EFFICIENT PARAMETER ESTIMATION IN PRECLINICAL ANIMAL PHARMACOKINETIC STUDIES

by

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GLOSSARY OF ABBREVIATIONS AND SYMBOLS

A	Amount of drug in the body at time t
Ao	Amount of drug in the body immediately after an intravenous dose
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
AUC	Area under concentration - time curve
C	Plasma concentration at time t
Co	Plasma concentration at time zero after an intravenous bolus dose
Cl	Clearance
c.f.	Compare with
CV	Coefficient of Variation
C_{max}	Maximum concentration
df	Degree of freedom
ED_{50}	Dose required to produce 50% of maximal response
HPLC	High performance liquid chromatography
IV	Intravenous
L	Likelihood
LLD	Log Likelihood Difference
MLE	Most Likely Estimates
N_A	Total number of animals
N_S	Total number of observations
NS	Not significant
NONMEM	Nonlinear Mixed Effects Model
NPD	Naive Pooled Data approach

OBJ	Objective function
PE	Prediction error
QSAR	Quantitative Structure Activity Relationship
SD	Standard deviation
SE	Standard error
STS	Standard Two Stage approach
T_{max}	Time to maximum concentration
t_{min}	The earliest sampling time
t_{end}	The latest sampling time
v	Variance
V	Volume of distribution
V_1	Volume of central compartment
k_e	First - order elimination rate constant of drug
k_{12}	First - order transfer rate constant associated with the movement of drug from compartment 1 (central compartment) to 2 (peripheral compartment)
k_{21}	First - order transfer rate constant associated with the movement of drug from compartment 2 to 1
k_{10}	First - order transfer rate constant associated with the elimination of drug from compartment 1
$t_{1/2}$	Elimination half - life of drug
η	Inter-animal (interindividual) variability
ϵ	Error in concentration measurement
σ_{Cl}	Population standard deviation in clearance (i.e., inter-animal (interindividual) variability in clearance)

σ_V	Population standard deviation in volume (i.e., inter-animal (interindividual) variability in volume)
σ_{V1}	Inter-animal variability in volume of central compartment
σ_ϵ	Intra-animal (intraindividual) variability
Φ_i	Design number for the estimation of an individual parameter
Φ_{ir}	Rescaled design number for the estimation of an individual parameter
Φ	Overall design number for the estimation of all parameters in a model as a set
Φ_r	Rescaled overall design number for the estimation of all parameters in a model as a set
α	First - order hybrid rate constant of distribution
β	First - order rate constant of elimination

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DECLARATION

I declare that this thesis has been written by myself and is a record of work performed by myself. It has not been submitted previously for a higher degree.

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SUMMARY

An estimation of the average value of pharmacokinetic parameters in a group of animals provides limited information if there is no good measure of the variability of each of the parameters. The traditional approach used in the analysis of animal pharmacokinetic data obtained from studies involving the use of small laboratory animals (rats or mice) in which each animal supplies only one concentration - time point does not provide this, nor can it assess the influence of physiology (or pathology) on pharmacokinetics. The consideration of variability within the same species during interspecies scaling has been advocated (Vocci & Farber, 1988). Thus, provision should be made for the estimation of variability inherent in an animal population in analysing data obtained by "destructive sampling". The NONMEM approach does, however, provide estimates of both average values of pharmacokinetic parameters and their statistical distribution within the population. In this thesis data were generated by simulation (assuming no covariance), and analysed using the NONMEM program. The efficiency of this approach is the focus of this thesis.

Experimental error, number of samples taken, and the arrangement of samples in time are factors which must be taken into account in designing experiments for efficient parameter estimation. In addition, appropriate methods of data analysis must be used to extract the required information from the data. Simulated data sets were used to investigate the effect of various design features on the efficiency of parameter estimation using the one observation per animal design. In addition, the efficiency with which parameters could be estimated given a range of parameter values and variability was investigated.

Several methods were used to determine the efficiency of parameter estimation. Prediction error (bias and precision) was useful in assessing the efficiency with which individual parameters were estimated. In addition, the 99% individual and joint confidence intervals containing the true parameter 95% of the

time for all parameters were introduced as aids to judging the efficiency of estimation of individual and all parameters of a model, considered as a set. Confidence interval tables were constructed to reveal the influence of bias and standard error on parameter estimation.

Also, the design number, a new statistic which combines the contributions of bias and precision in judging the efficiency of parameter estimation, was introduced to complement bias and precision, and confidence intervals methods of analysis. The design number also allowed the efficiency with which all parameters of a model were estimated as a set to be judged. The incidence of high pairwise correlations of parameter estimates was also taken into account in assessing the acceptability of estimates and the adequacy of model parameterization.

Assuming IV bolus injection with the monoexponential pharmacokinetic model, simulation studies were carried out to investigate the influence of interanimal variability on the estimation of population pharmacokinetic parameters and their variances. The range of variability investigated was similar to that expected in real studies, and sampling was done at set times. The efficiency of estimation of the structural model parameters (Cl and V) was good, on average, irrespective of the variability in Cl and V. However, the estimation of these parameters was associated with negative bias which was attributed to the nature of the NONMEM program (i.e. estimation error since negative bias was also observed in subsequent studies in which σ_{ϵ} was set to 0%). The variance parameters were mostly inefficiently estimated in this study and all other studies using the one observation per animal design. This was attributable to the lack of information in the data set about σ_{ϵ}.

When the effect of the arrangement of concentrations in time on parameter estimation was studied with the two sample point design, efficient parameter estimates were obtained when the first sample was obtained as early as

possible (5 min.) and the second sample was located at ≥ 1.4 times the simulated $t_{1/2}$ (84 min.) of the drug. When three or four sample points were used the exact location of the third or fourth sample was not critical to efficient parameter estimation.

The efficiency of parameter estimation was investigated given a range of parameter values, concentration measurement error, and sampling schedules with the two compartment model parameterized as A, α, B, β and assuming IV bolus injection with animals sampled at set times. The parameters, considered as a set, were efficiently estimated when α was in the range of 2.0 to 4.0 h^{-1}, and the A:B ratio in the range of 2.5 to 30.0. These results were attributed to the distribution of data points between the distribution and elimination phases of the plasma concentration - time profile. Concentration measurement error greater than 10% yielded variance parameter estimates with a greater degree of bias and imprecision. The inter-animal variability in parameters estimated was a composite of inter- and intra-animal variability. Some sampling schedules gave rise to more efficient parameter estimates than others. High correlation between some parameters led to instability in the estimates, and reparameterization of the model in terms of Cl, V_1, k_{12} and k_{21} led to more stable estimates.

The need for keeping the number of animals used in any study to a minimum, and the necessity for efficient parameter estimation led to the investigation of the effect of sample size on parameter estimation. With the monoexponential model (assuming IV bolus injection with one observation per animal) and sampling at ten time points, it was found that parameters of the model were estimated with equal efficiency when 6 to 15 animals were sampled per time. Since there was no loss in efficiency when 6 animals are sampled per time (i.e., a sample size of 60), the cost involved in such studies could be greatly reduced. However, similar results could be obtained with at least 30 animals sampled twice with the same traditional sampling strategy. Sampling an animal at

least twice allows the partitioning of inter- and intra-animal variability, almost eliminating bias in the estimation of the variance parameters.

Using the two compartment model, efficient parameter estimates were obtained when 15 observations were made at each of 10 time points (i.e., a sample size of 150), but there was no loss in efficiency when 10 animals were used at each time point. The use of the numbers of animals with the design specifications considered in this thesis would strike a good balance between cost and good science.

Given the results of the simulation studies, NONMEM was used to analyse data with the one observation per animal design for a drug under development. NONMEM permitted some explanation of variability in terms of sex, but efficient partitioning between inter- and intra-animal variability would have required an increase in the number of samples per animal.

Thus, inefficient estimates of inter-animal variability were obtained with the one observation per animal design, but sampling an animal at least twice significantly improved the efficiency of parameter estimation. The structural model parameters, on the other hand, were efficiently estimated. The individual and joint confidence intervals for parameter estimates, design number, incidence of high pair-wise correlations in addition to bias and precision were useful in judging the efficiency of parameter estimation.

CHAPTER 1

INTRODUCTION

1.1 SUMMARY

This chapter contains an overview of pharmacokinetics in drug research and development in the preclinical setting. The importance of pharmacokinetics in toxicity testing and drug safety evaluation is discussed , and the various methods used in the estimation of population pharmacokinetic parameters in the preclinical animal setting are examined. Variability has been reported to occur, even in homogeneous strains of animals, and the need to account for this in the estimation of population pharmacokinetic parameters is stressed. The need for the appropriate design of pharmacokinetic experiments for the efficient estimation of population pharmacokinetic parameters is highlighted.

1.2 INTRODUCTION

Preclinical testing of new xenobiotics in animals to predict their safety and efficacy in man is a very large industry. It has reached its present level of activity because of the growth in the number of compounds which have to be tested, the expansion of testing requirements which has occurred over the past few years, and the increase in data required from any one study. The fact that thousands of animals are used can only be justified if it ensures that life is made safer for humans who are subsequently exposed to these xenobiotics. However, if the testing explosion is to be controlled and the effort worthwhile, then urgent attention must be given to increase its scientific content.

The main purpose for conducting extensive animal studies is to help in predicting what will happen when xenobiotics are given to humans. These studies

encompass toxicity, efficacy, metabolism, pharmacokinetics and biopharmaceutics, and are done to accumulate information in the preclinical phase of drug development.

The 1964 Helsinki Declaration which was revised in 1975 states that sufficient well conducted and controlled animal studies should be performed prior to undertaking human studies, and that positive data are essential before subjecting humans to a drug (or procedure). In accordance with this, international regulatory authorities demand a dossier containing considerable amounts of data from animals before they will authorise the administration of new xenobiotics to man.

Many of the techniques and procedures used in conventional animal toxicity testing are empirically based. For example, the proper relationship between the duration of toxicity tests and the length of permitted treatment is a matter of opinion. Similarly, the selection of dose levels in toxicity tests often appears to be arbitrary. On the other hand, there are disquietening voices which even question the validity of animal testing to predict safety for man (Rowan & Andrutis, 1990).

Given the Helsinki Declaration, however, there is no doubt that new xenobiotics cannot be administered to man until sufficient evidence has been collected to indicate that there is no obvious risk. For example, understanding the pharmacodynamics of a new drug is very important, particularly from the point of view of anticipating the effects of overdosage. In the particular case of a new opiate analgesic, it would be vital to know whether or not any potential respiratory depressant effect could be reversed by an opiate antagonist such as naloxone. The development of new medicines and the use of animals in preclinical drug evaluation is therefore inextricably linked.

1.3 PHARMACOKINETICS IN DRUG RESEARCH AND DEVELOPMENT

Pharmacokinetics is an applied scientific discipline that achieves its greatest potential when considered during the early stages of drug development. It encompasses the relationships between the physicochemical properties of a drug and both its physiological disposition by the organism and its pharmacological response (Kaplan & Jack, 1980). The value of pharmacokinetic studies during early stages of drug research and development is to enable critical decisions to be made as to which form of active compound should be recommended for the time - consuming and costly animal toxicology, formulation design, and clinical studies.

1.3.1 Structure - Pharmacokinetic Relationship and Drug Design

The search for new drug molecules basically involves two steps: the setting up of a working hypothesis and the screening of molecules resulting from application of the hypothesis. A working hypothesis may be formulated in different ways (Balant, Roseboom, & Gundert-Remy, 1990):

(a) It may be postulated that the systematic synthesis of compounds differing progressively in their chemical structure and physicochemical properties will eventually lead to the discovery of novel and useful drugs.

(b) One may also start from known drugs and optimise their pharmacological properties by relying on receptor - binding studies.

(c) A more basic approach consists in the study of physiological

mechanisms and structure - activity analysis of specific enzyme activators or inhibitors.

Inherent in all of these hypotheses is the application of quantitative structure - pharmacokinetics relationships. Many an *in vivo* quantitative structure - activity relationship (QSAR) study of a series of compounds has related the dose required to produce a defined response, such as the dose required to produce 50% of maximal response (ED_{50}) at some predetermined time, to molecular modification. But these dose - effect relationships encompass not only the structure - effect relationship but also that between the dose administered and the unbound concentration of compound at the receptor sites which produces the pharmacologic response. Thus, pharmacokinetic events (the processes of and kinetics of absorption, distribution and elimination) determine the concentration of drug at receptor sites. Any movement from an empirical to a more rational design of drug molecules intended to be used *in vivo*, therefore, requires the application of pharmacokinetic principles (Tozer, 1981; Rowland, 1983).

The knowledge of pharmacokinetic parameters is essential for the calculation of effective doses, dosing intervals, estimation of bioavailability and correlation to pharmacodynamic effects. Pharmacokinetic parameters are, however, also an extremely valuable tool with which to derive quantitative structure - pharmacokinetic relationships. Variation in different pharmacokinetic parameters is explained mainly by lipophilicity, ionisation (pKa) and in some cases also by steric influences of substituents within various classes of drugs (Seydel, 1983). At least five pharmacokinetic consequences can be expected as a result of structural changes. These are: rate and order of absorption, volume of distribution, rate and type of metabolism, affinity constant for binding to serum proteins and other "unspecific" biopolymeric binding sites, and rate and type of elimination (clearance) (Seydel, 1983). Many examples of QSAR analysis used to describe variation in rate of absorption have been published (Seydel & Schaper,

1979; Lien, 1981; Schaper, 1982) showing in most cases nonlinear dependence on lipophilicity and on pKa.

Protein binding *per se* generally does influence many pharmacokinetic characteristics of a drug. This influence may be a positive or negative one, depending on the drug class and upon the pharmacokinetic process under investigation. For example the blood compartment (if one considers the body to be made of compartments and blood as one of them) is responsible for drug transport and distribution. Although serum protein binding increases the capacity of the blood compartment, at the same time it decreases the free unbound fraction which can diffuse to receptor sites. Protein binding and also partitioning in red blood cells can therefore influence the therapeutic dose, volume of distribution, rate and type of metabolism, rate and type of excretion (only unbound drug is glomerularly filtered), and serum protein binding of other drugs administered simultaneously (capacity limitation, competition). Therefore, knowledge about quantitative relationships between structure and "non-specific" binding is important in drug design. It is essential not only for understanding, but also for planning changes in pharmacokinetics. This is because of the restrictive influence of protein binding on capillary transport, glomerular filtration and membrane transport (Seydel, 1983).

Volume of distribution has been shown to be dependent on lipophilicity, degree of ionisation of drug molecules, and the degree of binding to serum and tissue constituents in a series of β - blockers (Ritschel, 1980). Elimination rate constant and clearance are not only very important pharmacokinetic parameters but, as they can be accurately and precisely determined are very valuable for QSAR analysis and drug design. The predictive power of such analysis is considerable. This has been demonstrated with a series of sulphapyridines (highly protein bound drugs) given to rats intravenously (Seydel *et al.*, 1980). A high

correlation between clearance and protein - binding constant (i.e. B_{max}) was demonstrated.

Another aspect of QSAR analysis in pharmacokinetics is interspecies scaling. This would be useful for a better transformation of results from experimental animal species to humans and for appropriate selection of animal species for screening. In comparing QSARs for clearance of sulphonamides in rats, goats and humans it was found that the regression coefficients from models relating elimination rate constant to high performance liquid chromatography (HPLC) retention index (a function of the structure of a compound) were surprisingly similar, only the intercepts were different, indicating differences in the capacity of the clearing organ, but no significant differences in dependence on lipophilicity (Seydel *et al.*, 1980). Thus QSAR analysis coupled with well designed pharmacokinetic studies can be used for a more rational drug design.

1.3.2 **Pharmacokinetics in Toxicity Testing**

Over the years a great deal of work has been performed on the kinetics of drug absorption, metabolism, and excretion. These studies have led to the development of a number of general pharmacokinetic principles and to an appreciation of the central role played by kinetic relationships in pharmacological responses (Levy, 1964; Levy & Nelson, 1965; Levy, 1966; Wagner, 1968; Gibaldi & Perrier, 1975). The application of these principles in the assessment of pharmacological activity in animals in the drug development process , and in the optimisation of therapy in man is becoming increasingly common. In contrast, much less use seems to have been made of pharmacokinetic principles in the design and interpretation of toxicological tests (Jollow *et al.*, 1982).

Preclinical animal pharmacokinetic and metabolic studies are essential to a better understanding of the subsequent clinical pharmacology and toxicology of

new drugs. Acute and sub-chronic animal studies are designed to determine the safety of a new drug compound by characterising its disposition and physiological effects, both therapeutic and toxic. Specifically of interest are the dose range over which the pharmacologically desired effect occurs, the dose level at which toxic effects are induced, the scope of toxic effects from gross physical changes (dehydration, lassitude) to biochemical and physiological effects (renal damage, enzyme changes), and the effect of a multiple dose regimen (accumulation, enzyme induction). Correct design and interpretation of animal toxicity experiments is necessary to ensure that human trials will be safely conducted. Determination of pharmacokinetic parameters, such as rates of absorption and elimination, bioavailability, maximal blood concentration (C_{max}), time to C_{max} (t_{max}), area under the concentration - time curve (AUC), renal, metabolic and / or total body clearance, provides a quantitative description of a drug's disposition profile and can be used to compare profiles across species. Pharmacokinetic data from single exposures can be used to help determine appropriate dosing regimens for sub-chronic and chronic studies. Correlating observed toxicity with appropriate pharmacokinetic parameters may allow the investigator to interpret toxicity test data more accurately and even predict at what dose toxicity should occur and help in the understanding of the mechanism responsible for the effect (Scheuplein, Shoaf, & Brown, 1990).

The complicated and widely varying pharmacokinetics of a drug (in animal) may seriously impinge on the very promising properties of a new derivative. If there is a choice between different compounds, selection for further development is based on both the pharmacokinetic profile (bioavailability, half-life, clearance, volume of distribution), and the metabolic profile. Indeed, the role of metabolism in the evaluation of safety of new drugs is of great importance. Understanding the metabolic profile of a new drug in several animal

species can be of predictive value to the clinical pharmacologist, helping him to understand the potential pharmacological effects of a xenobiotic in man. Comparative metabolic and kinetic studies in different species may provide an insight into mechanisms of toxicity perhaps due to over-exposure within a particular species or because of the formation of toxic or reactive products. This provides a basis for a species dependent metabolic effect, and its relevance for the human situation can then be assessed more readily. Accurate and precise determination of pharmacokinetic parameters and better characterisation of drug disposition may allow the investigator to design safer human studies. In Phase I studies, results from animal studies may be used to adjust the intervals between dose levels in dose escalation studies (Collins, 1987). For example, if the ratio between pharmacologically active and toxic doses is small or there is an abrupt increase in the dose response curve in animals, then the initial dose escalation studies in humans should use smaller increases between doses. Tracer pharmacokinetic studies in a number of animal species yield information about the tissue distribution of the drug, and this is of predictive value in Phase I clinical studies (Colburn & Matthews,1979; Hammer & Bozler, 1977).

1.3.3 **Commonly Used Animals in Preclinical Drug Evaluation**

It would be desirable if animals used for toxicity testing were selected so that they were similar to humans in both their intrinsic sensitivity and pharmacokinetic handling of the test compound. However, more often than not, the selection of an animal model is based on considerations of cost, size and availability of the animal, housing requirements and lifespan. In the absence of pharmacokinetic and metabolism data, animal selection has tended toward the use of animal test species that are most sensitive and / or for which there is an

availability of historical controls (Hill, 1987; Huff *et al.*, 1988).

Only four animal species are commonly used and accepted for pharmacokinetic, metabolic, and long - term toxicological studies, namely mouse, rat, dog, and monkey. From a review of the literature it has been observed that rat and mouse are the animal species most commonly used in toxicological studies with the dog a distant second while the monkey is used least. This is a reverse of the order of metabolic similarities of these animals to man (Smith & Caldwell, 1977). Pharmacokinetics is a tool that can be used to further our understanding of the biology of laboratory animals and improve our interpretation of toxicity data.

1.3.4 **Role of Metabolic and Pharmacokinetic Studies in Preclinical Drug Evaluation**

Metabolic and pharmacokinetic studies are essential for gaining an insight into the behaviour of a new drug and as an adjunct to preclinical (and clinical) safety studies. The main objectives of such studies are (Annex IV,1983; Chasseaud, 1988; Smith, 1988; Tse, 1988):

(a) the assessment of drug and metabolite(s) concentrations and kinetics in blood, body fluids and organs;

(b) the gathering of information on the relationship between target organ toxicity and blood, or body fluids or organ concentrations;

(c) the assessment of possible enzyme induction and drug accumulation upon repeated administration;

(d) the choice, when feasible, of the animal species to be used in toxicological studies on the basis of their similarity to man in the handling of the drug. This determines, in part, the human relevance of these studies.

(e) the development of appropriate dosage schemes to be used in Phase I clinical studies.

(f) determination of the relationship between the age and sex of the animal and the kinetics of the test drug;

(g) support for the pharmacology of the drug;

(h) screening of new dosage forms and formulations.

A major objective of animal metabolic studies - bearing in mind human metabolic studies - is the assessment of the validity of the animal model in qualitative, quantitative and kinetic terms. Ideally, the qualitative pattern of metabolism of the test drug in animal species should resemble that occurring in man so that both species are broadly exposed to a similar array of metabolites.

The prediction of species differences in the qualitative pattern of metabolism of drugs is far from being an exact science, and the best that can be achieved at present is described as a "forecast". Indeed, by taking into account chemical structure, metabolic pathways, species patterns and deficiencies it is often possible to arrive at a quite reasonable forecast as to what would be anticipated in terms of metabolic pattern in humans (Smith, 1988).

Most helpful in this predictive context is the knowledge that has been acquired concerning species defects with respect to particular metabolic pathways and certain substrates. Also of value is the recognition that, occasionally, species may exhibit uncommon reactions, particularly unusual conjugation reactions. Their occurrence is relatively unpredictable and arises from a particular combination of species and substrates.

Potential confounding factors such as dose and duration of exposure which may alter metabolic patterns have to be taken into account in the interpretation of animal metabolic studies. Dose size is particularly important as it is now known that high dose exposure may saturate detoxification pathways and result in an alternative pathway of metabolism or "metabolic switching". This can

result in the formation of a different array of metabolites, or at least in a change in the relative proportions of metabolites compared with that seen in low dose exposure conditions (Zangouras *et al.*, 1981; Sangster *et al.*, 1983; Sutton *et al.*, 1985).

Moreover, the interpretation of long - term toxicity studies is hampered by lack of pharmacokinetic data. The need for detailed pharmacokinetic studies to aid in the design and interpretation of toxicity tests has been emphasised (Mellet, 1969; Clark & Smith, 1984; Jollow *et al.*, 1982). Pharmacokinetic data should usually be developed in correlation with acute and sub-chronic testing of a xenobiotic before the initiation of chronic studies. Evidence of possible absorption problems, unusual toxic dose relationships, or notable species differences in the early toxicity studies suggest that additional pharmacokinetic experiments are useful in developing protocols for further short - or long - term toxicity studies (Glocklin, 1982). Questions which arise from effects observed from a particular xenobiotic during sub-chronic or chronic toxicity studies may warrant additional pharmacokinetic studies and / or retrospective reassessment of pharmacokinetic/toxicology correlates. This might, for example, include comprehensive characterisation of metabolite identity and reactivity (Glocklin, 1982; Levy, Galinsky, & Lin, 1982). Hottendorf et al (1976) have pointed out the inadequacy of safety extrapolations based upon the daily dose and suggested that comparative peak blood levels, AUCs, duration of dosing, clearance, were of equal or greater importance. Pharmacokinetic and metabolic data are therefore important in virtually every aspect of drug safety evaluation.

Most toxicological studies are conducted according to standard guidelines, and no effort is made to optimise experimental protocols on the basis of sound pharmacokinetic and metabolic knowledge (Zbinden, 1984). Some pitfalls of traditional toxicity testing methods (Rentsch, 1974) are as follows:

(a) The methods are qualitative and empirical.

(b) Complete absorption is assumed in data interpretation.

(c) Animals are assumed to handle high and low doses of drug similarly.

(d) Dosing conditions are different from those intended to be used clinically.

(e) Differences among species are usually ignored.

(f) A test compound is usually abandoned when an unusual toxicity is observed, without attempts to understand the fundamental reasons for toxicity.

One has to assume complete absorption of a drug in the interpretation of safety data when pharmacokinetic information is not available. Similarly, pharmacokinetic linearity between doses being evaluated must be assumed. Unless tested, these assumptions are groundless because the kinetics of absorption, distribution, and elimination of large doses of a drug as given in toxicity (safety evaluation) studies can be completely different from the kinetics of smaller doses for therapeutic purposes. A serious disadvantage of traditional toxicity testing is that when an unusual toxicity is observed, further development of the compound is generally stopped with no attempts made to understand the mechanism of its toxicity. In many cases, the toxicity is simply due to selection of a very high dose or the formation of a toxic metabolite that may be specific to the test species, with no relation to human beings at all (Batra & Yacobi, 1989). A knowledge of the concentrations of the parent compound and metabolites in plasma and tissue, allied to the accumulation of the drug on further dosing or the rate of elimination after cessation of administration, allows the opportunity to rationalise both the species of animal most appropriate for the testing of a compound and the extrapolation of any toxicity observed in animals to the likely risk for man (Anderson, Hoel, & Kaplan, 1980; Batra & Yacobi, 1989).

Observance of nonlinear pharmacokinetics of absorption, elimination, or

both is very common in toxicity studies because of the very high doses (relative to therapeutic doses) used in such studies. This has become a rule rather than an exception (Batra & Yacobi, 1989). Pharmacokinetics incorporated in dose ranging studies would help establish a dose range in which linear relationship between blood concentration and dosage exists. This relationship would introduce a quantitative measure in the study relating response to an accurate estimate of the amount of drug absorbed rather than the dose administered. The knowledge gained could be useful in correlating toxicity with blood concentrations and elucidating whether toxicity observed at any point during the short - or long - term toxicity studies was drug related.

It is well established that drug metabolising capacity generally diminishes with age (Yacobi, Kamath, & Lai, 1982). Whereas age - dependent metabolism is unlikely to be of consequence in shorter - term toxicity studies, it may be of importance in lifespan studies in rodents, particularly as the dose levels for such studies are often chosen on the basis of data obtained from younger animals in shorter - term studies. An obvious consequence of such age - dependent metabolisms is that the impact of a selected dose alters as the study progresses: what was a suitable dose at the start of the study may become less appropriate towards its conclusion. Consequently, doses should be selected for lifespan studies by making allowances for age - dependent metabolism. Whether this actually occurs can be evaluated by determining the kinetics of the test compound at appropriate intervals during the course of the study (e.g. at 3 and 6 months, 1 year and 2 years (Chasseaud, 1988)). During maturation, for instance, the developmental profiles of different drug metabolising enzymes are dissimilar (Gibson & Skett, 1986), and it is not unreasonable to suppose that there are differences in senescence. The amount of the important endogenous protective agent, glutathione, in the heart, liver and kidneys of mice has been shown to

diminish gradually by some 20 - 30% with increasing age (Hazelton & Lang, 1980). This would be of importance for compounds detoxified by reaction with glutathione. Since age - or sex - dependent alterations in drug disposition is important in humans (Schmucker, 1985) because of the variable manner in which individuals metabolise xenobiotics, these factors must be considered as pharmacokinetics' input in the design and interpretation of toxicity studies.

It has been a long recognised fact that the intensity and duration of the pharmacological effect of a systemically acting drug are functions not only of its intrinsic activity but also of its pharmacokinetic characteristics. Thus, pharmacokinetic data obtained from the pharmacological test species are often useful in the interpretation of drug effects. A typical example is a drug that is active following intravenous administration but is considerably less active after comparable oral dose. Possession of the appropriate pharmacokinetic data could reveal whether the drug is poorly absorbed to yield subtherapeutic circulating levels or is subject to presystemic biotransformation to an inactive metabolite. Such information would be invaluable in subsequent decisions, for example to improve drug absorption by altering the salt form or formulation, to investigate the possibility of making prodrugs, or to abandon the oral route of administration.

For many drugs there is a direct correlation between drug concentration at site of action and pharmacological effect. For instance, present knowledge suggests that the bactericidal action of antibiotics is directly related to drug levels at the site of infection, and the bactericidal effect is lost when antibiotic levels fall below the minimum inhibitory concentration for the invading micro-organisms. Also, the time course of drug - induced hypothermia in cold - room acclimatised rats parallel plasma bromocriptine concentrations but not total radioactivity levels following an intravenous dose of ^{14}C - labelled bromocriptine (Schran, Tse, & Bhuta, 1985).

Knowledge of the effective blood or plasma concentration in animals can

be used as a guide for later studies in humans as the drug trial progresses into the clinical phase. A drug should not be considered as inefficacious unless circulating levels approaching the effective concentrations in the pharmacological test species are achieved in man (Tse, 1988). Consequently, potentially valuable therapeutic agents will not fall into unwarranted disrepute because of underdosing.

There is sometimes a more complicated relationship in the time course of plasma levels and the pharmacological effect for drugs with an extravascular site of pharmacodynamic action. Simultaneous modelling of the pharmacokinetics and pharmacodynamics of such drugs is relatively complex, and numerous integrated models have been have been introduced (Dahlstrom *et al.*, 1978; Colburn, 1981; Holford & Sheiner, 1981). Although there is a greater accessibility of tissue drug concentration data in small laboratory animals which should render them attractive models for testing the applicability of this modelling approach, this type of elaborate analysis is usually not attempted during the preclinical phase of drug development.

In the process of developing a final drug product, the formulation scientist develops one or more formulations that demonstrate desirable disintegration and dissolution characteristics *in vitro*. The *in vivo* release pattern of the drug based on the resulting blood level curves in humans is studied, and the dosage form is accepted if an adequate blood level profile is obtained. With some sophisticated formulation designs, such as those used in controlled release drug delivery systems, repeated trial and error may be needed before an acceptable product is identified. Such a development pattern is not only costly but also time consuming, since a typical human bioavailability study requires the coordination of personnel from the various units involved in drug research and development (Tse, 1988).

A more direct and simpler approach is to perform *in vivo* screening tests

in animals. As a result, formulations with a desirable release pattern *in vitro* are submitted for definitive bioavailability or bioequivalence testing in humans only after yielding a favourable blood level profile in an animal model.

A proper model is the key to the successful use of animal data in this manner. The beagle dog has proven to be a useful indicator of potential human absorption and formulation problems when the animal studies are conducted under appropriate conditions for the following reasons (Smyth *et al.*, 1983; Tse, 1988):

(a) Generally, oral dosage forms intended for man can be administered intact to dogs.

(b) It is relatively easy to handle and maintain the beagle dog. Its body weight is sufficiently stable over time to allow repeated studies using the crossover study design. The normal physiology of a 10 kg beagle dog is not affected by the withdrawal of approximately 100 ml of blood weekly for 6 weeks.

(c) Although interspecies differences in metabolism (Mellet, 1969), protein binding (Vallner, 1977), and drug clearance (Boxenbaum, 1980) preclude absolute correlation of dog and human pharmacokinetics, similarities in anatomy and physiology (Hamilton, 1957; Anderson, 1970; Wilson, 1962) provide a basis for the use of the dog in relative bioavailability studies. Formulation - related absorption problems in the dog usually also exist in the human (Crouthamel & Bekersky, 1983).

The need to apply the knowledge of pharmacokinetics and biopharmaceutics in the design and interpretation of toxicological studies cannot be overemphasised. The advantages are as follows (Smyth & Hottendorf, 1980; Hawkins & Chasseaud, 1985; Bolt & Filser, 1987):

(a) Effect of changing formulations on bioavailability by different routes can be established.

(b) Data on the extent of absorption, achieved plasma concentrations and rates of elimination over the range of doses selected for toxicity studies become available.

(c) Any likelihood of accumulation of the parent compound and or its metabolites is identified prior to commencing chronic toxicity studies.

(d) The relative and / or actual exposure to test compound can be determined (i.e. a bioavailability of 1.0 is not assumed).

Proper characterisation of kinetic behaviour is a prerequisite for the selection of appropriate dosages in long - term studies, and is also useful for interpretation of dose - response relationships, especially when toxicity is mediated by metabolites rather than the parent compound. It is therefore appropriate to investigate the pharmacokinetic behaviour of xenobiotics over the range of dosages used for animal toxicity tests as well as at dosages approximating "in use" exposure for humans. Pharmacokinetic data are essential if there is to be better and more rational interpretation of information obtained from toxicity studies. In fact, without pharmacokinetic data, the actual or relative dose levels to which animals are exposed systemically during toxicity studies cannot be determined, and the assessment of safety margins based on administered doses alone becomes pure guess work. Thus, adequate and proper characterisation of the pharmacokinetics of a drug is important for prediction from one animal species to another, and most importantly man.

1.4 Parameter Estimation in Preclinical Pharmacokinetic Studies

Small laboratory animals (rats and mice) have been the animals of choice for pharmacokinetic and toxicological studies because of economic considerations and ease of handling. The collection of samples (blood or tissues)

from these animals usually involves "destructive" sampling at specified times.

The most commonly used method of analysing pharmacokinetic data obtained from small laboratory animals is the **N**aive **P**ooled **D**ata (NPD) approach. This is best illustrated by example. Suppose that 10 animals are sacrificed at each of 10 time points and the concentration measured (i.e. 100 animals), the data at each time point are averaged to give 10 (averaged) concentration - time points. These are then used for the estimation of model parameters (Fig. 1.1).

However, the averaging procedure, in general, may mask the most appropriate model, and allow a different model to be justified. No estimate of intra- or inter-animal variability is possible. Estimates of parameter errors bear no relationship to the variability of these parameters within the population of animal under test. In fact, variability (physiological, anatomical, and biochemical) within the study population can be considerable, and when considered in terms of clearance and volume of distribution can be expressed as coefficients of variation of the order of 50% (Lindstrom & Birkes, 1984). The NPD approach cannot, therefore, be recommended as a reliable method of kinetic data analysis, either for modelling or parameter estimation.

There are instances where animal pharmacokinetic data are obtained from large animals (such as dogs) by serial sampling in each animal, and these are analysed by the **S**tandard **T**wo **S**tage (STS) method. This method is, in a sense , the opposite of the NPD approach. It involves estimating individual animal parameters in the first stage with simple nonlinear regression, and combining these estimates in the second. Estimates of average parameters are then computed as means and their variances.

The STS method provides reasonable estimates of average population parameters, but the standard deviation of these parameter estimates will, in general, overestimate variability (Sheiner & Beal, 1980a; 1981; Sheiner, 1984).

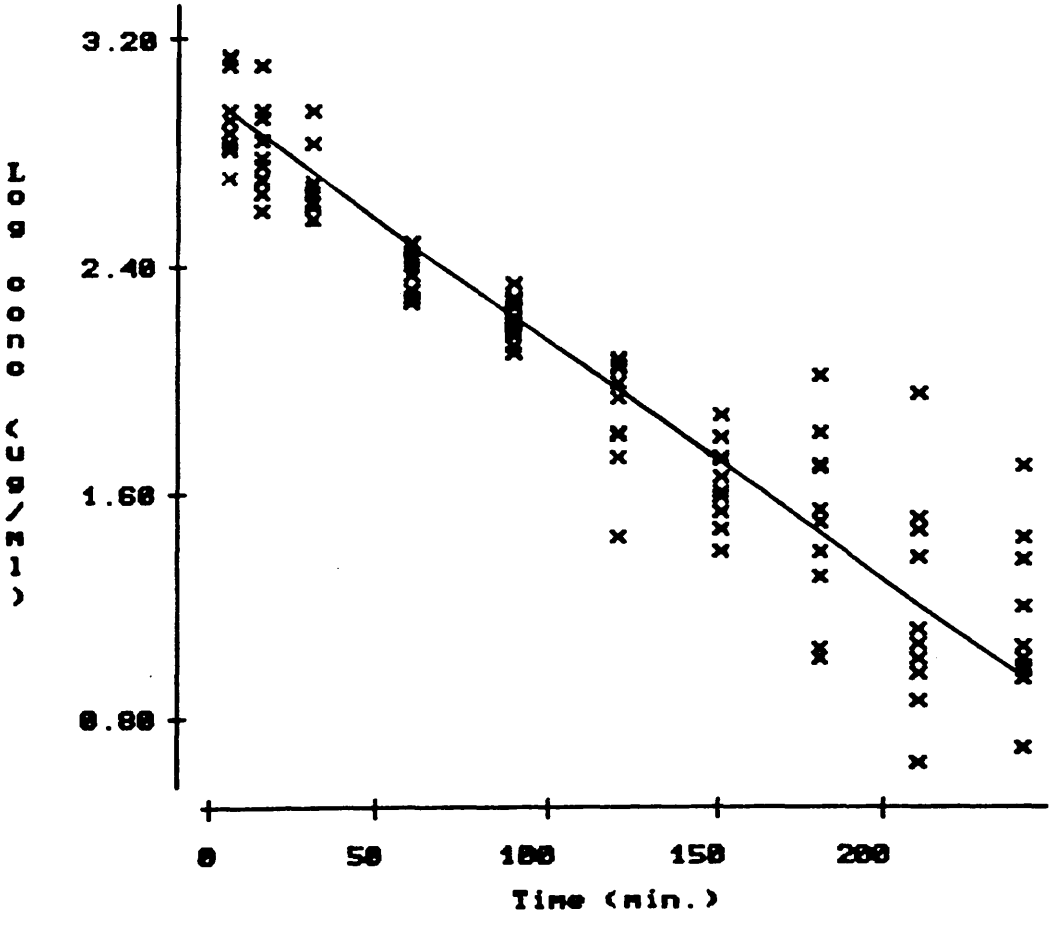


Fig. 1.1 An example of concentration - time plot from a typical animal pharmacokinetic study in which one observation is taken per animal. The parameters of the model are obtained by averaging concentrations at each time point and fitting a model to the averaged data (the NPD approach). The continuous line is the model fitted line.

This is because each parameter is estimated from the original drug level - time profile, which itself contains some measurement error and possibly model misspecification. This error adds variability to the parameter estimates that is not of biological origin. Hence random inter - animal variability will be over estimated.

Not only are the estimates of variability obtained with the STS method biased, but the use of this method is also not possible when the data from study animals are too few to permit the calculation of individual animal parameter estimates. Because of these reasons, the STS method, like the NPD method, cannot be regarded as ideal for population pharmacokinetic parameter estimation

Where data are not analysed by either the NPD or STS approaches, parameter estimates are obtained by the use of statistical moments analysis. Again, the estimates of AUCs obtained are devoid of estimates of error, and no information on variability is provided. Accuracy and precision at this stage of drug development is crucial, and these objectives are jeopardised by inefficient data analytical techniques.

There is a great need to incorporate in the analysis of data obtained from pharmacokinetic studies involving "destructive sampling" the fact that the data came from a population with more variability than the traditional experimental error. Once this provision is made the data should be analysed with a method which takes into account the inherent variability in the population sample. The precision of the parameter estimates is then a function of the underlying structural model and the sampling strategy (Balant *et al.*, 1990).

1.4.1 **Variability**

Comparison of pharmacokinetic data obtained from different animals

given the same dose of drug will indicate the degree of population variability in drug disposition; it may also indicate the source of variability. For example, comparison of the total amount of drug excreted unchanged into the urine can indicate whether metabolism or excretion is extremely variable. If the total amount of drug excreted is very different then this would indicate that the amount of drug metabolised was variable. If the total amount of drug excreted unchanged was the same but the rate at which it was excreted was different, then this would indicate variability in the excretion process. Brodie (1962) pointed out that different inbred strains of rats oxidise antipyrine at widely different rates (as much as a factor of 3). Vocci and Farber (1988) have advocated the consideration of pharmacokinetic differences within the same species in interspecies scaling. If population variability for a drug is high in laboratory animals, usually homogeneous and inbred populations, then even larger variations in response would be expected for humans (Scheuplein *et al.*, 1990). A large degree of unexplained inter-animal variability may suggest that other factors, as yet undetermined, may be affecting the pharmacokinetics of the drug.

Inter - animal variation in pharmacokinetics can be attributed to various factors. Some of these involve easily measurable animal characteristics (for example, weight, sex, age, protein binding). On the other hand, intra - animal pharmacokinetic variability involves the change in response of animal 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, and diurnal variation due to circadian rhythms. It is highly pertinent to accurate and precise pharmacokinetic parameter estimation that these variabilities be accounted for.

In contrast to the NPD, STS and statistical moments analysis approaches it is necessary to use the nonlinear mixed effects regression model approach (Beal

& Sheiner, 1979 - 1989) in estimating population mean parameters and their variability from data obtained from animal pharmacokinetic studies. This data analysis approach is usually carried out with the exportable software program NONMEM (**Non**linear **M**ixed **E**ffects **M**odel, Beal & Sheiner, 1979 - 1989). The statistical model used in NONMEM is based on the premise that individual (animal) pharmacokinetic parameters of a study (animal) population arise from a distribution which can be described by the population mean and inter-individual (animal) variance. Thus, each individual's (animal's) pharmacokinetic parameter can be expressed as a population mean and a deviation from the population mean, typical of that individual (animal). NONMEM is designed to handle relatively sparse data, in that it permits the use of unsystematically sampled plasma concentrations and few measurements per subject (animal), to determine population parameters and their variability. The strength of this approach, therefore, is the fact that a data set can be analysed at once to yield average values of pharmacokinetic parameters and their variances.

The NONMEM approach has proved itself in the human clinical setting (Vozeh *et al.*, 1982; Grasela *et al.*, 1983; Grasela & Sheiner, 1984; Grasela & Donn, 1985; Grasela *et al.*, 1986; Thomson & Whiting, 1987; Grevel, Thomas, & Whiting, 1989), and there is a need for the application of the NONMEM approach in the animal preclinical setting (Rahamani et al, 1988; Balant et al, 1990).

1.5 Experimental Design for the Estimation of Population Pharmacokinetic Parameters

The design of experiments is crucial in the analysis of a system under investigation. The design of pharmacokinetic experiments is usually based on

the immediate objective of the investigation, i.e., model discrimination or parameter estimation. Determining the correct structural model among alternatives (e.g., single versus multicompartmental) yields valuable insights into pharmacokinetic mechanisms, and estimating model parameters is the key to quantifying population variability. Pharmacokinetic analysis of data is informative only if the data themselves are informative, and that informative data could best be assured by appropriately designing the experiments from which the data are collected.

Animal pharmacokinetic experiments typically consist of administration of a test compound and measurement of the changing drug concentration in timed blood samples from either individual animals or groups of animals. It is established (Box, 1970; Landaw, 1985) that design decisions in human pharmacokinetic experiments can be at several levels:

1. the route of drug administration
2. the dose to be used (e.g., tracer versus large, single versus multiple, IV bolus versus continuous infusion)
3. sites, metabolites, or "pools" to be sampled
4. the number of samples to be collected
5. the spacing of sampling times

These decisions also apply to animal pharmacokinetic studies. Although "input" design can be quite important (Endrenyi, 1981; Mannervik, 1981), the route and dose of drug are often determined by the biopharmaceutical properties of the drug (Smyth & Hottendorf, 1980). Likewise the number of samples to collect may be limited to a large extent by the sample size in "destructive" animal pharmacokinetic studies in which one animal supplies only one observation. In situations which allow for serial sampling the total amount of blood that can be withdrawn is limited. The balance, particularly in small animals, between

providing realistic pharmacokinetic data and increasing the sample size to unmanageable proportions is narrow. Although items 4 and 5 are the most easily controlled aspects of animal pharmacokinetic studies examples abound in the literature of poor sampling strategy in animal pharmacokinetic studies designed for parameter estimation (Zbinden, 1984; Smith, Humphrey, & Charuel, 1990).

The information that can be derived from experimental data of pharmacokinetic studies is determined by three factors (Suverkrup, 1982): (1) accuracy, specificity and sensitivity of the assay, (2) number of samples taken, and (3) arrangement of samples in time.

The observations made in a pharmacokinetic study are subject to two types of error - errors due to analysis and errors due to biological variation during the course of the experiment. Both will contribute to the error of the parameters being estimated. The number of sample points taken and their timing will affect the errors in parameter estimation, hence it is important that sufficient samples are taken.

As with statistical estimations the larger the sample size, the better are the parameter estimates in the sense that the variances will be smaller. However, in animal and most pharmacokinetic studies the sample size is usually fixed so that the arrangement of samples in time should be given adequate consideration.

Generally, sampling times can be manipulated to improve the information content of the available concentration - time data. The benefits of attempting to obtain measurements at certain key time points which will contain the maximum pharmacokinetic information about model parameters have been highlighted by a number of authors (D'Argenio, 1981; DiStefano, 1981; Endrenyi, 1981; Endrenyi & Dingle, 1982; Landaw, 1985; Suvekrup, 1982). Theory suggests that two sampling times are needed for the efficient estimation of model parameters, clearance and volume of distribution, of the one compartment model (Box & Lucas, 1959). Using Monte Carlo simulation, D'Argenio (1981) found that a

repeating p point design led to a reduction in the parameter estimate variability when data were collected at optimal sequential sampling times from a group of 10 subjects. Using this simulation technique in population pharmacokinetic studies involving multiple sampling of subjects, Al-Banna, Kelman, and Whiting (1990) examined the impact of two sampling times (an early and a late sampling time) and three sampling times (where the first and the last samples were obtained at early and late times and the third time varied between the two) on parameter estimation. They concluded that variability was better estimated with the three point sampling strategy, and the exact location of the middle (third) sampling time was not critical. In animal pharmacokinetic studies involving the one observation per animal study design the situation is not so clear.

Even when sample size and the arrangement of samples in time are adequate, the parameterization of the model of choice may be crucial to efficient parameter estimation. Using the two compartment model with oral administration, Westlake (1971) pointed out that parameter estimates can be unreliable when the constants in the exponential terms (e.g., α and β) are nearly equal. He also noted that even when the parameter estimates are satisfactory for limited prediction purposes, they can be quite unreliable. Boxenbaum, Riegelman, and Elashoff (1974) noted the instability of regression parameter estimates and related this to high correlation between the estimates. In this type of model, reparameterization has been suggested to reduce correlation between parameter estimates, leading to more stable estimation (Boxenbaum *et al.*, 1974; Metzler, 1981; Laskerzewski, Weiner, & Ott, 1982). Reparameterization results in a transformation of the parameter space.

The philosophy behind this approach has been stated quite succinctly by Box (1980): 'Known facts (data) suggest a tentative model, implicit or explicit, which in turn suggests a particular analysis of data / or the need to acquire further

data; analysis may then suggest a modified model that may require further practical illumination and so on.' This is also the philosophy behind data driven linear regression transformations of the target variable (Box and Cox, 1964).

1.6 STUDY OBJECTIVES

The aim of the work described in this thesis is to investigate the efficiency with which NONMEM can estimate population pharmacokinetic parameters and their variances, using experimental design normally applicable to small laboratory animals. The effects of parameter variability, arrangement of samples in time, sample size, experimental error, and a range of parameter values are investigated.

1.7 OUTLINE OF THESIS

The chapters that follow have the following features:-

Chapter 2: methods of data acquisition and analysis;

Chapter 3: influence of inter-animal variability on parameter estimation;

Chapter 4: effect of sampling designs on parameter estimation;

Chapter 5: efficiency of parameter estimation given a range of parameter values of the 2 compartment model with an intravenous bolus administration, sample size, concentration measurement error, and sampling schedules;

Chapter 6: effect of reparameterization of the model used in Chapter 5 on parameter estimation;

Chapter 7: application of NONMEM to the analysis of real data from animal pharmacokinetic study;

Chapter 8: effect of sample size, error in concentration measurement,

sampling an animal twice on the efficiency of estimation of population pharmacokinetic estimates; and

Chapter 9: general discussion and conclusion.

With the exceptions of Chapters 5 to 7, all other experimental chapters deal with the one compartment model with intravenous bolus dose administration. All, but one, of the experimental chapters (Chapter 7) deal with simulated data sets.

CHAPTER 2

METHODS

2.1 SUMMARY

Pharmacokinetic principles, methods of estimation of pharmacokinetic parameters, population pharmacokinetic methods and data analysis methods used in this thesis are discussed in this chapter. The efficiency of parameter estimation is examined in terms of accuracy and precision (mean and SD of percent prediction error) and design number (a new statistic introduced). While the percent prediction error can be used to judge the efficiency with which an individual parameter is estimated, it cannot be used to determine the efficiency with which all parameters are estimated when different designs within a study are compared. The design number, on the other hand, not only measures the efficiency with which individual parameters are estimated but may measure that for all model parameters estimated as a set.

Since NONMEM, which is used throughout the course of this thesis, produces standard error estimates, individual and joint confidence intervals for parameter estimates were computed as measures of efficiency of parameter estimation. Also, incidence of pair-wise correlations were computed as an aid to judging the adequacy of the parameterization of a model.

2.2 INTRODUCTION

2.2.1 Parmacokinetics

Pharmacokinetics is concerned with the study and characterisation of the time course of drug absorption, distribution, metabolism and excretion, and the relationship of these processes to the intensity and time course of therapeutic and adverse effects of drugs. It involves the application of mathematical and

biochemical techniques in a physiological context (Gibaldi & Levy, 1976). In the preclinical setting the appropriate pharmacokinetic characterisation of a new xenobiotic is indispensable in the drug development process.

The pharmacokinetic behaviour of a drug is readily summarised with parameters which relate concentration to dose and time, and the two most useful parameters are clearance (Cl) and volume of distribution. Cl is defined as the volume of blood , plasma or serum (containing drug) which is cleared of drug per unit of time. In this thesis it is measured in units of millilitres per minute (ml/min).

The volume of distribution (V) of a drug corresponds generally to an apparent space, which may be defined as the volume it would occupy at a concentration equal to that at the site of measurement, often peripheral plasma (Gillete, 1973). In this thesis it is measured in millilitres (ml). Knowledge of volume enables calculation of the concentration of drug immediately after an intravenous bolus dose.

The elimination rate constant (K_e) is the fractional rate of removal of drug and is defined as the ratio of Cl to V (Eq. (2.1)).

$$K_e = Cl/V \tag{2.1}$$

It is measured in units of either per minute or hour (min^{-1} or h^{-1}).

2.2.2 Compartment Models

Generally, a compartment has no physiological or anatomical counterpart. Occasionally it does, such as circulating plasma, extravascular fluid space and

total body water space. A compartment may also correspond to a perfusion volume of tissue (Bischoff *et al.*, 1971). It can be defined as an ideal volume in which each molecule or particle of a substance (drug) has equal probability of leaving (Segre, 1986). Implied in this definition is the fact that the concentration of the material present in a compartment is uniform and that the rate of mixing within the compartment is rapid compared with transfer into or out of it (Segre, 1986). This definition emphasises the statement of Wagner (1971), that a compartmentalised system is an approximation of a biological system, being an "average" rather than an exact state.

Many biological systems can be modelled as a collection of homogeneous compartments, with material moving according to specified rate laws. In pharmacokinetics, an attempt is made to quantify the kinetics of absorption, distribution, metabolism and excretion of a drug. Quantification calls for a mathematical model, and "compartment models" have been extensively used in pharmaceutical and clinical pharmacology research. By modelling the body as a set of separate compartments and measuring the amount of drug in one or more of these over time, the parameters governing the movement of drug in the system can be estimated. The concentration of drug is assumed to be the same throughout all compartments at equilibrium, and the rates of transfer of drug between compartments are assumed to obey first order kinetics. The mathematical formulation of compartment models is a set of differential equations with constant coefficients.

The one compartment model is the simplest model which depicts the body as a single, kinetically homogeneous unit from which drug elimination is first order. This is a particularly useful model for the pharmacokinetic analysis of drugs that distribute relatively rapidly throughout the body. The schematic representation of this model is shown in Fig. 2.1.

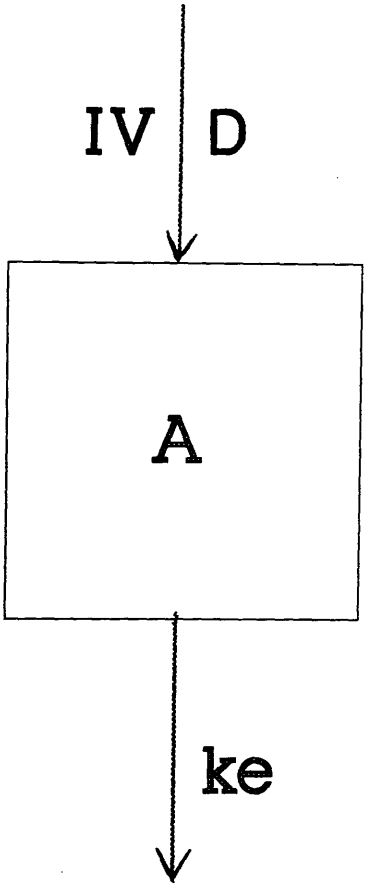


Fig. 2.1 A diagrammatic representation of one compartment model assuming instantaneous IV input with first order elimination

Assuming instantaneous input (IV bolus injection) the mathematical description of drug disposition with this model is given by the following differential equation:

$$dA/dt = -K_eA \qquad (2.2)$$

where A is the amount of drug in the body at time t after injection. K_e is the apparent first - order elimination rate constant for the drug. Eq. (2.2) can be solved by Laplace transformation (Gibaldi & Perrier, 1975) to give Eq. (2.3).

$$A = Ao.\exp(-K_e.t) \qquad (2.3)$$

Assuming that the relative binding of a drug to components of tissues and fluids is essentially independent of drug concentration, then the ratio of drug concentrations in various tissues and fluids is constant. Thus, there will exist a constant relationship between drug concentration in (for example) plasma, C, and the amount of drug in the body:

$$A = VC \qquad (2.4)$$

Thus, Eq. (2.3) can be expressed as

$$C = Co.\exp(-K_e.t) \qquad (2.5)$$

where Co is the drug concentration in plasma immediately after injection, and C is the drug concentration in the plasma at time t.

The two compartment model (Metzler, 1971) for drug kinetics is depicted

in Fig. 2.2. Compartment one is called the "central compartment" and incorporates circulating plasma from which the drug distributes into a second compartment which is sometimes referred to as the "tissue" or peripheral compartment. For a drug exhibiting two compartment kinetics the concentration - time profile shows an initial rapid decline in concentration which represents both distribution and elimination followed by a second slower decline.

Assuming all exchanges between compartments are first order processes, the mathematical description of this model is given by the following set of differential equations:

$$dA_1/dt = -(k_{12} + k_{10})A_1 + k_{21}A_2 \qquad (2.6)$$

$$dA_2/dt = k_{12}A_1 - k_{21}A_2 \qquad (2.7)$$

where A_1 and A_2 are the amounts of drug in compartments 1 and 2, respectively. The rate constants k_{10}, k_{12}, and k_{21} represent the rate of elimination from the central compartment, the rate of transfer from the central compartment to the peripheral compartment, and the rate of transfer from the peripheral to central compartment, respectively. These equations can also be solved by Laplace transformation to give:

$$C = [Dose/V_1(\alpha - \beta)][(\alpha - k_{21})EA + (k_{21} - \beta)EB] \qquad (2.8)$$

where $EA = \exp(-\alpha t)$, $EB = \exp(-\beta t)$

and $\alpha, \beta = 1/2\{k_{12} + k_{21} + k_{10} \pm [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2}\}$

α and β are hybrid rate constants describing distribution and elimination, and V_1 is the volume of the central compartment.

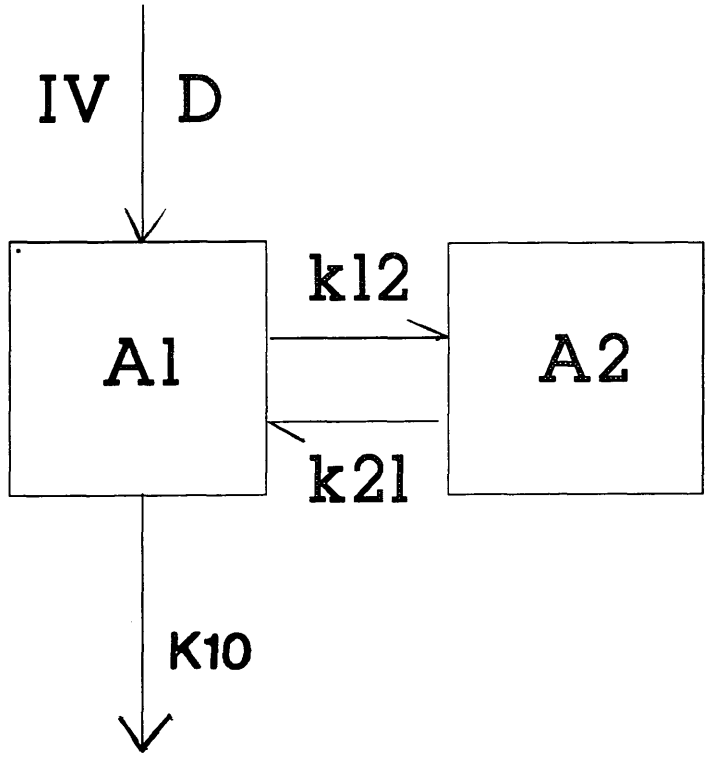


Fig. 2.2 A diagrammatic representation of the two compartment open model assuming instantaneous IV input with first order transfer and elimination processes.

2.2.3 **Nonlinear Regression**

Nonlinear regression methods are used in the analysis of data generated during the course of a pharmacokinetic study to estimate the parameters of the model. There is no unique solution for the model parameters. Initial parameter estimates are progressively altered until the best set of parameters is obtained corresponding to the minimisation of the sum of squared deviations between the observed and model predicted values.

For example, at each time t_i, i = 1, N, the expected drug concentration C^*_i will be given by an equation

$$C^*_i = f(\Theta, t_i) \qquad (2.9)$$

where Θ represents the structural model pharmacokinetic parameters (e.g., Cl and V) for the individual (or animal). The observed concentration, C_i may be represented by

$$C_i = C^*_i + \epsilon_i \qquad (2.10)$$

where the ϵ_i is a small random error. The distribution of the ϵ_i has zero mean and variance σ^2.

The probability density function of observing C_i at t_i is given by the normal distribution with a mean of C^*_i and variance, v_i,

i.e. $$p(C_i) = (1/\sqrt{2\Pi v_i}) \exp. -(C_i - C^*_i)^2 / 2v_i \qquad (2.11)$$

The joint probability density function (pdf) that all of the observations (C) occur, is given by p(C), where

$$p(C) = p(C_1).p(C_2).....p(C_n) \qquad (2.12)$$

and each $p(C_i)$ is as given above. Eq. (2.12) represents the probability function for obtaining the values of C, given the values of the parameters Θ.

The probability function can also be used to define the "likelihood" (L') function for the parameters, Θ, given the observations of C, so that

$$L'(\Theta) \alpha p(C) \quad \text{or} \quad L'(\Theta) = k.p(C) = k.L(\Theta)$$

where k is a constant, but $L(\Theta)$ is not a probability density function. $L(\Theta)$ can be used to obtain the most likely estimates (MLE) or estimators of Θ. These will be the set of parameter values which maximises $L(\Theta)$. Thus, by substituting for p(C), the problem reduces to maximising the product

$$L(\Theta) = [(1/\sqrt{2\Pi v_1})\exp -(C_1 - C^*_1)^2/2v_1]\ [(1/\sqrt{2\Pi v_2})\exp -(C_2 - C^*_2)^2/2v_2]... \qquad .....(2.13)$$

The logarithm of both sides gives

$$\ln(L(\Theta)) = -\{(C_1 - C^*_1)^2/2v_1 + 1/2\ln(2\Pi v_1)\} - \{(C_2-C^*_2)^2/2v_2 + 1/2\ln(2\Pi v_2)\} \qquad ....(2.14)$$

Thus maximising $\ln(L(\Theta))$ is equivalent to maximising $L(\Theta)$, or minimising $-\ln(L(\Theta))$, i.e.

$$-\ln(L(\Theta)) = \Sigma(C_i - C^*_i)^2/2v_i + 1/2\ln(2\Pi v_i) \qquad (2.15)$$

where -ln(L(Θ)) is called the negative log likelihood. Multiplying Eq. 2.15 by 2, and also removing the 2Π term reduces to minimising Eq. 2.15 to obtain the MLE of the parameters.

i.e. Objective function = $\Sigma(C_i - C^*_i)^2/v_i + \ln(v_i)$ (2.16)

This objective function is called the **E**xtended **L**east **S**quares (ELS) objective function.

The interest in the ELS regression method is due to the fact that the variance or weighting scheme can be included as part of the model for the data, and the parameters of the variance model may be estimated simultaneously. Consequently, a variance model or weighting scheme need not be chosen explicitly before the data are fitted. However, the form of the model used to describe the variance must be selected.

The most frequently used variance model is

$$v_i \propto C^*_i{}^{\varphi} \qquad (2.17)$$

where the value of φ is estimated along with other model parameters. Eq. (2.17) gives the general variance model in which various weighting schemes can be incorporated. When φ = 0, Eq. (2.17) yields the constant variance model and the objective function in Eq. (2.16) reduces to the **O**rdinary **L**east **S**quares (OLS) objective function which is given by

$$OLS_{OBJ} = \Sigma(C_i - C^*_i)^2 \qquad (2.18)$$

The assumption of a constant variance may be unjustifiable in cases in which concentration is measured over a large range of values (e.g. 0.01 to 100 μg/ml).

As the variance of each point is rarely known, there are several weighting schemes which are commonly used. Examples of these occur in the radioactive decay and dilution processes. In the former, $W_i \alpha V_i \alpha C^*$ and in the latter $W_i \alpha V_i \alpha C^{*2}$. Thus, $\varphi = 1$ and 2 for the respective processes (i.e. $W_i \alpha C^*_i$ or $C^*_i{}^2$, respectively) and the objective function in Eq. (2.16) reduces to the **W**eighted **L**east **S**quares (WLS) objective function. Thus,

$$WLS_{OBJ} = \Sigma W_i (C_i - C^*_i)^2 \qquad (2.19)$$

The contribution of each point to the WLS_{OBJ} is weighted by a function which reflects the certainty of the observation. More weight is placed on the data points about which there is the greatest confidence and vice versa.

2.3 POPULATION PHARMACOKINETICS

All drugs exhibit pharmacokinetic variability. Population pharmacokinetics describes this variability in terms of fixed and random effects. "The fixed effects are the population average values of pharmacokinetic parameters which may in turn be a function of various patient characteristics such as: (a) age, weight, height and sex; (b) underlying pathology such as renal or hepatic impairment; and (c) other influences on drug disposition such as concomitant drug therapy, smoking habits and alcohol intake. The random effects quantify the amount of pharmacokinetic variability which is not explained by the fixed effects, i.e., inter- and intrasubject variability" (Whiting, Kelman, & Grevel, 1986). In animals fixed effects are similarly a function of those characteristics listed for humans.

Thus inter-animal random effect parameters measure the magnitude of the random individual animal variability in relation to the fixed effects. Intra-animal variation includes measurement errors involved in quantifying drug concentration or response and random changes in an animal's parameter values over time. It also includes model misspecification errors which arise because all mathematical calculations of parameter values are simplifications of reality.

2.3.1 **Population Methods**

There are two standard approaches to estimating population pharmacokinetic parameters : the NPD and the STS approaches (Sheiner & Beal, 1980a). These approaches have been traditionally used in the estimation of population pharmacokinetic parameters from animal data. The NPD approach tends to ignore individual animal pharmacokinetic differences. The usual approach when an animal (e.g., rat or mouse) can be measured only once is to sample more than one animal at each of several time points and to treat the sample means as a time series of measurements from a "typical" animal. This procedure only gives estimates of population parameter means and ignores inter-animal variability in the parameters.

When animals are sampled serially all data are pooled at each time point to yield an arithmetic mean plasma concentration curve (Eq. 2.20) of all individual animal curves and a pharmacokinetic model fitted to the mean data as if it came from a "super" animal.

$$\mu\{C(t)\} = 1/n\ \Sigma C_i(t) \qquad (2.20)$$

Thus the NPD approach has several drawbacks:

(1) It totally ignores individual animal pharmacokinetic characteristics

and, by doing so, obscures important information on how xenobiotic substances are handled.

(2) The average concentration curve derived by the NPD approach, does not necessarily follow the individual model function. A wrong model may be obtained (Martin *et al.*, 1984). Undefined statistical uncertainties and large "unknown" animal variations might smooth the average response curve in an unpredictable manner.

The STS method is, in a sense, the exact opposite of the NPD method. At first it regards each animal as completely distinct from all others and estimates each animal's pharmacokinetic parameters from its data alone. In the second stage, the individual animal pharmacokinetic parameter estimates are often pooled to obtain population parameter estimates. If Cl, for instance, is to be related to physiology, linear regression is used. This, however, has only been used in human studies although it could be applied to animal studies. For inter-animal random effect parameters, the standard deviations of the individual animal parameters about the regression line (or the mean value) are used. When the residual error random effect parameter is estimated (which is rare) the square root of the sum of the pooled, squared residuals of the initial, nonlinear fits divided by the (pooled) residual degrees of freedom is usually used (Sheiner & Beal, 1980a).

When standard errors of the fixed effect parameter estimates are obtained with the STS approach they are usually taken to be the standard deviations of each animal's parameter estimates divided by the square root of the number of sampled animals. The standard errors of the inter-animal random effect parameter estimates are not computed.

The manner in which the random inter-animal effect parameters is estimated is a fundamental problem associated with the STS approach. They tend to be upward biased because each parameter is estimated from the original drug

concentration - time data with some error, and this error adds variability to the parameter estimates that is not of biological origin (Sheiner, 1984; Martin *et al.*, 1984). Accurate variance estimates can be achieved only through well designed and performed pharmacokinetic studies. In addition, this method cannot be used when data from some animals are too few to permit individual animal parameter estimates.

The statistical problems of pharmacokinetic data analysis are now being appreciated more often than formerly and alternative population - based methods of estimating population pharmacokinetic parameters have been elaborated. These methods focus on central tendency in response across a study population and the variability in response between individual members of the population studied. This difference in point of view requires a dramatically different approach to modelling and parameter estimation. A variety of approaches have been proposed (Steimer *et al.*, 1984), but the nonlinear mixed effects model has been studied in detail, and it is applied throughout the course of this thesis. Traditional compartmental pharmacokinetic models invariably assume that error or unexplained deviation from expected response is simply added to the predicted response. Such an error structure can be satisfactorily dealt with using simple least squares nonlinear regression. Population based methods assume a more complex error structure and are generally expressed as mixed effect models, indicating that complex interactions and effects are responsible for an observed response (Beal & Sheiner, 1984).

Expression of a population model in a form that lends itself to extended least squares analysis allows explicit estimation of components of variance as well as estimation of central tendencies. At the heart of population pharmacokinetic analysis is the explicit estimation of inter- and intra-individual (inter- and intra-animal) variability and the exploration of factors that account for this variability (Sheiner, Rosenberg, & Marathe, 1977; 1980a & b; 1981; 1983;

Beal & Sheiner, 1982, 1984).

2.3.2 Population Data Analysis Using NONMEM

The formal expression of a population based model for parameter estimation is accomplished through application of the basic principles of analysis of variance. The similarities between traditional analysis of variance (ANOVA) or, more accurately, analysis of covariance (ANCOVA) and nonlinear mixed effects modelling underscores the importance of variance in population modelling (Colburn & Olson, 1988).

In common with simple nonlinear regression models (OLS and WLS), mixed effects nonlinear regression models estimate a central tendency for parameters that predict average response. The principal difference between simple nonlinear regression and mixed effects nonlinear regression is the level of complexity allowed in the subsequent expression of variability. Simple nonlinear regression allows a single component of random error about the predicted response. This error is added to the predicted response to account for deviations from prediction and may or may not arise from a distribution of a constant variance. A more complex expression of variance models based on principles firmly established for traditional ANOVA is accomplished with mixed effects nonlinear regression (Beal & Sheiner, 1982).

Nonlinear mixed effects regression recognises two sources of deviation from a predicted response. Assuming that the central tendency in a population model represents the response of an average animal, any particular animal response will be different for the simple reason that the particular animal is not average. This is the source of inter-animal variability. The second source of variability (residual error) arises from deviations from predicted response after

accounting for inter-animal variability. The residual error is the same as that estimated in simple nonlinear regression. This analysis approach allows a generally correlated error structure, with varying error magnitude as a function of observable data (e.g., sex, weight, time of sample after dose, etc) and the fixed effect parameters.

Fewer samples are needed from each animal because the individual animal is no longer of central interest; this procedure should lend itself to the sparse data obtained with the one observation per animal study design. Observations are pooled to characterise a central tendency for the population rather than the individual animal. The characterisation of inter-animal variability preserves the fact that individual animal response is different from the population mean response. Each animal's contribution to the characterisation of this variance is adequately defined with fewer samples than are required for the characterisation of each animal's parameters.

The NONMEM program (Beal & Sheiner, 1979 - 1989) uses the ELS method to estimate population pharmacokinetic parameters and is designed to handle relatively sparse data from a large number of subjects. This feature makes it applicable in the analysis of data collected during animal pharmacokinetic studies in which as few as one observation is obtained per animal. It simultaneously analyses data from all animals in a study, provides estimates of average population parameters and partitions all sources of error into that arising from inter-animal variability and that arising from residual error. When analysing experimental data the ability to state a general parametric model for the error structure frees the analyst from the task of specifying weights for the data analysis. NONMEM provides estimates of standard errors for all parameters estimated and these can be used to construct confidence intervals for true parameter values, thereby allowing hypothesis tests for these. Under the

assumption of normality for the distributions of the random variables, NONMEM provides yet another possibly preferable method of testing hypotheses, the likelihood ratio test (Rao, 1965) which is used in comparing models.

Three input files are required to run NONMEM. These are: (1) the data file which contains the concentration - time data, (2) the "PRED" file, a FORTRAN subroutine which defines the structural and variance models, and (3) the control file which details information on the organisation 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 PRED files used in this thesis are shown in Appendix I.

The parameters of the structural model, (Θ_{ki}) for any animal are represented by the population mean, ($\bar{\Theta}_k$), plus the deviation from the mean which is relevant to the particular animal η_{ki} (where η_{ki} represents inter-animal variability), i.e.

$$\Theta_{ki} = \bar{\Theta}_k + \eta_{ki} \qquad (2.21)$$

η_{ki} values are often assumed to be normally distributed with zero mean and variance σ^2_k. The inter-animal variability expressed in this form is additive to the population mean, and σ_k approximates the inter-animal standard deviation for associated parameters. Alternatively, the inter-animal variability can be assumed to be proportional to the value of $\bar{\Theta}_k$, i.e.

$$\ln(\Theta_{ki}) = \ln(\bar{\Theta}_k) + \eta_{ki} \qquad (2.22)$$

The statistical model given in Eq. (2.21) is used throughout this thesis.

The residual error quantifies deviations of the plasma concentration measured in each animal, C_j, from the overall predicted concentration, C^*_j. The

predicted concentration is a function of structural model parameters (Θ_{ki}),

$$C^* = f(\Theta_{ki}, D, t) \qquad (2.23)$$

$$\text{and } C_j = f(\Theta_{ki}, D, t) + \epsilon_j = C^*_j + \epsilon_j \qquad (2.24)$$

The error is assumed to be normally distributed with zero mean and variance σ^2. This corresponds to a "constant or additive error" model. The "proportional error" (error proportional to concentration), a realistic assumption in pharmacokinetics, is an alternative model which can be obtained by assuming a log normal distribution of concentration, i.e.

$$\ln(C_j) = \ln f(\Theta_{ki}, D,t) + \epsilon_j \qquad (2.25)$$

Fig. 2.3 is an example of the PRED (for a drug which is administered by intravenous (IV) bolus dose injection and exhibits one compartment kinetics) used in the 1985 version of NONMEM (Beal & Sheiner, 1979 - 1989). It requires the provision of both the structural (pharmacokinetic) model (F) and the derivatives of the function with respect to each η (G array) by the user. The statistical nature of the inter-animal variability is defined by the G functions. The "H" function defines the statistical nature of the concentration error model. Appendix I contains examples of other PRED's and control files used in NONMEM analysis during the course of this thesis.

2.3.3 Model Comparison

NONMEM models are compared on the basis of the objective function

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C     1 COMP IV, 1ST DOSE, CL, V
C
C
C
      DIMENSION THETA(2),DATREC(3),H(1),G(2),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,DOSE,CL,V,EKT,XKE,EXPWCH
      CL=THETA(1)
      V=THETA(2)
      XKE=CL/V
      T=DATREC(2)
      DOSE=3000.
      EKT=EXPWCH(-XKE*T)
      F=DOSE*EKT/V
      G(1)=-T*F/V
      G(2)=(F/V)*(XKE*T-1)
C     H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

Fig. 2.3 The PRED subroutine used for parameter estimation with the one compartment model (IV bolus injection). Note that when more than one observation is obtained per animal "H" is included in the subroutine.

(twice negative log likelihood function). Hierarchical models can be compared using a chi- squared test with degrees of freedom equal to difference in the number of parameters (Sheiner, Rosenberg, & Marathe, 1977). Non-hierarchical models (where all models have the same number of parameters, see chapter 7) are compared by an examination of the objective function, the variances associated with each parameter, and the weighted residuals plot.

2.4 SIMULATION

Monte Carlo simulation is a numerical technique for conducting experiments with certain types of mathematical models describing the behaviour of the system under study (Naylor, Burdick, & Sasser, 1966). In a pharmacokinetic simulation study, it is assumed that both the form of the deterministic and the stochastic components (structural model parameters and error structures, respectively) of the pharmacokinetic model are known, and the sampling strategy specified.

Thus, simulation was carried out as described by Bard (1974). For studies involving the use of the one compartment model with IV bolus injection (Chapters 3, 4, and 8) population parameters of a drug having the characteristics of avicin, a cytotoxic agent (McGovern *et al.*, 1988) were used for the simulation. The parameter values were $\overline{Cl}$ = 1.3 ml/min.; $\overline{V}$ = 162.5 ml, σ_{Cl}, σ_V, and σ_ϵ were set to give coefficients of variation of 15%.

The half-life ($t_{1/2}$) of the simulated drug (using Cl and V) was 84 min., and ten sampling times were specified between 5 and 240 min. (i.e. 5 , 15, 30, 60, 90, 120, 150, 180, 210, and 240 min.). The first two time points were fixed in all cases while the other time points were sampled uniformly from a range of 15

min. about the stated times. This was considered to mimic a real study, and in parameter estimation with NONMEM the exact times were used. One observation was made on each animal. Variations of this sampling design are specified in chapters 4 and 8.

Individual Cl values (Cl_j's) were obtained by sampling from the population distribution ($\overline{Cl}$, σ^2_{Cl}) using a random number generator. V_j's were similarly generated. Using the appropriate sampling time (t_j) sampled from the uniform distribution ($t_j \pm 7.5$ min.), apart from the first two points, the expected concentration C^*_j was computed. A random error, proportional to C^*_j was then added to C^*_j to give the final observation. This was repeated for each animal comprising a data set.

For each study design, 30 such sets of data were generated and analysed assuming zero covariance between any two parameters. A similar procedure was used to simulate data for the two compartment open model with IV bolus injection using the parameters and variances specified in Chapters 5 and 6.

Simulation was carried out using the ICL main frame (ICL 3980). Appendix II contains the simulation programs used in this thesis. The data thus simulated were analysed with the NONMEM program.

2.5 DATA ANALYSIS

2.5.1 Prediction Error

Given that the "true" parameter values were known, the efficiency with which each model parameter is estimated could be judged. Let Θ^* represent the "true" value of the parameter Θ. Intuitively an estimate is "better" the closer it is to the "true" value.

This notion was formalised by defining the error (bias) of an estimate Θ as $\Theta - \Theta^*$. In order to express the accuracy and precision for all parameters on the same scale, percentage errors were computed. For each run and for each parameter, the difference between the "true" value and the "estimated" value was expressed as a percentage of the "true" value (i.e., percent prediction error, %PE). Thus,

$$\%PE = (\Theta_i - \Theta_i^*/\Theta_i^*) * 100 \qquad (2.26)$$

The mean of %PE for each of 30 replicates of data was used as a measure of the accuracy with which each parameter was estimated.

An estimate of the precision with which each parameter was estimated was obtained from the standard deviation of %PE, denoted SD of %PE. Bias and precision are illustrated in Fig. 2.4. The first estimate (I) of the parameter Θ is unbiased and precise, the second estimate (II) is unbiased and imprecise, the third estimate (III) is positively biased but precise, and the fourth estimate (IV) is positively biased and imprecise. In deciding on the acceptability of precision of estimates, an SD of %PE of 25% was used as the cut off. Statistical significance of nonzero %PE's was tested using the two - tailed t test.

In some studies reported in the course of this thesis some data sets gave rise to totally implausible estimates. Since these would be rejected from further analysis, criteria had to be adopted with which to judge acceptability. Thus, any parameter estimate which was smaller than 1/100th of the "true" value or larger than 10 times the "true" value was rejected. Also, if the estimated standard error of a parameter was greater than 10 times the "true" value, the result was rejected. This is similar to the criteria used by White *et al* (1991) in a simulation study with a drug exhibiting one compartment open model kinetics. These criteria were applied in Chapters 3, 5, and 8.

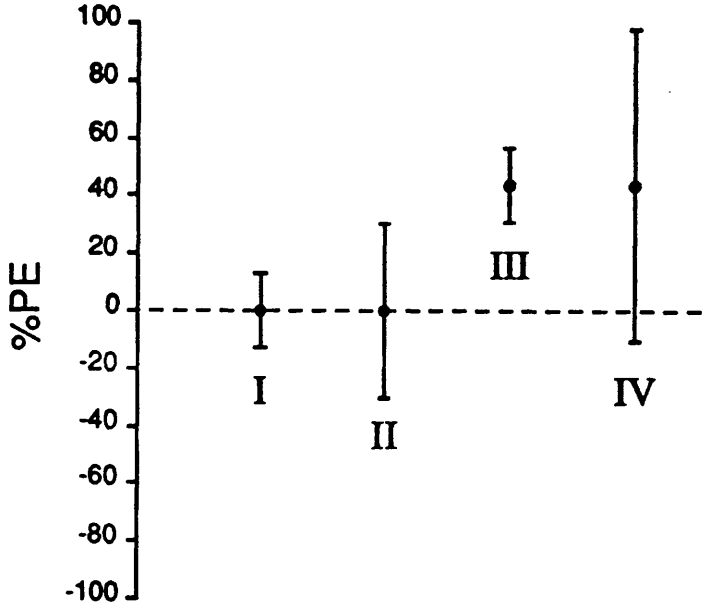


Fig. 2.4 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for a parameter, Θ. The first estimate (I) of Θ is unbiased and precise, the second estimate (II) is unbiased and imprecise, the third estimate (III) is positively biased but precise, and the fourth estimate (IV) is positively biased and imprecise.

2.5.2 Confidence Intervals

The reliability of estimates is important in parameter estimation. The usual way to approach this statistical problem is through the construction of a "confidence interval" for the parameter estimate. Briefly a 95% confidence interval is a region in the parameter space that is so constructed that in repeated trials the true parameter will lie in the confidence region (interval) in 95% of the cases.

The standard errors (SE) of parameter estimates (Θ_i) produced by NONMEM can be used for the construction of confidence intervals (Sheiner & Beal, 1980a). The approximate 95% confidence interval is given by $\Theta_i \pm 1.96(SE)$ for Θ. Efficient parameter estimation requires low standard errors for parameters. From preliminary experiments it was found that for any given amount of data, the variance parameters were estimated with considerably less precision than were the structural model parameters. Thus, a cut off rule was established as an aid to determining the impact of SE on confidence interval coverage for a parameter estimate, hence the efficiency with which such a parameter was estimated. For efficient estimation of Cl and V the "coefficient of variation" (i.e. $SE(\Theta_i)/\Theta_i$) associated with any estimate of any of these parameters for any given run had to be < 20% while that for the variance parameters had to be < 50%. Confidence intervals were calculated to determine the runs in a simulated data set which covered the "true" values. In addition, the 99% univariate confidence interval was used as suggested by Sheiner & Beal (1987) as a reasonable approximation for confidence interval estimates to contain 95% of the estimates produced using the ELS estimation procedure.

Bias in estimates production, and standard error of estimates are some of the factors that affect confidence interval coverage. Thus, there are three sections in confidence interval tables presented throughout the course of this thesis (e.g., Table 2.1). Section I, indicated by the ratio "success / total", shows confidence

Table 2.1 Individual and Joint Confidence Intervals Coverage for Parameter Estimates Obtained with the Two Sampling Times Design

2nd Sampling Time	Fraction Including True: Section I Success / Total					Section II (Success - Excluded) / (Total - Excluded)					Section III (Success - Excluded) / Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
90	177/180	179/180	154/180	174/180	145/180	177/180	179/180	148/171	174/180	137/171	177/180	179/180	148/180	174/180	137/180
150	178/180	178/180	180/180	169/180	166/180	178/180	178/180	179/180	169/180	166/180	178/180	178/180	179/180	169/180	166/180
210	178/180	175/180	177/180	169/180	160/180	178/180	175/180	138/139	169/180	133/139	178/180	175/180	138/180	169/180	133/180
240	177/180	177/180	180/180	160/180	155/180	177/180	177/180	110/110	168/176	100/110	177/180	177/180	110/180	168/180	100/180

intervals coverage for parameter estimates when the cut off rule is not applied. Section II, denoted by the ratio "success - excluded / total - excluded", shows interval estimates when the cut off rule is applied to both the numerator and denominator during confidence interval coverage computation. The estimates not used for the construction of these confidence intervals are herein referred to as "catastrophic" estimates. Thus, this section gives an indication of how good the coverage is if catastrophic estimates were deleted from the results. The last section of the table (Section III) shows the coverage when the catastrophic estimates are included in the denominator but discounted in the numerator for the computation of confidence interval coverage. With Section III the acceptability of an estimate can be judged in combination with the accuracy with which such an estimate is produced. From Fig. 2.5, for instance, the estimate of σ_{Cl} obtained with sampling time specification at 240 min. in a study in which the effect of the arrangement of sampling times on parameter estimation was studied, is almost unbiased. However, an examination of the confidence interval coverage (Table 2.1, Section III) which was computed from the results of the experiment presented in Fig. 2.5 shows that 70 NONMEM runs yielded catastrophic estimates of this parameter. Thus, Section III is helpful in determining the reliability of an estimate.

Parameters of a model are not estimated individually, and consideration should be given to this in results interpretation. Thus, the "joint confidence interval" for all parameter estimates was computed as an aid to the interpretation of the efficiency with which all parameters were estimated. The approximate 99% joint confidence interval for all parameter estimates was computed from the number of runs containing true parameter values for all parameters of the model. 99% individual and joint confidence intervals coverage for parameter estimates is used throughout the course of this thesis.

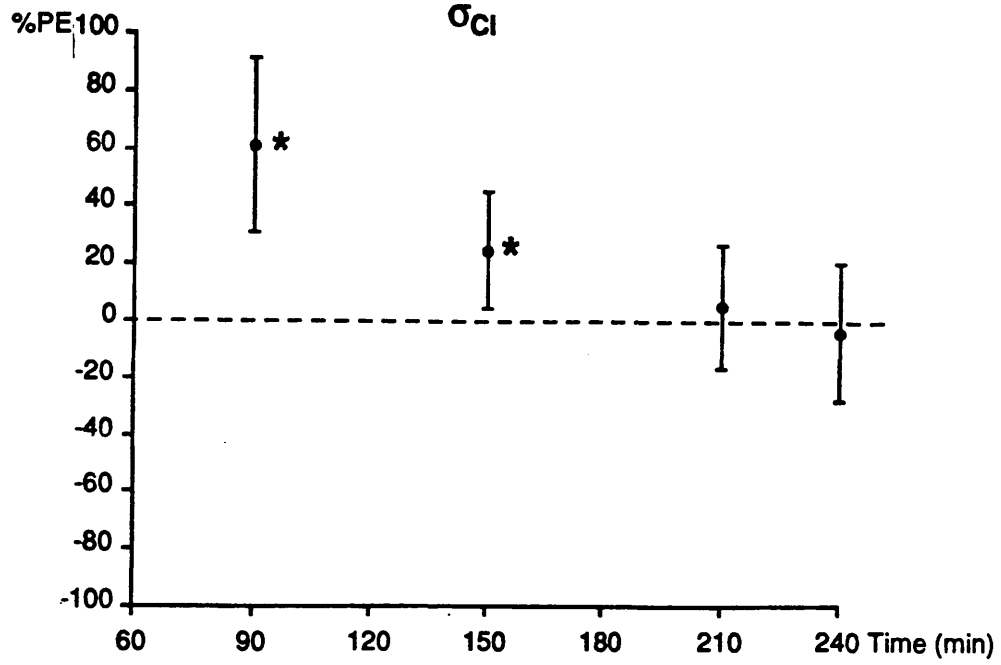


Fig. 2.5 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for σ_{Cl}. The horizontal axis represents the different sampling times for the two sampling times design. The first sampling time was fixed at 5 min. while the second time was varied. Each vertical bar expresses the bias and precision of the population parameter estimate for each design. Significant ($p < 0.05$) biases are indicated by asterisks.

The chi - squared test ($p < 0.01$) was used to determine whether the individual or joint confidence intervals coverage for parameter estimates was significantly different from the expected values (e.g., 0.95 and 0.81 (4 parameters only), for individual and joint confidence intervals coverage, respectively, for the parameters of the one compartment model with IV bolus injection).

2.5.3 **Design Number**

Most pharmacokinetic studies are carried out to obtain estimates of parameters which define an assumed pharmacokinetic model. Parameter estimation procedures (such as NONMEM which is used throughout the course of this thesis) produce sets of interrelated estimates. However, in the interpretation of the results, relationships between parameters are usually ignored. Thus, in the comparison of study designs used in parameter estimation, there is a need for an examination of the efficiency with which all model parameters are estimated singly and jointly from a design.

In Section 2.5.1 of this chapter the usual approach to judging accuracy and precision was presented. However, this method of analysis allows the investigator to judge the efficiency of estimation of only one parameter at a time. In the previous section the joint confidence interval was introduced as an aid to judging the efficiency with which all population pharmacokinetic parameters were estimated. Sometimes it may be difficult to choose the best sampling design, for instance, from a series of designs for the estimation of a parameter of interest. In addition, it was pointed out in the previous section that the reliability of a parameter estimate had to be judged with its SE taken into account. The %PE approach ignores the fact that NONMEM produces parameter estimates with SE's.

To this end, a new statistic was proposed with which the efficiency of parameter estimation from a study design could be measured. In it the elements of accuracy and precision in parameter estimation are combined. The statistic, a "design number", Φ_i for each parameter was defined:

$$\Phi_i = \{(\Theta_i - \Theta_i^*)/\Theta_i^*\}^2 * SE(\Theta_i)/\Theta_i^* \qquad (2.27)$$

Φ_i has two desirable properties which are useful for determining the most efficient parameter estimate. It should be recalled that $\Theta_i - \Theta_i^*$ measures the bias in the estimation of a parameter. SE, of course measures precision. The two terms on the right hand side of Eq. (2.27) are normalised to allow the comparison of different estimates of a parameter from different designs within a study.

Since accuracy is improved as Θ_i approaches Θ_i^*, the first term on the right hand side of Eq. (2.27) will approach 0 as this happens. This term is squared so that all computed values of Φ_i remain positive. As the parameter estimate becomes more precise, $SE(\Theta_i)$ becomes smaller. As the two right hand terms in Eq. (2.27) tend towards 0, Φ_i approaches 0 indicating greater efficiency with which the parameter is estimated. If a reasonably symmetrical distribution for Θ_i is assumed, then the distribution of Φ_i is skewed right. 95% confidence intervals can be calculated for different designs within a study.

From Eq. (2.27), Φ_i defines a design number for each parameter viewed independently. As earlier discussed, model parameters are estimated as a set, and an investigator may be interested in choosing a study design which produces the most efficient parameter estimates. This can be done by combining all design numbers to give the "overall design number". Thus,

$$\Phi = \Sigma\{[(\Theta_i - \Theta_i^*)/\Theta_i^*]^2 * SE(\Theta_i)/\Theta_i^*\} / n \qquad (2.28)$$

where n is the number of estimated parameters. The power and efficiency of Φ_i and Φ are outside the scope of this thesis.

A preliminary study was carried out in order to apply Φ_i (Φ) in determining the efficiency with which parameters were estimated with different sampling schedules using the two sampling times design.

Parameters of the drug, avicin (Section 2.4) were used to simulate data with the first sampling time fixed at 5 min. and the second sampling time specified at either 90, 150, 210, or 240 min. With the one observation per animal study design a sample size of 48 was used for each sampling strategy. 180 replicates of data were generated for each of the sampling schedules. Table 2.2 is a summary of the 95% confidence intervals for Φ_i and Φ for the different sampling schedules of this two sampling times design. It can be seen that Φ_i values for the variance parameters had more influence in Φ computed.

To give equal weighting to all parameters, Φ_i was rescaled as follows:

$$\Phi_{ir} = \{(\Theta_i - \Theta_i^*)/\Theta_i^*\}^2 * SE(\Theta_i)/\Theta_i^* \,/\, Max\,[\{(\Theta_i - \Theta_i^*)/\Theta_i^*\}^2 * SE(\Theta_i)/\Theta_i^*] \tag{2.29}$$

Therefore, the overall design number was computed as follows:

$$\Phi_r = 1/n \;\Sigma\{(\Theta_i - \Theta_i^*)/\Theta_i^*\}^2 * SE(\Theta_i)/\Theta_i^* \,/\, Max\,[\{(\Theta_i - \Theta_i^*)/\Theta_i^*\}^2 \; * \; SE(\Theta_i)/\Theta_i^*] \tag{2.30}$$

Φ_{ir}, Φ_r calculated using Eq. (2.29) and (2.30) were then used to compare the efficiency of parameter estimation from the different sampling schedules.

Table 2.2 Efficiency of Parameter Estimation: Two Sampling Times Design

2nd Sampling Time	Design Number (Φ_i, Φ) Median (95% Confidence Interval)				
	Cl	V	σ_{Cl}	σ_V	Overall
90	0.00013	0.00005	2.630	0.544	0.997
	(0.00009, 0.00018)	(0.00004, 0.00006)	(2.050, 3.430)	(0.395, 1.204)	(0.825, 1.204)
150	0.00004	0.00006	0.230	0.783	0.320
	(0.00003, 0.00006)	(0.00005, 0.00007)	(0.172, 0.324)	(0.585, 1.002)	(0.253, 0.404)
210	0.00004	0.00006	0.053	0.585	0.195
	(0.00003, 0.00005)	(0.00005, 0.00007)	(0.042, 0.067)	(0.413, 0.848)	(0.146, 0.274)
240	0.00006	0.00006	0.065	0.775	0.242
	(0.00005, 0.00007)	(0.00005, 0.00008)	(0.046, 0.083)	(0.531, 1.106)	(0.169, 0.315)

Table 2.3 summarises the 95% confidence intervals for Φ_{ir} and Φ_r obtained with the different sampling schedules for comparison with Table 2.2. The rescaling resulted in changes in Φ_i and Φ, therefore, giving equal weighting to all parameters. The Kruskal Wallis test ($p < 0.05$) with multiple comparisons was used to compare the efficiency with which parameters were estimated with the different sampling schedules. Thus, the most efficient parameter estimate(s) is obtained with the study design yielding the lowest average rank of Φ_{ir} (Φ_r).

The results of the multiple comparisons in this example are summarised in Fig. 2.6. The design numbers for the sampling times are ranked in increasing order from left to right, and this format is used in the presentation of results obtained using Φ_{ir} (Φ_r) throughout the course of this thesis. The design yielding the least efficient parameter estimate has the highest rank order. Where two sampling times in the Fig.2.6, for instance, are connected with a line it indicates that there was no significant difference in the efficiency with which a parameter was estimated with the designs considered. When two sampling times are not connected with each other by a line, it indicates that there was a significant difference in the efficiencies with which the parameters considered were estimated using the different sampling designs.

Thus, from Fig.2.6a Cl was most efficiently estimated with the specification of the second sampling time at 150 min., but this was not significantly different from the estimate obtained with the second sampling time specified at 210 min. However, the efficiency of Cl estimation with this two sampling times schedule was significantly better than the efficiency with which it was estimated when the second sample was at 90 or 240 min. The results obtained with the second sample at 90 min. yielded the most inefficient estimate of Cl. Although V was most efficiently estimated with the second sample at 90 min. (Fig.2.6b), this was not significantly better than results obtained with the other sampling

Table 2.3 Efficiency of Parameter Estimation: Two Sampling Times Design

2nd Sampling Time	Design Number (Φ_{ir}, Φ_r) Median (95% Confidence Interval) Cl	V	σ_{Cl}	σ_V	Overall
90	0.01194	0.03071	0.0365	0.0178	0.0338
	(0.00931, 0.01505)	(0.02056, 0.05208)	(0.0305, 0.0488)	(0.0149, 0.0217)	(0.0292, 0.0535)
150	0.00703	0.04043	0.0132	0.0256	0.0124
	(0.00504, 0.00817)	(0.03564, 0.05710)	(0.0116, 0.0187)	(0.0191, 0.0328)	(0.0092, 0.0186)
210	0.00703	0.03593	0.0099	0.0191	0.0114
	(0.00504, 0.00796)	(0.03064, 0.05013)	(0.0090, 0.0201)	(0.0105, 0.0277)	(0.0102, 0.0165)
240	0.01007	0.03683	0.0101	0.0253	0.0203
	(0.00801, 0.01208)	(0.02877, 0.05201)	(0.0091, 0.0147)	(0.0200, 0.0432)	(0.0145, 0.0362)

(a) Estimation of Cl

150	210	240	90	Sampling Times (min)

(b) Estimation of V

90	210	240	150	Sampling Times (min)

(c) Estimation of σ_{Cl}

240	210	150	90	Sampling Times (min)

(d) Estimation of σ_V

90	210	240	150	Sampling Times (min)

(e) Overall Design Efficiency

210	150	240	90	Sampling Times (min)

Fig. 2.6 [a]Summary of significant differences in the efficiency* with which parameters were estimated using the two sampling times design.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.
The connecting of sampling times with a line indicates a lack of significant difference between the designs.

schedules.

σ_{Cl} was "best estimated" with the second sample at 240 min. (Fig.2.6c), but this was not significantly better than when the second sample was at 210 min. These two sampling schedules produced more efficient estimates of this parameter than the design with the second sample at 90 min.

As with V, the efficiency with which σ_V was estimated was similar for all the study designs although the design with the lowest average rank of Φ_{ir} was the one with the second sampling time specified at 90 min. (Fig.2.6d).

Fig.2.6e gives the overall efficiency with which parameters were estimated. The parameters were estimated with a similar efficiency when the second sample was at 150, 210, or 240 min. More efficient parameter estimates were obtained with these sampling schedules than with the design having the second sample at 90min. Specifying the second sample at 210 min. yielded the most efficient parameter estimates. Φ_{ir} and Φ_r were applied in the analysis of data in Chapters 4, 5, 6, and 8 of this thesis.

2.5.3 **Correlation Analysis**

Model parameters are not estimated independently but as an interrelated set giving rise to the generation of a correlation matrix for parameters. The interpretation of this should be considered in the overall interpretation of the results of a study. In the course of this thesis, the incidence of "high" correlation between parameter estimates is used to examine the reliability of parameter estimates. Two parameters are judged to be highly correlated if the pair-wise correlation coefficient is ≥ 0.75. otherwise, it is termed low. Thus, the study reported in Section 5.3 yielded 0% incidence of high pair-wise correlations for the different parameter combinations (Table 2.4). When the incidence of high

Table 2.4 Incidence of Correlation Values Associated with Parameters for the Two Sampling Times Design

Sampling Times (min)	90		150		210		240	
Correlation	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs Cl	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs V	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs V	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs σ_{Cl}	0%	100%	0%	100%	0%	100%	0%	100%

a: High = Correlation coefficient ≥ 0.75

b: Low = Correlation coefficient < 0.75

pair-wise correlations between most parameters is high it is an indication of a "poor" fit of the model to the data since the data in the studies reported in this thesis were generated with the assumption that all model parameters were independent.

CHAPTER 3

INFLUENCE OF INTER-ANIMAL VARIABILITY ON THE ESTIMATION OF POPULATION PHARMACOKINETIC PARAMETERS

3.1 SUMMARY

Simulation studies were carried out to investigate the influence of inter-animal variability on the estimation of population fixed and random effects parameters. Data were simulated according to a monoexponential model with the one observation per animal study design and a range of inter-animal variability in Cl and V.

The efficiency with which the fixed effect parameters were estimated was good, on average, irrespective of the inter-animal variability in Cl and V. The estimates of the random effect parameters were sometimes imprecise and often inaccurate.

3.2 INTRODUCTION

Variability in pharmacokinetic parameters among homogeneous strains of small laboratory animals has been claimed to be between 30 and 50% in some cases (Lindstrom & Birkes, 1984; McArthur,1988). There is need to investigate the effect of this wide range of variability on the estimation of population pharmacokinetic parameters in a setting where each animal supplies only one concentration time point as is often the case in preclinical studies involving the use of small laboratory animals. The goal of this simulation - based study was to evaluate the influence of inter-animal variability on the estimation of population pharmacokinetic parameters using the one observation per animal study design. Specifically, the accuracy and precision with which these parameters were estimated, the "normality" of their sampling distributions, single and joint confidence intervals coverage of parameters estimates, and the incidence of high correlation between pairs of parameter estimates were examined.

3.3 METHODS

Parallel simulations were performed for two different studies. In the first study, three observations were obtained from 3 different animals at each time point. In the second study, 5 observations were obtained from 5 animals at each time point. In both studies only one observation was taken per animal. An intravenous bolus dose and sampling design previously described in Chapter 2 (Section 2.4) was used in these studies. Cl and V for the jth animal were sampled as previously described in Chapter 2 (Section 2.4), and the respective variances were selected to yield coefficients of variation of 15, 30, 45, and 60%. There were 4 * 4 combinations of variability in Cl and V (Table 3.1). These combinations of variability were chosen to cover the range of inter-animal variability likely to be encountered in real life (Lindstrom & Birkes, 1984; McArthur 1988). A 15% error was added to concentration measurements as previously described (Chapter 2, Section 4.1). 30 data sets were generated for each combination of σ_{Cl} and σ_V for each study. Thus, 480 data sets were generated for each study, and 960 data sets in all.

The chi - squared test ($p < 0.05$) was used to determine the normality of the distribution of the estimates obtained for the fixed and random effects parameters. When the assumption of normality was rejected further testing showed that the distributions were significantly positively skewed ($p < 0.05$), but tests of kurtosis were not appropriate since the test is only valid on sample sizes of greater than 50.

Table 3.1 Combinations of Inter-Animal Variability

Parameter	σ_V(%)			
σ_{Cl}(%)	15	30	45	60
15	*	*	*	*
30	*	*	*	*
45	*	*	*	*
60	*	*	*	*

Table 3.2 Runs with Acceptable Parameter Estimates: 3 Observations per Time Point

Parameter	σ_V(%)			
σ_{Cl}(%)	15	30	45	60
15	29	30	29	30
30	30	30	30	30
45	29	29	28	29
60	29	28	29	29

Table 3.3 Runs with Acceptable Parameter Estimates: 5 Observations per Time Point

Parameter	σ_V(%)			
σ_{Cl}(%)	15	30	45	60
15	30	30	30	30
30	29	30	30	30
45	30	30	27	26
60	30	30	30	26

3.4 RESULTS

3.4.1 Acceptable NONMEM Runs

Using the outlier criteria stipulated in Chapter 2 (Section 2.5.1) the number of times over 30 replications in each simulation that NONMEM produced acceptable estimates and corresponding standard errors was determined. All Cl and V estimates were acceptable (Chapter 2, Section 2.5.1). Estimates of σ_{Cl} and σ_V were acceptable (90 to 100%) in most of the combinations of inter-animal variability except for the 45% * 60% and 60% * 60% combinations of σ_{Cl} and σ_V where the acceptable estimates dropped to 86.7% (Table 3.3). Runs with unacceptable estimates were deleted, and the results presented are based on runs with acceptable estimates.

3.4.2 Bias and Precision

Three dimensional plots are used to summarise the relationships between the various combinations of σ_{Cl} and σ_V and the mean %PE while two dimensional plots are used to show both bias and precision for various values of σ_{Cl} at a specified value of σ_V. Thus, in the presentation that follows the bias and precision in parameter estimation are considered for each level of σ_V and at various levels of σ_{Cl} for each study. With three observations per time point, and setting σ_V at 15% while σ_{Cl} was varied between 15 and 60%, all estimates of Cl were negatively biased. The most biased estimate was obtained when σ_{Cl} was specified at 60% while the least biased estimate was obtained with σ_{Cl} specified

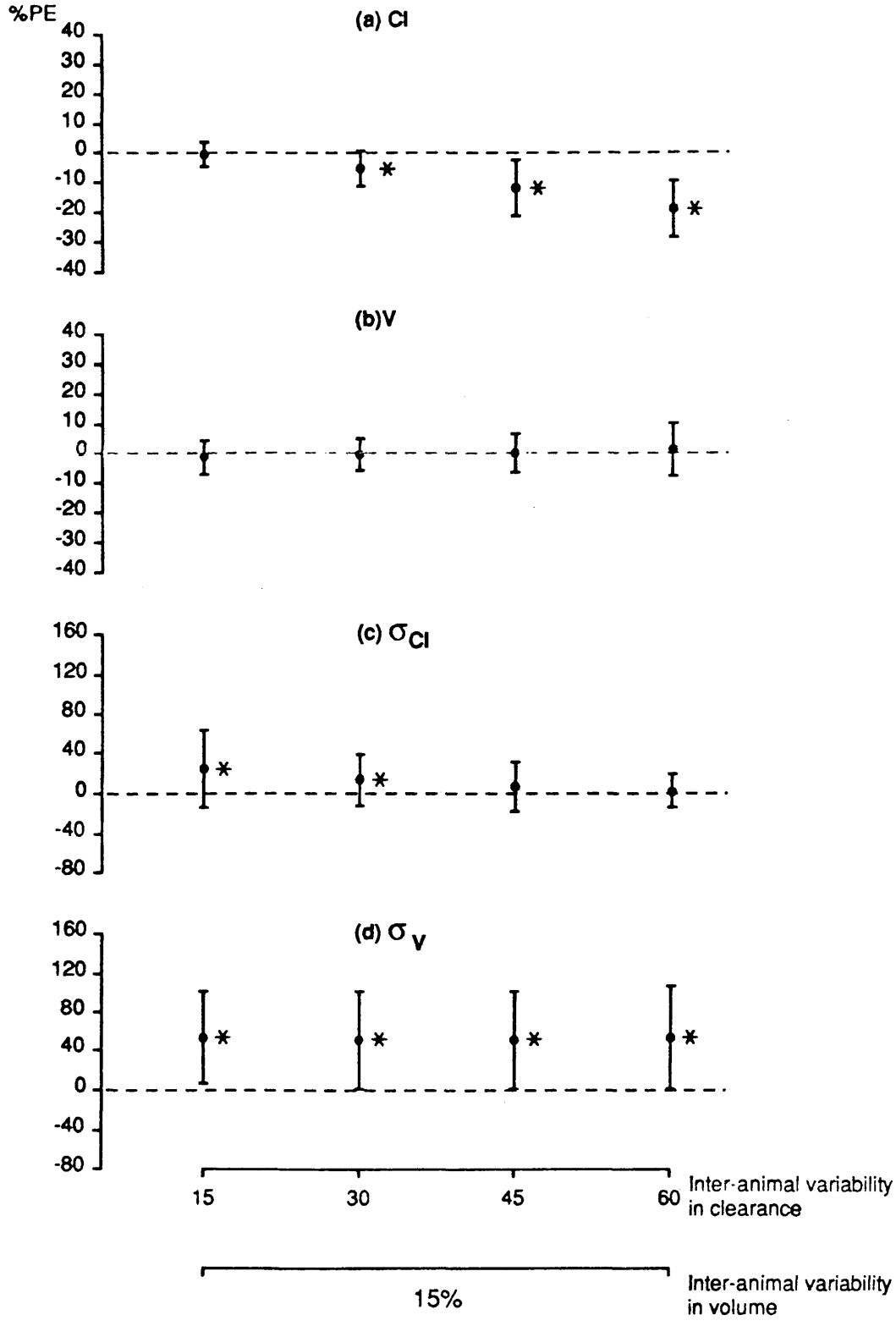


Fig. 3.1 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with a specified value of σ_V (15%). Each vertical bar expresses the bias and precision of the population parameter estimate. 3 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

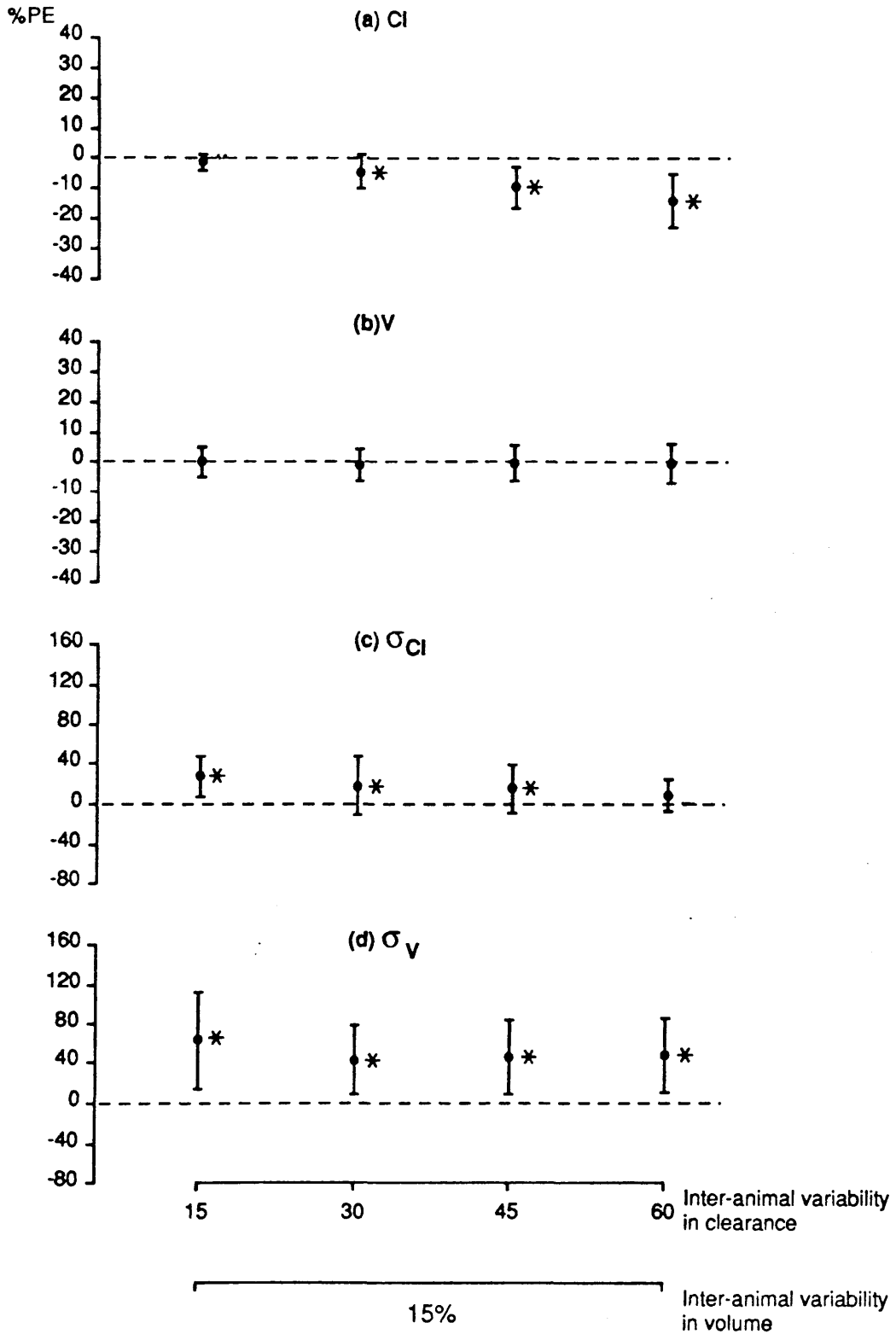


Fig. 3.2 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with σ_V set at 15% . Each vertical bar expresses the bias and precision of the population parameter estimate. 5 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

at 15%. The bias ranged from -0.5% (σ_{Cl} (σ_V) = 15%) to -18.7% (σ_{Cl} = 60%, σ_V = 15%) (Fig. 3.1a). All estimates of Cl were precise for all combinations of σ_{Cl} and σ_V (Fig. 3.1a). The SD of %PE ranged from 4.2% (σ_{Cl}, σ_V = 15%) to 9.6% (σ_{Cl} and σ_V = 60 and 15%, respectively).

The bias in Cl estimates obtained when 5 observations were made at each time point ranged from -1.2% (σ_{Cl}, σ_V = 15%) to -13.9% (σ_{Cl} = 60%, σ_V = 15%) (Fig. 3.2a), and all the estimates were more precise than those obtained when three observations were made per time point. The SD of %PE ranged from 2.7% (σ_{Cl} (σ_V) = 15%) to 8.9% (σ_{Cl} = 60%, σ_V = 15%).

When σ_V was 15% and σ_{Cl} varied between 15 and 60% relatively unbiased and precise estimates of V were obtained (Fig. 3.1b) when 3 observations were made per time point. The %PE ranged from -0.2 ± 5.4% (σ_{Cl} (σ_V) = 15%) to 1.4 ± 9.0% (σ_{Cl} = 60%, σ_V = 15%). Similar results were obtained when 5 observations were made per time point. All estimates of V were relatively unbiased and precise (Fig. 3.2b). The SD of %PE ranged from 5.2% (σ_{Cl} (σ_V) = 15%) to 6.6% (σ_{Cl} = 60%, σ_V = 15%).

Varying σ_{Cl} from 15 to 60% and fixing σ_V at 15% yielded estimates of σ_{Cl} at the different combinations of variability which were positively biased with 3 observations made per time point. As with Cl estimates there was a trend in the degree of bias associated with σ_{Cl} estimates. While Cl estimates showed a trend of bias from σ_{Cl} and σ_V combination of 15% * 15% yielding the least biased estimate to the 60% * 15% combination yielding the most biased estimate (Fig. 3.1a), the reverse was the case for estimates of σ_{Cl} (Fig. 3.1c). The 15% * 15% combination of inter-animal variability yielded the most biased and least precise estimate of σ_{Cl} while the 60% * 15% combination yielded the least biased and most precise estimate of this parameter (Fig. 3.1c). The %PE ranged from 2.44 ± 16.4% for the 60% * 15% combination to 24.4 ± 39.2% for the 15% * 15% combination. A similar trend was observed in the bias associated with the

estimation of σ_{Cl} when 5 observations were made per time point. The least biased estimate was observed with σ_{Cl} and σ_V set at 60 and 15%, respectively, and the most biased was obtained with σ_{Cl} and σ_V fixed at 15% (Fig. 3.2c). There was no clear cut pattern in the precision of the estimates (Fig. 3.2c). The SD of %PE ranged from 16.0% (σ_{Cl} = 60%, σ_V = 15%) to 28.9% (σ_{Cl} = 30%, σ_V = 15%).

Estimates of σ_V obtained by varying σ_{Cl} from 15 to 60% while setting σ_V at 15% were relatively stable across the different values of σ_{Cl} when 3 observations were made per time point. All the estimates were significantly positively biased and imprecise (Fig. 3.1d). Also, when 5 observations were made per time point all σ_V estimates were significantly positively biased and imprecise (Fig. 3.2d).

When σ_V was set at 30% and σ_{Cl} varied from 15 to 60% estimates of Cl which were biased with a positive to negative trend were obtained; positive at 15% and negative at σ_{Cl} of 60% when 3 observations were made per time point. The most biased estimate of Cl was obtained when σ_{Cl} was specified at 60% (Fig. 3.3a). All Cl estimates were precise (Fig. 3.3a). The SD of %PE ranged from 4.9% (σ_{Cl} = 15%, σ_V = 30%) to 10.9% (σ_{Cl} = 60%, σ_V = 30%). Similarly, the most biased estimate of Cl was obtained in the 5 observations per time point study with σ_{Cl} and σ_V specified at 60 and 30%, respectively (Fig. 3.4a). The bias ranged from 1.7% (σ_{Cl} = 15%, σ_V = 30%) to -12.7% (σ_{Cl} = 60%, σ_V = 30%). These estimates were precise with the SD of %PE ranging from 4.0% (σ_{Cl} = 15%, σ_V = 30%) to 10.0% (σ_{Cl} = 45%, 60%; σ_V = 30%).

All estimates of V were significantly negatively biased, relatively stable, but precise at all levels of σ_{Cl} when σ_V was 30% and 3 observations were made at each time point (Fig. 3.3b). The least precise estimate was obtained when σ_{Cl} and σ_V were 60 and 30%, respectively. In addition, similar results were obtained for V estimates when 5 observations were made at each time point.

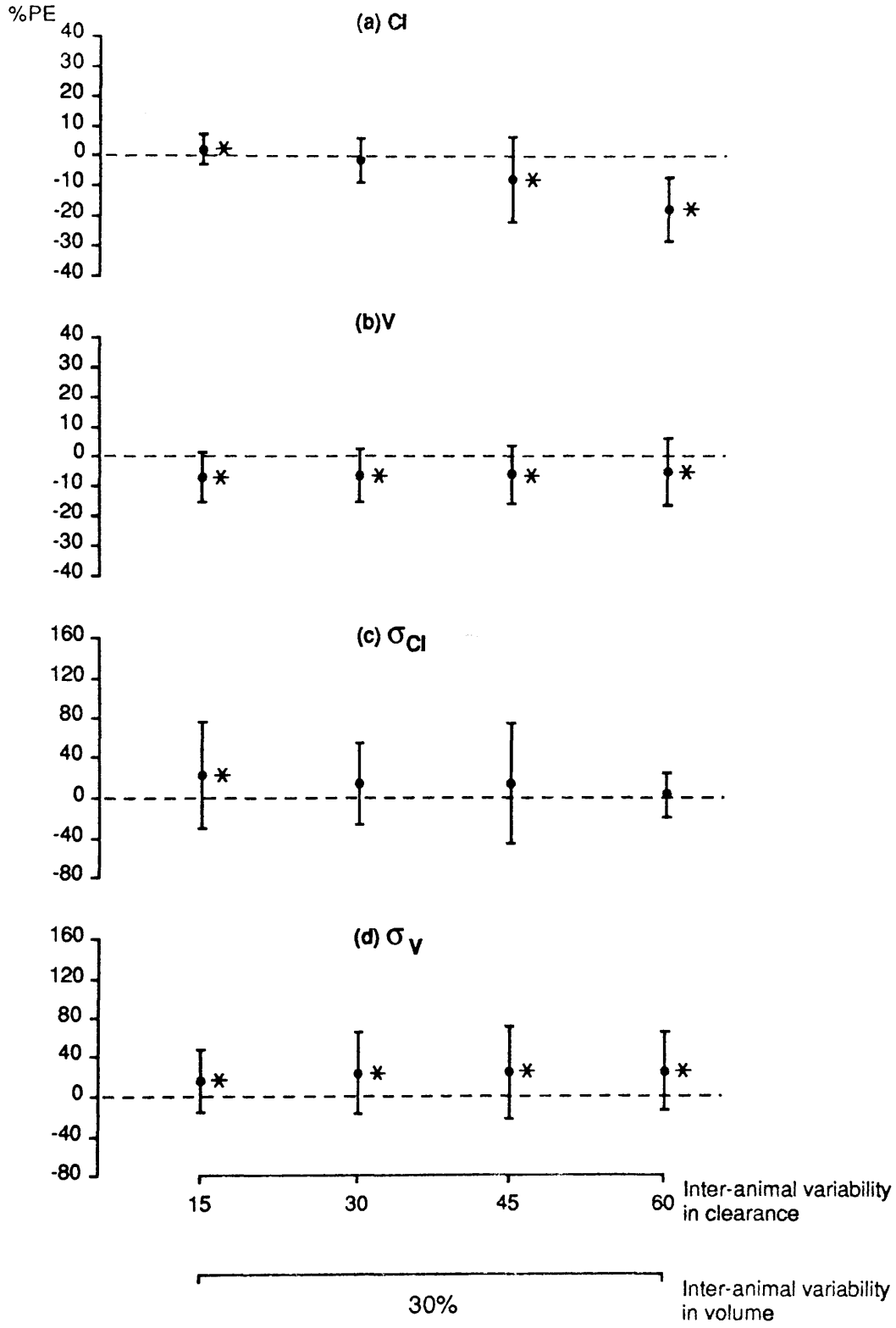


Fig. 3.3 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with a specified value of σ_V (30%). Each vertical bar expresses the bias and precision of the population parameter estimate. 3 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

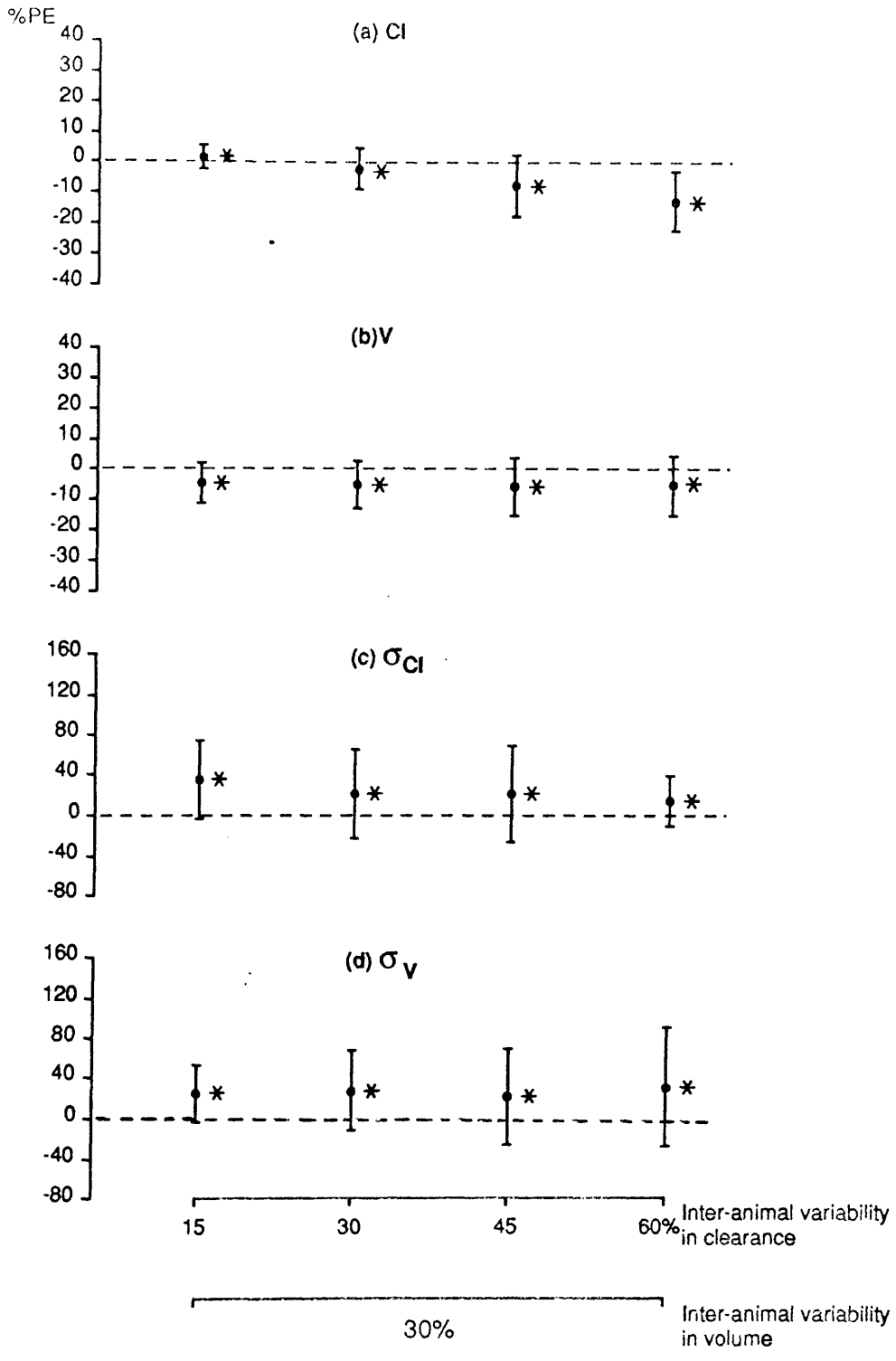


Fig. 3.4 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with σ_V set at 30% . Each vertical bar expresses the bias and precision of the population parameter estimate. 5 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

All estimates of this parameter were significantly negatively biased and precise (Fig. 3.3b). Setting σ_{Cl} and σ_V at 15 and 30%, respectively, produced the least biased estimate of V while the 60% * 30% combination produced the least precise estimate of this parameter.

All σ_{Cl} estimates were positively biased irrespective of the value of σ_{Cl} used in combination with σ_V of 30% when 3 observations were made per time point. When σ_{Cl} and σ_V were fixed at 15 and 30%, respectively, σ_{Cl} estimate was significantly biased and imprecise (%PE = 23.2 ± 53.0%; Fig. 3.3c). The least biased estimate (%PE = 2.7 ± 22.1%) was obtained with σ_{Cl} and σ_V set at 60 and 30%, respectively. At the latter combination of inter-animal variability the estimate was acceptably precise (Chapter 2, Section 5.1). All other estimates of σ_{Cl} were imprecise (Fig. 3.3c). Similar, results were obtained when 5 observations were made per time point. A 15% (σ_{Cl}) * 30% (σ_V) combination yielded the most biased estimate and 60% (σ_{Cl}) * 30% (σ_V) combination produced the least biased estimate (Fig. 3.4c). Only the 60% * 30% inter-animal variability combination produced an acceptably precise estimate (SD of %PE = 25.2%; Fig. 3.4c) since the SD of %PE is only 0.2% greater then 25%.

Significantly positively biased and imprecise estimates of σ_V were obtained at all levels of σ_{Cl} when σ_V was 30% and 3 observations were made per time point (Fig. 3.3d). The mean of %PE ranged from 15.8% (σ_{Cl} = 15%, σ_V = 30%) to 25.6% (σ_{Cl} = 60%, σ_V = 30%) while the SD of %PE ranged from 32.1% (σ_{Cl} = 15%, σ_V = 30%) to 46.4% (σ_{Cl} = 45%, σ_V = 30%). Taking 5 observations per time point yielded similarly biased and imprecise estimates of σ_V (Fig. 3.4d).

When σ_V was 45% and σ_{Cl} was in the range of 15 to 60% the estimates of Cl obtained in the 3 observations per time point study were biased but precise. The estimates were significantly positively biased when σ_{Cl} was set at 15%, almost unbiased at 30%, and significantly negatively biased at 45 and 60% (Fig. 3.5a). The most biased estimate was obtained with the 60% * 45% combination of σ_{Cl}

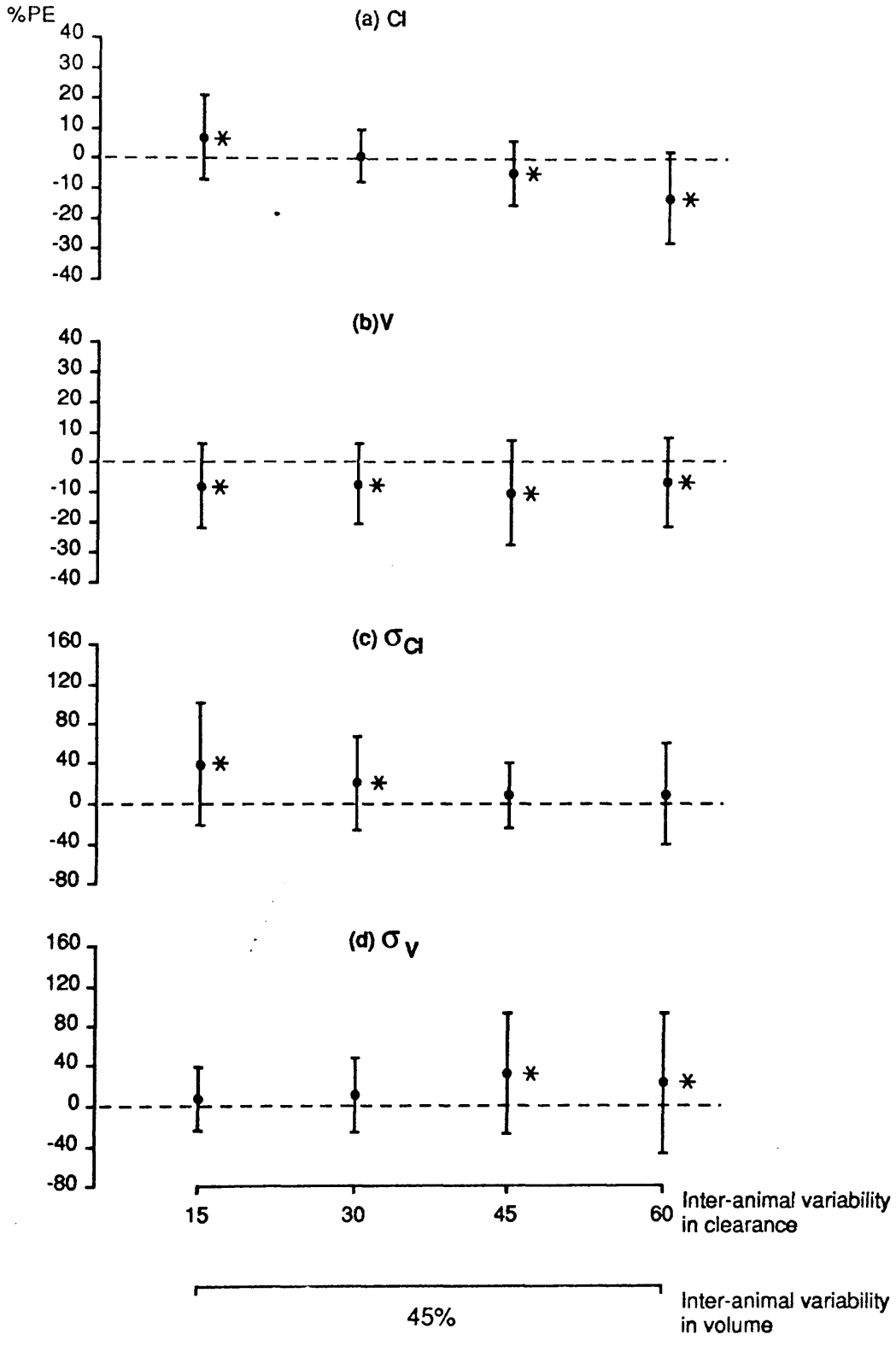


Fig. 3.5 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with a specified value of σ_V (45%). Each vertical bar expresses the bias and precision of the population parameter estimate. 3 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

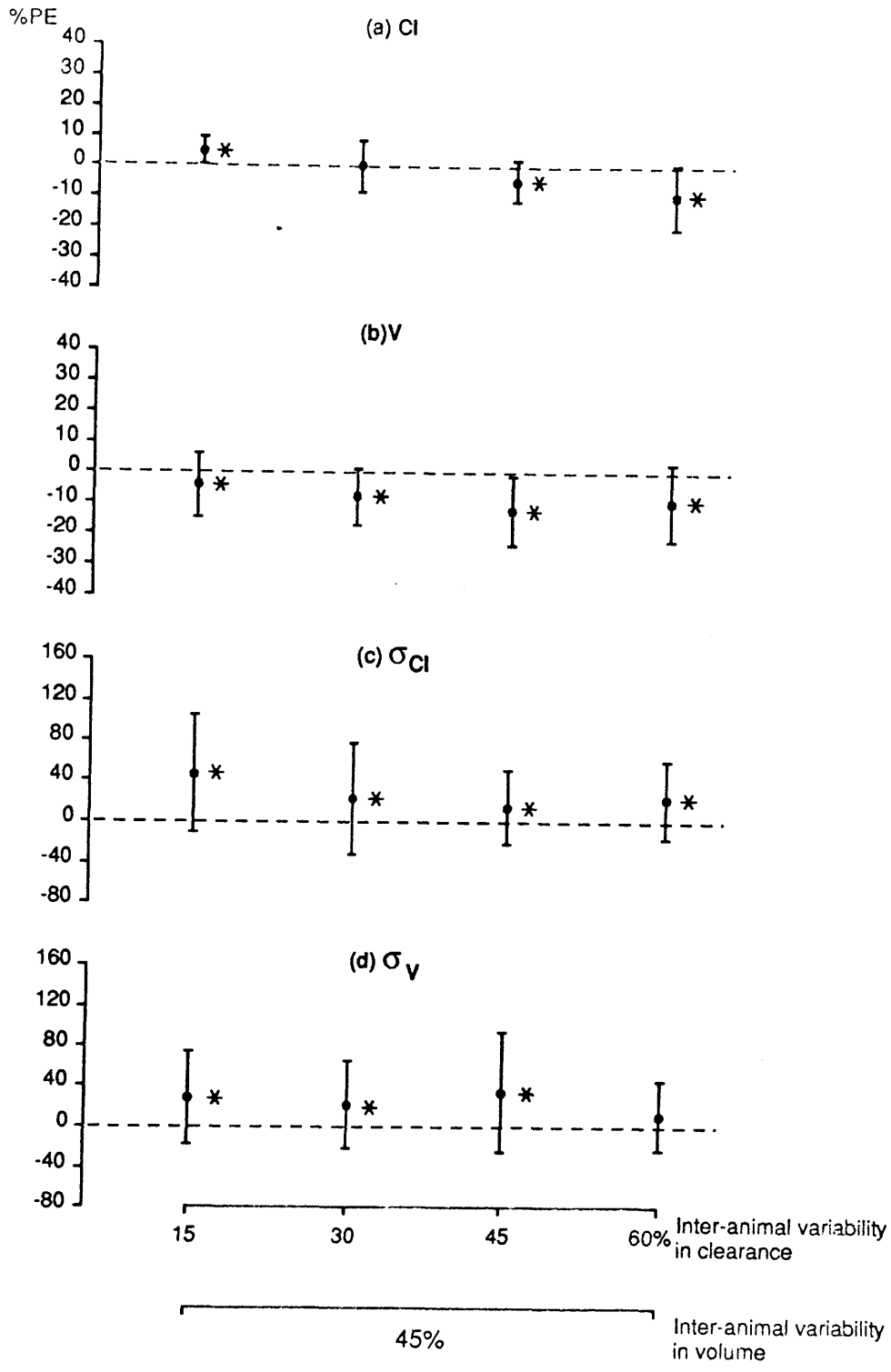


Fig. 3.6 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with σ_V set at 45% . Each vertical bar expresses the bias and precision of the population parameter estimate. 5 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

and σ_V. The SD of %PE ranged from 8.5% to 15.0%. For the 5 observations per time point study there was a trend in the bias from positive to the negative direction as σ_{Cl} was varied from 15 to 60%. The most biased estimate was obtained when σ_{Cl} and σ_V were specified at 60 and 45%, respectively (Fig. 3.6a). The SD of %PE ranged from 4.5% (σ_{Cl} = 15%, σ_V = 45%) to 10.6% (σ_{Cl} = 60%, σ_V = 45%).

V estimates obtained when 3 observations were made at each time point were significantly negatively biased at all levels of σ_{Cl} with σ_V fixed at 45% (Fig. 3.5b). The estimates were precise for each of the combinations of inter-animal variability with the SD of %PE ranging from 13.3 to 17.4% (Fig. 3.5b). When 5 observations were made per time point V estimates were significantly negatively biased and precise (Fig. 3.6b). The least biased estimate was obtained with the 15% (σ_{Cl}) * 45% (σ_V) combination.

With σ_V set at 45% the estimates of σ_{Cl} were positively biased when 3 observations were made at each time point. The most biased estimate was obtained when σ_{Cl} was specified at 15% and the least biased estimate when σ_{Cl} was fixed at 60% (Fig. 3.5c). All estimates of this parameter were imprecise (Fig. 3.5c). At this 45% level of σ_V all estimates of σ_V were significantly positively biased and imprecise (Fig. 3.5d). In addition, the estimates of σ_{Cl} when 5 observations were made per time point were imprecise and significantly positively biased (Fig. 3.6c). The estimates of σ_V obtained for this 5 observations per time point study at the different levels of σ_{Cl} with σ_V fixed at 45% were significantly positively biased and imprecise (Fig. 3.6d).

When σ_V was 60% and σ_{Cl} varied, estimates of Cl were positively biased when σ_{Cl} was set at either 15 or 30% and negatively biased when σ_{Cl} was set at either 45 or 60% with 3 observations made at each time point. The most biased and least precise estimate of this parameter was obtained with σ_{Cl} fixed at 60% (Fig. 3. 7a). All estimates of Cl were precise with SD of %PE ranging from

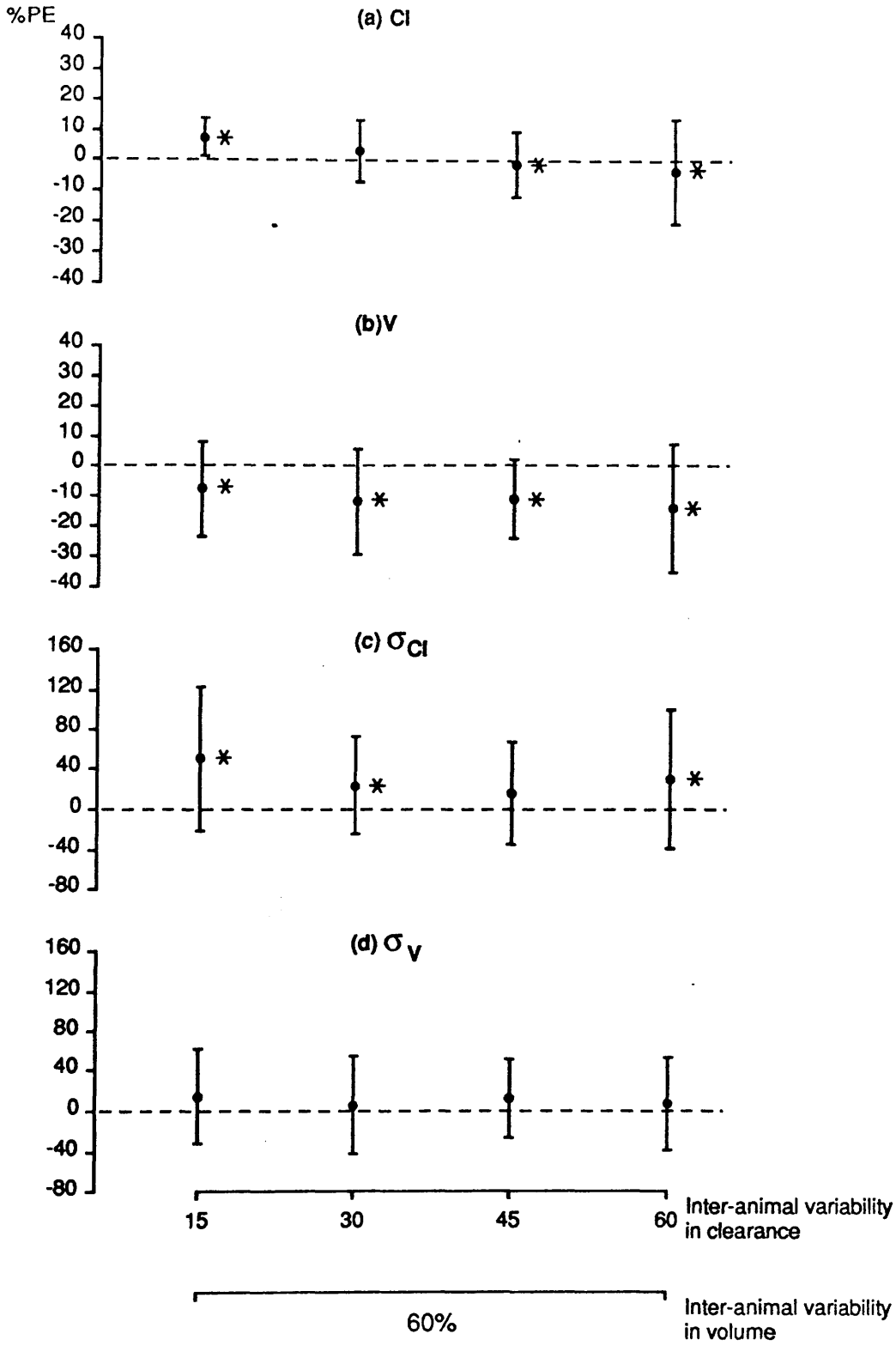


Fig. 3.7 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with a specified value of σ_V (60%). Each vertical bar expresses the bias and precision of the population parameter estimate. 3 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

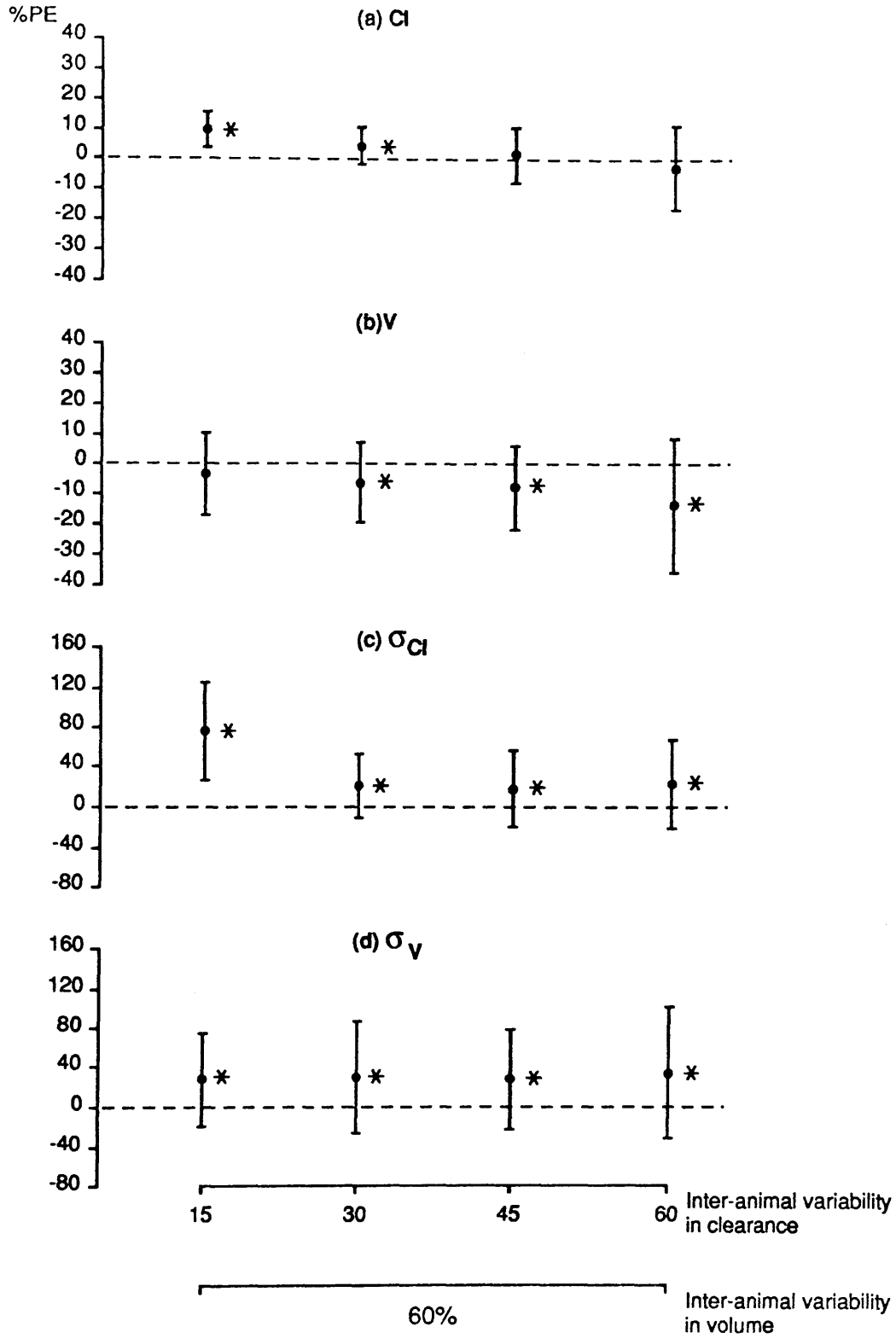


Fig. 3.8 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_{Cl} with σ_V set at 60% . Each vertical bar expresses the bias and precision of the population parameter estimate. 5 observations were made at each time point, and only one observation was made on each animal. Significant ($p < 0.05$) biases are indicated by asterisks.

6.0% (σ_{Cl} = 15%, σ_V = 60%) to 17.1% (σ_{Cl} (σ_V) = 60%; Fig. 3.7a). All estimates of V were significantly biased and precise at these specified values of σ_{Cl} and σ_V (Fig. 3.7b). The SD of %PE ranged from 13.0 to 21.3%. The most biased and least precise estimate of this parameter was obtained when σ_{Cl} and σ_V were set at 60%.

Making 5 observations at each time point when σ_V was 60% and σ_{Cl} varied between 15 and 60% yielded some estimates of Cl which were positively biased but precise (Fig. 3.8a). The %PE ranged from -2.4 ± 14.1% (σ_{Cl}, σ_V = 60%) to 9.6 ± 5.9% (σ_{Cl} = 15%, σ_V = 60%). Most of the estimates of V obtained with the various settings of σ_{Cl} when σ_V was fixed at 60% were significantly negatively biased (Fig. 3.8b). The mean of %PE ranged from -3.3% (σ_{Cl} = 15%, σ_V = 60%) to -13.6% (σ_{Cl} (σ_V) = 60%). All the estimates were acceptably precise with the SD of %PE ranging from 13.1 to 22.1%.

Most of the σ_{Cl} estimates obtained when 3 observations were made at each time point with σ_V at 60% and σ_{Cl} varied between 15 and 60% were significantly positively biased with poor precision (Fig. 3.7c). Similar findings were obtained when 5 observations were made per time point (Fig. 3.8c). The estimates of σ_V at these combinations of inter-animal variability were significantly positively biased and imprecise (Fig. 3.8d) when 5 observations were made per time point, but only positively biased and imprecise when 3 observations were made at each time point (Fig. 3.7d).

Overall, as the values of σ_{Cl} and σ_V were increased the bias in the estimation of Cl increased in both studies (Fig. 3.9a (3 observations per time point); Fig. 3.10a (5 observations per time point)). However, the bias in the estimates of V were relatively stable irrespective of the study considered (Fig. 3.9b (3 observations per time point); Fig. 3.10b (5 observations per time point)). In contrast to the results obtained with the estimation of Cl, the bias in the

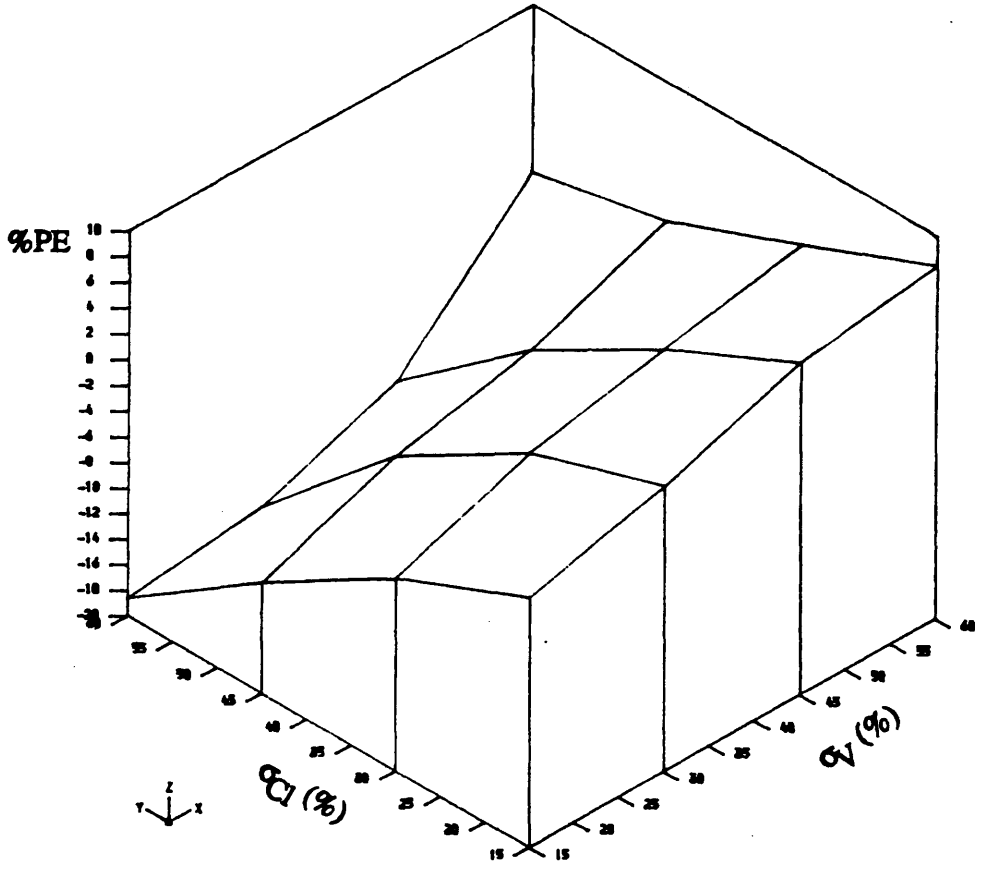


Fig. 3.9a Bias (expressed as mean %PE) in Cl estimation: three dimensional plot of the influence of varying σ_{Cl} and σ_V on the estimation of Cl. 3 observations were made per time point.

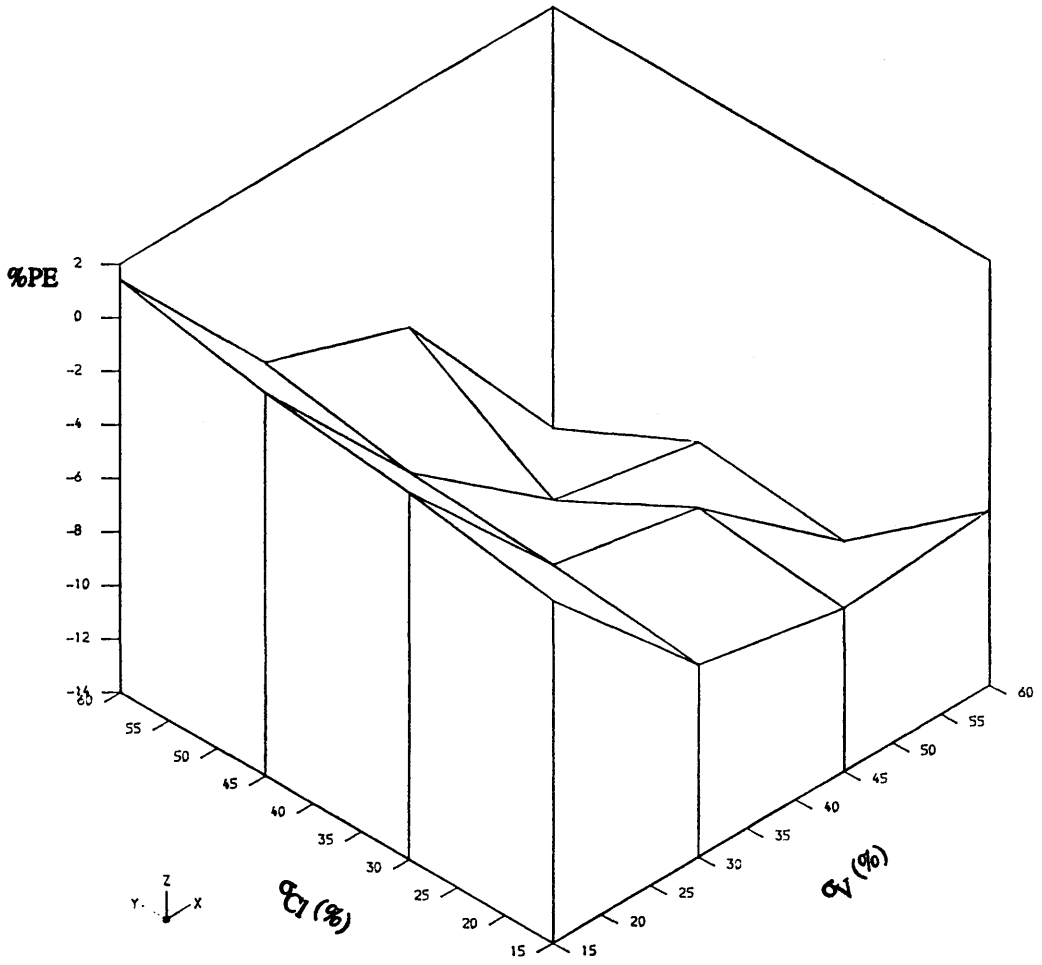


Fig. 3.9b Bias (expressed as mean %PE) in V estimation: three dimensional plot of the influence of varying σ_{Cl} and σ_V on the estimation of V. 3 observations were made per time point.

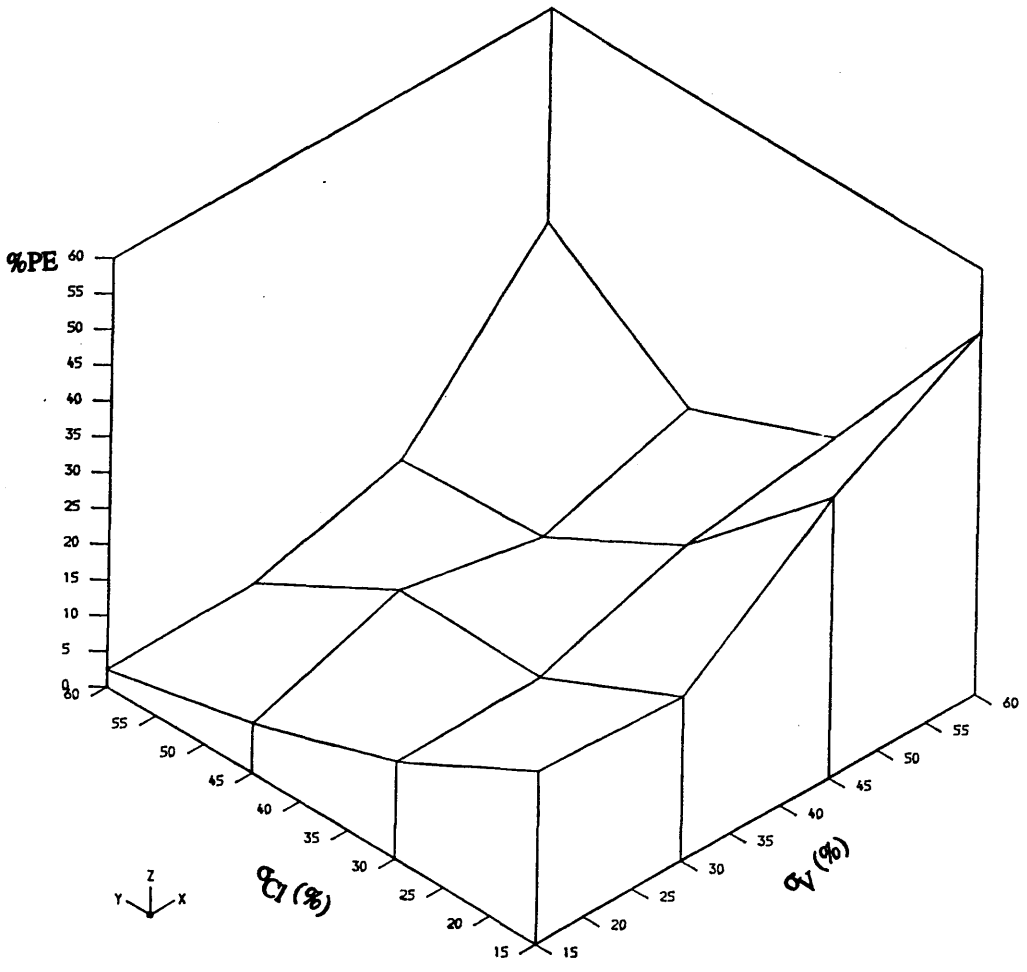


Fig. 3.9c Bias (expressed as mean %PE) in the estimation σ_{Cl}: three dimensional plot of the effect of varying σ_{Cl} and σ_V on the estimation of σ_{Cl}. 3 observations were made per time point.

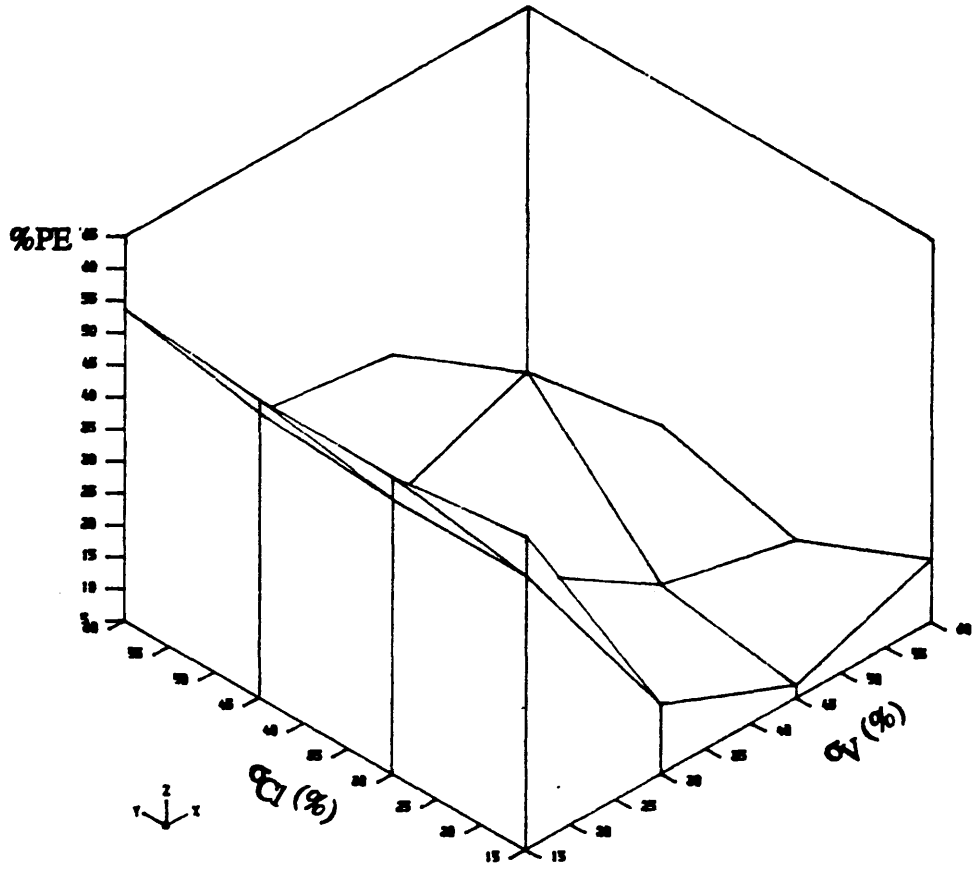


Fig. 3.9d Three dimensional plot of bias (expressed as mean %PE) in σ_V estimation as affected by varying inter-animal variability in Cl and V. 3 observations were made per time point.

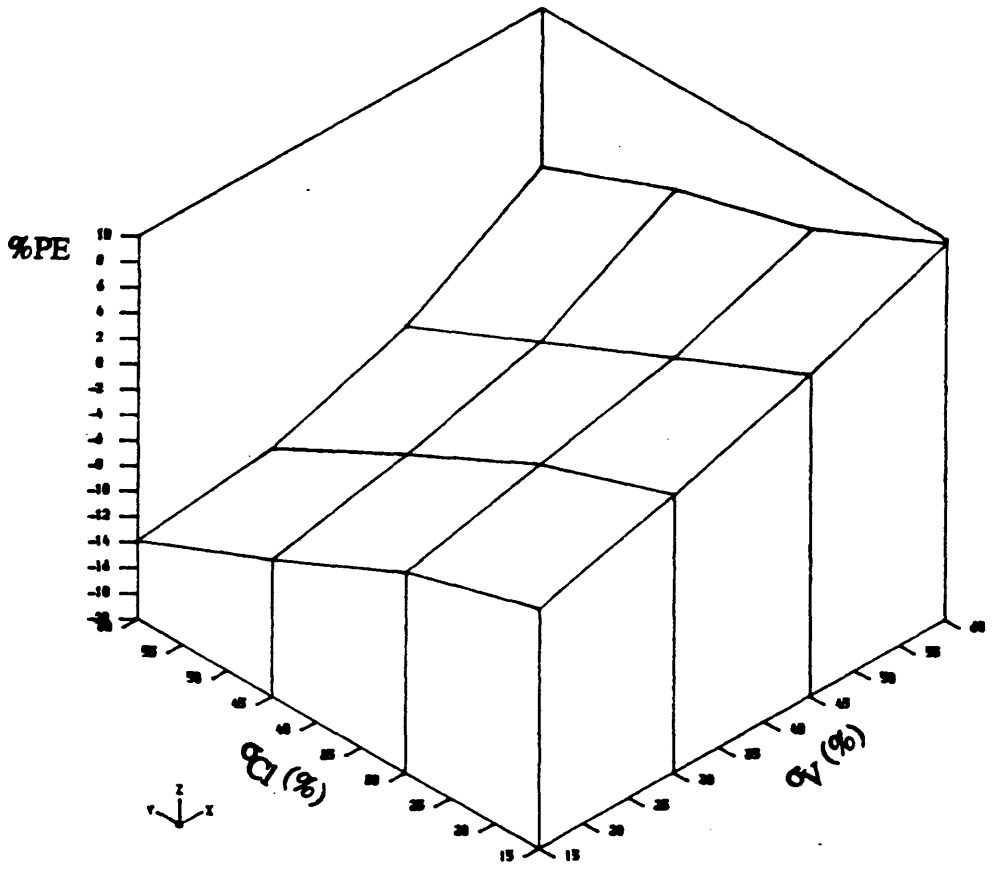


Fig. 3.10a Bias (expressed as mean %PE) in Cl estimation: three dimensional plot of the influence of varying σ_{Cl} and σ_V on the estimation of Cl. 5 observations were made per time point.

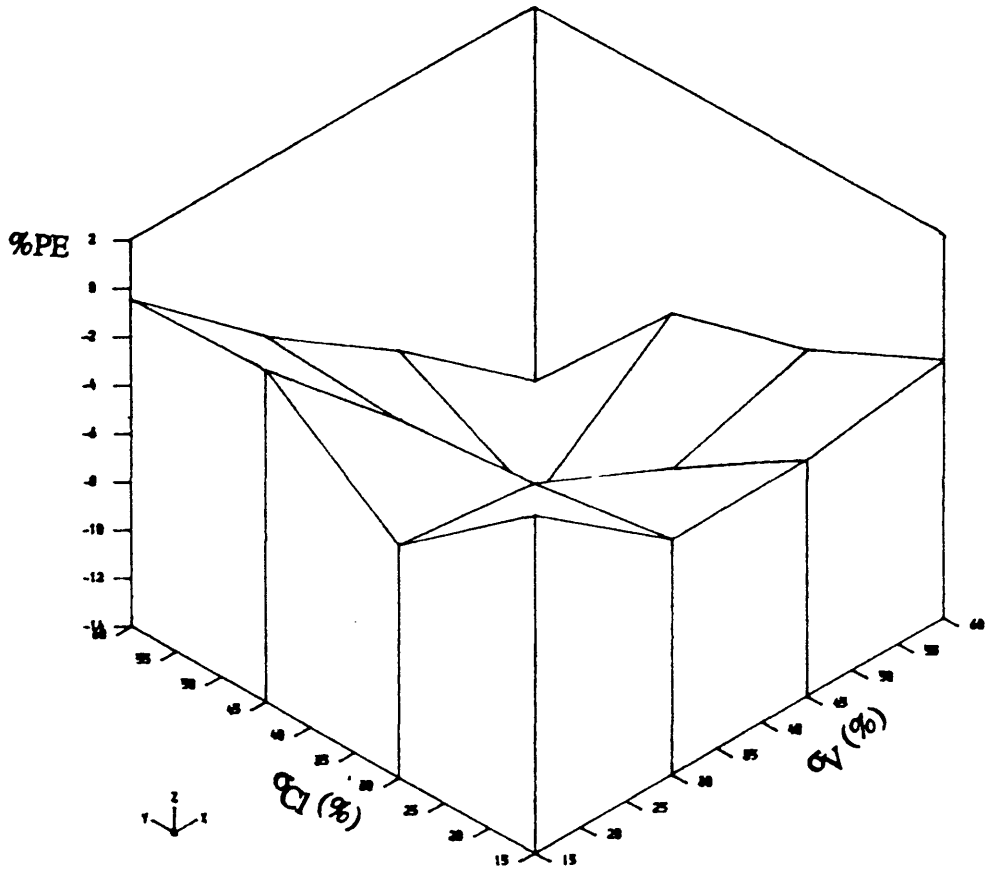


Fig. 3.10b Bias (expressed as mean %PE) in V estimation: three dimensional plot of the influence of varying σ_{Cl} and σ_V on the estimation of V. 5 observations were made per time point.

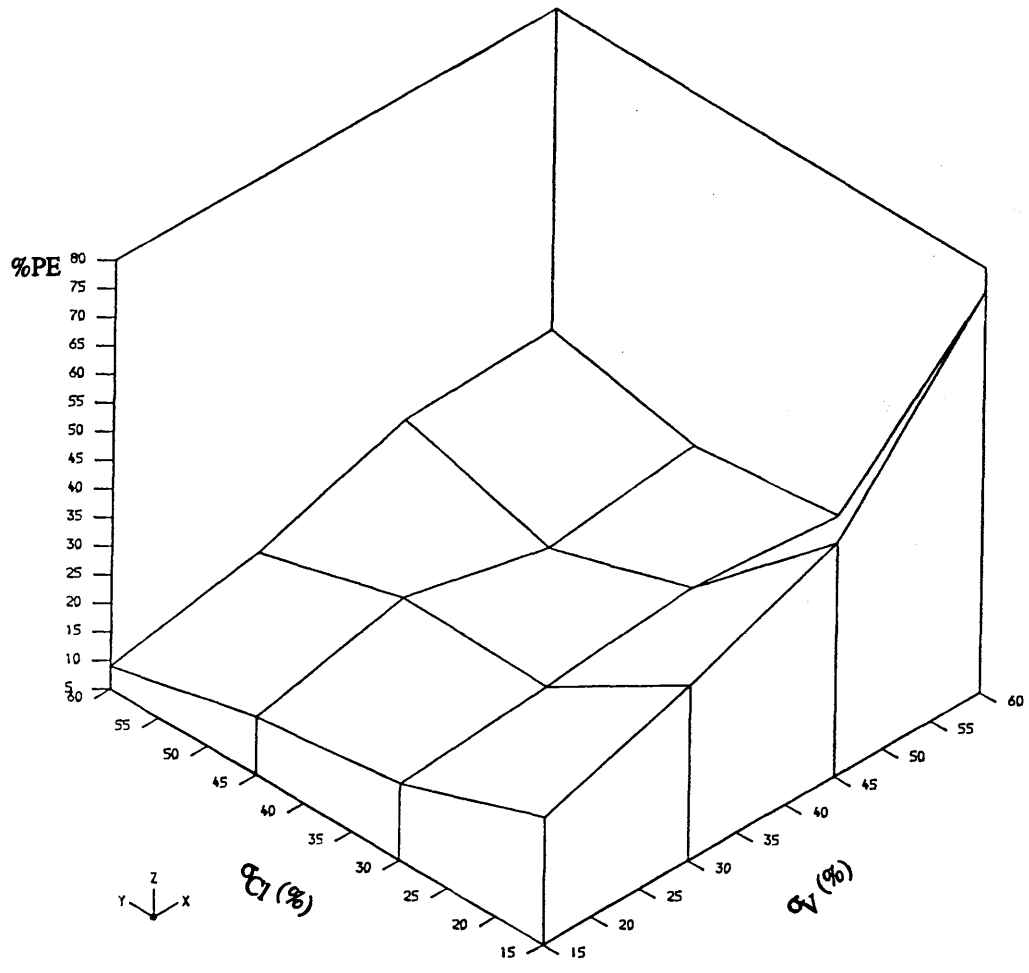


Fig. 3.10c Bias (expressed as mean %PE) in the estimation σ_{Cl}: three dimensional plot of the effect of varying σ_{Cl} and σ_V on the estimation of σ_{Cl}. 5 observations were made per time point.

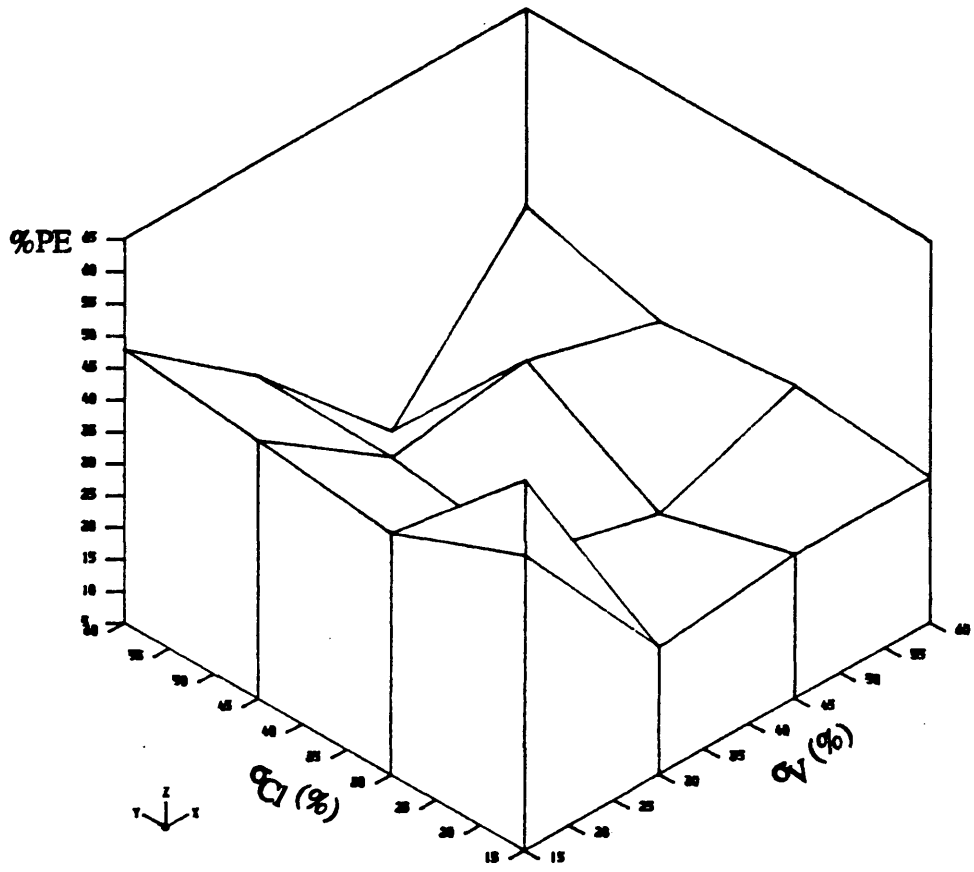


Fig. 3.10d Three dimensional plot of bias (expressed as mean %PE) in σ_V estimation as affected by varying σ_{Cl} and σ_V. 5 observations were made per time point.

Table 3.4 Distribution of Estimates: 3 Observations Per Time-Point

Inter-Animal Variability(%)		Parameter											
		Cl			V			σ_{Cl}			σ_V		
σ_{Cl}	σ_V	D	Skew.	Kurt.	D	Skew.	Kurt.	D	Skew.	Kurt.	D	Skew.	Kurt.
15	15	N	0.34	-0.70	N	0.10	-0.82	N	-0.06	-0.07	N	0.85	0.49
30	15	N	0.25	-0.95	N	0.28	-0.75	N	0.41	-0.69	N	0.55	-0.48
45	15	N	0.11	-0.18	N	0.37	-1.26	N	0.73	-0.15	N	0.54	-0.97
60	15	N	0.58	-0.30	N	0.06	-0.93	N	0.10	-0.75	N	0.86	-0.29
15	30	N	0.37	-0.85	N	-0.30	-0.51	N	-0.07	-0.44	N	0.88	0.41
30	30	N	0.65	-0.54	N	0.06	-0.13	N	0.86	0.61	N	0.68	-0.02
45	30	N	0.58	0.38	N	0.51	-0.56	NN	2.67	7.64	N	0.39	0.73
60	30	N	0.20	-0.49	N	0.42	-0.58	N	0.17	-0.80	N	0.34	-0.77
15	45	N	0.63	-0.55	N	0.76	0.40	N	-0.29	-0.04	NN	1.28	1.09
30	45	N	0.42	-1.04	N	0.31	-1.04	NN	1.20	2.51	N	0.82	-0.32
45	45	N	0.46	0.14	N	0.20	-0.18	N	0.23	-0.48	NN	1.28	1.29
60	45	N	-0.47	-0.14	N	0.75	0.13	N	0.10	-0.94	NN	1.63	2.01
15	60	N	-0.10	-0.99	N	0.45	-0.39	N	0.35	-0.43	NN	1.49	1.39
30	60	N	0.09	-0.94	N	0.73	0.31	N	0.48	-0.90	NN	3.21	11.65
45	60	N	-0.22	-0.34	N	0.31	-0.20	N	0.45	-0.41	N	0.74	-0.26
60	60	N	0.47	-0.13	N	0.62	-0.01	N	0.55	0.17	NN	1.22	0.47

D = Distribution
Skew. = Skewness
Kurt. = Kurtosis

N = Normal
NN = Nonnormal

Table 3.5 Distribution of Estimates: 5 Observations Per Time-Point

Inter-Animal Variability(%)		Parameter											
		Cl			V			σ_{Cl}			σ_V		
σ_{Cl}	σ_V	D	Skew.	Kurt.	D	Skew.	Kurt.	D	Skew.	Kurt.	D	Skew.	Kurt.
15	15	N	0.70	0.84	N	0.51	-0.91	N	-0.59	0.71	N	0.85	0.71
30	15	N	0.55	0.61	N	0.45	0.49	N	0.62	0.08	N	0.37	-0.73
45	15	N	0.49	-0.02	N	0.54	0.74	NN	1.14	1.68	N	0.36	-0.81
60	15	N	0.56	-0.72	N	0.62	0.03	N	0.37	-0.88	N	0.54	-0.58
15	30	N	0.59	0.08	N	0.26	-0.44	N	0.56	1.70	N	0.76	0.16
30	30	NN	1.60	5.25	N	0.57	-0.16	NN	1.78	5.11	N	0.70	0.13
45	30	NN	1.51	3.52	N	0.21	0.21	NN	2.69	8.91	NN	1.89	3.71
60	30	N	0.27	0.62	N	0.51	-0.13	N	0.52	-0.45	NN	1.85	3.95
15	45	N	0.20	-0.56	N	0.15	-0.73	N	0.15	0.73	NN	1.66	2.95
30	45	NN	2.09	5.74	NN	1.21	1.09	NN	2.38	8.17	NN	1.12	1.13
45	45	N	0.43	-0.28	N	0.56	0.19	N	-0.79	0.26	NN	1.59	1.92
60	45	N	-0.09	1.13	N	0.36	-0.20	N	0.85	0.09	N	1.00	0.54
15	60	N	0.86	0.15	N	0.19	-0.64	N	-0.45	0.47	NN	1.36	1.87
30	60	N	0.44	-0.71	N	0.25	-0.59	N	0.71	1.18	NN	1.99	4.66
45	60	N	0.56	-0.43	N	-0.10	0.36	N	0.15	0.50	N	0.83	-0.01
60	60	N	0.13	-0.23	N	0.17	-0.50	N	0.54	0.45	NN	1.22	1.02

D = Distribution
Skew. = Skewness
Kurt. = Kurtosis

N = Normal
NN = Nonnormal

estimation of σ_{Cl} decreased as the values of σ_{Cl} and σ_V were increased (Fig. 3.9c (3 observations per time point); Fig. 3.10c (5 observations per time point)). As with the estimation of V, σ_V were relatively stable and positively biased (Fig. 3.9d (3 observations per time point) ; Fig. 3.10d (5 observations per time point)).

3.4.3 Distribution of Estimates

The validity of the confidence interval coverage of parameter estimates is based on the assumption that parameter estimates follow a normal distribution. This assumption was validated for each of the estimates using the chi - squared test ($p < 0.05$). Accordingly, estimates of Cl and V for the 3 observations per time point study were normally distributed (Tables 3.4). 18.8 and 6.3% estimates of Cl and V, respectively, for the 5 observations per time point study were not normally distributed. Also, 12.5% (3 observations per time point) and 25.0% (5 observations per time point) of σ_{Cl} estimates were not normally distributed. 37.5 and 43.8% of σ_V estimates obtained in the 3 and 5 observations per time point studies, respectively, were not normally distributed. These estimates were significantly positively skewed (Tables 3.4 & 3.5).

3.4.4 Individual and Joint Confidence Intervals Coverage for Parameter Estimates

Individual and joint coverage for 99% interval estimates containing the true parameter value 95% of the time for all parameters are presented in Tables 3.6 to 3.9 for the 3 observations per time point study and Tables 3.10 to 3.13 for the 5 observations per time point study. The coverage for Cl and V was good for all combinations of σ_{Cl} and σ_V irrespective of the manner in which the coverage was computed and the study considered (Tables 3.6 - 3.9 & 3.10 - 13). For these two parameters the biases in the estimates were generally low (< 19%) and the

Table 3.6 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 3 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I					Section II						Section III				
		Success					(Success - Excluded)						(Success - Excluded)				
		Total					(Total - Excluded)						Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint		Cl	V	σ_{Cl}	σ_V	Joint
15	15	29/29	29/29	25/29	28/29	23/29	29/29	29/29	24/27	21/24	18/23		29/29	29/29	24/29	21/29	18/29
30	15	30/30	30/30	28/30	30/30	28/30	30/30	30/30	27/29	19/19	17/19		30/30	30/30	27/30	19/30	17/30
45	15	29/29	29/29	27/29	29/29	27/29	29/29	29/29	26/28	20/20	17/19		29/29	29/29	26/29	20/29	17/29
60	15	28/30	30/30	29/30	29/30	26/30	28/30	30/30	29/30	18/19	15/19		28/30	30/30	29/30	18/30	15/30

Table 3.7 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 3 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success Total						Section II (Success - Excluded) (Total - Excluded)					Section III (Success - Excluded) Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
15	30	27/28	25/28	24/28	26/28	22/28	27/28	25/28	17/19	20/22	12/15	27/28	25/28	17/28	20/28	12/28
30	30	30/30	28/30	28/30	27/30	23/30	30/30	28/30	21/22	20/20	14/17	30/30	28/30	21/30	20/30	14/30
45	30	30/30	30/30	22/30	28/30	22/30	29/30	30/30	25/26	17/17	15/16	29/30	30/30	25/30	17/30	15/30
60	30	29/30	30/30	27/30	29/30	27/30	29/30	30/30	27/30	18/18	16/17	29/30	30/30	27/30	18/30	16/30

Table 3.8 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 3 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success					Section II (Success - Excluded)					Section III (Success - Excluded)				
		Total					(Total - Excluded)					Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
15	45	27/29	23/29	27/29	26/29	20/29	27/29	23/29	14/16	21/23	9/16	27/29	23/29	14/29	21/29	9/29*
30	45	29/29	25/29	25/29	25/29	20/29	29/29	25/29	17/20	17/19	9/14	29/29	25/29	17/29	17/29	9/29*
45	45	28/28	28/28	23/28	27/28	20/28	28/28	28/28	15/15	15/15	7/8	28/28	22/28	15/28	15/28	7/28*
60	45	29/29	28/29	27/29	26/29	24/29	28/28	28/29	23/24	11/12	10/10	28/29	28/29	23/29	11/29*	10/29*

* $p < 0.01$

Table 3.9 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 3 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success / Total						Section II (Success - Excluded) / (Total - Excluded)					Section III (Success - Excluded) / Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
15	60	29/29	29/29	28/29	26/29	23/29	29/29	25/29	14/14	19/22	10/13	29/29	25/29	14/29	19/29	10/29*
30	60	28/28	23/28	28/28	27/28	25/28	28/28	23/27	12/12	18/19	11/12	28/28	23/28	12/28*	18/28	11/28*
45	60	29/29	26/29	26/29	27/29	23/29	29/29	26/29	18/20	16/18	10/11	29/29	26/29	18/29	16/29	10/29*
60	60	28/29	23/29	25/29	23/29	17/29	28/29	23/29	14/14	11/14	4/6	28/29	23/29	14/29	11/29*	4/29*

* $p < 0.01$

Table 3.10 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 5 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success Total					Section II (Success - Excluded) (Total - Excluded)						Section III (Success - Excluded) Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint		Cl	V	σ_{Cl}	σ_V	Joint
15	15	30/30	30/30	29/30	30/30	29/30	30/30	30/30	28/29	23/23	21/22		30/30	30/30	28/30	23/30	21/30
30	15	30/30	29/30	29/30	30/30	29/30	30/30	29/30	27/28	26/26	23/24		30/30	29/30	27/30	26/30	23/30
45	15	30/30	30/30	30/30	30/30	30/30	30/30	30/30	29/29	25/25	25/25		30/30	30/30	29/30	25/30	25/30
60	15	28/30	30/30	29/30	29/30	26/30	28/30	30/30	29/30	18/19	15/19		28/30	30/30	29/30	18/30	15/30

Table 3.11 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 5 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True: Section I Success / Total					Section II (Success - Excluded) / (Total - Excluded)					Section III (Success - Excluded) / Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
15	30	29/29	28/29	29/29	29/29	28/29	29/29	28/29	26/26	23/23	20/20	29/29	28/29	26/29	23/29	20/29
30	30	30/30	29/30	29/30	30/30	29/30	30/30	29/30	24/25	22/22	16/17	30/30	29/30	24/30	22/30	16/30
45	30	29/30	29/30	30/30	29/30	27/30	29/30	29/30	25/25	21/21	18/19	29/30	29/30	25/30	21/30	18/30
60	30	30/30	30/30	30/30	30/30	30/30	30/30	30/30	27/27	18/18	17/17	30/30	30/30	27/30	18/30	17/30

Table 3.12 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 5 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success Total						Section II (Success - Excluded) (Total - Excluded)					Section III (Success - Excluded) Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
15	45	30/30	27/30	29/30	30/30	27/30	30/30	27/30	18/18	20/20	15/15	30/30	27/30	18/30	20/30	15/29
30	45	30/30	30/30	29/30	28/30	27/30	30/30	30/30	26/28	15/17	14/17	30/30	30/30	27/30	15/30	14/30
45	45	27/27	25/27	25/27	27/27	24/27	27/27	25/27	19/19	18/18	12/13	27/27	25/27	19/27	18/27	12/27
60	45	25/26	24/26	26/26	25/26	23/26	25/26	24/26	20/20	14/14	11/11	25/26	24/26	20/26	14/26	11/26

Table 3.13 Confidence Interval Coverage for Individual & Joint Parameter Estimates: 5 Observations per Time Point

Inter-animal Variability (%)		Fraction Including True															
		Section I Success Total					Section II (Success - Excluded) (Total - Excluded)						Section III (Success - Excluded) Total				
σ_{Cl}	σ_V	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint		Cl	V	σ_{Cl}	σ_V	Joint
15	60	29/30	28/30	29/30	29/30	26/30	29/30	27/29	16/16	14/15	8/10		29/30	27/30	16/30	14/30*	8/30*
30	60	30/30	29/30	29/30	29/30	27/30	30/30	28/30	23/23	13/14	10/12		30/30	28/30	23/30	13/30*	10/30*
45	60	29/30	28/30	29/30	29/30	28/30	29/30	28/30	24/24	13/14	10/10		29/30	28/30	24/30	13/30*	10/30*
60	60	26/26	18/26	24/26	23/26	17/26	26/26	18/26	15/16	13/15	8/8		26/26	18/26	15/26	13/26	8/26*

* $p < 0.01$

"coefficient of variation" was generally less than 20%.

When catastrophic estimates were either included or excluded in the computation of coverage for all data sets good coverage was obtained for σ_{Cl} and σ_V (Tables 3.6 - 3.9 & 3.10 - 3.13, Section I & II). However, when catastrophic estimates were excluded from the numerator during the computation of coverage for all data sets, a reasonable coverage was obtained for σ_{Cl}, but significantly reduced coverage (less than the expected value of 0.95) was obtained for σ_V when σ_{Cl} was varied between 15 and 45% and σ_V set at 60% for the 5 observations per time point study (Table 3.13, Section III). This also occurred when either σ_{Cl} was 60% and σ_V, 45% or σ_{Cl} (σ_V) was 60% for the 3 observations per time point study (Tables 3.8 & 3.9, Section III).

The influence of bias in the estimation of confidence interval coverage was not marked for either σ_{Cl} or σ_V. Standard errors appeared to be the primary determinants of interval coverage for these parameters. As the values of the combinations of σ_{Cl} and σ_V became larger the coverage for these parameters was reduced irrespective of the sample size (Tables 3.6 - 3.9 & 3.10 - 3.13, Section III).

When catastrophic estimates were considered in the numerator in computing coverage for all data sets the joint coverage was reduced as the values for $\sigma_{Cl} * \sigma_V$ combinations became larger (Tables 3.6 - 3.9 & 3.10 - 3.13, Section III). The setting of σ_V at 45 and 60% for the 3 observations per time point study and σ_V at 60% for the 5 observations per time point study led to the production of joint confidence intervals coverage for all parameter estimates which were significantly lower than the expected value of 0.81.

3.4.5 Incidence of High Correlation between Parameter Estimates

The incidence of high correlation between parameter estimates was

relatively low when σ_V was 15% and σ_{Cl} varied between 15 and 60% for either study. The incidence of high correlation ranged from 0 to 20% when 3 observations were made per time point (Table 3.14), with the upper limit of this range being for the correlation between σ_V and V. On the contrary, incidence of high correlation when 5 observations were made per time point ranged from 0 to 10% (Table 3.15). The highest incidence occurred with the correlation between σ_V and V.

When σ_{Cl} was varied between 15 and 60% and σ_V was specified at 30% the incidence of high correlation ranged from 0 to 26.7% for the 3 observations per time point study (Table 3.16), and 0 to 16.7% for the 5 observations per time point study (Table 3.17).

With σ_V at 45%, varying σ_{Cl} resulted in incidence of high correlation ranging from 0 to 27.6% when 3 observations were made per time point (Table 3.18), and 0 to 22.2% when 5 observations were made per time point (Table 3.19). In both cases the upper limit of the ranges was for the correlation between σ_V and V.

When 3 observations were made per time point with σ_V at 60% and σ_{Cl} varied between 15 and 60% the highest incidence (34.5%) of high correlation was obtained with the σ_V versus V pair (Table 3.20). Similarly, the highest incidence (46.7%) of high correlation was obtained for this pair when 5 observations were made at each time point (Table 3.21).

Table 3.14 Incidence of Correlation Values Associated with Parameters: 3 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	15		15		15		15	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs V	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	13.8	86.2	13.3	86.7	17.2	82.8	20.0	80.0
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.15 Incidence of Correlation Values Associated with Parameters: 5 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	15		15		15		15	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	0.0	100.0	3.3	96.7	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	0.0	100.0	3.3	96.7	3.3	96.7	0.0	100.0
σ_{Cl} vs V	0.0	100.0	6.7	93.3	0.0	100.0	3.3	96.7
σ_V vs Cl	0.0	100.0	10.0	90.0	0.0	100.0	0.0	100.0
σ_V vs V	10.0	90.0	3.3	96.7	3.3	96.7	10.0	90.0
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.16 Incidence of Correlation Values Associated with Parameters: 3 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	30		30		30		30	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	14.3	85.7	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	7.1	92.9	3.3	96.7	6.7	93.3	3.3	96.7
σ_{Cl} vs V	10.7	89.3	10.0	90.0	16.7	83.3	6.7	93.3
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	10.7	89.3	16.7	83.3	23.3	76.7	26.7	73.3
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.17 Incidence of Correlation Values Associated with Parameters: 5 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	30		30		30		30	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	3.4	96.6	3.3	96.7	10.0	90.0	6.7	93.3
σ_{Cl} vs V	0.0	100.0	13.3	86.7	3.3	96.7	3.3	96.7
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	3.4	96.6	13.3	86.7	13.3	86.7	16.7	90.0
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.18 Incidence of Correlation Values Associated with Parameters: 3 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	45		45		45		45	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	3.4	96.6	0.0	100.0	10.7	89.3	0.0	100.0
σ_{Cl} vs Cl	0.0	100.0	3.4	96.6	3.6	96.4	0.0	100.0
σ_{Cl} vs V	3.4	96.6	0.0	100.0	17.9	82.1	20.7	79.3
σ_V vs Cl	3.4	96.6	10.3	89.7	3.6	96.4	0.0	100.0
σ_V vs V	3.4	96.6	3.4	96.6	17.9	82.1	27.6	72.6
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.19 Incidence of Correlation Values Associated with Parameters: 5 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	45		45		45		45	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	6.7	93.3	6.7	93.3	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	0.0	100.0	3.3	96.7	3.7	96.3	7.7	92.3
σ_{Cl} vs V	3.3	96.7	0.0	100.0	0.0	100.0	3.8	96.2
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	6.7	93.3	0.0	100.0	22.2	77.7	7.7	92.3
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.20 Incidence of Correlation Values Associated with Parameters: 3 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	60		60		60		60	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	6.9	93.1	3.6	96.4	6.9	93.1	10.3	89.7
σ_{Cl} vs Cl	13.8	86.2	0.0	100.0	3.4	96.6	24.7	75.3
σ_{Cl} vs V	20.7	79.3	7.1	92.9	13.8	86.2	31.0	69.0
σ_V vs Cl	3.4	96.6	7.1	92.9	0.0	100.0	0.0	100.0
σ_V vs V	24.1	75.9	21.4	78.6	24.1	75.9	34.5	65.5
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

Table 3.21 Incidence of Correlation Values Associated with Parameters: 5 Observations per Time Point

Inter-animal σ_{Cl}	15		30		45		60	
Variability(%) σ_V	60		60		60		60	
Correlation(%)	High[a]	Low[b]	High	Low	High	Low	High	Low
V vs Cl	3.3	96.7	3.3	96.7	3.3	96.7	3.8	96.2
σ_{Cl} vs Cl	13.3	86.7	3.3	96.7	10.0	90.0	7.7	92.3
σ_{Cl} vs V	3.3	96.7	3.3	96.7	10.0	90.0	3.8	96.2
σ_V vs Cl	3.3	96.7	0.0	100.0	0.0	100.0	15.4	84.6
σ_V vs V	46.7	53.3	36.7	63.3	40.0	60.0	15.4	84.6
σ_V vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a - High = > 0.75 b - Low = < 0.75

3.5 Discussion

The ranges of inter-animal variability used in these studies were based on their occurrence in preclinical situations (Lindstrom & Birkes, 1984; McArthur; 1988). Most Cl estimates were negatively biased irrespective of the values of σ_{Cl} and σ_V used to generate the data. This may be either due to estimation error or the nature of the NONMEM program because the fixed effect parameters enter the regression model nonlinearly and the random effect parameters linearly. This negative bias has been noted in the simulation studies reported in the literature which involved the use of a monoexponential pharmacokinetic model with multiple sampling (Sheiner & Beal, 1983; Al-Banna, Kelman & Whiting, 1990; White *et al.*, 1991) with no explanation. There was a tendency for the bias in Cl to increase as the value of σ_{Cl} was increased, irrespective of the values of σ_V. The relatively larger negative bias at higher values of σ_{Cl} compared with those obtained at smaller values of σ_{Cl} for any given value of σ_V was indicative of the fact that Cl was underestimated as σ_{Cl} was increased. This underestimation of Cl was coupled with the estimation of σ_{Cl} with either positive, minimally positive, or negligible bias in some cases. It is possible that the estimation error associated with Cl was partitioned to σ_{Cl}, hence the negative bias in Cl estimation and the positive bias associated with σ_{Cl} estimation. This may also be a consequence of the one observation per animal study design since this opposite trend in biases associated with the fixed and random effects parameters has not been reported with multiple sampling involving different combinations of variability using the monoexponential pharmacokinetic model (White *et al.*, 1991). Although most estimates of σ_{Cl} were minimally biased, they were mostly imprecise. This was possibly a consequence of the number of animals used in each study. Estimates of variability associated with structural model parameters are considerably less precise, given a fixed number of experimental units, than are estimates of their

means (Chapter 2, Section 5.3; Sheiner & Beal, 1981: Grasela *et al.*, 1986).

The negatively biased estimates of V were counterbalanced with positively biased estimates of σ_V. Most of the runs deleted were due to NONMEM yielding spurious estimates of σ_V and the associated standard error. The reasons for NONMEM producing negatively biased estimates of V and positively biased estimates of σ_V are probably the same as those advanced for the estimation of Cl and σ_{Cl}. It is pertinent to note that residual error was not estimated since there was no information in the data sets about error in concentration measurements. Thus, NONMEM was estimating composite inter-animal variability with error in concentration measurements incorporated since it had no information on σ_ϵ.

Confidence interval estimates are a function of three factors: bias, standard error estimates, and the distribution of parameter estimates. Good confidence interval coverage was obtained for Cl and V because of the small biases and high precision associated with the estimation of these parameters. There were no catastrophic estimates with these parameters even though the cut off criterion for the "coefficient of variation" was 20% as opposed to 50% for the for the variance parameters. For the variance parameters, the interplay of the three factors produced confidence intervals which, on average, were not different from the expected value of 0.95 when the cut off rule was not applied. With σ_V, for example, although there were large biases present with large standard errors, the nonnormality of some of the distributions brought in the confidence coverage back to the expected value of 0.95. A small increase is to be expected for a variable with a right-skew distribution. The import of large standard errors in the production of good confidence interval coverage could be observed when the exclusion criterion for NONMEM runs with large "coefficient of variation" was applied. The coverage was reduced when compared with the coverage obtained

with confidence intervals computed without the application of the exclusion criterion. Some of these confidence intervals were significantly different from the expected value of 0.95 (Tables 3.8 - 3.9 & 3.13, Section III). The standard error factor was also the major determinant in the joint coverage for all parameter estimates. However, it is difficult to anticipate what the results of the interplay between these three factors will be in any given data set.

The generally low incidence of high correlation between parameter estimates was an indication of the adequacy of the parameterization of the model. The relatively high correlation between σ_V and V at some combinations of σ_{Cl} and σ_V possibly contributed to the poor estimates of σ_V obtained.

In using NONMEM to analyse data in a realistic preclinical animal pharmacokinetic setting simulated in these studies no attempt was made to optimise conditions in regard to either experimental design. These results suggest that when magnitudes of inter-animal variability are in the range specified in these data sets, NONMEM produces estimates of fixed effect parameters which were relatively accurate and precise given the one observation per animal design. It often produced relatively accurate but imprecise estimates of σ_{Cl}, and mostly inaccurate and imprecise estimates of σ_V. It is worthy to note that when biases in σ_{Cl} and σ_V were large, they were positive and would require a more conservative approach to data interpretation. In addition, the usual confidence intervals computed may give an erroneous impression of the precision with which the random effect parameters were estimated because of the large standard errors associated with these parameters.

CHAPTER 4

EFFICIENT PARAMETER ESTIMATION: COMPARISON OF SAMPLING DESIGNS WITHIN A STUDY

4.1 SUMMARY

Simulation studies were carried out to evaluate the influence of sampling design on the efficiency of population pharmacokinetic parameter estimation when only one observation was obtained per animal. A finite number of observations and number of animals, as is always the case in practice, was used in the generation of data sets using the one compartment model with IV bolus administration.

The effect of arrangement of observations in time on the efficiency of parameter estimation was investigated using three different designs: the two sample point design, three sample point design, and four sample point design. The efficiency of parameter estimation obtained with the different sampling schedules within each design was compared to determine the "best" strategy.

The exact location of the third or fourth sample was not critical to the overall efficiency with which model parameters were estimated using either the three or four sample designs. However, in studies using the two sample design, the location of the second sampling time at approximately 1.4 times the elimination half-life of the drug or greater resulted in efficient estimation of population pharmacokinetic parameters.

4.2 INTRODUCTION

An optimal sampling strategy for monoexponential pharmacokinetic model with instantaneous IV input would require taking the first sample as early as possible after the dose (t_{min}) and the other (t_{end}) as late as possible (Edrenyi, 1981). The maximal feasible response is associated with t_{min} while the minimal

feasible response is associated with t_{end}.

In this chapter, simulated data sets were used to investigate the effect of arrangement of observations (drug concentrations) in time on parameter estimation. Data were generated assuming one compartment open model kinetics and IV bolus administration. Different sampling designs within a study were compared to identify the "best" sampling design for efficient parameter estimation involving the use of one observation per animal.

4.3 SAMPLING DESIGN

In this study, the optimal sampling strategy was applied in an ad hoc manner, and the drug was assumed to be administered by IV bolus injection. 15% error was added in the concentration measurements (see Chapter 2, Section 2.4). Sampling time ranged from as early as possible after the beginning of the experiment (t_{min} = 5 min.) to some value (t_{end} = 240 min.), the latest time that could be contemplated in actual experiment, taking into consideration the "average" $t_{1/2}$ of the drug. 48 observations corresponding to 48 animals were used in each design.

4.4 The Two Sample Point Design

In a series of experiments the first sampling time was fixed at 5 min. while the second was allowed to vary at 30 min. intervals from 90 to 240 min. after dose. The second sampling time was sampled uniformly within a range of

Table 4.1 Formulation of Sampling Schedules for the Two, Three, and Four Sample Point Designs

Two Sampling Times Design		Three Sampling Times Design			Four Sampling Times Design			
t_1(min)	t_2(min)	t_1(min)	t_3(min)	t_{end}(min)	t_1(min)	t_2(min)	t_4(min)	t_{end}(min)
5	90	5	30	240	5	30	60	240
5	120	5	60	240	5	30	90	240
5	150	5	90	240	5	30	120	240
5	180	5	120	240	5	30	150	240
5	210	5	150	240	5	30	180	240
5	240	5	180	240	5	30	210	240
		5	210	240				

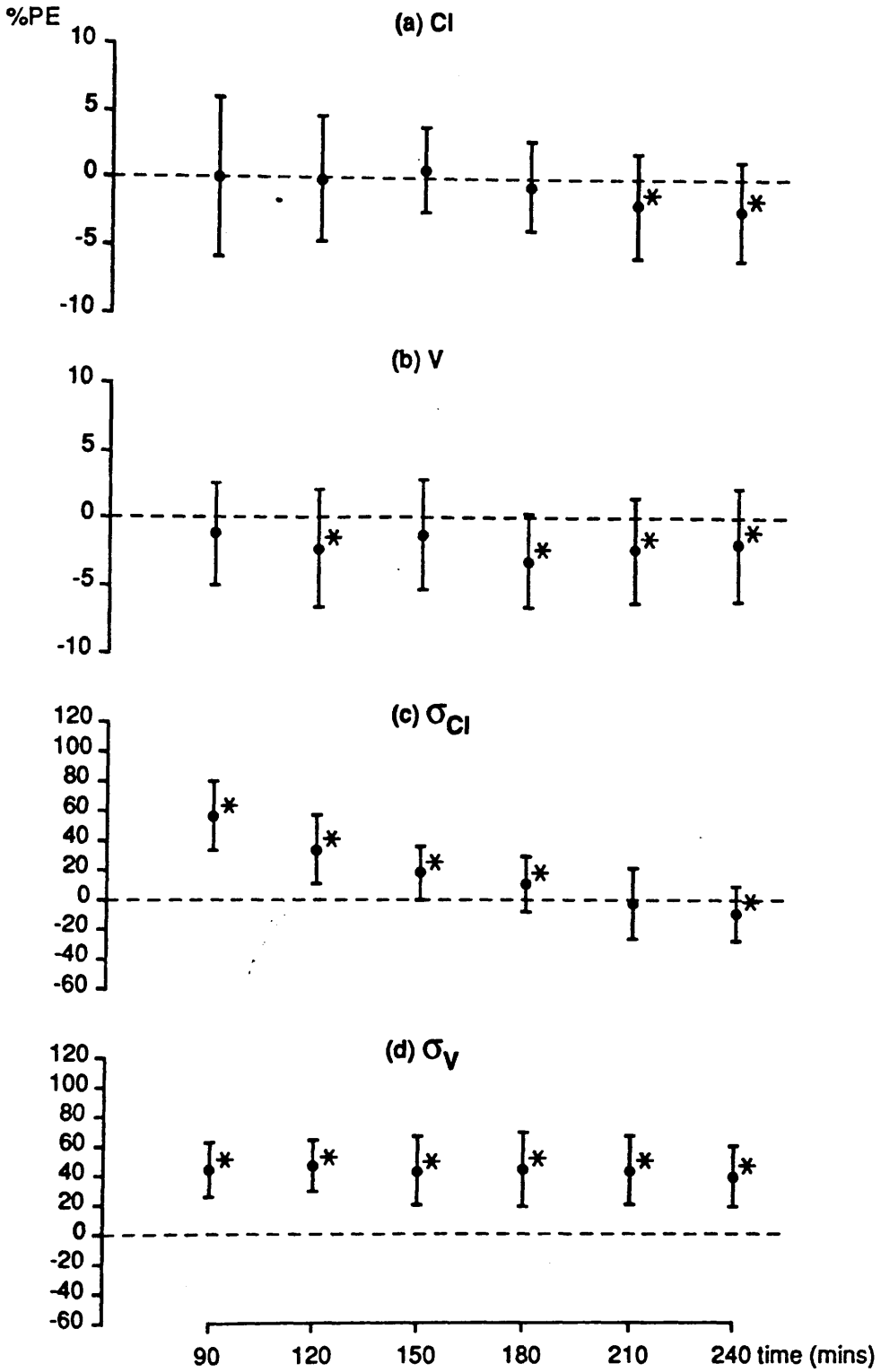


Fig. 4.1 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different samples for the two sample point design. Each vertical bar expresses the bias and precision of the population parameter estimate for each design. Significant ($p < 0.05$) biases are indicated by asterisks.

15 minutes centred on the stated time. This was considered to mimic a real study, and in the analysis the exact times were used. The six sampling schedules for this design specification are shown in Table 4.1.

4.4.1 Results

4.4.1a Bias and Precision

All designs yielded estimates of Cl which were precise. The SD of %PE ranged from 3.3% (180 min.) to 5.9% (90 min.). The bias ranged from approximately 0.0% (90 min.) to -2.3% (240 min.). Some of the sampling designs yielded estimates of Cl which were negatively biased (Fig. 4.1a).

All V estimates were relatively stable, negatively biased, but precise (Fig. 4.1b). The least biased estimate was obtained with the sampling design in which the second sample time was at 90 min. (mean of %PE = -1.2%) and the most biased estimate with the second sample at 180 min. (mean of %PE = -3.1%). The SD of %PE ranged from 3.5 to 4.4%.

σ_{Cl} estimates were highly positively biased when the second sample was at early times. As the second time point was specified at late times the bias was reduced, and tended to level off at 210 min. giving an almost unbiased estimate (Fig. 4.1c). However, the biases associated with most σ_{Cl} estimates were significant. The bias in the estimation of σ_{Cl} ranged from -2.3% (210 min.) to 56.4% (90 min.) although the precision was acceptable with the different designs. On the contrary, all estimates of σ_V were significantly positively biased, relatively stable, and acceptably precise (Fig. 4.1d).

(a) Estimation of Cl

180	150	210	240	120	90	Sampling Times (min)

(b) Estimation of V

90	150	180	240	210	120	Sampling Times (min)

(c) Estimation of σ_{Cl}

240	210	180	150	120	90	Sampling Times (min)

(d) Estimation of σ_V

240	210	180	90	150	120	Sampling Times (min)

(e) Overall Design Efficiency

150	240	180	210	120	90	Sampling Times (min)

Fig. 4.2 [a]Summary of significant differences in the efficiency[*] with which parameters were estimated using the two sample point design.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

4.4.1b Design Number

The design with the second sample at 180 min. produced the most efficient estimate of Cl (Fig. 4.2a). However, the efficiency with which Cl was estimated with this design was not significantly better than those with the second sample at 120, 150, 210, or 240 min. Also, the efficiency with which Cl was estimated with these designs was significantly better than that obtained when the second sample was at 90 min. The latter design produced the least efficient estimate of Cl.

V was estimated to a similar degree of efficiency with all sampling schedules although the design with the lowest rank order (on average) of Φ_{ir} was the one with the second sample at 90 min. (Fig. 4.2b).

σ_{Cl} was most efficiently estimated when the second sample was at 240 min. (Fig. 4.2c). The efficiency with which this parameter was estimated with this design was not significantly better than that obtained when the second sample was at either 150, 180, or 210 min. These designs yielded significantly better estimates of σ_{Cl} than designs having the second time point at either 90 or 120 min. The least efficient estimate of σ_{Cl} was obtained with the second sample at 90 min.

σ_V was poorly estimated with all sampling designs (Fig. 4.2d), and all produced similar results.

Overall, the most efficient estimates of fixed and random effects parameters were obtained with the specification of the second sample at 150 min. (Fig. 4.2e). The efficiency of parameter estimation with this design was not significantly better than when the parameters were estimated with designs having the second sample at 120, 180, 210, or 240 min. These designs (except when the second sample was at 120 min.) produced parameter estimates with a significantly better efficiency than the design with second time point at 90 min.

Table 4.2 Individual and Joint Confidence Intervals Coverage for Parameter Estimates Obtained with the Two Sample Point Design

Sampling Times	Fraction Including True: Section I Success / Total					Section II (Success - Excluded) / (Total - Excluded)					Section III (Success - Excluded) / Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
90	29/30	30/30	28/30	29/30	26/30	29/30	30/30	28/30	29/30	26/30	29/30	30/30	28/30	29/30	26/30
120	29/30	30/30	29/30	27/30	25/30	29/30	30/30	29/30	27/30	25/30	29/30	30/30	29/30	27/30	25/30
150	30/30	29/30	29/30	26/30	25/30	30/30	29/30	29/28	26/29	25/30	30/30	29/30	29/30	26/30	25/30
180	30/30	29/30	30/30	28/30	27/30	30/30	29/30	27/27	28/30	24/27	30/30	29/30	27/30	28/30	24/30
210	29/30	30/30	30/30	29/30	28/30	29/30	30/30	22/22	29/30	21/22	29/30	30/30	22/30	29/30	21/30
240	30/30	30/30	29/30	29/30	28/30	30/30	30/30	18/18	29/30	18/18	30/30	30/30	18/30	29/30	18/30

The least efficient estimates of model parameters and their variances were obtained with the second sample at 90 min.

Thus, Cl was best estimated with the second sample at 180 min. although this was not significantly better than estimates of this parameter obtained when the second sample was at 120, 150, 210, and 240 min., respectively. On the other hand, V was best estimated when the second sample was at 90 min., and this was not significantly better than the results obtained with the other designs. σ_{Cl} was best estimated with the second sample at 240 min. although not significantly better than when the second sample was at 150, 180, or 210 min. σ_V was poorly estimated at all specifications of the second time point.

The design with the second sample at 150 min. yielded the most efficient estimates of all parameters of the model, but this was not significantly better than when the second time point was at 120, 180, 210, or 240 min.

4.4.1c Individual and Joint Confidence Intervals for Parameter Estimates

Individual and joint confidence intervals for parameter estimates are summarised in Table 4.2. All designs produced good coverage for individual and joint confidence intervals for parameter estimates whether or not NONMEM runs with catastrophic estimates were included. The coverage for individual and joint parameter estimates were not significantly different from the expected values of 0.95 and 0.81, respectively. When NONMEM runs with catastrophic estimates were discounted in the numerator during confidence interval coverage computation a slightly reduced coverage, though not significantly different from the expected value, was obtained for σ_{Cl} and joint parameter estimates when the second sampling time was specified at 240 min. (Table 4.2, Section III).

Table 4.3 Incidence of Correlation Values Associated with Parameters for the Two Sample Point Design

Sampling Times (min)	90		120		150		180		210		240	
Correlation	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low
V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs V	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs V	3.3%	96.7%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_v vs σ_{Cl}	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%

a: High = Correlation coefficient ≥ 0.75

b: Low = Correlation coefficient < 0.75

4.4.1d Incidence of High Correlation between Parameter Estimates

Generally, 0% incidence of high correlation between parameter estimates was produced, except the design with the second sample at 90 min., this having an incidence of 3.3% for the correlation between σ_V and V (Table 4.3).

4.4.2 Discussion

The accuracy and precision with which fixed effect parameters were estimated were good with the bias in Cl and V not exceeding 3%, and the SD of %PE not exceeding 6%. However, most of the estimates of these parameters were negatively biased. This bias may be due to estimation error as discussed in Chapter 3. The best estimates of Cl and V in terms of bias and precision were obtained when the second sample was at 180 and 90 min., respectively.

The tendency for improvement in accuracy and precision in the estimation of σ_{Cl} as the second sampling time was specified at late times (150 to 240 min.) was due to the fact that information about this parameter was best obtained when the second sample was approximately two to three times the $t_{1/2}$ of the drug. Thus, an efficient estimate of σ_{Cl} could be obtained at either 180, 210, or 240 min.

σ_V was inefficiently estimated with all designs. Thus, all estimates of this parameter were biased but acceptably precise to the same extent. The positive bias associated with the estimation of σ_V with all designs and σ_{Cl} for some designs, could have been due to the lack of information in the data sets about concentration measurement error, since NONMEM was estimating composite inter-animal variability and concentration measurement error.

A comparison of Φ_{ir}'s obtained from the different sampling designs

showed that Cl was best estimated when the second sample was at 180 min. although this was not significantly better than when the second sample was at 120, 150, 210, or 240 min., respectively. Thus, specifying the second sampling time between 1.4 and 3 times the $t_{1/2}$ of the drug would produce efficient estimates of this parameter since information on drug elimination is contained in the late phase of the plasma concentration - time profile. This also explains why σ_{Cl} was better estimated at the late sampling times.

V was efficiently estimated when the second sample was at 90 min. However, this was not significantly different from the results obtained with the other designs. The lack of difference was due to the associated bias and precision with which this parameter was estimated with the different designs.

As with the estimation of V, all designs produced estimates of σ_V which were not different from one another. The reasons for this are as previously stated for V.

The design which yielded the most efficient estimates of all parameters was that with the second sample at 150 min. However, this was not significantly different from those obtained with the second time point at 120, 180, 210, or 240 min. The design with the second time point at 90 min. was significantly worse than others, except the one with the second time point at 120 min., due to the bias associated with the estimation of σ_{Cl}.

Bias and precision are some of the factors which determine the properties of interval estimates. The interplay of these factors produce confidence intervals for fixed effect parameter estimates which had coverage near the expected value of 0.95. The good coverage for the random effect parameters was essentially due to the good precision associated with these estimates. Also, the good coverage obtained for the joint confidence intervals coverage was due to all designs producing precise parameter estimates. The reduced coverage, though not

significant, obtained when the second sample was at 240 min., was due to 12 NONMEM runs producing estimates of σ_{Cl} with "coefficient of variation" > 50%. Moreover, the good coverage obtained for individual and joint confidence intervals for parameter estimates was associated with negligible incidence of high correlation.

Given the design specifications considered here, the "best" design for the efficient estimation of parameters was the one with the second sample at 150 min. However, this sampling time could be either 180, 210, or 240 min. to obtain parameters estimated with similar efficiency.

4.5 The Three Sample Point Design

The impact of introducing a third sample on parameter estimation was investigated. In this design t_{min} and t_{end} were fixed at 5 min. and 240 (± 7.5) min., respectively, and the third sampling time was at 30, 60, 90,....., or 210 (all ± 7.5) min. after dose, yielding the 7 schedules shown in Table 4.1.

4.5.1 Results

4.5.1a Bias and Precision

The estimates of Cl were mostly negatively biased with the mean of %PE ranging from -0.3% to -3.1% (30 min.). These estimates were precise with the SD of %PE ranging from 3.0% (150 min.) to 4.0% (60 min.) (Fig. 4.3a).

V estimates were precise and mostly negatively biased (Fig. 4.3b). The

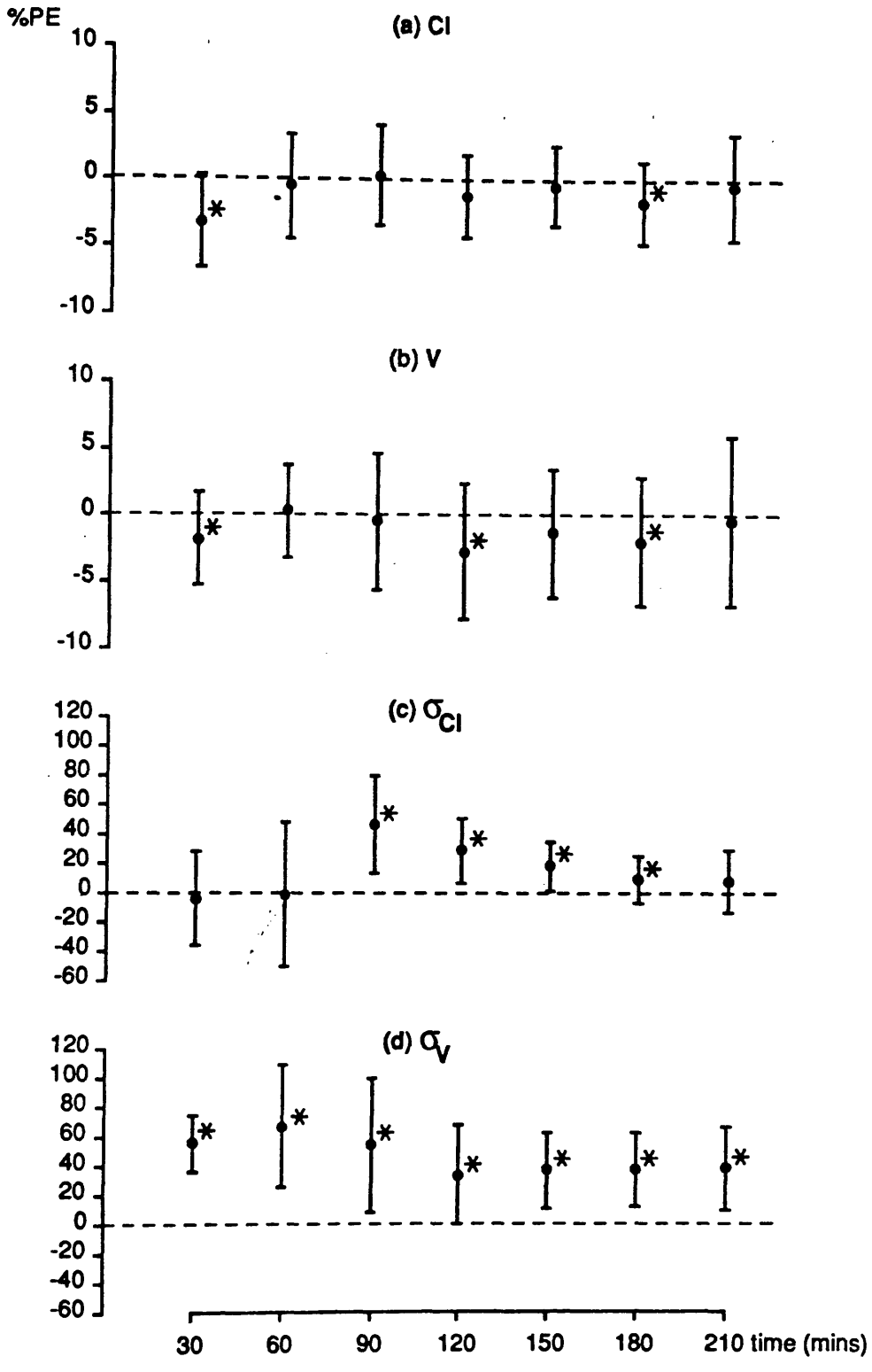


Fig. 4.3 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different samples for the three sample point design. Each vertical bar expresses the bias and precision of the population parameter estimate for each design. Significant ($p < 0.05$) biases are indicated by asterisks.

(a) Estimation of Cl

150 120 180 60 90 210 30 Sampling Times (min)

(b) Estimation of V

60 30 150 180 90 120 210 Sampling Times (min)

(c) Estimation of σ_{Cl}

180 210 150 30 120 60 90 Sampling Times (min)

(d) Estimation of σ_V

120 180 210 150 90 30 60 Sampling Times (min)

(e) Overall Design Efficiency

60 30 150 180 90 120 210 Sampling Times (min)

Fig. 4.4 [a]Summary of significant differences in the efficiency* with which parameters were estimated using the three sample point design.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

least biased estimate occurred with the third sample at 60 min. (mean of %PE = 0.3%) and the most biased estimate with the third sample at 120 min. (mean of %PE = -2.7%). The most precise estimate was obtained with the third sample at 60 min. (SD of %PE = 3.5%) while the least precise was at 210 min. (SD of %PE = 6.4%).

Except for the specification of the third sample at 30 or 60 min. there was a general trend for the bias in the estimation of σ_{Cl} to decrease as the third sample was shifted towards 240 min. (Fig. 4.3c). Estimates of σ_{Cl} were acceptably precise when the third sample was at $\geq$ 120 min., while the most imprecise estimate was obtained with the third sample at 60 min. (SD of %PE = 49.4%). The best estimate of this parameter was obtained with the third sample at 180 min. followed by sampling at 210 min.

As with the two sample design, σ_V estimates were significantly positively biased and imprecise (Fig. 4.3d). Specification of the third sample at 30 min. yielded acceptably precise estimates σ_V. The least biased estimate was obtained at 120 min. (mean of %PE = 33.8%) and the most biased estimate was obtained at 60 min. (mean of %PE = 67.2%).

4.5.1b Design Number

There was no significant difference when the efficiency with which Cl was estimated was compared for all sampling designs. This not withstanding, the design with the lowest rank order (on average) of Φ_{ir} was that with the third sample at 150 min. (Fig. 4.4a).

V was most efficiently estimated with the third sample at 60 min. (Fig. 4.4b), but this was not significantly better than when this sample was at 30, 90, 150, and 180 min. However, it was significantly better than the efficiency with

which estimates of V were produced with the third sample at 210 min.

The most efficient estimate of σ_{Cl} was obtained with the third sample at 180 min. (Fig. 4.4c). This, however, was not significantly better than when the third sample was at 30, 60, 120, 150, or 210 min., but was significantly better than results obtained with this sample at 90 min. Setting the third sampling time at 90 min. yielded the least efficient estimates of σ_{Cl}. However, when the efficiency with which σ_{Cl} was estimated with this design was compared with that obtained at either 60 or 120 min., there were no significant differences.

σ_V was estimated with similar efficiency at 30, 90, 120, 150, 180, and 210 min. (Fig. 4.4d). The design with the lowest rank order (on average) of Φ_{ir} was that with the third sample at 120 min. The efficiency of σ_V estimation at this time was significantly better than when the third sample was at 60 min.

Overall, the parameters were estimated with similar efficiency at all values of the third sample (Fig. 4.4e). There was no significant difference when the Φ_r's of all sampling designs were compared.

Consequently, Cl was efficiently estimated with all designs. However, the design with the third sample at 150 min. resulted in the lowest rank order of Φ_{ir} (i.e., least biased and most precise). Although V was efficiently estimated with the third sample at 30, 60, 90, 120, 150, or 180 min., the most efficient design was obtained when the third sample was at 60 min. σ_{Cl} was most efficiently estimated with the third sample at 180 min. The efficiency of the estimation of this parameter with this design was not significantly better than the results obtained with other designs, except the one with the third sample at 90 min. σ_V was badly estimated with all designs. Overall, the exact location of the third sample was not critical for the efficient estimation of the parameters.

Table 4.4 Individual and Joint Confidence Intervals Coverage for Parameter Estimates Obtained with the Three Sample Point Design

Sampling Times	Fraction Including True														
	Section I Success Total					Section II (Success - Excluded) (Total - Excluded)					Section III (Success - Excluded) Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
30	30/30	29/30	29/30	19/30	18/30	30/30	29/30	10/11	19/30	8/11	30/30	29/30	10/30*	19/30	8/30*
60	30/30	30/30	29/30	27/30	27/30	30/30	30/30	20/20	23/26	18/20	30/30	30/30	20/30	23/30	18/30
90	30/30	30/30	30/30	30/30	30/30	30/30	29/29	26/26	23/23	21/21	30/30	29/30	26/30	23/30	21/30
120	30/30	28/30	29/30	30/30	28/30	30/30	28/30	29/30	30/30	28/30	30/30	28/30	29/30	30/30	28/30
150	29/30	28/30	29/30	29/30	25/30	29/30	28/30	29/30	29/30	25/30	29/30	28/30	29/30	29/30	25/30
180	30/30	29/30	30/30	29/30	28/30	30/30	29/30	24/24	27/28	21/22	30/30	29/30	24/30	27/30	21/30
210	30/30	28/30	30/30	30/30	27/30	30/30	28/30	23/23	28/29	20/22	30/30	28/30	23/30	28/30	20/30

* $p < 0.01$

Table 4.5 Incidence of Correlation Values Associated with Parameters for the Three Sample Point Design

Sampling Times (min)	30		60		90		120		150		180		210	
Correlation	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	3.3%	96.7%
σ_{Cl} vs Cl	0%	100%	0%	100%	6.7%	93.3%	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs V	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	3.3%	96.7%
σ_V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs V	0%	100%	6.7%	96.3%	6.7%	93.3%	0%	100%	16.7%	83.3%	3.3%	96.7%	0%	100%
σ_v vs σ_{Cl}	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%

a: High = Correlation coefficient ≥ 0.75

b: Low = Correlation coefficient < 0.75

4.5.1c Individual and Joint Confidence Intervals for Parameter Estimates

When NONMEM runs with catastrophic estimates were included in the computation of confidence intervals, good coverage was obtained for all designs (Table 4.4, Section I). When catastrophic estimates were excluded in the numerator and denominator during the computation of confidence intervals coverage (see Chapter 2, Section 2.5.2) no significant effect was observed on the coverage (Table 4.4, Section II). However, when catastrophic estimates were discounted in the numerator during the computation of confidence intervals , the coverage for σ_{Cl} was reduced for the design with the third sample at 60 min. and significantly so with this sample at 30 min. (Table 4.4, Section III). Equally reduced coverage was observed for the joint confidence intervals for parameter estimates with these two designs compared to other designs. The coverage for σ_{Cl} and joint confidence intervals obtained for the design in which the third sample was at 30 min. was significantly different from the expected values of 0.95 and 0.81, respectively. All other designs yielded estimates with individual and joint confidence intervals coverage not significantly different from the expected value of 0.95 and 0.81, respectively.

4.5.1d Incidence of High Correlation between Parameter Estimates

The pair-wise correlations between V and Cl, σ_{Cl} and Cl, and σ_V and V for some designs yielded incidence of high correlation greater than 0% (Table 4.5). The incidence of high correlation between V and Cl was 3.3% for the design with the third sample at 210 min., but 0% for other designs. The incidence of high correlation for σ_{Cl} with Cl, and σ_{Cl} with V was 6.7% and 3.3% for the designs with the third sample at 90 and 210 min., respectively, but 0% for other designs. In the correlation between σ_V and V, the designs with the third sample at 30, 120,

or 210 min. produced an incidence of high correlation of 0%, while 6.7% was obtained with designs which had the third sample at 60 and 90 min. An incidence of 16.7% and 3.3% was obtained for designs with the third sample at 150 and 180 min., respectively.

4.5.2 Discussion

The estimation of Cl and V was associated with low bias and high precision for all designs. More precise estimates of Cl and V were obtained with the third sample at late and early times, respectively, where more information was available for the estimation of these parameters. The negative bias associated with the estimation of these parameters might be due to estimation error.

Although the estimates of σ_{Cl} obtained with the third sample at 30 and 60 min., respectively, were relatively unbiased, these estimates were associated with large "coefficient of variation". The improvement in precision when the third sample was obtained late was due to the increased amount of information (data points) available for σ_{Cl} (Cl) estimation. The most precise estimate of σ_V was obtained with the design having the third sample at 30 min. This was due to having more data points in the early times. The positive biases associated with the estimation of σ_{Cl} and σ_V were due to the lack of information in the data sets on σ_ϵ as earlier discussed for the two sample design.

The efficiency of Cl estimation was similar for all designs, although the lowest rank order (on average) of Φ_{ir} was obtained with the third sample at 150 min. The design with the highest rank order of Φ_{ir} was the one with the third sample at 30 min. However, the exact location of the third sample was not critical to the estimation of Cl.

On the contrary, the location of the third sample at early times (30 to 60 min.) led to a more efficient estimation of V, with the most efficient estimate obtained when the third sample was at 60 min., about two-thirds the drug $t_{1/2}$ (or as early as possible).

σ_{Cl} was most efficiently estimated with the third sample at 180 min., although the location of this sample at any time greater than 1.4 times the $t_{1/2}$ of the drug led to efficient estimation of this parameter. The best estimate of σ_V, obtained with the third time at 120 min., was associated with the least bias. The poor estimates of σ_V obtained with all designs could be a characteristic of the one observation per animal design.

The similar efficiency of estimation of all parameters with all the three sample designs indicated that the exact location of the third sample was not critical (Fig. 4.4e). The results obtained with the design number approach were in good agreement with those obtained using the bias and precision analysis.

The reduced confidence interval coverage obtained for the estimation of σ_V with the design with the third sample at 30 min. was due to the associated bias. On the other hand, the significantly reduced coverage obtained for σ_{Cl} estimates with designs having the third sample at 30 or 60 min., when NONMEM runs with catastrophic estimates were discounted in the numerator during confidence intervals computation (to reveal the influence of standard error on confidence intervals coverage), indicated that the estimates obtained for this parameter were not very reliable. However, the good coverage obtained for σ_{Cl} irrespective of the manner in which the confidence intervals were computed using the other designs indicated that those estimates were reliable. Apart from the design with the third sample at 30 min., the joint confidence intervals coverage for parameter estimates was good. The low incidence of high correlation between parameters was an indication of the adequacy of the parameterization of the model.

With the designs considered here, the exact location of the third sample was not critical to the efficiency with which the set of population pharmacokinetic parameters could be estimated.

4.6 The Four Sample Point Design

In this situation, t_{min} and t_{end} were fixed as in the previous case at 5 min. and 240 min., respectively. The second time point was fixed at 30 (± 7.5) min. and the fourth time point was varied between 60 and 210 (all ± 7.5) min. in steps of 30 min. (Table 4.1). The aim was to determine the efficiency with which fixed and random effects parameters could be estimated with this strategy.

4.6.1 Results

4.6.1a Bias and Precision

The estimate of Cl was least biased and most precise when the fourth sample was at 210 min. (Fig. 4.5a). All estimates of Cl were negatively biased ranging from approximately -0.2% to 3.1%, with the SD of %PE from 2.9% (210 min.) to 4.2% (60 min.).

On the contrary, the least biased and most precise estimate of V was obtained with the design in which the fourth sample was at 60 min. (Fig. 4.5b). All estimates of this parameter were also negatively biased with the mean of %PE ranging from -0.2% (60 min.) to 2.0% (120 min.) The SD of %PE ranged from 3.5% (60 min.) to 5.2% (180 min.).

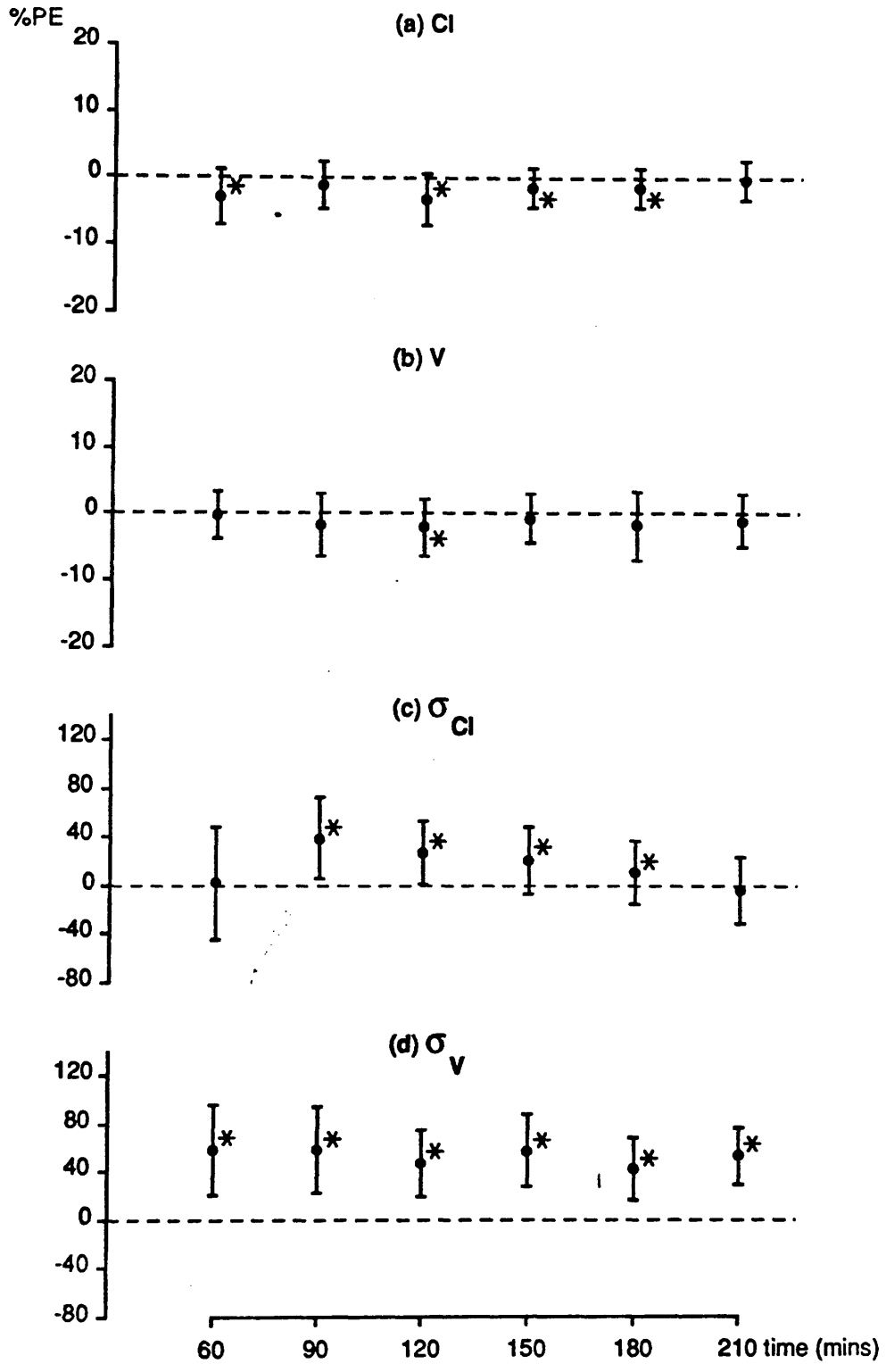


Fig. 4.5 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different samples for the four sample point design. Each vertical bar expresses the bias and precision of the population parameter estimate for each design. Significant ($p < 0.05$) biases are indicated by asterisks.

(a) Estimation of Cl

210	180	90	150	120	60	Sampling Times (min)

(b) Estimation of V

60	150	210	120	90	180	Sampling Times (min)

(c) Estimation of σ_{Cl}

180	210	150	120	60	90	Sampling Times (min)

(d) Estimation of σ_V

180	120	150	210	90	60	Sampling Times (min)

(e) Overall Design Efficiency

210	150	180	90	60	120	Sampling Times (min)

Fig. 4.6 [a]Summary of significant differences in the efficiency[*] with which parameters were estimated using the four sample point design.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

The estimates of σ_{Cl} were almost unbiased when the fourth sample was at 60 and 210 min. (Fig. 4.5c). As was the case with the two and three sample designs, there was a general trend of decrease in bias in the estimation of σ_{Cl} as the fourth sample occurred at later times. The estimates of σ_{Cl} were imprecise, except for the design with the fourth sample at 180 min. The most imprecise estimate was obtained when the fourth sample was at 60 min.

The estimation of σ_V was associated with a significant positive bias for all designs (Fig. 4.5d). The only acceptably precise estimate was obtained with the third sample at 210 min.

4.6.1b Design Number

The most efficient estimate of Cl was obtained with the fourth sample at 210 min. Cl was significantly better estimated with this design than the other designs. The least efficient estimate of Cl was obtained when the fourth sample was at 60 min. (Fig. 4.6a).

Although the design with the lowest average rank order of Φ_{ir} for the estimation of V was that with the fourth sample specified at 60 min., there was no significant difference when the Φ_{ir}'s for all designs were compared (Fig. 3.6b).

σ_{Cl} was most efficiently estimated with the fourth sample at 180 min. However, this was not significantly better than when this sample was at 60, 120, 150, and 210 min. (Fig. 4.6c). The design with the fourth sample at 90 min. produced the least efficient estimate of σ_{Cl}, and this was significantly worse than the results obtained with designs having the fourth sample at 180 min and 210 min., but not significantly worse than when this sample was at 60, 120 or 150 min.

σ_V was estimated with similar efficiency by all designs (Fig. 4.6d). The design with the lowest rank order of Φ_{ir} (on average) was the one with the fourth

sample at 180 min.

Similarly, there was no difference in the overall efficiency with which the population pharmacokinetic parameters were estimated (Fig. 4.6e).

Thus, Cl was most efficiently estimated when the fourth sample was at 210 min. V was efficiently estimated with all designs since there was no significant difference in the efficiency with which it was estimated when the Φ_{ir}'s were compared. However, the most efficient (least biased and most precise) estimate of this parameter was obtained with the fourth sample at 60 min. On the other hand, σ_{Cl} was most efficiently estimated when the fourth sample was at 180 min. The efficiency with which this parameter was estimated with this design was not significantly better than that with which it was estimated when the fourth sample was at 60, 120, 150, and 210 min., respectively. The efficiency of σ_V estimation obtained with the different designs was indistinguishable.

Equally, there was no significant difference in the overall efficiency with which all the parameters were estimated. Again, the results obtained using bias and precision were in agreement with those obtained using Φ_{ir}'s.

4.6.1c Individual and Joint Confidence Intervals Coverage for Parameter Estimates

The coverage for individual and joint confidence intervals for parameter estimates was good for all designs when the influence of bias alone was considered (Table 4.6, Section I &II). When the runs with catastrophic estimates were discounted in the numerator to examine the influence of standard errors on confidence intervals coverage (Table 4.6, Section III) the design with the fourth sample at 60 min. was found to yield estimates of σ_{Cl} with a confidence interval

Table 4.6 Individual and Joint Confidence Intervals Coverage for Parameter Estimates Obtained with the Four Sample Point Design

Sampling Times	Fraction Including True: Section I Success Total					Section II (Success - Excluded) (Total - Excluded)					Section III (Success - Excluded) Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
60	29/30	30/30	30/30	25/30	24/30	29/30	30/30	7/7	22/26	7/7	29/30	30/30	7/30*	22/30	7/30*
90	30/30	30/30	30/30	29/30	29/30	30/30	29/30	22/23	24/30	17/23	30/30	29/30	22/30	24/30	17/30
120	29/30	29/30	29/30	27/30	24/30	29/30	29/30	29/30	27/30	24/30	29/30	29/30	29/30	27/30	24/30
150	30/30	30/30	28/30	28/30	26/30	30/30	30/30	26/28	28/29	23/27	30/30	30/30	26/30	28/30	23/30
180	30/30	29/30	30/30	27/30	26/30	30/30	29/30	24/24	27/30	21/24	30/30	29/30	24/30	27/30	21/30
210	30/30	30/30	26/30	26/30	22/30	30/30	30/30	15/19	26/30	14/19	30/30	30/30	15/30	26/30	14/30

* $p < 0.01$

Table 4.7 Incidence of Correlation Values Associated with Parameters for the Four Sample Point Design

Sampling Times (min)	60		90		120		150		180		210	
Correlation	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low
V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs Cl	10%	90%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_{Cl} vs V	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs Cl	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
σ_V vs V	6.7%	93.3%	10%	90%	3.3%	96.7%	0%	100%	3.3%	96.7%	3.3%	96.7%
σ_V vs σ_{Cl}	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%

a: High = Correlation coefficient ≥ 0.75

b: Low = Correlation coefficient < 0.75

coverage significantly less than the expected value of 0.95. This design yielded 23 runs with catastrophic estimates of σ_{Cl}. On the other hand, the design with the fourth sample at 210 min. also produced estimates with reduced coverage, but this was not significantly different from the expected value of 0.95. In this case 11 NONMEM runs had catastrophic estimates of this parameter. Apart from the design with the fourth sample at 60 min. the other designs produced estimates of parameters whose joint coverage was not significantly different from the expected value of 0.81.

4.6.1d Incidence of High Pair-Wise Correlations

The incidence of high correlation between σ_V and V did not exceed 10% for any of the study designs (Table 4.7). With the exception of the correlation of σ_V and V all designs produced parameter estimates which were not highly correlated with one another.

4.6.2 Discussion

The production of the least biased and most precise estimates of Cl and V with the designs having the fourth sample at 210 and 60 min., respectively, was due to the fact that more information was contained in the data sets for the estimation of these parameters at late and early samples, respectively. The negative biases associated with the estimation of these fixed effect parameters were due to estimation error as earlier discussed.

Also, the production of efficient estimates of σ_{Cl} with late samples (180 min. (%PE = -3.6 ± 27.4%), and 210 min. (%PE = 11.3 ± 25.1%)) was due to the

same cause. The estimate of σ_{Cl} obtained with the fourth sample at 60 min., although almost unbiased, was associated with very poor precision (%PE = 2.5 ± 46.2%).

Using the design number approach, Cl was found to be most efficiently estimated when the fourth sample was at 210 min. The reason for this was previously stated when the result was discussed for bias and precision. The lack of difference in the estimation of V was due to the estimates being similarly biased and precise. No design produced estimates of V with "coefficient of variation" > 20% (Table 4.6).

σ_{Cl} was more efficiently estimated with the fourth sample at ≥ 1.4 times $t_{1/2}$ of the drug, since this provided more information on this parameter. The efficiency of estimation with the fourth sample at 60 min., although not significantly different from the results with the fourth sample at 120, 150, 180, and 210 min., was not acceptable. This was due to this design having 23 runs with the "coefficients of variation" > 50%. The similar poor efficiency with which all designs estimated σ_V could have been a consequence of the one observation per animal study design.

The exact location of the fourth sample was not critical in the overall estimation of parameters. The specification of two samples at not greater than one third the elimination $t_{1/2}$ of the drug (Table 4.1) and the fourth sample close to or greater than the $t_{1/2}$ of the drug, with the last sample at 240 min., might have contributed to this observation.

The influence of bias on confidence interval coverage was negligible. All designs produced good coverage for individual and joint parameter estimates when NONMEM runs with catastrophic estimates were included in the numerator during confidence intervals computation. The predominant factor governing confidence intervals coverage in the studies considered here, was standard errors.

Excluding NONMEM runs with estimates which had "coefficients of variation" > 50% in the numerator during confidence intervals computation led to reduced coverage for σ_{Cl} for most designs, and σ_V for two designs.The reduction in the coverage for σ_{Cl} was significant only for the design in which the fourth sample was at 60 min. With this design also, estimates of the joint confidence intervals coverage for parameter estimates was significantly different from the expected value because of the significantly reduced coverage for σ_{Cl} estimates. Setting the fourth sample at 210 min. was also associated with reduced coverage when NONMEM runs with catastrophic estimates were discounted in the numerator for confidence intervals computation. However, this was not significantly different from the expected value.

In addition, the estimation of parameters with this design was associated with low incidence of high correlation between parameter estimates. This might have contributed to the lack of significant difference in the overall efficiency with which model parameters were estimated.

Thus, the overall efficiency of parameter estimation obtained with all the four sample designs was similar. Although the exact location of this sample was not critical, the specification of the fourth sample at $\geq$ 2.5 times the elimination $t_{1/2}$ of the drug would result in more efficient parameter estimation.

CHAPTER 5

THE TWO COMPARTMENT POPULATION PHARMACOKINETIC MODEL: PARAMETER ESTIMATION WITH ONE OBSERVATION PER ANIMAL

5.1 SUMMARY

A simulation study was carried out using the two compartment model with IV bolus injection of a test drug. The efficiency with which model parameters and their variances were estimated was investigated given a set of parameter values, concentration measurement error with different sample sizes and sampling schedules. Data were simulated using one observation per animal.

Efficient parameter estimation was obtained when 15 observations were made per time point. Concentration measurement error greater than 10% yielded variance parameter estimates with greater degree of bias and imprecision. The inter-animal variability in parameters estimated was a composite of inter- and intra-animal variability.

When α was in the range of 2.0 and 4.0 h^{-1} and the A:B ratio between 2.5 and 40.0 efficient estimates of parameters were obtained. Some sampling schedules gave more efficient estimates of some parameters than others. High correlation between some parameters led to instability in the estimates.

5.2 INTRODUCTION

Equation (5.1) is the general equation for the disposition of a drug exhibiting two compartment open model kinetics and administered by IV bolus injection.

$$C = A.\exp(-\alpha.t) + B.\exp(-\beta.t) \qquad (5.1)$$

where A and B are regression coefficients; α and β are hybrid rate constants of distribution and elimination, respectively. Using the model expressed in Eq. (5.1)

the efficiency with which model parameters and their variances could be estimated was investigated given a set of parameter values, concentration measurement error with different sample sizes and sampling schedules.

5.3 METHODS

5.3.1 SAMPLING DESIGN

The individual values of A and B were randomly selected from normal distributions with means of 10000.0 and 500.0 IU/ml, respectively. The values of α and β, were similarly selected from distributions with means of 2.0 and 0.2 h^{-1}, respectively. The respective variances were chosen to yield a coefficient of variation of 15% for all parameters. A 15% error was added in concentration measurements as previously described (see Chapter 2, Section 2.4), except in (b) below.

An intravenous bolus dose of 200,000 IU was specified and data were sampled at ten time points, viz. 0.083, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h. The first point was fixed while the others were sampled from a uniform range of 0.25 h centred on the stated time. The simulation was carried out as previously described in Chapter 2 (Section 2.4) with 30 replicates of data for each simulation run.

(a) The effect of varying the number of observations at each time (i.e., number of animals used per time point) on the efficiency with which parameters were estimated was investigated using either 6, 10, or 15 observations per time point, yielding sample sizes of 60, 100, and 150, respectively.

(b) The influence of error in concentration measurements on parameter estimation was investigated by specifying σ_{ϵ} to be 0, 1, 5, 10, and 15%. A sample size of 150 was used in this study. The values A, α, B, and β were as previously stated.

(c) The efficiency with which model parameters were estimated with α in the range of 1.5 to 8.0 h^{-1} (i.e. 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, and 8.0 h^{-1}) was investigated. As with (b) a sample size of 150 was used in this study.

(d) The efficiency with which the parameters were estimated was investigated given a range of A:B ratios: 1.0, 2.5, 5.0, 7.5, 10.0, 20.0, 30.0, and 40.0. The A:B ratios were obtained by keeping B at a constant mean value of 500.0 IU/ml while the mean value of A was varied.

(e) From (a) and (b) above (α = 2.0 h^{-1}, β = 0.2 h^{-1}, and A:B ratio = 20.0) it was observed that the change over from the α to the β phase occurred after 2.0 h. Taking this demarcation of the α and β phases into consideration, the influence of varying sampling times in either the α or β phase of the plasma concentration time curve was examined in two separate studies:-

Study I:

12 sampling times were specified in the α phase. In this case the first time was fixed while the others were varied within a range of 0.033 h on the selected time (Table 5.1). The number was then reduced to 7, 5, and 3 (Table 5.1) with the total number of sampling times being 15, 10, 8, and 6, respectively. Consequently, the sample sizes were 150, 150, 152, and 150, respectively.

Table 5.1 Number and Specifications of Sampling Times

Number of Sampling Times			Sampling Times (h)													
Total	α Phase	β Phase														
6	3	3	0.083	1.50	2.0	3.0	4.0	6.0								
8	5	3	0.083	0.75	1.0	1.50	2.0	3.0	4.0	6.0						
10	7	3	0.083	0.25	0.50	0.75	1.0	1.50	2.0	3.0	4.0	6.0				
15	12	3	0.083	0.167	0.250	0.333	0.417	0.583	0.667	0.75	0.83	1.0	1.5	2.0	3.0	4.0	6.0
10	7	3	0.083	0.25	0.50	0.75	1.0	1.50	2.0	3.0	4.0	6.0				
13	7	6	0.083	0.25	0.50	0.75	1.0	1.50	2.0	3.0	4.0	5.0	6.0	7.0	8.0	
15	7	8	0.083	0.25	0.50	0.75	1.0	1.50	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0

Study II:

The number of sampling times in the β phase was increased from 3 to 6, and to 8 (Table 5.1), with a resultant total number of sampling times of 10, 13, and 15, respectively (i.e., 7 times in the α phase). The corresponding sample sizes were 150, 143, and 150, respectively. In each study, sample sizes were kept as close as possible to 150 to allow comparison of the results. α and the A:B ratio were set at 2.0 h^{-1} and 20.0, respectively.

5.4 RESULTS

NONMEM runs with estimates of parameters and / or their standard errors which did not satisfy the outlier criteria outlined in Chapter 2 (Section 2.5.1) were deleted. The results presented were based on runs with acceptable estimates.

5.4.1 Effect of Sample Size

The 60, 100, and 150 sample sizes had 27, 27, and 29 successful NONMEM runs, respectively. Most estimates of σ_β were infinitesimal and removing this parameter from the model did not alter the results. Model parameters were associated with minimal bias for the various sample sizes (Fig. 5.1(a - d)). Although the biases in the estimates of A and α were significant for the sample size of 150, these were less than 5% (Fig. 5.1(a & b)). The estimates of β were unbiased irrespective of the sample size (Fig. 5.1d). The estimates of A and α obtained with the different sample sizes were precise (SD of %PE < 9%), while the estimates of B (Fig.5.1c) and β (Fig. 5.1d) obtained with the 60 and 100 sample sizes were imprecise. Only the estimates of these parameters obtained with the 150 sample size were precise (SD of %PE < 13%). The estimates of σ_A,

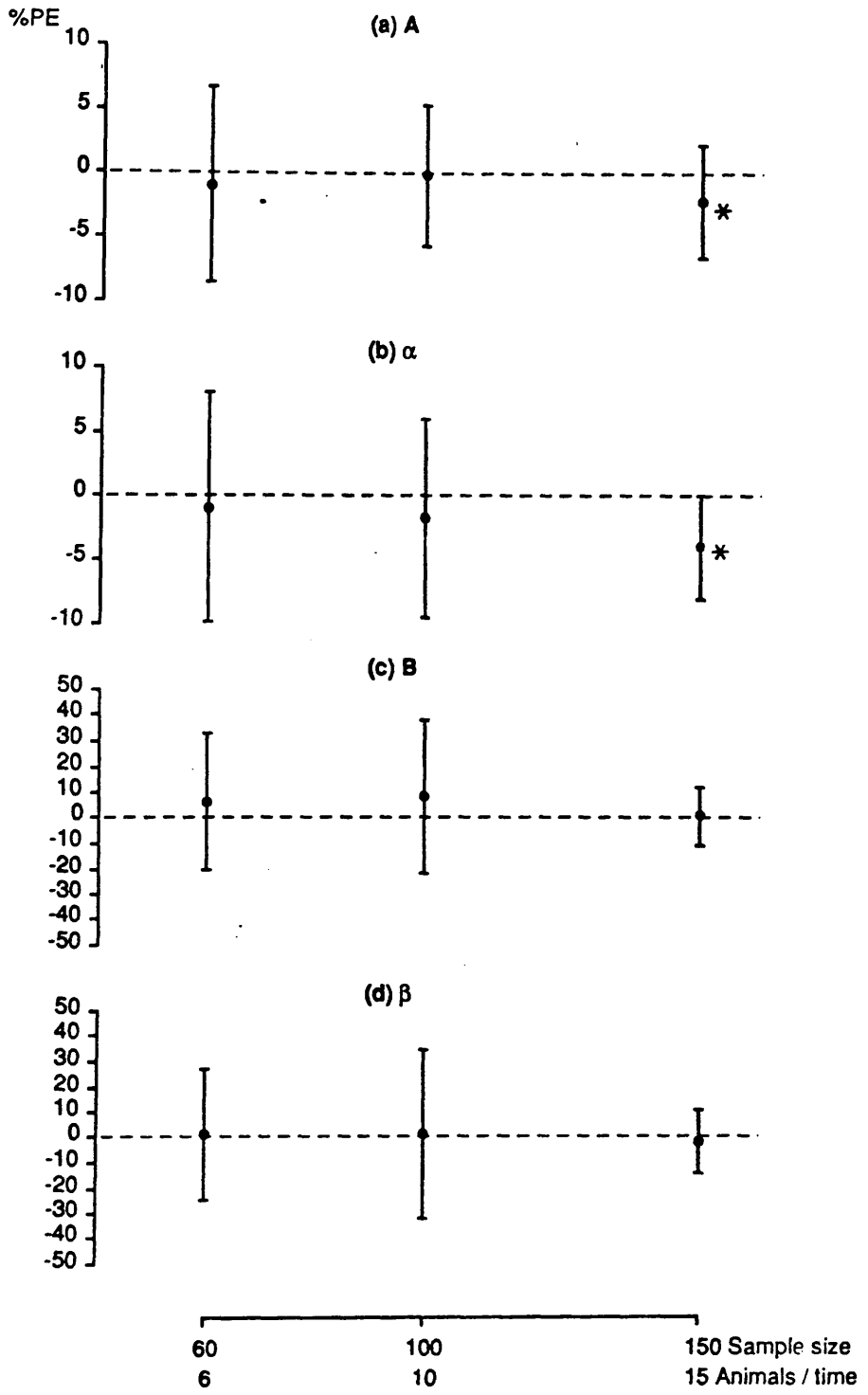


Fig. 5.1(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the number of animals used for observations at each time point. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

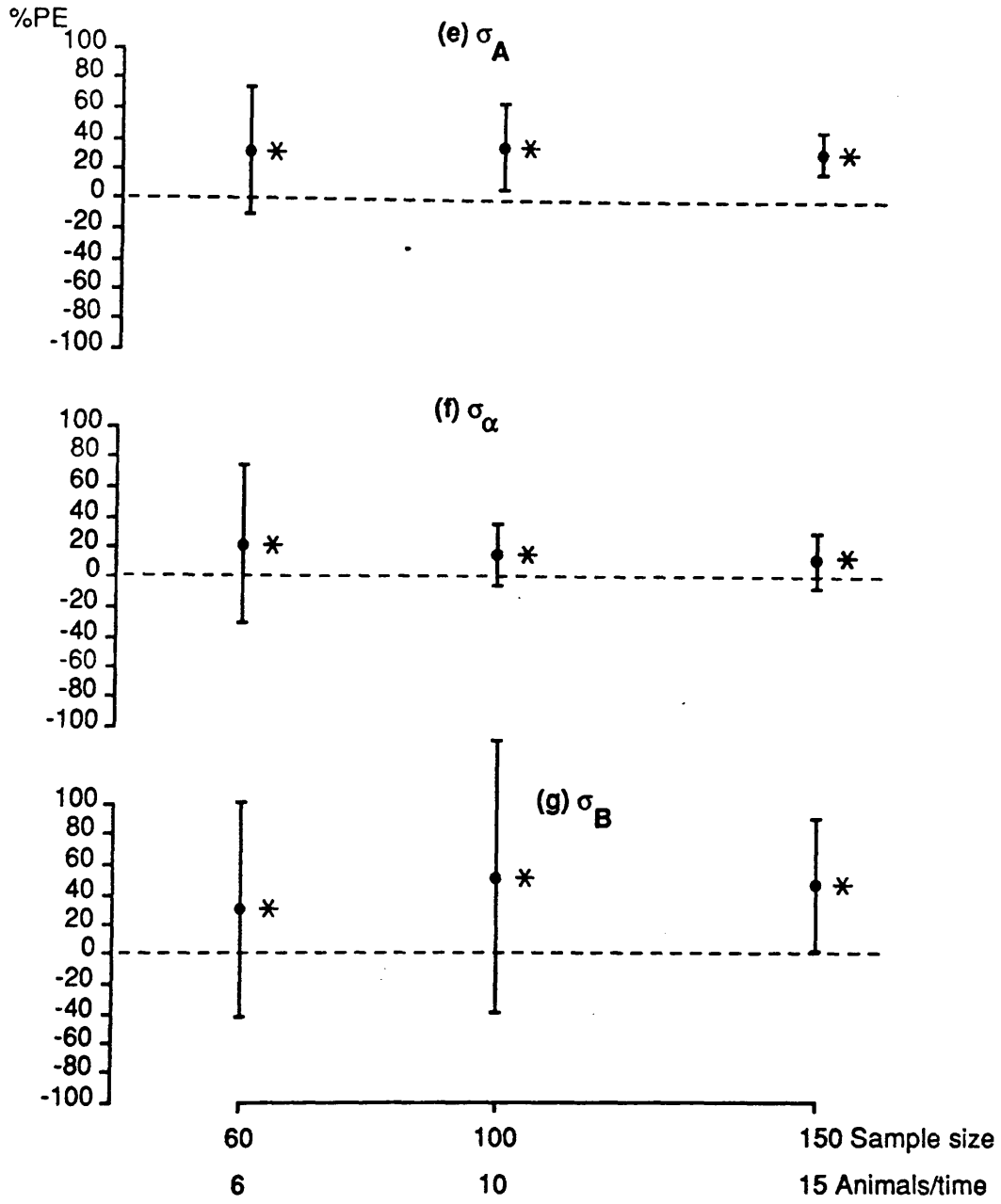


Fig. 5.1(e - g) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the number of animals used for observations at each time point. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

(a) Estimation of A

150 100 60 Sample size

(b) Estimation of α

150 60 100 Sample size

(c) Estimation of B

150 60 100 Sample size

(d) Estimation of β

150 60 100 Sample size

(e) Estimation of σ_A

150 100 60 Sample size

Fig. 5.2(a - e) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sample size.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

(f) Estimation of σ_α

150 100 60 Sample size

(g) Estimation of σ_B

150 100 60 Sample size

(h) Overall Design Efficiency

150 100 60 Sample size

Fig. 5.2(f - h) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sample size.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

σ_α, and σ_B were significantly positively biased (Fig. 5.1(e - g)). These estimates were associated with poor precision for the 60 and 100 sample sizes. Using the 150 sample size, acceptably precise estimates were obtained for σ_A and σ_α while an imprecise estimate was obtained for σ_B.

The Φ_{ir}'s were compared using the Kruskal Wallis test with multiple comparisons. The estimate of A produced with a sample size of 150 was significantly better than estimates produced with the sample size of 60 but not 100 (Fig. 5.2a). The most efficient estimate of A was obtained using a sample size of 150 while the least efficient estimate was obtained with a sample size of 60. Also, the most efficient estimate of α was obtained with the sample size of 150 while the least efficient estimate was obtained with a sample size of 100 (Fig. 5.2b). However, the efficiency with which α was estimated using the 100 sample size was not worse than that with the sample size of 60.

The best estimate of B was obtained using the 150 sample size. This was significantly better than the results obtained with the other two sample sizes which were not significantly different to each other (Fig. 5.2c). β was most efficiently estimated when the sample size was 150. This was significantly better than when the sample size was 100. Although the least efficient estimate of β was obtained with the latter sample size it was similar to that obtained with the 60 sample size.

σ_A (Fig. 5.2e) and σ_α (Fig. 5.2f) were most efficiently estimated with the 150 sample size, and these were significantly better than when the sample size was 60. The estimates obtained with a sample size of 100 had efficiencies similar to the other sample sizes. σ_B was estimated with similar efficiency using the three sample sizes (Fig. 5.2g).

Overall, parameters were most efficiently estimated when the 150 sample size was used. As expected, the sample size of 60 yielded the least efficient estimates of parameters when considered as a set. The results obtained with the

Table 5.2 Effect of Sample Size on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

Sample Size	A	α	B	β	σ_A	σ_α	σ_B	Joint
				Section I				
				Success / Total				
				Parameter				
60	27/27	27/27	27/27	27/27	25/27	27/27	27/27	25/27
100	27/27	27/27	27/27	27/27	26/27	27/27	27/27	26/27
150	29/29	29/29	29/29	29/29	25/29	29/29	29/29	25/29
				Section II				
				(Success - Excluded) / (Total - Excluded)				
60	27/27	27/27	27/27	26/26	20/22	9/9	1/1	0/0
100	27/27	27/27	26/26	23/23	26/27	16/16	1/1	0/0
150	29/29	29/29	29/29	29/29	25/29	28/28	0/0	0/0
				Section III				
				(Success - Excluded) / Total				
60	27/27	27/27	27/27	26/27	20/27	9/27*	1/27*	0/27*
100	27/27	27/27	26/27	23/27	26/27	16/27	1/27*	0/27*
150	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*

* - $p < 0.01$

Table 5.3 Incidence* of Correlation Associated with Parameters when Sample Size Was Varied

Correlation (%)	Sample Size					
	60		100		150	
	High[a]	Low[b]	High	Low	High	Low
α vs A	27.5	72.5	10.7	89.3	6.9	93.1
B vs α	20.0	80.0	13.8	86.2	13.9	86.2
β vs α	10.7	89.2	9.1	90.9	3.5	96.5
β vs B	100.0	0.0	100.0	0.0	100.0	0.0
σ_A vs A	25.0	75.0	3.6	96.4	0.0	100.0
σ_B vs α	9.1	90.9	6.9	93.1	6.9	93.1
σ_B vs B	14.6	85.4	10.7	89.3	3.5	96.5
σ_B vs β	10.7	89.3	6.9	93.1	0.0	100.0

* Incidence of pairwise correlations for combinations of parameters where the incidence of high correlation was greater than zero.

a - High > 0.75 b - Low < 0.75

sample size of 150 were significantly better than the results obtained with either 100 or 60 sample sizes.

When catastrophic estimates were included in the computation of individual and joint confidence intervals coverage for all parameters, good coverage was obtained (Table 5.2, Section I). However, when the catastrophic estimates were excluded in the computation of coverage, good coverage was obtained for A, α, B, β, and σ_A for all the sample sizes studied. Although good coverage was obtained for σ_α with 150 observations, the coverage obtained with 100 observations was reduced but not significantly lower than the expected value of 0.95, and poor coverage was obtained with 60 observations (Table 5.2, Section II & III). When the catastrophic estimates were excluded, very poor coverage was obtained for σ_B irrespective of the sample size (Table 5.2, Section III). Similar results were obtained for the joint coverage of all parameter estimates.

The incidence of high correlation was 100% for the correlation between β and B, but there was generally low incidence (< 30%) of high correlation between other parameter estimates (Table 5.3).

Overall, the use of 150 observations (15 animals per sampling time) yielded parameter estimates which were acceptably precise and least biased as expected.

5.4.2 Varying the Error in Concentration Measurements

With σ_ϵ specified at 0, 1, 5, 10, and 15% there were 29, 28, 28, 29, and 29, respectively, successful NONMEM runs. As in the previous section σ_β was removed from the model. Although the estimates of A and α were significantly negatively biased for all values of σ_ϵ, the magnitude of the bias was very small. The mean %PE ranged from -1.3 to -4.0% (Fig. 5.3(a & b). These estimates were

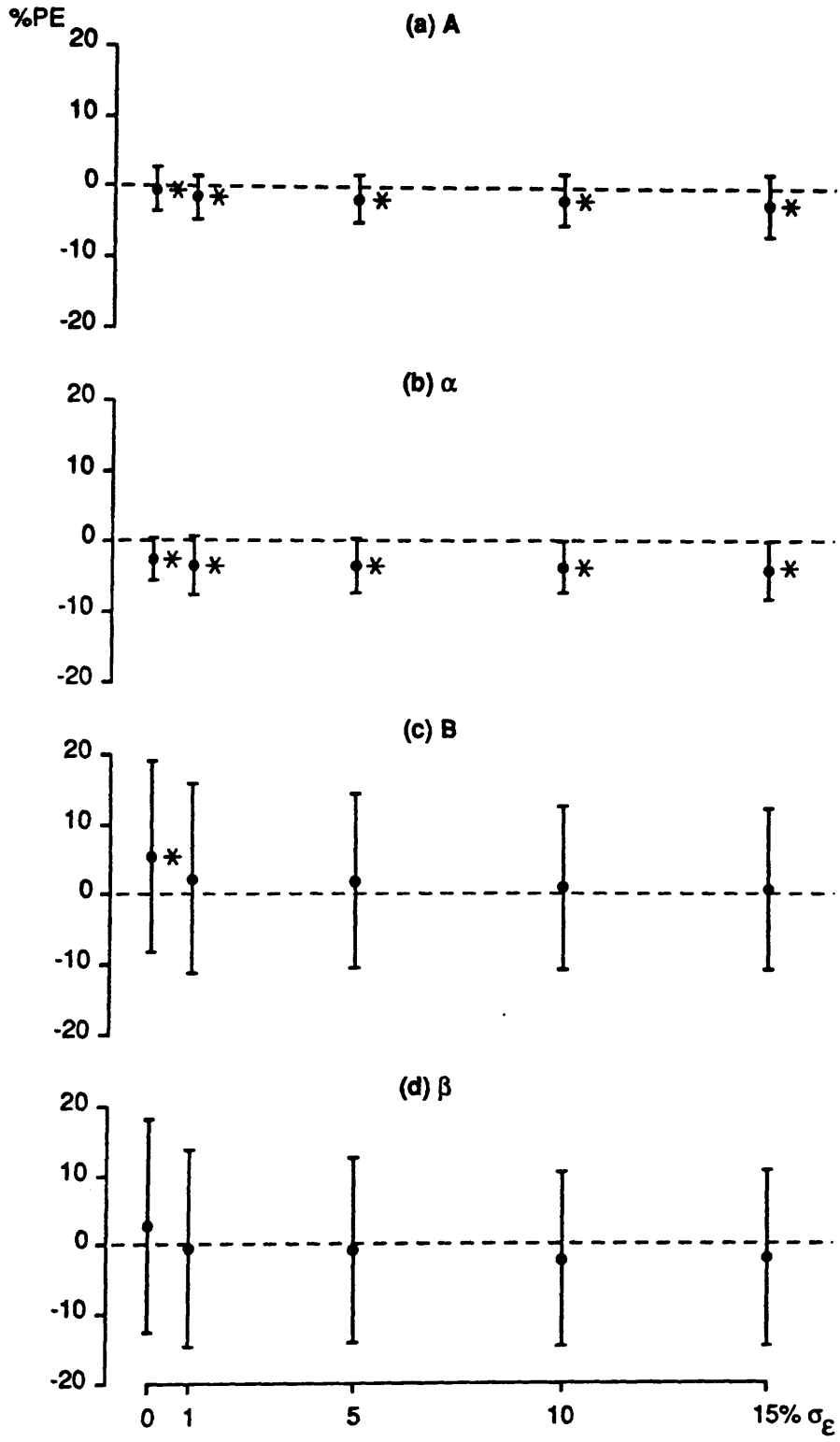


Fig. 5.3(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_ϵ used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

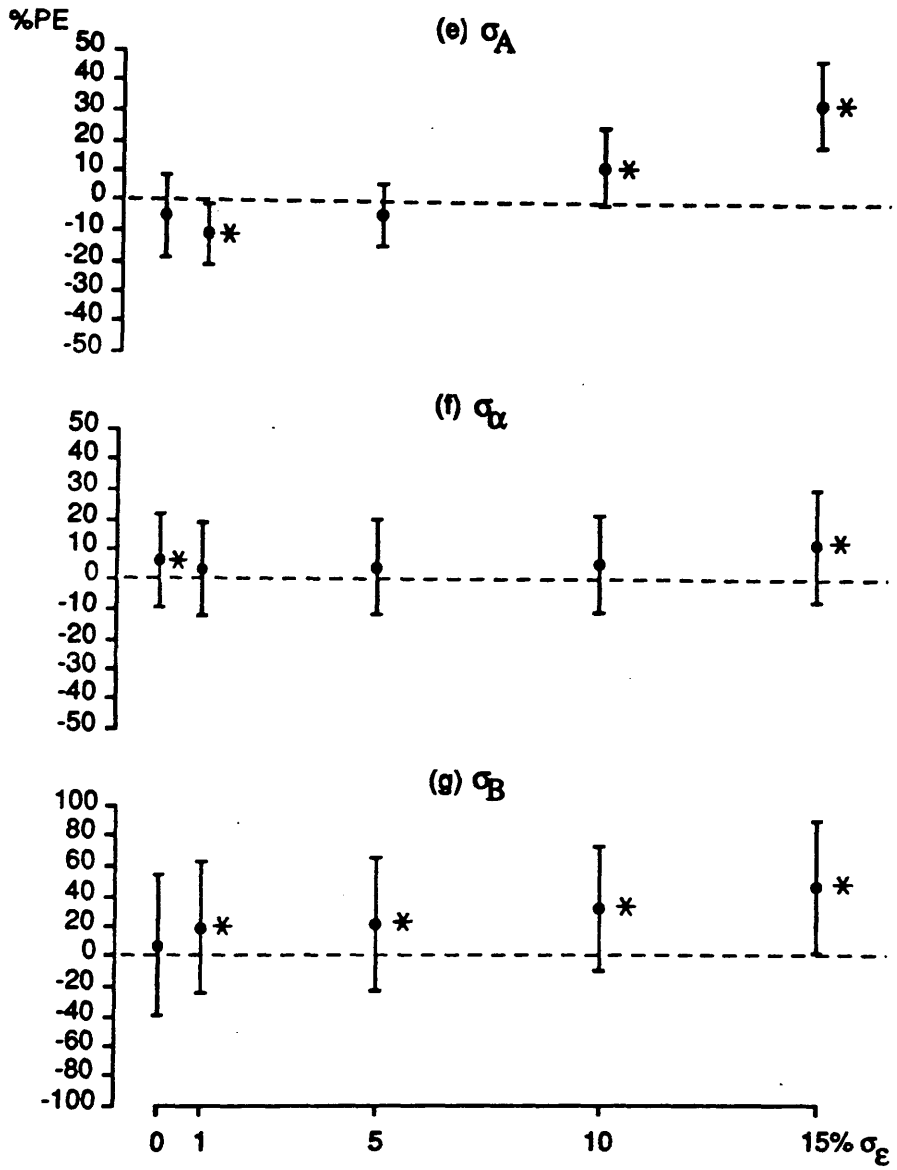


Fig. 5.3(e - g) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of σ_ϵ used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

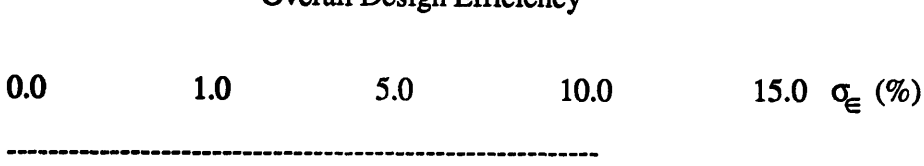


Fig. 5.4 [a]Summary of significant differences in the efficiency[*] with which all parameters were estimated: effect of error in concentration measurements.
a - Rank order of design numbers increasing from left to right..
* Efficiency measured with design number.

Table 5.4 Effect of Error in Concentration Measurements on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

σ_ϵ	Parameter							
(%)	A	α	B	β	σ_A	σ_α	σ_B	Joint
				Section I Success Total				
0.0	29/29	29/29	29/29	29/29	27/29	29/29	29/29	27/29
1.0	28/28	28/28	28/28	28/28	27/28	28/28	28/28	27/28
5.0	28/28	28/28	28/28	28/28	28/28	28/28	28/28	28/28
10.0	29/29	29/29	29/29	29/29	29/29	29/29	29/29	29/29
15.0	29/29	29/29	29/29	29/29	25/29	29/29	29/29	25/29
				Section II (Success - Excluded) (Total - Excluded)			.ls2	
0.0	29/29	29/29	29/29	29/29	27/29	29/29	5/5	5/5
1.0	28/28	28/28	28/28	28/28	27/28	28/28	2/2	2/2
5.0	28/28	28/28	28/28	28/28	28/28	27/27	1/1	0/0
10.0	29/29	29/29	29/29	29/29	29/29	28/29	1/1	0/0
15.0	29/29	29/29	29/29	29/29	25/29	28/29	0/0	0/0
				Section III (Success - Excluded) Total				
0.0	29/29	29/29	29/29	29/29	27/29	27/29	5/29*	5/29*
1.0	28/28	28/28	28/28	28/28	27/28	28/28	2/28*	2/28*
5.0	28/28	28/28	28/28	28/28	28/28	27/28	1/28*	0/28*
10.0	29/29	29/29	29/29	29/29	29/29	28/29	1/28*	0/28*
15.0	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*

* - $p < 0.01$

very precise (SD of %PE < 4.5%). All estimates of B were precise but positively biased (Fig. 5.3c). β estimation was associated with minimal bias and relatively good precision (Fig. 5.3d). The highest degree of bias was obtained for σ_A, σ_α, and σ_B when σ_ϵ was specified at 15% (Fig. 5.3(e - g)).

Parameter estimation was least efficient when σ_ϵ was set at 15% (Fig. 5.4) since Φ_r was significantly higher than when σ_ϵ was specified at 0, 1, 5, or 10%. As expected the best parameter estimates were obtained with σ_ϵ set as 0%, but the results obtained were not significantly better than the results obtained with σ_ϵ specified at 1, 5, and 10%.

Good individual and joint confidence intervals coverage was obtained for all levels of σ_ϵ used in this study (Table 5.4, Section I). However, discounting estimates, with "coefficient of variation" greater than 50% in the numerator for the computation of confidence intervals coverage, gave poor coverage for σ_B and joint parameter estimates (Table 5.4, Section III).

Thus, as the error in concentration measurements increased the efficiency with which parameters were estimated decreased as expected.

5.4.3 Varying the Distribution Rate Constant

The distribution rate constant (α) was varied between 1.5 and 8.0 h^{-1} (i.e. 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, and 8.0 h^{-1}) and the number of successful NONMEM runs were 27, 29, 29, 29, 30, 30, 29, and 28, respectively. As in the previous studies σ_β was removed from the model. The results show that A and α were associated with good precision and negative bias, irrespective of the value of α (Fig. 5.5 (a & b)). Except when α was 1.5 h^{-1}, B and β were unbiased and precise (Fig. 5.5 (c & d)). All estimates of σ_A, σ_α, and σ_B were significantly positively biased (Fig. 5.5 (e - g)). Poor precision was obtained in the estimation of

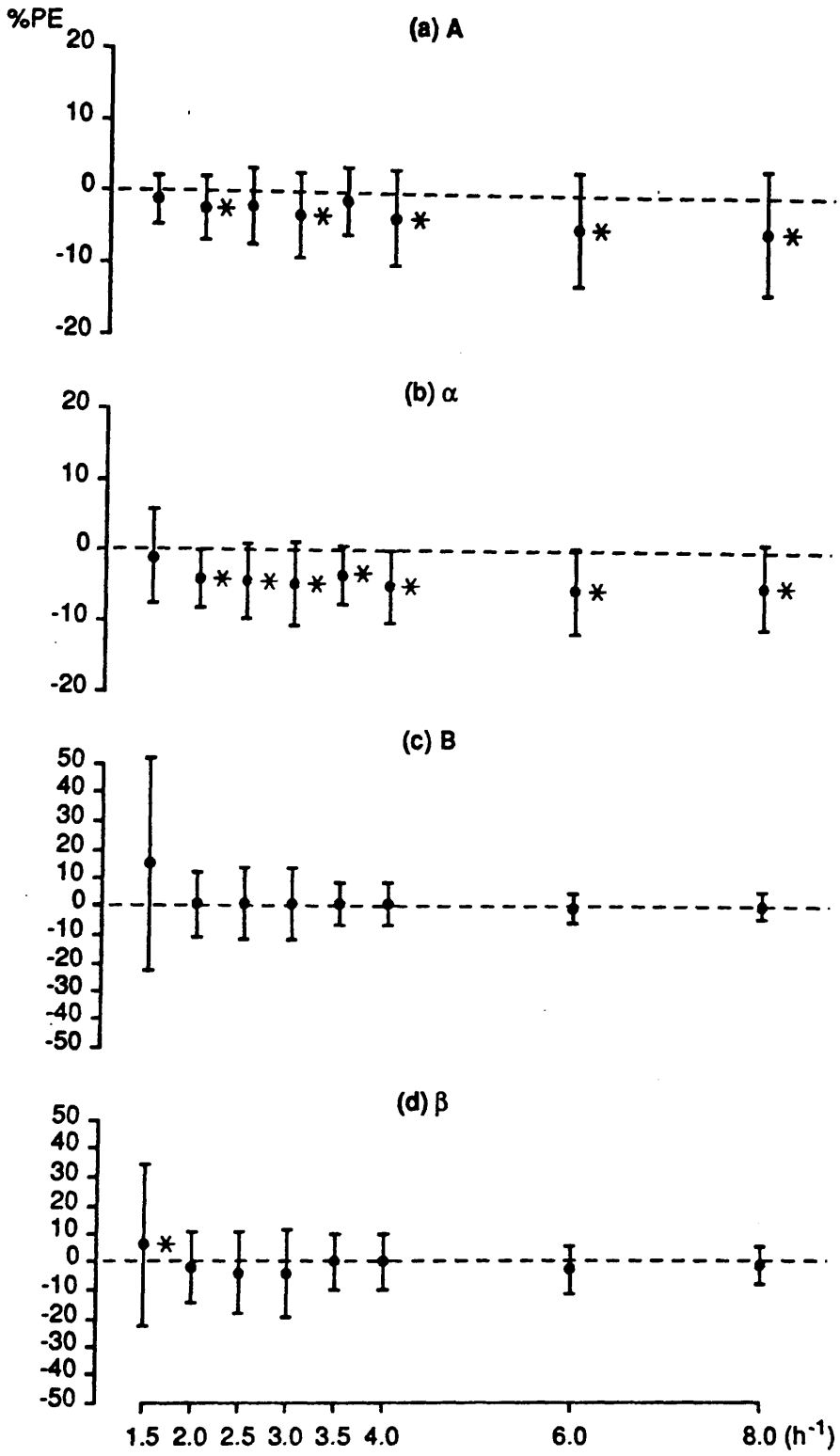


Fig. 5.5(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of α used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

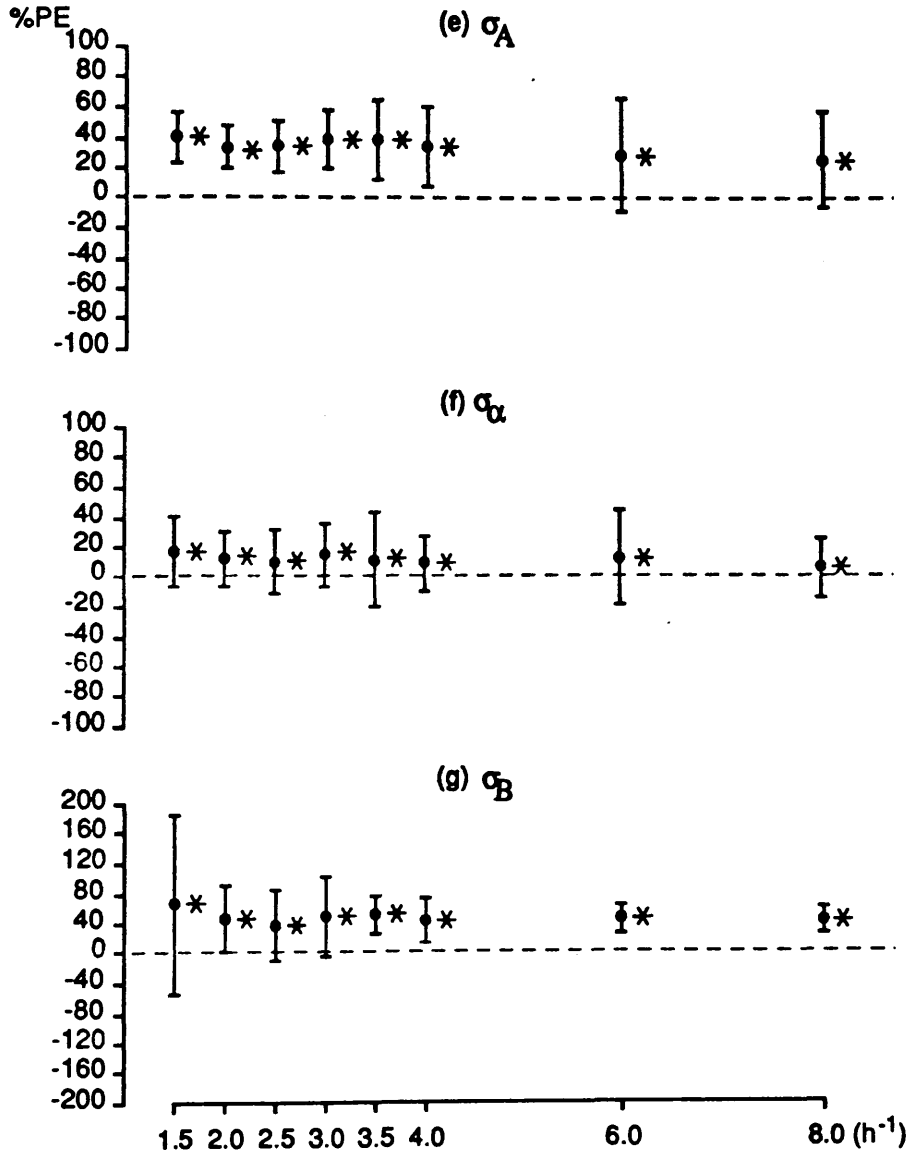
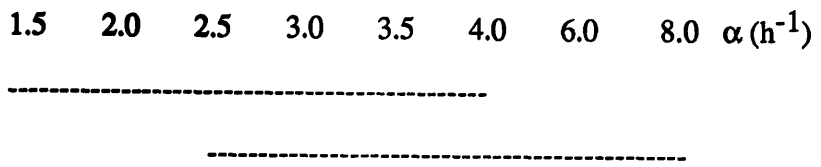
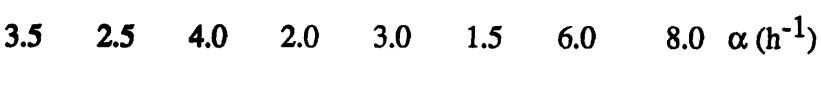


Fig. 5.5(e - g) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of α used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

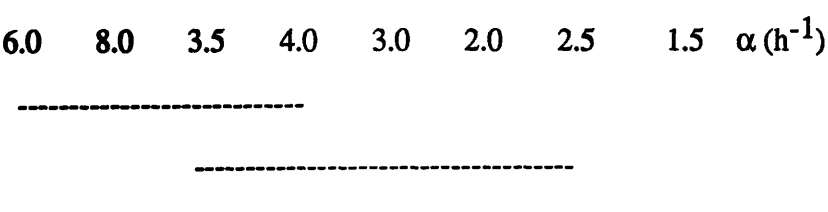
(a) Estimation of A



(b) Estimation of α



(c) Estimation of B



(d) Estimation of β

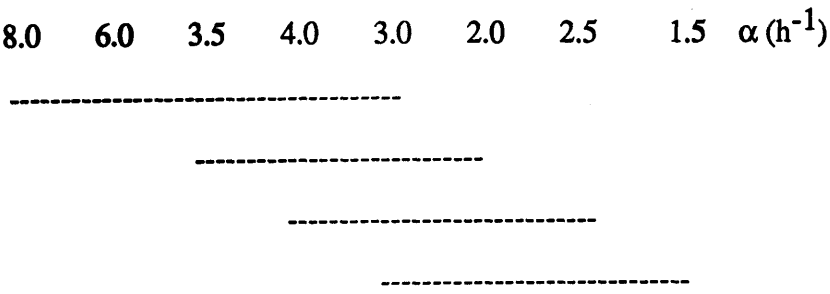


Fig. 5.6(a-d) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of different α values
a - Average rank of design number increasing from left to right.
* Efficiency measured with design number

(e) Estimation of σ_A

2.0 2.5 4.0 1.5 8.0 3.5 3.0 6.0 α (h^{-1})

(f) Estimation of σ_α

4.0 8.0 6.0 3.0 2.5 2.0 1.5 3.5 α (h^{-1})

(g) Estimation of σ_B

8.0 6.0 4.0 3.5 3.0 2.5 2.0 1.5 α (h^{-1})

(h) Overall Design Efficiency

2.0 2.5 4.0 3.5 3.0 6.0 8.0 1.5 α (h^{-1})

Fig. 5.6(e - h) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of different α values
a - Average rank of design number increasing from left to right.
* Efficiency measured with design number

σ_A (SD of %PE ranged from 26.8 to 32.2%) when α was varied between 3.5 and 8.0 h^{-1}. The estimates of σ_α were generally precise while those of σ_B were mostly imprecise, the most imprecise estimate being when α was 1.5 h^{-1}.

Using the Φ_{ir}'s, the best estimate of A was obtained with α of 1.5 h^{-1} (Fig. 5.6a). However, this was not significantly better than the efficiency with which A was estimated for α between 2.0 and 4.0 h^{-1}. The efficiency with which A was estimated with α of 1.5 and 2.0 h^{-1} was significantly better than when α was 6.0 or 8.0 h^{-1}. The least efficient estimate of A was obtained when α was 8.0 h^{-1}. α was estimated with similar efficiency over the range investigated (Fig. 5.6b).

B was most efficiently estimated when α was 6.0 h^{-1}, but this was not significantly better than the results obtained when α was 3.5, 4.0, and 8.0 h^{-1} (Fig. 5.6c). However, the B estimates obtained with α of 6.0 and 8.0 h^{-1} were significantly better than those obtained when α varied between 1.5 and 3.0 h^{-1}. Also, β was most efficiently estimated when α was 8.0 h^{-1} (Fig. 5.6d). The efficiency of β estimation when α equalled 8.0 h^{-1} was not significantly better than that when α varied between 3.0 and 6.0 h^{-1}. However, β estimates obtained when α was 6.0 and 8.0 h^{-1} were significantly better than those obtained when α varied between 1.5 and 2.0 h^{-1}.

σ_A (Fig. 5.6e) and σ_α (Fig. 5.6f) were estimated with similar efficiency for all values of α. However, the lowest rank order (on average) of Φ_{ir}'s was obtained when α was 2.0 h^{-1} for σ_A and α equalled 4.0 h^{-1} for σ_α. On the other hand, σ_B was best estimated when α was 8.0 h^{-1} and the worst estimate was obtained when α was 1.5 h^{-1} (Fig. 5.6g). The efficiency of σ_B estimation with α of 8.0 h^{-1} was not significantly better than that when α varied between 2.5 and 6.0 h^{-1}, but was significantly better than that obtained with α of 1.5 and 2.0 h^{-1}.

Overall, the parameters were best estimated when α was 2.0 h^{-1}

Table 5.5 Effect of Different Values of α on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

Section I

Success / Total

α (h^{-1})	Parameter A	α	B	β	σ_A	σ_α	σ_B	Joint
1.5	27/27	27/27	27/27	27/27	21/27	27/27	27/27	21/27
2.0	29/29	29/29	29/29	29/29	25/29	29/29	29/29	25/29
2.5	29/29	29/29	29/29	29/29	27/29	29/29	29/29	27/29
3.0	29/29	29/29	29/29	29/29	28/29	29/29	29/29	28/29
3.5	30/30	30/30	30/30	30/30	29/30	30/30	30/30	29/30
4.0	30/30	30/30	30/30	30/30	28/30	30/30	30/30	28/30
6.0	29/29	29/29	29/29	29/29	21/29	29/29	29/29	21/29
8.0	28/28	28/28	28/28	28/28	28/28	28/28	28/28	28/28

Section II

(Success - Excluded) / (Total - Excluded)

1.5	27/27	27/27	27/27	25/25	21/27	21/21	0/0	0/0
2.0	29/29	29/29	29/29	29/29	25/29	28/28	0/0	0/0
2.5	29/29	29/29	29/29	29/29	27/29	25/25	5/5	4/4
3.0	29/29	29/29	29/29	29/29	28/29	27/27	15/15	13/13
3.5	30/30	30/30	30/30	30/30	28/29	23/23	26/26	20/23
4.0	30/30	30/30	30/30	30/30	27/29	23/23	27/27	19/19
6.0	29/29	29/29	29/29	29/29	21/21	21/21	29/29	16/16
8.0	28/28	28/28	28/28	28/28	13/13	19/19	28/28	8/8

Table 5.5 Effect of Different Values of α on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

	Section III (Success - Excluded) / Total							
α	Parameter							
(h^{-1})	A	α	B	β	σ_A	σ_α	σ_B	Joint
1.5	27/27	27/27	27/27	25/27	21/27	21/27	0/27*	0/27*
2.0	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*
2.5	29/29	29/29	29/29	29/29	27/29	25/29	5/29*	4/29*
3.0	29/29	29/29	29/29	29/29	28/29	27/29	15/29	13/29
3.5	30/30	30/30	30/30	30/30	28/30	23/30	26/30	20/30
4.0	30/30	30/30	30/30	30/30	27/30	23/30	27/30	19/30
6.0	29/29	29/29	29/29	29/29	21/29	21/29	29/29	16/30
8.0	28/28	28/28	28/28	28/28	13/28	19/28	28/28	8/28*

* - $p < 0.01$

Table 5.6 Incidence* of Correlation Associated with Parameters for the Different Values of α

Correlation (%)	α (h^{-1})															
	1.5		2.0		2.5		3.0		3.5		4.0		6.0		8.0	
	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
α vs A	0.0	100.0	6.9	93.1	6.9	93.1	17.2	82.8	16.7	83.3	20.0	80.0	62.1	37.9	7.1	92.9
B vs α	81.5	18.5	13.8	86.2	13.8	86.2	0.0	100.0	0.0	100.0	3.3	96.7	0.0	100.0	0.0	100.0
β vs α	59.3	40.7	3.4	96.6	0.0	100.0	0.0	100.0	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0
β vs B	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
σ_A vs A	0.0	100.0	0.0	100.0	3.4	96.6	0.0	100.0	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0
σ_A vs α	0.0	100.0	0.0	100.0	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_α vs B	0.0	100.0	0.0	100.0	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_α vs β	0.0	100.0	0.0	100.0	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_B vs α	22.2	77.8	6.9	93.1	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_B vs B	29.6	70.4	3.4	96.6	17.2	82.8	10.3	89.7	6.7	93.3	3.3	96.7	0.0	100.0	0.0	100.0
σ_B vs β	25.9	74.1	0.0	100.0	13.8	86.2	6.9	93.1	0.0	100.0	3.3	96.7	0.0	100.0	0.0	100.0

* Incidence of pairwise correlations for combinations of parameters where the incidence of high correlation was greater than zero.

a - High > 0.75 b - Low < 0.75

(Fig. 5.6h). However, the parameters were estimated with similar efficiency when α was varied between 2.0 and 4.0 h^{-1}. The efficiency of parameters estimation with α of 2.0 h^{-1} was significantly better than the efficiency of parameters estimation when α was either 1.5 h^{-1} or between 6.0 and 8.0 h^{-1}.

Thus, A was efficiently estimated when α was in the range of 1.5 and 4.0 h^{-1}, with the most efficient estimate obtained when α was 1.5 h^{-1}. α was estimated with similar efficiency with the range of α considered in this study. B and β were best estimated with α in the range of 3.5 and 8.0 h^{-1} although the most efficient estimates of these parameters were obtained with α of 6.0 and 8.0 h^{-1}, respectively. While σ_A and σ_α were estimated with similar efficiency irrespective of the value of α, σ_B was more efficiently estimated when α was within the range of 2.5 to 8.0 h^{-1}. The best estimate of σ_B was obtained when α was 8.0 h^{-1}. All parameters were more efficiently estimated when α was between 2.0 and 4.0 h^{-1}, but the most efficient parameter estimates were obtained when α was 2.0 h^{-1}.

In addition, the estimation of all parameters was associated with good individual and joint parameters confidence intervals coverage when catastrophic estimates were included (Table 5.5, Section I). However, when runs with "coefficients of variation" > 50% were excluded, poor coverage was obtained for σ_B when α was between 1.5 and 2.5 h^{-1} (Table 5.5, Sections II & III). The joint confidence intervals coverage obtained was significantly lower than the expected value of 0.70 for α between 1.5 and 2.5 h^{-1}, and for α of 8.0 h^{-1} (Table 5.5, Section III).

The incidence of high correlation obtained for the correlation between B and α, β and α, and β and B when α was 1.5 h^{-1} was high. For all values of α high incidence of high pair-wise correlations was obtained between β and B, and for α and A when α was 6.0 h^{-1} (Table 5.6).

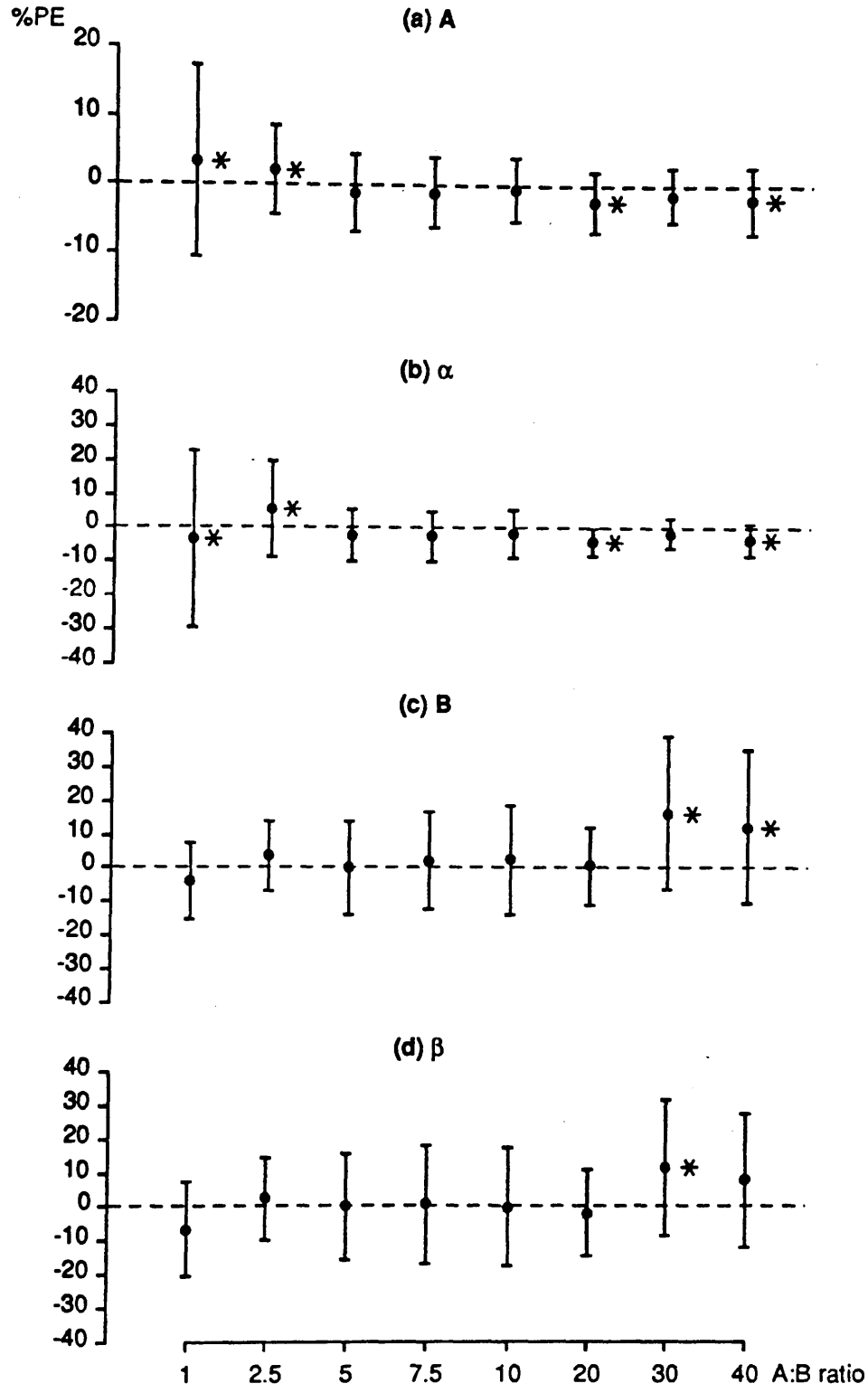


Fig. 5.7(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of A:B ratio used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

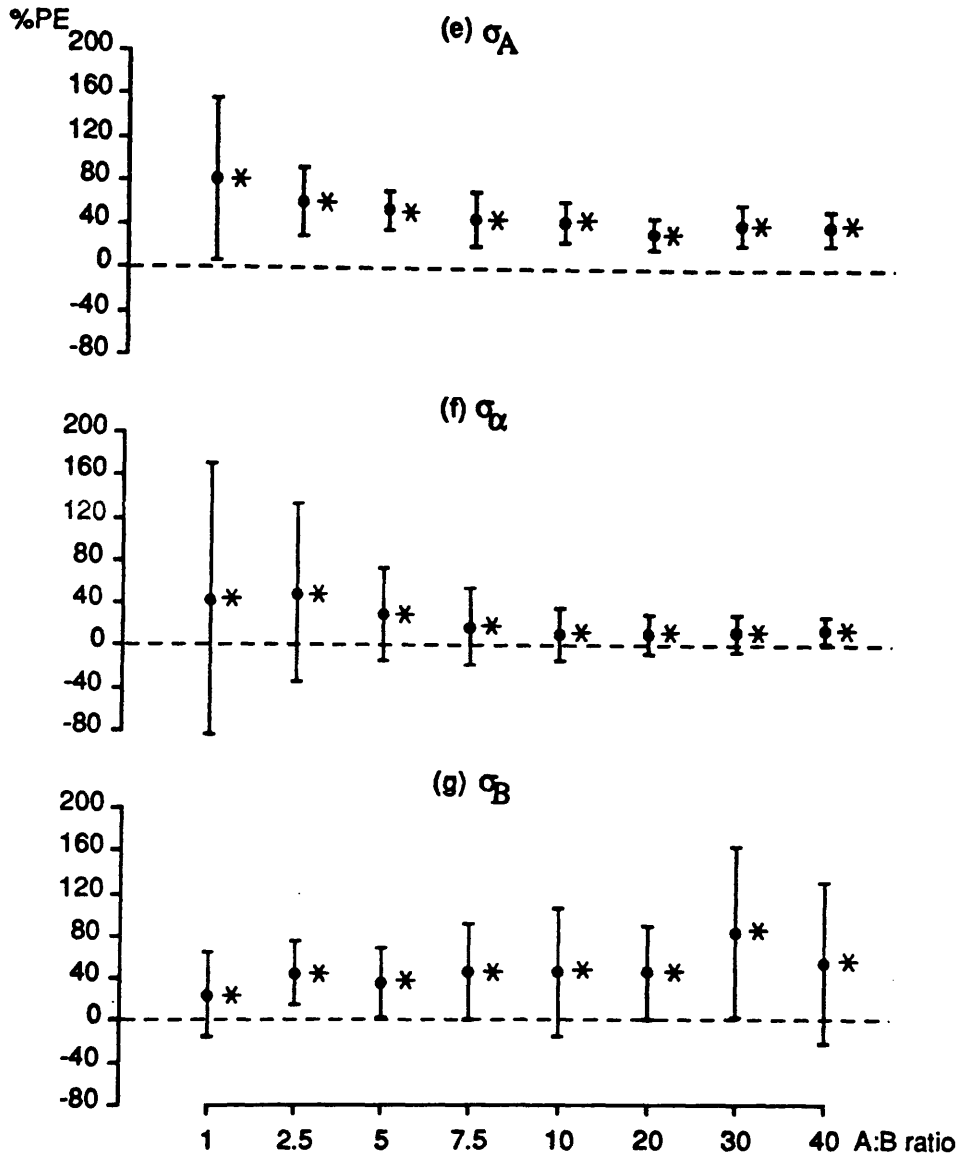


Fig. 5.7(e - g) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different values of A:B ratio used in the study. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

5.4.4 Varying A:B Ratio

When the ratio of A:B was varied between 1.0 and 40.0 (i.e. 1.0, 2.5, 5.0, 7.5, 10.0, 30.0, and 40.0) and α was 2.0 h^{-1}, the number of successful NONMEM runs were 27, 29, 30, 30, 29, 29, 29, and 27. As in the previous experiments, σ_β was excluded in the model and Fig. 5.7 shows the results as the A:B ratio varied.

All the estimates of A were minimally biased but relatively precise (Fig. 5.7a). The most biased and least precise estimate was obtained for the A:B ratio of 1.0. Most estimates of α were negatively biased. Apart from the estimate of this parameter obtained when the A:B ratio was 1.0 (SD of %PE = 26.2%) all other estimates were precise (Fig. 5.7b). All estimates of B and β were acceptably precise (Fig. 5.7 (c & d)). The estimates of B were significantly positively biased when the A:B ratio was 30.0 or 40.0. Also, greater bias was associated with estimates of β when the A:B ratio was 30.0 or 40.0. All estimates of σ_A, σ_α, and σ_B were significantly positively biased (Fig. 5.7 (e - g)). With the exceptions of estimates of σ_A obtained for A:B ratios of 1.0 and 2.5, other estimates of this parameter were acceptably precise. The estimates of σ_α were acceptably precise for A:B ratios of 20.0, 30.0, and 40.0, but all σ_B estimates were imprecise. The greater the A:B ratio the greater the precision in the estimation of σ_A and σ_α.

A was efficiently estimated when the A:B ratio was 30.0 (Fig. 5.8a), but this was not significantly better than that obtained with A:B ratio of 40 and between 2.5 and 20. However, this parameter was estimated with a significantly better efficiency with A:B ratio in the range of 2.5 and 40.0 than with A:B ratio of 1.0.

As with A, the most efficient estimate of α was obtained when the A:B ratio was 30.0, and the least efficient estimate when the A:B ratio was 1.0 (Fig. 5.8b). Estimates of α obtained with A:B ratio of 30.0 were significantly better than that with A:B ratio of 1.0, 2.5, 7.5, or 10.0. α was equally efficiently

(a) Estimation of A

30.0 40.0 20.0 10.0 5.0 7.5 2.5 1.0 A:B ratio

(b) Estimation of α

30.0 40.0 20.0 5.0 7.5 10.0 2.5 1.0 A:B ratio

(c) Estimation of B

1.0 2.5 5.0 20.0 7.5 10.0 30.0 40.0 A:B ratio

(d) Estimation of β

2.5 1.0 10.0 20.0 5.0 7.5 30.0 40.0 A:B ratio

Fig. 5.8(a - d) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of different A:B ratios
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

(e) Estimation of σ_A

20.0 40.0 30.0 10.0 7.5 5.0 2.5 1.0 A:B ratio

(f) Estimation of σ_α A:B ratio

40.0 20.0 30.0 10.0 7.5 5.0 2.5 1.0 A:B ratio

(g) Estimation of σ_B

1.0 2.5 5.0 7.5 20.0 10.0 40.0 30.0 A:B ratio

(h) Overall Design Efficiency

20.0 10.0 5.0 7.5 2.5 30.0 40.0 1.0 A:B ratio

Fig. 5.8(e - h) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of different A:B ratios.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

estimated with A:B ratios of 5.0, 20.0, 30.0, and 40.0, or 5.0, 7.5, 10.0, and 20.0, or between 2.5 and 10.0.

Unlike A and α, B was most efficiently estimated when the A:B ratio was 1.0 (Fig. 5.8c) and this was significantly better than when the A:B ratio was 30.0 or 40.0. The least efficient estimate of this parameter was obtained when the A:B ratio was 40.0.

β was best estimated when the A:B ratio was 2.5, and this was significantly better than the estimates obtained when the ratio was 40.0 (Fig. 5.8d) which resulted in the least efficient estimate of this parameter.

σ_A and σ_α were best estimated when the A:B ratio was 20.0 (Fig. 5.8e) and 40.0 (Fig. 5.8f), respectively. These estimates were significantly better than when the A:B ratio was between 1.0 and 5.0. The least efficient estimates were obtained when the A:B ratio was 1.0. On the contrary, σ_B was best estimated when the A:B ratio was 1.0 (Fig. 5.8g) and this was significantly better than when the A:B ratio was 30.0 and 40.0. The least efficient estimate of this parameter was obtained when the A:B ratio was 30.0.

All parameters were estimated with similar efficiency when the A:B ratio was in the range of 2.5 and 40.0 (Fig. 5.8h) and these were significantly better than when the A:B ratio was 1.0. The least efficient estimates of parameters overall were obtained when the A:B ratio was 1.0.

Consequently, A was efficiently estimated when the A:B ratio was in the range of 2.5 and 40.0, with the most efficient estimate when the A:B ratio was 30.0. Similarly, α was most efficiently estimated when the A:B ratio was 30.0. However, the efficiency of α estimation with this A:B ratio was similar to those obtained with A:B ratios of 5.0, 20.0, and 40.0. B was well estimated with A:B ratio in the range of 1.0 and 20.0. The most efficient estimate of this parameter was obtained when the A:B ratio was 1.0. β was efficiently estimated when the A:B ratio was between 1.0 and 30.0 with the most efficient estimate being when

Table 5.7 Effect of Different A:B Ratios on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

Section I

Success
Total

A:B Ratio	Parameter A	α	B	β	σ_A	σ_α	σ_B	Joint
1.0	27/27	27/27	27/27	27/27	27/27	27/27	27/27	27/27
2.5	29/29	29/29	29/29	29/29	29/29	29/29	29/29	29/29
5.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
7.5	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
10.0	29/29	29/29	29/29	29/29	26/29	29/29	29/29	26/29
20.0	29/29	29/29	29/29	29/29	25/29	29/29	29/29	25/29
30.0	29/29	29/29	29/29	29/29	20/29	29/29	29/29	20/29
40.0	27/27	27/27	27/27	27/27	22/27	27/27	27/27	27/27

Section II

(Success - Excluded)
(Total - Excluded)

1.0	26/26	24/24	26/26	26/26	11/11	6/6	14/14	0/0
2.5	29/29	29/29	29/29	29/29	24/24	1/1	26/26	0/0
5.0	30/30	30/30	30/30	30/30	30/30	10/10	14/14	3/3
7.5	30/30	30/30	30/30	30/30	29/29	17/17	16/16	8/8
10.0	29/29	29/29	29/29	29/29	26/29	19/19	7/7	4/4
20.0	29/29	29/29	29/29	29/29	25/29	28/28	0/0	0/0
30.0	29/29	29/29	28/28	29/29	20/29	28/28	1/1	1/1
40.0	27/27	27/27	27/27	26/26	22/27	26/26	1/1	1/1

Table 5.7 Effect of Different A:B Ratios on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

	Section III (Success - Excluded) / Total							
A:B	Parameter							
Ratio	A	α	B	β	σ_A	σ_α	σ_B	Joint
1.0	26/27	24/27	26/27	26/27	11/27*	6/27*	14/27	0/27*
2.5	29/29	29/29	29/29	29/29	24/29	1/29*	26/29	0/29*
5.0	30/30	30/30	30/30	30/30	30/30	10/30*	14/30*	3/30*
7.5	30/30	30/30	30/30	30/30	29/30	17/30	16/30	8/30*
10.0	29/29	29/29	29/29	29/29	26/29	19/29	7/29*	4/29*
20.0	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*
30.0	29/29	29/29	28/29	29/29	20/29	28/29	1/29*	1/29*
40.0	27/27	27/27	27/27	26/27	22/27	26/27	1/27*	1/27*

* - $p < 0.01$

Table 5.8 Incidence[-*] of Correlation Associated with Parameters for the Different A:B Ratios

Correlation (%)	A:B Ratio															
	1.0		2.5		5.0		7.5		10.0		20.0		30.0		40.0	
	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
α vs A	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	6.9	93.1	3.4	96.6	6.7	93.3
B vs α	7.4	92.6	75.9	24.1	86.7	13.3	56.7	43.3	62.1	37.9	13.8	86.2	20.7	79.3	6.7	93.3
β vs α	55.6	44.4	31.0	69.0	30.0	70.0	33.3	66.7	27.6	72.4	3.5	96.5	6.9	93.1	3.3	96.7
β vs B	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
σ_A vs α	3.7	96.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_A vs B	3.7	96.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_A vs β	3.7	96.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_α vs α	25.9	74.1	10.3	89.7	10.0	90.0	13.3	86.7	3.5	96.5	0.0	100.0	0.0	100.0	3.3	96.7
σ_α vs B	25.9	74.1	10.3	89.7	3.3	96.7	6.7	93.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_α vs β	22.2	77.8	3.3	89.7	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_B vs α	7.4	92.6	0.0	100.0	10.0	90.0	0.0	100.0	13.8	86.2	6.9	93.1	6.9	93.1	6.7	93.3
σ_B vs B	7.4	92.6	0.0	100.0	13.3	86.7	6.7	93.3	17.2	82.7	3.5	96.5	20.7	79.3	16.7	83.3
σ_B vs β	7.4	92.6	0.0	100.0	10.0	90.0	3.3	96.7	17.2	82.7	0.0	100.0	13.8	86.2	16.7	83.3.ls1

* Incidence of pairwise correlations of parameters where the incidence of high correlation was greater than zero. a - High > 0.75, b - Low < 0.75

A:B ratio equalled 2.5. σ_A and σ_α were well estimated when the A:B ratio was in the range of 7.5 and 40.0. The best estimates of σ_A and σ_α were obtained when the A:B ratio was 20.0 and 40.0, respectively. On the other hand, σ_B was well estimated when the A:B ratio was between 1.0 and 10.0, with the best estimate obtained when the A:B ratio was 1.0. The parameters when considered as a set were well estimated when the A:B ratio was between 2.5 and 40.0, with the best estimate obtained when the A:B ratio was 20.0.

Good individual and joint confidence intervals coverage was obtained with all A:B ratios when catastrophic runs were included (Table 5.7, Section I). When catastrophic runs were excluded to reveal the influence of standard errors on confidence intervals, poor coverage was obtained for of σ_α when the A:B ratio was between 1.0 and 5.0. However, good coverage was obtained for σ_B at A:B ratios of 1.5, and 2.5. The confidence interval coverage for σ_B when the A:B ratio equalled 5.0 was significantly lower than the expected value of 0.95. On the other hand, good coverage was obtained for the estimation of σ_α when the A:B ratio was in the range of 7.5 and 40.0. The reverse was true for the coverage of σ_B in this range of A:B ratios. All values of the A:B ratio produced joint confidence intervals coverage which were significantly lower than the expected value of 0.70 (Table 5.7, Section III).

A high incidence of high correlation was obtained between B and α with A:B ratios of 2.5, 5.0, 7.5, and 10.0, and for β and α with an A:B ratio of 1.0. 100% incidence of high correlation was obtained for β and B for all A:B ratios (Table 5.8). A lower incidence of high correlation between parameters was obtained when the A:B ratio was either 20.0 or 40.0.

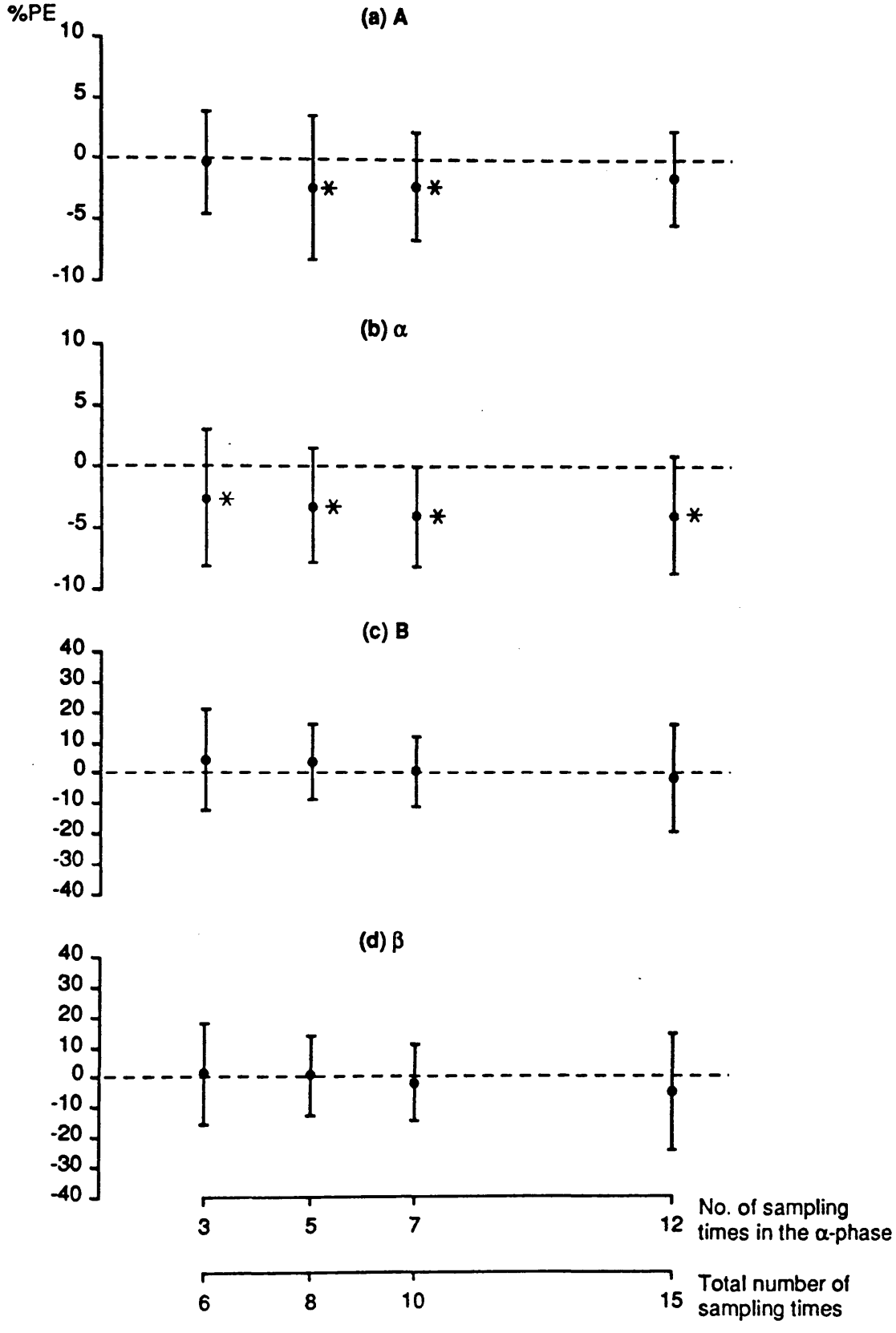


Fig. 5.9(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different number of sampling times in the α phase of the plasma concentration - time curve and the total number of sampling times. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

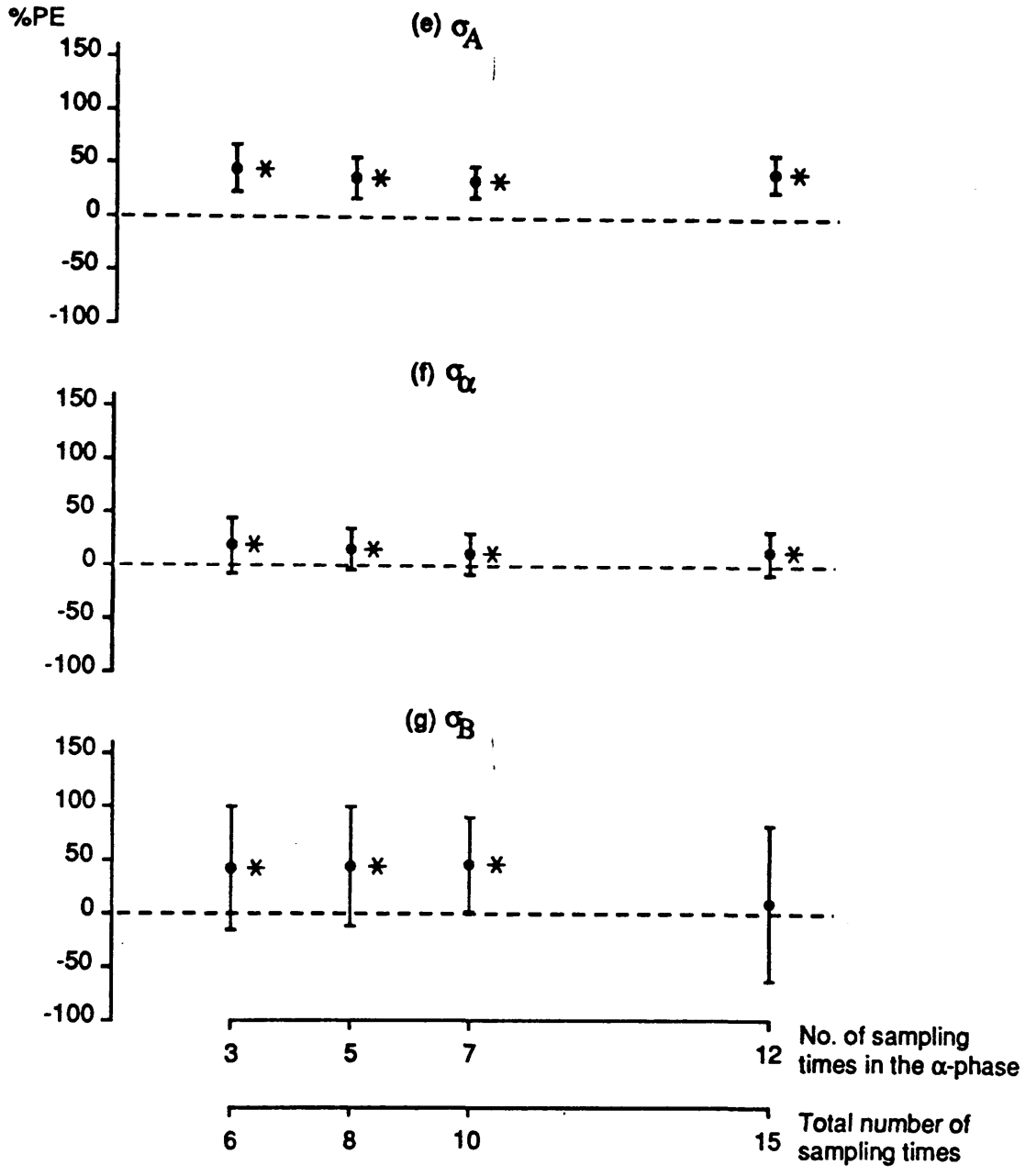


Fig. 5.9(e - g) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different number of sampling times in the α phase of the plasma concentration - time curve and the total number of sampling times. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

(a) Estimation of A

7	12	5	3	No. of sampling times in α phase

(b) Estimation of α

5	3	7	12	No. of sampling times in α phase

(c) Estimation of B

7	5	3	12	No. of sampling times in α phase

(d) Estimation of β

5	7	3	12	No. of sampling times in α phase

Fig. 5.10(a - d) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sampling times in the α phase.
a - Rank order of design numbers increasing from left to right
* Efficiency measured with design number

(e) Estimation of σ_A

7	5	12	3	No. of sampling times in α phase

(f) Estimation of σ_α

12	7	5	3	No. of sampling times in α phase

(g) Estimation of σ_B

3	5	7	12	No. of sampling times in α phase

(h) Overall Design Efficiency

7	5	3	12	No. of sampling times in α phase

Fig. 5.10(e - h) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sampling times in the α phase.
a - Rank order of design numbers increasing from left to right
* Efficiency measured with design number

5.4.5 Varying the Number of Samples in the α Phase

Setting 3, 5, 7, and 12 sampling times in the α phase of the plasma concentration - time curve, the number of successful NONMEM runs were 28, 28, 29, and 25, respectively. As in the previous studies σ_β was removed from the model. A and α were associated with negative bias and good precision irrespective of the number of sampling times in the α phase. The bias in A ranged from -0.2% (3 sampling times in the α phase) to -2.3% (5 times in the α phase) while the bias in α ranged from -2.6% (3 times in the α phase) to 4.0% (5 times in the α phase) (Fig. 5.9 (a & b)). Biased (mean %PE < 15%) but precise estimates of B and β were obtained with all schedules (Fig. 5.9 (c & d)). All estimates of σ_A and σ_α were significantly positively biased but precise (Fig. 5.9(e & f)). σ_A estimates were acceptably precise. σ_α estimates were precise for most of the schedules, except in the case where 3 time points were in the α phase (SD of %PE = 25.9%). σ_B was associated with a significant positive bias when 3, 5, and 7 sampling times were specified in the α phase and all estimates were imprecise (Fig. 5.9 g).

A was estimated with equal efficiency for all sampling schedules, although the schedule with 7 time points in the α phase had the lowest rank order (on average) of Φ_{ir}'s (Fig. 5.10a). α was most efficiently estimated with 5 time points in the α phase, but this was not significantly better than when 3 and 7 sampling times were in the α phase. However, it was significantly better than the design with 12 time points in specified in the α phase (Fig. 5.10b).

B was best estimated with 7 time points in the α phase (Fig. 5.10c) and this was significantly better than when 12 sampling times were in the α phase. β was best estimated with 5 time points in the α phase of the plasma concentration - time curve (Fig. 5.10d), and this was significantly better than when 12 time points

were in the α phase. The least efficient estimates of B and β were obtained with the latter sampling schedule.

σ_A was best estimated with 7 time points in the α phase (Fig. 5.10e) and the estimates of this parameter with this design were significantly better than when 3 or 12 times were set in the α phase. σ_α and σ_B were estimated with similar efficiency using the different designs (Fig. 5.10 f & g). However, the schedules with the lowest rank order (on average) of Φ_{ir}'s were the ones with 12 and 3 sampling times in the α phase for σ_α and σ_B, respectively.

Overall, all parameters were best estimated with the sampling schedule in which 7 sampling times were specified in the α phase (Fig. 5.10h), but designs with 3 and 5 time points in the α phase yielded similar results. The designs with 7 and 5 times in the α phase were significantly better than that with 12 time points.

All designs produced good individual parameters and joint confidence intervals coverage (Table 5.9, Section I). Also, examination of the impact of standard errors on confidence intervals coverage showed that all designs yielded coverage for σ_B and joint coverage for parameter estimates which were significantly lower than the expected values of 0.95 and 0.70, respectively (Table 5.9, Section III).

In addition, the incidence of high correlation between B and α when 3 (39.3%) and 12 (25.0%) time points were in the α phase was higher than the incidence for 5 (0%) and 7 (13.8%) sampling times in the α phase (Table 5.10). Also, the design with 12 time points in the α phase had an incidence of high correlation between α and A of 32.1%. As in all the other studies previously described for this pharmacokinetic model, a high incidence (100%) of high correlation between β and B was observed for all schedules.

Table 5.9 Effect of Varying the Number of Sampling Times in the α Phase on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

Section I

Success / Total

Number of Sampling Times		Parameter							
α phase	Total	A	α	B	β	σ_A	σ_α	σ_B	Joint
3	6	28/28	28/28	28/28	28/28	23/28	28/28	28/28	23/28
5	8	28/28	28/28	28/28	28/28	26/28	28/28	28/28	26/28
7	10	29/29	29/29	29/29	29/29	25/29	29/29	29/29	25/29
12	15	25/25	25/25	25/25	25/25	20/25	25/25	25/25	20/25

Section II

(Success - Excluded) / (Total - Excluded)

3	6	28/28	28/28	28/28	28/28	23/28	27/27	9/9	8/9
5	8	28/28	28/28	28/28	28/28	26/28	24/24	8/8	6/6
7	10	29/29	29/29	29/29	29/29	25/29	28/28	0/0	0/0
12	15	25/25	25/25	25/25	24/25	20/25	18/18	1/1	1/1

Section III

(Success - Excluded) / Total

3	6	28/28	28/28	28/28	28/28	23/28	27/28	9/28*	8/28*
5	8	28/28	28/28	28/28	28/28	26/28	24/28	8/28*	6/28*
7	10	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*
12	15	25/25	25/25	25/25	24/25	20/25	18/25	1/25*	1/25*

* - $p < 0.01$

Table 5.10 Incidence* of Correlation Associated with Parameters as the Specification of Sampling Times in the α Phase Was Varied

Correlation (%)	Number of Sampling Times in the α Phase							
	3		5		7		12	
	High[a]	Low[b]	High	Low	High	Low	High	Low
α vs A	0.0	100.0	0.0	100.0	6.9	93.1	32.1	67.9
B vs α	39.3	60.7	0.0	100.0	13.8	86.2	25.0	75.0
β vs A	0.0	100.0	10.7	89.3	0.0	100.0	0.0	100.0
β vs α	7.1	92.9	3.6	96.4	3.5	96.5	25.0	75.0
β vs B	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
σ_α vs B	0.0	100.0	0.0	100.0	0.0	100.0	7.1	92.9
σ_α vs β	0.0	100.0	0.0	100.0	0.0	100.0	7.1	92.9
σ_B vs α	0.0	100.0	0.0	100.0	0.0	100.0	14.3	85.7
σ_B vs B	7.1	92.9	3.6	96.4	3.5	96.5	25.0	75.0
σ_B vs β	0.0	100.0	0.0	100.0	3.5	96.5	21.4	78.6

* Incidence of pairwise correlations for combinations of parameters where the incidence of high correlation was greater than zero.

a - High > 0.75 b - < 0.75

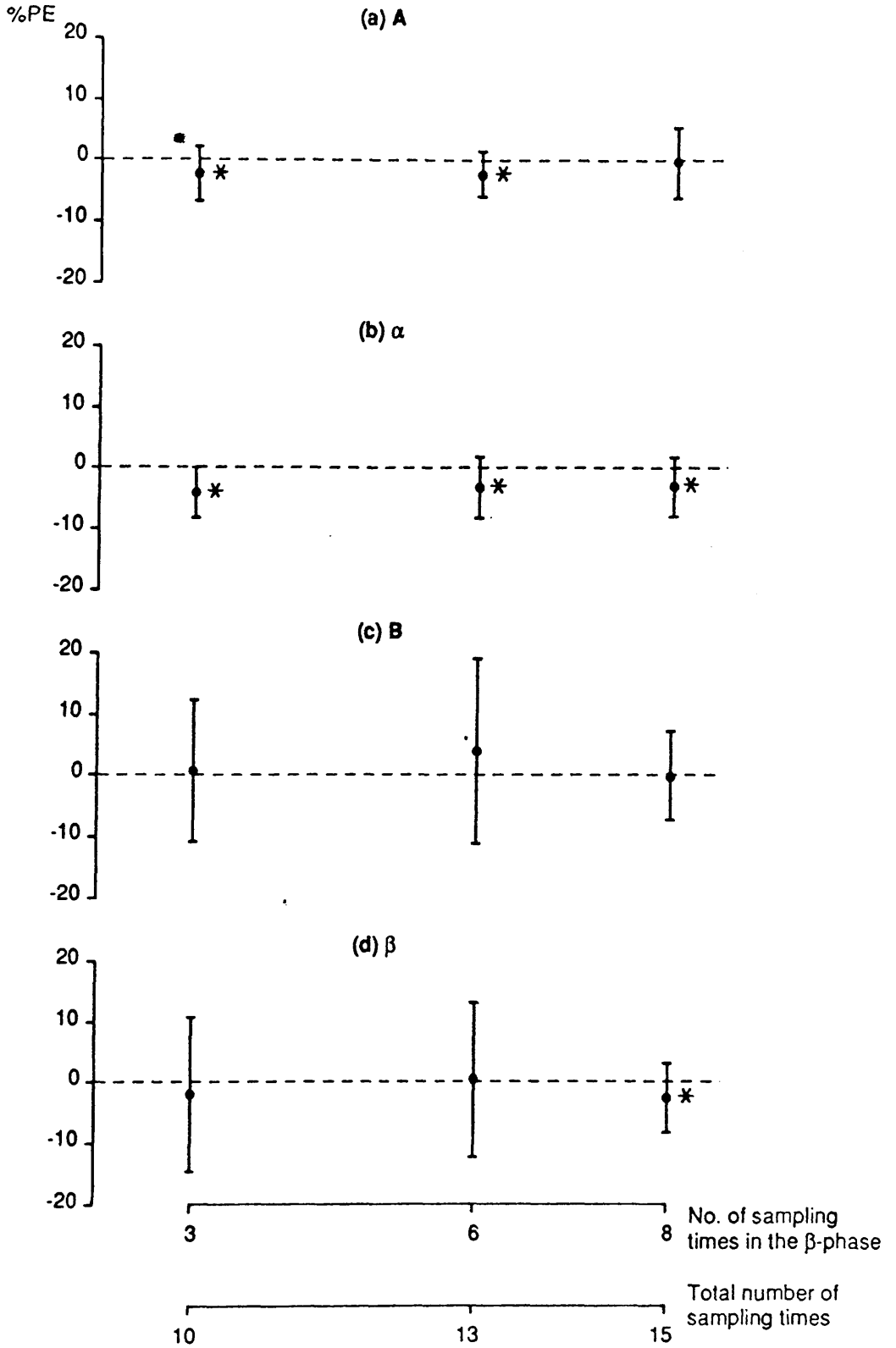


Fig. 5.11(a - d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different number of sampling times in the β phase of the plasma concentration - time curve and the total number of sampling times. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

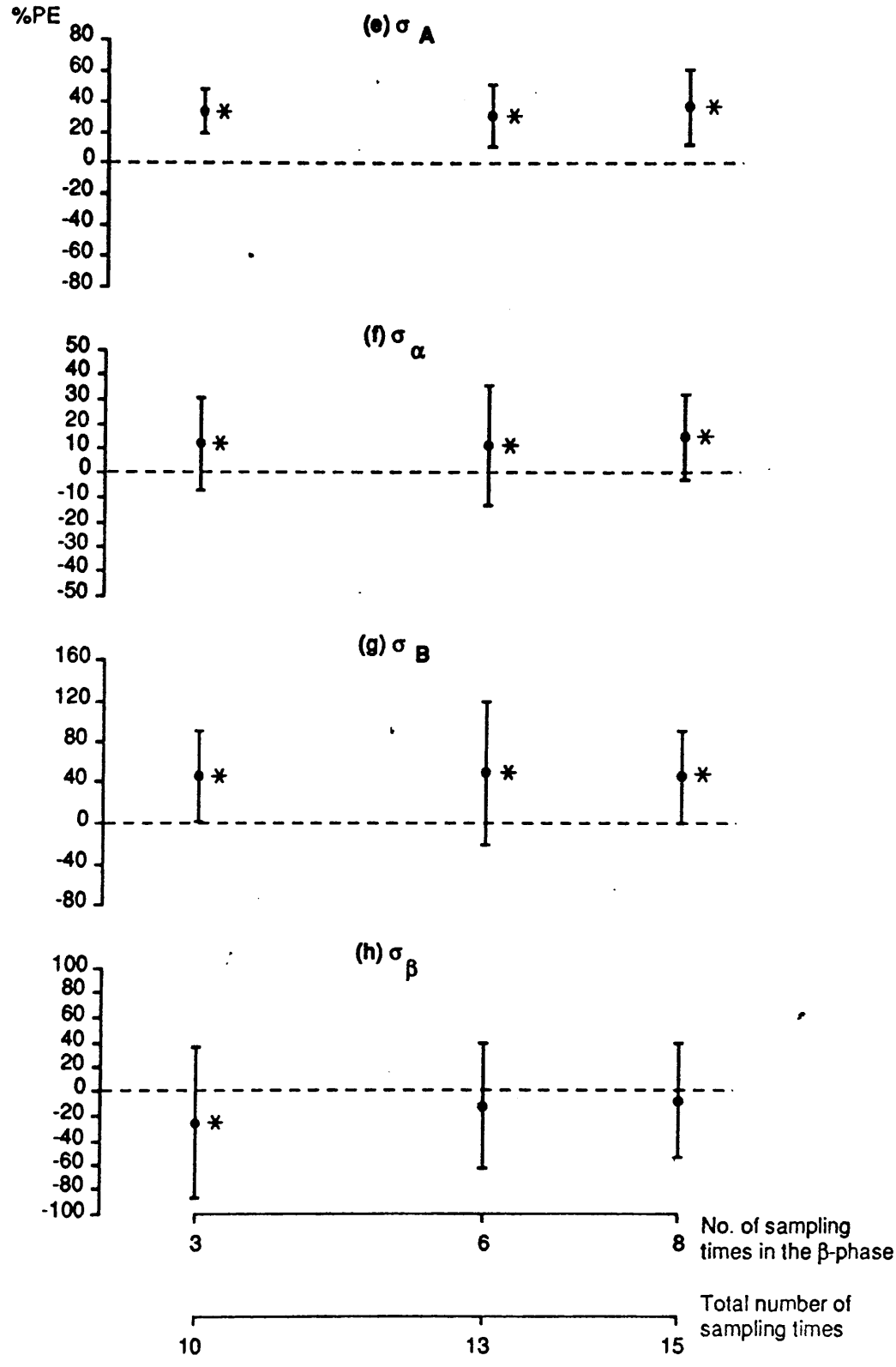


Fig. 5.11(e - h) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for estimated parameters. The horizontal axis represents the different number of sampling times in the β phase of the plasma concentration - time curve and the total number of sampling times. Each vertical bar expresses the bias and precision of the population parameter estimate. Significant ($p < 0.05$) biases indicated by asterisks.

(a) Estimation of A

6 3 8 No. of sampling times in β phase

(b) Estimation of α

8 6 3 No. of sampling times in β phase

(c) Estimation of B

8 3 6 No. of sampling times in β phase

(d) Estimation of β

8 6 3 No. of sampling times in β phase

Fig. 5.12(a - d) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sampling times in the β phase.
a - Rank order of design numbers increasing from left to right
* Efficiency measured with design number

(e) Estimation of σ_A

3	6	8	No. of sampling times in β phase

(f) Estimation of σ_α

8	6	3	No. of sampling times in β phase

(g) Estimation of σ_B

8	6	3	No. of sampling times in β phase

(h) Estimation of σ_β

8	6	3	No. of sampling times in β phase

(i) Overall Design Efficiency

8	6	3	No. of sampling times in β phase

Fig. 5.12(e - i) [a]Summary of significant differences in the efficiency* with which parameters were estimated: effect of varying sampling times in the β phase.
a - Rank order of design numbers increasing from left to right
* Efficiency measured with design number

5.4.6 Varying the Number of Sampling Times in the β Phase

The schedules with 3, 6, and 8 sampling times in the β phase had 29, 28, and 25 successful NONMEM runs, respectively, when the sampling times in the β phase were varied. The estimates of A and α were mostly significantly negatively biased but precise with the SD of %PE ranging from 3.8 to 5.8% (Fig. 5.11 (a & b)). B and β, on the other hand, were minimally biased and relatively precise (SD of %PE = 5.7 to 15.1%) (Fig. 5.11 (c & d)). σ_A and σ_α estimates were significantly positively biased, but acceptably precise (Fig. 5.11 (e & f)). σ_B was estimated with a significant positive bias and poor precision (Fig. 5.11g). σ_β was negatively biased and imprecise (Fig. 5.11h). The design with 3 time points in the β phase yielded the most biased estimate (mean %PE = 25.3%) and this was significant.

No sampling schedule was significantly better than any other for the efficiency with which A and α was estimated (Fig. 5.12 (a & b)). However, the lowest rank orders (on average) of Φ_{ir}'s were obtained with schedules having 6 and 8 time points in the β phase for A and α, respectively.

B (Fig. 5.12b) and β (Fig. 5.12c) were most efficiently estimated with 8 sampling times in the β phase. These estimates were significantly better than those obtained with other sampling schedules.

σ_A, σ_α, and σ_B were estimated with similar efficiency with all designs (Fig. 5.12 (e - g)). While the best estimate of σ_A was obtained with 3 time points in the β phase, the best estimates of σ_α and σ_B were obtained with 8 sampling times in the β phase. The most efficient estimate of σ_β was obtained with 8 time points in the β phase (Fig. 5.12h), but this was only significantly better than when 3 sampling times were in the β phase.

Overall, all sampling schedules did not differ in the efficiency in which

Table 5.11 Effect of Varying the Number of Sampling Times in the β Phase on Individual and Joint Confidence Intervals Coverage for Parameter Estimates

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Section I

Success / Total

Number of Sampling Times β phase	Total	A	α	B	β	σ_A	σ_α	σ_B	σ_β	Joint
3	10	29/29	29/29	29/29	29/29	25/29	29/29	29/29	29/29	25/29
6	13	28/28	28/28	28/28	28/28	28/28	28/28	28/28	28/28	28/28
8	15	25/25	25/25	25/25	25/25	24/25	25/25	25/25	24/25	24/25

Section II

(Success - Excluded) / (Total - Excluded)

3	10	29/29	29/29	29/29	29/29	25/29	28/28	0/0	0/0	0/0
6	13	28/28	28/28	28/28	28/28	26/26	23/23	11/11	5/5	8/8
8	15	25/25	25/25	25/25	25/25	23/24	19/19	9/9	11/11	6/6

Section III

(Success - Excluded) / Total

3	10	29/29	29/29	29/29	29/29	25/29	28/29	0/29*	0/29*	0/29*
6	13	28/28	28/28	28/28	28/28	26/28	23/28	11/28*	5/28*	0/28*
8	15	25/25	25/25	25/25	25/25	23/25	19/25	9/25*	11/25*	0/25*

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* - $p < 0.01$

Table 5.12 Incidence* of Correlation Associated with Parameters as the Specification of Sampling Times in the β Phase Was Varied

Correlation (%)	Number of Sampling Times in the β Phase					
	3		6		8	
	High[a]	Low[b]	High	Low	High	Low
α vs A	6.9	93.1	7.1	92.9	80.8	19.2
B vs α	13.8	86.2	3.6	96.4	0.0	100.0
β vs α	3.5	96.5	3.6	96.4	0.0	100.0
β vs B	100.0	0.0	100.0	0.0	100.0	0.0
σ_A vs A	0.0	100.0	3.6	96.4	0.0	100.0
σ_B vs α	6.9	93.1	3.6	96.4	0.0	100.0
σ_B vs B	3.5	96.5	10.7	89.3	0.0	100.0
σ_B vs β	0.0	100.0	10.7	89.3	0.0	100.0

* Incidence of pairwise correlations for combinations of parameters where the incidence of high correlation was greater than zero.

a - High > 0.75 b - Low < 0.75

parameters were estimated (Fig. 5.12i). The schedule with 8 time points in the β phase yielded estimates with the lowest rank order of Φ_r's, and the design with 3 sampling times in the β phase yielded estimates with the highest rank order of Φ_r's, on average.

Consequently, all sampling schedules produced similarly efficient estimates of A and α, and B and β were most efficiently estimated when 8 time points were in the β phase. While σ_A, σ_α, and σ_B were estimated with similar efficiency with the three sampling schedules studied, σ_β was better estimated with 6 or preferably 8 time points in the β phase. All designs produced parameter estimates with similar efficiency and not much could be gained by increasing the duration of sampling.

As with the other studies previously described, all sampling designs yielded good confidence intervals coverage for individual and joint parameter estimates when NONMEM runs with catastrophic estimates were included (Table 5.11, Section I). When runs with catastrophic estimates were excluded to reveal the impact of standard errors on confidence intervals coverage, poor coverage was obtained for σ_B, σ_β, and joint parameter estimates (Table 5.11, Section III). However, the coverage for σ_β was slightly better when 8 time points were in the β phase compared to the designs with 6 and 3 sampling times in this phase of the concentration time curve (Table 5.11, Section III).

The schedule with 8 sampling times in the β phase had 80.8%incidence of high correlation between α and A, while incidences of 6.9% and 7.1% were obtained for the correlation between these parameters when 3 and 6 time points, respectively, were in the β phase (Table 5.12). Although the incidence of high correlation between β and α was 13.8% (3 sampling times in the β phase) and 3.6% (6 sampling times in the β phase) it was 0% when 8 time points were in the β phase. In addition, 100% incidence of high correlation between β and B was

observed for all sampling schedules. Incidence of correlation of less than 11% was obtained for the correlation between β and α, σ_B and B for the designs having 3 and 6 sampling times in the β phase, but not the design with 8 time points in the β phase. The latter yielded 0% incidence for these pair-wise correlations. Also an incidence of 3.6% was obtained for the correlation between σ_A and A when 6 sampling times were specified in the β phase.

5.5 DISCUSSION

In the investigation of the effect of sample size on parameter estimation, the parameter values (A = 10000.0 IU/ml, $\alpha = 2.0\ h^{-1}$, B = 500 IU/ml, and $\beta = 0.2\ h^{-1}$) and sampling strategy were chosen to mimic a real study which is reported in detail in Chapter 7. With this sampling strategy, 70% of the data points were in the α phase. This yielded precise estimates of A and α, irrespective of the sample size, as would be expected with the partitioning. The effect of the partitioning was observed in the estimation of B and β. Only the use of a sample size of 150 (15 animals per time point) gave estimates of these parameters which were precise. With this sample size, 45 data points were located in the β phase of the concentration - time curve compared with 30 and 18 for the 100 and 60 sample sizes, respectively. The accuracy with which these parameters were estimated was not affected by sample size. Increase in the sample size led to an increase in the precision with which the variance parameters were estimated, as expected. In addition, the positive bias in the estimation of the variance parameters was probably a feature of the one observation per animal study design.

The estimation of the variance parameters (especially σ_B) was associated with large standard errors which led to poor individual and joint confidence intervals coverage when the runs with catastrophic estimates were excluded. The

contribution of bias to this poor coverage was negligible since good coverage was obtained when the runs with catastrophic estimates were included. Given the pharmacokinetic model and design specifications considered, the appropriate sample size necessary for efficient estimation of population pharmacokinetic parameters is 150 (i.e. 15 observations per time point) or more. This was associated with a lower incidence of high correlation between parameters. Although parameters were better estimated with 150 observations than 100 observations (i.e. 10 animals at each time point), the loss in estimation efficiency with the latter sample size was not very dramatic as seen in the individual and joint confidence intervals coverage when catastrophic runs were excluded (Table 5.2, Section III). Model parameters were least efficiently estimated with a sample size of 60 and with this sample size, more parameters were highly correlated with each other (Table 5.4).

NONMEM estimation of A, α, B, and β was often associated with negative bias. This could be due to either the study design, or a feature of the program (i.e. estimation error since negative bias in the estimation of these parameters was also observed when σ_{ϵ} was specified as 0%). Error in concentration measurements had negligible influence on the estimation of model parameters. However, it did have an influence on the estimation of the variance parameters. When σ_{ϵ} was greater than 10%, large biases were associated with the variance parameters. These were due to there being no information to allow the estimation of σ_{ϵ}. Thus, the inter-animal variability estimated was a composite of inter- and intra- animal variability. Setting σ_{ϵ} equal to 15% yielded the least efficient estimates of parameters. This specification of error in concentration measurements is the upper limit of error in concentration measurements generally acceptable in practice, and the need to minimise error in concentration measurements, especially with the one observation per animal study design cannot be over emphasised.

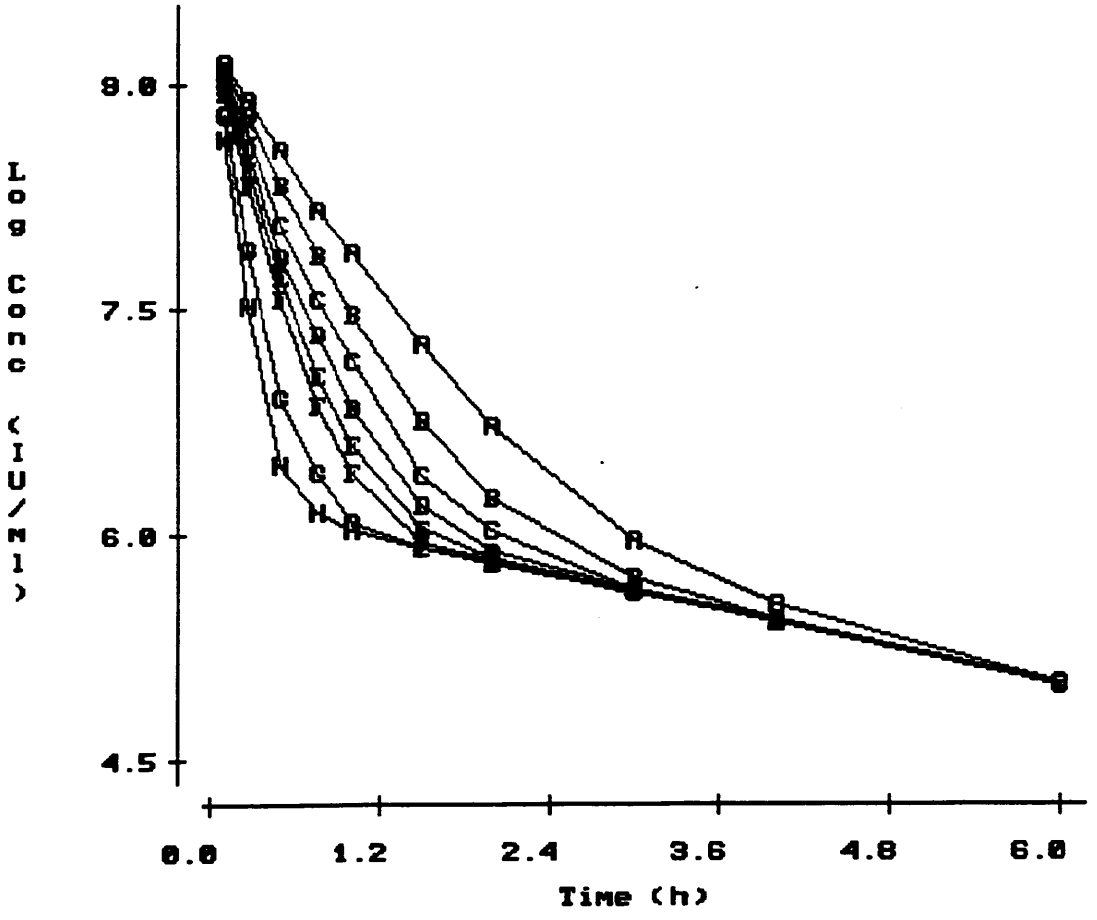


Fig. 5.13 Distribution of sampling times with different α values: A---A for α = 1.5 h^{-1}, B---B for α = 2.0 h^{-1}, C---C for α = 2.5 h^{-1}, D----D for α = 3.0 h^{-1}, E---E for α = 3.5 h^{-1}, F---F for α = 4.0 h^{-1}, G---G for α = 6.0 h^{-1}, H---H for α = 8.0 h^{-1}.

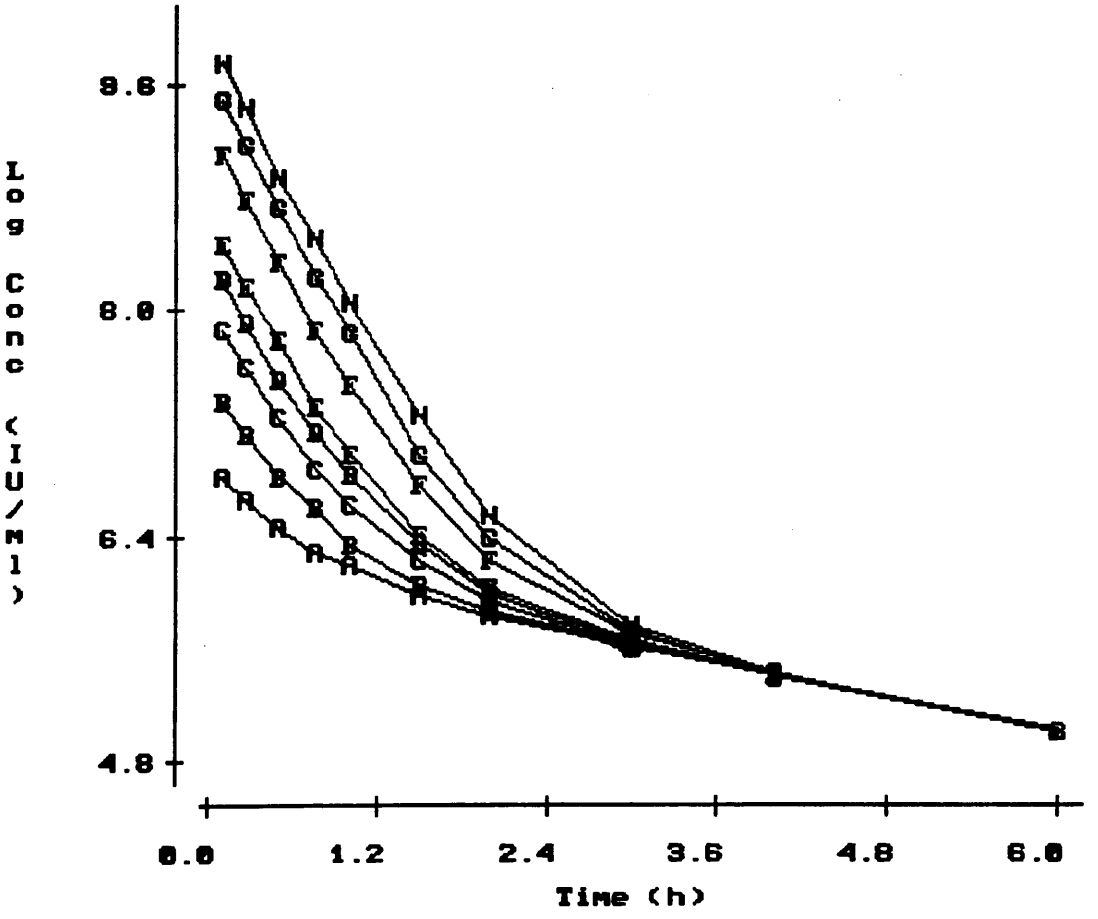


Fig. 5.14 Distribution of sampling times with different A:B ratios: A---A for A:B ratio = 1.0, B---B for A:B ratio = 2.5, C---C for A:B ratio = 5.0, D----D for A:B ratio = 7.5, E---E for A:B ratio = 10.0, F---F for A:B ratio = 20.0, G---G for A:B ratio = 30.0, H---H for A:B ratio = 40.0.

The negative bias associated with A and α observed in the study in which the efficiency of parameter estimation with a range of α values was determined, is a feature of the NONMEM program as previously discussed. The almost unbiased estimate of these parameters obtained when α was 1.5 h^{-1} was due to the fact that the slope of the α phase of the concentration - time curve was less steep, hence more data points were located in the α phase. Steeper slopes of the α phase of the concentration - time curve, a consequence of higher α values, yielded mostly efficient (unbiased and precise) estimates of B and β because more data points were partitioned into the β phase of the concentration - time curve (Fig. 5.13). Similar conclusions were arrived at using the design numbers.

Thus, A was more efficiently estimated when α was in the range of 1.5 and 4.0 h^{-1}, with the most efficient estimate obtained when α was 1.5 h^{-1}. Although α was estimated with similar efficiency irrespective of the value of α used, the best estimate was obtained when α was 3.5 h^{-1}. B and β were well estimated when α was in the range of 3.5 and 8.0 h^{-1}. Although the efficiency of σ_A and σ_α estimation was similar for α values, the best estimates were obtained for σ_A and σ_α when α was 2.0 h^{-1} and 4.0 h^{-1}, respectively. σ_B was better estimated when α was in the range of 2.5 and 8.0 h^{-1}. The relatively inefficient estimation of the variance parameters was due to the fact that there was no information in the data set on σ_ϵ.

With the range of A:B ratios considered and α of 2.0 h^{-1}, efficient estimation of A and α was obtained with the higher A:B ratios. The greater the A:B ratio the more precise were the estimates of these parameters. Given that the slope of the α phase of the concentration time curve remained constant irrespective of the A:B ratio, more data points were partitioned into the α phase of the concentration time curve with higher A:B ratios (Fig. 5.14). Thus, A and α were most efficiently estimated when the A:B ratio was 30.0. However, A was

estimated with similar efficiency irrespective of the value of the A:B ratio. α, on the other hand, was better estimated when the A:B ratio was between 20.0 and 40.0.

B and β, however, were better estimated when the A:B ratio was low. B was best estimated when the A:B ratio was between 1.0 and 20.0 while β was best estimated when the A:B ratio was between 1.0 and 30.0. The most efficient estimates of these parameters were obtained when the A:B ratio was 1.0 and 2.5, respectively.

Good estimates of σ_A and σ_α were obtained when the A:B ratio was in the range of 7.5 and 40.0 for the same reason advanced for the estimation of A and α. σ_B was well estimated when the A:B ratio was in the range of 1.0 and 20.0, and the best estimate when the A:B ratio was lowest (1.0). Interpreting the results using bias and precision, and the design number approach led to the same conclusions. All parameters were well estimated when the A:B ratio was in the range of 2.5 to 40.0 with the best estimates obtained when the A:B ratio was 20.0. It is worthy of note that fewer pair-wise high correlations were obtained when the A:B ratio was 20.0.

The poor confidence interval coverage observed for σ_B when the A:B ratios were high was due to large standard errors. There was no contribution of bias to this observation as seen in Table 5.11 (Section I & II). Similarly, the poor coverage observed for joint confidence intervals was due to large standard errors.

Although all schedules with the different specifications of sampling times in the α phase produced estimates of A and α that were negatively biased, some of which were significant, the mean %PE did not exceed 4%. All schedules produced precise estimates of A and α with the SD of %PE ranging from 4.0 to 5.9%. The difference in the efficiency with which these parameters were estimated lay in the contribution of the "standard error term" in the calculation of the design number. Thus, A was most efficiently estimated with 7 time points in

the α phase, while the design with 3 sampling times in the α phase produced the least efficient estimate. As in the case of bias and precision where the %PE values were very close for all schedules, there were no significant differences when the design numbers for the different sampling schedules were compared. That the sampling schedule with 5 time points in the α phase gave the most efficient estimate of α, while the sampling schedule with 12 time points in the α phase gave the most inefficient estimate, was also due to the effect of the "standard error term". The design with 7 sampling times in the α phase produced the least biased and most precise estimate of B, while the one with 5 time points produced the least biased estimate of β. The difference in the precision with which B and β were estimated with 5 and 7 time points in the α phase was only 1%. Thus, the most efficient estimates of B and β obtained with 7 and 5 sampling times in the α phase, respectively, were due to the influence of bias. Although all designs produced estimates of B and β that were precise and not significantly biased, the efficiency with which these parameters were estimated with the schedule having 12 time points in the α phase was significantly poorer than the rest. This was probably due to the fewer number of data points in the β phase which resulted in an estimate with a large standard error.

The positively biased estimates of variance parameters was a consequence of the one observation per animal study design. The least efficient estimates of σ_A and σ_α were obtained when 3 time points were in the α phase. This design was associated with estimates which were the least precise and the most biased. On the other hand, the most efficient estimates of σ_A and σ_α were obtained when 7 and 12 time points, respectively, were in the α phase. These schedules produced parameter estimates which were the most precise and least biased. Also, the "standard error term" in Φ_{ir} for these parameters generated with these designs was the lowest when compared with the other designs. σ_B was least efficiently

estimated when 12 sampling times were in the α phase, and this design produced the most imprecise estimate. Although this sampling schedule produced the least biased estimate of σ_B, the inefficiency was due to the imprecision of the estimate of this parameter. The efficient estimation of σ_B with 3 time points in the α phase was due to the location of a greater number of data points in the β phase. Overall, model parameters and their variances were most efficiently estimated when 7 time points were in the α phase.

The schedule with 12 time points in the α phase produced the least efficient parameter estimates. This could be attributed to the greatest incidence of high correlation between parameters (Table 5.10). This inefficiency in parameter estimation was also associated with large "coefficient of variation". However, large "coefficient of variation" was responsible for poor confidence interval coverage for σ_B and joint parameter estimates for all sampling designs when catastrophic runs were excluded in the calculation of confidence intervals.

In the study in which the effect of altering the number of time points in the β phase on parameter estimation was investigated, the bias and precision obtained in the estimation of A and α were similar for all sampling schedules; hence the lack of significant difference in the efficiency with which these parameters were estimated. All schedules produced negatively biased estimates of these parameters. The design with 8 time points in the β phase produced the most precise estimates of B and β. Thus, the production of the most efficient estimates of B and β with this sampling strategy was due to the estimates being the most precise. The lack of significant differences in the efficiency with which σ_A, σ_α, and σ_B were estimated by the different designs was due to estimates being biased and precise to a similar extent. On the other hand, the estimate of σ_β was the least biased and imprecise when 8 time points were in the β phase. The bias produced in the estimation of this parameter with this schedule was less than one third of that when 3 sampling times were in the β phase. Thus, the most efficient estimate

of σ_β was obtained when 8 time points were in the β phase.

Although the sampling schedule with 8 time points in the β phase gave the most efficient parameter estimates when the overall design efficiency was considered, this was not significantly better than the other schedules. This may have been due to the pair-wise correlations between parameter estimates. Whereas the incidence of high correlation between α and A was 6.9% for the design with 3 sampling times in the β phase, and 7.1% for the design with 6 time points in the β phase, it was 80.8% when 8 time points were in the β phase. Alternatively, the incidence of high correlation between B and α, β and α, σ_A and A, σ_B and α, σ_B and B, σ_B and β was less than 14% for the design with 6 sampling times in the β phase. A similar incidence was obtained for the sampling design with 3 sampling times in the β phase, but the incidence of high correlation for σ_A vs. A, and σ_β vs. β was zero. Except for the correlation of β and B, in which the incidence of high correlation was 100% for all sampling designs, the incidence of high correlation for other parameter pairs not previously discussed for the design with 8 time points in the β phase was zero. The instability in the estimates due to the pair-wise correlations was reflected in the poor coverage observed with the joint confidence intervals for parameter estimates.

In all studies described, a high incidence (100%) of high correlation occurred between β and B. There were some occasions of high incidence of high correlation between α and A, B and α, and α and β . These must have contributed to inefficient parameter estimation in some study designs. This was reflected in wide confidence intervals such that, when estimates with "coefficient of variation" greater than 50% were excluded in the computation of confidence intervals, poor coverage was obtained for σ_B and joint parameter estimates. The large "coefficients of variation" could be inherent to the model or to the relative magnitudes of the parameters used. A high correlation reduces the desirability of obtain-

ing parameter estimates (Boxenbaum *et al.*, 1974) and requires a reparameterization of the model.

CHAPTER 6

REPARAMETERIZATION OF THE TWO COMPARTMENT MODEL

6.1 SUMMARY

A simulation study was carried out using the one observation per animal design to examine the impact of reparameterization of the two compartment model (IV bolus dose injection) on the efficiency with which model parameters were estimated. The parameters of the model were Cl, V_1, k_{12}, and k_{21} instead of A, α, B, and β. The efficiency of parameter estimation was determined by examining accuracy and precision, design number, single and joint confidence intervals for parameter estimates, and the correlation between parameter estimates. Reparameterization led to the generation of more stable parameter estimates, and relatively lower incidence of high correlation between parameters.

6.2 INTRODUCTION

The results of the studies reported in Chapter 5 showed that the estimation of parameters of the two compartment model described by Eq. (6.1) below can be problematic.

$$C(t) = A.\exp(-\alpha.t) + B.\exp(-\beta.t) \qquad (6.1)$$

(Model I)

The instability in the estimation of some of the parameters was reflected in wide confidence intervals, and high correlation between parameter estimates. Consequently, the model in Eq. (6.1) (Model I) was reparameterized in terms of Cl, V_1,

k_{12}, and k_{21} and had the following structural form:

$$C^*_j = [D_j/V_{1j}(\alpha - \beta)][\alpha - k_{21})\exp(-\alpha t_j) + (k_{21} - \beta)\exp(-\beta t_j)] \quad (6.2)$$

(Model II)

where $\alpha, \beta = 1/2\{k_{12} + k_{21} + k_{10} \pm [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2}\}$, and $k_{10} = Cl_j/V_{1j}$. C^*_j is the true drug concentration in the jth animal , D_j, V_{1j}, Cl_j are the dose, volume of the central compartment and clearance in the jth animal, respectively, and t_j the corresponding sampling time. However, in the simulation α and β were parameterized in terms of the microscopic rate constants k_{12} and k_{21}.

The goal of this simulation study was to evaluate the impact of reparameterization of the two compartment open model with intravenous bolus dose administration on the estimation of population pharmacokinetic parameters using the one observation per animal design. Specifically, the efficiency with which these parameters were estimated was determined by examining the accuracy and precision, design number, single and joint confidence intervals for parameter estimates, and the incidence of high correlation between parameter estimates .

6.3 METHODS

6.3.1 SAMPLING DESIGN AND ANALYSIS

Using the following values of α: 2.0, 4.0, 6.0, and 8.0 h^{-1}; β, A and B values of 0.2 h^{-1}, 10000.0 and 500.0 IU/ml, respectively, used in Chapter 5, values of Cl, V_1, k_{12} and k_{21} were computed and used in the simulation

Table 6.1 Starting Values for Simulation on Reparameterization: Different α Values

	Parameter			
α (h^{-1})	Cl (l/h)	V_1 (l)	k_{12} (h^{-1})	k_{21} (h^{-1})
2.0	0.025	0.020	0.50	0.30
4.0	0.040	0.020	0.30	0.40
6.0	0.050	0.020	3.00	0.50
8.0	0.055	0.020	5.00	0.60

Table 6.2 Starting Values for Simulation on Reparameterization: Different A:B Ratios

	Parameter			
A:B	Cl (l/h)	V_1 (l)	k_{12} (h^{-1})	k_{21} (h^{-1})
1.0	0.070	0.200	0.75	1.00
10.0	0.040	0.035	0.70	0.35
20.0	0.025	0.020	0.50	0.30
30.0	0.020	0.012	0.40	0.25
40.0	0.016	0.010	0.30	0.20

(Table 6.1). The respective variances were selected to yield a coefficient of variation of 15% for all parameters. A 15% error was added in concentration measurements as previously described (see Chapter 2, Section 2.4).

An intravenous bolus dose of 200,000 IU was used, and animals were sampled over ten time points with 15 observations being made at each time point giving a sample size of 150. The sampling times used were 0.083, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h. As in Chapter 5 the first time point was fixed while others were sampled from a uniform range of 0.25 h. The simulation was carried out as previously described in Chapter 2 (Section 4) and 30 replicate data sets were generated for each experiment.

α was then kept constant at 2.0 h^{-1} and with β unchanged at 0.2 h^{-1}, the efficiency of parameter estimation with a range of A:B ratios was investigated. The A:B ratios used were 1.0, 10.0, 20.0, 30.0, and 40.0 (Table 6.2).

The chi-squared test was used to compare joint confidence intervals coverage for parameter estimates obtained using the reparameterized model (Model II) with those obtained with Model I in Chapter 5.

6.4 RESULTS

6.4.1 REPARAMETERIZATION WITH VARIATION IN α

6.4.1a Bias and Precision

In the results presented below σ_{k12} and σ_{k21} are not included since the estimates of these parameters were infinitesimal and their removal did not alter the NONMEM objective function or other parameters estimated. Fig. 6.1a shows

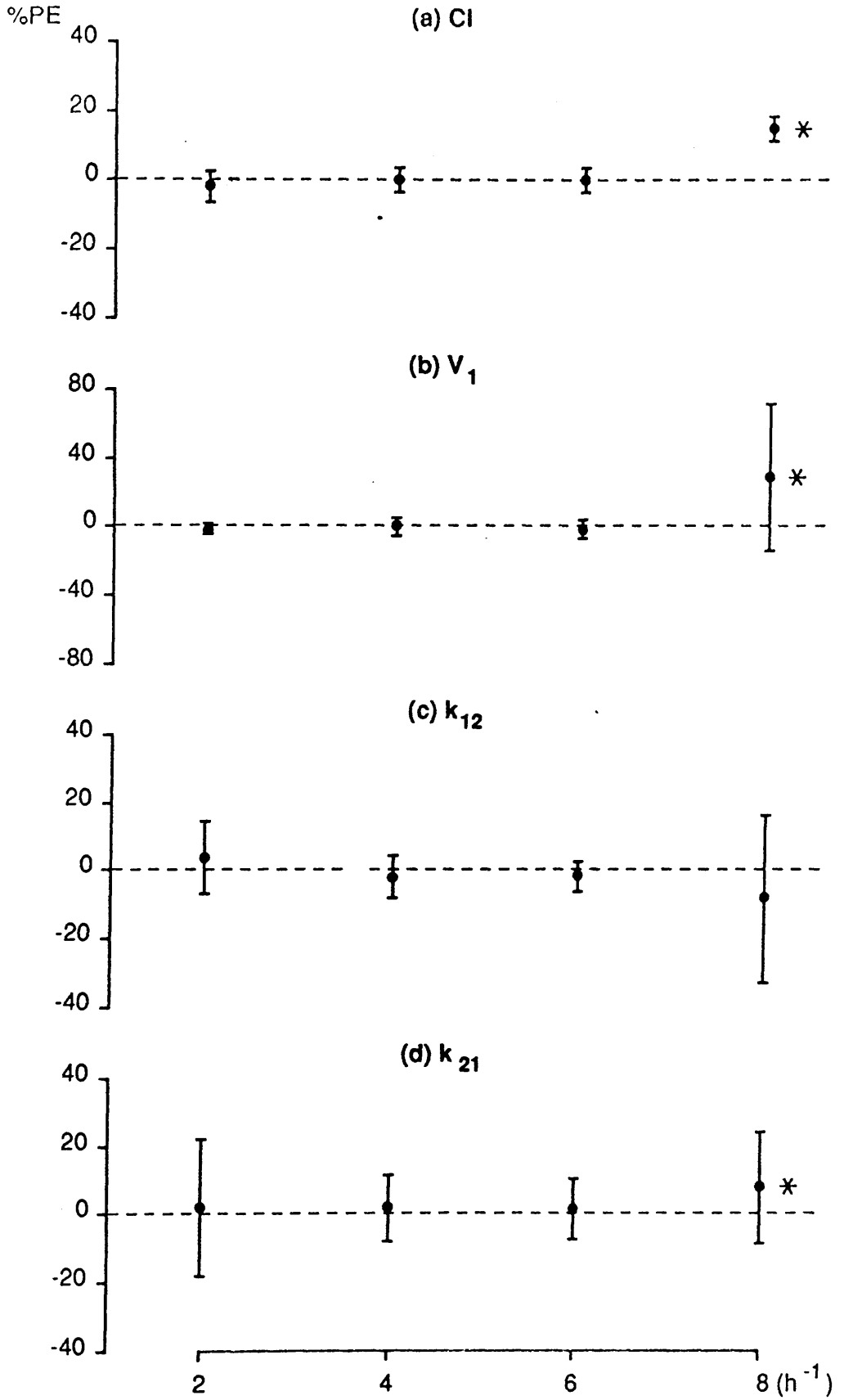


Fig. 6.1(a - d) Bias and precision in expressed as %PE (mean ± standard deviation, respectively) for (a) Cl, (b) V_1, (c) k_{12}, and (d) k_{21}. The horizontal axis represents the different values of α. Each vertical bar expresses the bias and precision of the population parameter estimate.

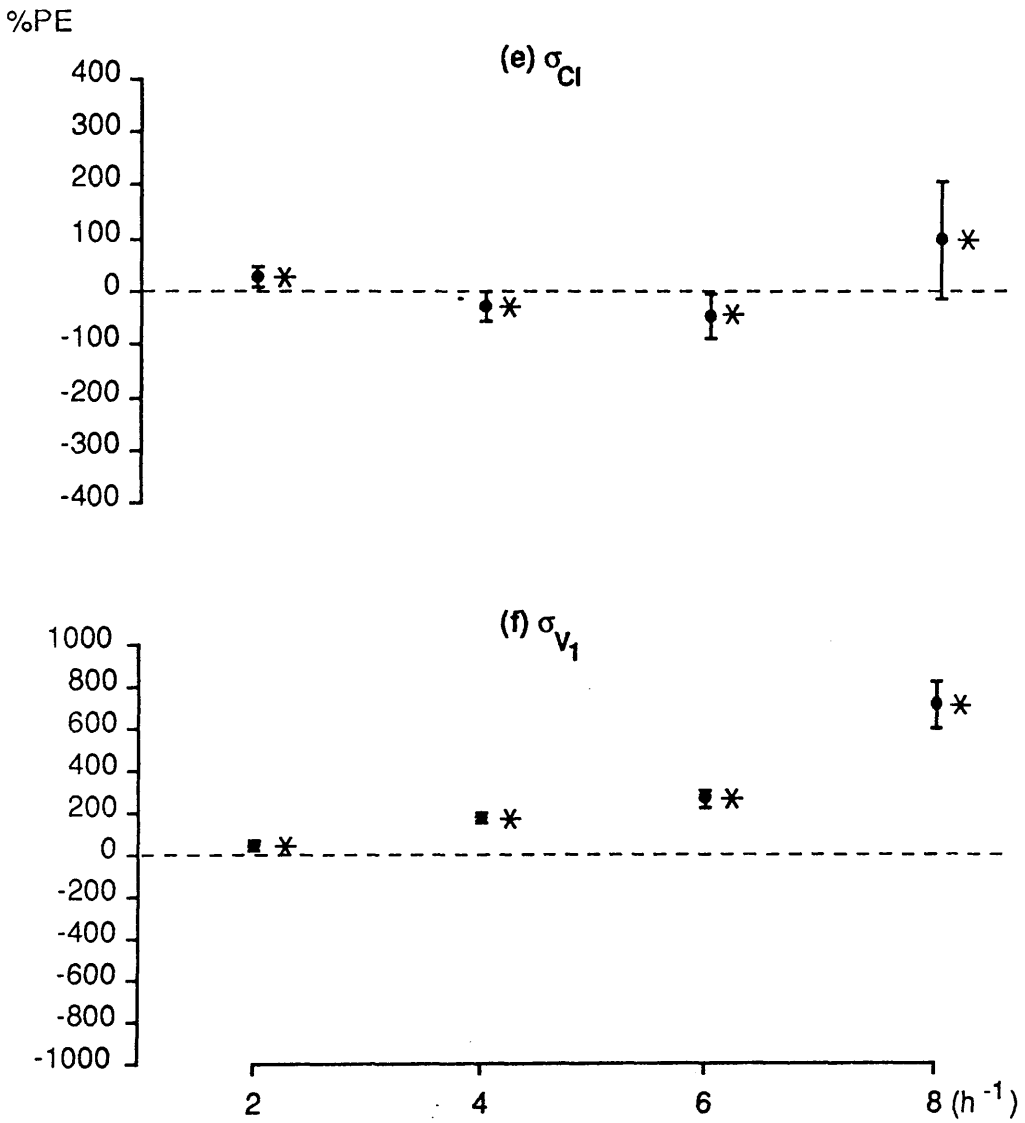
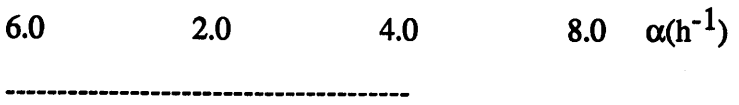
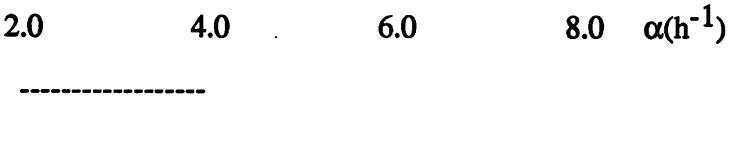


Fig. 6.1(e - f) Bias and precision in expressed as %PE (mean ± standard deviation, respectively) for (e) σ_{Cl}, and (f) σ_{V1}. The horizontal axis represents the different values of α. Each vertical bar expresses the bias and precision of the population parameter estimate.

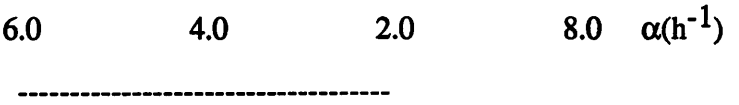
(a) Estimation of Cl



(b) Estimation of V_1



(c) Estimation of k_{12}



(d) Estimation of k_{21}

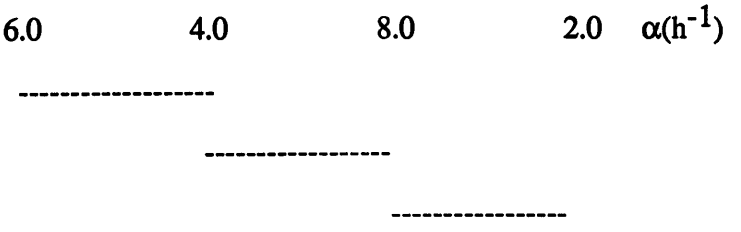


Fig. 6.2(a - d) [a]Summary of significant differences in the efficiency* with which parameters were estimated on reparameterization: effect of different values of α.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

(e) Estimation of σ_{Cl}

4.0 2.0 6.0 8.0 $\alpha(h^{-1})$

(f) Estimation of σ_{V1} [b]

2.0 4.0 6.0 8.0 $\alpha(h^{-1})$

(g) Overall Design Efficiency

4.0 6.0 2.0 8.0 $\alpha(h^{-1})$

Fig. 6.2(e - g) [a]Summary of significant differences in the efficiency* with which parameters were estimated on reparameterization: effect of different values of α.
a - Rank order of design numbers increasing from left to right.
b - All significantly different each other
* Efficiency measured with design number

that the estimates of Cl produced were very precise. The bias in the estimation of this parameter ranged from 0.19% (6.0 h^{-1}) to 15.08% (8.0 h^{-1}), with the most biased estimate obtained when α was 8.0 h^{-1}. The estimates of V_1 were unbiased and precise when α was in the range of 2.0 and 6.0 h^{-1}. When α was as 8.0 h^{-1}, the estimate of V_1 was biased and imprecise (Fig. 6.1b). The estimates of k_{12} were precise and not significantly biased (Fig. 6.1c). k_{21} estimates were precise except when α was 8.0 h^{-1} (Fig. 6.1d). In addition, the biases in the estimation σ_{Cl} and σ_{V1} were significant. The greater the value of α, the greater the bias (Fig. 6.1(e & f)). Imprecise estimates of σ_{Cl} were only obtained when α was 8.0 h^{-1}. Except when α was 2.0 h^{-1}, all estimates of σ_{V1} were imprecise.

6.4.1b Design Number

With reparameterization, efficient estimates of Cl were obtained when α was in the range of 2.0 and 6.0 h^{-1} (Fig. 6.2a) and these were significantly better than when α was 8.0 h^{-1}. The most efficient estimate of Cl was obtained when α was 6.0 h^{-1} while the least efficient estimate was when α was 8.0 h^{-1}.

V_1 was efficiently estimated when α was in the range of 2.0 and 4.0 h^{-1} with the best estimate obtained when α was 2.0 h^{-1} (Fig. 6.2b). Estimates obtained when α was 2.0 h^{-1} were significantly better than those when α was in the range of 6.0 to 8.0 h^{-1}.

k_{12} was more efficiently estimated when α was between 2.0 and 6.0 h^{-1}, and results obtained when α was in this range was significantly better than those obtained when α was 8.0 h^{-1} (Fig. 6.2c). The most efficient estimates of this parameter were obtained when α was 6.0 h^{-1}.

More efficient estimates of k_{21} were obtained when α was in the range of 4.0 to 6.0 h^{-1} than when it was 2.0 h^{-1}(Fig. 6.2d). The most efficient estimates

were when α was 6.0 h^{-1}, and these were significantly better than when α was either 2.0 or 8.0 h^{-1}.

Estimates of σ_{Cl} obtained when α was either 2.0 or 4.0 h^{-1} were significantly better than when α was in the range of 6.0 to 8.0 h^{-1} (Fig. 6.2e). The best estimate σ_{Cl} was obtained when α was 4.0 h^{-1} and the least efficient estimate when α was 8.0 h^{-1}. σ_{V1} was best estimated when α was 2.0 h^{-1} (Fig. 6.2f) and this was significantly better than when α was in the range of 4.0 to 8.0 h^{-1}. The least efficient estimate of σ_{V1} was obtained when α was 8.0 h^{-1}.

When the efficiency of estimation of all parameters was considered, efficient estimates were obtained with α in the range of 2.0 to 6.0 h^{-1} (Fig. 6.2g). These estimates were significantly better than when α was 8.0 h^{-1}. The most efficient estimates were when α was 4.0 h^{-1} and the least efficient when α was 8.0 h^{-1}. It should be noted that efficient estimates were obtained for parameters of Model I (see Chapter 5, Section 5.3.3) when α was 2.0 h^{-1} and not greater than 4.0 h^{-1}.

Thus, Cl was best estimated when α was in the range of 2.0 to 6.0 h^{-1}, and V_1 when α was between 2.0 to 4.0 h^{-1}. The most efficient estimates of Cl and V_1 were obtained when α was 6.0 and 2.0 h^{-1}, respectively. k_{12} and k_{21} were well estimated when α was in the range of 2.0 to 6.0 h^{-1}, and 4.0 to 6.0 h^{-1}, respectively. These micro transfer rate constants were best estimated when α was 6.0 h^{-1}. σ_{Cl} and σ_{V1} were most efficiently estimated when α was 4.0 and 2.0 h^{-1}, respectively. However, σ_{Cl} was estimated with a similar efficiency when α was either 4.0 or 2.0 h^{-1}. Considered as a set, all parameters were estimated with similar efficiency when α was in the range of 2.0 and 6.0 h^{-1} although the most efficient estimates were obtained when α was 4.0 h^{-1}.

Table 6.3 Effect of Reparameterization on Individual and Joint Confidence Intervals Coverage for Parameter Estimates: Variation in α

Section I

Success / Total

α	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
2.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
4.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
6.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
8.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30

Section II

Success - Excluded / Total - Excluded

α	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
2.0	30/30	30/30	30/30	30/30	29/29	30/30	29/29
4.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
6.0	30/30	30/30	30/30	30/30	28/28	30/30	28/28
8.0	30/30	30/30	30/30	30/30	16/16	30/30	16/16

Section III

Success - Excluded / Total

α	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
2.0	30/30	30/30	30/30	30/30	29/30	30/30	29/30
4.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
6.0	30/30	30/30	30/30	30/30	28/30	30/30	28/30
8.0	30/30	30/30	30/30	30/30	16/30	30/30	16/30

Table 6.4 Comparison of Joint Confidence Intervals Obtained with Models I and II: Effect of Different α Values

	Success - Excluded Total		
α (h^{-1})	Model I	Model II	
2.0	0/29	29/30	$p < 0.001$
4.0	19/30	30/30	$p < 0.001$
6.0	16/30	28/30	$p < 0.001$
8.0	8/28	16/30	$p < 0.05$

6.4.1c Individual and Joint Confidence Interval Estimates

After reparameterization, good confidence intervals coverage was obtained for individual and joint parameter estimates with or without excluding NONMEM runs with "coefficient of variation" > 50% for α of 2.0, 4.0, and 6.0 h^{-1} (Table 6.3, Section I - III). Unlike the 96% coverage (on average) for σ_{Cl} and joint parameter estimates obtained with α in the range of 2.0 to 6.0 h^{-1}, a value of 53% was obtained when α was 8.0 h^{-1} (Table 6.3, Section III). However, this was not significantly different from the expected values of 0.95 and 0.74, respectively.

6.4.1d Comparison of Joint Confidence Intervals Coverage for Parameter Estimates Obtained with Models I and II

Table 6.4 gives the joint confidence intervals coverage for parameter estimates obtained using the two models. Reparameterization led to a significant improvement in the joint confidence intervals coverage for parameter estimates irrespective of the α values.

6.4.1e Correlation between Parameter Estimates

A notable incidence of high correlation of 40.0%, 66.7%, and 30.0% was obtained for the correlation between k_{21} and Cl with α equal to 2.0, 4.0 and 6.0 h^{-1}, respectively. A 23.3% and 53.3% incidence of high correlation was obtained for the correlation between k_{21} and k_{12} , and σ_{Cl} and V_1, respectively, when α was 8.0 h^{-1} (Table 6.5).

Table 6.5 Effect of Reparameterization on the Incidence* of Correlation between Parameters: Variation in α

Correlation (%)	α (h^{-1}) 2.0		4.0		6.0		8.0	
	High[a]	Low[b]	High	Low	High	Low	High	Low
k_{21} vs Cl	40.0	60.0	66.7	33.3	30.0	70.0	0.0	100.0
k_{21} vs k_{12}	0.0	100.0	0.0	100.0	0.0	100.0	23.3	76.7
σ_{Cl} vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	6.7	93.3
σ_{Cl} vs V_1	0.0	100.0	0.0	100.0	6.7	93.3	53.3	46.7
σ_{Cl} vs k_{21}	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0
σ_{V1} vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	3.3	96.7
σ_{V1} vs k_{12}	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0

* - Incidence of high correlation greater than zero for at least a pair of parameters.

a - > 0.75 b - < 0.75

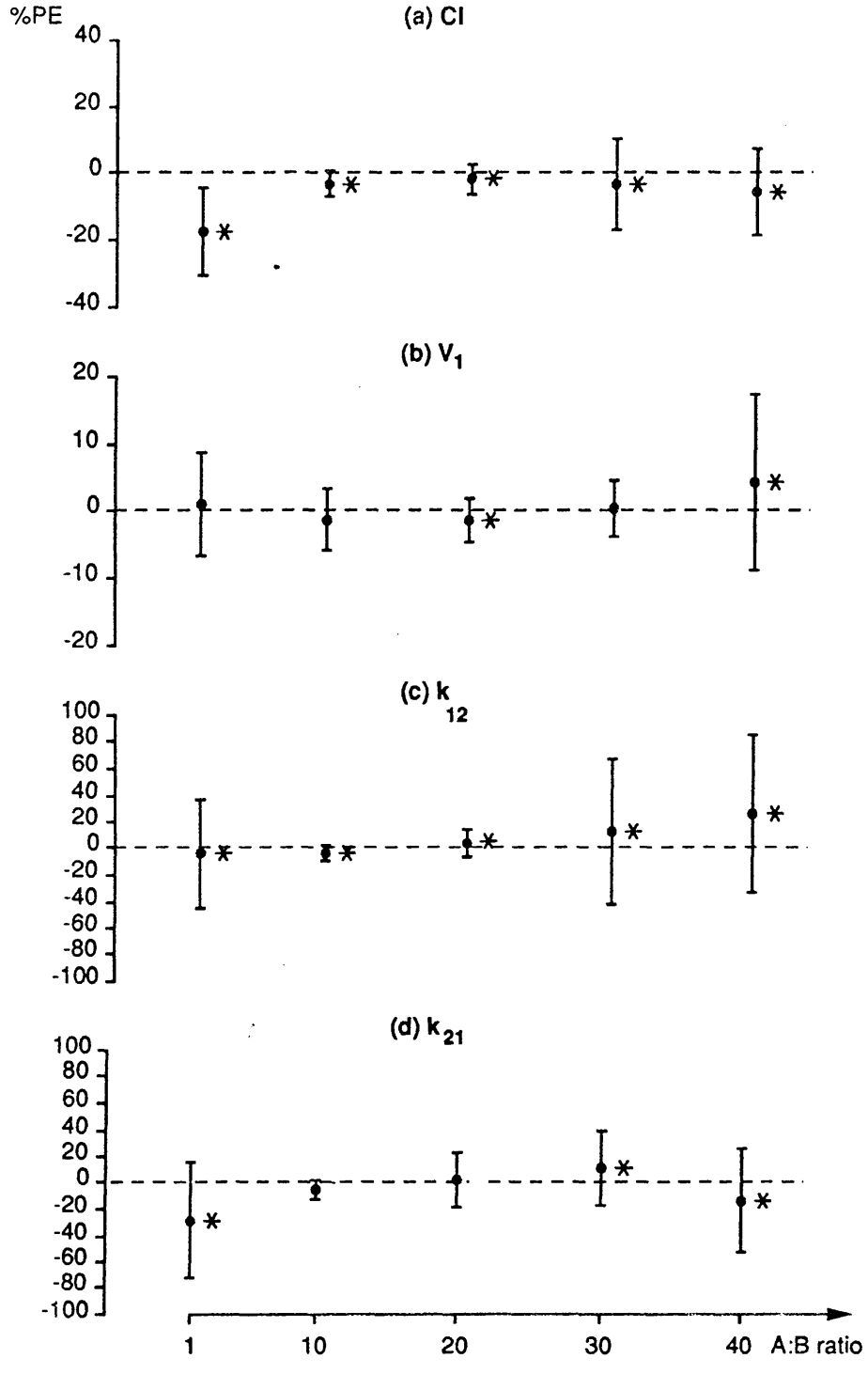


Fig. 6.3(a - d) Bias and precision in expressed as %PE (mean ± standard deviation, respectively) for (a) Cl, (b) V_1, (c) k_{12}, and (d) k_{21}. The horizontal axis represents the different values of A:B ratios. Each vertical bar expresses the bias and precision of the population parameter estimate.

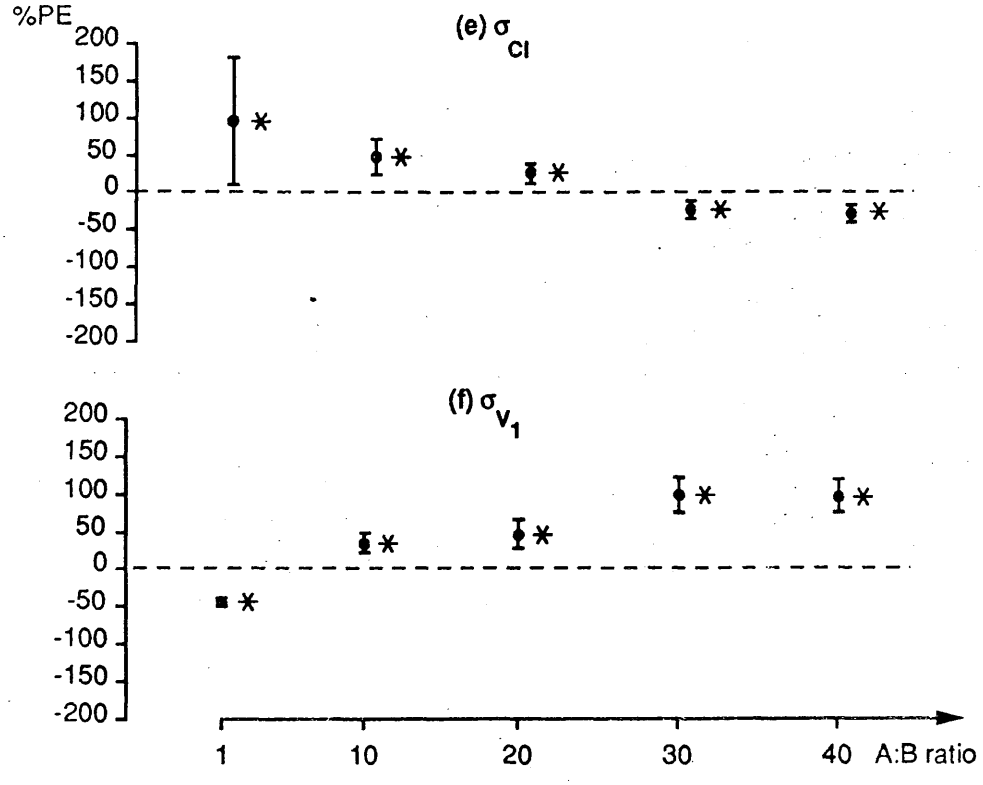


Fig. 6.3(e - f) Bias and precision in expressed as %PE (mean ± standard deviation,respectively) for (e) σ_{Cl}, and (f) σ_{V1}. The horizontal axis represents the different values of A:B ratios. Each vertical bar expresses the bias and precision of the population parameter estimate.

(a) Estimation of Cl

30.0 20.0 10.0 40.0 1.0 A:B ratio

(b) Estimation of V_1

20.0 30.0 10.0 40.0 1.0 A:B ratio

(c) Estimation of k_{12}

10.0 20.0 30.0 40.0 1.0 A:B ratio

(d) Estimation of k_{21}

10.0 20.0 30.0 40.0 1.0 A:B ratio

Fig. 6.4(a - d)[a] Summary of significant differences in the efficiency* with which parameters were estimated on reparameterization: effect of varying A:B ratio.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

(e) Estimation of σ_{Cl}

30.0 40.0 20.0 10.0 1.0 A:B ratio

(f) Estimation of σ_{V1}

1.0 10.0 20.0 30.0 40.0 A:B ratio

(g) Overall Design Efficiency

10.0 20.0 30.0 40.0 1.0 A:B ratio

Fig. 6.4(e - g)[a] Summary of significant differences in the efficiency* with which parameters were estimated on reparameterization: effect of varying A:B ratio.
a - Rank order of design numbers increasing from left to right.
* Efficiency measured with design number

6.4.2 REPARAMETERIZATION WITH VARIATION IN A:B RATIO

6.4.2a Bias and Precision

The estimation of Cl was associated with a significant negative bias irrespective of the A:B ratio (Fig. 6.3a). However, the least biased estimate was obtained when the A:B ratio was 20.0 (mean %PE = 1.7%), and the most biased estimate when the A:B ratio was 1.0 (mean of %PE = 17.4%). The estimates of V_1 were mostly unbiased (Fig. 6.3b) and all estimates of Cl and V_1 were acceptably precise. k_{12} and k_{21} estimates were biased and mostly imprecise, but the estimates obtained when the A:B ratio was 10 or 20 were precise (Fig. 6.3(c & d)). Fig. 6.3(e & f) shows the estimates of σ_{Cl} and σ_{V1} to be significantly biased. σ_{V1} was associated with acceptable precision, while σ_{Cl} was acceptably precise for most A:B ratios, except when the A:B ratio was 1.0. In this case the estimate of σ_{Cl} was greatly biased and imprecise (Fig. 6.3f).

6.4.2b Design Number

Cl was efficiently estimated when the A:B ratio was between 10.0 and 40.0, and these were significantly better than when the A:B ratio was 1.0 (Fig. 6.4a). The most efficient estimate of Cl was obtained when the A:B ratio was 30.0.

The most efficient estimate of V_1 was obtained when the A:B ratio was 20.0, and this was significantly better than estimates for other A:B ratios (Fig. 6.4b).

Good estimates of k_{12} and k_{21} were obtained when the A:B ratio was in the range of 10.0 to 30.0, with the most efficient estimate when the A:B ratio was

10.0. These estimates were significantly better than those obtained when the A:B ratio was either 1.0 or 40.0. (Fig. 6.4 (c & d)).

When the A:B ratio was in the range of 20.0 to 40.0, better estimates of σ_{Cl} were obtained than when the A:B ratio was in the range of 1.0 and 10.0 (Fig. 6.4e). The best estimates of σ_{Cl} were when the A:B ratio was 30.0. Estimates obtained when the A:B ratio was between 30.0 and 40.0 were significantly better than estimates obtained when the A:B ratio was in the range of 1.0 to 10.0.

Significantly better estimates of σ_{V1} were obtained when the A:B ratio was in the range of 1.0 to 20.0 than when the ratio was 30.0 to 40.0 (Fig. 6.4f)

Parameters were well estimated when the A:B ratio was in the range of 10.0 to 30.0, but the estimates when the A:B ratio was between 10.0 and 20.0 were significantly better than those for the A:B ratio of 1.0 or 40.0. The most efficient parameter estimates were obtained when the A:B ratio was 10.0. It should be recalled that the best parameter estimates using Model I (Chapter 5) were obtained when the A:B ratio was 20.0, but this result was not significantly better than when the A:B ratio was 2.5, 5.0, 7.5, 10.0, 30.0, and 40.0.

Thus, Cl was efficiently estimated when the A:B ratio was in the range of 10.0 to 40.0, with the most efficient obtained when the A:B ratio was 30.0. V_1 was most efficiently estimated when the A:B ratio was 20.0. Efficient estimates of k_{12} and k_{21} were obtained when the A:B ratio was in the range of 10.0 to 30.0, with the most efficient estimates of these parameters when the A:B ratio was 10.0. σ_{Cl} was most efficiently estimated when the A:B ratio was 30.0 although these estimates were not significantly better than the estimates obtained when the A:B ratio was either 20.0 or 40.0. On the other hand, σ_{V1} was better estimated when the A:B ratio was in the range of 1.0 to 20.0 than 30.0 to 40.0, with the best when the A:B ratio was 1.0. All parameters were well estimated when the A:B ratio was between 10.0 and 30.0 although the lowest rank order of Φ_r (on average) was obtained when the A:B ratio was 10.0.

Table 6.6 Effect of Reparameterization on Individual and Joint Confidence Intervals Coverage for Parameter Estimates: Variation in A:B Ratio

Section I
Success / Total

A:B	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
1.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
10.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
20.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
30.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
40.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30

Section II
Success - Excluded / Total - Excluded

A:B	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
1.0	30/30	30/30	22/22	20/20	27/27	30/30	20/20
10.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
20.0	30/30	30/30	30/30	30/30	29/29	30/30	29/29
30.0	29/29	30/30	30/30	30/30	30/30	30/30	29/29
40.0	30/30	30/30	30/30	21/21	29/29	30/30	20/20

Section III
Success - Excluded / Total

A:B	Parameter						
	Cl	V_1	k_{12}	k_{21}	σ_{Cl}	σ_{V1}	Joint
1.0	30/30	30/30	22/30	20/30	27/30	30/30	20/30
10.0	30/30	30/30	30/30	30/30	30/30	30/30	30/30
20.0	30/30	30/30	30/30	30/30	29/30	30/30	29/30
30.0	29/30	30/30	30/30	30/30	30/30	30/30	29/30
40.0	30/30	30/30	30/30	21/30	29/30	30/30	20/30

Table 6.7 Comparison of Joint Confidence Intervals Obtained with Models I and II: Effect of Different A:B Ratios

	Success - Excluded / Total		
A:B Ratio	Model I	Model II	
1.0	0/27	20/30	$p < 0.001$
10.0	4/29	30/30	$p < 0.001$
20.0	0/29	29/30	$p < 0.001$
30.0	1/29	29/30	$p < 0.001$
40.0	1/27	20/30	$p < 0.001$

Table 6.8 Effect of Reparameterization on the Incidence* of Correlation between Parameters: Variation in A:B Ratio

Correlation (%)	A:B Ratio 1.0		10.0		20.0		30.0		40.0	
	High[a]	Low[b]	High	Low	High	Low	High	Low	High	Low
k_{12} vs Cl	46.7	53.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
k_{21} vs Cl	93.3	6.7	56.7	43.3	40.0	60.0	33.3	66.7	90.0	10.0
k_{21} vs k_{12}	83.3	16.7	3.3	96.7	0.0	100.0	3.3	96.7	3.3	96.7
σ_{Cl} vs V_1	23.3	76.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{V1} vs Cl	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{V1} vs k_{12}	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	3.3	96.7
σ_{V1} vs k_{21}	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

* - Incidence of high correlation greater than zero for at least a pair of parameters.

a - > 0.75

b - < 0.75

6.4.2c Confidence Intervals for Individual and Joint Parameter Estimates

With reparameterization, good coverage was obtained for individual and joint confidence intervals for parameter estimates (Table 6.6, Section I). When runs with "coefficient of variation" greater than 50% were excluded, the coverage for k_{12} and k_{21} was reduced when the A:B ratio was 1.0 and 40.0, respectively. With these A:B ratios, the joint coverage for parameter estimates was similarly reduced (Table 6.6, Section (II & III)). However, the coverage obtained with these A:B ratios was not significantly lower than the expected values of 0.95 and 0.74 for individual and joint parameter estimates, respectively.

6.4.2d Comparison of Joint Confidence Intervals Coverage for Parameter Estimates Obtained with Models I and II

Reparameterization led to a statistically significant improvement in the joint coverage of parameter estimates irrespective of the value of the A:B ratio (Table 6.7). 62 to 93% improvement in coverage was observed as Model I was reparameterized to give Model II.

6.4.2e Correlation between Parameter Estimates

Incidence of high correlation was highest (93.3%) for k_{21} and Cl when the A:B ratio was 1.0, and was 90.0% when the A:B ratio was 40.0. Comparatively lower values were obtained when the A:B ratio was 10.0 (56.7%), 20.0 (40.0%), and 30.0 (33.3%). In addition, incidence of high correlation of 46.7%,

83.3%, and 23.3% for the pair-wise correlations of k_{12} and Cl, k_{21} and k_{12}, and σ_{Cl} and V_1, respectively, were obtained when the A:B ratio was 1.0 (Table 6.8).

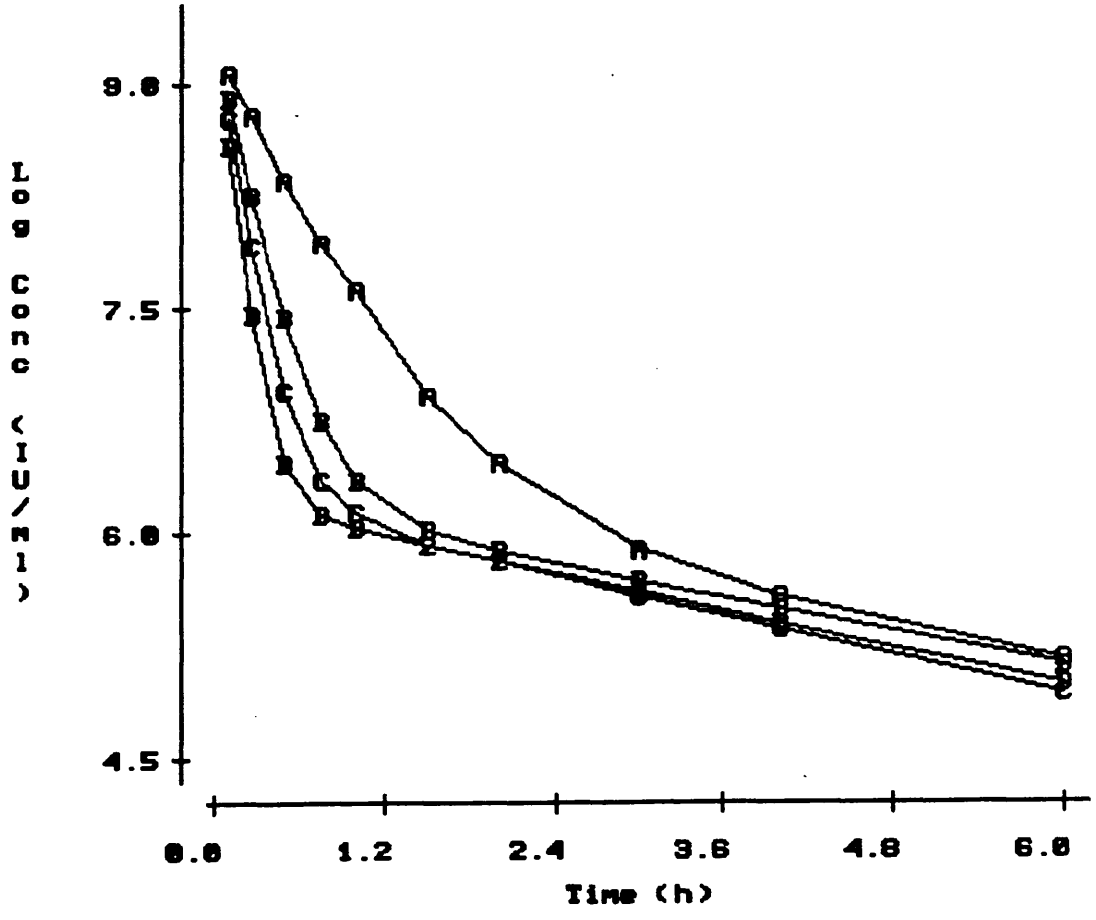


Fig. 6.5 Distribution of sampling times on reparmeterization with various values of α: A----A for $\alpha = 2.0\ h^{-1}$, B----B for $\alpha = 4.0\ h^{-1}$, C----C for $\alpha = 6.0\ h^{-1}$, and D----D for $\alpha = 8.0\ h^{-1}$.

6.5 DISCUSSION

The highly precise nature with which Cl was estimated when $\alpha \geq 4.0\ h^{-1}$ was due to the greater number of data points available in the elimination phase of the concentration - time curve. The greater the value of α, the steeper the slope of the distribution phase of the curve resulting in a fewer number of data points in this phase of the disposition curve (Fig. 6.5). However, the best estimate of Cl was obtained when α was $6.0\ h^{-1}$ and not $8\ h^{-1}$ because of the bias with which this parameter was estimated when α was $8.0\ h^{-1}$. V_1 was estimated with least bias and greatest precision when α was $2.0\ h^{-1}$ because more data points were located in the distribution phase of the plasma concentration - time curve. k_{12} and k_{21} were most efficiently estimated when α was $6.0\ h^{-1}$. The biases associated with the estimates of the variance parameters were due to the fact that the data contained no information on σ_ϵ. When α was $4.0\ h^{-1}$ the best estimate of σ_{Cl} was obtained. This estimate was associated with the highest precision. σ_{V1} was most efficiently estimated when α was $2.0\ h^{-1}$ for the same reason as V_1. As a whole, the best parameter estimates were obtained when α was in the range of 2.0 to 6.0 h^{-1} with the most efficient estimates obtained when α was $4.0\ h^{-1}$. This was probably a consequence of the even distribution of data points between the distribution and elimination phases of the concentration - time curve.

The inefficiency with which parameters were estimated when α was $8.0\ h^{-1}$ could be attributed to the correlation between parameter estimates since there were more cases of high correlation between parameters with this value of α, and this was reflected in the confidence intervals coverage. There were 14 NONMEM runs with catastrophic estimates of σ_{Cl} which led to a reduced joint confidence intervals coverage for all parameter estimates, and this was significantly different from all others.

However, when the joint confidence intervals coverages obtained after reparameterization (Model II) were compared with those obtained with Model I, significant improvements were obtained for all α values. The incidence of high pair-wise correlations on reparameterization did not exceed 67% compared with 100% obtained with Model I (Chapter 5, Section 5.4.3) for the same α values. Thus, reparameterization led to a reduced incidence of high pair-wise correlations and stability in the estimates as reflected in the significant improvement in joint confidence intervals coverage for all parameter estimates. This improvement was associated with "coefficients of variation" < 50% for most parameter estimates.

When the A:B ratio was 20.0 or 30.0, efficient estimates of Cl were obtained. The best estimate of Cl was when the A:B ratio was 30.0, due to the estimates being the least biased. The estimates obtained when the A:B ratio was 20.0 or 30.0 were equally precise. The most efficient (and most precise) estimate of V_1 was obtained when the A:B ratio was 20.0. The most efficient (least biased and most precise) estimates of the micro transfer rate constants were obtained when the A:B ratio was 10.0. The significant biases associated with the variance parameters were due to the lack of information about σ_{ϵ} in the data sets. The best estimates of parameters as a whole, were obtained when the A:B ratio was 10.0, although these were not significantly better than when the A:B ratio was 20.0 or 30.0. Inefficient parameter estimates were obtained when the A:B ratio was 1.0, and 40.0 with the most inefficient estimates obtained when the A:B ratio was 1.0.

The inefficiency of parameter estimation associated with the A:B ratios of 1.0 or 40.0 was associated with high pair-wise correlations. When the A:B ratio was 1.0, an incidence of high correlation of 93.3% and 83.3% was obtained for k_{21} and Cl, k_{12} and k_{21}, respectively. Also, parameter estimation when the A:B ratio was 40.0 was associated with a 90.0% incidence of high correlation between k_{21} and Cl. With these A:B ratios there were 10 NONMEM runs each with catas-

trophic estimates unlike the one NONMEM run each with catastrophic estimates obtained when the A:B ratio was 20.0 or 30.0, and none when the A:B ratio was 10.0.

However, reparameterization with these A:B ratios led to significant improvements in joint confidence intervals for parameter estimates when compared with results obtained with similar A:B ratios using Model I (Table 6.5). This improved coverage was due to the generation of parameter estimates with "coefficients of variation" mostly < 50%. Unlike the results obtained with Model I (Chapter 5) in which a 100% incidence of high correlation was obtained B and β irrespective of the A:B ratio, no such incidence was obtained with Model II. Thus, Model I reparameterized into Model II, resulted in a lower incidence of high pair-wise correlation between parameter estimates and more efficient estimation.

Given that parameters were efficiently estimated when α was in the range of 2.0 and 6.0 h^{-1} and the A:B ratio was 20.0, and when the A:B ratio was between 10.0 to 30.0 when α was 2.0 h^{-1}, it is reasonable to suggest that parameters would be efficiently estimated when the A:B ratio was in the range of 10.0 to 30.0 and α between 2.0 to 6.0 h^{-1}.

CHAPTER 7

PRECLINICAL PHARMACOKINETICS: AN APPLICATION OF THE POPULATION APPROACH

7.1 SUMMARY

Serum concentrations of a drug under development were obtained from an animal pharmacokinetic study using the one sample per animal design and analysed using the population data analysis program, NONMEM. A two compartment open model with IV administration was used as the basis of the analysis. Although sex and weight were not determinants of clearance (Cl), sex helped to explain the variability in the volume of the central compartment (V_1). The average values of Cl and V_1 were: Cl(ml/min) = 0.40, $V_1(ml/g)_{male}$ = 0.11, and $V_1(ml/g)_{female} = V_{1male} * 0.80$. The variability in Cl and V_1 were 23.5 and 23.2%, respectively.

7.2 INTRODUCTION

An estimation of the average value of pharmacokinetic parameters in a group of animals provides limited information if there is no good measure of the variability of each of the parameters. The traditional naive pooled data (NPD) approach used in the analysis of animal pharmacokinetic data does not provide this, nor can it assess the influence of physiology (or pathology) on pharmacokinetics.The nonlinear mixed effects model (NONMEM) approach (Sheiner & Beal, 1979 - 1989) does, however, provide estimates of both the average values of pharmacokinetic parameters and their statistical distribution within a population. Given the results of the simulation study described in Chapter 6, NONMEM was used to analyse data obtained during a preclinical pharmacokinetic study.

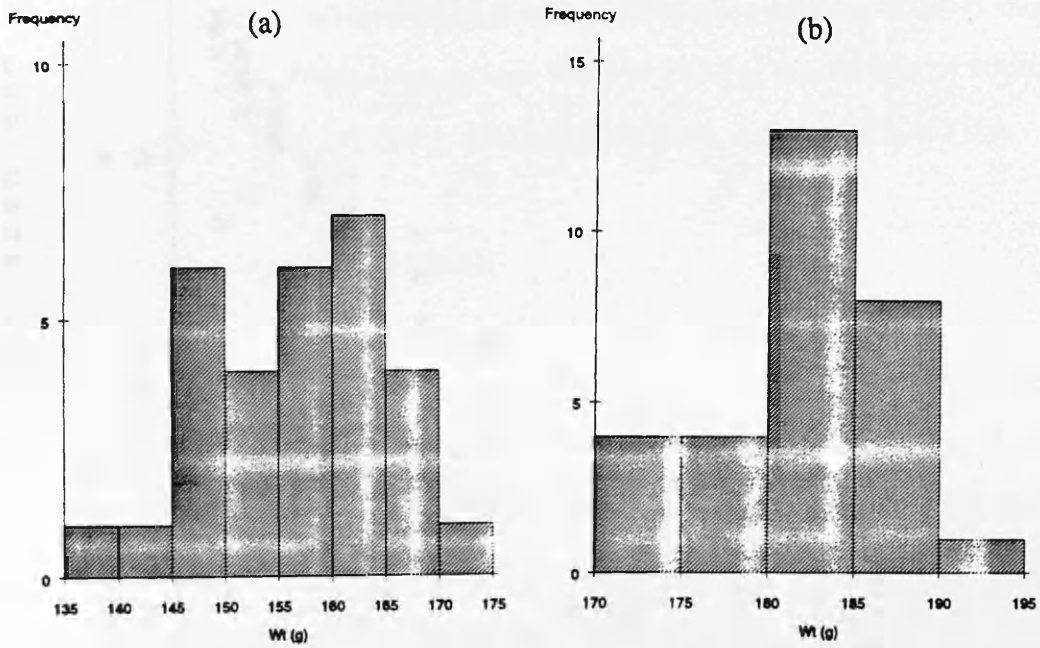


Fig. 7.1 Weight distribution for rats: (a) female, (b) male

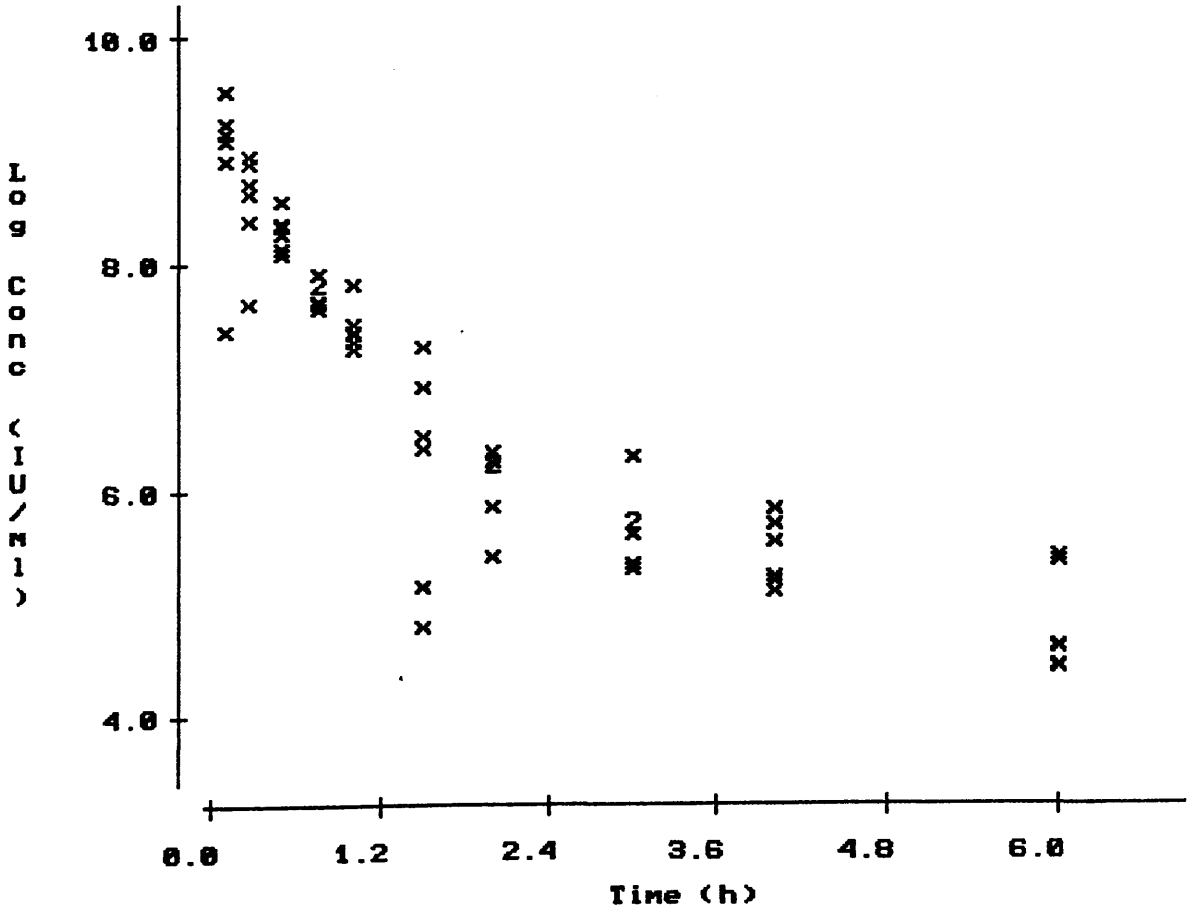


Fig. 7.2 Scatterplot of log. concentration versus time.

Data were supplied by Ares Serono (Italy): serum concentrations of a drug under development were measured in rats after single intravenous bolus injections.

7.3 METHODS

7.3.1 Animals

60 serum concentrations were obtained from 60 rats. Demographic data included weight and sex: the distribution of weight according to sex is shown in Fig. 7.1(a &b). Weight ranged from 139.0 to 192.0 g. The weight of female rats ranged from 139.0 to 171.0 g. and that of male rats, from 172.0 to 192.0 g.

7.3.2 Pharmacostatistical Models

A visual inspection of the data indicated that the disposition of the drug could, on average, be described by a two compartment open model (Fig. 7.2). The chi-squared test ($p < 0.005$) was used to examine the difference between the log likelihood values obtained from fitting the full (2 compartment) or reduced (1 compartment) models (Sheiner *et al.*, 1977). The model had the following structural form given in Eq. (7.1):

$$C^*_j = [D_j/V_{1j}(\alpha - \beta)][\alpha - k_{21})\exp(-\alpha t_j) + (k_{21} - \beta)\exp(-\beta t_j)] \qquad (7.1)$$

where $\alpha, \beta = 1/2\{k_{12} + k_{21} + k_{10} \pm [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2}\}$, and $k_{10} = Cl_j/V_{1j}$. C^*_j is the true drug concentration in the jth animal , D_j, V_{1j}, Cl_j are the dose, volume of the central compartment and clearance in the jth animal, respectively, and t_j the corresponding sampling time. In the analysis α and β were

parameterized in terms of the microscopic rate constants k_{12} and k_{21}. A bolus dose of 1.0M IU/kg was administered to each animal. The statistical model (see Chapter 2, Section 2.3.2) accounted for combined inter- and intra-animal variability.

7.3.3 Data Analysis

The data were analysed using the NPD approach and with NONMEM. For the NPD approach, the WLS estimation procedure (weight proportional to C^{*-2}) was used to estimate the mean pharmacokinetic parameters from the average concentration - time data.

Using NONMEM, the influence of demographic factors (fixed effects(FE)) was tested by relating them to the pharmacokinetic parameters (P) using linear models of the type:

$$P = \Theta(FE) \qquad (7.2)$$

where P is the expected value of pharmacokinetic parameter (e.g., Cl or V1) in any animal, FE is an identifiable animal factor (e.g., weight), and Θ is a regression coefficient. When quantifying the influence of a discontinuous variable such as sex, the model was of the type:

$$P = \Theta(FE) \quad \text{if male} \qquad (7.3a)$$

$$P = \Theta(FE).\ \Theta_{Sex} \quad \text{if female} \qquad (7.3b)$$

where Θ_{Sex} effectively allows different slopes for males and females.

Table 7.1 List of Models Tested and Log Likelihood Differences

Model No.	c.f.	OBJ	LLD	df	P
(i) Clearance Models					
1. $Cl = \Theta_1$		774.04			
2. $Cl = \Theta_1 * Wt$	1	774.04	0	NA	NA
3. $Cl = \Theta_1 * \Theta_{Sex}$	1	772.60	1.44	1	NS
4. $Cl = \Theta_1 * Wt * \Theta_{Sex}$	1	772.60	1.44	1	NS
(ii) Volume Models					
5. $V_1 = \Theta_2$		774.04			
6. $V_1 = \Theta_2 * Wt$	5	765.88	0	NA	NA
7. $V_1 = \Theta_2 * \Theta_{Sex}$	5	762.84	11.20	1	< 0.005
8. $V_1 = \Theta_2 * Wt * \Theta_{Sex}$	5	762.66	11.38	1	< 0.005

c.f. - compared with model number

OBJ - Objective function

LLD - Log Likelihood Difference

df - degree of freedom

NA - Not Appropriate

NS - Not Significant

Θ_{Sex} = 1.0 if male and is estimated for females

In the analysis, models that related weight and / or sex to Cl and V_1 were tested (Table 7.1). These models were embedded in the two compartment pharmacokinetic model. NONMEM estimated the values of Θ (equations (7.2) and (7.3)) and / or other kinetic parameters (if these were not specified as functions of demographic factors) simultaneously. Thus, the influence of these fixed effects was evaluated.

Theoretically, a data set could be analysed an infinite number of times with different regression models. Therefore, criteria were necessary to identify a useful analysis. One criterion was the value of the objective function which is normally calculated for each NONMEM run and is equal to -2 log likelihood. A difference in the objective function (log likelihood difference, LLD) between two NONMEM runs involving the use of two regression models (one of which was a restriction of the other; e.g., (a) a model which incorporated either Cl and V_1 as a function of sex, and (b) a model which incorporated Cl or V_1 without any explanatory factor) of more than 8 indicated a significant improvement ($p < 0.005$, assuming chi - square distribution) when the restricted model had one regression parameter less than the full model (Sheiner *et al.*, 1977). Other criteria were: (1) a minimum correlation between parameters; (b) small standard errors of parameter estimates; (c) weighted residuals which were randomly scattered around zero when plotted against predicted concentration; and (d) decrease in the estimate of the inter-animal variances (see Chapter 2, Section 2.3.3). For non-hierarchical models, where all models had the same number of parameters, model comparison was based on the objective function, and other criteria enumerated above. An LLD greater than zero indicated an improvement and the one with the smaller OBJ described the data better. A summary of the models tested is presented in Table 7.1.

Estimates were obtained for (1) population means for Cl, V_1, k_{12}, k_{21} and / or regression coefficients (Θ in equations (4) and (5)), (2) the variance

terms, (3) standard error of estimates, and (4) correlation matrix of the estimates.

7.4 RESULTS

An initial examination of NONMEM runs showed that the two compartment model was superior to the one compartment model. The population pharmacokinetic parameter values obtained with the NPD approach were similar to the population parameter values obtained with NONMEM when no covariates were modelled (Table 7.2). However, with NONMEM, estimates of inter-animal variability in Cl and V_1 were obtained in addition to the average population parameters.

Modelling V_1 without regard to animal size with an additive model for variability, the effects of modelling basic drug clearance with demographic factors were examined. The first regression model (Model 1, Table 7.1) simply defined Cl in ml/min. without an effect of animal size. To this was added an influence of weight (g) as in Model 2 (Table 7.1). Model 2 did not give any improvement in the estimation of Cl as seen from the objective function (Table 7.1). Thus, weight was not incorporated into the basic model for Cl.

Also, the inclusion of either sex (Model 3, Table 7.1), or sex and weight (Model 4, Table 7.1) in various regression models for clearance did not improve the value of the objective function (Table 7.1).

Using the basic Cl model, an additive model for variability, V_1 for the drug was initially modelled without regard to animal size (Model 5, Table 7.1), where Θ_2 equalled the volume of the central compartment in ml. To this was then added the influence of weight (g) (Model 6, Table 7.1). This was a significant improvement over V_1 without regard to animal weight (Table 7.1).

Table 7.2 Parameter Estimates (S.E)

NPD Approach

Structural Model Parameters

Cl	V_1	k_{12}	k_{21}
(ml/min)	(ml)	(min^{-1})	(min^{-1})
0.42	16.68	0.01	0.006
(0.15)	(5.20)	(0.003)	(0.005)

NONMEM Approach

0.41	15.80	0.01	0.005
(0.10)	(3.30)	(0.003)	(0.004)

NONMEM Variance Estimates

Cl $(ml/min)^2$	$V_1(ml)^2$
0.54	21.70
(0.60)	(16.6)

Table 7.3 Parameter Estimates (S.E.)

(i) Structural Model Parameters

Male		Female	
Cl (ml/min)	V_1 (ml/g)	Cl (ml/min)	V_1 (ml/g)
0.40	0.11	0.40	0.11 * 0.80
(0.03)	(0.01)	(0.03)	(0.08)

k_{12} (min^{-1})	k_{21}(min^{-1})
0.01	0.005
(0.002)	(0.002)

(ii) Variance Estimates

Cl $(ml/min)^2$	V_1 $(ml/g)^2$
0.40	0.20
(0.22)	(0.56)

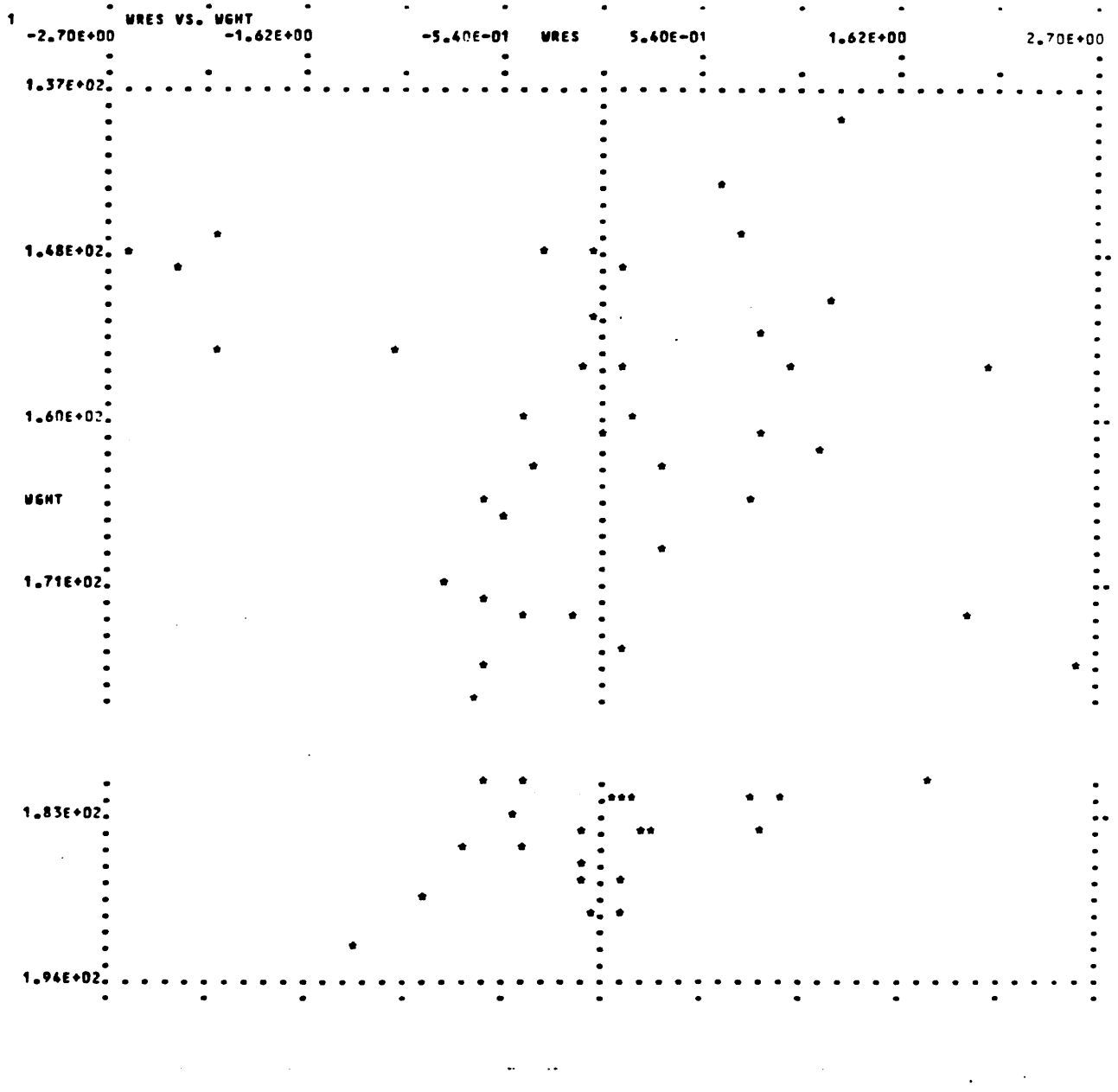


Fig. 7.3a Scatterplot of weighted residuals (upper axis) versus animal weight in grams (left axis) with volume of central compartment modelled as Model 5 (without regard to animal size).

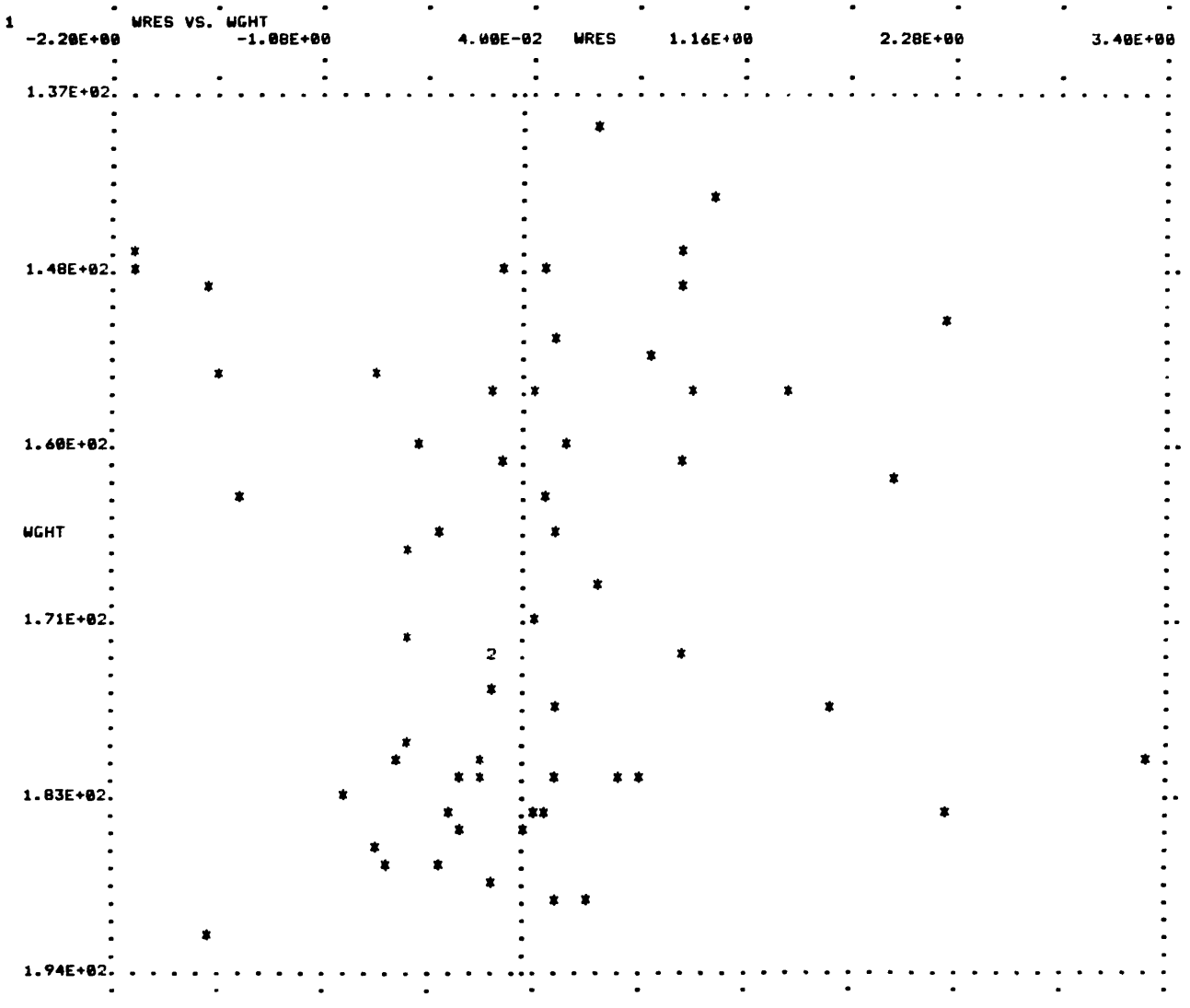


Fig. 7.3b Scatterplot of weighted residuals (upper axis) versus animal weight in grams (left axis) with volume of central compartment modelled as Model 6 (based on animal weight).

A method of assessing the "goodness of fit" is to the examine the scatterplots of weighted residuals generated by NONMEM. Predicted concentrations more closely equal observed concentrations as accuracy improves and the weighted residuals approach zero. Fig. 7.3a is the scatterplot of weighted residuals vs. animal weight, with volume modelled as Model 5 (Table 7.1), i.e. not including to animal weight. The pattern of the weighted residuals when volume was modelled with regard to weight (Model 6 (Table 7.1), Fig. 7.3b) was not different from that with Model 5 although the former model yielded a lower objective function.

Including either sex (Model 7, Table 7.1), or weight and sex (Model 8 , Table 7.1) as factors to explain the variability in V_1 led to a significant improvement in the objective function ($p < 0.005$) with a small reduction in variability (from 29.4% for the simple model (Model 5, Table 7.1) to 23.2% for the full model (Model 8, Table 7.1). The variability in Cl was 23.5%. The final model which best described the data is that specified in Model 8, with V_1 expressed as a function of weight and sex.

The variances for k_{12} and k_{21} could not be estimated: removal of these variance terms from the model resulted in no change in the objective function or parameter estimates. Table 7.3 gives a summary of the parameter estimates.

7.5 DISCUSSION

The similarity in the estimates of model parameters obtained using the NPD and NONMEM approaches was not surprising since the NONMEM approach, like the NPD approach, is focussed on the estimation of average (population) pharmacokinetic parameters. However, NONMEM gave additional information about the distribution of these population parameters by providing

estimates of variability.

Estimates of V_1 were improved by considering the demographic factors. Thus, weight and sex contributed significantly to the explanation of variability in volume of the central compartment. It should be noted, however, that all female rats weighed less than their male counterparts (Fig. 7.1). When estimating V_1 for this drug, animal sex alone appears to allow some reasonable estimation.

In practice the development of most drugs is abandoned when large variability is observed in the population pharmacokinetics of the drug, without any effort to explain the variability. With the NONMEM program, the relationship between physiology and pharmacokinetics has been determined as an aid to explain the inter-animal variability observed in Cl and V_1. The introduction of weight and sex in V_1 led to a reduction of the inter-animal variability in this parameter by approximately 6%. Other factors, as yet undetermined, may be affecting the pharmacokinetics of this drug. Vocci and Farber (1988) advocated the consideration of pharmacokinetic differences within species in interspecies scaling. With the inter-animal variability observed in a homogeneous population of rats, larger variations in response may be expected to occur in humans. The possibility of gender related drug response should be anticipated in man.

In conclusion, the NONMEM program has been used to obtain estimates of population pharmacokinetic parameters and their distributions for a drug under development in a group of rats. This analysis has taken into account the fact that samples came from a population with more variability than could be explained by simple experimental error. NONMEM has permitted some explanation of this variability in terms of sex, efficient partitioning between inter- and intra-animal variability would require an increase in the number of samples per animal.

CHAPTER 8

PARAMETER ESTIMATION IN PHARMACOKINETIC STUDIES INVOLVING THE USE OF SMALL LABORATORY ANIMALS

8.1 SUMMARY

A simulation study was carried out to determine the impact of various design factors on the efficiency with which population pharmacokinetic parameters could be estimated in an animal pharmacokinetic study. A drug which exhibits monoexponential disposition characteristics when administered by an intravenous bolus injection was used for the study. The factors investigated were: (1) number of animals sampled at specified time points with one observation taken per animal, (2) error in observed concentration measurements, and (3) doubling the number of observations per animal with varying number of animals. Increasing the error in the concentration measurement led to a significant worsening of the efficiency with which variability was estimated. The one point per animal design yielded biased and imprecise estimates of inter-animal variability. The limitation of this design is discussed and the importance of sampling an animal at least twice for unbiased and precise parameter estimation is highlighted.

8.2 INTRODUCTION

In earlier chapters (Chapters 3 & 4) the effect of inter-animal variability and sampling designs on parameter estimation with the one compartment model were examined. In Chapters 5 to 7 parameter estimation with the two compartment open model was examined. In this chapter the one compartment open model with IV bolus administration is reconsidered.

The results of simulation studies carried out to determine the impact of a

number of design features on the efficiency with which population pharmacokinetic parameters could be estimated in pharmacokinetic study involving the use of small laboratory animals are presented. The effects of the following design features: (a) number of animals sampled at specified time points with one observation taken per animal, (b) changing the error in observed concentration measurements, and (c) varying the total number of samples (i.e. doubling the number of samples per animal with or without halving the number of animals) on the estimation of population pharmacokinetic parameters were investigated.

8.3 METHODS

8.3.1 Sampling Design

The sampling design described in Chapter 2 (Section 2.4) was used in these studies. Briefly, there were ten sampling times (i.e. 5, 15, 30, 60, 90, 120, 150, 180, 210, 240 minutes). The first two times were fixed , but the other points were sampled uniformly from a range of 15 minutes centred on the stated time.

In the simulation, the parameter values were as given in Chapter 2 (Section 2.4). σ_{Cl} and σ_V were sequentially set to give coefficients of variation of 15%, 30%, 45%, and 60%, and σ_ϵ was set to 15% (except in section (b) below).

8.3.1a Varying the Number of Animals per Time Point

Each of j animals supplied one observation, and a different number of animals was used at each time point for different experiments. This design is denoted as the j * 1 design. Let the total number of animals used in each experiment be denoted by N_A, and the total number of observations, N_S. In the first set of experiments the effect of increasing the number of animals per time point (i.e increasing total sample size, N_S) on the efficiency with which parameters were estimated was investigated. There were nine sample sizes (20, 30, 40, 50, 60, 70, 80, 100, and 150) which involved the use of 2, 3, 4, 5, 6, 7, 8, 10 and 15 animals, respectively, at each time point, and this yielded nine j * 1 study designs.

8.3.1b Varying the Error in Concentration Measurements

The influence of specified intra - animal variability (or error in concentration measurement) on parameter estimation was studied for three cases: σ_ϵ = 0, 15 and 30% with three j * 1 designs of N_S and N_A = 30, 50, and 70. Inter- animal variability was set to 30%, i.e. σ_{Cl} = 30%; σ_V = 30%.

8.3.1c Keeping the Total Number of Observations Constant and Halving the Total Number of Animals

The effect of keeping N_S constant while halving N_A on parameter estimation was investigated by sampling each animal twice. The sampling regimen for this series of simulations involved dividing the ten sampling times into two independent blocks: the first five times (t_1 to t_5), and the later five times

Table 8.1 NONMEM Runs With Acceptable Estimates

Number of Animals Per Time -Point	Inter-Animal Variability(%)			
	15	30	45	60
2	29	26	27	23
3	29	30	28	29
4	29	30	27	27
5	30	30	27	27
6	30	30	29	28
7	30	29	29	29
8	30	30	29	30
10	30	30	29	30
15	30	30	29	30

(t_6 to t_{10}). Thus, each animal was sampled at, for example, the first times in each block (i.e. t_1 and t_6) or the second times in each block, etc. The study design in which each animal was sampled twice is denoted as j * 2. 15, 25, and 35 animals were used yielding three j * 2 designs with corresponding N_S of 30, 50, and 70, respectively. This allowed comparison with the j * 1 designs.

8.3.1d Doubling the Total Number of Observations without Changing the Total Number of Animals

The effect of keeping N_A constant while doubling N_S was investigated using N_A = 30, 50, and 70 animals. Each animal supplied two observations with resultant corresponding sample sizes of 60, 100, and 140 observations, respectively. Sampling was as described in the previous section.

8.4 RESULTS

8.4.1 Effect of Increasing the Number of Animals per Time Point

The outlier criteria outlined in Chapter 2 (Section 2.5.1) were applied to the data sets obtained. Table 8.1 is a summary of successful NONMEM runs used in the results presented below.

8.4.1a Bias and Precision

Fig. 8.1(a - d) summarises the results when σ_{Cl} and σ_V were 15%. As number of animals per time point increased, the precision of the estimates

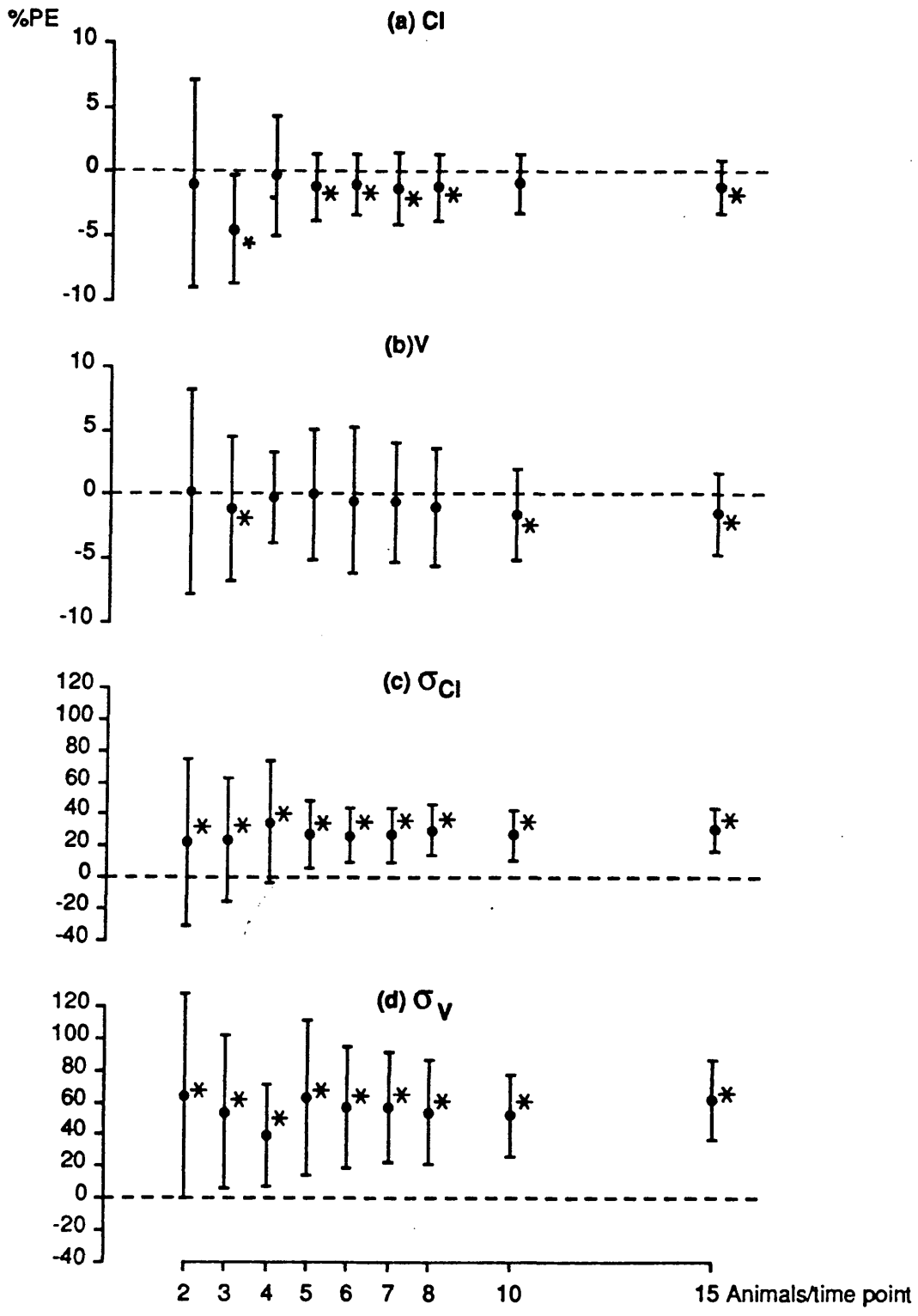


Fig. 8.1 Bias and precision expressed as %PE (mean ± standard deviation, respectively) for parameters. The horizontal axis represents the number of animals used at each time point. Each vertical expresses the bias and precision of the population parameter estimate. Only one observation was made on each animal. The inter-animal variability was set at 15%, and the error in concentration measurements was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

Table 8.2 Mean of %PE (SD) and Nonzero Significance of the Parameters Estimates from Simulated Data Sets for Different Study Designs (j * 1 Designs) at A CV of 30% in Inter-Animal Variability

Number of Animals Per Time Point	Parameter Cl	V	σ_{Cl}	σ_V
2	-6.70*	-2.65	15.86*	21.6
	(9.37)	(11.69)	(44.22)	(51.8)
3	-1.10	-6.16*	13.77	23.99*
	(7.57)	(8.80)	(41.27)	(42.88)
4	-0.09	-7.73*	17.76*	17.17*
	(6.57)	(8.36)	(42.90)	(46.41)
5	-1.91	-5.05*	20.66*	27.47*
	(6.95)	(7.95)	(44.55)	(39.96)
6	-2.65*	-4.51*	14.14*	27.04*
	(3.49)	(8.06)	(26.65)	(33.21)
7	-2.87*	-4.51*	14.44*	24.20*
	(5.28)	(7.32)	(22.17)	(27.93)
8	-3.22*	-4.41*	15.52*	22.78*
	(4.64)	(7.63)	(19.08)	(27.73)
10	-1.98*	-5.54*	16.38*	22.78*
	(3.41)	(5.40)	(17.18)	(24.59)
15	-3.77*	-5.42*	19.17*	31.20*
	(3.45)	(4.66)	(17.30)	(22.18)

*$p < 0.05$

Table 8.3 Mean of %PE (SD) and Nonzero Significance of the Parameters Estimates from Simulated Data Sets for Different Study Designs (j * 1 Designs) at A CV of 45% in Inter-Animal Variability

Number of Animals Per Time Point	Parameter Cl	V	σ_{Cl}	σ_V
2	-6.55	-6.84	12.60	21.1
	(16.74)	(21.99)	(63.60)	(83.5)
3	-5.07*	-10.13*	9.55	32.00*
	(10.88)	(17.41)	(51.32)	(60.30)
4	-2.04	-14.25*	15.81	23.0
	(7.89)	(13.88)	(44.78)	(65.10)
5	-4.16*	-12.06*	15.09*	34.20*
	(6.99)	(11.19)	(36.11)	(59.90)
6	-6.65*	-7.51*	23.98*	15.69*
	(10.56)	(10.51)	(22.54)	(34.00)
7	-5.74*	-6.84*	25.75*	17.31*
	(7.00)	(9.10)	(27.59)	(31.07)
8	-7.46*	-5.51*	22.07*	23.27*
	(7.84)	(9.89)	(22.44)	(35.60)
10	-7.64*	-5.90*	24.94*	18.21*
	(5.27)	(8.70)	(21.62)	(24.53)
15	-6.71*	-7.66*	26.71*	13.64*
	(5.69)	(7.77)	(21.79)	(21.41)

*$p < 0.05$

Table 8.4 Mean of %PE (SD) and Nonzero Significance of the Parameters Estimates from Simulated Data Sets for Different Study Designs (j * 1 Designs) at A CV of 60% in Inter-Animal Variability

Number of Animals Per Time Point	Parameter Cl	V	σ_{Cl}	σ_V
2	-12.72*	-3.74	22.00*	17.7
	(26.15)	(24.91)	(50.08)	(92.3)
3	-3.16	-13.81*	29.90*	6.91
	(17.13)	(21.29)	(69.0)	(46.77)
4	0.13	-12.99*	15.59	18.14*
	(11.93)	(19.01)	(46.07)	(39.74)
5	-2.44	-13.58	23.60*	34.30*
	(14.14)	(22.12)	(44.83)	(66.20)
6	-5.22*	-11.17*	26.53*	1.98
	(11.07)	(10.68)	(42.84)	(27.41)
7	-7.56*	-8.11*	22.43*	13.22
	(10.00)	(10.01)	(28.75)	(40.97)
8	-9.01*	-6.58*	19.77*	18.18*
	(9.18)	(8.17)	(23.82)	(37.75)
10	-8.62*	-6.07*	29.79*	14.48*
	(7.68)	(10.99)	(20.07)	(36.72)
15	-11.47*	-7.61*	34.95*	9.05*
	(6.02)	(7.51)	(22.59)	(21.33)

*$p < 0.05$

(indicated by the reduction in the error bars) also increased. However, the estimates of Cl and V were negatively biased, irrespective of the number animals used. It was also of some interest to consider the magnitude of the SD of %PE for the various parameters. j * 1 designs yielded relatively precise estimates for the fixed effect parameters. Estimates of σ_{Cl} were acceptably precise when the number of animals at each time point was 5 or greater, but the estimates of σ_V were acceptably precise only when the number of animals used at each time was 10 or greater. The estimates of inter-animal variability were, however, consistently positively biased and were relatively unaffected by increasing the number of animals.

When σ_{Cl} and σ_V were set at 30% the estimates of the fixed effect parameters were negatively biased, but precise (Table 8.2). As with the 15% inter-animal variability study, all estimates of σ_{Cl} and σ_V were positively biased and mostly imprecise. Estimates of σ_{Cl} with acceptable precision were obtained when the number of animals used at each time point was 7 or greater while σ_V estimates were acceptably precise when 10 animals or more were used at each time. As expected, the precision with which parameters were estimated increased as the number of animals per time point increased (i.e. precision increased with increased N_S).

With σ_{Cl} and σ_V equal to 45%, negatively biased, but precise estimates were obtained for Cl and V (Table 8.3). The estimates of σ_{Cl} and σ_V were positively biased and mostly imprecise as in the previous cases. Acceptably precise estimates of all parameters were obtained when $\geq$ 10 animals were used at each time point.

When σ_{Cl} and σ_V were set to 60%, the estimates of Cl and V were negatively biased, but mostly precise (Table 8.4) as in the previous cases considered. Imprecise estimates of Cl were obtained with the 2 observations per time point design. Again, the estimates of σ_{Cl} and σ_V were positively biased and

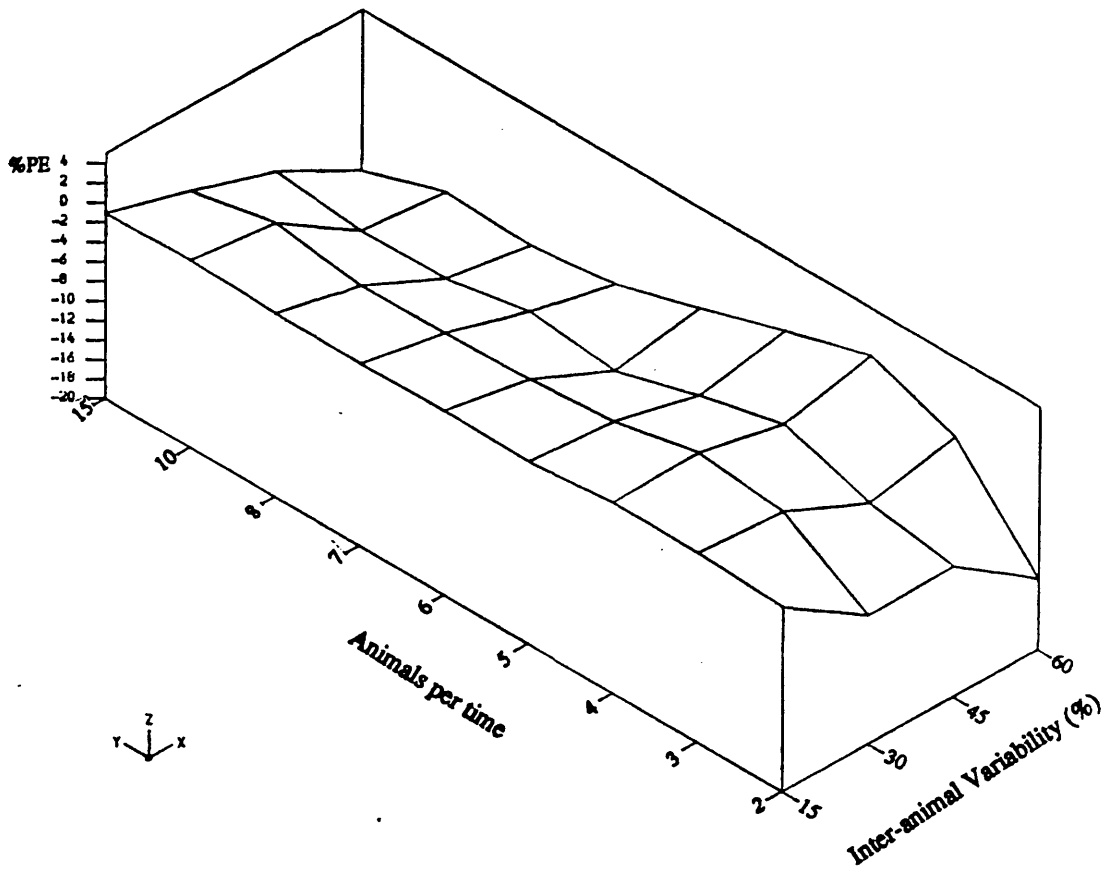


Fig. 8.2a Bias (expressed as mean of %PE) in the estimation of Cl: three dimensional plot of the influence of varying the number of animals sampled at each time point and inter-animal variability on Cl estimation.

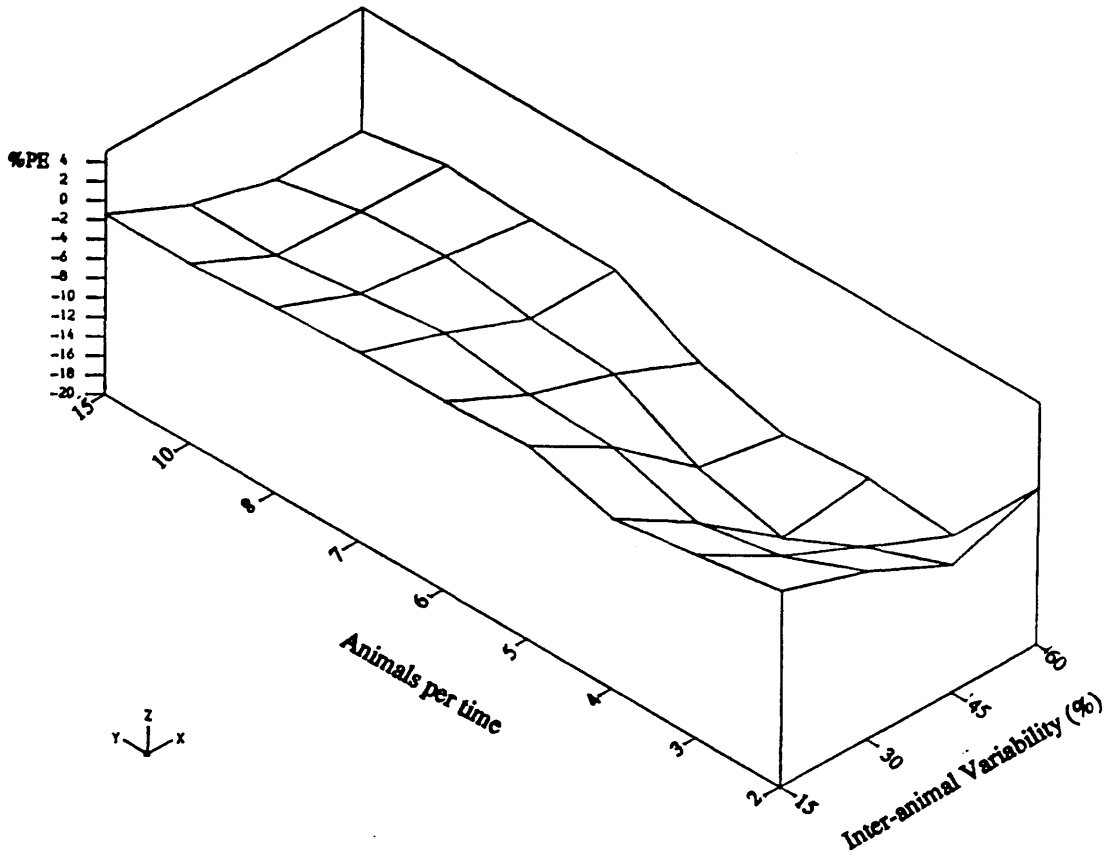


Fig. 8.2b Bias (expressed as mean of %PE) in the estimation of V: three dimensional plot of the influence of varying the number of animals sampled at each time point and inter-animal variability on V estimation.

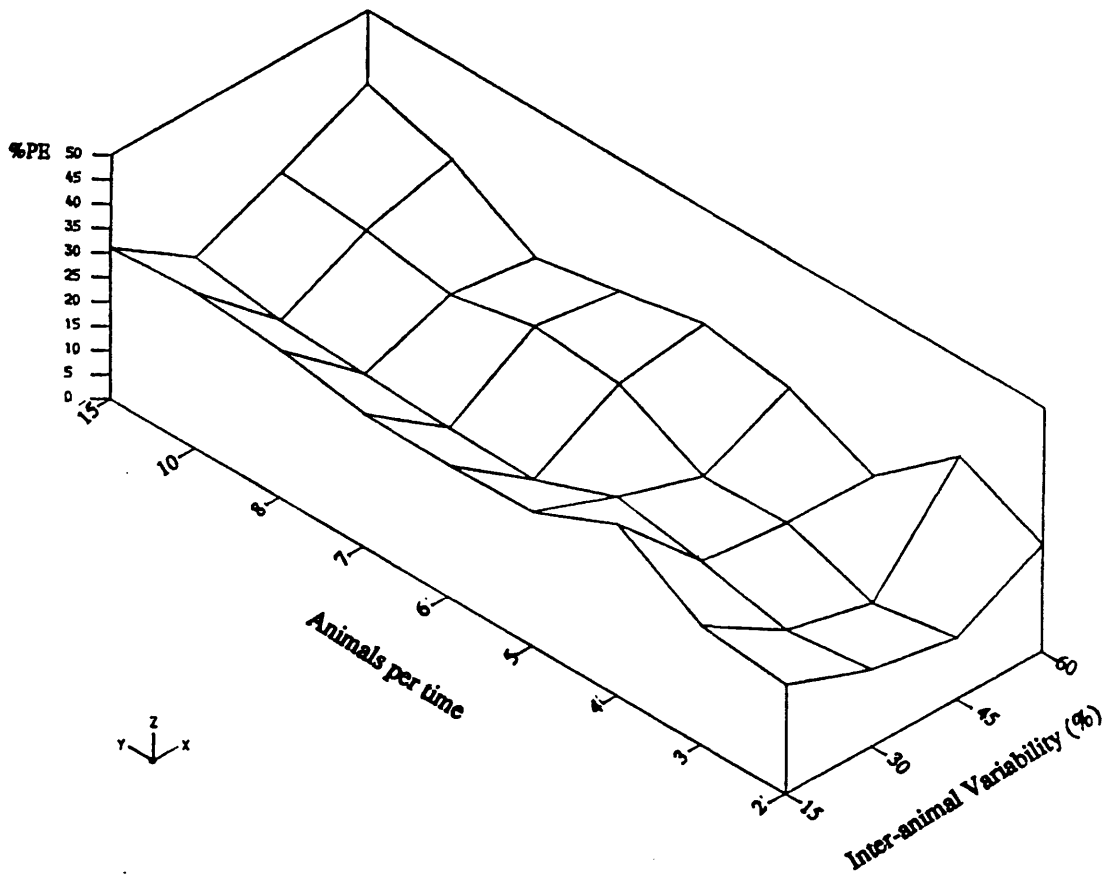


Fig. 8.2c Bias (expressed as mean of %PE) in the estimation of σ_{Cl}: three dimensional plot of the influence of varying the number of animals sampled at each time point and inter-animal variability on σ_{Cl} estimation.

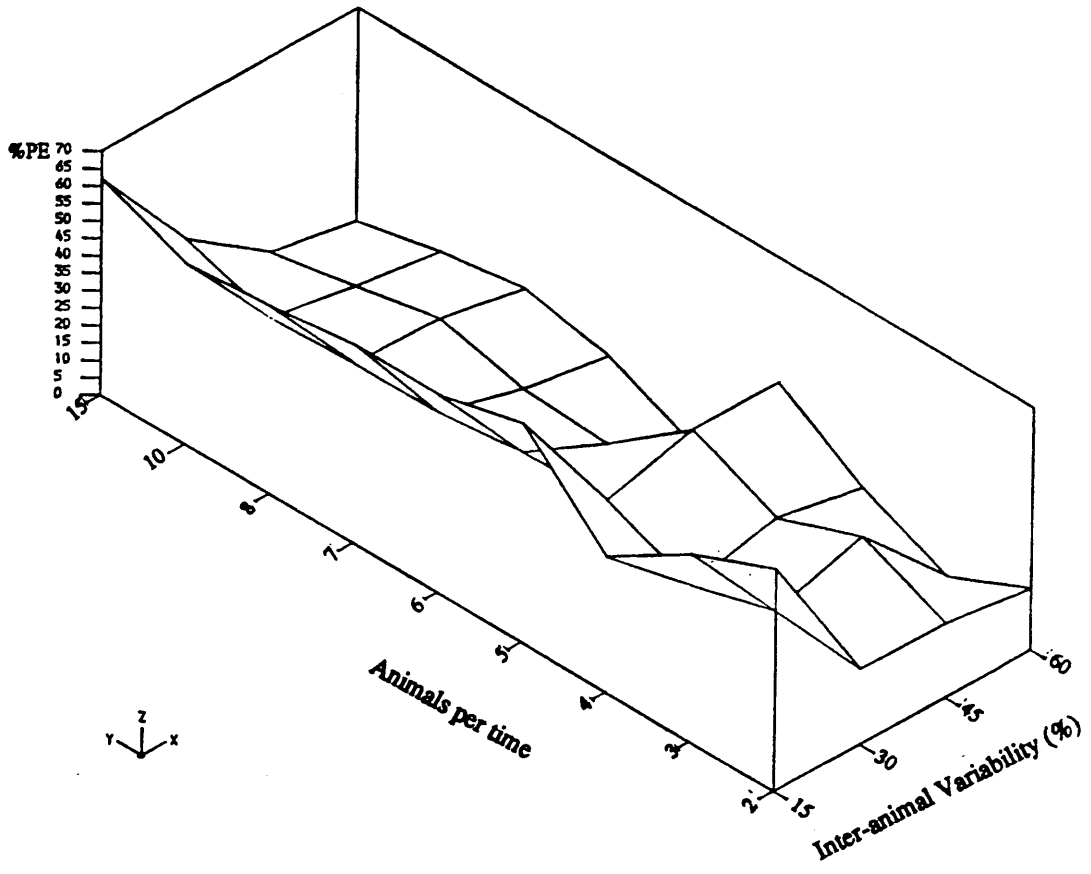


Fig. 8.2d Bias (expressed as mean of %PE) in the estimation of σ_V: three dimensional plot of the influence of varying the number of animals sampled at each time point and inter-animal variability on σ_V estimation.

mostly imprecise. Acceptably precise estimates of all parameters were obtained only when 15 animals were used at each time point.

These results are summarised in Fig. 8.2(a - d). The estimates of Cl obtained for each value of inter-animal variability were relatively stable. The mean of %PE ranged from -1.2 to -0.9% for σ_{Cl} and σ_V equal to 15%, -6.7 to -0.1% for σ_{Cl} and σ_V set to 30%, -7.5 to -2.0% for σ_{Cl} and σ_V equal to 45%, and -12.7 to 0.1% for σ_{Cl} and σ_V set to 60% (Fig. 8.2a). Similarly the estimates of V were relatively stable with mean of %PE ranging from -1.6 to 0.2%, -14.3 to -5.5%, -7.7 to -2.7%, and -13.8 to -3.7% for inter-animal variabilities of 15, 30, 45, and 60 %, respectively (Fig. 8.2b). Also, σ_{Cl} estimates were relatively stable with the difference between the most and least biased estimates for each level of variability not exceeding 12% (Fig. 8.2c). σ_V estimates were less stable with the difference between the most and least biased estimates ranging from 8% for an inter-animal variability of 60% to 25% for an inter-animal variability of 15% (Fig. 8.2d). There was a tendency for the bias in the estimation of the fixed effect parameters to increase with the increase in the inter-animal variability as would be expected.

8.4.1b Design Number

When σ_{Cl} and σ_V were set to 15%, Cl was efficiently estimated when the number of animals used per time were between 3 and 15. However, Cl estimates obtained when the number of animals per time point were between 4 and 15 were significantly better than the estimates obtained when 2 animals were used per time point (Fig. 8.3a). As expected, the most efficient estimates were obtained when 15 animals were used per time point. V was significantly better estimated when 4 to 15 animals were used than 2 animals per time point (Fig. 8.3b). Again, the most efficient estimates were obtained with 15 animals per time point, and the

(a) Estimation Cl

15	10	8	6	7	4	5	3	2	Animals per time point

(b) Estimation of V

15	10	8	7	5	6	4	3	2	Animals per time point

(c) Estimation of σ_{Cl}

7	15	6	8	3	10	5	4	2	Animals per time point

(d) Estimation of σ_V

4	10	8	3	6	7	15	5	2	Animals per time point

(e) Overall Design Efficiency

15	10	8	7	6	5	4	3	2	Animals per time point

Fig. 8.3 [a]Summary of significant differences in the efficiency* with which parameters were estimated by varying the number of animals sampled at each time point with inter-animal variability set at 15%.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

(a) Estimation Cl

10 7 6 15 5 8 4 3 2 Animals per time point

(b) Estimation of V

15 10 7 8 5 6 3 4 2 Animals per time point

(c) Estimation of σ_{Cl}

8 7 6 10 15 3 5 4 2 Animals per time point

(d) Estimation of σ_V

4 7 10 8 15 3 5 6 2 Animals per time point

(e) Overall Design Efficiency

15 10 7 8 6 5 4 3 2 Animals per time point

Fig. 8.4 [a]Summary of significant differences in the efficiency* with which parameters were estimated by varying the number of animals sampled at each time point with inter-animal variability set at 30%.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

least efficient estimates with 2 animals.

At the 15% level of inter-animal variability, σ_{Cl} (Fig. 8.3c) and σ_V (Fig. 8.3d) were inefficiently estimated with all j * 1 designs.

Overall, parameters were well estimated when the number of animals used at each time point was between 6 and 15 animals per time point (Fig. 8.3e). The use of between 5 to 8 animals per time yielded parameter estimates with similar efficiency. Parameter estimates obtained with 10 and 15 animals per time point were significantly better than those with 2 to 5 animals per time point. The best estimates of parameters was obtained with the 15 animals per time point design.

When σ_{Cl} and σ_V were set to 30%, Cl was more efficiently estimated with 4 to 15 animals used at each time point than 2 animals (Fig. 8.4a). V was well efficiently estimated using either 3, or 5 to 15 animals per time (Fig. 8.4b). V estimates with 3 animals per time were only marginally better than those with 4 animals. The difference lay in the bias term of Φ_{ir}, the estimates with 4 animals per time being more biased than those with 3 animals per time (Table 8.2). However, the results obtained using between 2 to 8 animals per time point were similar. Designs with 10 to 15 animals per time point yielded significantly better V estimates than those obtained with the design using 2 animals per time point. As with the 15% level of inter-animal variability, σ_{Cl} and σ_V were poorly estimated with all designs (Fig. 8.4c & d). All parameters were well estimated when 6 to 15 animals were used per time point, and the estimates with 10 and 15 animals per time point were significantly better than those with 2 to 5 animals per time (Fig. 8.4e). As expected, the least efficient estimates were obtained with the 2 animals per time point design.

Cl was efficiently estimated with the use of 3 to 15 animals per time point when the inter-animal variability was 45% (Fig. 8.5a). V was well estimated with designs having 6 to 15 animals per time (Fig. 8.5b). Estimates obtained with designs having 8 to 15 animals per time point were significantly better than those

(a) Estimation Cl

5	15	7	4	10	8	6	3	2	Animals per time point

(b) Estimation of V

10	15	8	7	6	5	4	3	2	Animals per time point

(c) Estimation of σ_{Cl}

8	10	15	6	7	3	5	4	2	Animals per time point

(d) Estimation of σ_V

15	7	4	10	6	8	5	2	3	Animals per time point

(e) Overall Design Efficiency

15	10	8	7	6	5	4	3	2	Animals per time point

Fig. 8.5 [a]Summary of significant differences in the efficiency* with which parameters were estimated by varying the number of animals sampled at each time point with inter-animal variability set at 45%.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

(a) Estimation Cl

8	10	4	6	7	5	15	3	2	Animals per time point

(b) Estimation of V

8	15	10	7	6	4	5	2	3	Animals per time point

(c) Estimation of σ_{Cl}

6	8	7	5	10	15	4	2	3	Animals per time point

(d) Estimation of σ_V

15	8	10	7	6	4	5	2	3	Animals per time point

(e) Overall Design Efficiency

15	8	10	7	6	5	4	3	2	Animals per time point

Fig. 8.6 [a]Summary of significant differences in the efficiency* with which parameters were estimated by varying the number of animals sampled at each time point with inter-animal variability set at 60%.
a - Rank order of design numbers increasing from left to right.
* - Efficiency measured with design number.

obtained with 2 to 5 animals per time point. σ_{Cl} and σ_V estimates were poorly estimated with all designs (Fig. 8.5c & d). Overall, parameters were better estimated with the use of 6 to 15 animals per time point (Fig. 8.5e). Estimates of similar efficiency were obtained with designs having 5 to 10 animals per time point. The most efficient estimates were obtained with the use of 15 animals at each time point. These estimates were significantly better than the estimates obtained with 2 to 5 animals per time point. Since the use of 6 to 15 animals at each time point produced parameter estimates with similar efficiency the use of 6 animals per time in this type of study does not result in any significant loss in efficiency.

With σ_{Cl} and σ_V equal to 60%, Cl was estimated with an equal efficiency with all the j * 1 designs (Fig. 8.6a). The designs with 6 to 15 animals per time point yielded more efficient estimates of V (Fig. 8.6b) than when 2 observations were used per time point. As with the previous results, σ_{Cl} and σ_V were inefficiently estimated with all designs (Fig. 8.6 c & d). When considered as a set, all parameters were most efficiently estimated with 6 to 15 animals used at each time point (Fig 8.6e). Estimates obtained with 8 to 15 animals per time point were significantly better than those using 2 to 4 animals. Again, the use of 6 animals per time yielded equally efficient parameter estimates as 15.

8.4.1c Individual and Joint Confidence Intervals for Parameter Estimates

At the 15% level of inter-animal variability, good coverage was obtained for individual and joint parameter estimates when NONMEM runs with catastrophic estimates were included (Table 8.5, Section I). However, reduced coverage for joint parameter estimates was obtained with the use of 15 animals at each time point due to the bias associated with the estimation of the variance parameters. The influence of standard errors on confidence intervals coverage

Table 8.5 Confidence Intervals Coverage for Individual & Joint Parameter Estimates: 15% Inter-Animal Variability in Cl and V

Number of Animals / Time Point	Fraction Including True: Section I Success — Total					Section II Success - Excluded — Total - Excluded					Section III Success - Excluded — Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
2	26/29	29/29	25/29	28/29	21/29	26/29	29/29	20/22	17/17	10/13	26/29	29/29	20/29	17/29	10/29*
3	29/29	29/29	25/29	28/29	23/29	29/29	29/29	24/27	21/24	18/23	29/29	29/29	24/29	21/29	18/29
4	29/29	29/29	29/29	29/29	29/29	29/29	29/29	28/28	25/25	24/24	29/29	29/29	28/29	25/29	24/29
5	30/30	30/30	29/30	30/30	29/30	30/30	30/30	28/29	23/23	21/22	30/30	30/30	28/30	23/30	21/30
6	30/30	28/30	30/30	29/30	27/30	30/30	28/30	30/30	26/27	24/27	30/30	28/30	30/30	26/30	24/30
7	30/30	30/30	30/30	27/30	27/30	30/30	30/30	30/30	26/29	26/29	30/30	30/30	30/30	26/30	26/30
8	30/30	29/30	29/30	26/30	25/30	30/30	29/30	29/30	26/30	25/30	30/30	29/30	29/30	26/30	25/30
10	30/30	29/30	28/30	26/30	23/30	30/30	29/30	28/30	26/30	23/30	30/30	29/30	28/30	26/30	23/30
15	30/30	29/30	23/30	18/30	15/30	30/30	29/30	23/30	17/29	15/29	30/30	29/30	23/30	17/30	15/30

* $p < 0.01$

Table 8.6 Confidence Intervals Coverage for Individual & Joint Parameter Estimates: 30% Inter-Animal Variability in Cl and V

Number of Animals / Time Point	Fraction Including True: Section I Success – Total					Section II Success - Excluded – Total - Excluded					Section III Success - Excluded – Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
2	26/26	25/26	24/26	21/26	19/26	26/26	25/26	15/15	16/17	7/15	26/26	25/26	15/26	16/26	7/26*
3	30/30	28/30	28/30	27/30	23/30	30/30	28/30	21/22	20/20	14/17	30/30	28/30	21/30	20/30	14/30
4	30/30	27/30	29/30	29/30	26/30	30/30	28/30	23/23	24/24	17/19	30/30	28/30	23/30	24/30	17/30
5	30/30	29/30	29/30	30/30	29/30	30/30	29/30	24/25	22/22	16/17	30/30	29/30	24/30	22/30	16/30
6	30/30	29/30	29/30	30/30	28/30	30/30	29/30	29/30	23/23	21/22	30/30	29/30	29/30	23/30	21/30
7	29/29	29/29	29/29	29/29	29/29	29/29	28/29	28/28	28/28	27/28	29/29	28/29	28/29	28/29	27/29
8	30/30	29/30	30/30	30/30	29/30	30/30	29/30	29/29	29/29	28/29	30/30	29/30	29/30	29/30	28/30
10	30/30	28/30	30/30	30/30	28/30	30/30	28/30	30/30	29/29	27/29	30/30	28/30	30/30	29/30	27/30
15	30/30	28/30	30/30	29/30	27/30	30/30	28/30	29/29	27/28	25/28	30/30	28/30	29/30	28/30	25/30

* $p < 0.01$

Table 8.7 Confidence Intervals Coverage for Individual & Joint Parameter Estimates: 45% Inter-Animal Variability in Cl and V

Number of Animals / Time Point	Fraction Including True: Section I Success – Total Cl	V	σ_{Cl}	σ_V	Joint	Section II Success - Excluded – Total - Excluded Cl	V	σ_{Cl}	σ_V	Joint	Section III Success - Excluded – Total Cl	V	σ_{Cl}	σ_V	Joint
2	26/27	24/27	25/27	22/27	19/27	23/24	23/26	10/10	7/8	2/3	23/27	23/26	10/27*	7/27*	2/27*
3	28/28	28/28	23/28	27/28	20/28	28/28	28/28	15/15	15/15	7/8	28/28	22/28	15/28	15/28	7/28*
4	27/27	23/27	24/27	26/27	20/27	27/27	24/27	17/17	21/22	13/16	27/27	24/27	17/27	17/27	13/27*
5	27/27	25/27	25/27	27/27	24/27	27/27	25/27	19/19	18/18	12/13	27/27	25/27	19/27	18/27	12/27*
6	29/29	28/29	29/29	29/29	28/29	29/29	25/29	25/25	20/20	18/19	29/29	25/29	25/29	20/29	18/29
7	29/29	28/29	29/29	29/29	28/29	29/29	28/29	26/26	23/23	22/23	29/29	28/29	26/29	23/29	22/29
8	29/29	27/29	29/29	28/29	28/29	29/29	28/29	27/27	22/23	22/23	29/29	28/29	27/29	22/29	22/29
10	29/29	26/29	29/29	29/29	26/29	29/29	28/29	29/29	28/28	25/28	29/29	28/29	29/29	28/29	25/29
15	29/29	24/29	28/29	29/29	23/29	29/29	24/29	28/29	28/28	22/28	29/29	24/29	28/29	28/29	22/29

* $p < 0.01$

was only significant when 2 animals were used at each time point (Table 8.5, Section III).

Similarly, significantly reduced coverage for joint confidence intervals was obtained with the 2 observation per time point study design when the inter-animal variability was set at 30% (Table 8.6, Section III). However, the joint coverage for parameter estimates was reduced (though not significantly lower than the expected value of 0.81) for designs with 3 to 5 animals per time point. When runs with catastrophic estimates were excluded in both the numerator and denominator during confidence intervals calculation, relatively good coverage was obtained for all study designs (Table 8.6, Section II), and the influence of bias was minimal (Table 8.6, Section I).

When the inter-animal variability was 45%, good coverage was obtained for individual and joint parameter estimates when catastrophic runs were included in the computation of confidence intervals coverage (Table 8.7, Section I). However, the coverage for the variance parameters and the joint coverage for parameter estimates were reduced when catastrophic runs were excluded in the numerator during the calculation of confidence intervals (Table 8.7, Section III). The reduced coverage obtained for σ_{Cl} and σ_V using the 2 animals per time point design was significantly lower than the expected value of 0.95. Designs in which 2 to 5 animals were used at each time point had joint coverage lower than the expected value of 0.81, due mostly to large standard errors.

Setting the inter-animal variability at 60% led to estimates whose confidence intervals coverage was good when NONMEM runs with "coefficient of variation" > 50% were included in the computation of the coverage (Table 8.8, Section I). However, reduced coverage was obtained for the variance parameters and the joint confidence intervals for parameter estimates when these NONMEM runs were excluded in the numerator during confidence intervals calculation (Table 8.8, Section III). The coverage obtained for σ_{Cl} (2 animals per time point)

Table 8.8 Confidence Intervals Coverage for Individual & Joint Parameter Estimates: 60% Inter-Animal Variability in Cl and V

Number of Animals / Time Point	Fraction Including True: Section I Success / Total					Section II Success - Excluded / Total - Excluded					Section III Success - Excluded / Total				
	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint	Cl	V	σ_{Cl}	σ_V	Joint
2	22/23	21/23	20/23	19/23	16/23	21/22	21/23	9/11	3/4	1/1	21/23	21/23	9/23*	3/23*	1/23*
3	28/29	23/29	25/29	23/29	17/29	28/29	23/29	14/14	11/14	4/6	28/29	23/29	14/29	11/29*	4/29*
4	27/27	21/27	23/27	26/27	19/27	27/27	21/27	12/13	14/15	5/5	27/27	21/27	12/27	14/27	5/27*
5	26/26	28/26	24/26	23/26	17/26	26/26	28/26	15/16	13/15	8/8	26/26	18/26	15/26	13/26	8/26*
6	28/28	25/28	27/28	26/28	22/28	28/28	25/28	22/23	19/20	13/17	28/28	25/28	22/28	19/28	13/28
7	29/29	26/29	28/29	28/29	24/29	29/29	26/29	23/24	21/22	17/20	29/29	26/29	23/29	21/29	17/29
8	30/30	27/30	29/30	28/30	25/30	30/30	27/30	27/28	22/24	19/23	30/30	27/30	27/30	22/30	19/30
10	30/30	27/30	30/30	27/30	26/30	30/30	27/30	28/28	25/27	22/25	30/30	27/30	28/30	25/30	22/30
15	29/30	28/30	30/30	30/30	27/30	29/30	28/30	30/30	27/27	24/27	29/30	28/30	30/30	27/30	24/30

* $p < 0.01$

Table 8.9 Incidence of Correlation Values Associated with Parameters at 15% Variability in Cl and V

		Number of Animals per Time Point																		
		2		3		4		5		6		7		8		10		15		
Correlation (%)	H[a]	L[b]	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0		
σ_{Cl} vs Cl	3.5	96.5	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0		
σ_{Cl} vs V	10.3	89.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0		
σ_{V} vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0		
σ_{V} vs V	24.1	75.9	13.8	86.2	13.3	86.7	10.0	90.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	3.3	96.7		
σ_{V} vs σ_{Cl}	17.2	82.8	0.0	100.0	10.3	89.7	0.0	100.0	3.3	96.7	3.3	96.7	3.3	96.7	3.3	96.7	3.3	96.7		

a: H = High, Correlation coefficient ≥ 0.75

b: L = Low, Correlation coefficient < 0.75

and σ_V (2 and 3 animals per time point) and the joint confidence intervals for parameter estimates (2 to 5 animals per time point designs) was significantly lower than the expected value of 0.95 and 0.81, respectively (Table 8.8, Section III).

Thus, as the inter-animal variability was increased, the coverage of interval estimates for the variance parameters and joint parameter estimates was reduced. At all levels of inter-animal variability, the joint coverage for parameter estimates was significantly lower than the expected value when 2 animals were used at each time point. On the other hand, significantly reduced coverage was only obtained for the joint confidence intervals at 45 and 60% level of inter-animal variability with study designs having 3 to 5 animals per time point.

8.4.1d Incidence of High Pair-Wise Correlations

Greater than 0% incidence of high pair-wise correlation occurred between σ_V and V, σ_V and σ_{Cl} for most of the designs when the inter-animal variability was 15% (Table 8.9). The greatest incidence of 24.1% was obtained for the correlation between σ_V and V with the 2 animals per time point design, and the incidence of high correlation between σ_{Cl} and Cl, and σ_{Cl} and V was 3.5 and 10.3%, respectively, for the same design.

At the 30% level of inter-animal variability the incidence of high correlation between σ_V and V ranged from 3.3 (15 animals per time point) to 21.4% (2 animals per time point) (Table 8.10). The incidence (> 0%) for the correlation between σ_{Cl} and V ranged from 3.3 (7 animals per time point) to 14.3 (2 animals per time point). Also, the 2 animals per time point design yielded the highest incidence of correlation between V and Cl (3.6%), σ_{Cl} vs. Cl (3.6%), and σ_V with σ_{Cl} (15.4%) (Table 8.10).

Table 8.10 Incidence of Correlation Values Associated with Parameters at 30% Variability in Cl and V

		Number of Animals per Time Point																	
		2		3		4		5		6		7		8		10		15	
Correlation (%)	H[a]	L[b]	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
V vs Cl	3.6	96.4	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	3.6	96.4	3.3	96.7	0.0	100.0	3.3	96.7	0.0	100.0	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs V	14.3	85.7	10.0	90.0	3.3	96.7	13.3	86.7	3.3	96.7	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	21.4	78.6	16.7	83.3	6.7	93.3	13.3	86.7	3.3	96.7	6.7	93.3	3.3	96.7	0.0	100.0	3.3	96.7	0.0	100.0
σ_V vs σ_{Cl}	15.4	84.6	0.0	100.0	13.3	86.7	0.0	100.0	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0

a: H = High, Correlation coefficient ≥ 0.75

b: L = Low, Correlation coefficient < 0.75

Table 8.11 Incidence of Correlation Values Associated with Parameters at 45% Variability in Cl and V

		Number of Animals per Time Point																			
		2		3		4		5		6		7		8		10		15			
Correlation (%)	H[a]	L[b]	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	
V vs Cl	7.4	92.6	10.7	89.3	7.1	92.9	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0			
σ_{Cl} vs Cl	14.8	85.2	3.6	96.4	10.7	89.3	3.7	96.3	3.4	96.6	3.4	96.6	0.0	100.0	0.0	100.0	0.0	100.0			
σ_{Cl} vs V	18.5	81.5	17.9	82.1	14.3	85.7	0.0	100.0	3.4	96.6	6.9	93.1	6.9	93.1	3.4	96.6	0.0	100.0			
σ_{V} vs Cl	11.1	88.9	3.6	96.4	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0			
σ_{V} vs V	22.2	77.8	17.9	82.1	10.7	89.3	22.2	77.8	24.1	75.9	20.7	79.3	24.1	75.9	10.3	89.7	0.0	100.0			
σ_{V} vs σ_{Cl}	14.8	85.2	0.0	100.0	11.1	88.9	0.0	100.0	3.3	96.7	3.3	96.7	0.0	100.0	0.0	100.0	0.0	100.0			

a: H = High, Correlation coefficient ≥ 0.75

b: L = Low, Correlation coefficient < 0.75

Table 8.12 Incidence of Correlation Values Associated with Parameters at 60% Variability in Cl and V

		Number of Animals per Time Point																	
		2		3		4		5		6		7		8		10		15	
Correlation (%)	H[a]	L[b]	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
V vs Cl	4.2	95.8	10.3	89.7	7.4	92.6	3.8	96.2	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
σ_{Cl} vs Cl	20.8	79.6	24.7	75.3	7.4	92.6	7.7	92.3	10.0	90.0	13.3	86.7	6.7	93.3	3.3	96.7	0.0	100.0	
σ_{Cl} vs V	20.8	79.6	31.0	69.0	7.4	92.6	3.8	96.2	0.0	100.0	6.7	93.3	20.0	80.0	10.0	90.0	6.7	93.3	
σ_V vs Cl	4.2	95.8	0.0	100.0	11.1	88.9	15.4	84.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	
σ_V vs V	41.7	58.3	34.5	65.5	22.2	77.8	15.4	84.6	26.7	73.3	26.7	73.3	10.0	90.0	10.0	90.0	13.3	86.7	
σ_V vs σ_{Cl}	21.7	78.3	0.0	100.0	11.1	88.9	0.0	100.0	3.6	96.4	3.4	96.6	6.6	93.3	0.0	100.0	0.0	100.0	

a: H = High, Correlation coefficient ≥ 0.75

b: L = Low, Correlation coefficient < 0.75

When the inter-animal variability was 45%, the highest incidence of high pair-wise correlations was obtained when 2 observations were made at each time point (Table 8.11). A greater incidence of high correlation was obtained for the correlation between σ_V and V for most study designs.

Similarly, a greater incidence of high pair-wise correlation was obtained for the correlation between σ_V and V irrespective of the study design when the inter-animal variability was set at 60% (Table 8.12). More parameters were highly correlated with each other using the 2 animals per time point design than other designs.

Irrespective of the study design and the inter-animal variability, a greater incidence of high pair-wise correlation was obtained for σ_V and V than any other parameter pair.

8.4.2 Effect of Varying the Error in Concentration Measurements

When 3 animals were used at each time point, there were 28, 29, and 27 successful NONMEM runs with σ_ϵ of 0, 15, and 30%, respectively. 30, 30, and 28 successful runs were obtained for σ_ϵ of 0, 15, and 30%, respectively when 5 animals were measured at each time point, and with the 7 animals per time point design, 30, 29, and 28 successful NONMEM runs were obtained for σ_ϵ of 0, 15, and 30%, respectively. The accuracy and precision of the fixed effect parameters were relatively unaffected by varying the error in concentration measurements. When σ_ϵ was 15%, the estimates of inter-animal variability were less precise, as expected, and biased, and this trend was maintained for σ_ϵ of 30%. Moreover, the estimates were significantly positively biased (Fig. 8.7 (a - d)). The bias in the estimation of inter-animal variability was unaffected by N_S.

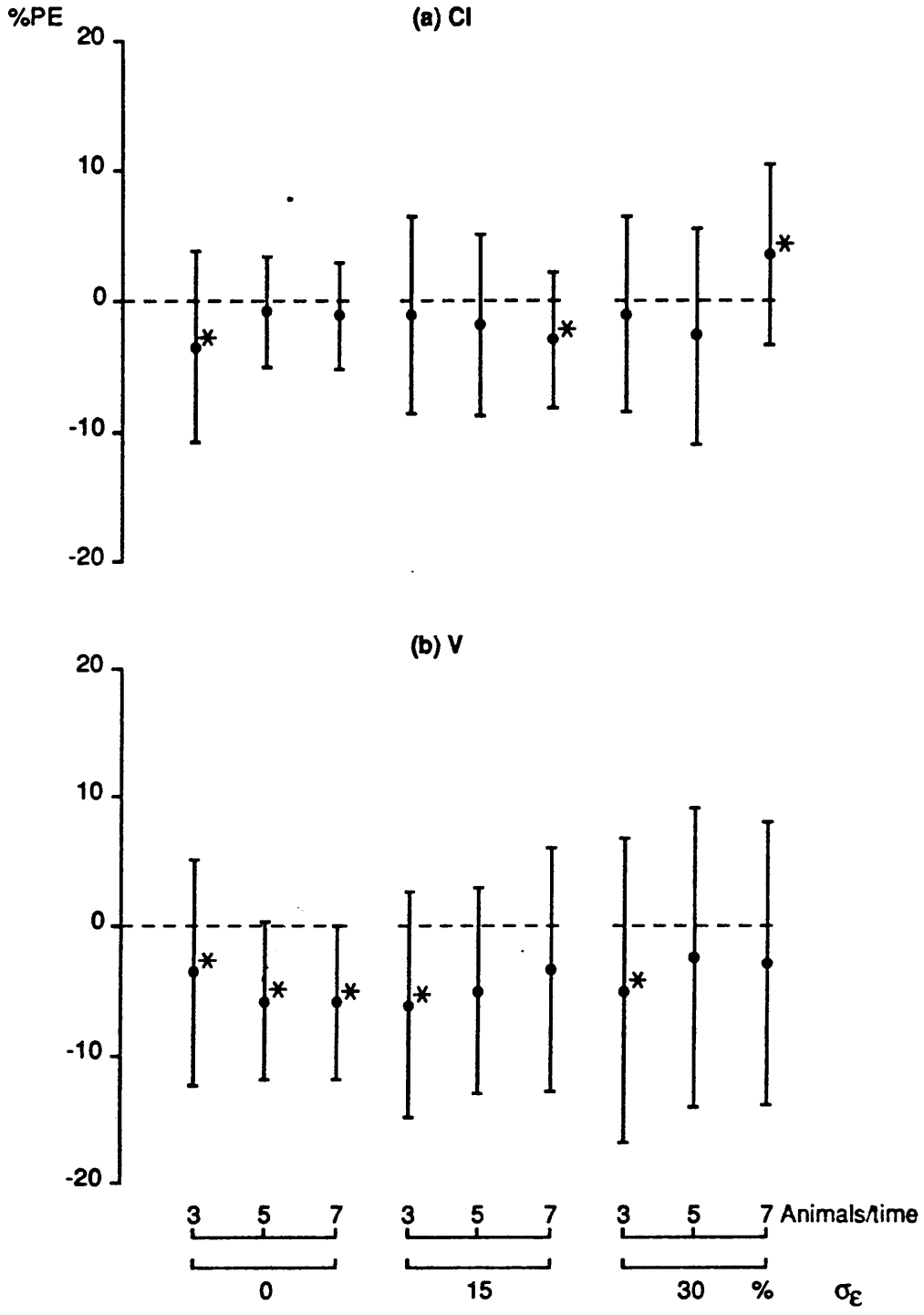


Fig. 8.7(a & b) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for parameters. The horizontal panels show data obtained using σ_ϵ = 0, 15, and 30%. Only one observation was made on each animal. Each vertical expresses the bias and precision of the population parameter estimate. The inter-animal variability used was 30% (see methods). Significant ($p < 0.05$) biases are indicated by asterisks.

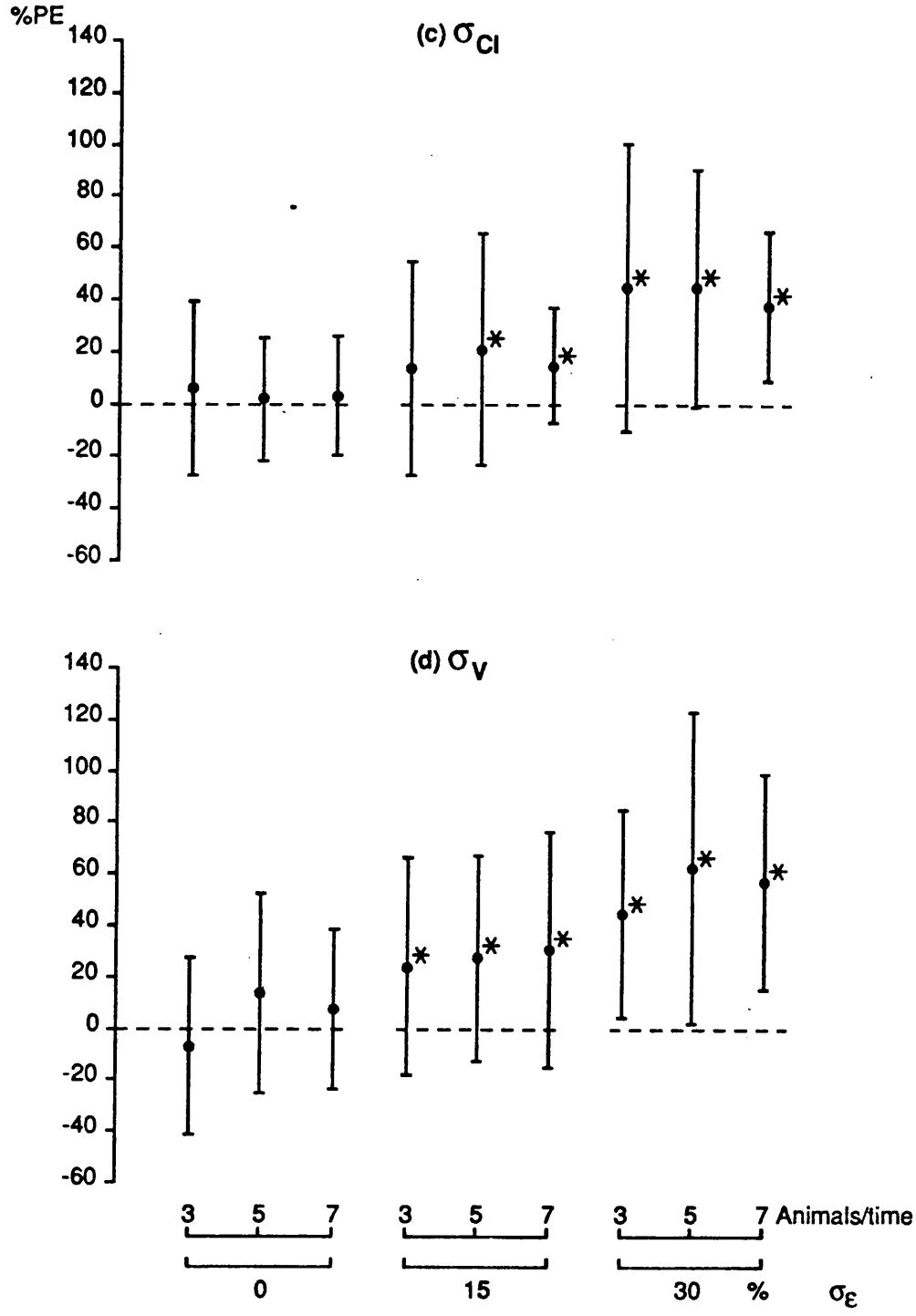


Fig. 8.7(c & d) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for parameters. The horizontal panels show data obtained using σ_ε = 0, 15, and 30%. Only one observation was made on each animal. Each vertical expresses the bias and precision of the population parameter estimate. The inter-animal variability used was 30% (see methods). Significant ($p < 0.05$) biases are indicated by asterisks.

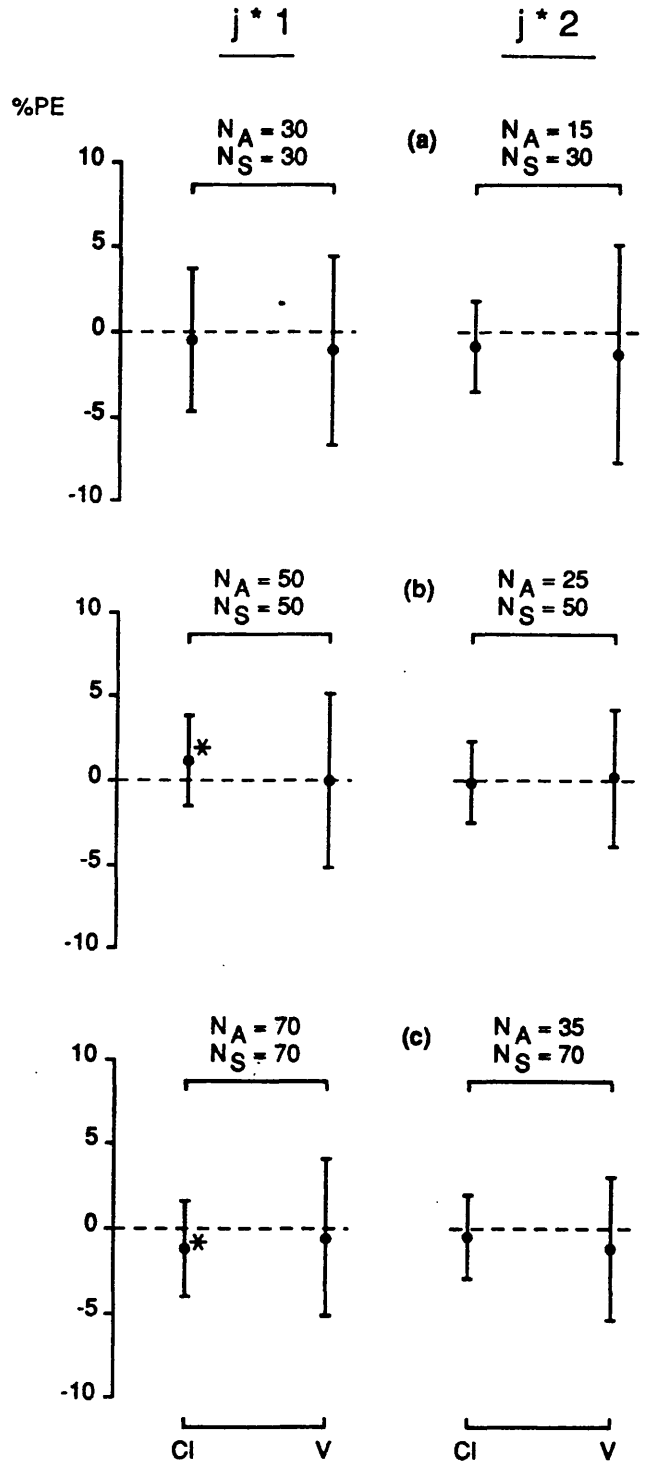


Fig. 8.8(a - c) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for parameters. The horizontal panels in each figure show results from different study designs. The first panel for each figure shows results with j * 1 designs which is used as a reference for comparing results obtained with the j * 2 designs (second panel, see methods). The j * 2 designs yielded total number of data points per data set equivalent to that obtained with the j * 1 designs but with the total number of animals halved. N_A represents the total number of animals used for each study design and N_S, the sample size for each study design. σ_ϵ was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

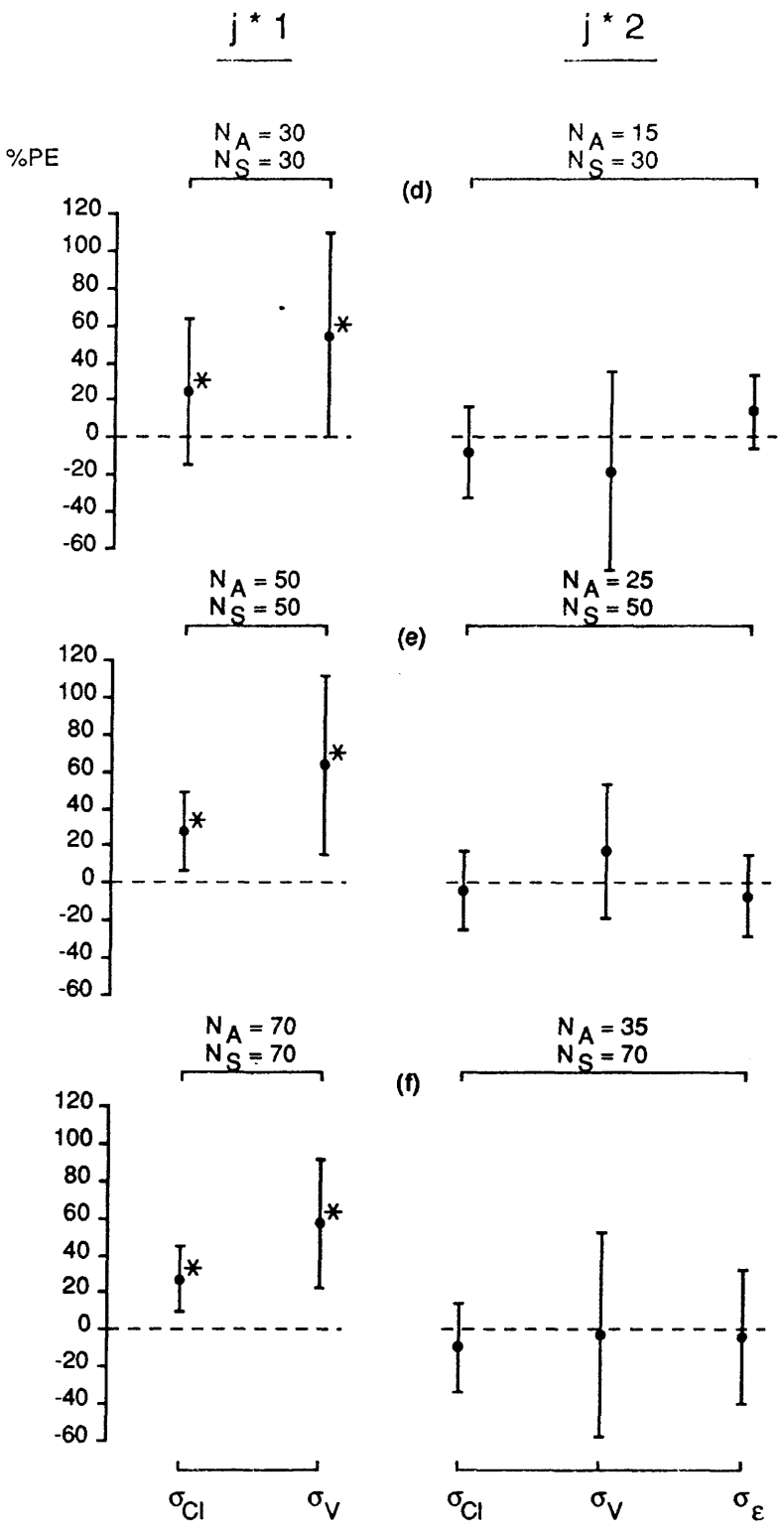


Fig. 8.8(d - f) Bias and precision expressed as %PE (mean ± standard deviation, respectively) for parameters. The horizontal panels in each figure show results from different study designs. The first panel for each figure shows results with j * 1 designs which is used as a reference for comparing results obtained with the j * 2 designs (second panel, see methods). The j * 2 designs yielded total number of data points per data set equivalent to that obtained with the j * 1 designs but with the total number of animals halved. N_A represents the total number of animals used for each study design and N_S, the sample size for each study design. σ_ϵ was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

8.4.3 Effect of Keeping N_S Constant while Halving N_A

8.4.3a Bias and Precision

N_S was kept constant while the N_A was reduced by a factor of 2 so that each animal supplied two concentration - time points (i.e., j * 2 designs) and N_A equalled 15, 25, and 35, preserving the total number of data points (N_S). There were 14, 18, and 24 successful NONMEM runs for N_A of 15, 25, and 35, respectively, compared to 29, 30, and 30 for the corresponding j * 1 designs. Most of the excluded NONMEM runs had spurious estimates of σ_ϵ. The results for the j * 2 designs are shown in Fig. 8.8 (a - f) with the j * 1 designs included for reference. The estimation of the fixed effect parameters were relatively unaffected (Fig. 8.8a & b). The bias in the estimation of σ_{Cl} and σ_V was significantly reduced (Fig. 8.8(d - f)) irrespective of N_S, but the precision of the estimates remained relatively unchanged. The relatively poorer precision for σ_V obtained with N_A of 35 (N_S = 70) as compared to 25 (N_S = 50) was due to the some estimates being at the ceiling of the cut off point for outliers. The bias in the estimation of σ_ϵ ranged from -2.9% (N_A = 35) to -13.7% (N_A = 15), and the SD of %PE from 19.5% (N_A = 15) to 35.9% (N_A = 35).

8.4.3b Incidence of High Pair-wise Correlations

A 100% incidence of high correlation was observed between σ_ϵ and Cl irrespective of N_A (Table 8.13). In addition, 13.3, 21.1, and 5.2% incidence of high correlation between σ_{Cl} and σ_V was obtained for N_A of 15, 25, and 35, respectively, while 40, 47.4, and 9.1% incidence was obtained with N_A equal to 15, 25, and 35, respectively, for the correlation between σ_ϵ and σ_V. Parameter estimates were more highly correlated with each other when N_A was 15 than 25 or 35.

Table 8.13 Incidence of Correlation Values Associated with Parameters at 15% Variability in Cl and V: Each Animal Sampled Twice

	Number of Animals Sampled Twice											
	15		25		30		35		50		70	
Correlation (%)	H*	L*	H	L	H	L	H	L	H	L	H	L
V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs Cl	13.3	86.7	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_{Cl} vs V	6.7	93.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs Cl	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_V vs V	6.7	93.3	0.0	100.0	6.3	93.7	5.2	94.8	0.0	100.0	0.0	100.0
σ_V vs σ_{Cl}	33.3	66.7	21.1	79.9	0.0	100.0	3.8	96.2	4.4	95.6	3.6	96.4
σ_ϵ vs Cl	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
σ_ϵ vs V	6.7	93.3	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_ϵ vs σ_{Cl}	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
σ_ϵ vs σ_V	40.0	60.0	47.4	52.6	6.3	93.7	9.1	90.9	13.0	87.0	14.3	85.7

a: H = High, Correlation coefficient ≥ 0.75 b: L = Low, Correlation coefficient < 0.75

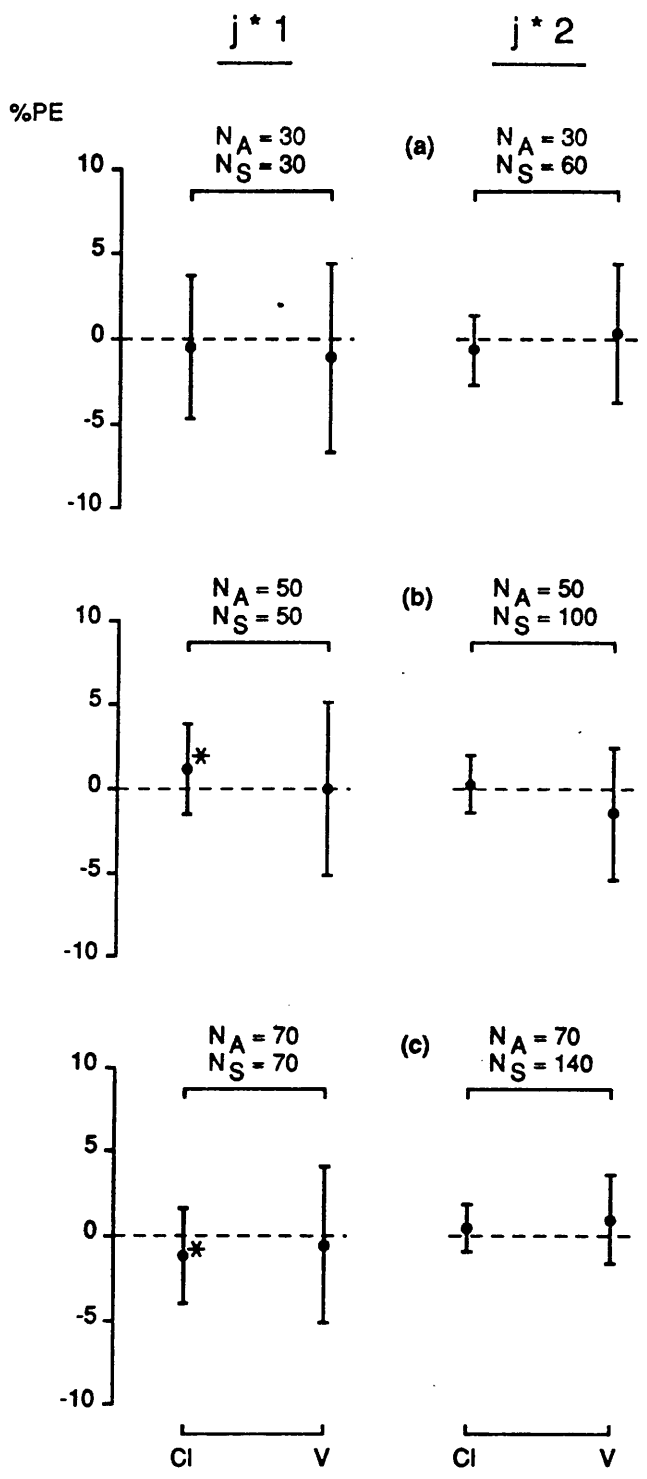


Fig. 8.9(a - c) Bias and precision expressed as %PE (mean ± standard deviation, respectively). The horizontal panels in each figure show results from different study designs. The first panel for each figure shows results with j * 1 designs which is used as a reference for comparing results obtained with the j * 2 designs (second panel, see methods). The j * 2 designs yielded total number of data points per data set twice that obtained with the j * 1 designs but with the total number of animals unchanged. N_A represents the total number of animals used for each study design and N_S, the sample size for each design. σ_ϵ was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

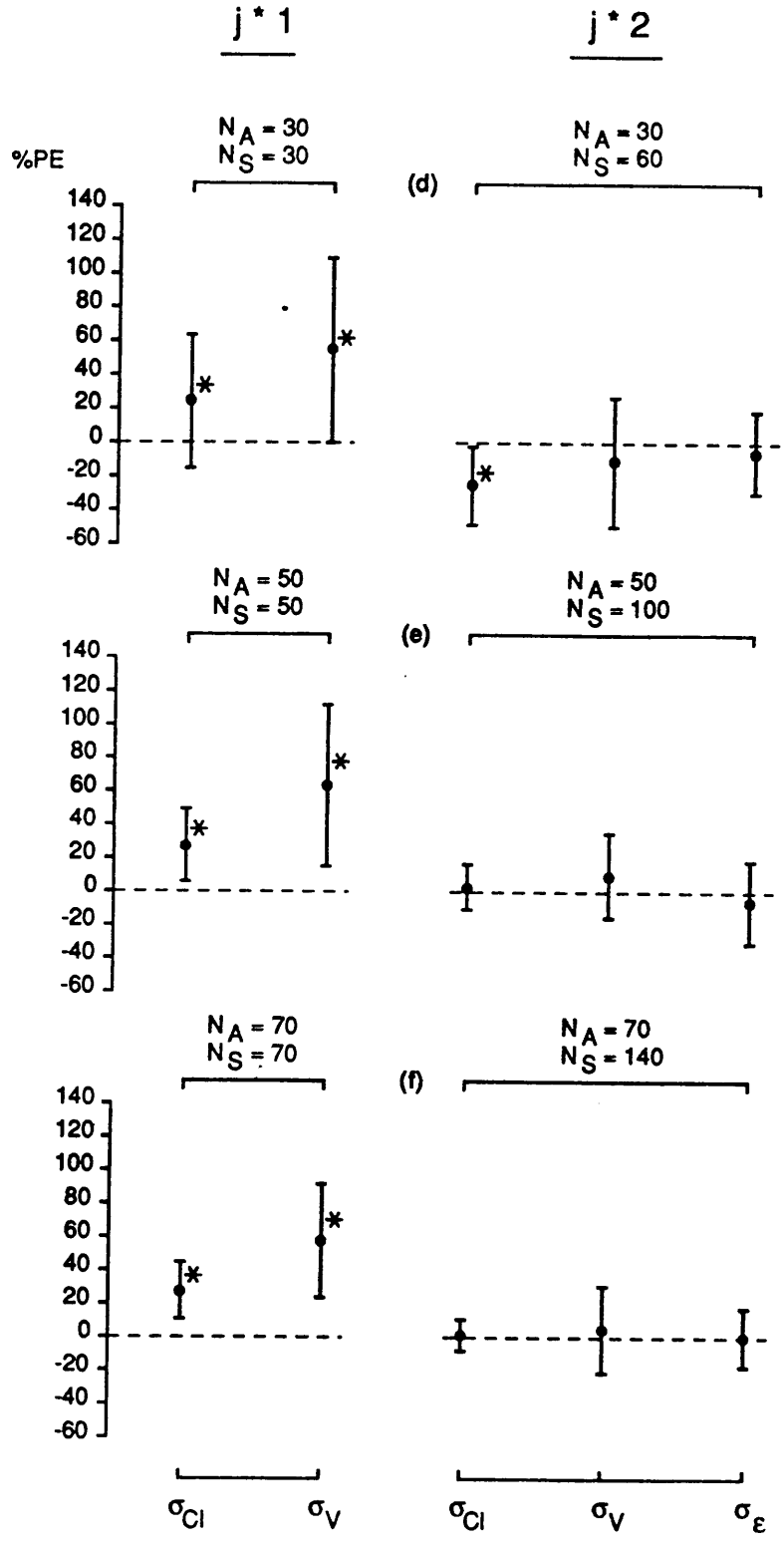


Fig. 8.9(d - f) Bias and precision expressed as %PE (mean ± standard deviation, respectively). The horizontal panels in each figure show results from different study designs. The first panel for each figure shows results with j * 1 designs which is used as a reference for comparing results obtained with the j * 2 designs (second panel, see methods). The j * 2 designs yielded total number of data points per data set twice that obtained with the j * 1 designs but with the total number of animals unchanged. N_A represents the total number of animals used for each study design and N_S, the sample size for each design. σ_ϵ was set at 15%. Significant ($p < 0.05$) biases are indicated by asterisks.

8.4.4 Doubling N_S without Changing N_A

8.4.4a Bias and Precision

Again, each animal supplied 2 concentration - time points but N_A equalled 30, 50, and 70 to keep the number of animals constant to allow comparison with the j * 1 designs. 16, 23, and 28 successful NONMEM runs were obtained with N_A of 30, 50, and 70, respectively, compared to 30 (N_A = 30), 30 (N_A = 50) and 30 (N_A = 70) for j * 1 designs. The results presented herein are based on the successful runs. As in the previous study the accuracy with which the fixed effect parameters were estimated was relatively unaffected, but the precision was improved as expected (Fig. 8.9(a - c). The bias in the estimates of σ_{Cl} and σ_V was almost completely eliminated and the precision greatly improved (Fig. 8.9(d - f)). However, acceptably precise estimates of σ_{Cl} and σ_V were only obtained with N_A = 50 and 70 (i.e. N_S = 100 and 140, respectively).

In all the j * 2 designs the estimates of σ_ϵ were minimally biased, but acceptably precise. The mean of %PE ranged from -0.2% (N_A = 70) to 6.0% (N_A = 50), and the SD of %PE from 17.7% (N_A = 70) to 24.6% (N_A = 50). Spurious values of σ_ϵ were responsible for the exclusion of most NONMEM runs.

8.4.4b Incidence of High Pair-wise Correlations

100% incidence of high correlation was obtained for the pair-wise correlation of σ_ϵ and Cl irrespective of N_A (Table 8.13). Except for the correlation between σ_ϵ and σ_V in which the incidence of high correlation ranged from 6.3% (N_A = 30) to 14.3% (N_A = 70), and the correlation between σ_V and V

where the incidence was 6.3% for N_A equal to 30, the incidence of all other pair-wise correlations was less than 5.0%.

8.5 DISCUSSION

The fixed effect parameters were well estimated irrespective of the inter-animal variability for most j * 1 designs. Inefficient estimates of Cl were obtained at the 60% level of inter-animal variability with the 2 animals per time point design. The accuracy of these estimates was relatively unaffected by increasing the number of animals sampled at each time. All inter-animal variability estimates were positively biased, and this highlights the difficulty when there is no information on one of the components of variability (in this case, $\sigma_{\in}$), emphasising the limitation of the one point per animal design. Estimates of variability associated with structural model parameters are considerably less precise, given a fixed number of experimental units, than are estimates of their means (Chapter 2, Section 2.5.3; Sheiner & Beal, 1981; Grasela *et al.*, 1986). Some significant biases, associated with parameter estimates obtained with designs having a greater number of animals compared to the ones with fewer animals at each time point, were due to sample sizes being large enough to detect bias.

Since estimates were considered acceptably precise when the SD of %PE < 25%, the minimum number of animals required for reasonable estimation of population pharmacokinetic parameters with the one observation per animal design was 10 per time point if the inter-animal variability was between 15 and

45%, and 15 when the inter-animal variability was 60%.

However, when the results were analysed using the design number approach in which the combined contributions of bias and precision are taken into account in determining the efficiency of parameter estimation, all parameters were estimated with similar efficiency when 6 to 15 animals were used per time point for all settings of σ_{Cl} and σ_V. Using this sampling strategy and the j * 1 design, studies could be performed with at least 6 animals per time with no loss in the efficiency with which population parameters are estimated. This would result in savings in terms of the number of animals used and the time spent on such studies.

When the inter-animal variability was between 15 and 30%, Cl and V were efficiently estimated when 4 to 15 animals were used at each time point. Thus, as few as 4 animals per time could be used for the estimation of the fixed effect parameters with these settings of inter-animal variability. σ_{Cl} and σ_V were inefficiently estimated with all j * 1 designs due to a lack of information about σ_ϵ.

When the inter-animal variability was 45% and the estimation of individual parameters were considered, Cl was well estimated using 3 to 15 animals per time point while V was efficiently estimated with designs having 6 to 15 animals per time point. As with previous levels of inter-animal variability considered, σ_{Cl} and σ_V were inefficiently estimated.

With σ_{Cl} (σ_V) being 60%, the efficiency of Cl estimation was similar for all designs since the contributions of the bias and standard error terms in Φ_{ir} counter balanced each other, such that a comparison of the designs revealed nonsignificant differences. However, V was better estimated with designs in which 6 to 15 animals were used per time point. Thus, efficient estimation of V would require more animals at each time point than Cl when the inter-animal variability is greater than 30%. Again, σ_{Cl} and σ_V were inefficiently estimated

irrespective of the j * 1 design. The inefficiency with which the variance parameters were estimated was due to lack of information in the data sets on σ_{ϵ} as previously discussed.

Thus, the use of 6 to 15 animals at each time point in the estimation of population pharmacokinetic parameters would result in the production of reasonable estimates when the inter-animal variability is between 15 and 60%. Inefficient estimates were obtained with the 2 animals per time point design for all levels of inter-animal variability due to poor precision as seen, in the confidence intervals coverage for the variance parameters and joint parameter estimates when catastrophic runs were excluded. Poor precision was also responsible for significantly reduced coverage for joint confidence intervals when 3 to 5 animals were measured at each time point with inter-animal variability set at 30 to 60%. The contribution of bias to the poor coverage (hence inefficient estimates) was minimal. However, bias was the major contributing factor to the reduced coverage obtained for σ_V and joint confidence intervals for parameter estimates when 15 animals were used at each time point with the inter-animal variability set at 15%. Poorer estimation of the variance parameters could be due to higher incidence of pair-wise correlation involving these parameters.

When σ_{ϵ} was varied to examine its effect on the estimation of σ_{Cl} and σ_V, the magnitude of the bias in σ_{Cl} and σ_V increased with the magnitude of σ_{ϵ}, as expected, indicating that a substantial fraction of this bias was due to an error, i.e., the intra-animal error, which could not be partitioned. This finding confirms earlier observation by Graves *et al.* (1989). Using Monte Carlo simulation techniques, these authors generated data sets with error in concentration measurements without introducing inter-subject variability, and concluded that error in concentration measurements contributes significantly to large standard deviations associated with structural model parameters which could be interpreted

as inter-individual variability in a real study situation.

Most NONMEM fixed effect parameter estimates derived from all studies with the j * 1 design showed a consistent significant negative bias. This was due to estimation error as negative biases in the estimation of these parameters were obtained even when σ_{ϵ} was set at 0%.

A trade - off between sample size and total number of animals (i.e., doubling the total number of observations (sampling an animal twice) while reducing the total number of animals sampled by half, produced a dramatic improvement in the estimation of inter-animal variability with a considerable reduction in bias. Accuracy was stable over the different population samples. The second sample practically eliminated bias and facilitated the partitioning of inter-animal variability and residual error, by introducing information about σ_{ϵ}. However, the estimates of σ_{ϵ} were unstable probably because of the correlation of σ_{ϵ} with Cl and σ_V. The correlation between σ_{ϵ} and σ_V was worse for N_A equal to 15 and 25.

Keeping N_A constant as in the j * 1 designs while doubling N_S (j * 2 designs) resulted in a significant improvement in the precision with which inter-animal variability was estimated. This had no effect on the accuracy and precision of fixed effect parameters. The estimates of σ_{ϵ} were more stable with significant high correlations occurring only between σ_{ϵ} and Cl.

Doubling of the number of observations per animal results in savings in terms of the number of animals that are needed in this type of study. The j * 2 design with N_A equal to 30 animals yielded acceptably precise estimates of inter-animal variability with no loss of efficiency. The use of this minimal number of animals with the j * 2 design and sampling strategy considered here would result in savings not only in animal number, but also in time and labour cost without sacrificing efficiency of parameter estimation.

The estimation of a set of population pharmacokinetic parameters

provides limited information if there is no measure of the variability of each of the parameter estimates. Given the design specifications considered here, accuracy and precision in the estimation of inter-animal variability is significantly improved when the data set is enhanced by taking 2 observations per animal. In recent years, experimental methods have become available which permit serial blood sampling in small laboratory animals (Migdalof, 1976) . These sampling methods combined with modern approaches to population data analysis should lead to much more informative pharmacokinetic studies in small animals.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSION

In pharmacokinetics, the rationale behind study design is the accurate and precise estimation of pharmacokinetic parameters. However, the overall objective of such studies is not only to learn about the average disposition of the drug in the study population, but also the interindividual variability. Indeed, the purpose of most animal pharmacokinetic studies is to estimate population parameters as a key step to quantifying individual animal response and population variability.

The traditional approaches (NPD and STS) to estimating population pharmacokinetic parameters in laboratory animals have been discussed in Chapters 1 and 2, and their limitations highlighted. The NPD approach provides no estimate of population variability, while the STS approach provides estimates of variability that are positively biased and requires a full concentration - time profile for each animal. On the other hand, NONMEM provides estimates of population parameters, their variances, and estimated standard errors of parameters (Sheiner & Beal, 1981; 1983). The efficiency of this approach is the focus of this thesis.

In studies involving the use of inbred strains of small laboratory animals (e.g., rats or mice), in which each concentration - time point usually represents one animal, the NPD approach is the most common method of analysis (Loscher & Esenwein, 1978; Roberts & Renwick, 1989; Pritchard, Holmes, & Kirschman, 1976). No estimate can be made of variability, although this may be up to 50% for some parameters (Lindstrom & Birkes, 1984; McArthur, 1988). Variability in the rate of oxidative metabolism of antipyrine by different inbred strains of rats has been reported, and Vocci and Farber (1988) advocated the consideration of variability within the same species in interspecies scaling. Thus, provision should be made for the estimation of variability inherent in the population sample in analysing data obtained by "destructive sampling". The NONMEM program was used in analysing data generated in the course of this thesis, and the majority of the data were simulated with the one observation per animal design.

Pharmacokinetic data analysis is informative only if the data themselves are informative, and this can best be assured by appropriate experimental design. In designing experiments for efficient parameter estimation the following factors are taken into account: experimental error, number of samples taken, and the spacing of samples (Suverkrup, 1982).

In this thesis simulated data sets were used to investigate the effect of the various design features on the efficiency of parameter estimation using the one observation per animal design. Several methods were used to determine the efficiency of parameter estimation. The 99% individual and joint confidence intervals containing the true parameter 95% of the time for all parameters were introduced as aids to judging the efficiency with which individual and all parameters as a whole were estimated. The confidence intervals tables were constructed to reveal the influence of bias and standard error on parameter estimation.

In addition, the design number, a new statistic which combines the contributions of bias and precision in judging the efficiency of parameter estimation, was introduced to complement bias and precision, and the confidence intervals methods of analysis. The design number also allowed the efficiency with which all parameters of a model were estimated as a set to be judged. The incidence of high pair-wise correlations of parameter estimates was also taken into account in assessing the acceptability of estimates and the adequacy of model parameterization. Data were simulated using population parameters of a drug having the characteristics of avicin, a cytotoxic drug (McGovern *et al.*, 1988), and assuming no covariance.

Using the one observation per animal design and assuming IV bolus injection with the monoexponential pharmacokinetic model, simulated data sets of different sample sizes (30 and 50) were employed to determine the influence of

inter-animal variability on parameter estimation (Chapter 3). The range of variability investigated was similar to that expected in real studies, and the traditional sampling strategy involving sampling animals at fixed times was used. Ten sampling times were specified between 5 and 240 min. using a simulated half-life (84 min.) of the drug (i.e., Cl and V of 1.3 ml/min. and 162.5 ml, respectively). It was observed that the fixed effect parameters (i.e., Cl and V) were precisely estimated at all combinations of inter-animal variability studied, but bias increased with increase in variability. These estimates were mostly negatively biased, and this was coupled with the overestimation of the variance parameters. The negative bias associated with the estimation of the fixed effect parameters was attributed to the nature of the NONMEM program (i.e. estimation error since negative bias was also observed in subsequent studies when σ_{ϵ} was set at 0%). The overestimation of the variance parameters was attributed to the one observation per animal design since there was no information in the data set about σ_{ϵ}.

The estimates of the fixed effect parameters were normally distributed while some of the variance parameters were nonnormal with right skewed distributions at large values of σ_{Cl} and σ_{V} (e.g., 60% * 60% combination). This right skewness was responsible for the good coverage of σ_{Cl} and σ_{V} when the influence of standard errors was not considered. When the influence of standard errors was considered, poor coverage was obtained at high variability irrespective of sample size.

In studying the effect on parameter estimation of the spacing of sampling times with a fixed sample size (Chapter 4) using the two sample point design (one compartment model with IV bolus injection), efficient parameter estimation was obtained when the second sample was located at $\geq$ 1.4 times the $t_{1/2}$ of the drug with the first sample obtained as early as possible (5 min.). When three or four samples were used, the exact location of the third or fourth sample was not

critical to efficient parameter estimation. The fixed effect parameters were efficiently estimated with all designs. Irrespective of the design considered (i.e., two, three, or four sample points design), σ_{Cl} was efficiently estimated when the second, third, or fourth sample were located at $\geq 1.4t_{1/2}$ of the drug. σ_V was poorly estimated with all sampling designs and this was attributable to lack of information about σ_ϵ in the simulated data sets. Metzler (1987) showed that NONMEM yielded poor estimates of volume, and although he did not estimate the variance parameters in his study, the poor estimates of σ_V found in this work may be a feature of the NONMEM program.

Using the two compartment model (assuming IV bolus injection and sampling animals at set times), the efficiency of parameter estimation was examined over a range of parameter values with the model parameterized in terms of A, α, B, and β (Chapter 5). A and α were efficiently estimated when α was between 1.5 and 4.0 h^{-1}, while B and β were efficiently estimated when α was in the range 6.0 to 8.0 h^{-1}. A and α, B and β were efficiently estimated at the higher (i.e. 20 to 40.0) and lower (i.e. 1.0 to 20.0) A:B ratios, respectively. The variance parameters were inefficiently estimated due to lack of information about σ_ϵ. The parameters, considered as a set, were efficiently estimated when α was in the range of 2.0 to 4.0 h^{-1}, and the A:B ratio in the range 2.5 to 30.0. These results were attributed to the distribution of the data points between the distribution and elimination phases of the plasma concentration - time curve.

Also, A and α were efficiently estimated when 3 to 12 and 3 to 7 times, respectively, were in the α phase. However, α was estimated with similar efficiency with designs having 3, 7, and 12 times in the α phase. Inefficient estimates of B, and β were obtained when 12 times were in the α phase because there were fewer samples in the β phase. Overall, designs with 5 to 7 times in the α phase yielded efficient parameter estimates. However, the variance parameters

were poorly estimated as a consequence of the one observation per animal design. The design with 12 times in the α phase had greater incidences of high pair-wise correlations than other designs and, overall, yielded the least efficient estimates of parameters.

When the number of sampling times in the β phase was increased and the duration of sampling extended without altering the total number of samples, parameters were estimated with equal efficiency when the overall performance of the different sampling schedules (3, 6, and 8 times in the β phase) was examined. There was no loss in efficiency when the duration of sampling was reduced from 10 h (8 times in β phase) to 6 h (3 times in the β phase). The schedule with 8 times in the β phase was only significantly better than others in the estimation B and β.

However, most parameter estimates of the A, α, B, and β parameterization of the two compartment model were unstable due to greater incidences of high pair-wise correlations. Reparameterization of the model (Chapter 6) in terms of Cl, V, k_{12}, and k_{21} resulted in more stable parameter estimates.

Observations made in a pharmacokinetic study are subject to two types of variability - biological variation (considered earlier) and errors in the analysis of samples. The influence of the latter on parameter estimation was also studied. Using both the one and two compartment open models with IV bolus injection, it was observed how the error in concentration measurements was added to the estimated inter-animal variability due to lack of knowledge about intra-animal variability. Thus, large inter-animal variability estimated in real studies involving the one observation per animal design could be misleading since it is actually a composite of inter- and intra-animal variability. Minimising experimental error is critical to efficient parameter estimation.

The 1986 Act on the protection of animals stipulates that use of animals for experimentation must be kept to the barest minimum, and, where possible,

alternatives should be found. In the preclinical pharmacokinetic setting, the use of animals for accurate and precise estimation of population pharmacokinetic parameters is inescapable. However, a balance must be struck between minimal use of animals and efficient parameter estimation since the parameters so determined are used for extrapolation from one species to another, and more importantly man. Thus, the effect of sample size on parameter estimation was investigated with both one and two compartment open models. It was found that with the design specifications considered, the parameters of the one compartment model were estimated with equal efficiency when 6 to 15 animals were sampled destructively at each of ten time points (Chapter 8). Since there was no loss in efficiency when 6 animals are sampled per time (i.e., a sample size of 60), the costs involved in such studies could be greatly reduced. However, with serial micro-sampling of small laboratory animals, similar results could be obtained with at least 30 animals sampled twice with the same traditional sampling strategy. Sampling an animal at least twice allows the partitioning of inter- and intra-animal variability, almost eliminating bias in the estimation of the variance parameters. Using the two compartment model, 15 animals were required at each of ten time points for efficient parameter estimation. However, the loss in estimation efficiency with 10 animals sampled at each time point for ten time points was not dramatic with this model. The use of these numbers of animals with the design specifications considered in this thesis would strike a good balance between cost and good science.

In all studies reported in this thesis, most estimates of fixed effect parameters were associated with negative bias. This was due to estimation error since negative bias in these parameters was also observed in studies in which σ_{ϵ} was set to 0%.

The design number was applied throughout the course of the thesis with

the assumption that it was efficient although the determination of the power and efficiency of this statistic was outside the scope of this thesis. The rescaling of design numbers could, perhaps, be better done using the median, and this needs to be explored further.

The results of the simulation studies led to the application of NONMEM in the analysis of data obtained with the one observation per animal design for a drug under development (Chapter 7). The NONMEM analysis took into account that the samples came from a population with more variability than could be explained with experimental error. Parameter estimation without estimates of variability is of little value. NONMEM permitted the explanation of inter-animal variability in V_1 in terms of sex: efficient partitioning between inter- and intra-animal variability would have required an increase in the number of samples per animal.

Thus, the influence of various design features on the efficiency of parameter estimation using the one observation per animal design has been investigated. Inefficient estimates of inter-animal variability are obtained with this design, but sampling an animal at least twice significantly improved the efficiency of parameter estimation. The fixed effect parameters, on the other hand, were efficiently estimated. The design number, individual and joint confidence intervals for parameter estimates, incidence of high pair-wise correlations in addition to bias and precision were found useful in judging the efficiency with which parameters were estimated individually or as a set.

APPENDIX

APPENDIX IA

PRED Subroutine for the 2 Compartment Model

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C
C     2 COMP IV, 1ST DOSE, A, AL, B, BE
C
C
C
      DIMENSION THETA(4),DATREC(3),H(1),G(4),INDXS(1)
      DOUBLE PRECISION THETA,F,G,H,T,EKT,XKE,EXPWCH,
     + A,AL,B,BE,EALT,EBET
      T=DATREC(2)
      A=THETA(1)
      AL=THETA(2)
      B=THETA(3)
      BE=THETA(4)
      EALT=EXPWCH(-AL*T)
      EBET=EXPWCH(-BE*T)
      F=(A*EALT)+(B*EBET)
      G(1)=EALT
      G(2)=-A*T*EALT
      G(3)=EBET
C     G(4)=-B*T*EBET
C     H(1)=F
      RETURN
      END
      DOUBLE PRECISION FUNCTION EXPWCH(XX)
      DOUBLE PRECISION XX
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=DEXP(XX)
      RETURN
      END
```

Note that G(4) was included in the subroutine when the effect of the number of sampling times in the β phase on parameter estimation was investigated.

APPENDIX IB

PRED Subroutine for the Reparameterized 2 Compartment Model

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C  2COMP, IV, CL, V1, K12, K21
C
      DIMENSION THETA(4),DATREC(3),INDXS(1),G(4),H(1)
      DOUBLE PRECISION THETA,G,H,F,CL,V1,K12,K21,
     +DOSE,T,T21,Q,C1,R,BE,AL,P,PP,C2,C3,C4,C5,C6,
     +C7,C8,A,B,DBE2,DBE1,DBE3,DBE4,DP1,DP2,DP3,DP4,
     +DAL1,DAL2,DAL3,DAL4,DA1,DA2,DA3,DA4,DB1,DB2,DB3,
     +DB4,EA,EB,EALT,EBET,AT,BT
      CL=THETA(1)
      V1=THETA(2)
      K12=THETA(3)
      K21=THETA(4)
      T=DATREC(2)
      DOSE=200000.
      T21=CL/V1
      Q=K12+K21+T21
      C1=T21*K21
      R=DSQRT(Q*Q-4.*C1)
      BE=0.5*(Q-R)
      AL=C1/BE
      P=1./(CL*K21-V1*BE**2)
      PP=P*P
      C2=(K21-BE)*BE
      C3=2.0*V1*BE
      C4=AL/BE
      C5=(C1-K21*BE)*DOSE
      C6=K21*P*DOSE
      C7=C2*DOSE
      C8=(K21-2.*BE)*P*DOSE
      A=DOSE*(C1-BE*K21)*P
      B=DOSE*C2*P
      DBE2=0.5*(1.0-(Q-2.*K21)/R)/V1
      DBE1=-T21*DBE2
      DBE3=-BE/R
      DBE4=DBE3-0.5*T21/R
      DP1=PP*(BE**2-C3*DBE1)
      DP2=PP*(C3*DBE2-K21)
      DP3=PP*C3*DBE3
      DP4=PP*(C3*DBE4-CL)
      DAL1=-C1*BE/V1-C1*DBE1
      DAL2=AL/CL-C4*DBE2
      DAL3=-C4*DBE3
      DAL4=AL/K21-C4*DBE4
```

```
      DA1=C5*DP1-C1*P*DOSE/V1-C6*DBE1
      DA2=C5*DP2+C6/V1-C6*DBE2
      DA3=C5*DP3-C6*DBE3
      DA4=C5*DP4-C6*DBE4+P*(T21-BE)*DOSE
      DB1=C7*DP1+C8*DBE1
      DB2=C7*DP2+C8*DBE2
      DB3=C7*DP3+C8*DBE3
      DB4=C7*DP4+C8*DBE4+DOSE*P*BE
      EA=AL*T
      EB=BE*T
      IF(EA.GE.50.) EA=50.
      IF(EB.GE.50.) EB=50.
      IF(EA.LE.-50.) EA=-50.
      IF(EB.LE.-50.) EB=-50.
      EALT=EXP(-EA)
      EBET=EXP(-EB)
      AT=A*T
      BT=B*T
      F=A*EALT+B*EBET
      G(1)=EALT*(DA1-AT*DAL1)+EBET*(DB1-BT*DBE1)
      G(2)=EALT*(DA2-AT*DAL2)+EBET*(DB2-BT*DBE2)
C     G(3)=EALT*(DA3-AT*DAL3)+EBET*(DB3-BT*DBE3)
C     G(4)=EALT*(DA4-AT*DAL4)+EBET*(DB4-BT*DBE4)
C     H(1)=F
      RETURN
      END
```

APPENDIX IC

2 Compartment Model: PRED Subroutine for Modelling the Influence of Weight on V_1

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C  2COMP. IV, CL,V1,K12,K21
C
      DIMENSION THETA(4),DATREC(4),INDXS(1),G(4),H(1)
      DOUBLE PRECISION THETA,G,H,F,CL,V1,K12,K21,WT,
     +DOSE,T,T21,Q,C1,R,BE,AL,P,PP,C2,C3,C4,C5,C6,
     +C7,C8,A,B,DBE2,DBE1,DBE3,DBE4,DP1,DP2,DP3,DP4,
     +DAL1,DAL2,DAL3,DAL4,DA1,DA2,DA3,DA4,DB1,DB2,DB3,
     +DB4,EA,EB,EALT,EBET,AT,BT
      CL=THETA(1)
      K12=THETA(3)
      K21=THETA(4)
      T=DATREC(2)
      WT=DATREC(4)
      DOSE=WT*1000.
      V1=WT*THETA(2)
      T21=CL/V1
      Q=K12+K21+T21
      C1=T21*K21
      R=DSQRT(Q*Q-4.*C1)
      BE=0.5*(Q-R)
      AL=C1/BE
      P=1./(CL*K21-V1*BE**2)
      PP=P*P
      C2=(K21-BE)*BE
      C3=2.0*V1*BE
      C4=AL/BE
      C5=(C1-K21*BE)*DOSE
      C6=K21*P*DOSE
      C7=C2*DOSE
      C8=(K21-2.*BE)*P*DOSE
      A=DOSE*(C1-BE*K21)*P
      B=DOSE*C2*P
      DBE2=0.5*(1.0-(Q-2.*K21)/R)/V1
      DBE1=-T21*DBE2
      DBE3=-BE/R
      DBE4=DBE3-0.5*T21/R
      DP1=PP*(BE**2-C3*DBE1)
      DP2=PP*(C3*DBE2-K21)
      DP3=PP*C3*DBE3
      DP4=PP*(C3*DBE4-CL)
```

```
      DAL1=-C1*BE/V1-C1*DBE1
      DAL2=AL/CL-C4*DBE2
      DAL3=-C4*DBE3
      DAL4=AL/K21-C4*DBE4
      DA1=C5*DP1-C1*P*DOSE/V1-C6*DBE1
      DA2=C5*DP2+C6/V1-C6*DBE2
      DA3=C5*DP3-C6*DBE3
      DA4=C5*DP4-C6*DBE4+P*(T21-BE)*DOSE
      DB1=C7*DP1+C8*DBE1
      DB2=C7*DP2+C8*DBE2
      DB3=C7*DP3+C8*DBE3
      DB4=C7*DP4+C8*DBE4+DOSE*P*BE
      EA=AL*T
      EB=BE*T
      IF(EA.GE.50.) EA=50.
      IF(EB.GE.50.) EB=50.
      IF(EA.LE.-50.) EA=-50.
      IF(EB.LE.-50.) EB=-50.
      EALT=EXP(-EA)
      EBET=EXP(-EB)
      AT=A*T
      BT=B*T
      F=A*EALT+B*EBET
      G(1)=EALT*(DA1-AT*DAL1)+EBET*(DB1-BT*DBE1)
      G(2)=EALT*(DA2-AT*DAL2)+EBET*(DB2-BT*DBE2)
C     G(3)=EALT*(DA3-AT*DAL3)+EBET*(DB3-BT*DBE3)
C     G(4)=EALT*(DA4-AT*DAL4)+EBET*(DB4-BT*DBE4)
C     H(1)=F
      RETURN
      END
```

APPENDIX ID

2 Compartment Model: PRED Subroutine for Modelling the Influence of Sex and Weight on V_1

```
      SUBROUTINE PRED(ICALL,NEWIND,THETA,DATREC,INDXS,F,G,H)
C
C     2COMP. IV, CL,V1,K12,K21
C
      DIMENSION THETA(5),DATREC(5),INDXS(1),G(2),H(1)
      DOUBLE PRECISION THETA,G,H,F,CL,V1,K12,K21,WT,
     +DOSE,T,T21,Q,C1,R,BE,AL,P,PP,C2,C3,C4,C5,C6,
     +C7,C8,A,B,DBE2,DBE1,DBE3,DBE4,DP1,DP2,DP3,DP4,
     +DAL1,DAL2,DAL3,DAL4,DA1,DA2,DA3,DA4,DB1,DB2,DB3,
     +DB4,EA,EB,EALT,EBET,AT,BT,SEX
      CL=THETA(1)
      V1=THETA(2)
      K12=THETA(3)
      K21=THETA(4)
      T=DATREC(2)
      WT=DATREC(4)
      SEX=DATREC(5)
      DOSE=WT*1000.
      V1=WT*THETA(2)
      IF(SEX.GT.1.0)V1=V1*THETA(5)
      T21=CL/V1
      Q=K12+K21+T21
      C1=T21*K21
      R=DSQRT(Q*Q-4.*C1)
      BE=0.5*(Q-R)
      AL=C1/BE
      P=1./(CL*K21-V1*BE**2)
      PP=P*P
      C2=(K21-BE)*BE
      C3=2.0*V1*BE
      C4=AL/BE
      C5=(C1-K21*BE)*DOSE
      C6=K21*P*DOSE
      C7=C2*DOSE
      C8=(K21-2.*BE)*P*DOSE
      A=DOSE*(C1-BE*K21)*P
      B=DOSE*C2*P
      DBE2=0.5*(1.0-(Q-2.*K21)/R)/V1
      DBE1=-T21*DBE2
      DBE3=-BE/R
      DBE4=DBE3-0.5*T21/R
      DP1=PP*(BE**2-C3*DBE1)
      DP2=PP*(C3*DBE2-K21)
```

```
      DP3=PP*C3*DBE3
      DP4=PP*(C3*DBE4-CL)
      DAL1=-C1*BE/V1-C1*DBE1
      DAL2=AL/CL-C4*DBE2
      DAL3=-C4*DBE3
      DAL4=AL/K21-C4*DBE4
      DA1=C5*DP1-C1*P*DOSE/V1-C6*DBE1
      DA2=C5*DP2+C6/V1-C6*DBE2
      DA3=C5*DP3-C6*DBE3
      DA4=C5*DP4-C6*DBE4+P*(T21-BE)*DOSE
      DB1=C7*DP1+C8*DBE1
      DB2=C7*DP2+C8*DBE2
      DB3=C7*DP3+C8*DBE3
      DB4=C7*DP4+C8*DBE4+DOSE*P*BE
      EA=AL*T
      EB=BE*T
      IF(EA.GE.50.) EA=50.
      IF(EB.GE.50.) EB=50.
      IF(EA.LE.-50.) EA=-50.
      IF(EB.LE.-50.) EB=-50.
      EALT=EXP(-EA)
      EBET=EXP(-EB)
      AT=A*T
      BT=B*T
      F=A*EALT+B*EBET
      G(1)=EALT*(DA1-AT*DAL1)+EBET*(DB1-BT*DBE1)
      G(2)=EALT*(DA2-AT*DAL2)+EBET*(DB2-BT*DBE2)
C     G(3)=EALT*(DA3-AT*DAL3)+EBET*(DB3-BT*DBE3)
C     G(4)=EALT*(DA4-AT*DAL4)+EBET*(DB4-BT*DBE4)
C     H(1)=F
      RETURN
      END
```

APPENDIX IIA

Simulation Program Used to Investigate the Effect of Sample Size and Inter-animal Variability on Parameter Estimation with the 1 Compartment Model

```
C     1ST TWO TIMES FIXED, REST RANDOMLY WITHIN 30 MINS
C     SAMPLING AT SET TIMES
C
      PROGRAM DATA2
C
C     CHAN 20 - INITIAL STARTING VALUES
C     CHAN 30 - NONMEM DATA FILE
C     CHAN 40 - MINITAB DATA FILE
C
      INTEGER RAN,GEN,BASE
      COMMON /MIRNG/RAN(10),GEN(10),NWRD,BASE,MMOD,FBASE,FMOD
      DIMENSION T(20)
C
C     CLBAR = POPN CL          VBAR = POPN VD
C     SDCL  = POPN SD CL       SDV  = POPN SD VD
C     SDC   = PROP ERROR IN CONC DOSE = DOSE
C     NT    = NO OF TIMES      NR = NO OF RATS/TIME
C     NSTART = RANDOM SEEDS
C
      READ(20,*) NSTART
      RMAX=2.0**15-1.0
      MAXINT=RMAX
      CALL RANSET(MAXINT,NSTART)
      READ(20,*) CLBAR,SDCL,VBAR,SDV,SDC,DOSE,NT,NR
      READ(20,*) (T(I),I=1,NT)
      DO 500 I=1,NT
      DO 100 J=1,NR
    5 CALL NGAUSS (CL,RN,CLBAR,SDCL)
      IF (CL.LE.0.0) GOTO  5
   10 CALL NGAUSS (V,RN,VBAR,SDV)
      IF (V.LE.0.0)  GOTO 10
      KE=CL/V
      DV=DOSE/V
      CALL URAND (T1)
      IF(I.EQ.1.OR.I.EQ.2) GO TO 30
      RE=15
      RM=-7.5
      GO TO 35
   30 RE=0
      RM=0
   35 T1= T1*RE + T(I) + RM
      EKET=EXPWCH(-KE*T1)
      C1=DV*EKET
```

```
      SDC1=SDC*C1
      CALL NGAUSS (CC,RN,C1,SDC1)
      C1=CC
      IF(C1.LT.0.1.OR.C1.GT.50.0) GO TO 5
      NN= ((I-1)*NR) +J
      RI=FLOAT (NN)
      WRITE(30,20) RI,T1,C1
      WRITE(40,25) RI,CL,V,T1,C1
  100 CONTINUE
  500 CONTINUE
   20 FORMAT (3F8.2)
   25 FORMAT (5F8.2)
      STOP
      END
      FUNCTION EXPWCH(XX)
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=EXP(XX)
      RETURN
      END
      SUBROUTINE RANSET(MAXINT,NSTRT)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      MAXI=MAXINT/4
      IB=0
      NBASE=1
   99 IF(NBASE.GT.MAXI)GO TO 100
      NBASE=NBASE*4
      IB=IB+1
      GO TO 99
  100 NBASE=2**IB
      FNBASE=NBASE
      NWRD=47/IB+1
      NREM=47-IB*(NWRD-1)
      MMOD=2**NREM
      FMOD=MMOD
      DO 101 N=1,10
      NRAN(N)=0
  101 NGEN(N)=0
      NGEN(1)=5
      DO 200 I=1,14
      NCARRY=0
      DO 190 N=1,NWRD
      NGEN(N)=NGEN(N)*5+NCARRY
      NCARRY=0
      IF(NGEN(N).LT.NBASE)GO TO 190
      NCARRY=NGEN(N)/NBASE
      NGEN(N)=NGEN(N)-NBASE*NCARRY
  190 CONTINUE
  200 CONTINUE
      NSTART=NSTRT
      IF(NSTART.LE.0)NSTART=2001
      NSTART=2*(NSTART/2)+1
      DO 300 N=1,NWRD
      NTEMP=NSTART/NBASE
      NRAN(N)=NSTART-NTEMP*NBASE
```

```
300 NSTART=NTEMP
    RETURN
    END
    SUBROUTINE URAND(FRAN)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    DIMENSION NSUM(10)
    DO 30 IS=1,NWRD
 30 NSUM(IS)=0
    DO 1 IG=1,NWRD
    N2=NWRD-IG+1
    DO 1 IR=1,N2
    IS=IR+IG-1
    NPROD=NRAN(IR)*NGEN(IG)
    NHPROD=NPROD/NBASE
    LPROD=NPROD-NHPROD*NBASE
    NSUM(IS)=NSUM(IS)+LPROD
    IF(IS.LT.NWRD)NSUM(IS+1)=NSUM(IS+1)+NHPROD
  1 CONTINUE
    N2=NWRD-1
    DO 5 IS=1,N2
    NCARRY=NSUM(IS)/NBASE
    NSUM(IS)=NSUM(IS)-NCARRY*NBASE
    NSUM(IS+1)=NSUM(IS+1)+NCARRY
  5 CONTINUE
    NSUM(NWRD)=NSUM(NWRD)-MMOD*(NSUM(NWRD)/MMOD)
    DO 20 IS=1,NWRD
 20 NRAN(IS)=NSUM(IS)
    FRAN=NSUM(1)
    DO 10 IS=2,NWRD
 10 FRAN=FRAN/FNBASE+NSUM(IS)
    FRAN=FRAN/FMOD
    RETURN
    END
    SUBROUTINE NGAUSS(X,RN,AM,SD)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    IF(SD.NE.0.0)GO TO 30
    X=AM
    RETURN
 30 SUM=0.0
    DO 20 I=1,100
    CALL URAND(R)
 20 SUM=SUM+R
    RN=(SUM-50.)/SQRT(25./3.)
    X=RN*SD+AM
    RETURN
    END
```

APPENDIX IIB

Simulation Program for Investigating the Effect of Arrangement of Concentrations in Time

```
C
C      ONLY TIME 1 IS FIXED
C
       PROGRAM DATA1
C
C      CHAN 20 - INITIAL STARTING VALUES
C      CHAN 30 - NONMEM DATA FILE
C      CHAN 40 - MINITAB DATA FILE
C
       INTEGER RAN,GEN,BASE
       COMMON /MIRNG/RAN(10),GEN(10),NWRD,BASE,MMOD,FBASE,FMOD
       DIMENSION T(20)
C
C      CLBAR = POPN CL            VBAR = POPN VD
C      SDCL  = POPN SD CL         SDV  = POPN SD VD
C      SDC   = PROP ERROR IN CONC DOSE = DOSE
C      NT    = NO OF TIMES        NR = NO OF RATS/TIME
C      NSTART = RANDOM SEEDS
C
       READ(20,*) NSTART
       RMAX=2.0**15-1.0
       MAXINT=RMAX
       CALL RANSET(MAXINT,NSTART)
       READ(20,*) CLBAR,SDCL,VBAR,SDV,SDC,DOSE,NT,NR
       READ(20,*) (T(I),I=1,NT)
       DO 500 I=1,NT
       DO 100 J=1,NR
     5 CALL NGAUSS (CL,RN,CLBAR,SDCL)
       IF (CL.LE.0.0) GOTO  5
    10 CALL NGAUSS (V,RN,VBAR,SDV)
       IF (V.LE.0.0)  GOTO 10
       KE=CL/V
       DV=DOSE/V
       CALL URAND (T1)
       IF(I.EQ.1) GO TO 30
       RE=15
       RM=-7.5
       GO TO 35
    30 RE=0
       RM=0
    35 T1= T1*RE + T(I) + RM
       EKET=EXPWCH(-KE*T1)
       C1=DV*EKET
       SDC1=SDC*C1
```

```
      CALL NGAUSS (CC,RN,C1,SDC1)
      C1=CC
      IF(C1.LT.0.1.OR.C1.GT.50.0) GOTO 5
      NN= ((I-1)*NR) +J
      RI=FLOAT (NN)
      WRITE(30,20) RI,T1,C1
      WRITE(40,25) RI,CL,V,T1,C1
  100 CONTINUE
  500 CONTINUE
   20 FORMAT (3F8.2)
   25 FORMAT (5F8.2)
      STOP
      END
      FUNCTION EXPWCH(XX)
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=EXP(XX)
      RETURN
      END
      SUBROUTINE RANSET(MAXINT,NSTRT)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      MAXI=MAXINT/4
      IB=0
      NBASE=1
   99 IF(NBASE.GT.MAXI)GO TO 100
      NBASE=NBASE*4
      IB=IB+1
      GO TO 99
  100 NBASE=2**IB
      FNBASE=NBASE
      NWRD=47/IB+1
      NREM=47-IB*(NWRD-1)
      MMOD=2**NREM
      FMOD=MMOD
      DO 101 N=1,10
      NRAN(N)=0
  101 NGEN(N)=0
      NGEN(1)=5
      DO 200 I=1,14
      NCARRY=0
      DO 190 N=1,NWRD
      NGEN(N)=NGEN(N)*5+NCARRY
      NCARRY=0
      IF(NGEN(N).LT.NBASE)GO TO 190
      NCARRY=NGEN(N)/NBASE
      NGEN(N)=NGEN(N)-NBASE*NCARRY
  190 CONTINUE
  200 CONTINUE
      NSTART=NSTRT
      IF(NSTART.LE.0)NSTART=2001
      NSTART=2*(NSTART/2)+1
      DO 300 N=1,NWRD
      NTEMP=NSTART/NBASE
      NRAN(N)=NSTART-NTEMP*NBASE
  300 NSTART=NTEMP
```

```
      RETURN
      END
      SUBROUTINE URAND(FRAN)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      DIMENSION NSUM(10)
      DO 30 IS=1,NWRD
30    NSUM(IS)=0
      DO 1 IG=1,NWRD
      N2=NWRD-IG+1
      DO 1 IR=1,N2
      IS=IR+IG-1
      NPROD=NRAN(IR)*NGEN(IG)
      NHPROD=NPROD/NBASE
      LPROD=NPROD-NHPROD*NBASE
      NSUM(IS)=NSUM(IS)+LPROD
      IF(IS.LT.NWRD)NSUM(IS+1)=NSUM(IS+1)+NHPROD
 1    CONTINUE
      N2=NWRD-1
      DO 5 IS=1,N2
      NCARRY=NSUM(IS)/NBASE
      NSUM(IS)=NSUM(IS)-NCARRY*NBASE
      NSUM(IS+1)=NSUM(IS+1)+NCARRY
 5    CONTINUE
      NSUM(NWRD)=NSUM(NWRD)-MMOD*(NSUM(NWRD)/MMOD)
      DO 20 IS=1,NWRD
20    NRAN(IS)=NSUM(IS)
      FRAN=NSUM(1)
      DO 10 IS=2,NWRD
10    FRAN=FRAN/FNBASE+NSUM(IS)
      FRAN=FRAN/FMOD
      RETURN
      END
      SUBROUTINE NGAUSS(X,RN,AM,SD)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      IF(SD.NE.0.0)GO TO 30
      X=AM
      RETURN
30    SUM=0.0
      DO 20 I=1,100
      CALL URAND(R)
20    SUM=SUM+R
      RN=(SUM-50.)/SQRT(25./3.)
      X=RN*SD+AM
      RETURN
      END
```

APPENDIX IIC

Simulation Program Used to Investigate the Effect of Sampling An Animal Twice on Parameter Estimation

```
C     THIS ASSUMES 10 TIMES
C     1ST TWO FIXED, REST RANDOMLY WITHIN 30MINS
C
C     THE VALUE PREVIOUSLY THE NO OF ANIMALS/TIME
C     IS NOW THE NO OF ANIMALS/BLOCK
C     EACH BLOCK HAS CONC TAKEN AT A DIFFERENT
C     PAIR OF TIMES
C     I.E. 1 & 6, 2 & 7, 3 & 8, 4 & 9, 5 & 10
C
C
      PROGRAM DATA3
C
C     CHAN 20 - INITIAL STARTING VALUES
C     CHAN 30 - NONMEM DATA FILE
C     CHAN 40 - MINITAB DATA FILE
C
      INTEGER RAN,GEN,BASE
      COMMON /MIRNG/RAN(10),GEN(10),NWRD,BASE,MMOD,FBASE,FMOD
      DIMENSION T(20)
C
C     CLBAR = POPN CL          VBAR = POPN VD
C     SDCL  = POPN SD CL       SDV  = POPN SD VD
C     SDC   = PROP ERROR IN CONC DOSE = DOSE
C     NT    = NO OF TIMES      NR = NO OF RATS/TIME
C     NSTART = RANDOM SEEDS
C
      READ(20,*) NSTART
      RMAX=2.0**15-1.0
      MAXINT=RMAX
      CALL RANSET(MAXINT,NSTART)
      READ(20,*) CLBAR,SDCL,VBAR,SDV,SDC,DOSE,NT,NR
      READ(20,*) (T(I),I=1,NT)
      NBL=NT/2
      DO 500 I=1,NBL
      DO 100 J=1,NR
    5 CALL NGAUSS (CL,RN,CLBAR,SDCL)
      IF (CL.LE.0.0) GOTO  5
   10 CALL NGAUSS (V,RN,VBAR,SDV)
      IF (V.LE.0.0)  GOTO 10
      KE=CL/V
      DV=DOSE/V
      CALL URAND (T1)
      IF(I.EQ.1.OR.I.EQ.2) GO TO 30
      RE=15
      RM=-7.5
```

```
    GO TO 35
 30 RE=0
    RM=0
 35 T1= T1*RE + T(I) + RM
    EKET=EXPWCH(-KE*T1)
    C1=DV*EKET
    SDC1=SDC*C1
    CALL NGAUSS (CC,RN,C1,SDC1)
    C1=CC
    IF(C1.LT.0.1.OR.C1.GT.50.0) GO TO 5
    II=I+5
    CALL URAND (T2)
    IF(II.EQ.1.OR.II.EQ.2) GO TO 300
    RE=15
    RM=-7.5
    GO TO 305
300 RE=0
    RM=0
305 T2= T2*RE + T(II) + RM
    EKET=EXPWCH(-KE*T2)
    C2=DV*EKET
    SDC2=SDC*C2
    CALL NGAUSS (CC,RN,C2,SDC2)
    C2=CC
    IF(C2.LT.0.1.OR.C2.GT.50.0) GO TO 5
    NN=((I-1)*NBL)+J
    RI=FLOAT (NN)
    WRITE(30,20) RI,T1,C1,RI,T2,C2
    WRITE(40,25) RI,CL,V,T1,C1,T2,C2
100 CONTINUE
500 CONTINUE
 20 FORMAT (3F8.2,/,3F8.2)
 25 FORMAT (7F8.2)
    STOP
    END
    FUNCTION EXPWCH(XX)
    IF(XX.LE.-50.) XX=-50.
    IF(XX.GE.50.) XX=50.
    EXPWCH=EXP(XX)
    RETURN
    END
    SUBROUTINE RANSET(MAXINT,NSTRT)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    MAXI=MAXINT/4
    IB=0
    NBASE=1
 99 IF(NBASE.GT.MAXI)GO TO 100
    NBASE=NBASE*4
    IB=IB+1
    GO TO 99
100 NBASE=2**IB
    FNBASE=NBASE
    NWRD=47/IB+1
    NREM=47-IB*(NWRD-1)
    MMOD=2**NREM
```

```
      FMOD=MMOD
      DO 101 N=1,10
      NRAN(N)=0
  101 NGEN(N)=0
      NGEN(1)=5
      DO 200 I=1,14
      NCARRY=0
      DO 190 N=1,NWRD
      NGEN(N)=NGEN(N)*5+NCARRY
      NCARRY=0
      IF(NGEN(N).LT.NBASE)GO TO 190
      NCARRY=NGEN(N)/NBASE
      NGEN(N)=NGEN(N)-NBASE*NCARRY
  190 CONTINUE
  200 CONTINUE
      NSTART=NSTRT
      IF(NSTART.LE.0)NSTART=2001
      NSTART=2*(NSTART/2)+1
      DO 300 N=1,NWRD
      NTEMP=NSTART/NBASE
      NRAN(N)=NSTART-NTEMP*NBASE
  300 NSTART=NTEMP
      RETURN
      END
      SUBROUTINE URAND(FRAN)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      DIMENSION NSUM(10)
      DO 30 IS=1,NWRD
   30 NSUM(IS)=0
      DO 1 IG=1,NWRD
      N2=NWRD-IG+1
      DO 1 IR=1,N2
      IS=IR+IG-1
      NPROD=NRAN(IR)*NGEN(IG)
      NHPROD=NPROD/NBASE
      LPROD=NPROD-NHPROD*NBASE
      NSUM(IS)=NSUM(IS)+LPROD
      IF(IS.LT.NWRD)NSUM(IS+1)=NSUM(IS+1)+NHPROD
    1 CONTINUE
      N2=NWRD-1
      DO 5 IS=1,N2
      NCARRY=NSUM(IS)/NBASE
      NSUM(IS)=NSUM(IS)-NCARRY*NBASE
      NSUM(IS+1)=NSUM(IS+1)+NCARRY
    5 CONTINUE
      NSUM(NWRD)=NSUM(NWRD)-MMOD*(NSUM(NWRD)/MMOD)
      DO 20 IS=1,NWRD
   20 NRAN(IS)=NSUM(IS)
      FRAN=NSUM(1)
      DO 10 IS=2,NWRD
   10 FRAN=FRAN/FNBASE+NSUM(IS)
      FRAN=FRAN/FMOD
      RETURN
      END
      SUBROUTINE NGAUSS(X,RN,AM,SD)
```

```
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      IF(SD.NE.0.0)GO TO 30
      X=AM
      RETURN
30    SUM=0.0
      DO 20 I=1,100
      CALL URAND(R)
20    SUM=SUM+R
      RN=(SUM-50.)/SQRT(25./3.)
      X=RN*SD+AM
      RETURN
      END
```

APPENDIX IID

Simulation Program for the 2 Compartment Model

```
C     SAMPLING AT SET TIMES
C
      PROGRAM DATA4
C
C     CHAN 20 - INITIAL STARTING VALUES
C     CHAN 30 - NONMEM DATA FILE
C     CHAN 40 - MINITAB DATA FILE
C
      INTEGER RAN,GEN,BASE
      COMMON /MIRNG/RAN(10),GEN(10),NWRD,BASE,MMOD,FBASE,FMOD
      DIMENSION T(20)
C
C     ABAR=POPN A          BBAR=POPN B          ALBAR=POPN AL
C     SDA=POPN SD A        SDB=POPN SD B        SDAL=POPN SD AL
C     BEBAR=POPN BE        SDBE=POPN SD BE
C     SDC   = PROP ERROR IN CONC DOSE = DOSE
C     NT    = NO OF TIMES      NR = NO OF RATS/TIME
C     NSTART = RANDOM SEEDS
C
      READ(20,*) NSTART
      RMAX=2.0**15-1.0
      MAXINT=RMAX
      CALL RANSET(MAXINT,NSTART)
      READ(20,*) ABAR,SDA,ALBAR,SDAL,BBAR,SDB,BEBAR,SDBE,SDC,
     +DOSE,NT,NR
      READ(20,*) (T(I),I=1,NT)
      DO 500 I=1,NT
      DO 100 J=1,NR
    5 CALL NGAUSS (A,RN,ABAR,SDA)
      IF (A.LE.0.0) GO TO  5
   10 CALL NGAUSS (AL,RN,ALBAR,SDAL)
      IF (AL.LE.0.0) GO TO 10
   15 CALL NGAUSS (B,RN,BBAR,SDB)
      IF (B.LE.0.0)  GO TO 15
   30 CALL NGAUSS(BE,RN,BEBAR,SDBE)
      IF (BE.LE.0.0) GO TO 30
      CALL URAND(T1)
      IF(I.EQ.1.OR.I.EQ.2) GO TO 35
      RE=0.25
      RM=-0.125
      GO TO 40
   35 RE=0
      RM=0
   40 T1= T1*RE + T(I) + RM
      EALT=EXPWCH(-AL*T1)
      EBET=EXPWCH(-BE*T1)
      C1=A*EALT+B*EBET
```

```
      SDC1=SDC*C1
      CALL NGAUSS (CC,RN,C1,SDC1)
      C1=CC
      IF(C1.LT.10.0.OR.C1.GT.20000.0) GO TO 5
      NN= ((I-1)*NR) +J
      RI=FLOAT (NN)
      WRITE(30,20) RI,T1,C1
      WRITE(40,25) RI,A,AL,B,BE,T1,C1
  100 CONTINUE
  500 CONTINUE
   20 FORMAT (3F10.2)
   25 FORMAT (7F10.2)
      STOP
      END
      FUNCTION EXPWCH(XX)
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=EXP(XX)
      RETURN
      END
      SUBROUTINE RANSET(MAXINT,NSTRT)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      MAXI=MAXINT/4
      IB=0
      NBASE=1
   99 IF(NBASE.GT.MAXI)GO TO 100
      NBASE=NBASE*4
      IB=IB+1
      GO TO 99
  100 NBASE=2**IB
      FNBASE=NBASE
      NWRD=47/IB+1
      NREM=47-IB*(NWRD-1)
      MMOD=2**NREM
      FMOD=MMOD
      DO 101 N=1,10
      NRAN(N)=0
  101 NGEN(N)=0
      NGEN(1)=5
      DO 200 I=1,14
      NCARRY=0
      DO 190 N=1,NWRD
      NGEN(N)=NGEN(N)*5+NCARRY
      NCARRY=0
      IF(NGEN(N).LT.NBASE)GO TO 190
      NCARRY=NGEN(N)/NBASE
      NGEN(N)=NGEN(N)-NBASE*NCARRY
  190 CONTINUE
  200 CONTINUE
      NSTART=NSTRT
      IF(NSTART.LE.0)NSTART=2001
      NSTART=2*(NSTART/2)+1
      DO 300 N=1,NWRD
      NTEMP=NSTART/NBASE
      NRAN(N)=NSTART-NTEMP*NBASE
```

```
300 NSTART=NTEMP
    RETURN
    END
    SUBROUTINE URAND(FRAN)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    DIMENSION NSUM(10)
    DO 30 IS=1,NWRD
 30 NSUM(IS)=0
    DO 1 IG=1,NWRD
    N2=NWRD-IG+1
    DO 1 IR=1,N2
    IS=IR+IG-1
    NPROD=NRAN(IR)*NGEN(IG)
    NHPROD=NPROD/NBASE
    LPROD=NPROD-NHPROD*NBASE
    NSUM(IS)=NSUM(IS)+LPROD
    IF(IS.LT.NWRD)NSUM(IS+1)=NSUM(IS+1)+NHPROD
  1 CONTINUE
    N2=NWRD-1
    DO 5 IS=1,N2
    NCARRY=NSUM(IS)/NBASE
    NSUM(IS)=NSUM(IS)-NCARRY*NBASE
    NSUM(IS+1)=NSUM(IS+1)+NCARRY
  5 CONTINUE
    NSUM(NWRD)=NSUM(NWRD)-MMOD*(NSUM(NWRD)/MMOD)
    DO 20 IS=1,NWRD
 20 NRAN(IS)=NSUM(IS)
    FRAN=NSUM(1)
    DO 10 IS=2,NWRD
 10 FRAN=FRAN/FNBASE+NSUM(IS)
    FRAN=FRAN/FMOD
    RETURN
    END
    SUBROUTINE NGAUSS(X,RN,AM,SD)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    IF(SD.NE.0.0)GO TO 30
    X=AM
    RETURN
 30 SUM=0.0
    DO 20 I=1,100
    CALL URAND(R)
 20 SUM=SUM+R
    RN=(SUM-50.)/SQRT(25./3.)
    X=RN*SD+AM
    RETURN
    END
```

APPENDIX IIE

Simulation Program for the Reparameterized 2 Compartment Model

```
C      SAMPLING AT SET TIMES
C
       PROGRAM DATA9
C
C      CHAN 20 - INITIAL STARTING VALUES
C      CHAN 30 - NONMEM DATA FILE
C      CHAN 40 - MINITAB DATA FILE
C
       INTEGER RAN,GEN,BASE
       COMMON /MIRNG/RAN(10),GEN(10),NWRD,BASE,MMOD,FBASE,FMOD
       DIMENSION T(20)
C
C      CLBAR=POPN CL          V1BAR=POPN V1          P12BAR=POPN P12
C      SDCL=POPN SD CL        SDV1=POPN SD V1     SDP12=POPN SD P12
C      P21BAR=POPN P21        SDP21=POPN SD P21
C      SDC    = PROP ERROR IN CONC DOSE = DOSE
C      NT     = NO OF TIMES       NR = NO OF RATS/TIME
C      NSTART = RANDOM SEEDS
C
       READ(20,*) NSTART
       RMAX=2.0**15-1.0
       MAXINT=RMAX
       CALL RANSET(MAXINT,NSTART)
       READ(20,*) CLBAR,SDCL,V1BAR,SDV1,P12BAR,SDP12,P21BAR,SDP21,
      +SDC,DOSE,NT,NR
       READ(20,*) (T(I),I=1,NT)
       DO 500 I=1,NT
       DO 100 J=1,NR
     5 CALL NGAUSS (CL,RN,CLBAR,SDCL)
       IF (CL.LE.0.0) GO TO  5
    10 CALL NGAUSS (V1,RN,V1BAR,SDV1)
       IF (V1.LE.0.0) GO TO 10
    15 CALL NGAUSS (P12,RN,P12BAR,SDP12)
       IF (P12.LE.0.0)  GO TO 15
    30 CALL NGAUSS(P21,RN,P21BAR,SDP21)
       IF (P21.LE.0.0) GO TO 30
       CALL URAND(T1)
       IF(I.EQ.1.OR.I.EQ.2) GO TO 35
       RE=0.25
       RM=-0.125
       GO TO 40
    35 RE=0
       RM=0
    40 T1= T1*RE + T(I) + RM
       P=P12+P21+CL/V1
       Q=SQRT(P**2-4.*P21*CL/V1)
       A=(DOSE/V1)*(0.5*(P/Q+1)-P21/Q)
```

```
      B=(DOSE/V1)*(P21/Q-0.5*(P/Q-1.0))
      EALT=EXPWCH(-0.5*(P+Q)*T1)
      EBET=EXPWCH(-0.5*(P-Q)*T1)
      F=A*EALT+B*EBET
      C1=A*EALT+B*EBET
      SDC1=SDC*C1
      CALL NGAUSS (CC,RN,C1,SDC1)
      C1=CC
      IF(C1.LT.10.0.OR.C1.GT.20000.0) GO TO 5
      NN= ((I-1)*NR) +J
      RI=FLOAT (NN)
      WRITE(30,20) RI,T1,C1
      WRITE(40,25) RI,CL,V1,P12,P21,T1,C1
  100 CONTINUE
  500 CONTINUE
   20 FORMAT (3F10.2)
   25 FORMAT (7F10.2)
      STOP
      END
      FUNCTION EXPWCH(XX)
      IF(XX.LE.-50.) XX=-50.
      IF(XX.GE.50.) XX=50.
      EXPWCH=EXP(XX)
      RETURN
      END
      SUBROUTINE RANSET(MAXINT,NSTRT)
      COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
      MAXI=MAXINT/4
      IB=0
      NBASE=1
   99 IF(NBASE.GT.MAXI)GO TO 100
      NBASE=NBASE*4
      IB=IB+1
      GO TO 99
  100 NBASE=2**IB
      FNBASE=NBASE
      NWRD=47/IB+1
      NREM=47-IB*(NWRD-1)
      MMOD=2**NREM
      FMOD=MMOD
      DO 101 N=1,10
      NRAN(N)=0
  101 NGEN(N)=0
      NGEN(1)=5
      DO 200 I=1,14
      NCARRY=0
      DO 190 N=1,NWRD
      NGEN(N)=NGEN(N)*5+NCARRY
      NCARRY=0
      IF(NGEN(N).LT.NBASE)GO TO 190
      NCARRY=NGEN(N)/NBASE
      NGEN(N)=NGEN(N)-NBASE*NCARRY
  190 CONTINUE
  200 CONTINUE
      NSTART=NSTRT
```

```
    IF(NSTART.LE.0)NSTART=2001
    NSTART=2*(NSTART/2)+1
    DO 300 N=1,NWRD
    NTEMP=NSTART/NBASE
    NRAN(N)=NSTART-NTEMP*NBASE
300 NSTART=NTEMP
    RETURN
    END
    SUBROUTINE URAND(FRAN)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    DIMENSION NSUM(10)
    DO 30 IS=1,NWRD
 30 NSUM(IS)=0
    DO 1 IG=1,NWRD
    N2=NWRD-IG+1
    DO 1 IR=1,N2
    IS=IR+IG-1
    NPROD=NRAN(IR)*NGEN(IG)
    NHPROD=NPROD/NBASE
    LPROD=NPROD-NHPROD*NBASE
    NSUM(IS)=NSUM(IS)+LPROD
    IF(IS.LT.NWRD)NSUM(IS+1)=NSUM(IS+1)+NHPROD
  1 CONTINUE
    N2=NWRD-1
    DO 5 IS=1,N2
    NCARRY=NSUM(IS)/NBASE
    NSUM(IS)=NSUM(IS)-NCARRY*NBASE
    NSUM(IS+1)=NSUM(IS+1)+NCARRY
  5 CONTINUE
    NSUM(NWRD)=NSUM(NWRD)-MMOD*(NSUM(NWRD)/MMOD)
    DO 20 IS=1,NWRD
 20 NRAN(IS)=NSUM(IS)
    FRAN=NSUM(1)
    DO 10 IS=2,NWRD
 10 FRAN=FRAN/FNBASE+NSUM(IS)
    FRAN=FRAN/FMOD
    RETURN
    END
    SUBROUTINE NGAUSS(X,RN,AM,SD)
    COMMON /MIRNG/NRAN(10),NGEN(10),NWRD,NBASE,MMOD,FNBASE,FMOD
    IF(SD.NE.0.0)GO TO 30
    X=AM
    RETURN
 30 SUM=0.0
    DO 20 I=1,100
    CALL URAND(R)
 20 SUM=SUM+R
    RN=(SUM-50.)/SQRT(25./3.)
    X=RN*SD+AM
    RETURN
    END
```

PRESENTATION

Analysis of animal pharmacokinetic data: limitations of the one point per animal design

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Cheltenham, November 9, 1990.

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