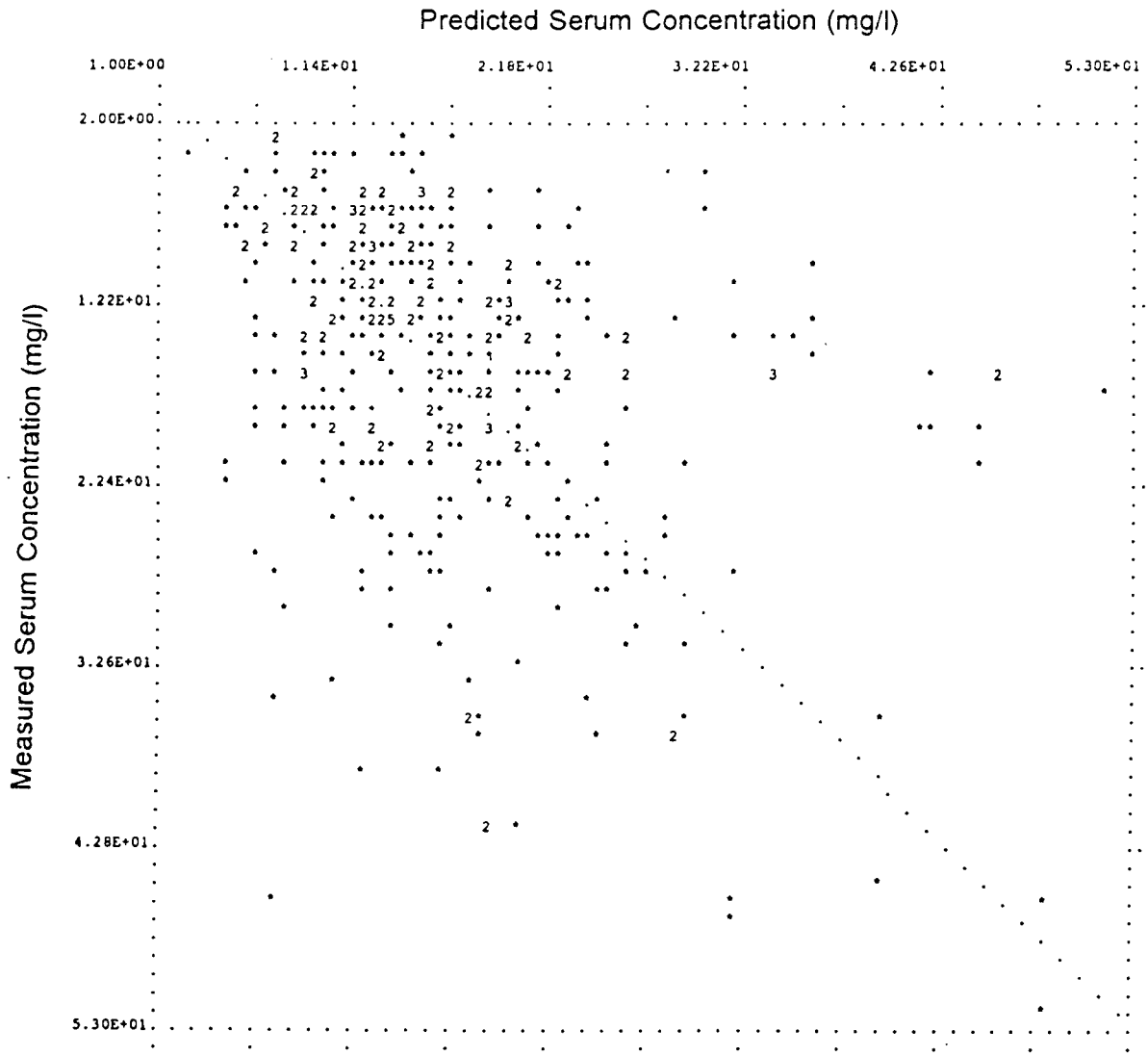

FIGURE 5: SCATTERPLOT OF PREDICTED SERUM PHENYTOIN CONCENTRATION (UPPER AXIS) VERSUS MEASURED SERUM CONCENTRATION (VERTICAL AXIS) OBTAINED FOR BLACK PATIENTS WITH THE FINAL MODEL

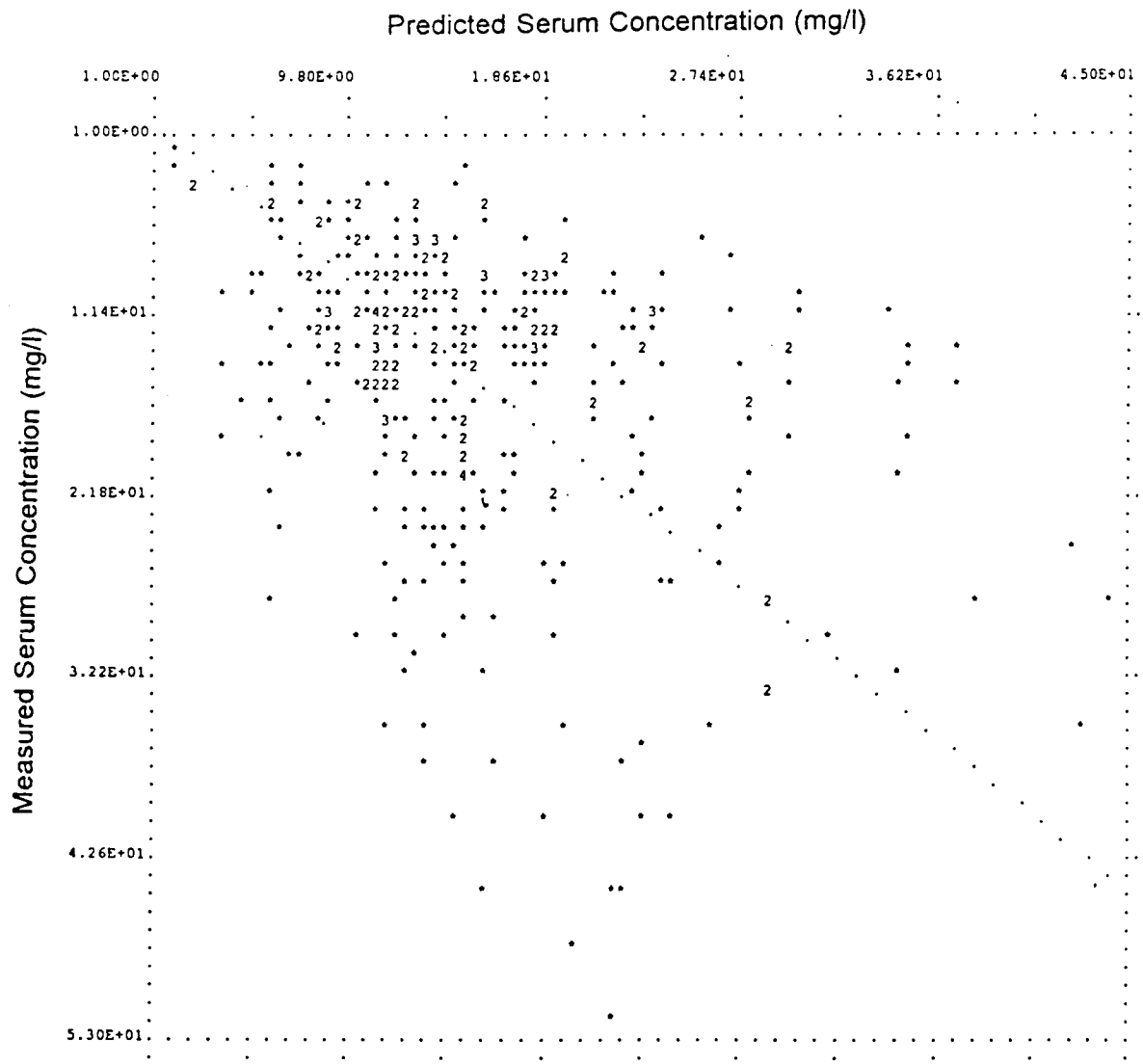

FIGURE 6: SCATTERPLOT OF PREDICTED SERUM PHENYTOIN CONCENTRATION (UPPER AXIS) VERSUS MEASURED SERUM CONCENTRATION (VERTICAL AXIS) OBTAINED FOR COLOURED PATIENTS WITH THE FINAL MODEL

TABLE 27: FINAL MEAN ESTIMATES OF THE POPULATION PHARMACOKINETIC PARAMETERS OBTAINED FOR EACH MODEL

PARAMETER	NONMEM ESTIMATES (± SE)		
	S1	S2	S3
Vm (mg/day)	366.7 (6.6) 353.8 - 379.6*	286.4 (12.3) 262.3 - 310.5*	338.3 (38.2) 263.4 - 413.2*
Vm (mg/kg/day)	5.71 (0.10) 5.51 - 5.91*	4.46 (0.19) 4.09 - 4.84*	5.27 (0.59) 4.10 - 6.44*
Km (mg/l)	3.45 (0.24) 2.98 - 3.92*	1.60 (0.31) 0.99 - 2.21*	6.81 (1.30) 4.26 - 9.36*
Cl (l/day)	-	2.09 (0.28) 1.54 - 2.64*	2.68 (0.78) 1.15 - 4.21*
% CV (Vm)	13.22	17.14	12.5
% CV (Km)	49.1	66.4	67.9
% CV (Cl)	-	28.5	46.3
% CV (ε)	5.0	4.87	20.1

* indicates the 95% confidence interval for the fixed effects
The values in parentheses are the standard errors of the estimates

model S1 was much lower. The mean Cl estimates of 2.09 and 2.68 l/day obtained with structural models S2 and S3 were comparable; a much lower % CV in Cl was, however, obtained with model S2. The high variability in Km and Cl was expected as no covariates were included in the model for these parameters.

Structural model S3, which was considered the best model in this study, was applied together with the FOCE method. The parameter estimates obtained represent improved parameter estimates, because the Bayesian feedback procedure was employed. The FOCE method calculated individualized Vm, Km and Cl values with all feedback serum phenytoin concentrations for every patient, and the mean Vm, Km and Cl values for the population were 370.9 mg/day (5.77 mg/kg/day), 6.48 mg/l and 2.15 l/day, respectively (Run 49, Table 25, page 102). The % CV for Vm was reduced from 12.5 (FO method) to

9.46 (FOCE method). Similarly, the % CV for Km was reduced from 67.9 (FO method) to 53.9 (FOCE method). The % CV for Cl, however, increased from 46.3 (FO method) to 96.3 (FOCE method)(Table 27, page 111).

The final regression model (Run 49, Table 25, page 102) obtained with the FOCE method can be represented as follows:

$$Vm = (\theta_1 * WT + \theta_3)RACE*SMK*AGE*EXP\eta_1$$
$$Km = \theta_2 * EXP\eta_2$$
$$Cl = \theta_6 * EXP\eta_3$$

where RACE = θ_4 if coloured, otherwise = 1
SMK = θ_5 if smoker, otherwise = 1
AGE = θ_7 if $\geq$ 65 years, otherwise = 1

θ_1 =	3.55	(0.396; 11.15)
θ_2 =	6.48	(0.653; 10.08)
θ_3 =	143.0	(35.5; 24.83)
θ_4 =	1.06	(0.0301; 2.84)
θ_5 =	1.12	(0.0329; 2.94)
θ_6 =	2.15	(0.668; 31.07)
θ_7 =	0.968	(0.077; 7.95)

The absolute and relative percentage standard errors of the estimates are given in brackets. The inter-individual variability estimated as variances of η^{Vm}, η^{Km} and η^{Cl} (random error terms) were 0.00895 (0.00262) mg/day^2, 0.29 (0.0426) mg/l^2 and 0.927 (0.299) l/day^2. The variance of ε was 7.67 (0.89) mg/l^2. The small standard errors indicated a good fit with reliable parameter estimates. Km and Cl parameters showed considerable variability which was excessively large for Cl - standard deviation of 44.8% of the mean.

In the final model, given the assumption of zero covariances among the individual parameters, seven population parameters are present: Vm, Km, Cl, σ_{Vm}, σ_{Km}, σ_{Cl} and σ_{ε}. The random effect parameters, the last 4, are often written as standard deviations (σ_{Vm}, σ_{Km}, σ_{Cl} and σ_{ε}) rather than variances.

Since ethnicity, smoking status and age were found to influence Vm, the patient population was subdivided into 8 subpopulation groups. Table 28 (page 114) summarizes the parameter models and mean Vm estimates for the different subgroups of patients. These estimates will be useful when using model S3 to predict a serum phenytoin level for a patient representative of a subgroup. In this way, the most appropriate Vm value will be used in the calculation. The θ values are given in Table 25 (Run 49, page 102).

This study yielded no incidence of pairwise correlations for different parameter combinations. Low Km and Vm values also occurred concurrently (Taylor *et al.*, 1983). The NONMEM control files for the best models for S1, S2 and S3 are given in Annexures 9, 10 and 11, respectively.

It should be noted that the Vm reported by NONMEM is in fact Vm/F. 'F' is a bioavailability factor and is usually assumed to have a value of one. However, any value of F less than one will increase the corresponding value of Vm.

The values of Vm obtained were normalized to standard body mass. Normalization is important for averaging data from individuals of markedly different sizes and for purposes of comparison with other data. Vm was expressed in mg/kg/day or mg/day.

4.9.5. VALIDATION OF THE POPULATION PARAMETER ESTIMATES AND STRUCTURAL MODELS

Predictions of the phenytoin dose or serum concentration were made with the prior knowledge of actual dose or measured serum phenytoin concentration and the patient variables which were found to influence the pharmacokinetics of phenytoin. The Vm value was calculated for each patient based on the individual's ethnic group, mass, smoking status and age group. The same Km and Cl estimates were used for all patients. The predictions obtained with the mean population estimates derived with FO and FOCE methods were compared. These population estimates were then used to predict dose or Cp_{ss} and compared with the actual dose or the measured concentrations (Fattinger *et al.*, 1991). The pharmacokinetic parameter estimates were obtained using the parameter models discussed previously (Tables 20 and 23), with estimates for the fixed effects obtained from Table 29 (page 115).

The influence of experimental error on the prediction performance was unlikely to be significant because in all the patients at least two serum phenytoin levels were obtained on the same dose, compliance was meticulously assessed, and at least a month elapsed before a blood sample for phenytoin concentration was taken to ensure that steady state was reached.

TABLE 28: FINAL MEAN Vm ESTIMATES OBTAINED FOR SUBPOPULATION GROUPS USING MODEL S3 AND THE CONDITIONAL ESTIMATION METHOD

(1)	Black nonsmoker, < 65 years:	
	Model: Vm	$= \theta_1 * WT + \theta_3$
		= 378.7 mg/day
		= 5.70 mg/kg/day
(2)	Black nonsmoker, ≥ 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_7$
		= 366.58 mg/day
		= 5.52 mg/kg/day
(3)	Black smoker, < 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_5$
		= 424.14 mg/day
		= 6.39 mg/kg/day
(4)	Black smoker, ≥ 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_5 * \theta_7$
		= 410.57 mg/day
		= 6.18 mg/kg/day
(5)	Coloured nonsmoker, < 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_4$
		= 384.51 mg/day
		= 6.21 mg/kg/day
(6)	Coloured nonsmoker, ≥ 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_4 * \theta_7$
		= 372.21 mg/day
		= 6.01 mg/kg/day
(7)	Coloured smoker, < 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_4 * \theta_5$
		= 430.65 mg/day
		= 6.96 mg/kg/day
(8)	Coloured smoker, ≥ 65 years:	
	Model: Vm	$= (\theta_1 * WT + \theta_3)\ \theta_4 * \theta_5 * \theta_7$
		= 416.87 mg/day
		= 6.73 mg/kg/day

Average mass of black and coloured patients was 66.4 and 61.9 kg, respectively. Refer to Run 49 (Table 25) for the parameter estimates

TABLE 29: PARAMETER ESTIMATES OBTAINED FOR SET 3 ANALYSIS (VALIDATION PROCEDURE)

RUN	MODEL	GROUP	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	θ_7	σ_{Vm}^2	σ_{km}^2	σ_{Cl}^2	σ_{ε}^2	CE*
50	S1	1	2.38	3.74	223	1.03	1.07	0.949	-	1950	3.40	-	257	No
51	S1	2	2.21	3.39	224	1.08	1.05	0.781	-	2640	2.77	-	228	No
52	S1	1	2.98	4.97	211	1.03	1.11	0.909	-	1.1×10^{-9}	0.366	-	388	Yes
53	S1	2	2.05	4.48	264	1.06	1.07	0.751	-	0.031	0.496	-	335	Yes
54	S2	1	2.25	2.0	160	1.03	1.10	1.83	0.928	2380	1.15	0.233	233	No
55	S2	2	2.20	1.5	137	1.12	1.08	2.28	0.706	2410	1.18	0.458	186	No
56	S2	1	1.55	2.35	233	1.02	1.07	0.89	0.951	2×10^{-8}	0.53	1.27	250	Yes
57	S2	2	2.21	1.57	154	1.12	1.04	1.71	0.671	1.8×10^{-8}	1.03	0.68	194	Yes
58	S3	1	3.30	7.72	202	1.02	1.11	0.945	0.91	1740	13.1	0.37	8.86	No
59	S3	2	3.46	4.93	49	1.22	1.20	3.64	0.724	2880	17.3	1.81	8.57	No
60	S3	1	2.66	8.04	290	1.04	1.06	0.162	0.911	0.011	0.204	6.09	8.18	Yes
61	S3	2	3.16	6.58	165	1.13	1.12	1.86	0.703	0.014	0.21	0.97	6.98	Yes

* denotes whether the conditional estimation (CE) method was used or not

4.9.5.1. PREDICTION PERFORMANCE EVALUATION

After excluding patients producing one steady-state concentration on a single dose of phenytoin, the patients were randomly divided into two equally matched groups of 122 patients each and stratified according to the following: ethnicity, smoking (non-smoker, smoking less than 10 cigarettes per day, 10 to 20 cigarettes per day, or more than 20 cigarettes per day) and age (less than 65 or 65 years or older). Refer to Section 3.7.5. in Chapter 3. The predictive performance was assessed using 770 (388 and 382 in Groups 1 and 2, respectively) paired predicted versus actual dose or measured serum concentrations.

The dose or serum concentration predictions were based on the estimated parameter values obtained with each structural model used (Table 29, page 115).

4.9.5.2. NAIVE FEEDBACK METHOD USING ESTIMATES OBTAINED WITH THE FO AND FOCE METHODS

The purpose of this part of the work was to compare the predictive performance of the 3 structural models using the mean population estimates obtained with the FO and FOCE methods.

Tables 30-32 present the previously described measures of bias (MPE) and precision (RMSE) obtained with the population estimates derived with the FO and FOCE methods. The separate and combined results are reported for Groups 1 and 2. Models S1 and S2, when the FO method was used, underpredicted the phenytoin dose by an average of 5.88 and 3.95 mg/day, respectively (Table 30, page 117). Model S3 overpredicted phenytoin concentration by an average of 2.54 mg/l (Table 31, page 118). The FOCE-derived parameter estimates were generally less biased than the FO-derived parameter estimates. When the FOCE method was used, models S1 and S2 underpredicted the phenytoin dose by an average of 1.32 and 3.73 mg/day, respectively (Table 32, page 118), and model S3 was found to overpredict phenytoin concentration by an average of 0.91 mg/l (Table 31, page 118). This was remarkable for a pharmacokinetic analysis where a number of variables can influence the results. It indicated that the prediction error was reduced in all 3 models when the FOCE-derived estimates were used. Reductions of 77.6, 5.7 and 64.2% in the MPE with the FOCE-derived estimates were obtained for models S1, S2 and S3, respectively. The MPEs of -0.43, -1.20 and 5.67% obtained with models S1, S2 and S3 with the FOCE-derived estimates were all considered acceptable (Tables 31 and 32). When the results of Groups 1 and 2 were combined, only the FOCE-derived estimates using model S1 were unbiased, as reflected by the 95% confidence interval which included zero (Toscano and Jameson, 1986)(Table 32, page 118). However,

when the groups were analyzed separately, Groups 1 and 2 (FO method - model S2; FOCE method - models S1 and S2) were unbiased (Tables 30 and 32). Only Group 1 predictions obtained with model S3 using the FOCE-derived estimates were unbiased (Table 31, page 118). The width of the 95% CI for MPE did not differ much between models S1 and S2 for the FO- and FOCE-derived estimates (Table 30). The width of the 95% CI for MPE was substantially shorter for model S3 when using the FOCE-derived estimates. This was expected with model S3 which predicted steady-state serum concentration. When model S3 was used for the combined data set, the 95% CIs of MPE when using the FO- and FOCE-derived estimates did not overlap. The bias had a wider range with the FO- (1.9 to 3.18 mg/l) than the FOCE-derived estimates (0.27 to 1.55 mg/l)(Table 31, page 118). This indicated a significant difference ($p < 0.05$) between the FO- and the FOCE-derived estimates when predicting steady-state serum concentrations with model S3.

TABLE 30: PREDICTIVE PERFORMANCE EVALUATION OF MODELS S1 AND S2 USING THE FO-DERIVED PARAMETER ESTIMATES

	MODELS					
	S1			S2		
	GROUP 1	GROUP 2	COMBINED	GROUP 1	GROUP 2	COMBINED
MPE (mg/day)	-5.80	-5.96	-5.88	-4.13	-3.77	-3.95
MPE (%)	-1.86	-1.92	-1.89	-1.32	-1.21	-1.27
MSE (mg/day)^2	2671.01	2857.43	2764.22	2671.0	2817.21	2744.11
RMSE (mg/day)	51.68	53.45	52.57	51.68	53.08	52.38
RMSE (%)	16.56	17.19	16.88	16.56	17.07	16.82
95% CI of BIAS (mg/day)	-10.92- - 0.68	-11.29 - -0.63	-9.56 - -2.18	-9.3 - 1.04	-9.15 - 1.61	-7.69 - -0.23
Does the 95% CI of BIAS include zero?	No	No	No	Yes	Yes	No
95% CI of RMSE	50.64 - 52.64	52.37 - 54.46	51.81 - 53.31	50.64 - 52.64	52.0 - 54.1	51.62 - 53.10

TABLE 31: PREDICTIVE PERFORMANCE EVALUATION OF MODEL S3 USING THE FO AND THE FOCE-DERIVED PARAMETER ESTIMATES

	MODELS					
	S3 (FO)			S3 (FOCE)		
	GROUP 1	GROUP 2	COMBINED	GROUP 1	GROUP 2	COMBINED
MPE (mg/l)	2.25	2.83	2.54	0.79	1.03	0.91
MPE (%)	14.23	17.50	15.87	4.97	6.37	5.67
MSE $(mg/l)^2$	95.12	125.92	110.52	93.30	122.2	107.75
RMSE (mg/l)	9.76	11.22	10.49	9.66	11.05	10.36
RMSE (%)	61.81	69.43	65.62	61.18	68.38	64.78
95% CI of BIAS (mg/day)	1.39-3.11	1.88-3.78	1.90 - 3.18	-0.07 - 1.65	0.08 - 1.98	0.27 - 1.55
Does the 95% CI of BIAS include zero?	No	No	No	Yes	No	No
95% CI of RMSE	9.56 - 9.93	10.99-11.43	10.36 - 10.66	9.46 - 9.84	10.83 - 11.26	10.23 - 10.52

TABLE 32: PREDICTIVE PERFORMANCE EVALUATION OF MODELS S1 AND S2 USING THE FOCE-DERIVED PARAMETER ESTIMATES

	MODELS					
	S1			S2		
	GROUP 1	GROUP 2	COMBINED	GROUP 1	GROUP 2	COMBINED
MPE (mg/day)	-1.59	-1.05	-1.32	-2.64	-4.81	-3.73
MPE (%)	-0.51	-0.34	-0.43	- 0.85	-1.54	-1.20
MSE $(mg/day)^2$	2962.65	3035.67	2999.16	2639.33	2877.35	2758.34
RMSE (mg/day)	54.4	55.1	54.75	51.37	53.64	52.51
RMSE (%)	17.44	17.72	17.58	16.46	17.25	16.86
95% CI of BIAS (mg/day)	-7.01 - 3.83	-6.58 - 4.48	-5.26 - 2.6	-7.72 - 2.44	-9.93 - 0.31	-7.33 - -0.11
Does the 95% CI of BIAS include zero?	Yes	Yes	Yes	Yes	Yes	No
95% CI of RMSE	53.34 - 55.44	53.98 - 56.14	53.96 - 55.52	50.34 - 52.33	52.55 - 54.65	51.75 - 53.25

The models S1 and S2 (FO and FOCE methods) tend to be slightly more conservative, usually underestimating the dose required (Tables 30 and 32). Given the nonlinear kinetics of phenytoin, this may be more clinically appropriate. All predictions lacked precision. The poor precision of the prediction obtained with model S3 was expected due to the nonlinear pharmacokinetics of phenytoin. The precision of all 3 models did not improve with the FOCE-derived estimates

The mean actual doses and the mean predicted doses were 311.2 and 307.6 and 311.2 and 307.4 for models S1 and S2, respectively. The mean measured and predicted steady-state concentrations were 15.97 and 17.7 mg/l, respectively. The predicted doses or steady-state serum concentrations represent a mean value obtained with the FO- or FOCE-derived parameter estimates. These results show a good relationship between the mean actual and predicted values.

For the combined data set, a significant difference (p = 0.028) was found between the actual and predicted doses for model S1 when using the FO-derived parameter estimates. No such difference (p = 0.64) was found when the FOCE-derived parameter estimates were used. This confirmed that the FOCE-derived estimates predicted better than the FO-derived estimates. For model S2, no significant differences were found for the FO (p = 0.142) and FOCE (p = 0.153) methods. This indicated the importance of Cl in the model. For model S3, a significant difference ($p = 4.39 \times 10^{-8}$) was found between the measured and predicted steady-state serum concentrations when the FO-derived estimates were used; however, no such difference (p = 0.0502) was found when the FOCE-derived estimates were used. When all the groups were analyzed separately, only the FO-derived estimates when used in model S3 showed a statistical difference between measured and predicted steady-state serum concentrations in Groups 1 ($p = 3.93 \times 10^{-5}$) and 2 ($p = 3.0 \times 10^{-4}$). The absence of a significant difference between most groups, particularly when the FO-derived estimates were used, indicated that the parameter and structural models were adequate. These results clearly indicate that model S3 can be used with confidence when the FOCE-derived parameter estimates are used.

Simply ranking the methods on the basis of bias and precision does not always predict their ranking in respect of superiority of clinical use (Toscano and Jameson, 1986). For this reason, our study also examined the relative frequencies of satisfactory predictions. The ability to predict a dose or steady-state serum concentration with precision within 10% of the actual dose or within 20% of measured serum concentration was tested for the various models (Yuen *et al.*, 1983a). These results were tabulated for each method and expressed as a percentage of the total predictions for that method (Table 33, page 120). Our conclusions are therefore also based on the latter.

TABLE 33: PREDICTIVE PERFORMANCE EVALUATION OF MODELS S1, S2 AND S3

	MODELS					
	S1 FO %	S2 FO %	S3 FO %	S1 FOCE %	S2 FOCE %	S3 FOCE %
UNDERPREDICTION	55.8	55.3	38.6	51.2	55.6	45.7
OVERPREDICTION	44.2	44.7	61.4	48.8	44.4	54.3
PE[1] > 10% FOR DOSE	54.9	55.7	-	58.2	54.5	-
PE[1] > 20% FOR Cp_{SS}	-	-	76.4	-	-	75.3

[1] denotes prediction error

No significant difference between the frequency of under- and overprediction of dose was found when comparing models S1 and S2. There was a trend towards conservative predictions, i.e. underpredictions of dose. This is an important attribute for a phenytoin dosing method since even a small overdosage may lead to substantial toxicity. Thus, in most clinical situations, underdosing is more desirable than overdosing. Model S3 tended to overpredict steady-state serum concentration in 61.4% of cases when the FO-derived estimates were used. With the FOCE-derived estimates, the tendency to overpredict was reduced to 54.3% which was considered satisfactory (Table 33). The percentage of prediction errors greater than 10% did not differ substantially between models S1 and S2. Using the latter models, on average 44.2% of predictions had errors less than 10%. With model S3, the percentage of prediction errors greater than 20% did not differ substantially when the FO- and FOCE-derived estimates were used. On average, only 24.2% of the predictions of steady-state serum concentrations had errors less than 20% (Table 33). The FOCE-derived estimates also resulted in fewer extreme dosing errors.

Although model S2 fitted the data better than model S1, the results showed that Cl was not clinically important when predicting the dose, as the percentage bias (% MPE) was not much lower when Cl was incorporated in the model. The frequency of prediction errors also did not differ between models S1 and S2. A definite statement about superiority cannot easily be made.

Because of the poor precision, the 3 models evaluated in this study should be applied with caution when predicting dose or serum concentration. As clearly demonstrated in this study, FOCE-derived estimates improved the bias to a variable extent but not to the point of precision. Given the larger variability in the kinetics of phenytoin, one or more feedback concentrations are probably required to improve precision. The more feedback serum

concentrations available, the more the precision of the predictions can be expected to improve.

4.9.5.3. PREDICTIONS OF STEADY-STATE SERUM CONCENTRATIONS USING THE MICHAELIS-MENTEN MODEL (S4)

In many cases, predictions could not be made since the maintenance dose administered exceeded the predicted Vm value for the individual, resulting in unrealistic steady-state serum concentration values. This made accurate assessment of prediction performance difficult. In such a case, the equation yielded a negative value for the steady-state concentration when conceptually it should have resulted in infinity. This would have confounded the analyses of bias and precision (Toscano and Jameson, 1986). For this reason, 53 dose-concentration pairs where the latter occurred were removed from the data set. Therefore, with the Michaelis-Menten model, dosing rate was used as a basis of comparison since it was always possible to calculate an estimated dosing rate required to achieve an observed steady-state concentration. When the maintenance dose administered exceeded the predicted Vm value, steady state would theoretically never have been reached (Yuen *et al.*, 1989).

The estimates of Vm and Km obtained with model S1 were used in this analysis. The results obtained when using model S4 are presented in Tables 34 and 35 (page 122). Model S4 tended to overpredict grossly the steady-state serum concentration when the FO-derived estimates were used - a % MPE of 76.2%. When linear clearance was taken into account (model S3), the % MPE decreased to 15.87% (Table 31, page 118), reflecting a 79.2% reduction. The precision of the prediction improved markedly when Cl was included from 379.4% (Table 34, page 122) for model S4 to 65.62% (Table 31, page 118) for model S3. Using the estimates obtained with the FOCE method, the RMSE was not substantially reduced (Table 34). The 95% CI for bias did not include zero when the FO- and FOCE-derived estimates were used. When the FO-derived estimates were used, model S3 tended to overpredict the Cp_{ss} (61.4%)(Table 33, page 120) more than model S4 (52.4%)(Table 35, page 122). However, the frequency of PEs greater than 20% did not differ much (Table 35). When interpreting the results, one should take into consideration that 53 dose : concentration pairs had to be removed from the data set when model S4 was used (Table 34).

TABLE 34: PERFORMANCE EVALUATION OF MICHAELIS-MENTEN MODEL (S4) TO PREDICT STEADY-STATE SERUM CONCENTRATION USING THE FO- AND THE FOCE-DERIVED PARAMETER ESTIMATES OBTAINED FOR MODEL S1

	MODELS					
	S4 (FO)			S4 (FOCE)		
	GROUP 1	GROUP 2	COMBINED	GROUP 1	GROUP 2	COMBINED
n	357	360	717	374	376	750
MPE (mg/l)	10.17	13.44	11.81	7.37	8.88	8.13
MPE (%)	67.49	84.9	76.2	48.0	55.81	52.0
MSE $(mg/day)^2$	4322.94	2555.77	3439.36	2961.63	2635.0	2798.3
RMSE (mg/l)	65.75	50.55	58.15	54.42	51.34	52.9
RMSE (%)	436.3	319.3	379.4	354.52	322.7	338.5
95% CI of BIAS (mg/l)	3.42 - 16.92	8.4 - 18.48	7.6 - 16.02	1.9 - 12.84	3.76 - 14.0	4.39 - 11.87
Does the 95% CI of BIAS include zero?	No	No	No	No	No	No
95% CI of RMSE	64.32 - 67.08	49.46 - 51.57	57.73 - 59.45	53.29 - 55.47	50.27 - 52.32	52.14 - 53.62
No. of predictions excluded	31	22	53	14	6	20
No. of unrealistic predictions > 75 mg/l	16	32	48	11	14	25

TABLE 35: PREDICTIVE PERFORMANCE EVALUATION OF MODEL S4

	MODELS	
	S4 (FO)	S4 (FOCE)
	n =717	n =750
	%	%
UNDERPREDICTIONS	47.6	50.5
OVERPREDICTIONS	52.4	49.5
PE > 20% FOR Cp_{ss}	80.2	79.7

When the FO-derived estimates were used (model S4), 48 of the predictions of Cp_{ss} were unrealistically greater than 75 mg/l. When estimates obtained with the FOCE method were used, the number of unrealistic predictions fell to 25. The combined number of patients excluded due to the dose being greater than Vm was reduced from 53 for the FO-derived estimates to 20 for the FOCE-derived estimates (Table 34). The standard deviation for the bias and predicted concentration was substantially lower for model S3 than for model S4. A statistical difference between the measured and predicted concentrations was found with model S4 when the FO- ($p = 7.56 \times 10^{-8}$) and the FOCE- ($p = 3.06 \times 10^{-5}$) derived estimates were used. With model S3, a statistical difference was only found when using the FO-derived estimates ($p = 4.39 \times 10^{-8}$).

The results clearly indicate that Cl in model S3 was clinically important when predicting steady-state serum concentrations, because the % MPE was much lower when clearance was incorporated in the model. Model S3 should therefore be used in preference to model S4. The estimates obtained with the FOCE method also performed better than those obtained with the FO method.

4.9.5.4. PREDICTIONS OF DOSE AND STEADY-STATE SERUM CONCENTRATION USING ESTIMATES OBTAINED FROM THE STUDY OF VOZEH *et al.* (1981) IN MODELS S1 and S4

The results obtained when using model S1 are presented in Tables 36 and 38. Only the FO-derived estimates were used in this part of the analysis. When using model S1, the % MPE of -1.89% obtained with our estimates was much lower than that of 11.4% obtained with the estimates of Vozeh *et al.* (1981). The estimates of Vozeh *et al.* (1981) also tended to overpredict the dose in 69% of cases (Table 38, page 125) and this may be problematic with phenytoin considering its nonlinear pharmacokinetics. When our estimates were used the dose was overpredicted in 44.2% of cases (Table 33, page 120). The precision of the prediction was superior with our estimates of Vm and Km. The width of the 95% CI for MPE was narrower with our estimates of the pharmacokinetic parameters. The frequency of PE greater than 10% for dose was 54.9% for our estimates, and 70.1% when the estimates of Vozeh *et al.* 1981 were used.

The results obtained with the estimates of Vozeh *et al.* (1981) in model S4 are presented in Tables 37 and 38 (page 123). When the estimates from the latter study were used, large differences were found in the % MPE and % RMSE between Groups 1 and 2. This indicated instability in the model or the estimates. When our estimates were used, model S4 overpredicted Cp_{ss} in 52.4% of cases (Table 35, page 122), in comparison with 30.7% (Table 38, page 124) with the estimates of Vozeh *et al.* (1981).

TABLE 36: PERFORMANCE EVALUATION OF THE MICHAELIS-MENTEN MODEL (S1) TO PREDICT PHENYTOIN DOSE USING THE ESTIMATES OF Vm AND Km OBTAINED BY VOZEH *et al.* (1981)

	S1		
	GROUP 1	GROUP 2	COMBINED
n	388	382	770
MPE (mg/day)	33.7	36.6	35.15
MPE (%)	10.9	11.9	11.4
MSE $(mg/day)^2$	6644	6525	6584.5
RMSE (mg/day)	81.51	80.78	81.15
RMSE (%)	26.13	25.97	26.05
95% CI of BIAS	26.5 - 40.9	29.6 - 43.6	30.33 - 40.67
Does the 95% CI of BIAS include zero?	No	No	No
95% CI of RMSE	79.88 - 83.03	79.13 - 82.30	79.96 - 82.28

TABLE 37: PERFORMANCE EVALUATION OF THE MICHAELIS-MENTEN MODEL (S4) TO PREDICT SERUM PHENYTOIN CONCENTRATION USING THE ESTIMATES OF Vm AND Km OBTAINED BY VOZEH *et al.* (1981)

	S4		
	GROUP 1	GROUP 2	COMBINED
n	380	373	753
MPE (mg/l)	0.13	7.42	3.78
MPE (%)	0.83	46.92	23.88
MSE $(mg/l)^2$	1104	12345	6724.50
RMSE (mg/l)	33	111.1	72.05
RMSE (%)	211.9	703.16	457.53
95% CI of BIAS	-3.22- 3.48	-3.85 - 18.69	-2.05 - 9.61
Does the 95% CI of BIAS include zero?	Yes	Yes	Yes
95% CI of RMSE	32.55 - 33.86	108.79 - 113.26	80.78 - 83.17

TABLE 38: PREDICTIVE PERFORMANCE EVALUATION OF MODELS S1 AND S4 USING THE ESTIMATES OF Vm AND Km OBTAINED BY VOZEH *et al.* (1981)

	MODELS	
	S1	S4
	n = 770	n =753
	%	%
UNDERPREDICTIONS	31.0	69.3
OVERPREDICTIONS	69.0	30.7
PE > 10% FOR DOSE	70.1	-
PE > 20% FOR Cp_{SS}	-	85.5

The frequency of PE greater than 20% did not differ much between the 2 evaluations (Tables 35 and 38). This analysis indicated that predictive performance improved when population estimates representative of a specific population were used.

In summary, the study demonstrated that the parallel Michaelis-Menten model with first-order elimination (model S3) was superior to the Michaelis-Menten model when predicting Cp_{ss}. When using the Michaelis-Menten model to predict dose, our estimates performed better than those of Vozeh *et al.* (1981). However, when predicting Cp_{ss}, our estimates did not perform substantially better than those of Vozeh *et al.* (1981).

The bias decreased and the precision increased as the number of individuals in the sample increased (Sheiner and Beal, 1983). When using the estimates determined in the whole population, improved estimates of bias and precision were obtained even though patients with one dose-concentration pair were not excluded. These results are not reported here. It is therefore emphasized that the estimates obtained in sets 1 and 2 analysis (i.e. 332 patients) should be used in clinical practice. The parameter estimates obtained in set 3 analysis (122 patients in each group) were solely for the purpose of validation of the parameter estimates and should not be used in the clinical setting.

CHAPTER 5

DISCUSSION

As no data are available on the population pharmacokinetics of phenytoin in the Western Cape, this study was undertaken to address this issue. This study was conducted prospectively primarily to: (1) investigate the influence of various patient variables on the population pharmacokinetic parameters of phenytoin, (2) assess whether the parallel Michaelis-Menten and first-order elimination model provides a better fit to the data than the Michaelis-Menten model, (3) determine population pharmacokinetic parameter estimates of phenytoin representative of the patient population, and (4) validate and compare the clinical applicability of the parameter estimates and the models.

More appropriate initial estimates of population pharmacokinetic parameters may greatly improve the performance of the Michaelis-Menten model or other models that use phenytoin population pharmacokinetics (Burton *et al.*, 1985) to predict phenytoin dose or serum concentration. However, before establishing the best estimates of the population pharmacokinetic parameters, it was necessary first to evaluate the influence of selected patient factors on these pharmacokinetic parameters. Generally, concomitant factors such as age, weight, serum albumin, liver and renal function, and concurrent drug therapy must be used to provide appropriate prior information (Ludden *et al.*, 1986). The importance of appropriate prior information for Bayesian regression analysis has been reported (Peck and Chen, 1985). Although many factors are known to influence the disposition of phenytoin, their relative effects on Vm and Km are difficult to quantify (Bryson *et al.*, 1988). Application of a model to a diverse patient population will require that the mean values for pharmacokinetic variables be defined appropriately for various subpopulations. The estimates of the proportionality factors that adjust the mean population parameters for inter-individual differences, the magnitudes of the inter-individual and residual variability of these parameters in the dose to serum concentration relationship should also be quantified. As mentioned before, it is important that the estimates of this variability be obtained in a patient population in whom the drug is used clinically (Vozeh *et al.*, 1982). The estimates of this variability are helpful in the development of dosage recommendations, because they enable calculation of the proportion of patients at risk of producing toxic or ineffective concentrations (Fattinger *et al.*, 1991). A recent conference report (Peck *et al.*, 1992) has once again emphasized the importance of the appreciation

of inter- and intra-patient variability and its causes, so that better individualization of treatment can be achieved.

Interpretation and comparison of practically all earlier studies are difficult because of their retrospective nature, small patient populations, multiple drug therapy, questionable compliance, different analytical techniques, different methods of data analysis and too many uncontrolled factors. The use of multiple regression analysis and the calculation of correlation coefficients to determine the effect of patient attributes on the pharmacokinetic parameters and predictive performance have been demonstrated by Sheiner and Beal (1981) to be an inappropriate method. In many earlier studies, the performance of a prediction method was evaluated by computing the correlation coefficient of the actual and predicted values. However, as demonstrated by Sheiner and Beal (1981), this statistical method provided a poor description of predictive performance. The correlation coefficient assesses the degree of association rather than the true closeness of the predicted and actual values (Beck *et al.*, 1987). This method was therefore not used in this study.

It is possible that in many studies the relationship between phenytoin dosage requirements and patient attributes was concealed by the presence of other anticonvulsants (Barot, 1978). How much confidence can be placed in these studies is not known (Whiting, 1986). There has been a need for investigations that include prospective evaluations of predictive performance (Pryka *et al.*, 1991). To our knowledge, this is the first prospective investigation to study population pharmacokinetics of phenytoin in South African patients. There is undoubtedly much to be gained from prospective planning in population pharmacokinetic studies (Fattinger *et al.*, 1991).

None of the predictive dosing methods based solely on population estimates has proven satisfactory. Ideally, these methods should apply before phenytoin therapy is initiated; alternatively, as soon as possible after therapy is started (Slattery *et al.*, 1980). Furthermore, predicting the proper maintenance dose early in therapy may be particularly valuable for a drug like phenytoin where the desired effect is delayed, a fact which complicates the temporal relationship between dose adjustment and observed clinical result. This study therefore concentrated on the naive feedback approach to validate the factors influencing the population pharmacokinetic parameters, the different structural models and the pharmacokinetic parameter estimates. Previous studies have shown the naive feedback method to be inaccurate. There is a need for a simple and general method

to predict individual dosage requirements, a method which extracts the maximum pharmacokinetic information about a given patient from the minimum of data obtained after an initial dose (Godley *et al.*, 1987; Slattery *et al.*, 1980).

Although nomograms (predictive algorithms) are useful as starting points for drug therapy, most are generally based on invalid assumptions of either a constant Vm or Km, and their error in achieving target concentration is considerable (Chiba *et al.*, 1980; Richens and Dunlop, 1975). Because of the large variability of both Vm and Km in a population, nomograms that assume either an average Vm or Km are inherently imprecise (Vozeh *et al.*, 1980). This imprecision is exacerbated by the fact that most of the algorithms have been developed using small populations of patients. The relationship between phenytoin pharmacokinetics and one or more clinical parameters is then applied to larger groups of patients. The groups from which the data are originally derived are often relatively homogenous, and the data do not necessarily apply to the population as a whole (Burton *et al.*, 1985). Since the therapeutic range usually exceeds the Km for phenytoin, the effects of this imprecision are magnified when phenytoin concentrations are above the lower limit of the therapeutic range. Algorithms are most successful when they are used in the same population for which they were developed (Miller *et al.*, 1989).

5.1. FACTORS INFLUENCING THE PHARMACOKINETICS OF PHENYTOIN

It is important to understand why variation in pharmacokinetic parameters occurs so that the dose can be tailored to achieve an optimal response in a given patient (Berman, 1976). Our study demonstrated that estimates of Vm improved when selected demographic factors were considered. Mass, ethnicity, smoking and age contributed significantly to explaining variability in Vm. Other factors, as yet undetermined, may also affect the pharmacokinetics of phenytoin.

It is clear that when phenytoin dose is individualized as a linear function of weight 60- 70% of patients attain steady-state concentrations outside the therapeutic range (Koch-Weser, 1975). Weight alone, therefore, cannot account for the wide variation seen in clinical practice (Richens, 1975). Other influential factors should also be taken into account, as demonstrated in this study.

Other than weight, the most influential factor related to Vm was smoking. To our knowledge, there are no studies in the literature that address this. Studies do address the effect of smoking on serum phenytoin concentration, but not on Vm. Serum phenytoin concentrations have been reported not to be appreciably affected by smoking (Benetello *et al.*, 1987; Crowley *et al.*, 1988; De Leacy *et al.*, 1979). Our study has demonstrated that smoking increased Vm significantly ($p < 0.0007$) on average by a factor of 12%.

With increased recognition that genetically determined polymorphism in drug metabolism is an important factor affecting drug disposition, interest has developed in the individualization of dose to account for racial differences (Wood and Zhou, 1991). Failure to take into account these differences may result in therapeutic failure and toxicity. Clearly discernible differences have been found to exist between South African blacks and coloureds in the kinetics of phenytoin. This may arise from the interplay of genetic as well as environmental factors, e.g. diet and alcohol intake (Branch *et al.*, 1978; Kalow, 1982). Our results are contrary to those of Grasela *et al.* (1983) in that our study showed that ethnicity influenced Vm. On the other hand, our study is in agreement with that of Grasela *et al.* (1983) in that ethnicity was found to influence Km in both studies. Our final model, however, did not include the influence of ethnicity in Km for the reasons discussed previously. Ethnicity has been reported to influence the effect of a number of drugs, e.g. ephedrine (Smith, 1989), beta-blockers (Seedat, 1980; Venter and Joubert, 1984), hydrochlorothiazide (Smith, 1989), theophylline (Driscoll *et al.*, 1989), diazepam (Ghoneim *et al.*, 1981), isoniazid (Sunahara *et al.*, 1961), procainamide, hydralazine, dapsone (Horai and Ishizaki, 1988) and mephenytoin (Jurima *et al.*, 1985; Nakamura *et al.*, 1985; Wedlund *et al.*, 1984).

Our study did not detect a continuous effect of age on Vm. The same effect was reported by Grasela *et al.* (1983). The latter investigators reported that discontinuity at 15 years of age provided the best fit of the model to the data, their patient population differing from ours in that their study included children. It is for this reason that our study included patients over the age of 15. Another reason for this is that the OPT^{R} computer programme has not been validated for persons under 15 years of age. Our study showed that discontinuity at 65 years of age provided the best fit. The conditional estimation method showed that the effect of age on phenytoin metabolism was less compared with that of smoking. These results are similar to those of Vestal *et al.* (1975) who showed that the effect of age on antipyrine clearance was insignificant compared with that of smoking. Our

results agree with those of Bauer and Blouin (1982) who showed that Vm values for geriatric patients are lower than those for younger adults. Our results also agree in that age did not influence Km. This study of Bauer and Blouin (1982) is discussed here as it is probably one of the few studies, if not the only one, on population pharmacokinetics of phenytoin performed prospectively in adult patients.

Our results also agree with Bauer and Blouin (1982) in that sex difference did not influence Vm and Km. Failure to find an effect of gender was also the case with other studies (Grasela *et al.*, 1983; Houghton *et al.*, 1975). This does not necessarily mean that such interaction may not occur on an individual patient basis. Gender-related differences in the disposition of drugs such as paracetamol, chlordiazepoxide, diazepam, oxazepam and theophylline have been reported. None of these gender-related differences has so far necessitated the modification of the dosage regimen (Driscoll *et al.*, 1989; Wilson, 1984).

A priori methods based on patient characteristics such as age, body mass, renal function, smoking status and degree of congestive heart failure have proved useful in predicting initial estimates of pharmacokinetic parameters and dose requirements. Greater precision has been obtained with predictive methods based on individual characterization of the pharmacokinetic parameters (Slattery *et al.*, 1980).

5.2. POPULATION PHARMACOKINETIC PARAMETER ESTIMATES

Further details of the studies cited below can be found in Chapter 2, and especially Table 1 in that chapter.

The information on the population pharmacokinetic parameter estimates was obtained mainly from Caucasian populations and information from other population groups seems to be fragmentary and scarce.

The Vm and Km values obtained in our study were compared with those obtained in other studies, especially where NONMEM was used. When comparing Vm and Km estimates obtained in other studies, it is important to consider the structural models used, the methods used to derive the parameter estimates, the number of patients in the study,

whether other drugs including anticonvulsants that may interfere with phenytoin pharmacokinetics were taken, and the compliance checks that were used.

A Km of 3.66 (inter-individual SD 1.69, CV 49.1%) obtained in our study for black patients compared favourably with that of 3.43 mg/l (inter-individual SD 2.27, CV 73%) obtained by Miller *et al.* (1987) for black patients attending the outpatient clinic at Baragwanath Hospital in Johannesburg, South Africa. In our study, a Vm of 5.71 mg/kg/day (inter-individual SD 0.73; CV 13.22%) was obtained for black patients when no covariates were taken in account. This was lower than that of 6.5 mg/kg/day (inter-individual SD 1.3, CV 20%) obtained by Miller *at al.* (1987) for black South African patients. The NONMEM approach was also used by Miller *et al.* (1987). In our study, a larger number of black patients was recruited (149 compared with 37 in Miller's study) and more serum phenytoin concentrations were measured (411 versus 100). Our study also had an advantage in that it was performed prospectively, reducing the chances of error.

The mean Vm value of 5.71 mg/kg/day obtained in our study with the Michaelis-Menten model compared well with that of 5.93 mg/kg/day reported in adult male European patients by Grasela *et al.* (1983). The Km value, on the other hand, differed markedly from that of adult European patients. A mean Km value of 3.45 mg/l was obtained in our study whereas a mean Km value of 5.7 mg/l was obtained in adult male European patients using the same model and procedure of analysis (i.e. NONMEM). Grasela *et al.* (1983) reported a mean Km value of 3.8 mg/l for Japanese patients over 15 years of age and this compared closely with the mean Km value obtained in our study. Grasela *et al.* (1983) reported that even after adjustments for covariates, the apparent Vm and Km varied unpredictably among individuals with a CV between 10 and 20% and approximately 50%, respectively. The % CV in our study varied from 12.5 to 17.14 and 49.1 to 67.9 for Vm and Km, respectively, depending on the structural model and the method (naive or conditional estimation) used. A CV of 13.22 and 49.1% for Vm and Km, respectively, obtained with the Michaelis-Menten model correlated well with that of Grasela *et al.* (1983). The estimate of the residual variability (5%) obtained in our patients was lower than that of 8% and 12% obtained in European and Japanese patients, respectively. In the same study, some patients in all the groups (German, Japanese and English) received other medication in addition to phenytoin. Information on these medications was unknown and it was assumed that they had no appreciable effect on the observations. This study

also assumed 100% compliance with the prescribed dosage regimen. There were large age differences between the groups.

The Vm value of 8.05 mg/kg/day obtained in Singapore Chinese patients was much higher than that obtained in our study. Ethnic differences may account for the greater metabolic capacity in the Chinese population. This study used the nonlinear mixed-effects model in the MULTI(ELS) programme. The possibility that the observed differences in Vm may have been due to differences in the bioavailability of phenytoin between different patient groups cannot be ruled out (Chan and Lee, 1990). On the other hand, the mean Km value of 3.45 mg/l obtained in our study was higher than that of 2.31 mg/l obtained in the study of Chan and Lee (1990). The latter study, however, included paediatric patients, which makes comparison difficult. Chan and Lee (1990) reported an inter-individual variability in Vm and Km of 28.5% and 65.7%, respectively, and a residual variability of 21.3%. The latter values are much higher than those obtained in our study.

Vozeh *et al.* (1981) studied 49 Swiss patients and reported the following parameter estimates: mean Vm = 7.22 mg/kg/day (inter-individual SD 1.72; CV 24%) and mean Km = 4.44 mg/l (inter-individual SD 2.4; CV 54%). The NONMEM method was used in this study. These estimates have been widely used in clinical practice for the calculation of phenytoin dose and prediction of steady-state concentration. These estimates were also used in the 'orbit plot' and in the OPT^{R} computer programme. Both Vm and Km estimates obtained in our study were lower than those reported by Vozeh *et al.* (1981).

In a retrospective study of 334 adult and paediatric patients, Yukawa *et al.* (1990) determined the population pharmacokinetics of phenytoin from routine clinical data in Japanese patients using the NONMEM approach. Only 101 of these patients were receiving phenytoin monotherapy. Duplicate steady-state phenytoin concentrations on the same dose were not obtained and therefore compliance was not confirmed. The data analysis using the Michaelis-Menten model estimated Vm and Km values of 5.42 mg/kg/day and 2.41 mg/l, respectively. In this study, the typical magnitude of inter-individual variability, as expressed by the coefficient of variation, was 17.2% for Vm and 53.3% for Km. The typical magnitude of the intra-individual variability, expressed as the coefficient of variation, was 11%. This study again confirmed that our estimates of Vm, Km, the inter-individual and residual variability in these parameters are acceptable. This study revealed that the Vm for patients taking phenytoin combined with other

anticonvulsants was 80% higher than that of patients taking phenytoin alone. Similarly, the Km for patients taking phenytoin combined with other anticonvulsants was 32% higher than that of patients taking phenytoin alone. It is, therefore, essential for the patients to be on phenytoin monotherapy in studies to assess population pharmacokinetic parameters of phenytoin.

5.3. PARALLEL MICHAELIS-MENTEN AND FIRST-ORDER KINETICS

Correct interpretation of phenytoin concentrations requires an accurate and precise method that takes the nonlinear and linear disposition of phenytoin into account (Bryson *et al.*, 1988).

Ludden *et al.* (1991) in a simulation based study, reported that the sensitivity of mean steady-state phenytoin concentrations to the change in effective dosing rate was influenced by a parallel, first-order elimination pathway, the magnitude of which was unknown. Our study determined that the magnitude of the mean Cl was 2.68 (0.78) l/day with the upper and lower bounds of the 95% confidence interval of 1.49 and 3.89 l/day, respectively. The standard error of the estimate is presented in parentheses.

In previous studies, the contribution of the parallel first-order elimination pathway was assumed to be negligible. This study proved that, on the contrary, taking linear clearance of phenytoin into account does indeed improve the fit of the model to the data. Such a model, especially when predicting serum phenytoin concentrations, provides predictions that are less biased.

5.4. VALIDATION OF POPULATION PARAMETERS AND STRUCTURAL MODELS

In the validation procedure, it was assumed that each patient behaved as the average. If a subsequently obtained serum concentration differed from that predicted, the patient clearly did not behave as average, and his/her pharmacokinetic parameters should be re-estimated to derive future dosing guidelines (Burton *et al.*, 1985).

For the validation of the population parameter estimates, only model S1 can be compared with other studies as, according to our knowledge, models S2 and S3 have never been validated. In our study, with the FO-derived parameter estimates a MPE of 5.88 (SD 52.31) mg/day were obtained using model S1. This is equivalent to a bias of 1.89%. Miller *et al.* (1989), also using the Michaelis-Menten model, obtained a MPE of 13.1 (SD 16.7) mg/day. In our study the MPE was 55% lower than that obtained by Miller *et al.* (1989). The latter study on average overpredicted the dosage rate whereas our study underpredicted the dosage rate. A positive bias in phenytoin dosage is a more serious error than a negative bias (Toscano and Jameson, 1986). Our results produced a RMSE of 52.57 mg/day, which was lower than that of 61.5 mg/day obtained by Miller *et al.* (1989), a 14.5% difference. A smaller prediction error was expected in our study as the Vm values were adjusted for ethnicity, weight, age and smoking status. The Vm value in the study of Miller *et al.* (1989) was not adjusted for any patient characteristic except for weight. The fact that the study was conducted in black patients only, however, automatically took ethnicity into account. The latter study was also not designed to study the influence of patient factors on Vm and Km values.

The predicted doses obtained with model S1 have a mean absolute deviation of 1.89% when compared with the actual doses. This was considered acceptable, as the mean error was approximately of the same magnitude as the assay error. Despite the relatively 'good' predictability of the models examined, it is disconcerting that there was a sizeable number of predictions with an error of greater than 10%. While this is trivial for most other drugs, such an error can become significant in the case of phenytoin because of the Michaelis-Menten characteristics of the drug (Mawer *et al.*, 1974). The majority of the predictions of dose were underpredictions, which minimized the risk of potentially toxic dosages.

The data of Flint *et al.* (1985) clearly demonstrated that two previous dose : serum concentration pairs are superior to one in the prediction of subsequent serum concentrations. The more information available about the patient, the more accurate the predictions. Such a finding is not surprising in view of the considerable variability associated with the clearance of phenytoin. However, the accuracy of the predictions cannot improve indefinitely because of assay variability and other factors that cannot be completely controlled. The constraints imposed by inter- and intra-patient pharmacokinetic variability will continue to limit prediction accuracy (Armijo *et al.*, 1989). Misspecification

of the pharmacokinetic model could be partly responsible for the difference in prediction accuracy (Vozeh *et al.*, 1984). In our study, the naive prediction method was used and therefore the worst scenario or highest prediction errors were expected. The forecasting of steady-state serum phenytoin concentrations from mean population parameter values was remarkably inaccurate (Bryson *et al.*, 1988). The bias and precision of our predictions would undoubtedly have improved if one or more feedback serum concentrations had been used.

Knowledge of the estimates of the pharmacokinetic parameters and their influential factors allows a prediction only of the mean concentration; concentrations in individual patients may differ substantially from this (Burton *et al.*, 1985). Only the additional knowledge of the variance components permits one to estimate how large the inter-individual differences in the actually achieved concentrations are expected to be, or in other words, how confident one can be about the predicted dose or concentration (Maitre *et al.*, 1987). Our study provides estimates of the inter- and intra-individual variability for the study population.

Few evaluative studies have been done to compare different models that may be useful in predicting dose or steady-state serum phenytoin concentrations. Most studies have used the same structural model, the Michaelis-Menten model, with different methods of analysis, (e.g. the Bayesian or naive prediction methods) and different estimates of Vm and Km. This study, in addition to the Michaelis-Menten model, also evaluated the parallel Michaelis-Menten and first-order elimination models.

5.5. THERAPEUTIC IMPLICATIONS

With more knowledge about factors influencing the pharmacokinetic parameters of phenytoin and the attainment of an improved pharmacokinetic model for prediction of serum phenytoin concentrations, the potential for further improvement is considerable. Using a simple model such as the Michaelis-Menten model has a drawback in that one must be satisfied with limited success.

A fundamental drawback with phenytoin is that it requires an extended period of initial evaluation to determine individual kinetics. The relationship between phenytoin dose and level is unpredictable if a patient's Vm and Km values are unknown (Dodson, 1982). This

study served to improve the understanding of this relationship by determining not only mean Vm and Km values but also these parameters in subsets of patients.

A lower Km value obtained in our patients in comparison with other population groups (e.g. Swiss and European patients) implied that our patients were particularly sensitive to changes in dosage when concentrations were in or near the therapeutic range. This also implies that toxic serum concentrations would be achieved in our population group at lower doses than in Swiss and European populations. It is therefore recommended that smaller increments of dose should be used in South African blacks and coloureds. The findings in the above studies suggested that the variability in Km was the main factor causing inter-individual differences in the dosage and steady-state concentration relationship. The magnitude of the inter-individual variability in Km emphasized the need for careful therapeutic drug monitoring employing Bayesian feedback. The lower Vm value obtained in our population in comparison with that obtained in other population groups (e.g. Swiss and Singapore Chinese) implied that lower doses of phenytoin were required for our patients to obtain the same serum phenytoin concentration. The implications of the present results are that if coloureds are to achieve the same serum level of total phenytoin as recommended for blacks, they must receive a higher dosage as their Vm is higher. For this reason, the side-effects of phenytoin should theoretically occur less frequently in coloured than in black patients receiving the same doses. However, it is important to emphasize that this difference alone may not be sufficiently relevant clinically to require an altered dosage policy. A combination of all the factors affecting the pharmacokinetics of phenytoin together will be clinically relevant.

The decline in Vm value for patients 65 years of age or older implies a decreased phenytoin metabolizing capacity in this age group. This probably reflects age-dependent changes in the amount or efficiency of drug-metabolizing enzymes in the liver. Because of this, caution should be used when prescribing phenytoin in patients 65 years of age or older.

Although a specific ethnic group (coloured patients) and a positive smoking history correlated with an increase in Vm (6% and 12% respectively), none of these correlations alone may be clinically significant. Similarly, the relationship of Vm to body weight alone indicated that there was a modest influence of weight on Vm. An age of 65 years or more was associated with a decrease in Vm of 3.2%. Therefore, in a coloured, non-smoking

patient 65 years of age or older, the overall effect on Vm may be minimal as the factors increasing or decreasing Vm may counterbalance each other. However, multiple factors, each of which is relatively minor, may together produce a significant contribution to the Vm value predicted by the parameter model (Mungall *et al.*, 1985).

It has been shown that systems utilizing Bayesian feedback techniques perform better than other methods previously reported (Burton *et al.*, 1985; Vozeh and Steimer, 1985). A prerequisite of such methods is good estimates of the population or subpopulation parameter distributions (means and variances). The greater the confidence in these (prior) distributions, the better will be the performance of the associated Bayesian system. This study has provided estimates of Vm, Km and Cl, and their inter- and intra-individual variability for use in a pharmacokinetic programme with Bayesian forecasting. Regardless of which dosing technique is used, some individuals will receive an inappropriate regimen. It is thus always important to monitor serum phenytoin concentrations and clinical status at appropriate intervals after initiation or alteration of a phenytoin regimen (Yuen *et al.*, 1983; 1989).

CHAPTER 6

CONCLUSIONS

The following conclusions were drawn from this investigation with regard to the pharmacokinetics of phenytoin:

(a) Mass, smoking, ethnicity and age (65 years or older) significantly influenced Vm of phenytoin in descending order of importance. Although a significant difference in Km between black and coloured patients was found, the incorporation of ethnicity in Km in the parameter model did not significantly improve the description of the data. The scaling factors for smoking, coloured patients and age 65 years or older in Vm were 1.12, 1.06 and 0.968, respectively when the parallel Michaelis-Menten and first-order elimination model and the FOCE method were used. Our study confirmed that different populations have differences in phenytoin disposition. These can be attributed to genetic differences associated with ethnic origin and/or to social and environmental factors.

(b) For the parallel Michaelis-Menten and first-order elimination model, the mean population parameter estimates (standard error in parentheses) for Vm, Km and Cl were 5.27 (0.59) mg/kg/day, 6.81 (1.30) mg/l and 2.68 (0.78) l/day, respectively. The inter-individual variability for these parameters was 12.5, 67.9 and 46.3%, respectively and the intra-individual variability was 20.1%. The mean population estimates for Vm and Km obtained with the Michaelis-Menten model were 5.71 (0.10) mg/day and 3.45 (0.24) mg/l, respectively. The inter-individual variability of these parameters was 13.2 and 49.1%, respectively, and the intra-individual variability was 5%. A set of mean Vm estimates for subpopulation groups stratified according to ethnicity, smoking status and age have been determined.

(c) The population parameters of Vm and Km obtained in this study were generally lower than those reported in the literature for other population groups.

(d) A parallel Michaelis-Menten and first-order elimination model provided a better fit of the model to the data than one comprising Michaelis-Menten metabolism only.

(e) The parallel Michaelis-Menten and first-order elimination model was not much better than the Michaelis-Menten model when predicting dose. However, when predicting steady-state serum concentration, the former model was superior to the latter.

(f) When representative parameter estimates (taking into account factors shown to influence these parameters) for a population were used, predictive performance improved. As expected, all predictions using the estimates obtained with the FO method were biased. When the estimates obtained with the FOCE method were used, the bias improved without an improvement in the precision of the predictions.

(g) This study has provided a predictive model that is useful for the design and adjustment of phenytoin regimens in clinical practice. However, none of the predictive methods was precise enough to substitute for confirmatory serum phenytoin concentrations.

(h) The findings of this study stressed the importance of accurate, population-based, pharmacokinetic parameter estimates for specific patient populations.

The following still needs to be accomplished:

(a) The estimates of Vm, Km and Cl, and their inter- and intra-individual variability, will be included in a pharmacokinetic computer programme that will be used to optimize phenytoin therapy in patients residing in the Western Cape. The Bayesian method will be incorporated into this programme to improve predictions.

(b) A Bayesian graphic method will be designed to assist clinicians who do not have immediate access to a computer to predict phenytoin doses or serum concentrations.

(c) An assessment of the factors which influence the Cl of phenytoin will be undertaken.

(d) Now that a better understanding of the characteristics of the Michaelis-Menten and the parallel Michaelis-Menten and first-order pharmacokinetic behaviour for phenytoin exists, non-steady-state serum phenytoin concentrations will be utilized as feedback.

(e) The improvement in the predictive performance using two or more feedback serum phenytoin concentrations will be assessed.

(f) Whether the difference in kinetics between the coloured and black patients is a result of genetic or environmental factors will be assessed.

(g) Now that more information is available on the pharmacokinetics of phenytoin in South African patients, a prospective trial assessing the clinical utility of the models with the patients' response as the clinical end-point needs to be performed.

Finally, it is hoped that this study will provide impetus for the establishment of clinical pharmacokinetic consulting services and for further research in the field of population pharmacokinetics. The clinician now has an additional predictive method available, which also takes into account Cl of phenytoin to predict steady-state serum concentrations more accurately. This study has contributed to an improvement in the population parameter estimates and should allow more accurate and precise dosage regimen design on limited concentration feedback.

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DEPARTMENT OF PHARMACOLOGY
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PHARMACOKINETIC GUIDELINES FOR ORAL PHENYTOIN USE

(1) Therapeutic range for total serum phenytoin concentration: 40-80 µmol/l
10-20 mg/l

(2) The therapeutic range should serve as a guideline only. Patients may respond well to treatment below and above the therapeutic range.

(3) To convert from mg/l to µmol/l: x 3.96

(4) When a patient has achieved steady state on an oral dosing regimen, the fluctuation in phenytoin concentration is relatively small. It therefore makes little difference when a sample is obtained. The most reliable value to be used for phenytoin dosage adjustments is the trough concentration obtained at the end of a steady state dosing interval.

(5) A blood sample for phenytoin assay should only be taken when the concentration of phenytoin in blood is at steady state. Due to the non-linear pharmacokinetics of phenytoin, the time taken to reach steady-state varies with the rate of administration and depends on the values of Vm and Km . The time to reach steady state can range from 5 days to 6 weeks and could be determined by calculation. Blood sampling before steady-state is of little value as phenytoin at this time is still accumulating except in cases where toxicity or non-compliance is suspected.

(6) Following a change in phenytoin dose, the time required to reach a new steady-state concentration should be determined.

(7) Blood sampling for phenytoin assay should be delayed if compliance is suboptimal. Do not increase the dose unless you are reasonably sure of the patient's drug compliance. If the dose is increased as a result of non-compliance and the patient becomes compliant thereafter, this may lead to toxicity especially with phenytoin (due to its non-linear pharmacokinetics).

(8) Due to the non-linear pharmacokinetics of phenytoin, a small increase in dose may lead to a disproportionately large increase in serum phenytoin levels. Similarly, a small decrease in dose may lead to a large decrease in serum phenytoin levels. It is therefore difficult to adjust empirically the dose based on serum phenytoin concentrations.

(9) The daily dose of phenytoin should not exceed an individual's Vm (maximum metabolic rate) value. If this occurs, then the patient will rapidly experience toxicity.

(10) It may be advisable to initially target serum phenytoin levels at the lower end of the therapeutic range, i.e. 48 µmol/l (12 mg/l) as there may initially be a tendency to

ANNEXURE 1: continued

overshoot the target concentration.

(11) If a patient shows evidence of seizures at a time corresponding to the trough serum concentration, seizure frequency may be decreased by shortening the dosage interval without necessarily changing the daily dose. Transient symptoms of toxicity associated with excessively high peak concentrations may also be avoided by decreasing the dose and shortening the dosing interval, without necessarily having to alter the daily dose.

(12) The total phenytoin concentration (which assumes normal protein binding) may be misleading in patients with altered protein binding. Total phenytoin concentrations should be carefully interpreted in the presence of the following conditions in which plasma protein binding of phenytoin is decreased: burns, hepatic cirrhosis, nephrotic syndrome, pregnancy, cystic fibrosis, renal failure, severe jaundice and uraemia.

In severe hepatic failure, total phenytoin concentration that is apparently therapeutic or subtherapeutic may actually be associated with phenytoin toxicity. Total phenytoin concentrations in the elderly should also be interpreted with caution.

The therapeutic range is decreased in uraemia or low blood protein to a level of 16-40 µmol/l (4-10 mg/l). No change in the daily dose of phenytoin may be required in uraemic patients despite observations of lower total phenytoin concentrations.

(13) Increased clearance of phenytoin may occur in pregnancy, head trauma and cerebral infection. Because children have higher phenytoin clearances, the daily phenytoin dose may have to be divided into two or three doses.

(14) Sudden withdrawal of phenytoin from epileptics may lead to status epilepticus. Phenytoin should be withdrawn over a few weeks.

(15) Regular monitoring of phenytoin levels is advisable, especially during the first two months after initiation of therapy so that the patient can be optimized on phenytoin as soon as possible. An upward adjustment of phenytoin dose may be required after 5 to 12 months as a result of phenytoin auto-induction.

(16) Different brands of phenytoin are not interchangeable. Because the formulation is such an important determinant of phenytoin bioavailability, patients who are stabilized on one brand of phenytoin should not be switched to another unless necessary. If a change is necessary, phenytoin concentrations should be monitored when a new steady-state is achieved.

(17) Since phenytoin sodium tablets (manufactured by Lennons Pty Ltd) on hospital code are available only in 50 and 100 mg strengths, it may be necessary to prescribe two different forms of phenytoin if small increments in dose is required. Phenytoin acid (Epanutin Infatabs^{R} and suspension) may be prescribed with phenytoin sodium provided that the difference in the salt form factor is taken into account.

ANNEXURE 1: continued

(18) It is preferable not to add a second anticonvulsant unless attempts to optimize the patient on phenytoin have failed.

(19) Common drugs interactions:

(a) **Drugs which may elevate phenytoin levels:**
ethosuximide, clonazepam, chloramphenicol, sulphonamides, isoniazid, disulfiram, chlordiazepoxide, nitrazepam, thioridazine, chlorpromazine, prochlorperazine, lithium, methylphenidate, propoxyphene, cimetidine, ranitidine, omeprazole, chlorpheniramine, tolbutamide, oestrogens, acute alcohol intake, warfarin, amiodarone and allopurinol.

(b) **Drugs which may decrease phenytoin levels:**
carbamazepine, reserpine, antacids, sucralfate, chronic alcohol abuse, nitrofurantoin, rifampicin, theophylline, dexamethasone, folic acid and pyridoxine.

(c) **Drugs which may either increase or decrease phenytoin levels:**
diazepam, phenobarbitone and valproate.

(d) **Phenytoin may decrease the efficacy of the following drugs:**
carbamazepine, clonazepam, primidone, valproic acid, tetracyclines, levodopa, mianserin, oxazepam, paracetamol, pethidine, methadone, oral contraceptives, glucocorticosteroids, quinidine, dysopyramide, verapamil, mexilitine, coumarin anticoagulants, furosemide, theophylline, thyroxine, folic acid, pyridoxine and vitamin D.

(e) **Other interactions**: tricyclic antidepressants.

PRANEET VALODIA M.Pharm.

ANNEXURE 2

DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN

DATA COLLECTION FORM

PATIENT'S SURNAME: ..... INITIALS: .....

FIRST NAME(S): ..... HOSPITAL: .....

ADDRESS: ..... OCCUPATION: .....

.....

.....

POSTAL ADDRESS: .....

FOLDER NUMBER: ..... TEL NO: (H) .....

HOME LANGUAGE: ..... (W) .....

DATE OF FIRST CONSULTATION: .....

ENTRY CRITERIA:

(1) AGE 15 years and older: YES □ NO □

(2) DISEASE: HEPATIC □ RENAL □

(3) EVIDENCE OF ABUSE: ALCOHOL □ DRUG □

(4) MAJOR DEPRESSIVE OR PSYCHIATRIC DISEASE: YES □ NO □

(5) INTAKE OF INTERFERING DRUGS: YES □ NO □

(6) TAKING PHENYTOIN AS THE ONLY ANTICONVULSANT: YES □ NO □

(7) PREGNANCY: YES □ NO □ N/A □

(8) BREASTFEEDING: YES □ NO □ N/A □

(9) ABLE TO UNDERSTAND INSTRUCTIONS: YES □ NO □

ANNEXURE 2: continued

AGE: .....

DATE OF BIRTH: .....

SEX: .....

RACE: .....

MASS (kg): ..... FRAME SIZE: .....

HEIGHT:.....cm SOCIAL CLASS: .....

CONCURRENT MEDICATION, DURATION OF THERAPY AND DOSAGES:

(1).....

(2).....

(3).....

SMOKING STATUS: YES □ NO □ If yes, how many cigarettes per day.....

ALCOHOL INTAKE: YES □ NO □ If yes, give details :.....

.....

DATE OF ENTRY INTO STUDY: .....

SEIZURE TYPE (If information available) .....

CAUSE OF SEIZURES: .....

PREVIOUS HISTORY OF OTHER DISEASES:

.....

.....

HISTORY OF SEIZURES AND FREQUENCY:

.....

.....

ANNEXURE 2: continued

NUMBER OF SEIZURES IN PREVIOUS WEEK:
NUMBER OF SEIZURES IN PREVIOUS MONTH:
NUMBER OF SEIZURES IN PREVIOUS 3 MONTHS:
NUMBER OF SEIZURES IN PREVIOUS YEAR:

DESCRIPTION OF SEIZURES PRIOR TO ENTERING STUDY:

.....

.....

PRESENT CLINICAL STATUS:

.....

.....

PREVIOUS ADMISSIONS TO HOSPITAL:

.....

.....

HISTORY OF ANTICONVULSANT THERAPY: (doses and duration of therapy)

.....

.....

PHENYTOIN HISTORY:

.....

.....

COMPLIANCE CHECKS:

.....

.....

ANNEXURE 2: continued

TABLET COUNTS:

Date dispensed	Quantity	Date checked	Quantity	Result
(1).....				
(2).....				
(3).....				

EVALUATION OF PATIENT'S DIARY:

Date	Accuracy		
	Good	Average	Poor
(1).....			
(2).....			
(3).....			

SERUM PHENYTOIN LEVELS:

Date	Accuracy of prediction		
	Good	Average	Poor
(1).....			
(2).....			
(3).....			

ANNEXURE 2: continued

CURRENT THERAPY:

Date	Dose	Seizure frequency	Time at steady state	Predicted serum conc.	Measured serum conc.
.....					
.....					
.....					
.....					
.....					

DATE	TIME BLOOD DRAWN	TIME ON THERAPY	TIME OF LAST DOSE BEFORE SAMPLING
(1).....			
(2).....			
(3).....			

DATE	DOSE PRESCRIBED BY DOCTOR	MEASURED Cp_{ss}	RECOMMENDED DOSE	PREDICTED Cp_{ss}
.....				
.....				
.....				

ANNEXURE 2: continued

ADVERSE EFFECTS: Type Mild Moderate Severe

Date Dose

.....(1).....

.....(2).....

.....(3).....

LABORATORY INVESTIGATION: NORMAL ☐ ABNORMAL ☐

If abnormal, give details:

.....

.....

REASON FOR REFERRAL FROM DOCTOR:

☐ Routine to establish optimal dose

☐ Insufficient control

☐ Toxicity

☐ Compliance in question

☐ Other (specify)

NAME OF REFERRING DOCTOR: .....

ADDITIONAL INFORMATION:

.....

.....

.....

COMMENTS AND DOSAGE RECOMMENDATIONS:

.....

.....

ANNEXURE 3: Patient Diary

MONTH / MAAND :							MONTH / MAAND :						
DATE DATUM	MORNING OOGEND	AFTERNOON MIDDAG	NIGHT SAANS	FITS AANVALLE	NEXT APPOINTMENT VOLGENDE AFSPRAAK	SIDE EFFECTS / COMMENTS NEWE EFFEKTE / KOMMETAAR	DATE DATUM	MORNING OOGEND	AFTERNOON MIDDAG	NIGHT SAANS	FITS AANVALLE	NEXT APPOINTMENT VOLGENDE AFSPRAAK	SIDE EFFECTS / COMMENTS NEWE EFFEKTE KOMMENTAAR
1							17						
2							18						
3							19						
4							20						
5							21						
6							22						
7							23						
8							24						
9							25						
10							26						
11							27						
12							28						
13							29						
14							30						
15							31						
16													

ANNEXURE 4

DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN

INFORMATION FOR PATIENTS TAKING PHENYTOIN

Remember:

* **For this medicine to work well, it must be taken exactly as instructed. Take the medicine at more or less the same time every day and for as long as prescribed by your doctor.**
* Do not stop taking this medicine without first checking with your doctor, nurse or pharmacist at the hospital.
* Before you begin taking any medicine that could be bought over the counter from a pharmacy, please first check with the investigator.
* If you become sick or pregnant whilst you are taking phenytoin, please tell the doctor, nurse or investigator.
* If you go to a doctor not working in this part of the hospital, please tell him/her that you are taking part in this study and that he/she should check with the investigator before giving you any other medicines.
* Many liquid medicines contain alcohol. Don't take such medicines during this study.
* If you have any questions, write them down and bring these questions with you when you visit the hospital again.

HOW TO USE THIS MEDICINE

If you missed taking phenytoin and you are taking your medicine as follows:
Once a day - Take the missed tablets as soon as you remember and as long as it is at least 12 hours before the next dose. If you remember the missed tablets within 12 hours before the next dose, then skip the dose. Do not use double your tablets.
More than once a day - Take the tablets you forgot to take as soon as you remember. If you remember when you have to take your tablets again, the tablets you had missed could be taken at that time. If you missed taking your tablets for 2 or more days in a row, do not stop taking your tablets, but contact the investigator.

For patients taking the tablets or capsules:
Swallow the tablet or capsule whole.

For patients taking the chewable tablets:
Chew or crush the tablet before swallowing.

For patients taking the liquid:
Shake the bottle well before using.
Use a medicine measuring spoon or plastic syringe to measure the medicine accurately. Do not use an ordinary teaspoon.

ANNEXURE 4: continued

HOW TO STORE THE MEDICINE

Keep your medicine in a cool, dry place. Do not store the medicine in the bathroom, near the kitchen sink or in other damp places. Heat or moisture may cause the medicine to break down and reduce its effect.
Do not keep the liquid form of this medicine in the fridge.
Keep all medicines away from children.

WHAT TO BE CAREFUL OF WHEN USING THIS MEDICINE

Your doctor should check your treatment regularly, especially during the first few months. During this time the amount of phenytoin that you need may have to be changed.

Do not start or stop taking any new medicines without checking with your doctor or pharmacist first. Other medicines may affect the way phenytoin works.

Phenytoin may worsen the drowsiness caused by alcohol and medicines normally used for colds, hayfever, allergies, pain, muscle cramps and sleeping. Check with the investigator before taking any other medicine while you are taking phenytoin.

Do not take phenytoin within 2 to 3 hours after taking antacids such as Eno or other medicines used for heartburn or upset stomach. The same applies for medicines used for running stomach.

Do not change the amount of phenytoin you are taking without first checking with your doctor or the investigator. Also do not change to a tablet made by another company, although the amount you are taking is the same. The effect of tablets made by different companies may be different, although the dose is the same. When you obtain a repeat prescription and the tablets do not look the same, check with the pharmacist.

Never stop taking this medicine suddenly. If necessary, your doctor will probably want you to reduce your dose gradually before stopping completely.

SIDE EFFECTS OF THIS MEDICINE

Occasionally phenytoin may cause some people to become dizzy, drowsy, light-headed and less alert than normal. After you have taken phenytoin for a while, this effect may not be troublesome any more. Make sure that you know how phenytoin will affect you before you drive a motor car, use machines, climb heights or buildings, or do other jobs that could be dangerous if you feel drowsy or dizzy.

Check with your doctor or investigator if any of the following side effects occur:
Behavioural changes; bleeding, tender or enlarged gums; clumsiness or unsteadiness; confusion; uncontrolled back-and-forth and/or rolling eye movements; enlarged glands in the

ANNEXURE 4: continued

neck or armpits; fever; increase in fits; mood or mental changes; muscle weakness; skin rash; slurring of speech; trembling of hands; unusual excitement; nervousness or irritability.

The following will happen if you take too much phenytoin:
Blurred or double vision; clumsiness or unsteadiness; confusion; severe dizziness or drowsiness; nausea; slurred speech; staggering walk and uncontrolled eye movements.

Other side effects may occur that are usually harmless. These side effects may go away during treatment as your body adjusts to the medicine.

NAME OF INVESTIGATOR: PRANEET VALODIA
ADDRESS: DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN
TELEPHONE: 4046036 (Office)
6385932 (Home)

ANNEXURE 5

DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN

INFORMED CONSENT

TITLE OF PROTOCOL: THE DETERMINATION AND VALIDATION OF POPULATION PHARMACOKINETIC PARAMETERS OF PHENYTOIN IN ADULT EPILEPTIC PATIENTS IN THE WESTERN CAPE USING NONLINEAR MIXED-EFFECTS MODELLING

SURNAME OF PATIENT: ..... INITIALS: .....

FIRST NAME(S): .....

FOLDER NUMBER: .....

DATE OF BIRTH: ..... AGE: ..... SEX: .....

ADDRESS: .....

TELEPHONE NO: .....

(1) **PURPOSE OF THIS STUDY**

Phenytoin is a medicine used for many years to treat fits. Best results are obtained when it is prescribed to meet the needs of each patient. Regular monitoring of blood levels of phenytoin is necessary to ensure that it is effective and to lessen the chance of taking too much or too little phenytoin.

Although two people may take the same amount of phenytoin, the amount of phenytoin in their bloodstream may not be the same. This difference is due to a number of factors, for example, age, amount of phenytoin absorbed, weight, genetics, or patients not taking the medicine as prescribed. The reason for this study is to determine the effect of factors, such as age, sex and race, on the parameters that are used to work out the amount of phenytoin needed.

(2) **WHAT IS GOING TO BE DONE**

In this study you will be asked to take your medicine regularly and not to miss a dose, if possible. At least two blood samples will be taken at specific times during this study. You will be taking part in this study for at least two months provided you follow all the instructions.

ANNEXURE 5: continued

(3) **RISKS**

Your medicine should be safe if used properly. It is, however, not entirely without side-effects as described in the information brochure. Your taking part in this study will not cause you to have any added risks that would not have happened if you were not part of this study. The medicine being given to you is the best available for the treatment of your illness. If problems develop at larger amounts of phenytoin that may be required to control your fits, then the amount of phenytoin will be reduced or another way to control your fits will be looked at.

(4) **BENEFITS**

This study will make sure that you will receive the greatest benefit from your medicine. Your taking part will improve the treatment and quality of life for all patients taking phenytoin.

(5) **IF PROBLEMS OCCUR**

If any serious problems occur, which is very unlikely, you will receive quick and proper medical treatment. Money will not be given for medical treatment elsewhere, loss of work or other expenses.

(6) **BASIS OF YOU TAKING PART**

You are free to stop taking part in this study at any time. If you do stop participating in this study, this will in no way affect the way you are being treated for your illness. This study has been approved by the Ethics Committee, Medical School, University of Cape Town.

I agree to take part in this study. I have been given a copy of this consent form.

Signature _____ Date_____
PATIENT

I, the undersigned, have fully explained the relevant details of this study to the patient named above.

Signature _____ Date _____
INVESTIGATOR

ANNEXURE 6

DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN

PROCEDURE FOR NURSES

CLINICAL PHARMACOKINETICS PROJECT

REQUEST FOR TAKING OF BLOOD SAMPLE

DATE: .....

PATIENT'S NAME: .....

ADDRESS: .....

HOSPITAL: .....

FOLDER NUMBER: .....

.....

PRANEET VALODIA

PROCEDURE:

(1) Collect blood for the following tests as indicated below:

- ☐ serum phenytoin concentration. 3 ml clotted blood (red-top tube)
- ☐ SMAC. 5 ml clotted blood. (tiger-top tube)
- ☐ full blood count. 3ml blood with anticoagulant (purple-top tube)

(2) DOSE: .....

(3) TIME BLOOD SAMPLE TAKEN: .....(use a 24-hr clock time e.g. 16h10)

(4) TIME OF LAST DOSE: ..... DATE: .....

(5) Ensure that the patient's initials and surname, as well as the hospital's name or number are on the label of the collection tube.

(6) Store the samples in a cool place.

(7) The blood samples for the SMAC and the full blood count should be processed as usual. Please hand the phenytoin blood sample and this form to the investigator at the end of this clinical session.

_____ (Please print)
NAME OF NURSE

ANNEXURE 7

DEPARTMENT OF PHARMACOLOGY
MEDICAL SCHOOL
UNIVERSITY OF CAPE TOWN

PHENYTOIN ASSAY RESULTS AND RECOMMENDATIONS

PATIENT'S NAME:
FOLDER NUMBER:

DATE	DOSE	CONCENTRATION (µmol/l)
(1).....		
(2).....		
(3).....		

Therapeutic range 10-20 mg/l (40-80 µmol/l)

COMMENTS AND RECOMMENDATIONS OF INVESTIGATOR

.....

.....

.....

.....

.....

.....

.....

....

PRANEET VALODIA DATE

TO BE COMPLETED BY THE DOCTOR: (Please tick)
(1) RECOMMENDATIONS ACCEPTED: □
(2) RECOMMENDATIONS NOT ACCEPTED: □

If recommendations are not accepted, please indicate what you have decided:

.....
.....
.....

ANNEXURE 8: NONMEM DATA SET

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
1	16	1	0	62.5	168.5	1.71	0	0	1	200	9.3	15.0
1	16	1	0	62.5	168.5	1.71	0	0	4	250	21.2	15.0
2	22	1	0	69.2	170.5	1.80	0	0	1	400	16.4	15.8
2	22	1	0	69.2	170.5	1.80	0	0	4	400	16.7	13.5
2	22	1	0	69.2	170.5	1.80	0	0	4	427	18.9	14.0
2	22	1	0	69.2	170.5	1.80	0	0	4	427	16.7	14.0
3	52	0	0	78.2	158.0	1.80	0	0	1	300	39.1	14.3
3	52	0	0	78.2	158.0	1.80	0	0	4	250	29.8	13.5
4	40	1	0	58.0	172.0	1.68	0	0	1	300	41.4	14.0
4	40	1	0	58.0	172.0	1.68	0	0	4	300	41.4	15.5
4	40	1	0	58.0	172.0	1.68	0	0	4	250	29.0	14.8
5	32	1	0	58.6	160.0	1.61	0	1	1	350	21.0	17.3
6	38	0	0	50.5	156.5	1.48	0	0	1	300	8.6	15.0
6	38	0	0	50.5	156.5	1.48	0	0	4	300	11.9	12.5
7	44	0	0	93.5	166.0	2.01	0	1	1	300	22.0	14.0
7	44	0	0	93.5	166.0	2.01	0	1	4	250	13.1	13.5
7	44	0	0	93.5	166.0	2.01	0	1	4	250	10.6	14.8
7	44	0	0	93.5	166.0	2.01	0	1	4	277	18.2	15.5
8	33	0	0	69.1	153.0	1.67	0	0	1	300	9.6	13.3
8	33	0	0	69.1	153.0	1.67	0	0	4	350	14.9	12.8
8	33	0	0	69.1	153.0	1.67	0	0	4	350	17.2	16.3
9	33	1	0	71.4	168.0	1.81	2	0	1	200	7.1	14.3
9	33	1	0	71.4	168.0	1.81	2	0	4	200	7.8	13.5
10	53	1	0	98.5	184.0	2.22	0	0	1	300	16.2	12.5
10	53	1	0	98.5	184.0	2.22	0	0	4	300	14.1	12.5
11	34	1	0	85.8	180.0	2.06	2	1	1	300	9.1	14.8
11	34	1	0	85.8	180.0	2.06	2	1	4	300	9.3	14.3
12	17	0	0	54.8	161.0	1.57	0	0	1	300	32.8	13.8
12	17	0	0	54.8	161.0	1.57	0	0	4	300	41.4	13.0
13	54	1	0	94.0	161.0	1.97	0	0	1	300	4.6	15.0
13	54	1	0	94.0	161.0	1.97	0	0	4	300	5.3	16.0
14	41	1	0	66.0	171.0	1.77	2	0	1	300	7.1	16.8
14	41	1	0	66.0	171.0	1.77	2	0	4	300	6.3	16.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
14	41	1	0	66.0	171.0	1.77	2	0	4	350	21.2	15.5
14	41	1	0	66.0	171.0	1.77	2	0	4	350	22.7	2.5
14	41	1	0	66.0	171.0	1.77	2	0	4	350	21.7	15.0
15	34	0	0	88.2	162.0	1.93	0	0	1	300	18.4	13.3
16	47	1	0	66.7	179.0	1.84	1	1	1	300	12.1	17.3
16	47	1	0	66.7	179.0	1.84	1	1	4	300	10.5	13.5
17	29	1	0	99.5	183.0	2.22	2	0	1	300	7.8	15.0
17	29	1	0	99.5	183.0	2.22	2	0	4	300	8.6	13.8
18	17	1	0	51.0	161.0	1.52	0	0	1	300	29.3	3.5
18	17	1	0	51.0	161.0	1.52	0	0	4	200	6.8	15.0
18	17	1	0	51.0	161.0	1.52	0	0	4	200	7.8	15.0
19	21	1	0	51.0	153.0	1.47	0	0	1	300	26.8	15.3
19	21	1	0	51.0	153.0	1.47	0	0	4	300	23.5	14.3
20	49	1	0	85.6	174.0	2.00	2	1	1	450	20.2	13.5
20	49	1	0	85.6	174.0	2.00	2	1	4	450	26.0	14.0
20	49	1	0	85.6	174.0	2.00	2	1	4	450	28.8	13.3
21	19	1	0	63.5	178.0	1.80	0	0	1	300	30.6	15.5
21	19	1	0	63.5	178.0	1.80	0	0	4	277	26.5	14.5
22	29	1	0	58.4	178.0	1.73	1	1	1	300	17.2	17.3
22	29	1	0	58.4	178.0	1.73	1	1	4	300	14.1	15.0
23	32	0	0	57.3	159.5	1.59	0	0	1	200	27.3	16.0
24	40	1	0	63.2	168.0	1.72	0	0	1	300	17.4	17.3
25	18	0	0	53.4	161.0	1.55	0	0	1	300	9.9	16.0
25	18	0	0	53.4	161.0	1.55	0	0	4	300	8.1	16.8
25	18	0	0	53.4	161.0	1.55	0	0	4	300	6.1	15.3
26	36	0	0	67.0	156.0	1.67	0	0	1	300	12.4	14.8
26	36	0	0	67.0	156.0	1.67	0	0	4	300	10.1	17.8
27	25	0	0	94.0	166.0	2.02	0	0	1	300	10.4	13.5
27	25	0	0	94.0	166.0	2.02	0	0	4	300	12.6	16.5
28	24	0	0	66.5	163.0	1.72	0	0	1	300	15.7	15.3
29	25	0	0	58.6	160.0	1.61	0	0	1	300	18.9	15.5
29	25	0	0	58.6	160.0	1.61	0	0	4	300	21.2	14.5
29	25	0	0	58.6	160.0	1.61	0	0	4	300	15.4	15.8
30	25	1	0	62.0	166.0	1.69	1	0	1	400	7.6	14.0
30	25	1	0	62.0	166.0	1.69	1	0	4	400	5.3	14.5
30	25	1	0	62.0	166.0	1.69	1	0	4	450	19.7	13.8

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
30	25	1	0	62.0	166.0	1.69	1	0	4	450	21.7	14.0
31	37	1	0	77.6	171.0	1.90	0	0	1	300	7.6	14.0
31	37	1	0	77.6	171.0	1.90	0	0	4	300	8.8	13.0
31	37	1	0	77.6	171.0	1.90	0	0	4	350	12.6	14.8
31	37	1	0	77.6	171.0	1.90	0	0	4	350	13.9	15.0
32	25	1	0	44.0	166.0	1.46	0	0	1	300	27.3	16.0
32	25	1	0	44.0	166.0	1.46	0	0	4	250	20.5	15.0
32	25	1	0	44.0	166.0	1.46	0	0	4	250	23.5	15.5
33	20	1	0	77.1	182.0	1.98	0	0	1	300	9.1	14.3
33	20	1	0	77.1	182.0	1.98	0	0	4	350	12.1	13.5
33	20	1	0	77.1	182.0	1.98	0	0	4	350	14.7	14.0
33	20	1	0	77.1	182.0	1.98	0	0	4	400	24.2	15.5
33	20	1	0	77.1	182.0	1.98	0	0	4	400	25.5	14.8
33	20	1	0	77.1	182.0	1.98	0	0	4	414	46.5	15.8
33	20	1	0	77.1	182.0	1.98	0	0	4	414	45.7	13.0
34	31	1	0	89.0	182.0	2.11	2	0	1	300	21.0	16.8
34	31	1	0	89.0	182.0	2.11	2	0	4	300	19.2	14.0
35	22	1	0	60.0	178.0	1.75	1	1	1	300	25.3	14.0
35	22	1	0	60.0	178.0	1.75	1	1	4	300	28.8	16.0
35	22	1	0	60.0	178.0	1.75	1	1	4	277	23.2	15.3
35	22	1	0	60.0	178.0	1.75	1	1	4	250	16.2	16.0
35	22	1	0	60.0	178.0	1.75	1	1	4	250	17.9	14.8
36	31	1	0	57.4	164.0	1.62	2	1	1	300	10.9	12.5
37	43	1	0	69.6	167.0	1.78	2	0	1	300	4.0	13.0
37	43	1	0	69.6	167.0	1.78	2	0	4	300	7.3	14.0
38	31	0	0	64.4	162.5	1.69	0	0	1	300	13.1	14.0
38	31	0	0	64.4	162.5	1.69	0	0	4	300	11.9	17.8
38	31	0	0	64.4	162.5	1.69	0	0	4	327	14.7	12.8
38	31	0	0	64.4	162.5	1.69	0	0	4	350	21.8	15.0
38	31	0	0	64.4	162.5	1.69	0	0	4	350	14.1	15.5
38	31	0	0	64.4	162.5	1.69	0	0	4	377	27.8	17.3
39	25	0	0	73.8	167.0	1.83	0	0	1	300	15.7	14.0
39	25	0	0	73.8	167.0	1.83	0	0	4	300	14.1	15.8
39	25	0	0	73.8	167.0	1.83	0	0	4	327	19.2	14.3
39	25	0	0	73.8	167.0	1.83	0	0	4	327	19.2	15.5
39	25	0	0	73.8	167.0	1.83	0	0	4	350	20.5	14.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
40	48	1	0	66.5	171.5	1.78	0	1	1	300	17.7	13.0
41	40	1	0	52.0	162.0	1.54	1	1	1	300	8.8	17.3
41	40	1	0	52.0	162.0	1.54	1	1	4	300	6.3	14.8
41	40	1	0	52.0	162.0	1.54	1	1	4	350	16.2	13.5
41	40	1	0	52.0	162.0	1.54	1	1	4	350	13.9	13.3
41	40	1	0	52.0	162.0	1.54	1	1	4	350	16.7	13.8
42	82	1	0	59.0	162.0	1.63	1	0	1	200	18.7	15.5
42	82	1	0	59.0	162.0	1.63	1	0	4	200	19.7	15.0
42	82	1	0	59.0	162.0	1.63	1	0	4	200	26.3	18.8
43	40	1	0	57.3	162.0	1.61	1	1	1	300	10.1	18.0
43	40	1	0	57.3	162.0	1.61	1	1	4	300	9.1	14.3
43	40	1	0	57.3	162.0	1.61	1	1	4	350	15.9	13.5
43	40	1	0	57.3	162.0	1.61	1	1	4	350	15.9	15.0
44	23	1	0	64.5	155.0	1.63	1	1	1	200	7.8	17.5
45	22	0	0	44.7	147.0	1.35	0	0	1	200	7.3	12.5
45	22	0	0	44.7	147.0	1.35	0	0	4	200	7.3	13.3
45	22	0	0	44.7	147.0	1.35	0	0	4	300	30.1	18.8
45	22	0	0	44.7	147.0	1.35	0	0	4	250	13.9	16.5
46	31	1	0	52.0	167.5	1.58	1	1	1	300	10.6	15.8
46	31	1	0	52.0	167.5	1.58	1	1	4	300	15.4	15.0
47	51	0	0	81.1	153.0	1.78	0	0	1	350	15.9	13.8
47	51	0	0	81.1	153.0	1.78	0	0	4	350	17.2	15.0
47	51	0	0	81.1	153.0	1.78	0	0	4	377	15.9	14.0
47	51	0	0	81.1	153.0	1.78	0	0	4	377	20.0	13.0
48	36	1	0	51.3	164.5	1.55	0	0	1	300	12.1	15.5
48	36	1	0	51.3	164.5	1.55	0	0	4	300	13.9	15.5
48	36	1	0	51.3	164.5	1.55	0	0	4	327	13.1	15.3
48	36	1	0	51.3	164.5	1.55	0	0	4	350	13.9	15.5
48	36	1	0	51.3	164.5	1.55	0	0	4	400	17.2	16.3
49	17	1	0	50.3	150.0	1.44	0	0	1	300	22.7	15.0
50	27	0	0	89.0	161.0	1.93	1	0	1	327	4.2	14.0
50	27	0	0	89.0	161.0	1.93	1	0	4	350	7.6	14.5
50	27	0	0	89.0	161.0	1.93	1	0	4	350	6.6	15.0
51	17	1	0	56.3	168.5	1.64	0	0	1	200	34.9	14.3
51	17	1	0	56.3	168.5	1.64	0	0	4	200	45.5	14.3
51	17	1	0	56.3	168.5	1.64	0	0	4	100	4.3	14.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
51	17	1	0	56.3	168.5	1.64	0	0	4	150	21.5	13.5
51	17	1	0	56.3	168.5	1.64	0	0	4	150	22.5	12.8
52	40	1	0	60.7	168.5	1.69	2	1	1	300	6.8	16.5
52	40	1	0	60.7	168.5	1.69	2	1	4	300	4.3	16.0
52	40	1	0	60.7	168.5	1.69	2	1	4	400	14.4	15.5
52	40	1	0	60.7	168.5	1.69	2	1	4	400	10.9	16.8
52	40	1	0	60.7	168.5	1.69	2	1	4	450	16.2	13.5
52	40	1	0	60.7	168.5	1.69	2	1	4	450	16.7	14.0
53	30	0	0	48.0	160.0	1.48	0	0	4	300	37.1	14.5
53	30	0	0	48.0	160.0	1.48	0	0	4	200	14.1	15.0
53	30	0	0	48.0	160.0	1.48	0	0	4	227	14.7	14.0
53	30	0	0	48.0	160.0	1.48	0	0	4	227	9.1	14.5
54	44	1	0	54.5	158.0	1.54	0	0	1	300	13.6	14.5
54	44	1	0	54.5	158.0	1.54	0	0	4	300	20.5	14.5
54	44	1	0	54.5	158.0	1.54	0	0	4	300	17.4	15.0
55	43	0	0	80.4	160.0	1.84	0	0	1	300	9.3	16.0
55	43	0	0	80.4	160.0	1.84	0	0	4	400	14.7	15.5
55	43	0	0	80.4	160.0	1.84	0	0	4	400	18.2	15.3
56	41	1	0	55.6	173.0	1.66	2	1	1	300	5.6	17.0
56	41	1	0	55.6	173.0	1.66	2	1	4	300	5.6	14.5
56	41	1	0	55.6	173.0	1.66	2	1	4	400	15.7	15.8
56	41	1	0	55.6	173.0	1.66	2	1	4	400	10.6	17.0
56	41	1	0	55.6	173.0	1.66	2	1	4	400	13.6	14.5
57	31	1	0	53.0	168.5	1.60	1	1	1	300	14.7	17.8
57	31	1	0	53.0	168.5	1.60	1	1	4	300	16.4	16.8
58	16	1	0	60.0	183.0	1.79	0	0	1	200	3.5	14.5
58	16	1	0	60.0	183.0	1.79	0	0	4	300	14.7	16.5
58	16	1	0	60.0	183.0	1.79	0	0	4	300	15.2	18.0
59	55	1	0	74.9	169.0	1.85	1	0	1	300	5.1	14.0
59	55	1	0	74.9	169.0	1.85	1	0	4	300	6.1	14.0
59	55	1	0	74.9	169.0	1.85	1	0	4	400	16.2	14.3
59	55	1	0	74.9	169.0	1.85	1	0	4	400	21.5	14.0
59	55	1	0	74.9	169.0	1.85	1	0	4	400	11.6	13.8
60	47	1	0	55.3	166.5	1.61	1	0	1	300	7.3	15.5
61	27	1	0	73.6	178.0	1.91	0	0	1	300	7.1	14.0
61	27	1	0	73.6	178.0	1.91	0	0	4	300	9.1	16.3

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
62	29	0	0	68.8	158.0	1.70	0	0	1	250	11.9	13.5
62	29	0	0	68.8	158.0	1.70	0	0	4	250	11.1	13.5
62	29	0	0	68.8	158.0	1.70	0	0	4	277	13.4	14.5
63	23	0	0	86.0	159.0	1.88	0	0	1	300	8.3	13.0
63	23	0	0	86.0	159.0	1.88	0	0	4	300	7.6	14.5
64	36	1	0	69.0	164.5	1.76	1	0	1	300	10.6	19.5
65	32	1	0	63.3	164.5	1.69	0	0	1	300	14.4	15.5
65	32	1	0	63.3	164.5	1.69	0	0	4	350	27.0	12.3
65	32	1	0	63.3	164.5	1.69	0	0	4	350	26.0	14.0
65	32	1	0	63.3	164.5	1.69	0	0	4	364	31.6	12.8
65	32	1	0	63.3	164.5	1.69	0	0	4	364	35.6	16.5
66	20	0	0	77.3	178.0	1.95	0	0	1	200	6.3	14.8
66	20	0	0	77.3	178.0	1.95	0	0	4	200	6.1	15.5
66	20	0	0	77.3	178.0	1.95	0	0	4	300	21.7	14.8
66	20	0	0	77.3	178.0	1.95	0	0	4	300	19.2	13.5
67	38	0	0	67.9	152.0	1.65	0	0	1	300	12.4	14.3
67	38	0	0	67.9	152.0	1.65	0	0	4	300	12.9	14.8
67	38	0	0	67.9	152.0	1.65	0	0	4	327	19.7	15.0
67	38	0	0	67.9	152.0	1.65	0	0	4	327	17.2	15.0
68	34	1	0	57.6	166.5	1.64	0	0	1	300	21.2	14.5
69	40	1	0	135	190.0	2.59	0	0	1	400	8.8	14.3
69	40	1	0	135	190.0	2.59	0	0	1	400	8.6	15.0
69	40	1	0	135	190.0	2.59	0	0	4	500	17.4	13.3
69	40	1	0	135	190.0	2.59	0	0	4	500	17.7	14.0
70	24	1	0	67.5	173.0	1.80	0	0	1	200	6.6	16.8
71	20	1	0	56.5	172.0	1.67	0	0	1	300	10.4	16.0
71	20	1	0	56.5	172.0	1.67	0	0	4	300	12.6	16.8
71	20	1	0	56.5	172.0	1.67	0	0	4	300	12.6	17.0
71	20	1	0	56.5	172.0	1.67	0	0	4	250	6.1	12.8
71	20	1	0	56.5	172.0	1.67	0	0	4	250	7.1	16.5
72	24	1	0	63.1	166.5	1.71	0	1	1	300	13.4	13.8
73	43	1	0	60.8	165.0	1.67	2	0	1	300	13.4	13.8
73	43	1	0	60.8	165.0	1.67	2	0	4	300	13.6	15.0
74	40	1	0	58.5	172.0	1.69	2	1	1	300	8.6	14.0
75	30	1	0	54.3	184.0	1.72	1	0	1	300	20.7	14.5
76	53	1	0	58.7	166.0	1.65	0	0	1	300	6.3	14.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
76	53	1	0	58.7	166.0	1.65	0	0	4	300	8.1	15.0
77	22	0	0	66.7	153.0	1.64	0	0	1	300	6.1	13.0
77	22	0	0	66.7	153.0	1.64	0	0	4	350	10.4	15.5
77	22	0	0	66.7	153.0	1.64	0	0	4	350	12.1	15.5
78	41	0	0	47.0	161.5	1.47	1	0	1	200	4.8	17.5
78	41	0	0	47.0	161.5	1.47	1	0	4	300	13.1	17.0
78	41	0	0	47.0	161.5	1.47	1	0	4	300	12.1	17.0
79	41	1	0	55.2	168.0	1.62	2	1	1	300	18.4	18.8
80	20	0	0	72.0	150.0	1.67	0	0	1	300	9.1	12.5
80	20	0	0	72.0	150.0	1.67	0	0	4	350	16.7	13.5
80	20	0	0	72.0	150.0	1.67	0	0	4	350	21.7	13.5
81	40	1	0	88.0	186.0	2.13	3	0	1	300	6.6	13.3
81	40	1	0	88.0	186.0	2.13	3	0	4	300	6.1	14.0
82	22	0	0	58.0	162.0	1.61	0	0	1	300	17.7	14.3
82	22	0	0	58.0	162.0	1.61	0	0	4	300	28.8	16.5
82	22	0	0	58.0	162.0	1.61	0	0	4	300	23.7	16.5
82	22	0	0	58.0	162.0	1.61	0	0	4	314	25.0	15.0
82	22	0	0	58.0	162.0	1.61	0	0	4	327	25.3	17.5
82	22	0	0	58.0	162.0	1.61	0	0	4	327	34.3	15.0
83	70	1	0	55.9	167.0	1.62	1	0	1	300	6.6	19.0
84	26	1	0	64.5	188.0	1.88	2	0	1	300	18.9	16.5
84	26	1	0	64.5	188.0	1.88	2	0	4	300	24.2	17.8
85	26	1	0	61.9	162.5	1.66	2	1	1	300	14.9	15.0
85	26	1	0	61.9	162.5	1.66	2	1	4	300	13.4	15.3
85	26	1	0	61.9	162.5	1.66	2	1	4	327	16.4	14.5
86	19	1	0	66.6	171.0	1.78	0	0	1	300	14.4	14.5
86	19	1	0	66.6	171.0	1.78	0	0	4	300	15.9	13.8
86	19	1	0	66.6	171.0	1.78	0	0	4	327	23.0	15.3
86	19	1	0	66.6	171.0	1.78	0	0	4	327	23.7	15.3
87	20	1	0	62.0	172.5	1.74	1	0	1	200	8.8	12.5
87	20	1	0	62.0	172.5	1.74	1	0	4	200	10.9	14.3
88	29	1	0	62.0	172.5	1.74	0	0	1	300	24.5	17.0
89	45	1	0	72.9	165.5	1.81	1	1	1	300	19.2	13.0
89	45	1	0	72.9	165.5	1.81	1	1	4	300	13.6	14.3
90	45	1	0	62.7	178.5	1.79	1	1	1	300	20.5	15.5
90	45	1	0	62.7	178.5	1.79	1	1	4	300	24.5	15.3

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
91	34	1	0	78.9	178.0	1.97	1	1	1	300	19.2	13.5
91	34	1	0	78.9	178.0	1.97	1	1	4	300	17.9	16.5
91	34	1	0	78.9	178.0	1.97	1	1	4	327	10.9	16.3
91	34	1	0	78.9	178.0	1.97	1	1	4	327	16.4	12.3
92	54	0	0	48.2	157.0	1.46	0	0	1	300	29.0	16.5
93	28	1	0	83.9	177.0	2.01	0	0	4	350	27.5	14.3
93	28	1	0	83.9	177.0	2.01	0	0	4	350	24.0	14.5
93	28	1	0	83.9	177.0	2.01	0	0	4	350	23.5	17.5
93	28	1	0	83.9	177.0	2.01	0	0	4	327	20.7	16.5
93	28	1	0	83.9	177.0	2.01	0	0	4	327	12.6	14.8
94	28	1	0	66.3	180.0	1.84	2	1	1	300	10.4	15.0
94	28	1	0	66.3	180.0	1.84	2	1	4	300	8.6	16.3
94	28	1	0	66.3	180.0	1.84	2	1	4	327	26.5	13.5
94	28	1	0	66.3	180.0	1.84	2	1	4	327	8.6	15.3
95	58	1	0	76.4	188.0	2.02	0	0	1	300	9.1	16.5
95	58	1	0	76.4	188.0	2.02	0	0	4	300	11.4	14.8
95	58	1	0	76.4	188.0	2.02	0	0	4	327	10.1	15.0
95	58	1	0	76.4	188.0	2.02	0	0	4	327	10.4	14.8
95	58	1	0	76.4	188.0	2.02	0	0	4	350	14.4	15.5
96	56	1	0	58.1	172.5	1.69	2	1	1	300	13.4	17.0
96	56	1	0	58.1	172.5	1.69	2	1	4	300	13.6	16.8
97	40	0	0	51.0	150.0	1.44	0	0	1	200	6.3	14.0
97	40	0	0	51.0	150.0	1.44	0	0	4	250	11.9	14.5
97	40	0	0	51.0	150.0	1.44	0	0	4	250	13.1	14.3
98	31	1	0	65.3	170.0	1.76	1	1	1	300	15.4	13.8
98	31	1	0	65.3	170.0	1.76	1	1	4	300	17.9	13.0
99	43	1	0	76.8	178.0	1.95	2	1	1	300	11.4	14.0
99	43	1	0	76.8	178.0	1.95	2	1	4	300	14.1	14.5
100	31	1	0	66.6	180.0	1.85	0	0	1	300	20.0	15.0
100	31	1	0	66.6	180.0	1.85	0	0	4	300	18.4	15.5
101	37	1	0	58.7	185.0	1.79	1	1	1	300	3.7	26.0
101	37	1	0	58.7	185.0	1.79	1	1	4	300	3.3	15.0
102	21	0	0	59.0	165.0	1.65	0	0	1	350	36.4	19.0
102	21	0	0	59.0	165.0	1.65	0	0	4	350	36.9	15.0
102	21	0	0	59.0	165.0	1.65	0	0	4	327	25.0	15.8
102	21	0	0	59.0	165.0	1.65	0	0	4	277	21.7	15.3

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
103	33	1	0	81.6	177.0	1.99	2	1	1	300	16.2	14.8
104	21	1	0	56.0	160.0	1.58	1	1	1	300	7.1	14.5
104	21	1	0	56.0	160.0	1.58	1	1	4	300	3.8	14.5
104	21	1	0	56.0	160.0	1.58	1	1	4	350	6.6	14.8
104	21	1	0	56.0	160.0	1.58	1	1	4	350	9.9	13.5
105	59	1	0	72.8	168.0	1.82	0	0	1	300	6.8	16.0
106	40	1	0	72.0	176.0	1.88	2	0	1	300	12.6	16.0
106	40	1	0	72.0	176.0	1.88	2	0	4	300	15.7	16.5
106	40	1	0	72.0	176.0	1.88	2	0	0	327	21.7	15.0
106	40	1	0	72.0	176.0	1.88	2	0	4	327	20.5	14.0
106	40	1	0	72.0	176.0	1.88	2	0	4	341	25.5	14.5
107	26	1	0	63.1	163.0	1.68	0	0	1	300	8.3	17.0
107	26	1	0	63.1	163.0	1.68	0	0	4	400	44.4	15.0
107	26	1	0	63.1	163.0	1.68	0	0	4	400	36.1	15.8
108	56	0	0	63.5	168.0	1.72	0	1	1	300	3.5	14.0
108	56	0	0	63.5	168.0	1.72	0	1	4	300	5.8	14.5
109	23	0	0	66.5	171.0	1.78	0	0	1	300	9.6	14.5
109	23	0	0	66.5	171.0	1.78	0	0	4	300	11.4	10.5
109	23	0	0	66.5	171.0	1.78	0	0	4	300	11.1	14.0
109	23	0	0	66.5	171.0	1.78	0	0	4	327	10.9	10.8
109	23	0	0	66.5	171.0	1.78	0	0	4	327	10.4	14.5
109	23	0	0	66.5	171.0	1.78	0	0	4	350	12.9	11.5
110	26	0	0	89.3	162.0	1.94	0	0	1	300	21.2	14.5
110	26	0	0	89.3	162.0	1.94	0	0	4	300	22.0	16.3
111	21	0	0	88.3	158.0	1.89	0	0	1	300	17.2	15.8
111	21	0	0	88.3	158.0	1.89	0	0	4	300	13.9	16.5
112	42	0	0	64.5	158.5	1.66	0	1	1	300	7.8	5.0
113	23	0	0	67.0	162.0	1.72	0	0	1	300	9.6	12.5
114	27	0	0	60.3	150.0	1.55	0	0	1	300	10.4	16.0
115	40	1	0	64.0	176.0	1.79	1	0	1	300	10.6	14.0
115	40	1	0	64.0	176.0	1.79	1	0	4	300	11.6	14.0
116	59	1	0	65.5	169.0	1.75	0	0	1	300	27.8	14.8
116	59	1	0	65.5	169.0	1.75	0	0	4	300	26.3	13.0
116	59	1	0	65.5	169.0	1.75	0	0	4	250	15.4	13.5
116	59	1	0	65.5	169.0	1.75	0	0	4	250	18.2	14.5
117	68	1	0	83.7	168.5	1.94	0	0	1	300	7.8	15.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
118	49	1	0	72.9	174.0	1.87	2	0	1	300	4.0	16.5
119	38	0	0	56.0	161.5	1.59	0	0	1	300	12.4	15.0
119	38	0	0	56.0	161.5	1.59	0	0	4	300	13.6	15.0
120	35	1	0	60.8	182.5	1.79	2	1	1	300	13.6	14.8
120	35	1	0	60.8	182.5	1.79	2	1	4	300	15.9	13.0
120	35	1	0	60.8	182.5	1.79	2	1	4	327	11.9	13.3
120	35	1	0	60.8	182.5	1.79	2	1	4	327	11.6	13.0
120	35	1	0	60.8	182.5	1.79	2	1	4	377	31.8	13.3
121	35	0	0	75.1	161.0	1.79	0	0	1	300	5.8	16.3
121	35	0	0	75.1	161.0	1.79	0	0	4	300	6.1	14.0
121	35	0	0	75.1	161.0	1.79	0	0	4	350	13.4	14.0
122	29	1	0	71.0	171.0	1.83	0	0	1	300	7.6	15.0
122	29	1	0	71.0	171.0	1.83	0	0	4	300	10.1	16.0
122	29	1	0	71.0	171.0	1.83	0	0	4	300	8.1	14.8
123	23	1	0	57.8	186.0	1.78	1	0	1	350	10.9	13.5
123	23	1	0	57.8	186.0	1.78	1	0	4	350	11.6	14.3
123	23	1	0	57.8	186.0	1.78	1	0	4	400	14.1	13.5
123	23	1	0	57.8	186.0	1.78	1	0	4	400	15.9	16.3
123	23	1	0	57.8	186.0	1.78	1	0	4	427	19.2	14.5
124	17	1	0	54.2	176.0	1.66	1	0	1	300	21.5	16.8
125	32	1	0	65.0	163.0	1.70	1	1	1	300	12.9	14.8
125	32	1	0	65.0	163.0	1.70	1	1	4	300	12.6	15.3
126	15	0	0	51.5	156.0	1.49	0	0	1	300	25.5	15.0
126	15	0	0	51.5	156.0	1.49	0	0	4	277	33.8	15.5
127	19	1	0	58.9	164.5	1.64	0	0	1	300	35.6	16.8
127	19	1	0	58.9	164.5	1.64	0	0	4	300	37.1	11.8
127	19	1	0	58.9	164.5	1.64	0	0	4	200	14.7	16.5
127	19	1	0	58.9	164.5	1.64	0	0	4	200	15.9	15.8
128	28	0	0	76.4	159.0	1.79	0	0	1	300	11.9	16.3
128	28	0	0	76.4	159.0	1.79	0	0	4	300	13.6	15.3
129	22	0	0	56.5	160.5	1.58	0	0	1	200	3.0	15.8
129	22	0	0	56.5	160.5	1.58	0	0	4	200	4.3	14.0
130	16	1	0	61.7	160.0	1.64	0	0	1	300	20.2	13.5
130	16	1	0	61.7	160.0	1.64	0	0	4	300	19.4	16.8
131	21	1	0	62.7	167.0	1.70	0	0	1	300	8.8	15.3
132	27	0	0	80.2	164.0	1.87	0	0	1	300	18.7	18.8

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
132	27	0	0	80.2	164.0	1.87	0	0	4	300	14.4	15.5
133	39	1	0	54.0	166.0	1.59	1	1	1	250	33.6	14.0
133	39	1	0	54.0	166.0	1.59	1	1	4	200	16.7	14.0
133	39	1	0	54.0	166.0	1.59	1	1	4	200	14.1	14.0
134	25	0	0	64.5	162.5	1.69	0	0	1	300	25.3	15.5
134	25	0	0	64.5	162.5	1.69	0	0	4	250	12.9	12.8
135	28	1	0	52.4	169.5	1.60	0	0	1	300	26.5	14.0
135	28	1	0	52.4	169.5	1.60	0	0	4	300	25.0	14.0
136	25	1	0	54.0	166.0	1.59	0	0	1	400	45.5	15.0
136	25	1	0	54.0	166.0	1.59	0	0	4	400	51.8	14.5
136	25	1	0	54.0	166.0	1.59	0	0	4	300	13.9	14.8
136	25	1	0	54.0	166.0	1.59	0	0	4	300	24.8	14.0
136	25	1	0	54.0	166.0	1.59	0	0	4	300	18.7	16.5
136	25	1	0	54.0	166.0	1.59	0	0	4	200	6.3	14.8
137	30	1	0	58.2	168.0	1.66	0	0	1	300	19.4	4.5
137	30	1	0	58.2	168.0	1.66	0	0	4	300	16.4	14.0
138	38	0	0	64.7	164.0	1.71	0	0	1	300	31.6	16.0
138	38	0	0	64.7	164.0	1.71	0	0	4	250	24.0	16.0
138	38	0	0	64.7	164.0	1.71	0	0	4	250	18.9	16.8
139	25	0	0	73.2	159.5	1.76	0	0	1	300	30.1	17.8
139	25	0	0	73.2	159.5	1.76	0	0	4	200	9.1	16.0
139	25	0	0	73.2	159.5	1.76	0	0	4	200	5.1	16.5
139	25	0	0	73.2	159.5	1.76	0	0	4	200	6.6	13.5
139	25	0	0	73.2	159.5	1.76	0	0	4	250	15.7	17.5
140	31	1	0	86.0	172.0	1.99	1	1	1	300	11.4	13.4
141	45	1	0	65.5	170.0	1.76	1	0	1	300	21.2	16.0
141	45	1	0	65.5	170.0	1.76	1	0	4	300	27.0	15.5
142	31	1	0	57.4	151.0	1.53	1	1	1	300	6.8	14.3
142	31	1	0	57.4	151.0	1.53	1	1	4	300	5.3	15.0
142	31	1	0	57.4	151.0	1.53	1	1	4	350	24.0	15.0
143	34	0	0	84.0	150.0	1.79	0	0	1	300	13.4	16.8
143	34	0	0	84.0	150.0	1.79	0	0	4	300	10.9	15.8
143	34	0	0	84.0	150.0	1.79	0	0	4	350	18.9	16.8
143	34	0	0	84.0	150.0	1.79	0	0	4	350	18.2	13.5
143	34	0	0	84.0	150.0	1.79	0	0	4	350	16.2	14.0
143	34	0	0	84.0	150.0	1.79	0	0	4	327	13.2	14.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
144	25	1	0	54.9	166.0	1.60	0	0	1	300	19.7	14.8
144	25	1	0	54.9	166.0	1.60	0	0	4	300	16.7	15.5
145	57	0	0	74.0	158.5	1.76	0	0	1	300	11.1	12.8
145	57	0	0	74.0	158.5	1.76	0	0	4	300	13.1	12.0
146	26	1	0	53.4	178.5	1.67	1	1	1	300	38.6	15.5
147	42	1	0	61.0	165.5	1.67	1	0	1	300	10.4	13.5
147	42	1	0	61.0	165.5	1.67	1	0	4	300	8.3	12.0
147	42	1	0	61.0	165.5	1.67	1	0	4	250	7.6	11.8
147	42	1	0	61.0	165.5	1.67	1	0	4	250	6.8	13.5
148	35	1	0	70.4	169.0	1.81	2	0	1	300	20.0	14.3
148	35	1	0	70.4	169.0	1.81	2	0	4	300	17.4	13.0
149	23	1	0	60.0	166.5	1.67	0	0	1	300	35.4	15.8
149	23	1	0	60.0	166.5	1.67	0	0	4	300	35.6	20.8
149	23	1	0	60.0	166.5	1.67	0	0	4	250	11.4	16.8
149	23	1	0	60.0	166.5	1.67	0	0	4	250	9.3	17.8
150	41	1	2	66.0	183.0	1.86	2	0	1	400	14.1	3.3
150	41	1	2	66.0	183.0	1.86	2	0	4	400	22.2	3.5
151	28	1	2	51.0	171.0	1.59	2	0	1	100	2.3	17.0
151	28	1	2	51.0	171.0	1.59	2	0	4	200	7.8	16.3
151	28	1	2	51.0	171.0	1.59	2	0	4	250	9.1	14.0
151	28	1	2	51.0	171.0	1.59	2	0	4	250	11.4	13.0
152	32	1	2	84.6	173.0	1.99	0	0	1	400	12.1	12.0
153	42	1	1	71.6	165.0	1.79	0	0	1	400	11.4	17.5
153	42	1	1	71.6	165.0	1.79	0	0	4	400	8.1	15.0
154	49	1	2	68.5	166.0	1.76	0	0	1	300	10.1	17.5
154	49	1	2	68.5	166.0	1.76	0	0	4	300	12.9	18.3
155	38	0	1	49.4	151.0	1.43	0	0	1	200	9.1	14.5
155	38	0	1	49.4	151.0	1.43	0	0	4	200	5.3	15.0
155	38	0	1	49.4	151.0	1.43	0	0	4	250	22.7	15.0
155	38	0	1	49.4	151.0	1.43	0	0	4	250	19.4	13.8
156	25	0	1	49.7	144.0	1.39	1	0	1	300	21.0	5.0
156	25	0	1	49.7	144.0	1.39	1	0	4	300	18.7	3.4
157	22	1	1	64.3	174.0	1.78	1	1	1	400	22.7	15.5
157	22	1	1	64.3	174.0	1.78	1	1	4	400	27.3	16.8
158	30	1	1	80.0	170.0	1.92	0	0	1	300	11.4	12.8
159	30	0	1	53.2	154.5	1.50	0	0	1	400	35.6	3.3

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
159	30	0	1	53.2	154.5	1.50	0	0	4	350	28.0	4.5
159	30	0	1	53.2	154.5	1.50	0	0	4	350	28.3	4.5
159	30	0	1	53.2	154.5	1.50	0	0	4	327	17.7	4.0
160	76	1	1	58.7	158.5	1.60	1	0	1	300	8.1	16.0
161	34	1	1	54.5	173.5	1.65	2	1	1	300	27.5	15.0
161	34	1	1	54.5	173.5	1.65	2	1	4	307	29.8	15.0
161	34	1	1	54.5	173.5	1.65	2	1	4	314	25.8	15.0
162	28	1	1	68.5	159.0	1.71	0	0	1	300	11.4	15.0
163	35	1	1	65.4	172.5	1.78	2	0	1	300	7.1	14.0
163	35	1	1	65.4	172.5	1.78	2	0	4	300	8.8	16.0
163	35	1	1	65.4	172.5	1.78	2	0	4	350	14.4	15.3
163	35	1	1	65.4	172.5	1.78	2	0	4	350	12.6	15.5
164	52	1	1	62.0	165.0	1.68	2	0	1	300	17.2	11.5
165	33	0	2	66.0	170.5	1.77	2	0	1	400	6.8	17.8
165	33	0	2	66.0	170.5	1.77	2	0	4	400	5.1	16.8
165	33	0	2	66.0	170.5	1.77	2	0	4	400	6.6	13.5
166	52	1	1	62.0	165.0	1.68	1	0	1	400	6.8	3.0
167	34	1	2	60.1	161.0	1.63	2	0	1	400	29.3	2.3
168	32	1	2	61.5	169.0	1.71	1	0	1	300	23.2	14.8
169	54	1	2	79.5	162.5	1.85	0	0	1	300	25.0	14.0
169	54	1	2	79.5	162.5	1.85	0	0	4	200	7.1	13.0
170	42	1	1	65.9	179.0	1.83	0	0	1	400	30.3	13.5
170	42	1	1	65.9	179.0	1.83	0	0	4	350	13.1	1.8
170	42	1	1	65.9	179.0	1.83	0	0	4	350	16.7	13.5
170	42	1	1	65.9	179.0	1.83	0	0	4	350	15.4	5.0
171	26	1	1	52.1	165.5	1.57	1	0	1	300	18.9	16.5
172	30	0	2	48.0	150.0	1.41	2	0	1	400	23.2	12.8
172	30	0	2	48.0	150.0	1.41	2	0	4	400	20.0	14.0
172	30	0	2	48.0	150.0	1.41	2	0	4	425	28.3	14.0
173	56	1	1	61.5	162.5	1.66	1	0	1	300	20.0	14.0
173	56	1	1	61.5	162.5	1.66	1	0	4	300	17.4	13.5
173	56	1	1	61.5	162.5	1.66	1	0	4	300	18.4	14.0
174	21	0	1	57.2	168.0	1.65	1	0	1	300	31.1	15.8
174	21	0	1	57.2	168.0	1.65	1	0	4	250	17.7	16.5
175	23	1	1	73.5	185.0	1.96	2	1	1	300	5.8	12.0
175	23	1	1	73.5	185.0	1.96	2	1	4	300	4.8	12.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
175	23	1	1	73.5	185.0	1.96	2	1	4	350	10.1	12.8
175	23	1	1	73.5	185.0	1.96	2	1	4	350	11.6	13.8
176	29	0	1	59.0	160.0	1.61	3	0	1	350	13.1	15.8
176	29	0	1	59.0	160.0	1.61	3	0	4	350	14.4	14.0
176	29	0	1	59.0	160.0	1.61	3	0	4	377	13.6	13.8
176	29	0	1	59.0	160.0	1.61	3	0	4	377	13.1	13.5
176	29	0	1	59.0	160.0	1.61	3	0	4	400	17.7	2.5
177	18	1	2	113	197.0	2.47	0	0	1	500	12.4	11.5
177	18	1	2	113	197.0	2.47	0	0	4	500	14.1	12.3
177	18	1	2	113	197.0	2.47	0	0	4	500	11.9	13.5
178	33	1	1	64.0	167.0	1.72	1	1	1	300	15.2	3.5
178	33	1	1	64.0	167.0	1.72	1	1	4	300	14.9	1.8
178	33	1	1	64.0	167.0	1.72	1	1	4	327	36.9	4.5
178	33	1	1	64.0	167.0	1.72	1	1	4	327	34.9	3.5
178	33	1	1	64.0	167.0	1.72	1	1	4	314	30.1	4.5
178	33	1	1	64.0	167.0	1.72	1	1	4	314	27.8	3.0
179	37	0	2	62.0	165.0	1.68	1	0	1	300	11.4	16.5
180	71	0	1	49.5	147.0	1.41	0	0	1	300	44.2	16.8
180	71	0	1	49.5	147.0	1.41	0	0	4	300	52.0	16.0
180	71	0	1	49.5	147.0	1.41	0	0	4	200	9.3	17.5
181	22	1	2	60.0	167.0	1.67	1	1	1	300	8.1	11.8
182	37	0	1	62.0	165.0	1.68	0	0	1	327	40.9	14.0
182	37	0	1	62.0	165.0	1.68	0	0	4	300	25.3	2.0
183	70	0	1	49.5	149.0	1.42	0	0	1	350	10.9	17.0
184	50	1	1	56.0	174.5	1.68	2	1	1	300	5.3	14.5
184	50	1	1	56.0	174.5	1.68	2	1	4	300	5.3	10.0
184	50	1	1	56.0	174.5	1.68	2	1	4	400	10.9	4.0
184	50	1	1	56.0	174.5	1.68	2	1	4	400	10.6	4.0
184	50	1	1	56.0	174.5	1.68	2	1	4	427	13.4	3.5
184	50	1	1	56.0	174.5	1.68	2	1	4	427	15.2	4.3
184	50	1	1	56.0	174.5	1.68	2	1	4	450	28.5	4.0
185	41	1	1	46.4	173.0	1.54	1	1	1	300	19.4	15.5
185	41	1	1	46.4	173.0	1.54	1	1	4	300	22.7	15.5
186	39	0	1	58.0	158.0	1.58	2	0	1	300	10.9	4.5
186	39	0	1	58.0	158.0	1.58	2	0	4	300	8.8	2.5
187	41	1	1	65.0	173.0	1.78	0	0	1	300	21.0	12.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
187	41	1	1	65.0	173.0	1.78	0	0	4	300	16.4	13.8
188	43	1	1	62.0	173.0	1.74	1	0	1	300	14.9	17.0
188	43	1	1	62.0	173.0	1.74	1	0	4	300	14.7	19.8
189	26	0	2	88.8	167.5	1.98	0	0	1	450	23.7	15.0
190	49	1	2	62.0	165.0	1.68	2	0	1	300	13.4	14.8
191	42	0	1	76.0	158.0	1.78	0	0	1	300	14.7	11.5
191	42	0	1	76.0	158.0	1.78	0	0	4	300	13.1	15.0
191	42	0	1	76.0	158.0	1.78	0	0	4	300	12.9	13.8
191	42	0	1	76.0	158.0	1.78	0	0	4	300	13.8	13.1
192	78	1	1	56.7	166.5	1.63	0	1	1	300	9.6	14.0
193	38	1	1	61.5	177.0	1.76	0	1	1	400	13.1	4.5
193	38	1	1	61.5	177.0	1.76	0	1	4	400	18.4	3.0
194	60	0	1	59.3	146.5	1.51	1	0	1	300	15.9	14.0
195	24	1	2	55.0	172.5	1.65	0	0	1	300	7.8	16.3
195	24	1	2	55.0	172.5	1.65	0	0	4	300	7.6	12.0
195	24	1	2	55.0	172.5	1.65	0	0	4	327	10.4	11.3
195	24	1	2	55.0	172.5	1.65	0	0	4	327	8.1	14.3
195	24	1	2	55.0	172.5	1.65	0	0	4	350	11.9	12.5
195	24	1	2	55.0	172.5	1.65	0	0	4	350	11.6	13.5
196	29	0	1	54.5	159.0	1.55	0	0	1	200	24.2	4.5
196	29	0	1	54.5	159.0	1.55	0	0	4	200	17.2	3.8
196	29	0	1	54.5	159.0	1.55	0	0	4	150	14.1	3.5
196	29	0	1	54.5	159.0	1.55	0	0	4	150	9.9	4.5
197	39	1	1	56.0	169.0	1.64	1	0	1	100	2.3	4.3
198	20	1	1	59.6	169.5	1.69	2	0	1	300	17.7	16.0
198	20	1	1	59.6	169.5	1.69	2	0	4	300	15.7	16.0
198	20	1	1	59.6	169.5	1.69	2	0	4	327	21.0	13.5
198	20	1	1	59.6	169.5	1.69	2	0	4	327	27.0	15.3
199	27	0	2	54.7	161.5	1.57	0	0	1	350	21.0	5.0
199	27	0	2	54.7	161.5	1.57	0	0	4	327	15.2	6.0
200	17	1	1	85.0	173.0	1.99	0	0	1	300	14.9	15.5
200	17	1	1	85.0	173.0	1.99	0	0	4	300	13.6	14.0
201	39	1	1	50.0	164.5	1.53	1	1	1	300	12.6	15.3
201	39	1	1	50.0	164.5	1.53	1	1	4	300	13.1	15.3
201	39	1	1	50.0	164.5	1.53	1	1	4	327	9.6	15.0
201	39	1	1	50.0	164.5	1.53	1	1	4	327	12.4	3.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
201	39	1	1	50.0	164.5	1.53	1	1	4	327	10.1	4.0
202	32	1	1	71.8	172.0	1.84	1	1	1	300	8.1	13.5
203	25	1	1	64.5	174.5	1.78	0	0	1	300	10.1	13.0
203	25	1	1	64.5	174.5	1.78	0	0	4	300	11.4	14.5
203	25	1	1	64.5	174.5	1.78	0	0	4	327	12.1	11.5
203	25	1	1	64.5	174.5	1.78	0	0	4	327	11.6	13.0
204	25	1	1	62.0	165.0	1.68	2	1	1	300	9.9	3.0
205	25	0	1	78.3	161.0	1.82	2	0	1	300	6.3	14.75
205	25	0	1	78.3	161.0	1.82	2	0	4	300	6.6	17.5
205	25	0	1	78.3	161.0	1.82	2	0	4	350	12.4	14.3
205	25	0	1	78.3	161.0	1.82	2	0	4	400	19.4	15.0
206	29	0	1	58.0	144.0	1.48	0	0	1	200	5.6	19.0
206	29	0	1	58.0	144.0	1.48	0	0	4	200	5.6	16.0
207	45	1	1	49.8	166.0	1.54	2	0	1	300	21.0	13.3
207	45	1	1	49.8	166.0	1.54	2	0	4	300	23.2	15.5
208	35	0	1	71.0	160.0	1.74	1	0	1	300	12.9	13.0
208	35	0	1	71.0	160.0	1.74	1	0	4	300	10.6	12.3
208	35	0	1	71.0	160.0	1.74	1	0	4	300	13.9	13.0
208	35	0	1	71.0	160.0	1.74	1	0	4	350	24.0	13.0
208	35	0	1	71.0	160.0	1.74	1	0	4	350	25.3	16.5
209	46	1	1	59.6	173.0	1.71	2	0	1	300	9.3	16.5
210	65	1	1	55.0	167.0	1.61	1	0	1	300	8.3	11.3
210	65	1	1	55.0	167.0	1.61	1	0	4	300	7.8	12.8
211	30	1	1	95.0	182.5	2.17	0	0	1	400	21.5	19.8
211	30	1	1	95.0	182.5	2.17	0	0	4	400	23.7	17.8
212	36	1	1	62.6	177.0	1.78	2	1	1	300	11.9	17.3
212	36	1	1	62.6	177.0	1.78	2	1	4	300	9.1	14.0
213	28	1	2	67.5	172.5	1.80	1	0	1	300	10.1	14.8
214	38	0	1	65.0	152.5	1.62	0	0	1	300	7.8	17.5
214	38	0	1	65.0	152.5	1.62	0	0	4	300	7.6	16.0
215	20	1	2	82.1	171.5	1.95	0	0	1	400	19.2	4.5
215	20	1	2	82.1	171.5	1.95	0	0	4	400	22.0	4.8
215	20	1	2	82.1	171.5	1.95	0	0	4	427	27.3	6.3
216	25	1	1	53.5	174.0	1.64	0	0	1	300	13.1	15.0
216	25	1	1	53.5	174.0	1.64	0	0	4	327	20.5	13.8
216	25	1	1	53.5	174.0	1.64	0	0	4	327	19.2	12.3

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
217	35	1	1	69.6	176.0	1.85	1	0	1	300	5.1	13.8
217	35	1	1	69.6	176.0	1.85	1	0	4	300	7.8	14.5
218	57	1	1	61.9	169.0	1.71	2	1	1	300	25.5	13.0
218	57	1	1	61.9	169.0	1.71	2	1	4	300	34.9	3.8
219	25	1	1	56.0	170.5	1.65	0	0	1	300	13.1	15.3
219	25	1	1	56.0	170.5	1.65	0	0	4	300	12.9	15.5
219	25	1	1	56.0	170.5	1.65	0	0	4	350	26.0	16.8
219	25	1	1	56.0	170.5	1.65	0	0	4	350	23.7	14.5
219	25	1	1	56.0	170.5	1.65	0	0	4	327	14.7	4.0
219	25	1	1	56.0	170.5	1.65	0	0	4	327	9.3	2.8
219	25	1	1	56.0	170.5	1.65	0	0	4	327	10.6	3.8
220	42	0	1	75.0	154.0	1.73	1	0	1	300	11.4	14.5
220	42	0	1	75.0	154.0	1.73	1	0	4	350	11.1	13.5
221	72	1	1	60.0	165.5	1.66	0	0	1	300	37.6	17.8
221	72	1	1	60.0	165.5	1.66	0	0	4	300	29.3	15.0
221	72	1	1	60.0	165.5	1.66	0	0	4	250	15.4	15.0
222	30	0	1	54.0	156.5	1.53	0	0	1	250	12.9	15.0
222	30	0	1	54.0	156.5	1.53	0	0	4	277	26.3	16.5
223	31	1	1	96.0	169.0	2.06	2	1	1	400	9.6	1.8
223	31	1	1	96.0	169.0	2.06	2	1	4	400	12.4	5.0
224	47	0	1	56.5	153.5	1.53	0	0	1	300	22.0	13.5
224	47	0	1	56.5	153.5	1.53	0	0	4	300	16.9	15.3
225	29	0	2	45.0	148.5	1.36	1	0	1	300	20.0	2.8
226	48	0	1	65.6	156.5	1.66	1	0	1	400	11.1	14.5
226	48	0	1	65.6	156.5	1.66	1	0	4	400	10.9	13.8
227	55	1	1	60.0	173.0	1.72	2	1	1	100	3.3	14.0
228	51	1	2	68.6	174.5	1.83	0	0	1	300	8.3	15.0
228	51	1	2	68.6	174.5	1.83	0	0	4	300	6.8	15.0
229	47	1	1	54.2	170.0	1.62	1	0	1	300	7.1	14.0
229	47	1	1	54.2	170.0	1.62	1	0	4	300	6.8	13.8
229	47	1	1	54.2	170.0	1.62	1	0	4	350	16.4	15.0
230	64	0	1	62.0	146.0	1.54	0	0	1	400	21.0	2.5
230	64	0	1	62.0	146.0	1.54	0	0	4	400	31.8	2.5
231	67	0	1	62.0	165.0	1.68	0	0	1	200	5.8	16.0
231	67	0	1	62.0	165.0	1.68	0	0	4	200	3.8	15.5
232	50	1	1	53.3	165.5	1.58	1	0	1	300	13.6	2.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
233	41	0	1	72.5	166.0	1.81	0	0	1	300	15.4	15.0
233	41	0	1	72.5	166.0	1.81	0	0	4	350	13.4	14.8
233	41	0	1	72.5	166.0	1.81	0	0	4	350	14.4	14.5
234	30	1	1	67.0	175.5	1.82	1	0	1	300	10.9	14.0
234	30	1	1	67.0	175.5	1.82	1	0	4	350	17.9	12.5
234	30	1	1	67.0	175.5	1.82	1	0	4	350	14.1	14.3
234	30	1	1	67.0	175.5	1.82	1	0	4	377	25.8	14.5
235	32	1	1	51.2	173.5	1.61	0	0	1	300	12.1	17.8
236	40	1	1	80.9	179.0	2.00	2	1	1	450	36.6	5.0
236	40	1	1	80.9	179.0	2.00	2	1	4	450	40.4	5.3
236	40	1	1	80.9	179.0	2.00	2	1	4	427	35.1	4.8
236	40	1	1	80.9	179.0	2.00	2	1	4	427	26.3	3.3
237	19	1	2	55.9	173.0	1.67	0	0	1	300	31.6	5.0
237	19	1	2	55.9	173.0	1.67	0	0	4	300	24.0	4.0
237	19	1	2	55.9	173.0	1.67	0	0	4	250	23.7	3.5
238	45	0	1	60.2	159.5	1.62	0	0	1	300	12.9	14.3
238	45	0	1	60.2	159.5	1.62	0	0	4	300	13.6	14.0
239	29	0	1	64.2	157.0	1.65	1	0	1	300	15.7	15.0
240	19	0	1	44.1	153.5	1.38	0	0	1	300	12.4	17.0
241	57	1	1	51.0	158.0	1.50	2	1	1	300	17.4	12.5
242	61	1	1	51.4	161.0	1.53	0	0	1	300	22.2	2.5
242	61	1	1	51.4	161.0	1.53	0	0	4	250	11.9	17.8
243	20	1	1	62.0	167.5	1.70	0	0	1	300	15.7	10.0
243	20	1	1	62.0	167.5	1.70	0	0	4	300	14.7	10.0
244	31	0	1	64.7	158.0	1.66	2	0	1	377	48.0	19.3
245	33	1	1	77.0	169.0	1.88	0	0	1	400	14.1	18.5
245	33	1	1	77.0	169.0	1.88	0	0	4	400	9.3	16.5
245	33	1	1	77.0	169.0	1.88	0	0	4	400	11.1	17.8
245	33	1	1	77.0	169.0	1.88	0	0	4	450	14.4	17.0
246	55	0	1	42.5	150.5	1.34	0	0	1	300	40.9	6.8
246	55	0	1	42.5	150.5	1.34	0	0	4	200	16.4	5.8
247	19	0	1	55.0	154.0	1.52	2	1	1	300	10.1	14.8
247	19	0	1	55.0	154.0	1.52	2	1	4	300	8.8	16.3
248	33	0	1	79.9	167.5	1.89	0	0	1	300	13.4	15.0
248	33	0	1	79.9	167.5	1.89	0	0	4	300	9.3	13.8
249	38	0	1	61.2	158.0	1.62	2	0	1	400	35.6	17.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
249	38	0	1	61.2	158.0	1.62	2	0	4	300	11.4	16.8
249	38	0	1	61.2	158.0	1.62	2	0	4	300	17.2	15.3
250	28	1	1	78.6	184.5	2.02	2	1	1	300	12.1	16.5
250	28	1	1	78.6	184.5	2.02	2	1	4	300	13.6	14.0
251	66	0	1	62.0	165.0	1.68	0	0	1	300	5.6	21.8
252	49	0	1	45.8	152.0	1.39	0	0	1	100	4.0	14.0
252	49	0	1	45.8	152.0	1.39	0	0	4	100	3.8	16.5
253	44	1	1	49.7	163.5	1.52	1	0	1	300	3.4	14.5
253	44	1	1	49.7	163.5	1.52	1	0	4	327	6.6	14.5
253	44	1	1	49.7	163.5	1.52	1	0	4	327	8.6	14.5
253	44	1	1	49.7	163.5	1.52	1	0	4	327	8.6	14.0
254	37	1	1	82.0	170.0	1.94	0	0	1	300	5.8	12.5
254	37	1	1	82.0	170.0	1.94	0	0	4	300	7.1	4.8
254	37	1	1	82.0	170.0	1.94	0	0	4	350	9.1	5.8
254	37	1	1	82.0	170.0	1.94	0	0	4	350	10.1	13.0
255	66	1	1	62.0	165.0	1.68	0	0	1	200	2.8	19.5
256	69	1	1	60.4	168.5	1.69	1	0	1	300	10.6	13.0
257	21	1	1	54.8	170.0	1.63	0	0	1	300	13.4	6.5
257	21	1	1	54.8	170.0	1.63	0	0	4	300	21.0	4.3
257	21	1	1	54.8	170.0	1.63	0	0	4	200	7.1	4.8
257	21	1	1	54.8	170.0	1.63	0	0	4	200	6.6	12.8
257	21	1	1	54.8	170.0	1.63	0	0	4	250	10.9	10.0
259	39	1	1	61.2	177.5	1.76	0	0	1	300	24.0	22.0
260	20	0	1	76.5	157.5	1.78	1	1	1	400	9.1	5.0
260	20	0	1	76.5	157.5	1.78	1	1	4	400	10.4	5.8
260	20	0	1	76.5	157.5	1.78	1	1	4	400	11.6	2.5
261	56	1	1	58.5	153.0	1.55	0	0	1	300	5.1	13.3
261	56	1	1	58.5	153.0	1.55	0	0	4	300	6.1	14.0
262	20	1	1	64.5	180.5	1.83	2	1	1	300	10.1	13.0
263	37	0	1	47.2	154.0	1.42	1	0	1	300	9.9	16.0
264	19	1	1	72.7	178.5	1.91	0	0	1	200	16.2	16.5
265	71	0	1	45.4	150.5	1.38	0	0	1	300	27.3	16.0
265	71	0	1	45.4	150.5	1.38	0	0	4	200	11.4	16.3
266	34	0	1	46.7	148.0	1.38	1	1	1	200	11.6	18.0
267	43	0	2	49.0	153.0	1.44	1	0	1	300	15.9	20.0
267	43	0	2	49.0	153.0	1.44	1	0	4	327	18.9	18.8

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
267	43	0	2	49.0	153.0	1.44	1	0	4	327	20.2	19.3
267	43	0	2	49.0	153.0	1.44	1	0	4	334	16.4	18.5
268	32	0	2	81.6	153.5	1.79	0	0	1	400	14.9	4.0
268	32	0	2	81.6	153.5	1.79	0	0	4	400	14.4	3.5
268	32	0	2	81.6	153.5	1.79	0	0	4	400	15.2	4.0
269	31	1	1	64.9	179.5	1.82	2	1	1	300	3.8	15.8
270	26	1	1	80.4	169.0	1.91	0	0	1	400	44.4	16.3
270	26	1	1	80.4	169.0	1.91	0	0	4	350	29.6	15.0
270	26	1	1	80.4	169.0	1.91	0	0	4	327	19.7	14.5
270	26	1	1	80.4	169.0	1.91	0	0	4	327	17.9	14.5
271	31	1	1	60.0	168.0	1.68	1	0	1	200	10.1	14.5
271	31	1	1	60.0	168.0	1.68	1	0	4	200	8.8	15.5
271	31	1	1	60.0	168.0	1.68	1	0	4	250	12.6	14.3
271	31	1	1	60.0	168.0	1.68	1	0	4	250	9.1	14.8
272	45	1	1	57.0	174.5	1.69	2	0	1	300	7.3	16.0
273	72	0	1	56.0	142.5	1.45	0	0	1	200	13.1	14.5
274	29	1	1	49.5	159.5	1.49	2	1	1	300	21.0	15.8
274	29	1	1	49.5	159.5	1.49	2	1	4	300	16.2	16.5
275	50	1	1	62.0	168.0	1.70	3	1	1	300	15.4	3.8
275	50	1	1	62.0	168.0	1.70	3	1	4	300	12.1	3.0
276	25	1	1	80.3	174.0	1.95	0	1	1	300	5.3	14.0
276	25	1	1	80.3	174.0	1.95	0	1	4	300	7.3	14.5
276	25	1	1	80.3	174.0	1.95	0	1	4	300	4.8	14.3
276	25	1	1	80.3	174.0	1.95	0	1	4	400	15.2	15.3
276	25	1	1	80.3	174.0	1.95	0	1	4	400	12.1	13.8
276	25	1	1	80.3	174.0	1.95	0	1	4	427	14.4	13.5
277	30	1	2	60.6	171.0	1.71	2	0	1	300	12.6	5.3
277	30	1	2	60.6	171.0	1.71	2	0	4	300	11.9	7.5
277	30	1	2	60.6	171.0	1.71	2	0	4	327	8.1	9.3
278	48	1	1	59.3	166.5	1.66	1	1	1	300	13.6	13.5
279	20	1	1	60.0	174.5	1.73	2	0	1	300	14.9	4.0
279	20	1	1	60.0	174.5	1.73	2	0	4	300	14.9	3.3
280	45	1	1	69.5	172.5	1.82	0	0	1	300	24.2	14.3
280	45	1	1	69.5	172.5	1.82	0	0	4	300	32.6	15.0
280	45	1	1	69.5	172.5	1.82	0	0	4	300	27.3	14.0
281	20	1	1	44.6	152.0	1.38	0	0	1	200	9.1	13.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
281	20	1	1	44.6	152.0	1.38	0	0	4	200	12.1	14.0
281	20	1	1	44.6	152.0	1.38	0	0	4	200	11.4	13.0
281	20	1	1	44.6	152.0	1.38	0	0	4	200	10.4	14.0
281	20	1	1	44.6	152.0	1.38	0	0	4	250	23.5	12.0
281	20	1	1	44.6	152.0	1.38	0	0	4	250	21.2	12.0
281	20	1	1	44.6	152.0	1.38	0	0	4	227	13.9	13.0
281	20	1	1	44.6	152.0	1.38	0	0	4	227	16.7	14.0
282	23	1	1	60.2	181.0	1.78	0	0	1	300	21.2	13.5
282	23	1	1	60.2	181.0	1.78	0	0	4	300	20.2	13.8
283	44	1	1	50.5	165.0	1.54	1	1	1	200	12.6	13.3
283	44	1	1	50.5	165.0	1.54	1	1	4	200	14.1	13.5
284	55	1	1	64.2	171.0	1.75	0	0	1	300	17.9	16.0
285	38	1	1	62.4	167.5	1.71	1	0	1	300	21.0	9.0
286	66	1	1	68.4	166.5	1.77	0	0	1	300	13.1	17.0
286	66	1	1	68.4	166.5	1.77	0	0	4	300	14.1	15.5
287	24	1	1	74.1	171.5	1.87	2	1	1	350	7.6	12.0
287	24	1	1	74.1	171.5	1.87	2	1	4	350	7.1	12.5
287	24	1	1	74.1	171.5	1.87	2	1	4	400	8.8	12.0
288	32	1	1	58.0	167.5	1.65	0	0	1	400	28.5	13.0
289	45	1	1	62.0	165.0	1.68	0	1	1	300	40.2	16.3
289	45	1	1	62.0	165.0	1.68	0	1	4	200	14.9	15.3
290	25	0	2	44.0	149.5	1.35	1	0	1	200	12.9	17.5
290	25	0	2	44.0	149.5	1.35	1	0	4	200	9.9	14.5
290	25	0	2	44.0	149.5	1.35	1	0	4	227	14.9	14.0
290	25	0	2	44.0	149.5	1.35	1	0	4	227	14.4	10.0
291	20	0	2	97.2	167.5	2.06	0	0	1	100	0.8	13.0
291	20	0	2	97.2	167.5	2.06	0	0	4	200	3.5	12.0
291	20	0	2	97.2	167.5	2.06	0	0	4	200	2.3	12.8
292	32	1	1	62.6	167.0	1.70	0	0	1	300	13.1	15.0
292	32	1	1	62.6	167.0	1.70	0	0	4	300	10.1	16.0
293	17	1	1	53.0	159.0	1.53	0	0	1	250	4.6	14.0
293	17	1	1	53.0	159.0	1.53	0	0	4	300	12.9	16.5
293	17	1	1	53.0	159.0	1.53	0	0	4	300	10.6	14.0
294	19	1	2	63.0	182.5	1.82	1	0	1	300	10.4	14.0
294	19	1	2	63.0	182.5	1.82	1	0	4	300	8.8	13.0
295	58	1	1	55.0	173.5	1.66	1	1	1	300	7.8	16.0

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
296	46	1	1	54.6	156.0	1.53	0	1	1	300	11.4	12.3
296	46	1	1	54.6	156.0	1.53	0	1	4	300	7.6	13.0
297	44	1	1	57.0	169.0	1.65	1	0	1	300	9.3	13.0
297	44	1	1	57.0	169.0	1.65	1	0	4	300	13.9	15.5
297	44	1	1	57.0	169.0	1.65	1	0	4	327	11.4	16.0
297	44	1	1	57.0	169.0	1.65	1	0	4	350	10.1	15.3
298	68	0	1	40.8	154.0	1.34	1	0	1	200	15.4	3.5
298	68	0	1	40.8	154.0	1.34	1	0	4	171	8.8	3.5
299	21	0	1	36.9	143.5	1.22	0	0	1	200	15.2	15.5
300	59	1	1	55.6	162.5	1.59	1	0	1	300	22.7	3.5
300	59	1	1	55.6	162.5	1.59	1	0	4	300	23.7	3.5
301	27	0	1	54.0	160.0	1.55	2	0	1	350	9.9	18.0
302	17	1	1	51.9	157.5	1.51	0	0	1	300	9.3	15.0
302	17	1	1	51.9	157.5	1.51	0	0	4	300	12.4	15.0
302	17	1	1	51.9	157.5	1.51	0	0	4	300	9.1	16.5
302	17	1	1	51.9	157.5	1.51	0	0	4	350	13.1	15.3
302	17	1	1	51.9	157.5	1.51	0	0	4	350	15.7	13.5
303	55	0	1	74.0	154.0	1.72	1	0	1	300	11.4	15.0
303	55	0	1	74.0	154.0	1.72	1	0	4	300	14.1	15.0
304	44	1	1	51.2	164.5	1.55	1	1	1	327	12.4	15.5
304	44	1	1	51.2	164.5	1.55	1	1	4	327	10.4	14.8
304	44	1	1	51.2	164.5	1.55	1	1	4	350	12.6	15.8
304	44	1	1	51.2	164.5	1.55	1	1	4	350	11.4	14.5
304	44	1	1	51.2	164.5	1.55	1	1	4	400	15.4	5.5
304	44	1	1	51.2	164.5	1.55	1	1	4	427	25.0	2.3
305	49	1	1	71.0	168.0	1.81	0	0	1	300	8.6	5.0
305	49	1	1	71.0	168.0	1.81	0	0	4	300	5.8	5.0
305	49	1	1	71.0	168.0	1.81	0	0	4	350	9.3	5.0
305	49	1	1	71.0	168.0	1.81	0	0	4	350	11.1	5.5
306	47	1	1	74.9	169.0	1.85	0	1	1	300	15.9	13.0
307	32	1	1	51.6	162.0	1.54	2	0	1	300	12.9	15.8
307	32	1	1	51.6	162.0	1.54	2	0	4	300	10.4	16.8
307	32	1	1	51.6	162.0	1.54	2	0	4	350	18.7	5.5
307	32	1	1	51.6	162.0	1.54	2	0	4	350	22.2	4.0
308	37	1	1	61.0	166.0	1.68	1	0	1	300	9.1	11.5
308	37	1	1	61.0	166.0	1.68	1	0	4	300	11.1	11.8

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
308	37	1	1	61.0	166.0	1.68	1	0	4	350	14.4	13.0
309	48	0	1	85.3	161.0	1.89	2	0	1	300	4.3	14.8
309	48	0	1	85.3	161.0	1.89	2	0	4	300	2.8	13.5
309	48	0	1	85.3	161.0	1.89	2	0	4	400	7.3	14.0
309	48	0	1	85.3	161.0	1.89	2	0	4	500	13.9	15.3
309	48	0	1	85.3	161.0	1.89	2	0	4	500	18.4	13.8
310	36	1	1	52.1	169.5	1.59	0	0	1	200	19.7	11.5
311	20	1	1	65.2	170.5	1.76	1	0	1	350	9.6	16.0
311	20	1	1	65.2	170.5	1.76	1	0	4	350	9.3	16.0
311	20	1	1	65.2	170.5	1.76	1	0	4	350	9.3	16.8
311	20	1	1	65.2	170.5	1.76	1	0	4	350	10.4	16.0
311	20	1	1	65.2	170.5	1.76	1	0	4	400	11.6	16.0
312	50	1	1	52.0	168.0	1.58	2	1	1	300	16.9	19.3
312	50	1	1	52.0	168.0	1.58	2	1	4	327	12.9	19.3
312	50	1	1	52.0	168.0	1.58	2	1	4	327	14.7	15.0
312	50	1	1	52.0	168.0	1.58	2	1	4	327	13.6	15.0
312	50	1	1	52.0	168.0	1.58	2	1	4	350	37.6	15.0
312	50	1	1	52.0	168.0	1.58	2	1	4	341	17.4	15.0
313	21	1	2	59.0	173.5	1.71	2	1	1	300	18.7	10.0
313	21	1	2	59.0	173.5	1.71	2	1	4	300	21.0	12.3
313	21	1	2	59.0	173.5	1.71	2	1	4	300	18.4	12.3
313	21	1	2	59.0	173.5	1.71	2	1	4	300	18.4	12.3
314	15	1	1	71.0	177.0	1.87	0	0	1	300	6.8	19.0
314	15	1	1	71.0	177.0	1.87	0	0	4	350	13.9	4.0
314	15	1	1	71.0	177.0	1.87	0	0	4	350	15.2	3.0
314	15	1	1	71.0	177.0	1.87	0	0	4	400	22.0	2.8
314	15	1	1	71.0	177.0	1.87	0	0	4	400	23.0	3.3
315	55	1	1	56.5	169.0	1.65	2	0	1	300	6.3	16.5
315	55	1	1	56.5	169.0	1.65	2	0	4	300	8.3	15.0
316	22	1	1	59.8	164.0	1.65	0	0	1	200	28.0	14.0
316	22	1	1	59.8	164.0	1.65	0	0	4	150	18.7	14.0
317	42	1	1	64.6	167.5	1.73	2	1	1	300	10.9	15.0
318	38	1	1	57.6	171.5	1.68	2	1	1	400	33.6	15.5
318	38	1	1	57.6	171.5	1.68	2	1	4	400	33.6	15.5
318	38	1	1	57.6	171.5	1.68	2	1	4	350	22.7	15.8
319	40	0	1	76.0	155.5	1.76	0	0	1	300	10.9	12.5

ID	AGE	SEX	RACE	WT	HT	BSA	SMK	ALC	EVID	DV	CP	TIME
319	40	0	1	76.0	155.5	1.76	0	0	4	300	8.3	15.0
320	64	1	1	64.2	157.2	1.65	0	1	1	300	12.1	6.3
321	16	1	1	54.4	168.5	1.62	0	0	1	350	21.2	3.8
321	16	1	1	54.4	168.5	1.62	0	0	4	350	16.2	14.0
321	16	1	1	54.4	168.5	1.62	0	0	4	350	16.4	14.0
322	71	1	1	53.6	154.5	1.51	0	0	1	200	19.2	15.3
323	40	1	1	51.7	166.0	1.56	2	1	1	300	11.4	17.3
324	28	0	1	47.8	149.5	1.40	2	0	4	300	44.4	15.0
324	28	0	1	47.8	149.5	1.40	2	0	4	250	29.8	4.5
324	28	0	1	47.8	149.5	1.40	2	0	4	200	22.0	13.5
324	28	0	1	47.8	149.5	1.40	2	0	4	200	16.4	13.5
325	60	0	1	59.0	157.5	1.59	0	0	1	300	14.9	15.0
325	60	0	1	59.0	157.5	1.59	0	0	4	300	13.9	15.0
326	60	1	1	61.0	161.5	1.64	0	0	1	300	17.9	15.0
326	60	1	1	61.0	161.5	1.64	0	0	4	300	18.9	18.8
326	60	1	1	61.0	161.5	1.64	0	0	4	300	20.0	13.8
326	60	1	1	61.0	161.5	1.64	0	0	4	327	22.2	14.0
326	60	1	1	61.0	161.5	1.64	0	0	4	327	30.6	18.8
326	60	1	1	61.0	161.5	1.64	0	0	4	327	26.8	17.3
327	63	1	1	49.7	166.5	1.54	0	0	1	200	8.1	15.0
327	63	1	1	49.7	166.5	1.54	0	0	4	250	11.1	15.3
328	20	1	1	88.0	172.0	2.01	0	0	1	300	12.1	17.0
328	20	1	1	88.0	172.0	2.01	0	0	4	300	10.6	21.3
328	20	1	1	88.0	172.0	2.01	0	0	4	350	21.0	17.5
328	20	1	1	88.0	172.0	2.01	0	0	4	350	18.4	19.5
329	34	1	1	63.0	171.0	1.74	2	1	1	300	8.8	15.8
329	34	1	1	63.0	171.0	1.74	2	1	4	300	11.9	16.5
330	44	1	1	64.0	171.5	1.75	2	1	1	300	22.5	17.5
331	37	1	1	68.0	175.0	1.83	1	1	1	300	7.3	16.5
332	39	1	1	56.0	169.0	1.64	1	0	1	300	15.7	16.0

ANNEXURE 9

NONMEM CONTROL FILE - MODEL S1

```
$PROB PHENYTOIN STEADY-STATE MODEL
$INPUT ID AGE SEX RACE WT HT BSA SMK ALC EVID DV CP TIME
$DATA Comb.prn
$MODEL
$PRED
 FLG1=0.D0
 FLG2=0.D0
 IF (RACE.EQ.0.D0)FLG1=1.D0
 IF (SMK.EQ.0.D0)FLG2=1.D0
 MV1=THETA(1)*WT+THETA(3)
 MV2=MV1*FLG1+MV1*THETA(4)*(1-FLG1)
 VM=MV2*FLG2+MV2*THETA(5)*(1-FLG2)+ETA(1)
 KM=THETA(2)+ETA(2)
 RI=VM*CP/(KM+CP)
 Y=RI+ERR(1)
$THETA (1.0,35.0,200.0)
$THETA (0.01,4.0,20.0)
$THETA (10.0,200.0,1000.0)
$THETA (0.1,1.0,20.0)
$THETA (0.1,1.0,10.0)
$OMEGA 2000.0 3.0
$SIGMA 200.0
$COV
;MSFI=INTER.OUT
$EST MAXEVAL=4000 MSFO=INTER.OUT
$SCAT PRED VS DV UNIT BY RACE
$SCAT WRES VS PRED BY RACE
$SCAT WRES VS WT
$SCAT WRES VS AGE
$SCAT WRES VS SEX
$SCAT WRES VS BSA
$SCAT WRES VS SMK
$SCAT WRES VS ID
;$TABLE ID WT
```

ANNEXURE 10

NONMEM CONTROL FILE - MODEL S2

```
$PROB PHENYTOIN STEADY-STATE MODEL
$INPUT ID AGE SEX RACE WT HT BSA SMK ALC EVID DV CP TIME
$DATA comb.prn
$PRED
 FLG1=0.D0
 FLG2=0.D0
 IF (RACE.EQ.0.D0)FLG1=1.D0
 IF (SMK.EQ.0.D0)FLG2=1.D0
 MV1=THETA(1)*WT+THETA(3)
 MV2=MV1*FLG1+MV1*THETA(4)*(1-FLG1)
 VM=MV2*FLG2+MV2*THETA(5)*(1-FLG2)+ETA(1)
 KM=THETA(2)+ETA(2)
 CL=THETA(6)+ETA(3)
 RI=VM*CP/(KM+CP)+CL*CP
 Y=RI+ERR(1)
$THETA (1.0,35.0,200.0)
$THETA (0.01,4.0,20.0)
$THETA (10.0,200.0,1000.0)
$THETA (0.1,1.0,20.0)
$THETA (0.1,1.0,10.0)
$THETA (0,1.5,5.0)
$OMEGA 2000.0 3.0 0.5
$SIGMA 200.0
$COV
;$MSFI=INTER.OUT
$EST MAXEVAL=4000 MSFO=INTER.OUT
$SCAT PRED VS DV UNIT BY RACE
$SCAT WRES VS PRED BY RACE
$SCAT WRES VS WT
$SCAT WRES VS AGE
$SCAT WRES VS SEX
$SCAT WRES VS BSA
$SCAT WRES VS SMK
$SCAT WRES VS ID
;$TABLE ID WT
```

ANNEXURE 11

NONMEM CONTROL FILE - MODEL S3

```
$PROB PHENYTOIN STEADY-STATE MODEL
$INPUT ID AGE SEX RACE WT HT BSA SMK ALC EVID RI  DV TIME
$DATA comb.prn
$PRED
 FLG1=0.D0
 FLG2=0.D0
 IF (RACE.EQ.0.D0)FLG1=1.D0
 IF (SMK.EQ.0.D0)FLG2=1.D0
 MV1=THETA(1)*WT+THETA(3)
 MV2=MV1*FLG1+MV1*THETA(4)*(1-FLG1)
 VM=MV2*FLG2+MV2*THETA(5)*(1-FLG2)+ETA(1)
 KM=THETA(2)+ETA(2)
 CL=THETA(6)+ETA(3)
 A=VM/CL+KM-RI/CL
 B=4*RI*KM/CL
 ROOT=DSQRT(A**2+B)
 CP=-0.5*(A-ROOT)
 Y=CP+ERR(1)
$THETA (1.0,35.0,200.0)
$THETA (0.01,4.0,20.0)
$THETA (10.0,200.0,1000.0)
$THETA (0.1,1.0,20.0)
$THETA (0.1,1.0,10.0)
$THETA (0,1.5,5.0)
$OMEGA 2000.0 3.0 0.5
$SIGMA 200.0
$COV
$MSFI=INTER.OUT
$EST MAXEVAL=4000 MSFO=INTER.OUT
$SCAT PRED VS DV UNIT BY RACE
$SCAT WRES VS PRED BY RACE
$SCAT WRES VS WT
$SCAT WRES VS AGE
$SCAT WRES VS SEX
$SCAT WRES VS BSA
$SCAT WRES VS SMK
$SCAT WRES VS ID
$SCAT RES VS PRED BY RACE
$SCAT RES VS WT
$SCAT RES VS AGE
$SCAT RES VS SEX
$SCAT RES VS BSA
$SCAT RES VS SMK
```

```
$SCAT RES VS ID
$TABLE ID VM KM CL
```