AN INVESTIGATION INTO THE EFFECTS OF EXTRACORPOREAL MEMBRANE OXYGENATION ON PHARMACOKINETICS

Hussain Mulla

July 2003

A thesis submitted to De Montfort University in partial fulfilment of the requirements of the degree of Doctor of Philosophy

If you believe, it will happen.....

ABSTRACT

There are limited reports describing the effects of extracorporeal membrane oxygenation (ECMO) on drug disposition with minimal emphasis on assessing the appropriateness of dosing regimens. The studies described in this thesis were designed to investigate sorption of drugs by the polymeric components of an ECMO circuit and the clinical pharmacokinetic consequences of both loss of drugs and the expanded circulating blood volume. Derived pharmacokinetic parameters were used to evaluate the significance of these effects and to develop improved dosing regimens.

In vitro investigations of diazepam, lorazepam, midazolam and propofol in contact with plasticised polyvinyl chloride (pPVC) circuit tubing and silicone membrane from the oxygenator, both as static solutions and during simulation of flow through an intact ECMO circuit, revealed a significant capacity for drug loss (range 40-98%, $p<0.005$). In contrast, a reduced loss of morphine was observed with pPVC (16%), whilst no loss was observed with silicone membrane ($p>0.05$). Fractional loss was correlated with the drug's log P value and ionisation status and consistent with a first order diffusion controlled process. Priming the circuit components with human albumin solution significantly decreased sorption by the silicone membrane ($p<0.05$). Sorption was shown to be a reversible process and analysis of *ex vivo* neonatal ECMO circuits confirmed sequestration of midazolam administered during normal clinical care.

The influence of sorption on midazolam pharmacokinetics was investigated in 20 neonates during ECMO. A population pharmacokinetic analysis revealed a one compartment model with time dependent increase in volume of distribution (V) to a maximum mean population value of 4.1 L/kg, three to four times greater than previously reported in neonates, and attributed to reversible sorption by the circuit. Consequently the median (range) steady state half-life of midazolam was substantially prolonged, 33.3 (7.4-178) hours. These results help explain elevated plasma midazolam concentrations observed in the group.

Population pharmacokinetic analysis of aminophylline and vancomycin in a wide age range ECMO population revealed a substantial impact of the expanded circulating blood volume. Estimates of V were significantly higher (e.g. mean 0.57 and 0.71 L/kg respectively in neonates) and clearance was lower (e.g. mean 0.023 and 0.041 L/kg/hr respectively in neonates), than previously reported in non ECMO patients of similar age. Pharmacokinetic parameters were correlated with demographic and clinical covariates such as age, weight and serum creatinine. Additional influences identified include blood recirculation during veno venous cannulation and altered renal and hepatic physiology in this critically ill group. Large interpatient variability in parameters reflected the heterogeneous nature of patients treated on ECMO. Similarly, linear regression analysis of plasma gentamicin concentrations from neonates revealed an enlarged V (0.8 L/kg), and the use of normal dosing regimens, 2.5-3.5mg/kg 8-12 hourly, resulted in a greater frequency of potentially toxic trough levels compared to a modified regimen of 2.5mg/kg 24 hourly (46% and 6.3% respectively, $p < 0.05$). The results of these studies are discussed in terms of their potential impact on pharmacotherapy during ECMO.

AKNOWLEDGEMENTS

A question on a medical ward round, sparked an idea that flamed a fascinating research project. However, a project of this nature would not be possible without the help and support of many people. First, I would like to extend my sincere thanks and gratitude to my supervisors, Dr Graham Lawson and Professor David Upton. Graham has been the ideal supervisor, for he provided guidance and encouragement in equal measures and allowed my ideas to be discussed and pursued. David's appreciation and facilitation of my desire to do research was of immense importance.

I would also like to thank the nurses and clinicians involved in the care of ECMO patients for their co-operation and assistance in collecting blood samples and circuits. In particular, those nurses who often called me at home when a new patient was cannulated or decannulated. My thanks also to Ms Hilliary Killer (Children Services Manager) and Mr Richard Firmin (Director of ECMO) for their support and part funding of the research project.

A special thanks to Dr Peter McCormack (AstraZeneca, R&D, Loughborough) and Dr Amin Rostami-Hodjegan (University of Sheffield) for their help in the pharmacokinetic analysis of midazolam. Thanks also to staff in the Faculty of Applied Sciences, De Montfort University for technical help and Mai Truong for help with the LC-MS analysis.

Special thanks also to the staff and other students of the Centre for Pharmacy Practice Research. In particular Carole Heubeck (for regular cups of coffee), Liz McKechnie (for managerial support), Melanie Squires (for gossip) and Laura Lee Leslie (for help in the collection of vancomycin data).

Finally, I would like to thank my family. To my wife Rabia, for her unstinting support, for recognising how important this has been to me and for sharing my vision. To my son Imran, who always lightened my mood at the end of a long thesis writing day. To my father, Mohammed Adam and my mother, Sara bi bi, without whom I would not be here.

Publications Arising from this Thesis

Papers

- Mulla H, Pooboni S, Jenkins D, Lawson G, Firmin RK, Upton DR. The effects of Extracorporeal Membrane Oxygenation on Vancomycin Pharmacokinetics. **Antimicrobial Agents and Chemotherapy**. (Submitted).

- Mulla H, McCormack P, Lawson G, Firmin R, Upton DR. Pharmacokinetics of Midazolam during Neonatal Extracorporeal Membrane Oxygenation. **Anesthesiology.** 2003; 99 (2): 275-282.

- Mulla H, Nabi F, Nichani S, Lawson G, Firmin RK, Upton DR. Population Pharmacokinetics of Theophylline during Paediatric Extracorporeal Membrane Oxygenation. **British Journal of Clinical Pharmacology**. 2003; 55: 23-31

- Mulla H, Lawson G, Peek G, Firmin RK, Upton DR. Plasma concentrations of Midazolam during Neonatal ECMO. **American Society of Artificial Internal Organs**. 2003; 49: 41-47

- Hawkes L, Mulla H. Acute Respiratory Distress Syndrome – Pathophysiology and Treatment. **The Hospital Pharmacist**. 2001; 8: 249-53.

- Mulla H, Lawson G, Firmin RK, Upton DR. Drug Disposition During Extracorporeal Membrane Oxygenation (Review). **Paediatric and Perinatal Drug Therapy**. 2001; 4(3): 2001.

- Mulla H, Lawson G, Woodland E, GJ Peek, Killer H, Firmin RK, Upton DR. Effects of Neonatal ECMO Circuits on Drug Disposition. **Current Therapeutic Research: Clinical and Experimental**. 2000. 61(11): 838-48.

- Mulla H, Lawson G, Von Anrep C, Burke MD, Upton DR, Firmin RK, Killer H. In vitro evaluation of sedative drug losses during ECMO. **Perfusion**. 2000; 15: 21-26.

Poster Presentations

- Mulla H, Pooboni S, Jenkins D, Lawson G, Firmin RK, Upton DR. The effects of Extracorporeal Membrane Oxygenation on Vancomycin Pharmacokinetics. **12th PAGE Meeting, Verona, Italy, June 2003**.

- Mulla H, McCormack P, Lawson G, Firmin RK, Upton DR. Population Pharmacokinetics of Midazolam during Neonatal ECMO. **SAPS/PKUK Roseno Meeting, Djuronaset, Stockholm, Sweden, October 17-19, 2002.**

- Mulla H, Nabi F, Nichani S, Lawson G, Firmin R, Upton DR. Population Pharmacokinetics of Theophylline during Paediatric Extracorporeal Membrane Oxygenation. **11th PAGE Meeting, Paris, France, 6th – 7th June 2002,**

- Mulla H, Lawson G, Roberts N, Westrope C, Poobani S, Peek GJ, Firmin RK, Upton DR. Plasma Concentrations of Midazolam in Neonates receiving ECMO. 18th Annual CNMC Symposium. **ECMO & the Advanced Therapies for Respiratory Failure, Keystone, Colorado, USA, February 24-28, 2002.**

- Mulla H, Firmin L, Lawson G, Upton DR. Gentamicin Pharmacokinetics in Neonatal ECMO patients: Optimisation of dosing (Abstract). **Care of the Critically Ill. 2001; 17(4): 143**. Presented at the Paediatric Intensive Care Society Spring Meeting, University of Leicester, Leicester, 27th April 2001.

- Mulla H, Lawson G, Upton DR. Chemists and Pharmacists 'stick' together like drugs to plastics. Special Reception for ***Britain's Top Younger Scientists, Engineers and Technologists, House of Commons***, **London, 19th March 2001. Runner up of Westminster Poster Prize.**

- Mulla H, Lawson G, Naheed G, Upton DR. In vitro evaluation of sedative drugs in neonatal ECMO circuits. **Mid year clinical meeting, American Society of Health System Pharmacists, Florida, USA, December 1999.**

ABBREVIATIONS

ACT	Activated Clotting Time
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMDPE	Median Absolute Prediction Error (Precision)
AUC	Area Under the Concentration time Curve between two time points
Cannulation	The surgical insertion of catheters into major venous and/or arterial vessels
CDH	Congenital Diaphragmatic Hernia
CL	Total plasma, serum or blood clearance of drug after intravenous administration (volume/time/kg)
CPB	Cardio Pulmonary Bypass
C_{SS}	Steady State Concentration
CV	Coefficient of Variation (%)
CVVH	Continuous veno venous haemofiltration
CYP 3A	Cytochrome P450 3A
Decannulation	Surgical removal of catheters from major venous and/or arterial vessels
Ex Vivo	Retrieval of clinically used ECMO Circuits
GA	Gestational Age
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IS	Internal Standard
Ke	Elimination rate constant ($Time^{-1}$)
K_{10}	Elimination rate constant from the central compartment ($Time^{-1}$)
k_p	Permeability constant for drug in plastic
K_{sor}	Rate constant for sorption
LC-MS	Liquid Chromatography-Mass Spectrometry
Log P	Octanol-Water Partition Coefficient
MAS	Meconium Aspiration Syndrome
MDPE	Median Prediction Error (Bias)
MIC	Minimum Inhibitory Concentration
MR	Metabolic Ratio of metabolite AUC and parent drug AUC
MS	Mass Spectrometer
OFV	Objective Function Value (ie. The least squares or the maximum likelihood estimator) at the end of each iteration.

OI	Oxygen Index
OST	Optimal sampling time
Peak Concentration	Maximum observed plasma or serum concentration during a dosing interval at steady state
PNA	Post natal Age
PPHN	Persistent Pulmonary Hypertension of the Newborn
pPVC	Plasticised Polyvinyl Chloride
Q	Intercompartmental Clearance (L/hr) between central compartment and another compartment
Residual	Observed minus predicted plasma concentrations
SA	Surface Area
SD	Standard Deviation
SE	Standard Error of mean
SIM	Single Ion Monitoring
$T_{1/2}$	Half-life (time)
$T_{1/2}(0)$	Initial Half-life
$T_{1/2}(ss)$	Half-life at steady State
TDM	Therapeutic Drug Monitoring
Trough Concentration	Minimum observed concentration at the end of a dosing interval at steady state (taken directly before next administration)
τ	Dosing Interval
UGT	Uridine-diphosphate glucuronosyl transferases
UV	Ultraviolet
V	Apparent Volume of Distribution (volume/kg)
V1	Apparent Volume of the Central Compartment
V_0	Apparent initial volume of distribution
V_{max}	Apparent maximum volume of distribution
VA	Veno Arterial
V_{ss}	Apparent volume of distribution at steady state
VT	Apparent tissue volume
$V\beta$	Apparent volume of distribution during the terminal elimination phase
Weighted Residuals	Weighted sum of the squared deviations between the calculated values of the model and the measured values

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

1.1 Background

Extracorporeal membrane oxygenation (ECMO) is often a final attempt at sustaining life, when conventional methods fail. The clinicians and researchers who pioneered this technique, did so in an attempt to push back the frontiers of critical care. Significant advances in oxygenator technology, surgery, and clinical and technical skills made this possible. In contrast, efforts to improve pharmacotherapy during ECMO remained limited.

It is perhaps quite obvious to the clinical pharmacist or pharmacologist during their first consultation with a patient supported by ECMO that drug disposition will be altered. But the important questions are how, to what extent, and will the alteration be clinically significant. Certainly the answer to the last question, at least empirically, appeared to be in the affirmative. Clinical staff often claimed altered therapeutic response particularly with sedative drugs and routinely monitored antibiotics. Though a number of 'theories' were put forward, in particular sorption in to the circuit, there appeared to be very little published evidence. More importantly, there were no suggestions as to how dosing regimens may be optimised.

The series of investigations described in this thesis start with the chemical analysis of drug loss in *in vitro* systems and combine this data with clinical pharmacokinetic studies to establish the influence on drug disposition of two ECMO circuit related phenomenon: drug sorption and an expanded circulating volume. An initial laboratory approach was essential to investigate the physico-chemical factors related to the drug, mobile phase and circuit polymers that may be important in predicting and quantifying the degree of sorption. But to assess the clinical impact of these phenomena, the drug concentration-time relationship in the body also needed to be investigated. Only once the pharmacokinetics were defined, could pharmacotherapy be improved. An additional advantage is that the basic concepts of pharmacokinetics are

common to all drugs; information gained about the pharmacokinetics of one drug during ECMO can help in anticipating the pharmacokinetics of another. Thus, clinical pharmacokinetic studies were planned for a number of drugs during ECMO. However, in order to avoid ethical difficulties associated with blood sampling in children and the rigours of traditional pharmacokinetic studies, a population pharmacokinetic approach was utilised.

1.2 History of ECMO

ECMO is a complex life support technique for critically ill patients, which has been developed through modification of the heart lung bypass machine (Peek *et al.*, 1998). ECMO is capable of sustaining life for days or weeks, permitting treatment and recovery during severe respiratory or cardiorespiratory disease. In practice, the average duration of ECMO support is 5 days for neonates and 10 days for adult patients (Mulla *et al.*, 1998). Over the last three decades, the technology has been developed and improved in the laboratory, investigated in clinical research settings and applied in routine clinical practice (Bartlett *et al.*, 1985). Although the equipment and its application have changed and evolved over time, the basic concept of continuous extracorporeal circulation of blood to provide gas exchange and perfusion remains unaltered (Bartlett *et al.*, 2000).

ECMO was first introduced as a treatment for respiratory failure in the 1970's. The earliest recorded clinical use was in an adult patient with acquired respiratory distress syndrome (Hill *et al.*, 1972). This generated widespread enthusiasm for the technique (Bartlett *et al.*, 1976a; Bartlett *et al.*, 1976b). However, a multicentre trial of ECMO for adult respiratory distress syndrome, reported in 1979, failed to demonstrate any improvement in survival compared with conventional medical therapy (Zapol *et al.*, 1979). As a result, the early enthusiasm for using ECMO in adults waned. In contrast,

however, Bartlett *et al* (1976b) and other groups were able to show the effectiveness of ECMO in neonatal respiratory failure.

Results in neonates have rapidly improved and, since 1985, ECMO has been considered the standard treatment for severe neonatal respiratory failure in the United States, with survival rates exceeding 90 % in some centres (Bartlett, 1990; Bartlett *et al.*, 1985).

ECMO support for neonates was first introduced in the UK in 1989 at Heartlink ECMO Centre, Groby Road Hospital, Leicester. The initial UK experience reported survival rates comparable with the United States (Pearson *et al.*, 1992). Despite this, some British clinicians were reluctant to refer potentially suitable neonates for ECMO because of uncertainty about its clinical and cost effectiveness. Although neonatal ECMO had been subjected to two modified design, randomised controlled trials in the United States comparing it with conventional medical management, there was varying interpretation, with considerable controversy and criticism (Elliott, 1991). Others were concerned that improved survival with the technique might be offset by an increased risk of long term disability.

As a result of this uncertainty in the UK, a multicentre randomised controlled trial of neonatal ECMO was conducted between 1993 and 1995. This study compared neonates referred to ECMO centres against continued conventional management without transfer. The findings of this study again conclusively demonstrated improved survival rates (68% versus 41%) achieved with a well staffed and organised neonatal ECMO service (*UK ECMO Collaborative Trial, 1996*).

1.3 Neonatal ECMO

There is no doubt that ECMO support should be actively considered for term or near term infants, who have severe cardiorespiratory disease that is potentially reversible, and who meet the eligibility criteria (Table 1.1) (*UK ECMO Collaborative Trial, 1996*). ECMO is currently contraindicated in premature infants because of the difficulties in cannulation and the inevitability of intracranial haemorrhage. It is also excluded in infants who have received mechanical ventilation for greater than 10 days. Such prolonged ventilation can cause bronchopulmonary dysplasia, resulting in chronic lung disease that is not easily reversed.

Table 1.1. Eligibility Criteria for Neonatal ECMO

- Gestational age greater than 34 weeks
- Birth weight greater than 2,000 g
- Reversible lung damage (high pressure mechanical ventilation less than 10 days)
- No major intracranial haemorrhage (grade 1 or less)
- No uncorrectable congenital cardiac lesions
- No lethal congenital anomalies
- No significant coagulopathy or uncontrolled bleeding complications

ECMO is not suitable for use in all neonates. Out of 1,000 neonatal intensive care admissions, approximately 0.5 % will be potential ECMO candidates. The vast majority do well on conventional treatment that includes other technological advances, such as high frequency ventilation and specific pulmonary vasodilators such as nitric oxide.

ECMO does not treat acute lung disease, but simply "buys time" for the lungs to recover spontaneously, and allows the implementation of pharmacological and other types of direct lung treatment (for example, physiotherapy and bronchoscopy) while

avoiding the baro and volutrauma caused by mechanical ventilation. The commonest diagnoses that ECMO is used to support are persistent pulmonary hypertension of the newborn (PPHN), associated with meconium aspiration (MAS), congenital diaphragmatic hernia (CDH), respiratory distress syndrome (RDS), isolated persistent foetal circulation, neonatal sepsis and pneumonia.

1.4 Paediatric and Adult ECMO

ECMO is also used as a life support for a wide range of diagnoses in older children and adults. Although the principal criterion remains unaltered, i.e. the underlying disease must be reversible, case selection is all important to prevent merely delaying the onset of death in cases of irreversible lung disease. It is more difficult to draw up definitive suitability criteria for adults than for neonates. Patients ventilated for more than seven days are normally excluded.

Worldwide, the number of older patients treated is much smaller than the number of neonates, and the success rate (to hospital discharge) is less dramatic. However, the programme in Leicester has made significant contribution to the total numbers, with a success rate of 80 % in paediatrics and 70 % in adults, compared with the worldwide average of 52 and 45 % respectively (Peek *et al.*, 1998).

1.5 Principles and Practice of ECMO

1.5.1 Cannulation

As the acronym suggests, the technique of ECMO involved oxygenating the blood outside the body and thus obviates the need for gas exchange in the lungs. The technique is categorised as either veno-venous (VV) or veno-arterial (VA), depending on the type of cannulation. In VV ECMO, deoxygenated blood is drained and oxygenated blood re-infused via venous sites. In neonates, this is achieved by placing a

double lumen cannula in the right internal jugular vein (Figure 1.1). In VA ECMO, deoxygenated blood drawn from the right internal jugular vein is returned oxygenated via the right common carotid artery. While VV ECMO provides support purely with gas exchange, VA ECMO also supports the heart.

1.5.2 Blood Circulation

Blood syphons from the venous cannula through tubing, driven by the right atrial pressure and a drop of one metre, into a distensible venous reservoir at floor level. A roller pump draws blood from the bladder and pushes it through artificial lungs (oxygenators) and then a heat exchanger (to compensate for the cooling effect of the oxygenators), before returning it to the patient (see Figure 1.1).

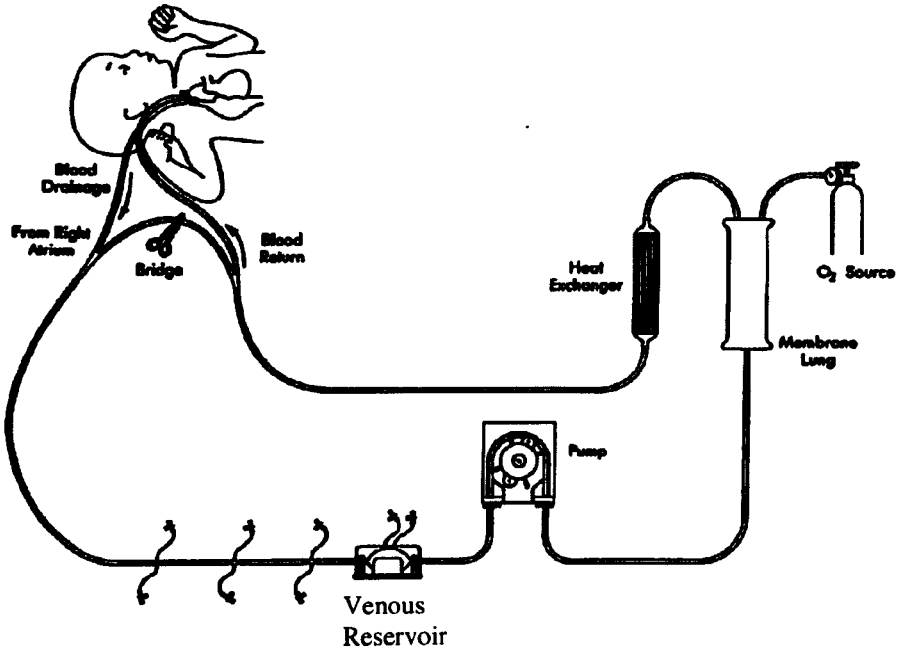


Figure 1.1. Schematic Diagram of a Paediatric ECMO Circuit

1.5.3 Gas Exchange

Gaseous exchange of oxygen and carbon dioxide occurs in the artificial lung oxygenator, and is a function of the rate of blood flow through the oxygenator and the

diffusion gradient between the gaseous phase and venous blood. The latter is maximised by operating a counter current flow of blood and gas through the oxygenator. Monitoring indices of blood oxygenation is an integral part of management, not only in the ECMO circuit, but also directly in the patient to ensure adequate oxygen delivery to tissues.

1.5.4 Pharmacotherapy

Extracorporeal circulation of blood requires anticoagulation to prevent thrombus formation in the circuit and oxygenator. Therefore, during cannulation, the patient is administered a loading dose of heparin, followed by a continuous infusion to maintain the whole blood activated clotting time (ACT) at approximately 180-200 seconds. While on ECMO, whole blood ACT is measured hourly, and more frequently if necessary, to minimise the risk of bleeding, clotting and embolisation. Anaemia and thrombocytopenia are common as a result of red blood cell and platelet destruction by oxygenators, and therefore regular transfusions are made.

All drugs are administered via the ECMO circuit, since the high anticoagulation status of the patient means direct interventions, e.g. intravenous line insertion, are kept to a minimum. Many patients develop renal failure and are managed with continuous veno venous haemofiltration (CVVH), which is attached directly to the circuit. Patients are sedated with continuous infusions of midazolam and morphine titrated to apparent patient comfort, and may be initially paralysed with atracurium to facilitate ventilation. There is a serious risk of infection during ECMO because of the underlying disease processes, compromised immune system, prolonged exposure of blood to the large plastic surfaces, cannulation of major blood vessels and multiple entries into the ECMO circuit. Broad spectrum antibiotics are therefore used prophylactically on a routine basis.

Nutrition is initiated as soon as possible after cannulation with total parenteral nutrition, again infusing it directly into the circuit. Calorific and protein requirements are calculated as in other critically ill patients, and the aim is to sustain positive nitrogen balance.

1.5.5 Complications

Complications during ECMO can be patient related or mechanical. Patient related complications include intracranial infarct or bleed, major bleeding, seizures, metabolic abnormalities (renal failure), and infection. Meticulous monitoring, anticipation and prevention and treatment of these complications is essential. Mechanical complications include clots and air in the circuit, tubing rupture, cannula problems and oxygenator failure. These technical problems require urgent corrective actions, and are kept to a minimum by highly trained ECMO specialists.

1.6 Components of the ECMO Circuit

John Gibbon (1937) was responsible for developing the first extracorporeal cardiopulmonary bypass (CPB) system. The machine provided oxygenation and removal of carbon dioxide via direct contact between the blood and oxygen. The same technique is still applied in the form of the bubble oxygenators, which are frequently used during CPB in the operating room. However, direct blood-gas contact for longer than approximately 6 hours results in red blood cell haemolysis, platelet consumption and protein denaturation (Bartlett *et al.*, 1976a). Bubble oxygenators are therefore not appropriate for use in prolonged applications such as ECMO.

In order to enable prolonged ECMO support, a means for separating the blood and oxygen is required. The ECMO circuit is comprised of four basic components: plasticised polyvinyl chloride (pPVC) tubing, roller pump, membrane lung and heat

exchanger (see Figure 1.1). These four components provide the goals of ECMO: perfusion of warmed, oxygenated blood into the patient.

1.6.1 Circuit tubing

The tubing utilised in the ECMO circuit is Tygon™ S-65-HL, a plasticised polyvinyl chloride (pPVC) construct that is hematocompatible, with extended flex life and low spallation (liberation of particles) properties (Peek *et al.*, 2000; Peek *et al.*, 1999). The 'raceway' tubing (the section within the pump housing which is swept by the rollers) is advanced at specified intervals (usually every 1-7 days) dependent on pump flow rates to avoid rupture due to wear (Zwischenberger *et al,* 2000a).

. Leaching of di-ethylhexyl-phthalate (DEHP), the most common plasticiser used in PVC medical devices (Tickner *et al.*, 2001), with subsequent accumulation in human tissue and serum has been frequently documented (Ganning *et al.*, 1984; Hillman *et al.*, 1975; Jaeger *et al.*, 1970; Lewis *et al.*, 1978), raising concerns (Hill *et al.*, 2001; Tickner *et al.*, 2001). Particular concern has been raised in the paediatric setting because of DEHP exposure in newborns, potentially receiving high doses from blood transfusions and ECMO (Schneider *et al.*, 1989; Sjoberg *et al.*, 1985). In comparison to other medical devices, infants on ECMO may be exposed to the greatest amount of DEHP, between 42-140 mg/kg during the period on ECMO (Schneider *et al.*, 1989).

1.6.2 Roller Pump

The roller pump has been integral to extracorporeal perfusion since the inception of ECMO. However, it requires continuous servo-regulation and monitoring to prevent application of high levels of negative pressure to the drainage circuit and high level of positive pressure, with the risk of circuit disruption, to the infusion circuit should occlusion occur. To prevent generation of negative pressures, a distensible 30ml

silicone venous reservoir, which compresses a spring loaded mechanical switching device that interrupts flow of power to the roller pump, is interposed between the drainage line and the roller pump. The reservoir remains distended as long as venous drainage is adequate for pump flow rate, and the reservoir pressure remains > -20 mmHg (26.7mbar). If pump flow rate exceeds venous drainage (for example due to hypovolaemia, a kink in the venous catheter or connector tubing), the reservoir will collapse, and the power to the pump will be interrupted (Zwischenberger *et al,* 2000a).

1.6.3 Oxygenator

The design of the oxygenator is central to the successful performance of prolonged ECMO support. Excellent gas exchange must be provided while a separation between the ventilating gas and the blood is maintained. The lung should also be non-compliant so that the blood volume remains constant. Finally, blood flow characteristics must be such that development of thrombosis and haemolysis is minimised. There are currently two types of oxygenators that are commercially available for ECMO: membrane lung and hollow fibre. Silicone membrane oxygenators are currently the standard device used for ECMO patients of all ages worldwide. In the year 2000, 76% of all adult ECMO runs were carried out using silicone devices (Peek *et al.*, 2002). However, recently a novel polymethyl pentene (PMP) hollow fibre oxygenator has been marketed, to overcome some of the limitations of silicone membrane oxygenators (see below). The Avecor® Silicone membrane oxygenator was in use at Glenfield Hospital until April 2002, after which it was replaced by the Medos Hilite® LT Hollow fibre oxygenator.

1.6.3.1 Silicone Membrane Oxygenator

First developed by Kobolow *et al* in 1963, this artificial lung consists of two sheets of silicone that are separated by a polypropylene diamond mesh and sealed at the edges.

Connector tubing segments at opposite ends are in continuity with the inside of the silicone envelope (Figures 1.2 and 1.3). The envelope is wound on to a polycarbonate spool. Blood flows through the connector tubing into a manifold region from which it is distributed around the envelope of a membrane lung. Oxygen, mixed with small amounts of carbon dioxide and/or room air, flows through the inside of the membrane envelope in a counter current direction to blood flow. Membrane lungs are available from $0.4m^2$ to $4.5m^2$ in surface area with relatively low priming volumes, $100cm^3$ and $665cm^3$ respectively (see Chapter 3, Section 3.6). They have excellent gas exchange properties because of the high residence time of blood as it flows between the coils of the spiral wound membrane. Although areas of stasis occur, membrane lungs are relatively non-thrombogenic with moderate levels of systemic anticoagulation. The main disadvantages of the membrane lung are a high resistance to blood flow, and a difficult and time consuming priming process (Zwischenberger *et al,* 2000b)

1.6.3.2 Medos Hilite® LT Hollow fibre oxygenator

This long term hollow fibre oxygenator designed for prolonged extracorporeal support was launched in 2001. The gas exchange plasma tight (non porous) hollow fibres are constructed from PMP and have an advantage over the older micro-porous capillaries in that plasma leakage (plasma components on the gas side during long term use) is prevented. The gas exchange efficiency of the hollow fibre lung is far superior to that of the membrane lung. In addition it has a small priming volume, is non-compliant, and is easy to prime and debubble. The hollow fibre oxygenator also has a lower resistance to blood flow, a smaller surface area and a reduced propensity for platelet consumption, compared to the silicone membrane oxygenator. This is as a result of a faster blood transfer rate.

Figure 1.2. Avecor™ Silicone Membrane Oxygenators (Neonatal and Infant)

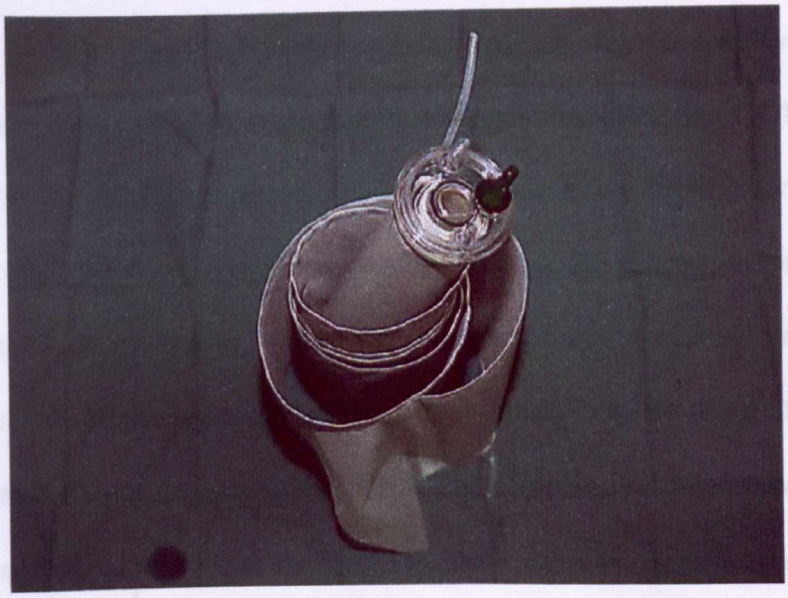


Figure 1.3. An unravelled Avecor™ Silicone Membrane Oxygenator

1.6.4 Heat Exchanger

Since heat is lost through the evaporation of water as blood flows through the artificial lung, it is necessary to warm the extracorporeal blood before return. This is especially important for newborn temperature maintenance. At times the heat exchanger may also be used to cool the patient to a preset temperature in order to effect a decrease in oxygen consumption, for example if cerebral hypoxia is a concern.

1.7 Drug Disposition During ECMO

Pharmacological therapy in the ECMO patient presents a challenge since the continuous extracorporeal circulation of blood may conceivably impact on pharmacokinetics and pharmacodynamics. Despite the rapid advancement in many of the technical aspects of ECMO, much remains to be discovered regarding pharmacotherapy. Many of the previous studies investigating the impact of extracorporeal circuits on drug disposition have been in the context of CPB. These studies provide a useful introduction to the complexities of the issues. However, whereas the CPB phase of cardiac surgery lasts a few hours, ECMO support for cardiorespiratory failure may run for days or weeks. Furthermore, many of the pharmacokinetic changes studied during CPB have been hampered by the short time period on bypass, at most a few hours. Pharmacokinetic analysis is often best conducted under steady state conditions, which for most drugs will not be reached during bypass. Moreover, pharmacokinetic evaluation and modelling assumes that the physiological processes remain fairly constant during the study period, which is certainly not the case during CPB. Therefore, only limited conclusions may be drawn from observations made during CPB, and cannot necessarily be extrapolated to ECMO. It is also important to draw a distinction between the likely effects on disposition of drugs administered prior to ECMO (bolus and continuous infusion), where the initial effects of haemodilution and protein binding changes may be greater,

and those administered during ECMO where sorption of drugs and altered hepatic and renal blood flow may play a greater role.

The overall paucity of clinical data available on drug disposition in ECMO presents a clinical challenge. Intensivists managing patients during ECMO should have an understanding of the potential effects that extracorporeal circuits may have on the disposition of drugs.

1.7.1 The Effects of Flow Rates and Injection Sites on Drug Delivery

One of the most fundamental differences between ECMO and non-ECMO patients is the site of drug delivery. The ECMO circuit has multiple ports available for infusion of drugs, blood products, parenteral nutrition, for blood sampling and for attachment of CVVH circuits although the exact location of these ports varies amongst centres. The method of drug administration during ECMO also varies amongst centres with no current accepted or published guidelines.

Although it is possible to administer drugs via direct access into the patient, systemic heparinisation necessitates minimal direct interventions, such as intravenous line insertion, in order to avoid excessive bleeding. Drugs administered via an umbilical venous catheter opening at the entrance of the right atrium may be recirculated to the ECMO circuit, though there is probably an element of drug recirculation during VV ECMO anyway (see section 1.7.4.2). Most drugs are therefore administered directly into the ECMO circuit, although the exact location may differ amongst centres.

The effects of circuit injection sites and flow rates on the distribution of injected solutions have been studied *in vitro* using isolated ECMO circuits with different sized venous reservoirs (30 and 50cm^3), minus the membrane oxygenator and heater (Hoie, 1993). A bolus of 0.8cm^3 of Bordeaux red, a water-soluble dye, followed by a 3cm^3 flush, was injected into the circulating fluid at sites proximal, distal and directly into the

venous reservoir. Samples taken at varying ECMO flow rates and intervals were analysed for dye concentration. Dye injected proximal and directly into the venous reservoir pooled at the top of the reservoir at flow rates of 75 cm^3/minute (such as during the weaning phase of ECMO). The concentration of dye at the top of the venous reservoir during stagnation was 30 times greater than in the ECMO circuit and this stagnation lasted approximately one hour. Furthermore, the investigators determined that some pooling of dye was possible at flow rates less than 250 cm^3/min. Dye injected distal to the reservoir did not pool in the venous reservoir at any flow rate studied (Hoie, 1993).

These results may be explained by the lack of turbulence at low ECMO flow rates, so that the dye, which has a lower specific gravity, stagnates at the top of the venous reservoir, an area of decreased flow. In fact, the specific gravity of most drugs used clinically is lower than blood and hence, pooling may contribute to incomplete drug delivery. Although these results suggest that drug administration distal to the reservoir may be optimal, this site increases the risk of air embolism. Thus many ECMO centres choose to administer drugs proximal to the reservoir, allowing the top of the venous reservoir to serve as an air trap.

1.7.2 Haemodilution

The most obvious alteration to pharmacokinetics occurs on initiation of ECMO, when the patients own blood volume mixes with the priming volume in the extracorporeal circuit. For example in neonates, the effective circulating volume will be approximately doubled to 900cm^3 on initiation of ECMO. One possible effect of this acute haemodilution is a decrease in the total blood concentration of any drug present. The pharmacological impact will depend on the apparent volume of distribution (V) of the drug, the degree of protein binding and the extent of equilibration between tissue

concentrations and plasma concentrations on initiation of ECMO. Drugs with a large V (e.g. fentanyl) would be expected to show only a slight change following the expansion of plasma volume, the initial lowering of plasma concentration from haemodilution being counteracted by the back diffusion of the drug into plasma from the large tissue reservoirs. In contrast, a drug with a small V (e.g. gentamicin) may be significantly affected, since the resultant enlarged apparent V may affect elimination of the drug.

The acute haemodilution on initiation of ECMO also produces a large reduction in circulating plasma protein concentration such as albumin and $\alpha 1$ acid glycoproteins. In blood, drugs exist as free (unbound) drug in equilibrium with protein bound drug and it is the free drug that interacts with the receptor site to exert a pharmacological effect. For drugs that are highly protein bound, decreased concentration of binding proteins will lead to an increase in the fraction of unbound drug. This favours transfer of drug from the plasma to the tissues and contributes to the lowering of plasma concentration. The pharmacodynamic result of this may be an increased effect because of an increased free fraction at the receptor sight. Another effect is acute anaemia, which may affect the degree of drug binding to red blood cells (Hynynen, 1987). This would be a transient effect in ECMO however, as following cannulation it is standard practice to normalise the effects of haemodilution by transfusing blood and related products, including albumin.

The effects of continuous heparin administration on plasma protein binding may also be of importance. In addition to displacing drugs bound to proteins, heparin induces release of lipoprotein lipase and hepatic lipase, increasing the plasma concentration of free fatty acids, which may further displace drugs from protein binding sites and increase free drug levels with resultant enhanced pharmacological effect.

The immediate effects on plasma concentration of haemodilution only (disregarding changes in protein binding effects) may be described by the formula (Mets, 2000):

$$\Delta Cp = \frac{Cp \times Vc}{(V1+Vc)}$$

ΔCp = Change in drug concentration Vc = Volume of circuit

Cp = Plasma concentration prior to haemodilution

V1 = Volume of distribution of the central compartment

1.7.2.1 The Effects of Haemodilution and Protein Binding changes on Drugs administered during ECMO

The expanded circulating volume should not significantly affect drugs with a high V and low protein binding administered during ECMO. However, it is possible that the properties that confer a higher V (e.g. lipid solubility) may result in greater interactions with the circuit (see Section 1.7.5). A drug with a low V administered during ECMO will tend to have a lower initial plasma concentration due to the dilution effect and its elimination may be significantly affected. The effect on a drug with high protein binding will depend on whether the drug has a high or low extraction ratio. A drug with high extraction ratio could have higher effective (free drug) plasma concentration (because of decreased protein binding) through heparin displacement and hence greater distribution into tissues resulting in a higher apparent V. A drug with a low extraction ratio could result in reduced free concentrations and therefore therapeutic effect due to a higher clearance.

1.7.3 Physiological Changes

Anderson *et al* (1992) noted an initial increase in bodyweight of between 5 and 30% in neonates with severe respiratory failure post cannulation for ECMO. They attributed this increase to the initial resuscitation prior to cannulation and intrinsic increases in intracellular and extracellular water. Such a fluid expansion could significantly affect the V of many water soluble drugs. Furthermore, many ECMO patients will require CVVH in an effort to diurese to dry weight and improve lung function. Although CVVH improves outcome, it adds uncertainty to the pharmacokinetics of drugs.

Perfusion of tissues may also be altered as a result of activation of the systemic inflammatory response syndrome releasing a variety of autonomic, endocrine and local cytokine reflexes that may affect not only tissue distribution of drugs but probably clearance mechanisms as well (Gravlee *et al.*, 2000).

1.7.4 Changes in Blood Flow

1.7.4.1 Pulsatile versus Non Pulsatile Blood Flow

Whereas VV ECMO results in pulsatile blood flow, VA ECMO at high low rates (> 100 cm^3/kg/min) may produce non-pulsatile flow. Non-pulsatile blood flow can alter perfusion of tissues, reducing capillary circulation and aerobic metabolism (Shevde *et al.*, 1987). Under experimental conditions, pulseless perfusion of the kidneys of dogs resulted in reduced urine production and impaired sodium excretion, although glomerular filtration was not affected (Many *et al.*, 1986). The kidneys interpret pulseless blood flow as hypotension and activate the renin-angiotensin system (Bartlett, 1990). Regional blood flow changes in the liver can also affect drug clearance, in particular those drugs with a high extraction ratio e.g. propranolol, lignocaine (Mckindley *et al.*, 1998).

1.7.4.2 Recirculation

The recirculation phenomenon is a major disadvantage of the VV mode of cannulation. A fraction of blood that has been oxygenated in the circuit flows directly from the reinfusion site to the drainage catheter and back into the circuit instead of the patient's circulation. Figure 1.4 demonstrates this for a double lumen catheter used in neonates. Four main factors affect recirculation: pump flow, catheter position, cardiac output and right atrial size (intravascular volume). The most important parameter and one that changes throughout the course of ECMO is pump flow. If the pump flow is high, the drainage of blood from the right atrium back into the ECMO circuit is higher, and streaming of blood from the reinfusion catheter to the venous drainage catheter is more likely to occur (Figure 1.4). Recirculation fraction increases linearly with increasing pump flow, whereas effective flow (oxygen delivery) versus pump flow reveals a non-linear relationship (Figure 1.5). The reason for the decreased effective oxygen delivery is that the recirculation proportion has limited the amount of oxygen delivered to the patient.

Effective Flow = Total Flow – (Total Flow × Recirculation Fraction)

When total flow is zero, the effective flow is zero. At some maximal flow the recirculation fraction is 100 %, and effective flow again becomes zero. The ideal pump rate is the one that provides the highest effective flow at the lowest revolutions per minute and the least degree of tubing wear and haemolysis (Zwischenberger *et al*, 2000c).

.

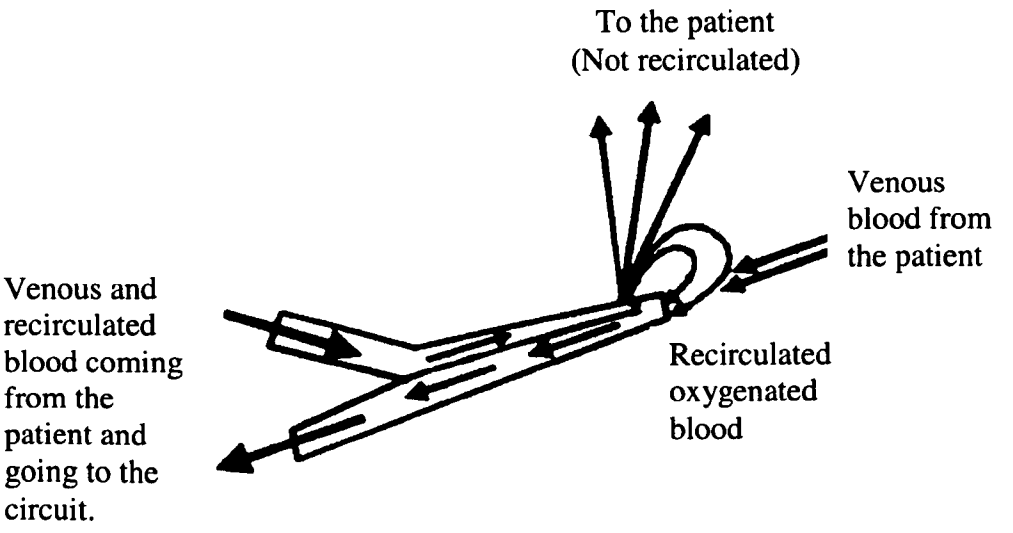


Figure 1.4. Recirculation in a double lumen VV Catheter

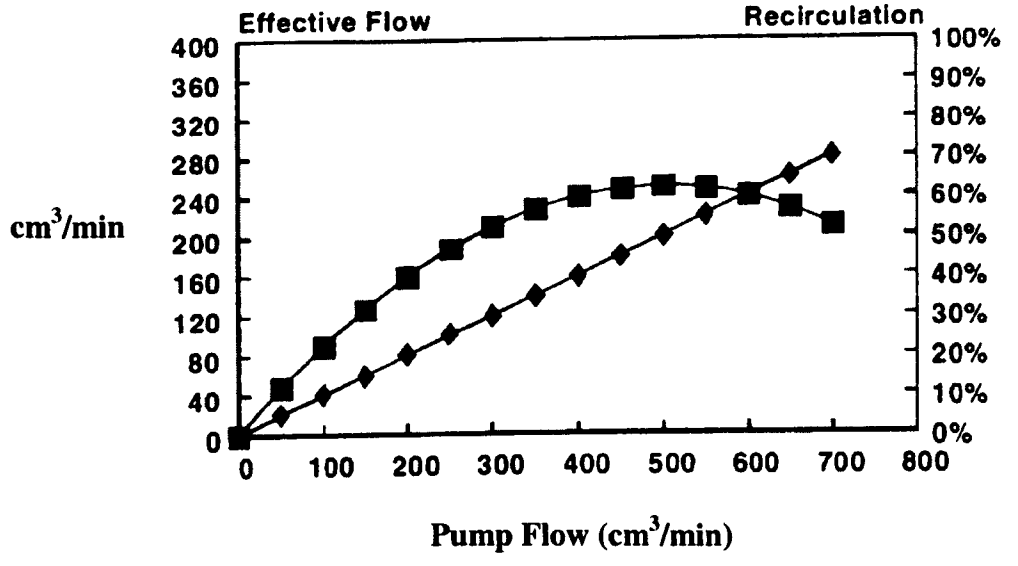


Figure 1.5. The effect of recirculation (⇦) on effective flow (■). As pump flow increases, recirculation increases, whereas effective pump flow increases initially to a maximum and then decreases.

It can be postulated that recirculation will also have a significant effect on pharmacokinetics. Consider a bolus dose of a drug administered into the circuit. At high flow rates and therefore high recirculation, only a proportion of the dose will be directed into the systemic circulation for distribution into tissues, the remainder will be redirected into the circuit. Sampling of blood immediately after the dose would reveal lower drug concentrations from systemic sites than from the circuit. At high flow rates however, equilibrium between circuit and systemic drug concentrations would be rapidly attained. The concentration-time profile would possibly reveal an ECMO distribution phase, distinct from the tissue distribution phase. As well as influencing distribution, elimination of drug is also expected to be reduced since less drug is available to organs such as liver and kidney. The impact of recirculation is discussed further in Chapters 5 and 7.

1.7.5 Drug Sorption

1.7.5.1 Interaction of drugs with Plastic Intravenous Delivery Systems

The use of plastic components in intravenous delivery systems is widespread in clinical practice. However, there has arisen the problem of drug sorption to the plastic and consequent reduction in bioavailability. For example, drugs such as insulin, glyceryl trinitrate and diazepam are well known to bind to plasticised polyvinyl chloride (pPVC) infusion bags, administration sets and filters (Baaske *et al.*, 1980; Cloyd *et al.*, 1980; Hirschl, 1981; Kowaluk *et al.*, 1981; Kowaluk *et al.*, 1982; Martens *et al.*, 1990; Parker *et al.*, 1980; Roberts *et al.*, 1980). Such interactions may result in reduced drug delivery to the patients and, in some cases, diminished therapeutic response (Kowaluk *et al.*, 1981). As the availability of medication through the intravenous route is of critical importance in patient therapy, it is essential to know not only which substances may be

lost during intravenous infusion, but also which drugs may be safely administered using plastic intravenous delivery systems.

Factors affecting the degree of uptake include the physico-chemical characteristics of the drug, the nature of the plastic, plastic surface area, solution volume, solution pH, time and temperature (Kowaluk *et al.*, 1982; Roberts *et al.*, 1991). The extent of ionisation and the lipid solubility of a drug are among the main physico-chemical determinants of its sorption by plastic components. Previous studies have shown that uptake of weakly ionised drugs by plastic infusion bags is pH dependent, the loss being accounted for by the preferential uptake of the non ionised species (Kowaluk *et al.*, 1981). The octanol/water partition coefficient (log P value) is a measure of a chemical compound's affinity for the organic or aqueous phase, or distribution between the two. Thus compounds with a high ratio will be very soluble in organic materials such as plastics and can be expected to exhibit considerable loss. Roberts *et al* (1991) showed it is possible to predict drug loss given the log P value of the drug of interest (Table 1.2). Differences in the predicted drug loss between $100cm^3$ and $500cm^3$ bags are probably due to differences in the surface area to volume ratios (see below).

Table 1.2. Observed and Predicted Losses of Drugs from Sodium Chloride 0.9% solutions stored in Polyvinyl Chloride Bags at 22°C for 8 hours

Drug	log P	Percent of Original Concentration Loss			
		100ml Bags		500ml Bags	
		Observed	Predicted	Observed	Predicted
Diazepam	2.7	59	41	31	27
Oxazepam	2.2	22	30	12	16
Nitrazepam	2.1	15	29	10	15
Glyceryl Trinitrate	2.2	54	31	—	16
Thiopentone	3.0	25	49	—	19
Pentobarbitone	2.0	0	27	0	14

Studies on different types of plastics show differing degrees of sorption. Sorption to containers, syringes and infusion lines can be avoided by using materials such as glass, polyolefin (polyethylene or polypropylene) whilst studies with polybutadiene manufactured and polyethylene lined pPVC administration sets have revealed significantly reduced sorption (Bianchi *et al.*, 1992; Kowaluk *et al.*, 1983; Lee, 1986; Martens *et al.*, 1990). The rate and extent of uptake for a given drug is significantly higher in the more flexible pPVC and may be dependent on the nature and concentration of the plasticiser or other additives used in the polymer (Yahya *et al.*, 1988).

Kowaluk *et al* (1980) and Baaske *et al* (1982) also demonstrated that the uptake of drugs by plastic infusion devices is dependent on the surface area of plastic-to-volume of solution ratio. The amount of sorption will increase when the ratio is higher. For example, small containers will increase the amount of sorption because the ratio is higher than in large containers (Table 1.2). This ratio and therefore consequent drug loss is higher still in tubing compared to containers (Cloyd *et al.*, 1980). Yliruusi *et al,* 1986 showed with simulations of diazepam infusions through pPVC intravenous administration sets that sorption of drug is a biexponential process and is dependent on duration of infusion as well as length of tubing. Furthermore, in a follow on study the same workers also revealed that sorption was highest when the diazepam infusion rate was lowest (Yliruusi *et al.*, 1986).

1.7.5.2 Drugs sorption by Extracorporeal Circuits

Much of the work investigating drug uptake by extracorporeal circuits has been carried out as *in vitro* experiments in CPB circuits. These report significant sequestration of opioids (alfentanil, fentanyl, morphine), benzodiazepines (midazolam, diazepam, lorazepam), glyceryl nitrate and propofol (Booth *et al.*, 1991; Dasta *et al.*, 1983; Dasta

et al., 1986; Hynynen, 1987; Hynynen *et al.*, 1994; Keaveny *et al.*, 1991; Koren *et al.*, 1984; Rosen *et al.*, 1988a; Rosen *et al.*, 1988b; Silvasi *et al.*, 1989; Skacel *et al.*, 1986).

The component with the greatest capacity for binding is the silicone membrane oxygenator (Rosen *et al.*, 1990). Furthermore, reversibility of this binding appears to be related to the drug's protein-binding characteristics, lipophilic drugs that are more highly protein bound displaying more reversibility (Rosen *et al.*, 1990). The majority of CPB oxygenators currently available on the market today are polypropylene based. They have a microporous structure in a microtubular or sheet design and appear to sequester drugs to a much lesser degree (Rosen *et al.*, 1990).

The component materials of an ECMO circuit are very similar to the CPB circuit. The circuit tubing is composed of pPVC whilst the most common oxygenator is the Avecor™ silicone membrane construction. It is therefore perfectly plausible that both of these components will lend themselves heavily to drug sorption and hence reduce bioavailability since the patient's blood is continuously exposed to a large surface area during ECMO. Comparatively fewer *in vitro* investigations have been conducted in ECMO circuits (Table 1.3) (Dagan *et al.*, 1993; Marx *et al.*, 1991).

The clinical significance of sorption may be difficult to predict. A susceptible drug administered as a bolus dose may result in attenuation of the expected plasma concentration. This is likely to be a first order process i.e. fractional uptake is independent of circulating concentration. The rate of uptake into the plastic is thought to be an equilibrium process between the mobile phase (blood) and the plastic components, although true equilibrium will not be attained in a flowing circuit. Thus, the level of drug reaching the patient will be at some intermediate concentration between that injected and the steady state equilibrium value. In a drug with a small V, the pharmacological impact of the decrease in plasma concentration will depend on the effect this will have on the concentration at the receptor site. Drugs with a large volume

of distribution may be less affected since any drug removed by the circuit will be replaced by the large tissue reservoirs.

Table 1.3. Sorption of Drugs by the ECMO Circuit

Drug	References	Component Tested			log P
		Circuit	pPVC tubing	Silicone Membrane Oxygenator	
Morphine Sulphate	*Dagan et al., 1993*	✓	–	–	0.8
Fentanyl	*Rosen et al., 1988a*	–	–	✓	2.9
Phenytoin Sodium	*Dagan et al., 1993*	✓	–	–	2.5
Phenobarbitone Sodium	*Marx et al., 1991*	–	✓	×	1.5
	Dagan et al., 1993	✓	–	–	
Gentamicin Sulphate	*Dagan et al., 1993*	✓	–	–	Very Low[a]
Vancomycin Hydrochloride	*Dagan et al., 1993*	✓	–	–	Very Low[a]

✓ = Loss of drug × = no loss of drug

a. Log P values not be determined since their partition coefficients are less than or equal to the minimum values (Jenke, 1994).

The impact of this phenomenon may also be different in CPB compared to ECMO because the two processes differ in terms of time span. For example, uptake of drugs is assumed to be a first order diffusion process with circuit polymers behaving as sinks (see Chapter 3). This assumption will only apply for the time before drug diffuses to the outside surface of the plastic. An estimate of the lag time for the appearance of the drug in the outside environment assumes a constant concentration of drug in solution and is defined by the thickness of the plastic and diffusivity of the drug in plastic. For most drugs this is only likely to be reached during prolonged administration (greater than 7 days), and therefore only in ECMO (Roberts *et al.*, 1991). Furthermore, in an ECMO circuit that has been used for several days it is possible that once infusion

of drug ceases, sequestered drug will be liberated back into the circulating blood, prolonging the pharmacological effect. In fact it is also conceivable that metabolites of parent drugs or another co-administered drug that has a higher affinity for the circuit plastic may affect the rate of uptake of the parent drug.

1.7.6 Photodegradation

The catalysis by light of drug degradation reactions such as oxidation or hydrolysis, has been reported for a number of drugs; amphotericin B, furosemide, dacarbazine, doxorubicin, vitamin A and sodium nitroprusside (Trissel, 2001). Sodium nitroprusside is so susceptible to photodegradation that during normal (non-ECMO) clinical use the intravenous container and administration set is protected from light by wrapping in foil. A variety of reactions may occur from the absorption of radiation energy, but the net effect is that energy concentrated at a chemical bond causes it to break or to rearrange into a new chemical entity. The energy imparted per photon of light increases as the wavelength increases. Consequently, ultraviolet light is more deleterious than visible light and daylight is more deleterious than fluorescent light. Photodegradation reactions also depend on the intensity of the light source, and therefore the closer the photolabile drugs are to these light sources, the greater the rate and degree of photodegradation (Trissel, 2001).

The patient supported by an ECMO circuit is in a unique clinical scenario, since the patient's blood circulation passes through a transparent pPVC tube circuit, exposed to light. Thus drug in circulating blood, periodically and for a length of time dependent on the pump flow rates, is exposed to light. The nature of the light will on the whole be fluorescent, though ECMO circuits may be exposed to some sunlight depending on their position in the intensive care unit. Such exposure of drugs in blood to light has not been investigated, but it is plausible that susceptible drugs may be significantly affected.

Although this is not an area explored, drugs investigated in this thesis are not acutely degraded by light in aqueous solutions (Trissel, 2001). Drugs used for *in vitro* evaluation in this thesis showed no loss when exposed to ambient light (See Chapter 2, Section 2.3.1), though photodegradation may be an issue with drugs such as amphotericin B, often used during ECMO to treat fungal infections and septicaemias.

1.8 The Effect of ECMO on the Disposition of Specific Drugs

1.8.1 Fentanyl

Although opioids are extensively used in infants during ECMO, very few studies have investigated their disposition. In contrast, the disposition of opioids (apart from morphine) during CPB has been extensively reported (Bovill *et al.*, 1980; Hug *et al.*, 1994; Hug *et al.*, 1982; Koren *et al.*, 1984; Kumar *et al.*, 1988; Lunn *et al.*, 1979; Robbins *et al.*, 1990; Sprigge *et al.*, 1982).

1.8.1.1 Haemodilution

Studies of single large bolus doses of fentanyl (60-75μg/kg) administered at induction of anaesthesia to adult cardiac surgical patients, have demonstrated reductions in plasma concentrations of fentanyl of between 30-60% of pre-CPB concentrations within minutes of the onset of CPB. This decrease was greater than that which could be attributed to haemodilution alone. The concentration of fentanyl remained stable during CPB. In these studies, bubble oxygenators, crystalloid primes and hypothermia to 25 - 30 °C were used (Bovill *et al.*, 1980; Hug *et al.*, 1982; Lunn *et al.*, 1979). Studies investigating the continuous infusions of fentanyl, started before CPB, at an initial high rate (30-50 μg/kg/min) followed by a lower maintenance infusion (0.15-0.5μg/kg/min), have demonstrated a smaller decrease (mean 30%) in plasma concentrations at the onset

of CPB compared with a single large bolus dose (Hug *et al.*, 1982; Sprigge *et al.*, 1982). This is presumably because the maintenance of continuous infusion counteracts the initial decrease. Furthermore, during CPB, the concentration of fentanyl returned to near pre-CPB values within 30min.

1.8.1.2 Sorption

In vitro investigations have demonstrated significant sorption of fentanyl by components of the circuits (Hynynen, 1987; Koren *et al.*, 1984; Rosen *et al.*, 1988a; Skacel *et al.*, 1986). Addition of fentanyl to the prime was shown to prevent the initial decrease in plasma concentration at the initiation of bypass, but after 2.5 minutes similar concentrations to those measured without the addition of prime were recorded (Hynynen, 1987). A decrease in concentration of 68% was observed after the first pass through a membrane oxygenator (Rosen *et al.*, 1985). Loss of fentanyl was greater at high pH, suggesting lipophilic (non ionised) binding to the circuit (Skacel *et al.*, 1986). The use of bubble or membrane oxygenators, or blood or crystalloid solutions for priming did not appear to influence the degree of sorption, although subsequent studies revealed that membrane oxygenator type does have a bearing (Hynynen, 1987; Rosen *et al.*, 1989).

In contrast to fentanyl, studies investigating the sequestration of alfentanil to bypass circuitry have shown zero or minimal loss using both membrane and bubble oxygenators (Hynynen, 1987; Skacel *et al.*, 1986). In fact, free alfentanil concentrations have been shown to remain relatively constant throughout CPB, as a result of an increase in the unbound fraction. The decrease in total alfentanil concentrations was explained by a dilution of α1 acid glycoprotein (Hug *et al.*, 1994; Kumar *et al.*, 1988). This data illustrates that is important to measure the active free concentrations of the drug.

1.8.1.3 Pharmacokinetic Studies

In a retrospective review of 37 newborn infants on ECMO, unexpectedly large doses of fentanyl to achieve adequate sedation were reported, with the infusion rate of fentanyl increasing with length of time on ECMO (Leushen *et al.*, 1993). Withdrawal symptoms were observed in 57% of the neonates, duration of ECMO being the most powerful predictor. In the same study, continuous infusion doses of fentanyl were correlated with plasma fentanyl concentrations in five neonates. Plasma concentrations climbed steadily during the period of infusion, suggesting the development of tolerance to the sedating effects and possibly explaining the large doses administered. The authors suggest that if binding to the ECMO circuit was the reason for the increased dosing requirements, the dose would have decreased over time as the membrane oxygenator became saturated (Leushen *et al.*, 1993).

In another prospective study, plasma levels of fentanyl were analysed in 12 infants undergoing VA ECMO who received a bolus dose followed by an infusion. Plasma levels were taken 6 hours following the initiation of infusion. This time lag was chosen as the researchers felt this would ensure the membrane oxygenator would reach saturation. Adequate sedation was achieved in all neonates with continuous infusion doses not exceeding 7μg/kg/hour. Overall, there was no correlation between the plasma fentanyl levels and either the time on ECMO or the fentanyl infusion rate. The plasma levels generally increased over the first four days and then tended to decrease thereafter without significant increase in infusion rates (Leushen *et al.*, 1993). The authors attributed the elevations in plasma levels to decreased clearance, and curiously correlated this with renal function despite evidence that fentanyl is primarily cleared via the liver (*AHFS Drug Information. 2001*). However, a rise in plasma levels is expected with continuous infusion as a steady state level is approached. Although not clear from

the data provided, the decrease in plasma levels beyond day 4 may have been related to the improvements in the patient's hepatic and pulmonary blood flow.

1.8.2 Morphine

Although there has been little work on the effect of CPB on plasma morphine concentrations, there have been 2 *in vivo* (Dagan *et al.*, 1994; Geiduschek *et al.*, 1997) and 1 *in vitro* (Dagan *et al.*, 1993) study investigating morphine disposition in ECMO (Tables 1.3 and 1.4).

1.8.2.1 Sorption

Dagan *et al* (1993) conducted a preliminary study of the *in vitro* effects of two ECMO circuits (primed with blood) on the disposition of common paediatric drugs (Table 1.3). One circuit was new and the other was used clinically for 5 days. Blood samples drawn from the circuit at 10, 30, 60 and 240 minutes revealed a 36% decrease in the morphine concentration in the new circuit and a lesser decrease of 16% in the used circuit. These results suggest that the process of drug uptake may be influenced by factors such as prior exposure of the circuit.

1.8.2.2 Pharmacokinetic Studies

In a prospective comparative study, Dagan *et al* (1994), investigated morphine pharmacokinetics during and after ECMO. They did not stipulate whether it was VA or VV ECMO, although stated that it was for cardiopulmonary support, implying VA ECMO. Data was collected on seven infants (age range 1 day to 12 months) receiving continuous infusion of morphine between 20 and 40μg/kg/hour. The opioid infusion rate remained unchanged for at least 22 hours during ECMO, and the infusion rate was not changed after decannulation. Blood samples were taken at 10 hour intervals whilst on ECMO and off ECMO. Mean morphine serum concentrations were found to be twice

those taken after ECMO was discontinued. Two of the study infants (neonates aged 1 and 2 days) who recorded the steepest decline in serum morphine concentrations post ECMO, experienced symptomatology consistent with opioid withdrawal. The calculated clearance of morphine approximately doubled when the infants were taken off ECMO (Table 1.4).

Table 1.4. Summary of mean (SD) pharmacokinetic values obtained in neonatal ECMO patients

Drug	References		V (L/kg)	CL (L/kg/hour)	T1/2 (hours)
Morphine	Dagan *et al.*, 1994*	On ECMO Off ECMO	- -	0.57 (0.3) 1.06 (0.73)	- -
	Geiduschek *et al.*, 1997	-	-	0.70 (0.56)	-
Gentamicin	Southgate *et al.*, 1989	-	0.51 (0.11)	0.05 (0.03)	9.5 (4.2)
	Cohen *et al.*, 1990*	On ECMO Off ECMO	0.58 (0.04) 0.45 (0.02)	0.04 (0.003) 0.06 (0.004)	10.0 (0.7) 5.7 (0.4)
	Munzenberger *et al.*, 1991	-	0.62 (0.25)	0.08 (0.03)	7.6 (5.3)
	Bhatt-Mehta *et al.*, 1992	-	0.67 (0.15)	0.05 (0.02)	10.4 (3.0)
	Dodge *et al.*, 1994*	On ECMO Off ECMO	0.75 0.47	0.24 litre/h 0.35 litre/h	9.24 3.87
Vancomcyin	Hoie *et al.*, 1990	-	0.68 (0.12)	0.066 (0.019)	7.71 (2.61)
	Amaker *et al.*, 1996	-	1.1 (0.5)	0.047 (0.011)	16.9 (9.5)
	Buck *et al.*, 1998	-	0.45 (0.18)	0.039 (0017)	8.29 (2.2)

* Comparison of values obtained during ECMO and after decannulation

The authors suggested that impaired hepatic metabolism and/or hepatic blood flow may be the reason for decreased clearance on ECMO, although there was no clinical evidence of this and no direct measurement of hepatic blood flow was

conducted. A renewal of blood flow to the lungs at the end of ECMO and therefore an increase in V causing an initial decrease in the serum concentration was also suggested as a possible reason. However, this would have been a transient effect and mean steady state concentrations should return to normal. The researchers conclude that the mechanisms leading to the changes in morphine disposition are unclear, but it is wise to carefully monitor pharmacological effects on and off ECMO (Dagan *et al.*, 1994).

In another prospective study by Geiduschek *et al* (1997), morphine sulphate pharmacokinetics during continuous infusion was determined in 11 neonates with severe persistent pulmonary hypertension and receiving VA ECMO support. All patients received 1 or 2 boluses of morphine before commencing an infusion of 10-20 μg/kg/hour. Blood samples were taken at baseline (before the cannulation procedure) and compared with samples obtained from sites immediately proximal and distal to the membrane oxygenator at 5 mins, and 1 and 3 hours after commencement of ECMO. Morphine clearance was also calculated using paired samples collected on days 1, 3 and 5 of ECMO, beginning at least 12 hours after the start of ECMO and any change in morphine infusion rate.

A baseline (pre ECMO) morphine concentration was only obtained in 5 patients. No significant decrease in serum morphine concentrations was seen in these 5 patients on initiation of ECMO. This is somewhat surprising since a decrease in concentration would have been expected due to the haemodilutional effect. It is possible that since the first sample was not collected until 5 minutes, the plasma levels had re-equilibrated from tissue stores. Morphine concentrations were also not significantly different in samples obtained simultaneously from sites immediately proximal and distal to the silicone membrane oxygenator, suggesting that morphine sulphate does not bind to the membrane oxygenator. Values for clearance were significantly depressed in 4 patients, although clinical information regarding their hepatic and renal function was not

supplied. Three patients in the study had a primary diagnosis of congenital diaphragmatic hernia, although it was not stated if these were included in any of the 4 patients with depressed clearance. Five patients had increased clearance during ECMO, contradicting the results of the previously discussed study. The authors conclude that the clearance for morphine in patients receiving ECMO is variable and the range of clearance values exceed those published previously for infants who are receiving morphine infusions (Geiduschek *et al.*, 1997).

1.8.3 Benzodiazepines

1.8.3.1 Haemodilution

The administration of a bolus dose of midazolam (0.15mg/kg) was found to suffer a decrease in concentration on establishing CPB and an increase in concentration post CPB with a prolonged elimination half-life (Kanto *et al.*, 1985). Dawson *et al* (1997) noted that although the total concentration of midazolam dropped on CPB, the unbound concentration remained stable, and so the unbound fraction had increased from 5.6% to 11.2%. Similar effects have been observed with lorazepam (Boscoe *et al.*, 1984).

1.8.3.2 Sorption

Although sorption of benzodiazepines in contact with pPVC intravenous administration containers and administration sets has been frequently demonstrated (see Section 1.6.5), no such studies with ECMO circuits have as yet been reported.

1.8.4 Propofol

Although the therapeutic use of propofol in paediatric or adult ECMO has not been described, it has been widely studied in the context of CPB, with conflicting results (Dawson *et al.*, 1997; Hammaren *et al.*, 1996; Massey *et al.*, 1990; Russell *et al.*, 1989). In two studies, the initiation of bypass, when propofol was being administered as a continuous infusion of 3-6mg/kg/hr, resulted in a decrease in total concentration of 50-78% (Hammaren *et al.*, 1996; Russell *et al.*, 1989). A corresponding increase in free fraction and free concentration was demonstrated, probably from haemodilution. In another study, no change in total concentration of propofol was observed (Massey *et al.*, 1990).

Propofol is a highly lipophilic (log P 3.7) and protein bound drug and therefore significant sequestration by the extracorporeal circuit is expected. In an *in vitro* evaluation of propofol interactions with closed CPB circuits, Hynynen *et al* (1994) reported that at 5 and 120 mins after addition of propofol into the circulating solution, only 65% and 25%, respectively, of the predicted levels were measurable in the solution. Hammaren and colleagues (1999) demonstrated that priming CPB circuits with heparin did not prevent loss of propofol during *in vitro* tests. Although the sorption of propofol to components of the ECMO circuit is anticipated, no such studies have as yet been conducted.

1.8.5 Anticonvulsants

Marx *et al* (1991), evaluated the interaction of phenobarbitone with the ECMO circuit prompted by a retrospective chart review of 20 neonates on ECMO, which showed that twice the normal doses were required to maintain therapeutic drug concentrations. However, specific doses and serum concentration data were not revealed.

The *in vitro* evaluation of phenobarbitone interactions from three repeat investigations revealed mixed results. In two of the three experiments, phenobarbitone concentrations were within 90% of expected values. However, the third circuit had only 47% of the expected level. Furthermore, the researchers were able to extract the sequestered phenobarbitone from the circuit using methanol.

Dagan *et al* (1993), have also demonstrated similar *in vitro* loss of phenobarbitone and phenytoin in ECMO circuits. This may explain an increased V for phenobarbitone observed in a neonate on ECMO (Elliott *et al.*, 1999).

1.8.6 Glyceryl Trinitrate.

GTN may be administered during ECMO to treat hypertension and/or to prevent cardiac ischaemia. It is possible however that bioavailability is reduced since there is substantial loss to the circuit and oxygenators (Booth *et al.*, 1991; Dasta *et al.*, 1983; Dasta *et al.*, 1986). This is anticipated since the drug is known to be sequestered by several plastics (Baaske *et al.*, 1980).

1.8.7 Antibiotics

1.8.7.1 Gentamicin

Gentamicin pharmacokinetics has been widely studied in ECMO with varying results (Table 1.4) (Bhatt-Mehta *et al.*, 1992; Cohen *et al.*, 1990; Dodge *et al.*, 1994; Munzenberger *et al.*, 1991; Southgate *et al.*, 1989). Although an increase in V is hypothesised, results from three studies did not support this (Bhatt-Mehta *et al.*, 1992; Munzenberger *et al.*, 1991; Southgate *et al.*, 1989). However, prolonged half-lives and decreased clearance was demonstrated in all but one of these studies (Muzenberger *et al.*, 1991). The choice of historical controls used in this one study was probably

inappropriate. A fourth study compared gentamicin pharmacokinetics in neonates who continued on gentamicin after coming off ECMO (Cohen *et al.*, 1990). They reported a decrease in V after cessation of ECMO and suggested that a loading dose was required to attain appropriate therapeutic levels. In a fifth study by Dodge *et al* (1994), data from 11 neonates who received gentamicin on ECMO, including 6 infants who received gentamicin both on and off ECMO, was presented. For six infants, while on ECMO their median V was 0.748 L/kg, considerably greater than the median V of 0.47 L/kg after ECMO was discontinued. Clearance was also shown to be reduced whilst on ECMO (median 0.239 L/hr versus 0.350 L/hr). Similarly, median half-life was 9.24 hr on ECMO compared with 3.87 hr when off ECMO. The range of target peak and trough plasma levels recommended by all the groups was 5-8 and <2.0 mg/L, respectively.

1.8.7.2 Vancomycin

Vancomycin pharmacokinetics during neonatal ECMO has also been described (Table 1.4) (Amaker *et al.*, 1996; Buck *et al.*, 1998; Hoie *et al.*, 1990). The results of these studies are described in detail elsewhere in this thesis (See Chapter 7).

1.9 Pharmacokinetic Investigations in Clinical Practice

The development of pharmacokinetic principles and subsequent application to the evaluation of drug disposition in man has resulted in an understanding of the processes of absorption, distribution, metabolism and elimination (Sheiner *et al.*, 1977).

It is now widely recognised that point estimates of pharmacokinetic and pharmacodynamic parameters are often inadequate, and it is more appropriate to describe parameters by a probability distribution. An important factor driving the change to probability based estimates of parameters is the recognition that there can be significant variability in dose-response in human populations. Conceptually, variability

in dose-response is caused by variability in pharmacokinetic and pharmacodynamic responses. The former relates to variability in tissue concentration-time profiles, whereas the latter to variability in the response on a given target tissue dose (Sheiner *et al.*, 1977). The influence of patient characteristics such as body weight, gender, age, physical and pathophysiologic states, genetics, environment and concurrent therapy on various pharmacokinetic parameters has received extensive attention. This appreciation has resulted in a proliferation of studies designed to quantify the effect of these factors on drug pharmacokinetics with the overall goal of minimising the extent of unexplained variability (Sheiner *et al.*, 1977).

Certain groups, however, including the elderly, critically ill and paediatric patient population have proven difficult to study because of the ethical and logistical issues which arise when one attempts to subject these patients to the rigors of a pharmacokinetic trial (see section below). The application of pharmacokinetics during normal clinical practice (outside the confines of a controlled study) is often limited to those drugs monitored routinely as part of the assessment of therapeutic effect. Pharmacokinetic parameters are estimated from limited observations using log linear regression analysis. Dosage adjustments are then based on these estimates. However, in order to address the effect of pharmacokinetic variability on dose response, it is necessary to progress from pharmacokinetic models for the 'average' individual to population pharmacokinetic models.

1.10 Problems in Performing Pharmacokinetic Studies in Children

The ethics of non-routine blood sampling from children has been much debated, and many studies have relied on the 'in practice approach' with samples being taken for routine therapeutic drug monitoring. Recently though, the Royal College of Paediatrics and Child Health (RCPCH) has assessed the taking of blood samples as a low risk

procedure. This aside, the Royal College emphasises that children do fear needles and so a careful explanation for the need of venepuncture and the application of an effective local anaesthetic cream is important. Informed consent from the patient or their guardian must be obtained and it is considered inappropriate to insist on taking blood samples where a child indicates otherwise (*RCPCH, 2000*). Pharmacokinetic investigations in neonates can be especially difficult due to the small blood volumes available. The use of urine and saliva samples has obvious attractions in children.

Blood sampling can be kept to a minimum by the use of Bayesian forecasting whereby only one or two blood samples are required. Bayesian programs combine measured concentrations and information from population studies to estimate *a posteriori* CL and V parameters and design dosage regimens for individual patients (Lannigan *et al.*, 2001). Although originally developed for adults, such programs have been adapted for use in neonates (Lannigan *et al.*, 2001). However, the paucity of paediatric population pharmacokinetic parameters is the main limitation to the use of this method. Sheiner and colleagues (1977) have advocated the use of information generated during the routine care of patients to obtain estimates of population pharmacokinetic parameters. These parameters can then be used to assist the clinician or clinical pharmacist in making decisions regarding drug prescribing for patients.

1.11 Population Pharmacokinetics

Population pharmacokinetics is the study of the time course of a drug and its metabolites in groups of patients having similar characteristics. The approach is to develop a model, identifying the measurable pathophysiologic factors that cause changes in the dose-concentration relationships and the extent of these changes so that dosages can be modified if there are clinically significant shifts in the therapeutic index (*Guidance for Industry: Population Pharmacokinetics. (1999). US Department of*

Health and Human Services Food and Drug Administration). Such guidelines, in an ideal scenario, would allow the clinician to select an appropriate dosage regimen once a patient has been characterised with respect to medical history, diagnosis, physical examination and clinical laboratory tests. Of course, essentially all models are imperfect and therefore a dosage regimen predicted for an individual may vary to some degree from the optimum regimen (Ludden, 1988).

The magnitude of unexplained (random) variability is important because the efficacy and safety of a drug may decrease as unexplainable variability increases. Sources of variability that contribute to differences between predicted and observed are categorised as interpatient and intrapatient in origin. The presence of interpatient variation suggests that even though expected parameter values can be calculated for an individual based on the population mean, the particular individual at hand may have parameter values that differ from the expected values (Ludden, 1988). Intrapatient variability (also known as residual error) is exemplified in the degree to which steady state drug concentrations within individuals typically vary when followed over a course of time. Concentrations may vary due to inexplicable day to day or week to week kinetic variability, or measurement errors or model misspecification. Estimates of residual error are particularly important for therapeutic drug monitoring.

1.12 Approaches to Estimating Population Pharmacokinetic Parameters

There are a number of methods for obtaining estimates of population pharmacokinetics parameters:-

1.12.1 Naïve Pooled Data

This method consists of pooling all drug concentration measurements from individuals, and analysing them together as though they all came from the same individual. These

yield point estimates and standard errors from which 95% confidence regions can be computed. No estimates of the random interpatient effect on parameters are obtained, and the estimate of interpatient variability that is obtained is actually an estimate of the magnitude of the variability from all random sources, and therefore is of limited interest.

1.12.2 Two Stage Method

This approach proceeds in two stages. First each individual's data are separately fitted, using nonlinear (weighted) least squares. For each individual, estimates of parameters and the residual sum of weighted squares are obtained. Population averages and standard deviations are then estimated from the sample of individual parameters using standard statistical methods. Although this approach gives unbiased estimates of population averages of pharmacokinetic parameters, estimates of variability are biased towards higher values. Furthermore the two stage approach is strictly applicable to data from balanced experiments, such as randomised controlled trials, and sufficient data from each individual are needed to estimate the pharmacokinetic parameters of the individual. This approach cannot therefore utilise the significant amount of sparse, unbalanced data that is routinely collected in clinical settings.

1.12.3 Non-Linear Mixed Effects Models

In sparse data situations, where the traditional two-stage approach is not applicable because estimates of individual parameters are, *a priori*, out of reach, a single-stage approach such as non-linear mixed effects modelling can be used (*Guidance for Industry: Population Pharmacokinetics. (1999). US Department of Health and Human Services Food and Drug Administration*). The major advantage of the approach is that it uses individual pharmacokinetic data of the observational type, which may be sparse,

unbalanced, and fragmentary, in addition to, or instead of, conventional pharmacokinetic data from traditional pharmacokinetic studies characterised by rigid and extensive sampling design (rich data situation).

In mixed effects modelling, the population pharmacokinetic model consists of mean parameter values (also known as fixed effect parameters) and their variability within the population (also known as random effects parameters). In contrast to the two stage method, the approach consists of estimating directly the fixed and random effects parameters of the population from the full set of individual concentration values. Importantly, the individuality of each subject is maintained and accounted for, even when the data are sparse (*Guidance for Industry: Population Pharmacokinetics. (1999). US Department of Health and Human Services Food and Drug Administration*).

As is expected, attention to sampling times can increase the quality of the information obtained. Samples obtained at the time of peak serum concentrations usually contain the most information about the V whereas samples obtained at later times are informative about CL (Ludden, 1988). Steady state peak and trough are useful when both V and CL are to be estimated. Data obtained prior to the attainment of steady state provide particularly useful in formation about V and CL (Ludden, 1988).

1.12.4 Computer Software for Population Pharmacokinetic Modelling

When there are not sufficient data to estimate regression parameters for each individual, population parameters may be estimated using methods based on first order linearisation of a hierarchical non-linear model (see Appendix II). These methods are based on approximations to the marginal likelihood within the relevant non-linear mixed model framework and are computationally, extremely intensive. A number of computer software packages are available for population pharmacokinetic analysis. The one used throughout this project is WinNonMix, Version 2.0.1. The output from the WinNonMix

program contains many important components. However, WinNonMix is only an aid or tool for the modelling process. As such, it can only use the model and data supplied by the researcher and find a 'best fit' of the model to the data. The program cannot determine the correctness of the model; nor can it determine the value of any decisions or interpretations based on the model. The program does, however, provide some information about the 'goodness of fit' of the model to the data and about how well the parameters are estimated. These include the objective function value (minus $2 \times \log$ likelihood), the population parameter estimates and their variances, the standard errors (indicating the precision of the estimates), the covariance matrix and correlation matrix of the estimates, scatter plots of parameters, weighted residuals and their relationships with covariates. The entire output is necessary in assessing the goodness of fit.

In addition the individuals' parameter estimates are also determined. These are derived based upon Bayes theorem for conditional probabilities. The population information and the patient specific data are combined to predict the most probable parameter values for an individual patient from the observed concentrations.

1.12.5 Assessing Goodness of Model Fits

Although statistical tests such as the objective function value (OFV) are useful in comparing hierarchical models, it is not the only test for model discrimination. The standard errors, covariance and correlation matrices of the estimates provide information about the parameter estimation procedure itself. For example, poorly estimated parameters may exhibit large relative standard errors or there may be high correlation between parameter estimates. Difficulties in parameter estimation may arise because there is insufficient data to support the parameters or because of inappropriate initial parameter estimates. Scatterplots are extremely useful for initial examination of the data set for input errors and for obvious correlations. Moreover, critical analysis of

plots of weighted residuals versus predicted concentrations, time and other covariates is essential. A non random distribution of weighted residuals indicates that the model used is not entirely appropriate, usually indicating that the model is not adequately parameterised. For example, the model needs to be changed from a one to two compartment model. A model is thought to be good when (Gabrielsson *et al.*, 2000):

- Predicted values mimic data and residuals show random scatter
- Parameter estimates are unbiased and have high precision
- Parameters have low correlation

1.13 Local Experience with Pharmacotherapy during ECMO and Background to the Research Project

In the paediatric intensive care unit at this institution, midazolam and morphine are the most commonly used agents for sedating ECMO and mechanically ventilated non-ECMO (mainly post-operative cardiac surgical) infants. It had been noted by clinicians and nurses that doses of sedative drugs required to achieve desired levels of sedation in ECMO patients were far greater (between 2-10 times) than for non-ECMO patients, a phenomenon that has previously been reported by other workers (Rosen *et al.*, 1991; Tobias *et al.*, 1995). These differences could not readily be accounted for by any variation in patient types, clinical status or sedation levels. Furthermore, once ECMO patients were decannulated, their sedative requirements often decreased considerably to more 'normal' levels, once again suggesting that the ECMO circuit may have an effect on drug disposition.

To test the hypothesis that neonates receiving ECMO require higher doses of sedation, a retrospective audit to determine and contrast doses of midazolam and morphine administered to comparable ECMO and post cardiac surgical neonates in the

PICU was undertaken. Inclusion criteria included comparability in terms of age and duration of ECMO and mechanical ventilatory support. The data was compiled as 12 hour cumulative doses for midazolam and morphine respectively. For direct comparison with the post cardiac surgical neonates only data from the first 72 hours was utilised. Data from the drug audit are shown in Figure 1.6 and Table 1.5.

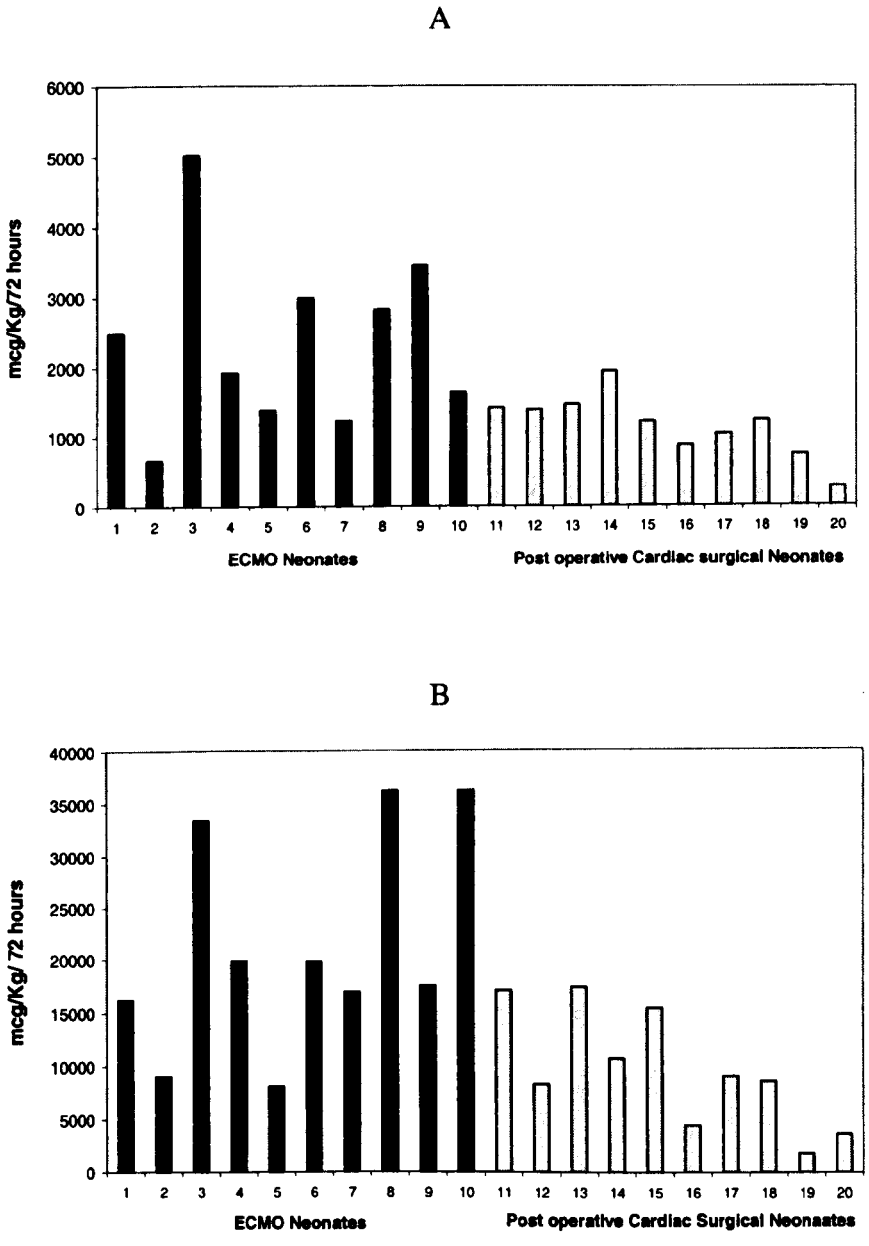


Figure 1.6. Total doses administered in the first 72 hours (A) Morphine (B) Midazolam

Table 1.5 Doses of Midazolam and Morphine administered over a 72 hour period

	Midazolam		Morphine	
	ECMO	**Cardiac**	**ECMO**	**Cardiac**
Mean Total dose (μg) (SD)	21283 (10446)	9599 (5504)	2364 (1280)	1158 (457)
***p*-value**	p = 0.0076		p = 0.017	
Estimate of Difference (μg) (95% Confidence intervals)	11684 (4365, 19003)		1206 (364, 2048)	
Min-Max doses (μg/kg/hour)	112 - 503	25 - 240	9 - 70	4 - 27
Recommended Dose Range (μg/kg/hr) [a]	30-200		10-30	

a. *Medicines For Children (2000).*

The results revealed that total doses of midazolam and morphine administered were higher in the ECMO neonates over the first 72 hours. Differences in the mean total doses administered to the two groups were significant for both midazolam (p=0.0076) and morphine (p=0.017) with the estimate of difference suggesting doses administered to the ECMO neonates were of the order 2-3 times the post cardiac surgical neonates. The range of midazolam doses administered to the ECMO and non-ECMO neonates (112-503 μg/kg/hour versus 25-240 μg/kg/hour, respectively) reflect not only the wide interpatient variability, but also differences in the hourly requirements between the two groups. Similar results are observed with the morphine. In addition, whereas doses administered to the cardiac patients reflected the 'normal' recommended doses for ventilated neonates (30-200 μg/kg/hour), the doses administered to the ECMO neonates were frequently above the upper normal range (Table 1.5) *(Medicines For Children,2000).*

Since the same clinical staff cares for both groups of patients on the same intensive care unit, it can be assumed that bias as a result of differing knowledge,

philosophy and practice had to a large extent been reduced. Although it could be argued that the goals of sedation may at times be different between neonates on ECMO and those requiring mechanical ventilation, such divergent aims could not explain the differences in total doses of the order of 2-3 times. These results suggested altered drug disposition during ECMO.

In addition to the sedation experience, it was felt that potentially toxic steady state gentamicin trough and aminophylline levels were observed with greater frequency amongst ECMO compared to non-ECMO neonates. This resulted in an opposite response to the sedation issue i.e. a dose reduction.

1.14 Aims of the Research

Patients supported by ECMO receive a myriad of pharmacological agents, yet there are few reports of drug administration during ECMO. As already outlined in this chapter, the effect of ECMO on drug disposition is a complex issue with the interplay of many factors, including physiological changes, sorption of drugs into components of the circuit and volume expansion. The broad scope of this research was to increase understanding of the effect of drug sorption and an expanded circulating volume on drug disposition during ECMO.

Although sorption of drugs by intravenous administration sets and lines has previously been reported, few such investigations have been conducted with ECMO circuit components. The availability of such data in the application of drug treatment during ECMO is vital since the circuit is an extension of the patient's blood circulatory system. Unlike drug sorption in intravenous delivery systems, drug-ECMO circuit interactions have the potential to dramatically affect pharmacokinetics.

In vitro investigations were designed not only to determine the rate and extent of sedative drug sorption, if any, but how this affects drug delivery and to delineate

physico-chemical factors of drugs and characteristics of plastic components that may influence the degree of loss. Since ultimately it is important to be able to demonstrate the *in vivo* sorption of drugs by ECMO circuits, *ex vivo* analysis of neonatal ECMO circuits for midazolam sorption was conducted. Next, the impact of potential midazolam sorption on plasma concentrations and pharmacokinetics was investigated in neonates treated on ECMO.

In the second part of this thesis, the effect of the expanded circulatory volume as well as other clinically significant covariates on pharmacokinetics of routinely monitored water soluble drugs (aminophylline, vancomycin and gentamicin) is explored and compared with similar non ECMO patients. Derived pharmacokinetic parameters could then be used to design more appropriate dosing regimens to achieve therapeutic goals.

CHAPTER II

MATERIALS AND METHODS

2.1 Introduction

Studies into drug disposition during extracorporeal membrane oxygenation followed two investigational routes: *in vitro* and Clinical. The aim of the *in vitro* investigation was to evaluate the sorption of commonly used intensive care sedatives by those polymeric components of an ECMO circuit that are in continuous contact with circulating blood: pPVC tubing and silicone membrane from the oxygenator. These experiments were designed so that sorption of known concentrations of aqueous drug solutions with components of the circuit could be determined, under static conditions and also during simulation of flow through an intact circuit. All analyses during this stage of the investigation were carried out using HPLC with UV detection. The final stage of these studies involved analysis of *ex vivo* circuits i.e. circuits that had previously been used to support neonates requiring ECMO. The purpose of this was to confirm the presence, or otherwise, of midazolam that had been administered as a continuous infusion during the period on ECMO. For this phase of the investigation and since potentially a variety of sequestered agents could interfere with the analysis, an LC-MS analytical method was developed.

The clinical investigations were designed to assess the impact on pharmacokinetics of two ECMO circuit associated phenomena: drug sorption to polymeric components and the expanded circulating volume. Plasma concentration observations for these studies were obtained either prospectively, through designed protocols (and therefore providing a rich data set) or retrospectively from therapeutic drug monitoring databases (sparse data set). The impact on the pharmacokinetics of midazolam as a result of sorption to neonatal circuit components was investigated through a prospective clinical study and population pharmacokinetic parameters were derived from a non-linear mixed effects model. The latter was developed using the software WinNonMix (Version 2.0.1). The impact of an expanded circulating volume

was assessed on three routinely monitored drugs that are water soluble and therefore have a relatively small volume of distribution. A population pharmacokinetic model was developed for aminophylline using data collected retrospectively from a database. Similarly, a population model was also developed for vancomycin, however during this investigation data collected both prospectively and retrospectively was utilised. All population models were validated using either internal or external methods. Finally, the consequence of an expanded circulating volume was also assessed through the comparison of two gentamicin dosing regimens for neonates. Pharmacokinetic parameters on this occasion were derived using log linear regression analysis.

2.2 Materials for *In Vitro* Investigations

2.2.1. Drugs and Other Chemicals

All drugs and chemicals were of a pharmaceutical and chemical standard:-

Diazepam Base:	Sigma-Aldrich Company Ltd, Dorset, UK
Diazepam injection ($10mg/2cm^3$):	Phoenix Pharmaceuticals Ltd, Gloucester
Lorazepam injection ($4mg/cm^3$):	Wyeth Laboratories, Maidenhead, Berkshire
Midazolam Base:	Hoffman La Roche, Basel, Switzerland (gift)
Midazolam injection ($10mg/2cm^3$):	Roche Products Ltd,
Morphine Sulphate ($10mg/cm^3$):	Martindale Pharmaceutics, UK
Nalorphine	Sigma-Aldrich Company Ltd, Dorset, UK
Propofol injection ($200mg/20cm^3$)	AstraZeneca, Wilmslow, Cheshire, UK
Plasma-Lyte A	Baxter Healthcare Corporation, USA
Human Albumin Solution 4.5%:	Bio Products Laboratory, Elstree, Herts, UK
Methanol (HPLC grade):	Fisher Scientific, Loughborough, UK
Disodium hydrogen phosphate:	Merck/BDH, Lutterworth, Leicestershire, UK
Sodium dihydrogen phosphate	Merck/BDH, Lutterworth Leicestershire, UK
Glacial Acetic Acid	Fisher Scientific, Loughborough, UK

2.2.2 Circuit Components

Tygon® S-65-HL pPVC tubing	Norton Performance Plastics, Corby, Northants,
(¼" luminal diameter)	UK
0800 Silicone membrane	Avecor, Cardiovascular Inc, Minneapolis, MN,
Oxygenator	USA
PMP Hollow fibre oxygenator	Medos Medizintechnik AG, Stolberg, Germany

2.3 Methodology for *In Vitro* Analysis

2.3.1 Preparation and Serial Dilutions of Stock Drug Solutions

For the *in vitro* analysis of circuit components using HPLC, pharmaceutical (injectable) preparations of the drug were used to prepare standard solutions. Diazepam 5mg/cm^3, lorazepam 4mg/cm^3, midazolam hydrochloride 5mg/cm^3, morphine sulphate 10mg/cm^3 and propofol 10mg/cm^{3}were serially diluted to volumes using double distilled water to make working standard solutions ranging 1μg/cm^3 to 50 μg/cm^3. At all times the solutions were kept in the dark. This was achieved by wrapping the glassware in aluminium foil. All solutions were stored at 4-8°C, and used for up to 2 weeks. For the flow simulation study, diazepam 5mg/cm^3, lorazepam 4mg/cm^3, midazolam hydrochloride 5mg/cm^3, and propofol 10mg/cm^{3}were used to prepare 3500cm^3 working standard solutions of 0.4μg/cm^3 using double distilled water. All solutions were used within one day.

For the ex-vivo analysis of circuit tubing using LC-MS, midazolam base was dissolved in double distilled water and then diluted in a serial format to concentrations ranging 10ng/cm^3 to 10μg/cm^3. Diazepam, the internal standard, was prepared by dissolving the powder form in methanol and then diluted in a serial format to 200ng/cm^3.

Stability of all drug solutions was monitored by storing aliquots of solution in glassware for 7 days. The samples were analysed in duplicate 0, 1 and 7 days after preparation. All the solutions were shown to be stable, with little change in concentration (<3%) over 7 days.

2.3.2 Preparation of Clear Prime Solution.

Clear prime solution was prepared by taking 20cm^3 of Human Albumin Solution 4.5% and diluting to 100cm^3 with Plasma-Lyte A. This ratio mimics normal clinical protocol relating to clear prime solutions used for ECMO circuits.

2.3.3 Preparation of Phosphate Buffer for HPLC Mobile Phase

Sodium dihydrogen phosphate 11.004g (0.092M) and disodium hydrogen phosphate 1.013g (0.008M) were dissolved in 100cm^3 of double distilled water separately and then made up together to 1000cm^3. The mobile phase was made up of methanol: water: phosphate buffer (800:100:100). The solution was vacuum filtered (0.22μ) and degassed in helium. The solution was stored at room temperature and used for up to 2 weeks.

2.3.4 Preparation of Ammonium Acetate Buffer for LC-MS Mobile Phase

Ammonium acetate 3.28g was dissolved in 500cm^3 double distilled water. Glacial acetic acid solution was then added to this solution to adjust pH to 5.4. The mobile phase was made up of methanol: water: ammonium acetate buffer (800:100:100). The solution was vacuum filtered (0.22μ) and degassed in helium. The solution was stored at room temperature and used for up to 2 weeks.

2.3.5 *In Vitro* Investigation of Drug Sorption into Circuit Components – Static Conditions

An 8cm length of ¼" pPVC tube was filled with 25μg/cm^3 of drug solution (diazepam, lorazepam, midazolam, propofol or morphine), sealed using glass stoppers and wrapped in aluminium foil. Triplicate samples (50μl) were then taken at 0, 5, 20 40 and 120 minutes with a glass syringe and analysed using HPLC.

A piece of silicone membrane, 6cm × 7cm, was cut from the 0800 Avecor oxygenator. This was achieved by opening the outer case of the oxygenator and unravelling and separating the membrane bilayer from the polypropylene mesh. The cut piece was stapled together in a cylinder shape and placed in a glass sample tube. The membrane was covered with approximately 27cm^3 of 25μg/cm^3 drug solution (diazepam, lorazepam, midazolam, propofol, morphine). The glass sample tube was wrapped in foil and triplicate samples (50μl) were then taken at 0, 5, 20 40 and 120 minutes with a glass syringe and analysed using HPLC.

2.3.6 *In Vitro* Investigation of Drug Sorption into Circuit Components Previously Primed.

An 8cm length of pPVC tube was filled with clear prime solution. The tube was sealed with glass stoppers and left to stand for one hour, to mimic priming of ECMO circuits prior to clinical use. The tube was then emptied out and filled with drug solution (diazepam, lorazepam, midazolam and propofol) and timed serial samples were taken as described in 2.3.5.

A piece of silicone membrane, 6cm × 7cm (obtained as detailed in 2.3.5), was placed in a glass sample tube. The membrane was covered with approximately 27cm^3 of clear prime solution and left to stand for one hour. The glass sample tube was then emptied out and filled with drug solution (diazepam, lorazepam, midazolam and propofol) and timed serial samples were taken as described in 2.3.5.

2.3.7 Extraction of Midazolam from Previously Exposed Circuit Components

pPVC tubing previously exposed to midazolam aqueous solution as detailed in 2.3.5 was rinsed thoroughly with double distilled water. The tubing was then refilled with methanol as an extraction solvent, sealed with glass stoppers and then timed serial

samples were taken as described in 2.3.5. This procedure was repeated with double distilled water as the extraction solvent.

2.3.8 Simulated Infusions of Sedative Drugs through the ECMO Circuit.

A complete neonatal ECMO circuit (minus double lumen cannula) was assembled in a linear fashion and consisted of the following components: 6 metres of Tygon S-65-HL pPVC tubing, a reservoir bag (Sac-R-14), silicone membrane oxygenator (0800 Avecor) and heat exchanger (ECMOTherm II, Avecor). The drainage end of the tubing was submerged in a 3500cm^3glass reservoir containing drug solutions (diazepam, lorazepam, midazolam, propofol) of 0.4$\mu g/cm^3$. A peristaltic pump was attached to tubing pre-reservoir and the drug solution was pumped through the circuit at a rate of 360cm^3/min. Freshly prepared solution was added to the reservoir solution to keep it topped up and to prevent air passing down the tube. The effluent leaving the circuit via the re infusion line was sampled at approximately 2 minute time intervals between 0-54 minutes. Triplicate samples (50μl) from each time point were analysed using HPLC.

2.3.9 Determination of Drug Sorption and Release from *In Vitro* Circuits by HPLC

HPLC was used to separate and quantify drug concentrations from the static and flow simulation sorption studies.

2.3.9.1 HPLC Apparatus and Conditions

HPLC pump:	Waters 600E System Controller: S/N 60EFR1069
Column:	Prodigy 5μ ODS (3) 100A 150 × 4.6mm Luna 5μ C_{18} (2) 150 × 4.6mm with integrated guard column (Phenomenex, UK)
Detector:	Severn Analytical SA 6500 UV/VIS Absorbance Detector S/N 00408
Flow Rate:	1.0cm^3/min
Injection volume:	50μl
Injection loop:	20μl
Wavelength:	Diazepam 230nm, Lorazepam 212 nm, Midazolam 219nm, Propofol 269 nm
Retention times:	Diazepam (3.3 mins), Lorazepam (2.6mins), Midazolam (3.1 mins) and Propofol (6.6 mins)
Chart Speed:	0.5cm/min
Peak Threshold:	200
Mobile Phase:	Methanol:Double Distilled Water:Phosphate Buffer solution (800:100:100 v/v)
Integrator:	Spectra Physics: Data Jet Injector

Calibration curves were constructed by plotting peak areas against concentration and for all drugs passed through the origin and were linear over the range 0 – 50mcg/cm^3 with r^2 values of 0.99. The lower limit of detection was 50ng/cm^3 and the intra-assay coefficient of variation was 6-8%.

2.3.10 Preparation of *Ex Vivo* Circuit for Analysis

All circuits investigated during this analysis comprised of the Medos Hilite® 800 LT infant hollow fibre oxygenator, as opposed to the silicone membrane oxygenator utilised in the *in vitro* investigations.

After surgical decannulation of neonates, the circuit was clamped via the double lumen cannula to prevent blood spillage. Infusion of all drugs and other infusions were

transferred from the separated circuit directly to the neonate. The roller pump was stopped and the circuit detached from the integrated heat exchanger. Using metal clamps to prevent excessive blood spillage, various components of the circuit were spliced. Circuit tube samples were taken from the drainage line (i.e where blood is normally drained from the patient), pre oxygenator, post oxygenator and reinfusion line. Blood was drained and any residues were washed out from the tubes using distilled water. The oxygenator was prepared by washing out excess blood by pushing water through the blood inlet port using a $50cm^3$ bladder syringe and allowing drainage from the blood outlet port. This was repeated until all visible blood had been removed. Excess water in the samples was shaken out and then allowed to dry off at room temperature. All samples were then placed and labelled individually in polythene bags and stored at -20°C until LC-MS analysis.

2.3.11 Extraction of Midazolam from *Ex Vivo* Circuits

Samples of tubing isolated from the clinically used circuit (detailed in 2.3.10) were cut into lengths of 10 to 20cm. The tubes were filled with a measured volume of double distilled water, sealed with glass stoppers. Serial samples were taken initially at 2, 4, 6, 24 hours and then 12 to 24 hourly thereafter. After each sampling occasion, the tube was completely emptied of the extraction solution, refilled with fresh double distilled water and resealed. The extraction solution was transferred into glass vials using glass pipettes ready for LC-MS analysis. Triplicate samples ($0.5cm^3$) of the extract solution was mixed with $0.5cm^3$ of diazepam $200ng/cm^3$ internal standard in a glass vial and sealed with caps prior to analysis by LC-MS.

The isolated oxygenator was filled with measured volume of double distilled water by infusing through the blood inlet port using a $50cm^3$ bladder syringe to the point water dripped out of the blood outlet port and then sealed using clips. All other ports on

the oxygenator were capped off. Serial samples were taken at 24 hour intervals. On each sampling occasion, the oxygenator was completely emptied of the extraction solution, refilled with fresh double distilled water and resealed. The extraction solution was transferred into glass vials using glass pipettes ready for LC-MS analysis. Triplicate samples ($0.5cm^3$) of the extract solution was mixed with $0.5cm^3$ of diazepam $200ng/cm^3$ internal standard in a glass vial, sealed with rubber bung caps prior to analysis by LC-MS.

2.3.12 Analysis of Drug Extraction from *Ex Vivo* Circuits by LC-MS

Acquisition was initially made in scan mode for both reference midazolam and diazepam (IS) to identify the fragmented ions in the full spectra. Following this, the extracted solution with added internal standard was identified and quantified using the selected ion monitoring (SIM) mode. Analytes were ionised by positive ion electrospray and detected by using an Agilent quadrople mass spectrometer (MS). MS parameters optimised for the analysis were fragmentor voltage to give the most intense protonated molecule for each analyte, capillary voltage for maximum signal, and spray chamber parameters for maximum signal with minimum noise.

2.3.12.1 Chromatographic Conditions

Column:	Luna 5μ C_{18} (2) 150×4.6mm with integrated guard column (Phenomenex, UK)
Mobile Phase:	Methanol:Double Distilled Water: Ammonium Acetate solution (800:100:100 v/v)
Flow rate:	$1cm^3$/min
Injection volume:	$20\mu l$
Run time:	10 minutes
Retention times:	Midazolam (2.9 mins), Diazepam (3.4 mins)

2.3.12.2 MS Conditions

Source:	ESI
Ionisation Mode:	Positive
Fragmentor:	70 V
Gain:	5
Vcap:	3500 V
Threshold:	20
Nitrogen drying gas flow:	10 L/min
Nebulizer:	35 psig
Quadropole temperature:	100°C
Mass range (Scan Mode):	200-400 daltons
Ions for identification and quantitation (SIM mode):	Midazolam (m/z 325, 326, 327) Diazepam (m/z 285, 286, 287).

Complete system control and data evaluation were carried out using the Agilent LC-MS Chemstation Software. Calibration curves were constructed by plotting peak area ratios of midazolam to the internal standard diazepam against concentration, using a linear regression model. The assay was linear (r^2 0.995) over the concentration range 10 – 500ng/cm^3 (See Chapter 3, Section 3.12.2). The inter and intra-day coefficient of variation was less than 3%.

2.4 Clinical Investigations

For all clinical studies, ethics approval was obtained from the Leicestershire Research and Ethics Committee, prior to the investigation. Data was collected both prospectively and retrospectively. For prospective investigations, signed assent was obtained from all parents or relatives and written information regarding the study was provided as soon as feasible.

2.4.1 Prospective Investigations

For prospective investigations, intensive care observation charts, twice daily serum biochemistry summaries, blood gases, prescription charts as well as other medical and nursing records were accessed on a daily basis. In the case of vancomycin administered intermittently, dosing regimens, including timing of administration was diligently noted from the prescription charts. Similarly for midazolam, administered as a continuous infusion, dosing was recorded on an hourly basis from the initiation to termination of infusion, noting any change in concentrations and infusion rates. Where obvious or substantiated modifications in the dosing regimen occurred, for example a delay in drug administration due to nursing priorities or clinical reasons, these were carefully noted. Any significant clinical occurrences during the period on ECMO, for example a change (renewal) in the ECMO circuit or surgical interventions requiring anaesthesia were also noted. Blood sampling times followed study protocol as far as possible. Unavoidable deviations were again carefully noted. Clarity was sought from the responsible clinician or nurse when uncertainty or ambiguity arose. All blood samples during VV ECMO were taken from indwelling arterial cannulas in the patient. During VA ECMO, all blood samples were taken from the circuit, proximal to drug infusions and the reservoir.

2.4.2 Retrospective Investigations

For retrospective investigations, suitable study populations were identified from an ECMO database dating back to April 1989. In reality though and in the interest of minimising confounding variables, patient records pre dating 1997 were not accessed since it was felt that practices and procedures (including components of the ECMO circuit) had altered significantly during this period. Inclusion criteria required at least one plasma drug concentration to be recorded per patient. Assay databases held in the Department of Biochemistry for aminophylline, and the Department of Microbiology

for gentamicin and vancomycin, were accessed and compared with the ECMO database. The medical records of matched patients were then traced and retrieved. Medical records older than two years at the time of study tended to be microfiche otherwise original paper records were found. As with prospective investigations, all relevant aspects of the medical and nursing records were accessed. Where dosing regimens could not be deciphered or were incomprehensible, patients were excluded from the analysis. All demographic and clinical data was recorded onto proformas and then collated in to MS Excel Spreadsheets. Patient identification was anonymised at this stage.

2.4.3 Determination of midazolam and 1-OH midazolam in plasma by GC-MS

This analysis was developed and carried out by the Department of Toxicology, Leicester Royal Infirmary, for the purposes of this study. The analysis identified and quantified midazolam and the primary metabolite 1-OH midazolam in blood samples collected from 20 neonates during ECMO (see Chapter 4).

2.4.3.1 Extraction

Midazolam was extracted from whole blood using solid-phase extraction (SPE) and then quantitated using gas chromatography-mass spectrometry (GC-MS). Nalorphine was used as an internal standard. Half a millilitre of whole blood was diluted to 3ml with deionised water. After addition of 20µl nalorphine (50µg/cm^3) internal standard, samples were centrifuged at 3000rpm for 5mins ready for the extraction process. Baker-Bond Narc-2 mixed phase SPE tubes were pre-conditioned with 3cm^3 methanol, followed by 3cm^3 water and finally 3cm^3 phosphate buffer. Samples (3cm^3) were then loaded onto the columns. After washing columns with 3cm^3 deionised water, followed by 0.25cm^3 10mmol/l acetic acid (pH3), midazolam and 1-OH midazolam were eluted from the columns with ammoniacal chloroform/isopropanol using low vacuum.

2.4.3.2 Derivatisation

The eluates were dried down under vacuum in a vortex evaporator at 70°C. The residues were reconstituted with 200µl ethyl acetate and then again evaporated to dryness in a vortex evaporator at 70°C. Pyridine (10µl) and MSTFA (30µl) was then added to the eluates and heated for 30 minutes in a hot block at 70°C to form Trimethylsilyl derivatives (TMS).

2.4.3.3 Analysis

Column:	HP1 (30m x 0.25mm x 22µm) methylsiloxane column
HP 6890 gas chromatograph with autosampler	
Carrier Gas:	Helium
Flow Rate:	1.2cm^3/min
Injection Volume:	20µl
Split Ratio:	10:1
Oven Program:	Initial Temp 90°C for 2mins Ramp 40°C min^{-1} up to 280°C, then by 20°C min^{-1} up to 320°C and held constant for 5 mins
Run time:	13.5 mins
Retention time:	Midazolam (9.2 min), Nalorphine Internal Standard (9.4 min), 1-OH Midazolam (9.6 min)
MS:	HP 5973 Mass Spectrometer

Derivatised ions used for identification and quantification (SIM mode):
midazolam (310, 312, 325), 1-OH midazolam (398, 413), nalorphine (260, 414, 455).

Calibration curves were constructed by plotting peak area ratios of target analyte to the internal standard against concentration, using a linear regression model. The assay was linear (r^2 0.999) over the concentration range 10 –10,000ng/cm^3. The inter and intraday coefficient of variation was less than 10%.

2.5 Pharmacokinetic Analysis

2.5.1 Linear Regression Analysis

Routine therapeutic gentamicin monitoring data was analysed using this approach. Peak (1 hour post dose) and trough (immediately prior to the next dose) plasma concentrations were fitted by linear regression analysis of log concentrations, assuming a one compartmental model with first order elimination. Assuming that peak and trough levels were determined in the elimination phase, the elimination rate constant (K_e in hr^{-1}) was determined directly from the slope.

$$K_e = (\text{Ln } C_{ss}\text{peak} - \text{Ln } C_{ss}\text{trough}) / \tau$$

The volume of distribution (V; L/kg) was determined using the Sawchuk and Zaske method (Sawchuk *et al.*, 1976):

$$V = (\text{Dose}/C_{ss}t).(1/1-e^{-Ke\tau}) \cdot e^{-Ke.t}$$

$C_{ss}t$ = Steady state plasma concentrations at time t

τ = time between peak and trough (hour)

t = 1 hour (peak) and trough

Half-life (hours) was calculated as ln 2 / K_e and clearance (L/kg/hr) was determined by K_e.Vd. Microsoft Excel 7.0 was used to perform pharmacokinetic calculations using the aforementioned equations.

2.5.2 Non-Linear Regression Analysis

Initial estimates of vancomycin pharmacokinetics were estimated from the rich data set by non-linear regression analysis, performed using WinNonLin Standard (Version 3). Initial exploratory analysis of concentration time profiles revealed a biexponential elimination phase. Thus for each individual, a two compartment model with first order elimination was fitted to the plasma concentrations. The mean and variability of parameter estimates from these individual were then used as initial estimates for the population model development.

2.5.3 Non-Linear Mixed Effects Modelling

Non-linear mixed effects modelling was performed using WinNonMix Professional (Version 2.0.1) bundled with Compaq Visual Fortran Compiler Professional Edition, (Version 6.5). This approach estimates not only the structural (mean) parameters of the model, but also the interpatient (population) variability in parameters and residual error (difference between observed and predicted drug plasma concentrations) (Sheiner *et al.*, 1977). In preliminary analyses, one, two and three compartment models with first order elimination were fitted to all data from all subjects simultaneously. The compartment model which demonstrated a more appropriate structural model on examination of the graphical diagnostic plots as well as the differences in the objective function value (OFV) i.e. two times the negative log likelihood value, was chosen for further analysis. Initial parameter estimates were obtained either from the mean parameter estimates from non linear regression analysis of rich data or previous reports of population pharmacokinetics in similar populations. Once the base model had been defined, the influence of various demographic and clinical covariables in the regression models for clearance and volume of distribution were evaluated. Any covariate present in less than 20% of the population was not tested. In the aminophylline and vancomycin study with

wide age and weight ranges and due to the multicollinearity of several clinical characteristics, body weight was included in the model before evaluation of all other covariates. Allometric scaling transformation of weight ($Weight^{0.75}$) was also assessed at this point. Following the inclusion of weight, each covariate was added sequentially and then (where significant) cumulatively to the initial regression model in a linear and non-linear manner. The impact of this model building process on the plotted weighted residuals and the change in the OFV was noted. The difference in the OFV obtained before and after the addition of covariables is approximately chi-squared distributed with degrees of freedom equal to the number of parameters that are set to the null hypothesis value. With two degrees of freedom, a change in the OFV > 7.88 ($p<0.005$) was accepted as statistically significant.

2.5.3.1 Interpatient Variability

The interpatient variability in clearance and volume of distribution was modelled as proportional (constant coefficient of variation) deviation from the estimated parameter values (assuming a log normal distribution), and in this way avoided negative estimates of parameters.

$$ßi = ß*e^{bi}$$

ßi = Estimate of structural parameter (clearance and volume) in the i^{th} individual

ß = Population parameter value predicted by the regression model

bi = Random variable, which is normally distributed with variance, ώ, mean zero and represents the interpatient variability in the study population.

2.5.3.2 Residual Error

The residual error (intrapatient variability), which describes the difference between the observed and the predicted concentrations, was also estimated:

$$Cij = \hat{C}ij + \varepsilon ij$$

Cij = The jth observed concentration in the ith individual

$\hat{C}ij$ = The jth predicted concentration in the ith individual

εij = Residual error, normally distributed with mean zero

The variance for εij, σ^2, was modelled as:

$$\text{Variance} = \sigma^2 \ (a + b \ \hat{C}ij^2)$$

Fixing *a* to 1 and *b* to 0, produces an additive error model, whereas fixing *a* to 0 and *b* to 1 produces a proportional error model. Both of these models were also compared to an additive plus proportional error model ($a>0$, $b=1$). Again, the diagnostic plots and OFV were used to search the most appropriate error model.

Initial modelling was carried out using the First Order linearisation method. The final fit of the basic model and subsequent covariate testing was conducted using the First Order Conditional Estimation method (See Appendix II).

2.5.4 Predictive Performance of Models

A measure of the predictive performance of models can be determined by calculating the median prediction error (MDPE) percent i.e. observed minus predicted concentrations divided by the predicted value, multiplied by 100, and the absolute

median prediction error (AMDPE) percent (Sheiner *et al.*, 1981). The MDPE describes the bias and the AMDPE the precision (variability) of the predictions.

2.5.5 Validation of Models

Validation of models is crucial since unlike scientific hypotheses, a model cannot be verified directly by an experiment. The validation of a model is not that it is true, but that it generates good testable hypotheses relevant to the problem in question (Levins, 1966). Two types of model validation methods were used: internal and external (Sun *et al.*, 1999).

2.5.5.1 Internal Validation

For the midazolam and aminophylline studies, it was not practical to collect new data prospectively to use as a validation set, and therefore the ability of the final model to perform in prospective tests was estimated using the cross-validation technique. Although cross validation is not a truly prospective validation method, it is a recognised and established approach to estimating model performance assuming identical experimental conditions (Fiset *et al.*, 1995; Kerbusch *et al.*, 2001; Zomorodi *et al.*, 1998). The index data set was divided into x smaller groups of y patients each. The pharmacokinetic model was fitted to the data whilst excluding one group. The estimated structural parameters from the sub models were then used to predict the concentrations in the excluded group. This process was repeated x times excluding each group in turn. Since the excluded group is not used to develop the model, the MDPE and the AMDPE are almost unbiased estimates of the predictive capabilities of the model (Zomorodi *et al.*, 1998).

2.5.5.2 External Validation

External validation provides the most stringent method for testing a developed model and this approach was used for the vancomycin study (Sun *et al.*, 1999). The final model developed from the index data set was applied to data collected in a separate group of patients (validation data set).

2.6 Statistical Analysis

2.6.1 Laboratory Data

All data is expressed as mean (SD). A difference between two means was investigated using the student's t-test and univariate regression analysis was conducted using SPSS (10.0). In all cases $p<0.05$ was taken as significant.

2.6.2 Clinical and Pharmacokinetic Data

All clinical and demographic characteristics were reported as mean (SD) or where the standard deviation was large, median (range). Population pharmacokinetic estimates are expressed as mean± SE. Parametric and non-parametric (Mann-Whitney U) statistical analysis was used for analysis of clinical and demographic data whilst all pharmacokinetic parameters were assumed to follow the normal distribution and thus only parametric analysis was applied. For comparing more than two means, one or two way analysis of variance was used depending on the number of classification variables. Univariate and multivariate regression analysis was conducted in WinNonMix (Version 2.0.1) and SPSS (Version 10.0) to reveal trends in the population studies.

CHAPTER III

IN VITRO EVALUATION OF SEDATIVE DRUG CONCENTRATIONS DURING ECMO

3.1 Introduction

Reduction of the amount of drug available to a patient resulting from interactions with plastics is a well recognised and documented phenomenon (Trissel, 2001). Previous studies have only reported loss of drugs from aqueous solutions in contact with infusion bags (Cloyd *et al.*, 1980; Kowaluk *et al.*, 1981; Roberts *et al.*, 1980), administration sets (Hirschl, 1981; Kowaluk *et al.*, 1982) and filters (Baaske *et al.*, 1980; Parker *et al.*, 1980). The potency loss appears to be dependent on the physico-chemical characteristics of the drug and solution, the nature of the plastic and the dynamics of the system (Kowaluk *et al.*, 1982). However, ECMO circuits are unique in sorption studies since unlike plastic containers and administration sets used for drug delivery, the ECMO circuit is an extension of the patient's circulatory system. Therefore in distinction to sorption in intravenous delivery devices, drug-circuit interactions *in vivo* will have a significant influence on pharmacokinetics and hence therapeutic efficacy. For drug sorption to occur *in vivo* not only will the drug have to distribute from the blood phase, at a buffered pH of approximately 7.4 and at body temperature, but the process will compete with binding to circulating plasma proteins and red blood cells.

The component materials of an ECMO circuit are mainly organic in nature. The circuit tubing is made from pPVC (Tygon S-65-HL) whilst the gas exchange membrane in the oxygenator is a silicone membrane construct (see Chapter 2, Section 2.2.2). It is perfectly plausible that both of these materials lend themselves heavily to drug sorption since the patient's blood is exposed to a large surface area of each plastic. However, to date there are only limited reports on the interaction of drugs with components of the ECMO circuit. Rosen *et al* (1988) demonstrated fentanyl uptake by the membrane oxygenator whilst Dagan *et al* (1993) in their preliminary *in vitro* evaluation revealed significant decreases in circuit drug concentration with time. The disposition of

commonly used critical care sedatives, diazepam, midazolam, lorazepam and propofol in the ECMO circuit has not been described.

The affect of different priming solutions used to modify the surface characteristics of the CPB circuit has also been investigated. Hynynen *et al* (1995) and Rosen *et al* (1986) reported a decrease in the drug loss when the a CPB circuit was primed with blood whereas Hammeren *et al* (1999) demonstrated that priming CPB circuits with heparin did not prevent loss of propofol during *in vitro* tests. No such tests have been reported for ECMO circuits.

3.2 Aims of the Study

The purpose of this phase of the study was to compare the sorptive profiles of morphine, diazepam, lorazepam, midazolam and propofol and to evaluate factors determining such losses, if they occurred.

The investigation was in several stages. First, the different polymeric components of a neonatal circuit were dismantled and a small portion of the pPVC tubing (¼" diameter) and silicone membrane (from the oxygenator) was isolated. The interaction between these two polymers with static solutions of the sedative drugs was then investigated to determine the relative degrees of sorption. Second, these preliminary studies were repeated after priming the circuit components with clear prime solution.

In clinical practice it is important to know the real amount of drug that a patient will receive during the infusion. The primary questions then are, what quantity of the drug will be lost to the ECMO circuit and what dose the patient will receive. To evaluate this, an intact neonatal circuit was assembled, and the administration of drug infusions through the circuit was simulated.

Previous investigations have not established or proven sorption of drugs in clinical practice. Therefore in the final investigation, *ex vivo* neonatal circuits were examined and evaluated for midazolam sorption.

3.3 Static Sorption Studies of Selected Sedative Drugs

3.4 Materials and Methods

3.4.1. Investigation of Drug Sorption into Circuit Components under Static Conditions

Stock drug solutions of morphine, diazepam, lorazepam, midazolam and propofol were prepared as detailed in Chapter 2, Section 2.3.1. Sorption tests were carried out on 8 different samples of the pPVC tube and 8 samples of the silicone oxygenator membrane taken from 2 nominally identical neonatal ECMO circuits of the same manufacturer. The method is described in Chapter 2, Section 2.3.5.

3.4.2. Investigation of Drug Sorption into Circuit Components Previously Primed.

Clear prime solution was prepared as described in Chapter 2, Section 2.3.2. pPVC tubes and silicone oxygenator membrane samples were treated with the clear prime solution for 1 hour as described in Chapter 2, Section 2.3.6. Tests were carried out on 8 different samples of the pPVC tube and 8 samples of the oxygenator membrane taken from 2 nominally identical neonatal ECMO circuits of the same manufacturer. The method is described in Chapter 2, Section 2.3.5.

3.4.3 Extraction of Midazolam from Previously Exposed Circuit Components

After the exposure experiments, pPVC tube and silicone samples were washed with double distilled water and then treated with methanol to extract midazolam from the polymeric material and confirm uptake by the specified component. This procedure was repeated with double distilled water as an extraction solvent. The method is described in Chapter 2, Section 2.3.7. This test was not carried out for the primed materials.

3.5 Statistical Analysis

All data is expressed as mean (SD). Differences in means between primed and unprimed data was explored using t-test. A p value < 0.05 was considered significant. Monoexponential curve fittings and determination of coefficients for flow simulation data, was achieved using MS Excel Solver (2000).

3.6 Results

The results obtained were reproducible within each category of test: drug, material and priming treatment. Reproducible differences between primed and unprimed components were observed for both the ECMO circuits investigated. Absorbance of the mobile phase was negligible over the range of wavelengths at which the study drugs were examined. Unused pPVC tubes filled with methanol caused leaching of plasticiser and resulted in increasing absorbance with time. A similar but reduced effect was seen with double distilled water.

3.6.1 Drug Sorption under Static Conditions

Table 3.1 presents the time dependent changes of the drugs in contact with the two principal plastics, pPVC tubing and the silicone oxygenator. In contact with pPVC and silicone membrane, concentrations of diazepam, lorazepam, midazolam and propofol

decreased significantly with time ($p<0.005$). Lorazepam decreased by ca 40%, midazolam by ca 68%, diazepam by ca 88% and propofol by 98%. Equilibrium in all cases was attained between 40 and 120 minutes (Figure 3.1). In contrast, morphine sulphate only decreased in contact with pPVC and the percentage decrease (16 %) was less. No appreciable loss seen with the silicone membrane ($p>0.05$).

3.6.2. Drug Sorption after Priming

The results from the primed pPVC samples revealed an increased loss of benzodiazepines by about 20% compared to the unprimed samples, although this was not significant at the $p<0.05$ level (Figure 3.2). In contrast, a decrease in sequestration to the primed silicone membrane was observed such that virtually no loss of lorazepam occurred ($p<0.0005$), whilst both midazolam and diazepam showed reduced losses of 40% and 60% (previously 68% and 88%) respectively ($p<0.05$).

Table 3.1. Decreasing concentrations ($\mu g/cm^3$) of a standard solution of drugs as a function of time in contact with the plastic components of an ECMO circuit[a].

Contact time (mins)	Morphine		Lorazepam		Midazolam		Diazepam		Propofol	
	pPVC[c]	Sil[b]	pPVC[c]	Sil[c]	pPVC[c]	Sil[c]	pPVC[c]	Sil[c]	pPVC[c]	Sil[c]
0	2.5	2.5	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
5	2.4	2.4	20.0	18.8	17.5	18.0	8.7	16.5	8.4	14.0
20	2.2	2.5	15.1	17.0	11.3	12.8	3.8	10.0	1.3	4.0
40	2.1	2.4	15.0	16.8	8.8	9.0	3.0	6.3	0.5	1.3
120	2.1	2.5	15.0	16.4	8.5	8.0	3.0	5.8	0.4	0.8

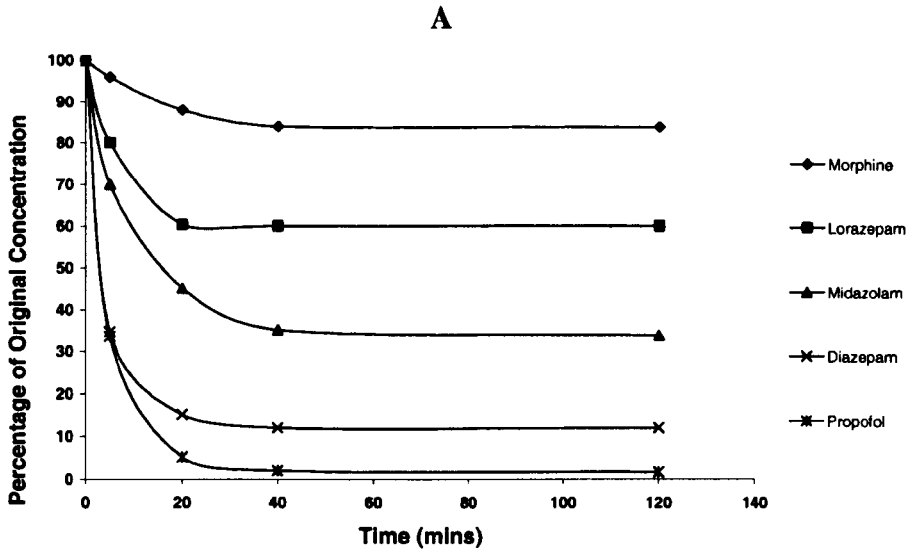
a. Results reported as the mean of eight determinations with a SD of +/- 5% of stated value.

b. $P>0.05$, difference between initial and final concentrations

c. $p< 0.005$, difference between initial and final concentrations

pPVC – Plasticised Polyvinyl Chloride tubing

Sil – Silicone membrane oxygenator



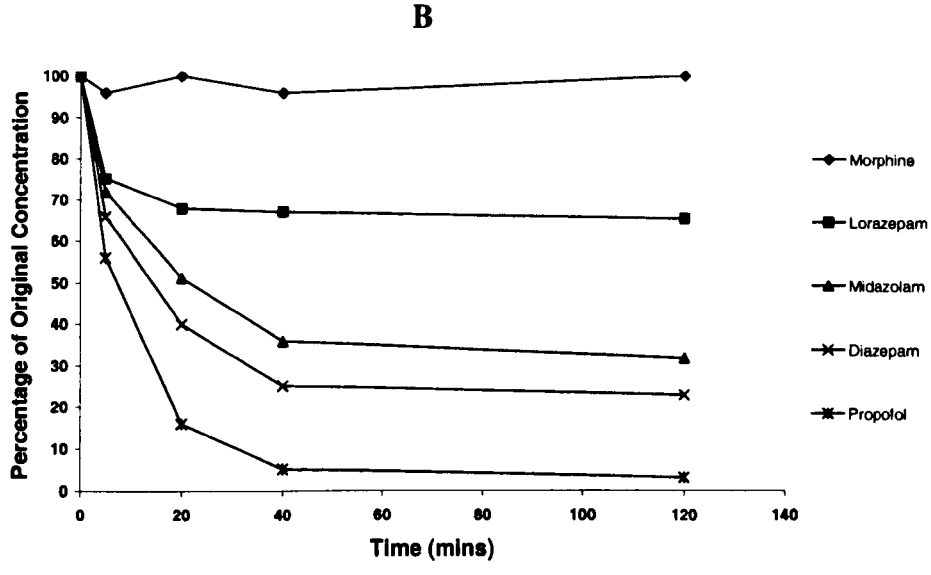
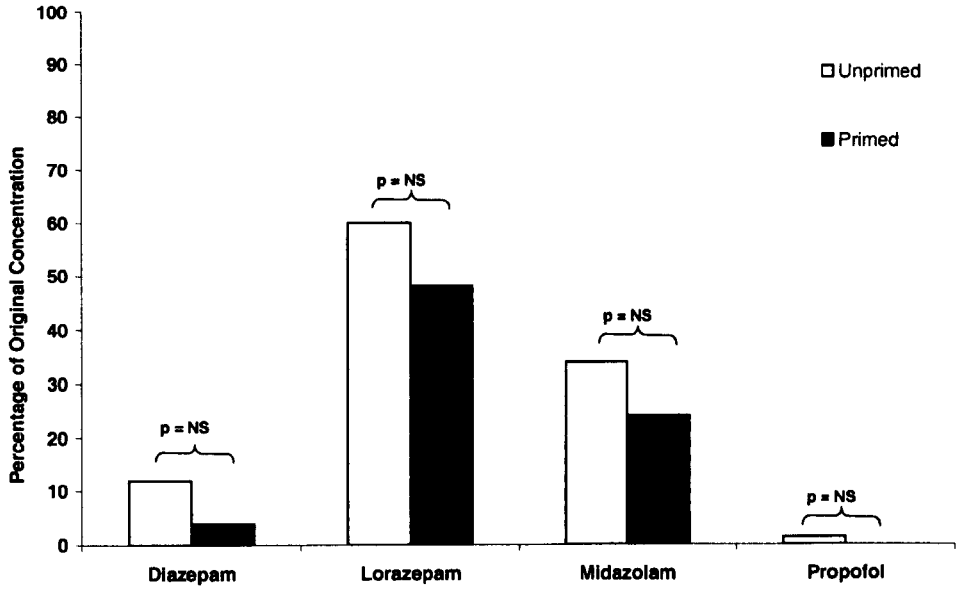


Figure 3.1. Percentage of original concentrations as a function of time for a range of standard drug solutions in contact with the (A) pPVC and (B) Silicone membrane components of an ECMO circuit. A mean of eight determinations is plotted at each time point. Standard deviation was +/- 5% of plotted value.

A



B

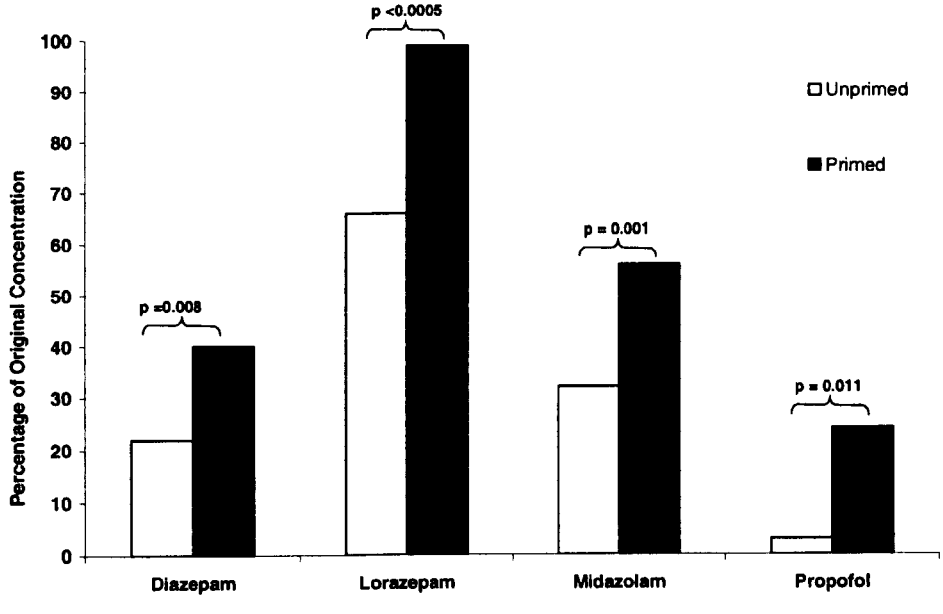


Figure 3.2. Percentage of original concentration remaining after 120 minutes in primed and unprimed (A) pPVC tubing (B) Silicone Membrane

3.6.3 Drug Extraction

Methanol extraction of midazolam from previously exposed pPVC tubing and silicone membrane also had an effect of leaching plasticiser, known to be DEHP, causing the HPLC detector to become overloaded with an off scale signal. This migrant had similar retention times to midazolam and interfered with the peak normally observed for the target analyte. Changing the mobile phase to a more inorganic composition (70% methanol) eliminated plasticiser contamination for the first 40 minutes of analysis. Using double distilled water as an extraction solution further delayed plasticiser contamination. The results revealed a cumulative increase in midazolam concentration with time, with almost total recovery of sequestered midazolam from the silicone membrane and more than 50% from pPVC tubes after 40 minutes in contact with 70% methanol/water (Figure 3.3)

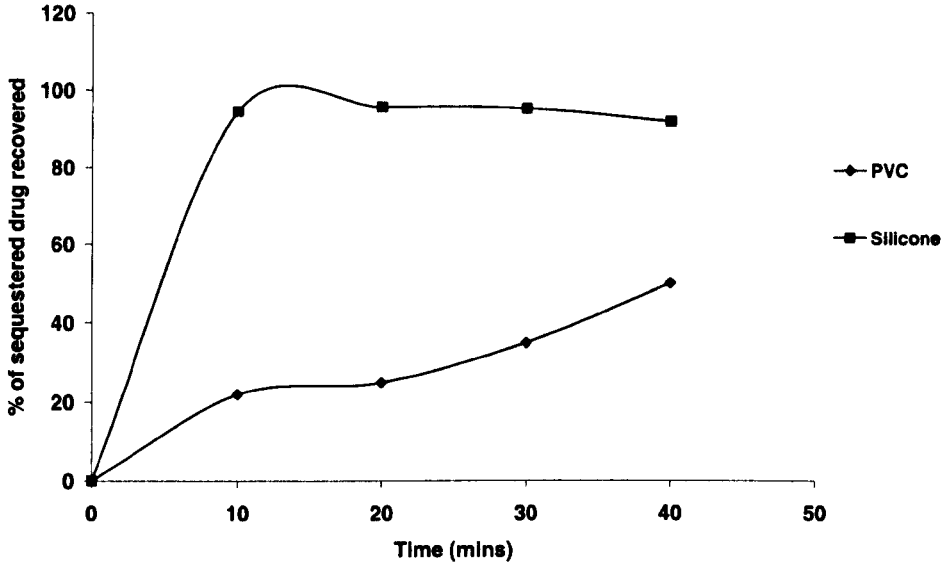


Figure 3.3. 70% Methanol/Water extraction of Midazolam from previously exposed unprimed circuit components

3.7 Investigation of Drug Sorption in a Flowing System

3.8 Materials and Methods

The effects of the ECMO circuit on continuous infusion of sedative drugs was investigated by injecting a therapeutic concentration of 0.4 $\mu g/cm^3$ solution of all four drugs into the circuit proximal venous line adjacent to the venous catheter connection (at the point blood is drained from the patient) and monitoring the level of drugs in the solution leaving the circuit post oxygenator (at the point blood would be reinfused into the patient). The flow rate of the circuit was set at $360cm^3/min$ and samples were taken at two minute intervals. The drug levels were determined by HPLC and all readings were replicated three times. The method is described in Chapter 2, Section 2.3.8.

3.9 Results

The investigations of a complete ECMO circuit showed that in the early stages, the concentration of drugs measured in the effluent post-oxygenator were considerably lower than anticipated. The data in figure 3.4 shows the effluent drug concentration versus time, and expressed as a percentage of the initial infusion concentration. The extent of loss of various drugs during simulated infusion through the circuit and the profile of effluent concentration over a period of time differed for all drugs examined. In the case of diazepam and midazolam, effluent concentrations were minimal at the onset of infusion and then increased gradually to a steady-state situation. Concentrations of lorazepam in the effluent were highest and reached almost 100% of the initial at the end of the experiment. In contrast, propofol was undetectable in the effluent for the first 36 minutes of infusion, increasing slightly thereafter with time. The fluctuations in the effluent drug concentration-time profiles shown in figure 3.4 are caused by small variations in the flow rate of drug solution through the circuit.

Figure 3.5 shows the cumulative amount of drug sorbed after various times. The sorption of diazepam and midazolam is slow and time dependent. At the end of the infusion period sorption is still continuing. In comparison, lorazepam sorption seemed to rapidly reach equilibrium with very little sorption occurring towards the end of the infusion period. Propofol sorption was extremely rapid and virtually linear for the duration of investigation.

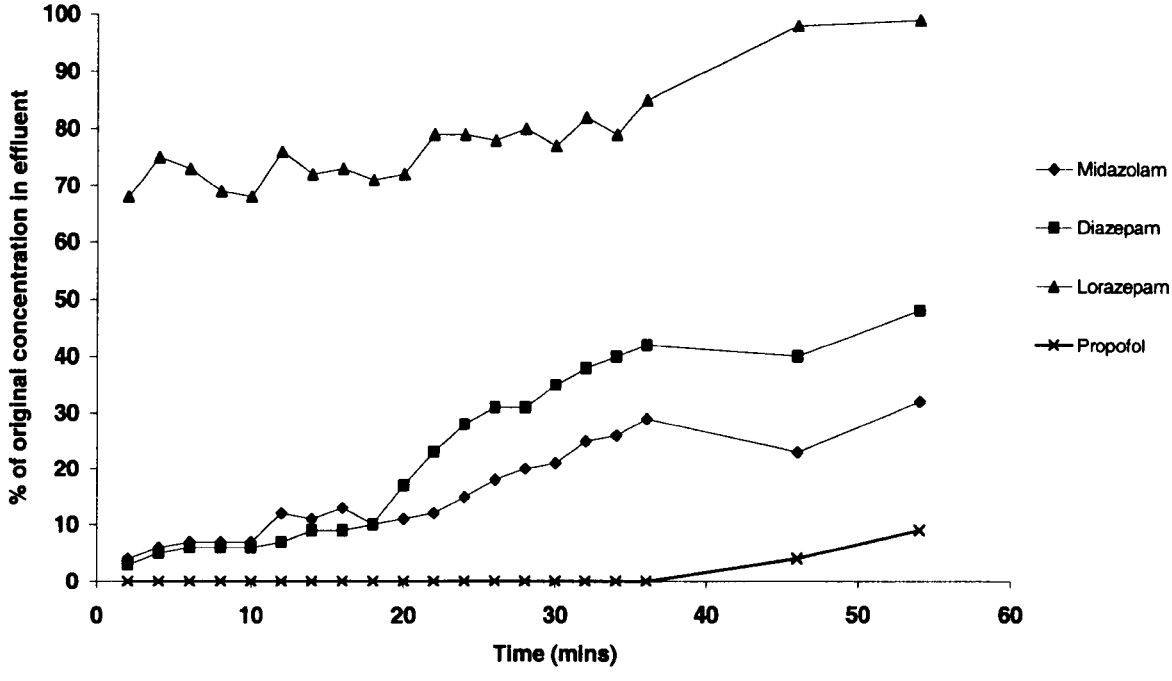


Figure 3.4. Percent of original drug in the effluent at various times during simulated infusion through a neonatal ECMO circuit at a flow rate of 360 cm^3/min

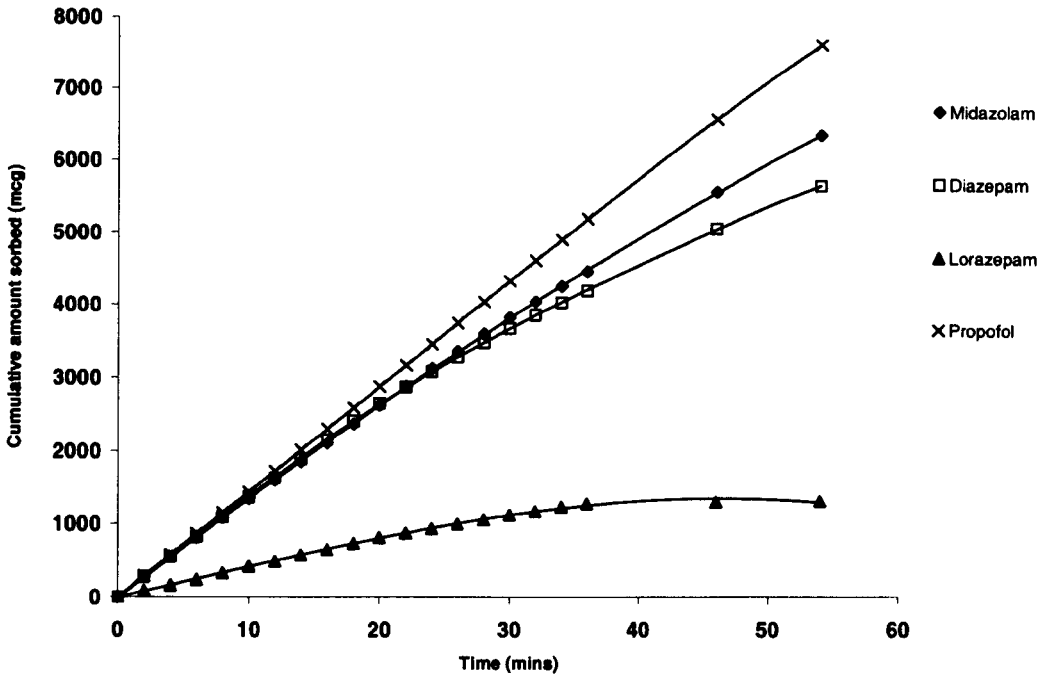


Figure 3.5. Cumulative drug sorption by a neonatal ECMO circuit during simulated infusion at a flow rate of 360 cm^3/min

The relationship between steady state effluent concentration of drug and time can be expressed in the form of a monoexponential equation:

$$C_{(e)} = C_{SS}.(1 - e^{-Ksor.ti})$$

Where $C_{(e)}$ is the effluent concentration of drug at time t, and K_{sor} is the first order rate constant for sorption (since fractional loss of drug is independent of concentration) and *ti* is the infusion time. The calculated coefficient values for curve fittings are listed in Table 3.2. Using these coefficient values, the percentage of dose delivered can then be extrapolated to various time intervals (Table 3.3). The results clearly show that in the case of lorazepam, after 1 hour of infusion the percentage of dose delivered will be

greater than 90%. Whereas with propofol, even after 24 hours of infusion only 75% of the dose will be delivered.

Table 3.2. Coefficient Values for Determining Sedative Drug Sorption to a Neonatal ECMO Circuit at a Flow Rate of 360 cm^3/min

Drug	Coefficient[a]	
	K_{sor} (min^{-1})	R^2
Diazepam	0.012	0.92
Lorazepam	0.9	0.74
Midazolam	0.007	0.91
Propofol	0.001	0.41

a. Coefficient values are given for the equation $C_{(e)} = C_{SS}.(1 - e^{-Ksor.ti})$

Table 3.3. Sedative Drug Bioavailability on Initiation of a 0.4 $\mu g/cm^3$ Continuous Infusion through a Neonatal ECMO Circuit

	% of dose delivered at flow rate of 360cm^3/min[a,b]			
Infusion Time (hrs)	**Diazepam**	**Lorazepam**	**Midazolam**	**Propofol**
0	0	0	0	0
0.5	31.1	82.0	20.2	1.6
1	54.3	90.9	35.3	4.5
1.5	69.7	95.4	47.4	7.4
3.0	91.2	99.4	71.9	15.3
6.0	99.3	100.0	92.0	29.3
12.0	100.0	100.0	99.3	50.7
24.0	100.0	100.0	100.0	76.0
48.0	100.0	100.0	100.0	94.3

a. Neonatal ECMO circuit = Tygon® S-65-HL pPVC tubing (691cm) and Avecor™ 0800 Silicone membrane oxygenator.
b. Calculated using equation and coefficient values listed in Table 3.2.

3.10 Investigation of Midazolam Release from *Ex Vivo* Neonatal Circuits

3.11 Materials and Methods

The *in vitro* studies indicated the amount of drug sorbed for a given dose and set of experimental conditions. This experiment was conducted in an attempt to extract and quantify sorbed midazolam from circuits exposed to the drug in a real clinical situation (and therefore very different conditions).

3.11.1 Patient Selection

Inclusion criteria included neonates who survived to decannulation and had received continuous infusions of midazolam during the period on ECMO. Patients who had replacement of circuit components during ECMO, for example oxygenator or raceway changes, were excluded. Circuits from 5 neonates were collected immediately after separation from patient. All midazolam infusions during ECMO were administered into the circuit proximal venous line, pre-reservoir. Clinical, demographic and dosing data were collected retrospectively from medical and nursing notes.

3.11.2 Preparation and Analysis of Circuit

Circuit samples were prepared immediately after collection of circuit as described in Chapter 2, Section 2.3.10. In order to obviate problems associated with plasticiser leaching and HPLC-UV analysis, midazolam was extracted with water (Chapter 2, Section 2.3.11) and analysed using LC-MS (Chapter 2, Section 2.3.12). The contact time between extraction solution and circuit components was preset at 2,4,6,12 and 24 hourly thereafter.

3.12 Results

3.12.1 Demographic and Clinical Data

Clinical, demographic characteristics and dosing data are displayed in Table 3.4. All patients underwent VV ECMO. No patient had received midazolam prior to the initiation of ECMO. All patients received concurrent morphine infusions as well as other enteral sedatives such as chloral hydrate.

Table 3.4. Demographic and Clinical Characteristics of Study Group.

Male/Female	3/2
Weight (kg)	3.6 (0.4)
Gestational Age (weeks)	40.04 (3.04)
Age at Cannulation (days)	0.9 (0.2)
Duration of ECMO (hours)	134 (57..3)
Cumulative midazolam dose (μg/kg)	18495 (12686)
ECMO Flow Rate (cm^3/kg/min)	72.7 (23.1)
Urea (mmol/L)	8.5 (4.2)
Creatinine (μmol/L)*	83.9 (34.9)
Liver Function	Normal in all patients

Data is expressed as Mean (SD)

3.12.2 LC-MS Analysis

The retention times of midazolam and diazepam (IS) were 2.9 and 3.4 minutes respectively, and the chromatographic peaks resolved on the total ion chromatogram after the pH of the mobile phase was adjusted to 5.4 (Figure 3.6). The full-scan mass spectra of midazolam and diazepam are presented in figure 3.7, and the major ions indicative of midazolam and diazepam were identified:

Midazolam (m/z): 326, 327, 328

Diazepam (m/z): 285, 286, 287

The within–day precison, as well as the between-day precision and accuracy were less than 3 % and satisfactory under all concentrations tested (see Chapter 2, Section 2.3.12.2). A calibration curve was constructed by plotting peak area ratios of midazolam to the internal standard diazepam against concentration, and this was used to quantify midazolam recovered from the circuit components (Figure 3.8).

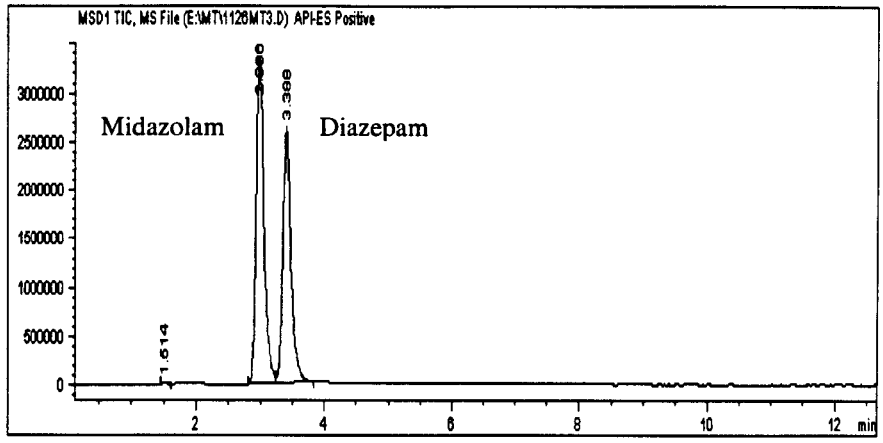


Figure 3.6. Total Ion Chromatogram of Midazolam and Diazepam (IS)

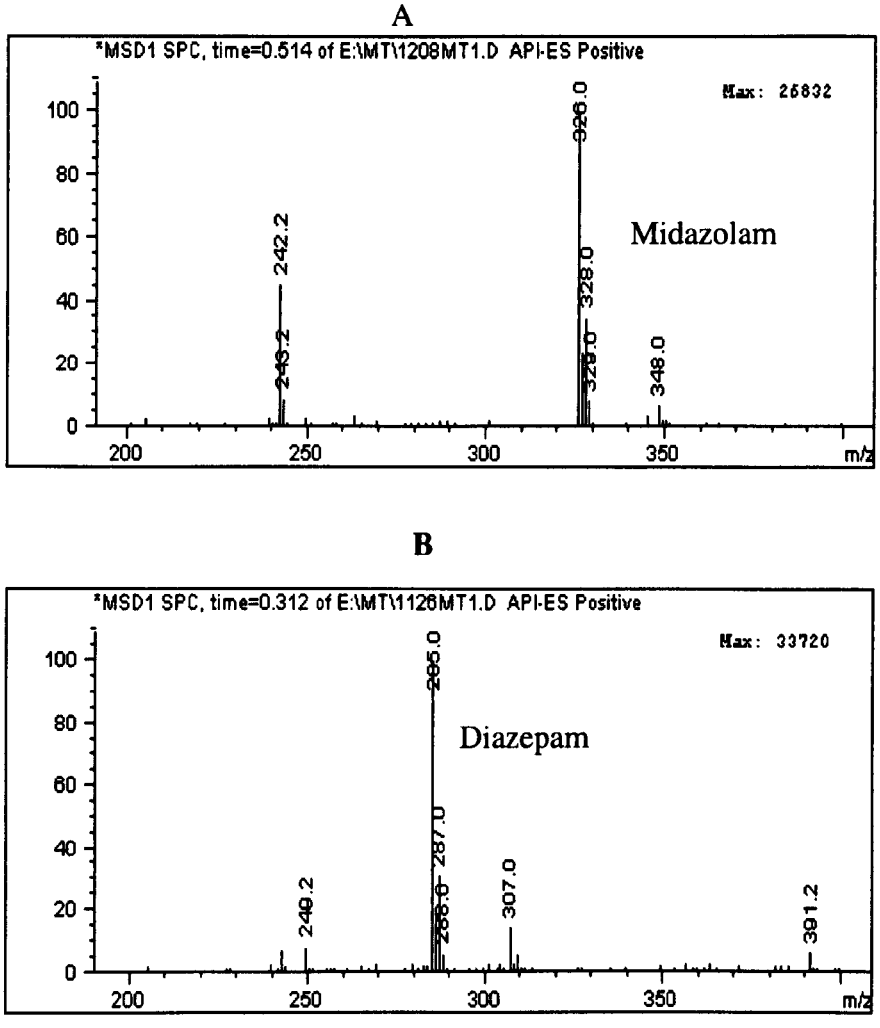


Figure 3.7. Full scan spectra of (A) Midazolam and (B) Diazepam

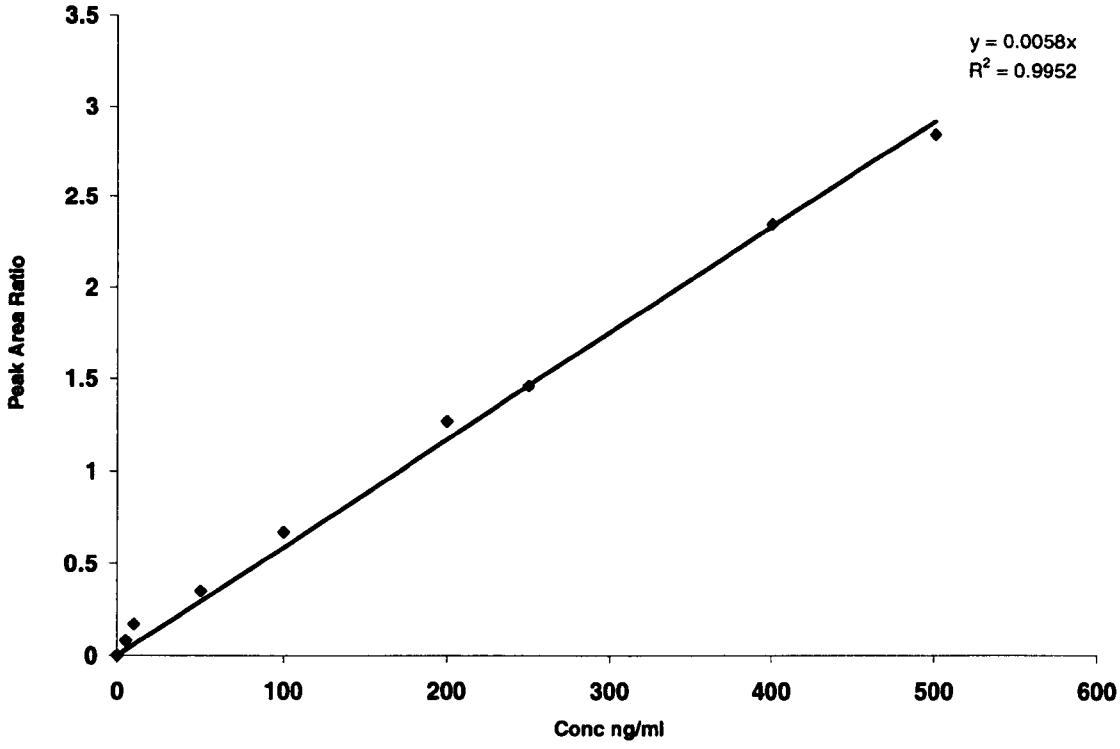


Figure 3.8. Calibration Curve Peak Area Ratio of Midazolam to Diazepam (IS) Against Concentration (ng/ml).

3.12.3 Midazolam Recovery from the *Ex Vivo* Circuits

A significant amount of midazolam was recovered from clinically used circuit components (Figure 3.9). The amounts extracted are mean values from three sections of the circuit pPVC tubing: drainage, pre oxygenator and post oxygenator. Data from the hollow fibre PMP oxygenators were not available because of significant contamination of the extracts with blood residues and other debris. As can be seen from Figure 3.9, the amount extracted increases with increasing doses administered. This presumes that the greater the doses administered, the higher the plasma concentrations achieved and since sorption from earlier investigations appears to be a first order process, a greater amount will be sorbed. The amount extracted as a percentage of total dose administered (and therefore circuit exposure) varied between 0.5-2.0%, although in only two patients did the amount extracted fall below the limit of detection (10ng/ml) suggesting that

significant amounts of drug was still present in the tubing. Based on cumulative amount of midazolam sorption estimated in the flow simulation studies, this may represent a fraction of total drug. It is expected that release of midazolam like uptake, is an exponential process and thus a limitation of this methodology i.e. it was not possible to determine total amount sequestered using this approach. Although the method of recovery was time consuming the major advantage was that the extraction and identification of midazolam was made relatively simple.

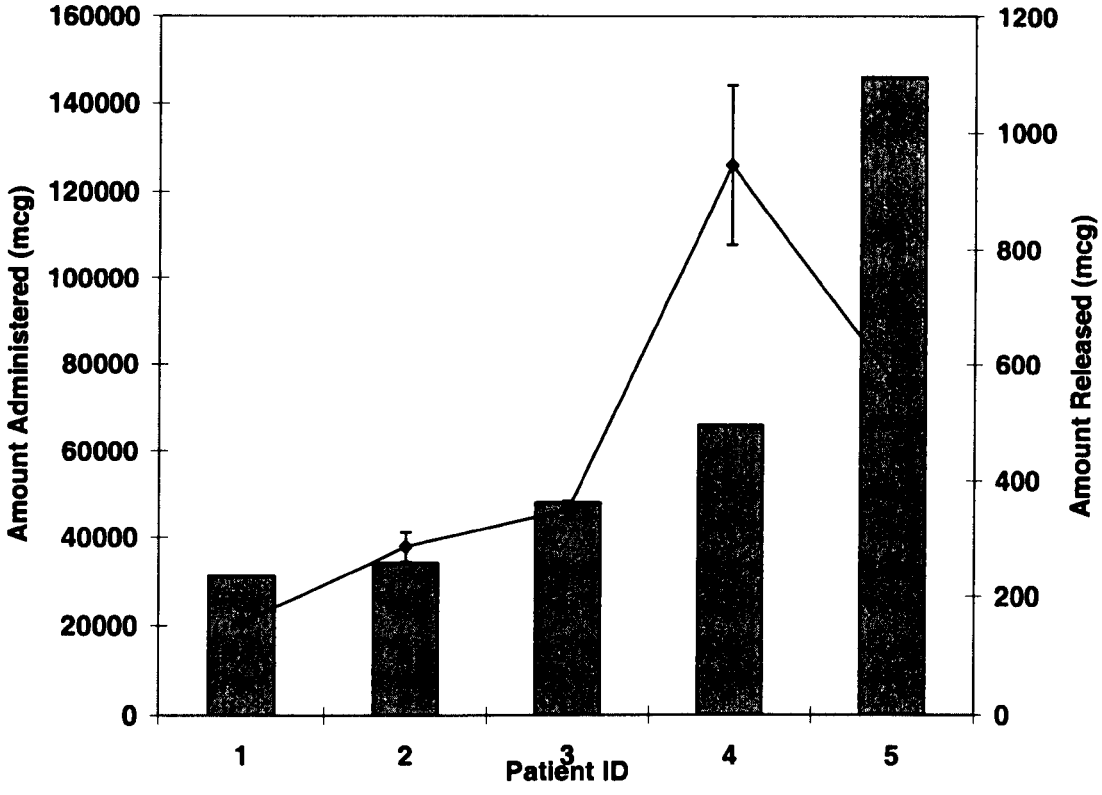


Figure 3.9. Comparison of amount of drug released (mean [SD]) from ex vivo circuit tubing (line) versus amount of drug administered (bars).

3.13 Discussion

Although there is limited data on drug sorption in ECMO circuits, the extent of the problem and predisposing factors affecting interaction between drugs and plastic components does not, however, appear to have been determined. Decreased levels of phenobarbital, heparin, vancomycin, gentamicin and phenytoin have been reported, however the clinical relevance of such losses has not been quantified (Dagan *et al.*, 1993; Marx *et al.*, 1991).

All of the sedative drugs examined in the present preliminary survey were lost from solution in contact with both the pPVC and silicone membrane components of the ECMO system. The extent of loss varied between drugs, and between the primed and unprimed components. In contrast, a reduced loss of morphine sulphate was observed in contact with unprimed pPVC, whilst no loss was observed in contact with unprimed silicone membrane. The lack of morphine sulphate sorption to the silicone membrane concurs with the *in vivo* findings of Geidushek *et al* (1997).

Table 3.5 shows that the percentage of drug loss from aqueous solution is a function of the log P value and the solution pH. All the sedative drugs exhibiting substantial losses have relatively high log P values in the non ionised form, whereas morphine sulphate has a very low log P value (Table 3.5). In accordance with the pH-partition hypothesis, a significant correlation was observed between percentage of drug lost from solution and the log P value of the drug, when in contact with both the pPVC tubing (r^2=0.83) and silicone membrane (r^2=0.89) (Figure 3.10). Although this relationship may be used for predicting probable losses of other drugs, it does not take account of the dynamic nature of the sorption process (Roberts *et al.*, 1991). For example, drugs with a high log P and a low percentage non ionised would show a lower degree of loss than that predicted by the equation. This is illustrated when the degree of sorption exhibited by midazolam and diazepam in static solution and during flow

simulation is compared. There was greater fractional loss of diazepam than midazolam in static sorption tests, but the converse is true during flow simulation (Figures 3.1 and 3.4).

Table 3.5. Loss of drugs from unbuffered aqueous solutions in contact with pPVC tubing and Silicone membrane components of ECMO circuit for 120 minutes

						% Loss	
Drug	**Acid/Base[a]**	**pH**	**PKa**	**% Non ionised**	**Log P**	**pPVC**	**Silicone**
Diazepam	Acid	6.8	3.4	99.99	2.8[b]	88	76.8
Lorazepam	Amphoteric	6.9	i) 1.3 ii) 11.5	100	2.5[b]	40	34.4
Midazolam Hydrochloride	Base	4.9	6.2	40	2.7[c]	66	68
Morphine Sulphate	Base	3.5	7.9	<0.001	0.8[d]	16	0
Propofol	Acid	7.0	11	99.99	3.7[b]	98.4	96.8

a. pH of unbuffered aqueous solutions determined in the lab.
b. Lund, 1994
c. Roche, 2000
d. Dollery, 1991

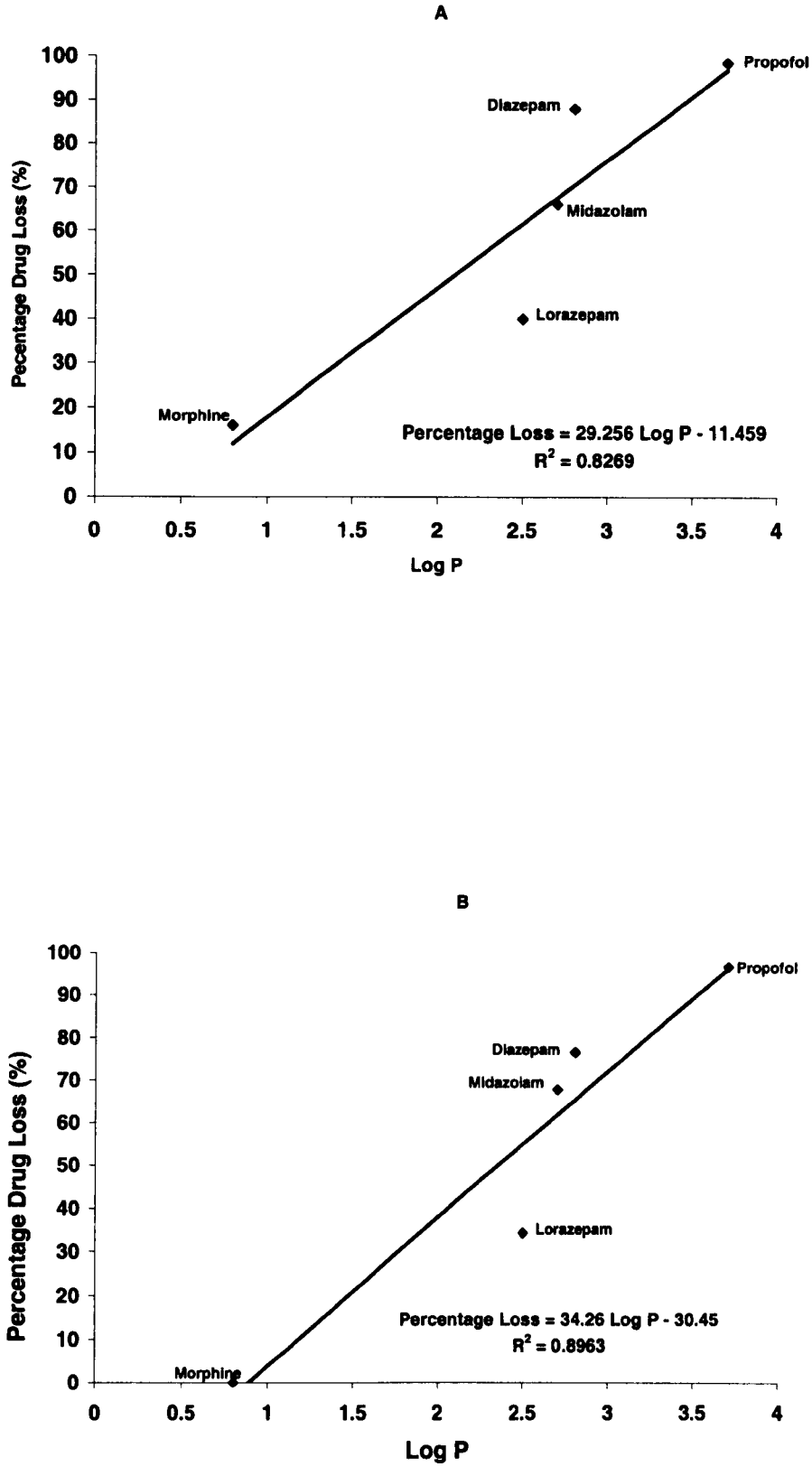


Figure 3.10. Linear Correlation of Log P value of Drug and Percentage Drug Loss During Static Conditions in (A) pPVC tubing (B) Silicone Membrane

The effect of the solution pH on the loss of drug is also significant. For a given drug, the extent of loss also appears to be directly related to the fraction non ionised (Kowaluk *et al.*, 1981; Kowaluk *et al.*, 1982). For a weak acid, as pH decreases the fractional loss of drug increases, in accordance with an increased percentage of drug in the non ionised form. Similarly for a basic drug, fractional losses will increase with increasing pH. Such a relationship was observed for the loss of drugs from solutions stored in plastic infusion bags (Kowaluk *et al.*, 1982). Since drugs administered into the ECMO circuit will be mixing into blood, the degree of ionisation of drug at physiological pH (buffered to 7.34 – 7.45) is an all important consideration. For example, at physiological pH the percentage of non ionised morphine will increase to about 25% and thus a greater propensity for sorption into the circuit. However, since it has a low log P value, the magnitude of increase is not expected to be great. With respect to midazolam, not only will the percentage non ionised increase to > 90%, but the molecule will exhibit increased lipophilicity. A pH dependent equilibrium exists between the closed-ring midazolam and an open-ring benzophenone structure, at position 4 of the benzodiazepine nucleus. At pH 4 or less, 80-85% closed-ring and 15-20% open-ring forms exist *(AHFS Drug Information. 2001)*. The open ring structure has much reduced lipophilic properties, whereas at physiological pH 7.4, midazolam is completely in closed-ring form, resulting in increased lipophilicity. In contrast to our findings, Bianchi *et al* (1992) and Martens *et al* (1990) observed little or no loss of midazolam stored in pPVC containers. They had prepared solutions in glucose 5% (pH 3.5 – 6.5) and sodium chloride 0.9% (pH 4.5 – 7.0), and thus potentially a higher degree of the ionised and open-ring species. A further explanation could be differences in the percentage of plasticiser present in the containers tested. Plasticiser content of pPVC medical devices can range between 30 to 80% by weight (Tickner *et al.*, 2001).

Priming an extracorporeal circuit is an attempt to coat the entire inner surface area with the primer, or in this case albumin. Albumin is noted for its ability to bind to drugs and the measured loss for a particular drug should therefore be the same irrespective of the nature of the primed component. Hammaren *et al* (1999) and How-Bow Su *et al* (1996) reported that priming with either heparin or dextrose/saline has no effect on drug loss in the circuit. Unger *et al* (2001) found that priming and perfusing pPVC tubes with foetal calf serum increased sorption of lignocaine, but not midazolam. Our results demonstrate that albumin priming does affect sedative drug loss such that uptake decreased significantly with the silicone membrane oxygenator but not the pPVC tubing. It is not clear why this should be but one explanation could be the differences in degree of priming achieved on both components. The albumin boundary layer may be contributing to an overall diffusional resistance into the silicone membrane. To relate the effect of priming ECMO circuits with albumin to clinical practice would require this work to be repeated with whole blood. The impact may be altered since binding to circuit components is probably competitive with binding to blood components, including red blood cells and plasma proteins. All the drugs investigated are significantly bound to albumin in blood: diazepam (99%), lorazepam (92%), midazolam (96%) and propofol (97%) (Dollery, 1991)

A number of workers have argued that the diffusion model is the most appropriate mechanistic model to describe the sorption of drugs by plastic devices (Roberts *et al.*, 1980; Roberts *et al.*, 1991). In this model, it is assumed only the non ionised species is taken up by the plastic. Also the aqueous solution is well stirred and the rate of drug uptake is controlled primarily by the diffusion in the plastic. Furthermore, the plastic is assumed to be an infinite sink. The flow simulation studies revealed continuous and time dependent cumulative sorption of the sedative drugs. The continuous loss of drug into the ECMO circuit is consistent with a diffusion controlled

sorption of drugs into the plastic matrix reported previously for plastic infusion bags and infusion systems (Kowaluk *et al.*, 1982). This is in contrast to drugs such as insulin and heparin (macromolecules), where loss to intravenous delivery systems is an adsorptive (surface) process, and dependent on the saturation of available binding sites (Hirschl, 1981). The kinetic profile would reveal an abrupt decrease in concentration followed by an equilibrium state.

Since the sorption of most drugs is a first-order process, controlled by the diffusion of drug in the plastic matrix, it is also expected to be concentration independent in buffered solution and blood (maintained at physiological pH). For a given drug, the amount taken up will increase with concentration, but the fraction taken up will remain constant. In unbuffered solutions where drug concentration affects the pH of the solution, the fractional loss may be affected. One other notable exception to this is chlormethiazole edisylate, where the fractional loss increases with concentration (Kowaluk *et al.*, 1981). This effect has been attributed to chlormethiazole enhancing its own sorption and possibly permeation by 'swelling' the pPVC resin.

Fractional loss of drugs to the circuit is also expected to be a function of the pump flow rates. Such a relationship has been observed for drugs infused through intravenous administration sets (Roberts *et al.*, 1980; Yliruusi *et al.*, 1986a). The drug concentration in the effluent is likely to be lowest at the slowest flow rates, since there is a longer contact time for the drug in solution with the circuit. The effluent drug concentration is then greatest at the fastest flow rate. However, it is also true that at the fastest flow rate more drug is presented to the circuit per unit time and therefore the rate of sorption will also be the greatest. Kowaluk *et al* (1982) described the relationship between steady-state effluent drug concentration (*Css*) and mean contact or transit time (*T*) of the solution in the tubing as:

$$C_{ss} = C_{o.}\, e^{-KsorT}$$

Where C_o is the initial concentration of the drug solution to be infused and K_{sor} is the first order rate constant for sorption. The contact time, T, may be derived from the volume of solution in the tubing (V) divided by the flow rate (F). Pump flow rates during neonatal ECMO are the highest at the onset of neonatal ECMO ($120cm^3/kg/min$) and the support is then weaned to a minimum ($50cm^3/kg/min$) as the patient's lungs recover. Therefore, drug loss through sorption will be least and percentage of dose delivered greatest at the onset of ECMO support. During normal clinical practice, where frequently midazolam is infused continuously from the onset of ECMO to a period post decannulation, the changing fractional loss will continuously alter its effect on pharmacokinetic parameters.

Steady state effluent concentration is also a function of the dimensions of the infusion tubing (Kowaluk *et al.*, 1982; Yliruusi *et al.*, 1986b). As the surface-area (SA) to volume ratio increases, the fractional loss through sorption increases. Yliruusi *et al* (1986b) showed that loss of diazepam was increased in pPVC tubing compared to pPVC containers and also loss to tubing increased with tubing length. The first order rate constant for sorption can then be expressed in terms of the SA in contact with solution and the volume of solution (V) (Kowaluk *et al.*, 1981):

$$K_{sor} = k_p\, (SA/V)$$

Where k_p is the permeability constant for the drug in plastic, (although these will be different for the pPVC and the silicone membrane components of the circuit, Table 3.6).

Table 3.6. Permeability coefficients for the plastic components of a neonatal ECMO circuit.

Polymer	Permeability Coefficient, $P_{10} \times 10^9$, $\frac{cm^3\ s.t.p.\ cm^2\ sec^{-1}}{cm\ Hg\ cm^{-1}}$
Unplasticised PVC	6
Plasticised PVC	200
Polypropylene [b]	6.8
Silicone (polydimethylsiloxane)	4300

a. Crank *et al.*, 1968

b. The polypropylene is not in contact with the drugs in solution

The internal surface area in a pPVC tube is given by the equation:

$$SA = 2\pi rl$$

Where r is the internal radius and l is the length of the tube. Thus as surface area increases, so does K_{sor}. According to these relationships, the highest drug delivery rate in an ECMO circuit will be achieved by maintaining high pump flow rates, ensuring that the tubing used has the smallest possible diameter and is of the shortest length possible. The implication here is that fractional loss of drugs will be different in different sized circuits. Table 3.7 shows that the neonatal ECMO circuit is the longest and has the highest overall SA to volume ratio. Furthermore, pump flow rates per bodyweight are greater in a neonate compared to an adult. Approximate pump flows at the onset of ECMO are 120 and 65 cm^3/kg/min and at the weaning stage, 65 and 15 cm^3/kg/min, in neonates and adults respectively.

Thus, fractional loss of drug is likely to be greatest in a neonatal ECMO circuit, and somewhat less in older children and adults. Pharmacokinetics in neonates are significantly different to older children and adults due to renal and hepatic immaturity,

and so loss of drug through sorption to the ECMO circuit will further add to the uncertainty of appropriate drug dosing in this group.

A major difference between drug sorption in polymeric containers and administration sets is that the ECMO circuit is an integral part or an extension of the patient's systemic circulation. Drug sorption can potentially alter pharmacokinetics and hence therapeutic response. The effect on drug disposition will depend on whether the process is reversible or not. Where drug sorption is a reversible process, a steady state equilibrium will be established in which a pool of bound drug is in equilibrium with a pool of unbound drug. In the classic compartmental model this would be modelled as an enlarged volume of distribution, with the ECMO circuit behaving as a peripheral tissue compartment. Irreversible sorption of drug to the circuit, therefore, appears as an increase in clearance (via a peripheral compartment).

The results of this investigation not only demonstrate that midazolam lost through sorption to pPVC tubes and silicone membranes is reversible through simple water extractions, but moreover, the identification of bound midazolam to ex-vivo circuits has for the first time confirmed the sorption of drug during clinical use. This suggests that the ECMO circuit behaves as a peripheral compartment, distinct from the patient, into which drugs can rapidly distribute to an equilibrium situation. It also suggests, once infusion of drug ceases and plasma concentrations decline, circuit bound drug will redistribute into circulating blood, prolonging the half-life and thus pharmacological effect.

Table 3 7. Dimensions of ECMO Circuits

	Tygon® S-65-HL pPVC Tubing					Avecor™ Silicone Membrane Oxygenator		
	Length (cm)	**Radius (cm)**	**Surface Area (cm^2)**	**Volume of blood cm^3/cm**	**SA:Volume ratio**	**Surface area (cm^2)**	**Volume of blood**	**SA:Volume ratio**
Neonate	691	0.635 (1/4 inch)	2756	0.28	14.3	8000	100	80
Infant	462	0.952 (3/8)	2763	0.68	8.8	15000	175	85.7
Paediatric	432 365	0.952 1.269 (1/2)	2584 2910	0.68 1.1	8.8 7.2	25000	455	54.9
Adult	411 485	0.952 1.269	2458 3867	0.68 1.1	8.8 7.2	45000	665	67.7

3.14 Conclusion

The results from these preliminary *in vitro* investigations reveal that ECMO circuits have the potential to sequester substantial amounts of the sedative drugs. This is a concentration independent process and the fractional loss will depend on physico-chemical properties of the drug (ionisation status, log P value), pH of solution, characteristics of the circuit (SA to volume ratio, pump flow rates) and whether the circuit is primed or not. Drug sorption was also shown to be a reversible process with the potential to significantly affect pharmacokinetics and therapeutic response.

These simple studies have examined the effect of an ECMO circuit on a single class of drugs. The results obtained suggest that further work be carried out to extend the range of drugs examined, to study the effect of various priming solutions and to use more realistic mobile phases e.g. blood.

CHAPTER IV

INVESTIGATION OF THE EFFECTS OF MIDAZOLAM SORPTION ON PHARMACOKINETICS DURING NEONATAL ECMO

4.1 Introduction

As with neonates supported by mechanical ventilation, adequate sedation of neonates receiving extracorporeal membrane oxygenation (ECMO) is essential to allay the physical, emotional and psychological distress that is inherent during intensive care. Furthermore, adequate sedation during ECMO prevents excessive movement, which can affect cannula position and hence blood drainage into the ECMO circuit thereby reducing gas exchange.

Analgesia and sedation are routinely administered in an opioid/benzodiazepine combination, for continuous intravenous sedation in neonatal and paediatric patients requiring mechanical ventilation. Midazolam is considered to be the drug of choice in most intensive care units in the United Kingdom. Midazolam, a benzodiazepine derivative with an imidazole [1,3] ring, is preferred over other benzodiazepines because of its water solubility and perceived rapid clearance (Jacqz-Aigrain *et al.*, 1992). It undergoes extensive metabolism by the cytochrome P450 3A (CYP3A) subfamily to a major hydroxylated metabolite (1-hydroxy-midazolam) and several minor metabolites (4-hydroxy and 1,4-hydroxy midazolam) before glucuronidation by uridine-diphosphate glucuronosyl transferases (UGT) and excretion in the urine (de Wildt *et al.*, 2001).

There have been several studies of continuous infusions of midazolam in critically ill neonates involving preterm neonates or neonates of wide gestational age range (Burtin *et al.*, 1994; Jacqz-Aigrain *et al.*, 1994; Jacqz-Aigrain *et al.*, 1992). Although its elimination half-life is significantly shorter than that of other benzodiazepines such as diazepam, elimination is delayed in preterm neonates compared with older infants and children (Lee *et al.*, 1999). This is due to significantly reduced hepatic CYP3A activity in neonates (de Wildt *et al.*, 2001).

The effective and appropriate use of midazolam during ECMO requires an understanding of its pharmacokinetics. However, it may not be appropriate to relate the

aforementioned studies to neonates on ECMO, who tend to be term or near term. Moreover, the disposition of drugs is known to be altered during ECMO. In chapter 3 of this thesis the sorption of midazolam by the polymeric components of the ECMO circuit was demonstrated with both *in vitro* and *ex vivo* studies (Chapter 3). The uptake was shown to be a first order diffusion process but also reversible. In addition, physiological changes as a result of the expanded circulating volume, intrinsic increase in intracellular and extracellular water, non-pulsatile blood flow during VA ECMO and reduced plasma protein concentrations may significantly affect pharmacokinetics and pharmacodynamics (see Chapter 1).

4.2 Aims of the Study

This study had two main purposes. First, to investigate the nature of the dose-concentration relationship for midazolam in neonates receiving ECMO and compare this to literature reports in non-ECMO neonates. Second, the pharmacokinetics of midazolam and 1-hydroxy midazolam were to be determined. Since significant midazolam sorption by polymeric components of the ECMO circuit has been demonstrated, the study investigated the influence of this phenomenon and whether pharmacokinetics differed if the drug was administered directly to the patient (intravenous) or into the circuit (extracorporeal).

An understanding of the pharmacokinetics of midazolam during ECMO helps promote the development of rational dosing approaches and titration to sedation scoring systems. Appropriate sedation levels reduce the incidence of haemodynamic side effects associated with over sedation, expediting return to normal mental status following cessation of the drug. Thus, the main goals were to improve the accuracy of midazolam administration by determining pharmacokinetic parameters and through simulations to suggest an appropriate dosing approach.

4.3 Materials and Method:

4.3.1 Patients

Entry criteria were neonates with severe respiratory or cardiorespiratory failure in need of ECMO and who received a continuous infusion of midazolam. Twenty neonates were randomised into two groups; group 1 received the infusion extracorporealy, pre-reservoir, via a pigtail catheter, whilst group 2 received the infusion via a central or peripheral venous catheter. Randomisation was completed as soon as the patient arrived on to the intensive care unit and the decision to cannulate for ECMO was made. The Department of Pharmacy carried out all randomisations using a sealed envelope technique.

4.3.2 Study Design

Midazolam was administered as a continuous infusion, at a rate usually between 50-250μg/kg/hr. Infusion rates were titrated in line with normal practice in the unit using the sedation scoring system: 1 = wide awake, 2 = awake but sleepy, 3 = asleep but moves spontaneously, 4 = asleep, but responds to stimulation, 5 =hard to rouse. The target sedation score in most cases was 4. Sedation scores were assessed when blood samples were taken for determination of midazolam concentrations. When levels of sedation were not satisfactory, rates of infusion were increased or decreased as indicated and additional bolus injections of 50-100μg/kg were given if necessary. Infusions of midazolam were initiated as soon as cannulation was achieved and extracorporeal blood flow established. Infusions were continued for the duration of ECMO, and weaned post decannulation and prior to extubation. If deemed clinically necessary by the responsible clinician (for assessment of neurological status or due to over sedation), infusions were temporarily stopped during ECMO.

As well as continuous heparinisation to ACT of between 160-200 seconds, various drugs such as antibiotics, inotropes, diuretics, H_2 blockers were co-administered during the study as indicated. ECMO circuits used in all neonates consisted of Tygon® S-65-HL pPVC tubing and the Avecor silicone membrane oxygenator. The priming volume of the circuit was approximately 500cm^3. The clear prime consisted of 100cm^3 of human albumin solution 20% and 400cm^3 Plasmalyte A, whilst the blood prime consisted of 500cm^3 donor blood, 120 units heparin, 15cm^3 sodium bicarbonate 8.4%, and 2.5cm^3 calcium chloride 10%.

4.3.3 Blood sampling and Analysis

Blood samples (1cm^3) for assay were collected from indwelling arterial lines or from the circuit proximal to drug infusions and the venous reservoir. Samples were drawn at baseline (prior to cannulation for ECMO), at 2hrs, 4hrs, 6hrs, 12hrs, 18 hrs, 24 hrs and 12 hourly thereafter. Samples were immediately stored at –20°C until analysis. Concentrations of midazolam and the free unconjugated form of its major metabolite 1-hydroxy midazolam were determined using GC-MS (see Chapter 2, Section 2.3.3). The assay was validated over the concentration range 10 ng/cm^3 to 10,000 ng/cm^3. The within and between day coefficient of variation was < 10%.

4.3.4 Pharmacokinetic Analysis

4.3.4.1 Model Development

Population pharmacokinetics of midazolam were estimated using WinNonMix Professional, Version 2.0.1, a non-linear mixed effects regression program, bundled with Compaq Visual Fortran Compiler, Professional Edition, Version 6.5 (see Chapter 2, Section 2.4.3).

In a preliminary analysis, the parameters of one, two, and three compartment models were fitted to the data using this approach. The models were parameterised in terms of volume of distribution (V) and clearance (CL). The interpatient variability in parameters was modelled as a proportional deviation from the 'true' parameter values (assuming a log normal distribution). Residual error was estimated with a combined proportional and additive model.

The influence of demographic and clinical variables on parameters of the model were also analysed and where significant added to the model to determine whether the overall variability in the model could be reduced. These included randomisation (extracorporeal or intravenous), gestational and postnatal age, weight, gender, creatinine, urine output, continuous veno-venous haemofiltration (CVVH), liver function tests, plasma albumin concentrations, co-medication, cannulation mode, duration of ECMO and ECMO pump flow rates.

4.3.4.2 Cross Validation and Predictive Performance

Since it was not practical to collect new data prospectively, the ability of the final model to perform in prospective tests was estimated using the cross-validation technique (see Chapter 2, Section 2.5.5). Although cross validation is not a truly prospective validation method, it is a recognised and established approach to estimating model performance assuming identical experimental conditions (Fiset *et al.*, 1995; Kerbusch *et al.*, 2001; Zomorodi *et al.*, 1998).

The study group was divided into 8 smaller groups of 2 patients each, and 1 group of 3 patients. The pharmacokinetic model was fitted to the data whilst excluding one group. The estimated structural parameters from the sub models were then used to predict the concentrations in the excluded group. This process was repeated 9 times excluding each group in turn. A measure of the predictive performance of models was

determined by calculating MDPE and AMDPE as described in Chapter 2, Section 2.5.4. Since the excluded group is not used to develop the model, the MDPE and the AMDPE are almost unbiased estimates of the predictive capabilities of the model (Zomorodi *et al.*, 1998).

4.3.5 Metabolic Ratio

In addition, the area under the curve (AUC) plasma metabolic ratio (1-hydroxymidazolam/midazolam, MR) was determined using the following equation (Johnson *et al.*, 2002):

$$MR = \frac{(C_{(t_1)\ 1\text{-hydroxyMDZ}} + C_{(t_2)1\text{-hydroxy MDZ}}) \times 0.5}{(C_{(t_1)\ MDZ} + C_{(t_2)\ MDZ}) \times 0.5}$$

where $C_{(t_1)1\text{-hydroxyMDZ}}$, $C_{(t_2)1\text{-hydroxy MDZ}}$, and $C_{(t_1)\ MDZ}$, $C_{(t_2)\ MDZ}$ are the concentrations of 1-hydroxy midazolam and midazolam respectively, at two time points. This ratio has been used as a marker of CYP3A activity (Johnson *et al.*, 2002).

4.4 Statistical analysis

Data are expressed as mean (SD), mean ± SE for population parameter estimates, and median (range). Differences in plasma concentrations and doses between the two groups were tested using student's t-test. A p value of <0.05 was considered to be significant. Selection of the optimal model was based on examination of graphical residuals plots and the OFV (-2 * the logarithm of the likelihood of the results). A change in the OFV > 7.88 ($p<0.005$) was considered statistically significant (See Chapter 2, Section 2.4.3). The impact of significant covariates was also evaluated using this approach. The MR was determined as the median MR from all patients.

4.5 Results

4.5.1 Demographic and Clinical Characteristics

The group characteristics of the study population are given in Table 4.1. Twenty neonates were recruited into the study; 10 patients received midazolam into the circuit, 10 patients via a peripheral or central venous line. There were no significant differences in characteristics between the two groups. Veno-venous ECMO is the preferred mode of ECMO in this unit and was performed in 17 patients; VA ECMO was performed in 3 of the patients. CVVH was employed in 7 patients in response to acute renal failure and/or to attain a negative fluid balance. Three patients died during ECMO, 1 patient died post decannulation. In 3 of the cases, deaths were as a result of pulmonary or cardiopulmonary failure, whilst myophosphorylase deficiency was the cause in another.

4.5.2 Dose-Plasma Concentration Analysis

The mean (SD) duration of ECMO for all patients was 6.2 (2.9) days. Data on dosing was collected for 120 hours or until cessation of infusion, whichever occurred sooner. In 9 patients, midazolam was infused for less than 120 hours as a result of early decannulation; none of these patients continued on midazolam infusion post-decannulation. Doses of midazolam administered for all patients during the entire study period were 250 (185) (range 50 –2200) μg/kg/hour. There were no significant differences between the two groups in total doses administered over 120 hours. However, the mean (SD) doses administered in the first 24 hours were significantly greater in the extracorporeal compared to intravenous group (361(300) versus 258(190) μg/kg/hour, $p<0.001$) (Table 4.2).

There was no significant correlation between duration of ECMO and mean doses administered or between postconceptional age and mean doses administered. However,

the lowest mean dose (130 μg/kg/hour) administered was to a neonate of 33-week gestation and 1 day of age, whilst the highest mean doses (>330 μg/kg/hour) were administered to 3 term babies greater than 7 days of age.

Table 4.1. Patient Demographics

	Randomisation	
	Intravenous (n=10)	**Extracorporeal (n=10)**
Sex	50% Males	60% Males
Gestational Age (weeks)	40.2 (1.2)	38.9 (2.4)
	(38 – 41.6)	(33-41)
Age at cannulation (days)	3.6 (5.4)	3.6 (5.6)
	(0.5 – 16)	(0.5 – 18)
Weight (kg)	3.6 (0.6)	3.2 (0.5)
APGAR Score		
1 minute	6.5 (3.5)	7.2(1.3)
5 minutes	7.8 (1.9)	9.1(0.9)
OI (at referral) *	41 (11.8)	58 (21.7)
Cannulation type		
VV	9	8
VA	1	2
ECMO Duration (days)	5.6 (2.9)	6.9 (2.9)
	(2 – 11)	(4.5 – 12)
Length of ICU stay (days)	12.3 (11.8)	11.3(4.3)
	(4 – 19)	(7 – 21)
CVVH (patients)	3	4
Elevated ALP / ALT (patients)	4	3
Diagnoses		
MAS/PPHN	7	5
Sepsis	0	1
CDH	2	2
Post Cardiac Surgery	0	2
Metabolic	1	0
Survival	90%	70%

Data are expressed as mean (SD) and (range)

OI (Oxygen Index) = (PaO2 * Mean Airway Pressure) / Fraction of Inspired Oxygen

CVVH = Continuous veno-venous haemofiltration

MAS = Meconium Aspiration Syndrome

PPHN = Persistent Pulmonary Hypertension of the Newborn

CDH = Congenital Diaphragmatic Hernia

Table 4.2. Infusion Rates and Plasma Concentrations

	All Patients	Intravenous	Extracorporeal
Infusion Rate over 120 hours (μg /kg/hour)	250 (185) (50 –2200)	250 (181) (50 –1600)	248 (187) (50 – 2200)
Infusion Rate over first 24 hours (μg /kg/hour)	312 (259)	258 (190) * (50 –1600)	361 (300) * (50 –2200)
Sedation Score at			
24 hrs	4.3 (1.0)	4.4 (1.0)	4.2 (1.0)
48 hrs	4.2 (0.9)	4.6 (1.0)	4.0 (1.0)
72 hrs	3.8 (1.0)	3.7 (1.0)	3.9 (1.0)
Plasma Concentrations (μg /cm^3) at			
24 hours	1.4 (0.9) (0.08 – 3.2)	1.4 (1.2) (0.3 – 3.2)	1.4 (0.9) (0.08 – 3.2)
48 hours	1.8 (1.2) (0.1 – 4.9)	1.7 (1.8) (0.1 – 4.9)	1.9 (0.7) (0.5 – 2.9)
72 hours	2.6 (1.8) (0.2 – 7.5)	2.7 (2.6) (0.2 – 7.5)	2.2 (1.0) (0.8 – 3.6)

Data are expressed as mean (SD) and (range)

* Intravenous vs Extracorporeal, $p<0.001$. No other significant differences between the two groups were found.

Plasma midazolam concentration profiles of samples analysed from neonates during ECMO at the set time points are shown in Figure 4.1. The mean profile reveals significant attenuation of plasma levels during the first 24 hours of ECMO and then a subsequent and significant increase suggestive of a reduced rate of elimination. The overall mean plasma concentration from all samples analysed in the first 72 hours was 1.2 (1.4) (range 0.01 – 9.8) μg/cm^3. Mean (SD) plasma concentrations at 24 hrs, 48 hrs and 72 hrs were 1.4 (0.9) (range 0.08 – 3.2) μg/cm^3, 1.9 (1.2) (range 0.1-4.9) μg/cm^3 and 2.6 (1.8) (range 0.2-7.5) μg/cm^3 respectively. A wide interpatient variability was

seen with values below 1 μg/cm^3 in 5 (25%) patients, and values greater than 2 μg/cm^3 in 10 (50%) of patients at 48hrs. There were no significant differences in the mean (SD) plasma concentrations achieved between the two treatment groups over 72 hours. Sedation scores for all patients at 24, 48 and 72 hours were 4.3 (1.0), 4.2 (0.9), 3.8 (1.0), and no significant differences were found between the two groups.

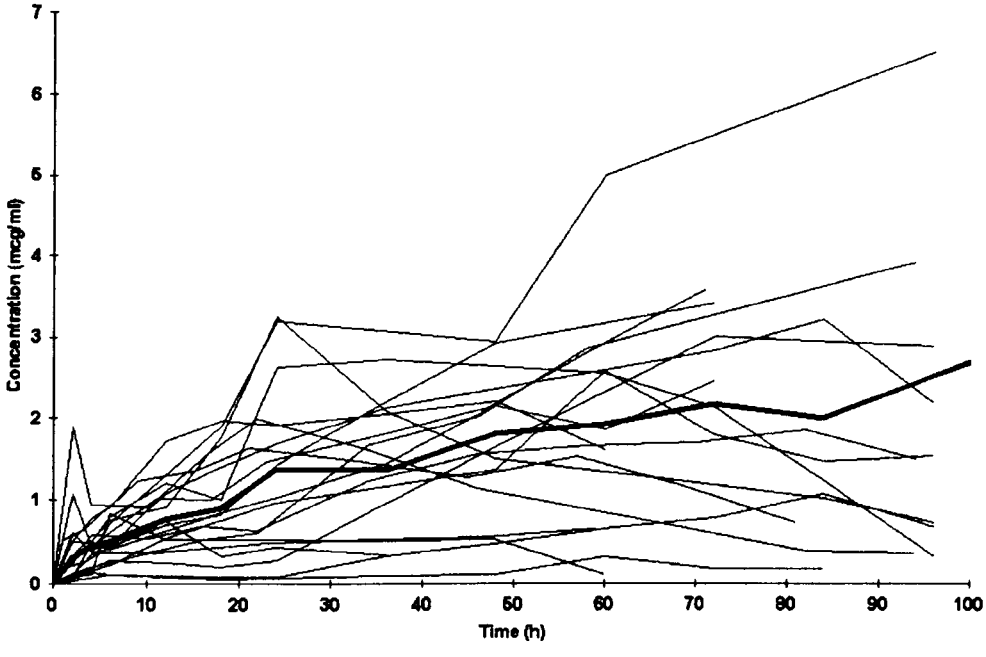


Figure 4.1. Plasma concentration-time profiles of Midazolam from the study population, with the mean profile in bold. The mean dose administered over this period was 250 μg/kg/hour.

4.5.3 Pharmacokinetic Analysis

From a total of 210 midazolam plasma concentrations analysed, 45 (21 %) observations were excluded from the pharmacokinetic analysis. Excluded observations included 13 observations from the patient with myophosphorylase deficiency who exhibited no decrease in plasma concentrations despite no drug being administered for 50 hours, 15 observations collected during the post-ECMO period as well as 17 observations that confounded normal physiological principles (e.g. increase in plasma midazolam concentrations when no drug had been administered).

The one compartment model was selected for further analysis of midazolam since it demonstrated a more appropriate structural model on examination of the graphical residual plots. Furthermore, the OFV was not significantly lower with the two and three compartment models and estimates of the pharmacokinetic parameters were less precise, probably a reflection of our limited sampling (particularly in the initial phase of ECMO) and inability to discern peripheral compartments. Plots of model parameters versus clinical and demographic covariates revealed no significant influences apart from randomisation allocation (i.e. whether the drug was administered extracorporealy or intravenously) with V. The latter suggested that those patients administered midazolam extracorporeally had a higher V (mean±SD: 13.8±5.2 L versus 10.9±5.9 L) (Figure 4.2). Although this improved the model by reducing the variability on the V and the OFV by 3.1U, this was not significant at the $p< 0.005$ level (Table 4.3) and thus was excluded from the final model.

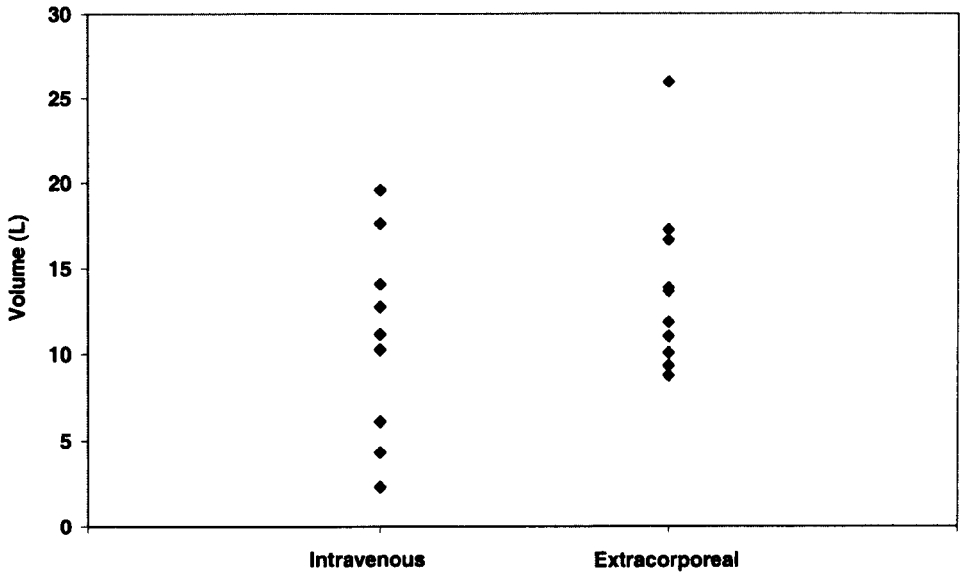


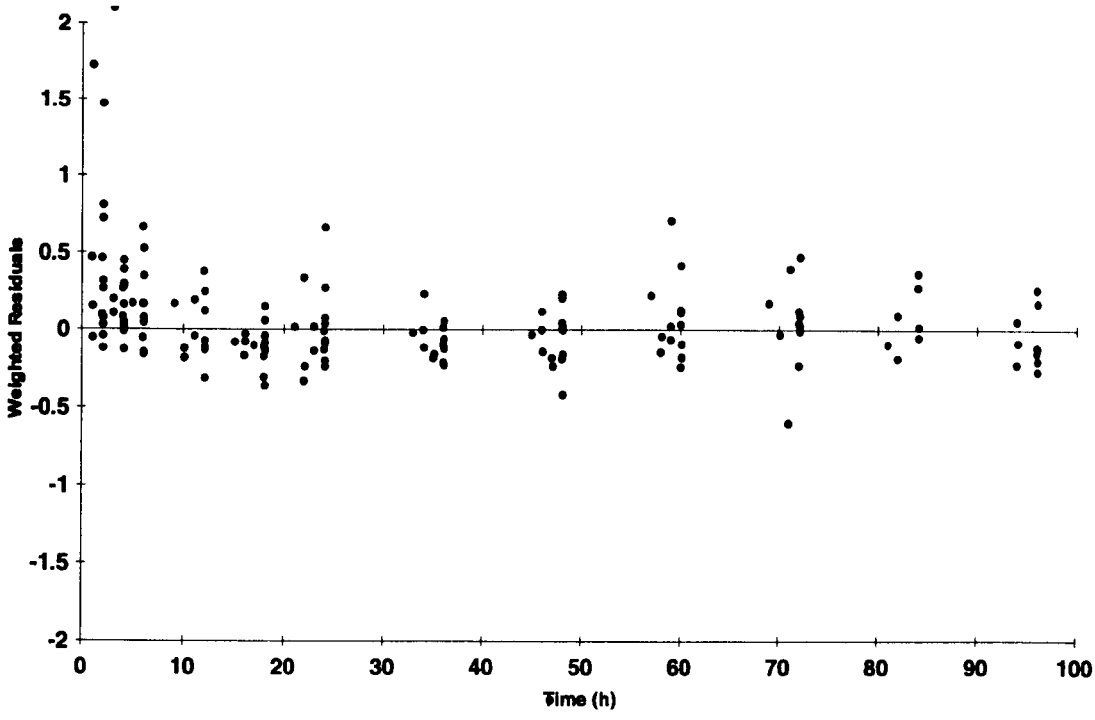
Figure 4.2. Volume of distribution versus route of administration

A closer inspection of the residuals against time plot (Figure 4.3a) suggested that the model under-predicted concentrations in the early phase of ECMO. Therefore the influence of various time covariates, added categorically to both CL and V (ranging 2 to 24 hours), were tested in the model. The model improved measurably with the inclusion of a 12-hour time covariate on V, producing a drop in the OFV of 16.6 U (Table 4.3). This time dependent change in V was attributed to reversible sorption of midazolam by components of the circuit. Thus, after detecting time-dependency as a significant covariate for V, the phenomenon was incorporated into the structural model. Midazolam uptake by the circuit was accommodated by allowing the initial value of volume (V_0) to increase mono-exponentially to an asymptotic value, V_{max}. This approach was analogous to that previously described by Rostami-Hodgegan *et al* (1999) for time dependent changes in CL due to enzyme induction.

$$\text{Volume (L)} = V_{max} - (V_{max} - V_0)e^{ksor.time}$$

(Where, K_{sor} = the rate constant for midazolam sorption into the circuit and time = duration of infusion. Initial estimates were taken from the *in vitro* results, see Chapter 3, Section 3.9).

The revised model was considerably better at fitting plasma midazolam concentrations, abolishing the early trend in residuals (Figure 4.3b) and reducing OFV by 21u, although it explained only 4% of the variance (Table 4.3).



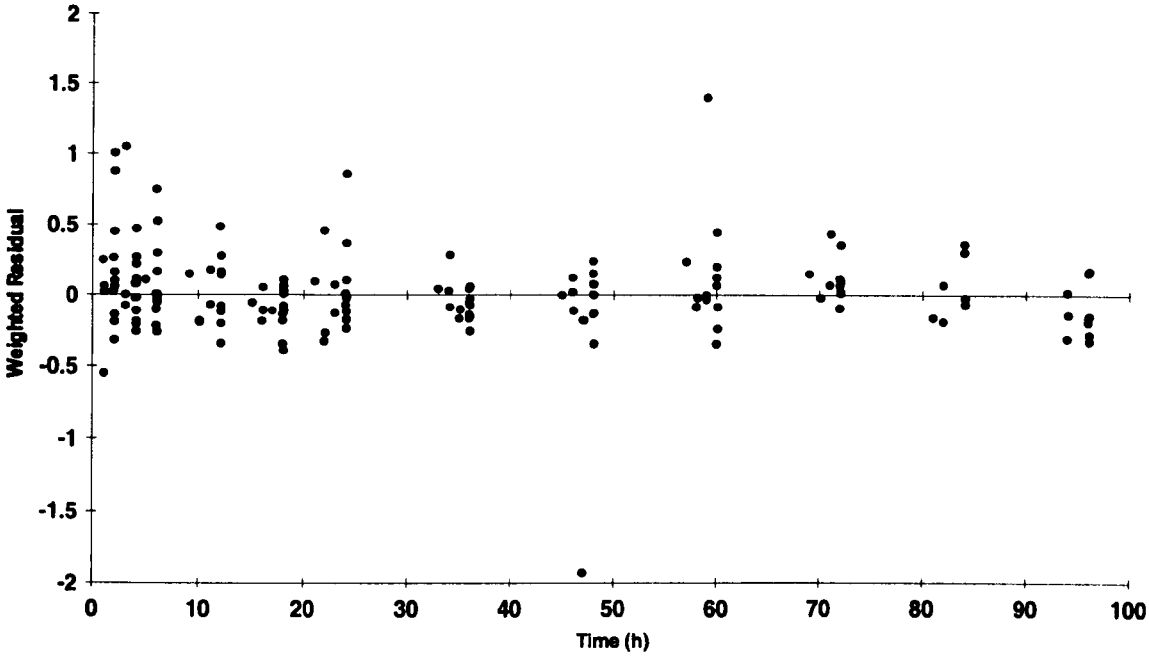


Figure 4.3. Weighted Residuals as a Function of Time. (A) Simple One Compartment Model. The model seems to under predict in the early phases of ECMO. (B) With the inclusion of a time dependent increase in volume of distribution, the early trend in the residuals diminishes.

Table 4.3. Model Development, Covariate Analysis and Predictive Performance

Model	OFV[b,c]	Comments	Bias (%)	Precision (%)
One compartment (Base Model)	242.3		-11.9 (-23.0, 0.8)	36.7 (30.3, 43.5)
Randomisation on Volume	239.2	Administration method does not significantly influence volume	0.2 (-7.6, 7.0)	19.8 (16.3, 25.3)
12 hour time covariable on Clearance	241.9	Clearance is not time dependent	0.3 (-6.4, 6.2)	22.0 (18.2, 25.4)
12 hour time covariable on Volume	225.7	Volume is time dependent	-1.4 (-5.2, 3.8)	20.9 (15.8, 25)
Time dependent volume[a] (Final Model)	204.7	Volume expansion with time (defining midazolam sorption) improves model	-0.3 (-5.2, 5.0)	20.0 (16.2, 23.1)
Cross Validation of Final Model			-0.3 (-5.6, 4.8)	19.3 (15.1, 23.3)

a. $V(L) = V_{ss} - (V_{ss} - V_0)e^{ksor.time}$
b. –2 * log Likelihood
c. Assuming a chi squared distribution, a change in the OFV > 7.88 ($p<0.005$) was accepted as statistically significant

Figures in parenthesis are 95% confidence intervals for the median.

The mean individual Bayesian posterior V increased by approximately five fold from the onset of ECMO to the steady state value (median (range)): 3.1 (1.0-8.0) L versus 14.2 (4.7-35.8) L, respectively (Figure 4.4). These changes were accompanied by a significant increase in the initial to steady state half-life (median (range)): 6.8 (2.2-39.8) hours versus 33.3 (7.4-178) hours, respectively (Figure 4.4). Interpatient variability in CL and V was 73% and 53%, respectively whilst residual error corresponded to a proportional error of 26% and an additive error of 0.17 $\mu g/cm^3$ (Table 4.4). The relationship between observed and model predictions and selected individual patient profiles before and after the addition of the time dependency model are shown in Figures 4.5 and 4.6, respectively. The individual Bayesian posterior parameters estimated from the model described the data well with bias of –0.3% and precision of 20%. The cross validation exercise revealed an overall bias and predictive precision for the sub models of –0.3% and 19.3% respectively, indicating that the full model is likely to perform well in a truly prospective trial during similar conditions (Table 4.3).

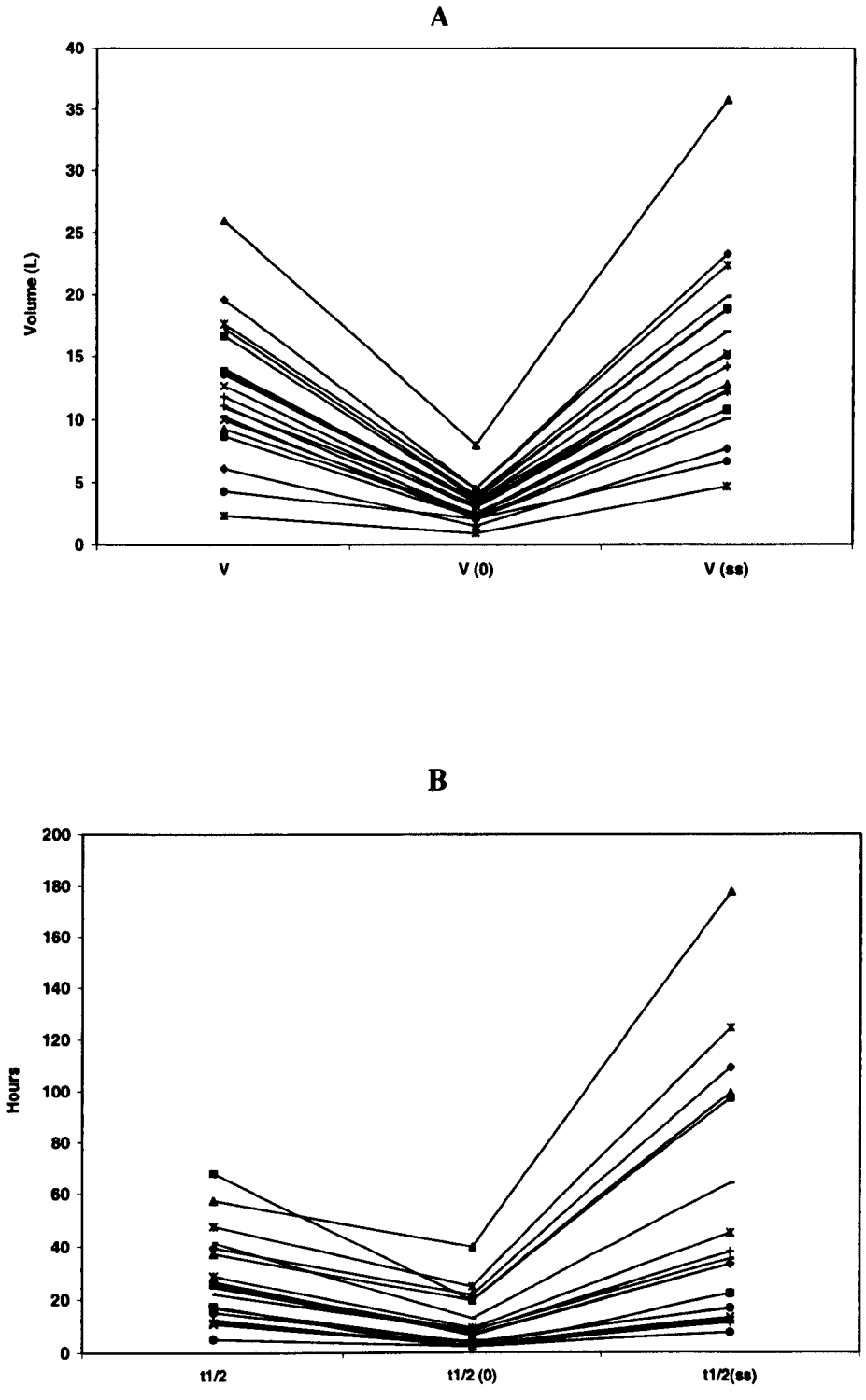
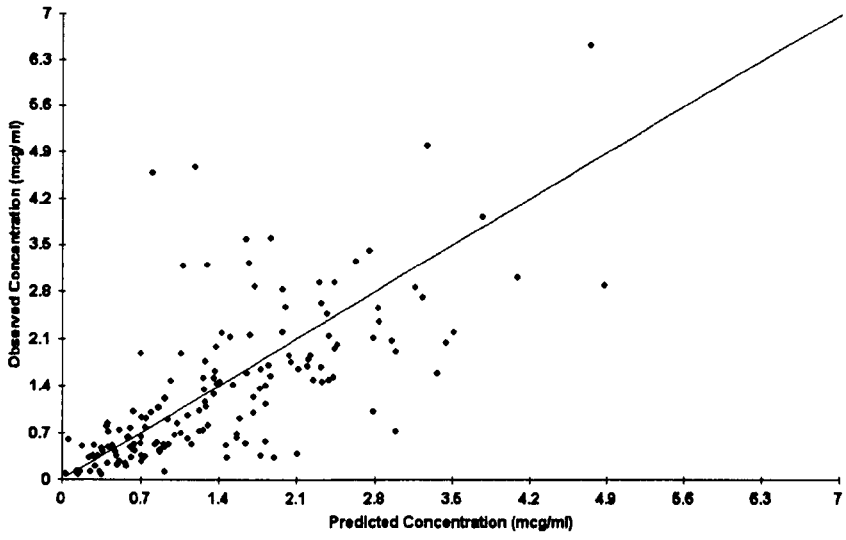


Figure 4.4. Individual estimates of (A) volume of distribution (V) and (B) half-life ($t_{1/2}$) obtained using the simple one compartment and time-dependent volume models: (0) initial values, (ss) steady state values.

A



B

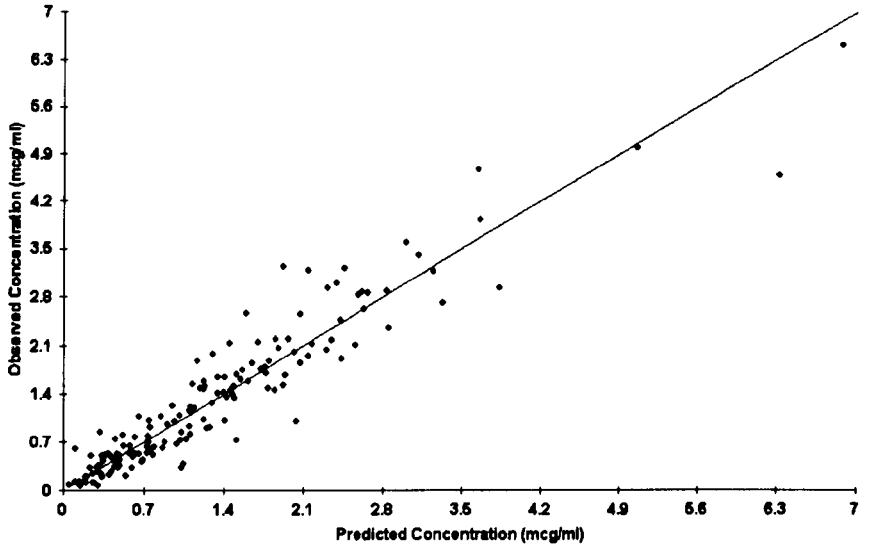


Figure 4.5. Observed versus (A) Population and (B) Individual Bayesian Posterior Predictions from the Final Time Dependent Model. Solid Line Represents Unity.

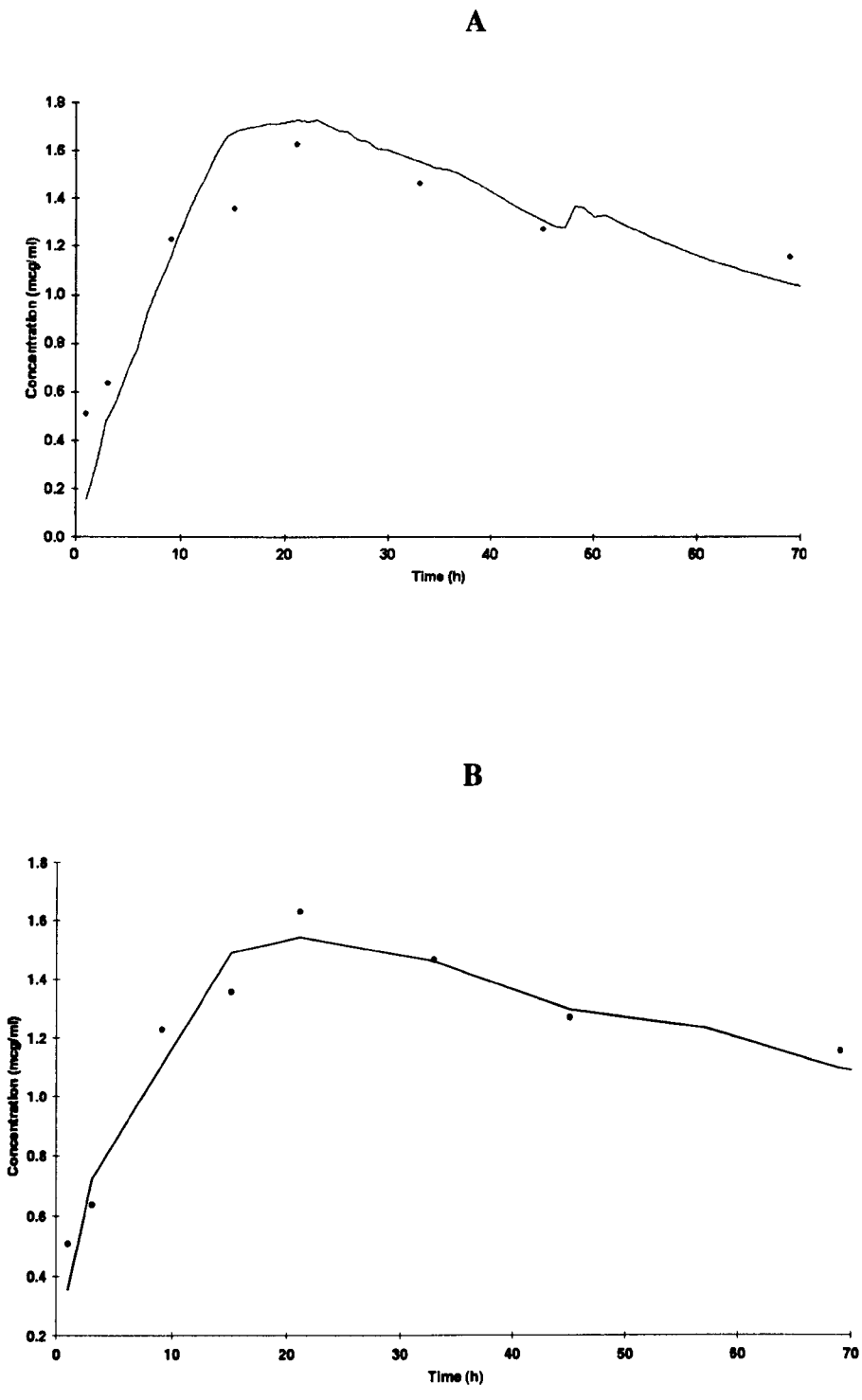


Figure 4.6. Model fits to the same patient, • observed concentrations, — individual Bayesian posterior predictions: (A) simple one compartment model, (B) with time dependent volume.

4.5.4 Metabolic Ratio

Plasma concentrations of the free unconjugated 1-hydroxy midazolam were assayed in 12 patients (106 observations with no exclusions). The median MR was 0.17 with a large interpatient variability (range: 0.03-0.9). Seven neonates (35%) exhibited biochemical signs of liver dysfunction with raised alkaline phosphatase (ALP) and alanine transaminse (ALT) though no correlation was observed with MR. However, the lowest MR (0.03) was observed in a patient with significantly deranged liver function tests (ALT 281 IU, ALP 922 IU, Bilirubin 100 mmol/L), suggesting impaired metabolism of midazolam. The highest MR (0.9) was observed in the patient with highest estimate of midazolam CL (0.23 $L.kg^{-1}.hr^{-1}$). Midazolam and 1-hydroxy midazolam pharmacokinetics are summarised in Table 4.4.

Table 4.4. Pharmacokinetic Parameters for Midazolam and 1-OH Midazolam in Neonatal ECMO Patients

V_0 (L)[a]	V_{max}(L)[a]	K_{sor} (hr^{-1}) [a]	CL ($L.hr^{-1}$)[a]	$t_{½(0)}$ (hr)[b]	$t_{1/2(ss)}$ (hr)[b]	MR[c]
2.8±1.7	13.9±1.6	-0.19±0.07	0.28±0.03	6.8(2.2-39.8)	33.3(7.4-178)	0.17(0.03-0.9)
53%[d]			*73%*[d]			

V_0=initial volume, V_{max}=maximum volume, K_{sor}=midazolam sorption rate constant CL=clearance, $t_{½(0)}$ =initial half-life, $t_{1/2(ss)}$=terminal half-life, MR= 1-hydroxy midazolam AUC/ midazolam metabolic AUC ratio

a. Population parameter estimates, expressed as Mean ±S.E.
b. Half-lives were not primary structural model parameters and were calculated as median (range) *a posteriori* from individual Bayesian parameter values.
c. Data from 12 patients.
d. Interpatient variability in parameter estimates, expressed as coefficients of variation.

4.6 Discussion

Following the introduction of midazolam into neonatal intensive care practice in the 1980's, there has been a paucity of published information, not only on efficacy and safety but also appropriate pharmacokinetic and pharmacodynamic data in critically ill neonates. The neonatal period is one of rapid and profound physiological changes, including maturation of renal and hepatic function and changes in body composition (Rowland *et al.*, 1995). Therefore, neonates present a particular challenge with respect to pharmacotherapy, with rapidly changing drug disposition (Jacqz-Aigrain *et al.*, 1996). It would also seem sensible therefore that caution is exercised when extrapolating data from one neonatal age group to another. Within such a background, the potential effects of ECMO on drug disposition further complicates and somewhat diminishes the applicability of such data.

To date there have been three studies investigating plasma concentrations and pharmacokinetics in neonates following bolus doses of midazolam for sedation in mechanically ventilated neonates (Harte *et al.*, 1997; Jacqz-Aigrain *et al.*, 1990; Lee *et al.*, 1999). Of these studies, only one was from near term or term neonates (mean gestational age 37 weeks) (Jacqz-Aigrain *et al.*, 1990); the other reports were of very premature infants (24 to 31 weeks and 25-30 weeks) (Harte *et al.*, 1997; Lee *et al.*, 1999). Two studies involved analysis following initial single bolus doses of 0.2mg/kg and 0.1mg/kg, producing peak plasma concentrations of 0.414 and 0.107 $\mu g/cm^3$ respectively (Harte *et al.*, 1997; Jacqz-Aigrain *et al.*, 1990). In the third bolus dose study, 0.1mg/kg doses were administered as a single dose or as multiple doses every 4 to 6 hours as necessary; mean plasma concentrations of 0.121 $\mu g/cm^3$ were reported (Lee *et al.*, 1999).

Following an earlier bolus dose study (Jacqz-Aigrain *et al.*, 1990), the same group report three studies investigating continuous infusions of midazolam in

mechanically ventilated neonates (Burtin *et al.*, 1994; Jacqz-Aigrain *et al.*, 1994; Jacqz-Aigrain *et al.*, 1992). In an initial pharmacokinetic profiling study of premature neonates (29-41 weeks), a bolus dose of 0.2mg/kg was followed by a constant continuous infusion of 60 μg/kg/hour for as long as sedation was considered necessary. The duration of midazolam infusion averaged 60 (23.3) hrs. Mean (SD) plasma concentrations at 24 and 48 hours were reported as 0.55 (0.24) and 0.83 (0.39) $\mu g/cm^3$ respectively (Jacqz-Aigrain *et al.*, 1992). In another report, the authors describe the use of a population pharmacokinetic approach to elucidate the influence of important co-variables on midazolam disposition in neonates (gestational age 29 – 42 weeks). The study included retrospective data from the previous profiling study, as well as a prospective analysis with subjects receiving a continuous infusion of 69 (63) μg/kg/hour. The authors report concentrations of midazolam ranging 0 to 7.1 $\mu g/cm^3$. Of these, 19.7% were above 1 $\mu g/cm^3$ and 4.7% above 2 $\mu g/cm^3$. It was not possible however, to determine from the report the average levels achieved at 24 and 48 hours (Burtin *et al.*, 1994). None of the studies mentioned thus far comment on the adequacy of sedation level achieved with the chosen regimen. However, in the third report on continuous infusions of midazolam in neonates (mean age 32 weeks), sedation and haemodynamic variables were assessed in a placebo-controlled study. The infusion dose was 60 μg/kg/hour, decreasing to 30 μg/kg/hr after 24 hours in infants below 33 weeks gestation. Mean midazolam concentrations were 0.634, 0.628 and 0.543 $\mu g/cm^3$ at 24, 48 hours and at the end of treatment. Nurses and physicians assessed sedation to be adequate in 78 and 65%, 78 and 65% and 75 and 55% of neonates at 24, 48 and 72 hours, respectively (Jacqz-Aigrain *et al.*, 1994).

The present investigation in neonates on ECMO has revealed some potentially important differences. Doses administered and plasma concentrations observed during this study appear to be significantly higher than previous reports, with the plasma

concentration-time profiles suggesting a reduced rate of elimination (Figure 4.1). The mean (SD) infusion rate for all patients was 250 (185) μg/kg/hour, approximately four times higher than those reported in any previous study of neonates. The average concentrations achieved at 24, 48 and 72 hours (1.4, 1.9 and 2.6 $\mu g/cm^3$, respectively) are approximately 2-3 times greater than those reported in the aforementioned continuous infusion studies. In fact 48% of all samples analysed in the first 72 hours were above 1 $\mu g/cm^3$, and 21 % were above 2 $\mu g/cm^3$. The results however, are skewed by a large proportion (35%) of infants who had evidence of liver dysfunction, a reflection of the severity of illness in this group. If data from these patients are omitted, then mean (SD) plasma concentrations at 24, 48 and 72 hours are 1.1 (0.9), 1.5 (0.8), 2.2 (1.0) $\mu g/cm^3$ respectively. Even so these values remain significantly higher than previous studies.

It must be borne in mind though that patients in the current study do differ in some important ways. First, almost all (except one) of the infants were term or near term. Thus, although plasma concentrations between 0.2 to 0.6 $\mu g/cm^3$ have been shown to produce adequate sedation in older children (Hartwig *et al.*, 1991; Tolia *et al.*, 1991), there have been no similar pharmacodynamic studies in term or near term neonates. Second, neonates referred for ECMO are in severe respiratory or cardiorespiratory failure with a high oxygen index at referral, often associated with shock and sepsis, all of which have been shown to affect drug metabolism (Gal *et al.*, 1982; Leppik *et al.*, 1986; Macnab *et al.*, 1986). ECMO itself may affect renal and hepatic blood flow and hence drug clearance (see Chapter 1, Section 1.6.4). Cerebral hypoperfusion, as a consequence of impaired autoregulation of cerebral circulation may occur during ECMO, especially after hypoxia and if VA ECMO is used, potentially reducing the efficacy of sedatives and analgesics (Burda *et al.*, 1999). Perfusion of tissues may also be altered as a result of activation of the systemic inflammatory response syndrome

releasing a variety of autonomic, endocrine and local cytokine reflexes that may affect not only tissue distribution of drugs, but also clearance mechanisms as well (Gravlee *et al.*, 2000). Furthermore, in comparison to the aforementioned study (Jacqz-Aigrain *et al.*, 1994), the adequacy of sedation was adjudged to be satisfactory in all patients during this study. Whilst on ECMO, and in order not to compromise blood flow through the circuit and hence oxygenation, adequate sedation to prevent excessive movement is maintained at all times. Higher plasma concentrations then may have been necessary to meet the goals of sedation for neonates during ECMO. However an important argument against this is that there were no significant differences in the sedation scores recorded at 24, 48 and 72 hours suggesting that adequate sedation was achieved at lower plasma concentrations of around 1 $\mu g/cm^3$.

It appears excessive plasma concentrations observed during this study are due to markedly altered midazolam pharmacokinetics exhibited by neonates treated with ECMO compared to previously reported values. Previous investigators have reported that a two compartment model is optimal for describing the pharmacokinetics of midazolam, in contrast to the one compartment model developed in this study (Burtin *et al.*, 1994; de Wildt *et al.*, 2001; Jacqz-Aigrain *et al.*, 1992; Jacqz-Aigrain *et al.*, 1990; Lee *et al.*, 1999). This may be a reflection of the study design, utilising continuous infusions of midazolam with limited sampling in the distribution phase and few samples captured in the elimination phase.

In this study, parameter estimates are based on a model where V expands with time as ECMO support progresses. The mean initial V on ECMO was estimated at 0.8L/kg, similar to that previously reported by Burtin *et al* (1994) in non-ECMO neonates of gestational ages 26 to 42 weeks (1L/kg). This initial V is perhaps lower than expected considering the expanded circulating volume for a child on ECMO. The normal intravascular volume for a neonate of $80cm^3/kg$ would be significantly diluted at

cannulation with the addition of a circuit volume of approximately 500cm^3. However, this parameter was imprecisely estimated with a coefficient of variation of almost 63%. A time dependent increase in V produced a maximum mean population value of 4.1L/kg, three to four times greater than previously reported in neonates (Burtin *et al.*, 1994; de Wildt *et al.*, 2001; Jacqz-Aigrain *et al.*, 1992; Lee *et al.*, 1999). The mechanism for this change is attributed to sorption of midazolam by the ECMO circuit. The uptake of drugs by polymeric components of the ECMO circuit has previously been demonstrated through *in vitro* studies (see Chapter 3). This is a first order, concentration independent process eventually reaching a state of equilibrium. Many physico-chemical factors will affect the time to equilibrium and the extent of drug loss including lipid solubility (or log P values) ionisation status of drugs, circuit surface area to volume ratio and ECMO flow rates (see Chapter 3). The model estimated the rate of midazolam sorption to be 0.19 hour^{-1}. This value is similar to 0.42 hour^{-1}, the estimated rate of midazolam sorption from the *in vitro* flow simulation study. Differences may be due to nature of the mobile phase (i.e. blood rather than aqueous solution), degree of ionisation and ECMO flow rates.

Inflation in V resulted in a mean terminal half-life of 33.3hours, substantially longer than previous reports in neonates (12±4.9) hours) (Jacqz-Aigrain *et al.*, 1992), older infants, children and adults (range, 1-7hours)(Hughes *et al.*, 1996; Matthews *et al.*, 1988; Payne *et al.*, 1989; Wills *et al.*, 1990). This accounts for raised plasma concentrations of midazolam observed during the latter phases of ECMO (Figure 4.1) with levels far in excess of those required for adequate sedation (<1μg/cm^3). Similar increases in plasma fentanyl levels and the development of tolerance during ECMO has been reported (See Chapter 1, Section 1.7.1.3) (Arnold *et al.*, 1991; Arnold *et al.*, 1990; Leushen *et al.*, 1993). Analogous to midazolam (log P 2.7), fentanyl, a highly lipophillic molecule with a log P value of 2.9 (Ngiam *et al.*, 1998) has frequently shown to be

sequestered by polymeric components of the cardiopulmonary bypass circuit (Hynynen, 1987; Koren *et al.*, 1984; Rosen *et al.*, 1988; Rosen *et al.*, 1990; Skacel *et al.*, 1986).

The enlarged V with associated prolonged half-life not only explains the observed accumulation of midazolam but may also explain the empirical experience of nurses and clinicians (described in Chapter 1, Section 1.13) in sedating these children. Sedation of the infant on ECMO is challenging, the desire to simultaneously achieve sedation, analgesia and relative immobility is paramount. The enlarged V will however delay time to effective plasma concentrations at conventional doses. Thus, ECMO specialists responding to sedation levels will increase infusion rates to achieve therapeutic levels more rapidly. However, once desired plasma concentrations are achieved and equilibrium is attained with tissues and the ECMO circuit, infusion rates need to be decreased. It is obvious from the dosing strategies used in this study that this does necessarily occur leading to excessive plasma concentrations. The latter may also then lead to tolerance and subsequently further dose increases as well as withdrawal symptoms after decannulation.

It is interesting to note that the group that received midazolam extracorporealy had a significantly higher mean doses administered in the first 24 hours and a tendency towards a higher V. Although the addition of this covariate did not significantly improve the fit, it is plausible that a higher V during extracorporeal drug administration exists due to greater distribution of drug into the polymeric components of the circuit. This implies that midazolam should ideally be administered directly into the patient via a peripheral or central venous access.

For ECMO neonates, the metabolic CL of midazolam is similar to that previously reported in non-ECMO neonates. Population estimates of CL in this study (0.084 $L.kg^{-1}.hr^{-1}$) was comparable to values previously reported in population studies of neonates by Burtin *et al* (0.072 $L.kg^{-1}.hr^{-1}$) and Lee *et al* (0.054 $L.kg^{-1}.hr^{-1}$), but

significantly lower than those reported in older infants and children (0.35-0.82 $L.kg^{-1}.hr^{-1}$) and adults (0.38-0.67 $L.kg^{-1}.hr^{-1}$) (Burtin *et al.*, 1994; Hartwig *et al.*, 1991; Lee *et al.*, 1999; Reves *et al.*, 1987). Reduced midazolam elimination in the current population was not unexpected, reflecting the pattern for the ontogeny of CYP3A4 (de Wildt *et al.*, 1999a). In adults, CL of midazolam has been delineated and correlated with CYP3A4/5 activity (Thummel *et al.*, 1994). Hepatic CYP3A4 is abundantly expressed in adults being responsible for catalysing the biotransformation of approximately 50% of currently used drugs. CYP3A5 expression shows large interpatient differences and has been shown to be present in only 10-30% of liver samples tested (Wrighton *et al.*, 1990). In contrast, CYP3A7 is the major isoform expressed in the foetal liver, with probably only marginal contribution to midazolam CL in the postnatal period. CYP3A4 expression is activated during the first few weeks after birth, increasing thereafter to reach adult values at 1 year of age, with a simultaneous decrease in CYP3A7 activity (de Wildt *et al.*, 1999a).

The MR (1-hydroxy midazolam/midazolam ratio) was used as a surrogate marker for CYP3A4/5 activity. The median (range) values in ECMO neonates (0.17 (0.03-0.9)) appear to be significantly higher than previously reported in preterm infants (0.09) and similar to that in than in older children and adults (0.13 – 0.25) (Lloyd-Thomas *et al.*, 1986; Mandema *et al.*, 1992). It has been reported that CYP3A4 expression is activated irrespective of gestational age (Lacroix *et al.*, 1997) and enzyme activity increases only marginally during the first 2 weeks of life. Though gestational age range in this group was higher than previous reports (33-42 weeks versus 26-34 weeks), postnatal age ranges were similar (0.5-18 days versus 3-11 days). Thus the higher MR in this group does not necessarily represent higher CYP3A activity. Rather, it may be indicative of reduced glucuronidation of 1-hydroxy midazolam by UGT and thus reduced renal elimination. The developmental ontogeny of UGT has not yet been

delineated (de Wildt *et al.*, 1999b). The wide interpatient variability in elimination of midazolam and metabolite, and MR, observed in this study concurs with previous reports (de Wildt *et al.*, 2001) and reflects not only the heterogeneous and critically ill nature of the ECMO population, but also indicates that CYP3A and glucuronidation activity in the neonate exhibits large variability.

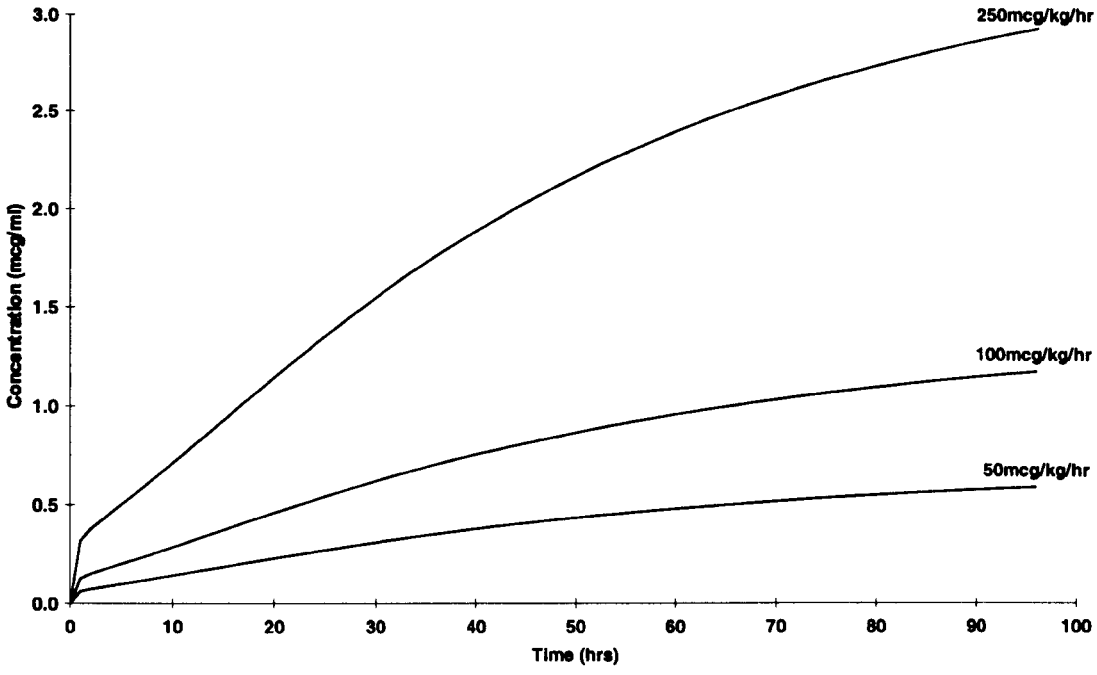
The clinical implications of the findings are illustrated in Figure 4.7A. The figure depicts simulated concentrations for a hypothetical neonate on ECMO receiving continuous infusions of midazolam at various dose rates, using the estimated population parameters. These simulations closely resemble the mean observed concentration profile in this population (Figure 4.1) revealing attenuation of plasma levels during the early phase of ECMO followed by a significant rise. The results also suggest that a higher initial dose rate in the early phase of ECMO needs to be followed by a significant decrease in the dose rate to prevent excessive plasma concentrations. Results from this investigation suggest that for optimum sedation, plasma concentrations of midazolam up to 1.0 $\mu g/cm^3$ are required. This was simulated using an initial infusion rate of 350 $\mu g/kg/hour$ for 6 hours followed by a drop in the dose rate to 50 $\mu g/kg/hour$ (Figure 4.7B). Cross validation provides a measure of the predictive ability of the model in the absence of prospectively collected test data. The exercise revealed an insignificant change in bias or precision, suggesting that the estimated model is likely to perform well in truly prospective trials.

4.7 Conclusion

The results of this study show that plasma midazolam concentrations of up to 1 $\mu g/cm^3$ may be required to achieve adequate sedation in neonates receiving ECMO, higher than previously reported in non ECMO studies. In addition, the pharmacokinetics of midazolam in neonates undergoing ECMO are significantly altered. The population

model reveals a significantly enlarged V, through reversible sorption to the circuit. Consequently, half-life was prolonged and hence midazolam must not be considered a short acting sedative agent in neonatal ECMO patients. The MR, a surrogate measure of CYP3A activity, was also found to be higher than previous reports in preterm neonates, probably attributable to a reduced CL of the metabolite. Using final model parameters, plasma midazolam concentration profiles were simulated at various dose rates and a strategy to achieve adequate plasma levels was suggested.

A



B

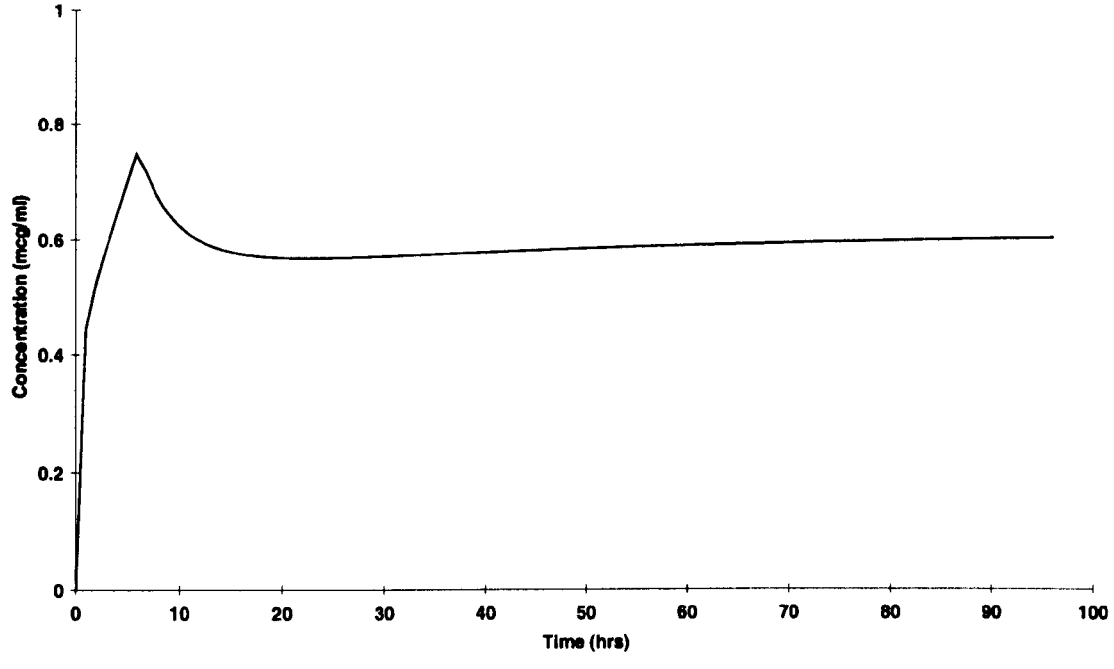


Figure 4.7. Simulated time-concentration profile using parameters from the final model. (A) Various continuous infusion rates (B) A regimen consisting of a continuous infusion rate of 350mcg/kg/hr for 6 hours and then 50mcg/kg/hr thereafter is utilised to maintain therapeutic plasma levels.

CHAPTER V

POPULATION PHARMACOKINETICS OF AMINOPHYLLINE DURING PAEDIATRIC ECMO

5.1 Introduction

Susceptibility of the kidney to hypoperfusion in the patient referred for ECMO is very high. At the time of initiation of ECMO, patients commonly have hypotension, decreased tissue perfusion or hypovolaemia secondary to volume loss or vascular redistribution. In addition ECMO itself may affect renal function. During ECMO, glomerular filtration rate is transiently reduced due to renal perfusion at a pressure outside the autoregulatory range. Non-pulsatile blood flow during VA ECMO can also affect renal cortical blood flow, renal venous return and renal tubular physiology. Other renal effects of ECMO have included the demonstration of elevated levels of atrial natriuretic factor, which affects fluid balance and urine production. Plasma renin activity also appears to be increased in infants during ECMO. This increased activity may affect blood pressure and fluid balance (*ECMO: Extracorporeal Cardiopulmonary Support, 2000*).

There is substantial evidence supporting the diuretic and naturetic effects of theophylline both in normal subjects and adult patients with congestive cardiac failure (Davis *et al.*, 1949; Sigurd *et al.*, 1977). More recently it has been shown to be beneficial in the management of fluid overload in critically ill patients (Lochan *et al.*, 1998; Pretzlaff *et al.*, 1999). Theophylline is a methylxanthine derivative and its primary mechanism of diruretic action is to cause vasodilation of the afferent arteriole and thereby increase renal blood flow and glomerular filtration rate. It appears that the resulting increased glomerular filtration rate can enhance the diuretic action of frusemide. In adults and older children, theophylline is extensively metabolised in the liver by CYP1A2, with only about 10% being excreted unchanged in the urine. In the premature neonate, theophylline is primarily excreted unchanged in the urine and approximately 10% is metabolised to caffeine (Dollery, 1991).

At this institution, continuous infusions of aminophylline are commonly employed during ECMO (in conjunction with conventional agents) for its diuretic properties and in response to renal failure or to manage fluid overload. However, it had been noted by clinicians, nurses and the researcher that plasma concentrations of theophylline reported in neonates on ECMO were frequently at potentially toxic levels (>20mg/L). Although the pharmacokinetics of theophylline in paediatric patients has been extensively studied (Aranda *et al.*, 1976; Driscoll *et al.*, 1989; du Preez *et al.*, 1999; Elliott *et al.*, 1976; Gilman *et al.*, 1986; Karlsson *et al.*, 1991; Lee *et al.*, 1996; Loughnan *et al.*, 1976; Moore *et al.*, 1989; Nassif *et al.*, 1981; Rosen *et al.*, 1979), suggesting age and weight as the most important determinants of CL and V, there are no similar studies in patients receiving ECMO

5.2 Aims of the Study

The purpose of this investigation was to determine the influence of the expanded circulating volume during ECMO on pharmacokinetics of theophylline. A major advantage of the population approach is that it enables estimates of pharmacokinetic parameters from sparse drug concentration data obtained from a large number of patients such as that available from routine therapeutic drug monitoring. This makes it particularly attractive in the paediatric population, as it does not require a large number of drug concentration points per patient. Furthermore, since it employs data generated during routine care, it does not require the patients to be managed under the rigors of a traditional study protocol.

The interpatient variability of derived parameters and any residual error arising from assay error and model misspecification was also investigated. Finally, the influence of patient factors (covariables) on these parameters was explored (Sheiner *et al.*, 1991; Sheiner *et al.*, 1977).

5.3 Materials and Methods

5.3.1 Patients

Data were collected retrospectively from medical records and routine therapeutic drug monitoring database for theophylline, in 75 children admitted for ECMO on the paediatric intensive care unit. Eligibility criteria included all patients receiving aminophylline during ECMO with at least 1 serum concentration measurement. The ECMO circuit used in the study population consisted of Tygon ® S-65HL polyvinyl chloride tubing and the Avecor™ silicone membrane oxygenator. The approximate priming volumes of the circuit depend on the patients' age and bodyweight and are displayed in Table 5. 1.

Table 5.1. Priming Volumes of Extracorporeal Circuits

Weight Range	Oxygenator (Avecor)	Priming Volume	Normal Circulating Volumes [a]	Approximate increase in circulating volume (%)
Up to 4.9 Kg	0800	500 cm^3	Up to 400 cm^3	125
5 – 9.9 Kg	1500	600 cm^3	Up to 792 cm^3	75
10 – 49 Kg	Ultrox II or Ultrox III	1200 cm^3 1000 cm^3	Up to 4000 cm^3	25
50 + Kg	2 x Ultrox I or 2 x Ultrox II	2200 cm^3 1500 cm^3	Up to 5-6000 cm^3	25 - 33

a. Calculated from assumed circulating volume of 80 cm^3/kg (Forfar *et al.1984)*

5.3.2 Study Design

Theophylline was administered as a continuous intravenous infusion of aminophylline into the ECMO circuit, pre-reservoir, initiated at a rate between 5-15μg/kg/min. Apart from 2 patients, no loading doses were administered. The decision to initiate treatment

once the patient had been cannulated for ECMO was made by the responsible clinician based on clinical and biochemical status. All blood samples were taken from an arterial line in the patient, or from the circuit proximal to drug infusions and venous reservoir, at time intervals determined by the attending clinician or clinical pharmacist. The precise date and time of sampling was recorded on the analysis request form and transferred with the final results of the analysis onto a computer database.

Demographic and clinical characteristics documented for each serum sample were: age (postnatal age in neonates), weight, gender, cannulation mode (VV or VA), pertinent medical history (congenital heart defect, sepsis/pneumonia, congenital diaphragmatic hernia), urea and creatinine, ECMO flow rates, co-medication (macrolide and quinolone antibiotics, imidazaole antifungals, phenytoin and phenobarbitone), liver function tests, requirements for CVVH and c-reactive protein.

5.3.3 Analytical Assay

Serum concentrations were analysed by the Olympus Theophylline enzyme immunoassay method (Olympus Diagnostica GmbH) in the Department of Biochemistry, Leicester Royal Infirmary. The limit of determination is 0.8mg/L and the intra-assay coefficient of variation at 5.5, 13.9 and 26.9 mg/L is 3.0, 1.9 and 1.5 % respectively.

5.3.4 Pharmacokinetic Analysis

Population pharmacokinetic modelling was performed using WinNonMix Professional, Version 2.0.1, a non linear mixed effects regression program, bundled with Compaq Visual Fortran Compiler, Professional Edition, Version 6.5 (See Chapter 2, Section 2.5.3).

In a preliminary analysis, one and two compartment models with first order elimination were fitted to all data from all subjects simultaneously. The one compartment model was selected for further analysis since it demonstrated a more appropriate structural model on examination of the graphical diagnostic plots as well as the differences in the OFV values (844 for one compartment model, 843 for two compartment model). Initial parameter estimates were obtained from a previous report of population pharmacokinetics in a paediatric population (Driscoll *et al.*, 1989). Once the base model had been defined, the influence of various demographic and clinical covariables in the regression models for clearance (CL) and volume of distribution (V) were evaluated (see Chapter 2, Section 2.5.3).

5.3.5 Cross Validation and Predictive Performance

The predictive performance of our model was tested using the cross-validation technique (Sun *et al.*, 1999) (See Chapter 2, Section 2.5.5). This method was used since it was not practical to collect new data prospectively. Although cross validation is not a truly prospective validation method, it is a recognised and established approach to estimating model performance assuming identical experimental conditions (Fiset *et al.*, 1995; Kerbusch *et al.*, 2001; Zomorodi *et al.*, 1998).

The study group was randomly divided into 5 smaller groups of 15 patients each. The pharmacokinetic model was fitted to the data whilst excluding one group. The estimated structural parameters were then used to predict the concentrations in the excluded group. This process was repeated 4 times excluding each group in turn. A measure of the predictive performance of models can be determined by calculating the MDPE and AMDPE as described in Chapter 2, Section 2.5.4 (Sheiner *et al.*, 1981). Since the excluded group is not used to develop the model, the MDPE and the AMDPE

are almost unbiased estimates of the predictive capabilities of the model (Zomorodi *et al.*, 1998).

5.4 Statistical Analysis

All clinical and demographic characteristics were reported as mean (SD) and (range). Population pharmacokinetic estimates are expressed as mean ± SE. Univariate and multivariate regression analysis was used to reveal trends in the population studies. A p value < 0.05 was taken as significant.

5.5 Results

5.5.1 Demographic and Clinical Characteristics

The clinical and demographic characteristics of the study population are shown in Table 5.2. A wide range of patients was included, with respect to age, weight and diagnoses. All neonates were term, and accounted for 49% of the total group. Patients were sedated with continuous infusions of a benzodiazepine and opioid combination and received antibiotics based on culture and sensitivity results or as prophylaxis. In 31% of cases aminophylline was initiated on day 1 of ECMO (range 1 –12 days). A mean of 2.2 (range 1 to 8) serum theophylline concentrations per patient were analysed. Samples were taken over a wide range of times (median 88.4 (range 9.8 to 431.7) hours) revealing a median concentration of 15 (range 1 to 38.8) mg/L. The distribution of sampling times and serum concentrations is presented in Figure 5.1. Serum concentration exceeded 20mg/L in 27% of cases. Although 10 patients required CVVH, no patient exhibited significant liver dysfunction apart from neonatal hyperbilirubinaemia.

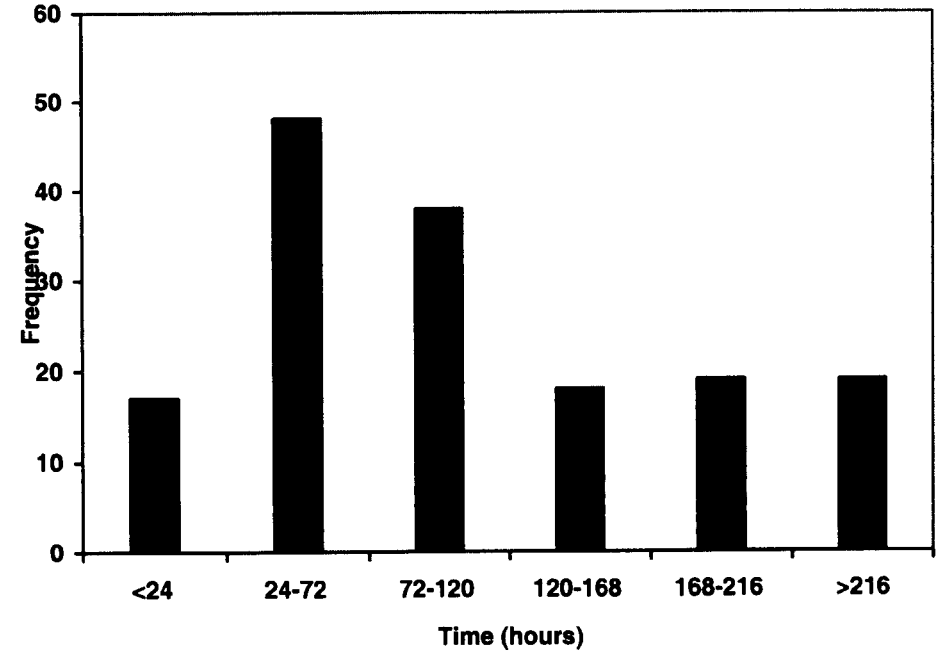
5.5.2 Development of a One Compartment Model

A one compartment model with first order elimination, associated with a proportional error model for interpatient variability and an additive error model for residual error produced the best scatter plots of weighted residuals (Figure 5.2 and 5.3) and the lowest OFV values. Figure 5.2A demonstrates that the weighted residual were randomly distributed around the zero ordinate with no systematic deviations related to predicted concentrations, suggesting that the error model chosen was appropriate. Figure 5.2B similarly resembles random scatter against time suggesting that the one compartment model was appropriate. During the covariate screening phase, only weight and age significantly reduced the OFV (>7.88) when added individually. Figure 5.3A shows the under prediction when weight is low, and over prediction when weight is high when weight has not been included in the basic model. Figure 5.3B shows the improvement in the weighted residuals on inclusion of weight in the model. No other covariate significantly contributed to explaining the variability in the structural parameters and in fact resulted in poor parameter estimates.

Table 5.2. Demographic and Clinical Characteristics

	Overall	Neonates (0 – 1month)	Infants (1 – 12 months)	Children (> 12 months)
Number of Patients	75	38	14	23
Gender				
Males	39	27	2	10
Females	36	17	11	8
Weight (kg)	10.3 (15.7) (2.1 – 85)	3.3 (0.5) (2.1 – 4.1)	4.8 (2.0) (2.7 – 9.0)	25.3 (22.1) (9.3 – 85)
Age (days)	713 (1487) (2-6205)	8.4 (5.9) (2 – 28)	122 (107) (29 - 368)	2236 (1980) (394 – 6205)
Cannulation				
VV	57	32	10	15
VA	18	12	3	3
Diagnosis				
Post Cardiothoracic Surgery	12	4	3	5
MAS/PPHN/RDS	22	22	—	—
CDH	10	9	1	—
ARDS	7	—	—	7
Pneumonia	11	2	3	6
Sepsis	2	1	—	1
RSV/CLD	5	—	4	1
Other	6	1	3	2
CVVH	10	7	2	1
Co-Medication	29	10	7	12
Number of Serum Concentrations	160	72	37	51
Number > 20mg/L	43	22	8	13
Infusion Dose (μg /kg/min)	9.2 (2.6) (4 – 16.6)	—	—	—

Data is expressed as Mean (SD) and Range.
VV – Venovenous, VA – Venoarterial, MAS – Meconium Aspiration Syndrome, PPHN – Persistent Pulmonary Hypertension of the Newborn, RDS – Respiratory Distress Syndrome, CDH – Congenital Diaphragmatic Hernia, ARDS – Acute Respiratory Distress Syndrome, RSV – Respiratory Syncitial Virus, CLD – Chronic Lung Disease, CVVH – Continuous Veno-Venous Haemofitlration



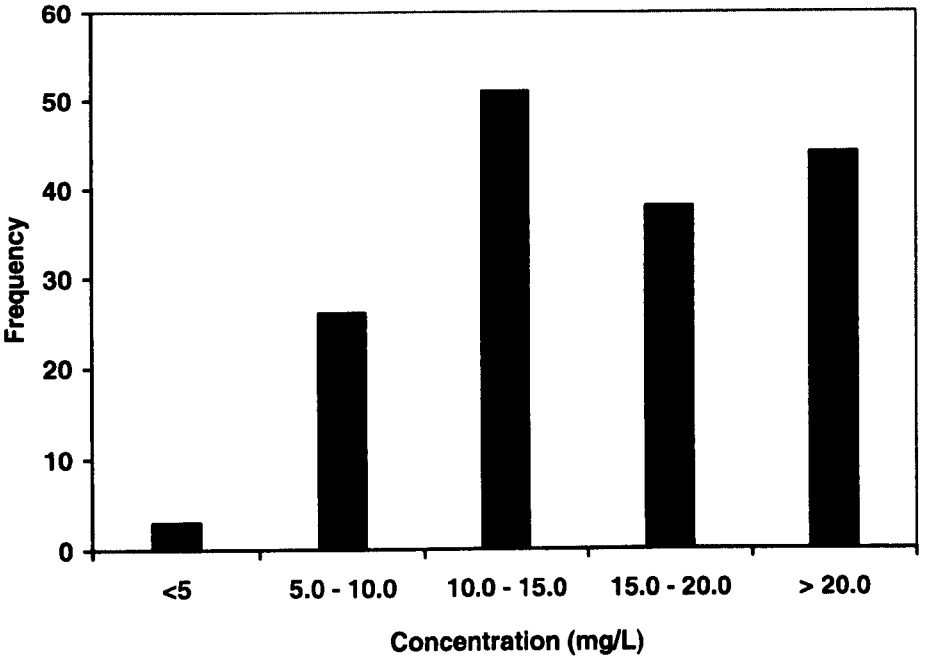
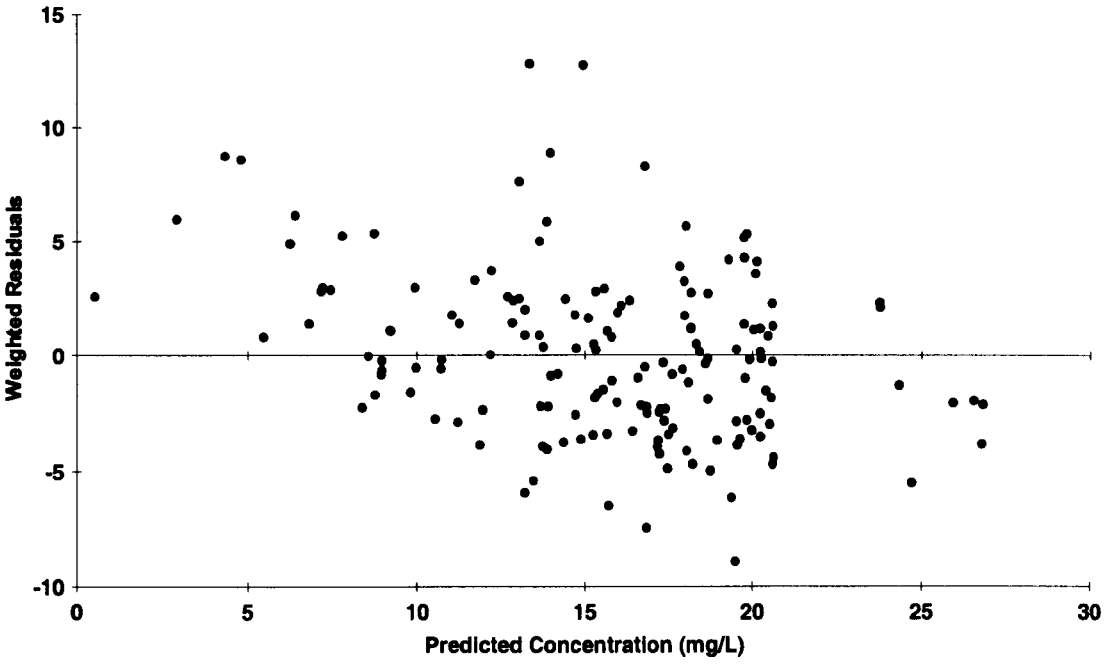


Figure 5.1. Distribution of Serum Theophylline Sampling times (hours) and Concentrations (mg/L)

A



B

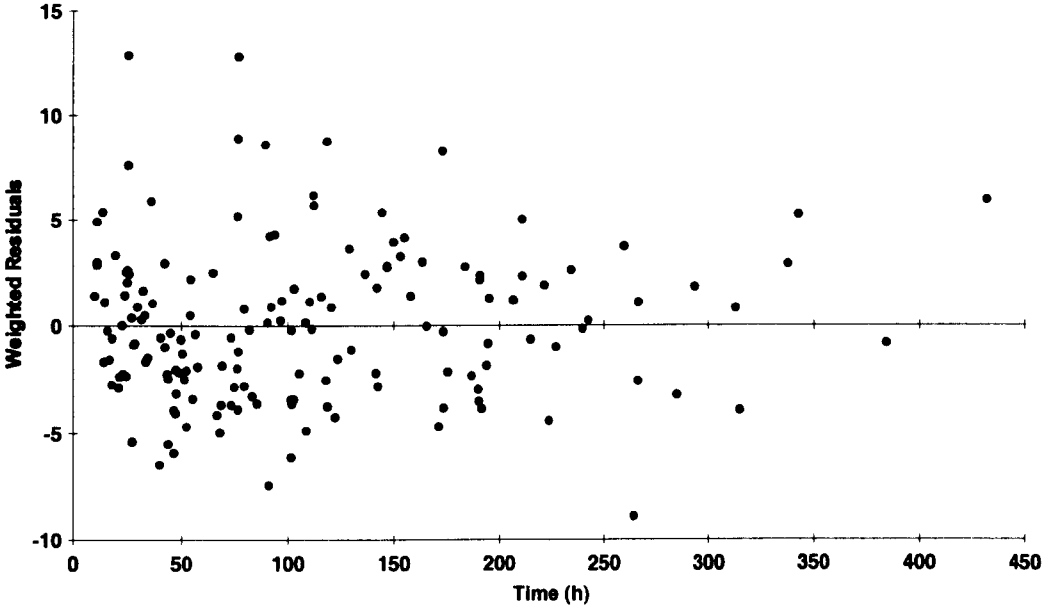
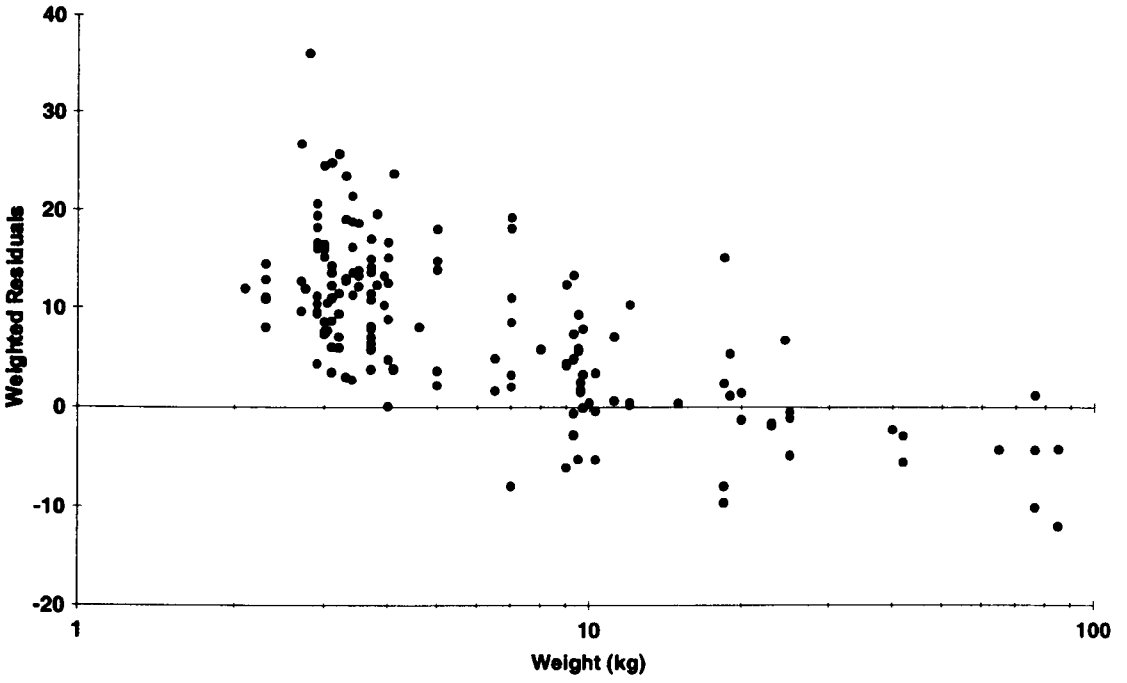


Figure 5.2. Weighted Residuals Versus (A) Predicted Concentration (mg/L) and (B) Time

A



B

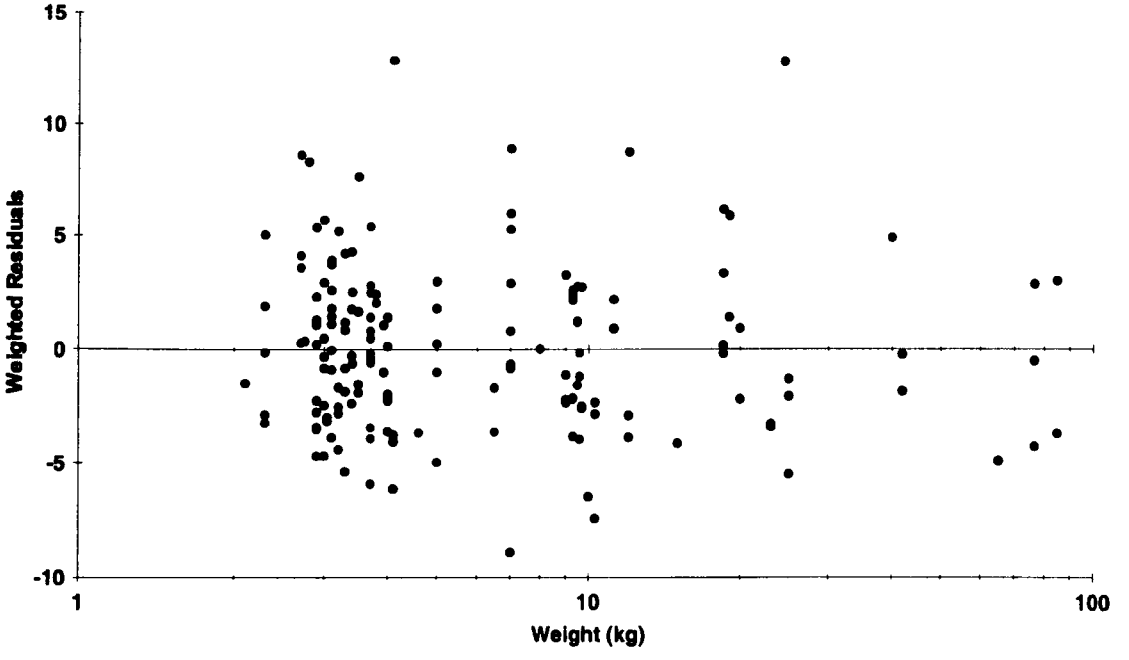


Figure 5.3. Weighted Residuals versus significant covariate Weight (A) before inclusion in the model and (B) after inclusion in the model

A significant correlation between CL and weight (r^2=0.97) as well as age (r^2=0.13) was demonstrable. In the cumulative model building process, weight was optimally modelled as a continuous function on CL and V. Exponential functions of weight resulted in very similar changes in OFV and thus the simpler model was chosen. Although the parameter estimate for age in CL was not estimated very precisely (coefficient of variation (CV) 96.4%), it's inclusion did significantly improve the model. In contrast, the combination of age with weight in V did not, and thus age was omitted from V (Table 5.3). The parameter estimates of the final structural and error models are shown in Table 5.4.

Table 5.3. Covariate Model Building: Effect on Objective Function Value of the Significant Covariables Weight and Age as Determinants of Clearance and Volume

Addition of Covariable	OFV[a]	P value[b]	Conclusion
CL, V (base model, with no covariate influence)	1154		
CL with Weight **V (with no covariate influence)**	1061	P < 0.005	Weight significantly influences clearance
CL (with no covariate influence) **V with weight**	1116	P < 0.005	Weight significantly influences volume
CL with weight **V with weight**	995	P < 0.005	
CL with Weight and Age **V with Weight**	985	P < 0.005	Age significantly influences clearance
CL with Weight **V with Weight and Age**	989	P > 0.005	Age does not significantly influence volume

a. - 2 x log likelihood
b. Assuming chi-squared distribution, a change in the OFV > 7.88 (p<0.005) was accepted as statistically significant.

Table 5.4. Population Pharmacokinetic Parameter Details for the Final Model of Theophylline in Paediatric ECMO Patients

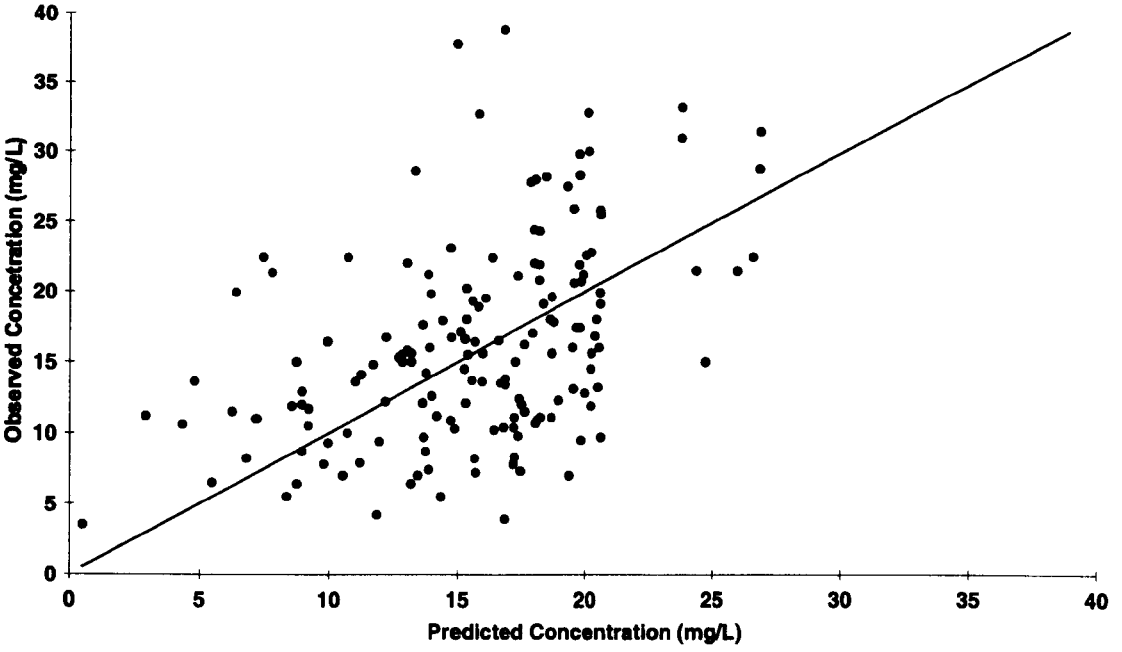
	Estimate[a]	CV (%)[b]
β_1	0.023 ± 0.0016	6.9
β_2	0.000057 ± 0.000055	96.4
β_3	0.57 ± 0.053	9.3
Interpatient variability in clearance	0.15 ± 0.038 (38.7 %)[c]	25.3
Interpatient variability in volume	0.16 ± 0.13 (40 %)[c]	81.2
Residual Error	13.0 ± 3.09 (S.D = 3.6mg/L)	23.7

a. Data is expressed as Mean ± SE
b. Precision of the parameter estimates expressed as percentage coefficient of variation (CV).
c. Parameters of interpatient variability expressed as coefficient of variation.

CL (L/hr) = β_1*Weight (kg) + β_2 × Age (days)
V (L) = β_3 × Weight (kg)

The values of CL and V were estimated with high precision (CV's of 6.9 and 9.3% respectively). The CV of interpatient variability for CL and V were 39 and 42% (precision 23 and 80% respectively). The additive error model for residual error estimated a standard deviation of 3.6mg/L. Since this error is constant over the concentrations studied, the CV in serum theophylline concentrations of 5,10 and 20mg/l would be 72, 36 and 18% respectively. The relationship between observed and final model predictions is shown in Figure 5.4.

A



B

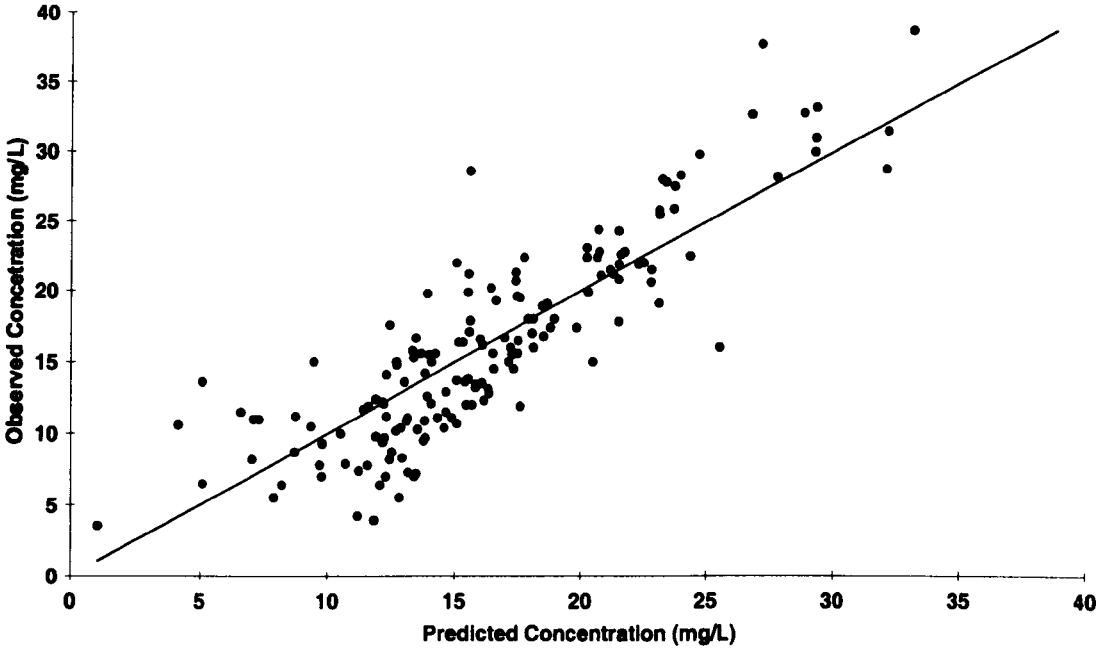


Figure 5.4. Observed versus (A) Population and (B) Individual Bayesian Posterior Predicted Concentrations (mg/L) from the Final Model

5.5.3 Predictive Performance and Cross Validation

The predictive performance of the base model (CL and V modelled with weight only) when compared to the final model (the addition of age in CL) further confirmed the superiority of the latter (Table 5.5). The bias of the model reduced from 6.4 to 4.3% whilst the precision improved from 30.6 to 27.6%. The worst and best predictive profiles from the final model are shown in Figure 5.5.

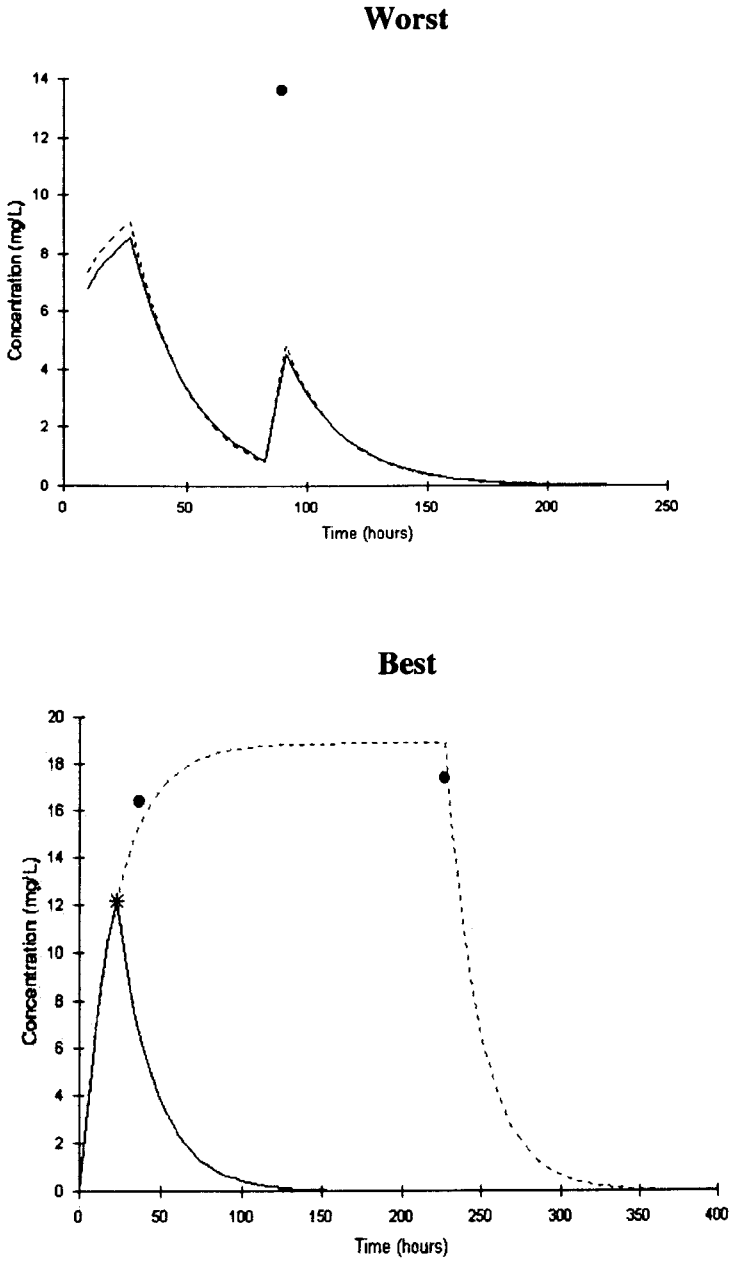


Figure 5.5. The worst and best time-concentration profiles predicted by the final model. Worst:● observed; —, predicted (P); ----, predicted (I). Best (two individuals):✳ observed; —, predicted (P); ● observed;----- predicted (I)

The cross validation analysis of the final model revealed estimates of structural parameters (and their CV's) comparable to those of the final model, suggesting that the model is likely to perform well in a truly prospective trial during similar conditions. The validation exercise revealed an increased bias of 9.2% indicating that the model had a small tendency to over predict. The predictive precision from the cross validation was determined to be very similar at 29.5% (Table 5.5).

Table 5.5. Predictive Performance and Cross Validation

	Base Model	**Full Model**	**Cross-Validation**
Bias[a] (%)	6.4 (-0.9, 16.3)	4.3 (-6.6, 13.2)	9.2 (2.9, 16.5)
Precision[b] (%)	30.6 (26.6, 34.8)	27.6 (22.2, 33.6)	29.5 (24.8, 36.0)

a. Bias (%) = median ([observed$_{ij}$-predicted$_{ij}$/predicted$_{ij}$]*100), i (ith individual), j (jth concentration)
b. Precision (%) = absolute median ([observed$_{ij}$-predicted$_{ij}$/predicted$_{ij}$]*100), i (ith individual), j (jth concentration)
Figures in parentheses are 95% Confidence Intervals for the median.

In the study by Pretzlaff *et al* (1999), increased diuresis was observed at theophylline levels between 6 and 12 mg/L, lower than that required in asthma therapy. Thus, a proposed new dosing regimen to achieve average steady state concentrations of 10mg/L following a loading dose are displayed in Table 5.6, based on calculations from the CL and V parameters determined in the final model.

Table 5.6. Maintenance Doses of Aminophylline (μg/kg/min) for Continuous Infusion to Achieve Average Steady State Concentration of 10mg/L

Age (days)	10^{th}, 50^{th} and 90^{th} Percentile Weight (Kg)	Maintenance infusion rate (μg/kg/min)
1	3	4.8
	3.5	4.8
	4	4.8
30	3.5	4.9
	4.3	4.9
	5	4.9
365	8.8	5.3
	10	5.2
	11.5	5.2
1095	12.9	5.8
	14.5	5.7
	17	5.6
1825	15.9	6.2
	18.5	6.0
	21.5	5.8
3650	25	6.5
	30	6.2
	37	6.0
5475	47	6.2
	56	6.0
	68	5.7

Recommended maintenance infusion rates following an initial Loading Dose (0.57 * Weight (kg) * 10mg/L). Maintenance infusion rate calculated from: Average steady state concentration = Rate of infusion / Clearance (using clearance parameters determined in the final model).

5.6 Discussion

ECMO patients comprise a group from the extreme spectrum of critical illness, supported with intensive medical interventions and multiple pharmacological agents. The use of bolus doses of theophylline as a diuretic in ECMO neonates has previously been reported, revealing an increased urine flow rate when compared with frusemide alone (Lochan *et al.*, 1998). However, this is the first time that population pharmacokinetics of theophylline has been reported in paediatric patients receiving ECMO. Furthermore, in contrast to previous population pharmacokinetic studies, the model was developed through data obtained from continuous intravenous infusions of aminophylline.

As with previous studies, weight and age emerge as the most important factors affecting CL (Aranda *et al.*, 1976; Driscoll *et al.*, 1989; du Preez *et al.*, 1999; Elliott *et al.*, 1976; Gilman *et al.*, 1986; Karlsson *et al.*, 1991; Lee *et al.*, 1996; Loughnan *et al.*, 1976; Moore *et al.*, 1989; Nassif *et al.*, 1981; Rosen *et al.*, 1979). Both these covariates are strong indicators of hepatic and renal maturation. However, in the study reported by Moore *et al* (1989), CL was modelled as a non-linear exponential function of weight showing a disproportionate increase in CL with weight. Even though this investigation included a wide weight and age range, the regression analysis suggested a linear relationship with CL. Moreover, exponential functions of weight did not improve the model. The inclusion of age in the CL model further improved the parameter estimate and reduced interpatient variability.

As can be seen from Table 5.7, the estimated CL of theophylline from this study appears to be significantly lower than previous reports of similar age groups. It is difficult to make a comparison of neonates, since previous studies involved premature infants of lower birth weights. Neonates in this investigation were full term, of greater birth weights and would be expected to demonstrate greater CL rates. Nevertheless, CL

rates determined in this investigation were similar to those found in premature neonates from traditional pharmacokinetic studies. Although previous population studies in premature infants by Lee *et al* (mean weight 1.15kg, mean gestational age 28.5 weeks) and Moore *et al* (mean weight 1.5kg, gestational age 31 weeks) have reported similar models, the differing neonatal populations make comparisons difficult (Lee *et al.*, 1996; Moore *et al.*, 1989). Estimates of CL in older infants and children are undoubtedly substantially lower than previous estimates (Figure 5.6) (Driscoll *et al.*, 1989; Elliott *et al.*, 1976; Loughnan *et al.*, 1976; Rosen *et al.*, 1979).

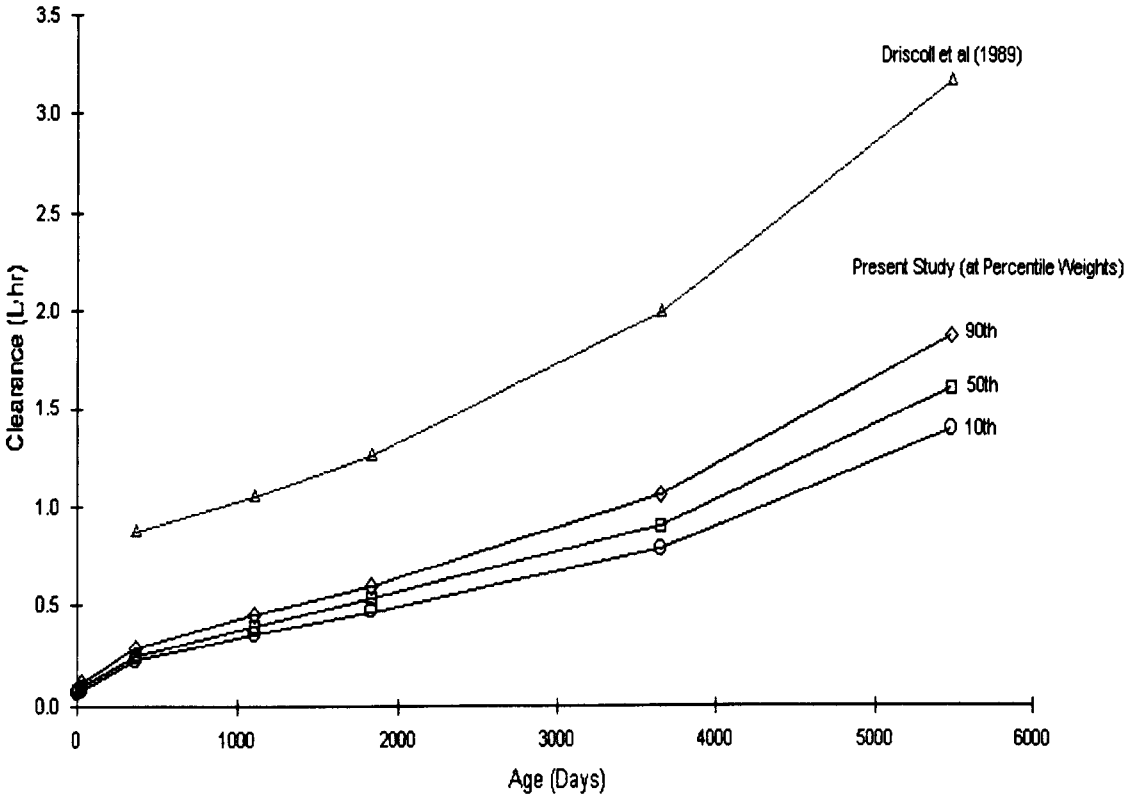


Figure 5.6. A comparison of the estimated theophylline clearances based on age determined by the present study (at 10th, 50th and 90th percentile weights) and a paediatric population study by Driscoll et al(1989).

Table 5.7. Previously Reported Estimates of Theophylline Clearance and Volume

Authors (Reference)	Number Of Patients		CL (L/kg/hr)	V (L/kg)
Traditional Studies				
Premature Neonates		*Mean Postnatal Age (weeks)*		
(Brazier et al., 1979)	20	2.6 (~ 1 – 1.5)	0.024 (SD=0.0051)	1.0 (SD=0.2)
(Jones et al., 1979)	11	~ 3.0 (~ 0 – 5.6)	0.019 (0.012 – 0.028)	0.7 (0.4 – 1.2)
(Hilligross et al., 1980)	17	7.1 (~ 3 –10.0)	0.023 (0.016-0.03)	0.6 (0.4 – 0.9)
(Gilman et al., 1986)	73[a]	2.3 (1.9)	0.016 (SD = 0.0053)	0.78 (SD= 0.16)
	106[b]	1.7 (1.3)	0.02 (SD=0.0054)	0.76 (SD = 0.17)
Infants & Children		*Mean Age (years)*		
(Elliott et al., 1976)	30	10.7 (6.08 – 16.75)	0.087 (0.031 – 0.22)	0.42 (0.28 – 0.52)
(Loughnan et al., 1976)	10	2.5 (1.4 – 4.4)	0.1 (0.057 – 0.163)	0.25 (0.11 – 0.57)
(Rosen et al., 1979)	13	0.9 (0.3 – 1.5)	0.089 (0.046 – 0.16)	0.56 (0.38 – 0.83)

Table 5.7 Continued...

Population Studies				
Premature Neonates		***Age (weeks)***	***Clearance Model***	***Volume (L) Model***
(Moore et al., 1989)	108	GA: 31 (24 – 42)	0.0175 (Weight)$^{1.28}$ +1.17 (PNA in weeks) L/hr	0.85 * Weight (kg)
(Karlsson et al., 1991)	138	PNA: 0.3 – 11.4	0.04 (SE=0.0019) L/kg/hr	1.31 * Weight (kg)
(Lee et al., 1996)		PNA: 0.6 (0.14 – 3.6)	0.0123 (Weight (kg)) + 0.000377 (PNA in days) L/hr	0.937 *Weight (kg)
(du Preez et al., 1999)		PNA: 0.16 (0.14 – 0.3)	0.0056 * (Weight$^{0.75}$) * 1.61 (if 0xygen support) or 1.0 (if no oxygen support)[c]	0.58 * Weight (kg)
Infants and Children		***Age (years)***		
(Driscoll et al., 1989)		0.3 – 15.2	Clearance, a function of age, colour (black or non-black), gender and asthma treated with oral β-agonists alone.	0.62 * Weight (kg)
Present Study		2 days - 17	0.023 * (Weight) + 0.000057 * Age (days) L/hr	0.57 * Weight (kg)

a. Asphyxiated newborns b Non-Asphyxiated newborns c Oxygen support by head box
PNA – Post Natal Age GA – Gestational Age

There may be a number of reasons for reduced theophylline CL in an ECMO population. First, the volume in the ECMO circuit significantly expands the total circulating blood volume in the patient and therefore would be expected to increase theophylline's V and may also reduce CL by affecting plasma protein binding (Table 5.1). Theophylline is 50-60% protein bound, and the fraction bound will increase with an expanded blood volume, decreasing the unbound pool. Since theophylline is a low extraction ratio drug, a decrease in fraction unbound will result in a reduced clearance. In the present study, V was modelled as a simple function of weight and the final estimate of 0.57L/kg may be indicative of an enlarged V in comparison to previous reports in similar age groups (Elliott *et al.*, 1976; Loughnan *et al.*, 1976; Rosen *et al.*, 1979). Although sorption to the circuit components may also contribute to an enlarged V, this is unlikely for theophylline since it has a low log P value of 0.96 (Dollery, 1991).

Second, the majority of these patients have significant and prolonged periods of hypoxia prior to cannulation for ECMO, often resulting in ischaemic insult to the kidneys and liver, potentially affecting excretion of unchanged drug in the urine (the main elimination route in neonates) and hepatic metabolism. Previous workers have reported significantly reduced theophylline CL in neonates with hypoxia and birth asphyxia (Estelle *et al.*, 1981; Gal *et al.*, 1982; Gilman *et al.*, 1986). Unfortunately it was not possible to test the influence of these covariables since an index of hypoxia at or before cannulation was not available in many patients nor was it possible to ascertain birth asphyxia in many of the neonates. There was also no discernible relationship between serum creatinine data and CL. As revealed in Table 5.2, 10 patients (13.3 %) in the study population required renal support in the form of CVVH but no significant influence on the model was demonstrable.

Third, although no overt manifestations of hepatic failure were seen, 9 patients (12%) in the group were referred with congenital diaphragmatic hernia (CDH), a surgical condition known to alter hepatic blood flow (MacGillivray *et al.*, 1994). Although this is most likely to affect those drugs with a high hepatic extraction ratio, significant changes in blood flow may also influence drugs of low to intermediate extraction such as theophylline (Mckindley *et al.*, 1998). No significant influence was demonstrable. It is known that cardiac failure can significantly decrease theophylline CL (Jusko *et al.*, 1979; Self *et al.*, 2000). Twelve (16%) ECMO patients in this study were postoperative cardiac surgical patients, often requiring multiple inotropes and vasopressor agents for cardiovascular support. Once again an influence could not be detected.

Finally, reduced CL (and higher V) may also be a consequence of the blood recirculation phenomena during VV ECMO (see Chapter 1). It is inevitable that a fraction of blood that has been oxygenated in the circuit flows directly from the reinfusion site to the drainage catheter and back into the circuit instead of the patient's circulation. The proportion of blood that recirculates is thought to depend on circuit pump flow, catheter position, cardiac output and right atrial size (*ECMO: Extracorporeal Cardiopulmonary Support, 2000*). Since drugs are administered into the circuit during ECMO, recirculation could significantly affect the removal of drugs. In addition, high flow rates during VA ECMO produce apulsatile blood flow that can also alter perfusion of tissues, reducing capillary circulation and aerobic metabolism (Shevde *et al.*, 1987). Although it was not possible to show a significant influence of cannulation method on the model, there was a tendency towards an increased V in the VV group.

The ECMO population consists of patients with extremely severe pulmonary or cardiopulmonary disease as a result of wide ranging primary diagnoses. The heterogeneous characteristics of the study population may help explain the wide

interpatient variability estimated for CL (39%) and V (42%). The study population included patients with congenital and acquired respiratory failure (infective and non-infective aetiology), congenital cardiac defects, septicaemia, CDH, post-operative cardiac and thoracic cases. As described above, the influences of these subgroups in the model were not detected probably as a result of limited numbers. Furthermore, since the ratio of extracorporeal:patient volume decreases in older children (see Table 5.1), this may have contributed to the increased variability in V. Age did not significantly affect V, however there was a tendency towards a negative relationship with age. It is also not surprising that the precision with which these were estimated was better for CL (23%) than V (80%) since a large majority of the serum theophylline concentrations were sampled at steady state, of which the major influential pharmacokinetic parameter is CL (Rowland *et al.*, 1995). The estimate of residual error (3.6mg/L) is higher than previously reported and reflects model misspecification, sampling and dosing errors as well as changing parameters during the period of analysis. The latter point may be particularly relevant during ECMO since the recirculation phenomenon (affecting CL and V) would be expected to be less prevalent towards the end of ECMO treatment when the pump flow rates are significantly lower.

The predictive accuracy and validity of the proposed final model was evaluated using the cross-validation method. It is evident from the methodology described (Chapter 2, Section 2.5.5) that cross-validation is a conservative measure of the expected performance since each sub-model is based on a reduced data set. The analysis revealed a greater bias than with the complete data set model, suggesting that the measured values tended to be on average slightly higher than predicted. There was no great change in the magnitude of the variability in the prediction error (27.6 vs. 29.5%) confirming that our final model seems to be able to predict with reasonable precision.

Although it was not *a priori* aim of this investigation to define an aminophylline dose regimen for diuresis in paediatric ECMO patients, since ideally this is only reliable in a well designed prospective pharmacokinetic-pharmacodynamic investigation, the results of this investigation provide a valuable dosing guide. A large proportion (20%) of the measured plasma concentrations in this investigation were potentially toxic (greater than 20mg/L) suggesting a reduction in the maintenance infusion dose rate prescribed in the study (Table 5.6). The long half-life in this study group (mean 16.6 hours) suggests that an initial loading dose is necessary to achieve effective plasma concentrations rapidly.

5.7 Conclusion

This is the first report of population pharmacokinetics of theophylline in paediatric ECMO patients. The estimated CL is significantly lower and the V higher than previously reported in this age group. These differences are probably as a result of the expanded circulating volume during ECMO, but also the extreme nature of critical illness this group comprises, as well as altered renal and hepatic physiology. The large interpatient variability observed reflects the heterogeneous nature of patients treated on ECMO. The validity of the derived model was substantiated using the cross validation method.

CHAPTER VI

GENTAMICIN PHARMACOKINETICS IN NEONATAL ECMO PATIENTS: OPTIMISATION OF DOSING

6.1 Introduction

Neonatal sepsis occurs at an estimated rate of 1 to 2 cases per 1000 live births, with positive blood cultures in up to 20% of infants in neonatal intensive care units. The organisms responsible for early onset (first week of life) sepsis include Group B *Streptococcus* (GBS) and *Escherichia coli* and account for 70 to 80% of blood and cerebrospinal fluid cultures. Although less common, enterococci, *Listeria monocytogenes,* and species of gram-negative enteric bacilli other than *Escherichia coli* are known to cause disease in neonates (Baltimore *et al.*, 2001).

The aminoglycoside gentamicin is commonly combined with a beta lactam for prophylaxis and treatment of early onset sepsis in preterm and term neonates(Fanos *et al.*, 1999). Since the inception of the ECMO programme at Glenfield Hospital in 1989, early onset sepsis has accounted for approximately 9% of the neonates treated on ECMO. However, a combination of benzyl penicillin and gentamicin is administered to all neonates cannulated for ECMO, unless otherwise indicated by microbiological culture and sensitivity reports or if deterioration in clinical condition necessitates a change in antibiotic therapy. As a result of gentamicin's low therapeutic index and large interpatient pharmacokinetic variability, plasma level monitoring during therapy is necessary (Zaske *et al.*, 1982; Zaske *et al.*, 1980). Furthermore, the heterogeneity of the neonatal population particularly with respect to maturation of renal function requires dosing regimens to be individualised in order to minimise toxicity (Murphy *et al.*, 1998).

The pharmacokinetics of gentamicin during ECMO has previously been investigated (see Chapter 1, Section 1.7.7.1) (Bhatt-Mehta *et al.*, 1992; Cohen *et al.*, 1990; Dodge *et al.*, 1994; Munzenberger *et al.*, 1991; Southgate *et al.*, 1989). In two of these studies, pharmacokinetic parameters were compared on and off ECMO (Cohen *et al.*, 1990; Dodge *et al.*, 1994). All apart from one study suggest altered gentamicin

disposition during ECMO (Munzenberger *et al.*, 1991). An increased volume of distribution (V), reduced clearance (CL) and prolonged half-life during ECMO led many of the investigators to suggest an altered dosing regimen (Cohen *et al.*, 1990; Dodge *et al.*, 1994). However, to date no study has evaluated a modified dosing approach during ECMO. At Glenfield Hospital, clinical staff had empirically noticed that initial dosing regimens quoted by formularies such as *Medicines for Children (2000)* and *Paediatric Formulary (5th Edition), Guy's, St. Thomas' and Lewisham Hospitals* for term neonates (2.5-3.5mg/kg 8-12 hourly) frequently resulted in potentially toxic trough levels (>2mg/L). This led to repeated measurement of plasma gentamicin concentrations and many dosage alterations. As a result and following review of studies undertaken by Cohen *et al* (1990) and Murphy *et al* (1998), a modified initial dosing regimen of 2.5mg/kg once a day was trialed over a six month period.

6.2 Aims of the Study

The aim of this investigation was a retrospective comparison of the steady state peak and trough levels achieved prior to and after modification of the dosing regimen. This would allow an evaluation of recommended starting doses. The pharmacokinetics of gentamicin in neonates during ECMO was also determined and where possible re-evaluated once the patient came off ECMO.

6.3 Materials and Methods

6.3.1 Patients

A retrospective analysis of neonates who received gentamicin therapy during ECMO over a 12 month period was conducted. For the first six months investigated, gentamicin doses of between 2.5-3.5mg/kg 8-12 hourly had been administered on initiation of therapy. In the subsequent six months, the initial dosing regimen had been modified to 2.5mg/kg once a day. Gentamicin was administered as a slow bolus over 3-5 minutes via the circuit, pre-reservoir and proximal to the oxygenator. Blood samples were collected just before (trough) and 1 hour after the end of the bolus dose (peak). Patients were selected by comparing an ECMO admissions database with a gentamicin assay database held by the Department of Microbiology. Inclusion criteria included all neonates who had received gentamicin during ECMO and had steady state (3^{rd} dose and beyond) peak and trough plasma levels recorded. In the event gentamicin treatment continued post ECMO, plasma levels repeated at a new assumed steady state were also included. Data on doses administered, demographic and clinical data (urea, creatinine, urine output, liver function tests) were also collected from the medical records, discharge summaries and prescription charts. All data was collated and entered into a Microsoft Excel spread sheet.

6.3.2 Assay Method

All samples were analysed in the Department of Microbiology using the INNOFLUOR™ Gentamicin Assay System (Opus Diagnostics Inc. Fort Lee, NJ) a fluorescence polarization immunoassay, and using the TDxFLx® analyser (Abbott Laboratories, Inc., Abbott Park, IL 60064). The coefficient of variation for this test is less than 3%.

6.3.3 Pharmacokinetic Analysis

Peak and trough plasma concentrations were fitted by log-linear regression analysis, assuming a one compartmental model with first order elimination. The elimination rate constant (K_e in hr^{-1}) was determined directly from the slope of the monoexponential terminal phase (see Chapter 2, Section 2.5)

6.3.4 Statistical Analysis

Clinical characteristics and pharmacokinetic parameter estimates were reported as mean (SD) or median (range). To ascertain whether clinical covariables were correlated with pharmacokinetic parameters, single linear regression analysis was performed for each covariate against V, CL and half-life. The covariates tested included gestational age (GA), postnatal age (PNA) at cannulation, duration of ECMO, urea, creatinine and urine output. Differences between the two dosing groups were investigated using the students t-test, a p value <0.05 was considered statistically significant. All statistical analysis was conducted using SPSS 10.0 software (SPSS, Chicago, Illinois, USA).

6.4 Results

6.4.1 Demographics and Clinical Characteristics

Clinical and demographic characteristics of the study population are shown in Table 6.1. Although 28 patients satisfactorily met the inclusion criteria, the medical records of only 22 neonates were located. Ten patients (group 1) received the normal dosing recommendation for term neonates ranging 2.5-3.5mg 8-12hourly (mean dose 4.5mg/kg/day). 12 patients (group 2) received the modified dose of 2.5mg/kg once a day. In 13 patients (59%) meconium aspiration syndrome was the primary diagnoses, 3

(13.6%) patients suffered persistent pulmonary hypertension of the newborn, 3 congenital diaphragmatic hernia and 3 patients with suspected congenital pneumonia. All patients were term; gestational ages ranging from 36-42 weeks. The majority of patients (86%) were cannulated for ECMO within 24 hours of birth. All patients underwent VVcannulation. There were no significant differences in clinical characteristics between the two groups, apart from serum creatinine concentration. The latter was lower in group 1 compared to group 2 (74.0 (16.5) versus 94.2 (18.6) μmol/L respectively, $P<0.005$). One patient in each group required renal support in the form of CVVH during gentamicin therapy. Liver function tests were normal in all patients.

6.4.2 Pharmacokinetic Analysis

A total of 29 peak and trough steady state levels were analysed from the 22 patients. Figure 6.1 shows the observed plasma concentrations from both groups. The agreed optimum therapeutic range for peak and trough plasma concentrations was 5-10mg/L and <2mg/L respectively. Peak serum concentrations were significantly higher in group 1 than in the group 2 (6.3 (1.5) versus 5.2 (2.0) mg/L, $p<0.05$) (Table 6.2). Similarly, the trough levels were also significantly higher in group 1 (2.0 (1.0) versus 1.5 (0.4) mg/L, $P< 0.05$). On further analysis, 2 (15.4%) peak levels in group 1 and 5 (31%) peak levels in group 2 were below the minimum effective concentration of 5mg/L. No levels greater than 10mg/L were reported in either group. With respect to the trough levels, 6 (46%) and 1 (6.3%) of reported concentrations were greater than 2mg/L in group 1 and 2 respectively. In terms of the percentage of combined peak and trough concentrations 'hitting' the target range, the dosing approach in group 2 was better than group 1 (62.5% versus 38.5% respectively).

Table 6.1. Clinical and Demographic Characteristics

	Group 1 (n=10)	Group 2 (n=12)
Male/Female	8/2	6/6
Weight (kg)	3.5 (0.8)	3.6 (0.7)
Gestational Age (weeks)	39.5 (1.4)	40.2 (1.3)
Age at Cannulation (days)	1 (1– 4)	1 (1 – 16)
Duration of ECMO (days)	7.5 (4 – 18)	5.5 (3 – 18)
Urea (mmol/L)	6.7 (2.8)	8.7 (10.6)
Creatinine (μmol/L)*	74.0 (16.5)	94.2 (18.6)
Urine Output (cm^3/kg/hr)	4.7 (6.5)	3.4 (2.5)
Survival	90%	75%

Data expressed as Mean (SD), Median (range)

* *p<0.005*

Table 6.2. Pharmacokinetic Analysis

	Overall	Group 1	Group 2	P
Peak (mg/L)	5.6 (1.4)	6.3 (1.5)	5.2 (2.0)	P<0.05
Trough (mg/L)	1.7 (0.8)	2.0 (1.0)	1.5 (0.4)	P<0.05
V (L/kg)	0.8 (0.3)	0.8 (0.2)	0.8 (0.4)	*p>0.05*
CL (L/kg/hr)	0.044 (0.01)	0.051 (0.009)	0.039 (0.008)	P<0.005
Half-Life (hrs)	12.8 (4.4)	11.6 (2.7)	14.1 (5.4)	*p>0.05*

Data expressed as Mean (SD)

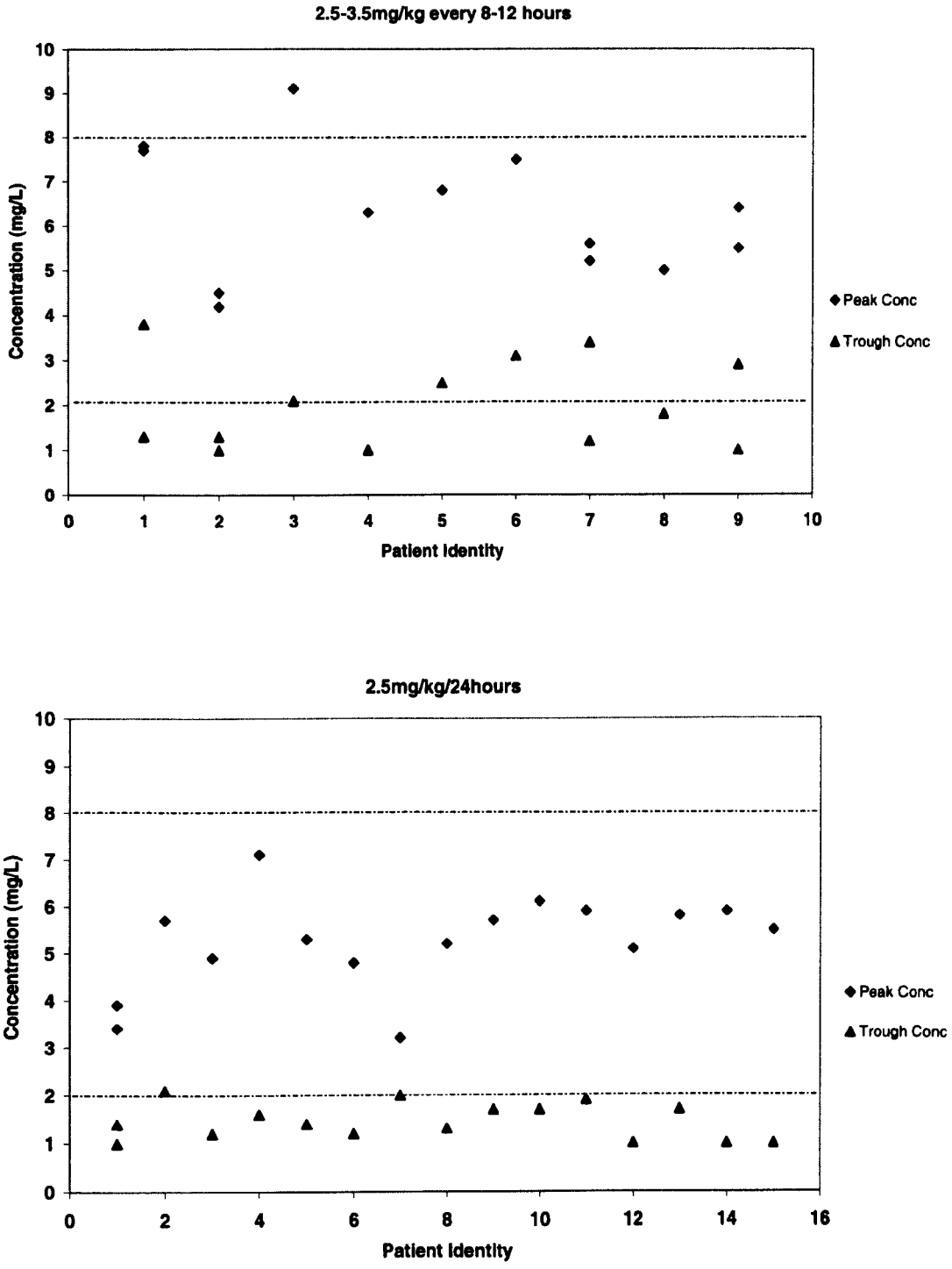


Figure 6.1. Steady State Peak and Trough Gentamicin Concentrations Achieved with the Two Dosing Regimens

The overall values of pharmacokinetic parameters, V, CL and half-life determined from the 22 patients were, 0.8 (0.3) L/kg, 0.044 (0.01) L/kg/hr and 12.8 (4.4) hours. The estimated parameters when regressed with covariates suggested significant correlation between serum creatinine and CL (r^2=0.22) and serum creatinine and half-life (r^2=0.28) ($p<0.05$) (Figure 6.2). In the sub analysis, CL was significantly greater ($p<0.05$) in group 1 (0.051 (0.009) L/kg/hr) compared to group 2 (0.039 (0.008) L/kg/hr). There were no significant differences in V or half-life.

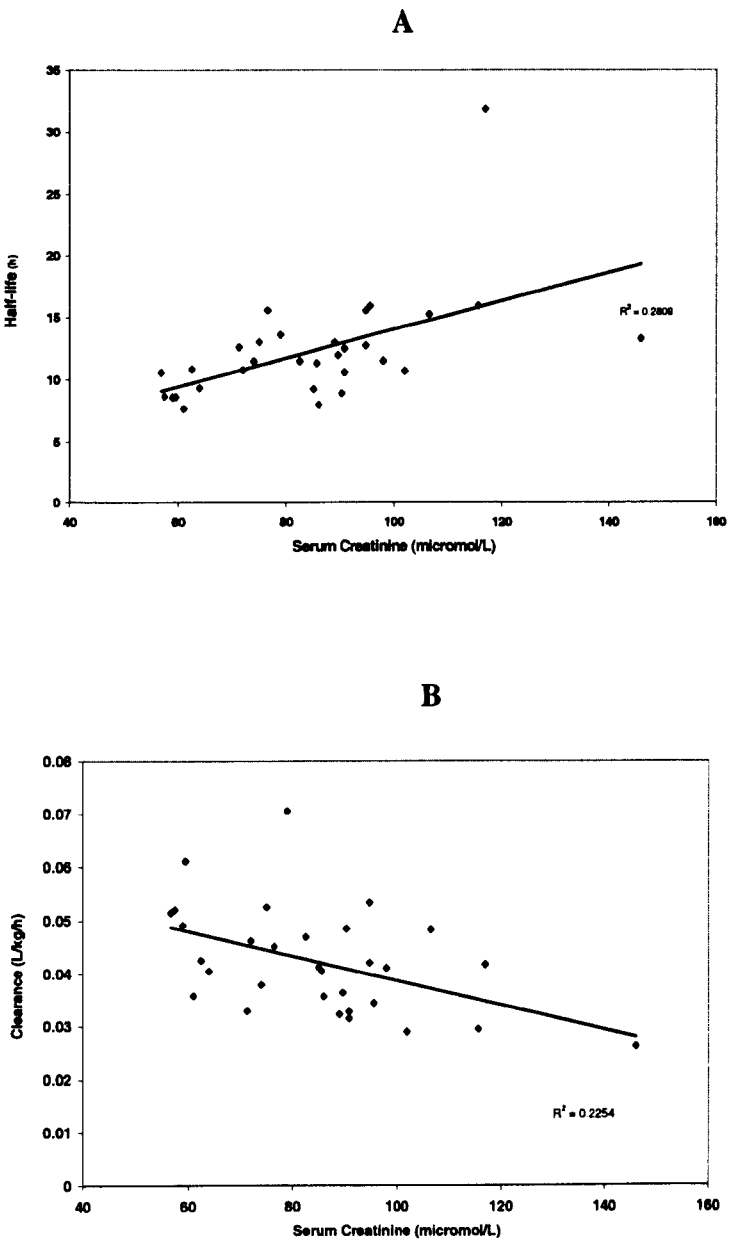


Figure 6.2. Linear Correlation between Serum Creatinine (μmol/L) and (A) Half-Life (hours) and (B) Clearance (L/kg/hr)

In 5 patients (two from group 1, three from group 2), steady state gentamicin levels were also collected after the child came off ECMO. Increase in peak concentration post ECMO were observed with no real differences in trough concentrations (Table 6.3). A comparison of parameters during and post ECMO revealed a significant decrease in V and half-life and a marginal decrease in CL (Table 6.3 and Figure 6.4).

Table 6.3. Change in Pharmacokinetic Parameters after Decannulation from ECMO

	On ECMO (n=5)	**Off ECMO (n=5)**
Peak (mg/L)	5.3 (1.6)	7.6 (2.2)
Trough (mg/L)	2.0 (1.1)	2.0 (1.6)
V (L/kg)	0.9 (0.5)	0.5 (0.07)
CL (L/kg/hr)	0.041 (0.008)	0.034 (0.005)
Half-Life (hours)	16.1 (9.0)	9.7 (2.3)

Data expressed as Mean (SD)

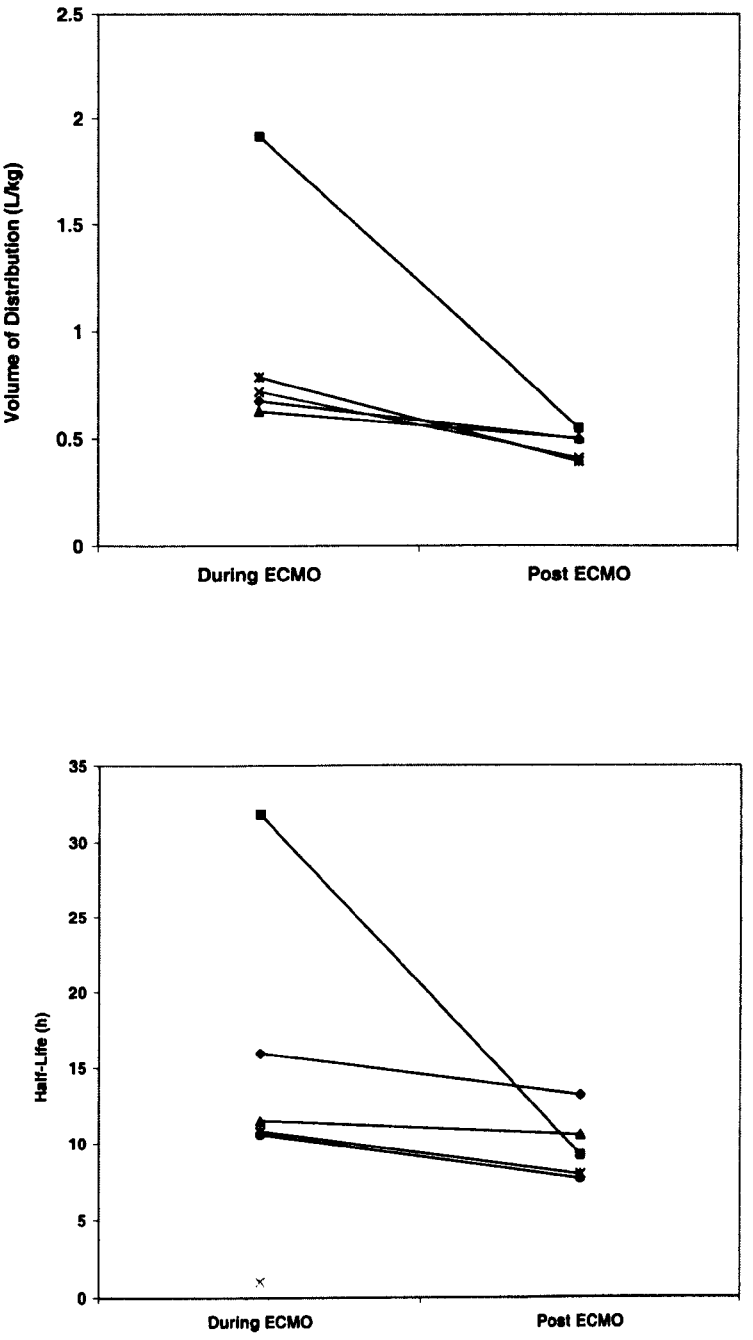


Figure 6.4. Change in Volume of Distribution (L/kg) and Half-Life (hours) after Decannulation from ECMO

6.5 Discussion

Gentamicin and other aminoglycosides exhibit concentration dependent bacterial killing and thus efficacy depends on the peak serum concentration and the ratio of peak serum concentration to minimum inhibitory concentration (MIC) of bacteria (Rotschafer *et al.*, 1992). In adults however, trough concentrations below 2mg/L are also desirable to reduce the incidence of nephrotoxicity and otovestibular toxicity (Munckhof *et al.*, 1996; Prins *et al.*, 1996; Triggs *et al.*, 1999). Similar evidence for gentamicin associated nephrotoxicity in neonates is derived from a study that showed raised *N*-acetyl-beta-glucosaminidase (a marker of proximal renal tubular injury) in neonates exposed to gentamicin compared with controls (Rajchgot *et al.*, 1984). In addition, *in vitro* studies suggest that exposing bacteria to high aminoglycoside concentrations for prolonged periods of time can result in down regulation of drug transport into bacterial cells, whereas a drug free period can increase penetration (Rotschafer *et al.*, 1992). The results of the present investigation reveal that gentamicin dosing regimens commonly recommended for term neonates are inappropriate during ECMO, frequently resulting in potentially toxic trough levels. Furthermore, the pharmacokinetics of gentamicin are significantly altered during ECMO.

Less than 40% of patients in group 1 achieved satisfactory combined peak and trough concentrations with the normal dosing recommendations. Although peak concentrations were adequate in 84.6% of patients, potentially toxic trough concentrations were observed in almost half of all patients. The dosing schedules used in these patients were derived from nationally recognised paediatric formularies. *Medicines for Children (2000)* recommends in term neonates a dose of 3.5mg/kg 8 hourly (PNA < 7 days old) and 3mg/kg 8hourly (PNA > 7days old). The *Paediatric Formulary (5th Edition), Guy's, St Thomas' and Lewisham,* recommends in term neonates a dose of 2.5mg/kg 12hourly (< 7 days old), and 8 hourly (> 7days old). The

high troughs observed in group 1 indicate that the dosing intervals were too short. Since ECMO patients may have severe hypoxic renal insult, reflected in the high serum creatinine values, it is important to avoid potentially nephrotoxic gentamicin trough concentrations. In contrast, more than 60% of patients in group 2 achieved satisfactory combined peak and trough concentrations. It must be noted though that the peak concentrations were lower, with steady state concentrations below 5mg/L in 31 % of patients.

This is of potential concern since peak concentrations have been correlated with outcome. It has been suggested that peak serum concentrations 6 to 10 times the MIC for the organism are required to optimise bactericidal activity (Blaser *et al.*, 1987; Moore *et al.*, 1987). A positive linear correlation between clinical response and the peak serum gentamicin concentrations to MIC (peak:MIC) ratio has been demonstrated in adults (Moore *et al.*, 1987). Compared with a peak:MIC ratio of 2:1, the relative odds of a clinical response were 4.4 times higher when the peak:MIC ratio was 6 to 8:1, and 8.4 times higher at a ratio of 10:1. It is also thought that a peak:MIC ratio of at least 10:1 may prevent the emergence of aminoglycoside-resistant pathogens (Karlowski *et al.*, 1994). Since the median MIC of most gram-negative organisms (apart from *Pseudomonas* and *Serratia*) is 0.8 to 1.6 mg/L, dosing regimens that attain high peak concentrations have been advocated (Rastogi *et al.*, 2002). The target peak range of 5-10mg/L during this investigation was agreed in conjunction with the Department of Clinical Microbiology. It was felt that slightly lower peaks were acceptable because of the synergistic effects when aminoglycosides are combined with beta lactam antibiotics. Moreover, a lower peak was particularly acceptable in neonatal ECMO patients at this centre, where gentamicin combined with benzylpenicillin is used primarily as prophylaxis. Indeed, neonates referred to the ECMO centre with suspected sepsis or pneumonia are usually initiated on second or third line agents (3rd generation

cephlasporins, 4-quinolones, carbapenems, glycopeptides), empirically or as guided by culture and sensitivity results from the referring hospital. Clearly a higher peak (>7mg/L) would be desirable when gentamicin is initiated for the purpose of treatment of an infection. This may be achieved with higher doses at less frequent intervals. From the parameters estimated during ECMO, a new dosing regimen was calculated using the equations stated in the methodology. It is predicted that a dose of 5mg/kg administered every 36 hours is required to achieve a peak (1hour post dose) plasma concentration of 7mg/L and a trough below 2mg/L.

The overall estimates of gentamicin pharmacokinetic parameters suggest that V is enlarged and half-life is prolonged during ECMO. In a recent population pharmacokinetic study in non ECMO neonates, the V in neonates of gestational age > 34 weeks, was estimated to be 0.50 L/kg (Stolk *et al.*, 2002). Similarly, Murphy *et al* (1998) in their sub analysis of a neonatal population estimated V in neonates of GA>38weeks to be 0.41L/kg. Our overall estimate of 0.8 L/kg (no differences between the two groups) suggests V is significantly expanded during ECMO. Clearance was estimated as 0.44L/kg/hr, and significantly higher in group 1 compared to group 2. This is a result of poorer renal function in group 2, reflected in the higher serum creatinine values. It is worthy of note that despite group1 having a higher gentamicin clearance, mean trough levels were higher than group 2. These estimates for CL do not differ significantly from previous reports involving term non-ECMO neonates (Murphy *et al.*, 1998; Stolk *et al.*, 2002). Secondary to the enlarged V, a significantly longer half-life was also estimated compared to previous reports in non-ECMO neonates (12.8 versus 8.8 hours respectively) (Hayani *et al.*, 1997).

However, it must be remembered that the use of linear regression can result in imprecise pharmacokinetic parameter estimates. For example a 1 hour post dose peak assumes complete distribution, which may not be the case in critically ill neonates and

particularly during ECMO. A change in the sampling times, for example 3 and 10 hours, may have improved the precision of estimates. In addition, errors in the recording of dosing and sampling times would be expected to affect V more than CL (Sun *et al.*, 1996). Also, Hoie (1993) reported that bolus doses administered pre-reservoir might stagnate at the top of the reservoir particularly at low pump flow rates (see Chapter 1, Section 1.6.1). Since all gentamicin doses administered during this investigation were by bolus injections into the circuit, pre-reservoir, parameter estimates may have been affected.

The influence of ECMO on pharmacokinetics and the response to drug therapy has been studied throughout this thesis by comparisons with previous reports from non-ECMO patients. Although this strategy has been necessary as a consequence of clinical and logistical practicalities, it is less than ideal. Such a design inevitably suffers from greater variability and loss of efficiency since many more patients are required to allow a firm conclusion to be drawn about the effect of ECMO. The ideal is a longitudinal cross-over design in which plasma concentration data is collected both during and after ECMO for each patient (Rowland *et al.*, 1995). That is to say, each patient acts as his or her own control, an opportunity that presented itself in this investigation. The five patients from whom it was possible to repeat steady state levels post cannulation clearly demonstrated a change in pharmacokinetics. There was a significant contraction in the V and a decrease in the half-life, a phenomenon previously reported (Cohen *et al.*, 1990; Dodge *et al.*, 1994). The difference in V (accounting for mean weight of 3.4kg) pre and post ECMO was 1.4 L and larger than previously reported by Cohen *et al* (1990) or Dodge *et al* (1994). This difference in V is not simply accounted for by the neonatal circuit volume of 500cm^3, but highlights the critically ill nature of these patients. Neonates referred for ECMO have often been aggressively fluid resuscitated with intrinsic increases in intracellular and extracellular water (Anderson *et al.*, 1992). It has

also been suggested that sequestration of gentamicin by components of the ECMO circuit may also contribute to the enlarged V (Dagan *et al.*, 1993). However, this is not substantiated by similar studies in non-ECMO polymeric devices. Kowaluk *et al* (1981) showed that gentamicin sulphate 40mg/L in sodium chloride 0.9% in pPVC bags did not exhibit significant sorption to the plastic during one week of storage at room temperature (15 to 20 °C). In another study, no loss due to sorption was observed during a seven-hour simulated infusion through an infusion set consisting of a cellulose propionate burette chamber and 170 cm of pPVC tubing (Kowaluk *et al.*, 1982). Although an actual log P value of gentamicin was not determined or located in the literature, it has previously reported as being very low (Jenke, 1994). Thus, on this basis one would not expect significant sorption to the circuit components. The change in parameters post ECMO suggests that continuation of gentamicin treatment after decannulation, requires dosing regimens to be altered in line with normal recommendations from non-ECMO neonates to maintain therapeutic plasma concentrations.

CHAPTER VII

POPULATION PHARMACOKINETICS OF VANCOMYCIN DURING ECMO

7.1 Introduction

As with other critically ill patients, ECMO patients are at an increased risk of serious infections because of invasive investigations and monitoring, central catheter related bacteraemias, multi-organ failure and immunosuppression. In addition, ECMO patients may have increased risks related to the circuit with prolonged exposure of blood to large polymeric surface areas and multiple ports of entry for organisms. At this institution, vancomycin is administered prophylactically as a single dose immediately post cannulation for ECMO, and also to treat serious infections caused by staphylococci, particularly coagulase-negative staphylococci. However the narrow therapeutic index of vancomycin, in particular the risk of nephrotoxicity, has lead to an interest in the pharmacokinetics of this antibiotic.

Previous studies of vancomycin pharmacokinetics during ECMO seem to provide conflicting results (Amaker *et al.*, 1996; Buck *et al.*, 1998; Hoie *et al.*, 1990). Although an expanded volume of distribution (V) with consequent reduced elimination is hypothesised, many of the studies did not seem to corroborate this. These studies involved neonates or infants and were limited by small numbers. Furthermore, pharmacokinetic parameters were determined using non-population approaches and thus population variability and the influence of significant covariates were not identified satisfactorily. Little or no emphasis was placed on dosage regimen design.

7.2 Aims of the Study

The purpose of this study was to determine the population pharmacokinetics of vancomycin in neonates, older children and adults treated on ECMO, and to characterise the population variability. In addition, by identifying significant covariates, new dosing guidelines could be recommended.

7.3 Materials and Method

7.3.1 Patients

This study was approved by the Leicestershire Research Ethics Committee and utilised both prospective rich and retrospective scant data. For those patients enrolled in the prospective study, assent was obtained from parents or relatives. All patients in whom vancomycin was administered during ECMO support were eligible for study. Vancomycin was administered as a one hour infusion and initial doses were determined according to age and weight. In the prospective study, plasma samples were taken from those patients administered vancomycin prophylactically as a single dose post cannulation or after the first and fourth doses when it was used to treat suspected or confirmed infection. Samples for analysis were taken at baseline prior to initiation of infusion and then 0, 30, 60, 120, 180, 360 minutes post infusion. Sparse and routine plasma concentration data collected during vancomycin treatment was obtained retrospectively from an assay database held in the Department of Microbiology, cross-referencing with the patients' medical records. All concentrations were steady state, usually obtained as peak (1hour post infusion) and trough levels (end of dose interval).

The following continuous and categorical clinical data were documented for all patients where possible: age (post natal, post- conceptional and gestational for neonates), gender, weight, diagnosis, duration of ECMO, cannulation mode, outcome, urea, creatinine, urine output and requirements for CVVH.

7.3.2 Analytical Assay

Vancomycin assays were performed by the Department of Microbiology using the Innofluor® fluorescence polarization immunoassay. The limit of determination is 1mg/L and the intraassay coefficient of variation is less than 6.0%.

7.3.3 Pharmacokinetic Analysis

Rich prospective and sparse retrospective data were collated, evaluated and entered into an Excel spreadsheet, and then analysed using the mixed effects non-linear regression modelling programme, WinNonMix Professional (Version 2.0.1) bundled with Compaq Visual Fortran Compiler Professional Edition (Version 6.5) (See Chapter 2, Section 2.4.3). Initial parameter estimates were derived from non linear regression analysis of the rich data set using WinNonLin (Version 3.0) as described in Chapter 2, Section 2.5.2).

7.3.4 Validation Data Set

To validate the model and the population pharmacokinetic parameter estimates, plasma concentrations of vancomycin observed during routine monitoring in a separate group of 20 patients were compared with the concentrations predicted by the model. A measure of the predictive performance of models can be determined by calculating the MDPE and AMDPE as described in Chapter 2, Section 2.5.4.

7.3.5 Statistical Analysis

All clinical and demographic characteristics were reported as mean (SD) and (range). Population pharmacokinetic estimates are expressed as mean± SE. For comparison of parameter estimates between the three age groups, one way analysis of variance was

used. Univariate and multivariate regression analysis was used to reveal trends in the population studies. A p value < 0.05 was taken as significant.

7.4 Results

7.4.1 Clinical and Demographic Characteristics

The clinical and demographic characteristics of the study group are summarised in Table 7.1. A total of 366 plasma vancomycin concentrations from 45 patients were included in the analysis. Of these, 26 patients were investigated prospectively, providing 227 (62%) plasma concentrations. In 9 of these patients plasma sampling followed a single dose that was administered following cannulation and once extracorporeal blood flow had been established, whilst in 17 patients sampling followed the first and fourth doses during the treatment of suspected or confirmed infections. Two patients were excluded from the original data set because of misplaced medical records.

The overall distribution of sampling times is shown in Table 7.2. Prophylactic doses of vancomycin were 15 mg/kg in neonates and children, and 1000mg in adults. For treatment, initial doses ranged between 10-15 mg/kg every 6 to 24 hours in children, and 750-1000 mg every 12 to 24 hours in adults. The overall mean (SD) peak and trough concentrations at steady state were 30.0 (10.1) and 11.9 (6.8) mg/L respectively. (The reference range for vancomycin serum levels at this institution is trough 5-15 mg/l, peak 20-30 mg/l). All neonates in the study were term or near term with median (range) gestational age 40.4 (34.3-42) weeks. Where determined (n=38), the majority of patients (84%) underwent VV ECMO, the most popular mode at this institution. The mean duration of ECMO was longer in adults (308.5hours) compared to neonates and older children (220.2 and 257.9 hours respectively), reflecting the typically longer and more

complicated course of illness amongst adult ECMO patients. Mean (range) serum creatinine levels were significantly higher amongst adults (125.1 (48.3-224.5)), compared to neonates and older children (79.6 (39-180) and 73.5 (26.5-158.9) μmol/L respectively). However, a greater number of children (42%) were supported with CVVH than neonates or adults (13% and 33% respectively).

Table 7.1. Clinical Characteristics of Study Group

	Overall	Neonates (0 – 1m)	Children (1m-18yrs)	Adults (> 18 years)
Number of Patients	45	15	12	18
Male	29	8	9	12
Females	16	7	3	6
Weight (kg)	36.0 (38.2) (2.5 – 116)	3.5 (0.5) (2.5 – 4.5)	14.3 (16.1) (2.4 – 57)	78.8 (20.3) (49.5 – 116)
Age (days)	4800 (5870) (0 – 22452)	8.2 (10.7) (0 – 28)	1358 (1712) (62 –5493)	11472 (4344) (6109 – 22452)
Sparse Data Group	19	2	5	12
Rich Data Group	26	13	7	6
Number of samples per patient				
Sparse	8.2 (4.1) (2 – 20)	8 (2.9) (4-12)	8 (4.2) (3-18)	6.9 (4.9) (2-20)
Rich	7.3 (2.8) (4-12)	5.8 (2.8) (4-12)	8.1 (2.5) (4-12)	9.5 (3.1) (5-12)
Serum Creatinine (μmol/L)	97.3 (54.2) (26.5 – 224.5)	79.6 (30.8) (39 – 180)	73.5 (38.9) (26.5-158.9)	125.1 (61.3) (48.3 –224.5)
CVVH	13	2	5	6
Cannulation				
VV	33	12	4	17
VA	6	2	3	1
Undetermined	6	1	5	0
Duration of ECMO (hrs)	264.4 (197.3) (50-907)	220.2 (148.9) (50-494)	257.9 (157.8) (73-550)	308.5 (243.1) (86-907)
Outcome				
Survived	27	11	5	11
Deceased	18	4	7	7

Data are expressed as Mean (SD) and (Range)

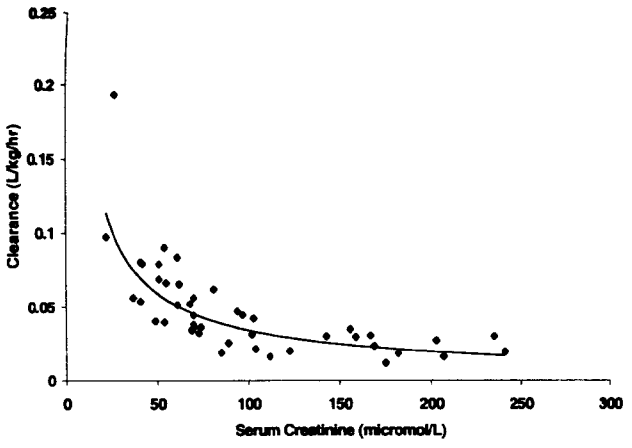
Table 7.2. Number of Vancomcyin Plasma Samples

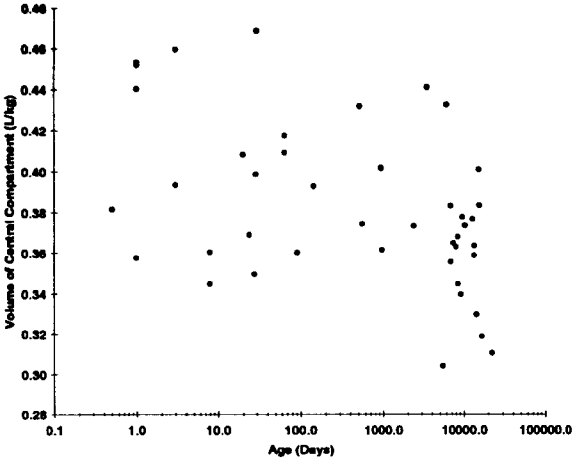
Sample Time (hours post infusion)	
Rich Data	**Number**
Baseline (pre-infusion)	20
0	5
0.5	35
1	36
2	36
4	35
6	36
Sparse Data	
1-2	16
2-4	32
4-6	4
6-8	4
8-10	8
10-12	24
12-18	38
18-24	6
24+	8

7.4.2 Development of a Two Compartment Model

The two compartment model was chosen in preference to the one compartment model since it demonstrated a more appropriate structural model on examination of the graphical residual plots and a lower OFV. Although a three compartmental model resulted in a further lowering of OFV, it was not explored because the parameter estimates were imprecise, probably reflecting insufficient sampling to allow three compartments to be discerned. Residual error was best described by a combined additive and proportional structure. Significant improvement in model fit was achieved when weight was associated linearly (without an intercept) with each parameter, and as stated in the methodology (Chapter 2, Section 2.5.3), before investigation of other covariates. Allometric transformation of weight$^{0.75}$ was also tested, but did not further improve the fit. Plots of Bayesian posterior estimates of parameters from the basic model versus the covariates revealed significant correlations (Figure 7.1): CL with

creatinine and age, central volume (V1) and intercompartmental clearance (Q) with age, Q with ECMO pump flow rates. The significance of these relationships was investigated by hypothesis testing of full and reduced model during the covariate screening stage (Table 7.3). The largest decrease in OFV (97U) was observed when creatinine was incorporated into a non-linear model of CL ($CL= \theta_1 * Cr^{\theta 2}$, where θ is to be estimated). Since the estimate of θ_2 was -0.9, the model was simplified to θ_1/Cr, without a significant change in OFV. Clearance was linearly related to age in neonates and children up to 1000 days, but beyond this, was independent of age. Surprisingly, there was no significant difference in CL between those patients on and off CVVH (p=0.183). Although V1 decreased with age, there appeared to be a bimodal distribution with a break point at 4000 days. Thus the influence of age on V1 when included into the model as a dichotomous variable resulted in a significant drop in OFV. Although the incorporation of an inverse relationship between age and Q in the model reduced OFV significantly, it was not included in the final model since it had little influence on the scatterplots or interpatient variability. By the same rationale, the positive influence of ECMO pump flow rates on Q was also omitted from the final model. The final structural model and parameter estimates are displayed in Table 7.4. The weighted residuals associated with the final model were randomly distributed (Figure 7.2).





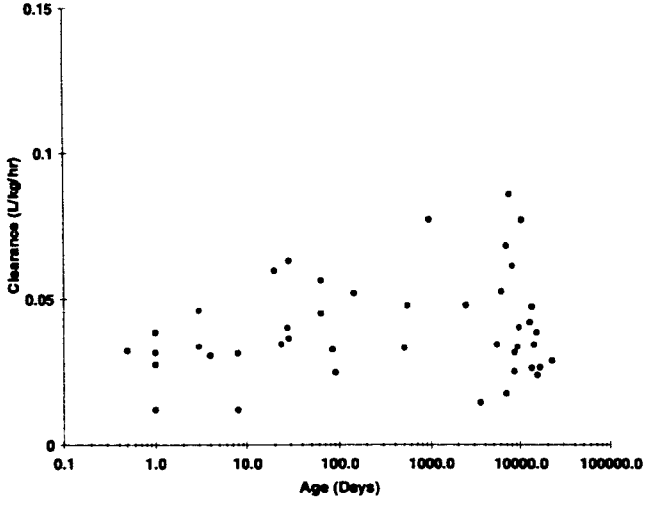


Figure 7.1. Plots of Bayesian posterior parameter estimates versus covariates.

Table 7.3. Summary of Univariate and Multivariate Analysis (2 compartment Model)

Hypothesis	Equation	Objective Function[a]	Conclusion
Basic Model	*CL×Weight* *Q×Weight* *V1×Weight* *VT×Weight*	*1981*	
Influence of creatinine on CL?	$(CL \times Cr^{\theta 1}) \times Weight$	1884	Creatinine significantly influences CL
Influence of 1/creatinine on CL?	$(CL/Cr) \times Weight$	1886	Influence of Cr on CL may be simplified to 1/Cr
Influence of age on CL[b]?	$(CL + \theta_2 * Age)/Cr \times Weight$	1965	Age significantly influences CL
Influence of age on Q?	$(Q + \theta_3 \times age) \times Weight$	1972	Age significantly influences Q
Influence of age on V1 (linear model)?	$(V1 + \theta_4 \times Age) \times Weight$	1968	Age added linearly significantly influences V1
Influence of dichotomous age on V1[c]?	$(V1 - \theta_5) \times Weight$	1966	Influence of age may be simplified to a dichotomous variable
Influence of pump flow rates on Q?	$(Q + \theta_6 \times Flow\ Rate) \times Weight$	1960	ECMO pump flow rates significantly influence Q
Final Model	$(CL + \theta_2 * Age)/Cr \times Weight$ $Q \times Weight$ $(V1 - \theta_5) \times Weight$ $VT \times Weight$	*1853*	

CL=Clearance, Q=Intercompartmental Clearance, V1=Central Volume, VT=Volume of Tissue Compartment, Cr=Serum Creatinine

a. A change in the OFV > 7.88 ($p<0.005$) were accepted as statistically significant.
b. θ_2 estimated when age was less than 1000 days.
c. θ_5 estimated when age was greater than 4000 days.

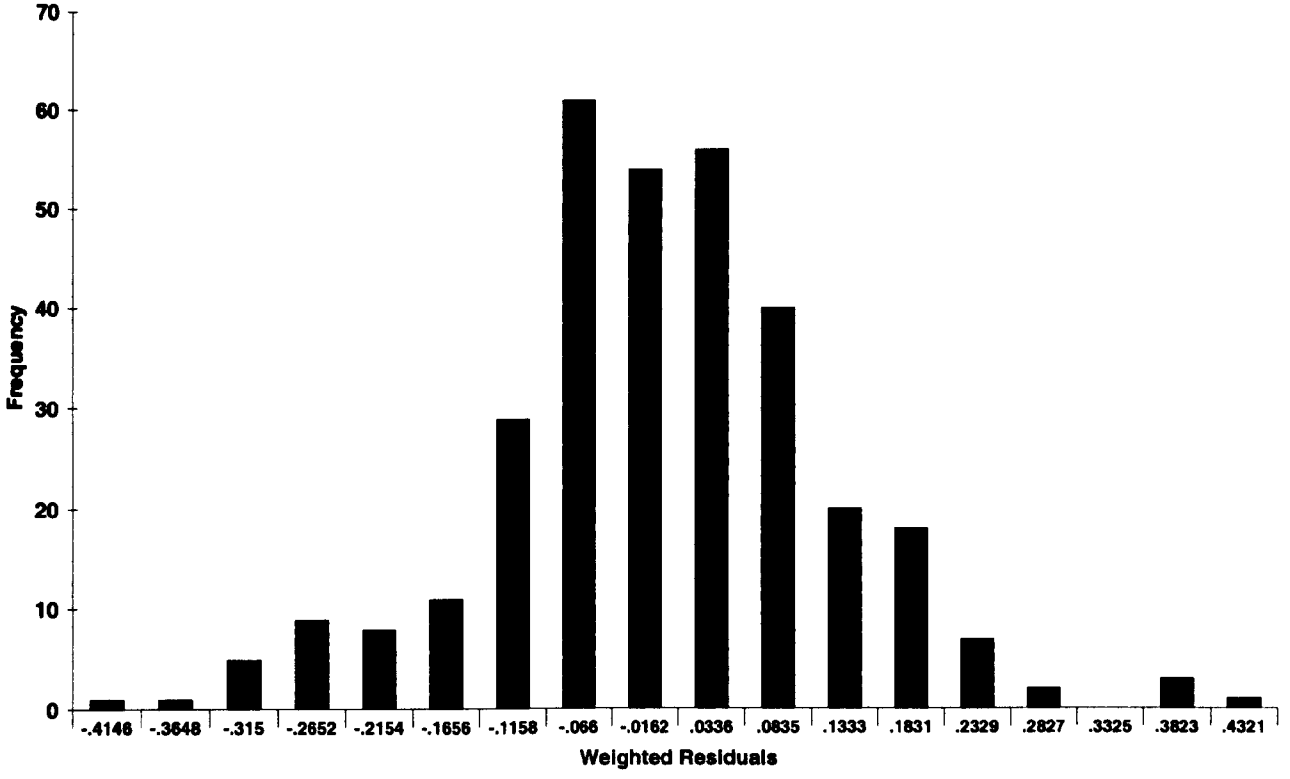
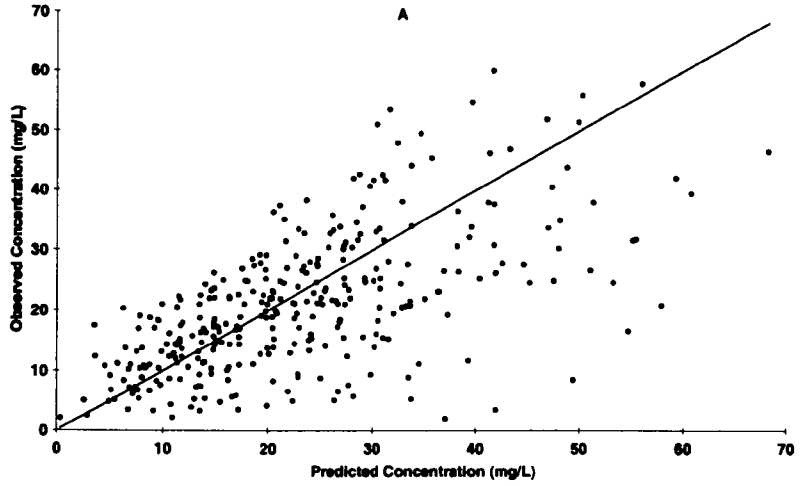


Figure 7.2. Frequency distribution of Weighted Residuals from the final model

The relationship between observed and model predictions and selected individual patient profiles obtained using the basic and final models are shown in Figures 7.3 and 7.4, respectively. The parameters estimated from the final model described the data well with a bias of –1.9% and precision of 20.3% (Table 7.7). The mean Bayesian posterior estimates of CL, V1, volume of distribution at steady state (V_{ss}) and half-life determined by the final model for each age category are displayed in Table 7.5. There were no significant differences apart from V1 (p=0.006).



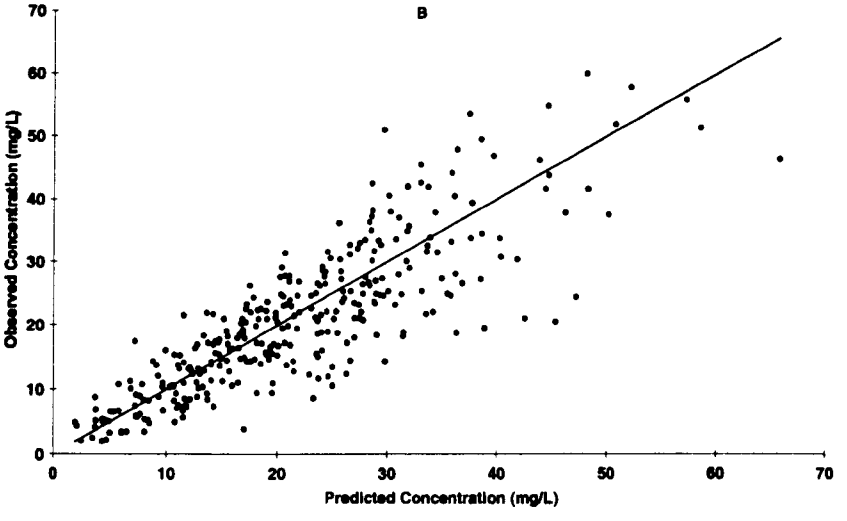
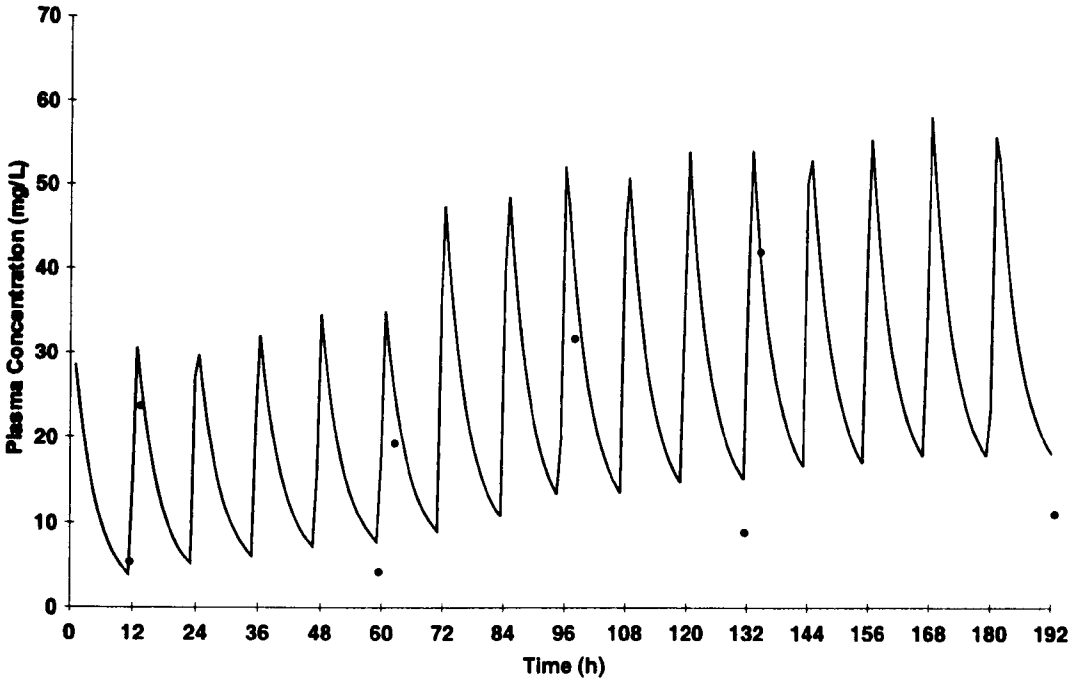


Figure 7.3. Scatterplot of observed versus population predicted plasma vancomycin concentrations (mg/L) indicated a closer distribution around the line of unity for the final model (B) versus the basic model (A).



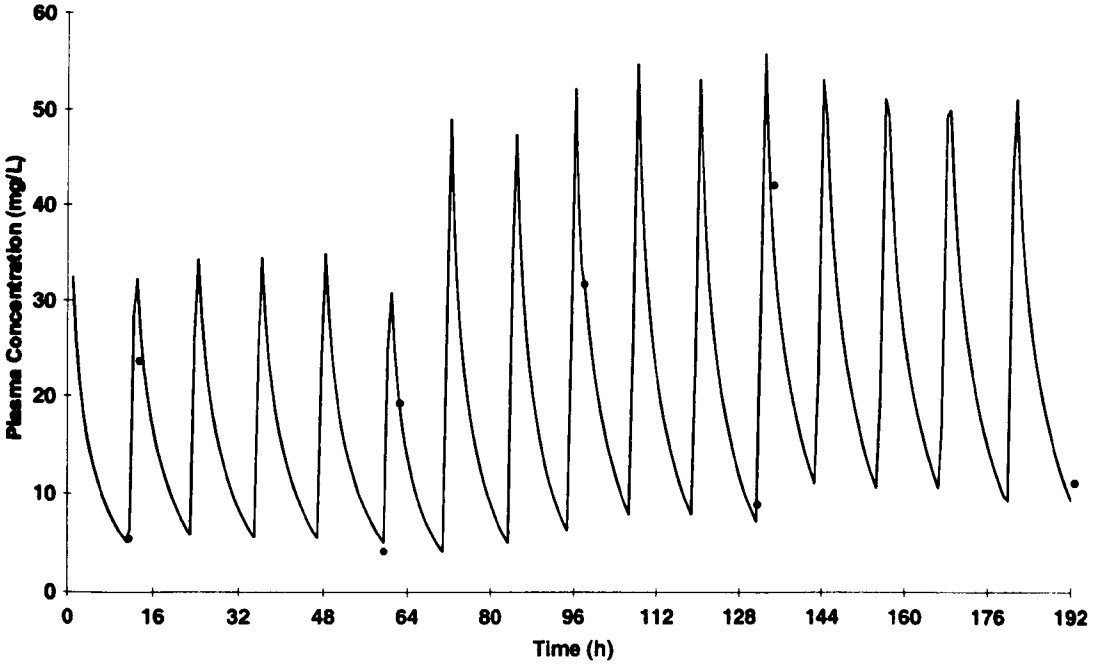


Figure 7.4. Model fits to the same patient, • observed concentrations, —population predictions: (A) Basic model (B) Final Model. Daily dose was increased from 1000mg twice daily to 1500mg twice daily at 71 hours.

Table 7.4. Population Pharmacokinetic Parameter details for the final model of Vancomcyin in ECMO patients

	Estimate	Standard Error
CL (L/kg/hr)	[2.4 + 0.0018*Age (days)]/Cr (μmol/L), if Age < 1000 days 4.3/Cr (μmol/L), if Age > 1000 days	0.18 0.24
Q (L/kg/hr)	0.09	0.023
V1 (L/kg)	0.45, if Age < 4000 days 0.37, if Age > 4000 days	0.038 0.042
VT (L/kg)	0.25	0.037
Population Variability[a]		
CL	25 %	
V1	25 %	
Q	91%	
VT	48 %	
Residual Error	12.1% ± 2.1 mg/L	

a. Expressed as coefficient of variation

Table 7.5. Summary of Bayesian posterior parameter estimates in the age related categories

	Overall	Neonates	Children	Adults	
V1 (L/kg)	0.42 (0.095)	0.45	0.46	0.37	P=0.006
Vss (L/kg)	0.71 (0.20)	0.67	0.71	0.73	*P=0.66*
CL (L/kg/hr)	0.047 (0.031)	0.041	0.057	0.044	*P=0.37*
Half-Life (hours)	8.44 (5.17)	10.40	6.18	8.55	*P=0.1*

7.4.3 Prospective Validation of Model

The 20 patients in the validation group (8 neonates, 4 children, 8 adults) provided a total of 108 plasma concentrations for evaluation (Table 7.6). These observed concentrations were compared with predictions made by the final model. A scatter plot of residuals (observed minus predicted concentrations) against the observed concentration revealed homogeneous scatter around the zero ordinate (Figure 7.5). The bias for the validation set was –7.7%, indicating that the model has a slight tendency to over predict. The predictive precision of the model was found to be 26.7%, indicating that on average the size of the error was less than 26.7% of the predicted concentration (Table 7.7).

Table 7.6. Clinical and Demographic Characteristics of Validation Group

	Overall	Neonates	Paediatric	Adult
Number of Patients	20	8	4	8
Male/Female	14/6	6/2	1/3	7/1
Weight (kg)	42.4 (44.5)	3.6 (0.6)	21.9 (23.0)	91.4 (20.7)
Age at cannulation (days)	6157 (7436)	5.5 (8.8)	2196 (2597)	14290 (4403)
Cannulation (VV/VA)	18/2	7/1	3/1	8/0
CVVH	13	4	3	6
Serum Creatinine (μmol/L)	111.5 (74.1)	65.6 (34.4.)	56.9 (17.9)	157.3 (79.6)
Number of samples per patient	5.4 (3.1)	4.9 (4.2)	4.3 (1.5)	6.5 (2.3)

Data are expressed as Mean (SD)

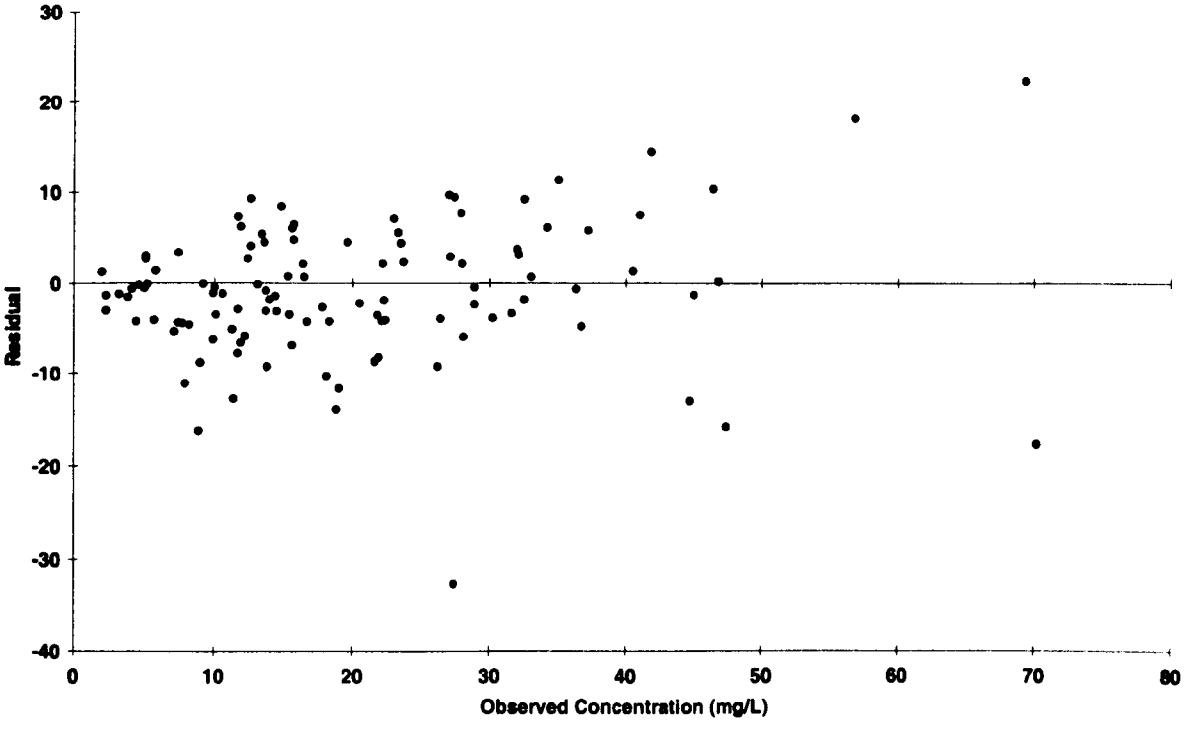


Figure 7.5. Assessment of predictive performance of final population model in a separate set of 20 patients (108 observations): Plot of residual (observed minus predicted) versus observed plasma vancomycin concentration.

Table 7.7. Model Validation and Predictive Performance

	Base Model	Full Model	Validation
Bias[a] (%)	-0.8 (-8.5, 5.4)	-1.9 (-6.4, 3.0)	-7.7 (-36.9, 58.3)
Precision[b] (%)	29.5 (25.8, 32.4)	20.3 (18.2, 22.5)	26.7 (17.5, 36.9)

a. Bias = median ([observed$_{ij}$-predicted$_{ij}$/predicted$_{ij}$]*100), i (ith individual), j (jth concentration)
b. Precision = absolute median ([observed$_{ij}$-predicted$_{ij}$/predicted$_{ij}$]*100), i (ith individual), j (jth concentration)
Figures in parentheses are 95% Confidence Intervals for the median.

The population parameter estimates from the final model and two compartment model equations, entered into Excel spreadsheets, were used to determine dosing regimens required to achieve approximate peak and trough concentrations of 30 and 10 mg/L respectively, for a range of age and creatinine values (Table 7. 8). For illustration purposes, these developed dosing regimens and final model parameters were used to simulate plasma profiles as displayed in Figures 7.6 and 7.7.

Table 7.8. Dosing Guidelines

Dose (mg/kg) / Interval (hours)	*Age (days)*				
Creatinine (μmol/L)	***1***	***365***	***1000***	***1000-4000***	***>4000***
25	15 / 8	20 / 8	20 / 6	20 / 6	20 / 6
50	10 / 12	10 / 8	10 / 8	10 / 6	10 / 6
100	10 / 24	15 / 24	10 / 12	15 / 18	10 / 12
150	15 / 48	15 / 36	12.5 / 24	15 / 24	12.5 / 24
200	Re dose after levels, approx every 72hrs	15 / 48	15 / 36	15 / 36	15 / 36

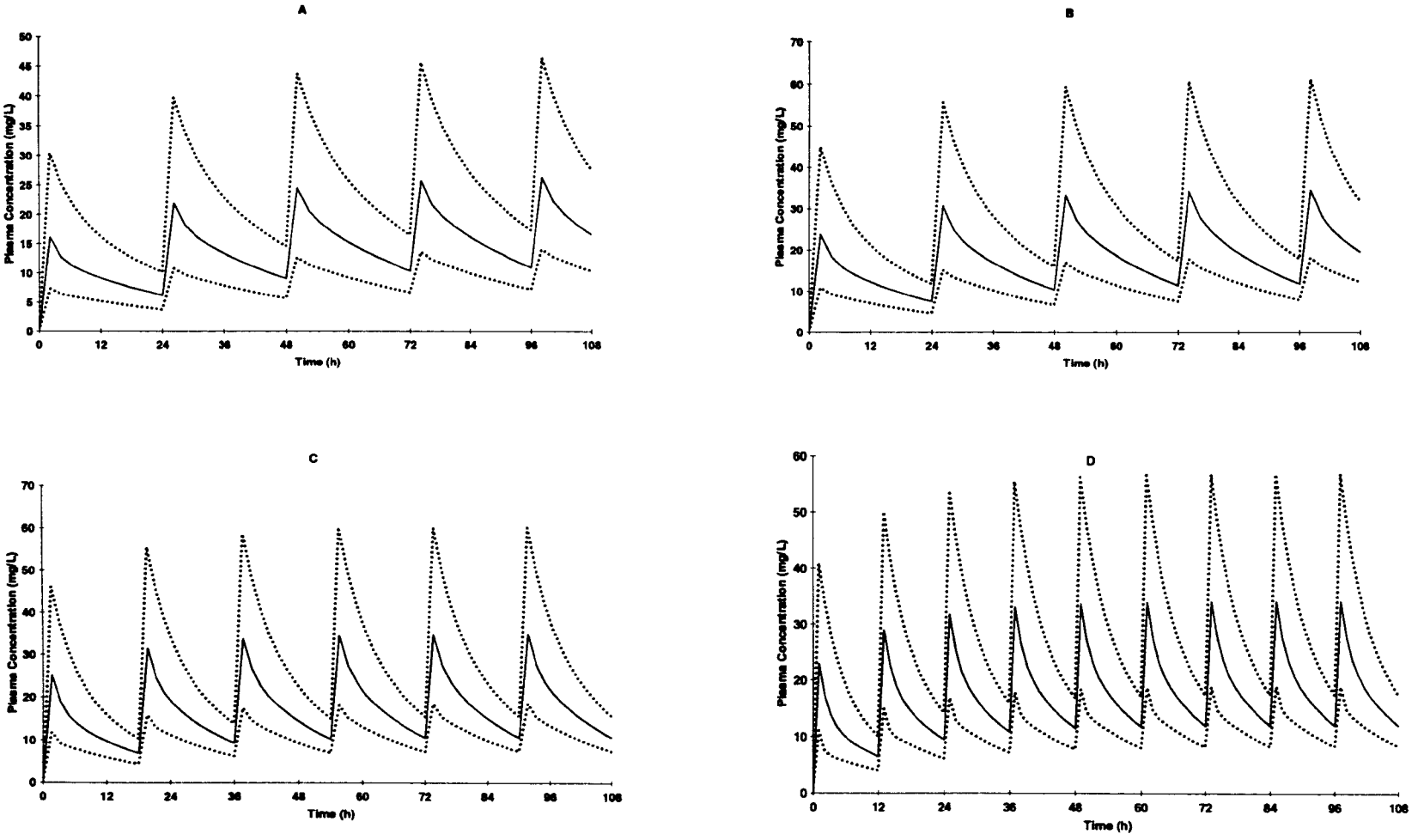


Figure 7.6. Simulated mean plasma profiles and 95% confidence intervals of interpatient variability in an age range ECMO population with serum creatinine of 100 μmol /L. (a) Neonate (1 day), 10mg/kg every 24 hours (b) Child (1 year), 15mg/kg every 24 hours (c) Child (10 years), 15mg/kg every 18 hours (d) Adult, 10mg/kg every 12 hours

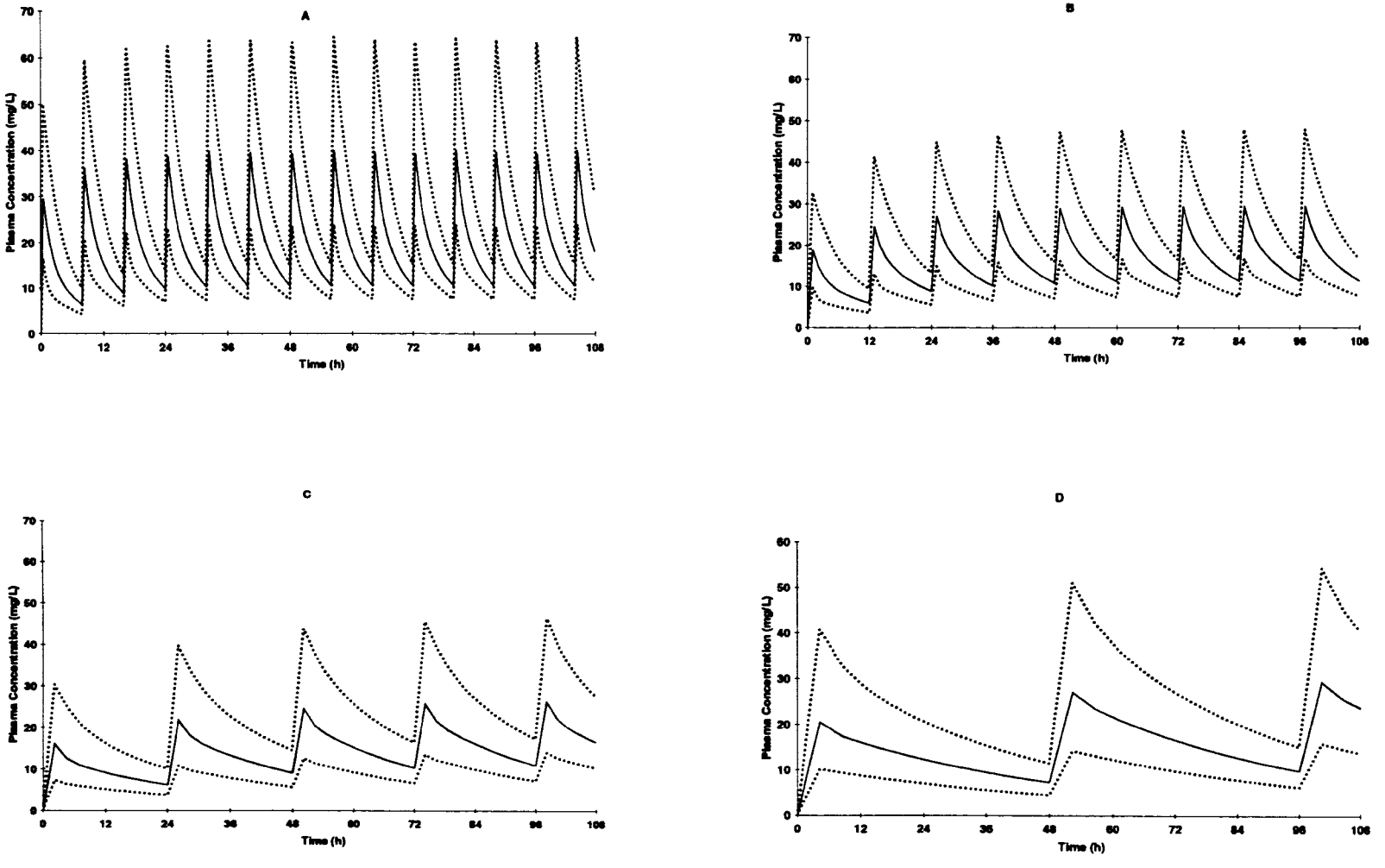


Figure 7.7. Simulated mean plasma profiles and 95% confidence intervals of interpatient variability for a neonate during ECMO and a range of serum creatinine values. (a) 25μmol/L, 15mg/kg every 8 hours (b) 50 μmol /L, 10mg/kg every 12 hours (c) 100 μmol /L, 10mg/kg every 24 hours (d) 150 μmol /L, 15mg/kg every 48 hours

7.5 Discussion

Recent years have seen an increased usage of intravenous vancomycin in intensive care patients. Nephrotoxicity and ototoxicity associated with vancomycin was originally related to impurities in the preparation as well as high serum concentrations but with purification and improvement in formulation, the incidence of toxicity is not thought to be as prevalent (Odio *et al.*, 1984). Furthermore, there are no definitive studies correlating toxicity with serum vancomycin concentrations, raising questions as to whether or not there is a need to monitor serum levels (Bhatt-Mehta *et al.*, 1999). However, since vancomycin exhibits concentration independent, time dependent bacterial killing, maintaining minimum plasma levels is crucial and monitoring of trough levels is still considered necessary, particularly in critically ill patients exhibiting wide interpatient variability (Duffull *et al.*, 1994; Lowdin *et al.*, 1998; Zimmermann *et al.*, 1995). Although pharmacokinetic data has become more readily available in this population, there have been limited and contradictory reports from ECMO patients.

There have been four previous reports of vancomycin pharmacokinetics during ECMO (Amaker *et al.*, 1996; Buck *et al.*, 1998; Capparelli *et al.*, 2001; Hoie *et al.*, 1990). Three of these studies were specifically designed to investigate ECMO patients and determined parameters utilising traditional pharmacokinetic approaches. Hoie *et al* (1990) investigated six term neonates, fitting either a one (n=3) or a two compartment model (n=3). They report a combined mean V1 and Vβ of 0.68 L/kg and CL of 0.066L/kg/hr, concluding values were similar to previous reports from non-ECMO infants. However, the dosage regimen of 20mg/kg 18 hourly they propose in patients with normal renal function suggests reduced CL and enlarged V. In contrast, Amaker *et al* (1996) investigated 12 infants undergoing ECMO (mean gestational age 39 weeks, mean creatinine 99 μmol/L), revealing a larger Vss (mean±SD; 1.06± 0.45 L/kg), a lower CL (0.047 ± 0.011 L/h/kg) and a prolonged $t_{1/2\beta}$ (16.9 ± 9.5 hours). Buck *et al*

(1998) compared ECMO patients (n=15) with a control group (n=15) utilising steady state peak and trough measurements, and found no significant differences in V and CL but a longer half-life in ECMO patients (8.29±2.23 versus 6.53±2.05 hours). A recent population study of 374 infants (including 15 ECMO patients) identified ECMO patients as displaying an enlarged V and reduced CL, although not significantly so, once serum creatinine and gestational age had been included in the final model (Capparelli *et al.*, 2001).

Vancomycin is excreted by glomerular filtration with 80 to 90% of the dose recovered unchanged in the urine 24 hours after administration in healthy adults (Rodvold *et al.*, 1997). The present study population involved wide, age range related, serum creatinine values. In total, 10 neonates (66%) and 5 children (42%) had creatinine values above 60 micromol/l, whilst 11 adults (61%) exhibited values greater than 120 micromol/L These creatinine values reflect the critically ill nature of ECMO patients with likely hypoxic ischaemic renal injury prior to the initiation of ECMO. A high proportion of patients (29%) also required CVVH support, often initiated to improve fluid balance. Similar to previous population studies in non-ECMO patients, vancomycin CL was strongly associated with renal function, the model improving substantially when serum creatinine values were associated with CL (Capparelli *et al.*, 2001; Grimsley *et al.*, 1999; Yasuhara *et al.*, 1998). Furthermore, estimates of creatinine corrected vancomycin CL during ECMO were substantially reduced compared to those reported in non-ECMO patients (Healy *et al.*, 1987; Lamarre *et al.*, 2000; Naqvi *et al.*, 1986; Polard *et al.*, 1999; Rotschafer *et al.*, 1982; Rybak *et al.*, 1990; Schaad *et al.*, 1980; Seay *et al.*, 1994).

Caparelli *et al* (2001) determined the CL in a typical study infant (creatinine= 53 μmol/L, weight = 1.8 kg, GA =33.5 weeks, age=27days) to be 0.066L/kg/hr. The mean Bayesian posterior estimate of CL for term neonates in this study was 0.041L/kg/hr

(creatinine =79.6 μmol/L, weight=3.5kg, GA= 39.8 weeks, age=8.2 days). Although serum creatinine values are higher in the present study, one would nevertheless expect a higher CL in term or near term neonates. Two previous studies involving infants and children, reported CL of 0.071L/kg/hr and 0.08L/kg/hr in their sub-analysis of term neonates (Naqvi *et al.*, 1986; Schaad *et al.*, 1980). Creatinine clearance in neonates has been shown to increase exponentially by a factor of 8 between 28 and 40 weeks conceptional age (Rowland *et al.*, 1995). Furthermore, 4 of the neonates in the present investigation were of postnatal age below 3 days, and so mean creatinine may have been skewed by residual maternal derived creatinine (Drukker *et al.*, 2002).

The observed increase in CL with age (up to 1000 days) during this study is in line with age related maturation of kidneys. Although renal clearance is slow in the neonate, it has been shown to increase rapidly with age so that clearance in children is considerably higher (Rowland *et al.*, 1995). Previous studies have reported total body clearance of vancomycin to be 2 to 3 times higher in the paediatric population (0.283L/kg/hr) compared with adults (0.084L/kg/hr) (Rodvold *et al.*, 1988; Schaad *et al.*, 1980). The mean Bayesian posterior estimate of CL for children and adults in this study were 0.057 and 0.044 L/kg/hr respectively. In the study by Rodvold *et al* (1988) non ECMO adults with mean serum creatinine of 97, 123, 185 μmol/L displayed a mean vancomycin CL of approximately 0.09, 0.047, 0.022 L/kg/hr respectively. In comparison and from a sub analysis of the present investigation, 9 adults with mean serum creatinine of 72 (21.3) μmol/L revealed a CL of 0.062 (0.02) L/kg/hr. Interestingly, there was no significant difference in CL or other parameters in those patients requiring CVVH, suggesting that creatinine is a more appropriate indicator of vancomycin CL even during CVVH.

Decreased creatinine corrected vancomycin CL in ECMO compared to non-ECMO patients of similar age may be due a number of factors. An obvious difference

during ECMO is the expansion in circulating blood volume. This will necessarily enlarge the V for a water soluble molecule like vancomycin, and may also reduce CL. Increase in total blood volume is related to circuit size and ranges between 25% (adults) and 125% (neonates) (see Chapter 5, Section 5.3.1). This was reflected in the population estimates of V1, determined to be higher in neonates and younger children (age < 4000 days) compared to older children and adults (0.45 versus 0.36L/kg respectively). The estimate of Vss (0.71L/kg) is less than previously reported in ECMO infants by Amaker *et al* (1.06L/kg), but higher than that reported in non-ECMO studies (0.38-0.64L/kg) (Naqvi *et al.*, 1986; Rodvold *et al.*, 1988; Schaad *et al.*, 1980). Changes in V may not be simply due to the extra circulating volume, but also due to increased extracellular fluid and increased renin and atrial natriuretic peptide release secondary to non-pulsatile renal blood flow during VA ECMO (Bartlett, 1990).

An additional but thus far little investigated phenomenon during VV ECMO is recirculation of blood, where a fraction of oxygenated blood from the circuit flows directly from the re-infusion site to the drainage catheter and back into the circuit instead of the patient's circulation (See Chapter 1, Section 1.6.4.2). At high flow rates (circa 120cm^3/kg/min) as much as 60% of blood returning from the circuit may recirculate. In the compartmental model, the ECMO circuit may constitute a peripheral compartment, distinct from tissues. During the early period of ECMO with high flow rates, recirculation of blood will significantly affect distribution of drug into systemic circulation and hence elimination of drug. However, as ECMO support is weaned with lower flow rates, recirculation is significantly reduced (<10%) and no significant influence is expected. Although the model developed in this investigation did not incorporate this phenomenon, intensive sampling during and immediately post infusion would enable the 'ECMO compartment' to be characterised. This phenomenon also

suggests that pharmacokinetic parameters will necessarily change with time, reflected in the residual error.

The validation group had similar characteristics to the study group. Although the proportion of paediatric patients in the data set was less, they were spread over the age range: one aged 44 days, weight 3 kg; one aged 2.3 years, weight 11kg; one aged 5 years, weight 18.5 kg; one aged 16 years, weight 55kg. However, there was a greater degree of renal dysfunction in the validation set with 65% of the patients having received CVVH support compared to 29% of the study group. The validation set also had higher serum creatinine levels (111.5 versus 97.3 μmol/L). This may explain the slight tendency of the model to over predict.

Dosing guidelines constructed using parameter estimates from the final model highlight the influence of two significant covariates, age and serum creatinine. Using these dosing regimens, plasma concentration profiles and associated 95% confidence intervals of the population variability were simulated. Since minimum plasma vancomycin concentration is all important for antimicrobial efficacy, the aim was to maintain trough levels between 10-15mg/L, and to avoid the lower and upper confidence intervals falling outside 5 and 20mg/L respectively (de Hoog *et al.*, 2000). It is also important to note, though mean plasma concentration profiles obtained in all simulations were acceptable, the confidence intervals highlight the difficulty in predicting vancomycin pharmacokinetics in an individual during ECMO.

7.6 Conclusion

In a study group consisting of the full spectrum of term neonates, older children and adults with a wide range of primary diagnoses, a population pharmacokinetic model for vancomycin during ECMO was developed for the first time, based on serum creatinine and age. Parameter estimates reveal significantly reduced vancomycin CL and expanded V when compared to critically ill non-ECMO patients of similar age and confirms altered disposition during ECMO. The pharmacokinetic parameter estimates were used to develop a guide to vancomycin dosing for those involved in the care of this specialist group of critically ill patients.

CHAPTER VIII

GENERAL DISCUSSION

Patients on ECMO support represent the extreme end of the critical illness spectrum. Often these patients are in multi-organ failure with accompanying haemodynamic compromise and a high predicted mortality with conventional management. As such, ECMO patients may require multiple drug therapy for organ support, analgesia, sedation and the treatment of systemic infections. It is thus crucial that consistent and predictable drug delivery is achieved to ensure optimal patient care, improving the prospects of recovery. The disposition of drugs during ECMO is a complex issue with the interplay of many factors: an expanded circulating volume with consequent haemodilution and protein binding changes, changes in physiology (hepatic and renal function), sorption by components of the circuit and the influence of injection sites and flow rates. Although literature in ECMO has become extensive in recent years, studies and discussions regarding pharmacotherapy has been, at best, minimal. Limited *in vitro* studies had suggested that drug sorption by the ECMO circuit may be an important influence on drug disposition, however the influence of this phenomenon on plasma drug concentrations and hence pharmacokinetics had not been investigated. Although a number of studies have determined the pharmacokinetics of gentamicin and vancomycin, these produced conflicting results and importantly, did not use the estimated parameters to develop more appropriate dosing regimens. The series of investigations described in this thesis start with the chemical analysis of drug loss in *in vitro* systems and combines this data with clinical pharmacokinetic studies to establish the influence on drug disposition of two ECMO circuit related phenomenon: drug sorption and expanded circulating volume. The parameters derived from these investigations were used to develop more appropriate dosing regimens for the major drugs used in the ECMO unit at Glenfield Hospital.

Static sorption studies revealed a time dependent decrease in sedative drug concentrations in contact with pPVC tubing and silicone membrane from the

oxygenator. In contrast, morphine sulphate showed limited losses in contact with pPVC and no loss in contact with the silicone membrane. As with previous studies investigating drug interactions with intravenous administration containers and sets, the degree of loss was correlated with the appropriate log P value, and ionisation status. The results reveal significant potential for drug loss in an ECMO circuit. These simple static sorption studies provide the basis for more physiological models to be developed.

The studies require to be repeated at normal body temperature. It is expected that the rate and extent of drug sorption will be much greater at higher temperatures as molecules interact with the polymer with increased frequency. In addition, drug sorption in more physiological solutions such as blood need to be compared. Indeed one could then determine the influence of drug binding to plasma proteins such as albumin and α_1 acid glycoprotein as well as red blood cells. Such binding may significantly reduce drug available for sorption by the polymers. Interestingly, priming the polymers with an albumin solution seemed to significantly decrease sorption of all drugs by the silicone membrane, but not pPVC. In fact, there seemed to be an increased loss of drugs with the latter. It is difficult to provide a mechanistic explanation for this. It is possible that differing levels of surface coating was achieved, such that a more uniform coating of the silicone membrane resulted in an 'albumin boundary layer' and resistance to the diffusion of drugs into the polymer. Future studies need to investigate the influence of priming times and changing the concentration of albumin in the priming solution on sorption of drug.

In order to explore drug sorption in a dynamic model, flow through an ECMO circuit was simulated by infusing drugs at constant concentration. The results from these studies revealed significant capacity of the circuit to sequester drugs and to affect drug delivery. In the case of propofol, the most lipophilic of all drugs tested, it was estimated that even after 24 hours of a constant infusion rate, only 75% of the original dose would

be delivered to the patient. Again, this simple model could be further developed with more physiological components. Using blood as a mobile phase, warmed to body temperature, is an important next step as already described. It would also be interesting to explore the effects on fractional loss of drug of different pump flow rates and circuit sizes (with differing surface area: volume ratios). A limitation of the current model is that flow through the circuit (and hence the drug) is not in a loop i.e. the patient is missing. Thus drug in the effluent is not recirculated (albeit at an expected lower concentration as drug distributes through the body), and time to equilibrium may have been artificially extended. Ultimately, future studies may be more creative and informative through the development of an animal ECMO model. Such a model would allow blood concentrations to be simultaneously monitored in the reinfusion cannula, arterial circulation and drainage cannula. This would then enable investigation of not only circuit related factors and physico-chemical properties of drugs, but also the effects of mode of cannulation (VV or VA).

In contrast to previous investigations of drug sorption in non-ECMO appliances, a primary aim at the outset of this research was to assess the effect of drug sorption on pharmacokinetics. The *ex vivo* analysis of circuits confirmed sorption of midazolam in vivo by the pPVC circuit tubing. Furthermore, simple water extractions showed that this is a reversible process. The evaluation of midazolam pharmacokinetics in neonates revealed a significant influence of reversible sorption to the circuit. The studies also revealed doses of midazolam administered that were substantially higher than previously reported in preterm neonates for mechanical ventilation, with consequently elevated plasma concentrations. Comparison of plasma midazolam concentrations with the sedation score suggested that levels up to 1$\mu g/cm^3$ might be required to achieve appropriate sedation in an ECMO patient. However, analysis of the sedation scores revealed poor correlation with plasma midazolam concentrations. Scores were noted at

the time of blood sampling only, and since this did not necessarily coincide with dose alterations, they were not a sensitive marker of pharmacodynamic effect. Nevertheless, the typical plasma concentration-time profile reveals plasma concentrations, as ECMO progresses, rising in excess of those normally required for adequate sedation.

It was also an aim of the study to see whether route of midazolam administration, intravenous or extracorporeal, affected dosage requirements and pharmacokinetics. Along with higher dosage requirements in the first 24 hours, the extracorporeal group also had a tendency towards a higher volume of distribution (V). This concurs with the midazolam sorption model since infusion through the circuit, as shown through the *in vitro* simulation studies, is expected to have an immediate effect on drug delivery. In contrast during intravenous administration sorption is likely to have a more gradual impact on arterial concentrations by virtue of lower initial circuit concentrations. It is however anticipated that final equilibrium concentrations, in blood and circuit, would be the same.

The population pharmacokinetic model developed included a time dependent V that was significantly larger than previously reported in neonates. Typically the residuals shown in figure 4.3, Chapter 4, indicates the need for an additional distribution compartment. The addition of a second compartment did improve from the base model but plots of residuals versus time, observed versus predicted concentrations, and residuals versus predicted concentrations revealed there was a greater tendency to under predict, particularly at later times and at higher concentrations. This was also borne out in the predictive performance evaluation, which revealed a bias of +6%. In addition, estimates of parameters were less precise. However, an alternative approach to an ordinary bi-exponential decay model is to represent V as a function of time, where drug distributes initially in a volume corresponding to Vo and then gradually reaching a pseudo-equlibrium at V_{max}, where V_{max} corresponds to V_{β} in a two-compartment model

and K_{sor} is the first-order sorption rate constant (Gabrielsson *et al.*, 2000). This approach provided more precise parameter estimates, and reduced bias, as evidenced by the plot of residuals and the predictive performance of the model. Although a less conventional approach, this resulted in a better model.

Though the model performed well during cross validation and in the simulations, this was an observational kinetic study and as such it can be argued that the experimental design determined the kinetic model. For example previous studies have shown that midazolam behaves as a two compartment model. Had it been possible to conduct more intensive blood sampling the kinetics may have been better characterised presumably as a three compartment model. The present study also did not collect samples after cessation of midazolam infusion during the elimination phase and this is reflected in the high interpatient variability for clearance (CL).

In 12 neonates it was also possible to determine the 1-hydroxy midazolam/midazolam MR as a surrogate marker for CYP3A4/5 activity. However, the higher MR obtained in term ECMO neonates as compared to preterm neonates, suggests reduced activity of uridine-diphosphate glucuronosyl transferases (UGT). In contrast to cytochrome P450, knowledge concerning the impact of development on UGT is far from complete. The lack of specific probe drugs capable of assessing the activity of individual UGT isoforms and their specificity for the biotransformation of important UGT substrate drugs has contributed to this lack of knowledge (de Wildt *et al.*, 1999). In addition, the influence of critical illness on UGT activity is not known so that these results may be a reflection of the critically ill nature of ECMO patients. Due to the importance of UGTs in human drug metabolism more complete characterisation of these critical enzymes is important and deserves attention. The results also highlight the fact that these studies were performed in ill patients in an intensive care unit. The disposition of midazolam is therefore more likely to be affected by both exogenous

factors, for example the administration of co-drugs capable of altering CYP3A activity or other pathways of metabolism (glucuronidation) and elimination (renal compromise) and endogenous factors (e.g. hepatic disease, altered hepatic blood flow). The development of an animal ECMO model, as already suggested, would allow for these covariates to be controlled and enable the ECMO impact on pharmacokinetics to be more clearly delineated.

The remainder of this thesis investigated the impact of the enlarged circulating volume on three water soluble molecules with consequent small V: aminophylline (theophylline), gentamicin and vancomycin. It is worthwhile bearing in mind when comparing pharmacokinetic parameters estimated in the studies presented in this thesis with published data, that ECMO neonates are unique in neonatal intensive care. Apart from being 'sicker', the majority of the babies are term or near term whereas the majority of non-ECMO neonates are preterm. The majority of reported pharmacokinetic studies are therefore in preterm neonates. Since gestational age has a major influence not only on renal and hepatic maturation but also the body water content, caution must be exercised when comparing one group to another. Not withstanding this point, there is little doubt that pharmacokinetics are significantly different compared to previous reports in non-ECMO neonates.

The pharmacokinetics of theophylline has previously been well characterised showing age and weight related CL. Although similar influences of covariates were detected on parameter estimates from the population model developed in the present study, there were also major differences. Theophylline CL during paediatric ECMO was shown to be significantly impaired. This was not only reflected in the empirical experience of staff involved in the care of these patients, but also in the proportion (27%) of potentially toxic (>20mg/L) plasma levels reported. Although the estimate of V did not appear to be significantly high and was in fact less than previous estimates in

preterm neonates, plasma observations used in this investigation were primarily steady state values obtained through routine therapeutic drug monitoring. As such, only CL can be estimated with a high degree of confidence as reflected in the precision with which the interpatient variability was estimated. The clinical implication of this is that the loading dose recommended may not be sufficient to achieve therapeutically effective levels. The impact of ECMO specifically on the V of theophylline warrants further study, preferably with sampling during the distribution phase.

Linear regression analyses of gentamicin peak and trough plasma concentrations not only highlighted the impact of an expanded circulating volume on V but also how this can result in potential toxicity if 'normal dosing regimens' are utilised. Furthermore, the study was also unique in that pharmacokinetic parameter estimates in 5 patients could be repeated after decannulation from ECMO. Although this study was limited in its design with inherent errors in the model, assumptions made and an inability to estimate variability around parameter estimates, it does reflect practice on the ECMO unit. The future development of a population model will provide accurate estimates of pharmacokinetic parameters and through the use of Bayesian forecasting programs, can then be utilised *a priori* in conjunction with one or two plasma observations to determine parameter values in individual patients. This is then a much more powerful tool to tailor individual dosing regimens.

The population pharmacokinetic investigation of vancomycin utilised both rich and sparse data and thus was able to characterise the impact of an expanded circulating volume more accurately. Although an enlarged V and reduced creatinine corrected CL was confirmed, the influence of blood recirculation during VV ECMO (Chapter 1, Section 1.7.4.2) was not elucidated. The importance of the recirculation phenomenon in affecting drug disposition is likely to be significant and requires urgent further work. At the onset of ECMO with high flow rates, recirculation will be prominent and delay

delivery of drug into the systemic circulation. Although more intensive sampling may be able to parameterise recirculation, such a study may not be ethically possible in children. Again the development of an animal model may be a more realistic approach. Recirculation may also provide a further explanation as to why during the midazolam study the extracorporeal group had a higher dose requirement at the onset of ECMO.

A limitation of all the pharmacokinetic studies presented in this thesis is that research involving collection of blood samples from children has inherent difficulties including ethical issues, sample size and sample availability (see Chapter 1, Section 1.9). These are legitimate concerns, protecting the health and overall well being of the child. The quantity of blood and the number of samples necessary are two extremely important variables to adequately describe and evaluate a drug's concentration-time profile. Pharmacokinetic models and parameters defined in this thesis can help circumnavigate blood sampling issues in follow up studies by using the optimal sampling theory (OST) (Reed, 1999). OST incorporates mathematical models (e.g. D-optimality criterion) to define the optimal sampling times that allow the investigator to obtain the least number of blood samples whilst targeting the most 'information-rich' areas of the drug concentration-time profile (Mentre *et al.*, 1997). Such designs increase the efficiency of pharmacokinetic studies, especially in the case of sparse data.

The clinical implications of findings in this research project will depend on the stage of ECMO that treatment is initiated and is perhaps better appreciated in Figure 8.1. In the pre-ECMO phase, the patient is likely to be significantly hypoxic, hypotensive and possibly also septic with consequent altered tissue perfusion and metabolism. On cannulation, hypoxia, hypotension and tissue perfusion will be resolved however the attachment of an extracorporeal circuit is associated with physiological and non-physiological consequences as described throughout this thesis. On decannulation and in the post-ECMO phase, the circulating volume has returned to normal but also the

patient is expected to be significantly 'less critical' with improved cardiovascular, hepatic and renal function. Thus, drugs administered pre-ECMO will have significantly different pharmacokinetics from that during ECMO and immediately post ECMO. Healthcare professionals involved in the care of these patients need to bear this is mind.

Whilst the studies described in this thesis help answer important questions regarding the disposition of drugs during ECMO, a vast amount of research remains to be undertaken. Continued *in vitro* and *in vivo* animal and human clinical studies are required in order to optimise drug therapy in this critically ill group of patients.

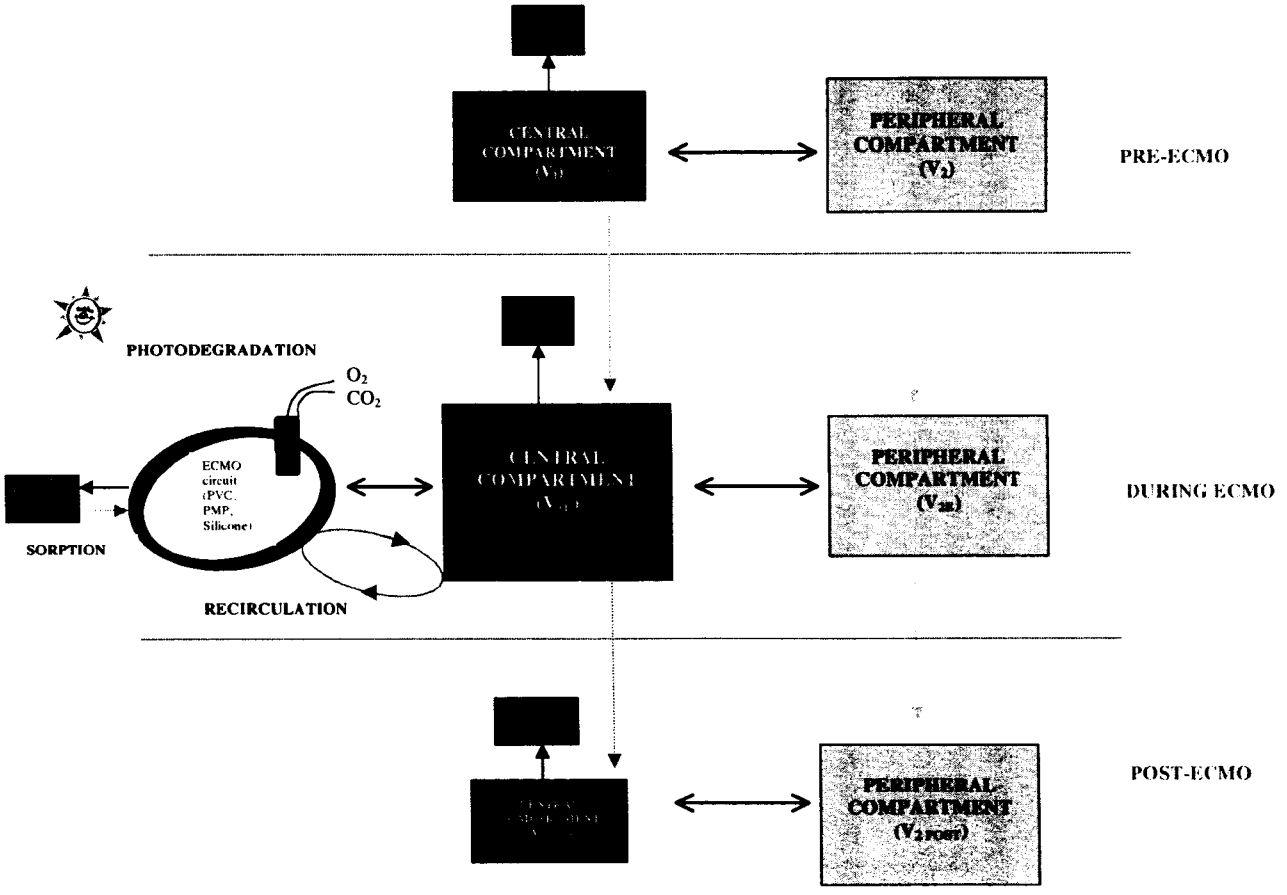


Figure 8.1. Theoretical Two Compartment ECMO Adjusted Pharmacokinetic Model

REFERENCES

AMAKER, R., DIPIRO, J. & BHATIA, J. (1996). Pharmacokinetics of vancomycin in critically ill infants undergoing extracorporeal membrane oxygenation. *Antimicrobial Agents and Chemotherapy*, **40,** 1139-1142.

AHFS Drug Information. 2001: American Society of Health-System Pharmacists, Bethesda, USA. Page 2330.

ANDERSON, H., CORAN, A., DRONGOWSKI, R., HA, H. & BARTLETT, R. (1992). Extracellular fluid and total body water changes in neonates undergoing extracorporeal membrane oxygenation. *Journal of Paediatric Surgery*, **27,** 1003-1008.

ARANDA, J., SITAR, D., PARSONS, W., LOUGHNAN, P. & NEIMS, A. (1976). Pharmacokinetic aspects of theophylline in premature newborns. *New England Journal of Medicine*, **295,** 413-416.

ARNOLD, J., TRUOG, R., ORAV, E., SCAVONE, J. & FENTON, T. (1991). Changes in the pharmacodynamic response to fentanyl in neonates during continuous infusion. *Journal of Paediatrics*, **119,** 639-643.

ARNOLD, J., TRUOG, R., ORAV, E., SCAVONE, J. & HERSHENSON, M. (1990). Tolerance and dependence in neonates sedated with fentanyl during extracorporeal membrane oxygenation. *Anaesthesiology*, **73,** 1136-1140.

BAASKE, D., AMANN, A., WAGENKNECHT, D., MOOERS, M., CARTER, J., HOYT, H. & STOLL, R. (1980). Nitroglycerin compatibility with intravenous fluid filters, containers, and administration sets. *American Journal of Hospital Pharmacy*, **37,** 201-205.

BALTIMORE, R., HUIE, S., MEEK, J., SCHUCHAT, A. & O'BRIEN, K. (2001). Early-Onset Neonatal Sepsis in the Era of Group B Streptococcal Prevention. *Pediatrics*, **108,** 1094-1098.

BARTLETT, R. (1990). Extracorporeal life support for cardiopulmonary failure. *Current Problems in Surgery*, **27,** 621-705.

BARTLETT, R. & GASANAGA, A. (1976a). The physiology and pathophysiology of extracorporeal circulation. In *Current techniques in extracorporeal circulation.* ed. Lonoseum, W.C. London: Butterworths.

BARTLETT, R., GAZZANIGA, A., JEFFRIES, R., HUXTABLE, R., HAIDUE, J. & FONG, S. (1976b). Extracorporeal membrane oxygenatio: cardiopulmonary support in infancy. *ASAIO Journal*, **22,** 80-88.

BARTLETT, R., ROLOFF, D. & CORNELL, R. (1985). Extracorporeal circulation in neonatal respiratory failure: a randomised perspective study. *Pediatrics*, **4,** 479-487.

BARTLETT, R., ROLOFF, D., CUSTER, J., YOUNGER, J. & HIRSCHL, R. (2000). Extracorporeal life support: The University of Michigan Experience. *Journal of the American Medical Association*, **283,** 904-908.

BAASKE, D., AMANN, A., WAGENKNECHT, D., MOOERS, M., CARTER, J., HOYT, H. & STOLL, R. (1980). Nitroglycerin compatibility with intravenous fluid filters, containers, and administration sets. *American Journal of Hospital Pharmacy*, **37,** 201-205.

BHATT-MEHTA, V., JOHNSON, C. & SCHUMACHER, R. (1992). Gentamicin pharmacokinetics in term neonates receiving extracorporeal membrane oxygenation. *Pharmacotherapy*, **12,** 28-32.

BHATT-MEHTA, V., SCHUMACHER, R., FAIX, R., LEADY, M. & BRENNER, T. (1999). Lack of vancomycin-associated nephrotoxicity in newborn infants: a case controlled study. *Pediatrics*, **103,** e48.

BIANCHI, C., AIRAUDO, C.B. & GAYTE-SORBIER, A. (1992). Sorption studies of dipotassium chlorazepate (Tranxene) and midazolam hydrochloride (Hypnovel) in polyvinyl chloride and glass infusion containers. *Journal of Clinical Pharmacy and Therapeutics*, **17,** 223-227.

BLASER, J., STONE, B., GRONER, M. & ZINNER, S. (1987). Comparative study with enoxacin and netilmycin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrobial Agents and Chemotherapy*, **31,** 1054-1060.

BOOTH, B., HENDERSON, M., MILNE, B., CERVENKO, F., MARKS, G., BRIEN, J. & NATSAKU, K. (1991). Sequestration of glyceryl trinitrate (nitroglycerin) by cardiopulmonary bypass oxygenators. *Anaesthesia and Analgesia*, **72,** 493-497.

BOSCOE, M., DAWLING, S., THOMPSON, M. & JONES, R. (1984). Lorazepam in open heart surgery - plasma concentrations before, during and after bypass following different dosing regimens. *Anaesthetics and Intensive Care*, **12,** 9-13.

BRAZIER, J., RENAUD, H., RIBON, B. & SALLE, B. (1979). Serum xanthine levels in low birthweight infants treated or not treated with theophylline. *Archives of Diseases in Childhood*, **54,** 194-199.

BOVILL, J. & SEBEL, P. (1980). Pharmacokinetics of high dose fentanyl. A study in patients undergoing cardiac surgery. *British Journal of Anaesthesia*, **52,** 795-801.

BUCK, M., KSENICH, R. & WOOLDRIDGE, P. (1998). Vancomycin pharmacokinetics in neonates receiveing extracorporeal membrane oxygenation. *Pharmacotherapy*, **18,** 1082.

BURDA, G. & TRITTENWEIN, G. (1999). Issues of pharmacology in paediatric cardiac extracorporeal membrane oxygenation with special references to analgesia and sedation. *Artificial Organs*, **23,** 1015-1019.

BURTIN, P., JACQZ-AIGRAIN, E., GIRARD, P., LENCLEN, R., MAGNY, J., BETREMIEUX, P., TEHIRY, C., DESPLANQUES, L. & MUSSAT, P. (1994). Population pharmacokinetics of midazolam in neonates. *Clinical Pharmacology and Therapeutics*, **56,** 615-625.

CAPPARELLI, E., LANE, J., ROMANOWSKI, G., MCFEELY, E., MURRAY, W., SOUSA, P., KILDOO, C. & CONNOR, J. (2001). The influences of renal function and maturation on vancomycin elimination in newborns and infants. *Journal of Clinical Pharmacology*, **41,** 927-934.

CLOYD, J., VEZEAU, C. & MILLER, K. (1980). Availability of diazepam from plastic containers. *American Journal of Hospital Pharmacy*, **37,** 492-496.

COHEN, P., COLLART, L., PROBER, C., FISCHER, A. & BLASCHKE, T. (1990). Gentamicin pharmacokinetics in neonates undergoing extracorporeal membrane oxygenation. *Paediatric Infectious Diseases*, **9,** 562-566.

Compaq Visual Fortran Professional Edition, September 2000: Compaq Computer Corporation, Houston, Texas, USA.

CLOYD, J., VEZEAU, C. & MILLER, K. (1980). Availability of diazepam from plastic containers. *American Journal of Hospital Pharmacy*, **37,** 492-496.

CRANK, J., PARK, G. (1968). *Diffusion in polymers*. London. Academic Press. Page 274-276.

DAGAN, O., KLEIN, J., GRUENWALD, C., BOHN, D., BARKER, G. & KOREN, G. (1994). Preliminary studies of the effects of extracorporeal membrane oxygenator on the disposition of common paediatric drugs. *Therapeutic Drug Monitoring*, **15,** 263-266.

DASTA, J., JACOBI, J., WU, L., SOKOLOSKI, T., BECKLEY, P., REILLEY, T. & HOWIE, M. (1983). Loss of nitroglycerin to cardiopulmonary bypass apparatus. *Critical Care Medicine*, **11,** 50-52.

DASTA, J., WEBER, R., WU, L., SOKOLOSKI, T., KAKOS, G., SMITH, D. & HOWIE, M. (1986). Influence of cardiopulmonary bypass on nitroglycerin clearance. *Journal of Clinical Pharmacology*, **26,** 165-168.

DAVIDIAN, M. & GILTINAN, D. (1995). *Monographs on Statistics and Applied Probability. Nonlinear Models for Repeated Mesurement data*. London: Chapman and Hall.

DAVIS, J. & SHOCK, N. (1949). The effect of theophylline ethylene diamine on renal function in control subjects and in patients with congestive heart failure. *Journal of Clinical Investigations*, **28,** 1459-1468.

DAWSON, P., BJORKSTEN, A., BLAKE, D. & AL, E. (1997). The effect of cardiopulmonary bypass on total and unbound plasma concentrations of propofol and midazolam. *Journal of Cardiothoracic and Vascular Anaesthesia*, **11,** 556-561.

DE WILDT, S., KEARNS, G., HOP, W., MURRY, D., ABDEL-RAHMAN, S. & VAN DEN ANKER, J. (2001). Pharmacokinetics and metabolism of intraveous midazolam in preterm infants. *Clinical Pharmacology and Therapeutics*, **70,** 525-531.

DE WILDT, S., KEARNS, G., LEEDER, J. & VAN DEN ANKER, J. (1999a). Cytochrome P450 3A: ontogeny and drug disposition. *Clinical Pharmacokinetics*, **37,** 485-505.

DE WILDT, S., KEARNS, G., LEEDER, J. & VAN DEN ANKER, J. (1999b). Glucuronidation in humans: Pharmacogenetic and developmental aspects. *Clinical Pharmacokinetics*, **36,** 439-452.

DODGE, W., JELLIFFE, R., ZWISCHENBERGER, J., BELLANGER, R., HOKANSON, J. & SNODGRASS, W (1994). Population pharmacokinetic models: Effect of explicit versus assumed constant serum concentration assay error patterns upon parameter values of gentamicin in infants on and off extracorporeal membrane oxygenation. *Therapeutic Drug Monitoring*, **16,** 522-559.

DOLLERY, C. (1991). *Therapeutic Drugs, Volume II*: Churchill Livingstone.

DRISCOLL, M., LUDDEN, T., CASTO, D. & LITTLEFIELD, L. (1989). Evaluation of theophylline pharmacokinetics in a pediatric population using mixed effects models. *Journal of Pharmacokinetics and Biopharmaceutics*, **17,** 141-168.

DRUKKER, A. & GUIGNARD, J. (2002). Renal Aspects of the term and preterm infant: a selective update. *Current Opinions in Pediatrics*, **2002,** 175-182.

DU PREEZ, M., BOTHA, J., MCFAYDEN, M. & HOLFORD, N. (1999). The pharmacokinetics of theophylline in premature neonates during the first few days after birth. *Therapeutic Drug Monitoring*, **21,** 598-603.

DUFFULL, S., BEGG, E., CHAMBERS, S. & BARCLAY, M. (1994). Efficacies of different vancomycin dosing regimens against Staphylococcus aureus determined with a dynamic in vitro model. *Antimicrobial Agents and Chemotherapy*, **38,** 2480-2482.

ELLIOTT, E. & BUCK, M. (1999). Phenobarbital dosing a pharmacokinetics in a neonate receiving extracorporeal membrane oxygenation. *Annals Of Pharmacotherapy*, **33,** 419-422.

ELLIOTT, E., KOYSOOKO, R. & LEVY, G. (1976). Pharmacokinetics of Theophylline in Children with Asthma. *Pediatrics*, **58,** 542-547.

ELLIOTT, S. (1991). Neonatal extracorporeal membrane oxygenation: how not to assess novel technologies. *Lancet*, **337,** 472-478.

ESTELLE, F., SIMONS, R., RIGATTO, H. & SIMONS, K. (1981). Pharmacokinetics of Theophylline in Neonates. *Seminars in Perinatology*, **5,** 337-345.

FANOS, V. & DALL'AGNOLA, A. (1999). Anitbiotics in Neonatal infections. *Drugs*, **58,** 405-427.

FISET, P., MATHERS, L., ENGSTROM, R., FITZGERALD, D., BRAND, S., HSU, F. & SHAFER, S. (1995). Pharmacokinetics of computer-controlled alfentanil administration in children undergoing cardiac surgery. *Anesthesiology*, **83,** 944-955.

FORFAR, J. & ARNEIL, G. (1984). *Textbook of Paediatrics. Volume 2.* Edinburgh: Churchill Livingstone.

GABRIELSSON, J. & WEINER, D. (2000). *Pharmacokinetic and Pharmacodynamic Data Analysis*: Swedish Pharmaceutical Press. Page 86.

GAL, P., BOER, H., TOBACK, J., WELLS, T. & ERKAN, N. (1982). Effect of asphyxia on theophylline clearance in newborns. *Southern Medical Journal*, **75,** 836-838.

GILMAN, J., GAL, P., LEVINE, R., HERSH, C. & VILDAN ERKAN, N. (1986). Factors influencing Theophylline Disposition in 179 Newborns. *Therapeutic Drug Monitoring*, **8,** 4-10.

GANNING, A., BRUNK, U. & DALLNER, G. (1984). Phthalate esters and their effect on the liver. *Hepatology*, **4,** 541-547.

GEIDUSCHEK, J., LYNN, A., BRATTON, S., SANDERS, J., LEVY, F., HABERKERN, C. & O'ROURKE, P. (1997). Morphine pharmacokinetics during continuous infusion of morphine sulphate for infants receiving extracorporeal membrane oxygenation. *Critical Care Medicine*, **25,** 360-364.

GIBBON JR, J. (1937). Artificial maintenance of circulation during experimental occlusion of pulmonary artery. *Archives of Surgery*, **34,** 1105.

GRAVLEE, G., R, D., KURUSZ, M. & UTLEY, J. (2000). *Cardiopulmonary Bypass: Principles and Practice*. Philadelphia; London: Lippincott Williams and Wilkins. Page 265

GRIMSLEY, C. & THOMSON, A. (1999). Pharmacokinetics and dose requirements of vancomycin in neonates. *Archives of Diseases in Childhood Fetal and Neonatal Edition*, **81,** F221-F227.

Guidance for Industry: Population Pharmacokinetics. (1999). US Department of Health and Human Services Food and Drug Administration.

HAMMAREN, E., ROSENBURG, P. & HYNYNEN, M. (1999). Coating of extracorporeal circuit with heparin does not prevent sequestration of propofol in vitro. *British Journal of Anaesthesia*, **82,** 38-40.

HAMMAREN, E., YLI-HANKALA, A. & AL, E. (1996). Cardiopulmonary bypass induced changes in plasma concentrations of propofol and in auditory evoked potientals. *British Journal of Anaesthesia*, **77,** 360-364.

HARTE, G., GRAY, P., LEE, T., STEER, P. & CHARLES, B. (1997). Haemodynamic responses and population pharmacokinetics of midazolam following administration to ventilated , preterm neonates. *Journal of Paediatrics and Child Health*, **33,** 335-338.

HARTWIG, S., ROTH, B. & THEISOHN, M. (1991). Clinical experience with continuous intravenous sedation using midazolam and fentanyl in the paediatric intensive care unit. *European Journal of Pediatrics*, **150,** 784-788.

HAW-BOW, S., CHIA-CHIH, T., CHERNG-THE, J. (1996). Changes of propofol levels in isolated cardiopulmonary bypass circuit. *Acta Anaesthesia Scandinavica*, **34,** 17-20.

HAYANI, K., HATZOPOULOS, F., FRANK, A., THUMMALA, M., HANTSCH, M., SCHATZ, B., JOHN, E. & VIDYASAGAR, D. (1997). Pharmacokinetics of once-daily dosing of gentamicin in neonates. *The Journal of Pediatrics*, **131,** 76-80.

HEALY, D., POLK, R., GARSON, M., ROCK, D. & COMSTOCK, T. (1987). Comparison of Steady-State Pharmacokinetics of Two Dosage Regimens of Vancomycin in Normal Volunteers. *Antimicrobial Agents and Chemotherapy*, **31,** 393-397.

HILL, J., O'BRIEN, T., MURRAY, J., DONTIGNY, L., BRAMSON, M. & OSBORN, J. (1972). Prolonged extracorporeal membrane oxygenation for acute post-traumatic respiratory failure (shock-lung syndrome): use of the Bramson membrane lung. *New England Journal of Medicine*, **286,** 629-634.

HILL, S., SHAW, B. & WU, A. (2001). The clinical effects of plasticisers, antioxidants, and other contaminants in medical polyvinylchloride tubing during respiratory and non-respiratory exposure. *Clinica Chimica Acta*, **304,** 1-8.

HILLIGROSS, D., JUSKO, W., KOUP, J. & GIACOIA, G. (1980). Factors affecting theophylline pharmacokinetics in premature infants with apnoea. *Developmental Pharmacology and Therapeutics*, **1,** 6-15.

HILLMAN, L., GOODWIN, S. & SHERMAN, W. (1975). Identification and mesurement of plasticiser in neonatal tissues after umbilical catheters and blood products. *New England Journal of Medicine*, **292,** 381-386.

HIRSCHL, J. (1981). Insulin adsorption to polyolefin infusion bottles and polyvinyl chloride administration sets. *American Journal of Hospital Pharmacy*, **38,** 995-997.

HOIE, E. (1993). Effects of injection site and flow rate on distribution of injected solutions in an extracorporeal membrane oxygenation circuit. *American Journal of Hospital Pharmacy*, **50,** 1902-1906.

HOIE, E., SWIGART, S. & LEUSCHEN, M. (1990). Vancomycin pharmacokinetics in infants undergoing extracorporeal membrane oxygenation. *Clinical Pharmacokinetics*, **9,** 711-715.

HUG, C., BURM, A. & DE LANGE, S. (1994). Alfentanil pharmacokinetics in cardiac surgical patients. *Anaesthesia and Analgesia*, **78,** 231-239.

HUG, C. & MOLDENHAUER, C. (1982). Pharmacokinetics and dynamics of fentanyl infusions in cardiac surgical patients. *Anaesthesiology*, **57,** A45.

HUGHES, J., GILL, A., MULHEARN, H., POWELL, E. & CHOONARA, I. (1996). Steady-state plasma concentrations of midazolam in critically ill infants and children. *Annals Of Pharmacotherapy*, **30,** 27-30

HYNYNEN, M. (1987). Binding of fentanyl and alfentanil to the extracorporeal circuit. *Acta Anaesthesia Scandinavica*, **31,** 706-710.

HYNYNEN, M. (1987). Binding of fentanyl and alfentanil to the extracorporeal circuit. *Acta Anaesthesia Scandinavica*, **31,** 706-710.

HYNYNEN, M., HAMMAREN, E. & ROSENBURG, P. (1994). Propofol sequestration within the extracorporeal circuit. *Canadian Journal of Anaesthesia*, **41,** 583-588.

HYNYNEN, M., SILTANEN, T., SAHLMAN, A. & AL, E. (1995). Continuous infusion of nimodipine during coronary artery surgery: haemodynamic and pharmacokinetic study. *British Journal of Anaesthesia*, **74,** 526-533.

JAEGER, R. & RUBIN, R. (1970). Plasticisers from PVC. *Lancet*, **2,** 778.

JACQZ-AIGRAIN, E. & BURTIN, P. (1996). Clinical Pharmacokinetics of sedatives in neonates. *Clinical Pharmacokinetics*, **31,** 423-443.

JACQZ-AIGRAIN, E., DAOUD, P., BURTIN, P., DESPLANQUES, L. & BEAUFILS, F. (1994). Placebo-controlled trial of midazolam sedation in mechanically ventilated newborn babies. *Lancet*, **344,** 646-650.

JACQZ-AIGRAIN, E., DAOUD, P., BURTIN, P., MAHERZI, S. & BEAUFILS, F. (1992). Pharmacokinetics of midazolam during continuous infusion in critically ill neonates. *European Journal of Clinical Pharmacology*, **42,** 329-32.

JACQZ-AIGRAIN, E., WOOD, C. & ROBIEUX, I. (1990). Pharmacokinetics of midazolam in critically ill neonates. *European Journal of Clinical Pharmacology*, **39,** 191-192.

JENKE, D. (1994). Drug Binding by Reservoirs in Elastomeric Infusion Devices. *Pharmaceutical Research*, **11,** 984-989.

JOHNSON, T., ROSTAMI-HODJEGAN, A., GODDARD, J., TANNER, M. & TUCKER, G. (2002). Contribution of midazolam and its 1-hydroxy metabolite to preoperative sedation in children: a pharmacokinetic-pharmacodynamic analysis. *British Journal of Anaesthesia*, **89,** 428-437.

JONES, R. & BAILLIE, E. (1979). Dosage schedule for intravenous aminophylline in apnoea of prematurity, based on pharmacokinetic studies. *Archives of Diseases in Childhood*, **54,** 190-193.

JUSKO, W., GARDNER, M., MANGIONE, A., SCHANTAG, J., KOUP, J. & VANCE, J. (1979). Factors affecting theophylline clearance: Age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates, and ethanol. *Journal of Pharmaceutical Sciences*, **68,** 1358-1366.

KANTO, J., HIMBERG, J., HEIKKILA, H., AROLA, M., JALONEN, J. & LAASONEN, V. (1985). Midazolam kinetics before, during and after cardiopulmonary bypass surgery. *International Journal of Clinical Pharmacology Research*, **2,** 123-126.

KARLSSON, M., THOMSON, A., MCGOVERN, E., CHOW, P., EVANS, T. & KELMAN, A. (1991). Population pharmacokinetics of rectal theophylline in neonates. *Therapeutic Drug Monitoring*, **13**.195-200.

KARLOWSKI, J., ZHANEL, G., DAVIDSON, R. & HOBAN, D. (1994). Once daily aminoglycoside dosing assessed by MIC reversion time with *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, **38,** 1165-1168.

KEAVENY, J., CLARKE, C., PLEUVRY, B. & WHITTAKER, D. (1991). Midazolam levels in the isolated cardiopulmonary bypass circuit. *Anaesthesia and Analgesia*, **72,** S133.

KERBUSCH, T., KRAKER, J.D., MATHOT, R. & BEIJNEN, J. (2001). Population Pharmacokinetics of Ifosfamide and its Dechloroethylated and Hydroxylated Metabolites in Children with Malignant Disease: A Sparse Sampling Approach. *Clinical Pharmacokinetics*, **40,** 615-625.

KOBOLOW, T. & BOMAN, R. (1963). Construction and evaluation of an alveolar membrane artificial heart lung. *Transactions ASAIO*, **9,** 238.

KOREN, G., CREAN, P., KLEIN, J., GORESKY, G., VILLAMATER, J. & MACLEOD, S. (1984). Sequestration of fentanyl by the cardiopulmonary bypass (CPBP). *European Journal of Clinical Pharmacology*, **27,** 51-56.

KOWALUK, E., ROBERTS, M., BLACKBURN, H. & POLACK, A. (1981). Interactions between Drugs and Polyvinyl Chloride Infusion Bags. *American Journal of Hospital Pharmacy*, **38,** 1308-1314.

KOWALUK, E., ROBERTS, M. & POLACK, A. (1983). Drug Loss in Polyolefin Infusion Systems. *American Journal of Hospital Pharmacy*, **40,** 118-119.

KOWALUK, E., ROBERTS, M. & POLACK, A. (1982). Interactions between Drugs and Intravenous Delivery Systems. *American Journal of Hospital Pharmacy*, **39,** 460-467.

KUMAR, K., CRANKSHAW, D., MORGAN, D. & BEEMER, G. (1988). The effect of cardiopulmonary bypass on plasma protein binding of alfentanil. *European Journal of Clinical Pharmacology*, **35,** 47-52.

LACROIX, D., SONNIER, M., MONION, A., CHERON, G. & CRESTEIL, T. (1997). Expression of CYP3A in the liver. Evidence that the shift beween CYP3A7 and CYP3A4 occurs immediately after birth. *European Journal of Biochemistry*, **247,** 625-634.

LAMARRE, P., LEBEL, D. & DUCHARME, M. (2000). A Population Pharmacokinetic Model for Vancomcycin in Pediatric Patients and Its Predictive Value in a Naive Population. *Antimicrobial Agents and Chemotherapy*, **44,** 278-282.

LANNIGAN, R. & THOMSON, A. (2001). Evaluation of 22 Neonatal Gentamicin Dosage Protocols Using a Bayesian Approach. *Paediatric and Perinatal Drug Therapy*, **2,** 92-100.

LEE, M. (1986). Sorption of four drugs to polyvinyl chloride and polybutadiene intravenous administration sets. *American Journal of Hospital Pharmacy*, **43,** 1945-1950.

LEE, T., CHARLES, B., HARTE, G., GRAY, P., STEER, P. & FLENADY, V. (1999). Population pharmacokinetic modeling in very premature infants receiving midazolam during mechanical ventilation: Midazolam Neonatal Pharmacokinetics. *Anesthesiology*, **90,** 451-457.

LEE, T., CHARLES, B., STEER, P., FLENADY, V. & GRANT, T. (1996). Theophylline population pharmacokinetics from routine monitoring data in very premature infants with apnoea. *British Journal of Clinical Pharmacology*, **41,** 191-200.

LEPPIK, I., FISHER, J., KRIEL, R. & SAWCHUK, R. (1986). Altered phenytoin clearance with febrile illness. *Neurology*, **36,** 1367-1370.

LEUSHEN, M., WILLETT, L., HOIE, E., BOLAM, D., BUSSEY, M., GOODRICH, P., ZACH, T. & NELSON, R. (1993). Plasma fentanyl levels in infants undergoing extracorporeal membrane oxygenation. *Journal of Thorasic and Cardiovascular Sugery*, **105,** 885-891.

LEVINS, R. (1966). *American Scientist*, **54,** 421-431.

LEWIS, L., FLETCHNER, T., KERKAY, J., PEARSON, K. & NAKAMOTO, S. (1978). Bis (2-ethylhexyl) phthalate concentrations in the serum of hemodialysis patients. *Clinical Chemistry*, **24,** 741-746.

LINDSTROM, M. & BATES, D. (1990). Nonlinear mixed effects models for repeated measures data. *Biometrics*, **46,** 673-687.

LLOYD-THOMAS, A. & BOOKER, P. (1986). Infusion of midazolam in paediatric patients after cardiac surgery. *British Journal of Anaesthesia*, **58,** 1109-15.

LOCHAN, S., ADENIYI-JONES, S., ASSADI, F., FREY, B., MARCUS, S. & BAUMGART, S. (1998). Coadministration of theophylline enhances diuretic response to furosemide in infants during extracorporeal membrane oxygenation. A randomized controlled pilot study. *Journal of Pediatrcs*, **133,** 86-89.

LOUGHNAN, P., SITAR, D., OGILVIE, R., EISEN, A., FOX, Z. & NEIMS, A. (1976). Pharmacokinetic analysis of the disposition of intravenous theophylline in young children. *Journal of Pediatrics*, **88,** 874-879.

LOWDIN, E., ODENHOLT, I. & CARS, O. (1998). In vitro studies of pharmacodynamic properties of vancomycin against Staphylococcus aureus and Staphylococcus epidermidis. *Antimicrobial Agents and Chemotherapy*, **42,** 2739-2744.

LUDDEN, T. (1988). Population Pharmacokinetics. *Journal of Clinical Pharmacology*, **28,** 1059-1063.

LUND, W. (1994). *The Pharmaceutical Codex: Principles and Practice of Pharmaceuticals*: The Pharmaceutical Press.

LUNN, J., STANLEY, T., EISELE, J. (1979). High dose fentanyl anaesthesia for coronary artery surgery: plasma fentanyl concentrations and influence of nitrous oxide on cardiovascular responses. *Anaesthesia and Analgesia*, **58,** 390-395.

MACNAB, M., MACRAE, D., GUY, E., GRANT, I. & FEELY, J. (1986). Profound reduction in morphine clearance and liver blood flow in shock. *Intensive Care Medicine*, **12,** 366-369.

MACGILLIVRAY, T., JENNINGS, R., RUDOLPH, A., RING, E., ADZICK, N. & HARRISON, M. (1994). Vascular Changes With In Utero Correction of Diaphragmatic Hernia. *Journal of Pediatric Surgery*, **29,** 992-996.

MANDEMA, J., TUK, B., VAN STEVENINCK, A., BREIMER, D., COHEN, A. & DANHOF, M. (1992). Pharmacokinetic-pharmacodynamic modeling of the central nervous system effects of midazolam and its main metabolite alpha-hydroxymidazolam in healthy volunteers. *Clinical Pharmacology and Therapeutics*, **51,** 715-728.

MANY, M., SOROFF, S., BIRTWELL, W., WISE, H. & DETERLING, R. (1986). The physiological role of pulsatile and nonpulsatile blood flow. *Archives of Surgery*, **97,** 917-923.

MARTENS, H., DE GOEDE, P. & VAN LOENEN, A. (1990). Sorption of various drugs in polyvinyl chloride, glass and polyethylene-lined infusion containers. *American Journal of Hospital Pharmacy*, **47,** 369-373.

MARX, C., LITMANOVITZ, I., KSENICH, R., CORNELL, D. & WALSH-SUKYS, M. (1991). Investigation of increased Phenobarbital dose requirement for newborn infants on ECMO: in vitro absorption to ECMO cicuit. *Pharmacotherapy*, **11,** 270.

MASSEY, N., SHERRY, K., OLDROYD, S. & PEACOCK (1990). Pharmacokinetics of an infusion of propofol during cardiac surgery. *British Journal of Anaesthesia*, **65,** 475-479.

MATTHEWS, H., CARSON, I., LYONS, S., ORR, I., COLLIER, P., HOWARD, P. & DUNDEE, J. (1988). A pharmacokinetic study of midazolam in paediatric patients undergoing cardiac surgery. *British Journal of Anaesthesia*, **61,** 302-307.

MCKINDLEY, D., HANES, S. & BOUCHER, B. (1998). Hepatic Drug Metabolism in Critical Illness. *Pharmacotherapy*, **18,** 759-778.

Medicines For Children (2000). London: RCPCH Publications Limited.

MENTRE, F., MALLET, A. & BACCAR, D. (1997). Optimal design in random-effects regression models. *Biometrika*, **84,** 429-442.

METS, B. (2000). The pharmacokinetics of anaesthetic drugs and adjuvants during cardiopulmonary bypass. *Acta Anaesthesiology Scandinavica*, **44,** 261-273.

MOORE, E., FAIX, R., BANAGALE, R. & GRASELA, T. (1989). The population pharmacokinetics of theophylline in neonates and young infants. *Journal of Pharmacokinetics and Biopharmaceutics*, **17,** 47-66.

MOORE, R., LIETMAN, P. & SMITH, C. (1987). Clinical response to aminoglycosides therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. *Journal of Infectious Diseases*, **155,** 93-99.

MULLA, H., PEEK, G., KILLER, H. & UPTON, D. (1998). Extracorporeal membrane oxygenation. *Hospital Pharmacist*, **5,** 163.

MUNCKHOF, W., GRAYSON, M. & TURNIDGE, J. (1996). A meta-analysis of studies on the safety and efficacy of aminoglycosides given either once daily or as divided doses. *Journal of Antimicrobial Chemotherapy*, **37,** 645-663.

MUNZENBERGER, P. & MASSOUD, N. (1991). Pharmacokinetics of gentamicin in neonatal patients supported with extracorporeal membrane oxygenation. *Trans American Society of Internal Organs*, **37,** 16-18.

MURPHY, J., AUSTIN, M. & FRYE, R. (1998). Evaluation of gentamicin pharmacokinetics and dosing protocols in 195 neonates. *American Journal of Health-System Pharmacy*, **55,** 2280-2288.

NASSIF, E., WEINBERGER, M., SHANNON, D., GUIANG, S., HENDELES, L., JIMENEZ, D. & EKWO, E. (1981). Theophylline disposition in infancy. *Journal of Pediatrics*, **98,** 158-161.

NAQVI, S., KEENAN, W., REICHLEY, R. & FORTUNE, K. (1986). Vancomcyin Pharmacokinetics in Small, Seriously Ill Infants. *American Journal Of Diseases in Childhood*, **140,** 107-110.

NGIAM, S. & CHONG, J. (1998). The addition of intrathecal sufentanil and fentanyl to bupivacaine for caesarean section. *Singapore Medical Journal*, **39,** 290-294.

ODIO, C., MCCRACKEN, G.J. & NELSON, J. (1984). Nephrotoxicity associated with vancomycin-aminoglycoside therapy in four children. *Journal of Pediatrics*, **105,** 491-493.

Paediatric Formulary (5th Edition). (1999). Guy's, St. Thomas' and Lewisham Hospitals.

PARKER, W. & MACCARA, M. (1980). Compatability of diazepam with intravenous fluid containers and administration sets. *American Journal of Hospital Pharmacy*, **37,** 496-500.

PAYNE, K., MATTHEYSE, F., LIEBENBERG, D. & DAWES, T. (1989). The pharmacokinetics of midazolam in paediatric patients. *European Journal of Clinical Pharmacology*, **37,** 267-72.

PEARSON, G., FIELD, D., FIRMIN, R. & SOSNOWSKI, A. (1992). UK experience in neonatal extracorporeal membrane oxygenation. *Archives of Diseases in Childhood*, **67,** 822-825.

PEEK, G., KILLER, H., REEVES, R., SOSNOWSKI, A. & FIRMIN, R. (2002). Early Experience with a Polymethyl Pentene Oxygenator for Adult Extracorporeal Life Support. *American Society of Artificial Internal Organs*, **48,** 480-482.

PEEK, G., KILLER, H., SOSNOWSKI, A. & FIRMIN, R. (1998). Extracorporeal membrane oxygenation: potiental for adults and children? (Review). *Hospital Medicine (London)*, **59,** 304-308.

PEEK, G., THOMPSON, A., KILLER, H. & FIRMIN, R. (2000). Spallation performance of extracorporeal membrane oxygenator tubing. *Perfusion*, **15,** 457-466.

PEEK, G., WONG, K., MORRISON, C., KILLER, H. & FIRMIN, R. (1999). Tubing Failure during prolonged roller poump use: a laboratory study. *Perfusion*, **14,** 443-452.

POLARD, E., LE BOUQUIN, V., LE CORRE, P., KEREBEL, C., TROUT, H., FEUILLU, A., LE VERGE, R. & MALLEDANT, Y. (1999). Non Steady State and Steady State PKS Bayesian Forecasting and Vancomycin Pharmacokinetics in ICU Adult Patients. *Therapeutic Drug Monitoring*, **21**. 395-403.

PRETZLAFF, R., VARDIS, R. & POLLACK, M. (1999). Aminophylline in the treatment of fluid overload. *Critical Care Medicine*, **27,** 2782- 2785.

PRINS, J., WEVERLING, G., DE BLOK, K., VAN KETEL, R. & SPEELMAN, P. (1996). Validation and nephrotoxicity of a simplified once-daily aminoglycoside dosing schedule and guidelines for monitoring therapy. *Antimicrobial Agents and Chemotherapy*, **40,** 2494-2499.

RAJCHGOT, P., PROBER, C., SOLDIN, S. (1984). Aminoglycoside-related nephrotoxicity in the premature newborn. *Clinical Pharmacology and Therapeutics*, **35,** 394-401.

RASTOGI, A., AGARWAL, G., PYATI, S. & PILDES, R. (2002). Comparison of two gentamicin dosing schedules in very low birth weight infants. *The Pediatric Infectious Disease Journal*, **21,** 234-240.

REED, M. (1999). Optimal Sampling Theory: An overview of Its Application to Pharmacokinetic Studies in Infants and Children. *Pediatrics*, **104,** 627-632

REVES, J., FRAGEN, R., VINIK, H. & GREENBLATT, D. (1987). Midazolam: Pharmacology and Uses. *Anesthesiology*, **62,** 310-324.

ROBBINS, G., WYNANDS, J., WHALLY, D. (1990). Pharmacokinetics of alfentanil and clinical responses after cardiac surgery. *Canadian Journal of Anaesthesia*, **37,** 52-57.

ROBERTS, M., COSSUM, P., GALBRAITH, A. & BOYD, G. (1980). The availability of nitroglycerin from parenteral solutions. *Journal of Pharmacy and Pharmacology*, **32,** 237-244.

ROBERTS, M., KOWALUK, E. & POLACK, H. (1991). Prediction of Solute Sorption by Polyvinyl Chloride Plastic Infusion Bags. *Journal of Pharmaceutical Sciences*, **80,** 449-455.

ROCHE, F.H.-L. (2000). Midazolam (base) Safety Data Sheet. Basel, Switzerland.

RODVOLD, K., BLUM, R., FISCHER, J., ZOKUFA, H., ROTSCHAFER, J., CROSSLEY, K. & RIFF, L. (1988). Vancomcyin Pharmacokinetics in Patients with Various Degrees of Renal Function. *Antimicrobial Agents and Chemotherapy*, **32,** 848-852.

RODVOLD, K., EVERETT, J., PRYKA, R. & KRAUS, D. (1997). Pharmacokinetics and Administration Regimens of Vancomcyin in Neonates, Infants and Children. *Clinical Pharmacokinetics*, **33,** 32-51.

ROSEN, J., DANISH, M., RAGNI, M., LOPEZ SACCAR, C., YAFFE, S. & LECKS, H. (1979). Theophylline Pharmacokinetics in the young infant. *Pediatrics*, **64.** 248-251.

ROSEN, K. & ROSEN, D. (1986). Factors which affect fentanyl uptake by the membrane oxygenator. *Anesthesiology*, **65,** A225.

ROSEN, D., ROSEN, K., DAVIDSON, B. & BROADMAN, L. (1988a). Fentanyl uptake by the Scimed Membrane Oxygenator. *Journal of Cardiothoracic Anaesthesia*, **2,** 619-626.

ROSEN, D., ROSEN, K., DAVIDSON, B., NAHRWOLD, M. & BROADMAN, L. (1985). Absorption of fentanyl by the membrane oxygenator. *Anesthesiology*, **63,** A281.

ROSEN, D., ROSEN, K., DAVIDSON, B. & BROADMAN, L. (1988b). Fentanyl uptake by the Scimed Membrane Oxygenator. *Journal of Cardiothoracic Anaesthesia*, **2,** 619-626.

ROSEN, D., ROSEN, K. & LEONG, P. (1988c). Uptake of lorazepam and midazolam by the Scimed Membrane Oxygenator. *Anaesthesiology*, **73,** A474.

ROSEN, D., ROSEN, K. & SILVASI, D. (1989). Factors which affect midazolam uptake by the Sci-med Membrane Oxygenator. *Anaesthesia and Analgesia*, **68**:S238

ROSEN, D., ROSEN, K. & SILVASI, D. (1990). In vitro variability in fentanyl absorption by the different membrane oxygenators. *Journal of Cardiothoracic Anaesthesia*, **4,** 332-335.

ROSTAMI-HODJEGAN, A., WOLFF, K., HAY, A., RAISTRICK, D., CALVERT, R. & TUCKER, G. (1999). Population pharmacokinetics of methadone in opiate users: characterisation of time-dependent changes. *British Journal of Clinical Pharmacology*, **48,** 43-52.

ROTSCHAFER, J., CROSSLEY, K., ZASKE, D., MEAD, K., SAWCHUK, R. & SOLEM, L. (1982). Pharmacokinetics of Vancomycin: Observations in 28 Patients and Dosage Recommendations. *Antimicrobial Agents and Chemotherapy*, **22,** 391-394.

ROTSCHAFER, J., ZABINSKI, R. & WALKER, K. (1992). Pharmacodynamic factors of antibiotic efficacy. *Pharmacotherapy*, **12,** 64S-70S.

ROWLAND, M. & TOZER, T. (1995). *Clinical Pharmacokinetics: Concepts and Applications*. Philadelphia: Lippincott Williams and Wilkins.

RUSSELL, G., WRIGHT, E., FOX, M. (1989). Propofol-alfentanyl anaesthesia for coronary artery sugery and cardiopulmonary bypass. *Anaesthesia*, **44,** 205-208.

RYBAK, M., ALBRECHT, L., BERMAN, J., WARBASSE, L. & SVENSSON, C. (1990). Vancomycin Pharmacokinetics in Burn Patients and Intravenous Drug Abusers. *Antimicrobial Agents and Chemotherapy*, **34,** 792-795.

SAWCHUK, R. & ZASKE, D. (1976). Pharmacokinetics of dosing regimens which utilize multiple intravenous infusions: gentamicin in burn patients. *Journal of Pharmacokinetics and Biopharmaceutics*, **4,** 183-195.

SCHAAD, U., MCCRACKEN, G. & NELSON, J. (1980). Clinical pharmacology and efficacy of vancomcyin in pediatric patients. *Journal of Pediatrics*, **96,** 119-126.

SCHNEIDER, B., SCHENA, J., TRUOG, R., JACOBSON, M. & KEVY, S. (1989). Exposure to di(2-ethylhexyl) phthalate in infants receiving extracorporeal membrane oxygenation. *New England Journal of Medicine*, **320,** 1563 (letter).

SELF, T., CHAFIN, C. & SOBERMAN, J. (2000). Effect of disease states on theophylline serum concentrations: Are we still vigilant? *American Journal of the Medical Sciences*, **319,** 2000.

SEAY, R., BRUNDAGE, R., JENSEN, P., SCHILLING, C. & EDGREN, B. (1994). Population pharmacokinetics of vancomycin in neonates. *Clinical Pharmacology and Therapeutics*, **56,** 169-75.

SHEINER, L., ROSENBERG, B. & ARATHE, V. (1977). Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. *Journal of Pharmacokinetics and Biopharmaceutics*, **5,** 445-479.

SHEINER, L. & BEAL, S. (1981). Some suggestions for measuring predictive performance. *Journal of Pharmacokinetics and Biopharmaceutics*, **9,** 503-512.

SHEINER, L. & GRASELA, T. (1991). An introduction to Mixed Effect Modelling: Concepts, Definitions, and Justification. *Journal of Pharmacokinetics and Biopharmaceutics*, **19,** 11S - 24S.

SHEVDE, K. & DUBOIS, W. (1987). Pro: Pulsatile flow is preferable to nonpulsatile flow during cardiopulmonary bypass. *Journal of Cardiothoracic Anaesthesia*, **1,** 165-168.

SIGURD, B. & OLESEN, K. (1977). The supra-additive natriuretic effect addition of theophylline ethylenediamine and bumetanide in congestive heart failure. *American Heart Journal*, **94,** 168-174.

SILVASI, D., ROSEN, D. & ROSEN, K. (1989). Absorption of midazolam by the Sci-med Membrane Oxygenator. *Anaesthesia and Analgesia*, **68,** S261.

SJOBERG, P., BONDENSSON, U., SEDIN, G. & GUSTAFSSON, J. (1985). Dispositions of di- and mono-(2-ethylhexyl) phthalate in newborn infants subjected to exchange transfusions. *European Journal of Clinical Investigations*, **15,** 430-436.

SKACEL, M., KNOTT, C., REYNOLDS, F. & APS, C. (1986). Extracorporeal circuit sequestration of fentanyl and alfentanil. *British Journal of Anaesthesia*, **58,** 947-949.

SOUTHGATE, M., DIPIRO, J. & ROBERTSON, A. (1989). Pharmacokinetics of gentamicin in neonates on extracorporeal membrane oxygenation. *Antimicrobial Agents and Chemotherapy*, **33,** 817-819.

SPRIGGE, J., WYNANDS, J., WHALLEY, D., BEVAN, D., TOWNSEND, G., NATHAN, H., PATEL, Y. & SRIKANT, C. (1982). Fentanyl infusion anaesthesia for aortocoronary bypass surgery: plasma levels and haemodynamic response. *Anaesthesia and Analgesia*, **61,** 972-978.

SUN, H., FADIRAN, E., JONES, C., LESKO, L., HUANG, S.-M., HIGGINS, K., HU, C., MACHADO, S., MALDONADO, S., WILLIAMS, R., HOSSAIN, M. & ETTE, E. (1999). Population Pharmacokinetics: A regulatory perspective. *Clinical Pharmacokinetics*, **37,** 41-58.

SUN, H., ETTE, E. & LUDDEN, T. (1996). On the recording of sample times and parameter estimation from repeated measures pharmacokinetic data. *Journal of Pharmacokinetics and Biopharmaceutics*, **24,** 637-650.

STOLK, L., DEGRAEUWE, P., NIEMAN, F., DE WOLF, M. & DE BOER, A. (2002). Population pharmacokinetics and relationship between demographic and clinical variables and pharmacokinetics of gentamicin in neonates. *Therapeutic Drug Monitoring*, **24,** 527-531.

THUMMEL, K., SHEN, D., PODOLL, T., KUNZE, K., TRAGER, W., HARTWELL, P., RAISYS, V., MARSH, C., MCVICAR, J. & BARR, D. (1994). Use of midazolam as a human cytochrome P450 3A probe: I. In vitro-in vivo correlations in liver trasnplant patients. *Journal of Pharmacology and Experimental Therapeutics*, **271,** 549-556.

TICKNER, J., SCHETTLER, T., GUIDOTTI, T., MCCALLY, M. & ROSSI, M. (2001). Health Risks Posed by Use of Di-2-Ethylhexyl Phthalate (DEHP) in PVC Medical Devices: A Critical Review. *American Journal of Industrial Medicine*, **39,** 100-111.

TOBIAS, J., DESHPANDE, J., PIETSCH, J., WHEELER, T. & GREGORY, D. (1995). Pentobarbital sedation for patients in the pediatric intensive care unit. *Southern Medical Journal*, **88,** 290-294.

TOLIA, V., BRENNAN S, ARAVIND, M. & KAUFFMAN, R. (1991). Pharmacokinetic and Pharmacodynamic study of midazolam in chidren during esophagogastroduodenoscopy. *Journal of Pediatrcs*, **119,** 467-471.

TRIGGS, E. & CHARLES, B. (1999). Pharmacokinetics and therapeutic drug monitoring of gentamicin in the elderly. *Clinical Pharmacokinetics*, **37,** 331-341.

TRISSEL, L. (2001). *Handbook on Injectable Drugs*: American Society of Health System Pharmacists.

UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation.(1996). *Lancet*, **348,** 75-81.

UNGER, J., KUEHLEIN, G., SCHROERS, A., GERLACH, J. & ROSSAINT, R. (2001). Adsorption of xenobiotics to plastic tubing incorporated into dynamic in vitro systems used in pharmacological research - limits and progress. *Biomaterials*, **22,** 2031-2037.

WILLS, R., KHOO, J., SONI, P. & PATEL, I. (1990). Increased volume of distribution prolongs midazolam half-life. *British Journal of Clinical Pharmacology*, **29,** 269-272.

WRIGHTON, S., BRIAN, W., SARI, M., IWASAKI, M., GUENGERICH, F., RAUCY, J., MOLOWA, D. & VANDENBRANDEN, M. (1990). Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). *Molecular Pharmacology*, **38,** 207-213.

WinNonLin Standard Version 3.0 (1993-2000): Pharsight Corporation. California 94040, USA.

WinNonMix Professional Version 2.0.1.(1998-2000): Pharsight Corporation. California 94040, USA.

WinNonMix Version 2.0. Reference Guide. (1998-2000): Pharsight Corporation. California 94040, USA.

YAHYA, A., MCELNAY, J. & D'ARCY, P. (1988). Drug Sorption to Glass and Plastics. *Drug Metabolism and Drug Interactions*, **6,** 1-45.

YASUHARA, M., IGA, T., ZENDA, H., OKUMURA, K., OGUMA, T., YANO, Y. & HORI, R. (1998). Population Pharmacokinetics of Vancomcyin in Japanese Adult Patients. *Therapeutic Drug Monitoring*, **20,** 139-148.

YLIRUUSI, J., UOTILLA, J. & KRISTOFFERSSON, E. (1986a). Effect of flow rate and type of i.v. container on adsorption of diazepam to i.v. administration systems. *American Journal of Hospital Pharmacy*, **43,** 2795-2799.

YLIRUUSI, J., UOTILLA, J. & KRISTOFFERSSON, E. (1986b). Effect of tubing length on adsorption of diazepam to polyvinyl chloride administration sets. *American Journal of Hospital Pharmacy*, **43,** 2789-2794.

ZAPOL, W., SNIDER, M. & HILL, J. (1979). Extracorporeal membrane oxygenation in severe respiratory failure. *Journal of the American Medical Association*, **242,** 2193-2196.

ZASKE, D., CIPOLLE, R. & ROTSCHAFTER, J. (1982). Gentamicin pharmacokinetics in 1640 patients: method for control of serum concentrations. *Antimicrobial Agents and Chemotherapy*, **21,** 407-411.

ZASKE, D., CIPOLLE, R. & STRATE, R. (1980). Gentamicin dosage requirements: wide interpatient variations in 242 surgery patients with normal serum function. *Surgery*, **87**, 64-69.

ZIMMERMANN, A., KATONA, B. & PLAISANCE, K. (1995). Association of vancomycin serum concentrations with outcomes in patients with gram-positive bacteremia. *Pharmacotherapy*, **15**, 85-91.

ZOMORODI, K., DONNER, A., SOMMA, J., BARR, J., SLADEN, R., RAMSAY, J., GELLER, E. & SHAFER, S. (1998). Population pharmacokinetics of midazolam administered by target controlled infusion for sedation following coronary artery bypass grafting. *Anesthesiology*, **89**, 1418-1429.

ZWISCHENBERGER, J., STEINHORN, R., BARTLETT, R. (2000a).*ECMO: Extracorporeal Cardiopulmonary Support in Critical Care*. Ann Arbor, Michigan: Extracorporeal Life Support Organization, page 210.

ZWISCHENBERGER, J., STEINHORN, R., BARTLETT, R. (2000b).*ECMO: Extracorporeal Cardiopulmonary Support in Critical Care*. Ann Arbor, Michigan: Extracorporeal Life Support Organization, page 219.

ZWISCHENBERGER, J., STEINHORN, R., BARTLETT, R. (2000c).*ECMO: Extracorporeal Cardiopulmonary Support in Critical Care*. Ann Arbor, Michigan: Extracorporeal Life Support Organization, page 121.

Appendix I

Tabulated Data from Chapter 3

Table A.I Mean data from triplicate determinations of drug samples after single passage through ECMO circuit.

		Ratio of the measured conc./initial conc. as %			
Sample	**TIME/min**	**Midazolam**	**Diazepam**	**Lorazepam**	**Propofol**
1	2	4	3	68	nd
2	4	6	5	75	nd
3	6	7	6	73	nd
4	8	7	6	69	nd
5	10	7	6	68	nd
6	12	12	7	76	nd
7	14	11	9	72	nd
8	16	13	9	73	nd
9	18	10	10	71	nd
10	20	11	17	72	nd
11	22	12	23	79	nd
12	24	15	28	79	nd
13	26	18	31	78	nd
14	28	20	31	80	nd
15	30	21	35	77	nd
16	32	25	38	82	nd
17	34	26	40	79	nd
18	36	29	42	85	nd
23	46	23	40	98	4
27	54	32	48	99	9

Mean of Triplicate Determinations
nd = not detected

Table A.II Cumulative amount of drug sorbed (μg).

Sample	Time (min)	Midazolam	Diazepam	Lorazepam	Propofol
1	2	276.5	279.4	92.2	288.0
2	4	547.2	553.0	164.2	576.0
3	6	815.0	823.7	241.9	864.0
4	8	1082.9	1094.4	331.2	1152.0
5	10	1350.7	1365.1	423.4	1440.0
6	12	1604.2	1633.0	492.5	1728.0
7	14	1860.5	1895.0	573.1	2016.0
8	16	2111.0	2157.1	650.9	2304.0
9	18	2370.2	2416.3	734.4	2592.0
10	20	2626.6	2655.4	815.0	2880.0
11	22	2880.0	2877.1	875.5	3168.0
12	24	3124.8	3084.5	936.0	3456.0
13	26	3361.0	3283.2	999.4	3744.0
14	28	3591.4	3481.9	1057.0	4032.0
15	30	3818.9	3669.1	1123.2	4320.0
16	32	4034.9	3847.7	1175.0	4608.0
17	34	4248.0	4020.5	1235.5	4896.0
18	36	4452.5	4187.5	1278.7	5184.0
23	46	5561.3	5051.5	1307.5	6566.4
27	54	6344.6	5650.6	1319.0	7614.7

Table A.III Mean Data from the Priming Studies

	Unprimed PVC	Unprimed Silicone	Primed PVC	Primed Silicone
Lorazepam				
0	25	25	25	25
5	20.18		20	24.94
20	15.18	16.95	12.23	25.09
40	14.96	16.7	12.1	24.84
120	14.98	16.4	12.05	24.66
Midazolam				
0	25	25	25	25
5	17.6	17.99	16.93	22.23
20	11.29	12.86	6.3	17.23
40	8.79	9.05	6	15.89
120	8.39	7.95	6	13.99
Diazepam				
0	25	25	25	25
5	8.73	16.55	7.24	21.36
20	3.81	9.9	1.23	14.78
40	2.99	6.2	1.06	12.39
120	2.91	5.61	1.01	9.98
Propofol				
0	25	25	25	25
5	8.15	14	3.86	19.49
20	1.16	3.93	0.04	11.5
40	0.53	1.24	0.04	7.55
120	0.34	0.86	0	6.01

Table A.IV Recovery of Midazolam from Ex Vivo Circuits

				Amount Extracted (μg)				
Patient	**Duration of ECMO**	**Duration of Infusion**	**Amount Infused (μg)**	**Pre oxygenator**	**Post Oxygenator**	**Drainage Line**	**Mean (μg)**	**% Recovered**
1	199.0	168	31298.0	176.7	161.2	131.0	156.3	0.5
2	66.0	66.0	34200.0	283.6	258.6	308.6	283.6	0.8
3	82.0	82.0	48008.0	353.4	331.0	359.5	348.0	0.7
4	167.0	132	65940.0	795.7	978.4	1062.9	945.7	1.4
5	156.0	156.0	146015.0	647.4	529.3	543.1	573.3	0.4

APPENDIX II

Estimation Algorithms for Population Pharmacokinetic Modelling

WinNonMix gives the option of two regression methods for performing the estimation: The Two Stage Method and Inference by First Order Linearisation. The latter method was used for all estimation during this project, since it is the method of choice when data are sparse or when a particular covariance structure is required. The linear approximation of the non-linear mixed effects model allows the linear mixed effects model, population model parameters and covariance parameters to be estimated via the same algorithm.

Three methods of inference based on linearisation are provided by WinNonMix: (Simple) First Order, (GLS) First Order, and First Order Conditional methods. Only (simple) First Order and First Order Conditional methods were used throughout this project. Each method employs an approximation to the marginal likelihood based on a Taylor series linearisation of the model (Davidian *et al.*, 1995). The conditional first order method (Lindstrom *et al*, 1990) employs a more sophisticated approximation which accommodates updating of the linearisation at each iterative step, based on the most recent estimate of the random effects. This yields a superior approximation, particularly when interpatient variability is large, however at the expense of computational complexity and longer model run times *(WinNonMix Version 2.0. Reference Guide. (1998-2000).*

Standard Error

Standard errors of fixed and random effects were calculated in WinNonMix by using the sandwich method *(WinNonMix Version 2.0. Reference Guide. (1998-2000).*

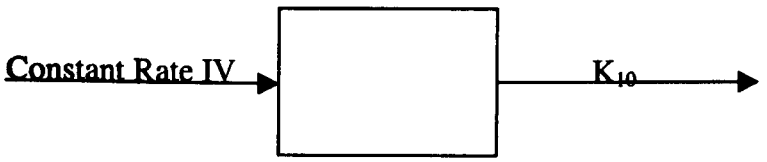
Optimisation Method.

The regression was optimised by using the Quasi-Newton algorithm (Davidian *et al.*, 1995). The objective function was used as the convergence criterion for regression: default tolerance was set at 0.0001.

APPENDIX III

Compartmental Models used by WinNonMix to Fit Plasma Concentration Data

1. One Compartment Model, Constant IV Infusion, First Order Output



$$C(t) = \text{Dose}/ti \times 1/V \times K_{10} \times (e^{(-K10 \times t^*)} - e^{(-K10^* t)})$$

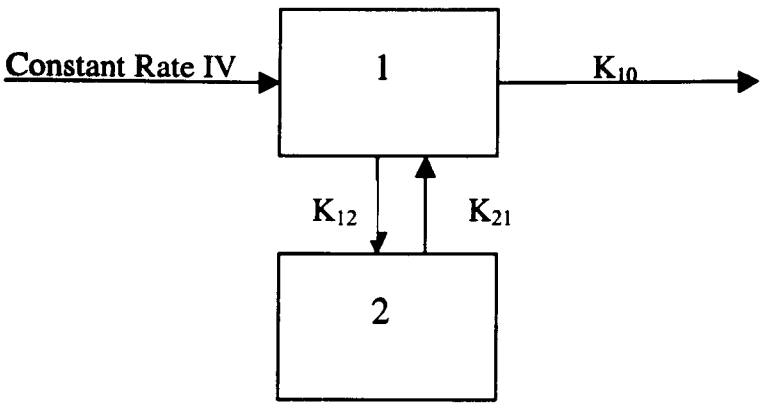
Where

C(t)= concentration in plasma at time, t
ti = length of infusion
$t^* = t - ti$ for t>ti
$t^* = 0$ for t</=ti

Estimated Parameters:

V
$CL = V \times K_{10}$

2. Two Compartment Model, Constant IV infusion, First Order Output



$$C(t) = (A.e^{-\alpha \times t} - e^{-\alpha \times t^*}) + (B.e^{-\beta \times t} - e^{-\beta \times t^*})$$

Where

$$A = (Dose/ti \times V)\,[(K_{21} - \alpha)/(\alpha - \beta) \times \alpha]$$

$$B = (-Dose/ti \times V)\,[(K_{21} - \beta)/(\alpha - \beta) \times \beta]$$

$$\alpha = \tfrac{1}{2}\,(K_{12} + K_{21} + K_{10} + \sqrt{((K_{12} + K_{21} + K_{10})^2 - 4.\,K_{21} + K_{10})})$$

$$\beta = \tfrac{1}{2}\,(K_{12} + K_{21} + K_{10} - \sqrt{((K_{12} + K_{21} + K_{10})^2 - 4.\,K_{21} + K_{10})})$$

$$\alpha.\,\beta = K_{21}\,.\,K_{10}$$

$$\alpha + \beta = K_{12} + K_{21} + K_{10}$$

Estimated Parameters:

$$V1 = D/A + B$$

$$Vss = [A.\beta^2 + B.\alpha^2 / (A.\beta + B.\alpha)^2].Dose$$

$$VT = Vss - V1 = (K_{21} / K_{12}).V1$$

$$CL = V1 \times K10$$

$$Q = VT \times K_{21}$$

APPENDIX IV

WinNonMix Final Model Outputs

Chapter 4: Midazolam

OFV

	A	B
1	Description	Value
2	Number of Subjects	19
3	Total Observations	165
4	Minimum Objective Function Value	-98.54141067
5	ML Log Likelihood	-102.3541526
6	Akaike's Information Criterion (AIC)	218.7083053
7	Schwarz's Bayesian Criterion (SBC)	240.4499236
8	-2 * ML Log Likelihood	204.7083053
9	Actual Number of Iterations	3
10	Convergence Achieved	Yes
11	Number of excluded observations	0
12	Hessian of Objective Function	Not Positive Definite

Final Fixed Effects

	A	B	C
1	Parameter	Estimate	StdError
2	V_0	13.92326391	1.64580627
3	V_1	2.7697781	1.73552704
4	V_2	-0.18560615	0.06751771
5	CL_0	0.27659139	0.03440851

Interpatient Variability

	A	B	C
1	Random Effect	V_ETA0	CL_ETA0
2	V_ETA0	2.80E-01	
3	CL_ETA0	7.98E-02	5.30E-01

Residual Error

	A	B
1	Parameter	Estimate
2	SIGMA^2	0.07481652
3	A	0.32918835
4	B	2

Chapter 5: Aminophylline

OFV

	A	B
1	Description	Value
2	Number of Subjects	75
3	Total Observations	160
4	Minimum Objective Function Value	691.7571768
5	ML Log Likelihood	-492.9087537
6	Akaike's Information Criterion (AIC)	997.8175074
7	Schwarz's Bayesian Criterion (SBC)	1016.26855
8	-2 * ML Log Likelihood	985.8175074
9	Actual Number of Iterations	3
10	Number of excluded observations	0
11	Convergence Achieved	Yes

Final Fixed Effects

	A	B	C
1	Parameter	Estimate	StdError
2	V_0	0.57139175	0.05330473
3	CL_0	0.02315136	0.00159435
4	CL_1	0.00005706	0.00005522

Interpatient Variability

	A	B	C
1	Random Effect	V_ETA0	CL_ETA0
2	V_ETA0	1.61E-01	
3	CL_ETA0	-1.03E-01	1.58E-01
4			
5	Standard Error	V_ETA0	CL_ETA0
6	V_ETA0	1.32E-01	
7	CL_ETA0	3.77E-02	3.81E-02

Residual Error

	A	B	C
1	Parameter	Estimate	StdError
2	SIGMA^2	13.02253239	3.09426006

Chapter 6: Vancomycin

OFV

	A	B
1	Description	Value
2	Number of Subjects	45
3	Total Observations	326
4	Minimum Objective Function Value	1254.446925
5	ML Log Likelihood	-926.7974242
6	Akaike's Information Criterion (AIC)	1879.594848
7	Schwarz's Bayesian Criterion (SBC)	1928.824514
8	-2 * ML Log Likelihood	1853.594848
9	Actual Number of Iterations	3
10	Convergence Achieved	Yes
11	Number of excluded observations	2
12	Hessian of Objective Function	Not Positive Definite

Final Fixed Effects

	A	B	C
1	Parameter	Estimate	StdError
2	V1_0	0.45187962	0.03799769
3	V1_1	0.09383609	0.04270538
4	CL_0	2.356579	0.18293873
5	CL_1	0.00175742	0.00069456
6	CL_2	4.30703004	0.23908122
7	V2_0	0.2505241	0.03324401
8	CLD2_0	0.08737405	0.02325717

Interpatient Variability

	A	B	C	D	E
1	Random Effect	CL_ETA0	V1_ETA0	V2_ETA0	CLD2_ETA0
2	CL_ETA0	6.37E-02			
3	V1_ETA0	1.08E-02	6.36E-02		
4	V2_ETA0			2.27E-01	
5	CLD2_ETA0			-4.35E-01	8.33E-01

Residual Error

	A	B
1	Parameter	Estimate
2	Sigma	0.01477491
3	A	286.4664686
4	B	2

Published papers not copied on instruction from the university