THE PHARMACOKINETICS/PHARMACODYNAMICS OF THEOPHYLLINE IN PREMATURE NEONATES DURING THE FIRST FEW DAYS AFTER BIRTH

by

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PREFACE

This study represents original work by the author and has not been submitted to any other University. Where use was made of the work of others it has been duly acknowledged.

The research described in this thesis was carried out in the Department of Experimental and Clinical Pharmacology, University of Natal, and the Nurseries of King Edward VIII Hospital, Durban, South Africa.

Prof Julia Botha from the Department of Experimental and Clinical Pharmacology, University of Natal and Prof Lynn McFadyen from the Department of Pharmacology University of Durban-Westville, Durban, Natal, supervised the thesis.

Prof Nick Holford from the University of Auckland, Auckland, New Zealand, assisted with the pharmacokinetic/pharmacodynamic modelling.

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Publications and congress presentations arising from this study:

Publications in refereed journals:

Du Preez MJ, Botha JH, McFadyen ML, Holford NHG.

The pharmacokinetics of theophylline in premature neonates during the first few days after birth.

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(See Appendix D.1 page 340)

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(See Appendix D.2 page 346)

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University of Natal, Medical School, Faculty Research Day, Durban, South Africa 1996.

Du Preez MJ*, Botha JH, McFadyen ML, Adhikari M.

The effect of theophylline on apnoea and oxygenation in neonates.

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The effect of theophylline on apnoea and hypoxaemic episodes in the premature neonate during the first three days after birth.

University of Natal, Medical School, Faculty Research Day, Durban, South Africa 1997.

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A count model for analysing PK/PD data for theophylline in neonatal apnoea.

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ABSTRACT

Theophylline is one of the few preparations available for the treatment of apnoea of prematurity. Currently little data is available on the pharmacokinetics and the pharmacokinetic/pharmacodynamic relationships of theophylline for premature neonates during the first few days of life, a time when neonates undergo profound physiological changes and when the drug is most often used. Furthermore, the influence of theophylline on hypoxaemic episodes has not yet been quantified.

The study aimed to investigate optimal theophylline dosing in this group by establishing pharmacokinetic parameters, assessing the effectiveness of the drug in abolishing apnoea and hypoxaemic episodes and investigating the concentration/effect relationship.

The project was conducted in the neonatal wards of King Edward VIII Hospital, Durban, South Africa. The study group comprised a total of 105 Black, apnoeic, premature neonates, with respiratory distress syndrome, who were receiving intravenous theophylline. Serum samples (263), collected from patients during routine care, were analysed for theophylline. Forty-six patients were monitored before and after theophylline therapy with a neonatal capnograph linked to a data acquisition.

Apnoea incidents were classified into total (all apnoea ≥5 seconds) and pathologic (all apnoea ≥20 seconds) and a hypoxaemic episode was defined as a ≥10% fall for >10 seconds in peripheral oxygen saturation. Within each of these groups patients were assessed as responders (≥50% reduction in the clinical effect from baseline to the last

recording) and non-responders. Patient characteristics were identified as possible markers of non-response to theophylline therapy.

The Nonlinear Mixed Effects Model (NONMEM) was used to derive population pharmacokinetic models and parameters for theophylline as well as to assess the concentration-effect relationship.

The pharmacokinetic analysis estimated a low clearance and volume of distribution, with oxygen support enhancing clearance. Relatively high inter-individual and residual variability values were obtained prompting testing for inter-occasion variability. This resulted in a decrease of inter-individual variability for clearance and volume of distribution as well as in residual variability.

In the theophylline doses used, a significant reduction in total and pathologic apnoea but not in hypoxaemic episodes occurred over the first three days after birth. The most positive improvement was seen on the first day of treatment after the loading dose. A statistically significant increase in the average pulse rate and a decrease in episodes of bradycardia from baseline to all three days of monitoring were recorded.

Most patients responded at serum theophylline concentrations of 3 to 9 mg/L. Most serum theophylline concentration measurements were also in this range and it was not possible to clearly define a concentration-effect relationship. The cumulative percentage of non-responders was relatively high for total apnoea (48%) and hypoxaemic episodes (45%), but low for pathological apnoea (13%). Being one of a set of twins was identified

as a marker of poor response for both total apnoea and hypoxaemic episodes. Other possible markers for poor response, in terms of total hypoxaemic episodes, were being born by caesarean section and having more than the 75th percentile pathologic apnoea per hour at baseline. It was interesting to note that, with regard to total apnoea, there were some features that seemed to predict a favourable response to theophylline. These were birth weight and 5 minute Apgar score below the 25th percentile, and patients with baseline total apnoea counts above the 75th percentile.

The cumulative graphs of the responders and non-responders resembled the fixed effect model, which is the simplest model to explain drug-effect relationships. More sophisticated analysis of the concentration-effect relationship, using NONMEM and the count model proved difficult. None of the models tested were found to be satisfactory, but that which included the influence of a hypothetical respiratory depressant factor gave the most realistic value of EC50. It is suggested that further even more complex modelling may be required to accurately define the concentration-effect relationship (and hence the therapeutic range) for theophylline in neonatal apnoea.

INTRODUCTION

The first report of the use of theophylline in neonates was published in 1973 (Kuzemco and Paala) and since then it has been widely used for the treatment of apnoea of prematurity. The development of specific serum assays for theophylline prompted therapeutic monitoring of the drug and provided information necessary for calculation of paediatric doses. Although a number of studies have been published on the use of theophylline in babies and infants, the therapeutic range and hence good dosing guidelines are not available for apnoeic premature neonates especially during the first few days after birth.

Premature neonates differ substantially from full term infants in the way they handle and respond to drugs (Rane 1992) and, therefore, form a subgroup of the population. Especially during the first few days after birth, the physiology of the premature neonate changes substantially to adapt to extra-uterine life. It has, for instance, been noted that the premature neonate has a markedly low clearance for theophylline and a unique methylation metabolic pathway to caffeine (Dothey *et al* 1989). Moreover, about 50% of the theophylline dose is excreted in the urine unchanged (Tserng *et al* 1983) compared to the 7 to 15% in children and adults. It is known that renal function develops slowly after birth and that this function is influenced by factors such as hypoxia and arterial pH (Chevalier 1996, Richter and Lam 1993). The pharmacokinetics of theophylline in premature neonates could, therefore, be expected to be very different to that in term babies and older infants.

The efficacy of theophylline in the treatment of apnoea in premature neonates is controversial. While Muttitt *et al* (1988) showed some efficacy, others have questioned this (Shannon *et al* 1975) and some even showed that the development of maturity is as important as drug therapy (Sims *et al* 1985). Most studies were undertaken in apnoeic but otherwise healthy neonates. However, in neonates with concomitant disorders the average reduction of apnoea incidents was reported to be only 58% (Roberts *et al* 1982). It is not known why certain neonates with apnoea do not respond to theophylline therapy and no markers or characteristics of non-response have been identified.

Even less is known about the effect of theophylline on hypoxaemia as such. Hypoxaemia may be a consequence of apnoea, but isolated hypoxaemic episodes are also common in the premature neonate (Miller and Martin 1992, Southall *et al* 1993). This could be due to ventilation/perfusion inequalities (Poets *et al* 1992). Theophylline has a wide spectrum of pharmacological actions and the potential to improve ventilation/perfusion and therefore oxygenation. As hypoxaemia may have serious consequences, the aetiology and management of these incidents have been investigated by a number of researchers (Poets *et al* 1992, Southall *et al* 1993).

Unfortunately theophylline has a narrow therapeutic range and toxic effects are dose related (Hendeles and Weinberger 1983, O'Donnell 1994). Currently various dosage guidelines are available for calculating theophylline dosages in neonates and infants. However, no consensus exists as to the best method to predict the appropriate dosage of theophylline in premature neonates. This is because (a) the therapeutic range at which it

is most effective and least toxic has not been established and (b) the pharmacokinetics have not been well described.

Some of the earlier investigators observed that apnoea could only be controlled with theophylline plasma concentrations greater than 5 mg/L and that cardiovascular side effect were associated with plasma concentrations greater than 13 mg/L (Shannon *et al* 1975, Jones and Baillie 1979). On the contrary, in other studies with neonates of comparable postconceptual ages, theophylline concentrations as low as 2 to 4 mg/L could control apnoea and also bradycardia (Milsap *et al* 1980, Myers *et al* 1980). However, in a dose-response study a poor response was demonstrated in neonates with theophylline serum concentrations between 4 to 8 mg/L (Muttitt *et al* 1988). After increasing mean serum concentrations to 12.7 mg/L, an additional 63% response rate was noted by the authors (Muttitt *et al* 1988).

Based on these studies Aranda *et al* (1992) in a subsequent review stated that the desired serum concentration of theophylline should range from 5 to 15 mg/L. However, recently a revised Food and Drug Administration (FDA) of the United States of America labelling guideline for theophylline oral dosage, recommended a therapeutic range of 5 to 10 mg/L for premature neonates less than 24 days old (Hendeles *et al* 1995). Despite the recommendations by the FDA, a number of different dosage schedules are found in the literature with serum theophylline concentrations ranging from 2 to 15 mg/L. Most of these guidelines do not take the very low birth weight neonate into consideration. Theophylline is also known as a drug with a high inter- and intra-patient variability

(Hendeles and Weinberger 1983, Milsap and Jusko 1994) and this could adversely affect the already fragile premature neonate (Bhatt-Mehta *et al* 1995).

Thus, a number of questions remain:

1. How effective is theophylline in reducing the number of apnoea and hypoxaemic episodes during the first few days after birth?

If so, then:

2. What serum theophylline concentrations are required?
3. What doses are required to achieve these concentrations in premature neonates?

If some neonates do not respond to theophylline therapy:

4. Can characteristics of non-response be identified?

Accordingly the objectives of the study were:

1. To determine, in a population of premature apnoeic neonates, the pharmacokinetic parameters, clearance and volume of distribution, for theophylline and investigate possible influences on these parameters.
2. To re-evaluate the effect of theophylline on neonatal apnoea and also hypoxaemia and, if appropriate, to identify markers of response and non-response.
3. To define the theophylline concentration-effect relationship in neonatal apnoea.

This investigation is described in three parts:

Section A: The pharmacokinetics of theophylline in premature neonates during the first few days after birth.

Section B: The efficacy of theophylline in apnoea and hypoxaemia.

Section C: Population concentration-effect modelling of theophylline in premature neonates suffering from apnoea during the first few days after birth.

SECTION A

THE PHARMACOKINETICS OF THEOPHYLLINE IN PREMATURE NEONATES DURING THE FIRST FEW DAYS AFTER BIRTH

SECTION A: CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 THE PHARMACOKINETICS OF THEOPHYLLINE IN THE PREMATURE NEONATE

Pharmacokinetics is defined as the study of the time course of drug absorption, distribution, metabolism and excretion. Mathematical models are used to describe these processes. These models allow estimation of a number of pharmacokinetic parameters such as clearance (CL), volume of distribution (V), half-life and rate of absorption of a drug. Use of these pharmacokinetic parameters allows for dose adjustment to target plasma concentrations in the therapeutic range thus leading to safer, more effective and appropriate management of patients.

One of the major clinical uses of theophylline is the treatment of apnoea of prematurity. In the neonate, and even older child, drug administration and the determination of a target concentration, is affected by a number of factors such as age and stage of development. The immaturity of the premature neonate, especially the relative inability to metabolise and excrete drugs, might have profound effects on the pharmacokinetics of theophylline.

1.1.1 **Absorption and bioavailability of theophylline**

Absorption is the passage of a drug from the site of administration through tissues or cell membranes to reach the systemic circulation. The bioavailability (F) of a drug is the

percentage of the dose administered that reaches the systemic circulation. Administration of a drug by intravenous (IV) bolus is assumed to provide full systemic availability, thus F = 1. With oral administration, drug absorption depends upon the physiochemical properties of the drug and a variety of patient factors. Some of these factors include surface area available for absorption, blood flow at absorption site, gastric emptying time, gastric and duodenal acidity, underlying disease states and the presence or absence of food in the intestine (Rowland and Tozer 1995). In the premature infant oral administration may result in aspiration and poor absorption especially during the first few weeks after birth (Behrman and Kliegman 1994).

The rate of drug absorption (k_a) is slower in the neonate than in the adult (Siegner and Fridrich 1975, Milsap and Jusko 1994). This could be due to the relative achlorhydria, the prolonged gastric emptying time and the unpredictable peristalsis that is characteristic of the premature neonate (Morselli 1976). The gastric pH is above 7 at birth but falls to values of 1 to 3 within a day or two (Rane 1992). Physiological factors that influence drug absorption develop slowly, but also at different rates after birth (Herngren *et al* 1983).

Theophylline, when administered orally to infants as a liquid formulation, is rapidly and completely absorbed (Giacoia *et al* 1976, Hendeles and Weinberger 1983), and the bioavailability is >0.9 (Moore *et al* 1989, Lee *et al* 1996). The presence of food may decrease the rate but not the extent of theophylline absorption (Heimann *et al* 1982): these researchers found that the mean time to peak serum concentration in premature infants was 1.7 hours in a fasting state and 4.7 hours in a fed state.

Some theophylline formulations are available for rectal administration either as enemas, solutions or suppositories. The rate and extent of absorption from rectally administered theophylline in neonates is more variable than the orally administered drug (Aranda *et al* 1992). In a recent population study with neonates the k_a for rectally administered theophylline was found to be 0.43 ($\pm$ 0.18) h^{-1} (Karlsson *et al* 1991).

1.1.2 **Distribution of theophylline in the neonate**

Once absorption is complete, the drug distributes into various tissues and other body fluids. The rate at which particular tissue-plasma concentration equilibrium is achieved depends on the rate of perfusion of the organ. The term V is used to describe the relationship between the amount of the drug in the body and its plasma concentration:

$$\text{Amount in the body} = V * C_p$$

Where C_p represents the plasma concentration.

The amount and character of plasma proteins, and the relative size of the fluid, fat and tissue compartments of the body, all influence distribution of the drug (Rowland and Tozer 1995). Some age-related differences in these factors are known. For example, total body water expressed as a percentage of total body weight is as much as 85% in premature neonates compared to 78% in full-term neonates and 60% in adults (Friis-Hansen 1971). Thus drugs that distribute in parallel with body water content have higher volumes for neonates than for adults when expressed per kilogram body weight.

The binding of drugs to plasma proteins is dependent on multiple factors, all of which may be underdeveloped in the neonate. The total plasma protein, plasma albumin and α_1-acid glycoprotein concentrations are decreased in the neonate and do not approach adult values until about one year of age (Herngren *et al* 1983). In addition, inadequate oxygenation due to an underdeveloped respiratory control system may cause acid-base disturbances that might affect distribution and binding of drugs. The neonate often develops neonatal jaundice thus competition for binding sites by increased circulatory concentrations of endogenous bilirubin may occur (Brodersen *et al* 1983). This may all contribute to an increased variability of V in neonatal patients compared to other population groups.

In the adult, after absorption of theophylline about 60% of the drug is bound to plasma proteins and the remaining free drug is distributed throughout the body water (Lesko *et al* 1981). In the neonate, the capacity of theophylline to bind to plasma proteins is only about 28% to 36% (Aranda *et al* 1976, Butts *et al* 1991). The results of Butts *et al* (1991) showed a significant negative correlation ($r = -0.825$, $p<0.001$) between unbound theophylline and serum albumin, suggesting that theophylline binds mainly to albumin. The binding of theophylline to plasma proteins in the premature neonate may change gradually as both the concentrations of total protein and albumin show an increase of 50% from 28 weeks to 40 weeks gestation (Reading *et al* 1990, Butts *et al* 1991).

In both adults and children the V of theophylline ranges from 0.3 to 0.7 L/kg (30% to 70% ideal body weight) and averages about 0.45 L/kg (Hendeles and Weinberger 1983). In the neonate mean V values ranging from 0.2 to 2.9 L/kg have been recorded in the

traditional pharmacokinetic studies (see Table A.1.1). In two of the published population studies the mean values were 0.94 L/kg (Lee *et al* 1996) and 0.86 L/kg (Moore *et al* 1989) respectively, and in a third population study, where theophylline was administered rectally, V/F was 1.3 L/kg (see Table A.1.2).

TABLE A.1.1

Theophylline pharmacokinetic data from traditional studies in neonates

Reference	Number of subjects and route of administration	Gestational age (wks)	Postnatal age (days)	CL (ml/kg/h) mean (SD) (Range)	V (L/kg) mean (SD) (Range)
Aranda *et al* 1976	6 IV	25 - 32	3 - 15	17.6 (12.1 - 25.9)	0.7 (0.4 - 1)
Giacoia *et al* 1976	8 Oral	26 - 32	25 - 57	39 (18) (23 - 68)	0.9 (0.7 - 2.9)
Neese *et al* 1977	12 Rectal	28 - 36	2 - 21	8.56 (4.3 - 12.4)	0.3 (0.2 - 0.54)
Latini *et al* 1978	7 IV	26 - 33	4 - 8	12.9 (6.3 - 29.9)	0.4 (0.2 - 1.0)
Brazier *et al* 1979	20 Oral	27 - 37	2.9 (± 2)	24.0 (5.1)	1.0 (0.2)
Jones *et al* 1979	14 IV	25 - 31	0 - 36	18.6 (4.8) (12 - 28)	0.7 (0.2) (0.4 - 1.2)
Hilligoss *et al* 1980	17 Oral	25 - 36	1 - 26	22.9 (3.9) (16 - 30)	0.6 (0.2) (0.4 - 0.9)
Gal *et al* 1982	30 IV	26 - 34	6 - 14	10.8 in asphyxia 20.1 in non-asphyxia	0.76 in asphyxia 0.82 in non-asphyxia
Lönnerholm *et al* 1983	17 Oral	28 - 34	6 - 11	16.8 (0.4)	-
Gilman *et al* 1986	179 IV	30 (± 3)	14 (± 10)	AGA 18.8 (5.8) SGA 17.9 (5.3) Asphyxia 16.4 (5.3) Nonasphyxia 20.2 (5.4)	0.77 (0.2)
Stile *et al* 1986	9 IV	25 - 30	1	46 (14)	1.0 (0.1)
Kraus *et al* 1993	52 IV and oral	24 - 40	14 - 483	30 - 40 wks:21.5 (6.9) 40 – 50 wks: 30.3 (10.3)	

Abbreviations: CL = clearance, V = volume of distribution, wks = weeks, IV = intravenous, AGA = average for gestational age, SGA = small for gestational age.

TABLE A.1.2

Theophylline pharmacokinetic data from population studies in neonates

Reference	Number of subjects and route of administration	Gestational age (wks)	Postnatal ages (days)	CL (ml/kg/h) mean (SD) (range)	V (L/kg) mean (SD) (range)
Moore *et al* 1989	108 IV and Oral	24 - 42	3 - 182	17.5 (15.5 - 19.5)	0.86 (0.79 - 0.92)
Karlsson *et al* 1991	35 Rectal	26 - 35	2 - 80	40 (2) CL/F	1.3 (0.2) V/F
Lee *et al* 1996	182 IV and Oral	24 - 32	1 - 111	12.3 (0.74)	0.94 (0.08)

Abbreviations: CL = clearance, V = volume of distribution, wks = weeks, IV = intravenous.

1.1.3 **Elimination of theophylline in the neonate**

A drug can be eliminated from the body by a number of organs with hepatic metabolism and renal excretion representing the major routes. The pharmacokinetic parameter CL is used to describe the overall elimination in terms of volume of plasma from which a drug is completely removed per unit of time:

$$\text{Rate of elimination (L/h)} = C_p * CL$$

The metabolism of a drug is dependent on the physiological variables of hepatic blood flow, binding in blood and intrinsic hepatocellular activity (Rowland and Tozer 1995). Most enzymatic microsomal systems responsible for drug metabolism are present at birth, but their activity is slow during the neonatal period (Rylance 1992) and increases with advancing postconceptual age (PCA) (Morselli *et al* 1980). In the adult, hepatic

metabolism is the main route of theophylline elimination, but in the premature neonate the hepatic metabolism of theophylline is relatively less important (Hendeles and Weinberger 1983, Tserng *et al* 1983, Baird-Lambert *et al* 1984). The differences between neonate, child and adult are depicted in Figure A.1.1. A number of different metabolic routes are found of which C-8-hydroxylation seems to be important in the neonate as well as the adult. In contrast, in the neonate about half of the theophylline is excreted unchanged.

It should be noted that, caffeine, which is also a central nervous system stimulant, is a major metabolite of theophylline in the premature neonate, but not in the adult. The metabolism of theophylline to caffeine reduces significantly with PCA as the infant matures (Tserng *et al* 1983, Rylance 1992, Kraus *et al* 1993). Apparently urine metabolite patterns reach adult values at approximately 55 weeks PCA (Kraus *et al* 1993).

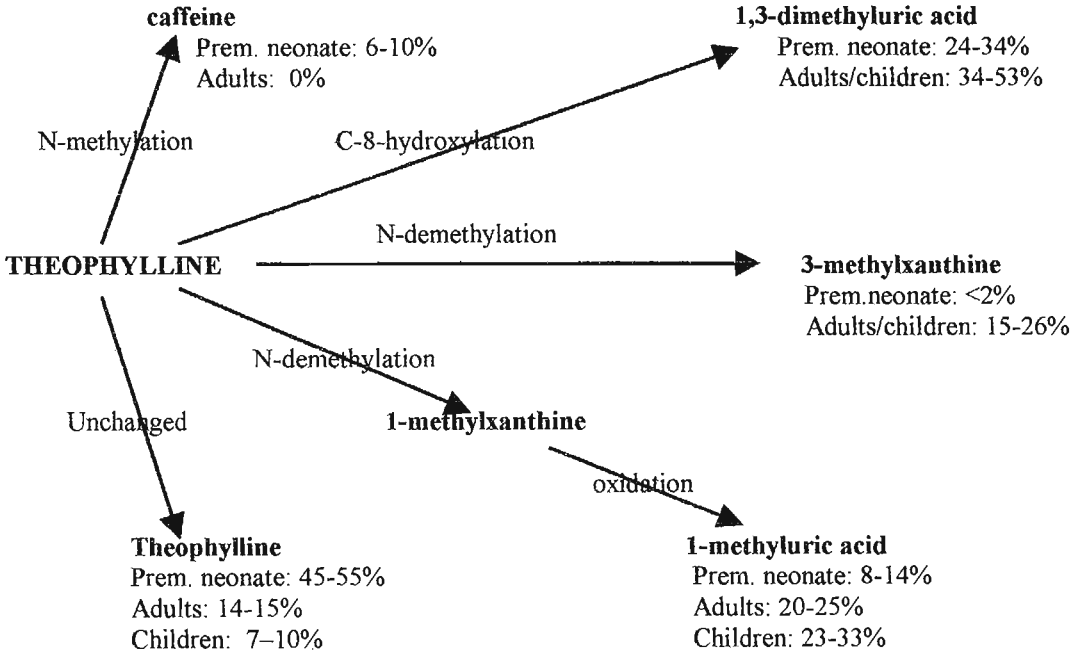

Fig. A.1.1 Hepatic metabolism of theophylline

References: Bonati *et al* 1981, Tserng and King 1981, Hendeles and Weinberger 1983, Tserng *et al* 1983 and Baird-Lambert *et al* 1984.

In the premature neonate, but not in the child or adult, renal clearance of theophylline is as important as hepatic degradation of the drug. Renal excretion is dependent on three processes, namely, glomerular filtration, tubular secretion and tubular reabsorption. Thus,

Rate of excretion = rate of filtration + rate of secretion - rate of reabsorption.

These processes differ between the premature neonate, the term infant and also the older infant. For example, in the premature neonate the glomerular filtration rate (GFR) at birth is 0.6 to 0.8 ml/min, whilst in term infants it is 2 to 4 ml/min (Van den Anker

1996). Adult GFR-values of approximately 130 ml/min are reached at 2 to 2.5 months of age. Van den Anker *et al* (1995) determined the GFR in premature neonates with both the inulin clearance and the reciprocal of the serum creatinine values. A positive linear relationship ($r = 0.6$) between GFR and gestational age (GA) on day three of life was found. On the contrary, Seikaly and Arant (1992) insisted that GFR does not change very much in premature neonates up to 34 weeks gestation, but at around 34 weeks after conception and regardless of postnatal age (PNA), the GFR increases very rapidly within a week (Arant 1978, Robillard *et al* 1979). Thirty-four weeks gestational age would be about the time nephrogenesis is complete. Factors responsible for this rapid increase include increasing mean arterial blood pressure, increasing renal blood flow, and increasing glomerular permeability and filtration surface area (Chevalier 1996). Apparently the renin-angiotensin system and prostaglandins modulate this change in GFR (Chevalier 1996). Thus, CL of a drug that is excreted by the kidneys is likely to be influenced by the changing GFR in the postnatal period.

Tubular function matures at a slower rate than glomerular function and tubular transport capacity reaches adult values by 30 weeks PCA (Kaapa *et al* 1995). The capacity to reabsorb drugs from the renal tubule seems to be reasonably developed in the neonate as drugs that are known to be reabsorbed in this way are excreted slowly during the first few days of life (Rylance 1992). It is also known that the premature neonate of less than 34 weeks gestational age reabsorbs 97%, 93% and 87% of filtered sodium, glucose and phosphate respectively (Arant 1978). Thus if the renal clearance of a drug is less than the amount of drug filtered then it could be assumed that some of the filtered drug is reabsorbed (see equation on page 14).

If a drug is only filtered, then renal clearance may be calculated as:

$$CL_R = f_u * GFR$$

Where CL_R = renal clearance, f_u = fraction unbound drug (Rowland and Tozer 1995).

As the GFR for the premature neonate is low (0.6 to 0.8 ml/min, Van den Anker 1996) and the fraction of unbound theophylline is approximately 0.7 (Aranda *et al* 1976, Butts *et al* 1991), the calculated renal clearance (taken as ± 0.7 x 0.7 = 0.49 ml/min or 0.0294 L/h, see page 15) would be larger than the estimated CL for most of the population studies reported above (e.g. Lee *et al* 1996, CL = 0.0123 L/h/kg). Therefore, the possibility exists that some of the filtered theophylline might be reabsorbed.

The mean values for theophylline CL in healthy non-smoking adult volunteers range from 40 to 52 ml/kg/h and in children less than 12 years old from 96 to 102 ml/kg/h (Hendeles and Weinberger 1983). However, the CL of theophylline is markedly less in neonates as the mean value for CL in the traditional studies ranged from 4.3 to 68 ml/kg/h. In two of the published population studies in neonates the mean values were 12.3 (Lee *et al* 1996) and 17.5 ml/kg/h (Moore *et al* 1989) respectively. The CL/F in neonates obtained from rectally administered theophylline was 40 ml/kg/h (Karlsson *et al* 1991).

Factors that may alter the CL of drugs include changes in severity of pulmonary obstruction, hypoxia and variation in arterial pH (Richter and Lam 1993). It is well known that GFR is lower than normal in infants with respiratory distress syndrome (RDS) (Guignard *et al* 1976). During RDS vascular resistance in the pulmonary circuit

remains elevated resulting in a distended right atrium (Kojima *et al* 1990). This is associated with an increase in circulating antinatriuretic peptide levels, which should cause diuresis. However, decreased GFR, tubular immaturity, and a generalised decrease in renal blood flow may attenuate the effects of this antinatriuretic peptide (Kojima *et al* 1990, Brem 1992).

Vasopressin (anti-diuretic hormone) is elevated in RDS (Kojima *et al* 1990) and free water clearance is diminished in parallel with the elevated plasma vasopressin levels (Robillard *et al* 1979, Wiriyathian *et al* 1986). In addition, hypoxia itself may also stimulate the release of vasopressin (Kojima *et al* 1990, Brem 1992). The neonatal ventilatory system responds to reduced oxygen availability by shunting the blood preferentially to the brain, heart and adrenal glands away from the intestine, kidney, lung and skin (Behrman *et al* 1994).

RDS usually resolves by day 3 to 4 after birth of the baby. Recovery from hypoxia and RDS may result in lower renal and systemic vascular resistance (Van den Anker 1996), which will then improve GFR. The variability in oxygen levels, and also the fact that pulmonary mechanisms vary up to 26% over the three days after birth (Goyal *et al* 1995), could contribute to an increased variability in theophylline CL in and also between premature neonates.

In three population studies with theophylline, it was found that PNA and weight (Moore *et al* 1989, Lee *et al* 1996) or weight alone (Karlsson *et al* 1991) accounted for variability in CL of theophylline. In traditional studies, Aranda *et al* (1976) found no

correlation between theophylline half-life and weight or age, whereas Lönnerholm *et al* (1983) and Gilman *et al* (1986) showed that PNA influenced CL. Driscoll *et al* (1989) and Kraus *et al* (1993) found that age was the major factor affecting CL. This indicated a close relationship between the demographic factors, age and weight, and the degree of development of the major drug clearance organs such as the liver and the kidneys. In addition to these demographic factors, attempts were also made to identify specific patient factors that may differentiate groups of patients with altered theophylline CL. Although Hilligoss *et al* (1980) found a weak correlation between CL and duration of therapy, it could not be shown in subsequent studies. Also, Gilman *et al* (1986) and Gal *et al* (1982) indicated that neonates who suffered birth asphyxia cleared theophylline slower than those who did not. However, these factors did not have a significant influence on the estimated values of the pharmacokinetic parameters obtained in the population studies.

Theophylline elimination appears to be a linear process but in some patients the metabolic processes may become saturated at serum concentrations within the therapeutic range (Lesko 1986). Thus, a disproportionate increase in serum drug concentration may occur for a given increase in dosage, resulting in toxicity, particularly in children (Sarrazin *et al* 1980). This is due to the fact that the C-8-hydroxylation and N-demethylation pathways, as depicted in Figure A.1.1, may exhibit saturable pharmacokinetics over the usual therapeutic range (Tang-Lui *et al* 1882).

The Michaelis-Menten equation for enzyme kinetics is used to describe how clearance may vary with the concentration of the drug:

$$\text{Total plasma clearance} = V_m / (K_m + C_p)$$

Where V_m is equal to the maximal rate of elimination (in units of mass/time), K_m is the plasma concentration at which half of the maximal rate of elimination is reached (in units of mass/volume) and C_p the plasma concentration of the drug (Rowland and Tozer 1995).

Recently, Anderson *et al* (1997) obtained a Michaelis-Menten constant (K_m) of 32.5 (±33.5) mg/L in a three month-old infant. This value is lower than the 67 mg/L that was obtained in an adult by Butts *et al* (1991). The lower value found in infants could indicate that non-linear elimination might occur at a lower plasma concentration in infants than in adults. No consensus has been reached regarding the linear- or dose-dependent pharmacokinetics of theophylline, as some investigators have found no evidence of non-linearity in dose-ranging trials (Rovei *et al* 1982). In the premature neonate the saturability of the C-8-hydroxylation process might however be significant, as it is responsible for 24% to 34% of metabolite formation.

Most premature infants undergo a phase of spontaneous diuresis during the first week of life (Lorenz *et al* 1995, Oliver *et al* 1995) with an eventual decrease in extracellular fluid volume (Heimler *et al* 1990, Ramiro-Tolentino *et al* 1996). In 87% of neonates the median age of onset and cessation of this diuresis is 24 and 96 hours respectively. In addition, theophylline may also cause a diuresis. In a recent study in a group of neonates, Mazkereth *et al* (1997) found that the initial loading dose of theophylline caused a marked diuresis with a loss of sodium, potassium, calcium and uric acid. This did not occur during maintenance therapy.

Theophylline is known as a drug with high inter- and intra-patient variability in CL (Hendeles and Weinberger 1983, Jenne 1986, Milsap and Jusko 1994). In the adult and child this appears to be due to differences in rate of hepatic biotransformation that changes with age, concurrent illness and other drugs, smoking habits and aberrations in diet (Hendeles and Weinberger 1983). In the premature neonate the variability in CL could be due to the immaturity of the hepatocellular activity and renal function, the level of oxygenation, and the physiological changes that take place after birth to adapt to extra-uterine life.

Although information on theophylline pharmacokinetics in the neonate is available, there is relatively little information specifically relating to premature neonates with apnoea during the immediate time after birth. Thus the objectives of the study were to estimate the pharmacokinetic parameters CL and V, of theophylline in premature neonates during the first few days after birth, and to identify influential effects such as demographic characteristics or disease states on these parameters.

1.2 PHARMACOKINETIC METHODOLOGY

In practice a number of methods can be followed to estimate the relevant pharmacokinetic parameters. These methods may be broadly classified into the traditional approaches, which use experimental data, and the alternative population approaches, using routine patient data (Sheiner and Beal 1980).

1.2.1 **The traditional approach to pharmacokinetic studies**

With traditional studies, also known as the standard two-stage (STS) approach (Sheiner

and Beal 1981), a small number of patients is selected and about ten to twenty serum samples for measurement of the study drug are collected from each patient according to a pre-set sampling time schedule. The patients may be healthy volunteers or patients carefully selected to represent a particular aspect or disease state that has to be investigated. The data collected from each patient is analysed using weighted or unweighted non-linear regression with the least-squares criterion to calculate that individual's pharmacokinetic parameters. The population values are then determined by pooling the values from each patient. The relationship between the pharmacokinetic parameters and the physiological factors such as age, weight and sex, which are most often statistically linear, are calculated using least-squares regression (Sheiner and Beal 1980, Sheiner and Beal 1980a). Extensions of the STS approach such as the iterative two-stage and the global two-stage method have been developed (Jelliffe *et al* 1993).

The traditional approach has several advantages (Sheiner and Beal 1980, Sheiner and Beal 1981a):

i) It has been used for a long time and is known to be reliable.

ii) Studies are relatively quick to perform.

iii) Data has little variability as the studies are stringently designed and adhered to.

iv) The statistical models are relatively simple and easily computed.

v) Data analysis with standardised computer programmes is generally rapid and inexpensive.

There are also several disadvantages of the method (Sheiner *et al* 1977, Sheiner and Beal 1980):

i) The source of the pharmacokinetic data may not be truly representative of the population for whom the drug would most often be used. Study subjects are usually healthy volunteers or carefully selected patients with a mild form of the disease who will be able to withstand the rigours of the trial.

ii) These studies may be expensive due to compensation of volunteers, temporary hospitalisation in clinical research wards and assaying numerous samples.

iii) As study conditions are strictly controlled the chance discovery of other factors influencing pharmacokinetics is limited.

iv) When ordinary least-squares methods are used for the first stage in the analysis, it is assumed that all errors between the predicted and the measured levels are independent from one concentration to another, are additive and of the same typical magnitude.

In the premature neonate the traditional approach has two very important further disadvantages:

i) It is often unethical to conduct a study where a large number of samples must be withdrawn during a relative short period of time from a patient at risk such as a neonate. It is also often impossible to get written informed consent from the parents of the neonate.

ii) The small blood volume of a premature neonate (approximately 90 - 100 ml in this age group) makes it impossible to withdraw adequate blood volumes for extensive concentration measurements.

1.2.2 **Population approach**

As a result of the limitations imposed by the traditional approach, alternative population methods, such as the Nonlinear Mixed Effects Model (NONMEM) (Sheiner *et al* 1977, Sheiner and Beal 1980), have been developed to utilise data generated during routine patient care (Sheiner *et al* 1977, Sheiner and Beal 1980, Whiting *et al* 1986). The alternative population approach treats the population as the unit of analysis and focuses on how measurable physiological and demographic features relate to the pharmacokinetic parameters. In these methods a few samples are collected from each of a large number of subjects. This usually occurs during routine therapeutic drug monitoring (TDM) where sampling times and dosing regimens may vary according to the judgement of the physician. The data collected in this manner can be used to determine the population pharmacokinetic parameters in a target population. Drug dose and dosing time must be recorded rigorously and it is important to record all clinical and demographic factors that are likely to influence the pharmacokinetic parameters.

The population approach has a number of advantages:

i) As the samples can be collected in the target treatment population, the data is more likely to be representative of the specific population. Rational dosage guidelines for specific risk groups can be developed.

ii) Data from different sources may be combined to accommodate varying spectra of disease states, dosing regimens, different routes of administration or different degrees of organ dysfunction.

iii) Few ethical problems arise, as few samples are required per patient.

iv) Costs are relatively low as samples are collected during patient care and sample analysis would form part of routine patient care.

v) As many types of patients form a population, the possibility of a chance discovery of a previously unknown influence, whether physiological or demographic, on drug kinetics exists.

Some of the most important disadvantages of the population approach include:

i) The data may be less reliable as it is collected routinely and not according to a strict experimental protocol. In the normal busy clinical setting it may not always be possible to accurately record times of dosing, serum sampling and all pertinent patient features.

ii) The analysis of routine clinical data requires a more sophisticated statistical approach than that required for experimental data.

iii) The possibility of bias due to the effects of unknown concomitant variables that are correlated with included variables, for example an undisclosed drug interaction, may lead to erroneous conclusions about the influence of included variables on CL (Sheiner *et al* 1977).

iv) The use of the wrong model, that is, model misspecification, may lead to incorrect results (Sheiner and Beal 1980).

Application of the population approach was for many years limited due to the complexity and the lack of general acceptance of the proposed statistical methodology. The available software was not user-friendly and expert guidance was needed to adequately use the programmes. The need for further development of the population approach was

recognised and, following a European Co-operation in Science and Technology (COST) meeting in 1991, a Working Party on Population Approaches was formed. Since then a number of meetings have been held and other groups interested in the population approach have been established for example PAGE (Population Approach Group Europe). The requirements of appropriate software were investigated by the Working Party and presented to interested parties. Some of the requirements stated were, for example, continuing update of programmes, complete and adequate documentation and regular training courses. As the population approach gained more support in drug development, the need to build it into the drug development plan by pharmaceutical companies was recognised and formalised.

There are various alternative methods commercially available to the pharmacokineticist. Examples include NPML (non-parametric maximum likelihood, Mallet 1986), P-Pharm (Mentré and Gomeni 1995), NPEM (non-parametric expectation maximisation method, Jelliffe *et al* 1993) and Non-linear mixed effects model (NONMEM, Beal and Sheiner 1992). Other new theoretical developments are the full Bayesian estimation method using the Gibbs sampler (Wakefield *et al* 1994) and the nonparametric EM algorithm (Schumitzky 1991, Mentré and Gomeni 1995).

A conference report of COST in 1996 (Aarons *et al*), stated that when only sparse data is available, as in studies in neonates, the population approach represents the only way to define pharmacokinetic/pharmacodynamic (PK/PD) models of a drug. The computer packages for population pharmacokinetic analyses are costly, sophisticated and training in their use is essential. NONMEM is established in our institution and trained

pharmacokineticists with experience are accessible. Additionally, a NONMEM repository site and users group is available on the Internet to assists in solving problems. It is also the programme that is most widely used and the statistical properties of the extended least squares estimation are well established. Thus NONMEM was chosen for use in the present study.

1.2.2.1 The NONMEM system

Sheiner and colleagues have extensively described the principles and mechanisms pertaining to NONMEM analysis (Sheiner *et al* 1977, Sheiner and Beal 1980). The programme analyses all the data simultaneously while taking into account the correlation among samples from the same individual. It is based on the premise that individual pharmacokinetic parameters arise from a random distribution, the first two statistical moments of which are directly estimated from the pool of individual data. The influence of patient covariates on drug handling can be assessed by incorporating regression relations of these to the pharmacokinetic parameters. The first-order method implemented in NONMEM estimates the inter-individual variability in the pharmacokinetic parameters not explained by the regression relations and the residual intra-individual variability in the drug concentrations.

The programme NONMEM (Beal and Sheiner 1992) is extensively used to estimate pharmacokinetic/pharmacodynamic parameters and has become an important tool in drug development and simulation studies (Jonsson 1998). Estimates for the PK parameters clearance and volume of distribution, as well as values for the PK/PD parameters, can be obtained. The programme models fixed (measurable) as well as

random effects and can determine which effects significantly influence the estimated PK parameters or the PK/PD measures. Dosing guidelines for specific patient population groups can be determined and the influence of disease states on the pharmacokinetics and the pharmacodynamics are identified. A step-wise model building procedure is followed to estimate the PK parameters and measures of drug effect. The analyst may use the available subroutines of the NONMEM programme or may construct his own subroutines.

1.2.2.1.1 The model building procedure

The NONMEM programme is based on the principle of extended least squares (ELS) as applied to a non-linear mixed effect statistical model. In this case the method of ELS is simply the maximum likelihood method. However, since most pharmacokinetic models to which it is applied are statistically non-linear, it is actually an approximate maximum likelihood method. Therefore the method can describe the time course of the amount of drug in the body using non-linear mixed effects models as follows (Beal and Sheiner 1992):

$$y_{ij} = f(x_{ij}, \phi_i) + \varepsilon_{ij}$$

Where y_{ij} is the j^{th} observation from the i^{th} individual, f() is the structural model that relates the independent variables, x_{ij}, such as time and dose, to the response given the i^{th} individuals vector of model parameters, ϕ_i. The random effects in the residual errors are denoted by ε_{ij}. This is usually assumed to be independently symmetrically distributed with a variance of σ^2.

The random effects (η_i) influencing the parameters ϕ_i, that is the parameter model, can be written as follows, assuming an additive structure:

$$\phi_i = g(z_i, \theta) + \eta_i$$

Where g is a structural, though non-kinetic type model that is a function of fixed effects z_i, and fixed effects parameter, θ.

Non-linear mixed effects models may be regarded as consisting of three sub-models:

i) The structural sub-model (PK or PD) which describes the main tendency in the data.

ii) The covariate sub-model which describes the relationships between the fixed effects parameters and the covariates.

iii) The statistical sub-model which includes the models for inter- and intra-individual variability.

Although these three sub-models overlap and also interact to some extent as shown by Wade *et al* (1994), each model is selected separately and the model building process usually proceeds in a step-wise fashion (Beal and Sheiner 1992, Mandema *et al* 1992, Ette and Ludden 1995).

At first the structural model is determined using the appropriate subroutines from the NONMEM PREDPP library, or a user-written subroutine. When analysing PK data the order in which the models are tested is usually defined by the number of compartments in the model. The difference in the objective function value (OFV) and plots of individual predictions versus observations as well as weighted residuals versus the

independent variable may be used to discriminate between rival structural models. The difference in the OFV approximates the χ^2 distribution.

Secondly, the covariate model is constructed. At this stage in the analysis of the data, a programme such as Xpose may be used to facilitate and expedite covariate identification. The Xpose programme[a] is an S-PLUS based model-building aid for population analysis using the NONMEM programme. It contains a stepwise-generalised additive modelling procedure (GAM) (Mandema *et al* 1992) which can be used to find a subset of the available covariates that could be most useful in explaining the variability in either CL or V. The building of the GAM is done using a stepwise multiple linear regression procedure allowing each covariate to enter the model in any of several functional representations. The model discrimination is made by comparison of the Akaike information criteria (AIC) (Jonsson and Karlsson 1997). The programme produces a plot of the 30 most important covariate models tried in a stepwise search for the final model. With this plot it is possible to evaluate how much better the final model is compared to the other models by evaluating the AIC. The AIC is the sum of the deviance and the product of the number of parameters and the dispersion factor:

$$AIC = D + p\phi$$

Where D = the deviance (the residual sum of squares), p = number of parameters and ϕ = dispersion factor.

In addition, the Bootstrap of the GAM can be used to assess the stability of covariate

[a] XPOSE 2.0 E.N. Jonsson and M.O. Karlsson "Xpose – an S-PLUS based model building aid for population analysis with NONMEM".

inclusion and the most common covariate combinations.

If the GAM or similar programme is not used to find the important covariates, a stepwise covariate model building procedure is followed. This process could be tedious and time-consuming as the covariates are added to the base model in turn and the change in OFV is noted. After selecting the most important single covariates, combination of these are then tested. To investigate which factors (covariates) might influence the population estimates of CL and V significantly, the weighted residual (WRES[b]) can be plotted against demographic and clinical data. If a clear trend in the plot is observed, it indicates that the factor might influence the pharmacokinetics of the drug and that it could be built into the population model. Stepwise selection depends very much on covariates selected in the early steps and on influential data points. Wade *et al* (1994) also pointed out the interaction between structural, statistical and covariate models and how the choice of the structural model may be affected by the choice of the covariate model and vice versa.

The alternative to the step-wise model building is the initial formation of a full model and then to reduce it to include only the relevant features. An advantage of this type of model building is that any change that is made to the model, will be representative of the possible true model. Disadvantages include the possibility that the data will not support the full model, and that computer run times could be very long (Jonsson 1998).

Thirdly, the inter-individual variability of CL and V, as well as the residual variability

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[b] WRES: The squared difference of the observed concentrations minus the predicted concentrations weighted by the reciprocal of the variance.

between the observed response and that predicted by the model, may be estimated according to a number of different error models. Residual variability is due to intra-patient variability such as differences in the timing of blood collections, drug assay errors, dosing times and model misspecification.

During initial data analysis, the default First Order method (FO) in NONMEM is usually used to estimate the typical values of the population parameters. The FO method makes expansions around the population average predicted value by using a first-term Taylor series expansion in the approximation. This expansion in eta takes place around eta equals to zero (Beal and Sheiner 1992). NONMEM can also obtain conditional estimates of eta variables as part of the computation of population parameter estimates. This is called the First Order Conditional Estimation Method (FOCE). This method is more accurate, but more time-consuming than the FO method (Beal and Sheiner 1992) and therefore not routinely used. When conditional estimates are obtained after estimation is carried out by the FO method, they are referred to as 'POSTHOC' estimates.

1.2.2.1.2 Inter-occasion variability

While building PK-models, it is important to investigate the possibility of inter-occasion variability (IOV). This is present when a parameter of the population model for example CL, varies within subjects between study occasions (Karlsson and Sheiner 1993). Failure to identify IOV, when present, may result in model misspecification, biased parameter estimates and false covariate relationships (Karlsson and Sheiner 1993). To account for and quantify IOV, Karlsson and Sheiner (1993) developed a new level of

random effects, π, between the inter-individual and the residual error variability. Thus to test for IOV a different type of eta is assigned to the parameter in question on each occasion studied. As the metabolic processes are changing continuously in the premature neonate as described above, the possibility of IOV should be investigated.

1.2.2.1.3 Assumption testing

Recently Karlsson *et al* (1998) discussed the problems associated with assumptions made during population pharmacokinetic modelling. As population PK and PK/PD models are now often used for simulation of clinical trials to optimise the design of the trials, a correct model as well as the acknowledgement of all assumptions made during the model building process is required. Most assumptions are related to the variability components of the population model. For example, a general assumption in population analyses is that the residual errors from all individuals arise from the same distribution. Assay imprecision was always given as the source of the residual error. It is now recognised that additional error sources such as model misspecification, imprecise dosing and sampling histories seem to be more important sources of variation (Jelliffe *et al* 1993, Jelliffe *et al* 1994, Karlsson *et al* 1995). The importance of assumptions should be recognised, as violations of these assumptions might have a major impact on the parameter estimates of the structural or covariate models. It is speculated that future population PK-programmes will automatically take care of most assumptions made during the model building process.

1.2.2.1.4 The importance of informative graphics

A well-designed graph or graphical technique is powerful diagnostics tool and may

assist the model builder to explore data effectively (Ette and Ludden 1995) and also to test assumptions (Karlsson *et al* 1998). Graphical displays can be used initially as exploratory data analysis to examine distributions and correlation between covariates. After a basic pharmacokinetic model has been constructed and Bayesian individual parameter estimates obtained, the distributions of the estimates of the parameters can be determined. Also, certain model building-aid programmes (such as Xpose) can graphically display the influence an individual or an observation has on the bulk of the data.

The goodness of fit of each NONMEM analysis can also be assessed by the visual examination of scatterplots. For example, predicted versus measured drug concentrations and weighted residuals may be plotted. The use of graphics is also extensively employed to test assumptions and the influence of assumptions on data analysis (Karlsson *et al* 1998).

1.2.2.1.5 Validation

The reliability of results obtained from population analysis depends on the quality of the data collected and on the correctness of the model building procedure. Providing evidence for the quality of the results is important for the application of the model in dose recommendations. Thus validation of the analysis is important and should be considered in any study. Validation can be defined as the evaluation of the predictive performance of the developed model and the model parameter estimates. Thus how close the model predictions are to the validation data. This may be judged in clinical rather

than statistical terms. A number of validation methods are available and are briefly discussed below.

a) External validation

This is a test of predictive accuracy of the developed population model on a new data set from another study. The objective of the validation is to determine how well the population model derived from an 'index' data set describes data, none of which was used to develop the model itself. In this case the data set is called the 'validation' or 'test' set. This is the most stringent validation test available at present. Often Bayesian predictions of serum concentrations are performed in an independent cohort of patients by fixing the structural and statistical parameters to the values obtained in the final model and invoking the POSTHOC function in the $ESTIMATION procedure without allowing NONMEM to iterate. Predictive performance in terms of bias and precision can then be calculated using a method developed by Sheiner and Beal (1981a) and described below.

b) Internal validation

i) Data splitting

Data splitting is an effective method when it is not practical to collect a new set of data to test the model. Thus usually about a third of the data is set aside for the test set. A disadvantage of this method is that the size of the data will be decreased, which may negatively affect the predictive accuracy of the model, as this is a function of the model size. Thomson *et al* (1996) in a population analysis of caffeine in neonates tried to overcome this disadvantage by combining the index and the test sets after validation of

the model to obtain the final parameter estimates. Although unbiased results were obtained when comparing the data sets, one of the covariates that had an influence on CL in the index set, could not be identified in the test set. The method of data splitting was also used by Lee *et al* (1996) in their population analysis of theophylline in neonates.

ii) Boot-strapping

This is another method of internal validation when no test data set is available. It has the advantage of using the entire data set that has been used for model development. In the bootstrap approach, sampling with replacement generates a large number of data points for example 200 bootstrap replicates of the original data set. It is of particular importance in the paediatric setting where ethical and medical concerns limit the number of individuals recruited into studies. Bootstrapping can be done using NONMEM by compiling it as a 'dynamic link library'.

iii) Jack-knifing

Population parameter estimates may be evaluated using the jack-knife technique. This technique involves a one-at-a-time omission and the creation of new data sets and then reanalysing them with NONMEM. A naïve Student t approximation for the standardised jack-knife estimator can be used. Ette (1997) calculated the magnitude of bias reduction as the reciprocal of the total number of blocks of individuals (10 individuals per block) omitted.

iv) Predictive performance using POSTHOC estimates of the final model as the "true" value

In the absence of a test data set the predictive performance in terms of bias and precision for the final and base model can be calculated using the population values and comparing these with the POSTHOC estimates of the final model (regarded as the "true" model) parameters (Sheiner and Beal 1981a). The prediction error is the difference between the predicted and the "true" value. This is a measure of bias and will show how well predictions match true values. The squared prediction error (or the absolute error) indicates the prediction precision. The smaller the prediction error the greater the precision of the model. The plausible range of these values is given by the Confidence Intervals of these values. The performance of models relative to each other may also be evaluated.

v) Posterior predictive check

This method has been described by Belin and Rubin (1995) and has been used by Girard *et al* (1998) for the validation of a compliance model. The purpose of this method is to simulate the posterior distribution of a non-sufficient statistic and to compare this distribution with the observed statistic on the actual data. If there is no contradiction between the two, the model may be accepted. Girard *et al* (1998) validated the compliance model by using either the longest drug holiday or the non-therapeutic coverage posterior distribution (the distribution of the percentage time during which the concentrations were within a therapeutic window). Unfortunately NONMEM does not give a posterior distribution of parameters. The software programmes POPKAN and PHARM-BUGS allow the defining of prior distributions of all parameters and thus the

estimation and computation of the posterior distributions of any statistic.

1.3 MEASUREMENT OF METHYLXANTHINE CONCENTRATIONS

A number of analytical methods for the determination of theophylline and caffeine in serum are available. These include spectrophotometry, gas-liquid chromatography, radioimmunoassay, high performance liquid chromatography and enzyme immunoassay. The various methods available differ in sensitivity, specificity, sample size needed, technical difficulty, amount of technician time required and initial equipment cost. The high performance liquid chromatography method remains the method of choice in laboratories whose goal is extreme accuracy. The enzyme multiplied immunoassay technique (EMIT) has become particularly popular as it is more rapid than most other conventional methods and also very accurate. Comparison of the EMIT® assay with high-performance liquid chromatography gave correlation coefficients of 0.95 to 0.98 (Syva Laboratories).

The equipment used for EMIT® is adaptable to processing large batches and the same equipment can be used for many other drugs. Commercial immunoassay methods for theophylline include the Syva EMIT® method the Abbott TDX® systems (fluorescence polarisation immunoassay).

An advantage of the EMIT® is that only a small volume of serum is necessary for the assay. This is particularly important in the premature neonate where only a very small sample of blood may be withdrawn at any time. A 200 μL serum sample is sufficient to

determine both theophylline and caffeine in duplicate. Thus the EMIT® Assay technique[c], which was also available locally, was selected for the assay.

1.4 OBJECTIVES

In view of the variable pharmacokinetics in the neonate and the likelihood that this will be especially problematic in the apnoeic premature neonate during the few days after birth, this part of the study aimed to determine the pharmacokinetic parameters, clearance and volume of distribution, for theophylline in premature neonates with apnoea during the first few days after birth. Because of the ethical constraints the population approach was used in the analysis.

[c] Syva Company, P.O.Box 10058, Palo Alto, California 94303.

SECTION A: CHAPTER 2

METHODS

2.1 THE PATIENTS

2.1.1 **Ethics approval and consent**

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Natal and was conducted in the Nursery of King Edward VIII Hospital Durban, South Africa. Informed written consent was obtained from the mother for each patient entered into the study (see Appendix A.1 for ethics approval and Appendix A.2 for the consent form).

2.1.2 **Inclusion and exclusion criteria**

Any premature neonate under two days old, for whom theophylline was prescribed to reduce neonatal apnoea, was eligible for the study. Exclusion criteria were congenital malformations and intra-ventricular haemorrhage of grade III or greater. Intra-ventricular haemorrhage was diagnosed and classified by the attending paediatrician and verified after an ultrasound scan.

2.1.3 **Demographic and clinical data**

The following demographic data was collected: birth weight, birth length, gestational age, postnatal age, and gender. Gestational age, if not available from sonar scans or menstrual dates, was estimated using the method described by Parkin *et al* (1976) and verified by comparison of birth weight, length and occipital frontal circumference on an

anthropometric chart which was developed by Lubchenco *et al* (1966). Body surface area (m^2) was calculated using the formula of Mosteller (1987) shown below:

$$BSA\ (m^2) = \sqrt{\frac{length(cm) \times weight(kg)}{3600}}$$

The following clinical data was captured and recorded: Apgar score at one minute and at five minutes, respiratory Apgar, and whether the neonate was asphyxiated at birth (determined by the attending paediatrician).

A daily record was kept of weight, development of neonatal jaundice or sepsis, presence of hypo- or hyperglycaemia, full blood count, values of urea and electrolytes, other drugs prescribed and all clinical interventions.

2.2 MEDICATION AND SERUM SAMPLING

2.2.1 **Drug administration**

Aminophylline[d] was administered through an IV-line and flushed in with 2 ml of normal saline over 2 minutes. Loading doses varied from 4 to 7.7 mg/kg. Maintenance doses ranged from 1.4 to 6 mg/kg per day and were given in two to four divided doses. All doses were determined by the physician in charge and not by any requirements of the study.

2.2.2 **Serum sample collection**

Accurately timed blood samples were collected approximately one hour after the loading dose. Thereafter, a sample was obtained each day if possible; these samples were drawn immediately prior to the next dose. A few samples were collected after the drug administration was stopped. All samples were immediately centrifuged and the resultant serum kept frozen at -70^0 C until analysis. Total serum theophylline concentrations were measured by Emit assay. The EMIT® assay is a homogenous enzyme immunoassay technique based on competition between the drug in the sample and drug labelled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) for antibody binding sites. The enzyme activity decreases upon binding to the antibody. Thus, the drug concentration in the sample can be measured in terms of enzyme activity. The active enzyme converts oxidised nicotinamide adenine dinucleotide (NAD) to the reduced form, NADH. This causes a change in the absorbance that is measured spectrophotometrically. Endogenous serum G6P-DH does not interfere, because the coenzyme functions only with the bacterial enzyme employed in the assay. The coefficient of variation is approximately 6% for both between run (7.5 mg/L) and within run (10 mg/L) measurements.

2.3 PHARMACOKINETIC ANALYSIS

Data analysis was performed using the computer package NONMEM version V level 1.0 double precision (Beal and Sheiner 1992). The analysis of the data set and the building of the models to describe the pharmacokinetic parameters were done in consecutive steps.

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[d] Sabax Aminophylline 250 mg/10ml, Adcock-Ingram,Sabax Rd, Isando, South Africa.

2.3.1 **The pharmacokinetic data**

2.3.1.1 The pharmacokinetic data file

A NONMEM data-file was constructed with the following parameters (abbreviation in parenthesis):

Patient number (ID)

Weight in kg (WGT)

Body surface area (BSA) m^2

Gestational age in weeks (GA)

Postnatal age (PNA) in days

Postconceptual (PCA) age in weeks

Gender (GEN) with males = 1 and females = 2

Apgar score at 1 minute (AP), and at 5 minutes (AQ)

Whether the neonate received respiratory support (OXY) at the time of sampling:

yes = 1 and no = 2

The time the sample was taken (TIME) in hours

The dose of theophylline in mg (AMT)

The serum theophylline concentration mg/L (DV)

Whether the mother received a corticosteroid before or during labour (DEX)

1 = yes, 2 = no.

The day i.e. day 0, 1, etc (DAY)

The route (RT): 1 = IV, 0 = none

The presence of neonatal jaundice (NNJ) at time of sampling: 1 = yes, 2 = no

(See Appendix A.3 for an example of the pharmacokinetic data file).

2.3.1.2 Data checkout

Initially the data was checked for outliers and encoding errors using the data checkout facility of the NONMEM programme.

2.3.2 **Model building**

2.3.2.1 The pharmacokinetic model

a) Selection of a one- or two compartment model

Initially one- and two compartment models were compared. For the one compartment model the ADVAN1 subroutine from the NONMEM PREDPP-library was implemented, using the TRANS2 subroutine to re-parameterise the models in terms of CL and V (Beal and Sheiner 1992). For the two compartment model the ADVAN2 and TRANS3 subroutines were used (see Appendix A.4 and A.5 for examples of the NONMEM control stream for the one and two compartment models respectively).

b) Selection of a bolus- or a rate model

Intravenous doses are usually modelled as bolus administration, but as theophylline was administered over two minutes a bolus administration model was compared with a rate model of drug administration. As the results of paragraph a) above indicated that a one compartment model is adequate to describe the data, a one compartment model was used for this comparison (see Appendix A.6 for an example of a control stream for the rate model).

c) Parallel first- and zero order elimination

The possibility of non-linear elimination was investigated by fitting the data to a control stream with parallel first and zero-order elimination (see Appendix A.7 for an example of a control stream for parallel first and zero-order elimination).

d) Construction of a base model

After the above initial analysis, a one compartment, bolus model with first-order elimination was used to construct a base model with no covariates on CL or V. Therefore:

$$CL = TVCL$$

$$V = TVV$$

Where TVCL and TVV are the typical values of CL and V respectively.

2.3.2.2 The statistical error models

Estimates for the inter-individual differences i.e. the deviations of the drug's CL_j and V_j of the jth individual from population mean values were estimated according to the following inter-patient error models:

Additive model: $CL_j = TVCL + \eta_j^{CL}$

Proportional model: $CL_j = TVCL * (1 + \eta_j^{CL})$

Exponential model: $CL_j = TVCL * EXP(\eta_j^{CL})$

Where CL_j is the estimate from the jth individual, and TVCL represented the population

mean estimate of CL. η_1 is a normally distributed random term with mean zero and variance ω^2. Similar models were used to describe the variability in V.

The residual error, which accounts for the difference between the observed concentrations and those predicted by the regression model, was modelled in five different ways:

Additive model:	$C_{ij} = C_{pred.ij} + \varepsilon_{ij}$
Proportional error:	$C_{ij} = C_{pred.ij} * (1+ \varepsilon_{ij})$
Combined error:	$C_{ij} = C_{pred.ij} + C_{pred.ij} * \varepsilon_{ij} + \varepsilon_{ij}$
Log model:	$\text{Log } C_{ij} = \text{Log } C_{pred.ij} + (\varepsilon_{ij} * \theta_3)$
Exponential model:	$C_{ij} = C_{predij} * \text{EXP } (ERR_1)$

Where C_{ij} was the observed and $C_{pred.ij}$ the predicted concentrations of the i^{th} individual at the j^{th} sampling time and θ_3 a factor estimated by the NONMEM programme.

The residual errors were assumed to be normally distributed with a mean of zero and a variance of σ^2. These differences (ε_{ij}) were attributable to intra-patient pharmacokinetic variability. Statistical model selection was based on assessment of goodness of fit, graphical analysis, evaluation of the size of the individual variability and the residual errors and the relative standard errors of the parameters.

2.3.2.3 The influence of possible outliers

The possibility of outliers was investigated as follows: Using the Xpose programme

(XPOSE 2.0), plots showing the Cooks distance versus leverage were constructed to determine the individual influence of the patients on the GAM fit. Cooks distance is a measure of the influence a certain data point has, that is, how much the fit will change if that data point is omitted from the analysis. A high value indicates a high influence. The leverage is a measure of how a data point influence the certainty with which the fit is obtained (Jonsson and Karlsson 1997). A point with a high value of Cooks distance and leverage is important to the fit and often affects the covariate selection. Thus if an individual point has a high leverage and influence on the GAM, this point will also be important for the covariate model in NONMEM (Jonsson 1998).

2.3.2.4 Covariate model building

The following covariates were available for testing WGT, BSA, GA, PNA, PCA, GEN, AP, AQ, OXY, DAY, NNJ, and DEX. The GAM in Xpose was used for the identification of covariates on CL and V (Jonsson and Karlsson 1997). Akaike plots were obtained of the most important models tested by the GAM with the corresponding AIC values. In addition, the bootstrap of the GAM was used to assess the importance of the covariates. Plots showing covariate inclusion frequency and most common covariate combinations were obtained.

Using NONMEM, the covariates selected by the GAM analysis were then tested, in a stepwise fashion, singly and in combination on CL and V, respectively. The covariates were built into the structural model in different ways:

(i) Linear model: $CL = \theta_1 + \theta_2 * \text{factor 1}$

$+ \theta_3 * \text{factor 2}$

or $CL = \theta_1 * \text{factor 1}$

Where the factors (1, 2, etc.) were continuous variables such as age, weight, body surface area etc., CL represented clearance and θ_1 .. θ_2 were the parameters to be estimated.

(ii) Nonlinear model: $CL = \theta_1 * (\text{factor or median of factor})^{\theta 2}$

Similar models were used for the estimation of V. Initially the best model for CL was determined, followed by the best model for V. Thereafter the best model for CL was combined with the best model for V. All covariate model building was performed using the default First Order estimation method (FO). Thereafter the base and final models were run using the first order conditional estimation method (FOCE).

2.3.2.5 Inter-occasion variability

The presence of inter-occasion variability (IOV) in CL and V was tested at the very end (Karlsson and Sheiner 1993). An 'occasion' was a different day, i.e. days 1, 2 and 3. All days after day 3 were grouped together as occasion 4 (see Appendix A.8 for the control stream for the final model).

2.3.2.6 Model evaluation

Successive models were evaluated and selected on the basis of:

(i) The differences in the objective function values (OFV). This value is minus twice the log likelihood of the data, approximately chi-squared (χ^2) distributed with q degrees of freedom equal to the difference in the number of parameters between the two models. A reduction in OFV of 6.8 ($p<0.01$) or greater was used.

(ii) Plots of DV vs PRED. These plots showed the distribution of the data and where the observations were lower or higher than the predictions.

(iii) The size of the relative standard errors of the estimates.

2.3.2.7 Predictive performance testing

As it was not possible to obtain enough patients for external validation, internal validation methods were used.

2.3.2.7.1 Predictive performance using POSTHOC estimates of the final model as the "true" value

The predictive performance in terms of bias (mean prediction error) and precision (mean squared prediction error) for the base and final models was calculated using the population values and comparing these with the POSTHOC estimates of the final model (regarded as the 'true' model) parameters (Sheiner and Beal 1981a).

To measure absolute bias, the following was calculated:

The difference in mean prediction error (Δme), thus

$$\Delta me = me_2 - me_1.$$

Where me_1 is the mean prediction error for the "true" model and me_2 is the mean prediction error for the base or final models. The percentage bias was calculated by dividing Δme by the mean value of the "true" model and multiplying the number by 100.

Absolute precision was calculated as follows:

Difference in mean squared prediction error (Δmse^2), thus

$$\Delta me^2 = me_2^2 - me_1^2.$$

Where mse_1^2 is the mean squared prediction error for the "true" model and mse_2^2 is the mean squared prediction error for the base or final models. The root mean squared prediction error was then calculated as $\sqrt{\Delta me^2}$. The percentage precision was calculated as above for bias.

The percentage standard errors and the corresponding 95% Confidence Intervals of the above parameters were calculated. If the Confidence Intervals derived did not overlap, the models were judged significantly different at the α level (0.05) used to compute the Confidence Intervals.

2.3.2.7.2 The Jack-knife

The stability of the developed pharmacokinetic models was also tested using the Jack-knife technique (Efron and Tibshirani 1993). This was done by leaving one patient out of the data set at a time and rerunning the final models with NONMEM. The resultant parameter estimates and their standard errors were noted and compared. If a patient has

a possible influence, the precision of the estimated parameters of the run without that specific patient will have a markedly different value compared with those of the other runs. Patients who might influence the results may thus be identified.

SECTION A: CHAPTER 3

RESULTS

3.1 THE PATIENTS

All the patients were Black premature neonates with apnoea. Demographic and clinical data are summarised in Table A.3.1 (see Appendix A.9 for an example of a data collection form).

TABLE A.3.1

Demographic and clinical data of the study population

Demographic and Clinical Data at Entry				
Patients = 105		Males = 52		
	Mean	SD	Median	Range
Birth weight (kg)	1.3	0.3	1.3	0.7 - 1.9
Gestational age (weeks)	30.8	1.8	31.0	26 - 34
*Postnatal age (days)	1.1	0.3	1.0	1 - 2
Postconceptual age (weeks)	31.0	1.8	31.1	26.1 - 34.1
Body surface area (m^2)	0.117	0.017	0.115	0.081 - 0.146
Apgar at 5 min	9	1	9	5 - 10

*Day of birth = day 1

Of the 105 patients, 97 (92%) had respiratory distress syndrome (RDS) at the time of serum sampling. Sixty-four, 59 and 48% of the neonates received oxygen support by headbox on days one, two and three respectively. The peripheral oxygen saturation was measured at intervals of 4 hours and was kept above 90%. Oxygen supply was removed

when the peripheral oxygen concentration was higher than 97%. Antenatal corticosteroids were given to 30% of the mothers. None of the mothers smoked or took caffeine-containing beverages during labour. Most of the neonates (96%) received beta-lactam and aminoglycoside antibiotic combinations for proven or suspected sepsis. See Appendix A.10 for frequency distribution graphs of the covariates such as patients' weight etc.

3.2 SERUM THEOPHYLLINE CONCENTRATION MEASUREMENTS

There were two hundred and sixty-three concentration measurements taken with a median of two samples per patient and a range of one to seven. See Figure A.3.1 for the frequency distribution of theophylline concentrations.

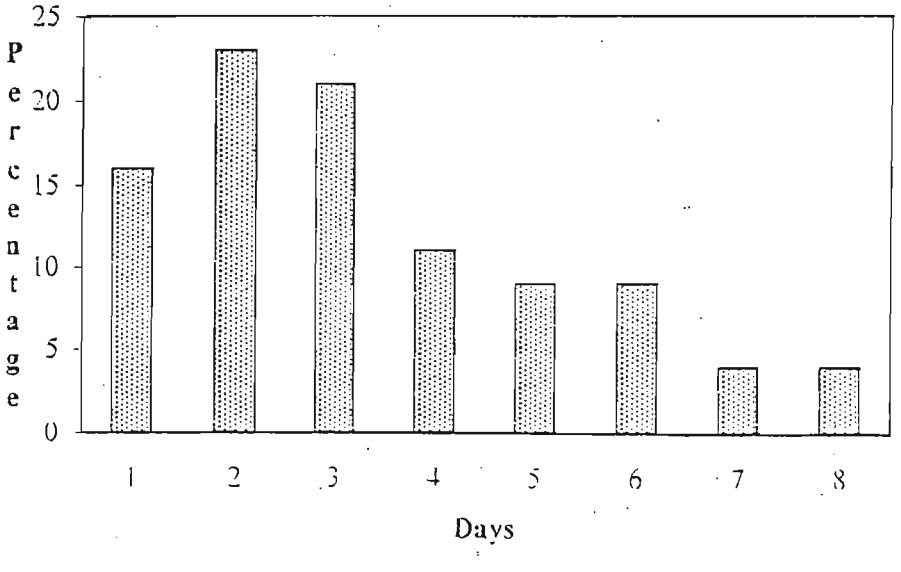

Fig.A.3.1 Frequency distribution of theophylline concentration measurements.

Of the theophylline measurements, approximately 60% and 94% were obtained in the first 3 days and within one week of birth respectively. Average (SD) serum theophylline concentrations for days 1, 2, and 3 were 5.6 (3.1), 7.5 (3.9), and 8.8 (5.7) mg/L respectively. Because of the long half-life of theophylline in neonates, very few of these samples could be expected to be steady state concentrations.

3.3 PHARMACOKINETIC ANALYSIS

3.3.1 **The pharmacokinetic data file**

The pharmacokinetic data file was constructed as described in Chapter 2 (see Appendix A.3 for an example of the pharmacokinetic data file). All errors indicated by the data checkout run were corrected on the data file.

3.3.2 **Model building**

3.3.2.1 The pharmacokinetic model

a) One- or two compartment model

As there was no significant difference in OFV between one- and two compartment models, the one compartment model was selected for further analysis. Previous studies of theophylline have shown that a one compartment model with first order absorption (Weinberger and Ginchansky 1977, Aranda *et al* 1981, Lee *et al* 1996) adequately describes the pharmacokinetics of the drug (see Table A.3.2 for results of the comparison of a one and two compartment model).

TABLE A.3.2

Results of a comparison of the one and two compartment models

Parameter	One compartment	Two compartment
OFV	1035.56	1034.52
CL (L/h)	0.0084	0.0078
V (L)	0.67	-
V1 (L)	-	0.00034
V2 (L)	-	0.574
Q (L/h)	-	0.1
Eta (CL)	83%	74%
Eta (V)	49%	-
Eta (V1)	-	260%
Eta (V2)	-	0.0004%
Eta (Q)	-	47%
Omega	32%	32%

OFV = Objective function value, CL = clearance, V = volume of distribution, V1 = central volume, V2 = peripheral volume, Q = inter-compartmental clearance, Eta = inter-individual variability, Omega = residual error.

b) Rate versus bolus model

The results of a comparison of a rate and a bolus model of theophylline administration showed no difference. The bolus model was thus chosen for further analysis (see Table A.3.3. for a comparison of the results of the analysis).

TABLE A.3.3

Results of a comparison of a rate and a bolus model (relative standard errors)

Parameter	Rate model	Bolus model
OFV	1035.56	1035.56
CL (L/h)	0.0084 (17%)	0.0084 (17%)
V (L)	0.66 (9%)	0.67 (9%)
Eta (CL)	83% (36%)	83% (36%)
Eta (V)	49% (23%)	49% (23%)
Rate	0.00017 (200%)	-
Residual error	32% (26%)	32% (26%)

OFV = Objective function value, CL = clearance, V = volume of distribution, Eta = inter-individual variability.

c) Parallel first- and zero-order elimination

The value obtained for the maximum elimination rate, V_m, was negligibly small. The first-order elimination model was selected for further analysis (see Table A.3.4 for results of parallel first- and zero-order elimination).

TABLE A.3.4

Results of the parallel first- and zero-order model.

Parameter	Value
OFV	1001.75
V (L)	0.721
K_{10} (h^{-1})	0.0104
V_m (mg/h)	1.38×10^{-11}
K_m (mg/L)	9.12

OFV = Objective function value, V = volume of distribution, K_{10} = elimination rate constant, V_m = maximum elimination rate, K_m = drug concentration at which metabolism proceeds at half its maximum rate.

Thus the basic pharmacokinetic model was a one compartment, bolus model with first order elimination (see Appendix A.4 for the control stream of the base model).

3.3.2.2 The statistical model

As both the additive and the proportional error models showed that certain estimated CL values were negative, inter-patient variability in CL and V were modelled as an exponential error model. The residual error was modelled as exponential, log, proportional, additive and a combination model. Inspection of frequency distribution graphs of weighted residuals of these various types of residual error models (prior to covariate inclusion) indicated that the exponential error model gave the more normal distribution and this was thus used for subsequent analysis. This was re-evaluated at the completion of the model building process, at which point the important covariates had been included (see Appendix A.11 for frequency distribution graphs of the various error models).

3.3.2.3 The influence of outliers

Graphs of the individual influence of patients on the GAM obtained by the Xpose programme, showed that 6 patients (numbers 9, 21, 86, 125, 140 and 142) might be outliers. This meant that their presence might influence the results of the pharmacokinetic analysis adversely (see Appendix A.12 and A.13 for the identification of influential individuals on the GAM fit for CL and V). Four of these patients (number 9, 86, 140 and 142) were on the extremes of the weight scale that could have an influence on CL and V. However, after inspection of the demographic and clinical data of these patients, no real reason could be found for the exclusion of these patients (see Appendix A.14 for demographic and clinical details of these patients). To determine the influence of each patient on the parameter estimation, they were deleted one at a time from the data file and the NONMEM base run was performed (see Appendix A.15 for

results of this NONMEM analysis). As shown in Appendix A.15 these patients did not have a significant influence on the NONMEM analysis. This confirmed their inclusion in the data set.

3.3.2.4 Covariate model building

The GAM on CL indicated that oxygen support and gestational age featured as significant covariates (see Appendix A.16 for the Akaike plots for CL). Additionally the bootstrap of the GAM showed that the most common two-covariate combinations for CL were oxygen support with gestational age or day, or day with gestational age or body surface area (see Appendix A.17 for the graphs of the most common covariate combinations for CL).

The GAM on V indicated that postconceptual age and gestational age were significant covariates (see Appendix A.18 for the Akaike plots for V). The bootstrap of the GAM showed that the most common two-covariate combinations for V were gestational age and postnatal age, neonatal jaundice and body surface area or postnatal age, and body surface area and postnatal age (see Appendix A.19 for the graphs of the most common covariate combinations for V).

Although not indicated by the GAM analysis, weight was also selected for testing on both CL and V. Because, besides being the most accurately and commonly measured covariate, it was also highly correlated (r^2 = 0.8) with both gestational age and postconceptual age (see Appendix A.20 for correlation matrix of the developmental covariates). In addition the presence or absence of antenatal corticosteroid therapy was

tested as a covariate.

3.3.2.4.1. Building the regression model for CL

At first the regression models for CL were built by keeping V constant, thus $V = \theta_2$. The influence of the covariates selected by the GAM, as well as weight, was investigated. Models incorporating each of these singly were constructed in a variety of ways: e.g. linear and non-linear functions (see page 47).

Weight was modelled as a continuous and as various exponential functions on CL. These exponential functions included the general model of $WGT^{0.75}$ as proposed by Holford (1996) and the specific value of $WGT^{1.28}$ found by Moore et al (1989). In addition an attempt was made to estimate an exponent on WGT. As the OFV of the WGT models did not differ significantly, various criteria were applied to assist in selecting the best WGT model. Visual inspection of the graphs, DV versus PRED, showed no obvious difference, but the relative standard errors of the $WGT^{0.75}$ model were marginally smaller than the other WGT models. Thus the $WGT^{0.75}$ model was selected for further model building. The other single covariate that decreased the OFV significantly was oxygen support. The presence or absence of antenatal corticosteroid therapy did not feature as a significant covariate.

Next the combinations of covariates on CL, as indicated by the bootstrap of the GAM, were tested in NONMEM. The resultant OFV of these combinations did not differ significantly from each other and decreased the OFV by approximately 20 points. This decrease in OFV was not significantly better than the decrease caused by the single

covariate, oxygen support. Therefore the best single covariates, as indicated by the NONMEM analysis, namely $WGT^{0.75}$ and oxygen support, were combined. When tested in NONMEM, this combination caused a larger decrease in the OFV than the other combinations indicated by the bootstrap of the GAM. The standard errors of the estimated parameters of this covariate combination were also slightly smaller than those of the other combinations. Thus the best covariate model for CL was $WGT^{0.75}$ plus oxygen support (full details of the model building process are shown in Appendix A.21).

3.3.2.4.2 Building regression model for V

Regression models for V were then developed in a similar fashion as for CL while keeping $CL = \theta_1$. At first the covariates selected by the GAM, postconceptual age and gestational age, were tested in NONMEM. Both caused a similar decrease in the OFV. For reasons mentioned above, WGT was then also tested. This covariate reduced the OFV to a greater extent than did postconceptual or gestational age and was thus selected as the best single covariate on V. Next, the combinations of covariates as indicated by the bootstrap of the GAM, were tested. In the NONMEM analysis all these combinations increased the OFV. Thus WGT, as a single covariate on V, was selected as the best covariate model for V (see Appendix A.21 for details of the model building process).

3.3.2.4.3 The full covariate model

The best models for CL and V were then combined with a resultant drop in OFV of 54 from the base model. As a result the full covariate models were:

$$\mathbf{CL\ (L/h) = \theta_1 * WGT^{0.75} * \theta_3}$$

$$\mathbf{V\ (L) = \theta_2 * WGT}$$

Where WGT = weight (kg) and θ_3 = with or without oxygen support.

Table A.3.5 summarises the main runs of the covariate model building process showing the base model, the covariate models on CL and V respectively, and the full covariate model. Full details of the model building process are shown in Appendix A.21.

TABLE A.3.5
Results of the covariate model building process showing selected models only and using the FO-method

CL Model	V Model	Parameter estimates (RSE %)			Eta1 (RSE %)	Eta2 (RSE %)	OFV	ΔOFV	Res error (RSE %)
		CL	V	θ_3					
θ_1	θ_2	0.0084 (17)	0.67 (9)	-	83 (36)	49 (23)	1035.56	-	32 (26)
θ_1*OXY	θ_2	0.0060 (22)	0.63 (9)	1.87 (22)	74 (41)	55 (24)	1019.52	16.1	29 (23)
θ_1*WGT **0.75	θ_2	0.0078 (17)	0.65 (9)	-	78 (35)	47 (25)	1024.02	11.6	32 (27)
θ_1*WGT **0.75*OXY	θ_2	0.0057 (21)	0.62 (10)	1.84 (20)	68 (37)	52 (23)	1007.12	28.48	30 (24)
θ_1	θ_2*WGT	0.0078 (13)	0.58 (7)	-	82 (34)	43 (28)	1000.62	35	31 (25)
θ_1*WGT **0.75*OXY	θ_2*WGT	0.0052 (20)	0.54 (8)	1.94 (21)	69 (38)	49 (29)	981.24	54.12	29 (24)

CL = clearance, V = volume of distribution, RSE = relative standard error, θ = parameter, Eta_1 = inter-individual variability CL, Eta_2 = inter-individual variability V, OFV = objective function value, ΔOFV = change in OFV,
Res error = residual error.

3.3.2.5 Finalisation of the full covariate model

a) FOCE-method

Parameter values appeared, in general, to be more precisely estimated when the FOCE estimation method of NONMEM was used. Therefore the base and full covariate models were next run with FOCE instead of the default FO-method.

b) Inter-occasion variability

Using FOCE, the inter-individual variability for CL, V and the residual variability were 60%, 53% and 29% respectively, which is relatively high (see Table A.3.5). As a result it was thought important to check for inter-occasion variability (IOV). Therefore implementing FOCE, the full covariate model was re-run with estimation of IOV. This resulted in the final model. On the introduction of IOV, the inter-individual variability for CL, V and the residual variability decreased to 56%, 47% and 16% respectively. The OFV decreased from 962.3 to 927.9. Parameter details for these models are presented in Table A.3.6. The relationship between the measured concentrations and those predicted for the base and the final models are shown in Figure A.3.2a and A.3.2b respectively.

TABLE A.3.6.

Parameter details for base, full covariate and final models (relative standard error %) using FOCE

	Base Model	Full Covariate Model (without IOV)	Final Model (with IOV)
θ_1	0.0074	0.0056	0.0060
	(16)	(19)	(14)
θ_3	-	1.61	1.47
		(23)	(18)
θ_2	0.75	0.58	0.63
	(9)	(8)	(7)
Inter-individual variability in CL (%)	73	60	56
	(27)	(29)	(25)
Inter-occasion variability in CL (%)	-	-	34
			(77)
Inter-individual variability in V (%)	57	53	47
	(20)	(23)	(28)
Inter-occasion variability in V (%)	-	-	35
			(29)
Residual error (%)	30	29	16
	(22)	(22)	(35)
OFV	1000.9	962.3	927.9

$\theta_3 = 1.0$ if no oxygen support.

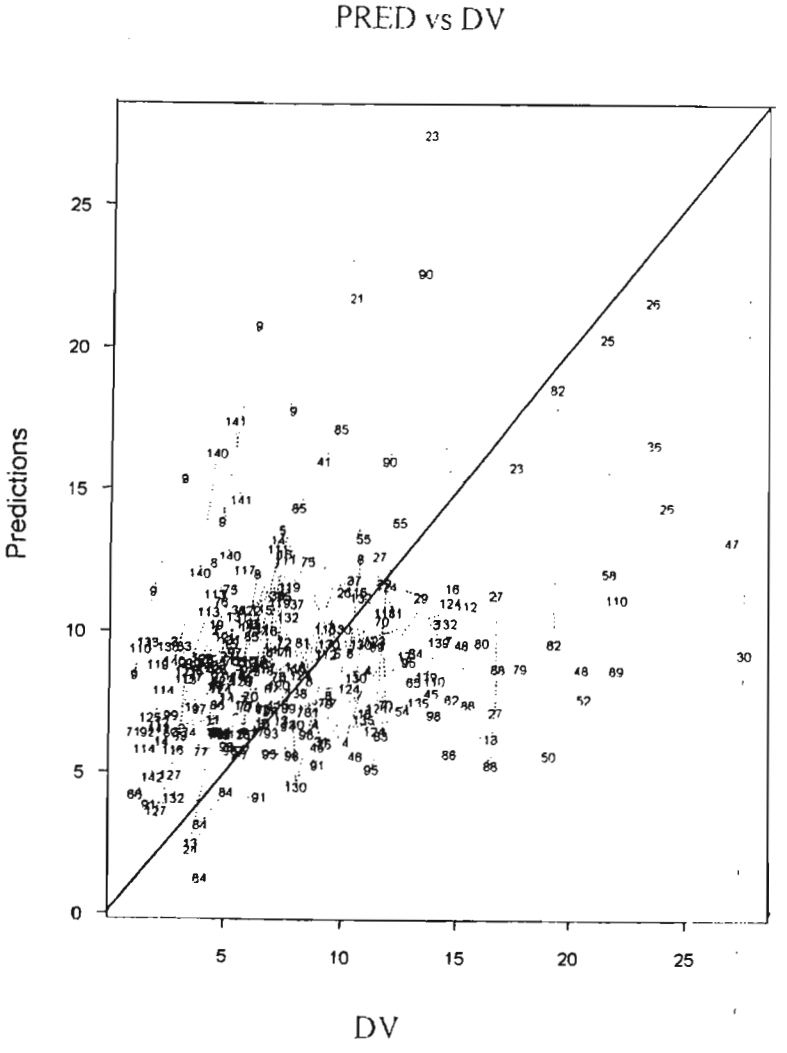

Fig.A.3.2a Predictions vs dependent variable for base model

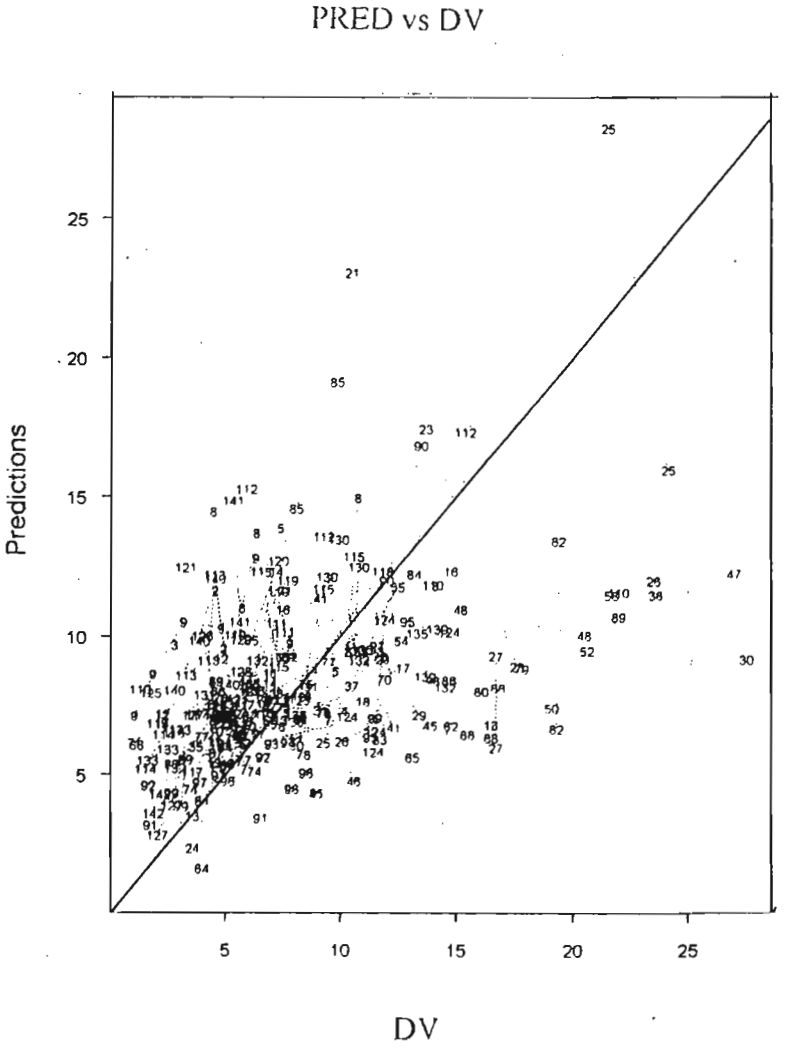

Fig.A.3.2b Predictions vs dependent variable for final model

3.3.2.6 Final pharmacokinetic values

Calculated using the final model, the mean values (95% Confidence Intervals) for CL in this population, were 0.0074 (0.0070, 0.0078) for neonates without oxygen support, and 0.0104 (0.0099, 0.0109) L/h for neonates receiving oxygen support. The mean V for the neonates was 0.81 (0.77, 0.85) L. This results in mean predicted population half-lives, without and with oxygen support, of 76 and 54 hours respectively. **For comparative purposes the weight normalised mean values for CL without and with oxygen support are 0.0056 and 0.0084 L/h/kg respectively; and for V 0.63 L/kg.**

3.3.2.7 Predictive performance testing

3.3.2.7.1 Bias and precision

The percentage bias (prediction error) and precision (squared prediction error) of the base and final models, when compared with the POSTHOC estimates of CL and V from the final model (taken as the "true value"), are given in Table A.3.7.

The results show that there is an improvement from the base to the final model in all categories. As the prediction errors of the final models were smaller than those of the base models, the precision of the final model is better than the base model.

3.3.2.7.2. Results of the Jack-knife

To check whether the developed pharmacokinetic models were stable, the Jack-knife technique was applied to the data set of the final model. The results, when each patient was left out one at a time, are shown in Appendix A22. When certain patients (3, 13, 27, 71, 82, 112, 114, 125 and 126) were left out of the data set the relative standard errors of

some of the parameter estimates increased. However, in no case did the parameter estimates change markedly. On inspection it was found that most of these individuals contributed 3 or more serum samples to the data set, or they had serum samples taken after the drug has been stopped. As a result the final pharmacokinetic model was considered not to be unduly dependent upon any particular individuals.

TABLE A.3.7

Percentage Bias and Precision (95% CI) for the Base and Final Models

CLEARANCE		
	Base Model	Final Model
Bias	24 (17, 31)	5 (0, 12)
Precision	51 (39, 58)	38 (29, 43)
VOLUME		
	Base Model	Final Model
Bias	14 (5, 22)	8 (0, 15)
Precision	52 (40, 58)	44 (35, 49)

SECTION A: CHAPTER 4

DISCUSSION

4.1 DISCUSSION

The present study differs from previous population pharmacokinetic analyses of theophylline in the premature neonate as it describes the pharmacokinetics of the drug during the first few days after birth. This is when theophylline is most often used for the treatment of apnoea of prematurity in our neonatal wards. Other unique features are the all Black population, and the high incidence of respiratory distress syndrome (92%).

The CL values normalised for weight, for neonates without and with oxygen support were 0.0056 and 0.0084 L/h/kg respectively. These values are lower than those reported in other NONMEM studies. For example, using the model by Lee *et al* (1996), CL for babies one day old would be 0.012 (± 0.00074) L/h/kg. Moore *et al* (1989) reported a CL value of 0.0175 (range 0.0155 to 0.0195) L/h/kg and Karlsson *et al* (1991) a CL/F of 0.040 (± 0.002) L/h/kg. The values obtained in the present study are closer to the lower end of the CL values reported in some of the traditional pharmacokinetic studies. In a traditional study with neonates with postnatal ages ranging from 4 to 8 days, which is only slightly older than the present study, the CL/F values ranged from 0.0063 to 0.0299 L/h/kg (Latini *et al* 1978). In another traditional study, using rectal data, the CL values ranged from 0.0043 to 0.0124 L/h/kg in neonates with postnatal ages ranging from 2 to 21 days (Neese and Soyka 1977). The value of V normalised for weight, 0.63 L/kg, is

also lower than that found in other population studies that reported a range from 0.8 to 0.9 L/kg (Moore *et al* 1989, Lee *et al* 1996). However, this lower value is well within the range (0.2 to 1.0 L/kg) recorded in some of the traditional pharmacokinetic studies with gestational- and postnatal-ages closer to those in the present study (Latini *et al* 1978, Stile *et al* 1986).

In the final model, weight was found to be an important determinant of CL and the $WGT^{0.75}$ (weight exponent of 0.75) model was accepted as the preferred weight model. This model was marginally better than the continuous weight model or the $WGT^{1.28}$ model proposed by Moore et al (1989). This 0.75 exponent model was based on the arguments by Peters (1983) who described the allometric ¾ power law as a good predictor of body functions. This concept was supported by subsequent work by Holford (1996) and Anderson *et al* (1997) who argued that clearances should be standardised using the ¾ power law. The results of the NONMEM analysis showed that the standard errors of the estimated parameters of the $WGT^{0.75}$ model were also smaller than the other weight models.

In the two other NONMEM studies of theophylline in neonates, weight was used as a continuous factor affecting CL (Karlsson *et al* 1991, Lee *et al* 1996). Weight as a covariate on CL in the neonate is important but complex, as the neonate loses weight during the first few days and then gains weight faster than any other type of patient.

In the present study postnatal age (range 1 to 9 days) did not have an influence on the estimation of CL. This correlates with the results of a traditional type analysis with a neonatal population with a similar small range (4 to 8 days) of postnatal ages (Latini *et al* 1978). However, in another traditional type analysis with a slightly wider range of postnatal ages (1 to 24 days), a weak but statistically significant correlation between CL and postnatal age ($p<0.005$) and postconceptual age ($p<0.01$) was found (Gilman *et al* (1986). In two of the published NONMEM studies of theophylline in neonates (Moore *et al* 1989, Lee *et al* 1996) postnatal age was one of the two important covariates on CL. In comparison with the present study, these other NONMEM studies had a much wider range of postnatal ages, namely from 1 to 111 days for the study by Lee *et al* (1996) and 3 to 182 days for the study by Moore *et al* (1989). Both Lee *et al* (1996) and Moore *et al* (1989) found that CL values increased steadily with age.

In premature neonates, renal clearance of theophylline is relatively more important than metabolic clearance. Approximately 50% of the drug is excreted unchanged compared to about 14% in children and adults (Tserng *et al* 1983, Baird-Lambert *et al* 1984, Kraus *et al* 1993). Theophylline clearance and urinary metabolite patterns apparently reach adult values at 55 weeks postconceptual age (Kraus *et al* 1993). Renal function could not be assessed in the present study and creatinine clearance could not be included as a candidate covariate for theophylline clearance, as it is not normally determined in our population.

In any event, creatinine excretion (Sertel and Scopes 1973) and glomerular filtration rate (Arant 1978, Robillard *et al* 1979) can vary considerably in the neonatal period.

Although it was at first believed to be a reflection of the mothers serum creatinine (Manzke *et al* 1980), it is now accepted that the higher plasma creatinine values in the neonate are due to the inability of the immature neonatal kidney to get rid of the excess creatinine (Bueva and Guignard 1994, van den Anker *et al* 1995). However, it was recently shown that creatinine reabsorption occurs along the renal tubule of the premature neonate (Guignard and Drukker 1999). The authors speculate that this temporary phenomenon is attributable to back-flow of creatinine across leaky immature tubular and vascular structures. Apparently, with time maturational changes will impose a barrier to creatinine reabsorption.

The possibility of renal tubular reabsorption of theophylline cannot be overlooked. It was previously shown that if a drug in the kidney is only filtered, then renal clearance might be calculated as $CL_R = f_u * GFR$ (page 16). If, for theophylline, the value for GFR is taken as 0.7 ml/min (range 0.6 to 0.8 ml/min), (van den Anker 1996) the fraction unbound theophylline as 0.7 (range 0.64 to 0.74), (Aranda *et al* 1976, Butts *et al* 1991), then the CL_R should be 0.49 ml/min or 0.0294 L/h. This is greater than the highest CL of 0.0104 L/h in this study. Therefore one might speculate that reabsorption of theophylline has occurred. This is supported by the finding of Guignard and Drukker (1999) who showed that creatinine is reabsorbed along the immature renal tubule of the premature neonate.

Glomerular filtration rate is lower than normal in infants with respiratory distress syndrome (Guignard *et al* 1976). This is a condition that is commonly found in the premature neonate during the first week of life due to the immaturity of lung tissue. The

low CL of theophylline found in the present study could also be due to the large number (92%) of neonates with respiratory distress syndrome at the time of sampling. It was previously shown that theophylline administration does not modify the course of respiratory distress syndrome (Hegyi *et al* 1986).

In the present study it was shown that neonates who received oxygen by headbox, cleared theophylline 47% faster than those who did not. Although some of the neonates may have been classified as suffering from asphyxia, it was not possible to record this accurately in our setting, and therefore asphyxia could not be tested as a covariate. It is known that hypoxia may decrease theophylline CL (Letarte and du Souich 1984, Kishimoto *et al* 1989, Richter and Lam 1993), and that CL is lower in asphyxiated neonates (Gal *et al* 1982, Gilman *et al* 1986). Gal *et al* (1982) originally reported a 46% lower CL in asphyxiated compared to non-asphyxiated neonates. In a subsequent study Gilman *et al* (1986) found a 19% lower CL in asphyxiated neonates. Asphyxia in these studies was defined as a 1- or 5-minute Apgar score of ≤ 3, a cardiac or respiratory arrest requiring resuscitation, apnoea longer than 1 minute requiring bag breathing, or an arterial oxygen pressure ≤ 3 torr. In the population study by Lee *et al* (1996), CL was reduced by about 10% in asphyxiated patients, but asphyxia as a covariate did not reduce the objective function value significantly. In the other population study by Moore *et al* (1989) asphyxia had no significant influence. However, it should be noted that in this study by Moore *et al* (1989), less than 10% of the serum samples were collected within the first week and any change in theophylline metabolism due to birth asphyxia may not have been detected.

Although it is now routine practice to administer corticosteroid treatment antenatally (Ryan and Finer 1995), only 31% of the mothers in the present study received a corticosteroid during labour. It is known that corticosteroids administered antenatally activate the hepatic microsomal metabolism of the neonate during the first week of life (Baird-Lambert *et al* 1984). However, theophylline CL value of these neonates was not different to those neonates whose mothers did not receive the drug. In children, the concomitant corticosteroid administration has also shown to be without influence on the clearance of theophylline (Leavengood *et al* 1983, Anderson *et al* 1984).

Karlsson and Sheiner (1993) reported that if inter-occasion variability is not recognised it may inflate inter-individual variability and/or residual variability. The results of the present study support this as the introduction of inter-occasion variability reduced inter-individual variability on both CL and V, and substantially decreased residual variability. The inter-occasion variability on CL and V were 34% and 35% respectively. An 'occasion' was taken as a day as theophylline sampling was done on consecutive days whenever possible. Therefore these values might reflect the changes due to the development of hepatic and renal function and the changes in body composition as well as changes in the clinical condition of the premature neonates.

Even after introduction of inter-occasion variability, the inter-patient variability in CL remained high at 56% for the final model, despite testing all the available covariates. This value is higher than those obtained in the other NONMEM studies. For example, Moore *et al* (1989), Karlsson *et al* (1991) and Lee *et al* (1996) reported inter-patient variability in CL of 16%, 25% and 30%, respectively. Various factors could account for

this large inter-individual variability in CL. The present study was conducted during the first few days after birth when the premature neonate undergoes profound changes to adapt from foetal to neonatal physiology (Behrman *et al* 1994, Lorenz *et al* 1995, Oliver *et al* 1995). All the other population studies followed the patients for longer periods of time thus allowing for maturation of the neonate and stabilisation of neonatal physiological processes such as elimination.

Some of the complicating factors that may contribute to the large inter-patient variability during the immediate time after birth, are the reabsorption of lung fluid (Behrman *et al* 1994) and the phase of spontaneous diuresis (Oliver *et al* 1995). The median age of onset of this diuresis is 24 hours in 87% of the patients with a cessation of diuresis at 96 hours median. Moreover, theophylline may also cause a diuresis. In neonates this effect will peak at 2 to 4 hours after a loading dose of 6 mg/kg and with the ratio of urinary output to water intake nearly doubled (Mazkereth *et al* 1997). However, this effect does not occur during maintenance therapy with theophylline in neonates. As sixteen percent of the theophylline measurements in the present study was made approximately an hour after the loading dose, the diuretic effect of theophylline could have affected the CL of the drug. Moreover, the second serum concentration measurement (24% of the total measurements) was done early the following day, thus during the phase of spontaneous diuresis that occurs in most but not all neonates (Oliver *et al* 1995). Another factor contributing to the large inter-patient variability is the possible reabsorption of theophylline previously mentioned that would also vary from patient to patient depending on the degree of renal maturity.

Also, during the immediate time after birth not all neonates are at the same stage of change. For example in the present study 20% of the patients had developed neonatal jaundice by day two, and another 30% from day three onwards. As lung function may also affect CL, the 26% variability of pulmonary mechanisms over the three days after birth (Goyal *et al* 1995) could also contribute to the variability found in CL. Another variable factor is respiratory distress syndrome. At entry to the study, the majority of patients suffered from respiratory distress syndrome. This usually resolves spontaneously, but slowly, after birth and therefore its time to resolution would have differed from patient to patient. This could have affected the glomerular filtration rate as discussed previously. These changes, as well as the different stages of maturity of the physiological and biochemical systems (Tserng *et al* 1981, Rylance 1992, Kraus *et al* 1993, Mazkereth *et al* 1997), may be responsible for much of the variation found in the estimation of CL. It could also perhaps be explained in part by the fact that the study was carried out in a very busy third world hospital where the prevailing circumstances, such as individual nursing care, ambient temperature etc, were sometimes variable. Despite attempts to ensure constant body temperatures of the patients during the study period, this was not always possible. It is known that very modest decreases in body temperature can affect the immature kidney causing significant decreases in urine flow, glomerular filtration rate and renal plasma flow (Guignard and Gilliéron 1997).

With regard to V, weight was found to be the most influential covariate. This supports the fact that in the neonate extra-cellular fluid volume is known to correlate better with weight than with gestational age (Aranda *et al* 1992). The inter-patient variability (47%) on V was high, but similar to that of 44% obtained by Lee *et al* (1996). The three phases

of fluid and electrolyte homeostasis (Lorenz *et al* 1995) and the shift of fluid from the extra- to the intra-cellular fluid compartments during the first week of life (Heimler *et al* 1990, Ramiro-Tolentino *et al* 1996), could all contribute to the high variability. Another possible contributing factor is the 50% increase in binding of theophylline to plasma proteins in the neonate from 28 to 40 weeks gestation (Reading *et al* 1990, Butts *et al* 1991).

The residual variability of 16% in the present study is similar to that of 14% recorded by Lee *et al* (1996) and within the range (9 to 25%, depending on concentration) found by Moore *et al* (1989).

Several limitations of the study have to be pointed out. Ideally more than one sample per patient should be obtained for accurate analysis of data (Jelliffe *et al* 1993, Aarons *et al* 1996, Jonsson *et al* 1996). Unfortunately this was not always possible as occasionally the neonate was too ill or permission for taking a sample was not granted. Thirty single serum samples were obtained out of a total of 263 samples from 105 patients.

It was assumed that an error free dosing history of theophylline was used for the analysis. Unfortunately not all the drug dosing and recording of the dosing times could be supervised at all times. However, the serum sampling times in the study were correct, as the researcher and assistant were responsible for these.

Under normal circumstances it is assumed that the covariate values are recorded/measured without error. The weights of the neonates were recorded whenever

possible, but we had to rely on the judgement of the clinicians for assessment of certain clinical conditions of the neonate such as hypotonia and asphyxia, as well as the Apgar 1- and Apgar 5 minute scores.

In the analysis of the data the assumption was made that the structural model was adequate and applied to all the subjects at all times. A one compartment first-order model was used, but non-linear kinetics have been described for theophylline especially at higher doses. Seventeen percent of the theophylline serum concentrations were higher than the suggested therapeutic range of 5 to 15 mg/L for the control of apnoea (Aranda *et al* 1992). Four percent of the serum concentrations were above 20 mg/L, which falls in the toxic range (Shannon *et al* 1975, Aranda *et al* 1992). Although parallel first-and zero-order elimination was tested and found deficient, saturation of some of the metabolic processes at the higher theophylline serum concentrations could have been possible.

It was assumed that the changes in individual parameter values between study occasions are random with a variability that is constant between occasions (Karlsson and Sheiner 1993, Karlsson *et al* 1998). However, if this variability is influenced by different underlying mechanisms, the variability will not be constant from occasion to occasion. This would be the case for premature neonates, with changing hepatic and renal function, changing lung function, gradual recovery from RDS, changing oxygen supply, developing and also recovery from neonatal jaundice, changing extra-cellular fluid volume and so forth.

An important feature of the development of pharmacokinetic models is the assessment of the model's predictive performance when applied to a separate but similar group of patients. Unfortunately we were unsuccessful in recruiting enough patients for a test data set. There are several reasons for this. For example, since the election of a democratic government, more suburban clinics were established and free medical care became available to all pregnant females. This improved prenatal care that resulted in more healthy term pregnancies. Therefore the number of patients admitted to the neonatal wards decreased. Also theophylline serum concentrations are not routinely monitored at the hospital due to lack of funds. Additionally, corticosteroids, which have been shown to improve lung function (Ryan and Finer 1995), are now routinely administered during labour. Moreover, the trend is to supplement oxygen using nasal prongs and prescribe the minimum of drugs. The data set was also not large enough to allow splitting into an index and a test set.

4.2 CONCLUSION

Clearance of theophylline in the premature neonate in the first few days after birth is low resulting in long half-lives. Small peak-to-trough fluctuations would be expected, even on once daily dosing. The estimated values of CL and V correlate better with those obtained in traditional studies with neonates of comparable postconceptual ages than with the results of the population studies that had a larger range of postconceptual ages.

The study confirms the high inter-individual variability in theophylline pharmacokinetics in the premature neonate, which is only partly explained by the contribution of inter-occasion variability. This makes it difficult to predict concentrations with the same

degree of accuracy as in other populations. The inter-occasion variability in CL of 34% is an indication of the variability in an individual that cannot be improved by therapeutic drug monitoring. The dramatic physiological changes in the neonate during the immediate time after birth, contribute to the higher variability recorded. Other confounding factors are the diuretic effect and the possibility of theophylline reabsorption, as well as the changing clinical condition of the premature neonate.

Therefore, serum concentration measurements should be used to determine the safety of administering additional loading doses of theophylline in the infant who fails to respond to initial therapy.

SECTION B

THE EFFICACY OF THEOPHYLLINE IN APNOEA AND HYPOXAEMIA

SECTION B: CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

"Neonatal apnoea represents the most common and probably most important disorder in the control of breathing in the newborn infant" (Aranda *et al* 1992). This statement aptly underlines the findings of the earlier research of Henderson-Smart (1981) who established that apnoea incidents of 20 seconds or longer occurred in 78% of infants born at 26 to 27 weeks gestation, 75% at 28 to 29 weeks and 54% at 30 to 31 weeks.

The most important physiological effects of apnoea are a deficiency of oxygen and the development of bradycardia. It has been suggested that a lack of oxygen might have severe long-term consequences and that it might be associated with poor neurodevelopment (Low *et al* 1993, Cheung *et al* 1996). Thus effective control of apnoea and of prolonged hypoxaemia in the neonate seems essential (Poets *et al* 1994, Poets *et al* 1995).

Pharmacological management of apnoea consists primarily of the administration of methylxanthines, theophylline or caffeine. Another respiratory stimulant, doxapram, is occasionally used as a second-line agent. Although theophylline is the drug that is most widely used, some researchers have questioned its efficacy. Moreover, the pharmacokinetic/pharmacodynamic relationship for theophylline in this population group

has been little studied. Therefore, this study investigated the efficacy and the concentration-effect relationship of theophylline in premature neonates with apnoea.

1.1 APNOEA AND RELATED CONDITIONS

1.1.1 **Definitions of apnoea**

Apnoea is defined as the intermittent absence of breathing, with hypoxaemia and bradycardia as major adverse effects (Aranda *et al* 1992). The definition of apnoea and other related and relevant pathophysiological conditions of the neonate have, over the years, been subjected to a wide range of interpretations and different researchers have used different definitions in their investigations. This may account for some of the divergent findings with respect to drug efficacy. Only in 1985 did the American Academy of Pediatrics Task Force on Prolonged Infantile Apnea, define pathologic apnoea as "a cessation of breathing for at least 20 seconds, or as a briefer episode of apnoea associated with bradycardia, cyanosis, or pallor" (American Academy of Pediatrics 1985). Two years later the American Academy of Pediatrics published a statement on the various definitions of infantile apnoea (Consensus Statement 1987). The definitions that have been used in this study are given in Table B.1.1 in summarised form.

1.1.2 **Aetiology of apnoea**

The aetiology of apnoea is unclear. A variety of factors and pathophysiological disorders are associated with the genesis of neonatal apnoea. Examples of such factors are: immaturity of the medullar centre, a depressed medullar centre, abnormal cortical

TABLE B.1.1

Clinical definitions used in this study

Term	Definition	Reference
Pathologic apnoea	A respiratory pause is abnormal if it is prolonged (≥20 seconds), or associated with cyanosis, abrupt, marked pallor or hypotonia, or bradycardia.	Consensus Statement, 1987
Apnoea	A cessation of respiratory airflow. The respiratory pause may be central or diaphragmatic (i.e., no respiratory effort), obstructive (usually due to upper airway obstruction), or mixed. Short central apnoea (<15 seconds) can be normal at all ages.	Consensus Statement, 1987
Periodic breathing	A breathing pattern in which there are three or more respiratory pauses of >3 seconds duration with <20 seconds of respiration between pauses. Periodic breathing can be a normal event.	Consensus Statement, 1987
Apnoea of prematurity	This is periodic breathing with pathologic apnoea in a premature infant. Apnoea of prematurity usually ceases by 37 weeks gestation (menstrual dating) but occasionally persists for several weeks past term.	Consensus Statement, 1987
Hypoxaemic episode	A fall in peripheral oxygen saturation ≥10 % from the previous baseline.	Based on alarm limits by Upton *et al*, 1991
Bradycardia	In premature neonates bradycardia is usually defined as a fall in heart rate below 100 beats per minute.	Hodgman *et al*, 1990
Cyanosis	The detection of cyanosis (or pallor) in premature neonates relies on the subjective assessment of the change in skin colour of the infant from pink or rosy to pale or pale-blue.	Samuels and Southall, 1993

input, airway obstruction, impaired central nervous system metabolism, low respiratory muscle function and hyperactive reflexes (Aranda *et al* 1992). Conditions such as infections, necrotising enterocolitis, hyper- or hypothermia, hypoxia, metabolic disorders, gastro-oesophageal reflux and the use of certain drugs by the mother, are also associated with apnoea (Eichenwald and Stark 1993). Factors associated with the development of neonatal apnoea are depicted in Figure B.1.1.

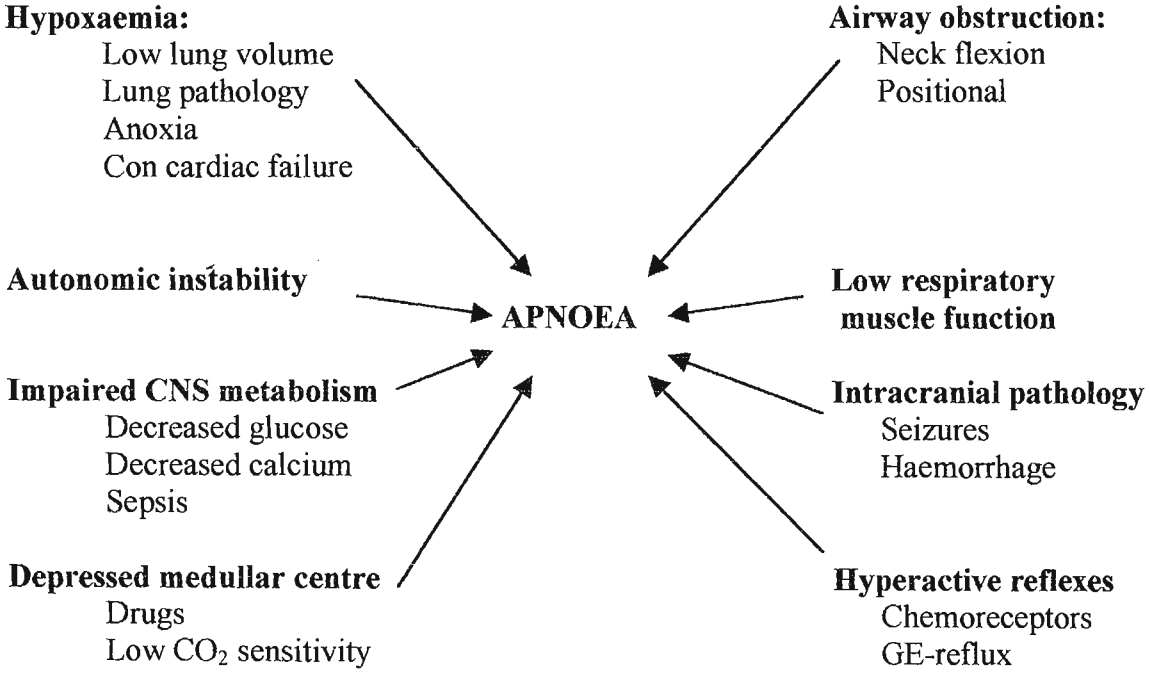

Fig. B.1.1 Factors associated with the development of neonatal apnoea (References: Aranda *et al* 1992, Eichenwald and Stark 1993).

At the molecular level it is known that the peripheral chemoreceptors play a major role in apnoea, as these are the only organs signalling hypoxia (Lahiri *et al* 1978). Thus failure of the satisfactory development of the chemoreceptors could contribute to respiratory disorders in the neonate (Lahiri 1994). This failure may reside in any of the steps involved in chemoreceptor regulation and response. This could be the initiation of oxygen-chemoreception involving respiratory and non-respiratory pigments (Duchen and Biscoe 1992, Buerk *et al* 1997), ion balance including hydrogen ions and calcium ions (Lahiri *et al* 1997), neurotransmitter mechanisms (Marchal *et al* 1992) and transduction (Higbee *et al* 1982).

In premature neonates, spells of apnoea usually begin during the first two days of life. If such spells do not occur in the first week of life, apnoea is unlikely to evolve later unless illness develops (Henderson-Smart 1981). Although apnoea attacks may persist after birth, for variable periods of time, they generally cease when the neonate has reached the postconceptual age of 37 weeks (Consensus Statement 1987, Eichenwald and Stark 1993).

1.1.3 **Definition of a hypoxaemic episode**

No consensus regarding the definition of a hypoxaemic episode has yet been reached. Arterial oxygen saturation of <80% (Jenni *et al* 1997), or <85% (Bolivar *et al* 1995), or ≤80% for ≥4 seconds (Poets *et al* 1993, Richard *et al* 1993), as well as a 5% (Finer *et al* 1992) or a 20% (Bucher and Duc 1988) fall in peripheral oxygen saturation from baseline, have been used. The limitations of the available equipment (giving a printout of the average value of the peripheral oxygen saturation over the previous ten seconds)

influenced the definition of a hypoxaemic episode as a fall in peripheral oxygen saturation of ≥10% from baseline, used in this study. The value of ≥10% fall in peripheral oxygen saturation is in accordance with the alarm limits for apnoea monitors suggested by Upton *et al* (1991).

1.1.4 **Aetiology of hypoxaemia**

Very little information on the aetiology of hypoxaemic episodes is available. In the premature neonate hypoxaemic episodes may occur associated with apnoea, or with bradycardia, or with apnoea and bradycardia, or not associated with either apnoea or bradycardia. The last-mentioned is referred to as *an isolated hypoxaemic episode*. Thus, isolated hypoxaemic episodes may occur despite both continued breathing movements and continuous airflow (Poets *et al* 1991). It has been shown that an active exhalation that produces a decrease in end-expiratory lung volume, which is followed by a decrease in tidal flow and volume, precedes episodes of hypoxaemia (Bolivar *et al* 1995). This reduction in lung volume, probably leading to the closure of small airways and the development of intra-pulmonary shunts, would explain the rapid development of hypoxaemia (Poets *et al* 1992, Samuels *et al* 1992, Southall *et al* 1993, Bolivar *et al* 1995). *Intrapulmonary shunts* is a collective term for all conditions in which deoxygenated venous blood passes through the pulmonary circulation into the systemic circulation without taking up oxygen. This could be due to ventilation-perfusion inequalities, anatomic right-to-left shunting in the lung, or blood flow through unventilated areas of the lung, or when gas diffusion is impaired (Poets *et al* 1992, Bolivar *et al* 1995). Another common cause of hypoxaemia in the neonate is periodic

breathing, which leads to low peripheral oxygen concentrations, causing alveolar hypoxia (Hunt *et al* 1996).

Many other factors, such as respiratory distress syndrome, local or central autonomic reflexes and others, are implicated in intrapulmonary shunting (Poets *et al* 1993). Only some of these causes can be prevented by clinical intervention or treated pharmacologically with drugs. See Figure B.1.2 for an illustration of the relationship between apnoea, intra-pulmonary shunts and hypoxaemia.

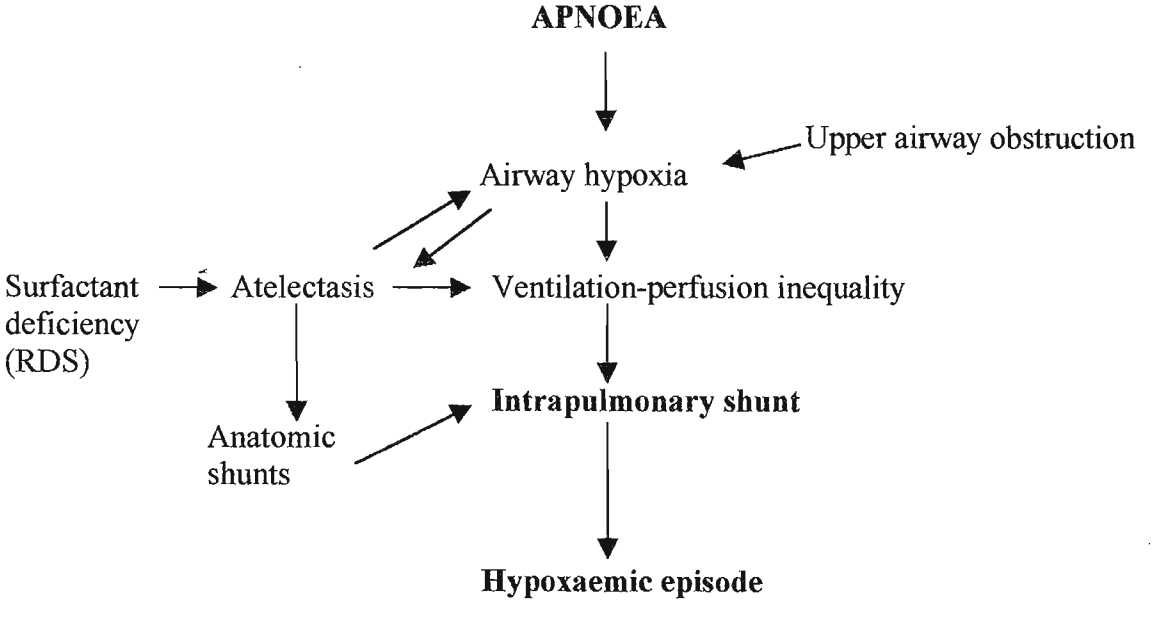

Fig. B 1.2. The relationship between apnoea and intrapulmonary shunts that may lead to hypoxaemic episodes (references: Poets *et al* 1992, Samuels *et al* 1992, Southall *et al* 1993, Bolivar *et al* 1995).

1.1.5. **Physiological and other consequences of apnoea and hypoxaemia**

The ventilatory consequences of apnoea are hypoxia and hypercarbia. These may produce derangement of central respiratory control, increased broncho-motor tone, depressed cardiac function and disturbed acid-base balance, which may all adversely affect the neonate (Miller and Martin 1992).

Finer *et al* (1992) showed that, regardless of the type of apnoea or treatment, oxygen saturation values decrease with an increase in duration of apnoea. A strong and very important relationship exists between apnoea and oxygenation (Samuels *et al* 1992, Southall *et al* 1993). Normally apnoea monitors detect most pathologic apnoea incidents by sounding an alarm after the neonate has stopped breathing for a number of seconds. These alarms are usually set at 20 seconds. However, depending on the type of equipment used and the availability of nursing staff, isolated hypoxaemic episodes may go unnoticed (Richard *et al* 1993, Poets *et al* 1995, Poets *et al* 1995a) and cause hypoxic injuries such as those described above by Miller and Martin (1992).

Additionally, hypoxaemia may have potentially harmful effects on early development (Poets *et al* 1993a, Poets *et al* 1994) and may even be associated with an increased risk of sudden death in premature neonates (Samuels *et al* 1992, Poets *et al* 1993a). Moderate grade hypoxaemia may also harm the function of the neonatal kidney that may affect the neonate adversely (Talosi *et al* 1996). The growing concern with hypoxaemia and its consequences is highlighted by the fact that some recent researchers tend to ignore the actual duration of the apnoea and rather concentrate on the duration and fall in oxygen saturation (Upton *et al* 1991, Bhandari *et al* 1992). Upton *et al* (1991) aptly

stated that "the degree of hypoxaemia is more important than the cessation of breathing per se". Other researchers who indicated that it would be more appropriate to measure oxygen saturation than count apnoea incidents in infants at risk also supported this view (Samuels *et al* 1992, Poets and Southall 1994).

The reflex effects of apnoea include changes in heart rate, blood pressure and pulse pressure. In the neonate, bradycardia usually occurs during apnoea as a response to decreasing oxygen saturation levels (Upton *et al* 1992). Bradycardia may result in a fall of anterior cerebral artery blood flow velocity that may lead to the development of periventricular leucomalacia (Perlman and Volpe 1985, Livera *et al* 1991). A close relationship between bradycardia, apnoeic pauses and oxygen desaturations has been demonstrated. For instance, 83% of bradycardia incidents are associated with apnoeic pauses and 86% of bradycardia incidents with oxygen desaturations (Poets *et al* 1993). Usually most bradycardia incidents commence after onset of fall in oxygen saturation (Poets *et al* 1993).

1.1.6 **Determination of apnoea and hypoxaemic episodes in the premature neonate**

The development since the 1970's of new techniques and tools to investigate breathing, contributed greatly to an understanding of the effects of apnoea on premature neonates. At first devices to pick up body movements associated with respiration were developed. These included apnoea mattresses, pressure sensitive devices that lie underneath the baby, pressure sensitive capsules attached to the abdominal skin and devices that measure change in the abdominal circumference. These devices had many disadvantages such as false positive alarms if the sensitivity is set too low, or failure to alarm if the

sensitivity is set too high and inability to distinguish between breathing and other body movements (Milner 1996).

With the development of electrodes to monitor transcutaneous oxygen pressure, researchers became aware of the previously unnoticed hypoxaemic episodes associated and not associated with apnoea. Development of the pulse oximeter refined the measurement of the oxygen concentration in the blood. These values could be recorded with electrodes attached to the finger or earlobe of an adult, or foot of a premature neonate. The continuous recording of plethysmographic pulse waveforms, peripheral oxygen saturation and breathing movements followed. This, in turn, led to the study of isolated hypoxaemic episodes in relation to apnoea (Poets *et al* 1991, Stebbens *et al* 1991), periodic breathing (Poets and Southall 1991), and bradycardia in the neonate (Poets *et al* 1993).

A number of monitors to record peripheral oxygen saturation, nasal airflow and heart rate in the neonate, are available. Examples are the Dinamap Oxytrack (Johnson and Johnson), the Spegas Neoset Neonatal Capnograph (Brittan Healthcare) and the Criticare Poet Te plus (Marcus Medical). These monitors differ in ease of operation, initial cost, reproducibility of recorded parameters and sensitivity. The Datex Oscar II capnograph was regarded as the most suitable for the requirements of the study as it gave a visual display of the essential parameters, a continuous plethysmograph, and it could also accommodate a data acquisition system. The following measurements could be recorded: peripheral oxygen saturation, breathing rate, pulse rate, carbon dioxide and nitrous oxide concentrations.

1.2 DRUG TREATMENT OF APNOEA AND HYPOXAEMIA

Only a few drugs are available for the treatment of apnoea of prematurity. They include the methylxanthines, theophylline and caffeine and the respiratory stimulant, doxapram (Aranda *et al* 1992). Other drugs being investigated are almitrine (Magny *et al* 1987) and the anti-epileptic, primidone (Miller *et al* 1992). The pharmacology of theophylline, which was used in this study, will be discussed in detail after a brief review of some of the other drugs mentioned above.

1.2.1 **Doxapram**

Doxapram, structurally unrelated to the methylxanthines, is an analeptic agent usually used as a respiratory stimulant in adults. The first study of doxapram in the treatment of apnoea of prematurity, was published in 1978 (Burnard *et al*). A number of studies followed, confirming the effectiveness of doxapram (Alpan *et al* 1984, Eyal *et al* 1985, Barrington *et al* 1986, Barrington *et al* 1987). Eyal *et al* (1985), in a double blind controlled study of 26 premature neonates, concluded that doxapram is as effective as aminophylline in abolishing apnoea spells. Effectiveness was judged by a reduction in frequency of apnoea during treatment. A full response was classified as complete cessation of apnoea, a partial response as a reduction of apnoea spells by more than 50% from pre-treatment frequency and failure as a reduction of less than 50% in apnoea frequency. In the doxapram group (n = 9) 66% of the neonates gave a full response and 22% failed. In the theophylline treated group (n = 7), 57% of the neonates gave a full response and 14% failed. The serum theophylline concentration was 16.1 ± 2.9 mg/L.

Barrington *et al* (1987), in a dose-finding study, used incremental doses of doxapram in a small group of premature neonates with idiopathic apnoea of prematurity refractory to therapeutic levels of theophylline. They defined a response as a ≥50% reduction in apnoea frequency. The optimum serum concentration for doxapram seemed to be about 2.9 mg/L (Barrington *et al* 1987). A loading dose of 2.5 to 3 mg/kg administered over 15 to 30 minutes followed by a continuous infusion of 1 mg/kg/h, with careful surveillance of blood pressure changes, are recommended (Aranda *et al* 1992). Adverse effects such as consistent increases in blood pressure as well as central nervous system irritability are frequent at serum concentrations above 5 mg/L (Barrington *et al* 1987). Another problem associated with doxapram use, is that the commercial preparations often contain benzyl-alcohol, a substance that is associated with the gasping syndrome in neonates (Jordan *et al* 1986).

Currently doxapram is used in cases where methylxanthines are not effective and before more aggressive forms of treatment such as mechanical ventilation are considered. It is also useful in weaning infants from ventilation, particularly if used in addition to theophylline (Samuels *et al* 1993).

1.2.2 **Investigational drugs**

1.2.2.1 Primidone

Recently it was shown that primidone is effective in neonates with apnoea resistant to theophylline treatment (Miller *et al* 1993). The dose used was 10 to 15 mg/kg per day and positive results were noticed 24 to 72 hours later. Although no toxic reactions were

observed, the authors warned against other possible effects related to the complex pharmacological characteristics of primidone.

1.2.2.2 Almitrine

Almitrine bismesylate is a triazine derivative that has a number of effects on respiration such as improvement of the hypoxic ventilatory response (Maxwell *et al* 1985) and ventilation-perfusion-matching in adults (Castaing *et al* 1986). Almitrine was shown to improve oxygenation in a small group of infants with bronchopulmonary dysplasia, but the response was highly variable, as were the serum concentrations of the drug (Magny *et al* 1987). The drug is being investigated for a possible role in bronchopulmonary dysplasia but is not yet available commercially.

1.2.3 **The methylxanthines**

The methylxanthines, theophylline, caffeine and theobromine, are naturally occurring alkaloids but only the first two are used in therapeutics. Theophylline is 1,3-dimethylxanthine and caffeine, 1,3,7-trimethylxanthine. The commonly used parenteral preparation of theophylline, aminophylline, is a water-soluble salt containing approximately 80% theophylline. The ability of caffeine to stimulate respiration has been known for a long time, but the potential role of theophylline to improve breathing in adult patients was first described in 1927 (cited by Aranda *et al* 1992). The role of these two drugs to control apnoea in the neonate was only recognised in the late 1970's and is discussed in more detail below.

1.2.3.1 The pharmacological mechanism of action of the methylxanthines

Several mechanisms have been proposed to account for the range of pharmacological effects of the methylxanthines. These include antagonism of adenosine, inhibition of phosphodiesterase (thereby increasing intra-cellular cyclic AMP), direct and indirect effects on intra-cellular calcium concentrations and interference with the intra-cellular translocation of calcium (Rall 1996). As theophylline was used in this investigation, its mode of action will be discussed in more detail.

Theophylline, at clinically relevant drug concentrations, may non-selectively relax smooth muscle in pulmonary arteries and airways by inhibition of phosphodiesterase isoenzymes, types III and IV, and thus increase the concentration of cyclic adenosine monophosphate (cAMP) (Schudt *et al* 1995, Banner and Page 1996). Apparently this effect is greater in the airways of asthmatics than in normal airways. This theory could explain why some of the early in vitro studies performed on non-asthmatic smooth muscle showed only weak phosphodiesterase activity (Polson *et al* 1978). It is not known whether relaxation of bronchial smooth muscle plays a role in the control of apnoea in the neonate, but it has been shown that theophylline can relax constricted bronchial smooth muscle of foetal lambs (Mehta *et al* 1991).

The recent findings of the anti-inflammatory properties of theophylline have resulted in a renewed interest in theophylline as an anti-inflammatory medication rather than as a bronchodilator (Vassallo and Lipsky 1998). Theophylline may act as an anti-inflammatory agent by numerous mechanisms of which the influence on cytokine production (Finnerty *et*

al 1996) and the late asthmatic response to antigen (Chung 1996) seem to be very important. It is not known whether similar effects occur in the neonate.

Theophylline has been shown to cause a statistically significant increase in adrenaline concentrations in healthy non-asthmatic subjects (Krzanowski and Polson 1988). The increase in cAMP production from adrenaline release and the decrease in cAMP breakdown from phoshodiesterase inhibition might result in a synergistic effect (Vassallo and Lipsky 1998). It is well known that cAMP acts as a second-messenger regulating many aspects of cellular function by activation of various protein kinases. This includes increased activity of voltage-activated calcium channels in heart muscle cells thereby increasing the force of contraction of the heart and improving circulation and oxygenation.

Theophylline may also improve the strength of muscle contractility, such as those of the diaphragm of neonates (Martin and Miller 1986). It is thought that theophylline acts through alterations in the calcium environment in the cells. The increase in muscle contractility is inhibited by calcium channel blockers and by removal of extracellular calcium, an indication that theophylline probably produces this effect through alterations of transmembrane calcium flux (Kolbeck and Speir 1989). Another possible effect of theophylline on calcium homeostasis is an interference with calcium release from the sarcoplasmic reticulum (Gayan-Ramirez *et al* 1995).

Theophylline blocks adenosine receptors (Lagercrantz *et al* 1984). Adenosine is a neuro-regulating substance released during hypoxaemia. It acts through A_1- and A_2-receptors, coupled respectively to inhibition and stimulation of adenylate cyclase (Ongini and

Fredholm 1996, Monin 1997). Theophylline's non-specific adenosine antagonism may be responsible for the increase in ventilation seen during hypoxia in adults and the decrease in diaphragmatic muscle fatigue (Barnes and Pauwels 1994), as well as the significant improvement in foetal breathing movements (Bissonnette *et al* 1990). Theophylline's effect on the A_1-receptors may be responsible for some of the drug's adverse effects such as the increase in psychomotor activity and heart rate (Barnes and Pauwels 1994).

However, the precise mechanism of action of theophylline in neonatal apnoea is still unknown. Gerhardt *et al* (1979) observed that theophylline decreased the incidence of apnoea, normalised minute ventilation and carbon dioxide pressure secondary to a 33% increase in oesophageal pressure change per breath. Theophylline administration shifted the position of the carbon dioxide curve to the left, but did not change the slope of the oxygen curve. The oxygen consumption of the neonates increased by 20% from the basal value (Gerhardt *et al* 1979). It was then postulated that apnoea in the neonate is related to an immature respiratory centre characterised by a decreased output. This was supported when it was shown that the ventral brainstem chemosensing function increases with post-conceptual age, and that a decreased sensitivity to carbon dioxide is found in premature, compared to full-term infants (Pan *et al* 1995). The role of theophylline is most probably to lower the threshold of the central chemoreceptors to carbon dioxide and thereby increase the output of the respiratory centre (Lahiri 1994). This suggested mechanism was later confirmed when it was shown that theophylline may enhance conduction along central auditory pathways and stimulate the regulatory effect on the respiratory centre of the brain stem (Chen *et al* 1994). Data from animal models suggest that this effect may be mediated through adenosine antagonism (Bissonnette *et al* 1990, Bissonnette *et al* 1991). Apnoea is

often triggered by hypoxaemia and it is possible that adenosine which is released during hypoxia, mediates this effect.

1.2.3.2 The pharmacological effects of methylxanthines on neonatal apnoea

Kuzemko and Paala (1973) first described the use of aminophylline in suppository form to treat apnoea in a group of ten neonates. Apnoea was recorded using mattresses with the apnoea alarm set at 30 seconds as well as nurses' recording of observed apnoea spells. Forty percent of the patients had no apnoea after theophylline treatment and another forty percent had 'very occasional' apnoea. One of the patients died due to hyaline membrane disease and pulmonary haemorrhage. These results prompted further studies of the potential role of theophylline in neonatal apnoea.

Fortunately recording of apnoea improved by using cardio-respiratory monitors and not only mattresses with apnoea alarms. The results of these early studies showed varying decreases in apnoea incidents after theophylline. Unfortunately comparison of results is difficult as no single definition of apnoea was used. Using a group of neonates (n = 7) with only apnoea of prematurity, Myers *et al* (1980) found an overall reduction of 62% in apnoea incidents (apnoea was defined as >15 seconds plus bradycardia). The serum theophylline concentration ranged between 2.8 and 3.9 mg/L. In a similar study (n = 11) an 81% reduction in apnoea incidents was recorded with serum theophylline concentration ranging from 1.5 to 15.3 mg/L (Milsap *et al* 1980). All the patients responded to therapy and one patient had no more apnoea after 48 hours of theophylline therapy. Finer *et al* (1984) recorded a 72% reduction of apnoea >10 seconds and only a

59% reduction in apnoea <10 seconds, in patients after seven days of theophylline treatment. No mention was made of how many patients did not respond to treatment.

The question arose whether theophylline would be effective in neonates with apnoea plus concomitant disorders that are common during the neonatal period. Results in a small group of patients (n = 10) with apnoea of prematurity as well as intra-ventricular haemorrhage (grade not specified) and/or hyaline membrane disease, found that 20% of the patients did not respond to therapy (Roberts *et al* 1982). The theophylline concentration was 7 to 13 mg/L. An overall reduction of only 58% in apnoea incidents was recorded. Apnoea was defined as cessation of breathing for ≥20 seconds or less if accompanied by bradycardia. Considerable patient variability was found and no correlation could be drawn between degree of response to theophylline, gestational age, postconceptual age, clinical diagnosis, or theophylline concentrations.

Non-responders to theophylline therapy were also observed in a group of neonates with apnoea of prematurity but no other disorders (Shannon *et al* 1975). In this study one of the eight patients with apnoea, 10 to 19 seconds in duration, showed an increase in apnoea incidents; the serum theophylline concentration ranged from 6.6 to 32 mg/L. In a small dose-effect study by Muttitt *et al* (1988), twenty-three percent of the patients (n = 22) were considered non-responders. A non-responder was a patient who had ≥0.33 significant apnoea per hour. Five patients did not respond even at the highest theophylline concentration (≥15.3 mg/L). These patients eventually required doxapram and/or continuous positive airway pressure (CPAP). Fourteen percent of the patients responded at a serum theophylline concentration of 4.2 mg/L, a further 14% at 8.5 mg/L,

then a further 45% at 12.7 mg/L and the remaining 4.5%, at 15.3 mg/L. These patients had apnoea of prematurity and no other disorders. Apnoea was defined as ≥20 seconds or less if accompanied by bradycardia and a 10% decrease in peripheral oxygen saturation.

In another study 27% of the patients (n = 60, gestational age 32.7 ± 0.4 weeks), after seven days of theophylline therapy, did not respond adequately. The author calculated the density of apnoea namely the percentage time spent in apnoea relative to the total monitoring time. An inadequate response was a density ≥3. These patients were then switched to caffeine therapy (Harrison 1992). Of these patients 12% did not respond to caffeine either. Serum theophylline concentrations were not given

Jones (1982) conducted a trial to compare the efficacy of theophylline with CPAP in the treatment of apnoea of prematurity. Although theophylline was found to be more effective than CPAP the response to both modes of treatment was 'disappointing'. Five of the 18 patients given theophylline needed intermittent positive pressure ventilation for apnoea compared with 12 of the 14 patients given CPAP, suggesting that theophylline treatment was better.

As it is often considered unethical to have a control group in a population at risk such as the premature neonate, only two studies to date used a control group to assess the efficacy of theophylline. In a study by Sims *et al* (1985) a definition of apnoea ≥20 seconds was used. On the first day a significant decrease in apnoea incidents was observed in both the control and the treated groups. Within one week 67% of the treated

and 33% of the control group, had no apnoea and this was considered as a significant difference. However, during the following week only another 17% of the treated but 33% of the control group registered no apnoea. After four weeks 17% of the treated and 20% of the control group continued to have apnoea. This difference was considered not significant and it was concluded that maturity played a significant role in decreasing the frequency of apnoea. Serum theophylline concentrations were between 9 and 13 mg/L.

A control group was also used by Merchant *et al* (1992) who administered theophylline prophylactically with the aim of preventing apnoea of prematurity. In the treated group 16% of the patients developed apnoea (defined as >15 seconds) with theophylline concentrations ranging from 5 to 40 mg/L. In the control group, 32% of the patients developed apnoea. See Table B.1.2 for a summary of studies of theophylline's effect on apnoea in neonates.

TABLE B.1.2 A SUMMARY OF THE EFFICACY OF THEOPHYLLINE IN NEONATAL APNOEA

Reference	Number, condition of patients	Gestational age (weeks) mean or range (SD)	Postnatal age (days) mean or range (SD)	Author's definition of apnoea	Theophylline dose and route of administration	Serum drug level mean and/or (range), and (SD) mg/L	Outcome of patients
Kuzemko et al 1973	10 RDS	26 - 34	1 - 6	>30 s with bradycardia and cyanosis	5 mg Rectally 6 hrly	Not measured.	1 died due to HMD and pulmonary haemorrhage. 4 had occasional apnoea spells and 4 had none after theophylline treatment
Shannon et al 1975	17 Apnoea	Not given	7	Pathological: >20 s Short apnoea 10-19s	4 mg/kg NG-tube 6 hrly	14.7 (6.6 - 32)	One patient had an increase in apnoea. Apnoea decreased in all the other patients.
Gerhardt et al 1978	14 Apnoea	30.2	1 - 21	>20 or < 20 with bradycardia (<100 bpm)	2 mg/kg IV 6 hrly	10.2 (0.7)	Apnoea decreased by 85%. All babies responded. No improvement in lung function. 20% increased tidal volume. Increased oxygen consumption and alveolar ventilation
Peabody et al 1978	10 Apnoea	27 - 34	2 - 10	≥ 15 s	Group1: 8 mg/kg 12 hrly for 2 doses Group 2: 8 mg/kg 12 hrly for 2 doses then 4 mg/kg for 8 doses. Rectal dosing	10 - 16	Apnoea decreased in all patients. Total duration of hypoxaemia decreased. No change in duration of apnoea episodes. Heart and respiratory rate more regular.
Myers et al 1980	7 Apnoea	28 - 34	1 - 21	1. >15 s and a HR <100 bpm 2. Also incidences >5 s	LD: 2.5 mg/kg MD:2 mg/kg/d Orally	2.8 - 3.9	After 1-2 days: 62 ± 9% reduction in incidences/hour. All neonates responded positively.
Milsap et al 1980	11 Apnoea	27 - 34	1 - 34	1. >15 s and a HR <100 bpm. 2. Also incidences >5 s were counted	LD: 2.5 mg/kg MD:2 mg/kg/d Orally	3.9 (0.2)	After 1-2 days: 81.4% reduction in incidences/hour. All neonates responded positively.
Jones 1982	32 Apnoea Compared theophylline (n=18) with CPAP (n=14)	25 - 32	1 - 28	≥10 s with bradycardia < 100 bpm or cyanosis	Oral choline theophyllinate 2.5 to 10.9 mg/kg or IV 5.1 to 7.8 mg/kg	Not recorded	5 of 18 (28%) theophylline treated babies needed intermittent positive pressure ventilation compared to 12 of 14 CPAP patients.

Table B.1.2 continued

Reference	Number, condition of patients	Gestational age (weeks) mean or range (SD)	Postnatal age (days) mean or range (SD)	Author's definition of apnoea	Theophylline dose and route of administration	Serum drug level mean and/or (range), and (SD) mg/L	Outcome of patients
Roberts et al 1982	10 Variety of disorders	26 - 34	1 - 30	≥20 s or less if HR = ≤ 100 bpm.	LD: 6 mg/kg MD:2 mg/kg 8 hrly Orally	6.6 - 13	No response in 20% of babies. 58% reduction in apnoea incidences. Total resolution of apnoea in 20% of babies.
Finer et al 1984	19 Apnoea	36.1 (5.1)	7 - 116	≥ 3 s	2.3 mg/kg 6 hrly	6 - 19	Significant reduction in number of apnoea; no significant reduction in falls of transcutaneous oxygen pressure, or number of bradycardia.
Brouard et al 1985	16 (8 per group) Apnoea	30.5 (0.4)	8 - 14	>10 s and HR< 80 for 30 s, or < 60 for 15 s Apnoea frequency = number/100 minutes	Theophylline: LD: 4.4 mg/kg IV MD: 0.64 - 2 mg/kg 8 hrly orally or IV Caffeine: LD: 10 mg/kg IM MD: 2.5 mg/kg/d orally	Theophylline: 4.5 - 6.8 Caffeine: 11.5 - 13	Day 1: Frequency decreased with theophylline from 1.02 to 0.12 and with caffeine from 1.42 to 0.13 Day 5: Frequency further reduced by theophylline to 0.06, and by caffeine to 0.07.
Sims et al 1985	43 Apnoea	31.4 (0.5)	2.5 (0.3)	20 s	LD: 6.8 mg/kg MD: 1.4 mg/kg 8 hrly or 6 hrly IV	Week 1: 10.0 (0.4) Week 2: 10.3 (0.3) Week 3: 11.7 (0.5)	No response in 17% of treated patients, and 21% of control patients. Total resolution by day 7: 67% in study group and 36% in control group.
Bairam et al 1987	20 Apnoea A double-blind study. Comparison of theophylline with caffeine.	30 (1.5)	6.2 (3.4)	>15 s and HR < 80 bpm. Apnoea incidences calculated as a factor of cardio-respiratory abnormalities/ 100 minutes.	LD: 6 mg/kg MD: 2 mg/kg 12 hrly IV Caffeine: LD: 10 mg/kg MD: 1.25 mg/kg 12 hrly	Theophylline 6 - 12 Caffeine 5 - 15	Cardio-respiratory abnormalities lowered by theophylline: By day 3: From 0.6 to 0.2 By day 7: to 0.3 Caffeine: By day 3: From 0.7 to 0.4 By day 7: 0.2.
Muttitt et al 1988	22 Apnoea	26 - 32	1 - 16.9	≥20 s plus 25% decrease in HR and 10% decrease in SpO_2. Incidences counted.	LD: 4 mg/kg IV - all four levels MD: 4 dosage groups 1; 1.5; 2; 2.5 mg/kg 8 hrly IV	Level 1: 4.2 Level 2: 8.5 Level 3: 12.7 Level 4: 15.3	No response in 23% of patients. Responded: At level 1: 14%. At level 2: A further 14% responded. At level 3: A further 45% responded. At level 4: The remaining 4.5% responded.

Table B.1.2 continued

Reference	Number, condition of patients	Gestational age (weeks) mean or range (SD)	Postnatal age (days) mean or range (SD)	Author's definition of apnoea	Theophylline dose and route of administration	Serum drug level mean and/or (range), and (SD) mg/L	Outcome of patients
Harrison 1992	60 Apnoea Initially on theophylline, if not sufficiently improved, caffeine was started.	32.7 (0.4)	19 (11)	≤19 s if HR <80 bpm for >2 s Shorter: 10-19 s Calculated densities: time spent in apnoea as a percentage of total monitoring time.	Density ≥ 5: Theophylline LD: 5 mg/kg MD: 1-1.5 mg/kg 8 hrly IV or orally When density still ≥ 3: Caffeine started LD: 10 mg/kg orally MD: 2.5 -3.75 mg/kg per day	Caffeine: 15 - 41.4	Theophylline: If density >5 treatment commenced. No response (density ≥ 3) in 27% of patients. 73% responded after day 2. A decrease in density from >5 to 0.4. Caffeine: No response in 12% of above 27%. 88% of above 27% responded; density = 0.8.
Merchant et al 1992	Group A: 56 Theophylline prophylactic. Group B: 25 in control group. Various disorders.	28 - 34	1	>15 s	Group A: 0.16 - 0.3 mg/kg/h infusion Group B: Control	5 - 40	Group A: 16% developed apnoea despite theophylline Group B: 32% developed apnoea Doxapram started in all patients who developed apnoea.
Scanlon et al 1992	36 Apnoea of prematurity	<31	2 - 12	No airflow plus stimulation of neonate and a fall in HR of ≥ 40 bpm. Incidences counted.	Group A (n =12): Caffeine LD: 12.5 mg/kg MD: 3 mg/kg/d Group B (n =12): Caffeine LD: 25 mg/kg MD: 6 mg/kg/d Group C(n =12): Theophylline LD: 7.5 mg/kg MD: 3 mg/kg 8 hrly Orally	Group A: 13 - 20 Group B: 26 - 40 Group C: 13 - 20	Group A: No response at all in 8% of patients 50% responded by day 1. 92% responded by day 2. Group B: 83% responded by day 1. 100% responded by day 2. Group C: 92% responded by day 1. 100% responded by day 2.

Abbreviations: RDS = respiratory distress syndrome, HMD = hyaline membrane disease, hrly = hourly, s = seconds, NG = naso-gastric, bpm = beats per minute, LD = loading dose, MD = maintenance dose, po = orally, IV = intravenous, IM = intramuscular, SpO_2 = peripheral oxygen saturation, CPAP = continuous positive airway pressure, PNA = postnatal age.

Aranda *et al* (1977) was the first to document the efficacy of caffeine, the other methylxanthine, in neonatal apnoea. Decreased incidents of apnoea, regularisation of breathing patterns, and an increase in alveolar ventilation was observed. Caffeine is usually given orally or intramuscularly but unfortunately a neonatal dosage form is not commercially available. The oral preparation is usually prepared by the resident pharmacist and often administered by naso-gastric tube. A loading dose of 10 mg/kg followed by daily maintenance doses of 2.5 mg/kg is recommended to prevent or decrease apnoea and increase respiratory drive (Aranda *et al* 1977, Rooklin 1989). No undesirable effects have been reported from this regimen and there appear to be no apparent adverse effects on the growth and development of infants given caffeine during the neonatal period. The target serum concentration of caffeine is 5 to 20 mg/L (Aranda *et al* 1992).

A number of clinical trials comparing the efficacy of theophylline with caffeine followed the initial research by Aranda *et al* (1977). No difference between the effects of theophylline and caffeine on neonatal respiratory function could be observed but caffeine seemed to have an earlier onset of action (Bairam *et al* 1987, Laubscher *et al* 1998). In a small group of neonates a 66% and 43% reduction in cardiorespiratory abnormalities (defined as apnoea ≥15 seconds, episodes of bradycardia <80 bpm, apnoea plus bradycardia <100 bpm) due to theophylline and caffeine respectively were found (Bairam *et al* 1987). In the study by Scanlon *et al* (1992) two different dosing regimens of caffeine were compared with a single dosing regimen of theophylline. In the 'usually' recommended dosing regimen for caffeine (i.e. a loading dose of 12.5 mg/kg and a maintenance dose of 3 mg/kg daily), 4 of the 12 patients responded to therapy within

eight hours. A response was regarded as a >50% reduction in number of apnoea over 8 hours. In the higher caffeine dosing regimen (loading dose of 25 mg/kg and maintenance dose of 6 mg/kg daily), 10 of the 12 patients (83%) responded within the first eight hours. In the theophylline group (loading dose 7.5 mg/kg and maintenance dose of 3 mg/kg three times daily) 11 of the 12 patients (92%) responded within the first eight hours. The serum theophylline concentration was 10.87 ± 3.52 mg/L. No failures were recorded in the theophylline and the higher caffeine group after 48 hours, whereas one patient in the lower caffeine group did not respond after 48 hours. In this study all patients with secondary apnoea were excluded. When Larsen *et al* (1995) compared theophylline and caffeine they found that 29% of the theophylline treated and 41% of the caffeine treated patients needed respiratory therapy some times during their ten-day treatment with either drug. The serum theophylline concentrations ranged between 4.8 and 16.8 mg/L and the caffeine concentrations between 9.6 and 23.8 mg/L.

Side effects such as tachycardia, arousal and gastrointestinal intolerance are more frequently observed with theophylline than with caffeine (Bairam *et al* 1987, Fuglsang *et al* 1989, Harrison 1992, Romagnoli *et al* 1992, Scanlon *et al* 1992, Larsen *et al* 1995). Caffeine seems to have a wider margin of safety than theophylline as no adverse effects are usually observed at concentrations as high as 50 mg/L (Aranda *et al* 1977).

In practice it seems that caffeine is preferred in the treatment of apnoea of prematurity (Aranda *et al* 1992). However, when bronchodilation is required, such as in patients with bronchopulmonary dysplasia, theophylline is still the drug of choice (Aranda *et al* 1992). Caffeine is not available in South Africa for neonatal use.

1.2.3.3 The pharmacological effects of the methylxanthines on hypoxaemic episodes in the neonate

Few studies mention an effect of the methylxanthines, beneficial or otherwise, on hypoxaemic episodes. One study with theophylline found a reduction in total duration of hypoxaemia and a significant reduction in the number of apnoea incidents after two days of drug administration. The number of apnoea as well as the duration of hypoxaemia increased forty-eight hours after withdrawal of the drug (Peabody *et al* 1978). In another study, Finer *et al* (1984) reported a significant reduction in apnoea incidents after 7 days of theophylline therapy (serum concentrations 6 to 19 mg/L), but no significant reduction in the number of falls of transcutaneous oxygen pressure greater than 3 mm (11.1 versus 7.2/h).

In a placebo-controlled trial, Bucher and Duc (1988) studied the preventative effect of caffeine by continuously monitoring transcutaneous oxygen tension and heart rate in premature neonates for fifty hours. They defined a hypoxaemic episode as a decrease in oxygen tension of 20% from the baseline within 20 seconds. They found no significant effect of caffeine on hypoxaemic episodes.

1.2.3.4 Other pharmacological effects of the methylxanthines in the neonate

Heart rate effects: In the above and other studies of theophylline and caffeine in neonates with apnoea, other pharmacological effects were observed. One of the most pronounced effects of these drugs is the ability to decrease the incidence of bradycardia that is often

associated with apnoea in the neonate (Shannon *et al* 1975, Aranda *et al* 1977, Howell *et al* 1981, Walther *et al* 1986, Bairam *et al* 1987, Nadkarni *et al* 1988).

Tachycardia is a common side effect of theophylline. Six of the 18 patients in the study by Jones (1982) had tachycardia >180 beats per minute (bpm); the serum theophylline concentrations were not mentioned. The effect of theophylline on heart rate seems to be inconsistent. Most results show an increase in heart rate, but reports of no significant effect on heart rate are also found. Finer *et al* (1984) studied premature neonates with gestational ages of 36.1 ± 5.1 weeks and postnatal ages 1 to 16.4 weeks. Theophylline was administered in doses of 2.3 mg/kg every six hours. Serum theophylline concentrations of 10.7 m/L (range 6 to 19 mg/L) were obtained. A significant decrease in the number of apnoea was found but the number of apnoea with bradycardia and bradycardia incidents without apnoea did not change significantly. This finding is supported in another study with comparable premature neonates (Muttitt *et al* 1988).

Respiratory effects: The ability of theophylline to stimulate the respiratory centre might lead to effects such as an increase in tidal volume, minute ventilation and respiratory timing (Muttitt *et al* 1988). The drug may also decrease the carbon dioxide threshold and increase carbon dioxide sensitivity in the neonate (Davi *et al* 1978). Gerhardt *et al* (1979) observed a parallel shift in the slope of the carbon dioxide response curve after theophylline administration. This is most probably due to an increase in ventilation (expired volume per minute) and a decrease in end-tidal carbon dioxide pressure, indicating a stimulating effect on the respiratory centre output. Theophylline may also cause respiration to become more regular in the neonate (Davi *et al* 1978, Weintraub *et al* 1992). In the foetus, it was

observed that maternal theophylline administration is associated with an increase in the incidence of foetal breathing movements (Ishikawa *et al* 1996). Theophylline and caffeine are also effective in apnoea that occurs during weaning from mechanical ventilation (Harris *et al* 1983, Viscardi *et al* 1985). The effect could be related to the improvement in respiratory muscle function and decreased pulmonary resistance (Blanchard *et al* 1987). Other effects in the neonate are the ability of theophylline to accelerate lung maturation (Karotkin *et al* 1976) and to influence surfactant production (Barrett *et al* 1978).

1.3 OBJECTIVES

As theophylline has been reported to have variable effects in the premature neonate with apnoea, and as its effect on hypoxaemic episodes is not at all clear, the objectives of Section B of this study were:

Firstly, to assess the response of premature neonates with apnoea to theophylline and to determine the effect of theophylline on hypoxaemic episodes; if indeed non-responders were identified, then predictors of non-response were to be determined; thirdly, the concentration of theophylline producing a response or toxicity was to be investigated.

SECTION B: CHAPTER 2

MATERIALS AND METHODS

2.1 THE PATIENTS

2.1.1 **Ethics approval and consent**

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Natal and was conducted in the Nursery of King Edward VIII Hospital. Informed written consent was obtained for each patient entered into the study. (See Appendix A.1 for ethics approval and Appendix A.2 for the consent form).

2.1.2 **Inclusion and exclusion criteria**

Any premature neonate under two days old, who was not receiving mechanical ventilation or continuous positive airway pressure support, and for whom theophylline was prescribed, was eligible for the study. The neonates were studied irrespective of concomitant diagnosis such as respiratory distress syndrome (RDS). Exclusion criteria were congenital malformations, intra-ventricular haemorrhage of grade III or greater. Intra-ventricular haemorrhage was diagnosed and classified by the attending paediatrician and verified after an ultrasound scan.

Before a patient was entered into the study, all identifiable causes of apnoea such as hypo-or hyperglycaemia, and hypothermia were addressed and corrected. Thus all the patients suffered from apnoea of prematurity and 96% had RDS.

2.1.3 **Demographic and clinical data**

The demographic and clinical data collected was similar to that of Section A. A daily record was kept of weight, development of neonatal jaundice or sepsis, presence of hypo- or hyperglycaemia, full blood count, values of urea and electrolytes, other drugs prescribed and all clinical interventions. The blood pressure and mean arterial pressure were recorded at regular intervals using a DINAMAP™ PLUS Vital Signs Monitor.[a] All data were captured on specially designed data sheets (see Appendix A.9 for an example of the data collection sheet).

2.2 DRUG ADMINISTRATION AND SERUM THEOPHYLLINE CONCENTRATION MEASUREMENTS

2.2.1 **Theophylline administration**

Aminophylline[b] was administered through an IV-line and flushed in with 2 ml of normal saline in over two minutes. The loading doses varied from 4 to 7.7 mg/kg. Maintenance doses ranged from 1.4 to 6 mg/kg per day and were given in two to four divided doses. All doses were determined by the physician in charge and not by any requirements of the study.

2.2.2 **Sample collection for serum drug concentration measurements**

Accurately timed blood samples were collected approximately one hour after the loading dose. Thereafter, a sample was obtained each day if possible in the morning during the pre-dose monitoring period. The samples were centrifuged immediately and the serum

[a] Critikon, Inc 4110 George Rd Tampa, Florida 33634.
[b] Sabax Aminophylline 250 mg/10ml, Adcock Ingram. Sabax Rd, Isando, South Africa.

kept frozen at -70^0 C until analysis. Total serum theophylline concentrations were measured by Emit Assay.[c] The coefficient of variation of the assay was approximately 6% for both between (7.5 mg/L) and within-run (10 mg/L) measurements.

2.3 RECORDING AND ANALYSIS OF CLINICAL EFFECTS

2.3.1 **Recording of clinical effects**

The neonates were monitored using a Datex Oscar II SC-123 Multigas Monitor (capnograph) and Pulse Oximeter[d] linked to a Datex Data Acquisition system. This is a combined airway gas monitor and pulse oximeter. It measured inspired and expired concentrations of carbon dioxide, nitrous oxide, saturation percent of haemoglobin, and respiratory and pulse rates. The monitor continuously pumped a small sample of the airway gas for analysis.

The accuracy of the peripheral oxygen saturation (SpO_2) measurement of the Datex Oscar II was % SpO_2 ± 1 standard deviation (SD), range: 100 to 80% ± 2 digits. Resolution was 1 digit (= 1%) and the measuring range 0 to 100%. The accuracy of the pulse rate measurement was ± 1%, ± 1 beat per minute and the range 30 to 250 beats per minute. The respiratory rate range was 4 to 60 breaths per minute with a detection of 1% (7.6 mm Hg) variation in carbon dioxide.

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[c] Syva Company, P.O. Box 10058, Palo Alto, California 94303.
[d] Datex interface cable from the Serial and Analog connector for data recording with a computer, Chicony 386, programmed to read the serial data output.

To record the peripheral oxygen saturation, the neonatal pulse oximeter electrodes were securely attached to the foot of the neonate and covered with a black sock to eliminate ambient light and possible interference with the signal. A neonatal sampling line taped in front of the nostrils, monitored airflow and respiratory rate. To obtain maximum sensitivity of airflow, a length of 150 cm of sampling line was selected as the most appropriate. The monitor drew a continuous gas sample from the sampling line for the measurement of carbon dioxide concentration. The respiratory rate was taken as the frequency of peak carbon dioxide measurements per minute. The values from the pulse oximeter were validated with the oxygen saturation of an arterial blood sample whenever such a sample was drawn for clinical purposes. The apnoea alarm setting was twenty seconds.

The neonates were monitored for as long as possible (usually 2 to 4 hours) before the loading dose of theophylline was given. These pre-dose recordings formed the baseline for analysis. After the loading dose, if possible, the neonates were again monitored for 2 to 4 hours and this period was termed day 1. The neonates were then monitored before the early morning maintenance dose of theophylline on the next two days when possible (day 2 and day 3). Monitoring of the neonates after the loading dose depended upon the clinical condition of the neonate.

All neonates were positioned to prevent neck flexion (Thach and Stark 1979). Oral secretions were removed whenever present (Ruggins and Milner 1991). All neonates were kept in the same position throughout the monitoring period, usually in the supine position. Handling and disturbance of the babies were kept to a minimum (Long *et al*

1980). If any neonate required oxygen during the monitoring period, then this supply was kept constant while monitoring. As a thermoregulated isolette was not always available, some of the neonates were monitored in an open cot. Therefore, the neonate's body temperature was measured in the morning before monitoring started and if necessary measures were taken to normalise the body temperature. As bolus feeding is known to cause apnoea, monitoring did not occur during or after a feed.

The following information was stored in a time ordered fashion on the computer by the Datex programme:

- Pulse rate per minute
- Volume percentage of inspired and expired carbon dioxide
- Respiratory rate per minute
- Percentage peripheral oxygen saturation, and
- Clock time in ten second intervals.

The monitor gave a beat to beat display of the above measures, a phlethysmographic pulse waveform, and a respiratory waveform in a breath-by-breath display. The instrument was calibrated each day before monitoring started. Monitoring was carried out at the same time each day to eliminate possible chronobiological influences.

In 1991, Ruggins pointed out the inadequacies of apnoea monitors, especially the problems of attachment, movement artefact, and the failure to detect obstructive apnoea. Therefore, a strict continuous manual record was kept of all incidents of cessation of breathing (shown on the video screen of the capnograph), any movements (Abu-Osba *et*

al 1982), mouth breathing, sneezes, yawning and any other interruptions and interventions. These were later used to confirm the computer printout. All apnoea events 20 seconds or longer were signalled by the monitoring system. It was possible to identify the pathologic apnoea events from the printout, and to confirm them using the manual recordings and the alarm. The printout only gave a ten second resolution, that is an average of the recordings per ten seconds. Thus, it was impossible to identify, from the printouts, the absolute length of the apnoea event, the precise change in oxygen saturation, pulse rate, and respiratory rate. The disadvantages of this system were overcome by using the manual recording as described above. Each patient was also used as his/her own control.

2.3.2 **Processing of recorded clinical effects**

A data file containing all the relevant information was constructed (see Appendix B.1 for an example of the data collection sheet for clinical effects).

From the printout and the manual recording the following clinical effects were calculated for each monitoring period and then averaged per hour, where appropriate:

- The total number of apnoea ≥5 seconds
- The number of pathologic apnoea incidents (cessation of nasal airflow ≥20 seconds)
- The number of pathologic apnoea associated with a hypoxaemic episode and a fall in pulse rate, or a fall in pulse rate only, or a hypoxaemic episode only

- The number of times the SpO_2 fell ≥10% for >10 seconds from the average over the previous uneventful minute (isolated hypoxaemic episode)
- The number of hypoxaemic episodes associated with movement, or pathologic apnoea, or a pathologic apnoea with a ≥20% fall in pulse rate

  "*Associated with" was defined as an event that occurred within 30 seconds of the onset of the hypoxaemic episode*
- The average SpO_2
- The number of times the pulse rate fell below 100 beats per minute (incidents of bradycardia)
- The average pulse rate
- Apnoea densities, for pathological and total apnoea, were calculated as the percentage of the time spent in apnoea compared with total monitoring time.

2.3.3 **Data analysis**

a) Statistical analysis

The mean, median and standard error values of all the recorded clinical effects on the data file as described above were calculated and tabulated. The Wilcoxon Sign Rank Test with a Bonferroni correction was used to compare the differences from baseline to days 1, 2, and 3 of all the recorded clinical effects. A probability value ≤0.017 indicated significance. All changes were noted with the serum theophylline concentration.

b) Exploratory graphical analysis

A graph of each patient's response of total apnoea per hour (i.e. ≥5 seconds) and total hypoxaemic episodes per hour (a fall in SpO_2 of ≥10% for >10 seconds) to theophylline therapy from baseline to day three with the measured serum theophylline concentrations was constructed. These graphs were visually analysed and compared.

c) Classification of patients as responders and non-responders

Each patient's response to pathologic apnoea (≥20 seconds), total apnoea and hypoxaemic episodes were separately analysed and the patient was then classified as a 'responder' or a 'non-responder' for that specific clinical effect. A 'responder' was classified as a neonate with ≥50% reduction in apnoea count (for pathologic as well as total apnoea) from baseline to the last recording of apnoea incidents. This is in accordance with the definition of a 'response' to theophylline therapy in apnoea used by Barrington *et al* (1987) and Scanlon *et al* (1992). The author used a similar ≥50% reduction in hypoxaemic episodes to classify patients as 'responders' and 'non-responders' to theophylline therapy.

SECTION B: CHAPTER 3

RESULTS

3.1 THE PATIENTS

The patients were all Black premature neonates who were prescribed theophylline for apnoea of prematurity. The demographic details are shown in Table B.3.1.

TABLE B.3.1.

Demographic and clinical data of patients on entry

Patients = 46			Males = 24	
	Median	Range	Average	SD
Birth weight (kg)	1.3	0.8 - 1.8	1.3	0.2
Gestational age (weeks)	30	28 - 34	30.6	1.3
Postnatal age (days)	1	1 - 2	1.1	0.3
Body surface area (m^2)	0.114	0.08 - 0.15	0.114	0.015
Haemoglobin (g/dL)	15.6	11 - 22.5	16.4	2.8
Apgar 1 minute	7	2 - 9	6	2
Apgar 5 minutes	9	4 - 10	9	1

Baseline recordings of apnoea, hypoxaemic episodes, pulse rate and other relevant clinical signs and symptoms were obtained from 46 neonates before the loading dose of

theophylline was given. These 46 patients formed part of the 105 patients used for the pharmacokinetic analysis described in Section A. Monitoring for baseline recordings started within 6.4 (± 5.8) hours after birth as soon as the patient was stabilised, the body temperature and other clinical signs satisfactory and informed consent obtained from the mother. Accurate time of birth was available for only 35 patients as some were born before the mother's arrival at the hospital.

Forty-four patients had mild-to-moderate respiratory distress syndrome diagnosed clinically and verified by X-ray examination. Thirteen neonates were born by caesarean section, and 12 were one of a set of twins. Most (96%) of the neonates received penicillin and an aminoglycoside for proven or suspected sepsis. Only 2 presented with a positive bacterial culture after the third day of the study. The appropriate antibiotics were then prescribed. Thirty-three percent of the mothers received antenatal corticosteroids. None of the mothers smoked and no caffeine containing beverages were ingested shortly before or during labour.

3.2 PATIENT MONITORING, DRUG ADMINISTRATION AND SERUM THEOPHYLLINE CONCENTRATION MEASUREMENTS

All the patients (46) were monitored before the loading dose of theophylline and this recording formed the baseline. Approximately an hour after the loading dose of theophylline was given, all the clinical effects were again recorded and this recording was termed 'day 1'. Only those patients whose clinical condition (determined by the attending physician) was satisfactory were monitored after the loading dose. The following day before the early maintenance dose of theophylline was given, recording of

the clinical effects for 'day 2' took place. Again only the patients whose clinical condition was satisfactory were monitored. Fortunately, all the patients who could not be monitored after the loading dose ('day 1') were then monitored ('day 2'). Recording of the clinical effects for 'day 3' occurred the next day at approximately the same clock time as 'day 2'. Figure B.3.1 illustrates when monitoring of the patients took place and the number of total apnoea per hour (that is, all apnoea ≥5 seconds) recorded at those times. The baseline recordings are shown on the y-axis at 0 hours. The recordings for 'day 1' follow within an hour of the baseline recordings, while the recordings for 'day 2' and 'day 3' follow about 20 hours and 40 to 49 hours after baseline. (See Appendix B.2 for the event types and incidences of the clinical effects from baseline to day 3).

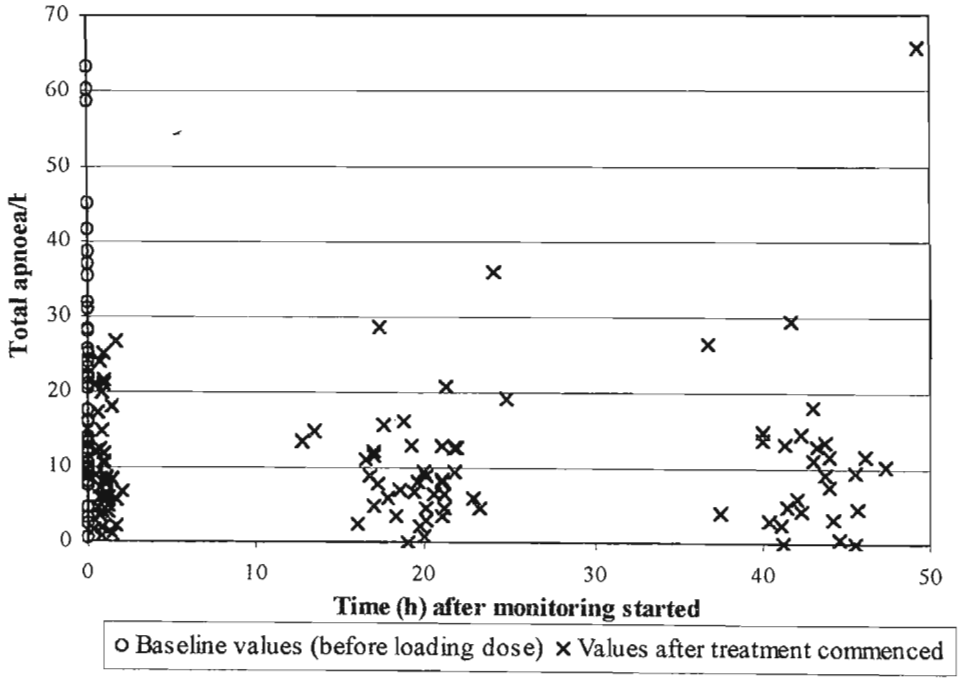

Fig.B.3.1 Scatter of number of total apnoea per hour recorded at the different monitoring times

Thirty-nine of the initial 46 neonates were monitored after the loading dose of theophylline was given. Thirty-nine neonates were monitored before the early morning maintenance dose of theophylline on the next day, 'day 2' and 29 neonates were monitored on day 3. As mentioned above, not all the neonates could be monitored on all the days due to deterioration in the condition of the neonate requiring positive ventilation. Seven neonates requiring ventilation from day 2 were then excluded from the study. Two of these died: one due to laryngomalacia and the other due to pneumothorax. Seven other neonates died subsequent to the 3 days of monitoring. Causes of death were extreme prematurity (3) and pneumonia (4).

There were 108 theophylline serum concentration measurements with a median of 2 and a range of 1 to 3 per patient. Average (SD) serum theophylline concentrations for days one, two, and three, were 5.8 (± 3.9), 8.8 (± 7.5), and 8.8 (± 5.7) mg/L respectively. The average caffeine concentrations on the three days were 0.2 (± 0.3), 0.5 (± 1.0) and 0.7 (± 1.0) mg/L respectively. As these concentrations of caffeine were low, the possible effect of caffeine was not considered in further analysis.

3.3 RECORDED CLINICAL EFFECTS

For clarity the recorded clinical effects are divided and presented in 5 major sections, namely total apnoea, pathologic apnoea, hypoxaemic episodes, heart rate and finally mean arterial pressure and peripheral oxygen concentration.

3.3.1 **Total apnoea (≥5 seconds) incidents and density**

3.3.1.1 Recorded total apnoea

Total apnoea included all apnoea ≥5 seconds. Baseline total apnoea recordings were obtained from 46 patients. Recordings at day 1, day 2 and day 3 were obtained from 39, 39 and 29 patients respectively.

A scattergram of the number of total apnoea per hour versus serum theophylline concentration recorded from baseline to day 3 is shown in Figure B.3.2. This gives an indication of the spread of the number of apnoea counts over the three days measured against the serum theophylline concentrations recorded at the same time.

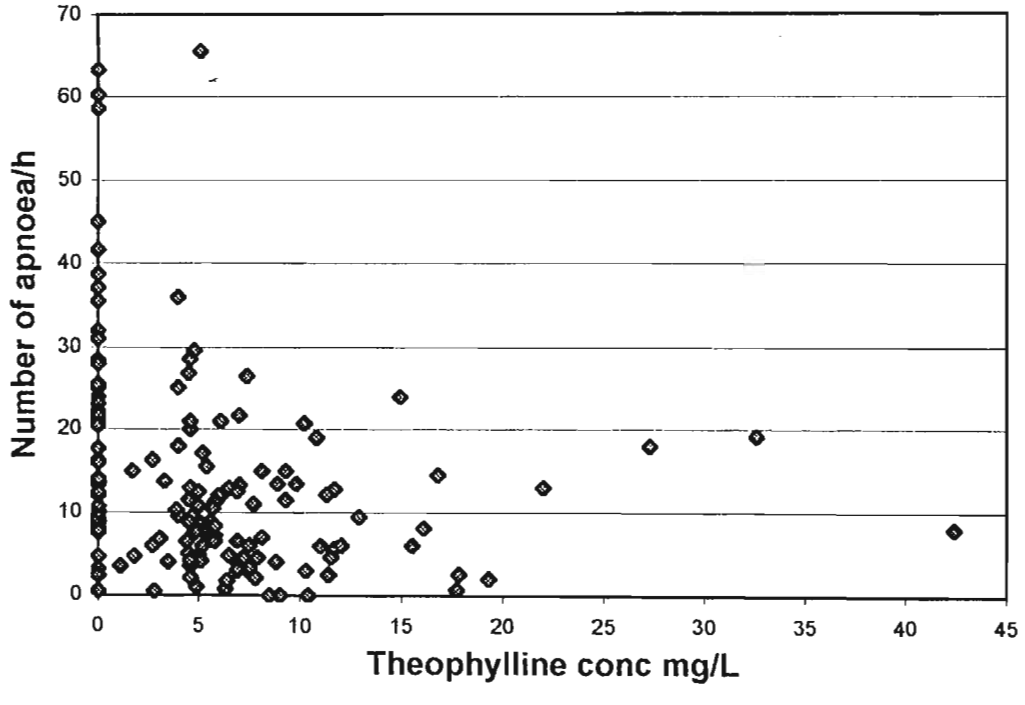

Fig. B.3.2 Number of total apnoea/h versus serum theophylline concentration (mg/L)

The results of the total apnoea incidents and density (percent time spent not breathing) from baseline to day 3 are given in Table B.3.2.

TABLE B.3.2

Incidents and densities of total apnoea (≥5 sec) per hour: median and (range) and average (SD) serum theophylline concentration (mg/L)

Day	Incidents of total apnoea/h	Densities of total apnoea (% time in apnoea)	Serum theophylline concentration mg/L (SD)
Baseline (n = 46)	16.0 (0.5 - 63)	4.8 (0.3 - 22.7)	0
1 (n = 39)	8.0 (1 - 27)*	2.3 (0.1 - 6.6)*	5.8 (3.9)
2 (n = 39)	8.8 (0 - 36)*	2.0 (0 - 11.4)*	8.8 (7.5)
3 (n = 29)	9.5 (0 - 66)*	1.8 (0 - 14.5)*	8.8 (5.7)

* Statistically significant ($p < 0.017$) compared with baseline, n = number of patients.

The incidents and density of total apnoea decreased significantly from baseline on all three days. The serum theophylline concentration increased from day 1 to day 2, but not from day 2 to day 3. (See Appendix B.3 for the total apnoea data file from baseline to day 3).

3.3.1.2 Graphical analysis of total apnoea

As the aim of the project was to investigate the influence of theophylline on apnoea as well as hypoxaemic episodes, both these clinical effects are shown in the individual graphs in Fig.B.3.3. However, the results of the effect of theophylline on hypoxaemic episodes will be presented in detail in section 3.3.3.

Although a significant decrease in the overall number of apnoea incidents from baseline was found, the response among patients was highly variable as shown in these graphs. For ease of graphical analysis, patients were divided into 4 groups according to the recorded number of total apnoea at baseline. Thus the scale of the Y-axis, showing the number of total apnoea/h, differs from group to group. The X-axis shows the days of monitoring, the secondary Y-axis the number of hypoxaemic episodes/h, and the secondary X-axis the serum theophylline concentration (mg/L). It should be noted that all the patients had apnoea at baseline, but not all of them had hypoxaemic episodes, thus some graphs show no hypoxaemic episodes.

Patients were classified as responders and non-responders according to the definition of a responder as a patient with a decrease equal to or more than 50% in apnoea incidents from baseline to the last recorded apnoea.

In order to identify markers of non-response it was thought appropriate to calculate the worst 25^{th} or 75^{th} percentile of the characteristics or clinical condition of the population to determine whether a non-responder belonged to the category or not. For gestational age the 25^{th} percentile was 30 weeks; a patient with the characteristic of low gestational

age would be one born at less than 30 weeks gestational age. (The postconceptual age was not used as the patients were monitored during the first three days after birth and the gestational age and the postconceptual age did not differ significantly). For total apnoea, the baseline 75th percentile was 27.4 total apnoea per hour. The 25th percentile for birth weight was 1.1 kg.

Group 1. Patients with >45 total apnoea/h at baseline:

There were four patients in this group with very high baseline counts. All four of them showed a decrease of more than 50% in the number of total apnoea/h from baseline and could thus be classified as responders.

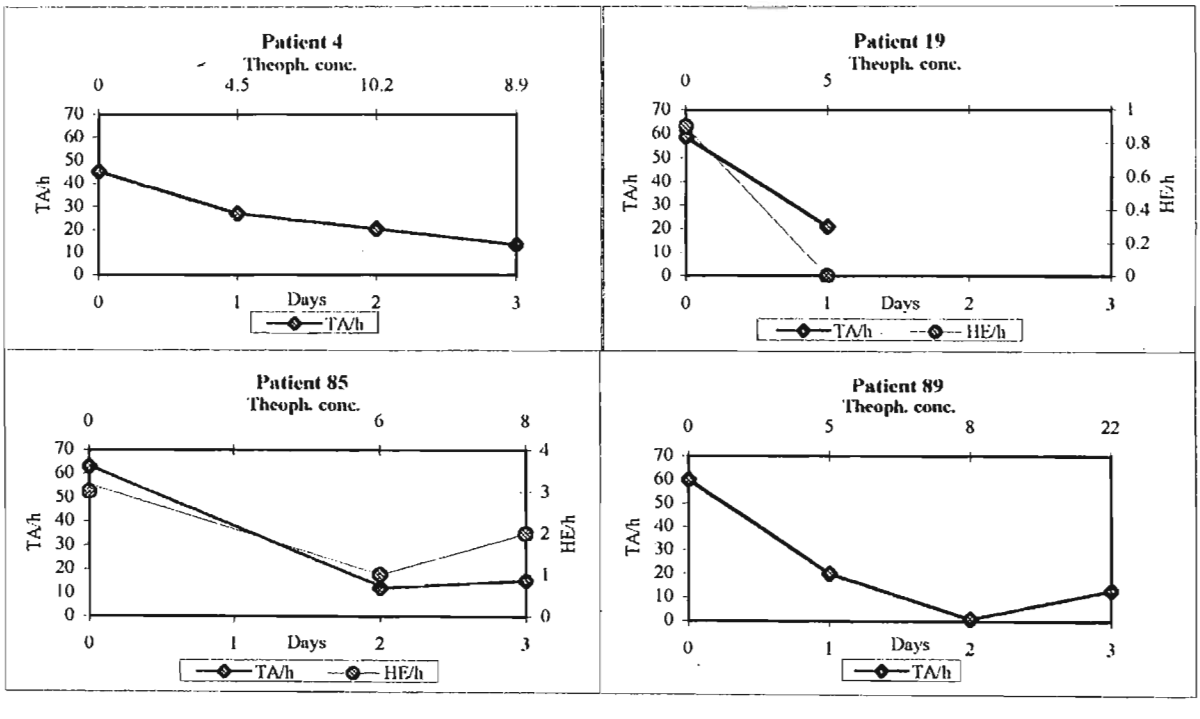

Fig. B.3.3. Individual graphs of patients, Group 1.

Group 2. Patients with between 30 and 45 total apnoea/h at baseline:

This group of six patients with intermediate baseline counts had 4 responders showing a ≥50% decrease in number of total apnoea/h, the two non-responders being patient numbers 5 and 84.

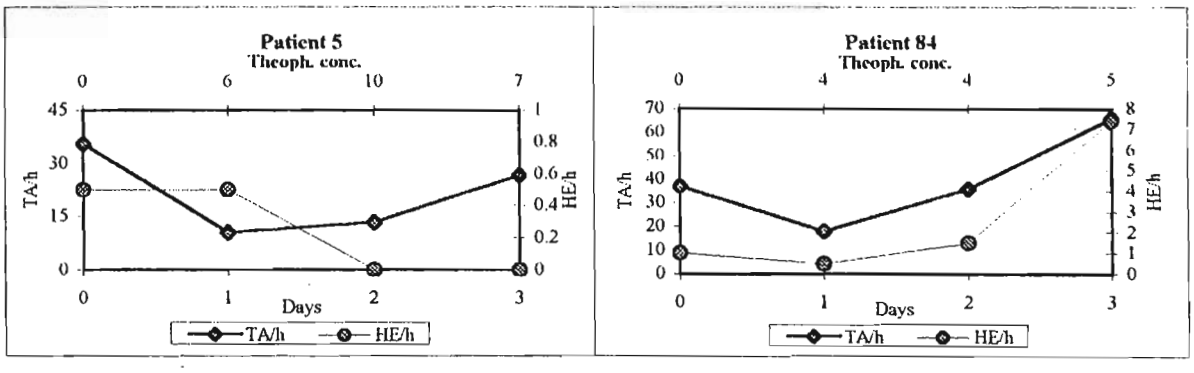

Fig.B.3.3 (cont.). Individual graphs Group 2, non-responders.

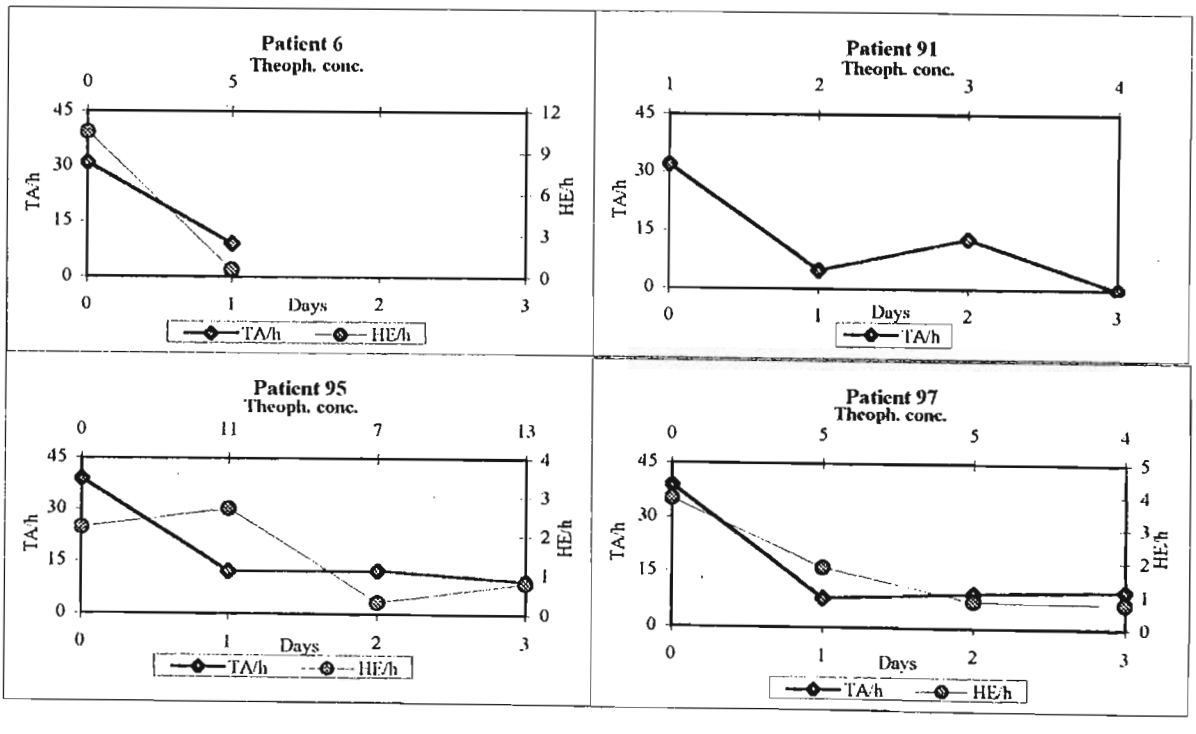

Fig.B.3.3 (cont.). Individual graphs Group 2, responders.

The demographics and clinical conditions of the non-responding patients will be briefly noted:

Patient 5 was a baby girl born at 29 weeks gestational age who weighed 1.1 kg at birth. She was born before her mother arrived at the hospital. She was in a poor condition and needed oxygen support. The total apnoea counts at first decreased and then increased after day 2. The serum theophylline concentration fell from 10 mg/L on day 2, to 7 mg/L on day 3. The value of the peripheral oxygen saturation remained stable at 98% over the three days.

Patient 84 was a baby boy who weighed only 800g at birth. He was the second of a set of twins born at 28 weeks gestational age. The mother suffered premature rupture of the membranes. The patient's apnoea incidents decreased after the loading dose of theophylline but thereafter increased steadily to day 3. The serum theophylline concentration was 5 mg/L on day 3. Despite oxygen support the peripheral oxygen concentration fell from 97% at baseline to 91% on day 3. Unfortunately the patient died on day 8 due to extreme prematurity.

If the denotations of markers of non-response are applied, both the patients in this group who did not respond, had gestational (postconceptual) ages less than the 25th percentile that is, <30 weeks, of the population and one had birth weight below the 25th percentile (<1.1 kg) of the population. Both patients had more than the 75th percentile (>27.4 total apnoea/h) of total apnoea at baseline and both patients needed oxygen support.

Group 3. Patients with between 15 and 30 total apnoea/h at baseline:

This group of fourteen patients with lower baseline counts than Group 2 had varying degrees of response. According to the definition of a non-responder, 7 patients, numbers 12, 70, 72, 73, 77, 93 and 124 qualified as non-responders.

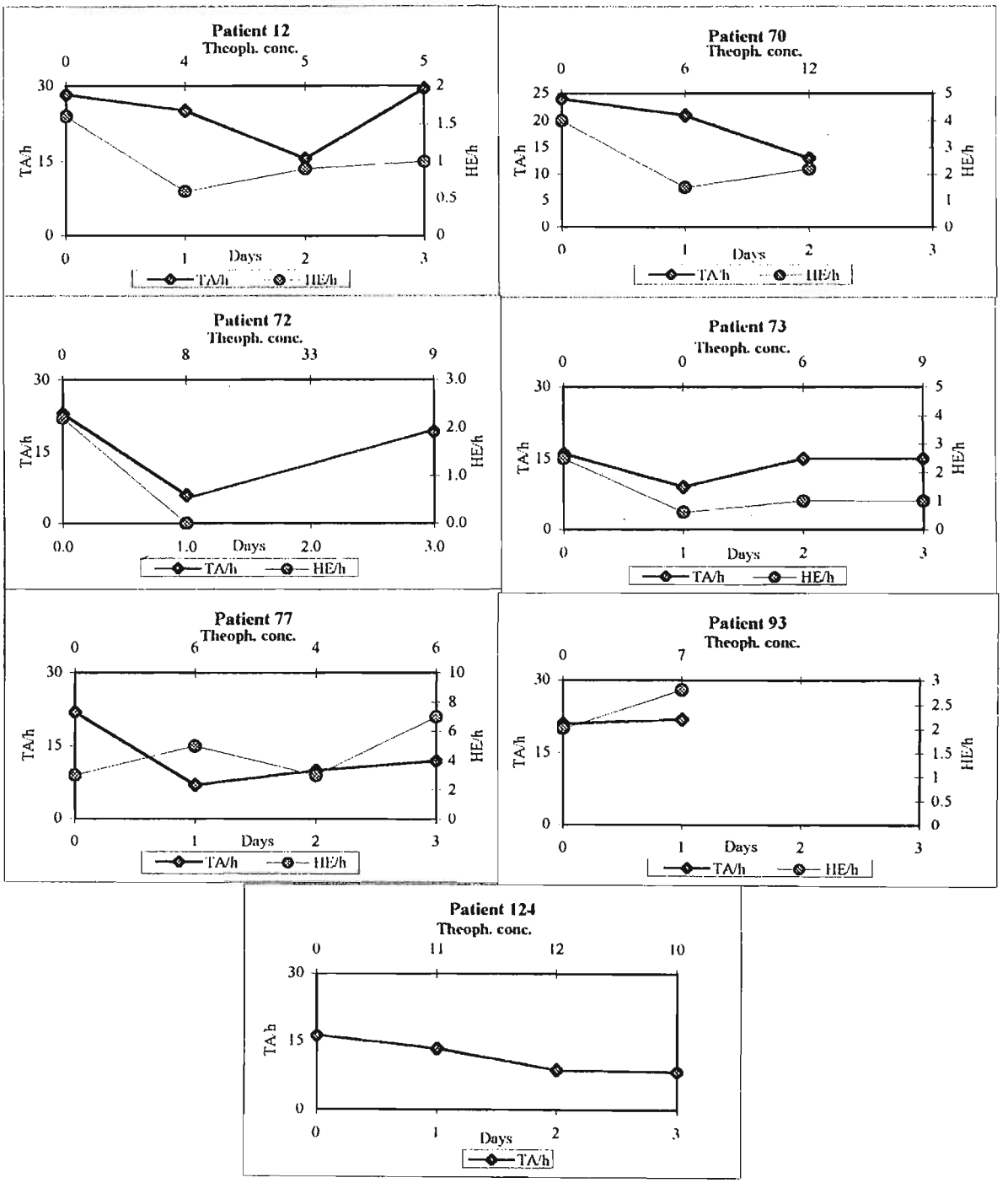

Fig.B.3.3 (cont.). Individual graphs Group 3, non-responders.

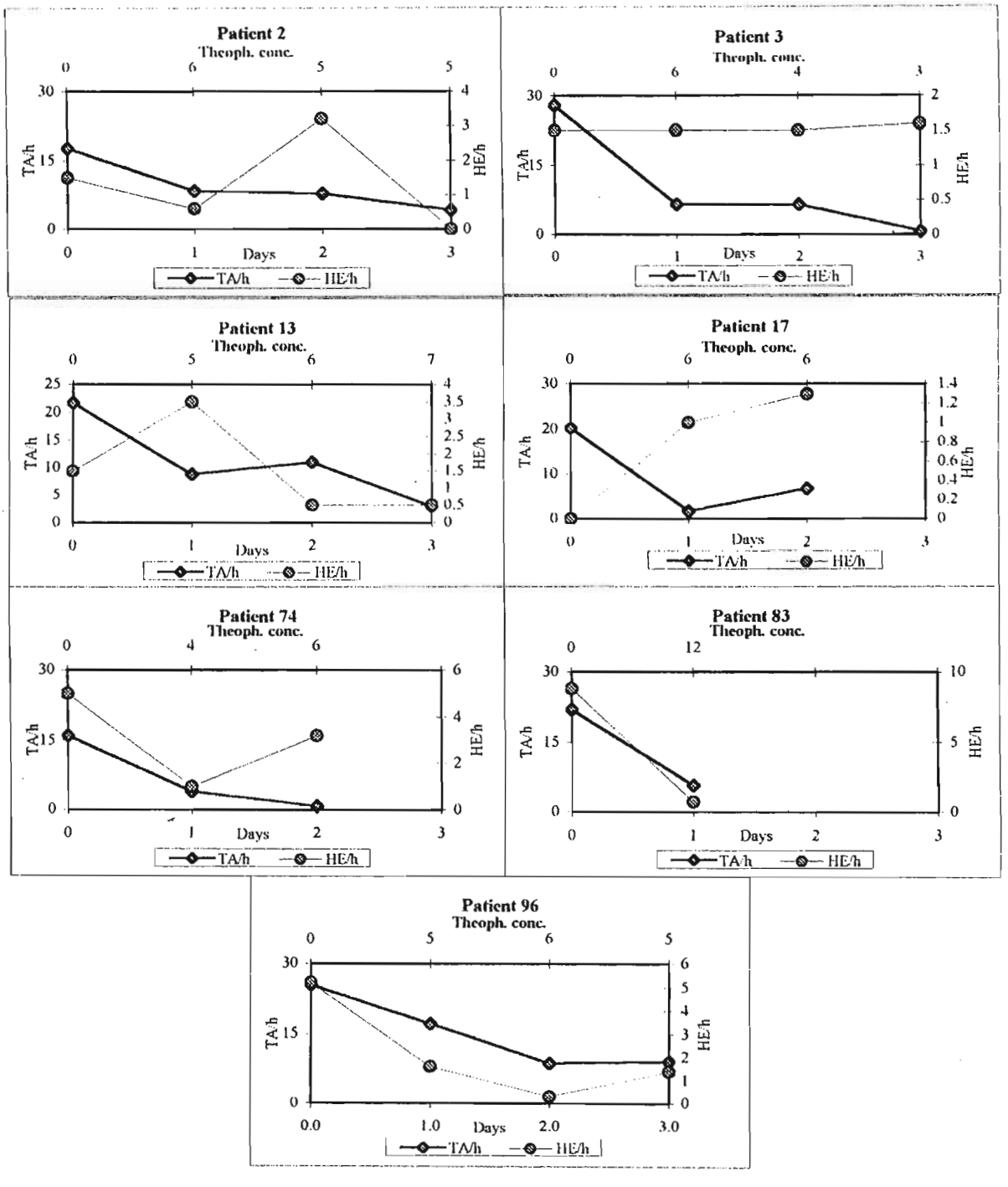

Fig.B.3.3 (cont.). Individual graphs Group 3, responders.

It is interesting to note the sharp decline in apnoea incidents from baseline to day 1, i.e. after the first dose of theophylline. This was seen in the responding as well as non-responding patients.

The demographics and clinical conditions of the non-responding patients will be briefly noted:

Patient 12 was a baby boy born at 30 weeks gestational age who weighed 1.3 kg at birth. The patient had grade 1 intra-ventricular haemorrhage. From baseline the patient received oxygen support but after the monitoring period on day 2 the oxygen support was stopped as the patient appeared satisfactory. The peripheral oxygen saturation was 96.8%. Hours later the patient developed tachypnoea and had to be resuscitated. Oxygen support was re-instated and on day 3 the peripheral oxygen saturation was 94.5%. The apnoea incidents showed a steady decline from day 1, but increased sharply from day 2 to day 3, reaching baseline values. The serum theophylline concentration was 5 mg/L on day 3. Unfortunately the patient died of pneumonia on day 10.

Patient 70 was a baby girl born at 30 weeks gestational age who weighed 1.25 kg at birth. She did not require oxygen support. A decrease of 46% in apnoea incidents occurred from baseline to the last recorded apnoea. Again this percentage is very close to a responder. The serum theophylline concentration was 12 mg/L.

Patient 72 was a baby boy born at 30 weeks gestational age who weighed 1.36 kg at birth. The total apnoea counts decreased significantly after theophylline administration but unfortunately the patient accidentally received an overdose of the drug on day 2, and the serum concentration increased to 32.6 mg/L. When noted theophylline administration was immediately stopped. The patient was observed and signs of jitteriness were recorded. The total apnoea incidents increased from 6/h on day 1 to 19/h on day 3. The patient's classification as a non-responder might have been erroneous as

he received the incorrect dose of the drug. The patient received oxygen support and the peripheral oxygen saturation increased from 92.8% at baseline to 96.4% at the last recording.

Patient 73 was a baby boy who weighed 1.5 kg at birth. He was born at 31 weeks gestational age by caesarean section from a teenage mother. The patient did not need oxygen support. The apnoea incidents decreased by 6% after baseline. The serum theophylline level was 9 mg/L on day 3.

Patient 77 was a baby boy who weighed 1.1 kg at birth. He was born at 31 weeks gestational age as one of a set of twins. The mother suffered premature rupture of membranes. A decrease of 48% in apnoea incidents occurred from baseline to the last recorded apnoea. He did not require oxygen support. The serum theophylline concentration was 6 mg/L.

Patient 93 was a baby boy who weighed 1.1 kg at birth. He was born at 29 weeks gestational age and was one of a set of twins. The mother suffered premature rupture of membranes. From day 2 the patient was ventilated due to poor perfusion and was thus excluded from further study. This patient's twin sister (patient 94, see group 4), also a non-responder, had less apnoea at baseline but did not require assisted ventilation although oxygen support was given. Both babies developed pneumonia after day three.

Patient 124 was a baby boy who weighed 1.2 kg at birth. He was born by caesarean section at 32 weeks gestational age. His mother suffered pregnancy-induced

hypertension. The patient's apnoea counts showed a steady decline after theophylline administration and was 48% less than baseline on day 3, very close to being a responder. The serum theophylline concentration was 10 mg/L.

Thus in summary, 5 of the seven patients in this group who did not respond, were males. One patient had gestational age less than the 25th percentile (<30 weeks) of the population. Only one patient had more than the 75th percentile (>27.4/h) total apnoea per hour at baseline. Two patients had pulse rates less than the 25th percentile (<120.5 bpm) of the population at baseline. Four patients required oxygen support; two were one of a set of twins; two mothers suffered premature rupture of membranes and one patient was born by caesarean section.

Group 4. Patients with equal to or less than 15 total apnoea/h at baseline:

Group 4 consisted of 22 patients with low starting baselines depicting varying degrees of response. All the patients had less than the 75th percentile total apnoea for the population at baseline. Thirteen patients, numbers 1, 10, 15, 16, 18, 76, 78, 80, 81, 87, 88, 94 and 99 were non-responders.

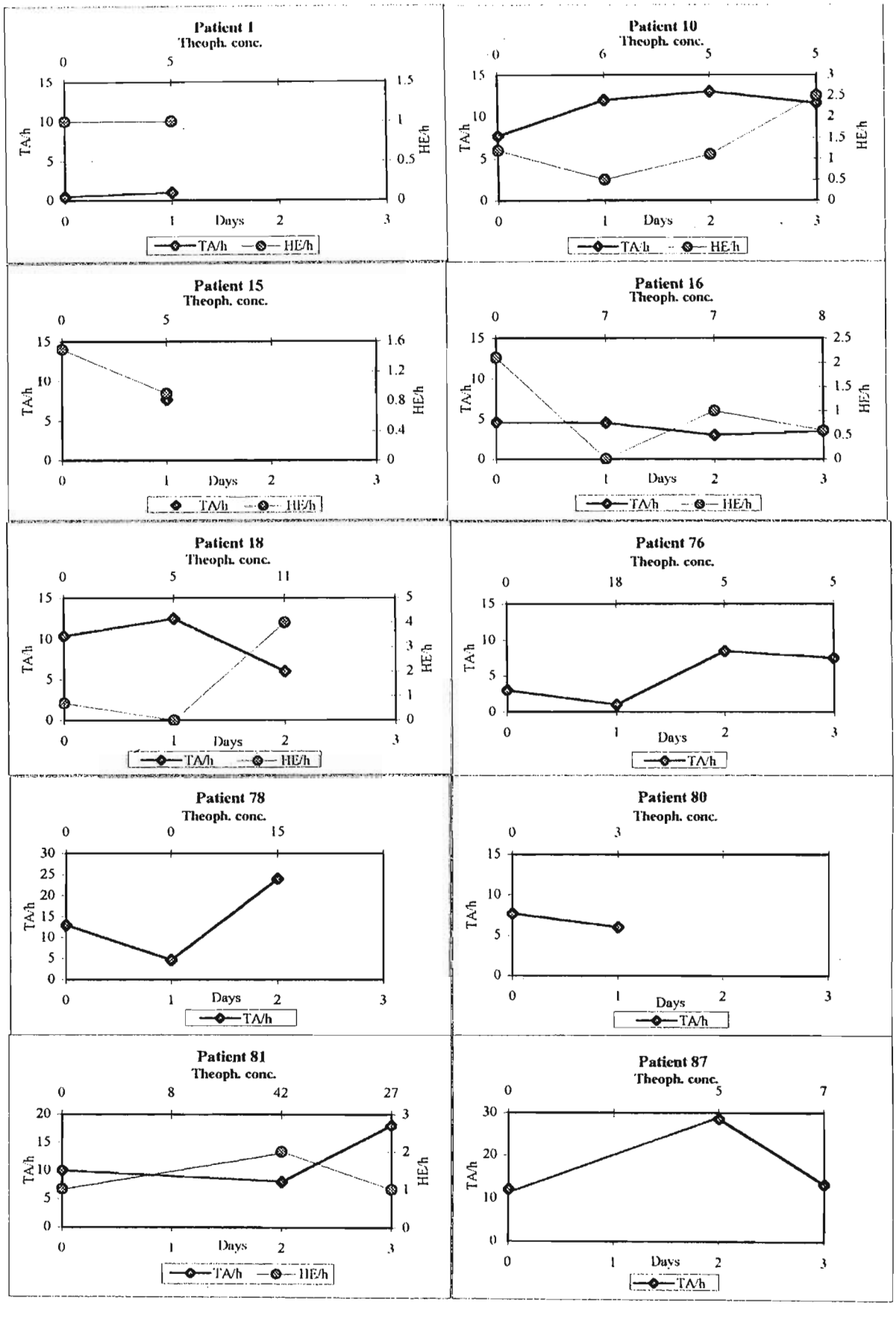

Fig.B.3.3 (cont.) Individual graphs Group 4, non-responders.

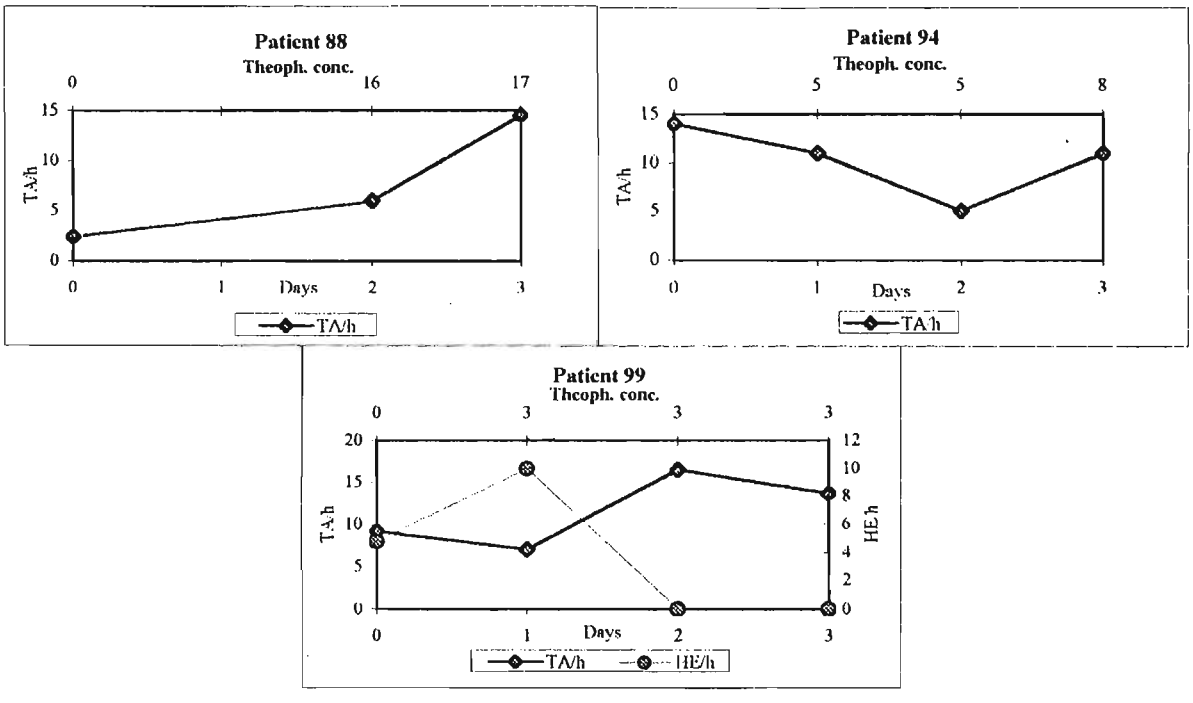

Fig.B.3.3 (cont.) Individual graphs Group 4, non-responders.

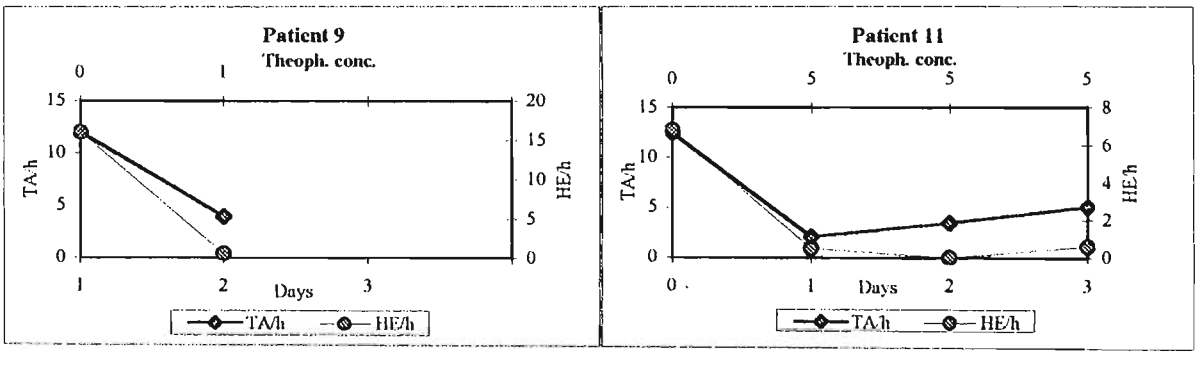

Fig.B.3.3 (cont.). Individual graphs Group 4, responders.

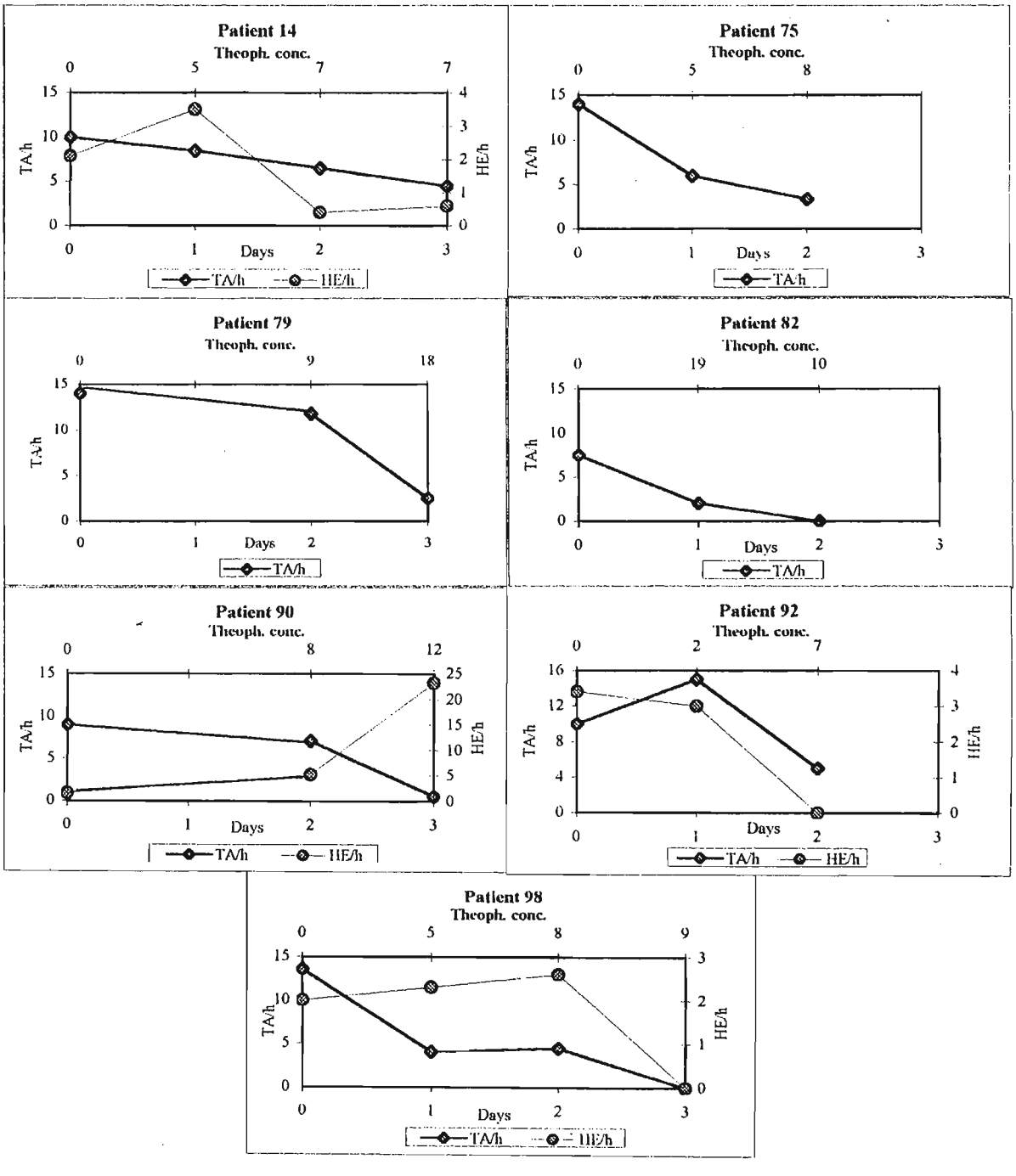

Fig.B.3.3 (cont.). Individual graphs Group 4, responders.

The demographics and clinical conditions of the non-responding patients will be briefly noted:

Patient 1 was a baby boy born at 30 weeks gestational age who weighed 1.2 kg at birth. The patient received oxygen support. A slight increase in apnoea incidents occurred after baseline; the serum theophylline level was 5 mg/L. Unfortunately the patient required assisted ventilation from day 1 and was thus excluded from further study.

Patient 10 was a baby girl who weighed 1.4 kg at birth. She was born by caesarean section at 32 weeks gestational age as one of a set of twins. The patient did not need oxygen support. The apnoea counts did not decrease after theophylline administration and the serum drug levels were 5 mg/L on day 2 and day 3. On day 3 the patient presented with grade 2 intra-ventricular haemorrhage.

Patient 15 was a baby boy born at 32 weeks gestational age who weighed 1.4 kg at birth. The patient received oxygen support. The total apnoea incidents decreased from 15/h to 8/h after the loading dose of theophylline. The serum drug concentration was 5 mg/L. Unfortunately the patient required assisted ventilation from day 1 and was thus excluded from further study.

Patient 16 was a baby boy born at 30 weeks gestational age who weighed 1.2 kg at birth. The patient received oxygen support. The total apnoea counts decreased by only 24% after theophylline administration. The serum theophylline concentration was 8 mg/L.

Patient 18 was a baby girl who weighed 0.95 kg at birth. She was born at 30 weeks gestational age as one of a set of twins. Her mother suffered premature rupture of membranes. After the loading dose of theophylline the total apnoea incidents did not decrease. The serum drug level was 5 mg/L. Unfortunately the patient's condition deteriorated and she died late afternoon on day 2.

Patient 76 was a baby girl who weighed 1.5 kg at birth. She was born by caesarean section at 31 weeks gestational age. At baseline the patient had three total apnoea/h and no pathologic apnoea (≥20 seconds). After the loading dose the serum theophylline concentration was 18 mg/L and the patient was restless. On day 2 the patient's perfusion was erratic. From day 2 the total apnoea incidents increased; the serum theophylline level was 5 mg/L. The patient received oxygen support.

Patient 78 was a baby girl who weighed 1.35 kg at birth. She was born by caesarean section at 31 weeks gestational age. Her mother suffered premature rupture of the membranes. On day 2 the patient experienced an increase in apnoea counts; the serum theophylline concentration was 15 mg/L and oxygen support was given.

Patient 80 was a baby boy who weighed 1.25 kg at birth. He was born at 30 weeks gestational age as one of a set of twins. The patient received oxygen support. After the loading dose of theophylline a slight drop in apnoea incidents occurred; the serum drug concentration was 3 mg/L. Unfortunately the patient developed pneumothorax on day 2 and died.

Patient 81 was a baby boy who weighed 1.45 kg at birth. He was born by elective caesarean section at 30 weeks gestational age and was in a poor condition after birth. He required oxygen support until day 2. On day 2 the patient unfortunately received an overdose of theophylline and the serum drug concentration was 42 mg/L. Drug administration was immediately stopped. The patient showed signs of jitteriness. On day 3 an increase in apnoea was experienced. The serum theophylline concentration was 27 mg/L. This patient was classified as a non-responder in terms of the definition. This classification might be erroneous if the patient had received the correct dose.

Patient 82 was a baby girl who weighed 1.2 kg at birth. She was born by caesarean section at 30 weeks gestational age. The patient was in a poor condition after birth and received oxygen support. On day 2 the patient experienced an increase in apnoea counts; the serum theophylline concentration was 5 mg/L. On day 3 the apnoea counts decreased to approximately baseline counts and the serum drug concentration was 7 mg/L.

Patient 88 was a baby girl who weighed 1.2 kg at birth. She was born by caesarean section at 30 weeks gestational age and was one of a set of twins. The total apnoea incidents increased steadily after birth and a sharp increase was experienced on day 3; the serum theophylline concentration was 17 mg/L. The patient received oxygen support.

Patient 94 was a baby girl who weighed 1.1 kg at birth. She was born at 29 weeks gestational age as one of a set of twins (patient 93 was her brother). She received oxygen support. Her total apnoea incidents decreased after baseline but increased on day 3. The

serum theophylline levels were 5 and 8 mg/L respectively. She developed pneumonia after day three.

Patient 99 was a baby boy who weighed 1.7 kg at birth. He was born at 32 weeks gestational age and received oxygen support. The patient experienced a slight drop in apnoea counts after the loading dose of theophylline and an increase in apnoea counts on day 2 and then a slight drop in counts on day 3. The serum theophylline concentration was 3 mg/L on all three days.

Thus in summary, 6 of the thirteen patients in this group who were classified as non-responders, were males. One patient had gestational age less than the 25th percentile (<30 weeks) and one had a birth weight less than the 25th percentile (<1.1 kg) of the population. Four patients had pulse rates below the 25th percentile (<120.5 bpm) of the population at birth. Ten patients required oxygen support, three were one of a set of twins, two mothers suffered premature rupture of membranes and six babies were born by caesarean section.

In order to identify possible markers of non-response, the characteristics of all the non-responders of the four groups with total apnoea (as depicted above) were analysed and compared with those of the rest of the patients with total apnoea and this is presented in Table B.3.3. As shown in the table, 10 (22%) of the patients (n = 46) weighed less than the 25th percentile (<1.1 kg) at birth; of these 10 patients, 8 (80%) were responders. Similarly there was a high percentage of responders in the group with low 5 minute

Apgar scores (80%) and in the group with high total apnoea counts at baseline (75%). Twelve patients were one of a set of twins and 8 of these (67%) were non-responders.

Thus, although the numbers are very small, being one of a set of twins might be regarded as an indicator of poor response. On the other hand, there is an indication that very low birth weight babies (<25th percentile), those with 5 minute Apgar scores less than the 25th percentile and those with high baseline total apnoea counts (>75th percentile) would be more likely to respond (at the defined level of 50%) to theophylline therapy.

Table B.3.3

Characteristics of patients with total apnoea (≥5 seconds): number and percentage of responding and non-responding patients with population characteristic

Characteristic	Number (%) of total population (n = 46) with this characteristic	Responders (n = 24) with this characteristic		Non-responders (n = 22) with this characteristic		
		Number	As % of responders (n = 24)	Number	As % of non-responders (n = 22)	As % of population with this characteristic
Birth weight <1.1 kg (25th percentile at baseline)	10 (22%) (10 out of 46)	8	33% (8 out of 24)	2	9% (2 out of 22)	20% (2 out of 10)
Gestational age <30 weeks (25th percentile at baseline)	8 (17%)	4	17%	4	18%	50%
Classified as 'in poor condition' on entry	28 (61%)	17	71%	11	50%	39%
Apgar at 5 minutes <8 (25th percentile at baseline)	5 (11%)	4	17%	1	5%	20%
Received oxygen support	32 (70%)	18	75%	14	64%	44%
Patients with >27.4 total apnoea/h (75th percentile at baseline)	12 (26%)	9	38%	3	14%	25%
Males	24 (52%)	11	46%	13	59%	54%
Pulse rate <120.5 bpm (25th percentile at baseline)	12 (26%)	5	21%	7	32%	58%
Corticosteroid given to mother before or during labour	16 (35%)	8	33%	8	36%	50%
Patient is one of a set of twins	12 (26%)	4	17%	8	36%	67%
Patient born by caesarean section	13 (28%)	6	25%	7	32%	54%
Developed NNJ	25 (54%)	15	63%	10	45%	40%

NNJ = neonatal jaundice, bpm = beats per minute.

A frequency distribution graph (see Fig.B.3.4) illustrates the number of responders and non-responders with total apnoea at the various serum theophylline concentrations. The cumulative number of responders and non-responders at each concentration are also shown. It should be noted that 6%, 45%, 26%, 12%, 2% and 8% of serum theophylline concentrations were in the 1-3, 3.1-6, 6.1-9, 9.1-12, 12.1-15 and >15 mg/L range.

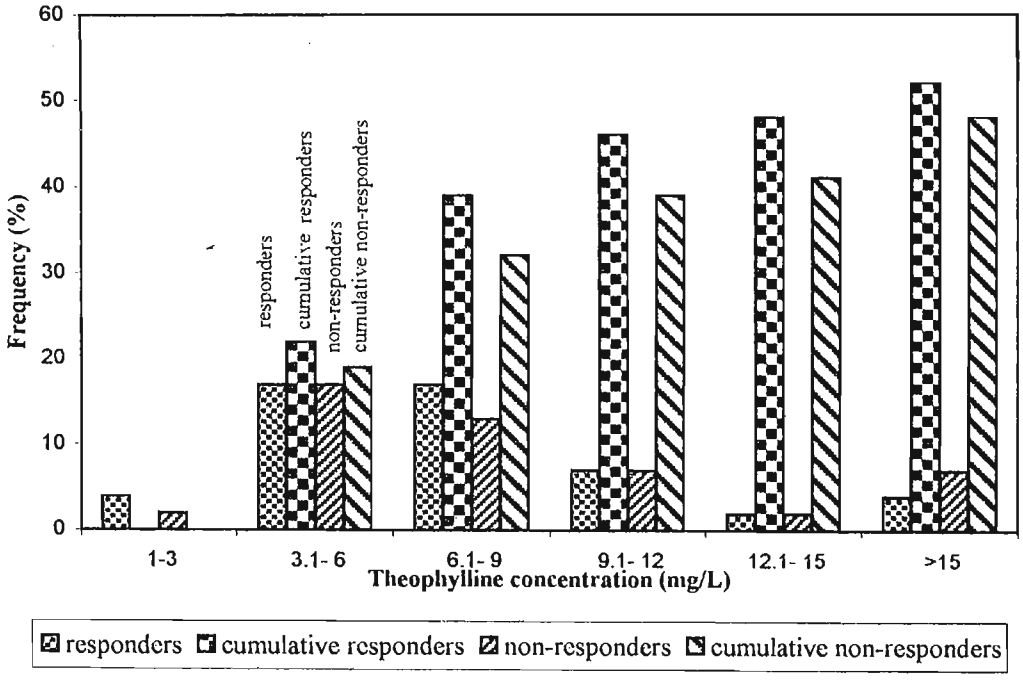

Fig.B.3.4 Frequency distribution of responders and non-responders with total apnoea at the various serum theophylline concentrations. Cumulative number of responders and non-responders at each concentration are also shown.

3.3.2 **Pathologic apnoea (≥20 seconds) incidents**

It was considered appropriate and of interest to analyse the results of the pathologic apnoea incidents separately as a number of studies report on the effect of theophylline on the course of longer apnoea (Shannon *et al* 1975, Gerhardt *et al* 1978, Roberts *et al* 1982, Sims *et al* 1985, Muttitt *et al* 1988, Harrison 1992). Thirty-eight of the initial 46 neonates monitored had pathologic apnoea at baseline. At the subsequent three monitoring times 32, 31 and 24 of these were followed. Figure B.3.5 illustrates when monitoring of these patients took place and the number of pathologic apnoea per hour recorded at those times. (See Appendix B.4 for the pathologic apnoea data file from baseline to day 3).

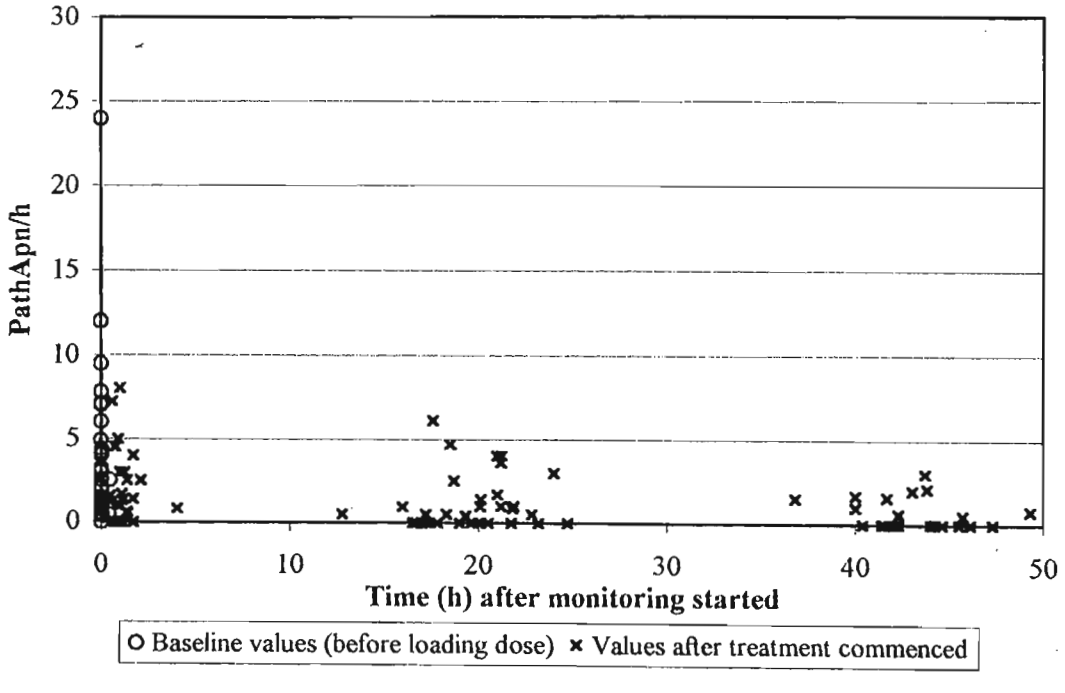

Fig.B.3.5. Scattergram of the number of pathologic apnoea per hour at the different monitoring times.

The number of pathologic apnoea per hour measured against serum theophylline concentration is shown in Fig.B.3.6.

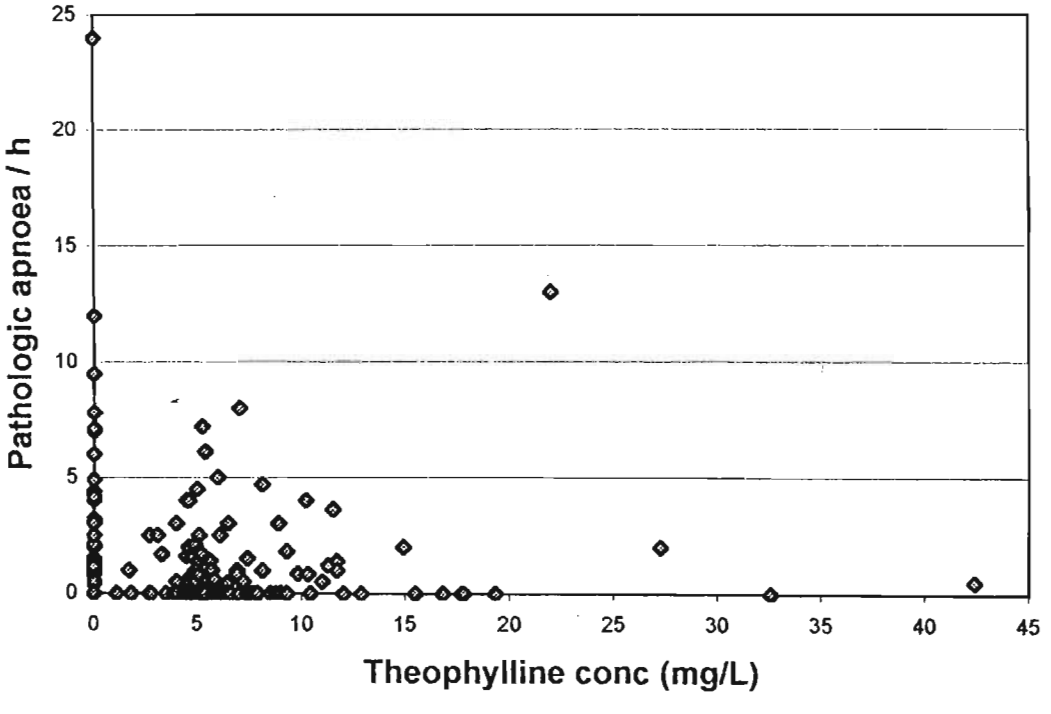

Fig.B.3.6 The number of pathologic apnoea per hour versus serum theophylline concentration (mg/L).

The results of the total pathologic apnoea incidents and density (percentage time spent not breathing) as well as all the different types of pathologic apnoea recorded are given in Table B.3.4. There was a significant decrease ($p < 0.017$) in the incidents and density

of the total pathologic apnoea, as well as the pathologic apnoea incidents associated with a fall in pulse rate and a hypoxaemic episode, on all 3 days when compared with baseline. However, the pathologic apnoea associated with a hypoxaemic episode decreased significantly on day 1 only. Also the pathologic apnoea associated with a fall in pulse rate decreased significantly on day 3 only.

TABLE B.3.4 The per hour recording of the median and (range) of the following apnoea counts at recorded average serum theophylline concentrations (SD): Total pathologic apnoea; density of total pathologic apnoea; pathologic apnoea associated with a fall in pulse rate and a hypoxaemic episode; pathologic apnoea associated with a hypoxaemic episode only; and pathologic apnoea associated with a fall in pulse rate only.

	Total pathologic apnoea	Density of total pathologic apnoea	Pathologic apnoea with a fall in pulse rate and a hypoxaemic episode	Pathologic apnoea with a hypoxaemic episode	Pathologic apnoea with a fall in pulse rate	Serum theophylline mg/L (SD)
Baseline (n = 38)	3.0 (0.4 - 24)	2.1 (0.3 - 13.5)	0.5 (0 - 2.3)	0.2 (0 - 3.0)	0 (0 - 2)	0
Day 1 (n = 32)	1.1 (0 - 8.0)*	0.8 (0 - 4.3)	0 (0 - 1.5)*	0 (0 - 2.0)*	0 (0 - 1.5)	5.8 (3.5)
Day 2 (n = 31)	0.5 (0 - 6.1)*	0.3 (0 - 3.4)	0 (0 - 1.1)*	0 (0 - 1.0)	0 (0 - 1)	8.2 (7)
Day 3 (n = 24)	0 (0 - 3.0)*	0 (0 - 1.6)	0 (0 - 1.0)*	0 (0 - 6.5)	0 (0)*	8 (5)

* Statistically significant ($p<0.017$) compared with baseline, h = hour, n = number of patients.

As the number of the different types of pathologic apnoea is very small, only the results for theophylline on the total number of pathologic apnoea will be reported further.

Analysis of the results showed that 5 of the 38 patients (numbers 1, 81, 93, 99 and 124) could be classified as non-responders, that is, a decrease of less than 50% in pathologic apnoea incidents from baseline to the last recorded apnoea.

The characteristics of the 5 non-responders were compared with those of the rest of the patients in this group and the results are summarised in Table B.3.5.

However, as the number of non-responders (n = 5) in this group (n = 38) was very small no markers of non-response could really be identified. It was noted that all the non-responders were males. A much better response to theophylline therapy was found for pathologic than for total apnoea.

Table B.3.5

Characteristics of patients with pathologic apnoea (≥20 seconds): number and percentage of responding and non-responding patients with population characteristic

Characteristic	Number (%) of total population (n = 38) with this characteristic	Responders (n = 33) with this characteristic		Non-responders (n = 5) with this characteristic		
		Num-ber	As % of responders (n = 33)	Num-ber	As % of non-responders (n = 5)	As % of population with this characteristic
Birth weight <1.1 kg (25th percentile at baseline)	9 (24%) (9 out of 38)	9	27% (9 out of 33)	0	0	0
Gestational age <30 weeks (25th percentile at baseline)	6 (16%)	5	15%	1	20%	17% (1 out of 6)
Classified as 'in poor condition' on entry	24 (63%)	22	67%	2	40%	8%
Apgar at 5 minutes <8 (25th percentile at baseline)	4 (11%)	3	9%	1	20%	25%
Received oxygen support	28 (74%)	26	79%	2	40%	7%
Patients with >4.2 pathologic apnoea/h (75th percentile at baseline)	9 (24%)	8	24%	1	20%	11%
Patients with >27.4 total apnoea/h (75th percentile at baseline)	10 (26%0	10	30%	0	0	0
Males	22 (58%)	17	52%	5	100%	23%
Pulse rate <120.5 bpm (25th percentile at baseline)	8 (21%)	8	24%	0	0	0
Corticosteroid given to mother before or during labour	11 (29%)	9	27%	2	40%	18%
Patient is one of a set of twins	12 (32%)	11	33	1	20%	8%
Patient born by caesarean section	11 (29%)	9	27%	2	40%	18%
Developed NNJ	21 (55%)	20	61	1	20%	5%

NNJ = neonatal jaundice, bpm = beats per minute.

A frequency distribution graph (see Fig.B.3.7) illustrates the number of responders and non-responders with pathologic apnoea at the various serum theophylline concentrations. The cumulative number of responders and non-responders at each concentration are also shown.

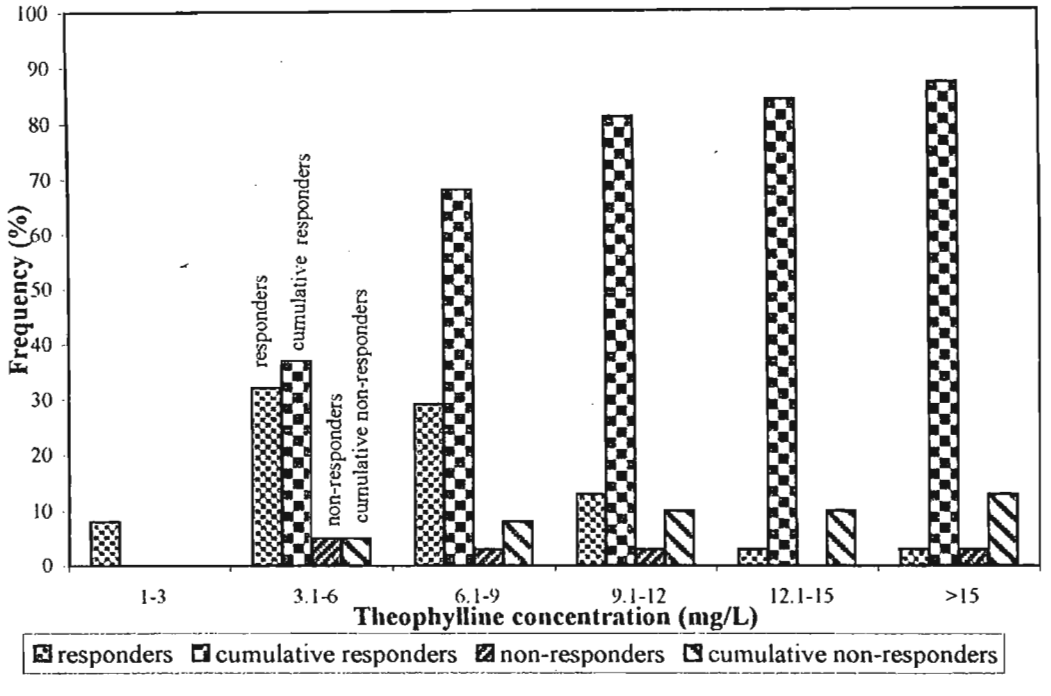

Fig.B.3.7. Frequency distribution of responders and non-responders with pathologic apnoea at the various serum theophylline concentrations. Cumulative number of responders and non-responders at each concentration are also shown.

3.3.3 **Hypoxaemic episodes**

Thirty-five of the 46 neonates who were monitored had hypoxaemic episodes ≥10 seconds at baseline. Thirty-one, 28 and 22 patients were followed at the subsequent monitoring times. The number of hypoxaemic episodes recorded per hour and the serum theophylline concentration measured at the same time is illustrated in Fig.B.3.8. (see Appendix B.5 for the hypoxaemic episodes data file from baseline to day 3).

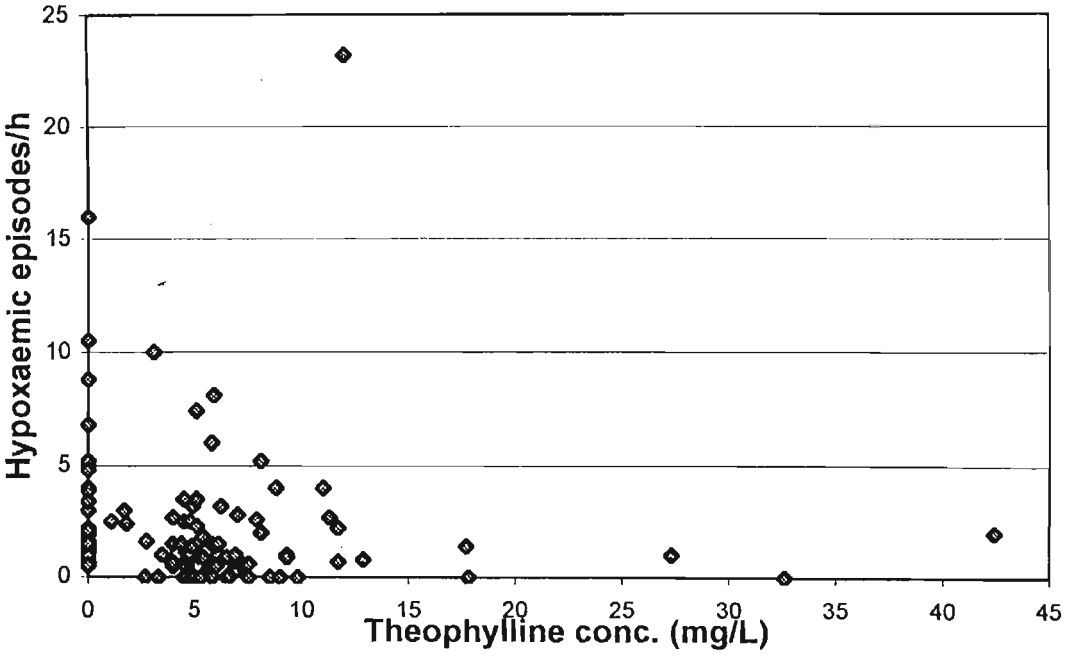

Fig.B.3.8 The distribution of hypoxaemic episodes/h versus serum theophylline concentration (mg/L).

The median (and range) of total as well as all the different types of hypoxaemic episodes/h with the serum theophylline concentration is shown in Table B.3.6.

TABLE B.3.6 The per hour recording of the median and (range) of the following hypoxaemic episodes at recorded average serum theophylline concentrations (SD): Total hypoxaemic episodes; isolated hypoxaemic episodes; hypoxaemic episodes associated with a fall in pulse rate; hypoxaemic episodes associated with a pathologic apnoea and a fall in pulse rate; hypoxaemic episodes associated with movement.

	Total hypoxaemic episodes	Isolated hypoxaemic episodes	Hypoxaemic episodes associated with a pathologic apnoea	Hypoxaemic episodes associated with a fall in pulse rate	Hypoxaemic episodes associated with a pathologic apnoea and a fall in pulse rate	Hypoxaemic episodes associated with movement	Serum theophylline mg/L (SD)
Baseline (n = 35)	2.0 (0 - 16)	1.7 (0 - 15)	0 (0 - 2)	0 (0 - 4.9)	0 (0 - 1.3)	0 (0 - 1)	0
Day 1 (n = 31)	1.0 (0 - 10)	0.7 (0 - 9)	0 (0 - 1)	0 (0 - 1.6)	0 (0 - 1)*	0 (0 - 2)	5.6 (3.3)
Day 2 (n = 28)	1.0 (0 - 8)	0.5 (0 - 8)	0 (0 - 0.9)	0 (0 - 3.5)	0 (0 - 0.3)*	0 (0 - 1.1)	8.6 (8.4)
Day 3 (n = 22)	0.9 (0 - 23)	0.3 (0 - 23)	0 (0 - 0.7)	0 (0 - 1.0)	0 (0 - 0.6)*	0 (0 - 1.6)	8.0 (5.5)

* Statistically significant ($p<0.017$) compared with baseline, h = hour, n = number of patients.

At baseline 65% of the total hypoxaemic episodes were isolated, that is, not associated with a pathologic apnoea or a fall in pulse rate or a fall in pulse rate with a pathologic apnoea or with movement. This remained the same for day 1 but changed to 71% and 75% on day 2 and 3 respectively. The only significant decrease in hypoxaemic episodes were those associated with pathologic apnoea and a fall in pulse rate from baseline to the subsequent days. As the number of the different types of hypoxaemic episodes was small, only the trend of the total number of hypoxaemic episodes was analysed further.

The influence of theophylline on hypoxaemic episodes (as well as on total apnoea) is shown in the individual graphs of Fig.B.3.3. The response is variable; occasionally a decrease is seen in the number of hypoxaemic episodes as well as total apnoea and occasionally in the number of the one clinical effect but not in the number of the other clinical effect. Not all the patients depicted in Fig.B.3.3 had hypoxaemic episodes; thus some of the graphs will not show any hypoxaemic episodes.

In order to identify possible markers of non-response the characteristics of all the non-responders with hypoxaemic episodes were analysed and compared with those of the rest of the patients with hypoxaemic episodes and this is presented in Table B.3.7.

Analysis of results for hypoxaemic episodes showed that 16 of the 35 patients could be classified as non-responders.

Table B.3.7

Characteristics of patients with hypoxaemic episodes ≥10 seconds: number and percentage of responding and non-responding patients with population characteristic

Characteristic	Number (%) of total population (n = 35) with this characteristic	Responders (n = 19) with this characteristic		Non-responders (n = 16) with this characteristic		
		Num-ber	As % of responders (n = 19)	Num-ber	As % of non-responders (n = 16)	As % of population with this characteristic
Birth weight <1.1 kg (25th percentile at baseline)	7 (20%)	4	21%	3	19%	43%
Gestational age <30 weeks (25th percentile at baseline)	6 (17%)	3	16%	3	19%	50%
Classified as 'in poor condition' on entry	19 (52%)	11	58%	8	50%	42%
Apgar at 5 minutes <8 (25th percentile at baseline)	4 (11%)	2	11%	2	13%	50%
Received oxygen support	22 (63%)	10	53%	12	75%	55%
Patients with >4.2 pathologic apnoea/h (75th percentile at baseline)	9 (26%)	3	16%	6	38%	67%
Patients with >27.4 total apnoea/h (75th percentile at baseline)	12 (34%)	7	37%	5	31%	42%
Males	20 (57%)	10	53%	10	63%	50%
Pulse rate <120.5 bpm (25th percentile at baseline)	14 (40%	8	42%	6	38%	43%
Corticosteroid given to mother before or during labour	10 (29%)	4	21%	6	38%	60%
Patient is one of a set of twins	8 (23%)	1	5%	7	44%	88%
Patient born by caesarean section	7 (20%)	2	11%	5	31%	71%
Developed NNJ	18 (51%)	10	53%	8	50%	44%

NNJ = neonatal jaundice, bpm = beats per minute.

Because of the non-significant response of hypoxia to theophylline (Table B3.6), it is not really relevant to be too concerned about predictors of response. However the results in table B3.7 revealed that those least likely to respond were twins (88%), those born by caesarean section (71%) and those with baseline pathologic apnoea per hour above the 75th percentile (67%). The frequency distributions of the responding and non-responding patients in relation to theophylline concentration are shown in Figure B.3.9.

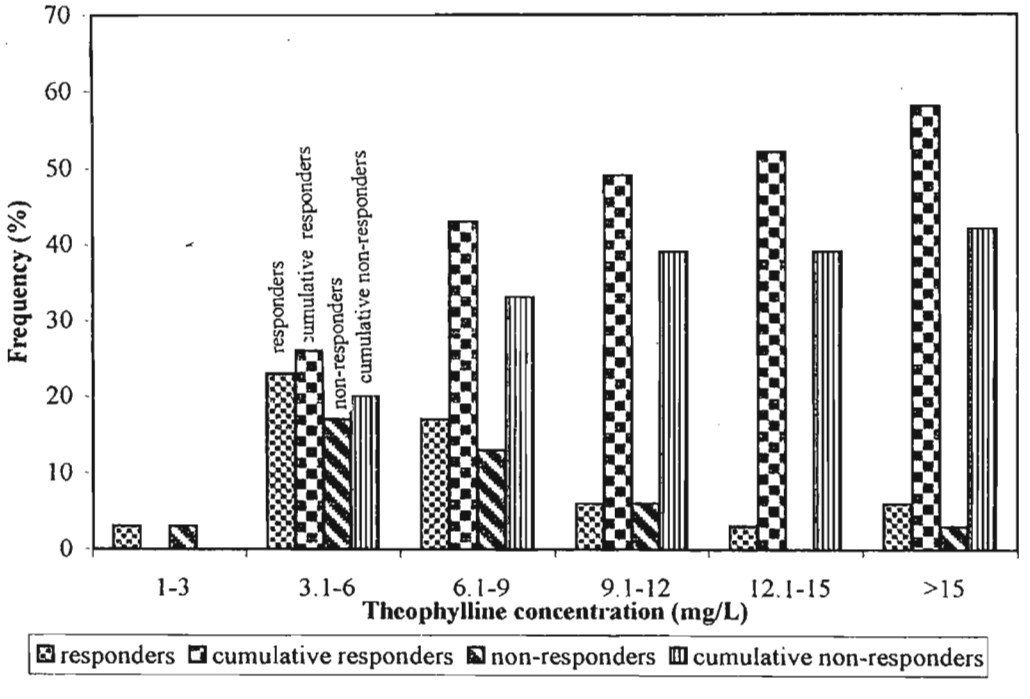

Fig.B.3.9 Frequency distribution of responders and non-responders with hypoxaemic episodes at the various serum theophylline concentrations. Cumulative number of responders and non-responders at each concentration are also shown.

Thus, in summary, twins seemed more likely to respond poorly in terms of both total apnoea and hypoxaemic episodes. Other markers of poor response in the hypoxia group were more than the 75th percentile pathologic apnoea at baseline and delivery by caesarean section. The babies most likely to respond in terms of total apnoea were those with very low birth weight (<25th percentile), those with 5 minute Apgar scores less than the 25th percentile at birth and those with high baseline total apnoea counts (>75th percentile).

3.3.4 **The effect of theophylline on pulse rate and episodes of bradycardia**

The results of measurements of pulse rate and recording of all incidents of bradycardia were obtained from all the 46 patients at baseline. Of these 39, 39 and 29 were followed at the other three monitoring times respectively. The results are shown in Table B.3.8.

The average pulse rate measured at each monitoring time increased significantly from baseline to all three days as shown in Table B.3.8. The biggest increase in pulse rate is seen after the loading dose of theophylline was given, thus from baseline to day 1. The number of incidents of bradycardia/h decreased significantly from baseline to all three days and on day 3 a median of null incidents was recorded.

TABLE B.3.8

Pulse rate (PR) per monitoring time, and number of episodes of bradycardia per hour: median and (range), with serum theophylline concentrations (mg/L)

Day	PR (bpm)	Episodes of bradycardia (<100 bpm /h)	Serum theophylline conc (mg/L)
Baseline (n = 46)	133.5 (99 - 159)	3.0 (0 - 173)	0
1 (n = 39)	143.1* (110 - 171)	0.7* (0 - 59)	5.8 (3.9)
2 (n = 39)	143.0* (117 - 188.5)	0.5* (0 - 19)	8.8 (7.5)
3 (n = 29)	146.0* (119 - 171.9)	0* (0 - 5.7)	8.8 (5.7)

Statistically significant: * $p \leq 0.017$ compared with baseline, n = number of patients, bpm = beats per minute, conc = concentration.

3.3.5 **Peripheral oxygen saturation and mean arterial pressure**

The results of the median (range) of the peripheral oxygen saturation (SpO_2) and the mean arterial pressure (MAP) recorded at each of the monitoring times are shown in Table B.3.9.

TABLE B.3.9

Peripheral oxygen saturation (SpO_2) and mean arterial pressure (MAP) per monitoring time: median and (range), with serum theophylline concentrations (mg/L)

Day	SpO_2 (%)	MAP (mm Hg)	Serum theophylline conc (mg/L)
Baseline (n = 46)	96.4 (83.6 - 98.5)	34.5 (29 - 45)	0
1 (n = 39)	96.5 (88.0 - 98.3)	38.0* (32 - 63)	5.8 (3.9)
2 (n = 39)	96.4 (87.6 - 98.3)	41* (33 - 50)	8.8 (7.5)
3 (n = 29)	96.5 (90.3 - 98.5)	46* (30 - 67)	8.8 (5.7)

Statistically significant: * $p \leq 0.017$ compared with baseline, n = number of patients.

For the whole population (n = 46) there was no significant change in the peripheral oxygen saturation (SpO_2) per monitoring time from baseline to any of the other days. The mean arterial pressure (MAP) showed a steady increase from baseline to the third day.

SECTION B: CHAPTER 4

DISCUSSION

4.1 DISCUSSION

The results of the study indicate that theophylline reduces apnoea in apnoeic infants with mild-to-moderate respiratory distress syndrome. There is, however, variability in response. Although hypoxaemic episodes associated with a pathologic apnoea (≥20 seconds) and a fall in heart rate decreased significantly, theophylline appears not to be effective in reducing isolated hypoxaemia.

One of the objectives of the study was to investigate the effect of theophylline during the first few days after birth, as this is the time when theophylline is most often used in the neonatal wards. In this way the study differs from other published studies as most of the patients in the present study were enrolled as soon as possible after birth, whereas the patients in most of the published studies had wider ranges of postnatal ages. Also, the patients in the present study had RDS, whereas those in most of the published studies suffered mostly from apnoea of prematurity only.

Comparison of the results with other studies is difficult due to the different definitions used for apnoea. In the present study, two different definitions of apnoea were used, namely total apnoea, which included all the apnoea equal to or longer than 5 seconds, and pathologic apnoea, which included all apnoea equal to or longer than 20 seconds. The effect of theophylline on shorter apnoea is important as Upton *et al* (1991) found

that apnoea of less than 10 seconds can cause reductions in peripheral oxygen saturation of up to 40%. The shorter apnoea (3 to 15 seconds) reflects a disturbance of the respiratory control system that may lead on to longer apnoea and to sudden infant death syndrome (Kelly and Shannon 1979, Lee *et al* 1987). The definition of pathologic apnoea (≥20 seconds) corresponded with the definition as determined by the Academy of Pediatrics Task Force on Prolonged Infantile Apnea (Consensus Statement 1987).

Theophylline significantly reduced the incidents of total apnoea from baseline to all three days. The biggest effect was seen after the loading dose. No difference was found between the average number as well as the densities of total apnoea between day one and day two, and day two and day three. As the definition for total apnoea included the shorter apnoea, comparison with other studies is difficult. Finer *et al* (1984) who reported on all apnoea ≥3 seconds, found a significant ($p<0.001$) decrease in apnoea 5 to 9.9 seconds and also those ≥10 seconds. They studied 19 infants with gestational ages 36.1 (±5.1) weeks at a mean age of 7.1 (±4 .3) weeks while asleep, before, and one week after theophylline administration. These infants had older gestational and postnatal ages than the present study. The dose of theophylline was 2.3 mg/kg 6 hourly; the serum theophylline concentrations were not reported.

In the present study, of the group of 46 patients with total apnoea, 22 (48%) could be classified as non-responders as they had a less than 50% decrease in total apnoea counts from baseline to the last recorded apnoea. An objective was to identify markers of non-response that could alert the attending physician to the possible problems associated with

the management of such a neonate. Such markers of non-response to theophylline therapy for apnoea have not been identified in other studies.

For the group with total apnoea, being one of a set of twins was found to be a characteristic of poor response to theophylline, as 67% of twin babies were non-responders. Greenough *et al* (1996) have reported that being one of a set of twins is a predisposing factor to RDS. This may be due to asphyxia (Arnold *et al* 1987) or faster maturation of the presenting twin (Weller *et al* 1976). A close relationship between RDS, asphyxia or hypoxaemia, and apnoea exists (Poets *et al* 1994, Greenough *et al* 1996), and hypoxaemia is often used to assess the severity of RDS (Greenough *et al* 1996). Recently Martín-Ancel et al (1995) analysed the relationship between the clinical and biochemical markers of asphyxia and multi-organ involvement and found Apgar scores at 1 and 5 minutes to be the only factors related to the number of organs involved. In the present study low 5 minute Apgar scores were a marker of favourable response (in terms of total apnoea) to theophylline therapy. Other features of babies more likely to respond were birth weight less than the 25^{th} percentile and baseline total apnoea/hour greater than the 75^{th} percentile.

The frequency distribution graph of serum theophylline concentrations for total apnoea (Fig B.3.4, page 140) shows that most patients in this group responded at concentrations of 3.1 to 9 mg/L. This might give a false impression, as most of the serum theophylline measurements in the present study were in the 3.1 to 9 mg/L range. The results show that 22% of the patients have responded at 3.1 to 6 mg/L, another 17% at 6.1 to 9 mg/L and another 7% at 9.1 to 12 mg/L. The cumulative values for the responders will be 22% at

3.1 to 6 mg/L, 39% at 6.1 to 9 mg/L, 46% at 9.1 to 12 mg/L and 48% at 12.1 to 15 mg/L. A maximum of 52% response was reached at >15 mg/L. Comparison of the results with other studies is not possible as the definition of total apnoea included a wider range of apnoea than any published study with dose or serum concentration information.

The results of the present study show that a significant reduction occurred in the number of pathologic apnoea (≥20 seconds) from baseline to all three days following theophylline therapy. Of the 38 patients in the group with pathologic apnoea, apnoea was completely abolished in 10 (26%) of the patients after the loading dose was administered. The results of the pathologic apnoea study may be compared with those recorded by Sims *et al* (1985) who used a definition for apnoea as 20 seconds with or without bradycardia. Also the postnatal ages and gestational ages of the patients of this study and those used by Sims *et al* are very similar. However, Sims *et al* only enrolled patients with apnoea of prematurity, while the patients of the present study also had RDS. In the study by Sims *et al*, theophylline administration was stopped when a 50% reduction of the baseline number of apnoea occurred. As in the present study, she found an overall significant ($p<0.025$) decrease in apnoea incidents. However, in her study, 3 of the 18 patients (17%) treated with theophylline and 3 of the control patients (21%) continued to have apnoea even after three weeks of theophylline administration. These six patients were not distinguishable from the other patients in her study in terms of birth weight, gestational and postnatal age, or the number of apnoea incidents at baseline.

Using a definition of apnoea as a cessation of breathing for ≥20 seconds or less if accompanied by bradycardia, Roberts *et al* (1982) recorded no response in 20% of

patients (n = 10) and an overall reduction of 58% in apnoea incidents. These patients (postnatal ages 1 to 31 days) had apnoea as well as a variety of other disorders. Apnoea was recorded before and then between 40 and 166 hours after theophylline therapy was started. Serum concentration levels of between 7 and 13 mg/L were obtained. Considerable patient variability was found and no correlation could be drawn between degree of response to theophylline, gestational age, postconceptual age, clinical diagnoses, or theophylline concentrations.

In the present study, thirteen percent of the patients with pathologic apnoea did not respond. This is lower than the twenty-three percent of the patients in the study by Muttitt *et al* (1988) who did not respond. It should however be noted that Muttitt *et al* used a more stringent definition of apnoea than the present study as well as the studies above, namely, ≥20 seconds plus a 25% decrease in heart rate and a 10% decrease in oxygen saturation. Muttitt *et* al defined a non-response as ≥0.33 apnoea per hour. Their patients had the same range of gestational ages than the patients of the present study, the study by Sims *et al* and also Roberts *et al*, but a wider range of postnatal ages than the present study.

Although the methods for recording of apnoea differed, the percentages of non-response for Muttitt *et al*, Roberts *et al* and Sims *et al* (23, 20 and 17% respectively), were higher than the 13% recorded in the present study. Although Muttitt *et al* used a more sophisticated recording system than Roberts or Sims, she agreed that all forms of artefact could not be recognised and could have been mistaken for an apnoea. Detection of apnoea in the present study was more accurate as computer printouts as well as manual

recordings of apnoea incidents were used. We were able to distinguish false apnoea recordings due to movement, sighs etc. whereas the above researchers could have misinterpreted these incidents. Another drawback in the study by Muttitt *et al* (1988) is the small number of patients, namely 22.

In the present study, in the group of 38 patients with pathologic apnoea, five (13%) were non-responders as they did not have a 50% decrease in number of pathologic apnoea from baseline to the last recording. These five patients were also non-responders in the total apnoea group, as total apnoea included all apnoea ≥5 seconds. For this population with pathologic apnoea, no outstanding characteristic of non-response could be identified, but all five non-responders were males.

It is known that boys are more likely to develop RDS than girls (Farrell and Avery 1975, Luerti *et al* 1993) but, as most of the patients in the study suffered from RDS, it could not be considered as a characteristic of non-response. However, RDS is a known and common cause of apnoea (Kercsmar 1994, Greenough *et al* 1996). Male foetuses have a delayed appearance of a mature lecithin: sphingomyelin ratio and phosphatidylglycerol (Fleisher et al 1985). This seems to be due to an androgen-induced delay in the maturation of the surfactant systems in the foetus (Torday 1992). Gender differences in lung function (Stocks *et al* 1997) as well as in growth of lung function was shown with girls generating greater maximal expiratory flows than boys (Hibbert *et al* 1995). It is not known whether this difference in lung function can be extrapolated to premature neonates.

The anticipated beneficial effect of corticosteroid administration to the mothers before or during labour was difficult to assess, as the numbers were small. Eleven of the mothers in the group of neonates with pathologic apnoea received a corticosteroid before or during labour and two of these neonates were non-responders. It has been shown that the use of antenatal corticosteroids in preterm labour halved the incidence of RDS when given between 24 hours and seven days before delivery (Crowley 1995). This may be due to an enhanced expression of protein and phospholipids of the surfactant system and enzymes of the antioxidant systems (Vyas *et al* 1997). Most of the clinical trials such as the Vermont-Oxford Trials Network (Horbar 1995) concentrated on antenatal corticosteroids and neonatal outcome and the emphasis was on the reduction of the incidences of RDS, intra-ventricular haemorrhage and necrotising enterocolotis (Crowley *et al* 1990) and not on apnoea as such. As apnoea and RDS are closely linked, a reduction in RDS should mean less apnoea.

After the loading dose of theophylline (recording of day 1) a sharp drop (50%) in both incidents and densities of both pathologic apnoea and total apnoea from baseline was observed. Others investigating the effect of theophylline (Peabody *et al* 1978, Brouard *et al* 1985, Sims *et al* 1985, Scanlon *et al* 1992) and also caffeine (Turmen *et al* 1981) have reported similar findings. The mode of action of theophylline is not clear. It is suggested that the peripheral chemoreceptors are inactive during the first 48 hours after birth (Barrington and Finer 1990) and are responsible for apnoea and hypoxaemia (Côté *et al* 1996). It was shown that a dopaminergic mechanism suppresses the sensitivity of the chemoreceptors and when oxygenation increases after birth the dopamine turnover decreases allowing the chemoreceptors to reset (Hertzberg *et al* 1990, 1993). In term

infants these chemoreceptors are more active in the presence of aminophylline (Cattarossi *et al* 1993). One might speculate that this dramatic effect is due to the stimulating effect of theophylline on the peripheral chemoreceptors (Cattarossi *et al* 1993) allowing them to 'reset' thus increasing their sensitivity to hypoxia (Alvaro *et al* 1992, Calder *et al* 1994). Subsequent beneficial effects on pathologic apnoea might be due to antagonism of adenosine that is released during hypoxia (Bissonnette *et al* 1990). Moreover, it could be due to theophylline's enhancement of conduction along the central auditory pathways with resultant improved regulatory effect on the respiratory centre of the brain stem (Chen *et al* 1994).

It is interesting to note that a sharp decline in apnoea incidents (20 seconds) during the first 24 hours after birth, has also been reported in neonates receiving no medication (Sims *et al* 1985, Barrington and Finer 1991). However, the apnoea incidents in the theophylline treated group in the study by Sims *et al* (1985), decreased more than in the control group of patients. The apnoea incidents of the treated group decreased significantly from 11 ± 3 to 4 ± 1, whereas in the control group, apnoea incidents dropped from 9 ± 3 to 6 ± 2 per day. Thus the decrease in apnoea incidents cannot with certainty only be attributed to theophylline. The profound changes in the first few days after birth in the physiology of the chemoreceptors and other systems controlling respiration (Hertzberg and Lagercrantz 1987, Hertzberg *et al* 1990, Rylance 1992) could play significant roles in regularising breathing.

The frequency distribution graph of serum theophylline concentrations for pathologic apnoea (Fig B.3.7, page 147) showed that most patients responded at concentrations of

3.1 to 9 mg/L. The steepest part of the cumulative response is between 3.1 and 12 mg/L; thereafter the curve flattens until an 87% cumulative response is reached. The results showed that 39% of the patients have responded at 3.1 to 6 mg/L, another 29% at 6.1 to 9 mg/L, and another 13% at 9.1 to 12 mg/L. It should be kept in mind that 48% of the serum theophylline concentration were between 3.1 to 6 mg/L and 25% between 6.1 to 9 mg/L. Comparison of these results with other studies is difficult, as most of the serum theophylline concentrations in the present study were below 9 mg/L. It should be noted that the dose of the drug was determined by the physician in charge and not by any requirements of the study. Muttitt *et al* (1988), in a dose-finding study, recorded a 14% response at a serum theophylline concentration of 4.2 mg/L, a further 14% response at 8.5 mg/L and a further 45% response at 12.7 mg/L. The postnatal ages of the patients in her study were older than the present study but the gestational ages overlapped. Sixty percent (6 of 10) of the patients in the study by Roberts *et al* (1982) had >50% decrease in apnoea incidents at serum theophylline concentrations of 6 to 10 mg/L. The other patients in the study responded at higher serum concentrations (13 mg/L), and 20% of the patients did not respond at all. These patients had similar gestational ages but older postnatal ages than the present study. Most of the other published studies, using a variety of definitions for apnoea, had responses at higher serum theophylline concentrations. For example, Scanlon *et al* (1992) obtained a 92% response by day 1 with serum theophylline concentrations of 13 to 20 mg/L, using a definition of no airflow plus stimulation of the neonate and a fall in heart rate of ≥40 beats per minute. A flaw in the present study (as in some of the others) is that, unlike Muttitt's study, there was a lack of titration of the dose within individual patients.

As yet no agreement regarding the definition of a hypoxaemic episode has been reached. The definition used in the present study was influenced by the limitations of the recording instruments used in the study and the alarm limits for apnoea monitors as suggested by Upton *et al* (1991). The study demonstrated a reduction in total hypoxaemic episodes although this was not significant. The only significant decrease was seen in the hypoxaemic episodes associated with a pathologic apnoea and a fall in pulse rate. This could be due to the effect of the drug on apnoea and heart rate as such. Comparison of the results with other studies is difficult due to experimental differences as well as differences in the definition of an episode. Finer *et al* (1984) reported a significant reduction in apnoea incidents after 7 days of theophylline therapy (serum concentrations 6 to 19 mg/L), but no significant reduction in the number of falls of transcutaneous oxygen pressure greater than 3 mm (11.1 versus 7.2/h). Peabody *et al* (1978), who also used transcutaneous oxygen pressure as a parameter, observed a significant decrease in the total duration of hypoxaemia after two days of theophylline treatment (n = 10, serum concentrations 10 to 16 mg/L). However, 48 hours after withdrawal of the drug, five of the six neonates who received theophylline for only two days, experienced an increase in apnoea incidents and hypoxaemia. It was also shown that caffeine the other methylxanthine used for apnoea of prematurity did not protect the neonate against the development of hypoxaemic episodes (Bucher and Duc 1988).

As reported in other studies many hypoxaemic episodes occurred without apnoea (Poets *et al* 1991, Poets *et al* 1992, Richard *et al* 1993). It has been suggested that isolated hypoxaemia could be due to intrapulmonary shunts (Poets *et al* 1992, Samuels *et al* 1992, Southall *et al* 1993). This topic has been extensively reviewed by Poets *et al*

(1992) who suggested that any change in ventilation-perfusion-ratio could lead to intrapulmonary shunting. Bolivar *et al* (1995) have shown that most hypoxaemic episodes in mechanically ventilated infants were triggered by an expiratory effort that produced a large decrease in lung volume. They suggested that this could lead to closure of small airways and the development of intrapulmonary shunts. Many factors are implicated in intrapulmonary shunting (Poets *et al* 1992) and theophylline, through its wide range of pharmacological effects, could possibly influence ventilation and/or perfusion. Theophylline could improve ventilation since it increases tidal volume (Davi *et al* 1978) and therefore enhances alveolar ventilation (Gerhardt *et al* 1979, Cordoba *et al* 1994). It also improves respiratory muscle function (Sherman *et al* 1996), and reduces diaphragmatic fatigue (Aubier 1986, Heyman *et al* 1989). During hypoxia, theophylline may prevent a fall in minute ventilation and respiratory rate (Bruce *et al* 1986). The drug may also improve the regulation of breathing through its stimulatory effect on the respiratory centre of the brain stem (Chen *et al* 1994). Theophylline might improve perfusion through its positive inotropic and chronotropic effects (Walther *et al* 1986). A lack of surfactant, as found in respiratory distress syndrome, can also lead to ventilation/perfusion inequalities (Poets *et al* 1992). Antenatal administration of theophylline has been shown to have a beneficial effect on surfactant production in foetal rabbits (Hegyi *et al* 1986) and lung maturation in humans (Granati *et al* 1984). However, post-natally the drug had no appreciable effect on respiratory distress syndrome (Hegyi *et al* 1986). Regardless of all the potential effects of theophylline to improve ventilation-perfusion, the results of the present study suggest that theophylline does not have a significant beneficial effect on hypoxaemia. Both increasing hypoxia and hypercarbia accompany prolonged apnoea and may produce derangement of central respiratory

control, increased bronchomotor tone, and depressed cardiac function (Miller and Martin 1992). Recently it was shown that even moderate grade hypoxaemia could harm the function of the neonatal kidney (Talosi *et al* 1996).

In the group of 35 patients who had hypoxaemic episodes at baseline, 16 (46%) did not respond to theophylline therapy. Possible markers of poor response for this population were being one of a set of twins, being born by caesarean section, and more than 4.2 pathologic apnoea per hour at baseline. However, determination of markers is not really important as hypoxaemia (unrelated to apnoea) was found to respond poorly to theophylline.

The frequency distribution graph of serum theophylline concentrations for hypoxaemic episodes (Fig B.3.9, page 152) shows that most patients responded at concentrations of 3.1 to 9 mg/L, but again, most of the serum theophylline concentrations were in this range. The cumulative response was 55% at >15 mg/L. Comparison of the results of the study with other studies is difficult. Finer *et al* (1984) found no significant reduction in the number of falls of transcutaneous oxygen pressure at serum theophylline concentrations 6 to 19 mg/L. Also, Peabody *et al* (1978) observed a significant decrease in the total duration of hypoxaemia at serum concentrations of 10 to 16 mg/L.

In the present study theophylline significantly increased the average pulse rate from baseline to all three days. Shannon *et al* (1975), in a study of 17 premature infants, noted a similar increase in heart rate (from 155 ±11.3 to 173 ±15.7 bpm) and noted tachycardia (>180 bpm) with serum theophylline concentration >13 mg/L. Tachycardia is a well-

known side effect of theophylline administration (Rall 1996), but in the present study the patients who accidentally received overdoses of theophylline did not develop heart rates above 180 bpm. This phenomenon was also observed by Nadkarni *et al* (1988) who conducted a study to investigate the relationship between theophylline, caffeine and heart rate in neonates; tachycardia did not occur even at theophylline concentrations >20 mg/L. In the present study only one patient presented with an average of 188 bpm on day 2 of monitoring, but the serum theophylline concentration was only 11 mg/L. The reason for this patient's tachycardia was unknown. Thus the practice of using tachycardia as an indicator of effective theophylline therapeutic concentrations by some clinicians (Samuels and Southall 1993) would be impractical and unsafe in the premature neonate during the first few days after birth. In the present study the relationship between pulse rate and serum theophylline concentration was weak with a correlation coefficient (r) of 0.14. This could be due to the changing dynamics of the neonate during the first few days after birth. Nadkarni *et al* (1988), in a small study with 12 neonates, found a good relationship of 0.56 for theophylline concentrations below 10 mg/L, but for concentrations above 10 mg/L, a poor one, namely 0.12. When all the serum theophylline concentrations above 10 mg/L were omitted from the data of the present study, the correlation coefficient changed from 0.14 to 0.15.

Sudden episodes of bradycardia are common in the neonate (Hodgman *et al* 1993) and if recurrent, may constitute a considerable problem in the clinical management of the neonate. In the present study theophylline significantly decreased the episodes of bradycardia from baseline to all three days. The episodes of bradycardia associated with a pathologic apnoea and a hypoxaemic episode also decreased with theophylline

administration. Using very stringent definitions for bradycardia, apnoea and desaturations, Poets *et al* (1993) showed a close relationship between episodes of bradycardia, apnoea and desaturations. The mechanism of action of theophylline is not clear but might be due to the drug's effect on A_1-receptors (Barnes and Pauwels 1994).

The mean arterial pressure showed a consistent increase from baseline to all three days. After treatment it remained above 30 mm Hg in all cases, which should ensure sufficient cerebral blood flow (Miall-Allen *et al* 1987). The average peripheral oxygen saturation over the three days showed no statistically significant change. These findings were consistent with those obtained by Peabody *et al* (1978) using transcutaneous oxygen pressure.

4.2 CONCLUSION

This study has shown that theophylline, in the doses used, appeared to significantly reduce total and pathologic apnoea. A reduction in hypoxaemic episodes occurred but this was not statistically significant. The most dramatic effect on apnoea and hypoxaemia was seen on the first day of treatment after the loading dose.

Twins were identified as being poor responders with regard to both total apnoea and hypoxaemic episodes. Other markers of non-response for hypoxaemic episodes were being born by caesarean section and more than the 75th percentile pathologic apnoea at baseline. For the population with total apnoea, there was a high percentage of responders in the group with low 5 minute Apgar scores, in the group with high total apnoea counts at baseline and in the group that weighed less than the 25th percentile

(<1.1 kg) at birth. No markers could be identified for pathologic apnoea, but all non-responders were boys.

A careful analysis of these results, as well as those of other researchers, highlights the uncertainty surrounding the mechanism of action of theophylline and the effect that other physiological factors have on the breathing pattern of the neonate. Increasing maturity cannot easily be separated from theophylline's effects. The inclusion of an untreated control group would have been ideal, but ethical considerations precluded this. Although any conclusions drawn from the study are confounded by the lack of a control group, the significant decrease in apnoea incidents and densities over the three days suggests that theophylline, in the concentrations used, is effective in reducing pathologic apnoea in most neonates with respiratory distress syndrome.

Although the results show that most of the patients responded at serum theophylline concentrations of 3.1 to 9 mg/L, no absolute conclusion regarding the effective dose range of theophylline could be drawn as most of the serum concentrations fell in this range. Information regarding toxic doses/serum concentrations is lacking as the three patients who accidentally received overdoses of theophylline only showed signs of jitteriness; the serum concentrations were >27 mg/L.

Assessments of concentration-effect relationships in this section were simplistic as they involved comparison of a baseline with other time-points in the patient. A more appropriate method of analysing repeated measures and using all the data, including that

for patients where some values are missing, would be a population analysis. Accordingly this was done in Section C.

SECTION C

POPULATION CONCENTRATION-EFFECT MODELLING OF THEOPHYLLINE IN PREMATURE NEONATES SUFFERING FROM APNOEA DURING THE FIRST FEW DAYS AFTER BIRTH

SECTION C: CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The term pharmacodynamics relates to the study of the physiological and biochemical effects of drugs and their mechanism of action. Towards this end, use may be made of either the overall detectable effect, such as a decrease in the number of apnoea, or the physiological effect, such as blocking of adenosine receptors. As in pharmacokinetics, mathematical models are used to describe the pharmacodynamics of a drug. Presently the trend is to combine the pharmacokinetics and pharmacodynamics of a drug and report on 'the PK/PD modelling' of a drug. The link between the pharmacodynamics and pharmacokinetics is the concentration of the drug as this forms a fundamental part of both studies. As it is not always possible to measure the concentration at the site of action, whether intra- or extra-cellular, the serum concentration of the drug is normally used. The correct measurement of the concentration of the drug is thus as important as the description of the effect.

Although the pharmacokinetics of theophylline in premature neonates have been studied using both traditional methods and population methods such as NONMEM (see Section A), very little has been published on the linked pharmacodynamics and pharmacokinetics of theophylline especially in the premature neonate with apnoea. In this section, the literature pertaining to receptor theory, PK/PD modelling and the proposed mechanism of drug action of theophylline and possible factors affecting this will be reviewed prior to the presentation of the experimental work.

The aim of this section of the study was thus to investigate the PK-PD relationship of theophylline in the treatment of apnoea in the premature neonate during the first few days after birth.

1.1 MECHANISMS OF DRUG ACTION

Drugs exert their pharmacological effects by interacting with macromolecular components of the organism. Thus the pharmacological effect may result through the activating or inhibiting action of a drug on these sites. The concept of a 'receptive system', based upon a drug-cell combination was first suggested by Langley in the late eighteenth century (Ross 1996). In the 1920's A. J. Clark developed this view further and the concept of a 'receptor' in the biological cell with which the drug may combine to trigger a response was born. It is now known that receptors are protein molecules usually located in the cell membrane. Many receptors have been cloned and their amino acid sequences determined (Ross 1996). Recently the International Union of Pharmacological Sciences (IUPHAR) published a compendium of receptor characterisation and classification (IUPHAR Media, London, 1998).

As the resultant effect of a drug-receptor interaction seldom equals the proportion of receptors occupied, Ariëns in 1954 introduced the term 'intrinsic activity' (α) to describe the relationship between the effect, E, elicited by a drug, D, and the concentration of drug-receptor complexes:

$$E = \alpha[D]$$

In 1956 Stephenson further advanced the concept of concentration-response relationships by introducing the concept of 'efficacy' to offer an explanation for non-linear relationships between receptor occupancy and drug response. Today the terms 'intrinsic activity' and 'efficacy' are commonly used interchangeably and are operationally synonymous (Ross 1996).

1.2 QUANTITATION OF DRUG-RECEPTOR INTERACTIONS

To understand drug-receptor interactions, it is necessary to quantify the relationship that exists between the drug and the biological effect it produces. As the degree of the effect produced by a drug is usually a function of the amount of drug administered, this relationship can eventually be expressed in terms of dose-response curves (or concentration-response curves).

The above concept may be illustrated as follows:

$$\text{Drug (D)} + \text{Receptor (R)} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \text{DR} \rightarrow \text{Effect}$$

This reaction sequence is similar to the interaction of substrate with enzyme and is identical in mathematical form with the Michaelis-Menten equation:

$$\text{Effect} = \frac{\text{Maximal Effect (D)}}{K_D + (D)}$$

Where (D) = free drug concentration and K_D (= k_2/k_1) = the dissociation constant for the drug-receptor complex. The fraction of receptors occupied by the drug is [D] / (K_D + [D]).

The scheme defines the drug's 'potency', that is, the dependency of effect on the drug's concentration. The equation describes a simple rectangular hyperbola. There is no effect if (D) = 0 and the effect is half-maximal when (D) = K_D, that is when half of the receptors are occupied. The maximal effect is approached asymptotically as (D) increases above K_D. If the effect is plotted against log (D), the familiar sigmoidal dose-effect curve is obtained (Ross 1996). A linear form of this relationship is obtained by taking the reciprocal of both sides of the expression and constructing the equivalent of a Lineweaver-Burk plot for enzyme kinetics. When a linear relationship exists between concentration and effect, the EC_{50} of the drug can be calculated. This is the concentration where it is half-maximally effective and is equal to the drug's K_D in many but not in all cases.

In certain situations receptor occupancy is not related to response and signal amplification may occur between receptor occupancy, effector activation and response. Thus the dose-response curve will be to the left of the receptor-occupancy curve. From these basic concepts drug-receptor theories have evolved that are widely applied to illustrate the drug's agonistic or antagonistic effects on receptors (Ross 1996).

1.3 PHARMACOKINETIC/PHARMACODYNAMIC MODELLING

The relationship between the dose of the drug and its eventual pharmacological effect can be summarised in the following diagram:

Dose – PK – Cp – PK/PD – CE – PD – E – PE – Effect

Where PK = pharmacokinetics, Cp = serum concentration of drug,

PK/PD = pharmacokinetic-dynamic relationship, CE = effect site concentration,

PD = pharmacodynamics, E = the observable effects,

PE = physiological effect at tissue concentration (Holford and Sheiner 1981).

A variety of mathematical models have been developed to describe the relationship between the pharmacological effect of a drug and its concentration. A brief description of these models follows.

1.3.1 **Types of PK-PD models**

1.3.1.1 Quantal dose response model (Categorical Data)

This is the simplest model to explain drug-effect relationships. The observed effect is either present or absent, or is defined by some criterion, such as a greater than 50% reduction in apnoea incidents. Thus the effect is 'fixed', but remains either present or absent; this model is thus applicable to dichotomous data.

With only two observations, the data is called dichotomous, and m = 2. Categories can be ordered, for example the drug effect can be classified into none, little, moderate or much. Thus, an observation, which can be one of m labels, is called an m-categorical observation. Analysis of categorical data uses logistic regression and gives a quantal dose-response curve. A number of observations can be in category one, category two etc. Thus, with each frequency observation, there are N underlying m-categorical observations, but they are not separately recorded. This is referred to as m-categorical frequency observations (Beal and Sheiner 1996).

No assumptions need to be made regarding the form of the relationship between the

concentration of the drug such as theophylline and the 50% reduction in apnoea counts. This is because only one degree of effect is considered, and the effect model has only one parameter, namely the concentration at which the 50% reduction occurs. The serum concentration, at which the desired effect appears, will vary among the patients. The degree of the effect is not important, but only whether it occurs or not (Holford and Sheiner, 1981 and 1982).

This model has been successfully applied in determining serum concentrations of alfentanil required to supplement nitrous oxide anaesthesia (Ausems *et al* 1986). Muttitt *et al* (1988) in a study of the dose concentration-effect relationship of theophylline in premature neonates determined whether a decrease in apnoea incidents occurred at a given concentration of theophylline. (The definition of response in Muttitt's study was a fall in number of apnoea to below 0.33 episodes per hour.) Although the authors did not analyse the pharmacodynamics of the drug, the results showed a cumulative frequency response curve with a sigmoidal shape. Most of the patients responded at a serum theophylline concentration of 12.7 mg/L.

1.3.1.2 Linear model

This model describes a continuous effect of the drug over the observed concentrations. Thus the data would be continuous variable data such as the measurement of blood pressure. When the drug concentrations are low in relation to EC_{50}, the effect becomes proportional to concentration and the slope of the line relates the effect to the concentration:

$$E = S * C$$

Where E = the intensity of the effect, S = slope of the curve, C = drug concentration.

The parameters can be estimated by linear regression. This model is derived from the E_{max} model and it predicts no effect when the drug is absent. The model cannot describe maximum effect, and is thus used to study effects in the range below 50% of the maximum effect. If however, the effect has some value when the drug is absent, such as blood pressure, the equation becomes:

$$E = S * C + E0$$

Where E0 = the effect without the drug, or the baseline effect.

Thus this model is applicable when it is not practicable to achieve maximum effect and when effects are studied in the range below 50% of the maximum effect (Oosterhuis and van Boxtel 1988).

This model was applied to the theophylline data collected by Falliers (1975). A linear relationship was found between forced expiratory volume (FEV_1), expressed as a percentage of predicted 'normal'. The theophylline concentrations ranged from 2 to 18 mg/L. The intercept value (E0) was estimated at 58.2%, which was close to the pre-drug mean FEV_1 of 55.2%. The slope of the line was estimated from pooled data of all the patients and was 1.23% per mg/L. This value was much lower than the 3.4% per mg/L estimated in a single patient. This difference occurred because the data was pooled and the intra-individual correlations were ignored (Holford and Sheiner 1982). It is possible that a population analysis approach may have been better in this case.

1.3.1.3 Log-linear model

This model is derived from the linear model and is often used to describe the relationship between concentration and effect; thus it may be applied to continuous variable data. As many drugs act over a wide concentration range, the concentration axis of the effect-concentration relationship is often converted to a log-scale, compressing the scale.

Thus:

$$E = S \log (C) + I$$

Where I is intercept and an arbitrary constant with no physical meaning.

This model cannot predict the effect when no drug is present, and cannot determine the maximum effect (Holford and Sheiner, 1981 and 1982). This is an important drawback and may obscure the existence of a maximal effect (Holford and Sheiner 1982).

The log-linear model has often been used to explain drug action, as the relationship between log concentration and effect, in the effect range 20% to 80%, is frequently linear. This effect range is also highly relevant under clinical conditions (Oosterhuis and van Boxtel 1988).

Singh *et al* (1980) used the log-linear model to describe the effects of timolol on heart rate during exercise and found only a weak correlation. However, when the same data was fitted to an inhibitory E_{max} model the existence of a maximal effect was shown (Holford and Sheiner 1982). (See 1.3.1.4 below.) This was one of the manipulations that highlighted the limitations of the log-linear model.

This model was used to describe the relationship between the decreasing plasma theophylline concentration and number of apnoea in 6 premature infants (Lagercrantz *et al* 1980). The apnoea frequency was monitored after cessation of theophylline treatment and plotted against the log plasma concentration of theophylline. The regression line crossed an arbitrarily chosen cut-off level of 2 apnoea per 12 hours at a mean theophylline concentration of 7 mg/L. Based on these results the authors recommended a plasma theophylline concentration >7 mg/L for effective control of apnoea. They defined apnoea as cessation of breathing for longer than 30 seconds or less, if accompanied by a decrease in heart rate below 100 beats per minute

1.3.1.4 E_{max} model

The E_{max} model is generally the most appropriate model to adequately describe the effect of a drug over the whole range of drug concentrations and the maximum effect a drug can achieve. It may be applied to continuous variable data as the log-linear model described above. It is based on a hyperbolic relationship:

$$E = E_{max} * C / (EC_{50} + C)$$

Where E_{max} = the maximum effect ascribed to the drug and EC_{50} = the concentration at half-maximal effect.

According to this equation, when the concentration is zero, there is no effect. This model can also accommodate a baseline effect as follows:

$$E = E0 + E_{max} * C / (EC_{50} + C)$$

Where E0 = baseline effect.

This model also includes the 'law of diminishing returns'; thus ever-higher concentrations are needed to increase the effect by a given amount (Holford and Sheiner 1981, 1982).

When the drug effect is measured as inhibition of a certain observation, for example disappearance of some effect (such as apnoea) from a baseline level, the formula can be written as follows:

$$E = E0 - (E_{max} * C) / (IC_{50} + C)$$

Where IC_{50} = the concentration producing half-maximal effect.

If a drug is able to completely abolish an effect, the value of E_{max} is equal to E0, which then becomes:

$$E = E0 * \{1 - C / (IC_{50} + C)\}$$

The expression $C / (IC_{50} + C)$ describes the relationship between the concentration and the fraction of maximal effect that can be attributed to the drug. This is the fractional E_{max} model (Holford and Sheiner 1981, 1982).

Mitenko and Ogilvie (1973) used the E_{max} model to illustrate the effects of theophylline on airway obstruction in six asthmatic patients. They determined the change in FEV_1 over a range of theophylline concentrations and expressed the effect as a percentage of the expected FEV_1 in a normal patient. The maximum effect predicted by the model was 63% and the EC_{50} of theophylline was 10 mg/L.

The E_{max} model is considered the basic pharmacodynamic model and is widely applied to describe concentration-effect relationships, using continuous variable data. When a particular effect is studied in the range below 50% of the E_{max} or where no maximum effect can be obtained, the linear model will provide a good approximation of the concentration-effect relationship.

1.3.1.5 Sigmoid E_{max} model

The Sigmoid E_{max} model is based on the E_{max} model with the addition of a parameter that changes the simple hyperbolic form of the E_{max} model:

$$E = E_{max} * C^N / (EC_{50}{}^N + C^N)$$

Where N = a number influencing the slope of the curve.

If N equals 1 the equation describes a hyperbolic function, and if N is greater than 1 the curve will be sigmoid-shaped (Holford and Sheiner 1981, 1982). The sigmoid E_{max} model has been used by a number of investigators such as Stanski *et al* (1979) who used the model to describe the effect of d-tubocurarine on muscle strength.

1.3.1.6 Count Model

The count model is based on the E_{max} model (Beal and Sheiner 1996) but is applied to 'count' data (discrete events) whereas the E_{max} model is normally applied to continuous variables. By a 'count' is meant the number of events occurring in a given volume of time or space, for example the number of apnoea per hour. The model describing the relationship when a baseline count is present, will be:

$$E = A + B \{1 - C / (D + C)\}$$

Where B = the effect due to the drug, A + B = the typical baseline count, C = the concentration of the drug, and 1 – C / (D + C) = the fractional reduction due to the presence of the drug.

For this count model the Poisson distribution is deemed suitable. The Poisson distribution is used to model discrete events that occur infrequently in time or space; thus it is sometimes called the distribution of rare events (Pagano and Gauvreau 1993). The underlying assumptions for the Poisson Distribution are:

1. The probability that a single event occurs is proportional to the length of the interval.
2. Theoretically within an interval an infinite number of events are possible.
3. The events occur independently both within the same interval and between consecutive intervals.

This Poisson distribution describes the probability (P) that the number K assumes the value k and is given by

$$P (K=k) \sim \exp (-\lambda_I) \lambda_1^k / k!$$

In this count model adapted for population modelling within NONMEM, the parameter λ_I is related to concentration (or time) as follows:

$$\lambda_I = \theta_1 - \theta_2 (CONC_I) + \eta_I$$

Where λ_I = the mean number of events, given η_I.

Thus with this model (where the drug reduces the number of events from some baseline (θ_1)), $\theta_1 - \theta_2 (CONC_I)$ is also the population mean for individuals with $CONC_I$. λ_I is also the variance of the number of events, given η_I. With this additive model, the population variance is $\theta_1 - \theta_2 (CONC_I) + \omega^2$ (Beal and Sheiner 1996).

In this area of very topical research, count models are being used in population analysis to describe various drug effects such as reduction in apnoea or seizure frequency, as well as for drugs that promote the occurrence of a positive event.

1.3.1.7 Models for indirect pharmacodynamic response (based on Dayneka *et al* 1993, and Jusko and Ko 1994)

Many drug responses, (R), may be considered indirect in nature. Four basic models to represent drug responses that are characterised by indirect mechanisms have been developed. Factors controlling the production or input (k_{in}) of the response variable may be either inhibited or stimulated; similarly, the determinants of loss, (k_{out}), of the response variable may also either be inhibited or stimulated.

The rate of change of the response over time with no drug present can be described as follows:

$$dR/dt = k_{in} - k_{out} * R$$

Where k_{in} = the zero-order constant for production of the response and k_{out} = the first-order constant for loss of the response, R = the measured response variable.

The effect site represents a separate site of drug action where the mode of action controls either stimulation or inhibition of the designated controlling process (k_{in} or k_{out}).

Model 1 and Model 2 represent inhibitory processes that operate according to the inhibitory function, I (t):

$$I(t) = 1 - Cp / (Cp - IC_{50})$$

Accordingly, the rate of change of drug response in Model 1 can be described as follows:

$$dR/dt = k_{in} * I(t) - k_{out} * R$$

Model 2 describes drug response that results from inhibition of the factors controlling the dissipation of the response variable:

$$dR/dt = k_{in} - k_{out} * I(t) * R$$

Model 3 and Model 4 represent processes that stimulate the factors controlling drug response and operate according to the stimulation function S (t):

$$S(t) = 1 + E_{max} * Cp / (EC_{50} + Cp)$$

Thus Model 3 describes drug response that occurs from stimulation of factors that control the production of the response:

$$dR/dt = k_{in} * S(t) - k_{out} * R$$

and Model 4 the dissipation of the stimulating drug response:

$$dR/dt = k_{in} - k_{out} * S(t) * R$$

The development of these models emphasised the importance of mechanism based pharmacokinetic/pharmacodynamic modelling. Therefore an understanding of the biochemical events involved in the pharmacological action of the drug should be a prerequisite for the development of appropriate drug-effect models. These models have been extensively discussed and applied to diverse drugs when time lags exist between plasma or biophase drug concentrations and the time course of pharmacodynamic responses (Jusko and Ko 1994).

It is not clear whether these indirect models might apply to theophylline and its effect on apnoea in the neonate and it appears as yet that none of them has been used. It is known that theophylline probably has diverse mechanisms of action for the relief of apnoea of prematurity. For example, the stimulating effect of theophylline on the medullar centre and the resultant increase in its sensitivity is most probably mediated through theophylline's blocking effect on adenosine receptors (Bissonnette *et al* 1991, Barnes and Pauwels 1994, Griffiths *et al* 1997). This action may be responsible for the increase in ventilation, increase in breathing movements and the decrease in diaphragmatic muscle fatigue. Theophylline also relaxes constricted bronchial smooth muscle via inhibition of phosphodiesterases (Mehta *et al* 1991, Schudt *et al* 1995, Banner and Page 1996) and this must certainly be an indirect effect. Furthermore, it has direct and indirect effects on intra-cellular calcium (Kolbeck and Speir 1989, Gayan-Ramirez *et al* 1995) which may also contribute to the decrease in the number of apnoea.

1.4 CONCENTRATION-EFFECT RELATIONSHIPS OF METHYLXANTHINES

The magnitude or intensity of a drug's effect is usually dependent on the dose administered and the resultant serum drug concentrations. In general, as the dose administered increases, the magnitude of the effect will increase in a gradual fashion until a maximum is reached. Therefore, quantitative dose-response relationships may be constructed and visualised using the pharmacodynamic models described above. This has been done for some of the effects of theophylline in certain populations and will be discussed briefly in relation to the models that were described in 1.3 above.

1.4.1 **Concentration-effect relationship of theophylline in asthma**

In adult asthmatic patients a significant relationship between the bronchodilating effect of theophylline and the serum theophylline concentration has been shown. This relationship has been defined as proportional to the logarithm of the serum concentration over the range of 5 to 20 mg/L (Levy and Koysooko 1975, Simons *et al* 1982). This range of serum concentrations has been termed the "therapeutic range" for theophylline in adults with asthma.

A similar concentration-effect relationship was obtained in asthmatic children. The pharmacological effect, described as an improvement of forced expiratory volume in the first second, showed a linear correlation between the intensity of the effect and the logarithm of plasma concentration of the drug (Levy and Koysooko 1975).

The therapeutic range or therapeutic window of a drug indicates the serum drug concentration limits between lowest effective and lowest toxic dose in a population of

patients (Rowland and Tozer 1995). As the drug concentration must always be interpreted in conjunction with the clinical assessment of the patient, the therapeutic range might bear little relationship to the effects of the drug at a particular concentration in a particular patient. The concept of target drug concentration was created to achieve the optimum concentration to produce the optimum effect in a particular patient. This concept is based on the target concentration strategy (TCS) for therapeutic drug monitoring developed by Sheiner and Tozer (1978). This is achieved by first selecting a target concentration for a specific patient and then applying prior determined population pharmacokinetic parameters to calculate the loading and maintenance doses as well as the rate of administration to achieve the target concentration. Using the measured serum concentrations of the drug, the individual values for volume of distribution and clearance may then be calculated. The selected target concentration might be revised, if necessary based on the clinical assessment of the patient. The frequency of concentration measurements depends on the clinical assessment of the patient and the drug's pharmacokinetic parameters. For example, the asthmatic patient with altered theophylline clearance due to congestive cardiac failure or pneumonia may require daily or more frequent serum concentration monitoring than the stable asthmatic patient. Target concentration strategy is important for drugs with a narrow therapeutic range such as theophylline. Therapeutic drug monitoring of theophylline has been successfully used in the treatment of asthmatic patients.

1.4.2. **Concentration-effect relationships of theophylline in neonates**

1.4.2.1 The concentration-effect of theophylline on auditory evoked potentials in neonates

No linear correlation could be found between the effects of theophylline, the dose and also the serum drug concentrations when the neuro-physiological effects of aminophylline on apnoea and brain stem auditory evoked potentials were evaluated (Chen *et al* 1994).

1.4.2.2 Concentration-effect of theophylline on cardiac function

The effect of theophylline on cardiac function in premature neonates was investigated by Walther *et al* (1986). A theophylline loading dose of 6.8 mg/kg and maintenance doses of 2 mg/kg every eight hours were administered. A mean theophylline concentration of 7.3 ± 0.8 mg/L was obtained. Stroke volume increased initially but returned to pre-treatment values after a few days of treatment. Mean arterial pressure did not change but cardiac output and heart rates were increased. No linear relationship between cardiac output changes and theophylline concentrations was observed.

In contrast Nadkarni *et al* (1988) in a study with premature neonates of comparable gestational and postnatal ages, found a good relationship between the increase in heart rate and serum theophylline concentrations up to 10 mg/L. No linear relationship was found at higher serum theophylline concentrations. No tachycardia (defined as >180 beats per minute) was observed at theophylline concentration greater than 20 mg/L, although it has been associated with serum concentrations above 15 mg/L in other studies (Shannon *et al* 1975, Jones and Baillie 1979, Aranda *et al* 1992). The effect of

theophylline on heart rate seems to be inconsistent. Most studies show an increase in heart rate (Aranda *et al* 1992), but reports of no significant effect on heart rate are also found (Finer *et al* 1984, Muttitt *et al* 1988).

1.4.2.3 The concentration-effect of theophylline on apnoea

The relationship between serum concentration and efficacy of theophylline in abolishing idiopathic apnoea in premature infants appears to be not well defined. Only a few publications are available, as it is difficult to perform such studies on patients as small and as frail as the premature neonate.

One of the most frequently quoted study was that of Muttitt *et al* (1988) who studied the effect of theophylline in 22 premature neonates with mean (range) gestational age of 30 (26 to 32) weeks and postnatal age 4 (1 to 17) days. Apnoea was defined as ≥20 seconds or less if accompanied by bradycardia and a 10% decrease in peripheral oxygen saturation. A loading dose of 4 mg/kg and maintenance doses of 1 to 1.5 mg/kg eight hourly were used to attain four different levels of serum concentrations namely 4.2, 8.5, 12.7 or 15.3 mg/L, depending on response in each individual. A non-response was defined as ≥0.33 apnoea per hour. Fourteen percent of the patients responded at a serum theophylline concentration of 4.2 mg/L, a further 14% at 8.5 mg/L, then a further 45% at 12.7 mg/L and the remaining 4.5%, at 15.3 mg/L. Twenty-three percent of the patients (n = 22) were considered non-responders and they eventually required doxapram and/or continuous positive airway pressure. Characteristics of these non-responders were not given. Respiratory function as well as apnoea incidents were recorded. No significant change was detected in measures of ventilation across the four levels of theophylline.

There was a significant increase in inspiratory pressure, tidal volume, and minute ventilation at the maximum dose. However, the significant improvement in ventilation measures did not ensure a concomitant decrease in apnoea frequency, nor did a decrease in apnoea frequency reflect an increase in ventilation. A cumulative response graph was constructed to show that most of the patients responded at a serum theophylline concentration of 12.7 mg/L.

Lagercrantz *et al* (1980) in a study of six premature neonates used the log-linear model to describe the relationship between plasma theophylline concentration and number of apnoea after cessation of theophylline treatment. The regression line crossed an arbitrarily chosen cut-off level of 2 apnoea per 12 hours (0.16 apnoea per hour) at a mean theophylline concentration of 7 mg/L.

Two other studies of theophylline in premature neonates with apnoea showed no linear correlation between effect and serum theophylline concentrations (Milsap *et al* 1980, Roberts *et al* 1982).

The theophylline concentration–response relationship is complicated by the fact that theophylline is converted to caffeine and both may stimulate the central nervous system (Bada *et al* 1979, Bory *et al* 1979, Boutroy *et al* 1979) which may cause a decrease in apnoea. Serum caffeine concentrations may be detected in the neonate from the first day of theophylline administration and as caffeine has a longer half-life than theophylline (Aranda *et al* 1992) these concentrations may increase gradually. Premature neonates treated with theophylline may achieve mean caffeine concentrations of 4.4 mg/L by the

eleventh day of treatment (Brazier *et al* 1979, Brazier *et al* 1981). A further factor influencing the concentration of caffeine is the consumption of caffeine-containing beverages by the neonate's mother immediately before or during birth as this could be transferred to the neonate. In the present study, however, none of the mothers took any caffeine containing substances (for example coffee or cola) before or during birth. In long-term investigations of more than three days the total methylxanthine concentration should be measured to determine the concentration-effect relationships on apnoea of prematurity. It has been shown in Section B that the concentrations of caffeine in these first three days after birth were very small and therefore the possible influence of this metabolite was not investigated further.

In the treatment of neonatal apnoea, many people are attempting to employ target concentration strategies as used successfully in the treatment of asthma. Some of the earlier investigators observed that apnoea could only be controlled with serum theophylline concentrations greater than 5 mg/L but cardiovascular side effects were associated with serum concentrations greater than 13 mg/L (Shannon *et al* 1975, Jones and Baillie 1979). On the contrary, in other studies with neonates of comparable postconceptual ages, theophylline concentrations as low as 2 to 4 mg/L appeared to control apnoea and also bradycardia (Milsap *et al* 1980, Myers *et al* 1980). However, a poor response (3 out of 22 neonates responded) was demonstrated in neonates with serum theophylline concentrations between 4 to 8 mg/L by Muttitt *et al* (1988). After increasing mean serum concentrations to 12.7 mg/L, an additional 63% response rate was noted. (a response was defined as <0.33 apnoea per hour). Based on these and other studies, Aranda *et al* (1992), in a subsequent review, stated that the desired serum

concentration of theophylline should range from 5 to 15 mg/L. To achieve and maintain these serum concentrations, a loading dose of 5 to 6 mg/kg of theophylline followed by maintenance doses of 2 to 4 mg/kg daily in two to four divided doses were proposed. Table C.1.1 below is a summary of the diverse therapeutic ranges and dosing guidelines for the treatment of neonatal apnoea.

TABLE C.1.1

Recommended dosages and target serum concentrations for theophylline in neonates for the treatment of apnoea

Theophylline base			
Loading dose IV or orally	Maintenance dose IV or orally	Therapeutic range mg/L	Reference
5 - 6 mg/kg	1 - 2 mg/kg, 8 or 12 hrly	5 - 15	Halliday 1998
5 mg/kg	2 mg/kg, 12 or 24 hrly	5 - 15	Rall 1996
Premature <24 d 1.0	1.0 mg/kg 12 hrly	5 - 10	Hendeles *et al* 1995
5 - 6 mg/kg	1 mg/kg 8 hrly	5 - 15	Aranda *et al* 1992
5.5 mg/kg	3.3 - 4, 8, 12 or 24 hrly	-	Besunder *et al* 1988
5 mg/kg	1.5 mg/kg 12 hrly	6 mg/L (target conc)	Gal and Gilman 1986
2.5 mg/kg	0.66 mg/kg 8 hrly	3 - 5	Milsap *et al* 1980
2.5 mg/kg	0.66 mg/kg 8 hrly	3 - 4	Myers *et al* 1980
5 mg/kg	1.2 8 hrly	2 - 10	Dietrich *et al* 1978

Abbreviations: d = days postnatal age, IV = intravenous, hrly = hourly, conc = concentration.

These disparate recommendations confirm that the concentration-effect relationship and hence the therapeutic targets are, as yet, not well defined in the treatment of neonatal apnoea.

1.5 OBJECTIVES

Accordingly the objective of this section of the study was to use NONMEM to do a much more sophisticated concentration-effect analysis of theophylline, in premature neonates with apnoea, during the first few days after birth.

SECTION C: CHAPTER 2

METHODS

2.1 THE DATA

Total apnoea (that is, all apnoea ≥5 seconds) incidents were used for the pharmacodynamic modelling. Patient data, theophylline administration and serum theophylline concentration measurements are the same as those described in Section A, Chapter 2. Recording and analysis of clinical effects are also as described in Section B, Chapter 2.

A data-file was constructed with the following parameters (abbreviation in parenthesis):

- Patient number (ID)
- Gender (GEN) with males = 1 and females = 2
- Weight in kg (WT)
- Gestational age in weeks (GA)
- Density of all apnoea ≥5 seconds in observation time (FAPT)
- Number of all apnoea ≥5 seconds in observation time (NAPN=DV)
- The serum theophylline concentration mg/L (CONC)
- Duration of observation time e.g. 4 hours on that day (DOBS)
- The time the sample was taken (TIME) in hours
- The day, that is day 0, 1, etc (DAY)
- The condition of the patients (COND)
- Whether the neonate received respiratory support (OXY) yes = 1 and no = 2

- The postconceptual age (PCA) in weeks.

(See Appendix C.1 for an example of the data file).

2.2 CONCENTRATION-EFFECT MODELLING

Modelling of the data to determine the relevant pharmacodynamic parameters was performed using the computer package NONMEM (version V Level 1.0, double precision. See Section A, Chapter 2). Estimates of the following were obtained: (i) the objective function value for each model (OFV), (ii) population means of the pharmacodynamic parameters E_{max}, EC_{50}, baseline number of apnoea and other relevant parameters as specified in the model and (iii) variances of estimated parameters.

Criteria used for selecting a model included: a change in OFV of 6.8 ($p<0.01$) or greater, visual inspection of scatter plots of predicted versus recorded number of apnoea, and a decrease in unexplained variability.

As the data dealt with a number of events occurring in a given volume of time, it was classified as count data where the intra-individual distributions of the data are discreet and/or very asymmetrical (Beal and Sheiner 1996). Therefore, all analyses were performed using the LaPlacian estimation method. In all cases the concentration used was the actual concentration measured during the period of apnoea recordings. Consequently no pharmacokinetic models were used to predict concentrations. A number of pharmacodynamic models (structural models) were tested as detailed below.

The inter-patient variability was modelled using an exponential model. For each structural model, various numbers and placements of inter-individual variability parameters on the PD parameters were tested.

2.2.1 The fractional E_{max} count model using theophylline concentration as the independent variable

A control stream was written based on the fractional E_{max}-model using concentration of theophylline as independent variable. As a decrease in apnoea is expected with the use of theophylline, the inhibitory model was used.

The base model was therefore built upon the following equation:

> APNOEA COUNTS per hour = BASELINE COUNT per hour * (1 - maximum fractional reduction * concentration of drug / (concentration of drug that will cause 50% inhibition of apnoea counts + concentration of drug)).

Using abbreviations for the parameters, the above equation was re-written as:

$$HAPN = E0 * (1\text{-}FEMX * CONC) / (IC_{50} + CONC)$$

Where HAPN = the number of apnoea counts per hour, E0 = baseline apnoea count per hour, FEMX = maximum fractional reduction of apnoea counts due to the drug, IC_{50} = concentration of theophylline (mg/L) that will cause a 50% inhibition of apnoea and CONC = concentration of the drug (mg/L). (See Appendix C.2 for an example of the NONMEM control stream).

All available covariates were tested by adding them to the base model as follows:

$$HAPN = E0 * P * (1 - FEMX * CONC / (IC_{50} + CONC))$$

Where P was a covariate such as weight, or PCA etc.

The following covariates were screened: weight (kg), gestational age (weeks), postconceptual age (weeks), respiratory support (oxygen supplied per head-box), and condition of the neonate. 'Condition' was noted as = 1 on the data sheet if any of the following was present at birth: hypoxia, hypothermia, hypo/hyperglycaemia, hypotonia, congenital infection, prolonged rupture of membranes. The attending physician recorded these findings in the clinical notes of the neonate. These conditions may contribute to an overall poor condition of the neonate. (See Appendix C.3 for the distribution of the available covariates).

2.2.2 The sigmoid E_{max} count model using theophylline concentration as independent variable

The sigmoid E_{max} model, has an additional parameter, N, which allows for differences in the shape of the relationship of E_{max} and EC_{50}. Therefore the basic structure of the model was as follows:

$$HAPN = E0 * (1 - FEMX * CONC^N / (IC_{50}^N + CONC^N))$$

Where N is the number influencing the slope of the curve.

All the other parameters are as described in the model above.

(See Appendix C.4 for an example of the NONMEM control stream).

2.2.3 **Time count models using time as independent variable**

It is acknowledged that the lapse of time from birth may have an effect on the number of apnoea counts due to physiological changes and maturation of the premature neonate, as described in the literature review part of Section B (see page 99). Therefore, models were written where time instead of concentration was the independent variable. In order to cancel the influence of the drug, it was assumed that the theophylline concentration was equal to zero. Three different types of time models were tested: an exponential, an E_{max}, and a sigmoid E_{max} model.

The exponential time model was based upon the following equation:

$$HAPN = E0 * (1- FEMX * EXP\ KD)$$

Where KD is an exponential change in time.

All the other parameters are as described above.

Therefore:

$$HAPN = E0 * (1-FEMX * EXP (-Log (2) / T_{50} + TIME))$$

The E_{max} time model was written using the E_{max} model described earlier but substituting 'CONC' with 'TIME':

$$HAPN = E0 * (1-FEMX * TIME / (ET_{50} + TIME))$$

The sigmoid E_{max} time model was also tested. This equation was based on that described in paragraph 2.2.2:

$$HAPN = Baseline * (1-FEMX * TIME^{N} / (ET_{50}^{N} + TIME))$$

(See Appendix C.5 for an example of the NONMEM control stream for the E_{max} time model).

2.2.4 **Time and concentration count models**

The influence of both concentrations of theophylline and time was modelled together based on disease progression models proposed by Holford *et al* (1993). In these models a hypothetical respiratory depression factor (RDF) was used to describe the effect of time on the apnoea counts. It was assumed that RDF is present at birth at a certain concentration. This RDF has to be removed over time – this concept could be compared with, for example maturation of receptor sites, or resetting of chemoreceptors to improve breathing control in the neonate. As the removal of RDF could be associated with an improvement, a decrease in the number of apnoea counts is expected to occur. It was assumed that the concentration of RDF = 1 at birth.

Thus the basic model was written as follows:

HAPN = E0 + EFFECT OF RESPIRATORY DEPRESSION FACTOR * (1-EFFECT OF THEOPHYLLINE)

Using abbreviations as above, this was again re-written as:

HAPN = E0 + (ERDF at time = Time * (1-ETHEO at CONC = CONC))

Where ERDF = effect of RDF, and ETHEO = effect of theophylline.

The E_{max} models for RDF and drug effect were then combined as follows:

HAPN = E0 + ERDF * (1 – ETHEO), thus

HAPN = E0 + ((RMAX * RDFT / (RDFT + RC50)) * (1- CONC / (CONC + TC_{50}))

Where RMAX = the maximum increase in apnoea due to RDF, RDFT = concentration of RDF at time = Time, RC50 = EC_{50} of RDF, CONC = concentration of theophylline and TC_{50} = IC_{50} of theophylline. (See Appendix C.6 for an example of the NONMEM control stream).

A control stream was written to do simulation of the time-concentration model. (See Appendix C.7 for an example of the NONMEM control stream). Estimates of the model parameters are used to simulate the offset model to enable visualisation of predictions from the proposed model. For the application of the simulation results, specific data files were constructed. (See Appendix C.8 for an example of this data sheet).

SECTION C: CHAPTER 3

RESULTS

3.1 THE DATA

The patients, drug administration and serum theophylline concentration measurements, are the same as those in Section B. Results of the recording and analysis of clinical effects are identical to those described in Section B (3.1). The data set available for pharmacodynamic modelling comprised of 46 subjects with 154 concentration-effect data points. The total apnoea/h (that is all apnoea ≥5 seconds) incidents were selected as the effect measurement to be modelled with the actual serum theophylline concentrations. The number of these apnoea events changed significantly from baseline over the three days of monitoring and a larger number of these events compared to pathologic apnoëas, were available for modelling.

3.2 MODELLING

3.2.1 Results of modelling the fractional E_{max} count model and the sigmoid E_{max} model with theophylline concentration as the independent variable

The results are given in Table C.3.1. For the base model (model 1) inter-individual variability, η, was estimated for each pharmacodynamic parameter viz. for the baseline counts, the fractional E_{max}, and EC_{50}. The estimated value for η_3 on EC_{50} was very small and estimation of η_3 was thus omitted from the next model, model 2. The resultant OFV did not change neither did the estimated values of the other parameters. Another possibility was that the small η_3 for model 1 was the result of a correlation between E_{max}

and EC_{50}. This was tested by using a covariance (block2) matrix, on the FEMAX and EC_{50} parameters (model 3). This resulted in a drop of 7.6 in the OFV compared with model 1. However when the initial estimates of parameters were changed (model 4), a further reduction of the OFV was obtained (change in OFV of 12 compared with model 1), suggesting instability possibly due to overparameterisation of the model.

Similar variations to the above were tried with the Hill equation (Sigmoid E_{max} model) in models 5, 6 and 7 (Table C.3.1). None of these models proved better than the simple E_{max} model (model 1) with respect to OFV.

Due to the sensitivity to initial estimates of models 3 and 4, model 1 was selected as the most appropriate model in this series. In addition visual inspection of the scatter plots of predicted versus recorded number of apnoea of the base model (Fig C.3.1), showed no obvious difference from model 4 (Fig C.3.2). For model 1, estimated population parameter values for baseline, EC_{50} and E_{max} fraction were 16.4 apnoea per hour, 0.37 mg/L and 0.47 respectively. The estimate of the population value for baseline of 16.4 was very similar to the simple average of 16.0 apnoea/hour (Table B.3.2) and was very stable across the various models.

The available covariates were tested on the base model (model 1) and only on their influence on the variability in baseline counts. The results given in Table C.3.2 showed no significant improvement in the fit of the data. See Appendix C.3 for plots of the distributions of the covariates.

TABLE C.3.1

Results of the E_{max} and sigmoid E_{max} count models

Model number and properties	OFV	Baseline counts (η%)	FEMAX (η%)	EC_{50} (η%)	N
1 (3 η's)	1283.563	16.4 (71)	0.474 (136)	0.371 (0.00003)	–
2 (2 η's)	1283.563	16.4 (71)	0.473 (136)	0.371 –	–
3 (Omega block2)	1275.918	16.5 (70)	0.471 (126)	0.099 (289) (η2,3 = -30)	–
4 (Omega block2)	1271.191	17.5 (71)	0.587 (63)	0.478 (406) (η2,3 = -90)	–
5 (3 η's)	1284.634	16.4 (71)	0.438 (121)	0.061 (49)	6.09
6 (2 η's)	1284.634	16.4 (71)	0.438 (121)	0.105 –	5.9
7 (3 η's and Omega block(2) on FEMAX and EC_{50})	1284.634	16.4 (71)	0.438 (121)	0.072 (48) (η2,3 = 4.1)	6.16

OFV = Objective function value, η = inter-individual variability, FEMAX = the fractional E_{max}, EC_{50} = the concentration of theophylline (mg/L) that decreases the apnoea counts by 50% of E_{max}, N = the Hill coefficient.

TABLE C.3.2

Testing of covariates on fractional E_{max} count model

Model number and (covariate) tested	OFV	Baseline Counts [covariate estimate] (η%)	FEMAX (η%)	EC_{50} (η%)
8 (Postconceptual age)	1279.017	18.0 [PCA = 0.043] (81)	0.566 (131)	0.47 (149)
9 (Oxygen support)	1283.562	16.4 [OXY = 0.994] (71)	0.474 (137)	0.37
10 (Weight)	1289.913	13.1 [WT on baseline] (81)	0.824	5.29 (224)
11 (Gender)	1279.864	17.8 [Males = 0.9] (71)	0.493 (122)	0.10 (192)
12 (Condition)	1280.040	17.3 [COND = 0.923] (71)	0.770	3.13 (249)

OFV = Objective function value, FEMAX = the fractional E_{max}, EC_{50} = the concentration of theophylline (mg/L) that will decrease the apnoea counts by 50%, η = inter-individual variability, PCA = postconceptual age (weeks), OXY = oxygen support at time of monitoring, WT = weight (kg), COND = condition of the neonate.

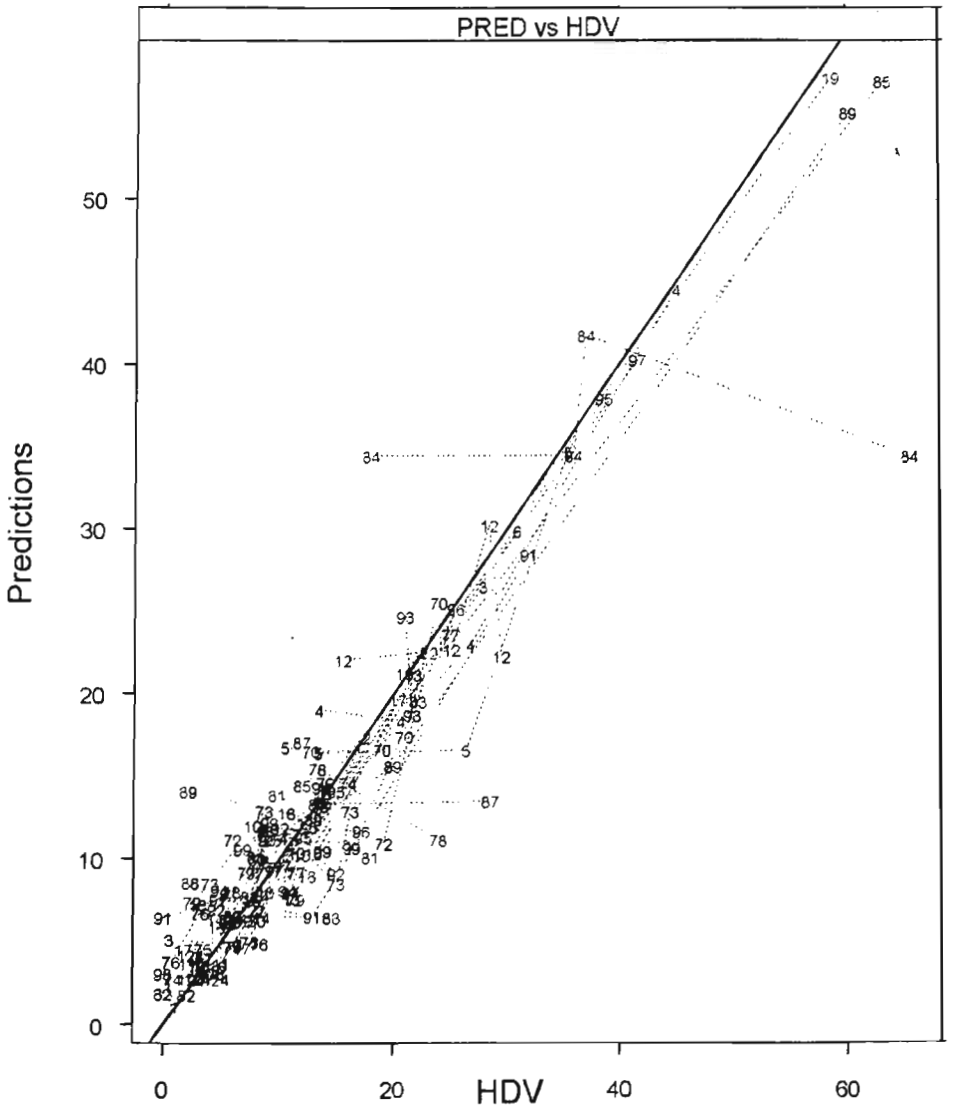

Fig.C.3.1 Predictions vs dependent variable, base model (Model 1)

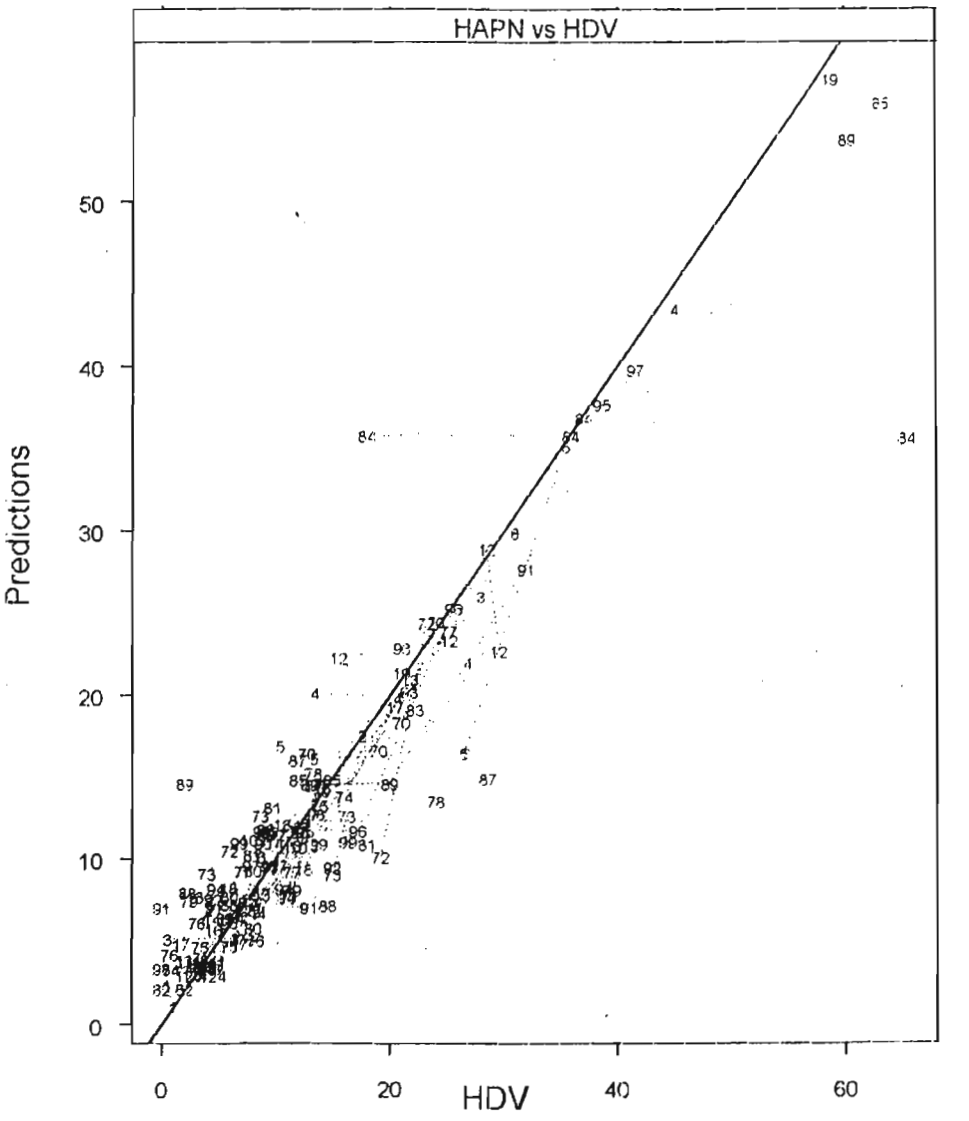

Fig.C.3.2 Predictions vs dependent variable, Model 4

3.2.2 **Results of time count models and time plus concentration count models**

Variations of the time model as described in Section C 2.2.3 were tested and the results are given in Table C.3.3.

TABLE C.3.3

Results of the time count model

Model number and properties	OFV	Baseline (η%)	FEMAX (η%)	ET_{50} (hour) (η%)	N (η%)
13 Exponential time model	1298.782	40.3 -	10 -	0.105 -	38.3
14 E_{max} time model	1276.863	17 (68)	0.565 (105)	0.0476 (237)	1 (Fixed)
15 Sigmoid E_{max} time model	1274.679	16.6 (67)	0.495 (109)	0.053 (169)	0.969 -
16 E_{max} time model with N fixed to 1 and Omega block(2)	1272.472	16.5 (71)	0.453 (133)	0.0206 (198) (η2,3 = 240)	1 (Fixed)

OFV = Objective function value, η = inter-individual variability,

FEMAX = the fractional E_{max} of time, ET_{50} = the time that will decrease the apnoea counts by 50%, N = the Hill equation, a number influencing the slope of the curve.

A successful minimisation could not be obtained with the exponential time model, model 13. The values of the estimated parameters of the E_{max} and sigmoid E_{max} time models did not differ significantly. The E_{max} time model with inter-individual covariance between FEMAX and ET_{50} (model 16) did not significantly improve the fit of the data and hence model 14 was the best of this series. The baseline counts were 17 per hour, the E_{max} fraction was 0.565 and the ET_{50} was 0.0476 hours. The value of ET_{50}, that is the time required to decrease the apnoea counts by 50%, was very small.

3.2.3 **Results of the time-concentration count model**

The results of the time-concentration models are given in Table C.3.4.

For the base model in this series (model 17) some of the parameter values (including the TC_{50} of 97.3 mg/L) appear to be unrealistically high. (The TC_{50} is the estimated value of the serum theophylline concentration that would decrease the apnoea counts by half in the presence of the hypothetical respiratory depressant factor (RDF).) Subsequent modelling revealed that the initial estimates of the parameters and the number and position of the ηs again influenced the estimated values of the parameters. However in all these subsequent models the baseline counts plus R_{MAX} (the maximum increase in apnoea due to RDF) was about 16. This is the same value as that of the baseline counts in the previous series. Covariance between the different parameters was tested by implementing Omega block (2), (3), (4) and (5). With the first two mentioned, minimisation was successful. However, successful termination could not be obtained with Omega block (4) and (5). The model selected as most appropriate from this series was model 18, because the OFV of this model was the lowest of those that terminated successfully.

TABLE C.3.4

Results of the time-concentration models

Model number and properties	OFV	Normal (η%)	RMAX (η%)	RC_{50} (η%)	Rthalf (η%)	TC_{50} (η%)
17 Base model with 4 η's	1278.673	6.39 (70)	33.9 (0)	0.723 (225)	0.053 (94)	97.3 (99)
18 3 η's and Omega block(2) between RMAX and RC_{50}	1263.968	8.08 (66)	7.8 (104)	0.037 (241) (η2,3:-150)	0.007 (0)	2.82
19 As model 18 but with Omega block(3) between RMAX, RC_{50} and Rthalf	1267.860	7.84 (66)	8.5 (90)	0.306 (183) (η2,3 = 27)	0.080 (83) (η3,4 = 17, 10)	2.97 0 fixed
20 As model 18 but with Omega block(4) between RMAX, RC_{50}, Rthalf, and TC_{50}	1269.595	7.38 (65)	8.43 (96) (η2,3 = -29)	0.235 (153) (η3,4 = 18,10)	0.069 (76) (η4,5 = 19, 11, 20)	2.36 (71)
21 As model 18 but with Omega block(5) between Normal, RMAX, RC_{50}, Rthalf and TC_{50}	1262.894	7.66 (70)	7.53 (108) (η1,2 = 16)	0.223 (170) (η2,3 = 9, -28)	0.053 (83) (η3,4 = 18, 16, 9)	2.66 (72) (η4,5= 21, 18, 11, 19)

OFV = Objective function value, η = inter-individual variability, Normal = the baseline count without the influence of RDF (respiratory depressant factor), RMAX = maximum increase in apnoea due to RDF, RC_{50} = the concentration of RDF that will have a 50% influence, Rthalf = half-life of RDT, TC_{50} = the concentration of theophylline (mg/L) that will decrease the apnoea counts by 50%.

Visual inspection of the scatter plots of predicted versus recorded number of apnoea of model 18 as depicted in Figure C.3.3 showed only marginal differences when compared with Model 1 or Model 4 of the fractional E_{max} series, where concentration was the independent variable.

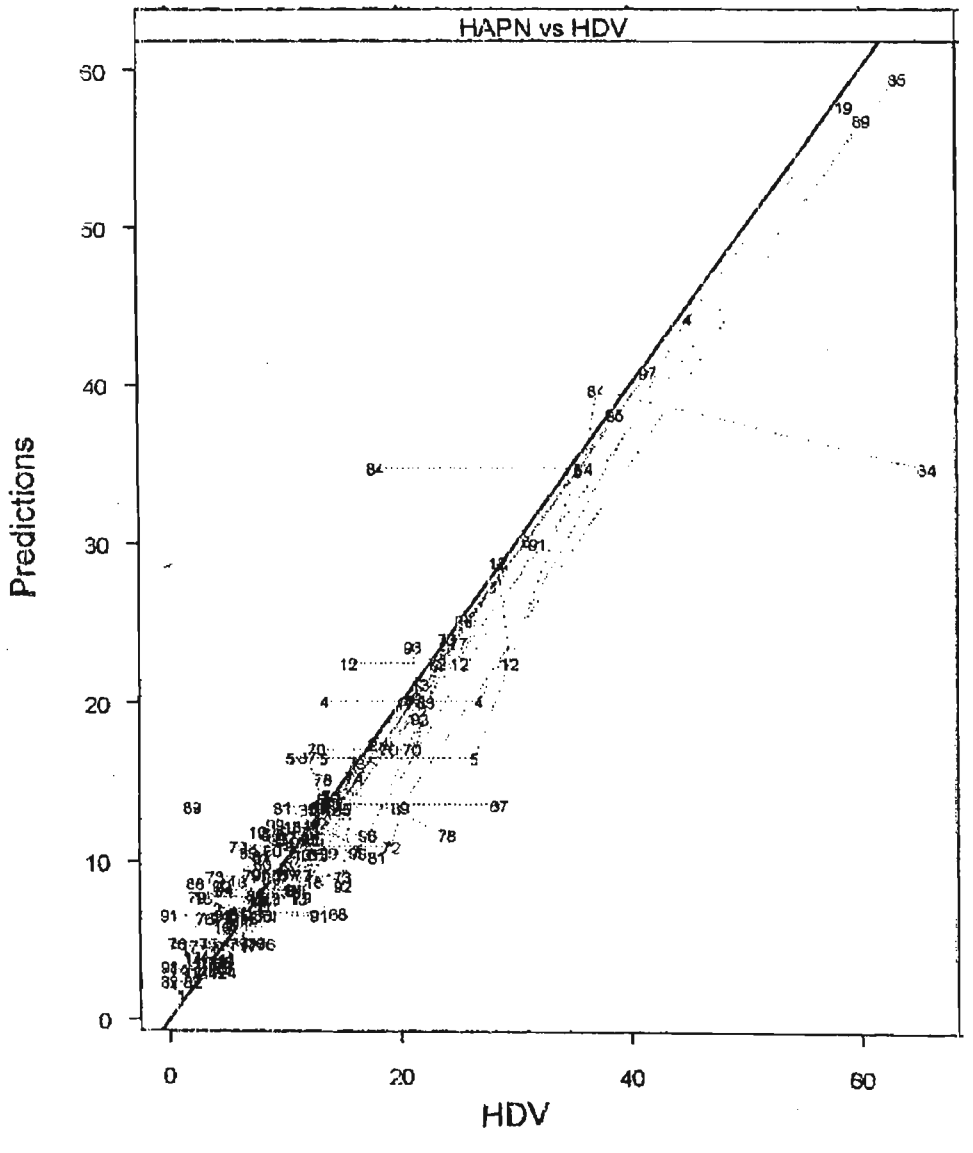

Fig.C.3.3 Predictions versus dependent variable for Model 18.

3.2.4 **Results of simulation of time-concentration model**

The result of a simulation of the time-concentration model is depicted in Figure C.3.4. The figure shows the ERDF (E_{max} model) of the respiratory depressant factor (RDF) when Rmax is taken as 20 and the half-time concentration of RDF ($RDFC_{50}$) as 1. The rapid fall of the respiratory depressant factor over time (RDFT) is clearly demonstrated. In this simulation the hourly apnoea counts (HAPN0) of the patient at baseline is the sum of the normal apnoea counts plus the influence of the respiratory factor, therefore HAPN0 = Normal + ERDF. When theophylline is administered, the effect of a serum concentration of 3 mg/L on the hourly apnoea (HAPN+T) becomes apparent and the apnoea counts decrease.

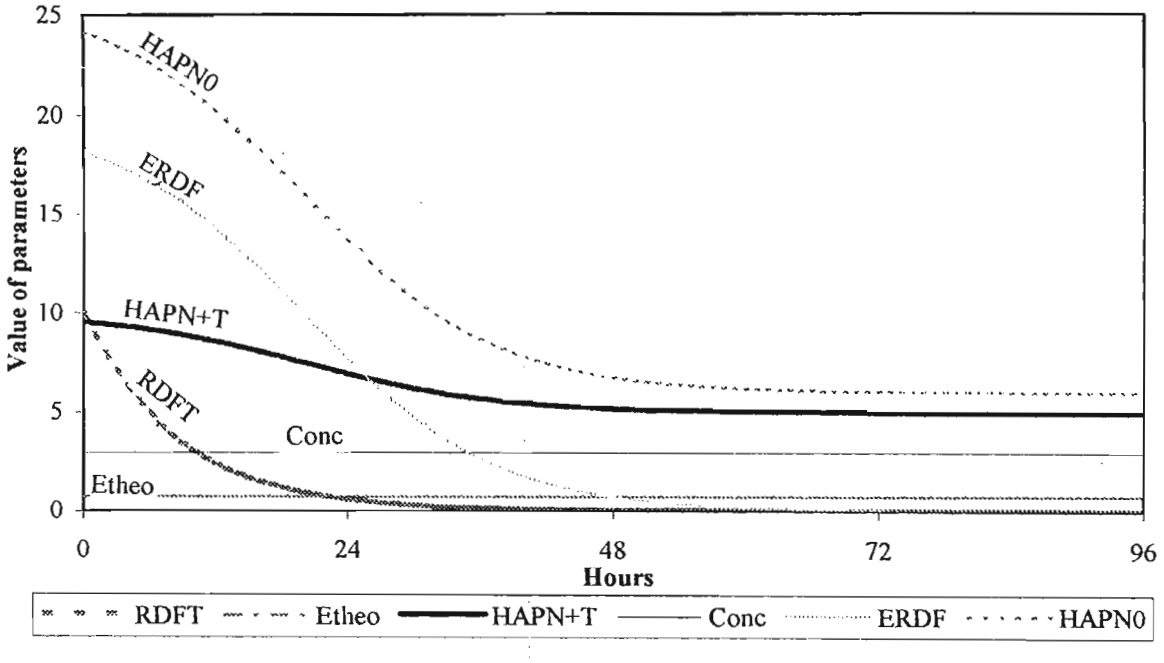

Fig. C.3.4 Simulation of the time-concentration model with serum theophylline concentration of 3 mg/L.

Figure C.3.5 is as above, but the 50% inhibitory concentration of theophylline (TC_{50}) was taken as equal to 10 mg/L.

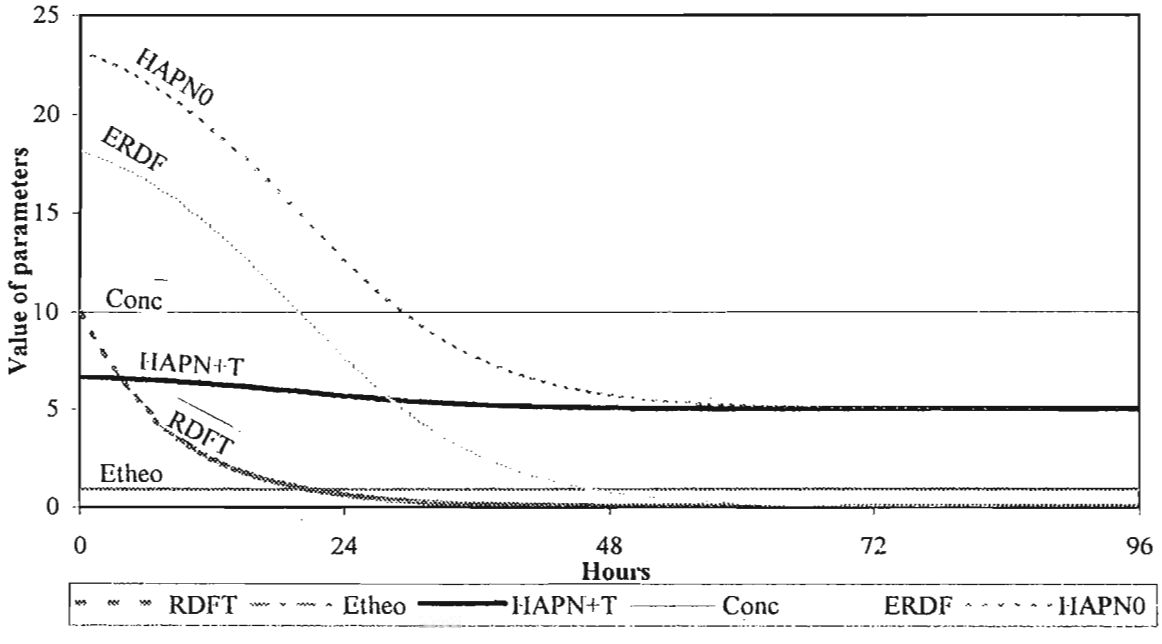

Fig. C.3.5 Simulation of the time-concentration model with serum theophylline concentration of 10 mg/L.

3.2.5 Model selection

There were 3 different series of models and within each series a best model could be selected (namely models 1, 14 and 18). However it was difficult to compare these best models across the three series and it is probably not appropriate to consider OFV differences.

Visual inspection of the graphs, of predictions versus dependent variables, showed no obvious difference between the models. With these types of models suitable diagnostic

graphs are not possible (Prof S Beal *personal communication*).

The inter-individual variabilities of the estimated parameters cannot easily be compared between models because the models are highly sensitive to the placement of variabilities as well as, in some cases, the value of the initial estimates. All the models tested are non-linear and therefore parameters are correlated and difficult to discriminate from one another. With the exception of the estimate of the baseline value, confidence in the parameter estimates is uncertain. With all models tested the covariance step aborted possibly due to instability of the models.

If we assume that theophylline has some pharmacological effect in apnoea, and that measurable concentrations are required for this, then Model 18 gives the only realistic EC_{50} value at 2.8 mg/L.

SECTION C: CHAPTER 4

DISCUSSION

4.1 DISCUSSION

One way of analysing the data in this section would have been to do a population analysis using the quantal dose response model as an extension of the assessment carried out in section B. However, Prof S Beal advised (*personal communication*) that a more appropriate analysis, which would not involve manipulation of the data, would be the count model.

Accordingly variations on the E_{max} model adapted for count data were tested on the data set involving total apnoeas. In fact, none of the models tested proved to be satisfactory either as descriptors, and certainly not for predictions and dosage recommendations. The models that considered only theophylline concentrations and took no account of the baseline improvement of apnoea, with maturation, all yielded unrealistically small values for EC_{50} for theophylline.

It has been shown that maturity could play a role in the disappearance of apnoea (Sims *et al* 1985). In the third series, which incorporated the possibility of a changing baseline, a more realistic value of 2.8 mg/L was obtained. Milsap *et al* (1980) and Myers *et al* (1980) suggested concentrations of 3 to 5 mg/L and 3 to 4 mg/L respectively to decrease apnoea.

Modelling drug concentration together with time, as in series 3, resembled a disease-progression model as described by Holford *et al* (1993). In this series a hypothetical respiratory depressant factor that is removed over time, as the neonate improves, was used. In the premature neonate respiratory symptoms are often the result of a complex interplay between RDS, intra-cranial haemorrhage, central hypoventilation and infection (Hegyi *et al* 1986) as well as low oxygen levels (Poets *et al* 1993). Thus, the respiratory depressant factor could be influenced by any condition that may contribute to the development of apnoea such as a lack of proper oxygenation, hypo- or hyper-thermia, fever, cardiac malfunction etc. The success of this model series would have been greatly facilitated by the inclusion of a control group that would have allowed for discrimination between the maturation and the theophylline effect.

Population concentration-effect modelling of theophylline in the treatment of neonatal apnoea proved to be very complex. As mentioned in the results-section, comparison between models was not really possible. Population modelling as applied to count data is in its infancy and techniques are still developing. Population count models were first described by Beal in the mid-1990s. The methodologies for assessing goodness of fit and model discrimination are currently still very unclear.

With all models tested, the number and position of the inter-individual variability parameter, η, influenced the estimated values of EC_{50} and the OFV, suggesting an unstable model (Prof S Beal, *personal communication*). Although the size of η on the estimated baseline apnoea counts model remained fairly consistent, it varied significantly on the estimated EC_{50} and on the fractional E_{max} of theophylline as well as on other

parameters. One possible explanation for this is that it involves overparameterisation of non-linear models and that the data set had relatively few repeated levels per individual. Covariate modelling was not carried further because of difficulties in identifying an appropriate structural model.

The analysis in section B clearly showed that, when considering total apnoeas, there were distinct groups in terms of response to theophylline (when defined as 50% reduction from baseline). A very recent development in analysing count data is the recognition that patients may fall into groups with different distributions i.e. responders and non-responders. Although it is understood that categorical and count data are different, the analysis of data in section B of the present study shows that patients could be categorised into responders and non-responders. Likewise the recognition of a group of non-responders to theophylline in apnoea appears frequently in the literature (Shannon *et al* 1975, Jones 1982, Roberts *et al* 1982, Sims *et al* 1985, Muttitt *et al* 1988, Harrison *et al* 1992). It is very likely that not taking this into account, in the present study, may have contributed to the poor modelling results when total apnoea was chosen. A few researchers are currently applying the mixture model feature in NONMEM to apply two distributions and to estimate the proportion of individuals falling into each (Piotrovsky and Van Peer 2000, Dr R Miller *personal communication*). This is one way in which this data could be further analysed. Modelling using the pathological apnoeas may have been more successful using only one distribution because in this group there were only 13% non-responders. In the light of the poor results of the modelling using the count model it might have been worthwhile to model the data using logistic regression, particularly since patients have already been categorised as responders or non-responders. Although

this approach is theoretically not as appropriate as the count model, it could have provided some indication of a concentration response relationship.

4.2 CONCLUSION

Modelling with count data is in its infancy and as a result the findings in this section were not conclusive. Possible future directions would be to do even more sophisticated modelling. This could involve using the mixture modelling facility in NONMEM with multiple distributions. Incorporation of a control group, as well as the option to increase dose in non-responders, would greatly improve study design.

CONCLUSION

This study has focussed on the pharmacokinetics, the clinical effects and the concentration-effect relationship of theophylline in apnoea during the first few days of life.

The results have shown that theophylline has a lower clearance and volume of distribution in the premature neonate than in older babies. The clearance is also influenced by oxygenation, as clearance is faster in neonates who receive oxygen support. The findings are comparable with pharmacokinetic results obtained from traditional pharmacokinetic studies in premature neonates with postnatal ages similar, or close to, those of the present study. The low clearance results in a long half-life thus small peak to trough fluctuations would be expected. Therefore, a once daily dosing is recommended for this population. This will ease the burden of the nursing staff and also improve compliance and accuracy.

The results also confirmed the high degree of variability in this population group as large inter- and intra-patient, as well as inter-occasion, variability were found. This may be explained by the changing physiology of the neonate after birth. Unfortunately this high variability makes it difficult to predict drug concentrations with the same degree of accuracy as in other populations. The inter-occasion variability in clearance of 34% is an indication of the size of variability that may be expected during the first few days. Because of this variability it is advised that serum drug levels should be determined and the dose adjusted accordingly.

The effect of theophylline on total apnoea (all apnoea ≥5 seconds) was not as dramatic as on pathologic apnoea (≥20 seconds). Although a statistically significant decrease was seen from baseline to all three days for both types of apnoea, only a 52% cumulative response was obtained in total apnoea compared to the 87% in pathologic apnoea. Approximate EC_{50} values were in the region of 3 to 9 mg/L. The most dramatic effect was seen after the loading dose (day 1) when a sharp drop in both incidents and densities of both pathologic and total apnoea, from baseline, was observed.

Regardless of all the potential beneficial effects of the drug on ventilation/perfusion inequalities, theophylline did not decrease hypoxaemic episodes significantly. A cumulative response of 56% was seen at serum theophylline concentration >15 mg/L. Only the hypoxaemic episodes associated with a pathologic apnoea (≥20 seconds) and a fall in heart rate decreased significantly.

Analysis of possible markers for non-response (<50% improvement from baseline) indicated that being one of a set of twins was indicative of a poor response for both total apnoea and hypoxaemic episodes. Other possible markers of poor response for hypoxia were being born by caesarean section and having more than the 75^{th} percentile pathologic apnoea at baseline. As there was such a good response to theophylline for pathological apnoea (only 13% non-response), no markers of poor response were identified for this group. It was noted however that all five of the babies who did not respond were boys. It was interesting that, with regard to total apnoeas, there were some features that seemed to predict a favourable response to theophylline. These were a birth weight and a 5

minute Apgar score below the 25th percentile, and a baseline total apnoea count above the 75th percentile.

The cumulative graphs of the responders and non-responders resembled the fixed effect model, which is the simplest model to explain drug-effect relationships.

The more sophisticated exploration of the concentration-effect relationship of theophylline in neonatal apnoea using the count model was complex. Although none of the models tested proved to be satisfactory, that which incorporated the influence of a hypothetical respiratory depressant factor gave the most realistic value of EC_{50}.

A control group (which was not possible in this study on ethical grounds) would be needed to distinguish the role of theophylline from the effect of maturation.

Population modelling as applied to count data is still undergoing development and it has only recently been recognised that, where patients fall into two distributions (e.g. responders and non-responders), the mixture model feature in NONMEM could be applied to the data. It is possible that, in future, such modelling could further define the concentration-effect relationship (and hence the therapeutic range) for theophylline in neonatal apnoea.

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APPENDICES

LIST OF APPENDICES

APPENDIX A.1

Ethics approval

UNIVERSITY OF NATAL

FACULTY OF MEDICINE

INTER-OFFICE MEMORANDUM

TO: Mrs M du Preez
Department of Experimental and Clinical Pharmacology

FROM: Mrs S McDonald
PostGraduate Administration
Faculty of Medicine

21 April 1993

PROTOCOL: AN INVESTIGATION INTO THE DRUG THERAPY (PHARMACOKINETICS AND PHARMACODYNAMICS OF THEOPHYLLINE) AND AETIOLOGY OF NEONATAL APNOEA

The Ethics Committee has considered the abovementioned application and has found it acceptable.

Your letter dated 9 March 1993 refers.

S McDONALD (Mrs)
PostGraduate Administration

McD/ethics/accept

APPENDIX A.2

Consent form

11. INFORMED CONSENT FOR INCLUSION IN A CLINICAL TRIAL*

1. I NAME:

hereby consent to the following procedure and/or treatment being conducted on the person indicated in (4) below.

A serum sample to be taken for theophylline concentration measurements when necessary.

2. I acknowledge that I have been informed by:

Mrs M. du Preez, Professor Adhikari

concerning the possible advantages and possible adverse effects which may result from the abovementioned procedure and/or treatment and of the ways in which it is different from the conventional procedure and/or treatment. The information which I was given and which I acknowledge I understand is shown on the reverse side of this form.

3. I agree that the above procedure and/or treatment will be carried out and/or supervised by:

Professor Adhikari

4. I acknowledge that I understand the contents of this form, including the information provided on its reverse and as the

SUBJECT	PARENT	GUARDIAN	OTHER (SPECIFY)

freely consent to the above procedure and/or treatment being conducted on:

NAME:

5. I am aware that I may withdraw my consent at any time without prejudice to further care.

SIGNED	SUBJECT:	DATE
SIGNED	WITNESS:	DATE
SIGNED	INFORMANT:	DATE
SIGNED	RESEARCHER:	DATE

* With the exception of the names and signatures in paragraphs 1, 4 and 5, please provide the above information.

12. INFORMATION GIVEN TO SUBJECTS

Please indicate what will be told to subjects in simple language. The procedure or treatment which will be applied should be described and reference should be made to possible side effects, discomfort, complications and/or benefits. It must be made clear to the patient that he/she is free at any time to withdraw without suffering any disadvantage.

Your baby is being given a medicine to help it breath. We are trying to find out more about how this medicine works and would therefore like to take some blood samples from your baby without changing his/her treatment.

You have the right to refuse and then we will not take these samples. However, we will not change the baby's treatment.

APPENDIX A.3

Pharmacokinetic data file

ID	WGT	BSA	GA	PNA	PCA	GEN	AP	AQ	OXY	TIME	AMT	DV	DEX	DAY	RT	NNJ
21	1.2	0.112	33	1	33.1	1	6	8	2	0	5.16	0	2	1	1	2
21	1.2	0.112	33	1	33.1	1	6	8	2	9.83	1.72	0	2	1	1	2
21	1.2	0.112	33	2	33.3	1	6	8	2	19.83	1.72	0	2	2	1	2
21	1.2	0.112	33	2	33.3	1	6	8	2	25.83	1.72	0	2	2	1	2
21	1.2	0.112	33	3	33.4	1	6	8	2	33.83	1.72	0	2	3	1	2
21	1.2	0.112	33	3	33.4	1	6	8	2	41.08	1.72	0	2	3	1	2
21	1.2	0.112	33	3	33.4	1	6	8	2	51.33	1.72	0	2	3	1	2
21	1.2	0.112	33	4	33.6	1	6	8	2	57.83	1.72	0	2	4	1	2
21	1.2	0.112	33	4	33.6	1	6	8	2	64.83	1.72	0	2	4	1	2
21	1.2	0.112	33	4	33.6	1	6	8	2	73.33	1.72	0	2	4	1	2
21	1.2	0.112	33	5	33.7	1	6	8	2	81.83	1.72	0	2	5	1	2
21	1.2	0.112	33	5	33.7	1	6	8	2	89.16	1.72	0	2	5	1	2
21	1.2	0.112	33	5	33.7	1	6	8	2	97.33	1.72	0	2	5	1	2
21	1.2	0.112	33	6	33.9	1	6	8	2	105.83	1.72	0	2	6	1	2
21	1.1	0.107	33	6	33.9	1	6	8	2	115.08	1.72	0	2	6	1	2
21	1.1	0.107	33	6	33.9	1	6	8	2	121.83	1.72	0	2	6	1	2
21	1.1	0.107	33	7	34	1	6	8	2	129.83	1.72	0	2	7	1	2
21	1.1	0.107	33	7	34	1	6	8	2	137.83	1.72	0	2	7	1	2
21	1.1	0.107	33	7	34	1	6	8	2	145.33	1.72	0	2	7	1	2
21	1.2	0.112	33	8	34.1	1	6	8	2	153.83	1.72	0	2	8	1	2
21	1.25	0.114	33	8	34.1	1	6	8	2	162.16	0	10.5	2	8	0	2
23	1.95	0.164	32	1	32.1	1	6	8	2	0	8.6	0	2	1	1	1
23	1.95	0.164	32	2	32.3	1	6	8	2	4	3.44	0	2	2	1	1
23	1.95	0.164	32	2	32.3	1	6	8	2	11.83	0	17.6	2	2	0	1
23	1.95	0.164	32	2	32.3	1	6	8	2	12.21	3.44	0	2	2	1	1
23	1.95	0.164	32	3	32.4	1	6	8	2	27.96	3.44	0	2	3	1	1
23	1.95	0.164	32	3	32.4	1	6	8	2	35.13	3.44	0	2	3	1	1
23	1.95	0.164	32	3	32.4	1	6	8	2	43.46	3.44	0	2	3	1	1
23	1.95	0.164	32	4	32.6	1	6	8	2	51.96	3.44	0	2	4	1	1
23	1.95	0.164	32	4	32.6	1	6	8	2	60.29	0	13.7	2	4	0	1
24	1	0.1	30	2	30.3	2	7	9	1	0	1.72	0	2	1	1	2
24	1	0.1	30	2	30.3	2	7	9	1	9.33	0	3.6	2	1	0	2
25	1.05	0.102	28	1	28.1	2	6	9	2	0	4.3	0	1	1	1	2
25	1.05	0.102	28	1	28.1	2	6	9	2	5.67	0	9.25	1	1	0	2
25	1.05	0.102	28	1	28.1	2	6	9	2	6.34	1.72	0	1	1	1	2
25	1.05	0.102	28	1	28.1	2	6	9	2	14.51	1.72	0	1	1	1	2
25	1.05	0.102	28	2	28.3	2	6	9	2	22.01	1.72	0	1	2	1	2
25	1.05	0.102	28	2	28.3	2	6	9	2	29.51	1.72	0	1	2	1	2
25	1.05	0.102	28	2	28.3	2	6	9	2	37.51	1.72	0	1	2	1	2
25	1.05	0.102	28	3	28.4	2	6	9	2	46.01	1.72	0	1	3	1	2
25	1.05	0.102	28	3	28.4	2	6	9	2	53.76	0	24.15	1	3	0	2
25	1.05	0.102	28	3	28.4	2	6	9	2	54.93	1.72	0	1	3	1	2
25	1.05	0.102	28	3	28.4	2	6	9	2	61.18	1.72	0	1	3	1	2
25	1.05	0.102	28	4	28.6	2	6	9	2	69.35	1.72	0	1	4	1	2
25	1.05	0.102	28	4	28.6	2	6	9	2	77.35	1.72	0	1	4	1	2
25	1.05	0.102	28	4	28.6	2	6	9	2	85.35	1.72	0	1	4	1	2
25	1.05	0.102	28	5	28.7	2	6	9	2	93.35	1.72	0	1	5	1	2
25	0.9	0.095	28	5	28.7	2	6	9	2	109.35	1.72	0	1	5	1	2
25	0.9	0.095	28	6	28.9	2	6	9	2	117.35	1.72	0	1	6	1	2
25	0.9	0.095	28	6	28.9	2	6	9	2	124.52	1.72	0	1	6	1	2
25	0.9	0.095	28	6	28.9	2	6	9	2	128.18	0	21.55	1	6	0	2
26	1.95	0.152	34	2	34.3	1	3	5	1	0	8.6	0	1	1	1	2

26	1.95	0.152	34	2	34.3	1	3	5	1	10.08	0	10.08	1	1	0	2
26	1.95	0.152	34	2	34.3	1	3	5	1	15.5	3.44	0	1	1	1	2
26	1.95	0.152	34	3	34.4	1	3	5	1	23.67	3.44	0	1	2	1	2
26	1.95	0.152	34	3	34.4	1	3	5	1	39.67	3.44	0	1	2	1	2
26	1.95	0.152	34	4	34.6	1	3	5	1	47.5	3.44	0	1	3	1	2
26	1.95	0.152	34	4	34.6	1	3	5	1	56.58	0	23.5	1	3	0	2
27	1.25	0.116	31	1	31.1	1	1	6	1	0	4.3	0	2	1	1	2
27	1.25	0.116	31	2	31.3	1	1	6	1	4.5	1.29	0	2	2	1	2
27	1.25	0.116	31	2	31.3	1	1	6	1	13.92	0	16.7	2	2	0	3
27	1.25	0.116	31	2	31.3	1	1	6	1	14.09	1.29	0	2	2	1	3
27	1.25	0.116	31	3	31.4	1	1	6	1	28.51	1.29	0	2	3	1	3
27	1.25	0.116	31	3	31.4	1	1	6	1	37.51	1.29	0	2	3	1	3
27	1.25	0.116	31	3	31.4	1	1	6	1	45.51	1.29	0	2	3	1	3
27	1.25	0.116	31	4	31.6	1	1	6	1	53.18	1.29	0	2	4	1	3
27	1.25	0.116	31	4	31.6	1	1	6	1	59.76	0	16.7	2	4	0	3
27	1.25	0.116	31	5	31.7	1	1	6	1	62.09	1.29	0	2	4	1	3
27	1.25	0.116	31	5	31.7	1	1	6	1	68.42	1.29	0	2	4	1	3
27	1.25	0.116	31	6	31.9	1	1	6	1	75.92	1.29	0	2	5	1	3
27	1.3	0.118	31	6	31.9	1	1	6	1	84.42	1.29	0	2	5	1	3
27	1.3	0.118	31	7	32	1	1	6	1	108.42	1.29	0	2	6	1	3
27	1.3	0.118	31	7	32	1	1	6	1	116.42	1.29	0	2	6	1	3
27	1.3	0.118	31	8	32.1	1	1	6	1	124.75	1.29	0	2	7	1	3
27	1.4	0.123	31	8	32.1	1	1	6	2	134.58	0	11.6	2	7	0	3
29	1.9	0.154	34	2	34.3	1	7	9	2	0	7.74	0	2	1	1	2
29	1.9	0.154	34	3	34.4	1	7	9	2	3.5	0.86	0	2	2	1	2
29	1.9	0.154	34	3	34.4	1	7	9	2	10.5	0.86	0	2	2	1	2
29	1.9	0.154	34	3	34.4	1	7	9	2	19.5	0.86	0	2	2	1	2
29	1.9	0.154	34	4	34.6	1	7	9	2	27.5	0.86	0	2	3	1	2
29	1.9	0.154	34	4	34.6	1	7	9	2	37.42	0	13.4	2	3	0	2
29	1.9	0.154	34	4	34.6	1	7	9	2	38	0.86	0	2	3	1	2
29	1.9	0.154	34	4	34.6	1	7	9	2	43.5	0.86	0	2	3	1	2
29	1.9	0.154	34	5	34.7	1	7	9	2	51.5	0.86	0	2	4	1	1
29	1.9	0.154	34	5	34.7	1	7	9	2	58.25	0.86	0	2	4	1	1
29	1.9	0.154	34	5	34.7	1	7	9	2	67.5	0.86	0	2	4	1	1
29	1.9	0.154	34	6	34.9	1	7	9	2	75.92	0.86	0	2	5	1	1
29	1.72	0.146	34	6	34.9	1	7	9	2	84.42	0	11.8	2	5	0	1
30	1.41	0.128	31	1	31.1	1	8	10	2	0	6.02	0	2	1	1	2
30	1.41	0.128	31	1	31.1	1	8	10	2	3	0.86	0	2	1	1	2
30	1.41	0.128	31	2	31.3	1	8	10	2	11	0.86	0	2	2	1	2
30	1.41	0.128	31	2	31.3	1	8	10	2	19	0.86	0	2	2	1	2
30	1.41	0.128	31	1	31.3	1	8	10	2	27	0.86	0	2	2	1	2
30	1.41	0.128	31	3	31.4	1	8	10	2	35	0.86	0	2	3	1	2
30	1.41	0.128	31	3	31.4	1	8	10	2	43	0.86	0	2	3	1	2
30	1.41	0.128	31	3	31.4	1	8	10	2	51	0.86	0	2	3	1	2
30	1.41	0.128	31	4	31.6	1	8	10	2	59	0.86	0	2	4	1	1
30	1.41	0.128	31	4	31.6	1	8	10	2	67	0.86	0	2	4	1	1
30	1.41	0.128	31	4	31.6	1	8	10	2	75	0.86	0	2	4	1	1
30	1.41	0.128	31	5	31.7	1	8	10	2	91	0.86	0	2	5	1	1
30	1.41	0.128	31	5	31.7	1	8	10	2	99	0.86	0	2	5	1	1
30	1.4	0.128	31	6	31.9	1	8	10	2	115.92	0	27.5	2	6	0	1
31	0.85	0.082	28	1	28.1	2	7	10	1	0	4.3	0	2	1	1	2
31	0.85	0.082	28	2	28.3	2	7	10	1	11	0.86	0	2	2	1	2
31	0.85	0.082	28	2	28.3	2	7	10	1	20.83	0	9.15	2	2	0	2
31	0.85	0.082	28	2	28.3	2	7	10	1	27	0.86	0	2	2	1	2
31	0.85	0.082	28	3	28.4	2	7	10	1	35	0.86	0	2	3	1	2
31	0.85	0.082	28	3	28.4	2	7	10	1	51	0.86	0	2	3	1	1
31	0.85	0.082	28	4	28.6	2	7	10	1	59	0.86	0	2	4	1	1
31	0.85	0.082	28	4	28.6	2	7	10	1	68.5	0	8.74	2	4	0	1

35	1.5	0.132	31	1	31.1	1	9	10	1	0	6.02	0	2	1	1	2
35	1.5	0.132	31	2	31.3	1	9	10	1	8.5	0.86	0	2	2	1	2
35	1.5	0.132	31	2	31.3	1	9	10	1	16	0	3.75	2	2	0	2
35	1.5	0.132	31	2	31.3	1	9	10	1	16.25	0.86	0	2	2	1	2
35	1.5	0.132	31	2	31.3	1	9	10	1	24.5	0.86	0	2	2	1	2
35	1.5	0.132	31	3	31.4	1	9	10	1	32.5	0.86	0	2	3	1	2
35	1.5	0.132	31	3	31.4	1	9	10	1	42.08	0.86	0	2	3	1	2
35	1.5	0.132	31	3	31.4	1	9	10	1	48.5	0.86	0	2	3	1	2
35	1.3	0.123	31	4	31.6	1	9	10	1	56.5	0.86	0	2	4	1	2
35	1.3	0.123	31	4	31.6	1	9	10	1	64.17	0	6.2	2	4	0	2
36	1.65	0.135	33	1	33.1	1	8	9	1	0	6.88	0	2	1	1	2
36	1.65	0.135	33	1	33.1	1	8	9	1	5.5	1.72	0	2	1	1	2
36	1.65	0.135	33	1	33.1	1	8	9	1	13.5	1.72	0	2	1	1	2
36	1.65	0.135	33	2	33.3	1	8	9	1	21.5	1.72	0	2	2	1	1
36	1.65	0.135	33	2	33.3	1	8	9	1	29.5	1.72	0	2	2	1	1
36	1.65	0.135	33	3	33.4	1	8	9	1	45.5	1.72	0	2	3	1	1
36	1.65	0.135	33	3	33.4	1	8	9	1	53.5	1.72	0	2	3	1	1
36	1.65	0.135	33	3	33.4	1	8	9	1	61.5	1.72	0	2	3	1	1
36	1.65	0.135	33	4	33.6	1	8	9	1	69.5	1.72	0	2	4	1	1
36	1.6	0.133	33	4	33.6	1	8	9	2	78.83	0	23.6	2	4	0	1
37	1.9	0.149	33	1	33.1	1	8	10	2	0	7.74	0	2	1	1	2
37	1.9	0.149	33	1	33.1	1	8	10	2	4.58	0	8.05	2	1	0	2
37	1.9	0.149	33	1	33.1	1	8	10	2	5	0.86	0	2	1	1	2
37	1.9	0.149	33	1	33.1	1	8	10	2	10	0.86	0	2	1	1	2
37	1.9	0.149	33	2	33.3	1	8	10	2	19.83	0.86	0	2	2	1	2
37	1.9	0.149	33	2	33.3	1	8	10	2	28	0.86	0	2	2	1	2
37	1.9	0.149	33	2	33.3	1	8	10	2	35.58	0.86	0	2	2	1	2
37	1.9	0.149	33	2	33.3	1	8	10	2	52.92	0.86	0	2	3	1	2
37	1.9	0.149	33	2	33.3	1	8	10	2	59.5	0.86	0	2	3	1	2
37	1.9	0.149	33	3	33.4	1	8	10	2	67.5	0.86	0	2	4	1	2
37	1.9	0.149	33	3	33.4	1	8	10	2	75.5	0.86	0	2	4	1	2
37	1.9	0.149	33	3	33.4	1	8	10	2	78.25	0	10.5	2	4	0	2
38	1.8	0.145	34	1	34.1	2	8	10	2	0	7.74	0	2	1	1	2
38	1.8	0.145	34	1	34.1	2	8	10	2	6.33	0	5.55	2	1	0	2
38	1.8	0.145	34	1	34.1	2	8	10	2	7.16	0.86	0	2	1	1	2
38	1.8	0.145	34	1	34.1	2	8	10	2	13.99	0.86	0	2	1	1	2
38	1.8	0.145	34	2	34.3	2	8	10	2	21.99	0.86	0	2	2	1	2
38	1.8	0.145	34	2	34.3	2	8	10	2	30.16	0.86	0	2	2	1	2
38	1.8	0.145	34	2	34.3	2	8	10	2	37.99	0.86	0	2	2	1	2
38	1.8	0.145	34	3	34.4	2	8	10	2	45.99	0.86	0	2	3	1	2
38	1.8	0.145	34	3	34.4	2	8	10	2	55.16	0	7.2	2	3	0	2
41	1.7	0.143	32	2	32.3	2	4	6	1	0	1.29	0	2	1	1	2
41	1.7	0.143	32	2	32.3	2	4	6	1	9.5	1.29	0	2	1	1	2
41	1.7	0.143	32	3	32.4	2	4	6	1	24	1.29	0	2	2	1	2
41	1.7	0.143	32	3	32.4	2	4	6	1	32	1.29	0	2	2	1	2
41	1.7	0.143	32	3	32.4	2	4	6	1	40	1.29	0	2	2	1	2
41	1.7	0.143	32	4	32.6	2	4	6	1	48	1.29	0	2	3	1	1
41	1.7	0.143	32	4	32.6	2	4	6	1	56	1.29	0	2	3	1	1
41	1.7	0.143	32	4	32.6	2	4	6	1	64	1.29	0	2	3	1	1
41	1.7	0.143	32	5	32.7	2	4	6	1	72	1.29	0	2	4	1	1
41	1.7	0.143	32	5	32.7	2	4	6	1	81	0	12.3	2	4	0	1
41	1.7	0.143	32	5	32.7	2	4	6	1	81.25	1.29	0	2	4	1	1
41	1.7	0.143	32	5	32.7	2	4	6	1	88	1.29	0	2	4	1	1
41	1.6	0.14	32	6	32.9	2	4	6	1	96	1.29	0	2	5	1	1
41	1.6	0.14	32	6	32.9	2	4	6	1	104	1.29	0	2	5	1	1
41	1.6	0.14	32	6	32.9	2	4	6	1	112	1.29	0	2	5	1	1
41	1.6	0.14	32	7	33	2	4	6	1	120	1.29	0	2	6	1	1
41	1.6	0.14	32	7	33	2	4	6	1	128	1.29	0	2	6	1	1

41	1.6	0.14	32	7	33	2	4	6	1	136	1.29	0	2	6	1	1
41	1.6	0.14	32	8	33.1	2	4	6	1	144	1.29	0	2	7	1	1
41	1.6	0.14	32	8	33.1	2	4	6	1	153.17	1.29	0	2	7	1	1
41	1.6	0.14	32	8	33.1	2	4	6	1	160	1.29	0	2	7	1	1
41	1.56	0.139	32	9	33.3	2	4	6	1	168	1.29	0	2	8	1	1
41	1.56	0.139	32	9	33.3	2	4	6	2	176.58	0	9.15	2	8	0	1
45	1.65	0.155	32	1	32.1	1	4	5	2	0	0.86	0	2	1	1	2
45	1.65	0.155	32	2	32.3	1	4	5	2	12	0.86	0	2	2	1	2
45	1.65	0.155	32	2	32.3	1	4	5	2	18	0.86	0	2	2	1	2
45	1.65	0.155	32	2	32.3	1	4	5	2	24	0.86	0	2	2	1	2
45	1.65	0.155	32	3	32.4	1	4	5	2	35	0.86	0	2	3	1	2
45	1.65	0.155	32	3	32.4	1	4	5	2	42	0.86	0	2	3	1	2
45	1.65	0.155	32	4	32.6	1	4	5	2	52	0.86	0	2	4	1	1
45	1.65	0.155	32	4	32.6	1	4	5	2	61.58	0	8.95	2	4	0	1
45	1.65	0.155	32	4	32.6	1	4	5	2	66	0.86	0	2	4	1	1
45	1.65	0.155	32	5	32.7	1	4	5	2	76	0.86	0	2	5	1	1
45	1.65	0.155	32	5	32.7	1	4	5	2	84	0.86	0	2	5	1	1
45	1.65	0.155	32	5	32.7	1	4	5	2	90	0.86	0	2	5	1	1
45	1.5	0.148	32	5	32.7	1	4	5	2	96	0.86	0	2	5	1	1
45	1.5	0.148	32	6	32.9	1	4	5	2	109.75	0	13.9	2	6	0	1
46	1.35	0.125	31	1	31.1	2	5	9	2	0	0.86	0	2	1	1	2
46	1.35	0.125	31	1	31.1	2	5	9	2	7.75	0.86	0	2	1	1	2
46	1.35	0.125	31	2	31.3	2	5	9	2	15.75	0.86	0	2	2	1	1
46	1.35	0.125	31	2	31.3	2	5	9	2	23.75	0.86	0	2	2	1	1
46	1.35	0.125	31	2	31.3	2	5	9	2	31.75	0.86	0	2	2	1	1
46	1.35	0.125	31	3	31.4	2	5	9	2	39.75	0.86	0	2	3	1	1
46	1.35	0.125	31	3	31.4	2	5	9	2	47	0	10.6	2	3	0	1
47	1.1	0.107	30	1	30.1	1	6	8	1	0	4.3	0	2	1	1	2
47	1.1	0.107	30	1	30.1	1	6	8	1	1.58	1.72	0	2	1	1	2
47	1.1	0.107	30	2	30.3	1	6	8	1	9.58	1.72	0	2	2	1	2
47	1.1	0.107	30	2	30.3	1	6	8	1	17.75	1.72	0	2	2	1	2
47	1.1	0.107	30	2	30.3	1	6	8	1	24.92	1.72	0	2	2	1	2
47	1.1	0.107	30	3	30.4	1	6	8	1	33.59	1.72	0	2	3	1	1
47	1.1	0.107	30	3	30.4	1	6	8	1	43.34	0	27	2	3	0	1
48	1	0.098	30	1	30.1	1	7	9	2	0	5.16	0	2	1	1	2
48	1	0.098	30	2	30.3	1	7	9	2	5.25	0.86	0	2	2	1	2
48	1	0.098	30	2	30.3	1	7	9	2	13.25	0.86	0	2	2	1	2
48	1	0.098	30	2	30.3	1	7	9	2	21.25	0.86	0	2	2	1	2
48	1	0.098	30	3	30.4	1	7	9	2	29.25	0.86	0	2	3	1	2
48	1	0.098	30	3	30.4	1	7	9	2	39.17	0	20.5	2	3	0	2
48	1	0.098	30	3	30.4	1	7	9	2	39.75	0.86	0	2	3	1	2
48	1	0.098	30	3	30.4	1	7	9	2	45.25	0.86	0	2	3	1	2
48	1	0.098	30	4	30.6	1	7	9	2	53.25	0.86	0	2	4	1	1
48	1.05	0.101	30	4	30.6	1	7	9	2	63.17	0	15.2	2	4	0	1
50	1	0.098	30	1	30.1	2	7	9	2	0	5.16	0	2	1	1	2
50	1	0.098	30	2	30.3	2	7	9	2	5.25	0.86	0	2	2	1	2
50	1	0.098	30	2	30.3	2	7	9	2	13.25	0.86	0	2	2	1	2
50	1	0.098	30	2	30.3	2	7	9	2	21.25	0.86	0	2	2	1	2
50	1	0.098	30	3	30.4	2	7	9	2	39.75	0.86	0	2	3	1	2
50	1	0.098	30	3	30.4	2	7	9	2	45.25	0.86	0	2	3	1	2
50	1	0.098	30	5	30.6	2	7	9	2	86.93	0	19.1	2	5	0	1
52	0.9	0.098	28	2	28.3	1	7	10	2	0	0.86	0	2	1	1	2
52	0.9	0.098	28	2	28.3	1	7	10	2	8	0.86	0	2	1	1	2
52	0.9	0.098	28	2	28.3	1	7	10	2	16	0.86	0	2	1	1	2
52	0.9	0.098	28	3	28.4	1	7	10	2	24	0.86	0	2	2	1	2
52	0.9	0.098	28	3	28.4	1	7	10	2	32.92	0.86	0	2	2	1	2
52	0.9	0.098	28	3	28.4	1	7	10	2	40	0.86	0	2	2	1	2
52	0.9	0.098	28	4	28.6	1	7	10	2	48	0.86	0	2	3	1	2

52	0.9	0.098	28	4	28.6	1	7	10	2	54.33	0.86	0	2	3	1	2
52	0.9	0.098	28	4	28.6	1	7	10	2	61.33	0.86	0	2	3	1	2
52	0.95	0.101	28	5	28.7	1	7	10	2	72	0.86	0	2	4	1	2
52	0.95	0.101	28	5	28.7	1	7	10	2	80.5	0	20.6	2	4	0	2
54	0.85	0.095	28	1	28.1	2	5	8	2	0	1.72	0	2	1	1	1
54	0.85	0.095	28	2	28.3	2	5	8	2	8.5	0.86	0	2	2	1	1
54	0.85	0.095	28	2	28.3	2	5	8	2	17	0.86	0	2	2	1	1
54	0.85	0.095	28	2	28.3	2	5	8	2	24.5	0.86	0	2	2	1	1
54	0.85	0.095	28	3	28.4	2	5	8	2	32.5	0.86	0	2	3	1	1
54	0.85	0.095	28	3	28.4	2	5	8	2	40.5	0.86	0	2	3	1	1
54	0.85	0.095	28	3	28.4	2	5	8	2	48.5	0.86	0	2	3	1	1
54	0.85	0.095	28	4	28.6	2	5	8	2	56.5	0.86	0	2	4	1	2
54	0.85	0.095	28	5	28.7	2	5	8	2	65.75	0	12.65	2	5	0	2
55	1.55	0.137	31	1	31.1	2	8	9	2	0	8.6	0	2	1	1	3
55	1.55	0.137	31	1	31.1	2	8	9	2	4	1.72	0	2	1	1	3
55	1.55	0.137	31	2	31.3	2	8	9	2	12.92	0	10.9	2	2	0	3
55	1.55	0.137	31	2	31.3	2	8	9	2	13.5	1.72	0	2	2	1	3
55	1.55	0.137	31	2	31.3	2	8	9	2	20	1.72	0	2	2	1	3
55	1.55	0.137	31	3	31.4	2	8	9	2	28	1.72	0	2	3	1	3
55	1.55	0.137	31	3	31.4	2	8	9	2	43.5	1.72	0	2	3	1	3
55	1.51	0.134	31	4	31.6	2	8	9	2	62	0	12.5	2	4	0	3
58	1.3	0.12	30	2	30.3	1	6	8	1	0	1.29	0	2	1	1	2
58	1.3	0.12	30	2	30.3	1	6	8	1	14.58	1.72	0	2	1	1	2
58	1.3	0.12	30	3	30.4	1	6	8	2	30.66	1.29	0	2	2	1	2
58	1.3	0.12	30	3	30.4	1	6	8	2	39.96	1.29	0	2	2	1	2
58	1.3	0.12	30	4	30.6	1	6	8	2	47.96	1.29	0	2	3	1	2
58	1.3	0.12	30	4	30.6	1	6	8	2	54.71	1.29	0	2	3	1	2
58	1.3	0.12	30	4	30.6	1	6	8	2	63.96	1.29	0	2	3	1	2
58	1.3	0.12	30	5	30.7	1	6	8	2	71.96	1.29	0	2	4	1	2
58	1.3	0.12	30	5	30.7	1	6	8	2	78.63	1.29	0	2	4	1	2
58	1.3	0.12	30	5	30.7	1	6	8	2	87.38	1.29	0	2	4	1	2
58	1.3	0.12	30	6	30.9	1	6	8	2	95.96	1.29	0	2	5	1	2
58	1.3	0.12	30	6	30.9	1	6	8	2	105.04	0	21.7	2	5	0	2
62	1.1	0.107	32	1	32.1	2	8	10	2	0	4.3	0	2	1	1	2
62	1.1	0.107	32	2	32.3	2	8	10	2	26.5	0.86	0	2	2	1	2
62	1.1	0.107	32	3	32.4	2	8	10	2	34.5	1.08	0	2	3	2	2
62	1.1	0.107	32	3	32.4	2	8	10	2	50.5	1.08	0	2	3	2	2
62	1.35	0.119	32	4	32.6	2	8	10	2	58.5	1.08	0	2	4	2	2
62	1.35	0.119	32	4	32.6	2	8	10	2	64.83	0	14.8	2	4	0	2
65	1.55	0.133	32	2	32.3	2	6	8	1	0	4.3	0	2	1	1	2
65	1.55	0.133	32	3	32.4	2	6	8	1	4.5	0.86	0	2	2	1	2
65	1.55	0.133	32	3	32.4	2	6	8	1	12.5	0.86	0	2	2	1	2
65	1.55	0.133	32	3	32.4	2	6	8	1	20.5	0.86	0	2	2	1	2
65	1.55	0.133	32	4	32.6	2	6	8	1	28.5	0.86	0	2	3	1	1
65	1.55	0.133	32	4	32.6	2	6	8	1	35.42	0	13.1	2	3	0	1
66	0.9	0.101	30	2	30.3	1	8	9	1	0	4.73	0	2	1	1	2
66	0.9	0.101	30	2	30.3	1	8	9	1	6.87	0.86	0	2	1	1	2
66	0.9	0.101	30	3	30.4	1	8	9	1	17.29	0.86	0	2	2	1	2
66	0.9	0.101	30	3	30.4	1	8	9	1	30.79	0.86	0	2	2	1	2
66	0.9	0.101	30	4	30.6	1	8	9	1	40.79	0.86	0	2	3	1	2
66	0.9	0.101	30	4	30.6	1	8	9	1	54.79	0.86	0	2	3	1	2
66	0.9	0.101	30	5	30.6	1	8	9	1	65.21	0	4.7	2	4	0	2
66	0.9	0.101	30	5	30.7	1	8	9	2	65.53	0.86	0	2	4	1	2
66	0.9	0.101	30	5	30.7	1	8	9	2	78.8	0.86	0	2	4	1	2
66	0.9	0.101	30	6	30.9	1	8	9	2	88.8	0.86	0	2	5	1	2
66	0.9	0.101	30	6	30.9	1	8	9	2	98.8	0.86	0	2	5	1	2
66	0.9	0.101	30	7	31	1	8	9	2	160.55	0	1.2	2	6	0	2
70	1.25	0.116	30	1	30.1	2	7	9	2	0	5.16	0	1	1	1	2

70	1.25	0.116	30	1	30.1	2	7	9	2	0.46	0	6.1	1	1	0	2
70	1.25	0.116	30	1	30.1	2	7	9	2	6.34	1.72	0	1	1	1	2
70	1.25	0.116	30	2	30.3	2	7	9	2	14.17	1.72	0	1	2	1	1
70	1.25	0.116	30	2	30.3	2	7	9	2	21.84	0	11.7	1	2	0	1
70	1.3	0.118	30	2	30.3	2	7	9	2	22.34	1.72	0	1	2	1	1
70	1.25	0.116	30	2	30.3	2	7	9	2	29.67	1.72	0	1	2	1	1
70	1.25	0.116	30	3	30.4	2	7	9	2	37.92	1.72	0	1	3	1	1
70	1.24	0.116	30	5	30.7	2	7	9	2	96.45	0	11.9	1	5	0	1
71	1.1	0.108	30	2	30.3	2	8	10	2	0	4.3	0	2	1	1	2
71	1.1	0.108	30	2	30.3	2	8	10	2	0.78	0	1.1	2	1	0	2
71	1.1	0.108	30	2	30.3	2	8	10	2	6.64	0.86	0	2	1	1	2
71	1.1	0.108	30	3	30.4	2	8	10	2	15.64	0.86	0	2	2	1	2
71	1.1	0.108	30	3	30.4	2	8	10	2	22.39	0.86	0	2	2	1	2
71	1.1	0.108	30	3	30.4	2	8	10	2	31.64	0.86	0	2	2	1	2
71	1.1	0.108	30	4	30.6	2	8	10	2	40.56	0.86	0	2	3	1	2
71	1	0.103	30	4	30.6	2	8	10	2	47.64	0.86	0	2	3	1	2
71	1	0.103	30	5	30.7	2	8	10	2	63.64	0.86	0	2	4	1	2
71	1	0.103	30	5	30.7	2	8	10	2	71.64	0.86	0	2	4	1	2
71	1	0.103	30	5	30.7	2	8	10	2	79.64	0.86	0	2	4	1	2
71	1	0.103	30	6	30.9	2	8	10	2	87.64	0.86	0	2	5	1	2
71	1	0.103	30	6	30.9	2	8	10	2	96.08	0	7.6	2	5	0	2
72	1.36	0.121	30	1	30.1	1	7	9	1	0	6.45	0	2	1	1	2
72	1.36	0.121	30	1	30.1	1	7	9	1	0.67	0	7.5	2	1	0	3
74	1.5	0.129	31	1	31.1	2	9	10	1	0	4.3	0	1	1	1	3
74	1.5	0.129	31	1	31.1	2	9	10	1	0.88	0	3.5	1	1	0	3
74	1.5	0.129	31	1	31.1	2	9	10	1	4.25	0.86	0	1	1	1	3
74	1.5	0.129	31	2	31.3	2	9	10	1	12.25	0.86	0	1	2	1	3
74	1.5	0.129	31	2	31.3	2	9	10	1	19.92	0	6.25	1	2	0	3
75	1.7	0.141	34	1	34.1	2	2	5	1	0	7.74	0	2	1	1	3
75	1.7	0.141	34	1	34.1	2	2	5	1	1.17	0	5.2	2	1	0	2
75	1.7	0.141	34	1	34.1	2	2	5	1	7.87	0.86	0	2	1	1	2
75	1.7	0.141	34	2	34.3	2	2	5	1	13.87	0.86	0	2	2	1	1
75	1.6	0.136	34	2	34.3	2	2	5	1	20.87	0	7.5	2	2	0	1
75	1.6	0.136	34	2	34.3	2	2	5	1	21.25	0.86	0	2	2	1	1
75	1.6	0.136	34	2	34.3	2	2	5	1	31.86	0.86	0	2	2	1	1
75	1.6	0.136	34	3	34.4	2	2	5	1	37.86	0.86	0	2	3	1	1
75	1.6	0.136	34	3	34.4	2	2	5	1	44.03	0.86	0	2	3	1	1
75	1.6	0.136	34	3	34.4	2	2	5	1	47.11	0	8.5	2	3	0	1
76	1.5	0.131	31	1	31.1	2	3	9	1	0	6.88	0	2	1	1	2
76	1.5	0.131	31	1	31.1	2	3	9	1	2.36	0.86	0	2	1	1	2
76	1.5	0.131	31	1	31.1	2	3	9	1	20.84	0	5.3	2	1	0	2
76	1.5	0.131	31	2	31.3	2	3	9	1	21.24	0.86	0	2	2	1	2
76	1.5	0.131	31	2	31.3	2	3	9	1	26.37	0.86	0	2	2	1	2
76	1.5	0.131	31	2	31.3	2	3	9	1	32.52	0.86	0	2	2	1	2
76	1.5	0.131	31	3	31.4	2	3	9	1	38.27	0.86	0	2	3	1	2
76	1.5	0.131	31	3	31.4	2	3	9	1	44.02	0	4.8	2	3	0	2
77	1.1	0.108	31	1	31.1	1	5	9	2	0	3.87	0	2	1	1	2
77	1.1	0.108	31	1	31.1	1	5	9	2	0.67	0	5.8	2	1	0	2
77	1.1	0.108	31	1	31.1	1	5	9	2	11.7	0.86	0	2	1	1	2
77	1	0.103	31	2	31.3	1	5	9	2	19.8	0	4	2	2	0	1
77	1	0.103	31	2	31.3	1	5	9	2	20.11	0.86	0	2	2	1	1
77	1	0.103	31	2	31.3	1	5	9	2	27.69	0.86	0	2	2	1	1
77	1	0.103	31	3	31.4	1	5	9	2	35.75	0.86	0	2	3	1	1
77	1	0.103	31	3	31.4	1	5	9	2	44.18	0	5.9	2	3	0	1
77	1	0.103	31	3	31.4	1	5	9	2	44.39	0.86	0	2	3	1	1
77	1	0.103	31	4	31.6	1	5	9	2	59.67	0.86	0	2	4	1	1
77	1	0.103	31	4	31.6	1	5	9	2	69	0	9.5	2	4	0	1
78	1.35	0.124	31	2	31.3	2	6	9	1	0	1.29	0	1	1	1	2

78	1.35	0.124	31	2	31.3	2	6	9	1	9.5	1.29	0	1	1	1	2
78	1.35	0.124	31	3	31.4	2	6	9	1	21.53	1.29	0	1	2	1	2
78	1.3	0.121	31	3	31.4	2	6	9	1	32.5	1.29	0	1	2	1	2
78	1.3	0.121	31	4	31.6	2	6	9	1	45.58	1.29	0	1	3	1	1
78	1.3	0.121	31	4	31.6	2	6	9	1	57.5	1.29	0	1	3	1	1
78	1.3	0.121	31	5	31.7	2	6	9	1	68.97	0	8.4	1	4	0	1
78	1.3	0.121	31	5	31.7	2	6	9	1	69.67	1.29	0	1	4	1	1
78	1.3	0.121	31	5	31.7	2	6	9	1	81.42	1.29	0	1	4	1	1
78	1.3	0.121	31	6	31.9	2	6	9	1	92.72	0	7.3	1	5	0	1
79	1.15	0.112	29	1	29.1	2	7	9	2	0	5.16	0	1	1	1	2
79	1.15	0.112	29	2	29.3	2	7	9	2	10	0.86	0	1	2	1	2
79	1.15	0.11	29	2	29.3	2	7	9	2	16.7	0	9.3	1	2	0	2
79	1.15	0.11	29	2	29.3	2	7	9	2	18.38	0.86	0	1	2	1	2
79	1.15	0.11	29	2	29.3	2	7	9	2	25.99	0.86	0	1	2	1	2
79	1.15	0.11	29	3	29.4	2	7	9	2	33.99	0.86	0	1	3	1	1
79	1.15	0.11	29	3	29.4	2	7	9	2	41.09	0	17.8	1	3	0	1
80	1.25	0.116	30	1	30.1	1	9	10	1	0	4.3	0	2	1	1	2
80	1.25	0.116	30	1	30.1	1	9	10	1	0.58	0	2.7	2	1	0	2
80	1.25	0.116	30	1	30.1	1	9	10	1	5.76	1.72	0	2	1	1	2
80	1.25	0.116	30	2	30.3	1	9	10	1	13.93	1.72	0	2	2	1	2
80	1.25	0.116	30	2	30.3	1	9	10	1	19.6	0	16.1	2	2	0	2
81	1.45	0.13	30	1	30.1	1	9	10	2	0	6.45	0	2	1	1	2
81	1.45	0.13	30	1	30.1	1	9	10	2	0.5	0	8.3	2	1	0	2
82	1.51	0.131	31	1	31.1	2	8	9	1	0	6.45	0	1	1	1	2
82	1.51	0.131	31	1	31.1	2	8	9	1	0.84	0	19.3	1	1	0	2
82	1.51	0.131	31	2	31.3	2	8	9	1	11.85	1.72	0	1	2	1	2
82	1.51	0.131	31	2	31.3	2	8	9	1	18.82	1.72	0	1	2	1	2
82	1.51	0.131	31	2	31.3	2	8	9	1	27.85	1.72	0	1	2	1	2
82	1.51	0.131	31	3	31.4	2	8	9	1	35.85	1.72	0	1	3	1	2
82	1.51	0.131	31	3	31.4	2	8	9	1	43.85	1.72	0	1	3	1	2
82	1.51	0.131	31	3	31.4	2	8	9	1	51.85	1.72	0	1	3	1	2
82	1.51	0.131	31	4	31.6	2	8	9	1	59.65	1.72	0	1	4	1	1
82	1.51	0.131	31	4	31.6	2	8	9	2	67.85	1.72	0	1	4	1	1
82	1.51	0.131	31	4	31.6	2	8	9	2	69.91	0	19.4	1	4	0	1
83	1.06	0.104	30	1	30.1	1	7	8	1	0	4.3	0	1	1	1	2
83	1.06	0.104	30	1	30.1	1	7	8	1	1.65	0	11.7	1	1	0	2
84	0.8	0.087	28	1	28.1	1	6	8	1	0	0.86	0	2	1	1	2
84	0.8	0.087	28	1	28.1	1	6	8	1	1.36	0	4	2	1	0	2
84	0.8	0.087	28	1	28.1	1	6	8	1	8.71	0.86	0	2	1	1	2
84	0.8	0.087	28	2	28.3	1	6	8	1	16.71	0.86	0	2	2	1	1
84	0.8	0.087	28	2	28.3	1	6	8	1	24.21	0	4	2	2	0	1
84	0.8	0.087	28	2	28.3	1	6	8	1	24.69	0.86	0	2	2	1	1
84	0.8	0.087	28	2	28.3	1	6	8	1	32.72	0.86	0	2	2	1	1
84	0.8	0.087	28	3	28.4	1	6	8	1	49.33	0	5.1	2	3	0	1
84	0.8	0.087	28	3	28.4	1	6	8	1	49.63	0.86	0	2	3	1	1
84	0.8	0.087	28	3	28.4	1	6	8	1	55.96	0.86	0	2	3	1	1
84	0.8	0.087	28	4	28.6	1	6	8	1	64.71	0.86	0	2	4	1	1
84	0.8	0.087	28	4	28.6	1	6	8	1	71.88	0.86	0	2	4	1	1
84	0.8	0.087	28	4	28.6	1	6	8	1	80.21	0.86	0	2	4	1	1
84	0.8	0.087	28	5	28.7	1	6	8	1	88.71	0.86	0	2	5	1	2
84	0.77	0.085	28	5	28.7	1	6	8	1	95.21	0.86	0	2	5	1	2
84	0.77	0.085	28	5	28.7	1	6	8	1	104.71	0.86	0	2	5	1	2
84	0.77	0.085	28	6	28.9	1	6	8	2	112.74	0.86	0	2	6	1	2
84	0.77	0.085	28	6	28.9	1	6	8	2	118.46	0	13.2	2	6	0	2
85	1.03	0.105	30	1	30.1	2	7	9	1	0	4.3	0	2	1	1	2
85	1.03	0.105	30	1	30.1	2	7	9	1	3.27	1.72	0	2	1	1	2
85	1.03	0.105	30	2	30.3	2	7	9	1	11.27	1.72	0	2	2	1	2
85	1.03	0.105	30	2	30.3	2	7	9	1	16.85	0	6.1	2	2	0	2

85	1.03	0.105	30	2	30.3	2	7	9	1	17.51	2.16	0	2	2	1	1
85	1.03	0.105	30	2	30.3	2	7	9	1	26.74	1.72	0	2	2	1	1
85	1.03	0.105	30	3	30.4	2	7	9	1	34.74	1.72	0	2	3	1	1
85	1.03	0.105	30	3	30.4	2	7	9	2	40.24	0	8.1	2	3	0	1
85	1.03	0.105	30	3	30.4	2	7	9	2	41.72	1.72	0	2	3	1	1
85	1.03	0.105	30	3	30.4	2	7	9	2	51.08	1.72	0	2	3	1	1
85	1	0.103	30	4	30.6	2	7	9	2	59.25	1.72	0	2	4	1	1
85	1	0.103	30	4	30.6	2	7	9	2	64.42	0	9.9	2	4	0	1
86	0.68	0.082	26	1	26.1	1	3	4	1	0	2.9	0	2	1	1	2
86	0.68	0.082	26	1	26.1	1	3	4	1	5.86	0.86	0	2	1	1	2
86	0.68	0.082	26	2	26.3	1	3	4	1	13.94	0.86	0	2	2	1	2
86	0.68	0.082	26	2	26.3	1	3	4	1	19.69	0	14.7	2	2	0	2
87	1.2	0.115	30	1	30.1	2	9	10	2	0	4.3	0	2	1	1	2
87	1.2	0.115	30	2	30.3	2	9	10	2	11.52	0.86	0	2	2	1	2
87	1.2	0.115	30	2	30.3	2	9	10	1	17.27	0	4.6	2	2	0	2
87	1.2	0.115	30	2	30.3	2	9	10	1	18.38	0.86	0	2	2	1	1
87	1.2	0.115	30	2	30.3	2	9	10	2	27.26	0.86	0	2	2	1	1
87	1.2	0.115	30	3	30.4	2	9	10	2	35.51	0.86	0	2	3	1	1
87	1.2	0.115	30	3	30.4	2	9	10	2	41.43	0	7	2	3	0	1
88	1.2	0.113	30	1	30.1	2	7	9	1	0	4.3	0	1	1	1	2
88	1.2	0.113	30	1	30.1	2	7	9	1	3.83	0.86	0	1	1	1	2
88	1.2	0.113	30	2	30.3	2	7	9	1	11.83	0.86	0	1	2	1	2
88	1.2	0.113	30	2	30.3	2	7	9	1	17.83	0	15.5	1	2	0	2
88	1.2	0.113	30	2	30.3	2	7	9	2	18.61	0.86	0	1	2	1	2
88	1.2	0.113	30	2	30.3	2	7	9	2	27.56	0.86	0	1	2	1	2
88	1.2	0.113	30	3	30.4	2	7	9	2	36.56	0.86	0	1	3	1	2
88	1.2	0.113	30	3	30.4	2	7	9	2	42.31	0	16.8	1	3	0	2
88	1.2	0.113	30	3	30.4	2	7	9	2	43.09	0.86	0	1	3	1	1
88	1.2	0.113	30	3	30.4	2	7	9	2	51.97	0.86	0	1	3	1	1
88	1.2	0.113	30	4	30.6	2	7	9	2	66.89	0.86	0	1	4	1	1
88	1.2	0.113	30	4	30.6	2	7	9	2	75.89	0.86	0	1	4	1	1
88	1.2	0.113	30	6	30.7	2	7	9	2	126.14	0	16.5	1	6	0	1
89	0.8	0.081	30	1	30.1	2	6	9	1	0	4.3	0	1	1	1	2
89	0.8	0.081	30	1	30.1	2	6	9	1	0.9	0	4.6	1	1	0	2
89	0.8	0.081	30	1	30.1	2	6	9	1	3.74	0.86	0	1	1	1	2
89	0.8	0.081	30	2	30.3	2	6	9	1	13.74	0.86	0	1	2	1	2
89	0.8	0.081	30	2	30.3	2	6	9	1	19.74	0	7.75	1	2	0	2
89	0.8	0.081	30	2	30.3	2	6	9	1	19.87	0.86	0	1	2	1	2
89	0.8	0.081	30	3	30.4	2	6	9	1	29.23	0.86	0	1	2	1	2
89	0.8	0.081	30	3	30.4	2	6	9	1	37.53	0.86	0	1	3	1	2
89	0.8	0.081	30	3	30.4	2	6	9	1	43.33	0	22	1	3	0	2
90	1.5	0.126	32	1	32.1	1	8	10	1	0	4.3	0	12	1	1	2
90	1.5	0.126	32	2	32.3	1	8	10	1	10.46	2.58	0	2	2	1	2
90	1.5	0.126	32	2	32.3	1	8	10	1	18.54	0	8.1	2	2	0	2
90	1.5	0.126	32	2	32.3	1	8	10	1	18.84	2.58	0	2	2	1	1
90	1.4	0.121	32	2	32.3	1	8	10	1	26.3	2.58	0	2	2	1	1
90	1.4	0.121	32	3	32.4	1	8	10	1	34.3	2.58	0	2	3	1	1
90	1.4	0.121	32	3	32.4	1	8	10	1	42.05	0	12	2	3	0	1
90	1.4	0.121	32	3	32.4	1	8	10	1	42.53	2.58	0	2	3	1	1
90	1.4	0.121	32	3	32.4	1	8	10	1	50.32	2.58	0	2	3	1	1
90	1.4	0.121	32	4	32.6	1	8	10	1	58.82	2.58	0	2	4	1	1
90	1.4	0.121	32	4	32.6	1	8	10	1	65.32	2.58	0	2	4	1	1
90	1.4	0.121	32	5	32.7	1	8	10	1	82.49	2.58	0	2	5	1	1
90	1.4	0.121	32	5	32.7	1	8	10	1	90.32	2.58	0	2	5	1	1
90	1.4	0.121	32	5	32.7	1	8	10	1	98.32	2.58	0	2	5	1	1
90	1.4	0.121	32	6	32.9	1	8	10	1	113.68	0	13.5	2	6	0	1
91	1.25	0.113	30	1	30.1	1	9	10	1	0	2.58	0	1	1	1	2
91	1.25	0.113	30	1	30.1	1	9	10	1	0.57	0	1.8	1	1	0	2

91	1.25	0.113	30	1	30.1	1	9	10	1	6.57	0.86	0	1	1	1	2
91	1.25	0.113	30	2	30.3	1	9	10	1	19.22	0	6.5	1	2	0	2
91	1.25	0.113	30	2	30.3	1	9	10	1	19.46	0.86	0	1	2	1	2
91	1.25	0.113	30	2	30.3	1	9	10	1	30.66	0.86	0	1	2	1	2
91	1.25	0.113	30	3	30.4	1	9	10	1	41.16	0	9	1	3	0	1
92	1.45	0.115	33	1	33.1	1	6	9	2	0	4.3	0	2	1	1	2
92	1.45	0.115	33	1	33.1	1	6	9	2	0.92	0	1.7	2	1	0	2
92	1.45	0.115	33	1	33.1	1	6	9	2	10.21	1.72	0	2	1	1	1
92	1.45	0.115	33	2	33.3	1	6	9	2	20.71	0	6.6	2	2	0	1
93	1.1	0.103	29	1	29.1	1	6	9	1	0	4.3	0	1	1	1	2
93	1.1	0.103	29	1	29.1	1	6	9	1	0.97	0	7	1	1	0	2
94	1.1	0.103	29	1	29.1	2	7	9	1	0	4.3	0	1	1	1	2
94	1.1	0.103	29	1	29.1	2	7	9	1	1.02	0	5	1	1	0	2
94	1.1	0.103	29	1	29.1	2	7	9	1	4.98	0.86	0	1	1	1	2
94	1.1	0.103	29	2	29.3	2	7	9	1	17.04	0	5	1	2	0	2
94	1.1	0.103	29	2	29.3	2	7	9	1	19.34	0.86	0	1	2	1	2
94	1.1	0.103	29	2	29.3	2	7	9	1	29.21	0.86	0	1	2	1	2
94	1.1	0.103	29	3	29.4	2	7	9	1	42.77	0	7.7	1	3	0	2
95	0.85	0.089	28	1	28.1	1	5	7	1	0	3.44	0	2	1	1	2
95	0.85	0.089	28	1	28.1	1	5	7	1	0.5	0	11.3	2	1	0	2
95	0.85	0.089	28	2	28.3	1	5	7	1	13.5	1.29	0	2	2	1	2
95	0.85	0.089	28	2	28.3	1	5	7	1	21.8	0	6.92	2	2	0	2
95	0.85	0.089	28	2	28.3	1	5	7	1	22.2	1.29	0	2	2	1	2
95	0.85	0.089	28	2	28.3	1	5	7	1	28.5	1.29	0	2	2	1	2
95	0.85	0.089	28	3	28.4	1	5	7	1	36.5	1.29	0	2	3	1	1
95	0.85	0.089	28	3	28.4	1	5	7	1	45.5	0	12.9	2	3	0	1
96	0.9	0.089	29	1	29.1	1	6	9	1	0	3.87	0	2	1	1	2
96	0.9	0.089	29	1	29.1	1	6	9	1	0.58	0	5.24	2	1	0	2
96	0.9	0.089	29	2	29.3	1	6	9	1	9.53	0.86	0	2	2	1	2
96	0.9	0.089	29	2	29.3	1	6	9	1	20.13	0	5.62	2	2	0	2
96	0.9	0.089	29	2	29.3	1	6	9	1	23.3	0.86	0	2	2	1	2
96	0.9	0.089	29	2	29.3	1	6	9	1	33.44	0.86	0	2	2	1	1
96	0.9	0.089	29	3	29.4	1	6	9	1	43.77	0	4.9	2	3	0	1
96	0.9	0.089	29	3	29.4	1	6	9	1	48.19	0.86	0	2	3	1	1
96	0.9	0.089	29	3	29.4	1	6	9	1	58.11	0.86	0	2	3	1	1
96	0.9	0.089	29	4	29.6	1	6	9	1	68.94	0.86	0	2	4	1	1
96	0.9	0.089	29	4	29.6	1	6	9	1	81.19	0.86	0	2	4	1	1
96	0.9	0.089	29	5	29.7	1	6	9	1	91.56	0	6.97	2	5	0	1
97	1.8	0.15	34	1	34.1	2	4	8	1	0	6.24	0	2	1	1	2
97	1.8	0.15	34	1	34.1	2	4	8	1	1.1	0	5.4	2	1	0	2
97	1.8	0.15	34	2	34.3	2	4	8	1	20.63	0.86	0	2	1	1	2
97	1.8	0.15	34	2	34.3	2	4	8	1	21.68	0	4.7	2	2	0	2
97	1.8	0.15	34	3	34.4	2	4	8	2	37.05	0.86	0	2	3	1	1
97	1.8	0.151	34	3	34.4	2	4	8	2	47.33	0	3.9	2	3	0	1
98	1.3	0.11	32	2	32.3	1	4	6	1	0	4	0	2	1	1	2
98	1.3	0.11	32	2	32.3	1	4	6	1	1.2	0	5.1	2	1	0	2
98	1.3	0.11	32	2	32.3	1	4	6	1	11.26	0.86	0	2	1	1	2
98	1.3	0.11	32	3	32.4	1	4	6	1	23.14	0	7.9	2	2	0	1
98	1.3	0.11	32	3	32.4	1	4	6	1	23.31	0.86	0	2	2	1	1
98	1.3	0.11	32	3	32.4	1	4	6	1	35.18	0.86	0	2	2	1	1
98	1.3	0.11	32	4	32.6	1	4	6	1	45.48	0	8.5	2	3	0	1
98	1.3	0.11	32	4	32.6	1	4	6	1	47.18	0.86	0	2	3	1	1
98	1.3	0.11	32	4	32.6	1	4	6	1	58.1	0.86	0	2	3	1	1
98	1	0.097	32	5	32.7	1	4	6	2	69.68	0.86	0	2	4	1	1
98	0.96	0.095	32	5	32.7	1	4	6	2	82.01	0.86	0	2	4	1	1
98	0.96	0.095	32	6	32.9	1	4	6	2	93.81	0	14	2	5	0	1
99	1.7	0.146	32	1	32.1	1	7	9	1	0	4.3	0	2	1	1	2
99	1.7	0.146	32	1	32.1	1	7	9	1	2.11	0	3.1	2	1	0	2

99	1.7	0.146	32	1	32.1	1	7	9	1	10.62	1.4	0	2	1	1	2
99	1.7	0.146	32	2	32.3	1	7	9	1	18.66	0	2.7	2	2	0	2
99	1.7	0.146	32	2	32.3	1	7	9	1	18.8	1.4	0	2	2	1	1
99	1.7	0.146	32	2	32.3	1	7	9	1	30.63	1.4	0	2	2	1	1
99	1.7	0.146	32	3	32.4	1	7	9	1	39.9	0	3.3	2	3	0	1
99	1.7	0.146	32	3	32.4	1	7	9	1	40.84	1.4	0	2	3	1	1
99	1.7	0.146	32	4	32.6	1	7	9	1	66	1.4	0	2	4	1	1
99	1.7	0.146	32	4	32.6	1	7	9	2	77.76	1.4	0	2	4	1	1
99	1.56	0.139	32	5	32.7	1	7	9	2	87.61	0	11.5	2	5	0	1
10	1.4	0.12	32	1	32.1	2	8	10	2	0	6.02	0	2	1	1	2
10	1.4	0.12	32	1	32.1	2	8	10	2	0.93	0	6	2	1	0	2
10	1.4	0.12	32	1	32.1	2	8	10	2	11.61	0.86	0	2	1	1	2
10	1.4	0.12	32	2	32.3	2	8	10	2	20.98	0	4.6	2	2	0	2
10	1.4	0.12	32	2	32.3	2	8	10	2	23.38	0.86	0	2	2	1	2
10	1.4	0.12	32	3	32.4	2	8	10	2	37.88	0.86	0	2	3	1	2
10	1.4	0.12	32	3	32.4	2	8	10	2	46.11	0	4.5	2	3	0	2
10	1.4	0.12	32	3	32.4	2	8	10	2	47.34	0.86	0	2	3	1	2
10	1.4	0.12	32	3	32.4	2	8	10	2	57.87	0.86	0	2	3	1	2
10	1.4	0.12	32	4	32.6	2	8	10	2	69.87	0.86	0	2	4	1	2
10	1.4	0.12	32	4	32.6	2	8	10	2	83.37	0.86	0	2	4	1	2
10	1.4	0.12	32	5	32.7	2	8	10	2	93.87	0.86	0	2	5	1	2
10	1.4	0.12	32	5	32.7	2	8	10	2	107.37	0.86	0	2	5	1	2
10	1.4	0.12	32	6	32.9	2	8	10	2	117.87	0.86	0	2	6	1	2
10	1.4	0.12	32	6	32.9	2	8	10	2	131.37	0.86	0	2	6	1	2
10	1.4	0.12	32	6	32.9	2	8	10	2	141.87	0.86	0	2	7	1	2
10	1.34	0.119	32	8	33.1	2	8	10	2	167.75	0	6.6	2	8	0	2
11	1.25	0.114	30	1	30.1	2	8	9	1	0	4.3	0	2	1	1	2
11	1.25	0.114	30	1	30.1	2	8	9	1	1.66	0	4.6	2	1	0	2
11	1.25	0.114	30	1	30.1	2	8	9	1	8.96	0.86	0	2	1	1	2
11	1.25	0.114	30	2	30.3	2	8	9	1	18.26	0	4.6	2	2	0	2
11	1.25	0.114	30	2	30.3	2	8	9	1	21.29	0.86	0	2	2	1	2
11	1.25	0.114	30	2	30.3	2	8	9	1	32.89	0.86	0	2	2	1	2
11	1.14	0.109	30	3	30.4	2	8	9	1	41.45	0	4.5	2	3	0	2
11	1.14	0.109	30	3	30.4	2	8	9	1	43.93	0.86	0	2	3	1	2
11	1.14	0.109	30	3	30.4	2	8	9	1	56.56	0.86	0	2	3	1	1
11	1.18	0.111	30	4	30.6	2	8	9	1	67.02	0.86	0	2	4	1	1
11	1.18	0.111	30	4	30.6	2	8	9	2	80.52	0.86	0	2	4	1	1
11	1.14	0.109	30	5	30.7	2	8	9	2	91.25	0	3.6	2	5	0	1
12	1.3	0.12	30	1	30.1	1	8	10	1	0	6.02	0	2	1	1	2
12	1.3	0.12	30	1	30.1	1	8	10	1	1	0	4	2	1	0	2
12	1.3	0.12	30	1	30.1	1	8	10	1	7.7	1.29	0	2	1	1	2
12	1.3	0.12	30	2	30.3	1	8	10	1	17.58	0	5.4	2	2	0	2
12	1.3	0.12	30	2	30.3	1	8	10	1	19.83	1.29	0	2	2	1	2
12	1.3	0.12	30	2	30.3	1	8	10	1	31.58	1.29	0	2	2	1	2
12	1.1	0.111	30	3	30.4	1	8	10	1	41.73	0	4.8	2	3	0	2
12	1.1	0.111	30	3	30.4	1	8	10	2	43.91	1.29	0	2	3	1	2
12	1.1	0.111	30	3	30.4	1	8	10	2	55.66	1.29	0	2	3	1	2
12	1.2	0.115	30	4	30.6	1	8	10	2	66.33	1.29	0	2	4	1	1
12	1.24	0.117	30	6	30.9	1	8	10	2	114.18	0	2.3	2	6	0	2
13	1.25	0.117	31	1	31.1	2	7	9	2	0	4.3	0	2	1	1	2
13	1.25	0.117	31	1	31.1	2	7	9	2	0.5	0	4.5	2	1	0	2
13	1.25	0.117	31	1	31.1	2	7	9	2	5.98	0.86	0	2	1	1	2
13	1.16	0.113	31	2	31.3	2	7	9	2	16.54	0	5.8	2	2	0	2
13	1.16	0.113	31	2	31.3	2	7	9	2	19.16	0.86	0	2	2	1	2
13	1.16	0.113	31	2	31.3	2	7	9	2	30.81	0.86	0	2	2	1	2
13	1.16	0.113	31	3	31.4	2	7	9	2	40.44	0	7.4	2	3	0	2
13	1.16	0.113	31	3	31.4	2	7	9	2	42.66	0.86	0	2	3	1	2
13	1.16	0.113	31	3	31.4	2	7	9	2	54.48	0.86	0	2	3	1	2

13	1.2	0.115	31	4	31.6	2	7	9	2	64.98	0.86	0	2	4	1	2
13	1.2	0.115	31	5	31.7	2	7	9	2	89.21	0	16.5	2	5	0	1
13	1.2	0.115	31	7	32	2	7	9	2	161.71	0	3.6	2	8	0	1
14	1.5	0.124	32	1	32.1	1	9	10	2	0	5.16	0	2	1	1	2
14	1.5	0.124	32	1	32.1	1	9	10	2	1.36	0	5.1	2	1	0	2
14	1.5	0.124	32	1	32.1	1	9	10	2	10.86	2.58	0	2	1	1	2
14	1.26	0.124	32	2	32.3	1	9	10	2	21.19	0	6.9	2	2	0	1
14	1.26	0.124	32	2	32.3	1	9	10	2	24.15	2.58	0	2	2	1	1
14	1.26	0.124	32	2	32.3	1	9	10	2	34.85	2.58	0	2	2	1	1
14	1.26	0.124	32	3	32.4	1	9	10	2	45.68	0	7.2	2	3	0	1
14	1.25	0.124	32	3	32.4	1	9	10	2	48.46	1.29	0	2	3	1	1
14	1.25	0.124	32	3	32.4	1	9	10	2	59.34	1.29	0	2	3	1	1
14	1.25	0.124	32	4	32.6	1	9	10	2	70	1.29	0	2	4	1	1
14	1.5	0.124	32	4	32.6	1	9	10	2	82.83	1.29	0	2	4	1	1
14	1.5	0.124	32	5	32.7	1	9	10	2	91.83	1.29	0	2	5	1	1
14	1.5	0.124	32	8	33.1	1	9	10	2	163.86	0	2.3	2	8	0	1
15	1.4	0.125	32	1	32.1	1	4	8	1	0	6.45	0	1	1	1	2
15	1.4	0.125	32	1	32.1	1	4	8	1	1.08	0	5.2	1	1	0	2
16	1.2	0.11	30	2	30.3	1	7	9	1	0	6.02	0	2	1	1	2
16	1.2	0.11	30	2	30.3	1	7	9	1	1.25	0	6.5	2	1	0	2
16	1.2	0.11	30	2	30.3	1	7	9	1	13.42	2.16	0	2	1	1	1
16	1.2	0.11	30	3	30.4	1	7	9	1	20.09	0	6.9	2	2	0	1
16	1.2	0.11	30	3	30.4	1	7	9	1	22.59	2.16	0	2	2	1	1
16	1.2	0.11	30	3	30.4	1	7	9	1	33.92	2.16	0	2	2	1	1
16	1.2	0.11	30	4	30.6	1	7	9	1	44.17	0	7.5	2	3	0	1
16	1.2	0.11	30	4	30.6	1	7	9	1	44.42	0.86	0	2	3	1	1
16	1.04	0.11	30	4	30.6	1	7	9	2	57.92	0.86	0	2	3	1	1
16	1.04	0.11	30	5	30.7	1	7	9	2	68.08	0	14.8	2	4	0	1
17	1.05	0.101	29	2	29.3	1	7	10	1	0	4.3	0	1	1	1	2
17	1.05	0.101	29	2	29.3	1	7	10	1	0.4	0	6.4	1	1	0	2
17	1	0.101	29	2	29.3	1	7	10	1	10.33	1.72	0	1	1	1	2
17	1	0.101	29	3	29.4	1	7	10	1	19.33	0	5.7	1	2	0	1
17	1	0.101	29	3	29.4	1	7	10	1	22.25	1.72	0	1	2	1	1
17	1	0.101	29	3	29.4	1	7	10	1	34.25	1.72	0	1	2	1	1
17	1	0.101	29	4	29.6	1	7	10	1	46.25	1.72	0	1	3	1	1
17	1	0.101	29	4	29.6	1	7	10	1	58.25	0.86	0	1	3	1	1
17	1	0.101	29	5	29.7	1	7	10	1	70.25	0.86	0	1	4	1	1
17	1	0.101	29	5	29.7	1	7	10	1	82.25	0.86	0	1	4	1	1
17	1	0.101	29	6	29.9	1	7	10	1	93	0.86	0	1	5	1	1
17	1	0.101	29	6	29.9	1	7	10	1	106.25	0.86	0	1	5	1	1
17	1	0.101	29	7	30	1	7	10	1	117.23	0	12.7	1	6	0	2
17	1	0.101	29	7	30	1	7	10	1	117.75	0.86	0	1	6	1	2
18	0.95	0.099	30	2	30.3	2	6	7	1	0	4.3	0	2	1	1	2
18	0.95	0.099	30	2	30.3	2	6	7	1	0.75	0	5	2	1	0	2
18	0.95	0.099	30	2	30.3	2	6	7	1	7.98	0.86	0	2	1	1	2
18	0.95	0.099	30	3	30.4	2	6	7	1	16.06	0.86	0	2	2	1	2
18	0.95	0.099	30	3	30.4	2	6	7	1	22.78	0	11	2	3	0	2
19	1.75	0.141	33	1	33.1	1	9	10	1	0	6.88	0	2	1	1	2
19	1.75	0.141	33	1	33.1	1	9	10	1	0.92	0	4.6	2	1	0	2
1	1.2	0.114	30	1	30.1	1	7	8	1	0	5.16	0	1	1	1	2
1	1.2	0.114	30	1	30.1	1	7	8	1	1.4	0	4.9	1	1	0	2
2	1.15	0.109	32	1	32.1	2	8	10	2	0	6.02	0	2	1	1	2
2	1.15	0.109	32	1	32.1	2	8	10	2	1.13	0	5.8	2	1	0	2
2	1.05	0.109	32	2	32.3	2	8	10	2	12.79	1.29	0	2	2	1	2
2	1.05	0.104	32	2	32.3	2	8	10	2	17.19	0	4.91	2	2	0	2
2	1.05	0.104	32	2	32.3	2	8	10	2	25.61	1.29	0	2	2	1	2
2	1	0.104	32	3	32.4	2	8	10	2	36.79	1.29	0	2	3	1	1
2	1	0.104	32	3	32.4	2	8	10	2	42.44	0	4.56	2	3	0	1

3	1.3	0.12	32	1	32.1	2	5	8	2	0	6.02	0	2	1	1	2
3	1.3	0.12	32	1	32.1	2	5	8	2	1.12	0	5.8	2	1	0	2
3	1.3	0.12	32	1	32.1	2	5	8	2	10.42	1.29	0	2	1	1	2
3	1.3	0.12	32	2	32.3	2	5	8	2	20.47	0	4.4	2	2	0	2
3	1.3	0.12	32	2	32.3	2	5	8	2	23.64	1.29	0	2	2	1	2
3	1.3	0.12	32	2	32.3	2	5	8	2	34.42	1.29	0	2	2	1	2
3	1.18	0.115	32	3	32.4	2	5	8	2	44.55	0	2.8	2	3	0	1
3	1.18	0.115	32	3	32.4	2	5	8	2	47.42	1.29	0	2	3	1	1
3	1.18	0.115	32	3	32.4	2	5	8	2	57.92	1.29	0	2	3	1	1
3	1.18	0.115	32	4	32.6	2	5	8	2	69.58	1.29	0	2	4	1	1
3	1.18	0.115	32	4	32.6	2	5	8	2	81.41	1.29	0	2	4	1	1
3	1.18	0.115	32	5	32.7	2	5	8	2	105.91	1.29	0	2	5	1	1
3	1.18	0.115	32	5	32.7	2	5	8	2	116.83	1.29	0	2	6	1	1
3	1.18	0.115	32	6	32.9	2	5	8	2	130.41	1.29	0	2	6	1	1
3	1.18	0.115	32	7	33	2	5	8	2	140.49	0	14.1	2	7	0	1
4	0.95	0.097	30	1	30.1	1	5	9	1	0	4.3	0	1	1	1	2
4	0.95	0.097	30	1	30.1	1	5	9	1	1.68	0	4.49	1	1	0	2
4	0.95	0.097	30	1	30.1	1	5	9	1	11.08	0.86	0	1	1	1	2
4	0.85	0.097	30	2	30.3	1	5	9	1	21.23	0	10.2	1	2	0	2
4	0.85	0.097	30	2	30.3	1	5	9	1	21.88	0.86	0	1	2	1	2
4	0.75	0.087	30	2	30.3	1	5	9	1	34.16	0.86	0	1	2	1	2
4	0.75	0.087	30	3	30.4	1	5	9	1	43.74	0	8.88	1	3	0	2
4	0.65	0.08	30	3	30.4	1	5	9	1	45.91	0.86	0	1	3	1	2
4	0.7	0.083	30	3	30.4	1	5	9	1	58.08	0.86	0	1	3	1	2
4	0.75	0.087	30	4	30.6	1	5	9	1	66.83	0.86	0	1	4	1	2
4	0.85	0.097	30	4	30.6	1	5	9	1	79.08	0.86	0	1	4	1	1
4	0.92	0.096	30	5	30.7	1	5	9	1	90.65	0.86	0	1	5	1	1
4	0.92	0.096	30	5	30.7	1	5	9	1	90.72	0	11.13	1	5	0	2
5	1.1	0.109	29	1	29.1	2	4	8	1	0	4.3	0	2	1	1	2
5	1.1	0.109	29	1	29.1	2	4	8	1	1	0	5.7	2	1	0	2
5	1.1	0.109	29	1	29.1	2	4	8	1	6.55	2.7	0	2	1	1	2
5	1.1	0.109	29	2	29.3	2	4	8	1	12.82	0	9.8	2	2	0	2
5	1	0.106	29	2	29.3	2	4	8	1	15.79	2.7	0	2	2	1	2
5	1	0.106	29	2	29.3	2	4	8	1	27.72	2.7	0	2	2	1	1
5	1	0.106	29	3	29.4	2	4	8	1	36.82	0	7.4	2	3	0	1
6	1	0.103	30	1	30.1	1	4	6	1	0	4.3	0	2	1	1	2
6	1	0.103	30	1	30.1	1	4	6	1	10.46	0.86	0	2	1	1	2
6	1	0.103	30	1	30.3	1	4	6	1	16.76	0	4.5	2	2	0	2
7	1.55	0.129	31	1	31.1	2	7	8	1	0	6.45	0	2	1	1	2
7	1.55	0.129	31	1	31.1	2	7	8	1	13.08	1.29	0	2	1	1	2
7	1.55	0.129	31	2	31.3	2	7	8	1	22.33	1.29	0	2	2	1	2
7	1.55	0.129	31	2	31.3	2	7	8	1	32.21	0	14.66	2	2	0	2
7	1.55	0.129	31	3	31.4	2	7	8	1	34.38	1.29	0	2	3	1	2
7	1.55	0.129	31	3	31.4	2	7	8	1	43.46	0	9.5	2	3	0	2
7	1.55	0.129	31	4	31.6	2	7	8	1	66.79	0	5.8	2	4	0	2
8	1.1	0.108	31	1	31.1	2	4	6	1	0	3.87	0	1	1	1	2
8	1.1	0.108	31	2	31.3	2	4	6	1	13.58	0.86	0	1	2	1	2
8	1.1	0.108	31	2	31.3	2	4	6	1	21.7	0.86	0	1	2	1	2
8	1.1	0.108	31	3	31.4	2	4	6	1	30.7	0.86	0	1	3	1	2
8	1.1	0.108	31	3	31.4	2	4	6	1	37.5	0.86	0	1	3	1	2
8	1.1	0.108	31	3	31.4	2	4	6	1	41.23	0	9.41	1	3	0	2
8	1.1	0.108	31	3	31.4	2	4	6	1	46.3	0.86	0	1	3	1	2
8	1.1	0.108	31	4	31.6	2	4	6	1	55.63	0.86	0	1	4	1	2
8	1.1	0.108	31	4	31.6	2	4	6	2	61.7	0	8.59	1	4	0	2
8	1.1	0.108	31	4	31.6	2	4	6	2	61.84	0.86	0	1	4	1	2
8	1.1	0.108	31	4	31.6	2	4	6	2	69.56	0.86	0	1	4	1	2
8	1.1	0.108	31	5	31.7	2	4	6	2	79.48	0.86	0	1	5	1	2
8	1.1	0.108	31	5	31.7	2	4	6	2	86.06	0	10.36	1	5	0	2

8	1.1	0.108	31	5	31.7	2	4	6	2	86.31	0.86	0	1	5	1	2
8	1.1	0.108	31	5	31.7	2	4	6	2	93.56	0.86	0	1	5	1	2
8	1.1	0.108	31	6	31.9	2	4	6	2	103.41	0.86	0	1	6	1	2
8	1.1	0.108	31	6	31.9	2	4	6	2	110.06	0.86	0	1	6	1	2
8	1.1	0.108	31	6	31.9	2	4	6	2	119	0.86	0	1	6	1	2
8	1.1	0.108	31	7	32	2	4	6	2	128	0.86	0	1	7	1	2
8	1.1	0.108	31	7	32	2	4	6	2	133.45	0.86	0	1	7	1	2
8	1.1	0.108	31	7	32	2	4	6	2	141.75	0.86	0	1	7	1	2
8	1.1	0.108	31	8	32.1	2	4	6	2	152	0.86	0	1	8	1	2
8	1.1	0.108	31	8	32.1	2	4	6	2	157.45	0.86	0	1	8	1	2
8	1.1	0.108	31	8	32.1	2	4	6	2	160.33	0	6.33	1	8	0	2
8	1.1	0.108	31	8	32.1	2	4	6	2	164.5	0.86	0	1	8	1	2
8	1.1	0.108	31	9	32.3	2	4	6	2	174.25	0.86	0	1	9	1	2
8	1.1	0.108	31	9	32.3	2	4	6	2	180.5	0.86	0	1	9	1	2
8	1.1	0.108	31	9	32.3	2	4	6	2	183.4	0	4.48	1	9	0	2
8	1.1	0.108	31	9	32.3	2	4	6	2	189.75	0.86	0	1	9	1	2
8	1.1	0.108	31	10	32.4	2	4	6	2	198.25	0.86	0	1	10	1	2
8	1.1	0.108	31	10	32.4	2	4	6	2	204.58	0.86	0	1	10	1	2
8	1.1	0.108	31	10	32.4	2	4	6	2	207.66	0	10.77	1	10	0	2
8	1.1	0.108	31	10	32.4	2	4	6	2	212.99	0.86	0	1	10	1	2
8	1.14	0.11	31	11	32.6	2	4	6	2	222.25	0.86	0	1	11	1	2
8	1.14	0.11	31	12	32.7	2	4	6	2	255.67	0	5.7	1	12	0	2
110	1.25	0.113	32	1	32.1	1	6	9	2	0	6.02	0	2	1	1	2
110	1.25	0.113	32	1	32.1	1	6	9	2	6.48	0.86	0	2	1	1	2
110	1.25	0.113	32	2	32.3	1	6	9	2	8.73	0	1.34	2	2	0	2
110	1.25	0.113	32	2	32.3	1	6	9	2	14.13	0.86	0	2	2	1	2
110	1.25	0.113	32	3	32.4	1	6	9	2	23.96	0.86	0	2	3	1	2
110	1.25	0.113	32	3	32.4	1	6	9	2	30.5	0.86	0	2	3	1	2
110	1.25	0.113	32	3	32.4	1	6	9	2	38.08	0.86	0	2	3	1	1
110	1.25	0.113	32	4	32.6	1	6	9	2	47.83	0.86	0	2	4	1	1
110	1.25	0.113	32	4	32.6	1	6	9	2	54.25	0.86	0	2	4	1	1
110	1.25	0.113	32	4	32.6	1	6	9	2	62.25	0.86	0	2	4	1	1
110	1.25	0.113	32	5	32.7	1	6	9	2	71.75	0.86	0	2	5	1	1
110	1.25	0.113	32	5	32.7	1	6	9	2	78.25	0.86	0	2	5	1	1
110	1.25	0.113	32	5	32.7	1	6	9	2	82.25	0.86	0	2	5	1	1
110	1.25	0.113	32	6	32.9	1	6	9	2	92.45	0.86	0	2	6	1	1
110	1.25	0.113	32	6	32.9	1	6	9	2	101.5	0	22	2	6	0	1
110	0.98	0.11	32	7	33	1	6	9	2	124.98	0	14	2	7	0	1
111	1.26	0.115	30	1	30.1	2	8	9	1	0	6.02	0	1	1	1	2
111	1.26	0.115	30	1	30.1	2	8	9	1	5.08	0.86	0	1	1	1	2
111	1.26	0.115	30	2	30.3	2	8	9	1	13.66	0.86	0	1	2	1	2
111	1.26	0.115	30	2	30.3	2	8	9	1	20.11	0	7.29	1	2	0	2
111	1.26	0.115	30	2	30.3	2	8	9	1	20.24	0.86	0	1	2	1	2
111	1.26	0.115	30	2	30.3	2	8	9	1	27.57	0.86	0	1	2	1	2
111	1.26	0.115	30	3	30.4	2	8	9	1	37.49	0.86	0	1	3	1	2
111	1.26	0.115	30	3	30.4	2	8	9	1	43.41	0	6.06	1	3	0	2
111	1.26	0.115	30	3	30.4	2	8	9	1	45.63	0.86	0	1	3	1	2
111	1.26	0.115	30	3	30.4	2	8	9	1	51.58	0.86	0	1	3	1	2
111	1.26	0.115	30	4	30.6	2	8	9	1	61.24	0.86	0	1	4	1	2
111	1.26	0.115	30	4	30.6	2	8	9	1	70.74	0.86	0	1	4	1	2
111	1.26	0.115	30	4	30.6	2	8	9	1	76.91	0.86	0	1	4	1	2
111	1.26	0.115	30	5	30.7	2	8	9	1	85.49	0.86	0	1	5	1	2
111	1.26	0.115	30	5	30.7	2	8	9	1	91.74	0.86	0	1	5	1	2
111	1.26	0.115	30	5	30.7	2	8	9	1	101.24	0.86	0	1	5	1	2
111	1.26	0.115	30	6	30.9	2	8	9	1	109.24	0.86	0	1	6	1	2
111	1.26	0.115	30	6	30.9	2	8	9	1	115.57	0.86	0	1	6	1	2
111	1.26	0.115	30	6	30.9	2	8	9	1	126.23	0.86	0	1	6	1	2
111	1.26	0.115	30	7	31	2	8	9	1	133.15	0.86	0	1	7	1	2

111	1.26	0.115	30	7	31	2	8	9	1	139.92	0.86	0	1	7	1	2
111	1.26	0.115	30	7	31	2	8	9	1	142.85	0	7.55	1	7	0	2
111	1.26	0.115	30	7	31	2	8	9	1	147.85	0.86	0	1	7	1	2
111	1.26	0.115	30	8	31.1	2	8	9	1	156.15	0.86	0	1	8	1	1
111	1.26	0.115	30	8	31.1	2	8	9	1	163	0.86	0	1	8	1	1
111	1.26	0.115	30	8	31.1	2	8	9	1	165.3	0	7.2	1	8	0	1
112	0.75	0.083	28	1	28.1	2	3	5	1	0	3.44	0	2	1	1	2
112	0.75	0.083	28	1	28.1	2	3	5	1	4.75	0.86	0	2	1	1	2
112	0.75	0.083	28	1	28.1	2	3	5	1	10.75	0.86	0	2	1	1	2
112	0.75	0.083	28	2	28.3	2	3	5	1	22.25	0.86	0	2	2	1	2
112	0.75	0.083	28	2	28.3	2	3	5	1	28.75	0.86	0	2	2	1	2
112	0.75	0.083	28	2	28.3	2	3	5	1	34.75	0.86	0	2	2	1	2
112	0.66	0.08	28	3	28.4	2	3	5	1	46.25	0.86	0	2	3	1	2
112	0.66	0.08	28	3	28.4	2	3	5	1	52.32	0.86	0	2	3	1	2
112	0.66	0.08	28	3	28.4	2	3	5	1	54.92	0	9.3	2	3	0	2
112	0.66	0.08	28	3	28.4	2	3	5	1	60.75	0.86	0	2	3	1	2
112	0.66	0.08	28	4	28.6	2	3	5	1	70.25	0.86	0	2	4	1	2
112	0.66	0.08	28	4	28.6	2	3	5	2	76.37	0.86	0	2	4	1	2
112	0.66	0.08	28	4	28.6	2	3	5	2	79.12	0	5.9	2	4	0	2
112	0.66	0.08	28	4	28.6	2	3	5	2	85.84	0.86	0	2	4	1	2
112	0.66	0.08	28	5	28.7	2	3	5	2	94.26	0.86	0	2	5	1	2
112	0.66	0.08	28	5	28.7	2	3	5	2	100.51	0.86	0	2	5	1	2
112	0.66	0.08	28	5	28.7	2	3	5	2	103.84	0	15.4	2	5	0	2
113	1.2	0.114	30	1	30.1	2	7	9	1	0	5.16	0	1	1	1	2
113	1.2	0.114	30	2	30.3	2	7	9	1	9.25	0.86	0	1	2	1	2
113	1.2	0.114	30	2	30.3	2	7	9	1	15.75	0.86	0	1	2	1	2
113	1.2	0.114	30	2	30.3	2	7	9	1	23.75	0.86	0	1	2	1	2
113	1.2	0.114	30	3	30.4	2	7	9	1	33.25	0.86	0	1	3	1	2
113	1.2	0.114	30	3	30.4	2	7	9	1	39.75	0.86	0	1	3	1	2
113	1.2	0.114	30	3	30.4	2	7	9	1	47.75	0.86	0	1	3	1	2
113	1.2	0.114	30	4	30.6	2	7	9	1	57.25	0.86	0	1	4	1	2
113	1.2	0.114	30	4	30.6	2	7	9	1	63.4	0.86	0	1	4	1	1
113	1.2	0.114	30	4	30.6	2	7	9	1	66.02	0	4.26	1	4	0	1
113	1.2	0.114	30	4	30.6	2	7	9	1	71.25	0.86	0	1	4	1	1
113	1.2	0.114	30	5	30.7	2	7	9	1	81.25	0.86	0	1	5	1	1
113	0.94	0.11	30	5	30.7	2	7	9	1	87.43	0.86	0	1	5	1	1
113	0.94	0.11	30	5	30.7	2	7	9	1	90.31	0	4.53	1	5	0	1
113	0.94	0.11	30	6	30.9	2	7	9	1	115.14	0	3.27	1	6	0	1
114	1.34	0.123	32	1	32.1	2	9	10	2	0	4.3	0	1	1	1	2
114	1.34	0.123	32	2	32.3	2	9	10	2	4	0.86	0	1	2	1	2
114	1.34	0.123	32	2	32.3	2	9	10	2	10.43	0.86	0	1	2	1	2
114	1.34	0.123	32	2	32.3	2	9	10	2	13.13	0	2.35	1	2	0	2
114	1.34	0.123	32	3	32.4	2	9	10	2	37.5	0	1.57	1	3	0	2
115	0.85	0.09	30	1	30.1	2	7	10	1	0	3.44	0	1	1	1	2
115	0.85	0.09	30	1	30.1	2	7	10	1	4.17	0.86	0	1	1	1	2
115	0.85	0.09	30	2	30.3	2	7	10	1	12.17	0.86	0	1	2	1	2
115	0.85	0.09	30	2	30.3	2	7	10	1	18.52	0.86	0	1	2	1	2
115	0.85	0.09	30	2	30.3	2	7	10	1	21.17	0	7.31	1	2	0	2
115	0.85	0.09	30	2	30.3	2	7	10	1	28.17	0.86	0	1	2	1	2
115	0.85	0.09	30	3	30.4	2	7	10	1	36	0.86	0	1	3	1	2
115	0.85	0.09	30	3	30.4	2	7	10	1	42.77	0.86	0	1	3	1	2
115	0.85	0.09	30	3	30.4	2	7	10	1	51.84	0.86	0	1	3	1	2
115	0.85	0.09	30	4	30.6	2	7	10	1	60.17	0.86	0	1	4	1	2
115	0.85	0.09	30	4	30.6	2	7	10	1	66.94	0.86	0	1	4	1	2
115	0.85	0.09	30	4	30.6	2	7	10	1	68.94	0	9.26	1	4	0	2
115	0.85	0.09	30	4	30.6	2	7	10	1	76.17	0.86	0	1	4	1	2
115	0.85	0.09	30	5	30.7	2	7	10	1	83.39	0.86	0	1	5	1	2
115	0.85	0.09	30	5	30.7	2	7	10	1	91.09	0.86	0	1	5	1	2

115	0.85	0.09	30	5	30.7	2	7	10	1	93.94	0	6.5	1	5	0	2
115	0.85	0.09	30	5	30.7	2	7	10	1	98.17	0.86	0	1	5	1	2
115	0.85	0.09	30	6	30.9	2	7	10	1	108.34	0.86	0	1	6	1	2
115	0.85	0.09	30	6	30.9	2	7	10	1	114.42	0.86	0	1	6	1	2
115	0.85	0.09	30	6	30.9	2	7	10	1	117.72	0	10.6	1	6	0	2
116	1.05	0.104	32	1	32.1	2	9	10	2	0	4.3	0	1	1	1	2
116	1.05	0.104	32	2	32.3	2	9	10	2	5	0.86	0	1	2	1	2
116	1.05	0.104	32	2	32.3	2	9	10	2	11.52	0.86	0	1	2	1	2
116	1.05	0.104	32	3	32.4	2	9	10	2	38.23	0	2.78	1	2	0	2
117	1.75	0.143	34	1	34.1	2	6	9	1	0	4.3	0	2	1	1	2
117	1.75	0.143	34	2	34.3	2	6	9	1	9.66	0.86	0	2	2	1	2
117	1.75	0.143	34	2	34.3	2	6	9	1	17.83	0.86	0	2	2	1	2
117	1.75	0.143	34	3	34.4	2	6	9	1	27.45	0.86	0	2	3	1	2
117	1.75	0.143	34	3	34.4	2	6	9	1	34.33	0.86	0	2	3	1	2
117	1.75	0.143	34	3	34.4	2	6	9	1	37.38	0	3.55	2	3	0	2
117	1.75	0.143	34	4	34.6	2	6	9	1	57.33	0.86	0	2	4	1	2
117	1.75	0.143	34	4	34.6	2	6	9	1	66.75	0.86	0	2	4	1	2
117	1.75	0.143	34	5	34.7	2	6	9	1	75.25	0.86	0	2	5	1	1
117	1.75	0.143	34	5	34.7	2	6	9	1	83.25	0.86	0	2	5	1	1
117	1.75	0.143	34	5	34.7	2	6	9	1	90.75	0.86	0	2	5	1	1
117	1.75	0.143	34	6	34.9	2	6	9	1	99.25	0.86	0	2	6	1	1
117	1.75	0.143	34	6	34.9	2	6	9	1	105.91	0.86	0	2	6	1	1
117	1.75	0.143	34	6	34.9	2	6	9	1	114.24	0.86	0	2	6	1	1
117	1.75	0.143	34	7	35	2	6	9	1	122.41	0.86	0	2	7	1	1
117	1.75	0.143	34	7	35	2	6	9	1	128.56	0.86	0	2	7	1	2
117	1.75	0.143	34	7	35	2	6	9	1	130.21	0.86	0	2	7	1	2
117	1.75	0.143	34	7	35	2	6	9	1	132.2	0	5.79	2	7	0	2
118	1.3	0.119	31	1	31.1	1	5	9	2	0	5.59	0	2	1	1	2
118	1.3	0.119	31	2	31.3	1	5	9	2	7.57	0.86	0	2	2	1	2
118	1.3	0.119	31	2	31.3	1	5	9	2	10.25	0	6.61	2	2	0	2
118	1.3	0.119	31	3	31.4	1	5	9	2	24.83	0.86	0	2	3	1	2
118	1.3	0.119	31	3	31.4	1	5	9	2	30.83	0.86	0	2	3	1	2
118	1.3	0.119	31	3	31.4	1	5	9	2	34.46	0	8.01	2	3	0	2
118	1.3	0.119	31	3	31.4	1	5	9	2	38.83	0.86	0	2	3	1	2
118	1.3	0.119	31	3	31.4	1	5	9	2	44.86	0.86	0	2	3	1	2
118	1.3	0.119	31	4	31.6	1	5	9	2	55.79	0.86	0	2	4	1	2
118	1.3	0.119	31	5	31.7	1	5	9	2	73.29	0.86	0	2	5	1	2
118	1.3	0.119	31	5	31.7	1	5	9	2	79.87	0.86	0	2	5	1	2
118	1.3	0.119	31	5	31.7	1	5	9	2	90.79	0.86	0	2	5	1	2
118	1.3	0.119	31	6	31.9	1	5	9	2	97.29	0.86	0	2	6	1	2
118	1.11	0.119	31	6	31.9	1	5	9	2	103.51	0.86	0	2	6	1	2
118	1.11	0.119	31	6	31.9	1	5	9	2	105.96	0	11.82	2	6	0	2
9	1.95	0.154	33	1	33.1	2	7	9	1	0	8.6	0	2	1	1	2
9	1.95	0.154	33	1	33.1	2	7	9	1	8.4	1.72	0	2	1	1	2
9	1.95	0.154	33	1	33.1	2	7	9	1	16.16	1.72	0	2	1	1	2
9	1.95	0.154	33	2	33.3	2	7	9	1	23.99	1.72	0	2	2	1	1
9	1.95	0.154	33	2	33.3	2	7	9	1	30.7	1.72	0	2	2	1	1
9	1.95	0.154	33	2	33.3	2	7	9	1	30.98	0	7.8	2	2	0	1
9	1.44	0.14	33	3	33.4	2	7	9	1	50.98	0	4.8	2	3	0	1
9	1.44	0.14	33	3	33.4	2	7	9	1	51.81	1.72	0	2	3	1	1
9	1.44	0.14	33	3	33.4	2	7	9	1	60.73	1.72	0	2	3	1	1
9	1.44	0.14	33	4	33.6	2	7	9	1	75.73	1.72	0	2	4	1	1
9	1.44	0.14	33	4	33.6	2	7	9	1	84.73	1.72	0	2	4	1	1
9	1.44	0.14	33	5	33.7	2	7	9	1	93.15	1.72	0	2	5	1	1
9	1.76	0.15	33	5	33.7	2	7	9	1	99.65	1.72	0	2	5	1	1
9	1.76	0.15	33	5	33.7	2	7	9	1	108.48	1.72	0	2	5	1	1
9	1.76	0.15	33	6	33.9	2	7	9	1	116.81	1.72	0	2	6	1	2
9	1.76	0.15	33	6	33.9	2	7	9	1	123.46	1.72	0	2	6	1	2

9	1.76	0.15	33	6	33.9	2	7	9	2	126.54	0	6.3	2	6	0	2
9	1.76	0.15	33	7	34	2	7	9	2	150.6	0	3.2	2	7	0	2
9	1.76	0.15	33	8	34.1	2	7	9	2	174.47	0	1.9	2	8	0	2
9	1.76	0.15	33	9	34.3	2	7	9	2	198.23	0	1.1	2	9	0	2
119	1.35	0.124	32	1	32.1	2	5	8	1	0	5.6	0	2	1	1	2
119	1.35	0.124	32	1	32.1	2	5	8	1	5.8	0.86	0	2	1	1	2
119	1.35	0.124	32	1	32.1	2	5	8	1	8.52	0	2.1	2	1	0	2
119	1.35	0.124	32	1	32.1	2	5	8	1	13.02	0.86	0	2	1	1	2
119	1.35	0.124	32	2	32.3	2	5	8	1	23.25	0.86	0	2	2	1	2
119	1.35	0.124	32	2	32.3	2	5	8	1	29.03	0.86	0	2	2	1	2
119	1.35	0.124	32	2	32.3	2	5	8	2	37.16	0.86	0	2	2	1	2
119	1.35	0.124	32	3	32.4	2	5	8	2	46.98	0.86	0	2	3	1	2
119	1.35	0.124	32	3	32.4	2	5	8	2	53.5	0.86	0	2	3	1	2
119	1.35	0.124	32	3	32.4	2	5	8	2	63	0.86	0	2	3	1	2
119	1.35	0.124	32	4	32.6	2	5	8	2	71.17	0.86	0	2	4	1	1
119	1.35	0.124	32	4	32.6	2	5	8	2	77.5	0.86	0	2	4	1	1
119	1.35	0.124	32	5	32.7	2	5	8	2	95	0.86	0	2	5	1	1
119	1.24	0.12	32	5	32.7	2	5	8	2	101.13	0.86	0	2	5	1	1
119	1.24	0.12	32	5	32.7	2	5	8	2	110.75	0.86	0	2	5	1	1
119	1.24	0.12	32	6	32.9	2	5	8	2	118.97	0.86	0	2	6	1	1
119	1.24	0.12	32	6	32.9	2	5	8	2	125.34	0.86	0	2	6	1	1
119	1.24	0.12	32	6	32.9	2	5	8	2	127.42	0	7.7	2	6	0	1
119	1.24	0.12	32	6	32.9	2	5	8	2	131.4	0	7.3	2	6	0	1
119	1.18	0.12	32	7	33	2	5	8	2	152.71	0	5.4	2	7	0	1
120	0.9	0.093	31	1	31.1	2	7	9	2	0	4.3	0	2	1	1	2
120	0.9	0.093	31	1	31.1	2	7	9	2	15.5	0.86	0	2	1	1	2
120	0.9	0.093	31	2	31.3	2	7	9	2	27.5	0.86	0	2	2	1	2
120	0.9	0.093	31	2	31.3	2	7	9	2	39.5	0.86	0	2	2	1	2
120	0.9	0.093	31	3	31.4	2	7	9	2	51.5	0.86	0	2	3	1	2
120	0.9	0.093	31	3	31.4	2	7	9	2	63.5	0.86	0	2	3	1	2
120	0.9	0.093	31	4	31.6	2	7	9	2	74	0.86	0	2	4	1	2
120	0.9	0.093	31	4	31.6	2	7	9	2	87.85	0.86	0	2	4	1	2
120	0.9	0.093	31	5	31.7	2	7	9	2	97.9	0.86	0	2	5	1	2
120	0.78	0.09	31	5	31.7	2	7	9	2	101.13	0	7.3	2	5	0	2
121	1.1	0.106	31	1	31.1	2	7	9	2	0	4.3	0	2	1	1	2
121	1.1	0.106	31	2	31.3	2	7	9	2	9.92	0.86	0	2	2	1	2
121	1.1	0.106	31	2	31.3	2	7	9	2	21	0.86	0	2	2	1	2
121	1.06	0.104	31	3	31.4	2	7	9	2	33	0.86	0	2	3	1	2
121	1.06	0.104	31	3	31.4	2	7	9	2	45	0.86	0	2	3	1	2
121	1.06	0.104	31	4	31.6	2	7	9	2	57	0.86	0	2	4	1	2
121	1.06	0.104	31	4	31.6	2	7	9	2	69	0.86	0	2	4	1	2
121	1.06	0.104	31	5	31.7	2	7	9	2	79.42	0.86	0	2	5	1	2
121	1.06	0.104	31	5	31.7	2	7	9	2	91	0.86	0	2	5	1	2
121	1.02	0.103	31	6	31.9	2	7	9	2	105.51	0.86	0	2	6	1	2
121	1.02	0.103	31	6	31.9	2	7	9	2	117.16	0.86	0	2	6	1	2
121	1.02	0.103	31	7	32	2	7	9	2	127.13	0.86	0	2	7	1	2
121	0.88	0.096	31	7	32	2	7	9	2	130.15	0	3.3	2	7	0	2
122	1.4	0.124	30	1	30.1	1	9	10	1	0	6.02	0	2	1	1	2
122	1.4	0.124	30	1	30.1	1	9	10	1	17.6	0.86	0	2	1	1	2
122	1.4	0.124	30	2	30.3	1	9	10	1	28.66	0.86	0	2	2	1	2
122	1.4	0.124	30	2	30.3	1	9	10	1	35.75	0.86	0	2	2	1	2
122	1.4	0.124	30	2	30.3	1	9	10	1	40.25	0.86	0	2	2	1	2
122	1.4	0.124	30	3	30.4	1	9	10	1	51.75	0.86	0	2	3	1	2
122	1.4	0.124	30	3	30.4	1	9	10	1	59.75	0.86	0	2	3	1	2
122	1.4	0.124	30	3	30.4	1	9	10	1	65.75	0.86	0	2	3	1	2
122	1.4	0.124	30	4	30.6	1	9	10	1	75.65	0.86	0	2	4	1	2
122	1.4	0.124	30	4	30.6	1	9	10	1	76.95	0	6	2	4	0	2
123	1.2	0.115	30	1	30.1	1	7	9	1	0	5.16	0	1	1	1	2

123	1.2	0.115	30	1	30.1	1	7	9	1	7.06	0.86	0	1	1	1	2
123	1.2	0.115	30	2	30.3	1	7	9	2	18.03	0.86	0	1	2	1	2
123	1.2	0.115	30	2	30.3	1	7	9	2	21.28	0	4.9	1	2	0	2
123	1.2	0.115	30	2	30.3	1	7	9	2	31.63	0.86	0	1	2	1	2
123	1.2	0.115	30	3	30.4	1	7	9	2	41.83	0.86	0	1	3	1	2
123	1.28	0.119	30	3	30.4	1	7	9	2	44.85	0	8.2	1	3	0	2
123	1.28	0.119	30	3	30.4	1	7	9	2	55.55	0.86	0	1	3	1	2
123	1.28	0.119	30	4	30.6	1	7	9	2	66.15	0.86	0	1	4	1	1
123	1.28	0.119	30	4	30.6	1	7	9	2	69.21	0	6	1	4	0	1
123	1.28	0.119	30	4	30.6	1	7	9	2	72.29	0.86	0	1	4	1	1
123	1.28	0.119	30	5	30.7	1	7	9	2	79.54	0.86	0	1	5	1	1
123	1.28	0.119	30	5	30.7	1	7	9	2	90.29	0.86	0	1	5	1	1
123	1.28	0.119	30	5	30.7	1	7	9	2	93.06	0	11.4	1	5	0	1
124	1.2	0.111	32	1	32.1	1	6	9	1	0	4.3	0	1	1	1	2
124	1.2	0.111	32	2	32.3	1	6	9	1	9.42	0.86	0	1	2	1	2
124	1.16	0.109	32	2	32.3	1	6	9	1	15.92	0	11.4	1	2	0	2
124	1.16	0.109	32	2	32.3	1	6	9	1	18.73	0.86	0	1	2	1	2
124	1.16	0.109	32	2	32.3	1	6	9	1	21.16	0	11.5	1	2	0	2
124	1.16	0.109	32	2	32.3	1	6	9	1	24.91	0.86	0	1	2	1	2
124	1.16	0.109	32	3	32.4	1	6	9	1	33.57	0.86	0	1	3	1	1
124	1.16	0.109	32	3	32.4	1	6	9	1	40.44	0	10.3	1	3	0	1
124	1.16	0.109	32	3	32.4	1	6	9	1	42.44	0.86	0	1	3	1	1
124	1.16	0.109	32	3	32.4	1	6	9	1	46.94	0.86	0	1	3	1	1
124	1.16	0.109	32	4	32.6	1	6	9	1	55.44	0.86	0	1	4	1	1
124	1.16	0.109	32	4	32.6	1	6	9	1	62.27	0.86	0	1	4	1	1
124	1.16	0.109	32	4	32.6	1	6	9	1	69.77	0.86	0	1	4	1	1
124	1.16	0.109	32	5	32.7	1	6	9	1	79.43	0.86	0	1	5	1	1
124	1.1	0.107	32	5	32.7	1	6	9	1	85.83	0.86	0	1	5	1	1
124	1.1	0.107	32	5	32.7	1	6	9	1	88.83	0	14.7	1	5	0	1
124	1.1	0.107	32	5	32.7	1	6	9	1	94.23	0.86	0	1	5	1	2
124	1.1	0.107	32	6	32.9	1	6	9	1	103.23	0.86	0	1	6	1	2
124	1.1	0.107	32	6	32.9	1	6	9	1	109.48	0.86	0	1	6	1	2
124	1.1	0.107	32	6	32.9	1	6	9	1	112.71	0	11.9	1	6	0	2
124	1.1	0.107	32	6	32.9	1	6	9	1	117.54	0.86	0	1	5	1	2
124	1.1	0.107	32	7	33	1	6	9	1	127.46	0.86	0	1	7	1	2
124	1.1	0.107	32	8	33.1	1	6	9	1	160.59	0	4.8	1	8	0	2
125	0.9	0.11	28	1	28.1	1	8	9	1	0	4.3	0	1	1	1	2
125	0.9	0.11	28	1	28.1	1	8	9	1	6.53	0.86	0	1	1	1	2
125	0.9	0.11	28	1	28.1	1	8	9	1	10.19	0	1.8	1	1	0	2
126	1.4	0.12	32	1	32.1	2	9	10	2	0	6.02	0	2	1	1	2
126	1.28	0.116	32	2	32.3	2	9	10	2	14.12	0.86	0	2	2	1	2
126	1.28	0.116	32	2	32.3	2	9	10	2	16.05	0	4.6	2	2	0	2
126	1.28	0.116	32	2	32.3	2	9	10	2	26.38	0.86	0	2	2	1	2
126	1.28	0.116	32	3	32.4	2	9	10	2	36.6	0.86	0	2	3	1	2
126	1.28	0.116	32	3	32.4	2	9	10	2	39.83	0	6.2	2	3	0	2
126	1.28	0.116	32	3	32.4	2	9	10	2	50.33	0.86	0	2	3	1	2
126	1.28	0.116	32	4	32.6	2	9	10	2	60.66	0.86	0	2	4	1	2
126	1.28	0.116	32	4	32.6	2	9	10	2	72.32	0.86	0	2	4	1	2
126	1.28	0.116	32	5	32.7	2	9	10	2	82.98	0.86	0	2	5	1	2
126	1.28	0.116	32	5	32.7	2	9	10	2	96.31	0.86	0	2	5	1	2
126	1.28	0.116	32	6	32.9	2	9	10	2	106.47	0.86	0	2	6	1	1
126	1.18	0.112	32	6	32.9	2	9	10	2	109.59	0	4	2	6	0	1
127	1.35	0.118	32	1	32.1	2	8	10	1	0	6.02	0	2	1	1	2
127	1.35	0.118	32	2	32.3	2	8	10	1	12.83	0.86	0	2	2	1	2
127	1.28	0.118	32	2	32.3	2	8	10	1	15.21	0	6.7	2	2	0	2
127	1.28	0.118	32	2	32.3	2	8	10	1	25.66	0.86	0	2	2	1	2
127	1.28	0.118	32	3	32.4	2	8	10	1	35.86	0.86	0	2	3	1	2
127	1.28	0.118	32	3	32.4	2	8	10	1	39.06	0	3.6	2	3	0	2

127	1.28	0.118	32	3	32.4	2	8	10	1	47.66	0.86	0	2	3	1	2
127	1.28	0.118	32	4	32.6	2	8	10	1	59.91	0.86	0	2	4	1	2
127	1.26	0.118	32	6	32.9	2	8	10	1	110.06	0	2.7	2	6	0	2
127	1.26	0.118	32	7	33	2	8	10	1	134	0	2.1	2	7	0	2
128	0.85	0.09	27	1	27.1	2	8	10	1	0	3.44	0	2	1	1	2
128	0.85	0.09	27	2	27.3	2	8	10	1	14.16	0.86	0	0	2	1	2
128	0.85	0.09	27	2	27.3	2	8	10	1	23.17	0.86	0	2	2	1	2
128	0.72	0.084	27	3	27.4	2	8	10	1	38.9	0.86	0	2	3	1	2
128	0.72	0.084	27	3	27.4	2	8	10	1	40.58	0	5.64	2	3	0	2
129	0.95	0.098	27	1	27.1	2	5	9	1	0	4.3	0	1	1	1	2
129	0.95	0.098	27	2	27.3	2	5	9	1	14.17	0.86	0	1	2	1	2
129	0.95	0.098	27	2	27.3	2	5	9	1	23.16	0.86	0	1	2	1	2
129	0.95	0.098	27	3	27.4	2	5	9	1	31.66	0.86	0	1	3	1	2
129	0.84	0.092	27	3	27.4	2	5	9	1	38.96	0.86	0	1	3	1	2
129	0.84	0.092	27	3	27.4	2	5	9	1	40.91	0	5.64	1	3	0	2
130	1	0.1	28	1	28.1	2	6	8	1	0	3.44	0	2	1	1	2
130	1	0.1	28	2	28.3	2	6	8	1	6	0.86	0	2	2	1	2
130	1.01	0.1	28	2	28.3	2	6	8	1	13.37	0.86	0	2	2	1	2
130	1.01	0.1	28	2	28.3	2	6	8	1	14.92	0	7.94	2	2	0	2
130	1.01	0.1	28	2	28.3	2	6	8	1	21.5	0.86	0	2	2	1	2
130	1.01	0.1	28	3	28.4	2	6	8	1	30	0.86	0	2	3	1	2
130	1.01	0.1	28	3	28.4	2	6	8	1	37.17	0.86	0	2	3	1	2
130	0.9	0.095	28	3	28.4	2	6	8	1	39.25	0	10.6	2	3	0	2
130	0.9	0.095	28	3	28.4	2	6	8	2	45.33	0.86	0	2	3	1	2
130	0.9	0.095	28	4	28.6	2	6	8	2	54	0.86	0	2	4	1	2
130	0.9	0.095	28	4	28.6	2	6	8	2	61.05	0.86	0	2	4	1	2
130	0.86	0.094	28	4	28.6	2	6	8	2	63.8	0	9.43	2	4	0	2
130	0.86	0.094	28	4	28.6	2	6	8	2	68.68	0.86	0	2	4	1	2
130	0.86	0.094	28	5	28.7	2	6	8	2	78.01	0.86	0	2	4	1	2
130	0.86	0.094	28	5	28.7	2	6	8	2	84.61	0.86	0	2	5	1	2
130	0.86	0.094	28	5	28.7	2	6	8	2	90.84	0	9.93	2	5	0	2
130	0.86	0.094	28	5	28.7	2	6	8	2	94.94	0.86	0	2	5	1	2
130	0.86	0.094	28	6	28.9	2	6	8	2	104.5	0.86	0	2	6	1	2
130	0.92	0.097	28	6	28.9	2	6	8	2	115.1	0	10.81	2	6	0	2
130	0.92	0.097	28	9	29.4	2	6	8	2	174.83	0	8.08	2	9	0	2
131	1.5	0.126	31	1	31.1	1	9	10	1	0	6.02	0	1	1	1	2
131	1.5	0.126	31	1	31.1	1	9	10	1	3.35	0.86	0	1	1	1	2
131	1.5	0.126	31	1	31.1	1	9	10	1	5.47	0	5.2	1	1	0	2
131	1.46	0.126	31	1	31.1	1	9	10	1	11.59	0.86	0	1	1	1	2
131	1.46	0.126	31	2	31.3	1	9	10	1	19.51	0.86	0	1	2	1	2
131	1.46	0.126	31	2	31.3	1	9	10	1	26.57	0.86	0	1	2	1	2
131	1.46	0.126	31	2	31.3	1	9	10	2	29.92	0	5.49	1	2	0	2
131	1.46	0.126	31	2	31.3	1	9	10	2	33.75	0.86	0	1	2	1	2
131	1.46	0.126	31	3	31.4	1	9	10	2	43.25	0.86	0	1	3	1	2
131	1.36	0.122	31	4	31.6	1	9	10	2	67.58	0.86	0	1	4	1	1
131	1.36	0.122	31	4	31.6	1	9	10	2	76.3	0	4.09	1	4	0	1
132	1.4	0.125	34	1	34.1	2	7	9	1	0	6.02	0	1	1	1	2
132	1.4	0.125	34	1	34.1	2	7	9	1	3.75	0.86	0	1	1	1	2
132	1.4	0.125	34	2	34.3	2	7	9	1	12.25	0.86	0	1	2	1	2
132	1.4	0.125	34	2	34.3	2	7	9	1	19.33	0.86	0	1	2	1	2
132	1.32	0.121	34	2	34.3	2	7	9	1	21.76	0	14.54	1	2	0	2
132	1.32	0.121	34	2	34.3	2	7	9	1	27.49	0.86	0	1	2	1	2
132	1.32	0.121	34	3	34.4	2	7	9	1	35.49	0.86	0	1	3	1	2
132	1.32	0.121	34	3	34.4	2	7	9	2	42.47	0.86	0	1	3	1	2
132	1.32	0.121	34	3	34.4	2	7	9	2	44.6	0	10.8	1	3	0	2
132	1.32	0.121	34	3	34.4	2	7	9	2	49.23	0.86	0	1	3	1	2
132	1.32	0.121	34	4	34.6	2	7	9	2	65.88	0.86	0	1	4	1	2
132	1.32	0.121	34	4	34.6	2	7	9	2	68.9	0	7.66	1	4	0	2

132	1.32	0.121	34	4	34.6	2	7	9	2	74.74	0.86	0	1	4	1	2
132	1.36	0.123	34	5	34.7	2	7	9	2	81.73	0.86	0	1	5	1	2
132	1.36	0.123	34	5	34.7	2	7	9	2	91.83	0	6.36	1	5	0	2
132	1.26	0.118	34	8	35.1	2	7	9	2	163.33	0	2.82	1	8	0	2
133	1.85	0.149	34	1	34.1	1	8	9	1	0	6.88	0	1	1	1	2
133	1.84	0.149	34	1	34.1	1	8	9	1	5.92	0	1.66	1	1	0	2
133	1.84	0.149	34	1	34.1	1	8	9	2	15.67	0.86	0	1	1	1	2
133	1.84	0.149	34	2	34.3	1	8	9	2	26.45	0.86	0	1	2	1	2
133	1.84	0.149	34	2	34.3	1	8	9	2	29.75	0	2.52	1	2	0	2
133	1.84	0.149	34	3	34.4	1	8	9	2	39.67	0.86	0	1	3	1	2
133	1.76	0.145	34	3	34.4	1	8	9	2	50.29	0.86	0	1	3	1	2
133	1.76	0.145	34	3	34.4	1	8	9	2	52.44	0	3.05	1	3	0	2
133	1.76	0.145	34	3	34.4	1	8	9	2	63.59	0.86	0	1	3	1	2
133	1.76	0.145	34	4	34.6	1	8	9	2	74.17	0.86	0	1	4	1	1
133	1.76	0.145	34	4	34.6	1	8	9	2	87.67	0.86	0	1	4	1	1
133	1.76	0.145	34	5	34.7	1	8	9	2	98.17	0.86	0	1	5	1	1
133	1.76	0.145	34	5	34.7	1	8	9	2	111.5	0.86	0	1	5	1	1
133	1.74	0.145	34	6	34.9	1	8	9	2	122.42	0.86	0	1	6	1	2
133	1.74	0.145	34	6	34.9	1	8	9	2	128.16	0	4.56	1	6	0	2
135	0.9	0.092	30	1	30.1	1	8	10	1	0	4.3	0	2	1	1	2
135	0.9	0.092	30	2	30.3	1	8	10	1	13.5	0.86	0	2	2	1	2
135	0.9	0.092	30	2	30.3	1	8	10	1	26.5	0.86	0	2	2	1	2
135	0.9	0.092	30	3	30.4	1	8	10	1	37.62	0.86	0	2	3	1	2
135	0.72	0.082	30	3	30.4	1	8	10	1	43.14	0	10.94	2	3	0	2
135	0.72	0.082	30	3	30.4	1	8	10	1	53.01	0.86	0	2	3	1	2
135	0.72	0.082	30	4	30.6	1	8	10	1	63.43	0.86	0	2	4	1	2
135	0.72	0.082	30	4	30.6	1	8	10	1	66.13	0	13.32	2	4	0	2
136	1.25	0.116	32	1	32.1	2	8	10	1	0	5.16	0	1	1	1	2
136	1.25	0.116	32	1	32.1	2	8	10	1	6.83	0.86	0	1	1	1	2
136	1.25	0.116	32	1	32.1	2	8	10	1	8.49	0	5.7	1	1	0	2
138	1.2	0.111	31	1	31.1	2	8	10	2	0	5.16	0	1	1	1	2
138	1.2	0.111	31	1	31.1	2	8	10	2	11.28	0.86	0	1	1	1	2
138	1.2	0.111	31	1	31.1	2	8	10	2	13.21	0	8.07	1	1	0	2
139	1.2	0.109	30	1	30.1	1	7	8	1	0	5.16	0	2	1	1	2
139	1.2	0.109	30	1	30.1	1	7	8	1	4.75	0.86	0	2	1	1	2
139	1.2	0.109	30	2	30.3	1	7	8	2	16.62	0.86	0	2	2	1	2
139	1.08	0.105	30	2	30.3	1	7	8	2	18.85	0	13.67	2	2	0	2
139	1.08	0.105	30	2	30.3	1	7	8	2	23.75	0.86	0	2	2	1	2
139	1.08	0.105	30	2	30.3	1	7	8	2	30.25	0.86	0	2	2	1	2
139	1.08	0.105	30	3	30.4	1	7	8	2	40.3	0.86	0	2	3	1	2
139	1.08	0.105	30	3	30.4	1	7	8	2	42.75	0	14.17	2	3	0	2
140	1.7	0.14	34	1	34.1	1	9	10	2	0	7.31	0	2	1	1	2
140	1.7	0.14	34	2	34.3	1	9	10	2	14	2.58	0	2	2	1	2
140	1.7	0.14	34	2	34.3	1	9	10	2	16.75	0	5.16	2	2	0	2
140	1.58	0.135	34	2	34.3	1	9	10	2	27.25	2.58	0	2	2	1	2
140	1.58	0.135	34	3	34.4	1	9	10	2	38.08	2.58	0	2	3	1	2
140	1.58	0.135	34	3	34.4	1	9	10	2	41.16	0	4.55	2	3	0	2
140	1.58	0.135	34	4	34.6	1	9	10	2	65.19	0	3.85	2	4	0	2
140	1.58	0.135	34	5	34.7	1	9	10	2	88.99	0	2.8	2	5	0	2
141	1.55	0.136	33	1	33.1	1	5	7	1	0	8.6	0	1	1	1	2
141	1.55	0.136	33	2	33.3	1	5	7	1	12.5	1.72	0	1	2	1	2
141	1.55	0.136	33	2	33.3	1	5	7	1	19.47	1.72	0	1	2	1	2
141	1.48	0.133	33	2	33.3	1	5	7	2	21.92	0	5.6	1	2	0	2
141	1.48	0.133	33	2	33.3	1	5	7	2	28.17	1.72	0	1	2	1	2
141	1.48	0.133	33	3	33.4	1	5	7	2	36.25	1.72	0	1	3	1	2
141	1.34	0.126	33	3	33.4	1	5	7	2	42.91	1.72	0	1	3	1	2
141	1.34	0.126	33	3	33.4	1	5	7	2	47.08	0	5.33	1	3	0	2
142	1.85	0.149	34	1	34.1	1	8	9	2	0	3.44	0	2	1	1	2

142	1.78	0.146	34	2	34.3	1	8	9	2	9.02	0.86	0	2	2	1	2
142	1.78	0.146	34	2	34.3	1	8	9	2	17.42	0.86	0	2	2	1	2
142	1.78	0.146	34	3	34.4	1	8	9	2	33.35	0.86	0	2	3	1	2
142	1.78	0.146	34	3	34.4	1	8	9	2	35.53	0	2.18	2	3	0	2
142	1.72	0.143	34	4	34.6	1	8	9	2	59.76	0	1.91	2	4	0	2

APPENDIX A.4

Control stream for one-compartment, bolus model.

```
$PROB Theophylline in Neonates run1
Runs NM5
1.Structural model: one compartment
2. Exponential etas on CL & V
3. Exp res error
4. Covariate Models none
5. Comment Base model

$INPUT ID WGT BSA GA PNA PCA SEX AP AQ OXY TIME AMT DV DVC DAY RT NNJ
$DATA theoiv.prn   ;Amino x.86=theop
$SUBROUTINE ADVAN1 TRANS2
$PK

IF (NEWIND.LE.1) THEN
  LN2=LOG(2)
ENDIF

FSZCL =1
FSZVD=1

TVCL=FSZCL*THETA(1)
TVV=FSZVD*THETA(2)

CL=TVCL*EXP(ETA(1))
V=TVV*EXP(ETA(2))
S1=V
IWRES=1

$ERROR
CP=F

Y=CP*EXP(ERR(1))+ERR(2)
IPRED=Y

$THETA (0,0.0001,10) ;CL 1
$THETA (0,0.5,2) ;Vss 2
;$THETA  1 ;FOXYCL 3

$OMEGA 0.5 ; CVCL 1
$OMEGA 0.5 ; CVV 2
$SIGMA .1 ;CVCP 1
$SIGMA .001 FIX ;SDCP 2

$EST MAXEVALS=3000 SIGDIG=3 POSTHOC NOABORT
$COVARIANCE
$TABLE ID TIME IPRED IWRES MDV NOPRINT ONEHEADER FILE=sdtab1
$TABLE ID AMT TIME IPRED IWRES NOPRINT ONEHEADER FILE=mutab1
$TABLE ID CL V TVCL TVV NOPRINT ONEHEADER FILE=patab1
$TABLE ID GA PNA PCA WGT BSA NOPRINT ONEHEADER FILE=cotab1
$TABLE ID SEX DAY NNJ AP AQ OXY NOPRINT ONEHEADER FILE=catab1
```

APPENDIX A.5

Control stream for two-compartment model.

```
$PROB Theophylline in Neonates run100
Runs NM5
1.Structural model: Two compartment
2. Exponential etas on CL & V
3. Exp res error
4. Covariate Models none
5. Comment Base model

$INPUT ID WGT BSA GA PNA PCA SEX AP AQ OXY TIME AMT DV DVC DAY RT NNJ
$DATA theoiv.prn   ;Amino x.86=theop
$SUBROUTINE ADVAN3 TRANS4
$PK

IF (NEWIND.LE.1) THEN
  LN2=LOG(2)
ENDIF

FSZCL =1
FSZVD=1

CL=FSZCL*THETA(1)*EXP(ETA(1))
V1=FSZVD*THETA(2)*EXP(ETA(2)) ;central vol
 Q=THETA(3)*EXP(ETA(3))       ;intercomp CL
V2=THETA(4)*EXP(ETA(4))       ;periph V

K=CL/V1  ;rate constant of elimination
K12=Q/V1 ;rate constant from central to peripheral
K21=Q/V2 ;rate constant from periph to central
 S1=V1
 S2=V2

IWRES=1

$ERROR
CP=F
Y=CP*EXP(ERR(1))+ERR(2)
IPRED=Y

$THETA (0,0.0001,10) ;CL 1
$THETA (0,0.005,2) ;Vss 2
$THETA (0,0.001,10)
$THETA (0,0.6,10)

$OMEGA 0.5 ; CVCL 1
$OMEGA 0.5 ; CVV 2
$OMEGA 0.5 ; CVV 3
$OMEGA 0.5 ; CVV 4
```

```
$SIGMA .1 ;CVCP 1
$SIGMA .001 FIX ;SDCP 2

$EST MAXEVALS=3000 SIGDIG=3 POSTHOC NOABORT
$COVARIANCE
$TABLE ID TIME IPRED IWRES MDV NOPRINT ONEHEADER FILE=sdtab100
$TABLE ID AMT TIME IPRED IWRES NOPRINT ONEHEADER FILE=mutab100
$TABLE ID CL V1 V2 Q NOPRINT ONEHEADER FILE=patab100
$TABLE ID GA PNA PCA WGT BSA NOPRINT ONEHEADER FILE=cotab100
$TABLE ID SEX DAY NNJ AP AQ OXY NOPRINT ONEHEADER FILE=catab100
```

APPENDIX A.6

Control stream for rate model.

```
$PROB Theophylline in Neonates run4
Runs NM5
1.Structural model: one compartment
2. Exponential etas on CL & V
3. Res Exp
4. Covariate Models
5. Comment RATE model

$INPUT ID WT BSA GA PNA PCA SEX AQ OXY TIME AMT RATE DV DAY RT
$DATA ..\theoivrt.prn ;Amino x.86=theop
$SUBROUTINE ADVAN1 TRANS2
$PK
IF (NEWIND.LE.1) THEN
  LN2=LOG(2)
ENDIF

FSZCL =1
FSZVD=1

TVCL=FSZCL*THETA(1)
TVV=FSZVD*THETA(2)
TVTK0=THETA(3)

CL=TVCL*EXP(ETA(1))
V=TVV*EXP(ETA(2))
TK0=TVTK0
S1=V
D1=TK0/60
IWRES=1
$ERROR
CP=F
Y=CP*EXP(ERR(1))+ERR(2)
IPRED=Y

$THETA (0,0.0001,10) ;CL 1
$THETA (0,0.5,2) ;Vss 2
$THETA (0,0.1,5) ;TK0 3

$OMEGA 0.5 ; CVCL 1
$OMEGA 0.5 ; CVV 2
;$OMEGA 0.5 ; CVTK0 3
$SIGMA .1 ;CVCP 1
$SIGMA .001 FIX ;SDCP 2

$EST MAXEVALS=3000 SIGDIG=3 POSTHOC NOABORT
$COVARIANCE
$TABLE ID TIME IPRED IWRES MDV NOPRINT ONEHEADER FILE=sdtab4
$TABLE ID AMT TIME IPRED IWRES NOPRINT ONEHEADER FILE=mutab4
$TABLE ID CL V TVCL TVV NOPRINT ONEHEADER FILE=patab4
$TABLE ID GA PNA PCA WT BSA NOPRINT ONEHEADER FILE=cotab4
$TABLE ID SEX DAY AQ OXY NOPRINT ONEHEADER FILE=catab4
```

APPENDIX A.7

Control stream for parallel first-and zero-order model

```
$PROB Theophylline in Neonates run 300
 control-stream  parallel 1st and 0-order elimination
 1.Structural model: one compartment
 2. Exponential etas on parameters
 3. Control stream by E Chatelut
 4. No Covariates
 5. Comment: Date 29.9.98

$INPUT ID WGT BSA GA PNA PCA SEX AP AQ OXY TIME AMT DV DVC DAY RT NNJ
$DATA THEOIV.prn   ;Amino x.86=theop
$SUBROUTINE ADVAN6 TRANS1 TOL=4
$MODEL COMP=(CENTRAL,DEFOBS)
$PK
V=THETA(1)*EXP(ETA(1))
K10=THETA(2)*EXP(ETA(2))
VM=THETA(3)*EXP(ETA(3))
KM=THETA(4)*EXP(ETA(4))
S1=V
$DES
C1=A(1)/S1
DADT(1)=-K10*A(1)-C1*VM/(KM+C1)
$THETA(0,0.1,10)(0,0.01,10)(0,0.01,10)(0,0.01,10)
$OMEGA 0.5 0.5 0.5 0.5

 $ERROR
 DEL=0
 IF(F.EQ.0)DEL=1
 W=F+DEL
 Y=F*(1+ERR(1))+ERR(2)
 IPRED=F
 IRES=DV-IPRED
 IWRES=IRES/W
 $SIGMA 0.04 .05
 $EST MAXEVALS=9000 POSTHOC
 $COVARIANCE
 $TABLE ID TIME IPRED IWRES NOPRINT ONEHEADER FILE=sdtab300
 $TABLE ID AMT TIME IPRED IWRES NOPRINT ONEHEADER FILE=mutab300
 $TABLE ID V K10 VM KM ETA(1)ETA(2)ETA(3)ETA(4) NOPRINT ONEHEADER FILE=patab300
 $TABLE ID GA PNA PCA WGT BSA NOPRINT ONEHEADER FILE=cotab300
 $TABLE ID SEX DAY NNJ AP AQ OXY NOPRINT ONEHEADER FILE=catab300
```

APPENDIX A.8

Control stream for the final model

```
$PROB THEOPHYLLINE IN NEONATES RUN31
1.structural model
 one compartment
2.Res error Exp
3.Interindividual variability
 EXP on CL and V
4.Covariate
5.Comments:Checking the interoccasion variability on CL and V

$INPUT ID WGT BSA GA PNA PCA SEX AP AQ OXY TIME AMT DV DVC DAY RT NNJ
$DATA THEOIV.prn ;Amino x.86=theop
$SUBROUTINE ADVAN1 TRANS2
$PK
IF (DAY.EQ.1) IOVCL=ETA(3)
IF (DAY.EQ.2) IOVCL=ETA(4)
IF (DAY.EQ.3) IOVCL=ETA(5)
IF (DAY.GT.3) IOVCL=ETA(6)
IF (DAY.EQ.1) IOVV=ETA(7)
IF (DAY.EQ.2) IOVV=ETA(8)
IF (DAY.EQ.3) IOVV=ETA(9)
IF (DAY.GT.3) IOVV =ETA(10)

IF (NEWIND.LE.1) THEN
  LN2=LOG(2)
ENDIF

FSZCL=WGT**0.75
FSZVD=WGT ;**1

IF (OXY.EQ.1) THEN
  FOXY=THETA(3)
ELSE
  FOXY=1
ENDIF

TVCL=FOXY*FSZCL*THETA(1)
TVV=FSZVD*THETA(2)
CL=TVCL*EXP(ETA(1))*EXP(IOVCL)
V=TVV*EXP(ETA(2))*EXP(IOVV)
S1=V
IWRES=1
$ERROR
CP=F
Y=CP*EXP(ERR(1))+ERR(2)
IPRED=Y

$THETA (0,0.001,10)  ;CL
$THETA (0,.1,10)     ;V
$THETA (0,1,10)      ;OXY
$OMEGA 0.5
$OMEGA 0.5
$OMEGA BLOCK(1) 0.1 ;IOVCL
```

```
$OMEGA BLOCK(1) SAME ;IOVCL
$OMEGA BLOCK(1) SAME ;IOVCL
$OMEGA BLOCK(1) SAME ;IOVCL
$OMEGA BLOCK(1) 0.1  ;IOVV
$OMEGA BLOCK(1) SAME ;IOVV
$OMEGA BLOCK(1) SAME ;IOVV
$OMEGA BLOCK(1) SAME ;IOVV

$SIGMA 1.0
$SIGMA 0.001 FIX
;$SIML (1)
$EST METHOD=CONDITIONAL MAXEVALS=3000 SIGDIG=3 POSTHOC
$COVARIANCE
$TABLE ID TIME IPRED IWRES NOPRINT ONEHEADER FILE=sdtab31
$TABLE ID AMT TIME IPRED IWRES NOPRINT ONEHEADER FILE=mutab31
$TABLE ID CL V ETA(1) ETA(2) NOPRINT ONEHEADER FILE=patab31
$TABLE ID GA PNA PCA WGT BSA NOPRINT ONEHEADER FILE=cotab31
$TABLE ID SEX DAY NNJ AP AQ OXY NOPRINT ONEHEADER FILE=catab31
```

APPENDIX A.9

Data collection form

NEONATAL THEOPHYLLINE STUDY

NAME: HOSPITAL NUMBER

ADDRESS: TELEPHONE:

MOTHER'S NAME: HOSPITAL NUMBER:

DATE OF BIRTH: BRT WEIGHT: GA: SEX:

APGAR: 1 min....5 min.... LENGTH: TERM/PRETERM

RESP.APGAR: 1min..... 5min.....

OBSTETRIC COMPLICATIONS:

DRUGS USED BEFORE & DURING LABOUR:

BEVERAGES CONSUMED BEFORE OR DURING LABOUR:

MATERNAL SMOKING HISTORY: Y/N/UNKNOWN

ADMISSION DIAGNOSIS:

EXCLUSION CRITERIA: 1. VSGA

2. CONGENITAL ABNORMALITIES

INCLUSION CRITERIA: 1. APNOEA. DEFINED AS NO BREATHING FOR >20s, HR<100/min, AND/OR CYANOSIS.
WHAT IS CONSIDERED THE CAUSE OF THIS APNOEA?

RESUSCITATION AT BIRTH: Y/N, DETAILS:

ASSISTED VENTILATION AT BIRTH: Y/N:

OTHER INTERVENTION AT BIRTH:

Appendix A.9 continued. Data collection form.

THEOPHYLLINE FLOW CHART

Name: ....

No.: ....

Ward: ....

Sex: ....

Date	Time	Theophylline				Mass	Apnoea	V?	RR	HR	BP	Ox sat	Capillary Blood			Ab Dist	Tone	Color	Feeding		Level	Time	Temp	Site	N Size	Concommitant Diseases	Other eg. Vomit gi-bleed etc.
		Dose	mg	ml	Route								pCO2	pH	HCO3				ml	Type							

Appendix A.9 continued. Data collection form.

THERAPY

Name: .....
Number: .....

	Route	Dose	Date Started	Date Stopped	Disease	Other
Plasma $NaHCO_3$						
Dopamine						
Antibiotics						
Phenobarb.						
IV-fluids						
Other						
Vit.K						

Clinical Picture:

RDS: y / n Details: .....

PDA: y / n Details: .....

INF: y / n Details: .....

Bld.sugar ↑ / ↓ Details: .....

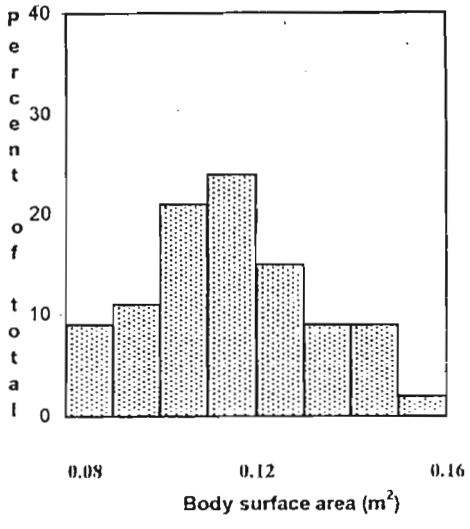
Asphyxiated y / n Details: .....

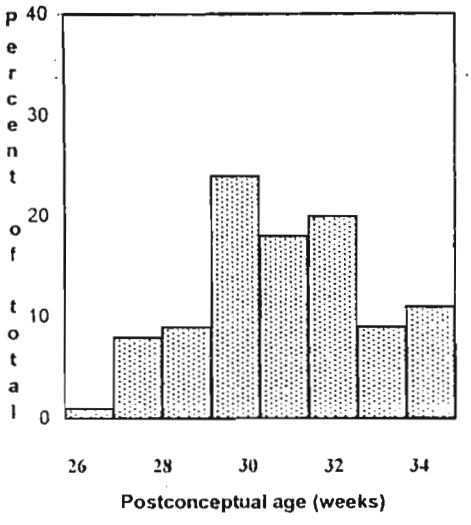
Other: .....
.....
.....
.....

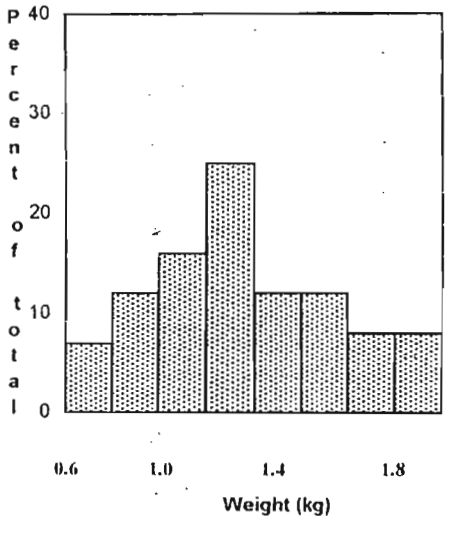
Final Diagnosis:	Date of Discharge	Follow-up visit
.....	.....	.....
.....		.....

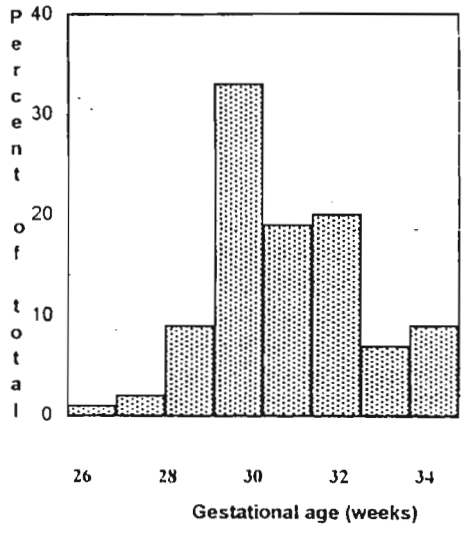
APPENDIX A.10

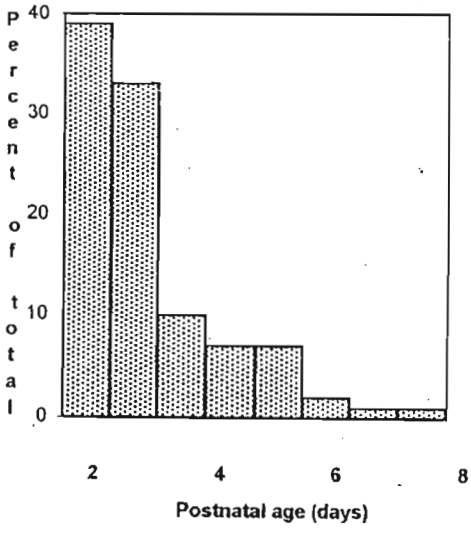
Distribution of available covariates

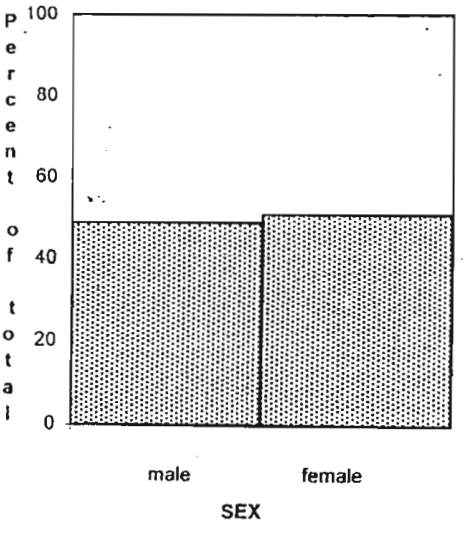




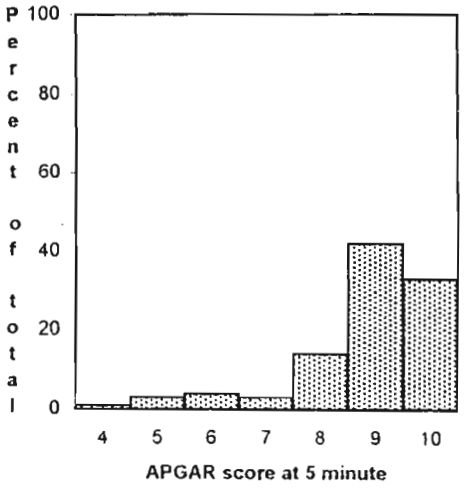


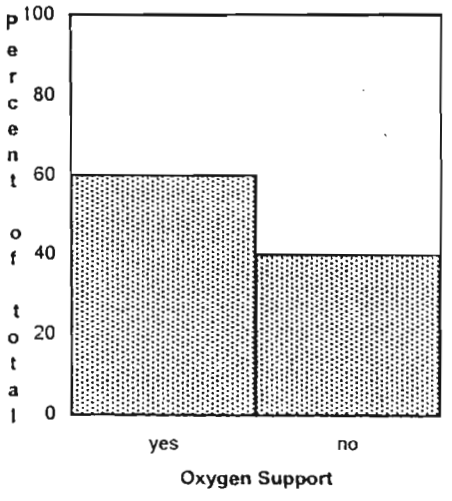


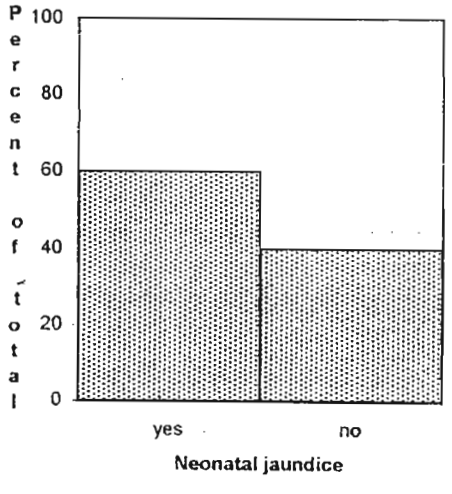


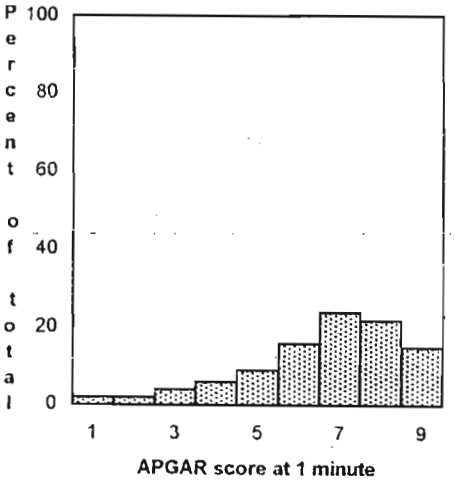


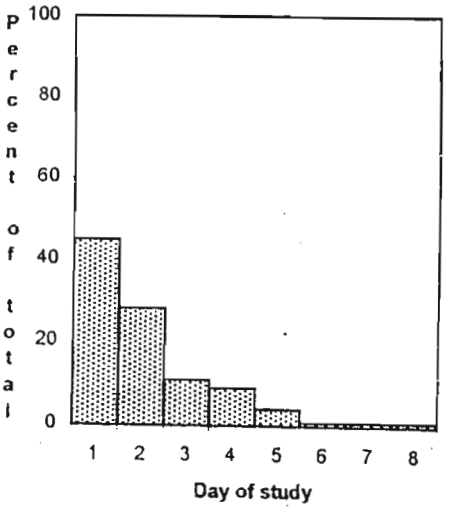
Distribution of available covariates.





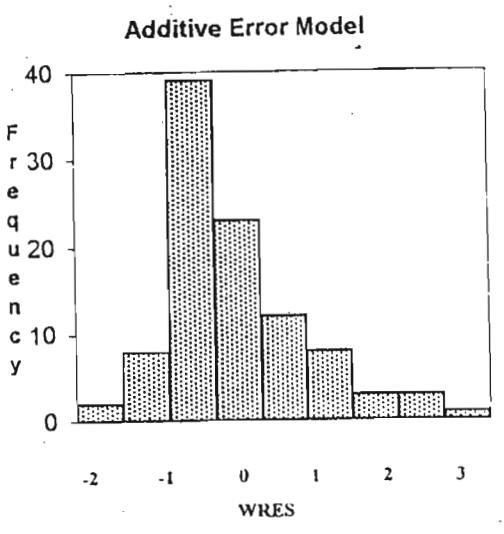


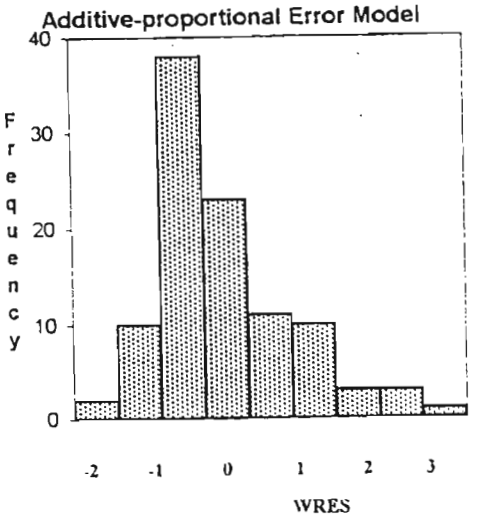


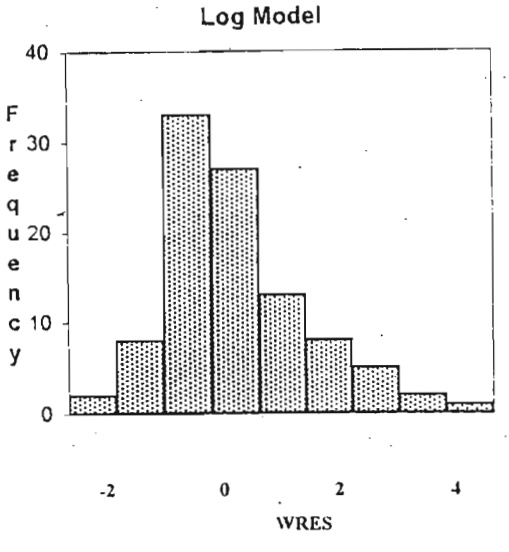


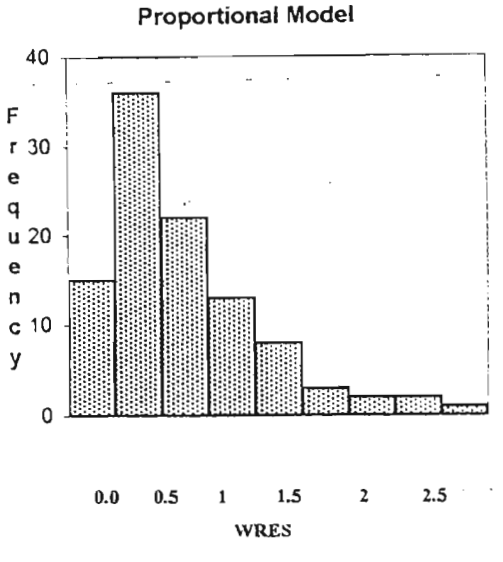
APPENDIX A.11

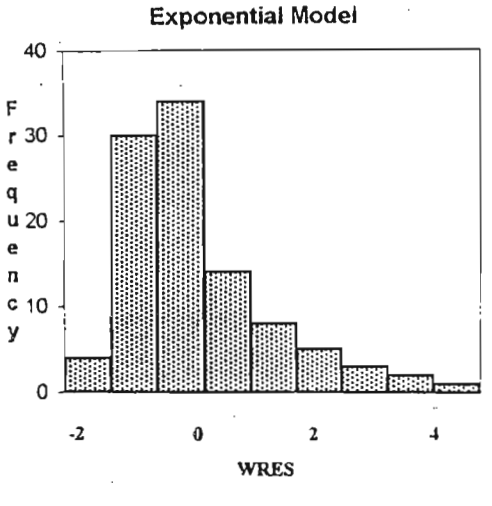
Frequency distribution graphs of various residual error models

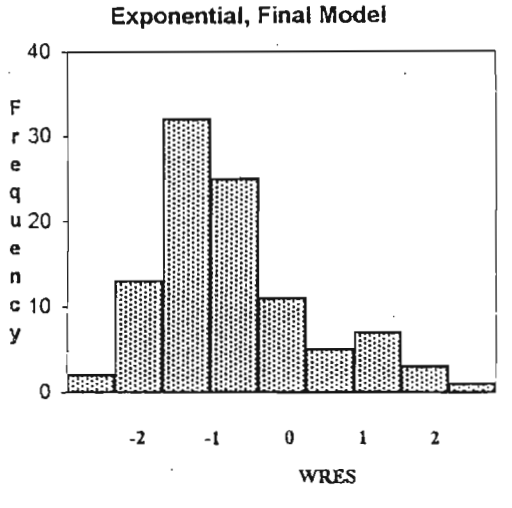






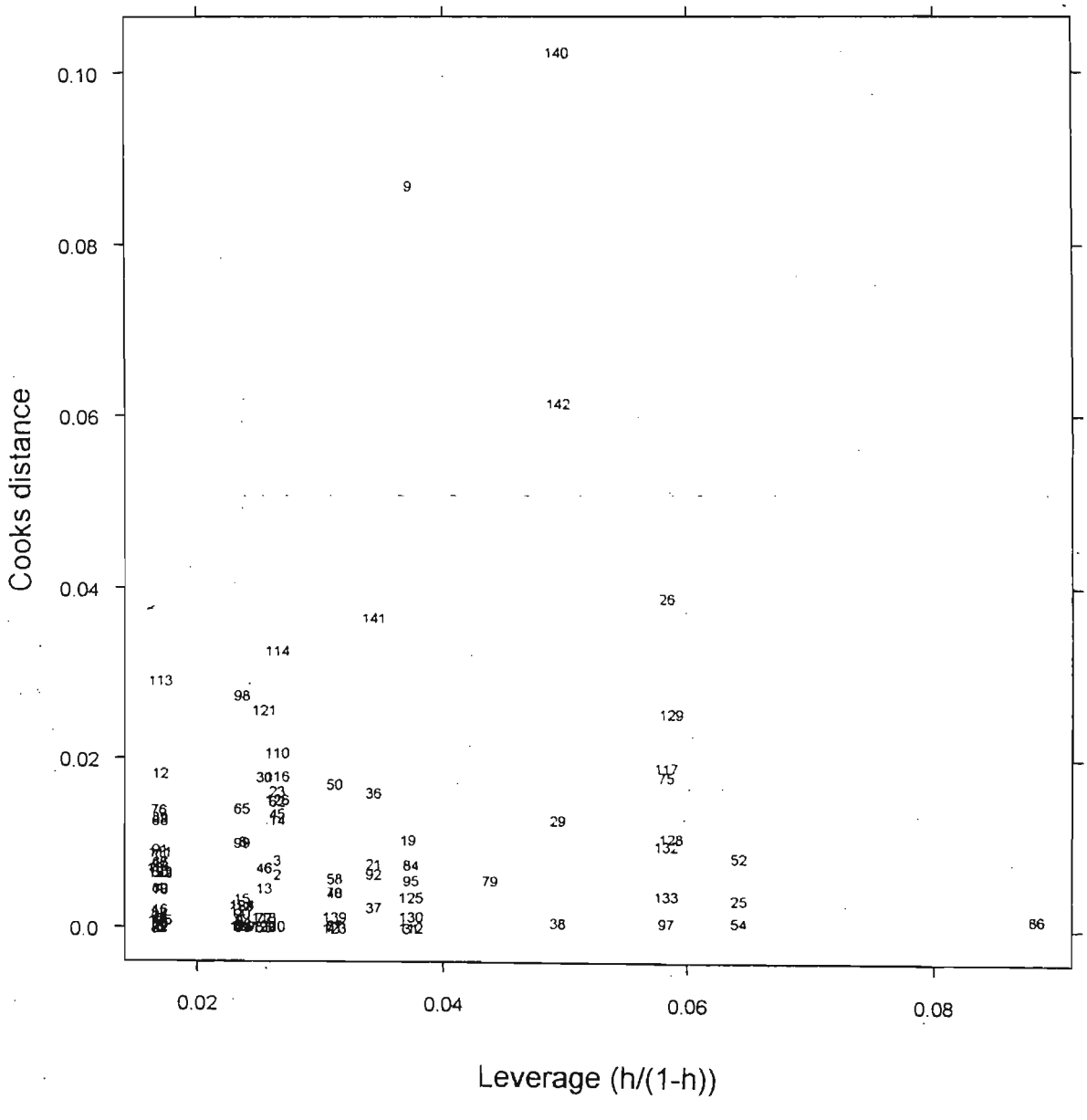






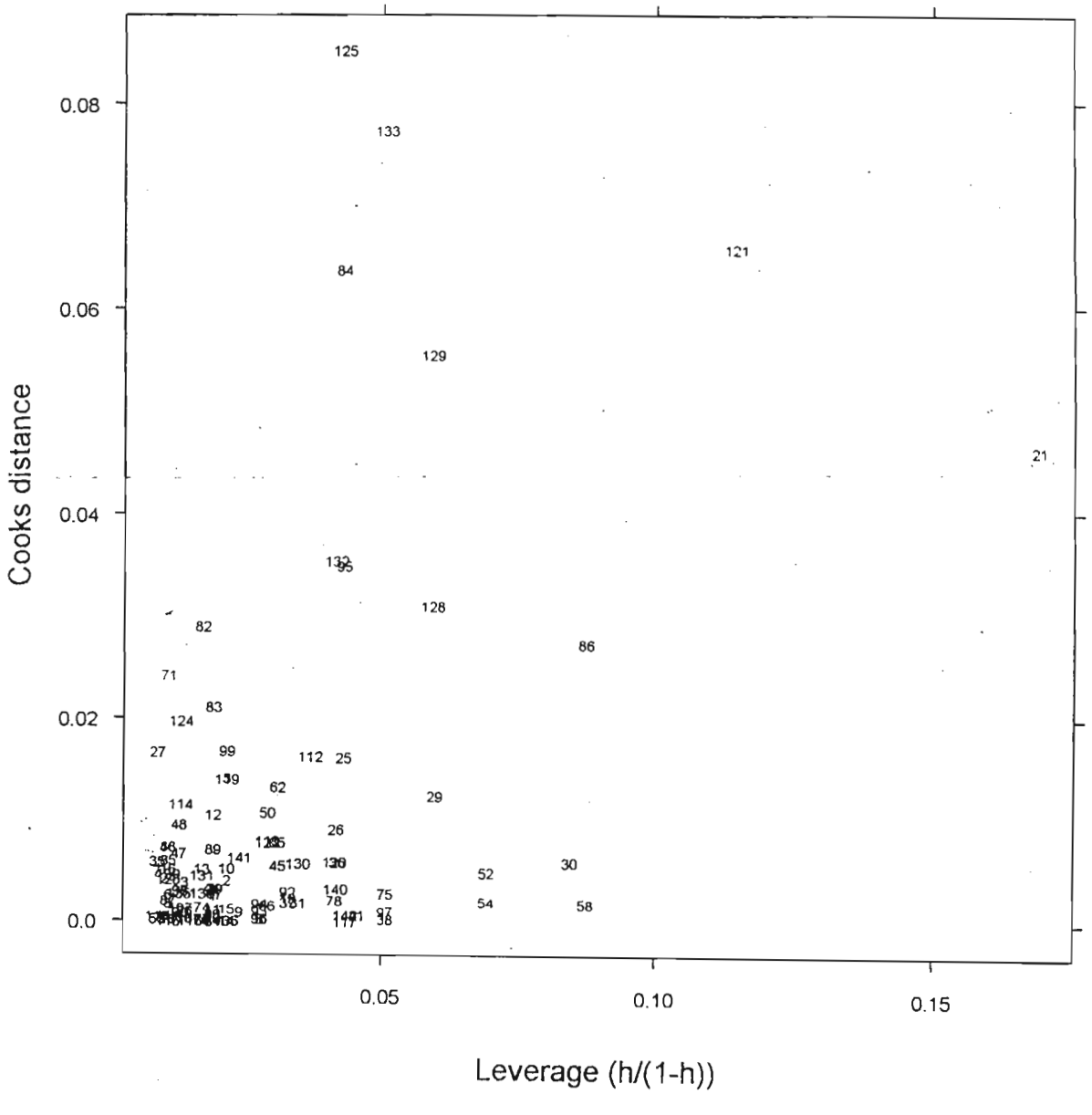
APPENDIX A.12

Individual influence on the GAM fit for CL base model



APPENDIX A.13

Individual influence on the GAM fit for V base model



APPENDIX A.14

Demographic details of possible outliers

Patient Number	Outlier on CL and/or V	WGT (kg)	GA (wks)	PCA (wks)	PNA (days)	GEN-DER	OXY	Number of drug samples
9	CL	1.95	33	33.1	1	F	y	6
21	V	1.2	33	33.1	1	M	n	1
86	CL and V	0.68	26	26.1	1	M	y	1
125	V	0.9	28	28.1	1	M	y	1
140	CL	1.7	34	34.1	1	M	n	4
142	CL	1.85	34	34.1	1	M	n	2

WGT = weight, GA = gestational age, PCA = postconceptual age,
PNA = postnatal age, OXY = oxygen support, CL = clearance, V = volume,
M = male, F = female, y = yes, n = no.

APPENDIX A.15

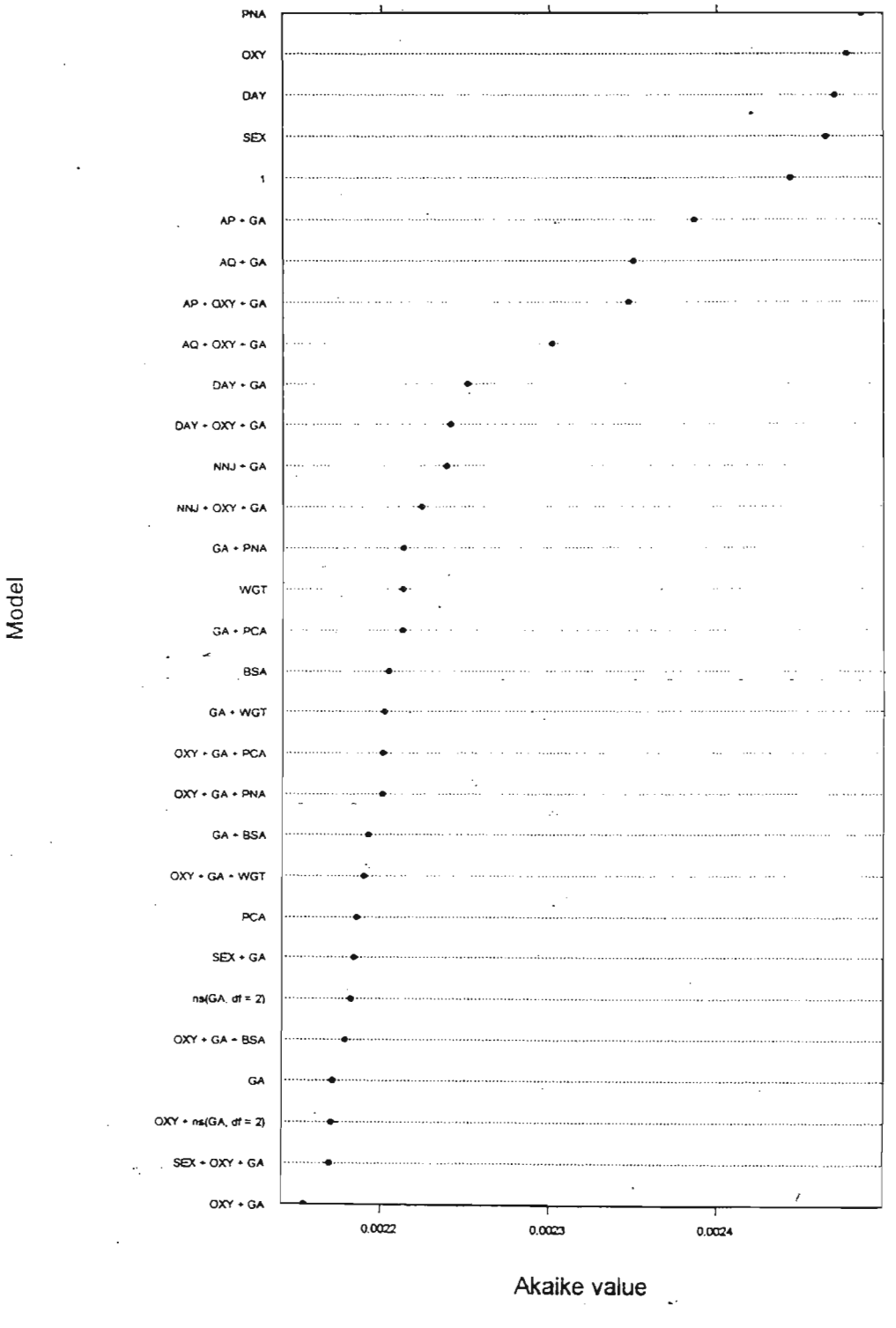
A summary of the NONMEM analysis of the possible outliers using the base model (Relative standard error)

	Parameter estimates (RSE %)		Eta_1 % (RSE %)	Eta_2 % (RSE %)	OFV	Res error (RSE %)
	CL	V				
Full data set	0.0084 (17)	0.67 (9)	83 (36)	49 (23)	1035.56	32 (26)
Data without pat 9	0.00797 (17)	0.67 (9)	82 (36)	49 (26)	1010.61	32 (26)
Data without pat 21	0.00829 (17)	0.67 (9)	83 (37)	49 (23)	1029.51	32 (26)
Data without pat 86	0.00849 (17)	0.68 (13)	85 (35)	46 (23)	1024.14	32 (26)
Data without pat 125	0.00834 (17)	0.66 (9)	83 (36)	32 (26)	1031.05	32 (26)
Data without pat 140	0.00827 (17)	0.66 (10)	82 (37)	49 (24)	1019.06	32 (26)
Data without pat 142	0.00828 (17)	0.66 (9)	82 (37)	49 (23)	1029.61	32 (26)

Pat = patient, CL = clearance, V = volume, RSE = relative standard error, Eta = inter-individual error, OFV = objective function value, Res error = residual error.

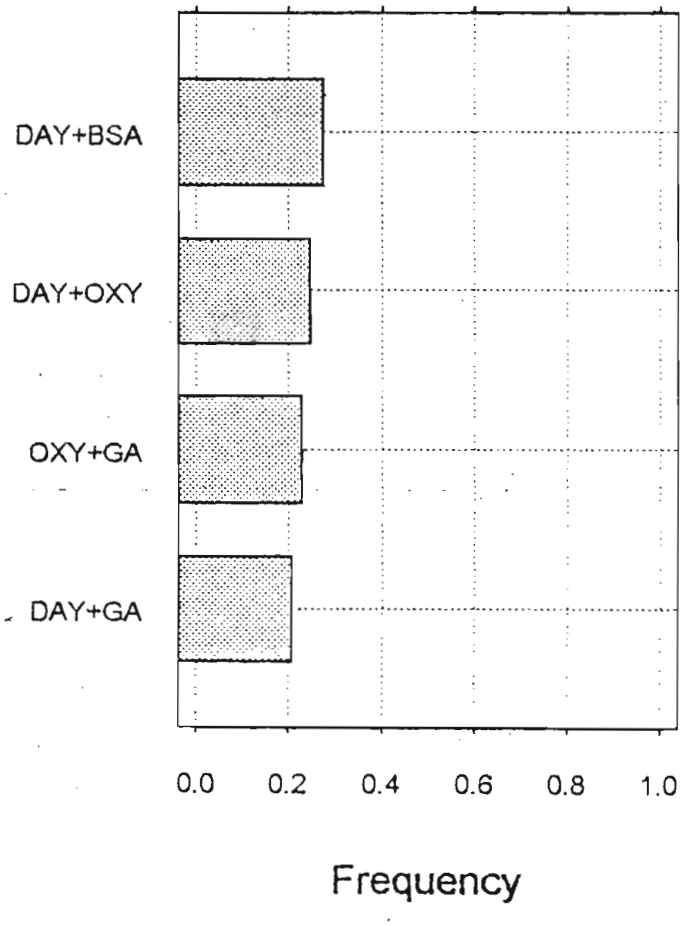
APPENDIX A.16

Akaike plot for CL



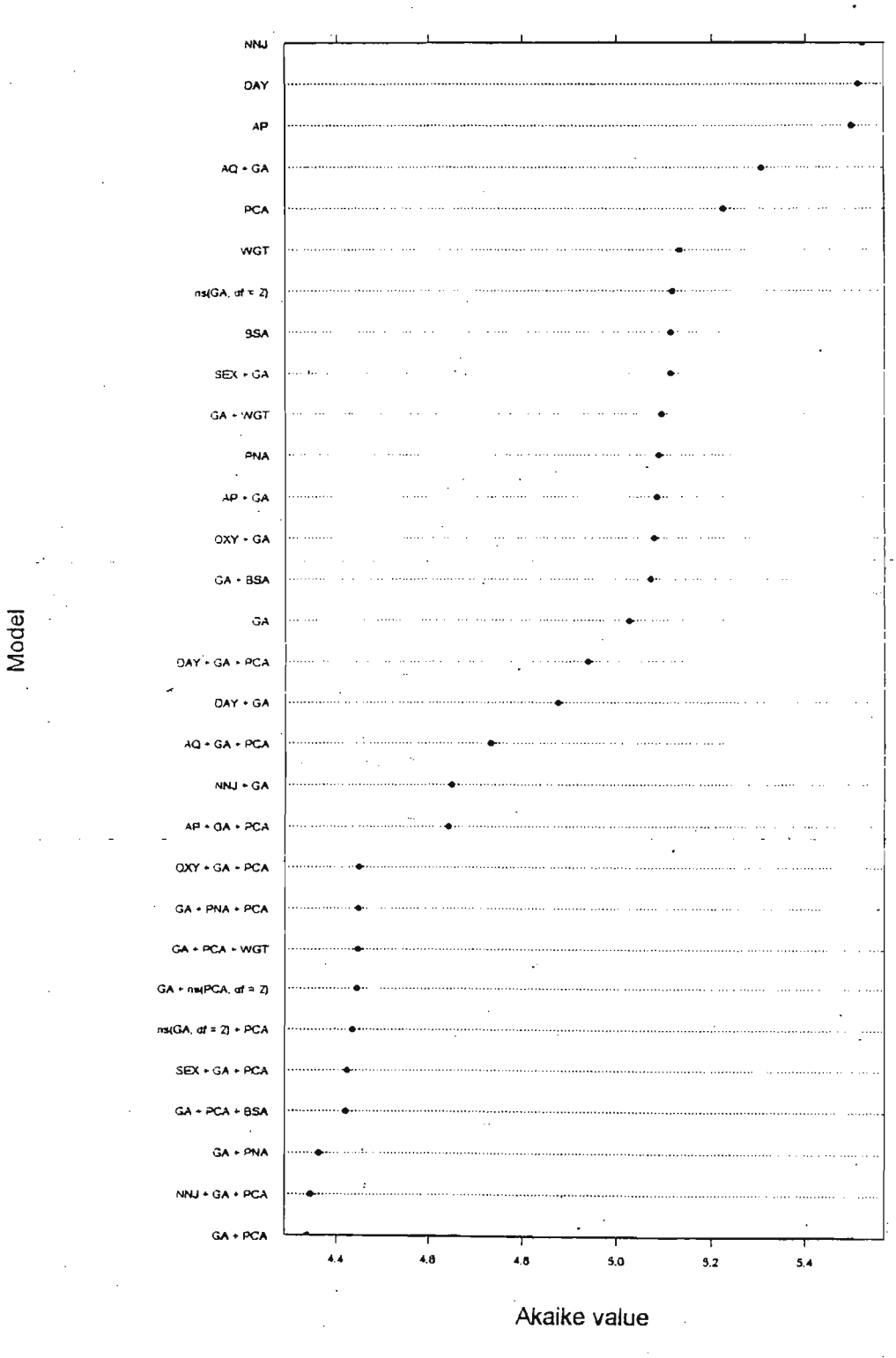
APPENDIX A.17

Most common covariate combinations for CL



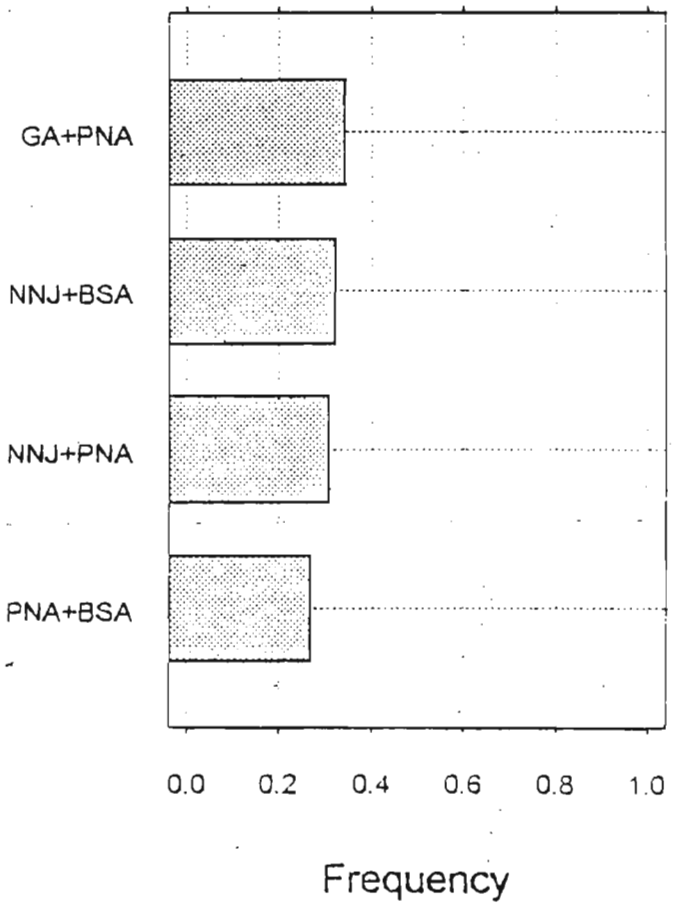
APPENDIX A.18

Akaike plot for V



APPENDIX A.19

Most common covariate combinations for V



APPENDIX A.20

Correlation matrix of the developmental covariates

	GA	PNA	WGT	BSA	PCA
GA	1.000				
PNA	-0.1095	1.0000			
WGT	0.7717	-0.0731	1.000		
BSA	0.7531	-0.0615	0.9781	1.000	
PCA	0.9628	0.1594	0.7461	0.7310	1.000

GA = gestational age (weeks), PNA = postnatal age (days), WGT = weight (kg), BSA = body surface area (m^2), PCA = postconceptual age (weeks).

Appendix A.21 Results of the population pharmacokinetic analysis with the FO estimation method.

CL Model	V Model	Parameter estimates (RSE %)				Eta1 (RSE %)	Eta2 (RSE %)	OFV	ΔOFV	Res error (RSE %)
		CL	V	θ_3	θ_4					
θ_1	θ_2	0.0084 (17)	0.67 (9)	-	-	83 (36)	49 (23)	1035.56	-	32 (26)
θ_1*OXY	θ_2	0.0060 (22)	0.63 (9)	1.87 (22)	-	74 (41)	55 (24)	1019.52	16.1	29 (23)
θ_1*GA	θ_2	0.0003 (17)	0.66 (9)	-	-	80 (36)	49 (24)	1030.83	4.7	32 (27)
θ_1*WGT	θ_2	0.0074 (17)	0.65 (9)	-	-	78 (36)	47 (25)	1021.78	13.8	33 (27)
θ_1*WGT**θ_3	θ_2	0.0066 (27)	0.66 (9)	1.51 (46)	-	79 (37)	45 (30)	1019.97	15.6	34 (29)
θ_1*$WGT^{0.75}$	θ_2	0.0078 (17)	0.65 (9)	-	-	78 (35)	47 (25)	1024.02	11.6	32 (27)
θ_1*$WGT^{1.28}$	θ_2	0.0070 (17)	0.65 (9)	-	-	78 (36)	46 (26)	1020.33	15.2	33 (28)
θ_1*OXY *GA	θ_2	0.0002 (22)	0.63 (9)	1.84 (21)	-	72 (39)	54 (23)	1014.58	21.02	30 (23)
θ_1*OXY *DAY	θ_2	0.0063 (20)	0.60 (10)	2.36 (38)	1.72 (21)	72 (37)	54 (26)	1015.02	20.58	30 (23)
θ_1*DAY*GA	θ_2	0.0003 (14)	0.60 (10)	3.49 (34)	-	73 (35)	48 (31)	1021.89	13.7	33 (27)
θ_1*DAY *BSA	θ_2	0.0743 (14)	0.59 (10)	3.7 (34)	-	71 (35)	46 (35)	1014.22	21.38	34 (28)
θ_1*$WGT^{0.75}$ *OXY	θ_2	0.0057 (21)	0.62 (10)	1.84 (20)	-	68 (37)	52 (23)	1007.12	28.48	30 (24)

Appendix A.21 continued: Results of the population pharmacokinetic analysis with the FO estimation method

CL Model	V Model	Parameter estimates (RSE %)				Eta1 (RSE %)	Eta2 (RSE %)	OFV	ΔOFV	Res error (RSE %)
		CL	V	θ_3	θ_4					
θ_1	θ_2*PCA	0.0079 (17)	0.022 (9)	-	-	87 (36)	47 (24)	1019.10	16.46	31 (26)
θ_1	θ_2*GA	0.0080 (16)	0.02 (9)	-	-	85 (36)	47 (24)	1019.18	16.42	31 (26)
θ_1	θ_2*WGT	0.0078 (13)	0.58 (7)	-	-	82 (34)	43 (28)	1000.62	35	31 (25)
θ_1	θ_2*GA *PNA	0.0021	0.14	-	-	1600	120	1243.64	-	460
θ_1	θ_2*NNJ *BSA	0.0067 (11)	2 (9)	1.15 (20)	-	47 (19)	81 (12)	1274.59	-	21 (26)
θ_1	θ_2*NNJ *PNA	1.93e-011	0.37	0.72	-	67	58	1152.66	-	43
θ_1	θ_2*PNA *BSA	0.0013 (140)	2 (17)	-	-	290 (270)	57 (31)	1199.23	-	29 (35)
θ_1*WGT$^{0.75}$ *OXY	**θ_2*WGT**	0.0052 (20)	0.54 (8)	1.94 (21)		69 (38)	49 (29)	981.24	54.12	29 (24)

Appendix A.21 continued: Results of the population pharmacokinetic analysis with the FOCE method

CL Model	V Model	Parameter estimates (RSE %)				Eta1 (RSE %)	Eta2 (RSE %)	OFV	ΔOFV	Res error (RSE %)
		CL	V	θ_3	θ_4					
θ_2 Base model	θ_2*	0.0074 (16)	0.75 (9)	-	-	73 (27)	57 (20)	1000.86	-	30 (22)
θ_1*WGT$^{0.75}$ *OXY Full covariate model	θ_2* WGT	0.0056 (19)	0.58 (8)	1.61 (23)	-	60 (29)	53 (23)	962.29	38.57	29 (22)
θ_1*WGT$^{0.75}$ *OXY Final model with IOV	θ_2* WGT	0.0060 (14)	0.633 (7)	1.47 (18)	-	56 (25) IOV 34 (77)	47 (28) IOV 35 (29)	927.94	72.92	16 (35)

CL = clearance, V = volume of distribution, RSE = relative standard error, θ = parameter, Eta_1 = inter-individual variability CL, Eta_2 = inter-individual variability V, OFV = objective function value, ΔOFV = change in OFV, Res error = residual error.

APPENDIX A.22

Results of the Jack-knife analysis of the final model

Number of patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
1	923.4	0.00597 (14)	0.631 (7)	1.47 (8)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
2	918.3	0.00593 (14)	0.63 (7)	1.49 (18)	56 (24)	46 (29)	34 (76)	35 (29)	16 (35)
3	913.4	0.0061 **(32)**	0.63 (7)	1.44 **(38)**	57 **(100)**	46 (56)	32 **(110)**	34 (30)	16 (35)
4	914.9	0.00598 (14)	0.63 (7)	1.48 (18)	56 (24)	47 (28)	34 (77)	35 (29)	16 (35)
5	917.9	0.00595 (14)	0.63 (7)	1.45 (18)	56 (24)	47 (28)	33 (78)	35 (29)	16 (35)
6	925.5	0.00596 (14)	0.63 (7)	1.45 (18)	56 (24)	47 (28)	33 (78)	35 (29)	16 (35)
7	915.7	0.00595 (15)	0.64 (7)	1.48 (20)	57 (28)	47 (30)	33 (77)	34 (31)	16 (35)
8	896.9	0.00595 (15)	0.62 (7)	1.54 (19)	61 (21)	48 (28)	37 (28)	37 (0)	14 (42)
9	913.8	0.00575 (15)	0.64 (7)	1.45 (20)	54 (35)	47 (28)	38 (72)	35 (29)	16 (36)
10	918.9	0.00593 (15)	0.63 (7)	1.48 (19)	57 (25)	47 (29)	34 (78)	35 (29)	16 (35)
11	919.8	0.00592 (14)	0.63 (7)	1.45 (18)	56 (24)	47 (28)	34 (78)	35 (29)	16 (35)
12	916.9	0.00585 (14)	0.63 (7)	1.45 (18)	55 (28)	47 (29)	35 (77)	35 (29)	16 (35)
13	904.5	0.00607 **(28)**	0.63 (7)	1.43 **(34)**	58 **(88)**	48 **(46)**	32 **(110)**	32 (33)	16 (37)
14	916.4	0.00574 (15)	0.63 (7)	1.53 (19)	55 (33)	48 (28)	36 (76)	35 (29)	16 (36)
15	925.3	0.00597 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
16	915.3	0.00602 (14)	0.63 (7)	1.45 (18)	57 (26)	47 (29)	33 (78)	35 (29)	16 (35)
17	918.5	0.00599 (14)	0.63 (7)	1.49 (18)	56 (24)	47 (28)	33 (76)	35 (29)	16 (35)
18	920.8	0.00596 (14)	0.63 (7)	1.48 (18)	56 (24)	47 (28)	33 (77)	34 (30)	16 (35)

Appendix A.22 continued. Results of the Jack-knife analysis of the final model

Number of Patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
19	925.6	0.00597 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
21	921.9	0.00579 (14)	0.63 (7)	1.51 (18)	56 (26)	47 (28)	34 (76)	35 (29)	16 (35)
23	916.4	0.00586 (14)	0.64 (7)	1.48 (18)	57 (24)	47 (28)	34 (77)	34 (30)	16 (36)
24	916.4	0.00586 (14)	0.64 (7)	1.48 (18)	57 (24)	47 (28)	34 (77)	34 (30)	16 (36)
25	913.0	0.00589 (15)	0.64 (7)	1.47 (19)	57 (24)	47 (28)	34 (77)	35 (29)	16 (35)
26	918.0	0.00597 (14)	0.64 (7)	1.49 (18)	56 (24)	46 (29)	34 (75)	35 (29)	16 (35)
27	911.0	0.00607 (21)	0.64 (7)	1.43 **(27)**	60 **(67)**	43 **(45)**	32 **(100)**	35 (29)	16 (35)
29	919.4	0.00602 (16)	0.64 (7)	1.45 (20)	57 (32)	46 (31)	34 (79)	35 (29)	16 (35)
30	916.9	0.00626 (130	0.64 (7)	1.41 (18)	54 (26)	46 (29)	31 (77)	35 (29)	16 (35)
35	923.3	0.00597 (14)	0.63 (7)	1.46 (19)	57 (26)	47 (29)	33 (78)	35 (29)	16 (35)
36	920.9	0.00599 (14)	0.64 (7)	1.49 (18)	56 (25)	46 (28)	34 (76)	35 (29)	16 (35)
37	921.5	0.006 (14)	0.64 (7)	1.45 (18)	57 (25)	47 (29)	33 (78)	35 (29)	16 (35)
38	923.1	0.00597 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
41	915.9	0.00588 (18)	0.64 (7)	1.5 (23)	58 (40)	46 (32)	31 (90)	34 (29)	16 (35)
45	920.4	0.00612 (18)	0.64 (7(	1.43 (23)	56 (24)	46 (31)	33 (77)	34 (29)	16 (35)
46	922.0	0.00604 (17)	0.64 (7)	1.44 (21)	56 (34)	46 (31)	33 (79)	35 (29)	16 (35)
47	920.0	0.00596 914)	0.64 (7)	1.5 (18)	55 (25)	46 (28)	34 (75)	35 (29)	16 (35)
48	917.4	0.00605 (16)	0.64 (7)	1.44 (21)	57 (33)	46 (31)	33 (80)	35 (29)	16 (35)

Appendix A.22 continued. Results of the Jack-knife analysis of the final model

Number of Patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
50	919.5	0.0062 (14)	0.64 (7)	1.42 (18)	55 (28)	46 (29)	33 (77)	35 (29)	16 (35)
52	920.7	0.0061 (16)	0.64 (7)	1.43 (20)	56 (34)	46 (31)	33 (79)	35 (29)	11 (35)
54	924.1	0.00598 (14)	0.64 (7)	1.46 (18)	56 (25)	47 (29)	33 (77)	35 (29)	16 (35)
55	920.9	0.00597 (14)	0.64 (7)	1.46 (18)	57 (24)	47 28)	33 (77)	35 (29)	16 (35)
58	921.4	0.00608 (16)	0.64 (7)	1.44 (20)	56 (31)	46 (30)	33 (79)	35 (29)	16 (35)
62	921.4	0.00607 (16)	0.64 (7)	1.44 (20)	56 (32)	46 (30)	33 (79)	35 (29)	16 (35)
65	921.1	0.00595 (14)	0.64 (7)	1.49 (18)	56 (25)	46 (28)	34 (75)	35 (29)	16 (35)
66	922.1	0.0058 (14)	0.64 (7)	1.48 (19)	56 (26)	47 28)	34 (76)	35 (29)	16 (35)
70	917.9 7	0.00609 (15)	0.633 (7)	1.44 (19)	56 (28)	47 (29)	33 (79)	35 (29)	16 (34)
71	919.8	0.00596 (29)	0.63 (7)	1.47 **(34)**	57 **(87)**	45 **(51)**	34 **(100)**	35 (29)	16 (35)
72	924.9	0.00596 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
74	923.7	0.00597 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
75	920.1	0.00599 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
76	923.4	0.00596 (14)	0.63 (7)	1.45 (18)	56 (24)	47 (28)	33 (78)	35 (29)	16 (35)
77	917.6	0.00595 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (78)	35 (29)	16 (35)
78	921.7	0.00594 (16)	0.64 (7)	1.47 (20)	57 (30)	47 (30)	34 (79)	34 (0)	16 (35)
79	918.6	0.00604 (15)	0.64 (7)	1.44 (19)	56 (27)	47 (30)	33 (77)	35 (29)	16 (35)
80	915.6	0.0059 (14)	0.63 (7)	1.5 (18)	56 (24)	48 (26)	33 (77)	33 (31)	16 (36)

Appendix A.22 continued. Results of the Jack-knife analysis of the final model

Number of Patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
81	924.7	0.00596 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
82	915.2	0.00596 **(29)**	0.64 (7)	1.45 **(35)**	58 **(89)**	45 **(50)**	34 **(100)**	35 (29)	16 (35)
83	923.0	0.00594 (14)	0.64 (7)	1.46 (18)	56 (24)	46 (29)	34 (77)	35 (29)	16 (35)
84	916.4	0.0059 (16)	0.64 (7)	1.44 (21)	57 (32)	47 (29)	36 (75)	34 (29)	16 (35)
85	917.2	0.00596 (14)	0.63 (7)	1.46 (18)	56 (24)	46 (29)	33 (78)	35 (29)	16 (35)
86	922.7	0.00595 (14)	0.64 (7)	1.48 (18)	56 (24)	46 (29)	34 (76)	35 (29)	16 (35)
87	923.5	0.00596 (14)	0.63 (7)	1.47 (18)	1.56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
88	910.8	0.00612 (14)	0.64 (7)	1.45 (18)	52 (31)	45 (31)	40 (82)	36 (28)	16 (34)
89	912.4	0.00594 (14)	0.63 (7)	1.51 (18)	55 (25)	48 (27)	33 (77)	33 (32)	16 (36)
90	917.1	0.00592 (14)	0.64 (7)	1.45 (19)	57 (25)	47 (28)	35 (74)	35 (29)	16 (35)
91	914.9	0.00592 (14)	0.63 (7)	1.51 (18)	55 (26)	48 (27)	34 (76)	33 (31)	16 (36)
92	921.2	0.00596 (14)	0.63 (9)	1.46 (18)	56 (25)	47 (28)	33 (77)	34 (30)	16 (35)
93	925.1	0.00596 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
94	921.0	0.00599 (14)	0.63 (7)	1.48 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
95	917.3	0.00594 (14)	0.64 (7)	1.46 (19)	57 (25)	47 (29)	34 (78)	35 (30)	16 (35)
96	919.2	0.00599 (15)	0.63 (7)	1.46 (19)	57 (27)	47 (29)	33 (78)	35 (29)	16 (35)
97	922.6	0.00596 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
98	914.9	0.00602 (13)	0.64 (7)	1.49 (18)	55 (25)	46 (29)	34 (76)	35 (29)	16 (34)

Appendix A.22 continued. Results of the Jack-knife analysis of the final model

Number of Patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
99	916.9	0.00605 (14)	0.63 (7)	1.47 (18)	56 (25)	47 (28)	32 (77)	34 (30)	16 (35)
110	898.4	0.00622 (15)	0.64 (7)	1.33 (20)	61 (43)	48 (31)	34 (79)	32 (26)	14 (29)
111	918.2	0.00593 (14)	0.64 (7)	1.43 (19)	57 (25)	47 (28)	35 (75)	35 (29)	16 (35)
112	913.1	0.00613 (34)	0.63 (7)	1.39 **(43)**	58 (13)	46 (64)	33 **(120)**	36 (29)	15 (41)
113	919.3	0.00594 (14)	0.64 (7)	1.41 (18)	56 (25)	47 (28)	33 (80)	34 (29)	16 (36)
114	921.8	0.00598 **(39)**	0.62 (8)	1.47 **(45)**	57 **(130)**	45 **(73)**	33 (12)	35 (29)	16 (35)
115	915.7	0.00594 (14)	0.63 (7)	1.46 (19)	56 (25)	47 (29)	35 (74)	35 (29)	16 (38)
116	924.2	0.0059 (14)	0.63 (7)	1.49 (18)	56 (24)	46 (28)	34 (77)	34 (29)	16 (35)
117	924.2	0.00595 (14)	0.63 (7)	1.45 (19)	56 (26)	47 (29)	34 (77)	35 (29)	16 (35)
118	919.6	0.00596 (14)	0.63 (7)	1.47 (18)	56 (24)	46 (28)	34 (77)	35 (29)	16 (35)
119	916.8	0.00586 (15)	0.63 (7)	1.5 (19)	57 (29)	46 (3O)	34 (78)	34 (29)	16 (35)
120	923.9	0.00586 (14)	0.63 (7)	1.5 (18)	56 (24)	47 (28)	34 (76)	34 (29)	16 (35)
121	921.3	0.00575 (14)	0.63 (7)	1.52 (19)	54 (31)	47 (29)	33 (76)	35 (29)	16 (35)
122	925.2	0.00596 (15)	0.63 (7)	1.45 (19)	57 (26)	47 (29)	33 (78)	35 (29)	16 (35)
123	914.3	0.00631 (15)	0.63 (7)	1.34 (21)	64 (42)	43 (36)	33 (77)	36 (29)	15 (39)
124	909.2	0.00595 (21)	0.64 (7)	1.46 (26)	57 (54)	45 (39)	36 (82)	35 (29)	16 (36)
125	922.1	0.00598 (27)	0.63 (7)	1.46 **(32)**	57 **(81)**	45 **(47)**	34 **(98)**	35 (29)	16 (35)
126	919.3	0.00593 **(33)**	0.63 (7)	1.46 **(44)**	60 **(140)**	45 **(73)**	33 **(110)**	36 (30)	16 (35)

Appendix A.22 continued. Results of the Jack-knife analysis of the final model

Number of Patients left out	OFV	θ_1 (SE%)	θ_2 (SE%)	θ_3 (SE%)	η^{CL} (SE%)	η^{V} (SE%)	η^{IOVCL} (SE%)	η^{IOVV} (SE%)	Res Err (SE%)
127	921.1	0.00594 (15)	0.63 (7)	1.42 (20)	57 (29)	47 (29)	33 (82)	34 (29)	16 (35)
128	924.9	0.00596 (14)	0.63 (7)	1.46 (18)	55 (26)	47 (28)	35 (78)	35 (29)	16 (35)
129	924.4	0.00596 (14)	0.63 (7)	1.45 (18)	56 (25)	47 (28)	33 (78)	35 (29)	16 (35)
130	911.6	0.00595 (15)	0.63 (7)	1.47 (19)	57 (25)	47 (28)	33 (81)	34 (30)	16 (34)
131	920.6	0.00591 (14)	0.63 (7)	1.47 (18)	56 (24)	47 28)	34 (77)	35 (29)	16 (35)
132	911.8	0.00577 (14)	0.64 (7)	1.52 (18)	57 (23)	46 (28)	34 (78)	34 (30)	16 (36)
133	921.3	0.00578 (17)	0.63 (7)	1.57 (20)	43 **(66)**	45 (31)	48 **(93)**	35 (29)	16 (35)
135	920.8	0.00597 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (29)	34 (76)	35 (29)	16 (35)
136	925.4	0.00596 (14)	0.63 (7)	1.46 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
138	924.957	0.00595 (14)	0.63 (7)	1.47 (18)	56 (24)	47 (28)	33 (77)	35 (29)	16 (35)
139	919.6	0.00598 (14)	0.64 (7)	1.46 (18)	56 (25)	46 (29)	34 (77)	35 (29)	16 (34)
140	916.1	0.00585 (15)	0.62 (7)	1.51 (19)	56 (35)	45 (32)	34 (78)	34 (29)	16 (36)
141	919.7	0.00595 (15)	0.63 (7)	1.47 (19)	56 (28)	46 (30)	33 (78)	35 (29)	16 (35)

APPENDIX B.1

Data collection sheet for clinical effects

Pat	Gen	GA	Birth	P Apn	P Apn	Tot Apn	Tot Apn	A+PR+O2	A+O2	A+PR	Dec PR	PR	DecOx	HypEp	Oxy	MAP	T rec	CpTheo	CpCaf	Theop
No		wks	wgt Kg	density	per h	density	per h	per h	per h	per h	per h	ave	per h	per h	ave		min	mg/L	mg/L	mg/kg

APPENDIX B.2

Events types and incidences: baseline to day 3

EVENT TYPES AND INCIDENCES ON DAY 0 or BASELINE															
Pat	Gen	GA	Birth	PApn	PApn	TotApn	TotApn	A+PR+O2	Ap+O2	AP+PR	Dec PR	PR	HypoxEp	Oxy	MAP
No		wks	wgt Kg	density	per h	density	per h	per h	per h	per h	per h	ave	>=10%	ave	
1	m	30	1.2	0.3	0.5	0.3	0.5	0.5	0	0	6.5	140.9	1	94.2	38
2	f	32	1.15	4.1	4.2	6.8	17.7	3	2	0	1.2	159.1	1.5	96.5	32
3	f	32	1.3	2.6	2.5	7.3	28	0	0	0	3	154.2	1.5	96.1	
4	m	30	0.95	4.5	7.1	13.1	45.1	0	0	1	0	140.5		97.76	35
5	f	29	1.1	2.5	3	8.7	35.5	0	0	0	0	132.8	0.5	98.1	29
6	m	30	1	0.93	1	7.43	31	0.5	0	0	8	131	10.5	96.4	
10	f	32	1.4	2.8	4.9	3.4	7.7	1.62	1.2	0.4	13	106.4	1.2		
11	f	30	1.25	0.21	0.4	2.61	12.4	0.4	0	0	1.1	153.1	6.8	92.9	
12	m	30	1.3	2.48	3.2	8.43	28.3	1	0	0	0	138.2	1.6	97.7	33
13	f	31	1.25	2.6	3.1	6.3	21.7	1	1.5	0	3.1	125.2	1.5	96.9	35
14	m	32	1.5	1.4	1.5	3.7	10	1	0	0	0.5	134.6	2.1	94.86	31
15	m	32	1.4	2.94	4.2	5.14	14.1	1	0.5	0	4	134.6	1.5	89.4	29
16	m	30	1.2	1.4	2.1	1.76	4.6	0.84	0	0.42	173	98.59	2.1	97.2	29
17	m	29	1.05	1.33	1.3	4.83	20.3	0	0.5	0	2.2	130.4	0	98.5	31
18	f	30	0.95	7.5	7.8	8.1	10.4	1.5	1.5	1.5	10	146.4	0.7	97.2	40
19	f	33	1.75	0.124	0.8	10.7	58.8	0	1	0	0.4	118.1	0.9	94.7	
70	f	30	1.25	3.6	4	9.24	24	2	0	1	14	124.9	4	97.9	38
71	f	30	1.1	1.7	3	4.4	12	0	3	0	12	149.3	16	91.6	
72	m	30	1.36	0	0	4.8	23	0	0	0	3	118.7	2.2	92.8	32
73	m	31	1.5	1.56	1.5	5	16	0	0	2	14	120.4	1.9	97	
74	f	31	1.5	1.1	2	3.6	16	0	2	0	89	113.6	5	94.8	38
75	f	34	1.7	0	0	2.8	14	0	1	0	0	121.7	0	92.4	34
76	f	31	1.5	0	0	0.3	3	0	0	0	25	136.1	0.6	98	
77	m	31	1.1	2.4	4.4	7.2	22	0	1	1	3	134.4	3	89	30
78	f	31	1.35	0	0	2.2	13.3	0	0	0	1.3	107.8	0	97	
79	f	29	1.15	0	0	5	14	0	1	0	0	141	5	90.1	34
80	m	30	1.25	0.5	0.4	1.75	7.7	0	0	1	151.6	99	0	96.9	45

81	m	30	1.45	1.8	3	3.6	10	0	0	0	2	137.4	1	95.8	39
82	f	31	1.51	0.46	0.83	1.3	7.5	0	0	0	0.8	130	0	98	39
83	m	30	1.06	2.5	4	5.6	22	0	0	0	0	144.6	8.8	90.2	37
84	m	28	0.8	1.7	2	6.9	37	1	1	0	44	105.1	1	97.6	36
85	f	30	1.03	13.5	24	22.7	63	1.6	1.85	1.85	16	120.8	3	96.8	36
87	f	30	1.2	0.6	1	2.61	12	0	2	0	0	142.6	0	93.9	36
88	f	30	1.2	0.64	1.2	0.8	2.4	0	0	0	2.3	129.3	0	98.2	42
89	f	30	0.8	0	0	4.6	60	0	0	0	0	133.2	0	96.9	34
90	m	32	1.5	4.2	6	4.7	9	0	0	0	3	147.6	1.5	83.6	41
91	m	30	1.25	0	0	6.4	32	0	0	0	16	113.8	1.3	94.7	22
92	m	33	1.45	2.13	4	3.4	10	1	0.5	0	40.8	109.9	3.4	92.4	30
93	m	29	1.1	7.4	12	9.3	21	2	2	0	4	136.5	2	96.4	38
94	f	29	1.1	0	0	2.51	14	0	0	0	0	145.6	0	99	37
95	m	28	0.85	3.61	7	9.21	39	1.26	0.3	1	3.3	133.4	2.2	96.2	24
96	m	29	0.9	5.3	9.5	8.1	25.5	2.3	0	2	80	106	5.2	94.4	31
97	f	34	1.8	0.5	1	6	39	0.4	0.4	0	1	139.2	3.9	96.3	27
98	m	31	1.3	2.1	2.1	4.5	13.6	1	1	0	2.5	145.8	2	97.8	27
99	m	32	1.7	2.4	3	3.7	9.2	0.73	0.73	0	2.2	139.1	4.8	93.4	35
124	m	32	1.2	0.63	1.14	4.11	16.3	0	0	0	0	133.6		96.6	
ave		30.6	1.3	2.1	3.2	5.3	20.0	0.6	0.6	0.3	18.9	128.5	1.3	95.0	34.0
SD		1.4	0.25	2.65	4.41	3.83	15.06	0.70	0.78	0.61	39.38	14.98	3.0324849	3.32	5.08
Med		30	1.3	1.6	2.0	4.7	14.0	0.0	0.3	0.0	3.0	133.2	1.6	96.3	34.5
Stats															
25or75		30.0	1.1	0.3	4.0	3.1	23.5	0.0	0.0	0.0	0.9	118.4		93.0	30.8
Wilcoxcin-Matched Pairs test for significant difference:							Baseline to day 1,2,3:								
Bonferroni adjustment, over 3 days: p<0.05/3							Therefore significant difference if p.0.(if p<0.017								

EVENT TYPES AND INCIDENCES ON DAY 1														
No	Theop	P Apn	P Apn	Tot Apn	Tot Apn	A+PR+O2	AP+O2	AP+PR	PR dec	PR	HypEpi	Oxy	MAP	Cp Th
	mg/kg	density	per h	density	per h	per h	per h	per h	per h	ave	>=10%	ave		mg/L
1	4	0.6	1	0.6	1	1	0	0	8	142.7	0	95.8	32	4.92
2	4.9	1.3	0.6	2.6	8.4	0	0	0	0.6	153	0	96.3	48	5.8
3	4.3	0	0	0.97	6.5	0	0	0	0	162.1	1.5	96.5	30	5.8
4	4.2	2.5	4	6.6	27	0	0	0	0	154		98	40	4.5
5	3.6	0.8	1	2.4	10.5	0	0	0	0.5	142.6	0.5	98.1	40	5.7
6	4										0.50			
10	4	3.2	5	4.6	12	0	1	1.5	3	112.4	0.5	95.5	41	6
11	3.2	0	0	0.3	2.1	0	0	0	0.5	170.7	0.5	94.6	37	4.6
12	4.3	1.7	3	6.55	25.2	0.6	0	0	0	142.9	0.6	98.3	33	4
13	3.2	0.86	1.6	2.36	8.8	0	1.6	0	0	148	3.5	96.5	40	4.5
14	3.2	2.5	2.5	3.7	8.5	1.5	0	0	4	132.7	3.5	95.9	40	5.1
15	4.28	1.2	1.7	6.34	7.7	0	0	0	0	136.2	0.9	88	39	5.2
16	4.7	1.75	3	1.98	4.5	0	1	0	0.5	121.7	0	97.06	40	6.5
17	3.8	0.24	0.43	1.94	1.73	0	0	0	5.6	151.1	1	97.4	37	6.4
18	4.2	4.3	4.5	5.9	12.5	0.5	1	0	6	159.2	0	98	34	5
19	3.7	0	0	3.1	21	0	0	0	0	134.5	0	97.3	29	4.61
70	3.84	1.4	2.5	6.1	21	0	2	0.5	0	124.9	1.5	97.9	63	6.1
71	3.6	0	0	0.8	4	0	0	0	1	149.5	2.5	96.4	48	1.1
72	4.4	0	0	1	6	0	0	0	1	135.6	0	96.6		7.5
73	0.53	0.3	0.54	1.8	8.7	0	0	0.5	2	124	0.54	97.8	39	0
74	2.7	0	0	0.6	4	0	0	0	36	118.1	1	96	36	3.5
75	4.2	0	0	1.1	6	0	0	0	2	129.5	3	96.4		5.2
76	4.26	0	0	0.1	1	0	0	0	0.7	147	1.4	96		17.7

77	3.6	0	0	1.2	7.2	0	0	0	0	145.1	6	92.4	36	5.8
78	0.88	0	0	1.53	4.7	0	0	0	0		0			0
79	4.17													
80	3.2	0	0	1.1	6	0	0	0	1.3	123.4	0.7	96		2.7
81	4.14													8.3
82	4	0	0	0.4	2	0	0	0	0	157		98	39	19.3
83	3.8	0.8	1.4	1.43	5.7	0	0.7	0	0	167.1	0.7	95.8	32	11.7
84	1	0.35	0.5	3.45	18	0	0	0	8.5	110.4	0.5	96.8	32	4
85	3.9												48	
87	3.5												47	
88	3.33												46	
89	5	1.1	2	2.6	20	0	0	0	0	149.5	1	96.7		4.6
90	2.7												40	
91	1.92	0	0	0.9	4.7	0	0	0	4	143.2	2.4	96.8		1.8
92	2.8	0.6	1	3.7	15	1	0	0	4	118	3	94.7	36	1.7
93	3.63	3.1	8	4.92	22	1	1.4	0	14	143	2.8	95.3	37	7
94	3.63	0	0	2.3	11	0	0	0	0	145.8	0	97.6		5
95	3.76	0.65	1.2	2.65	12.2	0.4	0	0	2.6	143.6	2.7	96.2	33	11.27
96	4	4	7.2	6.03	17.2	1.2	0.8	1.2	59	138.3	1.6	93.2	36	5.24
97	4	0	0	1.3	8	0	0	0	0	152.2	1.8	97	44	5.4
98	3.7	0.9	1.4	1.4	4.1	0	0	0.9	1	161.2	2.3	97.9	38	5.1
99	2.35	2.3	2.5	3.5	7	1	0.5	0	1	149.7	10	89.8	34	3.1
124	3.58	0.95	1.7	3	13.3	0	0	0	0	130.4		97.3	38	11.4
	3.5	0.9	1.5	2.6	9.7	0.2	0.3	0.1	4.4	140.2	0.5	95.9	39.1	5.7
	1.00	1.25	2.08	1.94	6.70	0.43	0.56	0.37	11.67	15.52	2.04	2.29	6.69	4.12

EVENT TYPES AND INCIDENCES ON DAY TWO														
No	Theop	PApn	PApn	Tot Apn	Tot Apn	A+PR+O2	AP+O2	A+PR	PR dec	PR	HypEpi	O2	MAP	CpTheo
	mg/Kg	density	per h	density	per h	per h	per h	per h	per h	ave	>=10%	ave		mg/L
1														
2	1.04	0.4	0.5	1.8	7.8	0	0	0	3	154.7	3.2	96.3	47	4.91
3	0.9	0	0	0.97	6.5	0	0	0	6.5	162.1	1.5	96.4	37	4.39
4	0.94	3	4	6.2	20.5	0	0	0	0	147.6		97.3	43	10.2
5	1.8	0.5	0.86	2.7	13.36	0	0	0	0.43	143.8		97.2	33	9.8
6	0.8	0	0	1.7	9	0	0	0	0	137.4		96.4	28	4.5
10	0.57	2.4	4	4	13	1.1	0.6	0.6	3.3	134	1.1	96.1	50	4.6
11	0.64	0.3	0.5	1	3.5	0.5	0	0	0.5	122.8	0	94.7	39	4.6
12	0.92	3.4	6.1	5.46	15.54	0	1	0.5	2	152.9	0.9	96.8	43	5.4
13	0.64	0	0	2.1	11	0	0	0	0.5	127	0.5	96.3	49	5.8
14	1.6	0.5	1	1.3	6.5	0	1	0	0	143.6	0.4	95.96	41	6.9
15														
16	1.7	0.68	1	0.96	3	0	0	1	0.5	131.05	1	98.15	41	6.9
17	1.5	0.4	0.4	1.6	6.8	0	0	0	1.3	148	1.3	96.8	36	5.7
18	1.7	0.3	0.5	1.3	6	0	0.5	0	4	188.5	4	95.7	38	11
19														
70	1.28	0.25	1	2.85	13	0.6	0.6	0.6	7.7	143	2.2	97	49	11.7
71											8			
72	0.53	0	0	2.6	15	0	0	0	0	143.6	1	97.7	33	9.3
73	0.53	0	0	0.1	0.8	0	0	0	2.4	140	3.2	88.9	38	6.25
74	0.47	0	0	0.5	3.4	0	0		1.14	139.3	0	95.6		7.5
75	0.53	0	0	1.4	8.4	0	0	0	1.1	143	0	93.9		5.3
76	0.73	0	0	2.1	9.5	0	0	0	19	116.9	2.7	94.3	41	4

77	0.9	2.2	2	11.4	24	0	1	0	0	141.6	4	98.2		14.9
78	0.7	0.98	1.8	2.3	11.8	1	0	0	4	122.5	0.9	93.8		9.3
79											0			
80	1.38	0.3	0.5	1.7	8	0	0	0	0	166	2	97.1	45	42.4
81	1.07	0	0	0	0	0	0	0	0	139.5		96.3	45	10.4
82														
83	1	1.7	3	9.4	36	0	0	0.8	2.4	125.4	1.5	97	33	4
84	1.55	0	0	1.9	12	0	1	0	0	129.8	1	98.3	50	6.1
85	0.7	0	0	4.4	28.6	0	0	0	0	144.4		87.6		4.6
87	0.7	0	0	1.2	6	0	0	0	0	131	3	94.2	41	15.5
88	1	0	0	0.34	1	0	0	0	2	163.4	2	96.1		7.8
89	1.6	3.07	4.7	3.52	7.1	0	0.5	0	0	153.9	5.2	91.8	34	8.1
90	0.64	0	0	2.7	12.9	0	0	0	3.4	155.4	0.9	97.1		6.5
91	1.1	0	0	1	5	0	0	0	0	118.6	0	94.3	38	6.6
92														
93	0.72	0	0	0.6	5	0	0	0	0	131	0	96.8		5
94	1.4	0.46	0.85	2.86	12.45	0.3	0	0	2	135.4	0.3	95.7	41	6.92
95	0.88	0.8	1.4	2.4	8.8	0	0.6	0	0.6	153	0.3	97		5.62
96	0.88	0	0	2	9.4	0	0	0	0	123.6	0.8	96.2	38	4.7
97	0.8	0	0	0.8	4.5	0	0	0	0.6	165.4	2.6	96.8	42	7.9
98	0.8	1.5	2.5	4.2	16.5	0.5	0.5	0.5	0	151.1	0	98	48	2.7
99														
124	0.72	1.6	3.6	2.97	8.7	0	0	0	0	145.7		97.2	43	11.5
65.7	1.0	0.6	1.0	2.5	10.1	0.1	0.2	0.1	1.8	141.4	2.4	95.6	41.4	8.2
31.9	0.39	0.95	1.54	2.46	7.85	0.29	0.37	0.28	3.58	15.89	1.799059	2.44	5.28	6.90

78.5	0.9	0.1	0.2	2.0	8.6	0.0	0.0	0.0	0.6	140.8	1.0	96.3	41.0	6.6
		0.0020	0.0008	0.0001	0.0001	0.0014	0.0194	0.0597	0.0016	0.0004	0.062	0.460	0.0001	
		Sig	Sig	Sig	Sig	Sig	NS	NS	Sig	Sig		NS	Sig	

EVENT TYPES AND INCIDENCES ON DAY THREE													
No	PApn	PApn	Tot Apn	TotAP	A+PR+O2	AP+O2	A+PR	PR dec	PR	HypEpi	Oxy	MAP	CpTheo
	density	per h	density	per h	per h	per h	per h	per h	ave	>=10%	ave		mg/l
1													
2	0.33	0.6	0.93	4.2	0	0	0	0	146.1	0	97	67	4.56
3	0	0	1.5	0.7	0	0	0	1	162.2	1.6	94.8	39	2.75
4	1.6	3	3.52	13.5	0	0	0	0	160.9		96	40	8.9
5	0.83	1.5	6.13	26.5	0	0	0	0	152.9		96.8	33	7.4
6													
10	0	0	2.2	11.6	0	0	0	0.5	124.8	2.5	96.6	57	4.5
11	0	0	1	5.1	0	0	0	0	139.1	0.6	94.8	55	4.5
12	0.9	1.6	7.1	29.5	0	1	0	0.5	148.5	1	94.5	40	4.8
13	0	0	0.78	2.9	0	0	0	0	145	0.5	96	53	7.4
14	0.06	0.5	0.93	4.5	1	0	0	0.5	143.6	0.6	96.5	47	7.2
15													
16	0	0	0.9	3.5	0	0	0	0	133.15	0.6	98	38	7.5
17													
18													
19													
70													
71													
72													
73	0	0	0.7	4	0	0	0	0	142.6	4	97.2	51	8.8
74													
75													
76	0	0	1.25	7.5	0	0	0	1.2	127	2.5	96.5		4.8

77	0	0	2.05	11.5	0	0	0	2	129.1	8.1	98.5	40	5.9
78													
79	0	0	0.5	2.5	0	0	0	0	144.4	0	95		17.8
80													
81	1.1	2	4.4	18	0	1	0	0	153.1	1	97.3	48	27.3
82													
83													
84	0.45	0.8	14.5	66		6.5	0	5.7	141.4	7.4	91		5.1
85	0.55	1	3.5	15	0	2	0	0	119.1	2	97.1	45	8.1
87	0	0	2.4	13.3	0	0	0	5.3	167.5		96		7
88	0	0	2.5	14.5	0	0	0	2	142.6	1	98.3	53	16.8
89	7.5	13	8.8	13	0	0	0	0	171.9		82.2		21.95
90	0	0	0.1	0.6	0	0	0	2.4	159.9	23.2	90.3	44	12
91	0	0	0	0	0	0	0	0	160.8	0	97.3		9
92													
93													
94	0	0	1.8	11	0	0	0	1	146	0	96.4		7.7
95	0	0	1.8	9.5	0	0	0	0	159.5	0.8	92.8	30	12.86
96	1.2	2.1	2.7	9.1	0.7	0	0	0	158	1.4	97		4.9
97	0	0	1.8	10.3	0	0	0	0	126.1	0.75	96.8	54	3.9
98	0	0	0	0	0	0	0	0	141.4	0	96.7	59	8.5
99	0.95	1.7	3.55	13.7	0	0	0	0	152.2	0	95.4	33	3.3
124	1.2	0.83	2.16	8.4	0	0	0	0	154.5		95.9		10.3
66.5	0.5	0.9	2.7	11.5	0.1	0.4	0.0	0.9	144.9	0.8	95.3	46.7	9.2
31.9	1.54	2.66	3.29	13.42	0.25	1.38	0.00	1.60	13.89	5.099	3.47	8.65	6.09

79.0	0.0	0.0	1.8	9.9	0.0	0.0	0.0	0.0	144.0	0.9	96.5	47.5	7.5
	0.0003	.0002	.0008	.0021	.0022	.1401	.0117	.0004	.0005	0.721	.759	.0003	
	Sig	Sig	Sig	NS	Sig	NS	Sig	Sig	Sig	NS		Sig	

APPENDIX B.3

Total apnoea per hour from baseline to day three with serum theophylline concentrations (mg/L)

Patient number	Baseline total apnoea	Day 1 total apnoea	Day 1 Theop conc	Day 2 total apnoea	Day 2 Theop conc	Day 3 total apnoea	Day 3 Theop conc	Percentage change
1	0.5	1	4.92					-100
2	17.7	8.4	5.8	7.8	4.91	4.2	4.56	76
3	28	6.5	5.8	6.5	4.39	0.7	2.75	98
4	45.1	27	4.5	20.5	10.2	13.5	8.9	70
5	35.5	10.5	5.7	13.36	9.8	26.5	7.4	25
6	31			9	4.5			71
10	7.7	12	6	13	4.6	11.6	4.5	-51
11	12.4	2.1	4.6	3.5	4.6	5.1	4.5	59
12	28.3	25.2	4	15.54	5.4	29.5	4.8	-4
13	21.7	8.8	4.5	11	5.8	2.9	7.4	87
14	10	8.5	5.1	6.5	6.9	4.5	7.2	55
15	14.1	7.7	5.2					45
16	4.6	4.5	6.5	3	6.9	3.5	7.5	24
17	20.3	1.73	6.4	6.8	5.7			67
18	10.4	12.5	5	6	11			42
19	58.8	21	4.61					64
70	24	21	6.1	13	11.7			46
71	12	4	1.1					67
72	23	6	7.5	19.2	32.6			17
73	16	8.7	0	15	9.3	4	8.8	6
74	16	4	3.5	0.8	6.25			95
75	14	6	5.2	3.4	7.5			76
76	3	1	17.7	8.4	5.3	7.5	4.8	-150
77	22	7.2	5.8	9.5	4	11.5	5.9	48
78	13.3	4.7	0	24	14.9			-80

80	7.7	6	2.7					22
81	10		8.3	8	42.4	18	27.3	-80
82	7.5	2	19.3	0	10.4			100
83	22	5.7	11.7					74
84	37	18	4	36	4	66	5.1	-78
85	63			12	6.1	15	8.1	76
87	12			28.6	4.6	13.3	7	-11
88	2.4			6	15.5	14.5	16.8	-504
89	60	20	4.6	1	7.8	13	21.95	78
90	9			7.1	8.1	0.6	12	93
91	32	4.7	1.8	12.9	6.5	0	9	100
92	10	15	1.7	5	6.6			50
93	21	22	7					-5
94	14	11	5	5	5	11	7.7	21
95	39	12.2	11.27	12.45	6.92	9.5	12.86	76
96	25.5	17.2	5.24	8.8	5.62	9.1	4.9	64
97	39	8	5.4	9.4	4.7	10.3	3.9	74
98	13.6	4.1	5.1	4.5	7.9	0	8.5	100
99	9.2	7	3.1	16.5	2.7	13.7	3.3	-49
124	16.3	13.3	11.4	8.7	11.5	8.4	10.3	48

APPENDIX B.4

Pathologic apnoea per hour from baseline to day three with serum theophylline concentrations (mg/L)

Patient number	Baseline path apnoea	Day 1 path apnoea	Day 1 Theop conc	Day 2 path apnoea	Day 2 Theop conc	Day 3 path apnoea	Day 3 Theop conc	Percentage change
1	0.5	1	4.92					-100
2	4.2	0.6	5.8	0.5	4.91	0.6	4.56	86
3	2.5	0	5.8	0	4.39	0	2.75	100
4	7.1	4	4.5	4	10.2	3	8.9	58
5	3	1	5.7	0.9	9.8	1.5	7.4	50
6	1			0	4.5			100
10	4.9	5	6	4	4.6	0	4.5	100
11	0.4	0	4.6	0.5	4.6	0	4.5	100
12	3.2	3	4	6.1	5.4	1.6	4.8	50
13	3.1	1.6	4.5	0	5.8	0	7.4	100
14	1.5	2.5	5.1	1	6.9	0.5	7.2	66
15	4.2	1.7	5.2					59
16	2.1	3	6.5	1	6.9	0	7.5	100
17	1.3	0.43	6.4	0.4	5.7			69
18	7.8	4.5	5	0.5	11			93
19	0.8	0	4.61					100
70	4	2.5	6.1	1	11.7			75
71	3	0	1.1					100
73	1.5	0.54	0	0	9.3	0	8.8	100
74	2	0	3.5	0	6.25			100
77	4.4	0	5.8	0	4	0	5.9	100
80	0.4	0	2.7					100
81	3		8.3	0.5	42.4	2	27.3	33
82	0.83	0	19.3	0	10.4			100
83	4	1.4	11.7					65
84	2	0.5	4	3	4	0.8	5.1	60

85	24			0	6.1	1	8.1	95
87	1			0	4.6	0	7	100
88	1.2			0	15.5	0	16.8	100
90	6			4.7	8.1	0	12	100
92	4	1	1.7	0	6.6			100
93	12	8	7					33
95	7	1.2	11.27	0.85	6.92	0	12.86	100
96	9.5	7.2	5.24	1.4	5.62	2.1	4.9	78
97	1	0	5.4	0	4.7	0	3.9	100
98	2.1	1.4	5.1	0	7.9	0	8.5	100
99	3	2.5	3.1	2.5	2.7	1.7	3.3	43
124	1.14	1.7	11.4	3.6	11.5	0.8	10.3	30

APPENDIX B.5

Hypoxaemic episodes per hour from baseline to day three with serum theophylline concentrations

Patient number	Baseline hypox epis	Day 1 hypox epis	Day 1 Theop conc	Day 2 hypox epis	Day 2 Theop conc	Day 3 hypox epis	Day 3 Theop conc	Percentage change
1	1	0	4.92					100
2	1.5	0	5.8	3.2	4.91	0	4.56	100
3	1.5	1.5	5.8	1.5	4.39	1.6	2.75	-6
5	0.5	0.5	5.7	0	9.8	0	7.4	100
6	10.5			0.5	4.5			99
10	1.2	0.5	6	1.1	4.6	2.5	4.5	-200
11	6.8	0.5	4.6	0	4.6	0.6	4.5	99
12	1.6	0.6	4	0.9	5.4	1	4.8	37
13	1.5	3.5	4.5	0.5	5.8	0.5	7.4	67
14	2.1	3.5	5.1	0.4	6.9	0.6	7.2	71
15	1.5	0.9	5.2					40
16	2.1	0	6.5	1	6.9	0.6	7.5	71
18	0.7	0	5	4	11			-570
19	0.9	0	4.61					100
70	4	1.5	6.1	2.2	11.7			45
71	16	2.5	1.1					84
72	2.2	0	7.5	0	32.6			100
73	1.9	0.54	0	1	9.3	4	8.8	-210
74	5	1	3.5	3.2	6.25			46
76	0.6	1.4	17.7	0	5.3	2.5	4.8	-416
77	3	6	5.8	2.7	4	8.1	5.9	-270
79	5			0.9	9.3	0	17.8	100
81	1		8.3	2	42.4	1	27.3	0
83	8.8	0.7	11.7					99
84	1	0.5	4	1.5	4	7.4	5.1	-750

85	3			1	6.1	2	8.1	33
90	1.5			5.2	8.1	23.2	12	-1547
91	1.3	2.4	1.8	0.9	6.5	0	9	100
92	3.4	3	1.7	0	6.6			100
93	2	2.8	7					-140
95	2.2	2.7	11.27	0.3	6.92	0.8	12.86	64
96	5.2	1.6	5.24	0.3	5.62	1.4	4.9	73
97	3.9	1.8	5.4	0.8	4.7	0.75	3.9	81
98	2	2.3	5.1	2.6	7.9	0	8.5	100
99	4.8	10	3.1	0	2.7	0	3.3	100

APPENDIX C.1

Pharmacodynamic data file

# ID	GEN	WT	GA	FAPT	NAPN	CONC	DOBS	TIME	DAY	COND	OXY	PCA
70	2	1.25	30	9.24	24	0	1	0	0	2	2	30
70	2	1.25	30	6.1	42	6.1	2	0.46	1	2	2	30.1
70	2	1.25	30	2.85	23	11.7	1.8	21.84	2	2	2	30.3
70	2	1.25	30	3.4	19	10.8	1	68.8	4	2	2	30.6
71	2	1.1	30	4.4	12	0	1	0	0	1	2	30
71	2	1.1	30	0.8	7	1.1	2	0.78	1	1	2	30.1
72	1	1.36	30	4.8	32	0	1.38	0	0	1	1	30
72	1	1.36	30	1	12	7.5	2	0.67	1	1	1	30.1
72	1	1.36	30	3.5	24	32.6	1.25	24.7	2	1	1	30.3
73	1	1.5	31	5	26	0	1.6	0	0	1	1	31
73	1	1.5	31	1.8	16	0	1.83	1.03	1	1	1	31.1
73	1	1.5	31	2.6	15	9.3	1	13.45	2	1	1	31.3
73	1	1.5	31	0.7	4	8.8	1	37.5	3	1	1	31.4
74	2	1.5	31	3.6	16	0	1	0	0	1	1	31
74	2	1.5	31	0.6	4	3.5	1	0.9	1	1	1	31.1
74	2	1.5	31	0.1	1	6.3	1.27	20	2	1	1	31.3
75	2	1.7	34	2.8	10	0	0.72	0	0	1	1	34
75	2	1.7	34	1.1	6	5.2	1	1.2	1	1	1	34.1
75	2	1.7	34	0.5	6	7.5	1.75	21	2	1	1	34.3
76	2	1.5	31	0.3	5	0	1.6	0	0	2	1	31
76	2	1.5	31	0.1	1	17.7	1.4	0.9	1	2	1	31.1
76	2	1.5	31	1.4	15	5.3	1.8	21	2	2	1	31.3
76	2	1.5	31	1.25	6	4.8	0.8	44	3	2	1	31.4
77	1	1.1	31	7.2	23	0	0.916	0	0	2	2	31
77	1	1.1	31	1.2	9	5.8	1.25	0.7	1	2	2	31.1
77	1	1.1	31	2.1	7	4	0.73	20	2	2	2	31.3
77	1	1.1	31	2	10	5.9	0.87	44.2	3	2	2	31.4
78	2	1.35	31	2.2	20	0	1.5	0	0	2	1	31
78	2	1.35	31	11.4	6	14.9	0.25	0.75	2	2	1	31.3
79	2	1.15	29	5	14	0	1	0	0	2	2	29
79	2	1.15	29	2.3	13	9.3	1.13	17	2	2	2	29.3
79	2	1.15	29	0.5	2	17.8	0.8	41.1	3	2	2	29.4
80	1	1.25	30	1.75	10	0	1.23	0	0	1	1	30
80	1	1.25	30	1.1	9	2.7	1.5	1	1	1	1	30.1
80	1	1.25	30	4.8	10	16.1	1.23	19.6	2	1	1	30.2
81	1	1.45	30	3.6	9	0	0.916	0	0	2	2	30
81	1	1.45	30	1.7	16	42.4	2	21	2	2	2	30.3
81	1	1.45	30	4.4	18	27.3	1	43	3	2	2	30.4
82	2	1.51	31	1.3	9	0	1.2	0	0	1	1	31
82	2	1.51	31	0.4	3	19.3	1.5	0.8	1	1	1	31.1
82	2	1.51	31	0	0	10.4	0.77	19	2	1	1	31.3
83	1	1.06	30	5.6	10	0	0.45	0	0	1	1	30
83	1	1.06	30	1.43	8	11.7	1.43	1.7	1	1	1	30.1

#ID	GEN	WT	GA	TDEN	DV	CONC	FRAC	TIME	DAY	COND	OXY	PCA
84	1	0.8	28	6.9	39	0	1.05	0	0	1	1	28
84	1	0.8	28	3.45	36	4	2	1.4	1	1	1	28.1
84	1	0.8	28	9.4	45	4	1.25	24	2	1	1	28.3
84	1	0.8	28	14.5	80	5.1	1.22	49.3	3	1	1	28.4
85	2	1.03	30	22.7	39	0	0.616	0	0	2	1	30
85	2	1.03	30	1.9	10	6.1	0.83	17	2	2	1	30.3
85	2	1.03	30	3.5	15	8.1	1	40	3	2	1	30.4
87	2	1.2	30	2.61	12	0	1	0	0	1	2	30
87	2	1.2	30	4.4	10	4.6	0.35	17.3	2	1	2	30.3
87	2	1.2	30	2.4	10	7	0.75	41.4	3	1	2	30.4
88	2	1.2	30	0.8	2	0	0.87	0	0	1	1	30
88	2	1.2	30	1.2	3	15.5	0.5	17.8	2	1	1	30.3
88	2	1.2	30	2.5	8	16.8	0.55	42.3	3	1	1	30.4
89	2	0.8	30	4.6	41	0	0.68	0	0	1	1	30
89	2	0.8	30	2.6	20	4.6	1	0.9	1	1	1	30.1
89	2	0.8	30	0.34	1	7.8	0.48	19.7	2	1	1	30.3
89	2	0.8	30	8.8	13	22	1	43.3	3	1	1	30.4
90	1	1.5	32	4.7	6	0	0.67	0	0	2	1	32
90	1	1.5	32	3.5	12	8.1	1.72	18.5	2	2	1	32.3
90	1	1.5	32	0.1	10	12	1.67	42.1	3	2	1	32.4

89	2	0.8	30	0.34	1	7.8	0.48	19.7	2	1	1	30.3
89	2	0.8	30	8.8	13	22	1	43.3	3	1	1	30.4
90	1	1.5	32	4.7	6	0	0.67	0	0	2	1	32
90	1	1.5	32	3.5	12	8.1	1.72	18.5	2	2	1	32.3
90	1	1.5	32	0.1	10	12	1.67	42.1	3	2	1	32.4
91	1	1.25	30	6.4	24	0	0.75	0	0	1	1	30
91	1	1.25	30	0.9	4	1.8	0.85	0.6	1	1	1	30.1
91	1	1.25	30	2.7	15	6.5	1.16	19.2	2	1	1	30.3
91	1	1.25	30	0	0	9	1	41.2	3	1	1	30.4
92	1	1.45	33	3.4	21	0	2.08	0	0	1	2	33
92	1	1.45	33	3.7	15	1.7	1	0.9	1	1	2	33.1
92	1	1.45	33	1	5	6.6	1.1	20.1	2	1	2	33.3
93	1	1.1	29	9.3	19	0	0.9	0	0	1	1	29
93	1	1.1	29	4.9	31	7	1.43	1	1	1	1	29.1
94	2	1.1	29	2.51	15	0	1.1	0	0	2	1	29
94	2	1.1	29	2.3	10	5	0.93	1	1	2	1	29.1
94	2	1.1	29	0.6	7	5	1.46	17	2	2	1	29.3
94	2	1.1	29	1.8	11	7.7	1	43	3	2	1	29.4
95	1	0.85	28	9.2	122	0	3.15	0	0	2	1	28
95	1	0.85	28	2.65	31	11.3	2.55	0.5	1	2	1	28.1
95	1	0.85	28	2.86	44	6.9	3.5	21.8	2	2	1	28.3
95	1	0.85	28	1.8	26	12.9	2.75	45.5	3	2	1	28.4
96	1	0.9	29	8.1	78	0	3.05	0	0	2	1	29
96	1	0.9	29	6.03	43	5.2	2.5	0.6	1	2	1	29.1
96	1	0.9	29	2.4	31	5.6	3.5	20.13	2	2	1	29.2
96	1	0.9	29	2.7	13	4.9	1.42	43.8	3	2	1	29.4
97	2	1.8	34	6	97	0	2.33	0	0	1	2	34
97	2	1.8	34	1.3	18	5.4	2.27	1.1	1	1	2	34.1
97	2	1.8	34	2	23	4.7	2.4	21.7	2	1	2	34.3
97	2	1.8	34	1.8	16	3.9	1.55	47.3	3	1	2	34.4
98	1	1.3	31	4.5	28	0	2.06	0	0	1	2	31
98	1	1.3	31	1.4	9	5.1	2.18	1.2	1	1	2	31.1
98	1	1.3	31	0.8	7	7.9	1.55	23.14	2	1	2	31.3
98	1	1.3	31	0	0	8.5	1.65	45.5	3	1	2	31.4
99	1	1.7	32	3.7	25	0	2.73	0	0	2	1	32
99	1	1.7	32	3.5	14	3.1	2.05	2.1	1	2	1	32.1
99	1	1.7	32	4.2	34	2.7	2.08	18.7	2	2	1	32.3
99	1	1.7	32	3.55	16	3.3	1.16	40	3	2	1	32.4
10	2	1.4	32	3.4	19	0	2.47	0	0	1	2	32
10	2	1.4	32	4.6	24	6	2	0.93	1	1	2	32.1
10	2	1.4	32	4	23	4.6	1.77	21	2	1	2	32.3
10	2	1.4	32	2.2	23	4.5	2	46.1	3	1	2	32.4
11	2	1.25	30	2.61	33	0	2.67	0	0	1	1	30
11	2	1.25	30	0.3	4	4.6	1.87	1.7	1	1	1	30.1
11	2	1.25	30	1	7	4.6	2.03	18.3	2	1	1	30.3
11	2	1.25	30	1	9	4.5	1.8	41.5	3	1	1	30.4
12	1	1.3	30	8.43	35	0	1.23	0	0	1	1	30
12	1	1.3	30	6.6	42	4	1.67	1	1	1	1	30.1
12	1	1.3	30	5.5	33	5.4	2.12	17.6	2	1	1	30.3
12	1	1.3	30	7.1	55	4.8	1.86	41.7	3	1	1	30.4
13	2	1.25	31	6.3	42	0	1.93	0	0	2	2	31
13	2	1.25	31	2.36	17	4.5	1.93	0.5	1	2	2	31.1
13	2	1.25	31	2.1	21	5.8	1.88	16.5	2	2	2	31.3
13	2	1.25	31	0.78	6	7.4	2	40.4	3	2	2	31.4
14	1	1.5	32	3.7	12	0	1.2	0	0	1	2	32
14	1	1.5	32	3.7	17	5.1	2	1.4	1	1	2	32.1
14	1	1.5	32	1.3	15	6.9	2.31	21.2	2	1	2	32.3
14	1	1.5	32	0.93	8	7.2	1.78	45.7	3	1	2	32.4
15	1	1.4	32	5.14	37	0	2.62	0	0	2	1	32
15	1	1.4	32	6.34	9	5.2	1.16	1.1	1	2	1	32.1
16	1	1.2	30	1.76	11	0	2.38	0	0	2	1	30
16	1	1.2	30	1.98	9	6.5	1.9	1.25	1	2	1	30.1
16	1	1.2	30	0.96	6	6.9	2.03	20.1	2	2	1	30.3
16	1	1.2	30	0.9	6	7.5	1.82	44.2	3	2	1	30.4
17	1	1.05	29	4.83	47	0	2.3	0	0	1	1	29
17	1	1.05	29	1.94	4	6.4	2.32	0.4	1	1	1	29.1

17	1	1.05	29	1.6	16	5.7	2.33	19.3	2	1	1	29.3
18	2	0.95	30	8.1	16	0	1.5	0	0	2	1	30
18	2	0.95	30	5.9	25	5	2	0.75	1	2	1	30.1
18	2	0.95	30	1.3	12	11	2.02	22.8	2	2	1	30.3
19	2	1.75	33	10.7	145	0	2.47	0	0	2	1	33
19	2	1.75	33	3.1	42	4.6	2	0.92	1	2	1	33.1
1	1	1.2	30	0.3	1	0	2	0	1	1	1	30
1	1	1.2	30	0.6	2	4.9	2	1.4	1	1	1	30.1
2	2	1.15	32	6.8	46	0	2.6	0	0	2	2	32
2	2	1.15	32	2.6	14	5.8	1.67	1.13	1	2	2	32.1
2	2	1.15	32	1.8	18	4.9	2.33	17.2	2	2	2	32.3
2	2	1.15	32	0.93	7	4.6	1.67	42.3	3	2	2	32.4
3	2	1.3	32	7.3	56	0	2	0	0	1	2	32
3	2	1.3	32	0.97	13	5.8	2	1.12	1	1	2	32.1
3	2	1.3	32	0.97	13	4.4	2	20.5	2	1	2	32.3
3	2	1.3	32	1.5	1	2.8	1.83	44.6	3	1	2	32.4
4	1	0.95	30	13.1	114	0	2.53	0	0	1	1	30
4	1	0.95	30	6.6	55	4.5	2.05	1.7	1	1	1	30.1
4	1	0.95	30	6.2	42	10.2	2.03	21.23	2	1	1	30.3
4	1	0.95	30	3.52	28	8.9	2.08	43.7	3	1	1	30.4
5	2	1.1	29	8.7	71	0	2	0	0	1	1	29
5	2	1.1	29	2.4	21	5.7	2	1	1	1	1	29.1
5	2	1.1	29	2.7	31	9.8	2.31	12.8	2	1	1	29.3
5	2	1.1	29	6.13	53	7.4	2	36.8	3	1	1	29.4
6	1	1	30	7.43	62	0	2	0	0	1	1	30
6	1	1	30	1.7	8	4.5	0.9	16.7	2	1	1	30.3
124	1	1.2	32	4.11	6.2	0	2.63	0	0	1	1	32
124	1	1.2	32	3	5.7	11.4	2.33	16	1	1	1	32.1
124	1	1.2	32	2.97	6.3	11.5	1.38	21.2	2	1	1	32.3
124	1	1.2	32	2.16	5.04	10.3	1.7	40.4	3	1	1	32.4

APPENDIX C.2

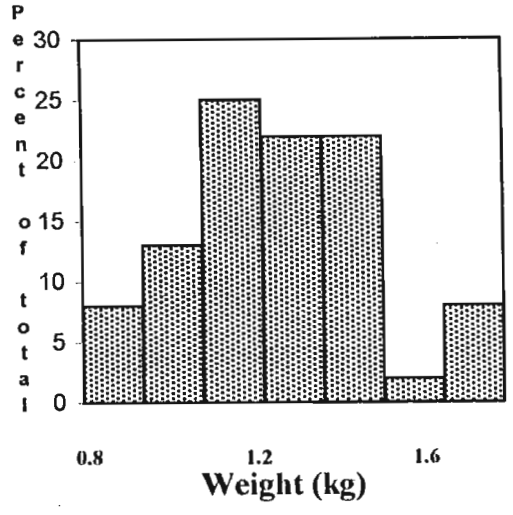
Summary of results and control stream for fractional E_{max} count model

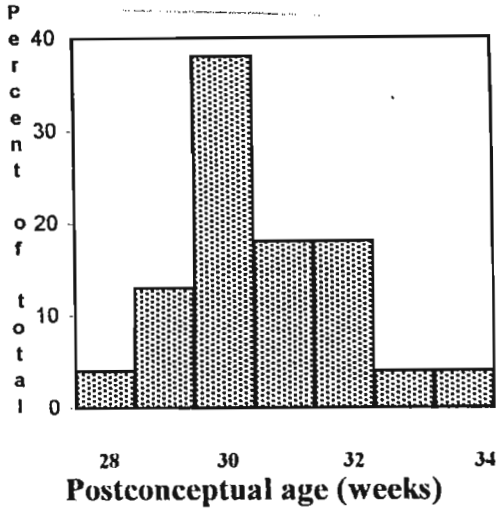
```
THETA:       E0            FEMAX         EC50
ETA:         CVE0          CVEMAX        CVEC50
ERR:
THPD5.LST    1271.191      eval=394 sig=4.2 sub=46 obs=154 CIL=YNNY NV1.0
THETA      = 17.5          0.587         0.478
ETASD      = 0.711337      0.634823      4.06202
ETAR23     =-0.931
ERRSD      =
MINIMIZATION SUCCESSFUL
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS
0.
ETABAR:    -.48E-01     .93E-02     .11E+00
P VAL.:     .63E+00     .90E+00     .82E+00


   user 0.0 real 0.0    tcl 0.0

$PROB POP COUNT DATA
;FAPT=percent of time in observation time (DOBS) that infant was
apnoeic
;NAPN=number of apnoeas in observation time (DOBS)
;DOBS=duration of observation e.g. 2 h on that day (DAY)
;CONC=theophylline conc (mg/L)
;TIME=time of sampling for theophylline conc (h)
;COND=diagnosis
$INPUT ID GEN WT GA FAPT NAPN=DV CONC DOBS TIME DAY COND OXY PCA
$DATA c:\users\marie\theoivpd.prn IGNORE=#

$ESTIMATION METH=COND LAPLACE -2LL MAXEVALS=1000 PRINT=5
;$COV

$THETA (0,15)     ; E0 1
$THETA (0,.4,1)   ; FEMAX 2
$THETA (0,.1,)    ; EC50 3

$OMEGA 0.25       ; CVE0 1
$OMEGA BLOCK (2)
0.25        ; CVEMAX 2
.01 0.25         ; CVEC50 3


$PRED

E0      =THETA(1)*EXP(ETA(1))   ;baseline

TVFEMX =THETA(2)
;Transform Femax into -INF to +INF before adding ETA
;This ensures that an individual value of Femax will be in 0-1 space
TMP=TVFEMX/(1-TVFEMX)
TMP2=LOG(TMP)+ETA(2)
;Transform Femax into 0-1 space
```

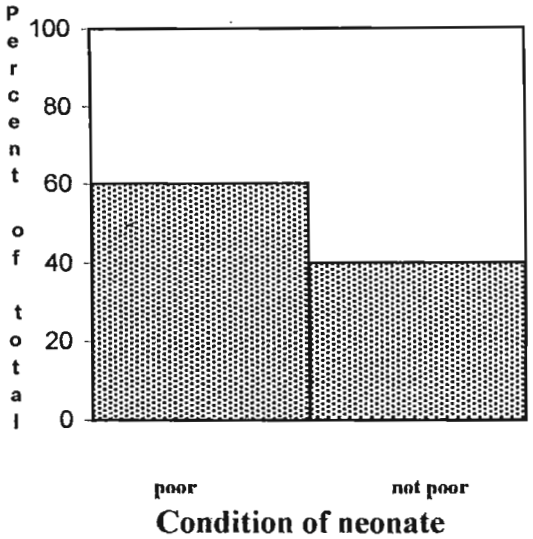
```
TMP3=EXP(TMP2)
FEMX    =TMP3/(1+TMP3)          ;maximum fractional reduction

EC50    =THETA(3)*EXP(ETA(3))   ;EC50

E       =FEMX*CONC/(EC50+CONC)
HAPN    =E0*(1-E)

;Predict number of apnoeas in the observation period
;NONMEM needs actual integer number of observations not a nominal rate
APN=DOBS*HAPN

;Stirlings formula for log DV factorial
IF (DV.GT.0) THEN
  LDVFAC=(DV+.5)*LOG(DV)-DV+.5*LOG(6.283185)
ELSE
  LDVFAC=0
ENDIF
LOGAPN=LOG(APN)
Y=-2*(-APN+DV*LOGAPN-LDVFAC)

HDV=DV/DOBS
;REX=1
;WREX=1
;IPRED=HAPN
;IWRES=1

$TABLE DV CONC HAPN WT GA DAY PCA ETA1 ETA2 ETA3 NOPRINT FILE=THPD5.fit
$SCAT HAPN VS DV   UNIT

;REX=1 ETA(2
;WREX=1 ETA(2
;IPRED=HAPN ETA(2
;IWRES=1 ETA(2

;$TABLE ID TIME IPRED IWRES
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=sdtab002
;$TABLE ID TIME IPRED IWRES
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=mutab002
;$TABLE ID FEMX E0 EC50
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=patab002
;$TABLE ID GA WT PCA
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=cotab002
;$TABLE ID GEN OXY COND
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=catab002
```

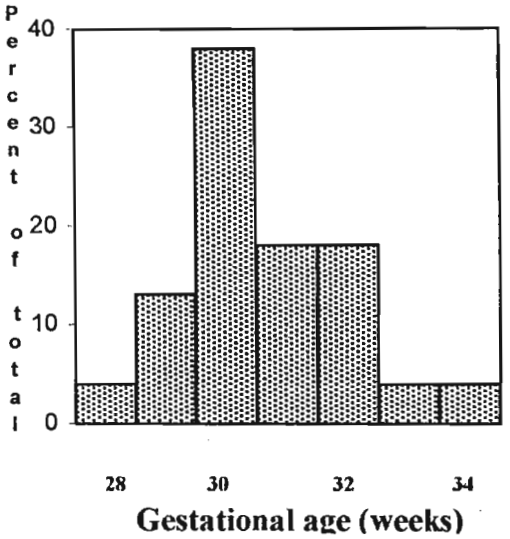
APPENDIX C.3

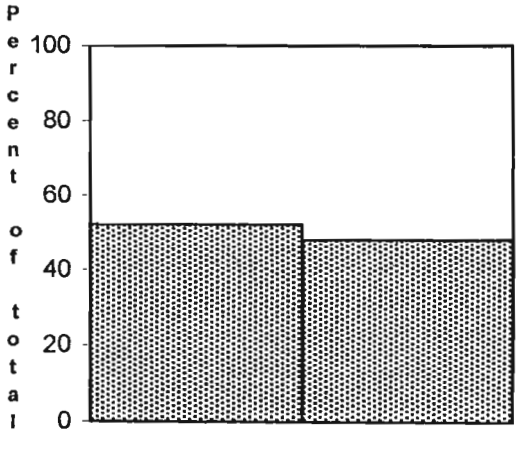
Distribution of the available covariates

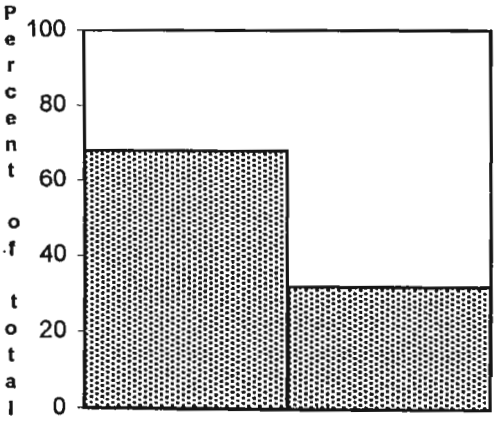












APPENDIX C.4

Summary of results and control stream of sigmoidal E_{max} count model

```
THETA:       E0            FEMAX         EC50          HILL
ETA:         CVE0          CVEMAX        CVEC50
ERR:
CEMXOM2.LST 1269.566     eval=477 sig=4.5 sub=46 obs=154 CIL=YNNY NV1.0
THETA     = 17.2         0.59          0.645         1c
ETASD     = 0.692098     0.583095      3.6606
ETAR23    =-0.834
ERRSD     =
MINIMIZATION SUCCESSFUL
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS
0.
ETABAR:    -.30E-01    .36E-01    .12E+00
P VAL.:     .76E+00    .58E+00    .76E+00


   user 0.0 real 0.0    tcl 0.0

$PROB POP COUNT DATA
;FAPT=percent of time in observation time (DOBS) that infant was
apnoeic
;NAPN=number of apnoeas in observation time (DOBS)
;DOBS=duration of observation e.g. 2 h on that day (DAY)
;CONC=theophylline conc (mg/L)
;TIME=time of sampling for theophylline conc (h)
;COND=diagnosis
$INPUT ID GEN WT GA FAPT NAPN=DV CONC DOBS TIME DAY COND OXY PCA
$DATA c:\users\marie\theoivpd.prn IGNORE=#
$ESTIMATION METH=COND LAPLACE -2LL MAXEVALS=1000 PRINT=5
;$COV

$THETA (0,15)     ; E0 1
$THETA (0,.4,1)   ; FEMAX 2
$THETA (0,.1,)    ; EC50 3
$THETA 1 FIX ; HILL

$OMEGA 0.25       ; CVE0 1
$OMEGA BLOCK (2)
0.25        ; CVEMAX 2
.01 0.25         ; CVEC50 3


$PRED

E0      =THETA(1)*EXP(ETA(1))   ;baseline

TVFEMX =THETA(2)
;Transform Femax into -INF to +INF before adding ETA
;This ensures that an individual value of Femax will be in 0-1 space
TMP=TVFEMX/(1-TVFEMX)
TMP2=LOG(TMP)+ETA(2)
;Transform Femax into 0-1 space again
TMP3=EXP(TMP2)
```

```
FEMX    =TMP3/(1+TMP3)          ;maximum fractional reduction

EC50    =THETA(3)*EXP(ETA(3))   ;EC50

CN=CONC**THETA(4)
E       =FEMX*CN/(EC50**THETA(4)+CN)
HAPN    =E0*(1-E)

;Predict number of apnoeas in the observation period
;NONMEM needs actual integer number of observations not a nominal rate
APN=DOBS*HAPN

;Stirlings formula for log DV factorial
IF (DV.GT.0) THEN
  LDVFAC=(DV+.5)*LOG(DV)-DV+.5*LOG(6.283185)
ELSE
  LDVFAC=0
ENDIF
LOGAPN=LOG(APN)
Y=-2*(-APN+DV*LOGAPN-LDVFAC)

HDV=DV/DOBS
REX=1
WREX=1
IPRED=HAPN
IWRES=1

$TABLE ID TIME IPRED IWRES
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=sdtab002
$TABLE ID TIME IPRED IWRES
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=mutab002
$TABLE ID FEMX E0 EC50
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=patab002
$TABLE ID GA WT PCA
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=cotab002
$TABLE ID GEN OXY COND
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=catab002
```

APPENDIX C.5

Summary of results and control stream for time model

```
THETA:       E0          FEMAX       EC50        HILL
ETA:         CVE0        CVEMAX      CVEC50
ERR:
TEMXOM2.LST 1272.472     eval=303 sig= sub=46 obs=154 CIL=YNNY NV1.0
THETA     = 16.5         0.453       0.0206      1c
ETASD     = 0.711337     1.33417     1.98242
ETAR23    = 0.242
ERRSD     =
MINIMIZATION TERMINATED
DUE TO ROUNDING ERRORS (ERROR=134)
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS
0.
ETABAR:     .31E-01    .17E+00    .24E+00
P VAL.:     .75E+00    .28E+00    .86E-01


   user 0.0 real 0.0    tcl 0.0

$PROB POP COUNT DATA
;FAPT=percent of time in observation time (DOBS) that infant was
apnoeic
;NAPN=number of apnoeas in observation time (DOBS)
;DOBS=duration of observation e.g. 2 h on that day (DAY)
;CONC=theophylline conc (mg/L)
;TIME=time of sampling for theophylline conc (h)
;COND=diagnosis
$INPUT ID GEN WT GA FAPT NAPN=DV CONC DOBS TIME DAY COND OXY PCA
$DATA c:\users\marie\theoivpd.prn IGNORE=#
$ESTIMATION METH=COND LAPLACE -2LL MAXEVALS=1000 PRINT=5
;$COV

$THETA (0,15)     ; E0 1
$THETA (0,.4,1)   ; FEMAX 2
$THETA (0,.1,)    ; EC50 3
$THETA 1 FIX ; HILL

$OMEGA 0.25       ; CVE0 1
$OMEGA BLOCK (2)
0.25        ; CVEMAX 2
.01 0.25         ; CVEC50 3


$PRED

E0      =THETA(1)*EXP(ETA(1))   ;baseline

TVFEMX =THETA(2)
;Transform Femax into -INF to +INF before adding ETA
;This ensures that an individual value of Femax will be in 0-1 space
TMP=TVFEMX/(1-TVFEMX)
TMP2=LOG(TMP)+ETA(2)
;Transform Femax into 0-1 space again
```

```
TMP3=EXP(TMP2)
FEMX    =TMP3/(1+TMP3)          ;maximum fractional reduction

EC50    =THETA(3)*EXP(ETA(3))   ;EC50

CN=TIME**THETA(4)
E       =FEMX*CN/(EC50**THETA(4)+CN)
HAPN    =E0*(1-E)

;Predict number of apnoeas in the observation period
;NONMEM needs actual integer number of observations not a nominal rate
APN=DOBS*HAPN

;Stirlings formula for log DV factorial
IF (DV.GT.0) THEN
  LDVFAC=(DV+.5)*LOG(DV)-DV+.5*LOG(6.283185)
ELSE
  LDVFAC=0
ENDIF
LOGAPN=LOG(APN)
Y=-2*(-APN+DV*LOGAPN-LDVFAC)

HDV=DV/DOBS
REX=1
WREX=1
IPRED=HAPN
IWRES=1

$TABLE ID TIME IPRED IWRES
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=sdtab002
$TABLE ID TIME IPRED IWRES
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=mutab002
$TABLE ID FEMX E0 EC50
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=patab002
$TABLE ID GA WT PCA
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=cotab002
$TABLE ID GEN OXY COND
HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=catab002
□
```

APPENDIX C.6

Summary of results and control stream for time-concentration model

```
THETA:       NORMAL       RMAX         RC50         RTHALF       TC50
RDF0
ETA:         CVNORM       CVRMAX       CVRC50       CVRTHF       CVTC50
ERR:
TCEMOM2.LST 1263.968     eval=460 sig=3.4 sub=46 obs=154 CIL=YNNY NV1.0
THETA     = 8.08         7.8          0.037        0.00691      2.82
1c
ETASD     = 0.663325     1.04403      2.40832c     0c           0
ETAR23    =-0.146
ERRSD     =
MINIMIZATION SUCCESSFUL
ETABAR IS THE ARITHMETIC MEAN OF THE ETA-ESTIMATES,
AND THE P-VALUE IS GIVEN FOR THE NULL HYPOTHESIS THAT THE TRUE MEAN IS
0.
ETABAR:    -.12E-01    .18E-01    .26E+00    .00E+00    .00E+00
P VAL.:     .90E+00    .89E+00    .82E-01    .10E+01    .10E+01
R MATRIX ALGORITHMICALLY SINGULAR
AND ALGORITHMICALLY NON-POSITIVE-SEMIDEFINITE
COVARIANCE STEP ABORTED


   user 0.0 real 0.0    tcl 0.0

$PROB POP COUNT DATA
;FAPT=percent of time in observation time (DOBS) that infant was
apnoeic
;NAPN=number of apnoeas in observation time (DOBS)
;DOBS=duration of observation e.g. 2 h on that day (DAY)
;CONC=theophylline conc (mg/L)
;TIME=time of sampling for theophylline conc (h)
;COND=diagnosis
$INPUT ID GEN WT GA FAPT NAPN=DV CONC DOBS TIME DAY COND OXY PCA
$DATA c:\users\marie\theoivpd.prn IGNORE=#
$ESTIMATION METH=COND LAPLACE -2LL MAXEVALS=1000 PRINT=5
MSFO=TCEMOM2.msf
$COV

$THETA (0,7.7,)    ; NORMAL
$THETA (1,10,50)    ; RMAX
$THETA (0.001,0.209,5)    ; RC50
$THETA (0.001,.0646,5)    ; RTHALF
$THETA (.01,2.09,100)    ; TC50
$THETA 1 FIX       ; RDF0

$OMEGA 0.431       ; CVNORM
$OMEGA BLOCK(2)
0.537          ; CVRMAX
-.309 1.79       ; CVRC50
$OMEGA 0 FIX       ; CVRTHF
$OMEGA 0 FIX       ; CVTC50

$PRED
```

```
NORMAL    = THETA(1)*EXP(ETA(1))    ;normal
RMAX      = THETA(2)*EXP(ETA(2))      ;max increase in APN due to RDF
RTHF      = THETA(4)*EXP(ETA(4))    ;RDF half-life
TC50      = THETA(5)*EXP(ETA(5))      ;theo EC50
RDF0     = THETA(6)
IF (RTHF.LE.0) EXIT 1 1

KD=LOG(2)/RTHF
RC50=THETA(3)*EXP(ETA(3))

;Predict conc of RDF at time=TIME
RDFT=RDF0*EXP(-KD*TIME)

;Predict effect of RDF at time=TIME
ERDF=RMAX*RDFT/(RDFT+RC50)
IF (TC50.LE.0) EXIT 1 3
BASE=NORMAL+RMAX*RDF0/(RC50+RDF0)

;Predict theophylline effect at conc=CONC
ETHEO=CONC/(CONC+TC50)

;Hourly APN is normal plus effect of RDF reduced by theo
HAPN=NORMAL + ERDF *(1-ETHEO)

;Predict number of apnoeas in the observation period
;NONMEM needs actual integer number of observations not an hourly rate
APN=DOBS*HAPN

;Stirlings formula for log DV factorial
IF (DV.GT.0) THEN
  LDVFAC=(DV+.5)*LOG(DV)-DV+.5*LOG(6.283185)
ELSE
  LDVFAC=0
ENDIF
LOGAPN=LOG(APN)
Y=-2*(-APN+DV*LOGAPN-LDVFAC)

HDV=DV/DOBS
REX=1
WREX=1
IPRED=HAPN
IWRES=1

$TABLE ID TIME CONC RDFT ERDF ETHEO ETA(1) ETA(2) HAPN DOBS
NOPRINT ONEHEADER FILE=TCEMOM2.fit
$SCAT HAPN VS DV
;$TABLE ID TIME IPRED IWRES
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=sdtab3
;$TABLE ID TIME IPRED IWRES
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=mutab3
;$TABLE ID NORMAL BASE RMAX RC50 RTHF TC50
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=patab3
;$TABLE ID GA WT PCA
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=cotab3
;$TABLE ID GEN OXY COND
;HDV HAPN REX WREX NOAPPEND NOPRINT ONEHEADER FILE=catab3
□
```

APPENDIX C.7

Control stream of simulation of time-concentration model

```
THETA:      NORMAL      RMAX        RC50        RTHALF      TC50
 RDF0
ETA:        CVNORM      CVRMAX      CVRC50      CVRTHF      CVTC50

ERR:
HAPNSIM.LST     2911.144        eval=0 sig=0 sub=46 obs=154 CIL=YNNY NV
1.0
THETA     = 5           20          1           6           10
 1c
ETASD     = 0c          0c          0c          0c          0
ERRSD     =
ESTIMATION OMITTED

   user 0.0     real 0.0        tcl 0.0

$PROB POP COUNT DATA
;FAPT=percent of time in observation time (DOBS) that infant was apnoei
c
;NAPN=number of apnoeas in observation time (DOBS)
;DOBS=duration of observation e.g. 2 h on that day (DAY)
;CONC=theophylline conc (mg/L)
;TIME=time of sampling for theophylline conc (h)
;COND=diagnosis
$INPUT ID GEN WT GA FAPT NAPN=DV CONC DOBS TIME DAY COND OXY PCA
$DATA c:\users\marie\theoivpd.prn IGNORE=#
$ESTIMATION METH=COND LAPLACE -2LL MAXEVALS=0 PRINT=5
MSFO=HAPNSIM.msf
;$COV

$THETA (0,5,)    ; NORMAL
$THETA (1,20,50)    ; RMAX
$THETA (0.001,1,5)    ; RC50
$THETA (0.001,6,20)    ; RTHALF
$THETA (.01,10,100)    ; TC50
$THETA 1 FIX       ; RDF0

$OMEGA 0 FIX       ; CVNORM
$OMEGA BLOCK(2) FIX
0          ; CVRMAX
0 0          ; CVRC50
$OMEGA 0 FIX       ; CVRTHF
$OMEGA 0 FIX       ; CVTC50

$PRED

NORMAL    = THETA(1)*EXP(ETA(1));normal
RMAX      = THETA(2)*EXP(ETA(2))   ;max increase in APN due to RDF
RTHF      = THETA(4)*EXP(ETA(4))   ;RDF half-life
TC50      = THETA(5)*EXP(ETA(5)) ;theo EC50
IF (RTHF.LE.0) EXIT 1 1
```

```
KD=LOG(2)/RTHF
RC50=THETA(3)*EXP(ETA(3))

;Predict conc of RDF at time=TIME
RDFT=THETA(6)*EXP(-KD*TIME)

;Predict effect of RDF at time=TIME
ERDF=RMAX*RDFT/(RDFT+RC50)
IF (TC50.LE.0) EXIT 1 3
RDF0=THETA(6)
BASE=NORMAL+RMAX*RDF0/(RC50+RDF0)

;Predict theophylline effect at conc=CONC
ETHEO=CONC/(CONC+TC50)

;Hourly APN is normal plus effect of RDF reduced by theo
HAPN=NORMAL + ERDF *(1-ETHEO)

;Predict number of apnoeas in the observation period
;NONMEM needs actual integer number of observations not an hourly rate
APN=DOBS*HAPN

;Stirlings formula for log DV factorial
IF (DV.GT.0) THEN
  LDVFAC=(DV+.5)*LOG(DV)-DV+.5*LOG(6.283185)
ELSE
  LDVFAC=0
ENDIF
"IF(APN.LE.1D-10)APN=1D-10
LOGAPN=LOG(APN)
Y=-2*(-APN+DV*LOGAPN-LDVFAC)

HDV=DV/DOBS
REX=1
WREX=1
IPRED=HAPN
IWRES=1

$TABLE ID TIME CONC RDFT ERDF ETHEO HAPN
NOPRINT ONEHEADER
FILE=hapnsim.fit
```

APPENDIX C.8

Data sheet for simulation of time-concentration model

Parameter and value	Time (h)	Conc (mg/L)	RDFT	ERDF	ETheo	HAPN+T	HAPN0
rdf0 = 10							
Normal = 5							
Rmax = 20							
RDFC50 = 1							
RT50 = 6							
TEC50 = 1							
Theoc0 = 5							
Kd							

Where:

rdf0	concentration of respiratory depressant factor (RDF) at time = 0
Normal	apnoea count of any patient before theophylline administration
Rmax	the maximum increase in apnoea due to RDF
RDFC50	concentration of RDF at 50% of maximum effect of RDF
RT50	time when RDF is half its original value
TEC50	concentration of theophylline at which half of RDF effects are removed
Theoc0	serum theophylline concentration (mg/L)
Kd	dissociation, calculated as LN(2)/RT50
Time	time in hours from 1 to 99 to depict changes in relevant parameters over time
Conc	Theoc0 = serum theophylline concentration (mg/L)
RDFT	concentration of RDF at time = TIME, calculated as rdf0*EXP(-kd*TIME)
ERDF	the Emax model of the RDF factor calculated as = Rmax*RDFT/(RDFT + RDFC50)
Etheo	Emax model of theophylline calculated as = Conc/(Conc + TEC50)
HAPN+T	hourly apnoea counts with effect of theophylline, calculated as = Normal + ERDF*(1 – Etheo)
HAPN0	Hourly apnoea counts before drug is given, calculated as = Normal + ERDF

APPENDIX D.1

Therapeutic Drug Monitoring
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The Pharmacokinetics of Theophylline in Premature Neonates During the First Few Days After Birth

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Summary: The aims of the study were to estimate the pharmacokinetic parameters, clearance rate (CL), and volume of distribution (V) of theophylline in premature neonates during the first few days after birth, and to identify factors contributing to interindividual variability. The authors obtained 263 serum concentrations from 105 apneic premature neonates receiving intravenous (IV) theophylline. Mean (SD) birth weight and postnatal ages were 1.3 (0.3) kg and 1.1 (0.3) days, respectively. The data were analyzed using the nonlinear mixed effects model (NONMEM). A one-compartment model with first order elimination was used. The final models were:

$$CL\ (L/h) = 0.006 * WGT^{0.75} * P$$
$$V\ (L) = 0.63 * WGT$$

WGT = weight (kg)

P = 1.47 with oxygen support and
1.0 without oxygen support.

The CL in the study population was low, resulting in long half-lives. After inclusion of the above covariates, as well as interoccasion variability, the interindividual variability in CL was 56% and in V was 47%. Interoccasion variability in CL and V was 34% and 35% respectively. Theophylline pharmacokinetics are variable in the premature neonate during the first week of life, and this high variability makes it difficult to predict drug concentrations with the same degree of accuracy as in other populations.
Key Words: Theophylline—Premature neonates—Population pharmacokinetics.

The pharmacokinetics of theophylline in premature neonates have been studied using either individual analysis methods (1–8) or population methods such as the Nonlinear Mixed Effects Model (NONMEM) (9–11). In the NONMEM studies, the postnatal ages of the neonates ranged from 1 to 182 days, and in the individual analysis studies from 1 to 36 days. In our setting, neonates invariably receive theophylline for only a few days after birth. There has been very little emphasis in the literature on pharmacokinetics in these very young babies at a time when physiologic parameters are changing rapidly. As a result, the models derived in the above studies may be inappropriate for predicting the doses required in our patients. The objective of this study was to investigate the population pharmacokinetics of theophylline in premature, apneic neonates within the first week of life. In view of the ethical constraints, a population approach was deemed appropriate, as few serum samples per patient would be required.

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METHODS

The study was conducted in the Nursery of King Edward VIII Hospital, Durban, South Africa. It was approved by the Ethics Committee of the Faculty of Medicine, University of Natal and informed written consent

was obtained from the mother for each patient entered into the study.

Any premature neonate under 2 days old, for whom theophylline was prescribed to reduce neonatal apnea, was eligible for the study. Exclusion criteria were congenital malformations and intraventricular hemorrhage of grade III or greater.

All relevant demographic and clinical information was recorded. Gestational age, if not available from sonar scans or menstrual dates, was estimated using the method described by Parkin (12) and verified by comparison of birth weight, length, and occipital frontal circumference on an anthropometric chart that was developed by Lubchenco et al (13). Body surface area was calculated using the formula of Mosteller (14).

Aminophylline (Sabax Aminophylline 250 mg/10 mL, Adcock-Ingram, Isando, South Africa) was administered through an IV line and flushed in with 2 mL normal saline over 2 minutes. Loading doses varied from 4–7.7 mg/kg. Maintenance doses ranged from 1.4–6 mg/kg per day and were given in 2–4 divided doses. All doses were determined by the physician in charge and not by any requirements of the study.

Accurately timed blood samples were collected approximately 1 hour after the loading dose. Thereafter, a sample was obtained each day if possible: these samples were drawn immediately prior to the next dose. The samples were centrifuged immediately and the serum kept frozen at −70°C until analysis. Total serum theophylline concentrations were measured by Emit Assay (Syva, Palo Alto, CA, USA). The coefficient of variation is approximately 6% for both between-run (7.5 mg/L) and within-run (10 mg/L) measurements.

Population Pharmacokinetic Modeling

Data analysis was performed using the computer package NONMEM (NONMEM Project Group, University of California, San Francisco, CA, USA) (version V Level 1.0, double precision) which models fixed and random effects to determine which effects influence the pharmacokinetic parameters.

One- and two-compartment pharmacokinetic models were compared using the appropriate subroutines from the NONMEM library. In the first phase of the analysis, a base model with no covariates on CL or V was used. The interpatient variability in CL and V was generated from exponential models. Various residual error models were tested, namely: exponential, log, proportional, additive, and a combination model. The following covariates were available for testing: Weight, body surface area, gestational age, postnatal age, postconceptual age, Apgar score at 5 minutes, the presence of neonatal jaundice, respiratory support (i.e., oxygen supplied per headbox at time of sampling), and antenatal corticosteroid administration.

The Xpose program (XPOSE 2.0), was then used for model diagnostics and covariate identification (Jonsson EN and Karlsson MO. Xpose - an S-PLUS based model-building aid for population analysis with NONMEM. Department of Pharmacy, University of Uppsala, Uppsala, Sweden). The GAM (stepwise-generalized additive modeling) function in Xpose was used for the identification of covariates of CL and V (15). The bootstrap of the GAM was used to test the stability of the covariate inclusion. Using NONMEM, the covariates selected by GAM analysis were then tested singly and in combination on CL and V, respectively. In the model-building process, criteria used for selecting a model included a reduction in objective function value (OFV) of 6.8 ($p > 0.01$) or greater.

All covariate model-building was performed using the first order estimation method. Thereafter the base and final models were run using the first order conditional estimation method. The presence of interoccasion variability in CL and V was tested at the very end (16). An "occasion" was a different day, i.e., days 1, 2, and 3. All days after day 3 were grouped together as occasion 4. The predictive performance, in terms of bias (mean prediction error) and precision (root mean square prediction error) for the base and final models, was calculated using the population values and comparing these with the post hoc estimates of the final model parameters (17).

RESULTS

The Patients

All the patients were black premature neonates. Demographic and clinical data are summarized in Table 1. Of the 105 patients, 97 (92%) had respiratory distress syndrome at the time of serum sampling. Oxygen support

TABLE 1. *Demographic and clinical data of the study population*

	Demographic data at entry			
	Patients = 105		Males = 52	
	Mean	SD	Median	Range
Birth weight (kg)	1.3	0.3	1.3	0.7–1.9
Gestational age (weeks)	30.8	1.8	31.0	26–34
Postnatal age (days)*	1.1	0.3	1.0	1–2
Postconceptual age (weeks)	31.0	1.8	31.1	26.1–34.1
Body surface area (m^2)	0.117	0.017	0.115	0.081–0.146
Apgar at 5 min	9	1	9	5–10

* Day of birth = day 1.

by head box was given to 64%, 59%, and 48% of the neonates on days 1, 2, and 3 respectively. The peripheral oxygen saturation was measured at intervals of 4 hours and was kept above 90%. Oxygen supply was removed when the concentration was higher than 97%. Antenatal corticosteroids were given to 30% of the mothers. None of the mothers smoked or took caffeine-containing beverages during labor. Most of the neonates (96%) received beta-lactam and aminoglycoside antibiotic combinations for proven or suspected sepsis.

Concentration Measurements

There were 263 concentration measurements taken, with a median of 2 samples per patient and a range of 1 to 7. Of the theophylline measurements, 60% and 94% were obtained in the first 3 days and within 1 week of birth respectively. Average (SD) serum theophylline concentrations for days 1, 2, and 3 were 5.6 (3.1), 7.5 (3.9), and 8.8 (5.7) mg/L respectively. Because of the long half-life of theophylline in neonates, very few of these samples could be expected to be steady state concentrations.

Population Analysis

As there was no significant difference in OFV between one- and two-compartment models, the one-compartment model was selected for further analysis. The exponential residual error model was selected because a frequency distribution of the weighted residuals gave a more normal distribution than did the other error models.

The GAM on CL indicated that oxygen support and gestational age featured as significant covariates. For V, postconceptual age and gestational age were significant covariates. The bootstrap of the GAM showed inclusion frequencies for all the above covariates of greater than 30%.

Weight was also selected for testing on both CL and V because, besides being the most accurately and commonly measured covariate, it was also highly correlated ($r^2 = 0.8$) with both gestational age and postconceptual age. Weight (WGT) was modeled as a continuous function on V and CL and, in addition, as various exponential functions on CL. These exponential functions included the general model of $WGT^{0.75}$ as proposed by Holford (18) and the specific value of $WGT^{1.28}$ found by Moore et al (9) in a study of theophylline in neonates. In addition, an attempt was made to estimate an exponent on weight.

Testing these covariates singly in the NONMEM analysis showed that oxygen support and weight (all models) on CL; and weight, gestational age, and postconceptual age on V all significantly ($p > 0.01$) decreased the OFV. With respect to the covariates on V, gestational age and postconceptual age produced the same reduction in OFV (15.5) while weight produced a larger reduction (35.0). Therefore weight was chosen for further model-building on V. The exponential functions of weight on CL all gave similar changes in OFV (11.6–15.6). Therefore, various combinations of these models on CL were tested together with oxygen support (while weight was on V). At this stage the $WGT^{0.75}$ combination was marginally better than the others and, as a result, the best covariate models were:

$$CL\ (L/h) = \theta_1 * WGT^{0.75} * \theta_3$$

$$V\ (L) = \theta_2 * WGT$$

$$WGT = \text{weight (kg)}$$

$$\theta_3 = \text{with or without oxygen support.}$$

In general, parameter values appeared to be more precisely estimated when the first order conditional estimation method was used. As a result of the relatively high values obtained for interindividual and residual variability, it was thought important to check for interoccasion variability. This resulted in the final model. Parameter values are presented in Table 2. Before testing for interoccasion variability, interindividual variability for CL, V, and residual variability were 60%, 53%, and 29% respectively. On the introduction of interoccasion variability, these decreased to 56%, 47%, and 16% respectively. The OFV changed from 962.3 to 927.9. Calculated using the final model, the mean values (95% Confidence Intervals) for CL in our population, were 0.0074 (0.0070, 0.0078) L/h for neonates without oxygen support, and 0.0104 (0.0099, 0.0109) L/h for neonates receiving oxygen support. The mean (95% CI) value for V for our neonates was 0.81 (0.77, 0.85) L. This results in mean predicted population half-lives, without and with oxygen support, of 76 and 54 hours respectively. For comparative purposes the weight-normalized mean values for CL without and with oxygen are 0.0056 and 0.0084 L/h/kg respectively, and for V is 0.63 L/kg. The relationship between the measured concentrations and those predicted using the final model is shown in Figure 1. The bias and precision of the base and final models, when compared with the post hoc estimates of CL and V from the final model (taken as the "true value"), are given in Table 3. It is evident that there is an improve-

TABLE 2. *Parameter details for base, best covariate, and final models (relative standard error %)*

	Base model	Best covariate model	Final model (with interoccasion variability)
θ_1	0.0074 (16)	0.0056 (19)	0.0060 (14)
θ_3	—	1.61 (23)	1.47 (18)
θ_2	0.75 (9)	0.58 (8)	0.63 (7)
Interindividual variability in CL (%)	73 (27)	60 (29)	56 (25)
Interoccasion variability in CL (%)	—	—	34 (77)
Interindividual variability in V (%)	57 (20)	53 (23)	47 (28)
Interoccasion variability in V (%)	—	—	35 (29)
Residual error (%)	30 (22)	29 (22)	16 (35)
OFV	1000.9	962.3	927.9

θ_3 = 1.0 if no oxygen support.

ment from base to final model in all categories, especially in the bias of CL.

DISCUSSION

The present study differs from previous population analyses of theophylline in the premature neonate as it describes the pharmacokinetics of the drug only during the first few days after birth. This is when theophylline is most often used for the treatment of apnea of prematurity. Other unique features of the present study are the all-black population and the high incidence of respiratory distress syndrome (92%).

In the final model, an exponential function of weight was found to be an important determinant of CL. This is in accordance with the other NONMEM studies of theophylline in neonates (9–11). In two of these studies (9,11) postnatal age was also important, but this was not the case in the present study. In comparison with the present study, these other NONMEM studies had much wider ranges of both postnatal ages (from 1 to 182 days) and weights (0.37 to 7.6 kg).

The weight-normalized CL values for neonates without and with oxygen support were 0.0056 and 0.0084 L/h/kg respectively. These values are lower than those reported in other NONMEM studies. Both Lee et al (11) and Moore et al (9) found CL values greater than 0.012 L/h/kg. The values obtained in the present study are closer to the clearances reported in some of the individual analysis studies. These values ranged from 0.0043 to 0.0299 L/h/kg (2,3,7). In these studies gestational ages and postnatal ages were close to those in the present study.

In neonates, renal clearance of theophylline is relatively more important than metabolic clearance. Approximately 50% of the drug is excreted unchanged compared to about 14% in children and adults (19–21). Theophylline clearance and urinary metabolite patterns apparently reach adult values at 55 weeks postconceptual age (21). Unfortunately we were unable to assess renal function in our study. However, glomerular filtration rate is lower than normal in infants with respiratory distress syndrome (22); therefore the low CL values may be related to the large number (92%) of neonates with respiratory distress syndrome.

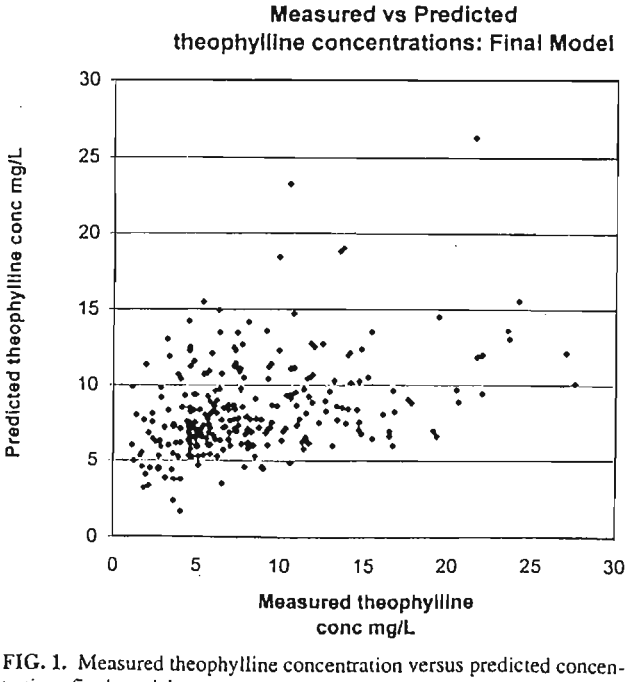

FIG. 1. Measured theophylline concentration versus predicted concentration: final model.

TABLE 3. *Percentage bias and precision (95% CI) for the base and final models*

	Clearance		Volume	
	Base model	Final model	Base model	Final model
Bias	24 (17, 31)	5 (0, 12)	14 (5, 22)	8 (0, 15)
Precision	51 (39, 58)	38 (29, 43)	52 (40, 58)	44 (35, 49)

In our study it was shown that neonates who received oxygen by headbox cleared theophylline 47% faster than those who did not. Although some of our neonates may have been classified as suffering from asphyxia, it was not possible to record this accurately in our situation, and therefore asphyxia could not be tested as a covariate. It is known that hypoxia may decrease theophylline CL (23–25), and that CL is lower in asphyxiated neonates (6,8). Gal et al (6) originally reported a 46% lower CL in asphyxiated than in nonasphyxiated neonates. In a subsequent study Gilman et al (8) found a 19% lower CL in asphyxiated neonates. Asphyxia in these studies was defined as a 1- or 5-minute Apgar score of ≤3, a cardiac or respiratory arrest requiring resuscitation, apnea > 1 min requiring bag breathing, or an arterial oxygen pressure ≤3 torr.

The weight-normalized value of V of 0.63 L/kg is lower than that found in other population studies that reported a range from 0.8 to 0.9 L/kg (9,11). However, this lower value is within the range (0.18 to 0.95 L/kg) recorded in some of the individual analyses (2,3,5) that reported on babies with lower postnatal ages (1 to 26 days).

Karlsson et al (16) reported that if interoccasion variability is not recognized, it may inflate interindividual variability and/or residual variability. Our results support this, as the introduction of interoccasion variability reduced interindividual variability on both CL and V and substantially decreased residual variability.

However, the interpatient variability in CL remained high at 56% for the final model, despite testing all the available covariates. This value is higher than those obtained in the other NONMEM studies. For example, Moore et al (9), Karlsson et al (10), and Lee et al (11) reported interpatient variability in CL of 16%, 25%, and 30%, respectively. Various factors could account for this large interindividual variability in CL. The study was conducted during the first few days after birth when the premature neonate undergoes profound changes to adapt from fetal to neonatal physiology (26–28). During this time not all the neonates were at the same stage of change. For example 20% of the patients had developed neonatal jaundice by day 2, and another 30% from day 3 onward. On entering the study, the majority of the neonates had respiratory distress syndrome. This usually resolves spontaneously, but slowly, after birth and therefore would have differed from patient to patient. These changes, as well as the different stages of immaturity of the physiologic and biochemical systems, may be responsible for much of the variation found in the estimation of CL (19,21,29,30). It could also perhaps be explained in part by the fact that the study was carried out in a busy third world hospital and thus the prevailing clinical circumstances (such as individual nursing care, ambient temperature, etc.) were sometimes variable.

The interpatient variability (47%) on V was also high, but similar to the 44% obtained by Lee et al (11). The three phases of fluid and electrolyte homeostasis (27) and the shift of fluid from the extracellular to the intracellular fluid compartments during the first week of life (31,32) could all contribute to the high variability.

The residual variability of 16% in the present study is similar to the 14% recorded by Lee et al (11) and within the range (9%–25%, depending on concentration) found by Moore et al (9).

The interoccasion variability on CL and V were 34% and 35% respectively. These values have been expressed as if they were random effects, but most likely they reflect systematic changes resulting from development of hepatic and renal function and changes in body composition. Because of the relatively short follow-up period and small sample size, we were unable to describe these changes as a function of time. With more data one would hope to be able to characterize these other systematic factors and thus minimize the interoccasion variability.

In conclusion, CL of theophylline in the premature neonate in the first week of life is low, resulting in long half-lives. Small peak-to-trough fluctuations would be expected, even on once-daily dosing. Our study confirms the high interindividual variability in theophylline pharmacokinetics in the premature neonate, which is only partly explained by the contribution of interoccasion variability. This makes it difficult to predict concentrations with the same degree of accuracy as in other populations. The interoccasion variability in CL of 34% is an indication of the size of variability in steady state concentration in an individual that cannot be improved by therapeutic drug monitoring.

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APPENDIX D.2

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The effect of theophylline on apnoea and hypoxaemic episodes in the premature neonate during the 1st 3 days after birth

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Summary Although the effect of theophylline on apnoea is well documented, its influence on hypoxaemic episodes in premature neonates is less well known. To investigate the influence of the drug on both parameters, 37 apnoeic neonates were monitored before and after theophylline treatment. Incidents and densities of pathological apnoea (cessation of nasal airflow ≥ 20 seconds) were recorded. A fall of $\geq 10\%$ for > 10 seconds in peripheral oxygen saturation was classified as a hypoxaemic episode. Ethical constraints precluded the inclusion of a control group. Each infant served as its own control. Theophylline serum concentrations were 5.6 (3.4), 8 (7.1) and 8 (5.3) mg/l on days 1, 2 and 3, respectively. The apnoea incidents and densities decreased significantly ($p = 0.0001$) from baseline on all 3 days. The total number of hypoxaemic episodes, as well as those not associated with pathological apnoea, decreased, though not significantly. However, those hypoxaemic episodes associated with pathological apnoea and a fall in pulse rate of $\geq 20\%$ decreased significantly from baseline on day 2 only. Throughout the study period, over 80% of hypoxaemic episodes were not associated with apnoea. It is concluded that in the doses used, theophylline was more effective in reducing apnoea than hypoxaemic episodes in premature neonates.

Introduction

Apnoea and hypoxaemic episodes are common in the premature neonate. Most pathological apnoea episodes, i.e. those lasting 20 seconds or longer, are detected by apnoea monitors. However, the hypoxaemic episodes that are unassociated with apnoea may go unnoticed,[1-3] depending on the type of equipment used and the availability of nursing staff. Many studies have shown that theophylline is effective for treating apnoea in neonates.[4-15] However, only two studies have included a control group.[16,17] Very few studies mention any influence of the drug, beneficial or otherwise, on hypoxaemic episodes. One study found a significant reduction in the number of apnoeic spells and total duration of hypoxaemia after theophylline was administered for 2 days but an increase in both parameters 48 hrs after withdrawal of the drug.[9] In another study, after 7 days of theophylline therapy, the number of hypoxaemic episodes decreased, although not significantly.[10] The effects on the neonate of intermittent hypoxaemia of moderate intensity and relatively short duration are as yet unclear. However, some authorities advise that intermittent hypoxaemia and abnormalities in peripheral oxygen saturation should be recognized and treated.[1,18]

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Hypoxaemic episodes unassociated with apnoea or bradycardia could be due to the development of intrapulmonary shunts.[2,19-21] Many factors are implicated in intrapulmonory shunting[2] and, through its wide range of pharmacological effects, theophylline might affect one or more of these factors. The present study investigated the influence of theophylline on apnoea as well as on hypoxaemic episodes, unassociated and associated with apnoea, in a group of premature neonates.

Material and methods

The study was approved by the Ethics Committee of The Faculty of Medicine, University of Natal and was conducted in the nursery of King Edward VIII Hospital. Informed written consent was obtained for each infant entered into the study. Any premature neonate who was not receiving mechanical ventilation or continuous positive airway pressure, and for whom theophylline was likely to be prescribed, was eligible for the study. The neonates were studied irrespective of concomitant diagnoses such as respiratory distress syndrome (RDS). Exclusion criteria were congenital malformations, intraventricular haemorrhage of grade III or greater, and the administration of other drugs known to interact with theophylline. If at least one pathological apnoea (a cessation of nasal airflow equal to or longer than 20 seconds) was detected during an initial monitoring period of at least 2 hours, the neonate was entered into the study.

Most of the neonates received penicillin and an aminoglycoside for proven or suspected sepsis. Only two presented with a positive bacterial culture after the 3rd day of the study. The appropriate antibiotics were then prescribed. Six of the mothers received dexamethasone just before or during labour. None of the mothers smoked and no caffeine-containing beverages were ingested shortly before or during labour.

Full demographic details were recorded. Gestational age, if not available from sonar scans or menstrual dates, was estimated using the method described by Parkin[22] and verified by comparison of birthweight, length and occipital frontal circumference on an anthropometric chart developed by Lubchenco *et al.*[23] Apgar score, development of neonatal jaundice or sepsis, presence of hypo- or hyperglycaemia, full blood count, values of urea and electrolytes, other drugs prescribed and all clinical interventions were recorded. Any factors known to precipitate apnoea were identified and corrected if possible before monitoring. As bolus feeding is known to cause apnoea, monitoring was not done during or after a feed. Blood pressure and mean arterial pressure were recorded at regular intervals using a Dinamap™ Plus Vital Signs Monitor. (Critikon, Tampa, Florida.)

The neonates received aminophylline (Sabax Aminophylline 250 mg/ml, Adcock-Ingram) into the intravenous line, flushed in slowly with 2 ml normal saline. The loading doses varied from 4 to 7.7 mg/kg. Maintenance doses ranged from 1.4 to 6 mg/kg per day and were given in two to four divided doses. All doses were determined by the physician in charge and not by any requirements of the study. The neonates were monitored for as long as possible (usually 2–4 hrs) before the loading dose was given. These pre-dose recordings formed the baseline for the analysis. After the loading dose, the neonates were again monitored for 2–4 hrs and this period was termed day 1. The neonates were then monitored before the early morning maintenance dose on the next 2 days when possible (days 2 and 3).

All neonates were positioned to prevent neck flexion.[24] Oral secretions were removed whenever present.[25] All neonates were kept in the same position throughout the monitoring period, usually in the supine. Handling and disturbance of the babies were kept to a minimum.[26] If any neonate required oxygen during the monitoring period, then this supply was kept constant while monitoring. The neonates were monitored using a Datex Oscar II SC-123 Multigas Monitor (capnograph) and Pulse Oximeter (Datex, Helsinki, Finland) linked to a Datex Data Acquisition system,

(Datex interface cable from the Serial and Analog connector for data recording with a computer, Chicony 386, programmed to read the serial data output.) Peripheral oxygen saturation (SpO_2), pulse rate, inspired and expired carbon dioxide and respiratory rate were recorded. The neonatal pulse oximeter electrodes were attached securely to the foot of the neonate and covered with a black sock to eliminate ambient light and possible interference with the signal. A neonatal sampling line taped in front of the nostrils monitored airflow and respiratory rate. The values from the pulse oximeter were validated with the oxygen saturation of an arterial blood sample whenever such a sample was drawn for clinical purposes. The apnoea alarm setting was 20 secs.

The following information was stored in a time ordered fashion on the computer by the Datex program and later downloaded for analysis: the volume percentage of inspired and expired carbon dioxide, respiratory rate per minute, percentage peripheral oxygen saturation, pulse rate per minute, and the clock time in 10-sec intervals. The monitor gave a beat-to-beat display of the above parameters, a phlethysmographic pulse waveform and a respiratory waveform in a breath-by-breath display. The instrument was calibrated each day before monitoring started.

In 1991, Ruggins pointed out the inadequacies of apnoea monitors, especially the problems of attachment, movement artefact and the failure to detect obstructive apnoea.[27] Therefore, a strict continuous manual record was kept of all incidents of cessation of breathing (shown on the video screen of the capnograph), any movements, mouth breathing, sneezes, yawning and any other interruptions and interventions. These were later used to confirm the computer printout. All apnoea events 20 secs or longer were signalled by the monitoring system. It was possible to identify the pathological apnoea events from the printout and to confirm them using the manual recordings and the alarm. The printout only gave a 10-sec resolution, i.e. an average of the recordings per 10 secs. Thus, it was impossible to identify from the printouts the absolute length of the apnoea event, the precise change in oxygen saturation, pulse rate and respiratory rate. The disadvantages of this system were overcome by using each infant as his/her own control.

From the printout the following were calculated for each monitoring period and then averaged per hour, where appropriate:

- —the number of pathological apnoea incidents (cessation of nasal airflow ≥ 20 seconds);
- —the number of times the SpO_2 fell $\geq 10\%$ for > 10 secs from the average over the previous uneventful minute (hypoxaemic episode);
- —the number of hypoxaemic episodes associated with movement, or pathological apnoea or pathological apnoea with a fall in pulse rate $\geq 20\%$ ('associated with' was defined as an event that occurred within 30 secs of the onset of the hypoxaemic episode);
- —the average SpO_2;
- —the number of times the pulse rate fell below 100 beats per minute (episodes of bradycardia);
- —the average pulse rate;
- —apnoea densities were calculated as the percentage of the time spent in apnoea compared with total monitoring time.[14]

Carefully timed blood samples were collected approximately 1 hr after the loading dose and each morning thereafter during the pre-dose monitoring period. The samples were centrifuged immediately and the serum kept frozen at $-70°C$ until analysis. The theophylline and caffeine concentrations were measured by Emit Assay (Syva Company, Palo Alto, California). The Wilcoxon Sign Rank Test with a Bonferroni correction was used to compare the differences from baseline to days 1, 2, and 3. A probability value ≤ 0.017 indicated significance.

Results

Baseline recordings were obtained from 37 neonates, 21 of whom were boys. The demographic details are shown in Table I. On days

TABLE I. Demographic data of infants (n = 37, 21 boys)

	Median	Range
Weight (kg)	1.25	0.8–1.7
Gestational age (weeks)	30	28–34
Postnatal age (days)*	1	1–2
APGAR—1 min.	7	2–9
APGAR—5 min.	9	4–10

*Postnatal age at start of monitoring. Day of birth taken as day 1.

1, 2 and 3, respectively, 31, 30 and 23 neonates were monitored. The entry diagnosis for all patients was apnoea of prematurity. All infants also had mild-to-moderate respiratory distress syndrome. Ten had been born by caesarean section and ten were one of a set of twins. Seven neonates required ventilation from day 2 and were then excluded from the study. Two of these died, one due to laryngomalacia and the other due to pneumothorax. Six other neonates died subsequent to the 3 days of monitoring. Causes of death were extreme prematurity (2) and pneumonia (4).

Drug serum concentrations

Average (SD) serum theophylline concentrations for days 1, 2 and 3 were 5.6 (3.4), 8.0 (7.08) and 8.0 (5.3) mg/l, respectively. The average caffeine concentrations on the 3 days were 0.1 (0.17), 0.5 (0.95) and 0.7 (1.02) mg/l, respectively.

Pathological apnoea

The results of the pathological apnoea densities and incidents per hour are given in Table II. Although there was a statistically significant decrease ($p = 0.0001$) in the densities and incidents on all 3 days when compared with baseline, individual responses varied. Seven infants showed an increase in the episodes of pathological apnoea on some days, at which times the theophylline concentrations ranged between 4 and 6.5 mg/l. These increases in apnoea incidents were not accompanied by hypoxaemic episodes.

Hypoxaemic episodes

Table II shows that the total number of hypoxaemic episodes decreased from baseline, but not significantly. Likewise, the number of hypoxaemic episodes unassociated with apnoea also decreased, but not statistically significantly. At baseline, 79% of the hypoxaemic episodes were unassociated with pathological apnoea. This changed to 87%, 92%, and 97% on days 1, 2 and 3, respectively. The hypoxaemic episodes associated with pathological apnoea and a $\geq 20\%$ fall in pulse rate decreased significantly ($p \leq 0.003$) on day 2 only.

As theophylline is a central nervous system stimulant, it can increase motor activity. Because increased motor activity has been associated with increased hypoxaemia,[28] we examined hypoxaemic episodes associated with movement. Overall, over the 3 days there was no significant change in the number of hypoxaemic episodes associated with movement. However, one infant experienced a significant increase in these episodes on day 3. His theophylline concentration was 12 mg/l.

Considering total hypoxaemic episodes, nine, eight and six infants had an increase on days 1, 2 and 3, respectively. Each of the latter six infants was one of twins. Nine infants developed neonatal jaundice, three on day 2 and six on day 3. The total number of hypoxaemic episodes, and those hypoxaemic episodes associated with movement, increased in four of these infants.

Average SpO_2, pulse rate, mean arterial pressure (MAP) and episodes of bradycardia

The average SpO_2, pulse rate and MAP per monitoring time, as well as episodes of bradycardia per hour, are shown in Table III. There was no significant change in the average SpO_2 per monitoring time from baseline on any of the other days. As expected for infants on theophylline, the number of episodes of bradycardia per hour decreased significantly ($p \leq 0.001$) over the 3 days, and the average pulse rate per monitoring time increased

TABLE II. Incidents and densities of pathological apnoea and hypoxaemic episodes per hour, median (range)

Day	Incidents of pathological apnoea	Densities of pathological apnoea (%)	Hypoxaemic episodes	Hypoxaemic episodes unassociated with apnoea
Baseline ($n = 37$)	3 (0.4–24)	2.1 (0.3–13.5)	1.8 (0–16)	1.2 (0–15)
1 ($n = 31$)	1 (0–8)*	0.8 (0–4.3)*	1.0 (0–6)	0.7 (0–9)
2 ($n = 30$)	0.5 (0–6.1)*	0.3 (0–3.4)*	1.1 (0–5.2)	0.5 (0–8)
3 ($n = 23$)	0 (0–2.1)*	0 (0–1.6)*	1 (0–23.2)	0 (0–23.2)

*Statistically significant ($p < 0.0001$) compared with baseline.

significantly ($p \leq 0.002$) from baseline. MAP increased significantly ($p \leq 0.002$) on all days.

Discussion

The results of this study, which indicate that theophylline reduces apnoea in apnoeic infants with mild-to-moderate RDS, are consistent with those obtained in premature infants with neonatal apnoea,[6,8,10,14,16] as well as in neonates with concomitant disorders.[9,15,17] Some of the neonates in this study experienced a small increase in pathological apnoea incidents at some stage during treatment. Other researchers have also found a lack of response in some healthy neonates.[13,14,16] Thus, although any conclusions drawn from our study are confounded by the lack of a control group, the significant decrease in apnoea incidents and densities over the 3 days suggests that theophylline in the concentrations used is effective in reducing apnoea in neonates with apnoea plus mild-to-moderate RDS.

A sharp drop in both incidents and densities of pathological apnoea from baseline to day 1 was observed. Similar findings have been reported by others investigating the effect of theophylline in neonates of a comparable gestational age.[6,11,15,16] It is interesting that a sharp decline in apnoea incidents during the 1st 24 hours after birth has also been reported in neonates receiving no medication.[16,29] The profound changes in the physiology of the chemoreceptors and other systems controlling respiration in the 1st few days after birth[30–32] make it difficult to attribute with certainty the decreases in apnoea in the present study to the influence of theophylline alone. Increasing maturity could have influenced the results over the 3 days. Sims *et al.* have suggested that maturity plays a more significant role in decreasing the frequency of apnoea than does theophylline.[16] However, Chen *et al.* showed that brain stem conduction time decreased more in apnoeic neonates receiving theophylline than in an age-matched control group with no apnoea.[33]

As yet, no consensus regarding the definition of a hypoxaemic episode has been reached. Our definition was influenced by the limitations of our equipment which gives a printout of the average over the previous 10 secs. Our value of a fall in SpO_2 of $\geq 10\%$ is in accordance with the alarm limits for apnoea monitors suggested by Upton *et al.*[34] Our study demonstrated a reduction in hypoxaemic episodes but it was not significant. Comparison with other studies is difficult due to experimental differences as well as differences in the definition of an episode. However, Finer *et al.* reported a significant reduction in apnoea attack rates after 7 days of theophylline therapy (serum concentrations 6–19 mg/l) and no significant reduction in the number of falls of $tcpO_2$ greater than 3 mm (11.1 vs 7.2/h).[10] Peabody *et al.*, who also used $tcpO_2$, observed significant decreases in the total duration of hypoxaemia after 2 days of theophylline treatment ($n = 10$, serum concentrations 10–16 mg/l). However, five of the six infants who received theophylline for only 2 days experi-

TABLE III. Average peripheral oxygen saturation (SpO_2), pulse rate and mean arterial pressure per monitoring time, and number of episodes of bradycardia per hour, median (range)

Day	Average SpO_2 (%)	Average pulse rate (bpm)	Mean arterial pressure (mmHg)	Episodes of bradycardia (< 100 bpm)
Baseline ($n = 37$)	96.4 (83.6–98.5)	134.4 (99–159)	34 (29–45)	3.0 (0–151)
1 (n = 31)	96.4 (89.8–98.3)	143.0* (110–170)	38.5* (32–63)	1** (0–59)
2 (n = 30)	96.4 (88.9–98.3)	143.3* (117–188.5)	41* (33–50)	0.5** (0–19)
3 (n = 23)	96.6 (90.3–98.5)	145* (119–162.2)	46* (30–67)	0** (0–5.7)

*$p \leq 0.002$ and **$p \leq 0.001$ compared with baseline.

enced an increase in apnoeic spells and hypoxaemia 48 hours after drug withdrawal.[6] It was reported recently that caffeine, the other methylxanthine used for apnoea of prematurity, did not protect the neonate against the development of hypoxaemia.[35]

As reported in other studies,[2,3,16] many hypoxaemic episodes occurred without apnoea and it has been suggested that these desaturations unassociated with apnoea could be due to intrapulmonary shunts.[2,19,20] This topic has been extensively reviewed by Poets *et al.* who suggest that any change in ventilation-perfusion-ratio (V/Q) could lead to intrapulmonary shunting.[2] Bolivar *et al.* have shown that most hypoxaemic episodes in mechanically ventilated infants are triggered by an expiratory effort that produces a large decrease in lung volume. They suggest that this could lead to closure of small airways and the development of intrapulmonary shunts.[21] Many factors are implicated in intrapulmonary shunting[2] and theophylline, through its wide range of pharmacological effects, could possibly influence ventilation and/or perfusion. Theophylline could improve ventilation through its possible enhancement of alveolar ventilation[37] or its improvement of respiratory muscle function[38] and reduction of diaphragmatic fatigue.[39,40] During hypoxia theophylline may prevent a fall in minute ventilation and respiratory rate.[41] The drug may also improve the regulation of breathing through its stimulatory effect on the respiratory centre of the brain stem.[33] Theophylline might improve perfusion through its positive inotropic and chronotropic effects.[42] A lack of surfactant, as found in RDS, can also lead to V/Q inequalities.[2] Antenatal administration of theophylline has been shown to have a beneficial effect on surfactant production in fetal rabbits[43] and lung maturation in humans.[44] Postnatally, however, the drug had no appreciable effect on RDS.[45] Our results suggest that theophylline did not have a significant beneficial effect on hypoxaemic episodes. The limitation of the study, once again, was the lack of a control group.

The average peripheral oxygen saturation over the 3 days showed no statistically significant change. These findings were consistent with those obtained by Peabody *et al.* using $tcpO_2$.[6] Not surprisingly, theophylline significantly decreased the episodes of bradycardia (pulse rate < 100 bpm) and increased the average pulse rate over the 3 days. Similar effects were noted by Shannon *et al.*[5] The mean arterial pressure showed a consistent increase from baseline on all 3 days. After treatment it remained above 30 mmHg in all cases, ensuring sufficient cerebral blood flow.[46]

In summary, this study has shown that

theophylline, in the doses used, is more effective in reducing apnoea than hypoxaemic episodes. The most pronounced effects on apnoea and hypoxaemia were seen on the 1st day of treatment after the loading dose. The inclusion of a control group would have been ideal, but ethical considerations precluded this.

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GLOSSARY

AP	Apgar score at one minute
AQ	Apgar score at five minutes
bpm	beats per minute
C	concentration
CI	confidence intervals
CL	clearance
CL_R	renal clearance
COND	condition of the neonate
C_p	plasma drug concentration
CPAP	continuous positive airway pressure
DAY	day 1, or 2, or 3 of monitoring
DV	dependent variable
EC_{50}	the concentration of half maximal effect
E0	effect without the drug or baseline effect
F	bioavailability
FEMAX	maximum fractional reduction of apnoea counts
FO	first order estimation method
FOCE	first order conditional estimation method
f_u	fraction unbound drug
GA	gestational age (weeks)
GAM	generalised additive modelling
GEN	gender

GFR	glomerular filtration rate
HAPN	hourly apnoea count
IC_{50}	the inhibitory concentration producing half maximal effect
IOV	inter-occasion variability
IV	intravenous
k_a	rate of drug absorption
LD	loading dose
MAP	mean arterial pressure
MD	maintenance dose
NNJ	neonatal jaundice
OFV	objective function value
OXY	oxygen support
P	covariate such as PCA or WGT etc
PCA	postconceptual age (weeks)
PK/PD	pharmacokinetic and pharmacodynamic
PNA	postnatal age (days)
PR	pulse rate
PRED	predictions
RDF	respiratory depressant factor (hypothetical)
RDS	respiratory distress syndrome
SpO_2	peripheral oxygen saturation (%)
V	volume of distribution
WGT	weight