

POPULATION PHARMACOKINETICS AND BAYESIAN FORECASTING OF VANCOMYCIN IN  
NEONATES REQUIRING INTENSIVE CARE

by

REBECCA ELLEN WRISHKO

B.Sc., The University of Alberta, 1993

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Faculty of Pharmaceutical Sciences  
The University of British Columbia  
Vancouver, Canada

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## **ABSTRACT**

### **PURPOSE**

The primary objective of this investigation was to develop a population-based pharmacokinetic model of vancomycin in neonates that can be utilized in the individualization of drug therapy. The second objective was to evaluate the accuracy and precision of a Bayesian forecasting method, based on an optimum population pharmacokinetic model, for predicting serum vancomycin concentrations in neonates.

### **METHODS**

#### **Patients**

All neonates with a post-conceptual age (PCA) of  $\leq 44$  weeks admitted to the special care nursery (SCN) of Children's and Women's Health Centre of British Columbia (C & W) between January 01, 1996 and December 31, 1999 and prescribed vancomycin by their attending physicians were eligible for enrollment.

#### **Population Pharmacokinetic Modeling**

Population pharmacokinetic models, using an iterative stepwise approach, were developed for vancomycin with data from 185 patients using a nonlinear mixed effects modeling program (NONMEM). Significant covariates were those that resulted in a decrease in the minimum value of the objective function (MOF) of  $> 6.6$  points. Final one- and two-compartment models were evaluated with data from a naïve cohort of 65 patients.

Following model validation, combined population pharmacokinetic models were fully developed using data from all 250 patients. As with the original model development, an iterative process was implemented to generate base, full, and final models.

#### **Bayesian Forecasting**

Serum vancomycin concentration predictions based on Bayesian estimates were provided in a NONMEM generated output using the POSTHOC function. Vancomycin concentrations were independently supplied as feedback observations to the final, one- and two-compartment models to obtain case-specific predictions of vancomycin peak and trough concentrations. The

precision and accuracy of Bayesian predictions were assessed using mean absolute error and mean error, respectively, and compared using 95% confidence intervals.

## RESULTS

At all sequential stages, the one-compartment model appeared inferior to the two-compartment model. The minimum values of the objective function (MOF) from the one-compartment unadjusted, base model and revised model, were respectively, 438.52 and 29.84 points greater than the comparable two-compartment values. Weight and PCA (relative to term gestation), modeled as power functions, yielded significant reductions in the MOF when included as covariates on vancomycin clearance. Dopamine exposure was associated with a 34% decrease in vancomycin clearance. Patient weight was modeled as a linear function on the central volume of distribution. Chronic lung disease was associated with a 276% increase in the peripheral volume ( $V_p$ ). The  $V_p$  represented 50% of the volume of distribution at steady-state in the youngest patients, but only 9% in the oldest patients. Model validation demonstrated better accuracy of the two-compartment model. The final, combined models were similar, except that indomethacin was associated with a 16% decrease in vancomycin clearance in the two-compartment model.

The two-compartment model was more accurate than the one-compartment model in the Bayesian prediction of initial peak and trough concentrations in neonates < 36 weeks PCA. Bayesian predictions using trough samples as feedback yielded relative mean errors of < 3% for both initial and future peak concentrations. Relative mean absolute error was 6% and 12% for initial and future peak concentrations, respectively.

## CONCLUSIONS

The two-compartment model was superior to the one-compartment model, particularly in neonates < 36 weeks PCA. The better specified two-compartment model also generated more accurate Bayesian predictions of peak and trough concentrations in neonates < 36 weeks PCA. Single trough samples using the two-compartment model and Bayesian forecasting appear to be clinically useful for therapeutic drug monitoring of vancomycin in the SCN population.



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## LIST OF GENERAL ABBREVIATIONS AND DEFINITIONS OF TERMS

$\tau$	dosing interval
AGA	appropriate for gestational age
BPD	bronchopulmonary dysplasia
C & W	Children's and Women's Health Centre of British Columbia
<i>ca.</i>	approximately
Cl	plasma clearance of drug
CLD	chronic lung disease
C <sub>max</sub>	maximum peak concentration extrapolated back to the time immediately post-infusion
C <sub>min</sub>	minimum trough concentration extrapolated to the end of the dosing interval
CONS	Coagulase-Negative Staphylococci
C <sub>p</sub>	measured peak concentration
C <sub>pss</sub>	peak concentration at steady-state
C <sub>t</sub>	measured trough concentration
C <sub>tss</sub>	trough concentration at steady-state
CV	coefficient of variation
DA	ductus arteriosus
ELBW	extremely low birth weight
EMIT	enzyme multiplied immunoassay technique
FIA	fluorescence immunoassay
FPIA	fluorescence polarization immunoassay
GA	gestational age
GFR	glomerular filtration rate
HPLC	high-pressure liquid chromatography
IMR	infant mortality rate
K <sub>e</sub>	elimination rate constant
LBW	low birth weight
LGA	large for gestational age
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NMR	neonatal mortality rate

(continued)

**LIST OF GENERAL ABBREVIATIONS AND DEFINITIONS OF TERMS** (concluded)

PaO <sub>2</sub>	arterial oxygen tension
PCA	post-conceptional age
PDA	patent ductus arteriosus
PNA	postnatal age
PNMR	post-neonatal mortality rate
PO <sub>2</sub>	partial pressure of oxygen
RDS	respiratory distress syndrome
RIA	radioimmunoassay
SCN	special care nursery
sd	standard deviation
SGA	small for gestational age
T	time interval between Cp and Ct
t <sub>1/2</sub>	elimination half-life (one-compartment)
t <sub>1/2α</sub>	distribution half-life (two-compartment)
t <sub>1/2β</sub>	elimination half-life (two-compartment)
TDM	therapeutic drug monitoring
t <sub>i</sub>	time of infusion
V <sub>c</sub>	central compartment volume of distribution
V <sub>d</sub>	volume of distribution
V <sub>ss</sub>	volume of distribution at steady-state
VLBW	very low birth weight
V <sub>p</sub>	peripheral compartment volume of distribution
VRE	vancomycin-resistant enterococci

## LIST OF NONMEM ABBREVIATIONS AND DEFINITIONS OF TERMS

$\theta$	THETA, fixed effect
$\varepsilon$	EPSILON, random effect
$\eta$	ETA, random effect
ADVAN TRANS	PREDPP subroutines
CL	plasma clearance
CLD	chronic lung disease variable
COV	covariate variable
DOP	dopamine pharmacotherapy variable
EVID	event identification
FO	first order estimation method
IND	indomethacin pharmacotherapy variable
IPRED	individual predicted value
MAE	mean absolute error, measure of precision
ME	mean error, measure of accuracy
MOF	minimum value of the objective function
NM-TRAN	NONMEM translator
NONMEM	Nonlinear Mixed Effects Modeling
NPEM	Nonparametric Expectation and Minimization
NPML	Nonparametric Maximum Likelihood
OMEGA	variance of $\eta$ , interindividual variability
P	pharmacokinetic parameter estimate
PCA	post-conceptional age variable
POSTHOC	posterior conditional estimate
PRED	population predicted value
PREDPP	prediction for population pharmacokinetics, library of common population pharmacokinetic models
Q	intercompartmental clearance
SIGMA	variance of $\varepsilon$ , intraindividual variability
TV	typical value
V	volume of distribution

(continued)

**LIST OF NONMEM ABBREVIATIONS AND DEFINITIONS OF TERMS** (concluded)

V1	volume of distribution of central compartment
V2	volume of distribution of peripheral compartment
Vss	volume of distribution at steady-state
WT	patient weight variable

**LIST OF BAYESIAN ABBREVIATIONS AND DEFINITIONS OF TERMS**

$\sigma_{Ci}^2$	residual variance of the measured drug concentrations
$\sigma_i$	Standard deviation from random error model
$\sigma_{pj}^2$	interindividual variance of the set of pharmacokinetic parameters
C	measured drug concentrations
$C_i$	observed drug concentrations
$\hat{C}_i$	predicted drug concentrations
LS	least-squares
OBJ	objective function
P	set of pharmacokinetic parameters
$p(C)$	probability distribution of observed concentrations
$p(P)$	population parameter probability distribution
$p(P C)$	conditional probability distribution of a set of pharmacokinetic parameters
$p(C P)$	conditional probability distribution of measured drug concentrations
$P_j$	mean pharmacokinetic parameters
$\hat{P}_j$	estimate of individual's pharmacokinetic parameters

## **INTRODUCTION**

### **1.1. NEONATAL MEDICINE**

Neonatal medicine, although focused on the care of the infant after birth, requires a continuum of understanding of the physiology of normal pregnancy; placental and fetal growth, function and maturity; and any extrauterine or intrauterine pathologic events that affect the mother, placenta or fetus (Kliegman, 1998). These latter adverse events, which may result in an untoward neonatal outcome, often are interrelated and include such influences as low socioeconomic status, black race, extremes of maternal age (< 16 years, > 35 years), physical or psychological stresses, acute or chronic maternal illness, obstetric complications during the antepartum and intrapartum periods, and genetic predisposition of the fetus (Kliegman, 1998).

#### **1.1.1. Neonatal Assessment**

Every newborn is evaluated and classified at birth according to birth weight, gestational age and intrauterine growth status. Together, these factors influence patient outcome and prognosis.

Gestational age (GA) assessment is commonly a clinical estimate based upon maternal menstrual history and it represents the number of weeks from the onset of the last menstrual period until birth (Wilkins-Haug and Heffner, 1998). GA is also determined by assessing physical and neurologic characteristics that vary according to fetal age and maturity (Ballard *et al*, 1991). Physical characteristics that mature with advancing fetal age include: increasing firmness of the pinna of the ear; increasing size of the breast tissue; decreasing fine, immature lanugo hair over the back; and decreasing opacity of the skin (Kliegman, 1998). Neurologic characteristics that mature with GA include increasing flexion of the legs, hips, and arms, and decreasing laxity of the joints (Kliegman, 1998). These signs are determined during the first days of life and are assigned scores. The cumulative score provides an estimate of GA that is generally accurate to within two weeks (Kliegman, 1998). Historical maternal data, when accompanied by physical examinations, are the baseline criteria for estimating GA. Whereas, GA represents the time from conception until birth, postnatal age (PNA) reflects the chronological age (days) after birth, and post-conceptual age (PCA) is the sum of the GA and PNA. Corrected age represents PNA if the neonate had been born at 40 weeks GA (term) and



can be calculated by subtracting 40 from the PCA (weeks). This adjusted parameter is used for assessing growth parameters and developmental milestones until 2½– 3 years of age (Kliegman, 1998).

Preterm birth occurs through the end of the last day of the 37<sup>th</sup> week (259 days) following onset of the last menstrual period (Cochran, 1998). Births occurring between weeks 38 – 42 of gestation are considered term; whereas, post-term reflects births  $\geq 42$  weeks of gestation.

Although the birth weight, length, and head circumference of premature newborns may differ from those measurements of normal, undelivered fetuses at the same GA, reference fetal growth curves have usually been derived from anthropometric measurements made soon after birth (Alexander *et al*, 1996; Appendix 1). An universal birth weight classification has not been agreed upon; however, the commonly accepted definitions are as follows: macrosomia ( $> 4000$  g); normal birth weight (2700 – 4000 g); low birth weight ([LBW]  $< 2500$  g); very low birth weight ([VLBW]  $< 1500$  g); and extremely low birth weight ([ELBW]  $< 1000$  g) (Cochran, 1998). Infants can be further classified by maturity and appropriateness for GA. Birth weights between the 10<sup>th</sup> and 90<sup>th</sup> percentiles are referred to as appropriate for GA (AGA), those  $< 10^{\text{th}}$  percentile as small for GA (SGA), and those  $> 90^{\text{th}}$  percentile as large for GA (LGA) (Ehrenkranz, 2000).

Reports of longitudinal growth of VLBW infants during the past 10 – 15 years have demonstrated that, once birth weight is regained, most VLBW infants grow at rates that approximate the intrauterine growth rate of about 15 g/kg/day (Ehrenkranz, 2000). However, almost all of these infants fail to achieve “catch-up” growth, and, although growing at the targeted rate, they remain below the 10<sup>th</sup> percentile weight of the reference fetus of the same PCA at hospital discharge (Ehrenkranz, 2000). Infants who experience major morbidities, such as chronic lung disease or late-onset sepsis, also tend to gain weight more slowly (Ehrenkranz, 2000).

Resuscitation efforts at delivery are designed to assist the newborn to make the respiratory and circulatory transitions that must be rapidly accomplished. The Apgar examination, a rapid scoring system based on physiologic responses to the birth process, is an initial method for assessing resuscitation need (Kliegman, 1998). At intervals of one- and five-minutes, each of five identifiable characteristics (heart rate, respiratory effort, muscle tone, reflex irritability, and color) is assessed and assigned a value of “0” to “2” (Casey *et al*, 2001). The

total score is the sum of the five components, and a score of  $\geq 7$  indicates that the condition of the newborn is good to excellent (Casey *et al*, 2001). Term neonates with normal cardiopulmonary adaptation should score between 8 and 9 at one- and five-minutes (Kliegman, 1998). Apgar scores of 4 – 7 warrant careful observation to determine if the status will improve and to ascertain the cause, such as a pathologic condition resulting from labor, delivery, or a congenital problem that is contributing to the low Apgar score (Kliegman, 1998). By definition, an Apgar score of 0 – 3 represents either a cardiopulmonary arrest or a condition due to severe bradycardia, hypoventilation, and/or central nervous system depression (Kliegman, 1998). The five-minute score has come to be regarded as a better predictor of infant survival (Casey *et al*, 2001). Among both preterm and term infants, those with five-minute Apgar scores of 0 – 3 had the highest risk of neonatal death. In term newborns, the risk of neonatal death was 0.2 per 1000 for those with Apgar scores of 7 – 10, as compared with 244 per 1000 for those with scores of 0 – 3 at birth (Casey *et al*, 2001).

#### **1.1.2. Neonatal Mortality and Morbidity**

Most published reports provide survival as a function of birth weight or GA. In fact, both birth weight and GA exert independent effects on survival (Lorenz, 2000). The infant mortality rate ([IMR], deaths in the first year of life per 1000 live births) has demonstrated a notable decline during the 20<sup>th</sup> century (Guyer *et al*, 2000). In 1915, approximately 100 white infants per 1000 live births died in the first year of life; the rate for black infants was almost two-fold higher (Guyer *et al*, 2000). In 1996, the IMR in Canada was 5.6 per 1000 live births whereas, that in the United States was 7.3 per 1000 live births (Joseph, 2000). In 1997, the infant mortality rates in Canada and the United States were 5.5 and 7.2 per 1000 live births, respectively (Joseph, 2000). During the same year, in the United States, the IMR was 6.0 for white infants and 14.3 for black infants, a greater than two-fold difference (Guyer *et al*, 2000). Between the years 1915 and 1998, the overall IMR in the United States decreased by 93%, the neonatal mortality rate ([NMR], deaths in the first 28 days of life) by 89%, and the post-neonatal mortality rate ([PNMR], deaths from 29 days through 11 months) by 96% (Guyer *et al*, 2000).

During the early part of the century, efforts to improve environmental and living conditions in urban areas were believed to have contributed to the decline in the IMR (Guyer *et al*, 2000). The decline in the IMR slowed during the 1950s, despite medical advances, the

greater availability of prenatal care, and increases in the percentage of births that occurred in hospitals (Guyer *et al*, 2000). The decline in IMR received new momentum in the 1960s with the availability of more effective neonatal management (Guyer *et al*, 2000). After a slow down in the decline in IMR in the 1980s, two important changes were noted in the 1990s related to birth weight specific mortality rates. First, there was a large decrease in mortality for VLBW infants between 1989 and 1990, believed to be a consequence of the widespread adoption of surfactant use to prevent or reduce respiratory distress syndrome (Guyer *et al*, 2000). The second change was a drop in the PNMR for normal birth weight infants after 1989, following the American Academy of Pediatrics recommendation for the prevention of sudden infant death syndrome (Guyer *et al*, 2000).

Birth weight specific survival of infants has been reported to be < 70% for those with a birth weight of < 700 g (Cochran, 1998). For those infants with a birth weight of 700 – 800 g and > 800 g survival was found to be 80% and > 90%, respectively (Cochran, 1998). A 99% survival was documented for those infants with a birth weight exceeding 1500 g (Cochran, 1998).

Survival of extremely premature infants has been considerably higher in the last decade than previously (Lorenz, 2000). Survival varies from 5 – 41% at 23 weeks GA; from 33 – 57% at 24 weeks GA; and 60 – 79% at 25 weeks GA (Lorenz, 2000). Survival then plateaus at 26 and 27 weeks of gestation, ranging from 71 – 78% (Lorenz, 2000). Reports of survival of infants < 23 weeks GA or < 500 g birth weight are not unique; however, currently available data do not permit survival of extremely premature infants to be predicted with clinically acceptable accuracy (Lorenz, 2000). Approximately 10% of all births in the United States are preterm, almost 2% are < 32 weeks of gestation and 1% < 1500 g; however, about 75 – 85% of neonatal deaths of normally formed infants are related to preterm delivery (Chescheir and Hansen, 1999).

Infants born at < 24 weeks GA are at high risk for developmental delay. The incidence of sensory impairment, specifically visual impairment, is 25 – 30% in this group (Kliegman, 1998). Approximately 30% of these infants will have mental retardation, some of whom will be multihandicapped (Kliegman, 1998). Approximately 30% demonstrate delays in learning and learning disabilities (Kliegman, 1998), and educational disadvantage associated with VLBW persists into early adulthood (Hack *et al*, 2002). Most LBW infants survive neonatal illnesses without long-term sequelae (Kliegman, 1998). Between 10% and 25% of survivors have mild

developmental problems, and 5 – 10% exhibit severe developmental problems (Kliegman, 1998). The lower the birth weight of the infant, the higher the illness indices and the higher the risk for more pronounced delay.

### **1.1.3. Sequelae of Prematurity**

Problems of prematurity related to difficulty in extrauterine adaptation due to immaturity of organ systems include respiratory, cardiovascular, gastrointestinal, and immunologic complications. Other common sequelae include neurologic, hematologic, nutritional, metabolic, renal, thermoregulation, and ophthalmologic disorders.

#### *1.1.3.1. Respiratory Distress Syndrome*

Respiratory distress syndrome (RDS) is the major cause of morbidity and mortality in preterm neonates (Kliegman, 1998). The primary cause of RDS is inadequate pulmonary surfactant. In addition to the developmental deficiency, surfactant synthesis may be reduced as the result of hypovolemia, hypothermia, acidosis, and hypoxemia (Kliegman, 1998). Pulmonary surfactant (lecithin) decreases the surface tension at the air/fluid interface in the alveoli and prevents alveolar collapse. Surfactant also facilitates the clearance of pulmonary fluid, prevents pulmonary edema, and stabilizes alveoli during aeration (Kliegman, 1998). At birth, the clearance of residual fetal lung fluid is accompanied by an increase in pulmonary blood flow that facilitates the transition from fetal to adult circulation (Liley and Stark, 1998).

The timing of surfactant production in quantities sufficient to prevent alveolar collapse (atelectasis) is dependent upon an increase in fetal cortisol levels that begins at 32 -34 weeks of gestation (Kliegman, 1998). Therefore, the incidence and severity of RDS increase as GA decreases. Nonetheless, RDS develops in only 30 – 60% of infants between 28 and 38 weeks of gestation, but in 60 – 80% of neonates born at 26 – 28 weeks GA (Kliegman, 1998). Other risk factors include: delivery of a previous preterm infant with RDS, maternal diabetes, hypothermia, asphyxia, male gender, Caucasian race, delivery by cesarean section without labor (Kliegman, 1998).

The manifestations of this disease are caused by diffuse alveolar atelectasis, resulting in poor gas exchange (hypoxemia, hypercapnia) (Kliegman, 1998). As atelectasis increases, lung compliance decreases and compensatory respiratory pressures are increased (Kliegman, 1998).

The extremely compliant neonatal chest wall does not permit the large negative inspiratory pressure necessary to open the alveoli, resulting in increased work of breathing and erratic ventilation (Kliegman, 1998). Aeration of the surfactant-deficient lung also results in the cyclic collapse and distention of bronchioles, with resultant cell injury and death (Liley and Stark, 1998). This epithelial damage causes pulmonary edema by allowing fluid and proteins to leak from the intravascular space into the air spaces and interstitium of the lung (Liley and Stark, 1998). The necrotic epithelial debris and proteins then form fibrous hyaline membranes (Liley and Stark, 1998). Atelectasis is well documented by the chest radiograph, which demonstrates a "ground-glass" haze in the lung surrounding air-filled bronchi (Kliegman, 1998). Severe RDS may demonstrate an airless lung field or a "whiteout" on x-ray, even obliterating the distinction between the atelectatic lungs and the heart (Kliegman, 1998).

Significant advances made in the management of RDS include the development of prenatal diagnosis, disease prevention by maternal glucocorticoid treatment in pregnancies < 34 weeks of gestation, improvements in perinatal care, advances in respiratory support, and surfactant replacement therapy (Liley and Stark, 1998). Synthetic (lecithin, tyloxapol, hexadecanol) or natural (lecithin-fortified bovine lung extract) surfactants can be administered repeatedly during the course of RDS in patients receiving endotracheal intubation, mechanical ventilation, and oxygen therapy (Kliegman, 1998). Acute complications of RDS include: pulmonary barotrauma (pneumothorax, interstitial emphysema); infections (primary or secondary to invasive monitoring devices); intracranial hemorrhage; and patent ductus arteriosus. Also, chronic lung disease in neonates occurs in 5 – 30% of survivors of respiratory therapy for RDS, neurologic impairment is estimated to occur in 10 – 15% of RDS survivors and these patients are at risk for retinopathy of prematurity (Liley and Stark, 1998).

#### *1.1.3.2. Patent Ductus Arteriosus*

Patent ductus arteriosus (PDA) is not particularly common in term newborns and rarely causes congestive heart failure in this patient population (Kirsten, 1996). However, the frequency with which premature neonates will develop a hemodynamically significant left-to-right shunt through a PDA is inversely proportional to advancing GA and weight. In a study of almost 1700 infants with birth weights < 1750 g, a hemodynamically significant PDA was noted in 80% of infants with birth weights < 1000 g, 21% of infants with birth weights of

1000 – 1500 g, and only 7% of those with birth weights of 1500 – 1750 g (Burns Wechsler and Wernovsky, 1998).

The ductus arteriosus (DA) is a shunt blood vessel of fetal life; it extends between the pulmonary artery and aorta (Appendix 2). During fetal life, the DA is the primary outflow channel for blood flow from the main pulmonary artery and acts to divert blood into the descending aorta and placenta (Kirsten, 1996). Pulmonary artery pressure is high and aortic pressure is low therefore, the flow is right-to-left (Smith, 1998).

Patency of the DA *in utero* appears to be maintained through the combined effects of a low partial pressure of oxygen ( $PO_2$ ) and a high level of circulating vasodilatory prostaglandins ( $PGE_1$ ,  $PGE_2$ , and prostacyclin) (Kirsten, 1996).  $PGE_2$  and prostacyclin ( $PGI_2$ ) are formed within the wall of the DA and may exert their action locally on muscle wall; however, the ductus appears to be more sensitive to  $PGE_2$  (Kirsten, 1996). Prostaglandin concentrations are elevated in the fetus because blood flow through the lungs, where prostaglandins are metabolized, is minimal and there is increased production in the placenta (Kirsten, 1996).

After birth, pulmonary arterial pressure falls following inflation of the lungs and aortic pressure rises with the removal of the low-resistance placental vascular bed (Smith, 1998). The decrease in pulmonary vascular resistance and an increase in systemic vascular resistance results in an increased pulmonary blood flow and a rise in the arterial oxygen tension ( $PaO_2$ ), and  $PGE_2$  is metabolized from the circulation that perfuses the pulmonary vessels (Kirsten, 1996). The combined effect of the greater  $PaO_2$  and decreased concentration of circulating prostaglandins is constriction of the DA (Kirsten, 1996). Functional closure or constriction of the DA occurs soon after birth in healthy, term infants, usually within the first few days of life, and it anatomically closes within three months (Kirsten, 1996).

Failure of the DA to constrict after birth may occur as a result of structural or biochemical abnormalities, which may be genetic or environmental in origin (Kirsten, 1996). In the premature neonate, the smooth muscle of the immature ductus demonstrates a diminished response to oxygen and a greater sensitivity to the dilating actions of prostaglandins (Kirsten, 1996). Circulating concentrations of  $PGE_2$  are often elevated in premature neonates because pulmonary metabolism of prostaglandins is reduced (Kirsten, 1996). These two factors contribute to the delayed closure of the DA in this fragile population (Kirsten, 1996). Further, when RDS improves and pulmonary vascular resistance declines, flow through the DA increases

in a left-to-right direction (Kliegman, 1998). Also, excessive intravenous fluid administration may increase the incidence of PDA (Kliegman, 1998).

There are certain classical physical findings that are often diagnostic of a PDA in premature neonates, which become evident during the first week of life (Kirsten, 1996). The most common symptom of PDA is a heart murmur, which may be continuous in systole and diastole, but usually only the systolic component can be auscultated (Kliegman, 1998). Additional signs of a PDA include increased pulse amplitude, decreased urinary output, and a widened pulse pressure (Kirsten, 1996). As the left ventricular function begins to deteriorate and the neonate develops signs of congestive heart failure, systemic hypoperfusion becomes evident as blood to the peripheral circulation decreases (Kirsten, 1996). A chest x-ray demonstrates cardiomegaly and pulmonary edema; a two-dimensional echocardiogram demonstrates patency; whereas, Doppler studies demonstrate markedly increased left-to-right flow through the ductus (Kliegman, 1996).

Initial medical management includes increased ventilatory support, fluid restriction, and diuretic therapy (Burns Wechsler and Wernovsky, 1998). Indomethacin, a prostaglandin synthetase inhibitor, is administered intravenously (0.2 mg/kg) every 12 hours for three doses or 0.1 mg/kg for five doses at 24-hour intervals. Routine prophylaxis with indomethacin during the first days of life to prevent the development of symptomatic PDA in mechanically ventilated newborns weighing < 1500 g is still controversial (Schmidt *et al*, 2001; Fowlie, 1996). Although prophylactic indomethacin reduces the frequency of PDA and severe periventricular and intraventricular hemorrhage, it does not improve the rate of survival without neurosensory impairment (Schmidt *et al*, 2001). Contraindications to indomethacin include: thrombocytopenia (< 80,000); hemorrhage, serum creatinine > 100  $\mu$ mol/L; blood urea nitrogen > 7 mmol/L, oliguria ( $\leq$  0.5 mL/kg/hr), necrotizing enterocolitis (NEC) and evolving intraventricular hemorrhage (Kliegman, 1998). Indomethacin is effective in approximately 80% of symptomatic patients (Burns Wechsler and Wernovsky, 1998). Since 20 – 30% of neonates initially do not respond to indomethacin and, of those who do, the DA reopens in 10%, a repeated course of indomethacin or surgical ligation is required in a large number of patients (Kliegman, 1998).

### 1.1.3.3. *Chronic Lung Disease*

Bronchopulmonary dysplasia (BPD) is a form of chronic lung disease (CLD) in neonates that often follows RDS in the VLBW newborn. Infants are considered to have BPD if they continue to require supplemental oxygen to maintain adequate oxygenation after 28 days of life or at 36 weeks PCA, with radiographic changes consistent with abnormal lung parenchyma (Parad and Berger, 1998). Failure of RDS to improve after two weeks and the need for prolonged mechanical ventilation are characteristic of patients who develop BPD (Parad and Berger, 1998). Recent reports suggest that morphological changes are present throughout life and that BPD does not start at 28 days of age (Hislop, 1997).

Approximately 1% of all infants develop RDS, reflecting pulmonary immaturity (Zimmerman, 1995). Generally, 20 – 30% of patients with RDS develop BPD, the most common form of CLD. Despite advances in the prevention and management of RDS, BPD still presents as one of the major complications in mechanically ventilated premature infants (Eber and Zach, 2001). Acceptance of modest hypercapnia with less aggressive application of positive pressure ventilation and reduction in the use of high oxygen concentrations has led to a decrease in the incidence of BPD in infants with a birth weight > 1500 g (Eber and Zach, 2001). However, with increased survival of extremely premature infants (24 – 26 weeks GA), who are most likely to develop BPD, the overall incidence has remained high (Eber and Zach, 2001). The risk of developing BPD increases with decreasing birth weight and GA, ranging from 50% in neonates 700 – 900 g to 5% in those with birth weights > 1250 g (Eber and Zach, 2001).

Since the original description of BPD by Northway *et al* (1967), its pathogenesis has included the combined iatrogenic insults of oxygen toxicity and barotrauma inflicted on an immature lung over a prolonged period of time. Although the etiology of BPD is unclear, several factors likely contribute to its development: prematurity, positive pressure ventilation, protracted use of endotracheal tubes, pulmonary edema, and pulmonary air leak (Parad and Berger, 1998). Oxygen concentrations above 40% are toxic to the neonatal lung (Kliegman, 1998). Inadequate antioxidant enzyme activity or deficiency of free-radical sinks, or both, may predispose the lung to oxygen toxicity (Parad and Berger, 1998). *In utero* or perinatal acquisition of microorganisms may contribute to BPD etiology or modification of the clinical course (Parad and Berger, 1998).



The pathogenesis of acute lung injury follows that cellular and interstitial injury results in the release of mediators that cause secondary changes in alveolar permeability and recruit inflammatory cells into interstitial and alveolar spaces; this in turn causes leakage of water and protein (Parad and Berger, 1998). In the chronic phase of lung injury, the interstitium may be altered by fibrosis and cellular hyperplasia that has resulted from excessive release of growth factors and mediators; interstitial fluid clearance is disrupted, resulting in pulmonary fluid retention (Parad and Berger, 1998). The histopathology of BPD reveals interstitial edema, atelectasis, mucosal metaplasia, interstitial fibrosis, necrotizing obliterative bronchiolitis, and overdistended alveoli (Kliegman, 1998).

The clinical manifestations of BPD are oxygen dependence, hypercapnia, compensatory metabolic alkalosis, pulmonary hypertension, and the development of right-sided heart failure (Kliegman, 1998). Increased airway resistance, with reactive airway bronchoconstriction, is also noted (Kliegman, 1998). Severe chest retractions produce very negative interstitial pressure that draws fluid into the interstitial space (Kliegman, 1998).

The goals of treatment are to minimize further lung injury, maximize nutrition, and diminish oxygen consumption. Ventilator adjustments are made to minimize airway pressures while providing adequate gas exchange (Parad and Berger, 1998). Diuretics indirectly attenuate symptoms of respiratory distress and result in decreased respiratory system resistance and increased dynamic compliance; gas exchange is variably affected (Parad and Berger, 1998). The clinical improvement is likely due to decreased lung water content, with decreased interstitial and peribronchial fluid resulting in less resistance and better compliance (Parad and Berger, 1998). Acute obstructive episodes or chronically increased resistance may be related to increased airway tone or bronchospasm and may respond to bronchodilator therapy (Parad and Berger, 1998).

Treatment with glucocorticoids in infants who remain ventilator-dependent for two to three weeks results in an improvement in pulmonary mechanics and gas exchange, facilitating the discontinuation of mechanical ventilation and possibly reducing the duration of oxygen therapy and the progression to severe BPD (Bancalari, 1998). The mechanism of action may be related to diminished inflammation and fibrosis or increased functional surfactant (Parad and Berger, 1998). In spite of this, steroid therapy does not appear to have a substantial impact on long-term outcomes, such as duration of supplemental oxygen requirement, length of hospital

stay, or mortality (Parad and Berger, 1998). Dexamethasone is administered intravenously or orally at a dose of 0.25 mg/kg/dose twice daily for three days, followed by a slow taper, depending on clinical response and complications (Parad and Berger, 1998). Systemic steroid administration is frequently associated with acute, and occasionally long-term adverse effects. Common acute complications include glucose intolerance, systemic hypertension, hyperkalemia, hypocalcemia and a transient catabolic state (Parad and Berger, 1998). Total neutrophil counts, band counts, and platelet counts increase during steroid treatment (Parad and Berger, 1998). Hypertrophic cardiomyopathy and adrenal suppression are transient (Parad and Berger, 1998). Dexamethasone treatment has been reported to have both transient and sustained negative effects on growth (Stark *et al*, 2001).

Infants with BPD survive (> 80%) with significant pulmonary sequelae (Eber and Zach, 2001). Tachypnea, retractions, dyspnea, cough, and wheezing can be seen for months to years in seriously affected children (Parad and Berger, 1998). Although complete clinical recovery can occur, underlying pulmonary function, gas exchange, and chest x-ray abnormalities may persist beyond adolescence (Parad and Berger, 1998). The rehospitalization rate for respiratory illness during the first two years of life is approximately twice that of matched control infants (Parad and Berger, 1998).

#### 1.1.3.4. Neonatal Infectious Disease

Systemic and local infections are common in the newborn period. Bacterial sepsis and meningitis continue to be major causes of morbidity and mortality in the newborn (Guerina, 1998). The overall incidence of neonatal sepsis varies between 1 and 8 cases per 1000 live births (Guerina, 1998). Multiple obstetric and neonatal risk factors for perinatal infections have been identified. Obstetric factors include premature onset of labor, premature rupture of membranes, and maternal peripartum infection (Guerina, 1998). The single most important neonatal risk factor is LBW. The frequency of sepsis is reportedly eight times higher in VLBW neonates (1000 – 1500 g) than in LBW neonates (1500 – 2500 g), and meningitis occurs 3 – 17 times more often in newborns weighing < 2500 g than those weighing ≥ 2500 g (Guerina, 1998). This increased prevalence among the very premature is a consequence of their more immature immunologic system and their prolonged period of hospitalization, which conveys the added risk of nosocomially acquired infectious diseases (Kleigman, 1998). The overall incidence of

nosocomial infections in neonates is  $< 5\%$ ; however, factors including PNA, LBW, foreign bodies, nursery crowding, surgery, and prolonged treatment with broad-spectrum antibiotics increase the risk of infection (Guerina, 1996).

## 1.2. VANCOMYCIN

### 1.2.1. Development

Since their discovery, antimicrobial drugs have demonstrated effectiveness for the control of bacterial infections. As the mechanisms and epidemiology of resistance to antimicrobial agents have been elucidated, it appears that bacteria develop resistance through an array of mechanisms. Initially, the problem of staphylococcal resistance to penicillins was overcome by the discovery of new classes of drugs, such as aminoglycosides, macrolides, and glycopeptides, in addition to chemical modification of existing therapeutic agents (Greenfield and Smith, 1983; Milliken, 1988; Gold and Moellering, 1996).

Vancomycin is a glycopeptide antibiotic first isolated in 1956 from a strain of *Streptomyces* (now *Amycolatopsis*) *orientalis* found in soil samples from a Borneo jungle (Greenfield and Smith, 1983; Cheung and DiPiro, 1986; Milliken, 1988; Wilhelm and Estes, 1999). Following the clinical introduction of vancomycin in 1958, it became an important agent for use against increasingly prevalent penicillin-resistant staphylococci and other gram-positive bacteria (Wilhelm and Estes, 1999). In this regard, vancomycin was so-named for its ability to vanquish the emerging strains of  $\beta$ -lactamase producing staphylococci (Griffith, 1981; Matzke, 1986).

Early preparations of the compound were named "Mississippi mud" due to the appearance of visible impurities (Griffith, 1981). These impurities were thought to be responsible for the thrombophlebitis reported following intravenous administration (Griffith, 1981; Milliken, 1988). Despite improvement of the vancomycin formulation, development of semisynthetic penicillins and cephalosporins that demonstrated equivalent activity and less toxicity resulted in a marked reduction in the clinical use of vancomycin (Cunha and Ristuccia, 1983; Greenfield, 1983; Matzke, 1986; Wilhelm and Estes, 1999). The subsequent emergence of increasingly resistant gram-positive bacteria, particularly methicillin-resistant staphylococci, led to a resurgence of interest in vancomycin (Wilhelm and Estes, 1999). In this regard, vancomycin

use increased 20-fold, in a university hospital, from 1981 to 1991 (Ena *et al*, 1993). Also, vancomycin is finding new applications as medical technology has advanced the disciplines of neonatology and oncology (Levine, 1987).

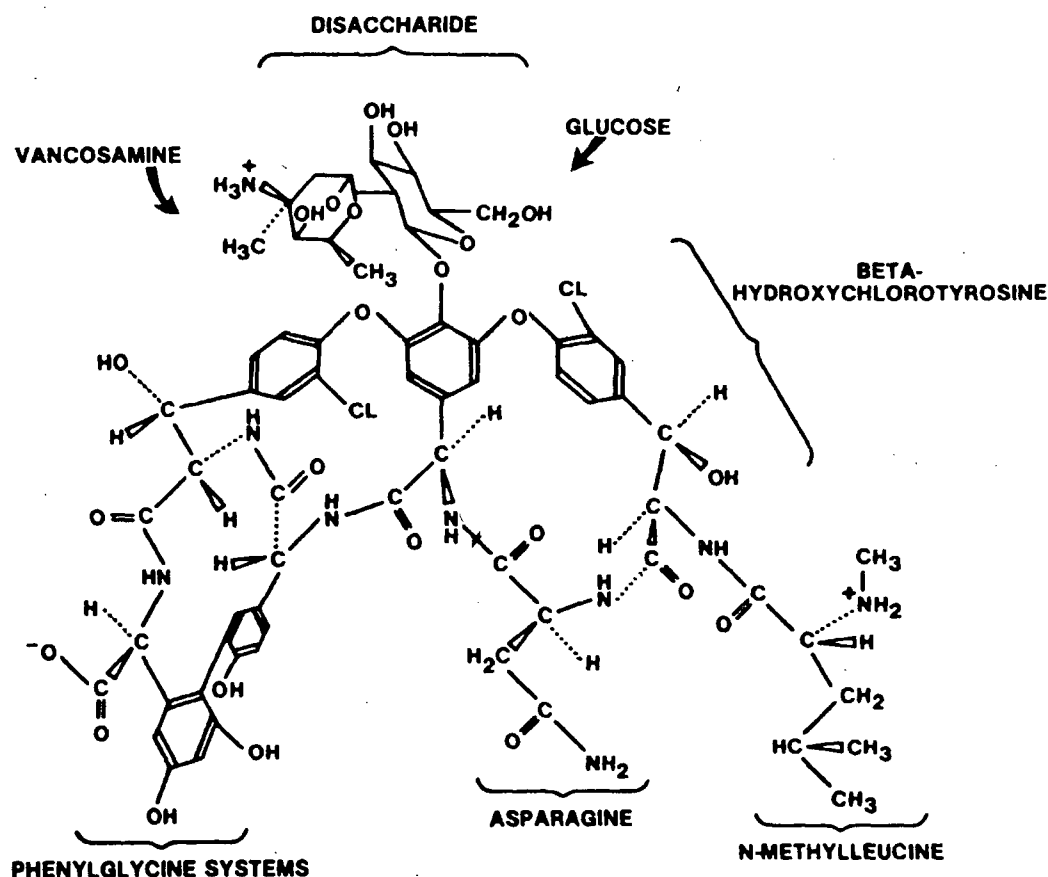
### 1.2.2. Chemistry

Although vancomycin was first introduced in 1956, its structure was not fully elucidated until 1982 (Barna and Williams, 1984). Vancomycin has an empirical formula of  $C_{66}H_{75}Cl_2N_9O_{24}$  (Figure 1) and a molecular weight of 1448 D (Sheldrick *et al*, 1978). It is a tricyclic glycopeptide in which two chlorinated  $\beta$ -hydroxytyrosine units, three substituted phenylglycine systems, N-methyllucine and asparagine are interconnected in a seven-member peptide chain (Sheldrick *et al*, 1978). One of the phenylglycine units possesses a disaccharide composed of glucose and the unique amino sugar, vancosamine (Cheung and DiPiro, 1986).

Vancomycin exerts its primary bactericidal effect by inhibiting the biosynthesis of peptidoglycan, the major structural polymer of the bacterial cell wall (Reynolds, 1989). Loss of the vancosamine disaccharide results in only minimal loss of activity; however, substitution of the amide groups in the asparagine substituent causes complete loss of bactericidal activity (Marshall, 1965). The drug complexes with the D-alanyl-D-alanine component of peptide precursor units at the site of attachment and thereby interferes with the utilization of the lipid-phosphodisaccharide-pentapeptide complex in glycopeptide synthesis (Levine, 1987; Wilhelm and Estes, 1999). Vancomycin inhibits the second stage of peptidoglycan synthesis at a site antecedent to the penicillin site of action; thus, no cross-resistance occurs (Wilhelm and Estes, 1999).

### 1.2.3. Spectrum of Activity

Pharmacodynamic studies, both *in vitro* and *in vivo*, suggest that vancomycin exhibits concentration-independent killing (Larsson *et al*, 1996). Once vancomycin concentrations exceed the minimal bactericidal concentrations (MBC) or are approximately four to five times the minimal inhibitory concentration (MIC), further increases in serum concentrations do not increase the kill rate (Wilhelm and Estes, 1999). The time during which the concentration exceeds the MIC of the organism may be the most important pharmacodynamic factor in predicting efficacy of this agent (MacGowan, 1998).



**Figure 1. Structure of Vancomycin.** A tricyclic glycopeptide with an empirical formula of  $C_{66}H_{75}Cl_2N_9O_{24}$  and a molecular weight of 1448 D (Adapted from Greenfield and Smith, 1983).

Vancomycin and teicoplanin, the other glycopeptide antibiotic in clinical use, differ from the  $\beta$ -lactams in that they exert their effect in the second stage of cell wall synthesis. An organism is defined as being sensitive to vancomycin if the MIC is  $\leq 5$  mg/L; whereas, an intermediate level of vancomycin resistance is defined by a MIC of 8 – 16 mg/L (Milliken, 1988). The majority of strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* (methicillin-sensitive and methicillin-resistant) are sensitive to vancomycin (Appendix 3). Anaerobic and microaerophilic streptococci are usually sensitive to vancomycin, as are most

clostridia; susceptibility to vancomycin among *Actinomyces* species is not predictable, however (Wilhelm and Estes, 1999). Gram-negative bacteria are generally resistant, except for occasional isolates of *Neisseria gonorrhoeae* (Jaffe *et al*, 1981). For *Enterococcus faecium* and *Enterococcus faecalis*, vancomycin concentrations of  $\geq 100$  mg/L may be required for a bactericidal effect (Geraci and Hermans, 1983). The addition of streptomycin provides a synergistic bactericidal effect against 40% to 70% of enterococcal isolates (Wilhelm and Estes, 1999). Excluding isolates that exhibit high-level gentamicin resistance ( $\geq 500$  mg/L), the combination of vancomycin and gentamicin is bactericidal against most vancomycin-sensitive enterococcal organisms and is indicated to treat the most serious infections, such as endocarditis and meningitis (Moellering, 1981; Wilhelm and Estes, 1999).

Although vancomycin-resistant strains of most gram-positive microorganisms encountered in clinical practice remain rare, there has been a relatively dramatic increase in the prevalence of vancomycin-resistant enterococci (VRE) during the past 20 years (CDC, 1993). Data reported to the National Nosocomial Infection Survey of the Centers for Disease Control and Prevention revealed that vancomycin resistance had increased more than 20-fold among nosocomial isolates of enterocci, from  $< 0.5\%$  in 1989 to  $> 10\%$  in 1995 (Gaynes *et al*, 1996). Almost 15% of enterococci isolated from intensive care units currently exhibit vancomycin resistance (Moellering, 1998). Among patients with VRE bacteremia, many of whom have serious underlying pathology, the mortality rate attributable to the sepsis may approach 50% (Shay *et al*, 1995).

Outbreaks of VRE may be monoclonal (Handwerger *et al*, 1993) or due to multiple strains (Boyle *et al*, 1993). Four vancomycin-resistant phenotypes, *vanA*, *vanB*, *vanC*, and *vanD*, have been observed (Wilhelm and Estes, 1999). Most of the strains identified in the United States express the *vanA*-resistance phenotype, which is characterized by high-level resistance to both vancomycin and teicoplanin, inducible by either compound (Wilhelm and Estes, 1999). The *vanB* phenotype typically displays inducible vancomycin resistance with preservation of teicoplanin sensitivity (Wilhelm and Estes, 1999). Genes determining the *vanA* and *vanB* phenotypes are located on transmissible genetic elements that may be located on plasmids or may insert into bacterial chromosomes (Wilhelm and Estes, 1999). Acquired glycopeptide resistance in enterococci is mediated by complex operons encoding an alternative biosynthetic pathway for the production of a modified cell wall component (peptidoglycan precursor) that

binds vancomycin with a small fraction of the avidity of the normal precursor (Walsh, 1993; Arthur, 1995). In this context, polymerization of the cell wall peptidoglycan proceeds unimpeded (Walsh, 1993; Arthur, 1995).

#### 1.2.4. Toxicity and Adverse Effects

Several side effects have been associated with vancomycin use; some of them attributed to the presence of substantial impurities in the early preparation of the drug. Fever, chills, and phlebitis at the site of infusion are less frequent with the present purified formulation and better awareness of proper administration (Fekety, 1982).

An infusion-associated reaction that is peculiar to vancomycin is referred to as the "red man" syndrome (Wilhelm and Estes, 1999). It appears to be a dose-related phenomenon in that it occurs as a result of rapid infusion of vancomycin and is associated with a rapid increase in serum drug concentration. The reaction is thought to be mediated by a nonimmunological release of histamine and is characterized by one or more of the following: pruritus; an erythematous rash involving the face, neck, and upper torso; and, occasionally, hypotension (Healy *et al*, 1990; Polk *et al*, 1988, Wilhelm and Estes, 1999). This complication can be avoided by administering vancomycin over at least one-hour.

Ototoxicity, considered to be the major systemic side effect of current vancomycin therapy, is characterized by damage to the auditory nerve, eventually leading to permanent hearing loss (Bailie and Neal, 1988). Although a correlation between ototoxicity and serum concentration has not been established, it is generally considered that serum concentrations in the range of 80 to 100 mg/L are associated with auditory toxicity (Kirby *et al*, 1960). Further, the risk of ototoxicity appears to be increased when vancomycin is administered in combination with an aminoglycoside (Wilhelm and Estes, 1999).

Nephrotoxicity has been associated with vancomycin administration; however, it is unclear whether this is due to underlying pathology, concurrent therapy with other nephrotoxic agents, or to the antibiotic itself (Bailie and Neal, 1988). Evidence suggests that the risk increases with increasing serum concentrations, but a well-defined association has not been established (Bailie and Neal, 1988).

Reports of acute nephrotoxicity following a single overdose of vancomycin in neonates and preterm infants are rare (Bhatt-Mehta *et al*, 1999; Müller *et al*, 1999). Burkhart *et al* (1992)

described an infant who was treated with vancomycin for necrotizing enterocolitis and who was inadvertently administered a 20-fold overdose for six doses. The patient exhibited only transiently altered renal function, which returned to normal values after oral treatment with activated charcoal. Other reports of transient pediatric vancomycin nephrotoxicity were complicated by concomitant aminoglycoside therapy (Odio *et al*, 1984; Tissing *et al*, 1993).

Other side effects reported with the use of vancomycin include neutropenia and thrombocytopenia (Linder *et al*, 1993). These resolved following cessation of therapy and were not correlated with vancomycin serum concentrations.

### **1.3. THERAPEUTIC INDICATIONS FOR VANCOMYCIN**

#### **1.3.1. General Uses**

The resurgence in the use of vancomycin has been partially due to the increased prevalence of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (Wilhelm and Estes, 1999). These organisms are important pathogens in patients with prosthetic devices, in whom they have produced significant morbidity and mortality. The resultant infections include prosthetic valve endocarditis, prosthetic joint infections, and cerebrospinal fluid shunt infections (Inman *et al*, 1984). Empiric antimicrobial therapy for seriously ill patients with these infections includes vancomycin in the initial management, pending culture and sensitivity results (Levine, 1987). Vancomycin is also used to treat serious infections caused by gram-positive microorganisms, staphylococci and streptococci, when the use of a penicilloyl derivative is precluded due to hypersensitivity reactions (CDC, 1994)

Although vancomycin is effective for the treatment of staphylococcal endocarditis, it may be considerably less effective than nafcillin for the treatment of methicillin-sensitive *Staphylococcus aureus* endocarditis (Small and Chambers, 1990). The combination of vancomycin and rifampin, with adjuvant gentamicin for the first two weeks, is used in the treatment of prosthetic valve endocarditis due to methicillin-resistant Coagulase Negative Staphylococci (CONS) (Wilhelm and Estes, 1999). Cerebrospinal fluid shunt-related infections due to CONS are occasionally successfully treated with a combination of intravenously and intrathecally administered vancomycin; however, removal of the shunt is often necessary for clinical cure (Bayston *et al*, 1987; Swayne *et al*, 1987).



Vancomycin is recommended for endocarditis prophylaxis in high-risk patients who must undergo invasive genitourinary or gastrointestinal procedures likely to be associated with transient bacteremia (CDC, 1994). Also, it may be an effective prophylactic agent during surgical procedures involving implantation of prosthetic materials or devices in medical centers where methicillin-resistant staphylococcal infections are common (CDC, 1994).

Vancomycin is the drug of choice for infections caused by resistant corynebacteria, including *Corynebacterium jeikeium* and multiple resistant strains of *Streptococcus pneumoniae* (Wilhelm and Estes, 1999). Metronidazole has replaced orally administered vancomycin as the drug of choice for treating antibiotic-associated *Clostridium difficile* colitis (CDC, 1994). Oral vancomycin is reserved for instances of metronidazole failure and for use in seriously ill patients.

Nosocomial infections are a significant cause of morbidity and mortality in the neonatal intensive care unit (NICU) (Stoll *et al*, 1996). The distribution of pathogens causing sepsis in a specific medical center is usually considered when empiric antibiotics are selected, and during the past decade CONS have emerged as the major pathogen in NICUs (Baier *et al*, 1998). In a study of the epidemiology of vancomycin use at a children's hospital, Sinkowitz *et al* (1997) reported that the highest frequency of vancomycin use was on the neonatology service and was reported to be 28 per 100 admissions.

### 1.3.2. Neonatal Sepsis

Neonatal sepsis presents during two periods. Early onset sepsis presents in the first seven days of life; it often begins *in utero* and is usually caused by an ascending bacterial infection from the maternal genitourinary tract (Polin and St. Geme, 1992). Generally, early-onset sepsis is a multi-system fulminant illness with prominent respiratory symptoms; however, early manifestations may be nonspecific (Polin and St. Geme, 1992). Among VLBW neonates, the incidence of early-onset sepsis increases with decreasing GA, 19 cases per 1,000 live births compared to 2.5 cases per 1,000 full-term live births (Polin, 2001). Early-onset sepsis is associated with a mortality of 10% to 20%; a higher frequency is observed in premature neonates (Baker, 1997). Risk factors for early-onset sepsis include: vaginal colonization with group B streptococcus; prolonged rupture of membranes (> 24 hours); chorioamnionitis; maternal fever or leukocytosis, fetal tachycardia, and preterm birth (Kliegman, 1998). The predominance of neonatal sepsis in males suggests the possibility of a sex-linked factor related to host

susceptibility (Polin and St. Geme, 1992). Investigators have postulated a gene located on the X chromosome involved with thymus function or immunoglobulin synthesis; however, definitive evidence has not been obtained (Polin and St. Geme, 1992).

Late-onset sepsis may occur as early as five days of age; however, it is more common after the first week of life and is generally caused by a nosocomial infection (Karlowitz *et al*, 2000). Approximately, 25% of VLBW neonates will develop one or more episodes of confirmed late-onset sepsis (Polin, 2001). The most frequently identified contributing factors to nosocomial infections are PNA, LBW, invasive devices for monitoring and support, and prolonged treatment with broad-spectrum antibiotics (Poin and St. Geme, 1992). Over the past 20 years, there has been a shift in the predominant pathogens for neonatal late-onset sepsis. Hemming *et al* (1976) reported that, during the period from 1970-1974, *Staphylococcus aureus* and gram-negative enteric bacilli accounted for the majority of late-onset infections. Recently, CONS have emerged as the most frequently isolated pathogens, responsible for 30% of late-onset sepsis cases (Karlowitz, *et al*, 2000). Other pathogens associated with late-onset sepsis include: *Staphylococcus aureus*, *Enterococcus*, *Klebsiella sp.*, *Enterobacter*, *Pseudomonas aeruginosa*, and fungi (especially *Candida albicans* (Polin, 2001).

The initial diagnosis of sepsis is, by necessity, a clinical one, because it is imperative to begin treatment before results of cultures are available. Clinical signs and symptoms of sepsis are nonspecific, and the differential diagnosis is broad, including RDS, metabolic diseases, hematologic disease, central nervous system disease, cardiac disease, and other infectious processes (Appendix 4) (Polin, 2001).

Treatment is most often begun before a definite etiologic agent is identified. For neonates who become infected during the first week of life, empiric therapy must cover group B streptococci, enterococci, *Listeria*, and Enterobacteriaceae. A combination of ampicillin and gentamicin is generally effective against all of these microorganisms. In late-onset sepsis, the pathogens of the institution must be considered when antibiotics are selected; however, generally, antimicrobial coverage with vancomycin and cefotaxime is initiated, as 40% to 80% of CONS are methicillin-resistant (Guerina, 1998). Continuing therapy is based on culture and sensitivity results.

### 1.3.3. Neonatal Necrotizing Enterocolitis

Neonatal necrotizing enterocolitis (NEC) is an acquired disorder representing an expression of serious intestinal injury following a combination of vascular, mucosal, and possibly toxic insults to a relatively immature gut (Faix and Adams, 1994). Necrotizing enterocolitis is the most common cause of intestinal perforation during the neonatal period; however, not all cases of NEC result in perforation (Faix and Adams, 1994). A large, multicenter survey resulted in an estimated incidence of 10.1% for definite NEC and 17.2% for suspected NEC among VLBW infants (Uauy *et al*, 1991). In most centers, NEC occurs in 2% to 5% of all NICU admissions and 5% to 10% of VLBW neonates (McAlmon, 1998). Overall mortality is 9% to 28%, regardless of surgical or medical intervention and has declined over time; however, it trails only RDS as a leading cause of neonatal death (Brans *et al*, 1982; McAlmon, 1998).

The underlying mechanisms by which NEC develops appear to involve complex interactions between mucosal injury and poor host protective mechanisms in response to injury (McAlmon, 1998). Various insults known to cause mucosal disruption have been implicated as potential factors in causing NEC. These include hyperosmolar enteral medications or nutrition, cold stress, infectious diarrhea, abdominal surgery, milk protein allergy, hypomotility, and hypoxia-ischemia (Book *et al*, 1975; Barlow and Santulli, 1975). Importantly, it appears increasingly likely that prematurity itself is the most common source of compromised enteric mucosal integrity (McAlmon, 1998). Typically, NEC occurs in neonates with a PCA of 30 – 32 weeks at a mean PNA of 12 days, when most premature neonates are on progressive enteral feedings (McAlmon, 1998). Although it remains unclear which organisms and conditions contribute to the development of NEC, it is evident that microorganisms play a major role in this disease (McAlmon, 1998). It is equally clear that the severe enteric mucosal disruption observed in NEC might permit invasion by organisms that are present near the injured sites. The presence of enteric organisms appears to be necessary, but not sufficient for the development of NEC (McAlmon, 1998).

The clinical presentation of NEC is quite variable. Abdominal distension, bloody stool and other features of gastrointestinal dysfunction are the most common first indications (Appendix 4) (Faix and Adams, 1994). Some infants may present with a fulminant course

including respiratory failure, cardiovascular collapse, and rapid death, similar to that observed with overwhelming sepsis.

Once the diagnosis of NEC is made or suspected, therapy is usually medical. Medical interventions are intended to ablate suspected inciting factors, preserve mesenteric perfusion, decrease invasion and dissemination by enteric microorganisms, and provide adequate nutrition for metabolic requirements (Faix and Adams, 1994). Surgery is reserved for infants with evidence of visceral perforation, intestinal gangrene, or inexplicable deterioration that is unresponsive to medical therapy (McAlmon, 1998).

Since bacteria play a role in the etiology NEC and blood cultures are positive in 30% of patients, the treatment of NEC includes the initiation of broad-spectrum antibiotics as well as gastrointestinal decompression with parenteral alimentation (Polin, 2001). Empiric parenteral antimicrobial therapy is initially selected to provide coverage for common microorganisms associated with NEC (Faix and Adams, 1994). Although a large survey indicated that gram-positive cocci are the most common isolates from blood in suspected or mild NEC, as are gram-negative rods in advanced NEC, there is considerable overlap (Uauy *et al*, 1991). Enteral administration of aminoglycosides for NEC has fallen into disfavor following a report of a controlled trial in which no apparent benefit was observed, despite a higher incidence of potentially toxic serum aminoglycoside concentrations (Hansen *et al*, 1980). Scheifele *et al* (1987) reported better outcomes in a cohort of infants with NEC who were treated with vancomycin and cefotaxime than in an historical comparison group treated with ampicillin and gentamicin. Hence, the antimicrobial regimen of vancomycin and cefotaxime has become standard therapy at C & W and other tertiary care centers. Parenteral antibiotic therapy is subsequently modified according to culture results, susceptibility reports, serum drug concentrations, and clinical developments.

#### **1.4. VANCOMYCIN PHARMACOKINETICS**

##### **1.4.1. Fundamental Properties**

Vancomycin is usually administered via the intravenous route. It may also be administered intraperitoneally, and there are data on the intrathecal use of the drug (Moellering, 1984). Intramuscular injection of vancomycin results in severe pain, consequently, the

recommended method for parenteral administration is as a slow intravenous infusion over 60 minutes. Vancomycin is not well absorbed, and oral administration typically does not result in measurable serum concentrations (Tedesco *et al*, 1978; Matzke, 1987).

In adults with normal renal function vancomycin pharmacokinetics have been characterized in different studies as being mono-, bi-, and triexponential (Appendix 5) (Rotschafer *et al*, 1982; Rodvold *et al*, 1988). This pattern indicates that the disposition of vancomycin cannot be explained by a simple first-order process. Based upon two-compartment analyses, vancomycin disposition demonstrates considerable interpatient variability with elimination half-lives ( $t_{1/2\beta}$ ) ranging from 2.9 to 9.1 hours in subjects with normal renal function (Appendix 5) (Rotschafer *et al*, 1982; Brown *et al*, 1983; Rodvold *et al*, 1988; Golper *et al*, 1998). Three studies of vancomycin disposition in healthy adults, described disposition with a three-compartment model (Krogstad *et al*, 1980; Comstock, 1988; Tann *et al*, 1990). In these investigations, an initial distribution phase with a half-life ( $t_{1/2\alpha}$ ) of approximately 0.2 hours was followed by a second distribution phase with a half-life of approximately 1.2 hours and finally an elimination phase with a half-life of approximately 7.3 hours. Similar results were reported following analysis of data from adults with end-stage renal disease using a three-compartment model (Comstock, 1988; Tann *et al*, 1990). However, these authors reported an elimination half-life of approximately 150 hours. In the presence of anuria, the elimination half-life can be prolonged to six days (Tan *et al*, 1990).

In adults, the pharmacokinetics of vancomycin are characterized by moderately extensive distribution of the drug throughout the various body compartments following intravenous administration (Krogstad *et al*, 1980). The central compartment volume of distribution ( $V_c$ ) derived from two- and three-compartment analyses is approximately 0.2-0.6 L/kg and 0.13 L/kg, respectively (Rotschafer *et al*, 1982; Rodvold *et al*, 1988; Healy *et al*, 1987; Wilhelm and Estes, 1999). The total volume of distribution at steady-state ( $V_{ss}$ ) is highly variable but is approximately 0.7 L/kg and can be affected by factors such as age, gender, and body weight (Ducharme *et al*, 1994). Although early evidence suggested that vancomycin was minimally bound (< 10%) to plasma proteins, recent observations in healthy volunteers and patients with normal renal function suggest that approximately 30% to 50% of circulating vancomycin is bound (Rodvold *et al*, 1988). The degree of binding in patients with end-stage renal disease is somewhat lower (0-30%) (Tan *et al*, 1990).

After intravenous administration of vancomycin, 40% to 90% of the dose is excreted unchanged by glomerular filtration within 24 hours (Wilhelm and Estes, 1999). The liver may also be involved to a lesser extent with vancomycin elimination, and some evidence suggests that dose adjustments may be required in patients with severe liver dysfunction (Rotschafer *et al*, 1982; Brown *et al*, 1983). Brown *et al* (1983) postulated that hepatic conjugation, perhaps glucuronidation, could account for these findings. The presence of vancomycin in the bile and feces after intravenous administration also supports the existence of extrarenal routes of elimination (Geraci *et al*, 1957; Moellering *et al*, 1981; Wilhelm and Estes, 1999).

#### **1.4.2. Influence of Renal Impairment and Age**

In the last 15 years, the disposition of vancomycin has been characterized in patients of different ages with various acute and chronic illnesses. The serum concentration-time profiles of vancomycin in these studies have been described in terms of one-, two-, and three-compartment pharmacokinetic models (Brater *et al*, 1986; Rybak *et al*, 1990; Matzke, 1986).

Regardless of the pharmacokinetic model used to assess vancomycin disposition, the terminal elimination half-life ( $t_{1/2\beta}$ ) of vancomycin is prolonged and the total body clearance is reduced in patients with impaired renal function (Matzke, 1986). The degree of decline in vancomycin total body clearance (Cl) associated with particular degrees of renal impairment has been characterized by numerous investigators (Appendix 5).

In two studies of burn patients, those with thermal injury required higher vancomycin doses than non-burn patients to achieve similar target serum concentrations (Brater *et al*, 1986; Rybak *et al*, 1990). Vancomycin Cl was observed to be increased in burn patients and this correlated with renal function (Brater *et al*, 1986; Rybak *et al*, 1990). The protein binding of vancomycin was not altered in the burn patients studied; furthermore, the increase in vancomycin Cl and renal excretion was predominantly due to enhanced tubular secretion (Brater *et al*, 1986; Rybak *et al*, 1990). The disposition of vancomycin has also been evaluated in intravenous drug abusers and critically ill patients (Rybak *et al*, 1990). A considerably increased vancomycin Cl was observed in these patients.

Vancomycin pharmacokinetics have been evaluated at the two ends of the age spectrum, in pediatric and geriatric patients (Matzke, 1986). Moreover, during the last 15 years there has been increased use of intravenous vancomycin in pediatric and neonatal patients (Guerina, 1998).

Therefore, more information has become available describing the vancomycin disposition for these specific populations.

#### 1.4.2.1. Renal Impairment

As vancomycin is primarily excreted unchanged by the kidneys, the progressive prolongation of  $t_{1/2\beta}$ , based upon a two-compartment assumption, and the reduction of vancomycin Cl noted as renal function declines is not unexpected (Matzke *et al*, 1986). Mean vancomycin Cl declined from a range of 74.6 - 158.6 mL/min in subjects with creatinine clearance  $>80$  mL/min, to 4.0-6.8 mL/min in patients with end-stage renal disease undergoing hemodialysis (Matzke *et al*, 1986).  $V_{ss}$  did not change significantly with declining renal function, with mean values ranging from 0.39-0.92 L/kg in subjects with creatinine clearance  $>80$  mL/min and 0.80-0.90 L/kg in patients with end-stage renal disease undergoing hemodialysis (Matzke *et al*, 1986). Vancomycin is not removed by conventional hemodialysis or peritoneal dialysis, but high-flux methods of hemodialysis and continuous renal replacement therapies may remove considerable quantities of the drug (De Bock *et al*, 1989).

Although marked variability in vancomycin Cl within a defined range of renal function has been observed, a number of investigators have reported an association between vancomycin Cl and creatinine clearance (Nielson *et al*, 1975; Moellering *et al*, 1984; Matzke *et al*, 1984). These relationships have been utilized to calculate dosing regimens for vancomycin use in patients with renal insufficiency.

Inherent processes of renal maturation, an increase in extracellular fluid volume in relation to body weight and a decrease in the percentage of total body weight as adipose tissue in children make extrapolation of data from adults to pediatric patients difficult (Rodvold *et al*, 1997). Similarly, it is not advisable to extrapolate data from pediatric patients to term neonates or from term to premature neonates (Rodvold *et al*, 1997).

#### 1.4.2.2. Age

In 1984, Cutler *et al* evaluated vancomycin disposition in six geriatric patients (61 to 77 years) and six healthy adult males with normal renal function (20 to 26 years). Elderly males were noted to have increased  $V_{ss}$  and  $t_{1/2\beta}$ , and reduced Cl values compared to younger males. These pharmacokinetic changes did not correlate with creatinine clearance and there were no

differences in serum protein binding observed between the two groups. The investigators postulated that the volume of distribution and half-life differences observed in the geriatric population may be the result of altered tissue binding and/or tissue distribution volume.

During childhood, broadly defined as the time from birth through adolescence, rapid developmental changes occur that can have a profound effect on the pharmacokinetics and pharmacodynamics of therapeutic agents (Kearns, 2000). The most dramatic pharmacokinetic changes occur during the first 12 months of life and affect absorption, protein binding, renal elimination, and drug biotransformation (Kearns, 2000).

The effects of age on vancomycin pharmacokinetics have been evaluated in neonates (Table 1) (Schiabbe *et al*, 1986; James *et al*, 1987; Reed *et al*, 1987; Leonard *et al*, 1989; Asbury *et al*, 1993; McDougal *et al*, 1995; Seay *et al*, 1994; Grimsley and Thomson, 1999; de Hoog *et al*, 2000), infants (Table 2) (Gross *et al*, 1985; Naqvi *et al*, 1986; Lisby-Sutch and Nahata, 1988; Kildoo *et al*, 1990; Gous *et al*, 1995), and children (Table 2) (Schaad *et al*, 1980; Chang *et al*, 1994; Chang, 1995; Krivoy *et al*, 1998; Yasuhara *et al*, 1998; Lamarre *et al*, 2000; Wrishko *et al*, 2000).

The distribution of most compounds within the body is influenced by a number of age-dependent factors, most notably body water and fat content and the quantity and binding capacity of plasma proteins (Kearns and Reed, 1989). During development, marked changes in body composition occur; the most dynamic changes occur in the first year of life (Kearns, 2000). Total body water, as a percentage of total body weight, has been estimated to be 94% in the fetus, 85% in premature neonates, 78% in term neonates, and 60% in adults (Friis-Hansen, 1971). Similarly, the extracellular fluid volume approximates 65% of body weight in preterm neonates, 50% in term neonates, 35% in four- to six-month-old infants, 25% in children one-year of age, and 20% in adults (Friis-Hansen, 1971). The intracellular fluid volume increases from 25% of body weight in the fetus to 33% in the term neonate to 37% by four months of age and 40% in adults (Friis-Hansen, 1971). Also, total body fat in preterm neonates may represent only 1% of total body weight compared with 15% in term neonates and 20% in adults (Kearns, 2000). Furthermore, neonatal adipose tissue may contain as much as 57% water and 35% lipids, whereas adult values approach 26% and 72%, respectively (Kearns, 2000).



The renal excretion of many drugs is directly proportional to age-dependent development of renal function, primarily glomerular filtration and active tubular secretion (Kearns, 2000). In the preterm neonate, renal function is dramatically lower because of the continued development of functioning nephron units (nephrogenesis) that continues until 36 weeks gestation (Kearns, 2000). At birth, the kidney replaces the placenta as a major organ responsible for elimination and fluid and electrolyte homeostasis; this transition occurs with changes in renal blood flow, glomerular filtration rate, and tubular functions.

Renal blood flow remains low in the fetus, accounting for 2% to 3% of cardiac output (Besunder *et al*, 1988). Renal blood flow and plasma flow increase with age as a result of a decrease in vascular resistance, which is proportionately greater in the kidney compared to other organs, and an increase in cardiac output (Besunder *et al*, 1988). The kidneys of the neonate receive 5% to 6% of total cardiac output compared with 15% to 20% for adults (Besunder *et al*, 1988). Glomerular filtration begins soon after the first nephrons are formed and the glomerular filtration rate (GFR) increases in parallel with body and kidney growth (Kim and Emma, 1998). At birth, GFR is directly proportional to GA (Besunder *et al*, 1988). The GFR for full-term neonates at birth ranges from 2 to 4 mL/min; in contrast, the GFR is approximately 1 mL/min prior to 34 weeks gestation (Besunder *et al*, 1988). For both term and preterm neonates with birth weights > 1500 g, the GFR increases dramatically during the first two weeks of postnatal life to rates between 8 and 20 mL/min (Besunder *et al*, 1988). The increase in neonates < 34 weeks gestation with increasing PNA is from 1 mL/min to 2 to 3 mL/min (Besunder *et al*, 1988). The increase in GFR after birth has been demonstrated to be dependent on PCA and not PNA (Besunder *et al*, 1988). Generally, the complete maturation of glomerular and tubular function is achieved around six to eight months of age (Morselli, 1989). Therefore, lower Cl and longer  $t_{1/2\beta}$  in neonates can be expected for drugs that rely on renal excretion for elimination (Besunder *et al*, 1988).

## **1.5. THERAPEUTIC DRUG MONITORING OF VANCOMYCIN**

### **1.5.1. Analytical Methods**

Six assay methods are available for determining vancomycin concentrations in biologic fluids. They include the microbiological assay, radioimmunoassay (RIA), fluorescence

**Table 1. Vancomycin Pharmacokinetics in Neonates.**

Reference	n	PCA <sup>a</sup> (weeks)	Model	$t_{1/2}\alpha^a$ (hours)	$t_{1/2}\beta^a$ (hours)	$V_{ss}^a$ (L/kg)	CI <sup>a</sup> (L/h/kg)
Schaad <i>et al</i> , 1980	7 7 7	32 34 40	2 2 2	0.15 0.05 0.25	9.8 5.9 6.7	0.74 0.71 0.69	0.05 0.14 0.12
Gross <i>et al</i> , 1985	3 6	30 33	2 2		9.9 5.3	0.97 0.45	0.07 0.06
Naqvi <i>et al</i> , 1986	2 2	32-41 40-62	2 2		4.9 3.0	0.48 0.38	0.08 0.10
James <i>et al</i> , 1987	20	25 - 41	1		3.5-24	0.69	0.02 - 0.11
Reed <i>et al</i> , 1987	15	25-34	none		6.6	0.52	0.06
Lisby-Sutch and Nahata, 1988	7 6	29 - 35 39 - 56	1 1		7.0 2.9	0.48 0.47	0.05 0.13
Kildoo <i>et al</i> , 1990	17	30.5	2		5.5	0.48	0.07
Asbury <i>et al</i> , 1993	23	26 - 46	1		5.6	0.52	0.07
McDougal <i>et al</i> , 1995	16 15 13	27 - 30 31 - 36 ≥ 37	1 1 1		6.6 5.6 4.9	0.55 0.56 0.57	0.06 0.07 0.08
Rodvold <i>et al</i> , 1995	29	24 - 48	1		6.4	0.55	0.06
Seay <i>et al</i> , 1994	192	31.7	2	2.6	22.1	0.76	0.06
Grimsley and Thompson, 1999	59	32.0	2			0.67	0.07
deHoog <i>et al</i> , 2000	108	30.9	1			0.43	0.06

<sup>a</sup> Values expressed as mean.

**Table 2. Vancomycin Pharmacokinetics in Infants and Children.**

Reference	n	Age <sup>a</sup> (years)	Model	$t_{1/2\alpha}$ <sup>a</sup> (hours)	$t_{1/2\beta}$ <sup>a</sup> (hours)	$V_{ss}$ <sup>a</sup> (L/kg)	Cl <sup>a</sup> (L/h/kg)
Schaad <i>et al</i> , 1980	12	3.1 mos	2	0.27	4.1	0.60	0.10
	4	4.3 mos	2	0.49	4.1	0.96	0.16
	5	3.9	2	0.23	2.4	0.82	0.24
	7	5.6	2	0.50	3.0	0.76	0.20
	6	7.6	2	0.31	2.2	0.54	0.17
Chang, 1994	28	4.0	2		3.0	0.63	0.15
Chang <i>et al</i> , 1986	31	4.2	2		3.1	0.62	0.11
	33	5.7	2		4.0	0.64	0.15
Gous <i>et al</i> , 1995	15	0.5 – 10 mos	1		3.4	0.44	0.07
Krivoy <i>et al</i> , 1998	30	6.0	1		10.5	0.62	0.11
	8	5.4	1		14.9	1.30	0.06
Yasuhara <i>et al</i> , 1988	49	2.4	1		3.0	0.52	0.12
Lamarre <i>et al</i> , 2000	78	7.0	2		3.9	0.43	0.10
Wrishko <i>et al</i> , 2000	6	6.9	2	0.80	5.6	0.63	0.11

<sup>a</sup> Values expressed as mean.

polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme multiplied immunoassay technique (EMIT), and high-pressure liquid chromatography (HPLC) (Matzke, 1986). HPLC is the only chemical method available for determining vancomycin concentrations. Although microbiologic plate assays require minimal capital investment and do not require large sample volumes, they are subject to interference from other antimicrobials and require an extended period of time to perform (Pfaller *et al*, 1984). Some of the potentially interfering antibiotics can be inactivated; however, this is precluded when the patient is receiving multiple antimicrobial agents including erythromycin, trimethoprim-sulfamethoxazole, and  $\beta$ -lactamase-resistant penicillins and cephalosporins (Pfaller *et al*, 1984). Radioimmunoassay can be performed with high throughput and is rapid, sensitive, and accurate. Either the EMIT or the

FPIA is recommended for established large clinical services, as both techniques are rapid, specific, and automated (Matzke, 1986).

### 1.5.2. Routine Monitoring of Serum Vancomycin Concentrations

Therapeutic drug monitoring (TDM) refers to the use of measured drug concentrations and pharmacokinetic and pharmacodynamic principles to regulate drug dosages in individual patients, with the goal of enhancing the probability of therapeutic efficacy and minimizing toxicity. The lack of uniformity in the pharmacokinetic model used and the definition of peak vancomycin concentrations has made it more difficult to evaluate the relationship between serum concentrations and efficacy or toxicity (Pryka, 1994). Although a multicompartment model may best characterize the pharmacokinetic profile of vancomycin, a one-compartment model has been reported to be adequate for predicting vancomycin concentrations in the clinical setting (Matzke, 1986). Further, the suggested times to measure peak concentrations include 15 minutes, one-hour, and three hours post-infusion (Rodvold *et al*, 1987). Theoretically, there should be no difference in defining peak vancomycin concentrations among investigators, as by definition, the peak concentration occurs immediately at the end of the infusion (Rodvold *et al*, 1987; Pryka, 1994). Uniformity of peak serum vancomycin concentration sampling would permit rational comparisons between large populations and application of a one-compartment model (Pryka, 1994). However, for a drug that obeys two-compartment kinetics the immediate post-infusion peak concentration in serum does not reflect actual post-distributional concentrations.

There is substantial controversy concerning the optimal method of monitoring parenteral vancomycin therapy (Edwards and Pancorbo, 1987; Rodvold *et al*, 1987; Freeman *et al*, 1993; Pryka RD, 1994; Welty and Copa, 1994; del Mar Fernandez de Gatta *et al*, 1996). Geraci (1977) suggested 30 - 40 mg/L and 5 - 10 mg/L as the accepted ranges for measured peak and trough concentrations, respectively. More recently, Lisby-Sutch and Nahata (1988) reported that measured peak concentrations ranging from 25 to 35 mg/L and measured trough concentrations of 5 to 10 mg/L resulted in bactericidal titres of >1:8 and 1:2 to 1:8, respectively. Currently, the therapeutic range of serum vancomycin concentrations is commonly reported as a peak concentration of 25 - 40 mg/L and a trough concentration of 5 - 10 mg/L (Wilhelm and Estes, 1999). However, this therapeutic range was derived primarily from observations that these

concentrations did not produce toxicity and that the trough concentrations exceeded the MIC for most organisms (Wilhelm and Estes, 1999).

The most common method of therapeutic monitoring of vancomycin has been to measure peak and trough concentrations at steady-state to individualize the dose to achieve target concentrations (Wilhelm and Estes, 1999). In this regard, the desired percentage increase or decrease in concentration achieved can be assessed using an equivalent proportional change in dose (Rodvold *et al*, 1987). The use of a one-compartment model requires the assumption or knowledge that all serum concentrations used to calculate pharmacokinetic parameters reflect post-distribution values. Failure to consider this assumption may result in overestimation of the elimination rate constant, underestimation of volume of distribution (Vd), and perhaps, overestimation of Cl. To minimize the effect of incomplete distribution on the calculation of vancomycin pharmacokinetic parameters, it has been recommended that peak serum concentrations should not be obtained during the distribution phase (Rodvold *et al*, 1987). In neonates, to minimize the effect of incomplete distribution on the calculation of vancomycin pharmacokinetic parameters, peak serum concentrations have been obtained one-hour following the completion of a one-hour infusion of the third dose (James *et al*, 1987; Lisby-Sutch and Nahata, 1988; Asbury *et al*, 1993; McDougal *et al*, 1995). These guidelines are based upon the relatively short distribution and elimination half-lives reported by Schaad *et al* (1980) suggesting that distribution is complete and steady-state achieved by these sampling times. Trough concentrations are commonly obtained 30 minutes prior to the third dose.

Recently, many institutions have begun monitoring only trough concentrations (Wilhelm and Estes, 1999). Proponents of this method cite the lack of evidence that vancomycin toxicity is associated with peak concentrations, the difficulty in interpreting peak concentrations because of multicompartment pharmacokinetics, and that adequate trough concentrations may be associated with efficacy, whereas high trough concentrations may increase the risk of nephrotoxicity (Wilhelm and Estes, 1999). Also, when trough concentrations are in the therapeutic range, it is unlikely that peak concentrations would exceed 40 mg/L (Wilhelm and Estes, 1999). Should a method of trough only or minimal serum concentration sampling be selected, it may be reasonable to perform more intensive monitoring in high-risk patients (Wilhelm and Estes, 1999).

Therapeutic drug monitoring is commonly performed during the administration of vancomycin in pediatric and neonatal populations. Due to the pronounced interindividual variability in vancomycin pharmacokinetic parameters, serum concentrations have been monitored in selected pediatric patients, including: patients with renal impairment; intensive care patients; patients with severe gram-positive infections receiving doses  $> 15$  mg/kg/dose; oncology, burn, and meningitis patients requiring higher doses; patients receiving concomitant nephrotoxic medications; and patients with changing renal function (eg. neonates) (Rodvold *et al*, 1997).

## **1.6. POPULATION PHARMACOKINETICS**

Quantification of the typical pharmacokinetic behavior and interindividual variability in patient populations is an important aspect of drug development. Based on traditional methods, to study the typical pharmacokinetics of a drug in a normal or patient population, the drug is administered to a small homogeneous sample of population members, and drug concentrations are measured from each individual at various times after a dose. Data from these serum samples are then used in the traditional, two-stage approach (Sheiner and Beal, 1981; Peck *et al*, 1986; Ludden, 1988; Jelliffe *et al*, 2000).

### **1.6.1. Two-Stage Method**

Traditionally, mean population pharmacokinetic parameter values have been estimated by performing intensive studies in a limited number of individuals. This method requires at least one serum concentration data point for each parameter to be estimated (Jelliffe *et al*, 2000). Parameter values are estimated using unweighted or weighted nonlinear least squares regression analysis and an appropriate compartmental or noncompartmental pharmacokinetic model (Ludden, 1988). Subsequently, individual values are used to calculate the mean and variance of the parameter for the sample population (Stage 2a) (Ludden, 1988). Relationships between patient characteristics and the estimated pharmacokinetic parameters are then established by categorization or regression techniques (Stage 2b) (Peck *et al*, 1986). The frequency distributions of the individual parameters can be examined; thus, this method can be regarded as nonparametric, as no assumptions are made with respect to the nature of the frequency distribution of the individual parameters (Jelliffe *et al*, 2000).

The two-stage approach offers several advantages. Weighted nonlinear regression is a familiar technique that may provide a reliable method of estimating pharmacokinetic parameters. Moreover, a variety of computer software applications are available for performing the required calculations (Peck *et al*, 1986). When sufficient data are available for each individual and a large number of individuals are included in the analysis, Stage 2a and 2b analyses of data provide reasonable, but potentially biased, estimates of population pharmacokinetic parameter distributions (Peck *et al*, 1986).

However, there are limitations to the two-stage method of population pharmacokinetic analysis. The individual parameter estimates may be imprecise estimates of the true individual parameter value due to intraindividual variability (Ludden, 1988). Intraindividual variability in parameter estimates should be small when there is a large number of observations per subject, the observations are made at times that provide information about the parameter, and the parameter is time invariant (Ludden, 1988). However, it is often difficult to obtain large numbers of blood samples from patients and parameter values can vary daily, even in a generally stable clinical situation. Ethical constraints on the number and timing of blood samples from seriously ill, elderly, and pediatric patients often preclude the use of the two-stage method. Thus, population pharmacokinetic information from the two-stage method is often obtained, primarily, from investigations of healthy, relatively homogeneous, groups or small numbers of patients who often inadequately represent those undergoing routine therapy (Peck *et al*, 1986). Accordingly, information generated by the two-stage method constitutes a limited foundation upon which to base strategies for drug regimen design or individualization of drug therapy in high-risk patient populations (Reed, 1999).

### **1.6.2. Population-Based Methods**

Sheiner *et al* (1977) advocated an alternative approach to the problem of estimating population pharmacokinetic parameters by the use of data generated during the routine clinical care of patients. This approach, implemented in the first true population modeling computer program NONMEM, an acronym for nonlinear mixed effects modeling, provides accurate and precise estimates of population pharmacokinetic parameters from such data in both simulation studies and in analysis of clinical data (Sheiner and Beal, 1981; Jelliffe *et al*, 2000). A nonparametric maximum likelihood (NPML) approach has also been proposed as a method for

analyzing population pharmacokinetic data (Steimer *et al*, 1984; Mallet, 1986). Like the parametric NONMEM method, NPML can function with only one sample per patient; however, no prior assumptions about the shape of the parameter distributions are made (Mallet, 1986; Jelliffe *et al*, 2000). The only assumption made with respect to the shape of the discrete parameter distribution is that the shape is the same for all subjects in the population (Jelliffe *et al*, 2000). The method gives rise to discrete distributions for the likelihood function and the parameter distributions (Whiting *et al*, 1986). These are then smoothed to give continuous distributions that may be skew or multimodal (Whiting *et al*, 1986). A nonparametric expectation and maximization (NPEM) method has been developed by Schumitzky (1991). Like the NONMEM and NPML methods, NPEM can operate with only one sample per patient; and like NPML, NPEM makes no parametric assumptions about the shape of the probability distribution (Jelliffe *et al*, 2000). Essentially, both the NPML and NPEM methods converge to the same results (Jelliffe *et al*, 2000).

#### 1.6.2.1. *Nonlinear Mixed Effects Modeling*

Nonlinear mixed effects modeling is a method of population pharmacokinetic estimation developed specifically to address some disadvantages inherent in the two-stage approach (Beal, 1984; Beal and Sheiner, 1980, 1982; Sheiner, 1984; Sheiner *et al*, 1979). This method has evolved from a strategy for extracting population parameters, means and variances, from sparse data collected during routine patient care. Mixed effects modeling treats the population, rather than the individual, as the unit of analysis and generally requires fewer data points per individual, but many more individuals, than are required with the two-stage method (Whiting *et al*, 1986; Ludden, 1988). In this regard, a much more representative sample of the target population can be obtained and quantitative relationships between pharmacokinetic parameters and pathophysiological features can be determined in a single step (Whiting *et al*, 1986; Maitre *et al*, 1991). In general, there are no restraints on sampling times and data can be collected at times after routine doses over a period of several days (Ludden, 1988; Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). The method can extract whatever information is in the data regarding the parameters (Ludden, 1988).

The mathematical term used in parameter estimation includes both fixed and random effects that describe the data (Ludden, 1988; Sheiner and Beal, 1989; Boeckmann *et al*, 1992;



Boeckmann *et al*, 1998). The analysis must often be performed in an exploratory manner thus; the regression model is developed by utilizing the forward inclusion and backward elimination method (Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). In forward inclusion, all fixed effects producing a relatively large change in the objective function are used to construct the full regression equation (Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). During backward elimination, each factor is individually eliminated from the regression equation, provided that it does not produce a significant change in the objective function (Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). Minimizing the objective function is equivalent to maximizing the likelihood of the model, and thus monitoring changes in the objective function can provide a basis for determining the parameter values that render the data most probable (Ludden, 1988; Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). By implementing a stepwise procedure the investigator chooses the appropriate model for the fixed and random effects and decides which covariates have to be included in the regression model to describe the interindividual variability that can be explained by observable patient characteristics (Sheiner and Beal, 1989; Vozech *et al*, 1990; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998).

The output from NONMEM does not provide patient-specific parameter estimates; therefore, the relationship between pharmacokinetic parameters and demographic factors such as age, gender, and body weight cannot be assessed (Maitre *et al*, 1991). Maitre *et al* (1991) have advocated a three-step approach addressing this limitation. First, an initial NONMEM analysis provides the population pharmacokinetic parameters with no assumptions of the demographic factors of importance. Second, individual posthoc (Bayesian) estimates using the individual measured drug concentrations and population pharmacokinetic parameters from step one are obtained. The relationships between the demographic factors and the individual Bayesian parameter estimates may then be examined through graphical interpretation of the data. Finally, the NONMEM analysis is resumed, and the relevant demographic factors are sequentially entered into the NONMEM regression model.

Nonlinear mixed effects modeling describes pharmacokinetic variability in terms of a number of factors termed fixed and random effects (Ludden, 1988; Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). The fixed effects ( $\theta$ ) are the mean values of the population parameters that may be a function of various patient characteristics including:

(a) age, weight, height, and sex; (b) underlying pathology such as renal or hepatic insufficiency; and (c) other influences on drug disposition such as concomitant drug therapy, smoking, and alcohol consumption (Whiting *et al*, 1986). Although all of these data may contain some error, it is usually small as compared to the other sources of variability (Ludden, 1988). The random effects quantify the sources of variation that contribute to differences between expected and actual results and are categorized as interindividual and intraindividual in origin (Ludden, 1988).

One of the strengths of NONMEM is partitioning of inter- and intraindividual variability (Ludden, 1988). The most relevant source of variability in pharmacokinetic evaluations arises from differences between patients. To reflect the interindividual variability, the pharmacokinetic parameters ( $\phi_j$ ) must be described as arising from a population where  $\theta$  is (are) population

$$\phi_j = \theta + \eta_j$$

parameter(s) and  $\eta_j$  is (are) the difference(s) between the individual from the population parameter value(s) (Peck *et al*, 1986). The presence of interindividual variability suggests that although expected parameter values can be calculated for an individual patient based on previous research and experience, the individual's parameters may differ from expected values.

Knowledge about the quantitative aspects of interindividual variability can provide information to assess the predictive performance of the model from patient characteristics and other factors (Ludden, 1988). Also, it improves the accuracy or precision of the prediction (Ludden, 1988).

The measured concentration cannot be determined without errors (Peck *et al*, 1986). The intraindividual variation ( $\epsilon$ ) or residual error includes measurement errors involved in quantitating drug concentration or response and random changes in individuals' parameters over time (Ludden, 1988). This residual error may be expressed as:

$$\epsilon_{ij} = y_{ij} - f(\phi_j, x_{ij})$$

where  $y_{ij}$  is the  $i^{\text{th}}$  measurement of the drug concentration in the  $j^{\text{th}}$  individual,  $\phi_j$  is the expected set of pharmacokinetic parameters for individual  $j$ , and  $x_{ij}$  includes information such as drug doses and times for measurement in the  $j^{\text{th}}$  individual (Peck *et al*, 1986).

Fundamental, therefore, in population pharmacokinetic studies with NONMEM is the estimation of  $\theta$ ,  $\eta$ , and  $\epsilon$  values for each pharmacokinetic parameter which then summarize the population distribution of pharmacokinetic parameters (Peck *et al*, 1986). The model can now be expressed as:

$$y_{ij} = f_{ij}(\theta + \eta_j) + \varepsilon_{ij}$$

whereby the predicted drug concentration ( $y_{ij}$ ) is a function of the pharmacokinetic parameters ( $\theta$ ), the interindividual variability ( $\eta_j$ ), as well as the intraindividual variability ( $\varepsilon_{ij}$ ).

The NONMEM technique uses all data as one set to separate intraindividual from interindividual sources of variation. Thus, in contrast to the two-stage method, the variability among individuals and the variability arising from observational error are both estimated, which permits a less biased and more precise estimate of certain population parameters (Sheiner and Beal, 1981). During the analysis the samples from a given patient remain identified with that patient, and yet the entire data set is computationally available, which permits the estimation of both the mean parameters and variances (Ludden, 1988). The clinical goal of these analyses is to apply the pharmacokinetic results to the ongoing care of patients by forming the foundation for the design of an optimal drug dosing regimen (Reed, 1999). To use population-based pharmacokinetic data in the determination of individual pharmacokinetic parameters for a specific patient using sparse sampling, Bayesian methods are integrated with population-based methods (Reed, 1999).

Despite these advantages, there are limitations to the NONMEM method. Although the NONMEM generated estimates specify the probability density function of the parameters when the distribution is normal or lognormal, they provide inadequate information in situations where the density is nonsymmetric or multimodal (Best *et al*, 1995). In addition, the method implemented by NONMEM uses a first-order linear approximation to solve the objective function (Sheiner and Beal, 1989; Boeckmann *et al*, 1992; Boeckmann *et al*, 1998). The accuracy of this linearization depends on the degree of dispersion of individual patients about the population mean (Best *et al*, 1995). Large intraindividual variability can lead to potential inaccuracies; moreover, the NONMEM estimate of the population variance may be biased if the pharmacokinetic parameters are highly correlated (Best *et al*, 1995).

#### 1.6.2.2. *Pediatric Considerations for Population Modeling*

In contrast to the approach often used in adults, a number of problems confront investigators when performing pharmacokinetic evaluations in pediatric patients (Reed, 1999). The constraints that complicate the performance of detailed, pharmacokinetic assessments in ill

infants and children represent legitimate safeguards protecting the health and well-being of pediatric patients (Reed, 1999).

The volume of blood necessary for each sample and the number of samples necessary to describe the drug disposition are important factors that directly impact the ability to perform pharmacokinetic evaluations (Reed, 1999). These factors are of particular importance in pediatric patients because the volume of blood that can be safely procured is limited by patient age, size, and underlying pathology (Reed, 1999). The risks associated with repeated venous sampling and/or venipuncture may include excessive blood loss, pain, bruising, and infection (Kauffman and Kearns, 1992). Venipuncture in neonates and infants can be difficult, even for skilled personnel; when repeated samples are required, this problem can become more pronounced, as satisfactory venous access decreases after repeated venipuncture (Koren, 1997). Full-term neonates have a blood volume of 80 – 100 mL/kg, therefore the least mature neonates may have a total blood volume of only about 50 mL (Long *et al*, 1987). Although the volume of blood that can be safely procured from neonates varies, repeated blood sampling is associated with depletion of circulating blood volume and may increase the requirement for transfusion, when the total sampling volume exceeds 10% of the estimated circulating blood volume (Kauffman and Kearns, 1992).

A balance must be therefore achieved between obtaining an accurate determination of pharmacokinetic parameters vital to describing the dose-concentration-effect relationship and minimizing the number of blood samples obtained. Clearly, the most effective way to achieve this goal is through the application of analytical and pharmacokinetic techniques that minimize risk and discomfort to the patient while meeting the demands of a given investigation (Reed, 1999). In this regard, population-based methods, like NONMEM, are ideally suited to the pediatric population, as well as other compromised populations (Reed, 1999). These methods characterize pharmacokinetic parameter estimates and variances similar to those obtained by the traditional method, without extensive blood sampling from any individual patient (Whiting *et al*, 1986). Several investigations using NONMEM to estimate the population pharmacokinetics of drugs, including as theophylline (Driscoll *et al*, 1989), propofol (Kataria *et al*, 1994) and valproate (Botha *et al*, 1995) have been undertaken in the pediatric population.

#### 1.6.2.2.1. Neonatal Considerations

The physiologic processes that determine drug disposition undergo radical changes during maturation. There are important differences in pharmacokinetics, not only between neonates and between adults, but also among premature neonates, full term neonates, infants and children (Morselli, 1989). The normal, dynamic changes that occur in organ function with age will dramatically influence the drug disposition profile (Reed, 1999). Similarly, the ontogeny of body water content and its anatomic distribution can directly influence the distribution of a drug within the body (Reed, 1999). During the first year of life the development of various physiological factors important for pharmacokinetic behavior is not predictable, and various external factors may mutually interact, leading to further alterations in pharmacokinetic parameters (Morselli, 1989).

The utilization of NONMEM in the neonatal population is particularly attractive since it does not require the patient to undergo the rigors of a traditional protocol. Moreover, NONMEM allows estimation of pharmacokinetic parameters, their inter- and intraindividual variability and the influence of factors on these parameters from routinely collected data (Collart *et al*, 1992). The population pharmacokinetics of zidovudine (Collart *et al*, 1992), midazolam (Burtin *et al*, 1994), phenobarbital (Grasela and Donn, 1985), theophylline (Moore *et al*, 1989; Driscoll *et al*, 1989; Martin, 1991; Karlsson *et al*, 1991), netilmicin (Fattinger *et al*, 1991), and gentamicin (Weber *et al*, 1993; Jensen *et al*, 1992; Thomson *et al*, 1988) have been evaluated in neonates using NONMEM.

#### 1.6.3. Population Pharmacokinetics of Vancomycin in Neonates

Numerous vancomycin pharmacokinetic analyses in infants and neonates have been undertaken (Tables 1 and 2). However, only three reports of a vancomycin population-based pharmacokinetic study in neonates have been published (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000). The most comprehensive population-based analysis of vancomycin in neonates to date was completed by Seay *et al*, 1994. The purpose of their investigation was to determine population pharmacokinetic parameters for neonates. Retrospective data from 1987 - 1989 for 192 neonates were collected sequentially and evaluated with NONMEM. Vancomycin dosing history, serum concentrations, and data from 28

covariates were collected. Thirty additional patients were included in the validation component of the study.

A two-compartment pharmacokinetic model and those covariates present in  $\geq 20\%$  of the population were used in population model development. Seven predictors were reported to significantly improve the model during forward inclusion. Only GA and dopamine exposure changed the objective function during backward elimination and thus were included in the final model. GA was incorporated into the model as a dichotomous variable in response to a bimodal distribution in the data with a break point at 32 weeks. In the validation component of the study, there was not a significant difference in the predictive performance between the one- and two-compartment pharmacokinetic models.

Although the investigators identified those patients with a GA  $\leq 32$  weeks and receiving dopamine as groups with significantly different Cl values, the authors failed to account for maturational differences. PCA represents an important covariate as it produces a continuous change in Cl; therefore, PCA should be addressed in addition to GA. Additionally, NONMEM may not provide adequate information in a bimodal distribution. Furthermore, neither the dose nor the time of initiation of dopamine in relation to vancomycin therapy was documented, and dopamine use may have been a marker for some underlying hemodynamic factor leading to decreased drug elimination.

In contrast to Seay et al (1994), Grimsley and Thompson (1999) and de Hoog *et al* (2000) implemented population analyses for the purpose of generating vancomycin dosing guidelines. The latter authors did not incorporate thorough model building or covariate screening; rather, only a one-compartment model with limited age and weight was constructed for 115 neonates. Furthermore, model evaluation was based upon measured peak and trough concentrations in a small group (22) of patients given the vancomycin regimen developed from their model. Although the authors concluded that adequate vancomycin trough serum concentrations were obtained, the accumulation index did not support the estimated half-life in some patients.

Grimsley and Thompson (1999) conducted a more comprehensive model building process in a small sample of 59 neonates than de Hoog et al (2000); however, a conventional validation analysis was not implemented. Like Seay et al (1994), Grimsley and Thompson (1999) implemented covariate selection with their best, two-compartment model and these factors were assumed to apply to the one-compartment model. Only weight and serum creatinine

were included in the final model; PCA offered no advantage on vancomycin Cl or Vd. Insufficient data from neonates receiving dopamine were collected to permit analyses, and no information with respect to RDS, CLD and indomethacin was collected. Although the two-compartment model was identified by Grimsley and Thompson (1999) to be superior, a one-compartment model was used to develop the dosing guidelines. Examination of the measured peak and trough concentration data in 25 neonates following implementation of the new dosing regimens revealed only a 11% improvement in initial concentration measurements and the need for vancomycin concentration monitoring was not alleviated.

Indomethacin was not identified as a covariate in any of the previous population-based analyses (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000). This is not consistent with reports suggesting that indomethacin decreases vancomycin Cl through reduced renal perfusion (Spivey and Gal, 1986; Kumar, 1985). Based on the increased use of indomethacin in premature neonates for the treatment of PDA it is possible that this medication may represent an important factor affecting vancomycin Cl. Another limitation of the aforementioned model-building processes was that they were conducted only with the two-compartment model, and those factors determined to be significant were assumed to apply to the one-compartment model. A population model developed using a one-compartment pharmacokinetic model may include covariates other than those described in the two-compartment model. Other limitations of these studies include the use of different vancomycin assays (Seay *et al*, 1994) and the retrospective nature of the data analysis.

In recent years, there has been an increased number of lower GA ( $\leq 30$  weeks) patients admitted to neonatal intensive care units with various pathophysiological disturbances (Lorenz, 2000). This, in addition to the limitations of the previous investigations (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000), necessitates a more detailed population pharmacokinetic study of vancomycin in this population. The results of a NONMEM analysis of vancomycin pharmacokinetics in neonates requiring intensive care may have direct applicability to future dosing of vancomycin and therapeutic drug monitoring in subgroups among this population.

**The primary objective was to develop a population-based pharmacokinetic model of vancomycin in neonates that can be utilized in the individualization of drug therapy.**

## 1.7. INDIVIDUALIZATION OF DRUG THERAPY

The goal in therapeutics is to determine the most appropriate drug dose that will enhance the probability of efficacy and minimize toxicity in an individual patient. The serum concentrations observed in an individual frequently differ from the desired therapeutic range when the regimen is based upon typical population pharmacokinetic parameters (Peck *et al*, 1986). Consequently, for those medications that possess a narrow therapeutic index, therapeutic drug monitoring permits individualization of drug dosage regimens. The pharmacokinetic individualization of dose and dosage intervals in response to measured serum drug concentrations improves the ability to achieve target concentrations (Peck *et al*, 1986).

Many methods have been proposed to achieve desired serum concentrations, including: predictive algorithms that do not use serum drug concentrations (Burton *et al*, 1986); one compartment pharmacokinetic models (Sawchuk and Zaske, 1976), and least-squares or Bayesian methods that do use serum drug concentrations to individualize dosing (Cropp *et al*, 1998; Andrés *et al*, 1997; Rodvold *et al*, 1989; Garrelts *et al*, 1987; Sheiner *et al*, 1979).

### 1.7.1. Standard Methods

#### 1.7.1.1. Sawchuk and Zaske Method

The method of Sawchuk and Zaske (1976) is commonly employed in the clinical setting and requires at least two serum vancomycin concentrations drawn around a dose at steady-state to calculate individual pharmacokinetic parameters based on a one compartment, first-order elimination pharmacokinetic model. The volume of distribution (Vd) is calculated by:

$$Vd = \frac{(Dose/t_i)}{Ke} * \frac{(1 - e^{-Ke t_i})}{[C_{max} - (C_{min} * e^{-Ke t_i})]} \quad (\text{Equation 1-1})$$

where dose is the administered dose (mg);  $t_i$  is the infusion time (h);  $C_{max}$  is the peak concentration (mg/L) measured one hour post infusion extrapolated back to the time immediately postinfusion; and  $C_{min}$  is the measured trough concentration (mg/L) extrapolated to the end of the dosage interval. The elimination rate constant ( $Ke$ ), clearance (Cl) and half-life ( $t_{1/2}$ ) are determined by:



$$Ke = \frac{\ln(Cp/Ct)}{T} \quad (\text{Equation 1-2})$$

$$Cl = Ke * Vd \quad (\text{Equation 1-3})$$

$$t_{1/2} = \frac{0.693}{Ke} \quad (\text{Equation 1-4})$$

where Ct is the measured trough concentration (mg/L); Cp is the measured peak concentration (mg/L) one hour following a one hour infusion; and T is the interval (h) between Cp and Ct. Based upon the individual's pharmacokinetic parameters, the following equations are used to predict the peak and trough serum vancomycin concentrations:

$$Cp_{ss} = \frac{(Dose/t_i)}{(Ke * Vd_{ss})} * \frac{(1 - e^{-Ket_i})}{(1 - e^{-Ke\tau})} * e^{-Ket_i} \quad (\text{Equation 1-5})$$

$$Ct_{ss} = Cp_{ss} * e^{-Ke[\tau - (t_i + t_1)]} \quad (\text{Equation 1-6})$$

where Cp<sub>ss</sub> is the predicted peak concentration one-hour following an one-hour infusion; Ct<sub>ss</sub> is the predicted trough concentration immediately prior to the infusion; τ is the dosing interval (h); and t<sub>1</sub> is the time between the end of the infusion and measured peak concentration (h).

This method requires that distribution be complete prior to obtaining a serum sample and that the patient be at steady-state. In this regard, the method can develop dosage regimens only to achieve target concentrations at a subsequent steady-state (Jelliffe *et al*, 1998). Also, the method can only analyze data obtained during a typical single dose interval; as soon as new serum concentrations become available, all previous data are ignored (Jelliffe *et al*, 1998).

#### 1.7.1.2. Least-Squares Methods

Least-squares (LS) methods, under certain statistical assumptions, can be derived from a more general estimation method known as maximum likelihood (Peck *et al*, 1986). A LS analysis involves a computer search for parameter values of the pharmacokinetic model that minimize an objective function (OBJ) expressed as:

$$OBJ = \sum_{i=1}^n \frac{(C_i - \hat{C}_i)^2}{\sigma_i^2} \quad (\text{Equation 1-7})$$

where  $C_i$  and  $\hat{C}_i$  denote the observed and predicted drug concentrations, respectively, and  $\sigma_i$  are the standard deviations from the random error model for  $i=1$  to  $n$  available drug concentrations (Peck *et al*, 1986). Thus, the LS computer search selects parameter values for the pharmacokinetic model that yield estimates,  $\hat{C}_i$  that most closely correspond to the measured concentrations. The  $\sigma_i$  can either be entered in the fitting as actual values or estimated automatically in the procedure under explicit assumptions about the functional form of the random model (Peck *et al*, 1986). The requirement to weight observations with the appropriate  $\sigma_i$  is a consequence of the varying absolute error for different values of concentrations measured. To minimize the sum of the squared residuals  $(C_i - \hat{C}_i)^2$ , a reliable estimate for the variance ( $\sigma_i^2$ ) is critical to obtaining valid estimates (Peck *et al*, 1986).

Certain inherent assumptions and characteristics of the LS method limit its suitability for estimating individualized pharmacokinetic parameters in patients. This method requires multiple, appropriately timed drug concentrations to provide accurate and precise estimates of the parameters (Peck *et al*, 1986). The minimum number of measurements for LS is determined by the number of parameters in the model (Peck *et al*, 1986). The variability in the observations, random error, requires additional measurements for adequate precision (Peck *et al*, 1986). However, clinical realities often preclude collecting the desired number of drug concentrations at informative times, though optimum sampling strategies can facilitate use of this method (Reed, 1999).

The LS method can be modified to accommodate fewer available drug concentrations by fixing one (or more) parameters at assumed values, or defining a proportional relationship between  $Cl$  and observations obtained at a fixed time, allowing few parameters for individualized estimation (Slattery, 1981; Bahn and Landaw, 1987). However, inherent in this approach is either an assumption of limited variability of the fixed parameter(s) and/or an optimistic estimate of the precision of the observations (Peck *et al*, 1986). The LS method, like all non-Bayesian methods, derives all of its information regarding the values of the pharmacokinetic parameters entirely from the measured serum drug concentrations (Peck *et al*, 1986). Thus, any prior knowledge regarding the pharmacokinetic parameters from the individual that the clinician may possess from patient characteristics and population pharmacokinetic data are excluded from the LS analysis. Despite these limitations, the LS method has been successfully employed in various forms to estimate individual patient pharmacokinetic parameters (Peck *et al*, 1986).

### 1.7.2. Bayesian Forecasting

The basic philosophical differences between classical and Bayesian statistical estimation or inference concerns the use of prior information or beliefs. Classical statisticians contend that inference, to be defensible, must be based only on observation or measurement of current data and must not be biased by prior information or the beliefs of the investigator (Feinstein, 1977). Conversely, Bayesian statisticians contend that the prior information and beliefs of the investigator are relevant data and should be considered, in addition to current experimental data, in making inferences (Feinstein, 1977).

Sheiner *et al* (1979) were the first to apply Bayesian principles to the forecasting of digoxin serum concentrations. They demonstrated that by using only one serum concentration as feedback, in conjunction with the prior probability distribution for the pharmacokinetic parameters, provided more accurate and precise predictions than did a naïve estimate approach. The use of one or two measured drug concentrations, as opposed to none, improved the forecast precision of subsequent drug concentrations by 40% and 67%, respectively, compared to using mean population pharmacokinetic parameters adjusted for patient characteristics. The authors reported no significant difference in the mean error of the predictions when two measured drug concentrations were used regardless of whether or not patient physiological factors were included. However, the same data set was used to derive population parameter estimates and then to test the predictive performance of Bayesian forecasting using these estimates; consequently, the predictions were not properly validated.

Bayesian forecasting alters prior estimates of multiple parameters based on one or more measured serum concentrations (Sheiner *et al*, 1979). Forecasting individual serum concentrations includes: formulating a model for the patient system that links dosage, time, and observable features; initiating the model for the individual patient; and adjusting the model accounting for observed patient responses (Sheiner *et al*, 1979).

All models require a number of parameters that are divided into observable features of patients (age, sex, weight) and population pharmacokinetic parameters (Sheiner *et al*, 1979). Uncertainty about individual parameters and measurement error will always be present, and the model accounts for this uncertainty in its statistical framework (Sheiner *et al*, 1979; Jelliffe *et al*, 1998). The magnitude of these types of variability can be expressed by introducing variance

terms into the kinetic model. Parameter means and variances as well as and intra-individual variance obtained by application of NONMEM are ideally suited for the development of a Bayesian regression algorithm for optimization of therapy (Ludden 1988). The coupling of NONMEM and Bayesian forecasting, resulting in true model-based, goal-oriented drug therapy, permits achievement of carefully selected targets, where the targets are individualized for each patient's perceived need for the drug (Jelliffe *et al*, 1998).

Model initiation begins with substitution of the observable features of the individual into the pharmacokinetic parameter expressions (Sheiner *et al*, 1979). The set of individual parameter values is regarded as a random variable characterized as a prior probability distribution. Model revision consists of applying Bayes' formula to adjust the prior probability distribution of the individual's parameters in light of the measured serum concentration and thus, arrive at a revised posterior probability distribution. The posterior probability will likely have a different mode than the prior probability and will be used, as before, to produce a revised forecast (Sheiner *et al*, 1979).

Estimating individual pharmacokinetic data constrained by population priors in terms of Bayesian forecasting, may be conceptualized as follows (Peck *et al*, 1986):

$$p(P|C) = \frac{p(P) * p(C|P)}{p(C)} \quad (\text{Equation 1-8})$$

where  $p(P|C)$  is the conditional probability distribution of the set of pharmacokinetic parameters (P) of the individual accounting for the measured drug concentrations (C), the probability of the individual's parameters to be within the expected population parameter distribution  $p(P)$  (i.e. population priors), and the probability distribution of measured concentrations  $p(C|P)$  in the context of the pharmacokinetic model, random errors, and the unconditional probability distribution of the observed concentrations  $p(C)$  (Peck *et al*, 1986). When the population distributions of pharmacokinetic parameters are approximately Gaussian, application of maximum-likelihood estimation to the Bayes' theorem results in the following objective function:

$$OBJ = \sum_{j=1}^n \frac{(P_j - \hat{P}_j)^2}{\sigma_{P_j}^2} + \sum_{i=1}^m \frac{(C_i - \hat{C}_i)^2}{\sigma_{C_i}^2} \quad (\text{Equation 1-9})$$

where  $P_j$  represents the population pharmacokinetic parameters and  $\hat{P}_j$  denotes the estimate of the individual's pharmacokinetic parameters,  $\sigma_{P_j}^2$  is the interindividual variance of the  $j^{\text{th}}$  set of population pharmacokinetic parameters,  $C_i$  and  $\hat{C}_i$  denote the observed and predicted drug concentrations, respectively, and  $\sigma_{C_i}^2$  is the residual error variance of the  $i^{\text{th}}$  measured drug concentration that encompasses assay error and intraindividual variability (Sheiner *et al*, 1979). Minimization of the Bayesian objective function results in estimates of pharmacokinetic parameters unique to the individual. These account for measured and predicted drug concentrations in addition to information on measurement error and the typical variability values of pharmacokinetic parameters in the population (Peck *et al*, 1986). Hence, the role of Bayesian fitting is to provide an individualized model of drug behavior based on dosage, serum concentration, and other relevant clinical descriptors or covariates.

Bayes' theorem and the Bayesian objective function encompass all of the usual methods for estimating individual pharmacokinetic parameters, assuming independent and normally distributed population parameters (Peck *et al*, 1986). When no measured drug concentrations are available from a patient,  $m=0$ , the second term does not exist and the prior population distribution alone determines the model (Sheiner *et al*, 1979). Hence, the equation is minimized when the objective function represents the set of mean population pharmacokinetic parameters (Sheiner *et al*, 1979). When abundant measured drug concentrations are available,  $m$  is very large, the second term dominates the expression; prior information is less important, and observed concentrations alone determine the model. The objective function represents the set of pharmacokinetic parameter estimates that minimizes the weighted sum of the residual error variance of the measured drug concentrations. When prior expectations are admitted and drug concentrations are available, the complete Bayesian method is expressed; both terms contribute in weighted proportion, taking advantage of current data in relation to expected probability distribution parameters, resulting in a revision of the objective function (Schumacher and Barr, 1984). If observed drug concentrations vary from the predicted values, feedback control is provided by using the Bayesian adjustment, to simultaneously modify the pharmacokinetic parameter set in proportion to the degree to which they are generally expected to vary from their initially predicted values (Sheiner *et al*, 1979). This approach balances observed outcomes with prior expectations by adjusting the prior probability distribution of the individual's pharmacokinetic parameters following incorporation of observed serum concentrations to arrive

at a revised, posterior distribution for the parameters (Schumacher and Barr, 1984; Sheiner *et al*, 1979). Therefore, the method fits both the estimated serum concentrations to the measured concentrations, and simultaneously fits the model parameter values as near as possible to the parameter values in the prior population of similar patients (Jelliffe *et al*, 1998).

Regarding the theoretical advantages of the Bayesian approach, the most important one is its use of population information at all times (Sheiner and Beal, 1982). *A priori* population data are used to determine initial pharmacokinetic parameters, but as serum concentration data become available, the predictions are refined to fit the pharmacokinetic profile, thus producing a more individualized assessment. By using population information even when individual observations are available, the Bayesian method performs better than methods that do not (Jelliffe *et al*, 1993). The non-Bayesian method implicitly assumes the correctness of the observation, and will often magnify small differences from expectation in these to produce large estimation errors (Sheiner and Beal, 1982). Conversely, the Bayesian method discounts observations, especially when they are in considerable conflict with prior parameter expectations (Sheiner and Beal, 1982). In some cases this conservatism will mean that a parameter truly different from the expected value will be incorrectly regarded as closer to expected than it really is, until further drug concentrations are obtained.

Despite these advantages, Bayesian methods pose potential problems. Adequate representativeness of the population of interest is required to generate population prior estimates (del Mar Fernandez de Gatta *et al*, 1996). Therefore, the population parameters need to be estimated from a sufficient number of patients, including the pathological and physiological factors affecting the pharmacokinetics of the drug (del Mar Fernandez de Gatta *et al*, 1996). In this sense, NONMEM permits population pharmacokinetic analysis using routine clinical data from representative patients for inclusion in Bayesian methods.

#### 1.7.2.1. *Bayesian Forecasting in Pediatrics*

The utilization of Bayesian forecasting in children may be advantageous for several reasons. It addresses the problem of limited sampling, and permits the use of more complex models accounting for the dynamic changes that will occur over their developmental period (del Mar Fernandez de Gatta *et al*, 1996). Furthermore, Bayesian methods may minimize the

necessity for aggressive monitoring, thus optimizing therapeutic drug monitoring in this patient population.

The drugs that have been commonly subjected to Bayesian forecasting approaches are those with a narrow therapeutic index that are routinely monitored by serum drug concentrations. Bayesian forecasting for neonates has been used for gentamicin (Kelman *et al*, 1984; Lui *et al*, 1991; Rodvold *et al*, 1993), and theophylline (Murphy *et al*, 1990). Most pediatric studies have reported that Bayesian forecasting can provide predictions of serum concentration-dose relationships that are as good or better than the standard method with regard to accuracy and precision (Rodvold *et al*, 1993; Rodvold *et al*, 1995; del Mar Fernandez de Gatta *et al*, 1996). Moreover, in these studies the Bayesian method tended to be more robust over a broad range of situations.

Computer software applications have been developed to assist dose optimization based on Bayesian methods; however, only a few programs include analysis and treatment guidelines for patients of all ages, ranging from premature neonates to geriatric patients (Poirier and Guidier, 1992; Ensom *et al*, 1998). Most of them do not permit the modification of population pharmacokinetic parameters, or inclusion of unlisted drugs. The introduction of specific pediatric population parameters in clinical pharmacokinetic software programs is hampered by the complexity of population pharmacokinetic models, particularly in neonates, which may include a large number of covariates (del Mar Fernandez de Gatta *et al*, 1996).

### **1.7.3. Bayesian Forecasting of Vancomycin in Neonates**

An investigation by Rodvold *et al* (1995) compared mean population parameters with Bayesian forecasting in predicting vancomycin concentrations in neonates. Retrospective data were collected from 47 neonates who received vancomycin between 1989 and 1992. Twenty-nine patients, having at least one set of steady-state peak and trough concentrations, were used to estimate population parameters by nonlinear least-squares analysis. Eighteen patients with both initial and subsequent (on a revised dose) peak and trough concentrations were used to test the predictive performance of the model with and without Bayesian forecasting.

Multiple stepwise linear regression identified PNA and creatinine clearance as predictors of vancomycin Cl. No significant covariates were identified for the volume of distribution. Hence, a one-compartment model was constructed using the associations of PNA and creatinine

clearance with vancomycin Cl. When predicted concentrations occurred within 30 days of feedback concentrations, the Bayesian method tended to be more accurate and precise than the population-based parameters. Conversely, population-based parameter estimates were more accurate in predicting both peak and trough concentrations obtained more than 30 days from the initial set of concentrations. Overall, the Bayesian method was significantly less biased for prediction of peak concentrations while population parameters were superior for prediction of trough concentrations.

There were discrepancies between the two groups of patients in this study. Given the retrospective nature of the data analysis, one group was necessary to determine population parameters and another to evaluate these parameters. The primary difference between groups was the number of available vancomycin concentrations. Accordingly, those patients included in the second group had to have more vancomycin concentrations available than group one. Hence, it is likely that patients in this second group were more likely to be older than those in the first thereby, introducing a selection bias in generating the predictor. Moreover, Bayesian forecasting permits the utilization of non-steady-state serum concentrations as feedback; however, it appears that the investigators used steady-state serum concentrations to predict steady-state concentrations either for the same dose or at a subsequent dose.

Due to the continuous maturation process and influences of other factors, dosage individualization in neonates is particularly difficult. Accordingly, the Bayesian method offers a clinical advantage in that limited drug concentration data may predict future drug concentration: dose relationships that are as good or better than the standard methods. Moreover, Bayesian forecasting can use both steady-state and non-steady-state drug concentrations, whereas the standard method requires steady-state data for reliable predictions. Bayesian forecasting offers an opportunity to minimize the number of measured drug concentrations that must be procured when therapeutic drug monitoring is indicated. Despite these potential benefits, no prospective studies assessing the predictive performance of Bayesian forecasting, using a pharmacokinetic model derived from a NONMEM population-based analysis, of vancomycin in neonates have been realized.

**The second objective was to evaluate the accuracy and precision of a Bayesian forecasting method based on an optimum population pharmacokinetic model for predicting serum vancomycin concentrations in neonates.**



## **METHODS**

### **2.1. POPULATION PHARMACOKINETIC MODELING**

#### **2.1.1. Study Design**

This component of the investigation was a prospective, observational study. A database of patient demographic and clinical characteristics potentially affecting vancomycin disposition was maintained for each patient meeting entry criteria. Subsequently, pharmacokinetic and pharmacostatistical models were developed to characterize vancomycin disposition in neonates.

#### **2.1.2. Study Setting**

This element, like all segments, of the investigation was conducted in the Special Care Nursery (SCN) of Children's and Women's Health Centre of British Columbia (C & W). In 1997, C & W was created in Vancouver, British Columbia, Canada through the merger of British Columbia's Children's Hospital, British Columbia's Women's Hospital and Health Centre, and Sunny Hill Health Centre for children. Presently, C & W has more than 400 in-patient beds and is the major referral center in the province for acutely ill or injured children. C & W has the largest maternal-fetal-newborn clinical service in Canada, and the SCN comprises a 50-bed tertiary-care unit that admits approximately 625 newborns each year. The SCN is a neonatal intensive care unit (NICU) that exclusively provides acute and chronic care to newborns requiring medical intervention.

#### **2.1.3. Patient Enrollment**

All neonates with a PCA of  $\leq 44$  weeks admitted to the SCN between January 01, 1996 and December 31, 1999 and prescribed vancomycin by their attending physicians comprised this study sample. Data were collected during each course of vancomycin treatment; hence, each patient may have been represented more than once in the database. All patients were included unless they met any exclusion criteria.

##### **2.1.3.1. *Exclusion Criteria***

1. Standard set of peak and trough concentrations not quantified or reported

2. Vancomycin dosing history incomplete
3. Post-conceptual age > 44 weeks.

The objective of this component of the investigation was to develop a population-based model of vancomycin pharmacokinetics in neonates. Accordingly, all patients presenting with various pathophysiological disturbances were included to permit a comprehensive analysis.

#### 2.1.4. Ethical Approval

The study protocol was approved by the British Columbia's Children's Hospital Research Review Committee and the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects. The Certificates of Approval are attached (Appendix 6). Informed consent was not required, as no therapeutic intervention affecting patient care was employed.

#### 2.1.5. Vancomycin Administration

Vancomycin hydrochloride (Vancocin<sup>®</sup>, Eli Lilly and Co., Indianapolis, IN, USA) was administered according to the current SCN dosage guidelines (Table 3), based upon four PCA groups: < 27 weeks; 27 – 30 weeks; 31 – 36 weeks; and ≥ 37 weeks (McDougal *et al*, 1995). The antibiotic was infused (antegrade) intravenously at a concentration of 5 mg/mL in D<sub>5</sub>W over 60 minutes, using a Medfusion<sup>®</sup> syringe pump (Ardus Medical Inc., Cincinnati, OH, USA) (Appendix 7).

**Table 3. Vancomycin Dosage Guidelines.**

Post-Conceptual Age (weeks)	Weight (g)	Dose (mg/kg/dose)	Dosing Interval (h)
< 27	< 800	18	36
27 - 30	800 - 1200	16	24
31 - 36	1200 - 2000	18	18
≥ 37	> 2000	15	12

## 2.1.6. Sample and Data Collection

### 2.1.6.1. *Biological Sampling*

Throughout the study period the standard practice in the SCN of C & W required the use of at least two steady-state serum vancomycin concentrations to optimize therapy. Trough samples (0.5 mL) were routinely drawn 30 minutes prior to the third dose, with a target concentration of 5 – 10 mg/L. Peak samples were obtained 60 minutes following a 60-minute infusion of the third dose, with a target concentration of 30 – 40 mg/L.

### 2.1.6.2. *Bioanalytical Methods*

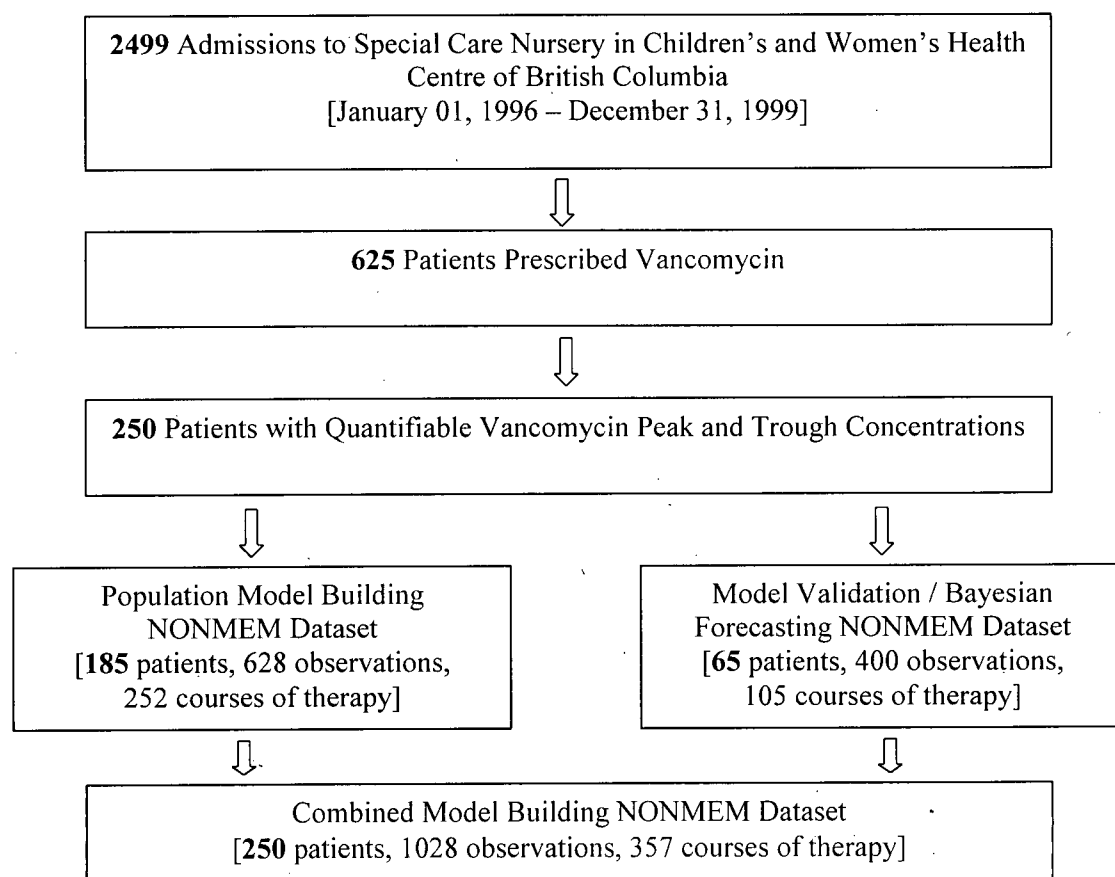
Serum samples were analyzed for vancomycin using a fluorescence polarization immunoassay (TDX, Abbott Diagnostics, Irving, TX, USA) validated over the concentration range of 2.0 to 100 mg/L. The coefficients of variation (CV) of the assay were 4 %, 3 %, and 3 % for vancomycin concentration ranges of 6 – 8 mg/L, 31.5 – 38.5 mg/L, and 67.5 – 82.5 mg/L, respectively (McDougal *et al*, 1995). The samples were analyzed in the Clinical Laboratory of C & W by staff technicians.

### 2.1.6.3. *Clinical Data Collection*

Data collection for this study included patients admitted to the SCN between January 01, 1996 and December 31, 1999. Clinical data were collected prospectively on all patients enrolled in this component of the investigation using a pre-specified data collection form (Appendix 8). Data were collected from information routinely recorded during the course of patient care and from samples obtained in accordance with standard therapeutic intervention or care. All data were recorded and maintained in a manner that ensured confidentiality. Eligible patients were followed prospectively during each course of vancomycin therapy. Information not available during daily data collection was collected retrospectively through patient chart review in Medical Records.

### 2.1.7. Dataset Preparation

Vancomycin serum concentrations were combined with dosing information, demographic, laboratory, physiological and therapeutic data and entered into a Microsoft Excel<sup>®</sup> 97 data file. The accuracy, consistency, completeness, and reliability of data was assured by the author who entered all data and reviewed the dataset. The final dataset for the population modeling component of the investigation contained 628 serum concentration results from 185 patients (Figure 2).



**Figure 2. Data Disposition: Vancomycin Concentration Data Included in the Pharmacokinetic Analyses.**

To produce the analysis datasets, relevant data from the original, validated, datasets were exported into separate Microsoft Excel<sup>®</sup> 97 files and saved with Excel extensions (.xls). These files, containing data values only, were then saved with formatted text, space delimited (.prn) extensions to permit viewing in a word processing application. The space delimited datasets

were then opened in Microsoft Notepad or Wordpad Versions 5.0, and the consistency and accuracy of the information were verified and saved as text files (.txt) to produce the analysis datasets for the nonlinear mixed effects modeling program (NONMEM V, version 1.1, NONMEM Project Group, UCSF). Appendix 9 provides definitions of the variables used in the NONMEM data input file.

### **2.1.8. Population Pharmacokinetic Modeling Strategy**

NONMEM is a parametric approach that can provide estimates of pharmacokinetic parameters based upon limited data from individual subjects who are representative of the population (Kauffman and Kearns, 1992). Fundamental in population pharmacokinetic studies with NONMEM is the estimation of fixed and random effects.

A population pharmacokinetic model was initially developed for vancomycin by fitting concentration-time data from 185 patients (252 courses of therapy) using NONMEM and PREDPP. Figure 3 illustrates the general process for development of a population pharmacokinetic model based upon the methods advocated by Sheiner and Beal (1992) and Maitre *et al* (1991). The relevant terminology is summarized in the preface list of NONMEM abbreviations.

Similar to other nonlinear regression applications, NONMEM does not contain preset models with which it can compute a predicted value given the current values of the regression parameters (Beal and Sheiner, 1989). Rather, NONMEM calls a subroutine having entry name PRED (prediction) to obtain predicted values and compute NONMEM partial derivatives with respect to the random error effects eta ( $\eta$ ) and epsilon ( $\epsilon$ ) (Beal and Sheiner, 1989). Prediction for Population Pharmacokinetics (PREDPP) is a collection of PRED subroutines for use with NONMEM. Whereas, NONMEM is a general regression tool, PREDPP is specialized to the type of predictions that arise in pharmacokinetic data analysis (Beal and Sheiner, 1989). It can compute predictions according to a variety of different pharmacokinetic models and dosing regimens.

Two important subroutines of PREDPP are called PK and ERROR (Beal and Sheiner, 1989). The first routine, PK, computes the values of pharmacokinetic parameters of a given model (i.e. clearance, in terms of the values of the covariates) and accounts for differences

between individual and population values ( $\eta$ ). The second routine, ERROR, functions essentially to specify the statistical error between predicted and observed values ( $\epsilon$ ).

NONMEM Translator (NM-TRAN) is a separate stand-alone control language translator and data processor. When NM-TRAN is used, a NONMEM execution includes two steps: first, the NM-TRAN process, in which a file of NM-TRAN records (begin with \$) are translated into several NONMEM input files, and second, the NONMEM step itself (Beal and Sheiner, 1989).

#### 2.1.8.1. *Unadjusted (Base) Model Development*

The dataset compiled from 185 patients with 628 observations was used for model development. The interoccasion variability, arising from random variation between study occasions, is often greater than the interindividual variability in human data; hence, Karlsson and Sheiner (1993) advocated treating each occasion as though it were a distinct individual. Furthermore, individual variability that may be linked to physiological processes by means of surrogate variables such as age (neonates) is predictable and not random (Karlsson and Sheiner, 1993). Therefore, in the present investigation, each course of vancomycin therapy (252) was assigned a unique identification number and treated as a separate patient to account for dynamic changes in the neonatal period. One- and two-compartment pharmacokinetic models were systematically evaluated to identify the model that best described vancomycin pharmacokinetics in these patients. The first order estimation method (FO) was implemented for each structural model tested (Beal and Sheiner, 1992).

One-compartment models were parameterized in terms of clearance (CL) and volume of distribution (V). Data analyses were conducted using the PREDPP subroutine ADVAN1 (TRANS2). Two-compartment models were evaluated with PREDPP subroutine ADVAN3 (TRANS4) and were parameterized in terms of CL, central volume of distribution (V1), peripheral volume of distribution (V2), and intercompartmental clearance (Q).

#### 2.1.8.2. *Covariate Model Development*

Patient factors examined as covariates affecting vancomycin disposition are listed (Table 4). Gestational age (GA), postnatal age (PNA), post-conceptual age (PCA), Apgar scores, weight, blood urea nitrogen, serum creatinine, urine output, and total fluid balance were

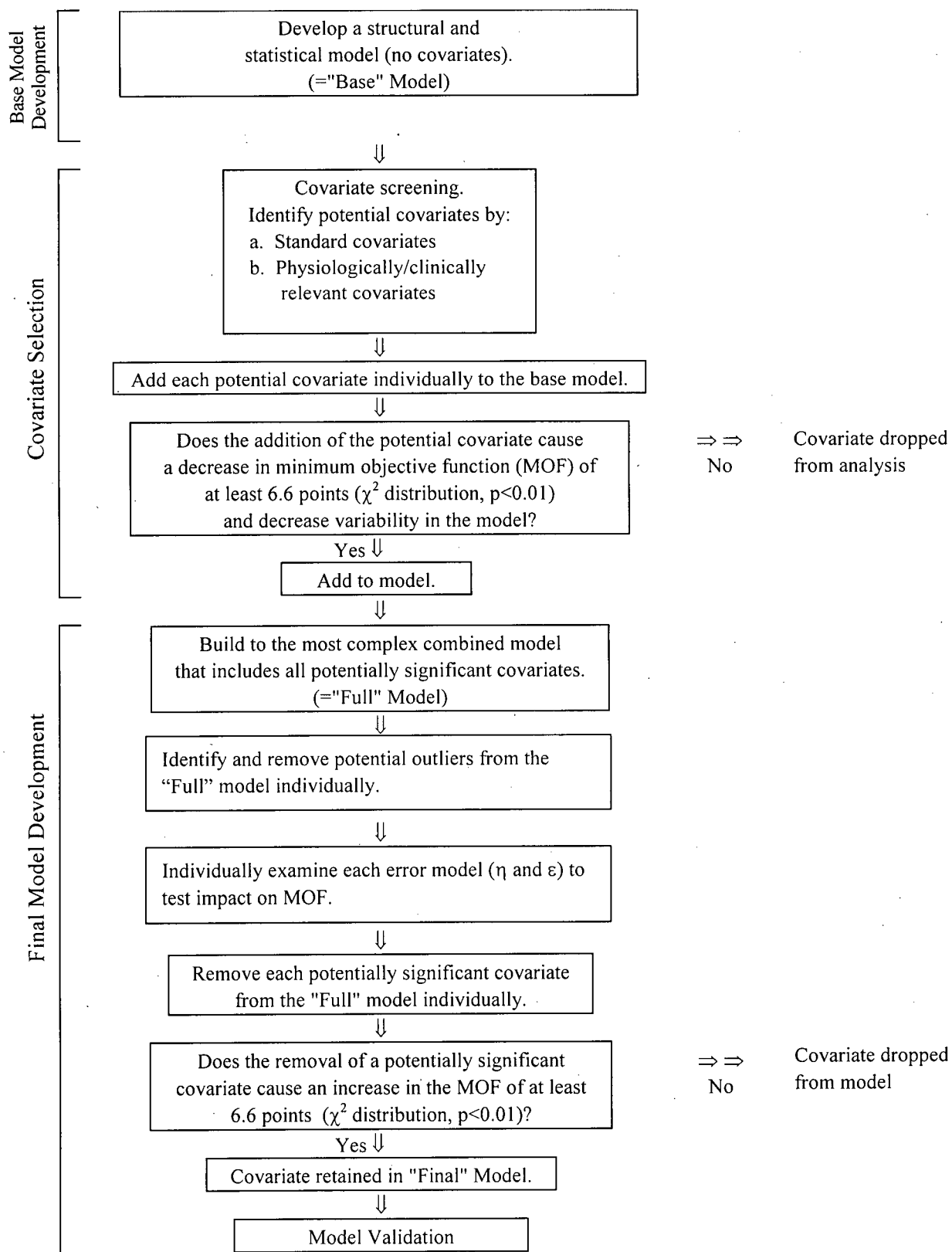


Figure 3.

**General Process for Pharmacokinetic Modeling.**

classified as continuous variables. Gender, pharmacotherapy within 72 hours of vancomycin concentration determination, preterm birth, chronic lung disease, Coagulase Negative Staphylococcus (CONS) sepsis, Necrotizing Enterocolitis (NEC), Patent Ductus Arteriosus (PDA), and Respiratory Distress Syndrome (RDS) were coded as dichotomous (categorical) variables. For all dichotomous variables, the presence of the variable was designated by a "1" and the absence was assigned a value of "0". If any physiologic measure, for example, weight, was missing during the course of vancomycin therapy, the value from the preceding day was used. For patients with a 100 g change between measured weights an interpolated weight was calculated as:

$$\left[ \frac{\text{Difference Between Last and Next to Last Measured Value}}{\text{Number of Days Between Measurements}} \right] + \text{Next to Last Measured Value}$$

Only those variables that were identified in at least 5% of the patient population were assessed as potential covariates.

Univariate analyses were used to reduce the initial list of patient factors that might be individually affecting vancomycin pharmacokinetics. For dichotomous covariates, univariate analysis was performed with the student t-test for analyses of means with unequal variances; whereas, continuous covariates were evaluated by regression analysis (Microsoft Excel® 97). Variables were selected as candidates for NONMEM analysis when the p-value was  $\leq 0.15$  by the appropriate univariate test (Table 4). Patient factors that exceeded this criterion, but were thought to be clinically important were also selected (Table 4).

Furthermore, individual Bayesian regression analysis using the measured serum vancomycin concentrations from each subject and the population parameters obtained in the unadjusted, base model development was performed with NONMEM. The use of a POSTHOC routine in the ESTIMATION record directs Bayesian estimation to be performed with each individual's record; Bayesian estimates of all  $\eta$  values, for each individual, are obtained, conditional on the population parameter values (Boeckman *et al*, 1992). This provided individual (Bayesian) estimates of the pharmacokinetic parameters, which were plotted against demographic factors to identify possible correlations (Maitre *et al*, 1991). Also, the plots illustrated the shape of the relationship, which facilitated the assignment of a mathematical equation to describe the association. The NONMEM analysis was resumed, whereby the influence of the patient factors of interest were entered into the pharmacokinetic model



sequentially, first for those factors that appeared strongly correlated with the pharmacokinetic parameters, then for those relationships that were less obvious.

**Table 4. Patient Factors Assessed in the Population Pharmacokinetic Analyses.**

Demographic	Clinical <sup>a</sup>	Therapeutic <sup>b</sup>	Laboratory <sup>b</sup>	Physiological <sup>a</sup>
Gender	Chronic Lung Disease (CLD) <sup>c</sup>	Budesonide <sup>c</sup>	Blood Urea Nitrogen	Daily weight <sup>c</sup>
Gestational Age (GA) <sup>c</sup>	Coagulase Negative Staphylococcus (CONS)	Dexamethasone <sup>c</sup>	Serum Creatinine	Urine Output
Postnatal Age (PNA) <sup>c</sup>	Sepsis	Diuretic		Total Fluid Balance
Post-Conceptional Age (PCA) <sup>c</sup>	Necrotizing Enterocolitis (NEC) <sup>c</sup>	Dopamine (DOP) <sup>c</sup>		
Preterm Birth	Patent Ductus Arteriosus (PDA) <sup>c</sup>	Gentamicin <sup>d</sup>		
APGAR Scores	Respiratory Distress Syndrome (RDS)	Indomethacin (IND) <sup>c</sup>		/
		Opioid <sup>d</sup>		
		Pavulon		

<sup>a</sup> Clinical diagnoses, continuing medical conditions, or physiological parameters during vancomycin therapy and concentration determinations.

<sup>b</sup> Pharmacotherapy or laboratory assessment within 72 hours of vancomycin concentration determination.

<sup>c</sup> Covariates that satisfied statistical criterion for testing in NONMEM analysis.

<sup>d</sup> Covariates tested in NONMEM analysis, but exceeded statistical criterion.

In NONMEM, continuous covariates were tested for their relationships with typical values (TV) of: CL (TVCL), V (TVV), V1 (TVV1), and V2 (TVV2) using linear, multiplicative, proportional, and power models (Equations 1-9 through 1-13). Categorical factors were tested with indicator variables (Equations 1-13 and 1-14).

Linear Model	$P = \theta_1 + (\theta_2 * [\text{COV}])$	(Equation 1-9)
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Multiplicative	$P = \theta_1 * [\text{COV}]$	(Equation 1-10)
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Proportional Model	$P = \theta_1 * (1 + \theta_2 * [\text{COV}])$	(Equation 1-11)
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Power Model (1)	$P = \theta_1 * (\theta_2 ** [\text{COV}])$	(Equation 1-12)
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Power Model (2)	$P = \theta_1 * ([\text{COV}] ** \theta_2)$	(Equation 1-13)
-----------------	---	-----------------

$$\text{Categorical Model (1)} P = \theta_1 * (\theta_2 ** [\text{IND}]) \quad (\text{Equation 1-14})$$

$$\text{Categorical Model (2)} P = \theta_1 * (1 + \theta_2 ** [\text{IND}]) \quad (\text{Equation 1-15})$$

Where P is the estimate of the pharmacokinetic parameter,  $\theta_1$  represents the typical base value of the parameter, and  $\theta_2$  estimates the effect of a covariate (COV). The indicator variables (IND) were designated by values of "0" or "1". Potentially significant covariates were identified as those which, when added to the unadjusted, base model individually, resulted in a decrease in the minimum value of the objective function (MOF; -2 log likelihood of the data) of > 6.6 points ( $\chi^2$  distribution for 1 degree of freedom,  $p < 0.01$ ) (Jensen *et al*, 1992).

#### 2.1.8.3. Refined (Final) Model Development

Selected covariates, when tested individually, were added sequentially to the unadjusted, base model to establish a full model containing all possible exploratory covariates. Inspection of the weighted residual (prediction error adjusted for interindividual variability) plots as a function of PCA permitted the identification of potential outliers. These outliers were individually removed and those that resulted in a > 6.6 point improvement in the MOF and whose clinical presentation was not consistent with the majority of the patient sample were removed. Next, the appropriateness of the interindividual ( $\eta$ ) and intraindividual ( $\epsilon$ ) variability was tested on the individual parameters (CL, V, V1, V2, and Q). Two interpatient variability models were tested in the one-compartment model:  $\eta$  on CL, and  $\eta$  on CL and V. The interpatient variability models tested with the two-compartment structural model included:  $\eta$  on CL,  $\eta$  on CL and V1,  $\eta$  on CL, V1, and V2, and  $\eta$  on CL, V1, V2, and Q. With each combination of structural model and interpatient variability, three residual ( $\epsilon$ ) error models were evaluated: additive, exponential, and combined additive and exponential. Finally, each covariate was individually removed from the full model. Covariates retained in the final, revised model were those resulting in a significant increase in the MOF of > 6.6 points, when removed from the full model.

#### 2.1.9. Population Model Validation

A database constructed from a naïve cohort of 65 patients with 400 observations (105 courses of therapy) (Figure 2) consisting of demographic, laboratory, physiological and

therapeutic data (Appendix 8) collected from admissions to the SCN during the same period as those obtained for the purposes of the aforementioned model building was used for validation analyses. Data collected during each course of vancomycin treatment from neonates with a PCA of < 44 weeks prescribed vancomycin by their attending physicians and having at least one set of standard peak and trough concentrations were included in this component of the investigation. In addition to the standard set of vancomycin concentrations, this patient sample was comprised of neonates with strictly timed midinterval and near-midinterval (residual) concentrations during a single course of vancomycin therapy (Section 2.2.3). These additional samples, collectively termed intradose interval concentrations, were obtained prior to or following the third dose of vancomycin therapy; thereby permitting assessment of the predictive performance of single non-steady-state and possible steady-state concentration predictions. Data assembly and preparation followed the method provided in Section 2.1.7. Consistent with model development (Section 2.1.8), each course of vancomycin therapy (105) was assigned a unique identification number and treated as a separate patient.

#### *2.1.9.1. Validation Analyses*

The revised, final one- and two-compartment models were evaluated with data from this naïve cohort of patients, which was not used to develop the models themselves. Model parameter values of THETA, OMEGA, and SIGMA were fixed by setting the MAXEVALS = 0 in the ESTIMATION record, thereby preventing the population parameters from changing. The purpose of this external validation was to examine the precision (mean absolute error) and accuracy (mean error) of the predicted concentrations generated by the final models (Sheiner and Beal, 1981). The population prediction, given by the population model with  $\eta = 0$ , was made without the benefit of using any concentration observations from the individual (feedback); that is, without Bayesian estimation.

The predictive performance of peak, trough, and intradose interval predictions based upon one- and two-compartment models was assessed according to the method of Sheiner and Beal (1981). Also, 95% confidence intervals were constructed around the difference between two- and one-compartment prediction error to indicate possible differences in predictive performance.

### **2.1.10. Combined Model Development**

Following model validation, a combined population pharmacokinetic model was fully developed using the patient samples comprising the model building (185) and validation (65) groups. As with the original model development (Section 2.1.8), an iterative process was implemented to generate unadjusted, full, and final models. Vancomycin pharmacokinetics were characterized for the combined dataset comprised of 1028 observations from 250 patients (Figure 2). As previously described (Sections 2.1.6.3; 2.1.9), data were collected (Appendix 8) from neonates with a PCA of < 44 weeks admitted to the SCN between January 01, 1996 and December 31, 1999 and prescribed vancomycin. Data were collected during each course of therapy providing exclusion criteria were not met. Analysis datasets for implementation in NONMEM were compiled as previously presented (Section 2.1.7).

## **2.2. BAYESIAN FORECASTING**

### **2.2.1. Study Design**

Similar to the design of the population pharmacokinetic modeling element, the Bayesian forecasting component reflected a prospective, observational study. Again, a database of patient demographic and clinical characteristics (Appendix 8) potentially affecting disposition was maintained for each patient meeting entry criteria. Subsequently, the predictive performance of one- and two-compartment Bayesian methods using single and two-point sampling strategies supplied to the population pharmacokinetic models previously described (Section 2.1) were evaluated.

### **2.2.2. Study Setting**

Like previously described (Section 2.1.2), this component of the investigation was conducted in the SCN of C & W. This facility is a NICU that exclusively provides acute and chronic care to newborns requiring medical intervention.

### **2.2.3. Patient Enrollment**

All neonates with a PCA  $\leq$  44 weeks admitted to the SCN between January 01, 1996 and December 31, 1999 and prescribed vancomycin by their attending physicians were eligible for

entry into this study. Data were collected during each course of vancomycin treatment in order to complete a comprehensive validation analysis (Section 2.1.9); however, only samples obtained during a single course of therapy were supplied as feedback concentrations in a Bayesian method. Vancomycin administration (Section 2.1.5) was identical to that previously described.

In order to evaluate the predictive performance of single, midinterval, feedback concentrations, two additional serum samples (0.5 mL each) were procured from each neonate (35) following either the first or second vancomycin dose and after the third dose (Appendix 10). As these samples did not constitute routine care or monitoring, informed parental consent was required (Appendix 11). All parents or legal guardians were personally approached by the investigator to provide informed consent at the time of initiation of vancomycin therapy or shortly thereafter, unless the patients met any exclusion criteria.

#### *2.2.3.1. Exclusion Criteria*

1. Hemodynamic instability
2. Pathological renal or cardiovascular disease
3. Vancomycin dosing history incomplete
4. Post-conceptual age > 44 weeks.

Originally, the intent of this component of the investigation was restricted to exclusive midinterval sample collection in addition to the standard set of peak and trough vancomycin concentrations, following parental consent. However, recruitment of 60 patients was difficult for the following reasons: consent refused due to the requirement for additional blood sampling with no immediate benefit to the patient, investigator could not contact parents or legal guardians prior to time of additional sampling, and investigator not informed of initiation of vancomycin therapy.

During the conduct of the investigation, an additional source of patients for Bayesian forecasting inclusion and evaluation was identified. To ensure a reasonable sample size for Bayesian assessments, a cohort of patients (30) with residual blood samples that were collected for other clinical purposes was identified. Residual samples collected within 10% of the mid-point of the dosing interval, following the first or second vancomycin dose and after the third dose, if available, were analyzed for vancomycin (Appendix 12). In this cohort, informed

consent was not required as samples were obtained during routine monitoring and care, and no therapeutic intervention affecting patient care was employed.

#### **2.2.4. Ethical Approval**

The study protocol was approved by the British Columbia's Children's Hospital Research Review Committee and the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects. The Certificates of Approval are attached (Appendix 6). The parental informed consent document required for the group with strictly timed midinterval sampling is appended (Appendix 11).

#### **2.2.5. Sample and Data Collection**

Bioanalytical methods (Section 2.1.6.2) and clinical data collection (Section 2.1.6.3) were identical to those previously described. Similarly, data assembly and preparation followed the method described in Section 2.1.7, with one exception. In order to identify an event record other than dose and observation, an identifier, EVID, was required in the NM-TRAN dataset for all records. Whereby, a value of "0" and "1" was assigned to a record containing a measured (feedback) concentration and dose event, respectively. A value of "2" was assigned to those records at which a predicted concentration was desired without associated dose or observation data. In this regard, user-specified vancomycin concentrations (pre-third dose, peak, trough, and post-third dose) were sequentially used as feedback in Bayesian estimation to obtain individual predictions of other, non-feedback, concentrations.

#### **2.2.6. Bayesian Estimation**

Feedback predictions for a given individual are based on estimates of individual-specific pharmacokinetic parameter values that are generated from individual (feedback) observations other than those that are being predicted. As the amount of data per individual increases, the Bayesian term becomes less influential, and the individual specific Bayesian estimates become extended least squares estimates (Boeckman *et al*, 1992).

Most Bayesian methods offer uncomplicated modeling with individual data; however, most do not permit the flexibility offered by NONMEM in terms of dataset and model definition. The individual predictions were generated from estimates of the individuals' parameters. These

individual-specific parameters were computed from Bayesian estimates of individuals'  $\eta$ , and thus the difference between the population parameter estimate and the individual parameter was a consequence of the Bayesian estimate of  $\eta$ . In contrast, population predictions for model validation that did not use feedback observations, assign an  $\eta = 0$ .

The NONMEM method to obtain feedback predictions, one used with data from this 65 patient cohort, implemented Bayesian estimation as follows. The ESTIMATION record included MAXEVALS = 0 and POSTHOC commands, by setting MAXEVALS = 0 the estimation step was not implemented. The population parameter values of THETA, OMEGA, and SIGMA were fixed to those given in the revised, final one- and two-compartment models. Both the THETA and SIGMA values remained unchanged in the NONMEM execution. Estimation of  $\eta$  was completed after estimates of the pharmacokinetic parameters were obtained using case-specific dosing and covariate data, rather than as part of the population parameter estimation. This estimate of  $\eta$  is therefore called a posthoc (conditional) estimate. The use of POSTHOC directs Bayesian estimation to be performed with each individual's record; Bayesian estimates of all  $\eta$  values, for each individual, were obtained, conditional on the population parameter values.

#### 2.2.6.1. *One- and Two-Compartment Comparisons*

Concentration predictions based on Bayesian estimates were provided in a NONMEM generated output (TABLE) by including an IPRED = F statement in the ERROR record, and using the IPRED label in the TABLE record. Pre-third dose, trough only, and post-third dose vancomycin concentrations were independently supplied as feedback observations in the revised, final, one- and two-compartment models to obtain case-specific predictions of vancomycin peak concentrations. Similarly, pre-third dose, peak only, and post-third dose vancomycin concentrations were independently applied as feedback in the revised, final one- and two-compartment models to obtain Bayesian predictions of trough concentrations.

The predictive performance of peak and trough concentrations based upon one- and two-compartment models was assessed according to the method of Sheiner and Beal (1981). The precision and accuracy of the Bayesian predictions were assessed by the measure of mean absolute error and mean error, respectively.

#### 2.2.6.2. *Bayesian Predictions of Follow-Up Concentrations*

A number of patients (16) required a dosage adjustment based upon measured vancomycin concentrations and clinical condition. Accordingly, two serum concentrations were drawn around the third dose of the initial course of therapy to calculate individual pharmacokinetic parameters based upon a one-compartment model for dose individualization (Sawchuk and Zaske, 1976). The predictive performance of the Bayesian method was also evaluated in patients from whom follow-up third dose peak and trough vancomycin concentrations were quantified following a dosage adjustment. Again, concentration predictions based on Bayesian estimates were presented in a NONMEM generated output (TABLE) by including an IPRED = F statement in the ERROR record, and using the IPRED label in the TABLE record (Section 2.2.2.1).

First, both peak and trough vancomycin concentrations obtained from the initial dosing regimen were supplied as feedback observations in the revised, final two-compartment model to obtain individual predictions of the follow-up peak and trough concentrations. In comparison, predictions of follow-up peak and trough concentrations calculated by the method of Sawchuk and Zaske (1976) were generated. This latter method assumes a one-compartment open model and requires both peak and trough concentrations as feedback. The predictive performance of both methods was assessed (Sheiner and Beal, 1981). Also, 95% confidence intervals were constructed around the difference between Bayesian and Sawchuk-Zaske prediction errors to indicate possible differences in predictive performance.

To compare the predictive ability of single and two-point sampling strategies, individual (pre-third dose, peak, trough, post-third-dose) and combined (peak and trough) vancomycin concentrations obtained from the initial dosing regimen were supplied as feedback observations in the revised, final two-compartment model to obtain case-specific predictions of follow-up peak and trough concentrations. Again, the predictive performance of follow-up peak and trough predictions using pre-third, trough only, peak and trough, and post-third dose feedback obtained during the initial dosing regimen was evaluated (Sheiner and Beal, 1981).



## **RESULTS**

### **3.1. POPULATION PHARMACOKINETIC MODELING**

#### **3.1.1. Demographic Characteristics of the Model Building Patient Sample**

The SCN in C & W admitted  $625 \pm 24$  (mean  $\pm$  sd) patients annually between January 01, 1996 and December 31, 1999. The demographic data from the 2499 admissions are presented in Table 5. Fifty-eight percent of the patients were male, and the mean ( $\pm$  sd) gestational age upon admission was  $33.4 (\pm 5.0)$  weeks. Of the 2499 admissions, 625 patients (25%) were prescribed vancomycin therapy by their attending physicians. Consistent with the general population demographics, 59% of those patients prescribed vancomycin were male, and the mean ( $\pm$  sd) gestational age upon admission was  $29.5 (\pm 4.6)$  weeks. Of these 625 patients, 40% were enrolled in the model building, validation analyses, or Bayesian forecasting investigation. Sixty percent of patients were excluded for the following reasons: standard set of peak and trough concentrations not quantified or reported, vancomycin dosing history incomplete, and failure of investigators to prospectively identify patients. Often, vancomycin therapy is empiric, based upon clinical presentation, and thus may be discontinued within three days or when infection no longer appears to be a concern. Therefore, standard sets of concentrations are not obtained for every patient.

Table 6 summarizes the demographic characteristics of the 185 patients enrolled in the model building component of this investigation. Similar to the general admission population, 58% of this sample were male, and the mean ( $\pm$  sd) gestational age upon admission was  $29.9 (\pm 4.5)$  weeks. The overwhelming majority of patients were preterm with a history of respiratory distress syndrome. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) Apgar scores at one- and five-minutes were 6 (4, 7) and 8 (7, 9), respectively. The prevalence of medical diagnoses and pharmacotherapy was indicative of fragile patients admitted to a NICU. Together, empiric sepsis therapy and confirmed Coagulase Negative Staphylococcal sepsis represented  $> 80\%$  of indications for vancomycin. Thirty percent of this patient sample were prescribed multiple courses of vancomycin, and 628 serum vancomycin concentrations were quantified.

**Table 5. Demographic Characteristics of Patients Admitted to the Special Care Nursery in the Children's and Women's Health Centre of British Columbia from 1996 through 1999.**

Demographic Characteristics	Number (%)
<b>Number of Patients</b>	<b>2499</b>
Male	1446 (57.9)
Female	1048 (41.9)
<b>Number of Patients Prescribed Vancomycin</b>	<b>625 (25.0)</b>
Male	370 (59.2)
Female	255 (40.8)
<b>Number of Vancomycin Treated Patients</b>	<b>250 (40.0)</b>
<b>Enrolled in the Investigation</b>	
Male	148 (59.2)
Female	102 (40.8)

**Table 6. Demographic Characteristics of Patients Enrolled in the Model Building Component of the Investigation.**

Demographic Characteristics	Number (%)
<b>Number of Patients</b>	<b>185</b>
Male	107 (57.8)
Female	78 (42.2)
<b>Admission History</b>	
Preterm Birth	169 (91.4)
Respiratory Distress Syndrome	149 (80.5)
<b>Indication for Vancomycin Therapy</b>	
Empiric Therapy - Sepsis	129 (51.2)
Coagulase Negative Staphylococcal Sepsis	76 (30.2)
Necrotizing Enterocolitis	20 (7.9)
Empiric Therapy - Necrotizing Enterocolitis	19 (7.5)
Other	8 (3.2)
<b>Clinical Presentation at the Initiation of Each Course</b>	
Chronic Lung Disease	155 (61.5)
Coagulase Negative Staphylococcal Sepsis	76 (30.2)
Dopamine <sup>a</sup>	24 (9.5)
Indomethacin <sup>a</sup>	20 (7.9)
Necrotizing Enterocolitis	20 (7.9)
<b>Number of Courses of Vancomycin</b>	<b>252</b>
<b>Number of Patients with Multiple Courses of Vancomycin</b>	<b>55 (29.7)</b>
Number of Patients with Two Courses	43 (23.2)
Number of Patients with Three Courses	12 (6.5)
<b>Number of Routine Serum Drug Concentration Determinations</b>	<b>628</b>
Number of Peak or Trough Concentration Determinations	624
Number of Random Concentration Determinations	4

<sup>a</sup> Pharmacotherapy within 72 hours of serum concentration determination.

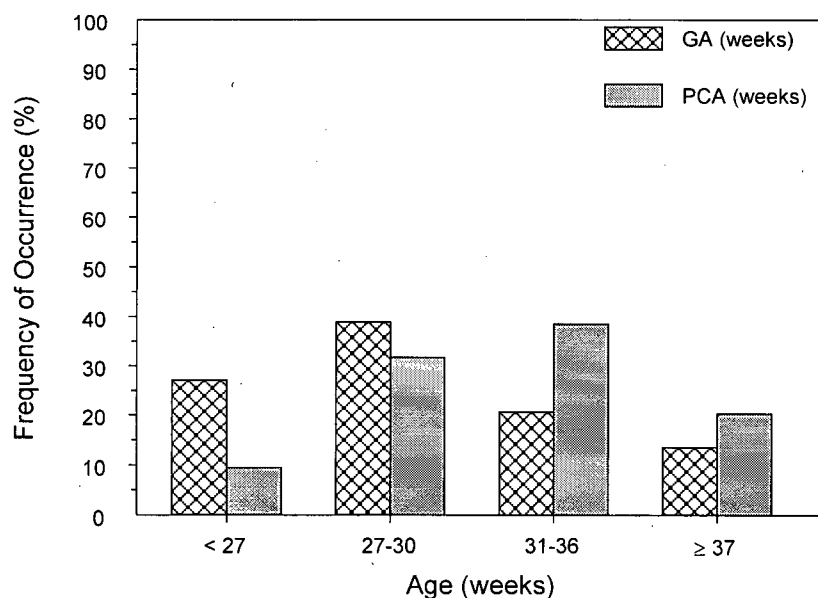
Figure 4 illustrates the gestational and post-conceptual age distribution of the 185 patients at the initiation of the 252 courses of vancomycin therapy. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) post-natal age at the start of each course was 15 (8, 26) days, and this is reflected the right-shift in the distribution pattern between gestational and post-conceptual age. The mean ( $\pm$  sd) post-conceptual age and weight at the initiation of each vancomycin course were 32.3 ( $\pm$  4.6) weeks and 1.5 ( $\pm$  0.9) kg, respectively. Figure 5 demonstrates a dynamic pattern of increasing weight and apparent variability with increasing post-conceptual age, suggesting that weight be incorporated as a continuous variable in the model. The frequencies of medical diagnoses and pharmacotherapy illustrated in Figure 6 are consistent with prior expectations. In this regard, the incidence of chronic lung disease remained high throughout the preterm period, dopamine therapy declined with maturation reflecting the prevalence of hemodynamic instability in the youngest patients, and Coagulase Negative Staphylococcal infection was frequent among all age groups. The distributions of vancomycin peak and trough concentrations are presented in Figure 7, as are those from patients later identified as outliers (Section 3.1.2). The mean peak and trough concentrations were within the target ranges of 25 - 40 mg/L and 5 - 10 mg/L, respectively, with considerable variability.

### 3.1.2. Two-Compartment Model Building

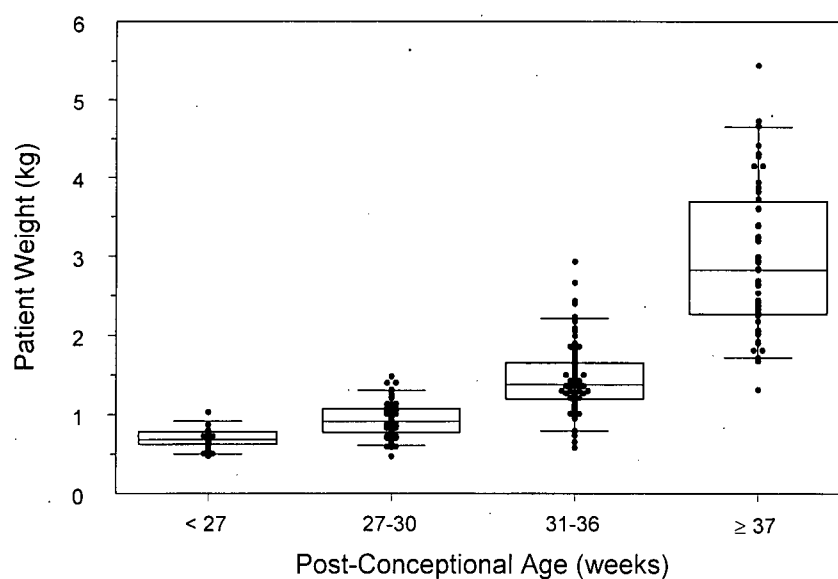
As previously described (Section 2.1.8), an iterative, stepwise, process to model building was implemented. To illustrate the development of the model, data are presented in a sequential manner for the modeling of covariates and error functions that individually resulted in a reduction in the objective function of  $> 6.6$  points ( $p < 0.01$ ). Data illustrated for each model reflect estimates determined for each patient on each day for which there was an event record.

Figure 8 illustrates the predicted and measured concentrations, and presents the pharmacokinetic parameters with respect to patient weight from the initial, unadjusted, model (Section 2.1.8.1). The absolute clearance increased with patient weight. In neonates  $< 2$  kg, the absolute value and variability of the central volume appeared to be large. The peripheral volume did not demonstrate a clear pattern; however, the absolute values were higher than anticipated.

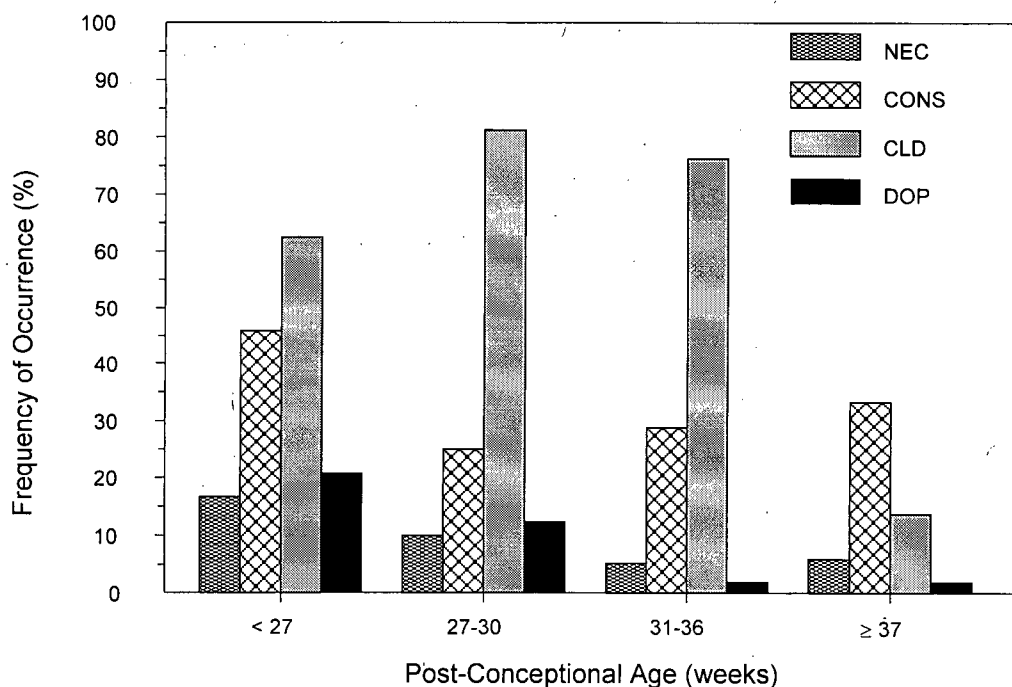
The next model (2b) reflects the influence of patient weight on clearance, alone (Figure 9). An observable improvement in the predicted concentrations over the initial model



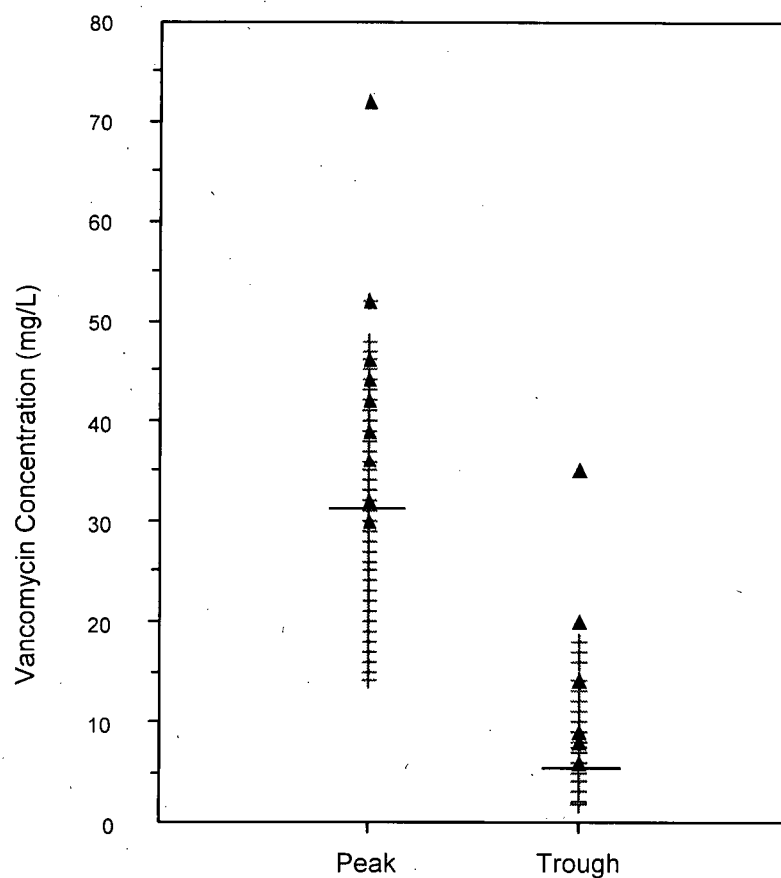
**Figure 4. Distribution of Gestational and Post-Conceptional Age by Groups.** Gestational age distribution reflects the age at birth of the 185 patients. Post-conceptional age reflects the age at birth plus the post-natal age from the time of birth to the initiation of each course (n = 252) of vancomycin therapy.



**Figure 5. Distribution of Patient Weight among the Post-Conceptional Age Groups at the Initiation of Each Course of Vancomycin Therapy.** Vancomycin courses numbered 252 in 185 patients. The Box-Whisker plots illustrate the median weights, the 25<sup>th</sup> to the 75<sup>th</sup> percentiles (Box), the 5<sup>th</sup> to the 95<sup>th</sup> percentiles (Whisker), and all data points (•).

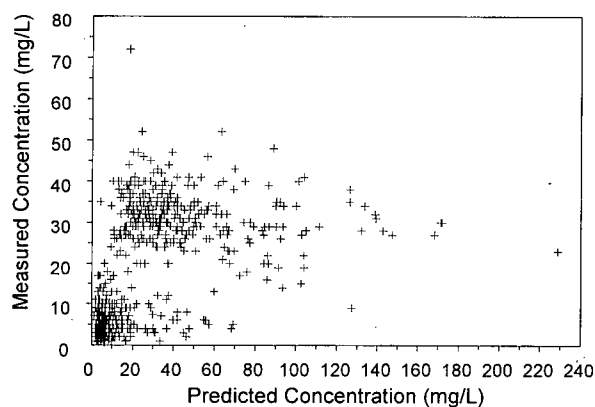


**Figure 6. Distribution of Clinical Diagnoses and Concurrent Pharmacotherapy by Post-Conceptual Age Groups.** Illustrates the frequency of Necrotizing Enterocolitis (NEC), Coagulase Negative Staphylococcal sepsis (CONS) and chronic lung disease (CLD) clinical diagnoses and concurrent Dopamine (DOP) pharmacotherapy at the initiation of each course (n = 252) of vancomycin therapy.

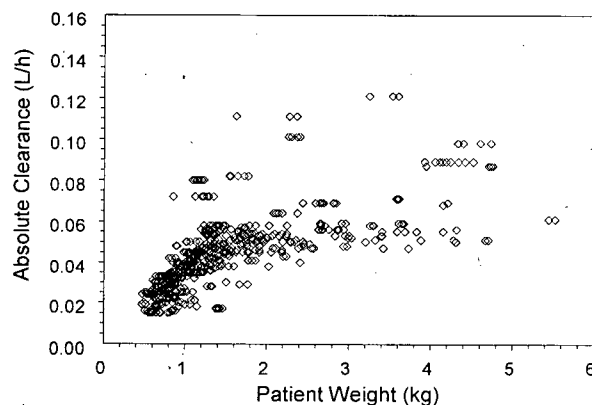


**Figure 7. Distribution of Measured Vancomycin Peak and Trough Concentrations.** Routine peak and trough serum vancomycin concentrations were analyzed from 185 patients prescribed 252 courses of therapy. Vancomycin concentrations from all patients included in the refined population model (+) and those later identified as outliers (▲) (Section 3.1.2) are presented with mean ( $\pm$  sd) peak and trough concentrations of 31 ( $\pm$  6) mg/L and 5 ( $\pm$  3) mg/L, respectively.

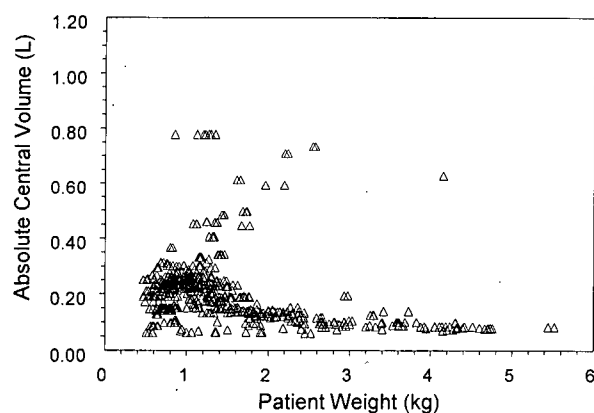
(A) Measured Versus Predicted Concentration



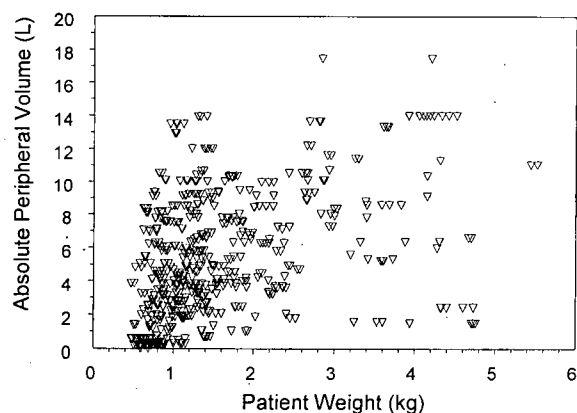
(B) Absolute Clearance Versus Patient Weight



(C) Absolute Volume of the Central Compartment Versus Patient Weight



(D) Absolute Volume of the Peripheral Compartment Versus Patient Weight



**Figure 8. Measured Versus Predicted Concentrations and Pharmacokinetic Parameters Versus Patient Weight for Model 2a.** Predicted concentrations (A), individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

$$TVCL = \theta_1$$

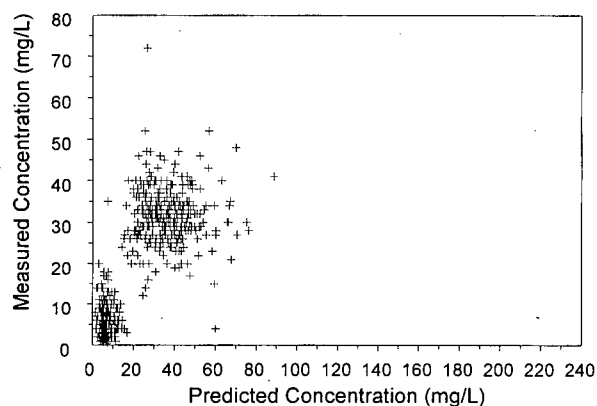
$$TVV1 = \theta_2$$

$$TVV2 = \theta_3$$

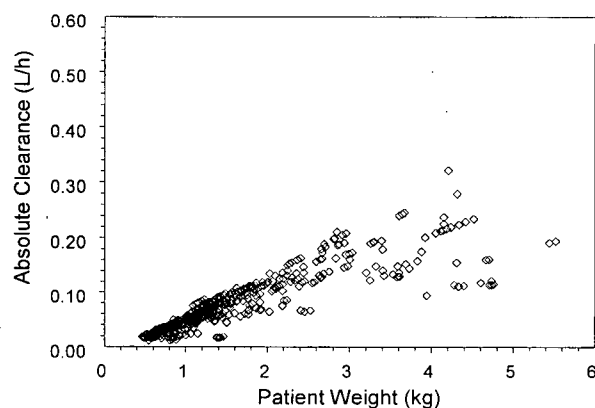
$$Q = \theta_4$$



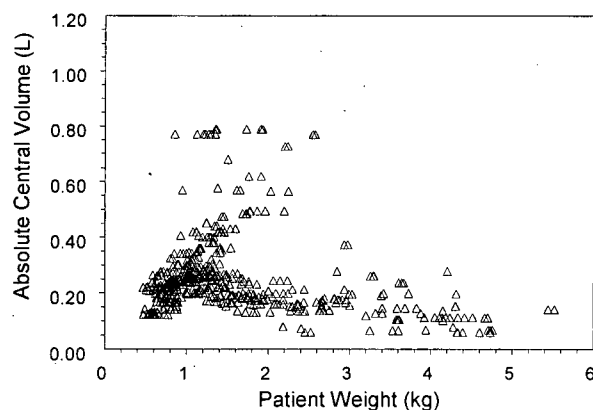
(A) Measured Versus Predicted Concentration



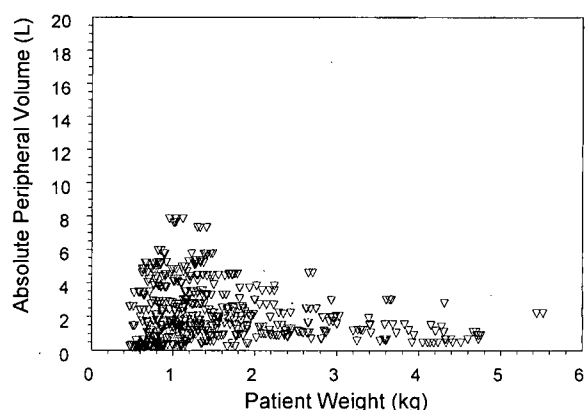
(B) Absolute Clearance Versus Patient Weight



(C) Absolute Volume of the Central Compartment Versus Patient Weight



(D) Absolute Volume of the Peripheral Compartment Versus Patient Weight



**Figure 9. Measured Versus Predicted Concentrations and Pharmacokinetic Parameters Versus Patient Weight for Model 2b.** Predicted concentrations (A), individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

$$TVCL = \theta_1 * (WT ** \theta_2)$$

$$TVV1 = \theta_3$$

$$TVV2 = \theta_4$$

$$Q = \theta_5$$

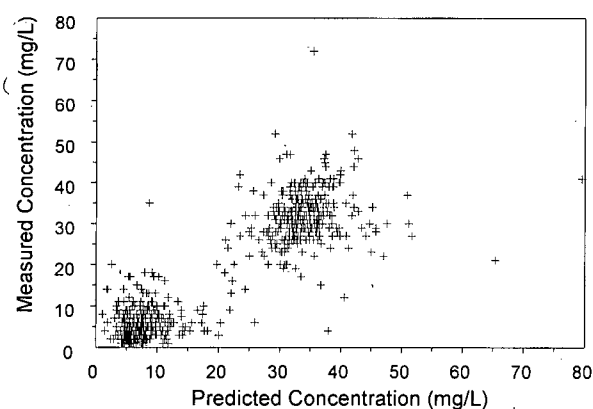
(2a) was discerned. The absolute clearance continued to demonstrate a relationship with weight. The absolute value and variability of the central volume continued to be greater in neonates  $< 2$  kg. The degree of variability in the peripheral volume was slightly reduced, particularly in neonates  $< 2$  kg; however, the absolute values were still higher than previously expected.

Model 2c incorporates mathematical functions of patient weight on both clearance and central volume. Figure 10 illustrates the predicted and measured concentrations, and the pharmacokinetic parameters with respect to post-conceptual age. Weight-normalized clearance demonstrated an increasing trend with increasing post-conceptual age, suggesting maturation in kidney function with increasing post-conceptual age. Weight-normalized central volume remained relatively constant ( $0.4 - 0.6$  L/kg) across post-conceptual age. Conversely, weight-normalized peripheral volume appeared to demonstrate greater variability, with larger values in the youngest patients, particularly those  $< 36$  weeks post-conceptual age.

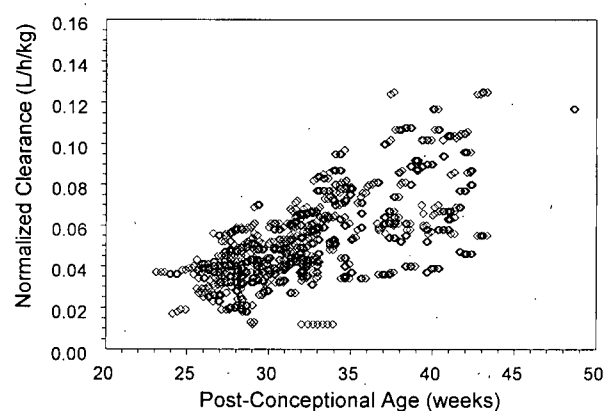
The next step involved the inclusion of post-conceptual age in the clearance term of the model. Various mathematical functions were attempted; the optimum method was to consider the continuous variable of post-conceptual age relative to term gestation modeled as a power function (model 2d). As depicted in Figure 11, weight-normalized clearance was higher in older patients, again suggesting a maturational component. Similar patterns for both central and peripheral volume were observed as with model 2c (Figure 10).

Continuous and dichotomous clinical covariates were selected for their potential to affect drug disposition, and their possible effects on vancomycin pharmacokinetic parameters was explored in univariate analyses (data not shown). Those covariates that met the criterion ( $p < 0.15$ ) were chosen sequentially and each one that individually demonstrated an improvement in the model ( $> 6.6$  point reduction in the objective function) was retained. In the SCN, patients are typically treated with corticosteroids for bronchopulmonary dysplasia. These therapeutic agents were found to be potential explanatory covariates in the univariate analyses; however, they failed to improve the model. Rather than recent exposure to the particular drug, it was considered that the diagnosis of chronic lung disease, itself, may affect the pharmacokinetic disposition of vancomycin. A new variable, chronic lung disease, was created to identify those patients presenting with the medical diagnoses of bronchopulmonary dysplasia and/or apnea of prematurity, common complications of the preterm neonate. In this regard, chronic lung disease

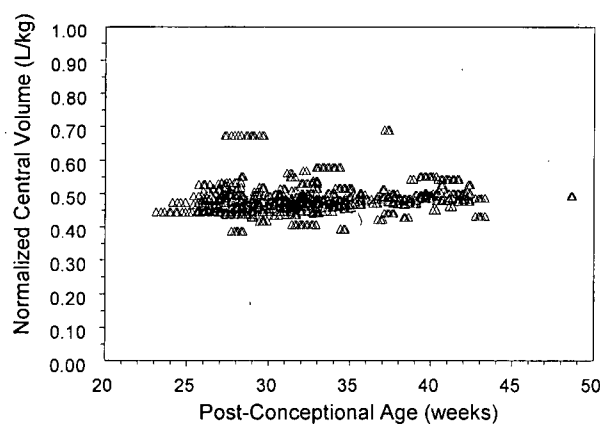
(A) Measured Versus Predicted Concentration



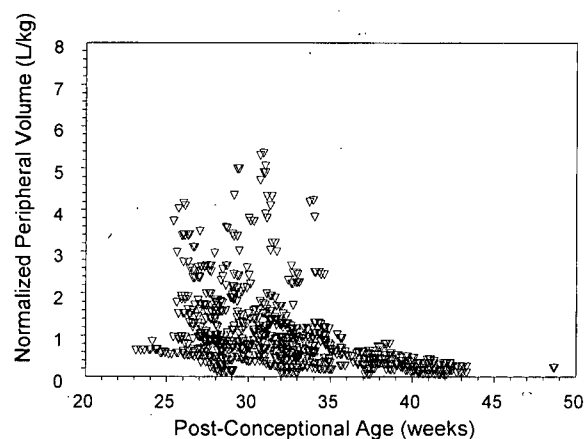
(B) Normalized Clearance Versus Post-Conceptional Age



(C) Normalized Volume of the Central Compartment Versus Post-Conceptional Age



(D) Normalized Volume of the Peripheral Compartment Versus Post-Conceptional Age



**Figure 10. Measured Versus Predicted Concentrations and Pharmacokinetic Parameters Versus Post-Conceptional Age for Model 2c.** Predicted concentrations (A) and individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

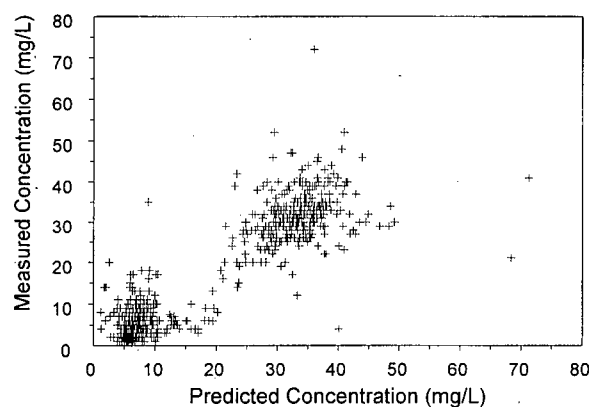
$$TVCL = \theta_1 * (WT ** \theta_2)$$

$$TVV1 = \theta_3 * WT$$

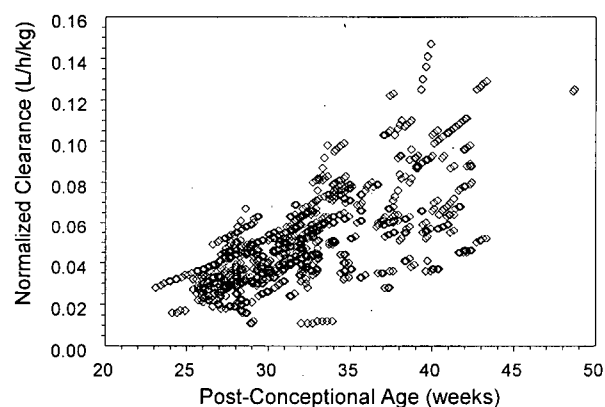
$$TVV2 = \theta_4$$

$$Q = \theta_5$$

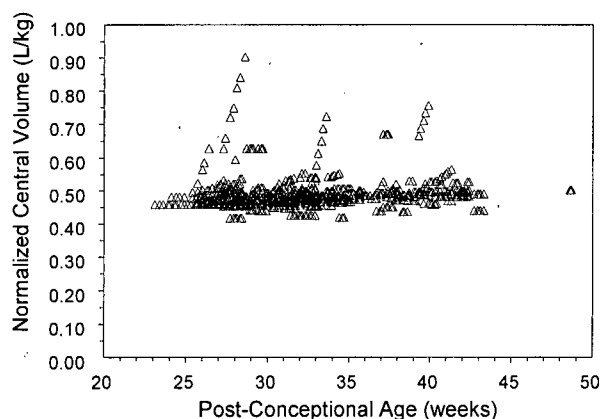
(A) Measured Versus Predicted Concentration



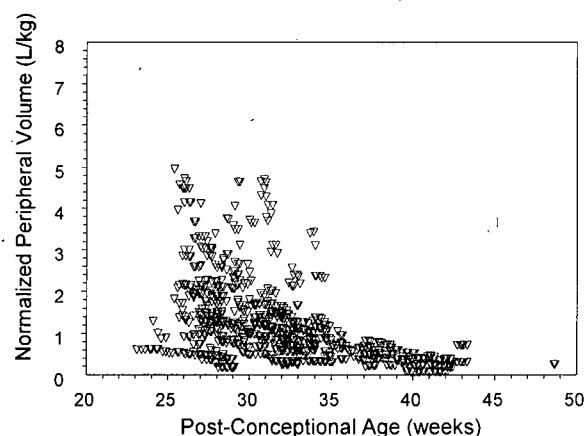
(B) Normalized Clearance Versus Post-Conceptional Age



(C) Normalized Volume of the Central Compartment Versus Post-Conceptional Age



(D) Normalized Volume of the Peripheral Compartment Versus Post-Conceptional Age



**Figure 11. Measured Versus Predicted Concentrations and Pharmacokinetic Parameters Versus Post-Conceptional Age for Model 2d.** Predicted concentrations (A), individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

$$TVCL = \theta_1 * (WT ** \theta_2) (PCA/40 ** \theta_3)$$

$$TVV1 = \theta_4 * WT$$

$$TVV2 = \theta_5$$

$$Q = \theta_6$$

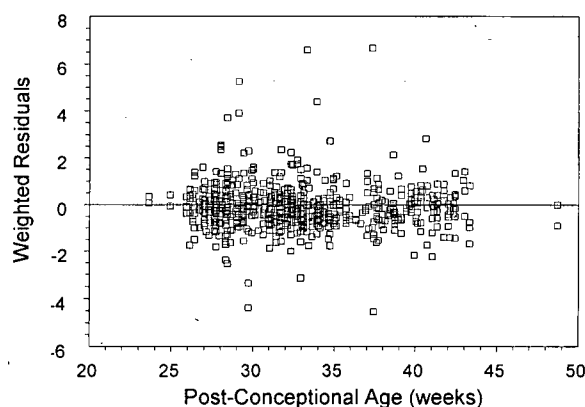
was included in the peripheral volume term of the model (2e) and produced a significant reduction of the objective function. Figure 12 illustrates the weighted residuals and pharmacokinetic parameters with respect to post-conceptual age for model 2e. The weighted residual plot (Figure 12A) facilitated the identification of outliers with weighted residuals  $\geq 4$  or  $\leq -4$ . Weight-normalized clearance and central volume demonstrated similar trends to those observed with previous models (2c and 2d). A notable reduction in weight-normalized peripheral volume and its variability was observed; however, a pattern of increased absolute value and variability of this covariate was discerned in the youngest age groups.

The potential outliers observed in model 2e (Figure 12A) were individually removed to elucidate their impact on the objective function value. Those that resulted in an improvement in the model and whose clinical presentation was not consistent with the preponderance of the patient sample were removed (model 2f), and the results are presented in Figure 13. Exclusion criteria included: death within 24 hours of serum drug concentration measurement (1 case), renal failure with serum creatinine  $> 150 \mu\text{mol/L}$  and blood urea nitrogen  $> 10 \text{ mmol/L}$  (3 cases), and congestive heart failure with or without congenital heart defects (2 cases). Three patients concurrently exhibited hydops fetalis, edema with cardiac decompensation and  $> 500 \text{ mL}$  fluid imbalance. Following removal of the outliers, the overall pattern of the pharmacokinetic behavior with respect to post-conceptual age remained unchanged.

Next, the appropriateness of the inter- and intraindividual variability error terms was tested. Each error term was individually and sequentially modified to determine its impact on the objective function value. The intraindividual error was optimally modeled as a mixed additive and exponential function ( $Y = F * \text{EXP}(\epsilon_1) + \epsilon_2$ ) whereas, the interindividual error terms ( $\text{EXP}(\eta_x)$ ) continued to be modeled as exponential functions (model 2g).

Finally, model 2g was refined through a backwards elimination technique, each covariate was removed sequentially and then replaced if the objective function value increased by  $> 6.6$ . Through implementation of this procedure, the dopamine covariate modeled in the peripheral volume term was removed from the refined model (2h), as the objective function value did not increase appreciably. To illustrate the improvement in the model through the sequence of model building, Figure 14 depicts a comparison between the unadjusted model (2a) and the refined model (2h). An observable improvement in the predicted concentrations and a reduction in the

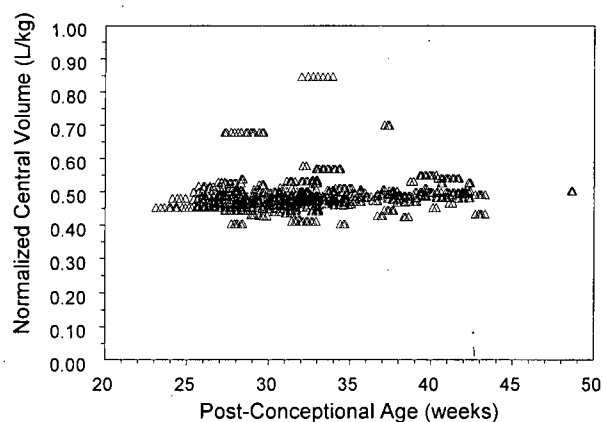
(A) Weighted Residuals Versus  
Post-Conceptual Age



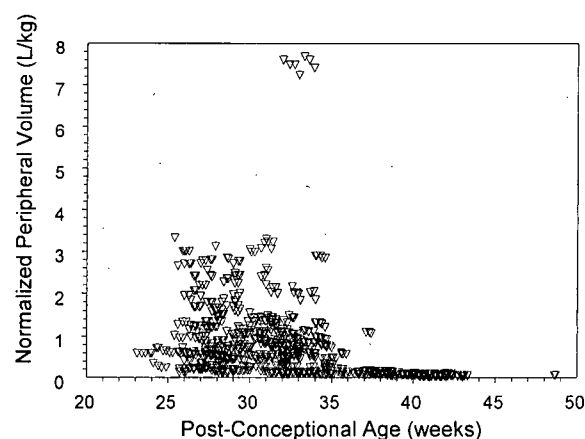
(B) Normalized Clearance Versus  
Post-Conceptual Age



(C) Normalized Volume of the Central  
Compartment Versus Post-Conceptual Age



(D) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



**Figure 12. Weighted Residuals and Pharmacokinetic Parameters Versus Post-Conceptual Age for Model 2e.** Weighted residual concentrations (A), individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

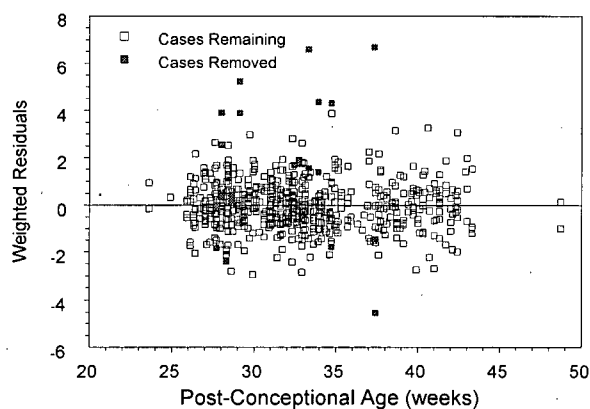
$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP)$$

$$TVV1 = \theta_5 * WT$$

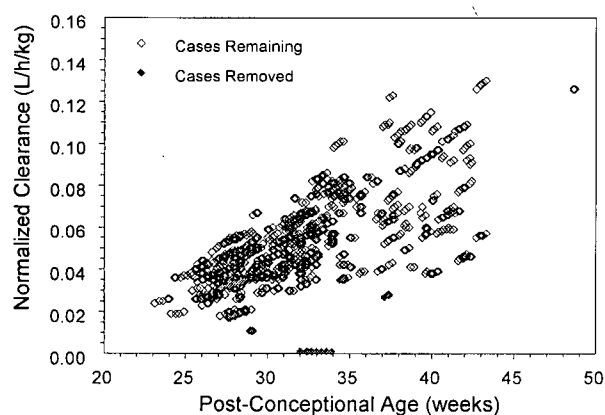
$$TVV2 = \theta_6 * (1 + \theta_7 ** CLD) * (\theta_8 ** DOP)$$

$$Q = \theta_9$$

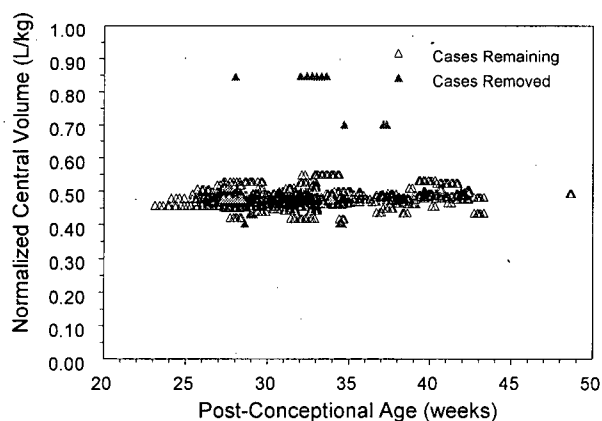
(A) Weighted Residuals Versus  
Post-Conceptual Age



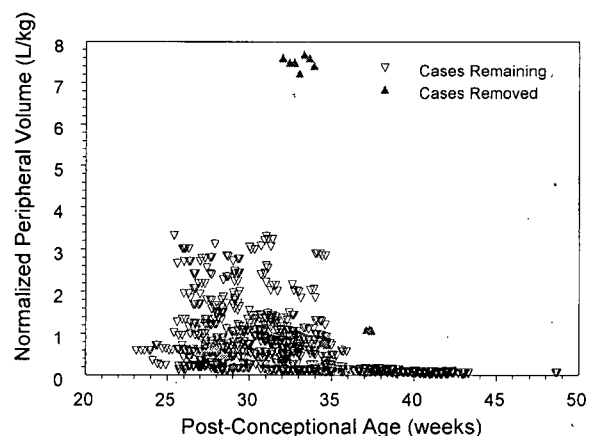
(B) Normalized Clearance Versus  
Post-Conceptual Age



(C) Normalized Volume of the Central  
Compartment Versus Post-Conceptual Age



(D) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



**Figure 13. Weighted Residuals and Pharmacokinetic Parameters Versus Post-Conceptual Age for Model 2f.** Weighted residual concentrations (A), individual clearance (B), central volume (C), and peripheral volume (D) pharmacokinetic parameters generated with a two-compartment model with exponential inter- and intra-individual variability in which:

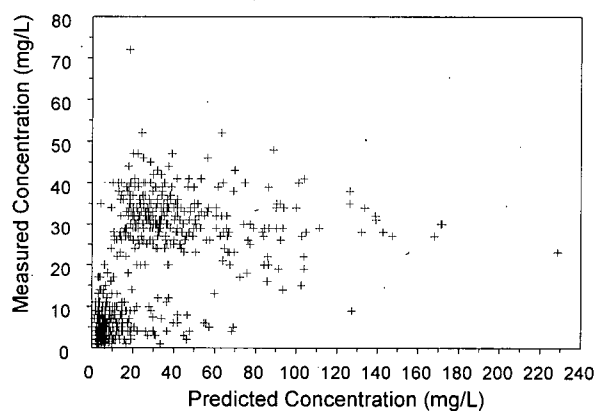
$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP)$$

$$TVV1 = \theta_5 * WT$$

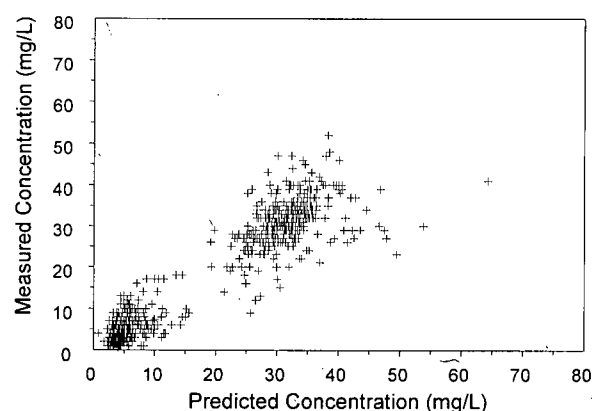
$$TVV2 = \theta_6 * (1 + \theta_7 ** CLD) * (\theta_8 ** DOP)$$

$$Q = \theta_9$$

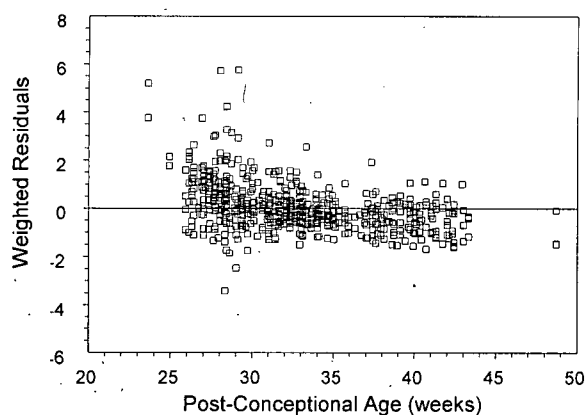
(A) Measured Versus Predicted Concentration  
(Model 2a)



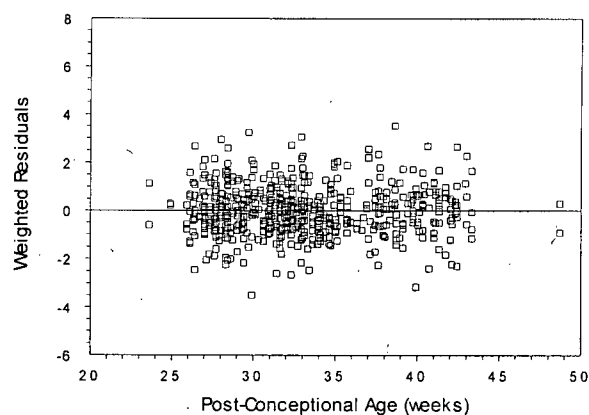
(B) Measured Versus Predicted Concentration  
(Model 2h)



(C) Weighted Residuals Versus  
Post-Conceptional Age (Model 2a)



(D) Weighted Residuals Versus  
Post-Conceptional Age (Model 2h)



**Figure 14. Measured Versus Predicted Concentrations and Weighted Residuals Versus Post-Conceptional Age for Models 2a and 2h.** Predicted concentrations (A, B) and weighted residuals (C, D) generated with two-compartment models. Model 2h with exponential interindividual variability and mixed (exponential and additive) intraindividual variability given:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP)$$

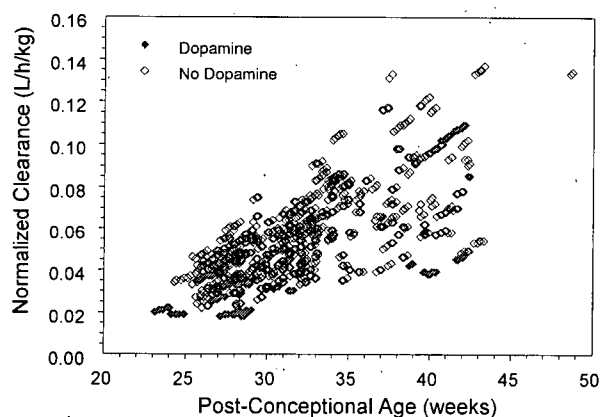
$$TVV1 = \theta_5 * WT$$

$$TVV2 = \theta_6 * (1 + \theta_7 ** CLD)$$

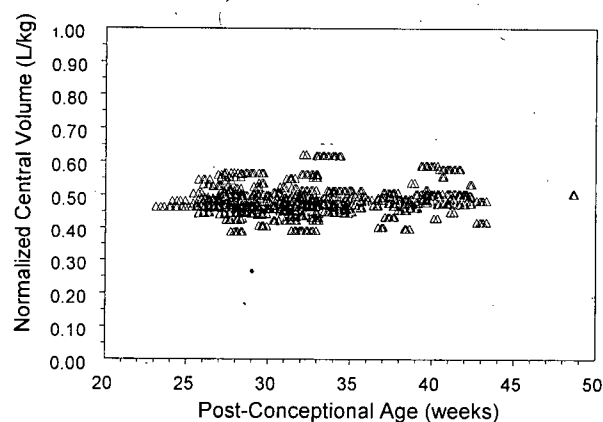
$$Q = \theta_8$$



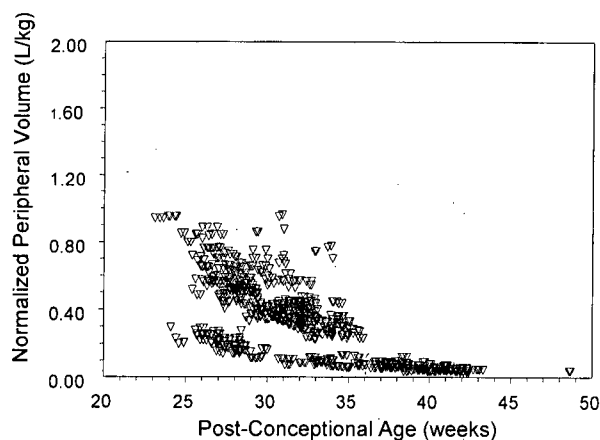
(A) Normalized Clearance Versus  
Post-Conceptual Age



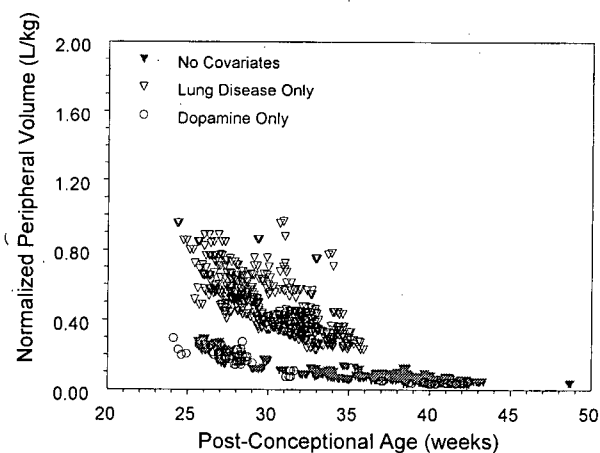
(B) Normalized Volume of the Central  
Compartment Versus Post-Conceptual Age



(C) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



(D) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



**Figure 15. Pharmacokinetic Parameters Versus Post-Conceptual Age for Model 2h.** Individual parameters of clearance (A), central volume (B), and peripheral volume (C, D) generated with a two-compartment model with exponential interindividual and mixed (exponential and additive) intraindividual variability in which:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP)$$

$$TVV1 = \theta_5 * WT$$

$$TVV2 = \theta_6 * (1 + \theta_7 ** CLD)$$

$$Q = \theta_8$$

weighted residual concentrations among the youngest patients in the final relative to the initial model can be discerned. Figure 15 illustrates the pharmacokinetic parameters with respect to post-conceptual age from model 2h. Dopamine continued to be an important factor in the clearance term, but was no longer required to explain peripheral volume. Weight-normalized central volume remained constant (0.4 – 0.6 L/kg) across all post-conceptual age groups. The model distinguished between patients with and without chronic lung disease: higher weight-normalized peripheral volume was associated with chronic lung disease patients and, to some degree, patients < 30 weeks post-conceptual age.

The summary of the incremental improvement of fit is presented in Table 7, wherein the mean posthoc parameter estimates and changes in the objective function are reported (Pharmacostatistical codes of models 2a - 2h are presented in Appendix 13). The mean weight-normalized central volume increased somewhat from the unadjusted model (2a) to the refined model (2h). Conversely, the mean weight-normalized peripheral volume, and thereby volume of distribution at steady-state, was markedly reduced in model 2h compared to 2a.

The parameter and error estimates generated by NONMEM for the refined model (2h) are reported in Table 8. The point estimate associated with patient weight in the clearance term results in a 79% increase in clearance with a doubling of patient weight. Further, those patients < 30 weeks post-conceptual age exhibited a 50 - 70% lower clearance than neonates at 40 weeks post-conceptual age, independent of weight. Exposure to dopamine (mean dose = 7.5 µg/kg/min) within 72 hours of serum vancomycin concentration determination, which occurred in 9% of cases, was associated with a 33% lower vancomycin clearance. A diagnosis of chronic lung disease (62% of cases) can be calculated to be associated with a 178% increase in peripheral volume. The coefficients of variation with respect to interindividual variability in clearance, central volume, and peripheral volume were 27%, 11%, and 11%, respectively. The combined coefficient of variation (exponential and additive) for intraindividual variability was approximately 51%.

The mean pharmacokinetic estimates calculated from typical values for model 2h are presented in Table 9. The distribution half-life remained long (> 4 hours) across all post-conceptual age groups. The observed elimination half-life decreased slightly between 24 and 36 weeks post-conceptual age, followed by a dramatic reduction in neonates ≥ 37 weeks post-conceptual age. Weight-normalized clearance increased by 167% from the youngest to the

**Table 7. Summary of Changes in Objective Function Values and Mean Posthoc Parameter Estimates from Two-Compartment Model Building<sup>a</sup>.**

Model	Cumulative $\Delta$ Objective Function Value	Mean Posthoc Parameter Estimates			
		Cl (L/h/kg)	V <sub>c</sub> (L/kg)	V <sub>p</sub> (L/kg)	V <sub>ss</sub> (L/kg)
2a		0.03	0.19	3.65	3.84
2b	-487.92	0.05	0.21	1.75	1.96
2c	-994.29	0.05	0.48	0.97	1.45
2d	-1021.89	0.05	0.49	1.15	1.63
2e	-1073.92	0.05	0.49	1.34	1.83
2f	-1575.08	0.05	0.48	0.69	1.17
2g	-1609.09	0.06	0.48	0.33	0.81
2h	-1608.83	0.06	0.48	0.31	0.70

<sup>a</sup> The mean posthoc estimates generated by NONMEM of clearance (Cl), central volume of distribution (V<sub>c</sub>), peripheral volume of distribution (V<sub>p</sub>), and steady-state volume of distribution (V<sub>ss</sub>) are reported for two-compartment models. The change in the minimum value of the objective function value is presented for each model relative to the objective function of the basic model (model 2a, objective function = 3579.21) without any covariates. Posthoc estimates were determined for each patient on each day for which there was an event record for a total of 971 determinations (models 2a – 2e) and 947 determinations (models 2f – 2h).

**Table 8. Two-Compartment Model Building: Parameter and Error Estimates<sup>a</sup>.**

	Population Point Estimates	Standard Error
<b>Structural Parameters</b>		
$\theta_1$	0.093	0.007
$\theta_2$	0.839	0.078
$\theta_3$	2.370	0.291
$\theta_4$	0.665	0.036
$\theta_5$	0.482	0.006
$\theta_6$	0.081	0.024
$\theta_7$	4.55	1.810
$\theta_8$	0.013	0.003
<b>Interindividual Variability</b>		
$\eta_1$ (% CV)	0.073 (27.1)	0.007
$\eta_2$ (% CV)	0.012 (10.9)	0.003
$\eta_3$ (% CV)	0.012 (11.0)	< 0.001
<b>Intraindividual Variability</b>		
$\epsilon_1$ (% CV)	0.007 (8.4)	0.001
$\epsilon_2$ (% CV)	0.261 (51.1)	0.176

<sup>a</sup> NONMEM point estimates and standard errors generated with a two-compartment model (2h) given:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP)$$

$$CL = TVCL * EXP(\eta_1)$$

$$TVV1 = \theta_5 * WT$$

$$V1 = TVV1 * EXP(\eta_2)$$

$$TVV2 = \theta_6 * (1 + \theta_7 ** CLD)$$

$$V2 = TVV2 * EXP(\eta_3)$$

$$Q = \theta_8$$

$$Y = F * EXP(\epsilon_1) + \epsilon_2$$

**Table 9. Mean Pharmacokinetic Estimates Derived from the Refined Two-Compartment Population Model<sup>a</sup>.**

PCA Group	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Cl (L/h/kg)	Vc (L/kg)	Vp (L/kg)	Vss (L/kg)
< 27 weeks	5.31	32.81	0.03	0.48	0.47	0.95
27 – 30 weeks	5.34	31.44	0.04	0.48	0.44	0.92
31 – 36 weeks	4.85	26.26	0.06	0.48	0.30	0.78
≥ 37 weeks	4.12	10.01	0.08	0.48	0.06	0.54
<b>All</b>	<b>4.84</b>	<b>25.34</b>	<b>0.05</b>	<b>0.48</b>	<b>0.31</b>	<b>0.79</b>

<sup>a</sup> The mean values of distribution half-life ( $t_{1/2\alpha}$ ), elimination half-life ( $t_{1/2\beta}$ ), clearance (Cl), central volume of distribution (Vc), peripheral volume of distribution (Vp), and steady-state volume of distribution (Vss) are reported for 246 courses of vancomycin therapy in 179 patients. Parameter estimates were determined from typical values (Model 2h) for each patient on each day for which there was an event record (947 determinations) and the mean values for each case were calculated over the course of therapy.

oldest patients. The weight-normalized central volume remained constant across all age groups; whereas, peripheral volume decreased appreciably. Consequently, the peripheral volume represented 50% of the volume of distribution at steady-state in the youngest patients, but only 9% in the oldest patients. This suggested that a one-compartment model may be a close approximation of vancomycin pharmacokinetics in neonates  $\geq 37$  weeks post-conceptual age.

### 3.1.3. One-Compartment Model Building

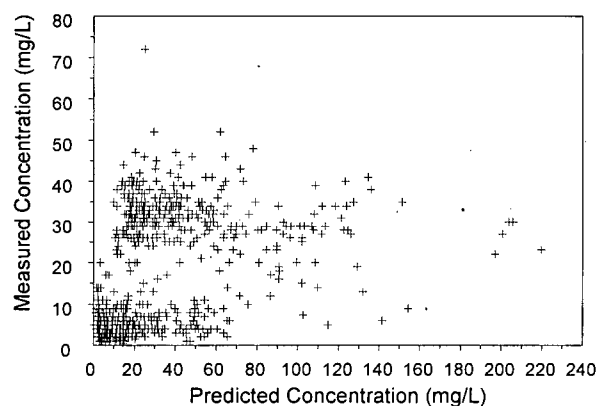
As with the two-compartment model building, an iterative process was implemented to generate a one-compartment model of vancomycin disposition. All covariates and error terms that demonstrated a reduction in the objective function of  $> 6.6$  points ( $p < 0.01$ ) were retained in the model. Data illustrated reflect estimates determined for each patient on each day for which there was an event record. Since the process replicated that of the two-compartment approach (Section 2.1.8), for brevity, only the data for the unadjusted (1a) and refined (1h) models are presented (Pharmacostatistical codes of models 1a – 1h are presented in Appendix 14).

Figure 16 illustrates a comparison between the unadjusted model (1a) and the refined model (1h). An improvement in the predicted concentrations and a reduction in weighted residual concentrations among the youngest patients was noted. The pharmacokinetic parameters with respect to post-conceptual age are presented in Figure 17. The influence of patient weight and post-conceptual age was reflected in the inclusion of these covariates in the refined model (1h). Chronic lung disease was an important factor in both the clearance and volume of distribution terms. Chronic lung disease was associated with a lower vancomycin clearance, and volume of distribution remained constant (0.4 – 0.6 L/kg) across all age groups, with a slightly lower weight-normalized value observed in patients with chronic lung disease.

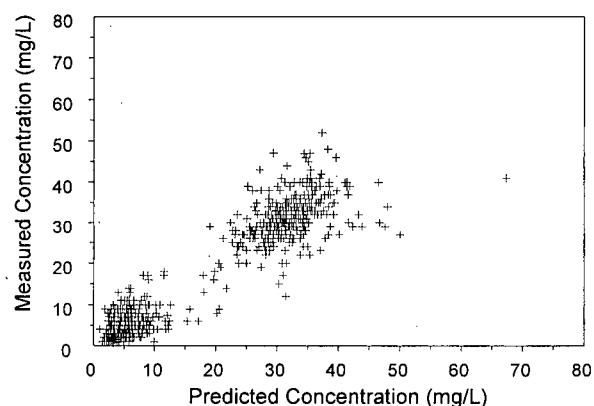
The summary of the incremental improvement of fit is presented in Table 10, wherein the mean posthoc parameter estimates and changes in the objective function are reported. The mean weight-normalized volume of distribution decreased somewhat from the unadjusted model (1a) to the refined model (1h). Conversely, the mean weight-normalized clearance increased between models 1a and 1h.

The parameter and error estimates generated by NONMEM for the refined model (1h) are reported in Table 11. The point estimate associated with patient weight in the clearance term results in a 73% increase in clearance with a doubling of patient weight. Further, those patients

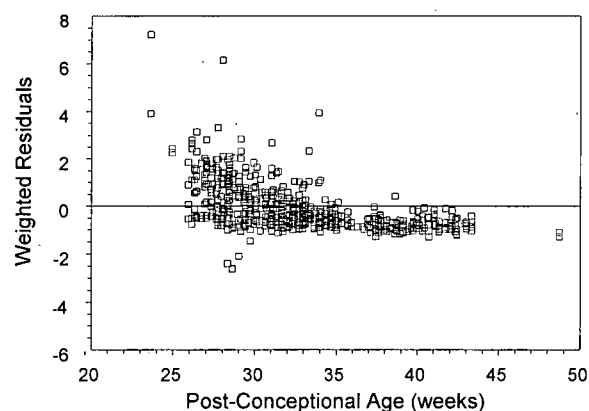
(A) Measured Versus Predicted Concentration  
(Model 1a)



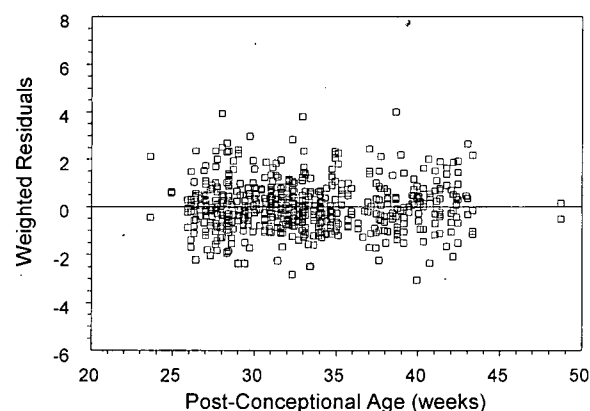
(B) Measured Versus Predicted Concentration  
(Model 1h)



(C) Weighted Residuals Versus  
Post-Conceptional Age (Model 1a)



(D) Weighted Residuals Versus  
Post-Conceptional Age (Model 1h)

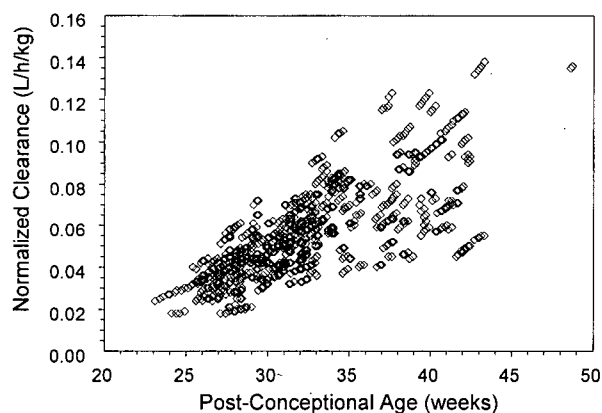


**Figure 16. Measured Versus Predicted Concentrations and Weighted Residuals Versus Post-Conceptional Age for Models 1a and 1h.** Predicted concentrations (A, B) and weighted residuals (C, D) generated with one-compartment models. Model 1h with exponential interindividual variability and mixed (exponential and additive) intraindividual variability given:

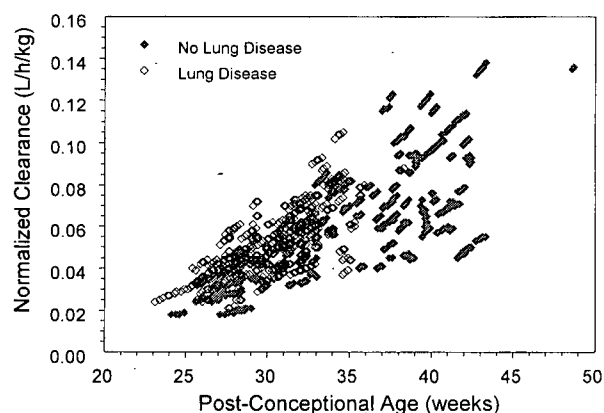
$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** CLD)$$

$$TVV = \theta_5 * WT * (\theta_6 ** CLD)$$

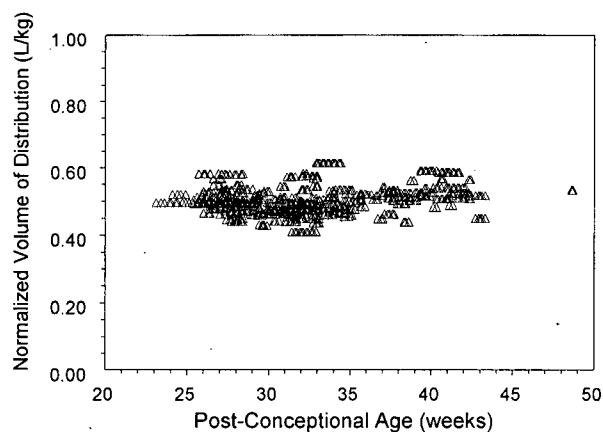
(A) Normalized Clearance Versus  
Post-Conceptual Age



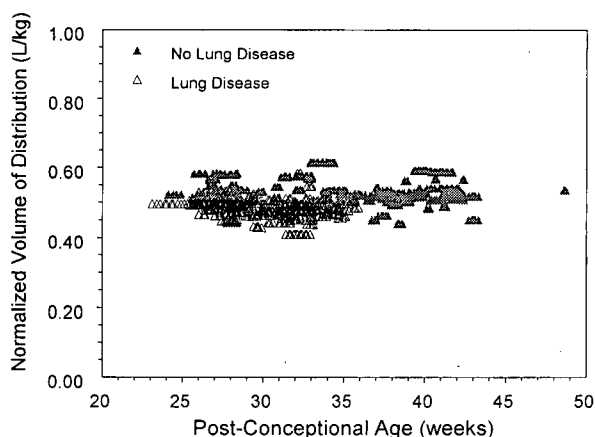
(B) Normalized Clearance Versus  
Post-Conceptual Age



(C) Normalized Volume of Distribution  
Versus Post-Conceptual Age



(D) Normalized Volume of Distribution  
Versus Post-Conceptual Age



**Figure 17. Pharmacokinetic Parameters Versus Post-Conceptual Age for Model 1h.** Individual parameters of clearance (A, B) and volume of distribution (C, D) generated with a one-compartment model with exponential interindividual and mixed (exponential and additive) intraindividual variability in which:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** CLD)$$

$$TVV = \theta_5 * WT * (\theta_6 ** CLD)$$



**Table 10. Summary of Changes in Objective Function Values and Mean Posthoc Parameter Estimates from One-Compartment Model Building<sup>a</sup>.**

Model	Cumulative $\Delta$ Objective Function Value	Mean Posthoc Parameter Estimates	
		Cl (L/h/kg)	Vd (L/kg)
1a		0.04	0.59
1b	-489.10	0.05	0.53
1c	-939.48	0.05	0.53
1d	-961.81	0.05	0.59
1e	-1214.25	0.05	0.56
1f	-1928.77	0.06	0.51
1g	-2017.50	0.06	0.50
1h	-2017.50	0.06	0.50

<sup>a</sup> The mean posthoc estimates generated by NONMEM of clearance (Cl) and volume of distribution (Vd) are reported for one-compartment models. The change in the minimum value of the objective function value is presented for each model relative to the objective function of the basic model (model 1a, objective function = 4017.72) without any covariates. Posthoc estimates were determined for each patient on each day for which there was an event record for a total of 971 determinations (models 1a – 1e) and 947 determinations (models 1f – 1h).

**Table 11. One-Compartment Model Building: Parameter and Error Estimates<sup>a</sup>.**

	Population Point Estimates	Standard Error
<b>Structural Parameters</b>		
$\theta_1$	0.093	0.008
$\theta_2$	0.794	0.079
$\theta_3$	3.10	0.302
$\theta_4$	1.27	0.064
$\theta_5$	0.524	0.011
$\theta_6$	0.919	0.021
<b>Interindividual Variability</b>		
$\eta_1$ (% CV)	0.066 (25.7)	0.007
$\eta_2$ (% CV)	0.008 (8.8)	0.003
<b>Intraindividual Variability</b>		
$\varepsilon_1$ (% CV)	0.007 (8.4)	0.002
$\varepsilon_2$ (% CV)	0.918 (95.8)	0.303

<sup>a</sup> NONMEM point estimates and standard errors generated with a one-compartment model (1h) given:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** CLD)$$

$$CL = TVCL * EXP(\eta_1)$$

$$TVV = \theta_5 * WT * (\theta_6 ** CLD)$$

$$V = TVV * EXP(\eta_2)$$

$$Y = F * EXP(\varepsilon_1) + \varepsilon_2$$

< 30 weeks post-conceptual age exhibited a 60 – 80% lower vancomycin clearance than neonates at 40 weeks post-conceptual age, independent of weight. A diagnosis of chronic lung disease (62% of cases) can be calculated to be associated with a 27% increase in clearance and a 9% reduction in volume of distribution. The coefficients of variation with respect to interindividual variability in clearance and volume of distribution were 26%, and 9%, respectively. The combined coefficient of variation for intraindividual variability (exponential and additive) was approximately 96%.

The mean pharmacokinetic estimates calculated from typical values for model 1h are presented in Table 12. The half-life decreased markedly with age, 56% from the youngest to the oldest patients. Moreover, the half-life estimates were considerably less than the elimination half-life estimates generated in the refined two-compartment model (Table 9). As observed with the two-compartment model (2h), the one-compartment weight-normalized clearance increased by 167% from the youngest to the oldest patients. The weight-normalized volume of distribution in the youngest patients was notably lower with the one-compartment model; however, both the one- and two-compartment models generated similar values for the oldest patients.

At all sequential stages, the one-compartment model appeared inferior to the two-compartment model. The objective function values from the one-compartment unadjusted model (1a) and revised model (1h) were, respectively, 438.52 and 29.84 points greater than the comparable two-compartment values. To test the appropriateness of the refined two-compartment model (2h), validation analyses were completed in a naïve cohort of patients.

#### **3.1.4. Demographic Characteristics of the Validation Sample of Patients**

Data were collected (Section 2.1.9) from a naïve cohort of patients admitted to the SCN during the same period as those obtained for the purposes of model building. The presence of this patient sample permitted the opportunity, not only to validate the refined one- (1h) and two- (2h) compartment models, but also assess a Bayesian forecasting method as applied to neonates.

Table 13 summarizes the demographic data of the 65 patients enrolled in the validation analyses component of this investigation. Sixty-three percent of this cohort were male, and the mean ( $\pm$  sd) gestational age upon admission was 29.0 ( $\pm$  3.8) weeks. Similar to the model building patient sample, the majority of patients were preterm, with a history of respiratory

**Table 12. Mean Pharmacokinetic Estimates Derived from the Refined One-Compartment Population Model<sup>a</sup>.**

PCA Group	$t_{1/2}$ (h)	Cl (L/h/kg)	Vd (L/kg)
< 27 weeks	11.25	0.03	0.51
27 – 30 weeks	8.40	0.04	0.49
31 – 36 weeks	5.94	0.06	0.49
≥ 37 weeks	4.99	0.08	0.52
<b>All</b>	<b>4.84</b>	<b>0.06</b>	<b>0.50</b>

<sup>a</sup> The mean values of half-life ( $t_{1/2}$ ), clearance (Cl), and volume of distribution (Vd), are reported for 246 courses of vancomycin therapy in 179 patients. Parameter estimates were determined from typical values (Model 1h) for each patient on each day for which there was an event record (947 determinations) and the mean values for each case were calculated over the course of therapy.

**Table 13. Demographic Characteristics of Patients Enrolled in the Validation Analyses Component of the Investigation.**

Demographic Characteristics	Number (%)
<b>Number of Patients</b>	<b>65</b>
Male	41 (63.1)
Female	24 (36.9)
<b>Admission History</b>	
Preterm Birth	61 (93.9)
Respiratory Distress Syndrome	56 (86.2)
<b>Indication for Vancomycin Therapy</b>	
Empiric Therapy - Sepsis	62 (59.0)
Coagulase Negative Staphylococcal Sepsis	31 (29.5)
Necrotizing Enterocolitis	6 (5.7)
Empiric Therapy - Necrotizing Enterocolitis	5 (4.8)
Other	1 (1.0)
<b>Clinical Presentation at the Initiation of Each Course</b>	
Chronic Lung Disease	71 (67.6)
Coagulase Negative Staphylococcal Sepsis	31 (29.5)
Dopamine <sup>a</sup>	15 (14.3)
Indomethacin <sup>a</sup>	11 (10.5)
Necrotizing Enterocolitis	6 (5.7)
<b>Number of Courses of Vancomycin</b>	<b>105</b>
<b>Number of Patients with Multiple Courses of Vancomycin</b>	<b>31 (47.7)</b>
Number of Patients with Two Courses	23 (35.4)
Number of Patients with Three or Four Courses	8 (12.3)
<b>Number of Routine Serum Drug Concentration Determinations</b>	<b>400</b>
Number of Peak or Trough Concentration Determinations	272
Number of Intradose Interval Concentration Determinations	128

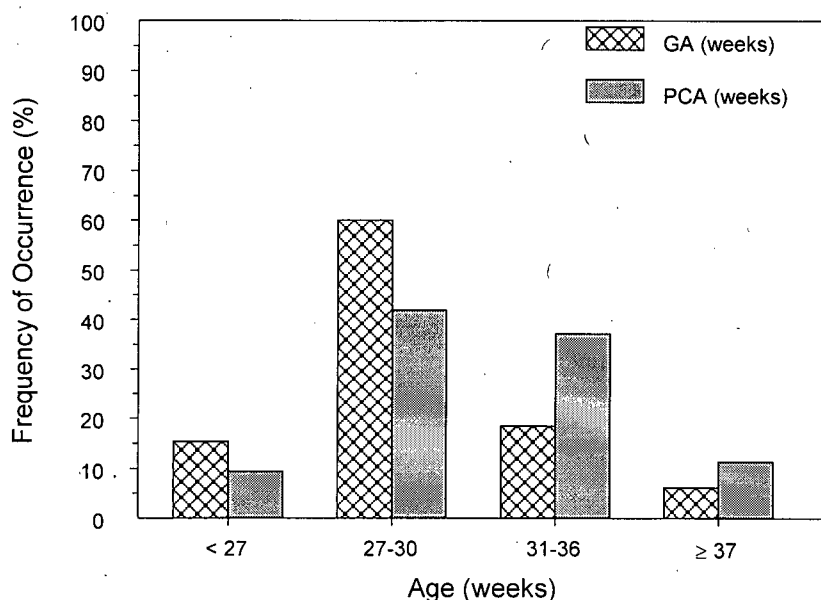
<sup>a</sup> Pharmacotherapy within 72 hours of serum concentration determination.

distress syndrome. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) Apgar scores at one- and five-minutes were 5 (3, 7) and 8 (7, 9), respectively. Together, empiric sepsis therapy and confirmed Coagulase Negative Staphylococcal sepsis represented > 85% of indications for vancomycin. The prevalence of medical diagnoses and pharmacotherapy was consistent with both the model building patient sample and expectations of patients admitted to a NICU. Forty-eight percent of this patient sample were prescribed multiple courses of vancomycin, and 400 serum vancomycin concentrations were quantified.

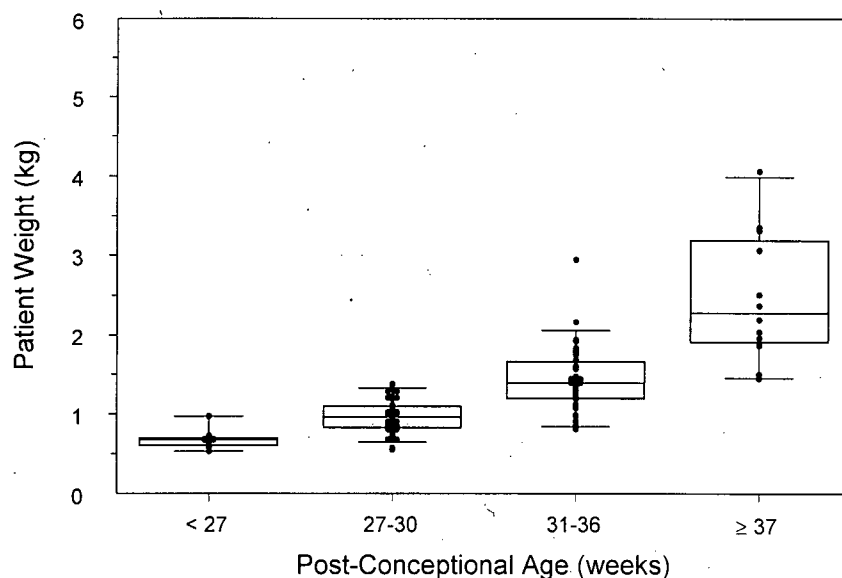
Figure 18 illustrates the gestational and post-conceptual age distribution of the 65 patients at the initiation of the 105 courses of vancomycin therapy. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) post-natal age at the start of each course was 15 (7, 30) days, this reflects the right-shift in the distribution pattern between gestational and post-conceptual age. The mean ( $\pm$  sd) post-conceptual age and weight at the initiation of each vancomycin course were 32.3 ( $\pm$  4.6) weeks and 1.5 ( $\pm$  0.9) kg, respectively. Figure 19 demonstrates a dynamic pattern of increasing weight and apparent variability with increasing post-conceptual age, similar to that observed in the model building cohort (Figure 5). The frequencies of medical diagnoses and pharmacotherapy illustrated in Figure 20 are consistent with prior expectations and the model building patient sample. In this regard, the incidence of chronic lung disease remained high throughout the preterm period, dopamine therapy declined with maturation, and Coagulase Negative Staphylococcal infection was frequent among all age groups. As data from this cohort were also utilized in the assessment of a Bayesian Forecasting method as applied to neonatal patients, intradose interval concentrations were procured in addition to routine peak and trough concentrations (Appendices 10 and 12). The distributions of vancomycin peak, trough, and intradose concentrations are illustrated in Figure 21, as are those from the 10 patients later identified as outliers (Section 3.1.2). The mean peak and trough concentrations were within the target ranges of 25 - 40 mg/L and 5 - 10 mg/L, respectively, with considerable variability.

### 3.1.5. Validation Analyses

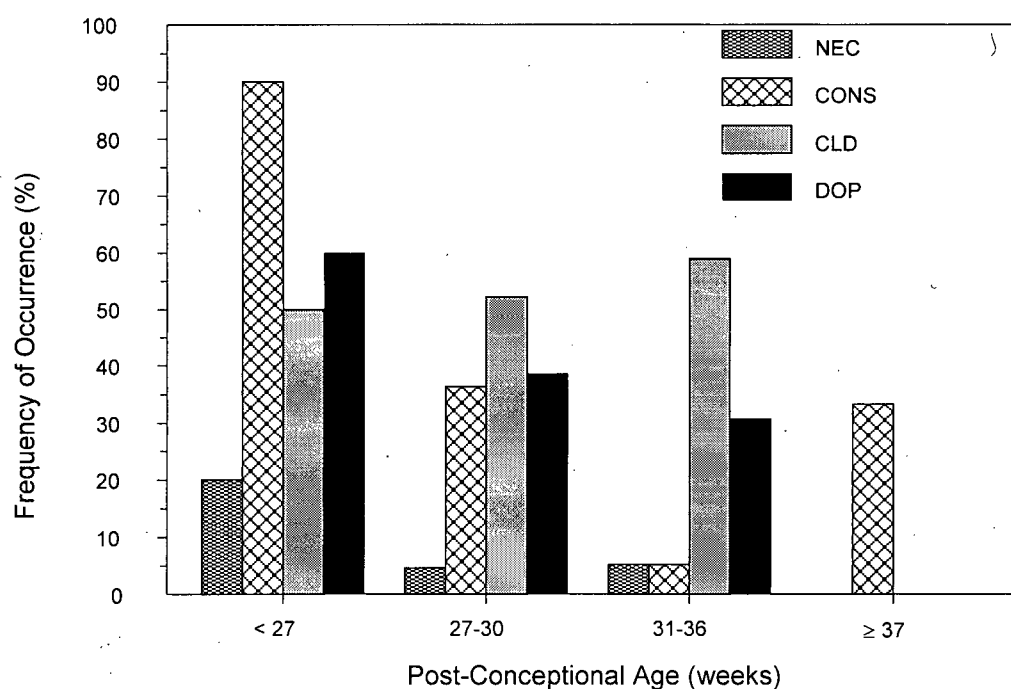
Figure 22 summarizes the error associated with predictions of vancomycin concentrations. These population-based predictions were generated from patient-specific data supplied to the optimal one- (1h, Table 11) and two- (2h, Table 8) compartment models, without the benefit of estimation or Bayesian implementation.



**Figure 18. Distribution of Gestational and Post-Conceptional Age by Groups.** Gestational age distribution reflects the age at birth of the 65 patients. Post-conceptional age reflects the age at birth plus the post-natal age from the time of birth to the initiation of each course (n = 105) of vancomycin therapy.

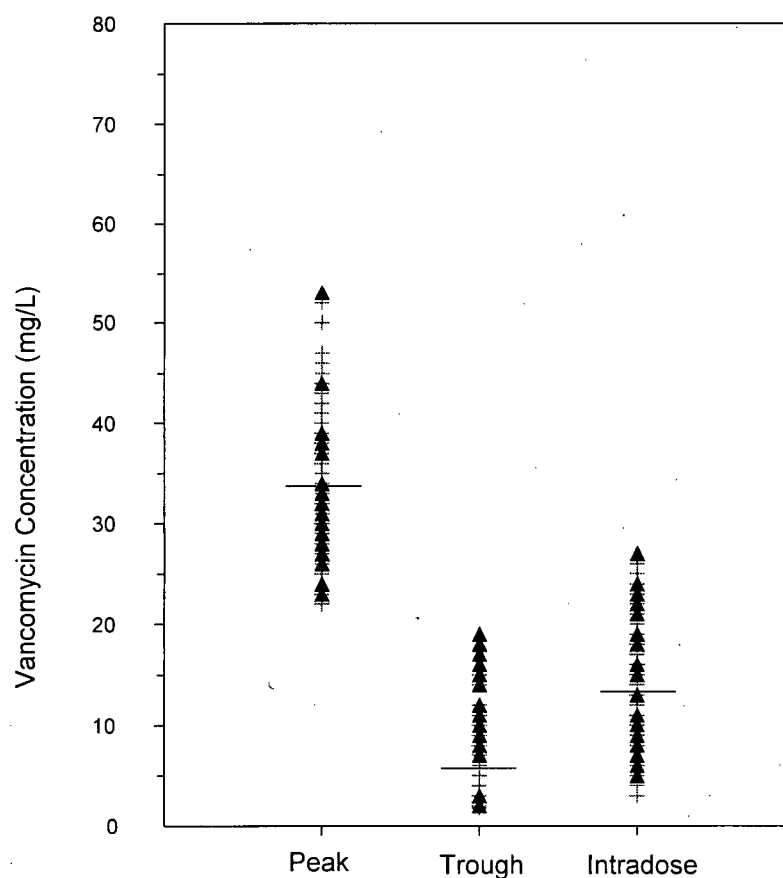


**Figure 19. Distribution of Patient Weight among the Post-Conceptional Age Groups at the Initiation of Each Course of Vancomycin Therapy.** Vancomycin courses numbered 105 in 65 patients. The Box-Whisker plots illustrate the median weights, the 25<sup>th</sup> to the 75<sup>th</sup> percentiles (Box), the 5<sup>th</sup> to the 95<sup>th</sup> percentiles (Whisker), and all data points (•).



**Figure 20. Distribution of Clinical Diagnoses and Concurrent Pharmacotherapy by Post-Conceptual Age Groups.** Illustrates the frequency of Necrotizing Enterocolitis (NEC), Coagulase Negative Staphylococcal Sepsis (CONS) and chronic lung disease (CLD) clinical diagnoses and concurrent Dopamine (DOP) pharmacotherapy at the initiation of each course (n = 105) of vancomycin therapy.



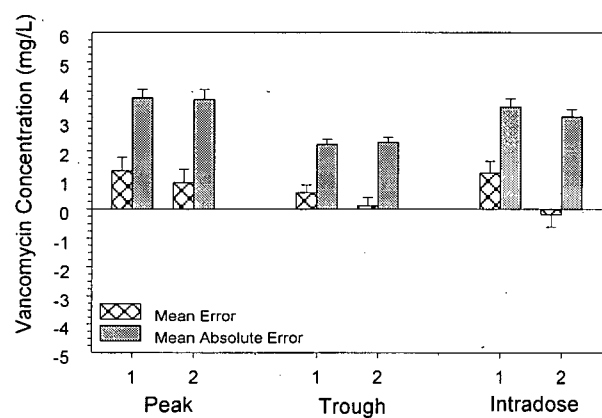


**Figure 21. Distribution of Measured Vancomycin Concentrations.** Peak, trough, and intradose interval serum vancomycin concentrations were analyzed from 65 patients prescribed 105 courses of therapy. Vancomycin concentrations from all patients included in the final validation analysis (+) and those identified as outliers (▲) (Section 3.1.2) are presented with mean ( $\pm$  sd) peak, trough, and intradose interval concentrations of 34 ( $\pm$  7) mg/L, 5 ( $\pm$  6) mg/L, and 14 ( $\pm$  6) mg/L, respectively.

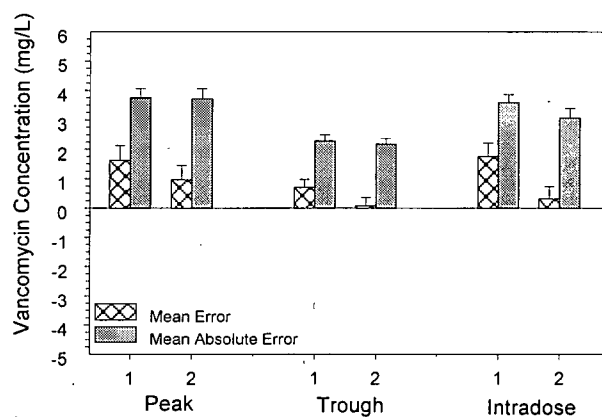
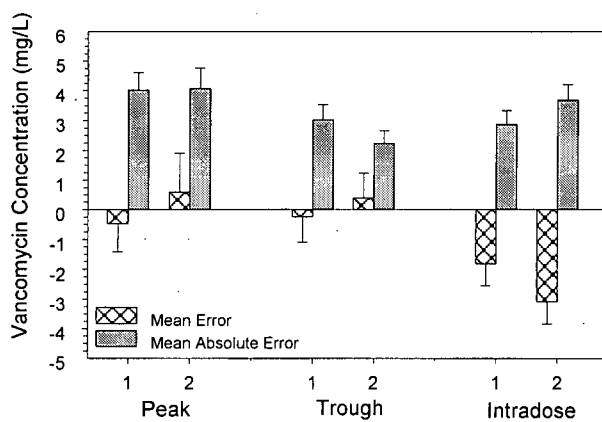
**Figure 22. Error Associated with Population-Based Predictions of Vancomycin Concentrations.**

ME ( $\pm$  se) and MAE ( $\pm$  se) of peak, trough, and intradose interval predictions of vancomycin concentrations based upon one- (1h) and two-compartment (2h) models were evaluated in all cases (A), mean ( $\pm$  sd) peak, trough, and intradose concentrations were 34 ( $\pm$  7) mg/L, 6 ( $\pm$  3) mg/L, and 14 ( $\pm$  6) mg/L, respectively. For cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) peak, trough, and intradose concentrations were 35 ( $\pm$  6) mg/L, 6 ( $\pm$  3) mg/L, and 15 ( $\pm$  6) mg/L, respectively. In cases  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) peak, trough, and intradose concentrations were 28 ( $\pm$  8) mg/L, 5 ( $\pm$  2) mg/L, and 10 ( $\pm$  4) mg/L, respectively.

(A) Prediction Error in All Cases (n = 91)



(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 79)

(C) Prediction Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 12)

For all cases (Figure 22A), the mean error (accuracy) of one- and two-compartment predictions was similar and represented 4% and 3%, respectively, of measured peak concentrations (Figure 22A). However, the two-compartment model exhibited lower mean error in predicting both trough and intradose vancomycin concentrations. In this regard, the mean error of two-compartment predictions represented 2% and 1% of trough and intradose concentrations, respectively; whereas, the mean error of one-compartment predictions represented 9% of both trough and intradose concentrations. The mean absolute error (precision) was similar for both models in predicting peak, trough, and intradose concentrations.

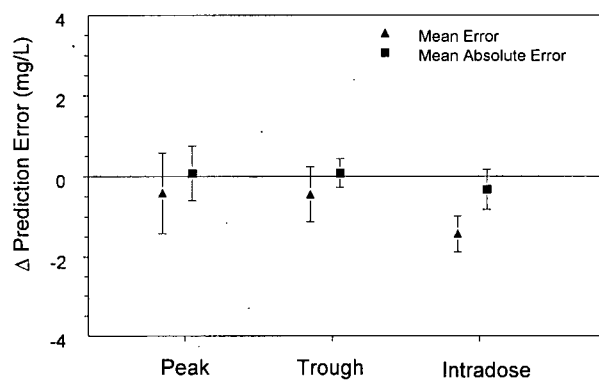
In those cases < 36 weeks post-conceptual age, the prediction error pattern can be discerned from Figure 22B. The mean error of two-compartment predictions represented 3%, 1%, and 2% of peak, trough, and intradose vancomycin concentrations, respectively. Whereas, the mean error of one-compartment predictions represented 5%, 12%, and 12% of peak, trough, and intradose vancomycin concentrations, respectively. Based on these data, the two-compartment model exhibited lower mean error for the three concentrations than the one-compartment model. The two models were similar with respect to mean absolute error for each of the predicted (peak, trough, and intradose) vancomycin concentrations.

In those cases  $\geq$  36 weeks post-conceptual age (Figure 22C), the mean error of one- and two-compartment predictions was similar and represented 2% of measured peak concentrations, and, respectively, 5% and 8% of trough concentrations. The mean error was somewhat larger for two-compartment predictions of intradose concentrations and represented 31% of this measurement compared to 18% for the one-compartment predictions. Again, the mean absolute error was similar for both models for each of the predicted concentrations.

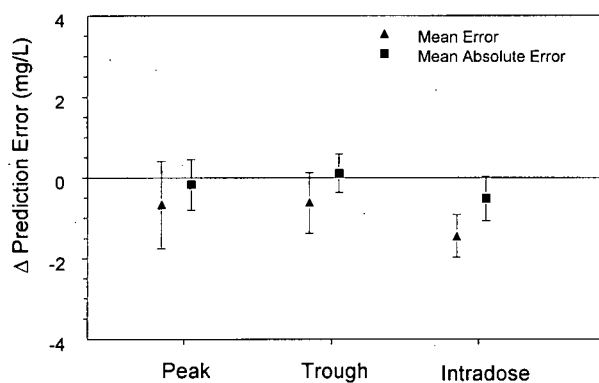
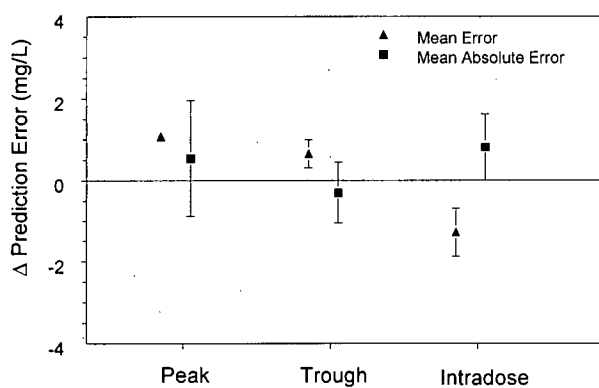
To illustrate the range of differences in prediction error between one- and two-compartment models, the 95% confidence intervals were constructed around these differences and are presented in Figure 23. As reported (Figure 22A), the two-compartment model exhibited lower mean error in predicting peak, trough, and intradose concentrations. The 95% confidence intervals constructed for all cases (Figure 23A) suggests that the mean error and mean absolute error associated with one- and two-compartment predictions of peak, trough, and intradose concentrations were similar. The two-compartment model demonstrated lower mean error and superior accuracy in predictions of intradose concentrations, as the confidence interval failed to

**Figure 23. Confidence Interval (95%) Constructed Around the Difference Between Two- and One-Compartment Population-Based Predictions.** Mean difference (two-compartment error minus one-compartment error) and confidence intervals of predictions of peak, trough, and intradose interval vancomycin concentrations are depicted for all cases (A), cases  $< 36$  (B) and  $\geq 36$  (C) weeks post-conceptual age. The two-compartment model was favored for all cases in which the confidence interval did not include zero, except for the mean error associated with predictions of trough and intradose interval concentrations in cases  $\geq 36$  weeks post-conceptual age (C).

(A) Difference in Prediction Error in All Cases (n = 91)



(B) Difference in Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 79)

(C) Difference in Error in Cases  $\geq$  36 weeks Post-Conceptional Age (n = 12)

cross zero. Similarly, in cases < 36 weeks post-conceptual age (Figure 23B), the two-compartment model demonstrated superior accuracy in predicting intradose concentrations. As reported in cases  $\geq 36$  weeks post-conceptual age (Figure 22C), the mean error of one-compartment predictions vancomycin concentrations was generally lower than that of the two-compartment model. The 95% confidence intervals constructed for this group (Figure 23C) suggest that the one-compartment model demonstrated lower mean error, superior accuracy, in predictions of trough and intradose concentrations. These confidence intervals indicated a trend toward better predictive performance of the one-compartment model in older patients, which may have moderated the advantage of the two-compartment model evident in the youngest patients in the overall pattern.

### **3.1.6. Demographic Characteristics of the Combined Model Building Patient Sample**

Table 14 summarizes the demographic characteristics of the 250 patients enrolled in the combined model building component of this investigation. This group is comprised of the 185 patients enrolled in the original model building component and those 65 patients included in the validation analyses. Fifty-nine percent of the combined patient sample were male, and the mean ( $\pm$  sd) gestational age upon admission was 29.7 ( $\pm$  4.3) weeks. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) Apgar scores at one- and five-minutes were 6 (4, 7) and 8 (7, 9), respectively. Together, empiric sepsis therapy and confirmed Coagulase Negative Staphylococcal sepsis represented > 80% of indications for vancomycin. The prevalence of medical diagnoses and pharmacotherapy was consistent with that reported for the original model building (Figure 6) and validation (Figure 20) patient samples. Thirty-four percent of the combined group were prescribed multiple courses of vancomycin, and over 1000 serum vancomycin concentrations were quantified.

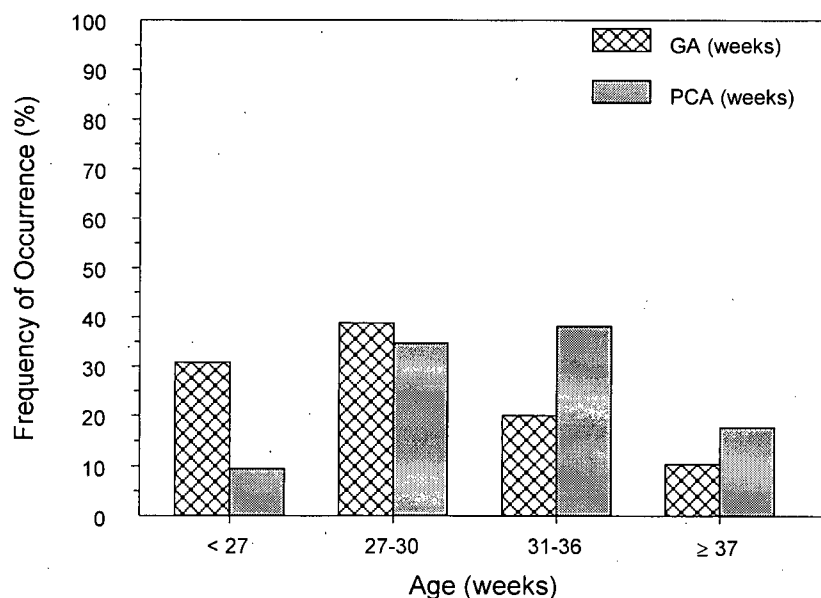
Figure 24 illustrates the gestational and post-conceptual age distribution of the 250 patients at the initiation of the 357 courses of vancomycin therapy. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) post-natal age at the start of each course was 15 (7, 28) days, again reflecting the right-shift in the distribution pattern between gestational and post-conceptual age. The mean ( $\pm$  sd) post-conceptual age and weight at the initiation of each vancomycin course were 32.1 ( $\pm$  4.5) weeks and 1.5 ( $\pm$  0.9) kg, respectively. Figure 25 demonstrates a dynamic pattern of increasing weight and apparent variability with increasing post-conceptual age, again

**Table 14. Demographic Characteristics of Patients Enrolled in the Combined Model Building Component of the Investigation.**

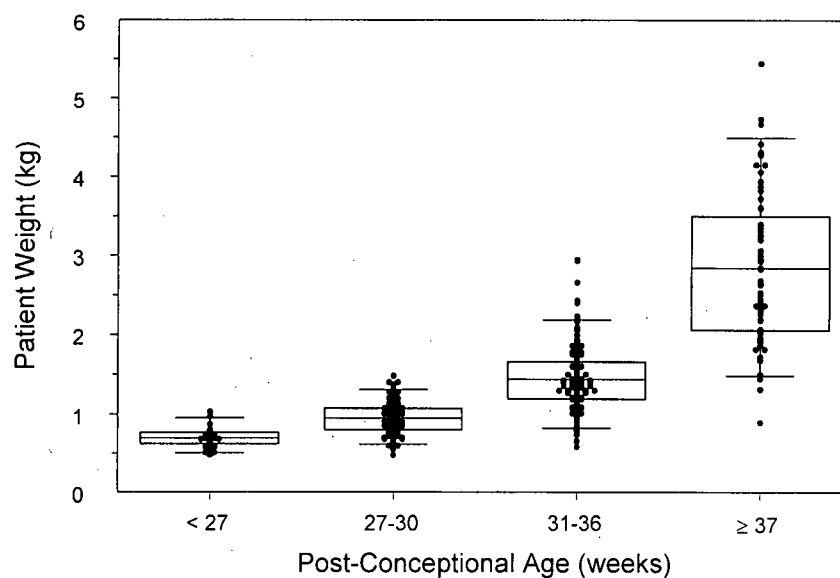
Demographic Characteristics	Number (%)
<b>Number of Patients</b>	<b>250</b>
Male	148 (59.2)
Female	102 (40.8)
<b>Admission History</b>	
Preterm Birth	230 (92.0)
Respiratory Distress Syndrome	205 (82.0)
<b>Indication for Vancomycin Therapy</b>	
Empiric Therapy - Sepsis	191 (53.5)
Coagulase Negative Staphylococcal Sepsis	107 (30.0)
Necrotizing Enterocolitis	26 (7.3)
Empiric Therapy - Necrotizing Enterocolitis	24 (6.7)
Other	12 (3.4)
<b>Clinical Presentation at the Initiation of Each Course</b>	
Chronic Lung Disease	226 (63.3)
Coagulase Negative Staphylococcal Sepsis	107 (30.0)
Dopamine <sup>a</sup>	39 (10.9)
Indomethacin <sup>a</sup>	31 (8.7)
Necrotizing Enterocolitis	26 (7.3)
<b>Number of Courses of Vancomycin</b>	<b>357</b>
<b>Number of Patients with Multiple Courses of Vancomycin</b>	<b>86 (34.4)</b>
Number of Patients with Two Courses	67 (26.8)
Number of Patients with Three or Four Courses	19 (7.6)
<b>Number of Serum Drug Concentration Determinations</b>	<b>1028</b>
Number of Peak or Trough Concentration Determinations	896
Number of Intradose Interval Concentration Determinations	132

<sup>a</sup> Pharmacotherapy within 72 hours of serum concentration determination.





**Figure 24. Distribution of Gestational and Post-Conceptional Age by Groups.** Gestational age distribution reflects the age at birth of the 250 patients. Post-conceptional Age reflects the age at birth plus the post-natal age from the time of birth to the initiation of each course (n = 357) of vancomycin therapy.



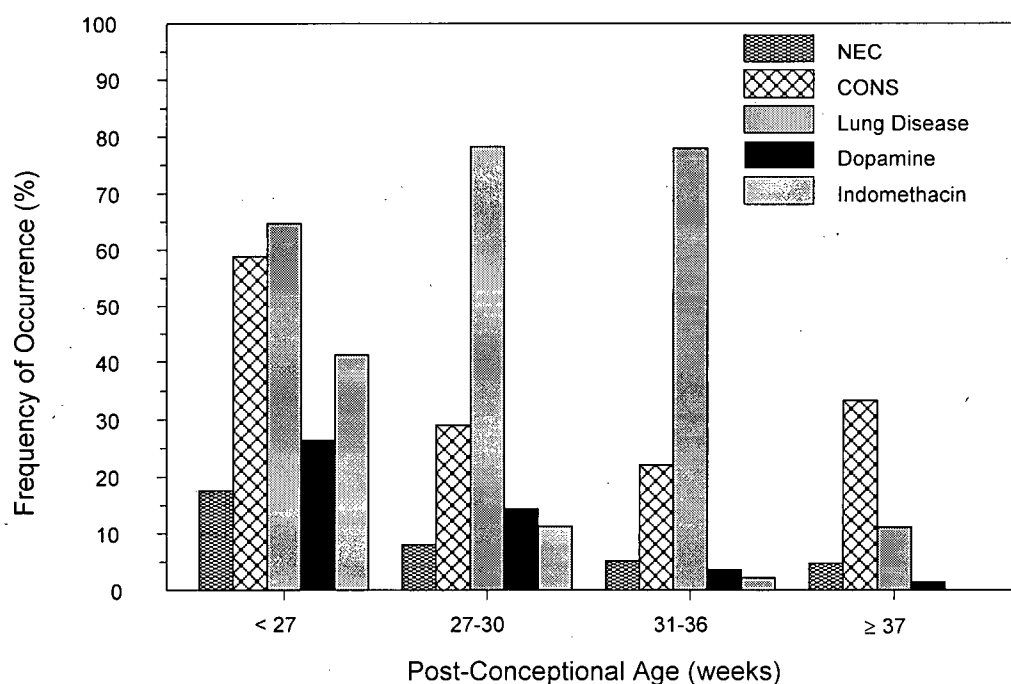
**Figure 25. Distribution of Patient Weight among the Post-Conceptional Age Groups at the Initiation of Each Course of Vancomycin Therapy.** Vancomycin courses numbered 357 in 250 patients. The Box-Whisker plots illustrate the median weights, the 25<sup>th</sup> to the 75<sup>th</sup> percentiles (Box), the 5<sup>th</sup> to the 95<sup>th</sup> percentiles (Whisker), and all data points (•).

suggesting that weight be incorporated as a continuous variable in the model. The frequencies of medical diagnoses and pharmacotherapy are presented in Figure 26. As 10% of the combined group received indomethacin therapy within 72 hours of serum vancomycin concentration determination, the frequency of indomethacin exposure among the age groups is illustrated in Figure 26; whereas, only 8% of those patients enrolled in the original model building component received indomethacin. As observed with dopamine, indomethacin therapy declined with maturation reflecting hemodynamic and cardiovascular complications in the youngest patients. The incidence of chronic lung disease remained high throughout the preterm period, and Coagulase Negative Staphylococcal infection was common among all age groups. The distributions of vancomycin peak and trough concentrations are presented in Figure 27, as are those from patients later identified as outliers (Section 3.1.2). The mean peak and trough concentrations were within the target ranges of 25 - 40 mg/L and 5 - 10 mg/L, respectively, with considerable variability.

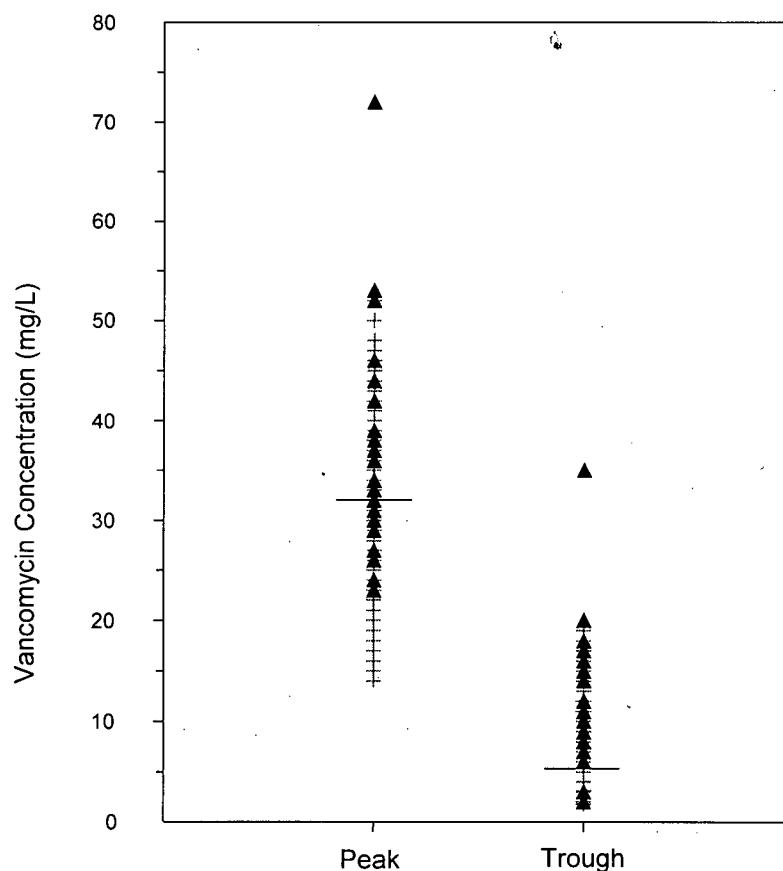
### 3.1.7. Combined Model Building

As with the original one- and two-compartment model building, an iterative process was implemented to generate a two-compartment model of vancomycin disposition for the combined, full dataset. All covariates and error terms that demonstrated a reduction in the objective function of  $> 6.6$  ( $p < 0.01$ ) were retained in the model. Data illustrated reflect estimates determined for each patient on each day for which there was an event record. Since the process replicated that of the previous approach, for brevity, only the data for the initial (c2a) and final (c2h) models are presented (Pharmacostatistical codes of models c2a – c2h are presented in Appendix 15).

Figure 28 illustrates a comparison between the initial model (c2a) and the final model (c2h). An improvement in the predicted concentrations and a reduction in weighted residual concentrations among the youngest patients was noted. The pharmacokinetic parameters, with respect to post-conceptual age, are presented in Figure 29. The influence of patient weight and post-conceptual age was reflected in the inclusion of these covariates in the final model (c2h). Dopamine continued to be an important factor in the clearance term; however, indomethacin was also associated with reduced clearance. This observed association may be attributed to the larger sample size. Weight-normalized central volume remained constant (0.4 – 0.6 L/kg) across all

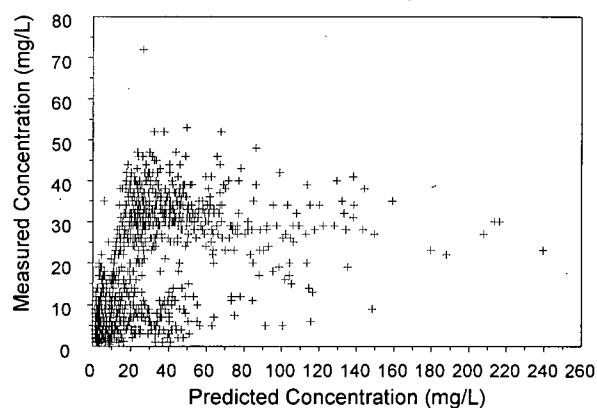


**Figure 26. Distribution of Clinical Diagnoses and Concurrent Pharmacotherapy by Post-Conceptual Age Groups.** Illustrates the frequency of Necrotizing Enterocolitis (NEC), Coagulase Negative Staphylococcal Sepsis (CONS) and Chronic Lung Disease (Lung Disease) clinical diagnoses and concurrent Dopamine and Indomethacin pharmacotherapy at the initiation of each course (n = 357) of vancomycin therapy.

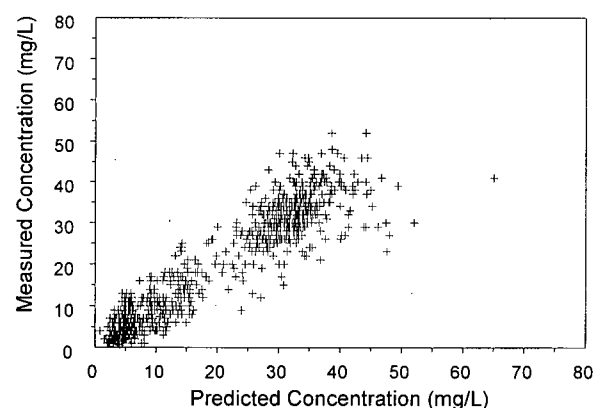


**Figure 27. Distribution of Measured Vancomycin Peak and Trough Concentrations.** Routine peak and trough serum vancomycin concentrations were analyzed from 250 patients prescribed 357 courses of therapy. Vancomycin concentrations from all patients included in the refined model (+) and those later identified as outliers (▲) (Section 3.1.2) are presented with mean ( $\pm$  sd) peak and trough concentrations of  $32 (\pm 6)$  mg/L and  $6 (\pm 4)$  mg/L, respectively.

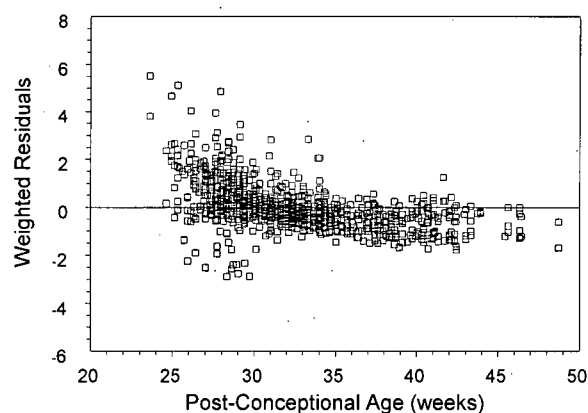
(A) Measured Versus Predicted Concentration  
(Model c2a)



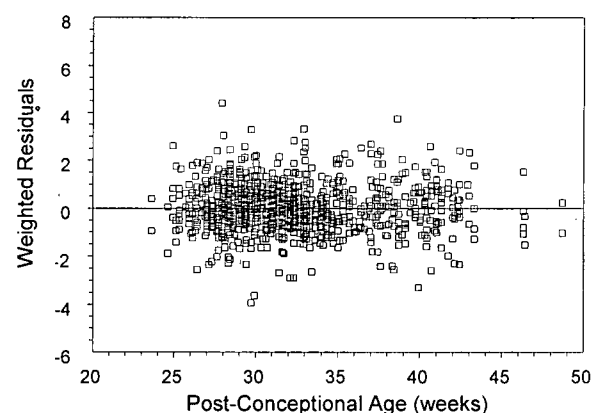
(B) Measured Versus Predicted Concentration  
(Model c2h)



(C) Weighted Residuals Versus  
Post-Conceptional Age (Model c2a)



(D) Weighted Residuals Versus  
Post-Conceptional Age (Model c2h)



**Figure 28. Measured Versus Predicted Concentrations and Weighted Residuals Versus Post-Conceptional Age for Models c2a and c2h.** Predicted concentrations (A, B) and weighted residuals (C, D) generated with two-compartment models. Model c2h with exponential interindividual variability and mixed (exponential and additive) intraindividual variability given:

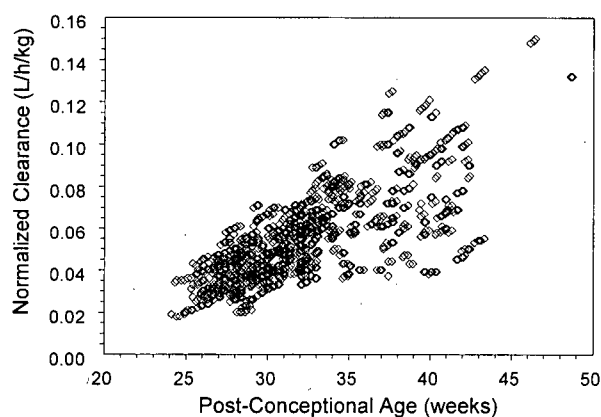
$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP) * (\theta_5 ** IND)$$

$$TVV1 = \theta_6 * WT$$

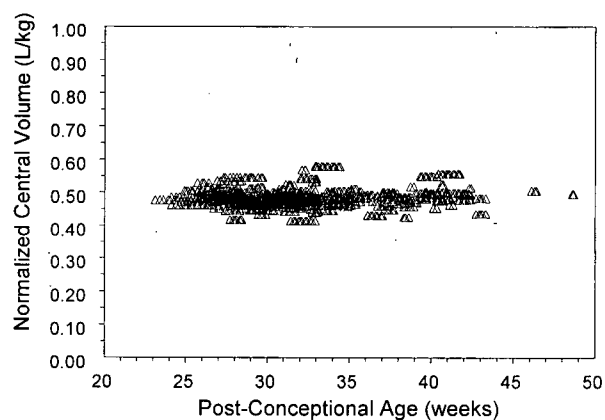
$$TVV2 = \theta_7 * (1 + \theta_8 ** CLD)$$

$$Q = \theta_9$$

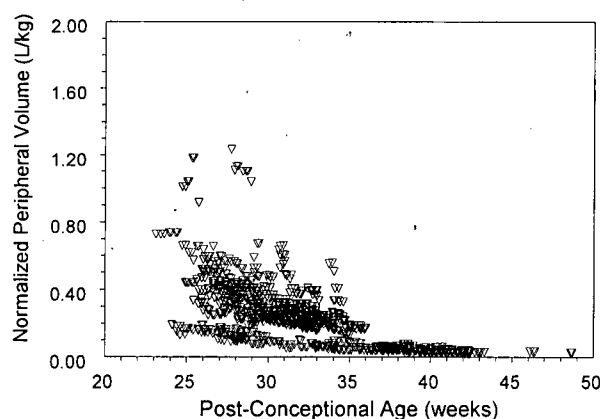
(A) Normalized Clearance Versus  
Post-Conceptual Age



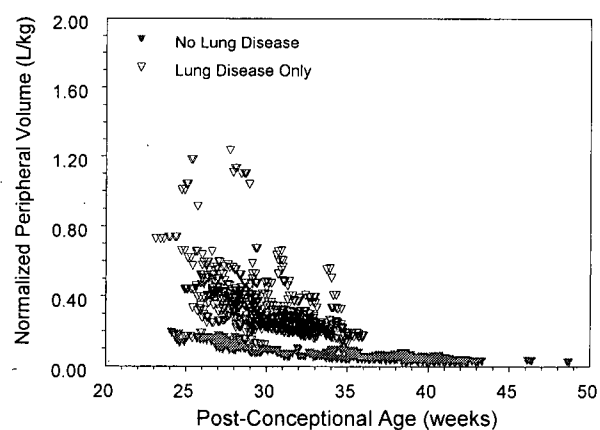
(B) Normalized Volume of the Central  
Compartment Versus Post-Conceptual Age



(C) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



(D) Normalized Volume of the Peripheral  
Compartment Versus Post-Conceptual Age



**Figure 29. Pharmacokinetic Parameters Versus Post-Conceptual Age for Model c2h.** Individual parameters of clearance (A), central volume (B), and peripheral volume (C, D) generated with a two-compartment model with exponential interindividual and mixed (exponential and additive) intraindividual variability in which:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP) * (\theta_5 ** IND)$$

$$TVV1 = \theta_6 * WT$$

$$TVV2 = \theta_7 * (1 + \theta_8 ** CLD)$$

$$Q = \theta_9$$

post-conceptual age groups. The model distinguished between patients with and without chronic lung disease: higher weight-normalized peripheral volume was associated with chronic lung disease patients and, to some degree, patients < 36 weeks post-conceptual age.

The summary of the incremental improvement of fit is presented in Table 15, wherein the mean posthoc parameter estimates and changes in the objective function are reported. The mean weight-normalized central volume increased from the initial (c2a) to the final model (c2h). Conversely, the mean weight-normalized peripheral volume, and thereby volume of distribution at steady-state, was markedly reduced in model c2h compared to c2a.

The parameter and error estimates generated by NONMEM for the final model (c2h) are reported in Table 16. The point estimate associated with patient weight in the clearance term results in a 75% increase in clearance with a doubling of patient weight. Further, those patients < 30 weeks post-conceptual age exhibited 50 - 70% lower clearance than neonates at 40 weeks post-conceptual age, independent of weight. Exposure to dopamine (mean dose = 8.0  $\mu\text{g/kg/min}$ ) within 72 hours of serum vancomycin concentration determination, which occurred in 10% of cases, was associated with a 30% lower vancomycin clearance. Also, exposure to indomethacin (10% of cases, mean dose = 0.1 mg/kg/day) was associated with a 16% lower clearance. A diagnosis of chronic lung disease (62% of cases) can be calculated to be associated with a 88% increase in peripheral volume. The coefficients of variation with respect to interindividual variability in clearance, central volume, and peripheral volume were 25%, 8%, and 75%, respectively. The combined coefficient of variation (exponential and additive) for intraindividual variability was approximately 68%.

The mean pharmacokinetic estimates calculated from typical values for model 2h are presented in Table 17. The distribution half-life remained long (> 4 hours) across all post-conceptual age groups. The modeled elimination half-life decreased slightly between 24 and 36 weeks post-conceptual age, followed by a dramatic reduction in neonates  $\geq 37$  weeks post-conceptual age. Weight-normalized clearance increased by 133% from the youngest to the oldest patients. The weight-normalized central volume remained constant across all age groups; whereas, peripheral volume decreased appreciably. Consequently, the peripheral volume represented 41% of the volume of distribution at steady-state in the youngest patients, but only 8% in the oldest patients. These mean estimates are consistent with the trends observed in the original model building (Table 9).

**Table 15. Summary of Changes in Objective Function Values and Mean Posthoc Parameter Estimates from the Final Two-Compartment Model<sup>a</sup>.**

Model	Cumulative $\Delta$ Objective Function Value	Mean Posthoc Parameter Estimates			
		Cl (L/h/kg)	Vc (L/kg)	Vp (L/kg)	Vss (L/kg)
c2a		0.04	0.49	2.07	2.56
c2b	-771.12	0.05	0.48	0.63	1.11
c2c	-1626.72	0.05	0.51	0.59	1.10
c2d	-1679.33	0.05	0.50	0.68	1.23
c2e	-1831.48	0.05	0.51	3.38	3.88
c2f	-2830.96	0.05	0.48	0.35	0.83
c2g	-2896.17	0.06	0.48	0.24	0.72
c2h	-2891.85	0.06	0.48	0.24	0.72

<sup>a</sup> The mean posthoc estimates generated by NONMEM of clearance (Cl), central volume of distribution (Vc), peripheral volume of distribution (Vp), and steady-state volume of distribution (Vss) are reported for two-compartment models. The change in the minimum value of the objective function value is presented for each model relative to the objective function of the basic model (model c2a, objective function = 5819.50) without any covariates. Posthoc estimates were determined for each patient on each day for which there was an event record for a total of 1431 determinations (models c2a – c2e) and 1304 determinations (models c2f – c2h).



**Table 16. Final Two-Compartment Model: Parameter and Error Estimates<sup>a</sup>.**

	Population Point Estimates	Standard Error
<b>Structural Parameters</b>		
$\theta_1$	0.095	0.007
$\theta_2$	0.806	0.070
$\theta_3$	2.390	0.254
$\theta_4$	0.724	0.041
$\theta_5$	0.837	0.038
$\theta_6$	0.483	0.005
$\theta_7$	0.108	0.046
$\theta_8$	2.770	1.410
$\theta_9$	0.007	0.002
<b>Interindividual Variability</b>		
$\eta_1$ (% CV)	0.061 (24.7)	0.006
$\eta_2$ (% CV)	0.007 (8.1)	0.002
$\eta_3$ (% CV)	0.556 (74.6)	1.690
<b>Intraindividual Variability</b>		
$\varepsilon_1$ (% CV)	0.008 (8.8)	0.001
$\varepsilon_2$ (% CV)	0.460 (67.8)	0.262

<sup>a</sup> NONMEM point estimates and standard errors generated with a two-compartment model (c2h) given:

$$TVCL = \theta_1 * (WT ** \theta_2) * (PCA/40 ** \theta_3) * (\theta_4 ** DOP) * (\theta_5 ** IND)$$

$$CL = TVCL * EXP(\eta_1)$$

$$TVV1 = \theta_6 * WT$$

$$V1 = TVV1 * EXP(\eta_2)$$

$$TVV2 = \theta_7 * (1 + \theta_8 ** CLD)$$

$$V2 = TVV2 * EXP(\eta_3)$$

$$Q = \theta_9$$

$$Y = F * EXP(\varepsilon_1) + \varepsilon_2$$

**Table 17. Mean Pharmacokinetic Estimates Derived from the Final Two-Compartment Population Model<sup>a</sup>.**

PCA Group	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Cl (L/h/kg)	Vc (L/kg)	Vp (L/kg)	Vss (L/kg)
< 27 weeks	6.70	33.61	0.03	0.48	0.33	0.81
27 – 30 weeks	6.20	31.90	0.04	0.48	0.28	0.76
31 – 36 weeks	5.32	27.88	0.06	0.48	0.18	0.66
≥ 37 weeks	4.32	10.87	0.07	0.48	0.04	0.52
<b>All</b>	<b>5.56</b>	<b>26.74</b>	<b>0.06</b>	<b>0.48</b>	<b>0.16</b>	<b>0.64</b>

<sup>a</sup> The mean values of distribution half-life ( $t_{1/2\alpha}$ ), elimination half-life ( $t_{1/2\beta}$ ), clearance (Cl), central volume of distribution (Vc), peripheral volume of distribution (Vp), and steady-state volume of distribution (Vss) are reported for 336 courses of vancomycin therapy in 236 patients. Parameter estimates were determined from typical values (Model c2h) for each patient on each day for which there was an event record (1304 determinations) and the mean values for each case were calculated over the course of therapy.

Although not reported, one-compartment models were also generated using the combined dataset, and consistent with the original model building results, the one-compartment model appeared inferior to the two-compartment model at all sequential steps.

### **3.2. Bayesian Forecasting**

#### **3.2.1. Demographic Characteristics of the Bayesian Forecasting Patient Sample**

Data were collected (Section 2.2.5) from this cohort of patients admitted to the SCN during the same period as those obtained for the purposes of model building. This permitted the opportunity to evaluate the predictive performance of Bayesian forecasting in a patient sample representative of the general admissions population and model building cohort. This patient sample was comprised of neonates with strictly timed midinterval (Midinterval) and near-midinterval (Residual) vancomycin concentrations quantified prior to or following the third dose of vancomycin therapy, in addition to the routine set of peak and trough concentrations (Section 2.2.3). For all patients in the Residual subset, the additional vancomycin concentrations were obtained within 10% of the midpoint of the dosage interval.

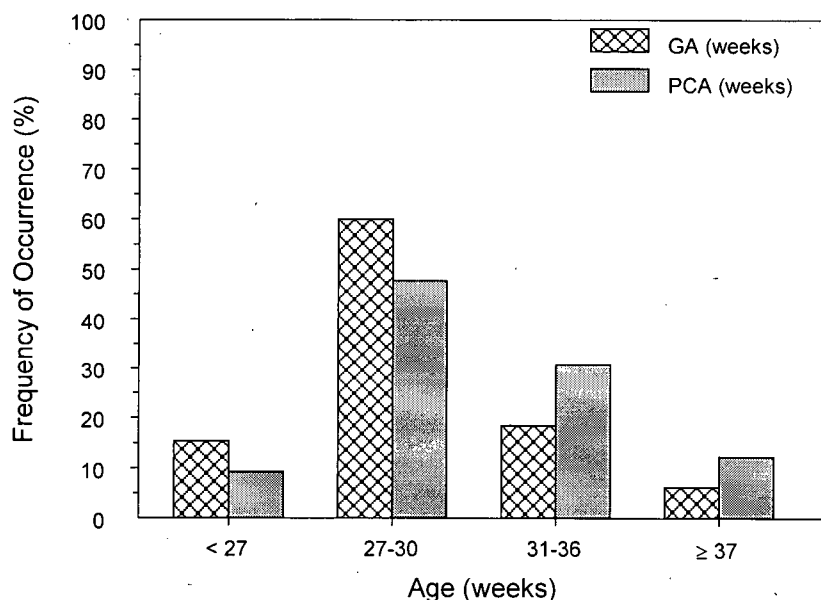
Table 18 summarizes the demographic data of the 65 patients enrolled in the Bayesian forecasting component of this investigation. Sixty-three percent of this cohort were male, and the mean ( $\pm$  sd) gestational age upon admission was 28.3 ( $\pm$  3.8) weeks. As with the original model building patient sample (Table 5), the majority of patients were preterm with a history of respiratory distress syndrome. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) Apgar scores at one- and five-minutes were 5 (3, 7) and 8 (7, 9), respectively. Together, empiric sepsis therapy and confirmed Coagulase Negative Staphylococcal sepsis represented > 85% of indications for vancomycin. The prevalence of medical diagnoses and pharmacotherapy was consistent with the original model building patient sample (Table 5). Only one course of therapy for each patient was implemented in the Bayesian analyses and 299 serum vancomycin concentrations were quantified.

Figure 30 illustrates the gestational and post-conceptual age distribution of the 65 patients at the initiation of each course of vancomycin therapy. The median (25<sup>th</sup>, 75<sup>th</sup> percentile) post-natal age at the start of each course was 9 (7, 30) days, this reflects the right-shift in the distribution pattern between gestational and post-conceptual age. The mean ( $\pm$  sd) post-

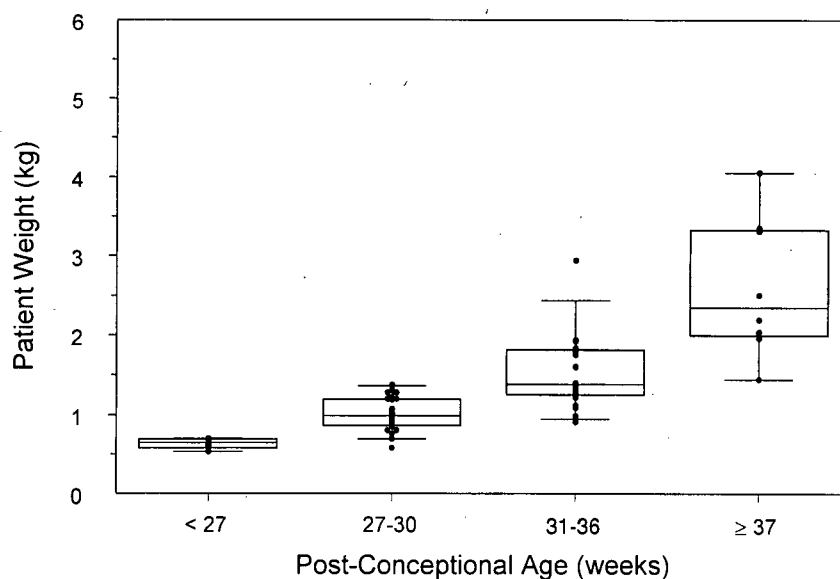
**Table 18. Demographic Characteristics of Patients Enrolled in the Bayesian Forecasting Component of the Investigation.**

Demographic Characteristics	Number (%)		
	Midinterval	Residual	Combined
<b>Number of Patients</b>	<b>35</b>	<b>30</b>	<b>65</b>
Male	22 (62.9)	19 (63.3)	41 (63.1)
Female	13 (37.1)	11 (36.7)	24 (36.9)
<b>Admission History</b>			
Preterm Birth	32 (91.4)	29 (96.7)	61 (93.9)
Respiratory Distress Syndrome	31 (88.6)	25 (83.3)	56 (86.2)
<b>Indication for Vancomycin Therapy</b>			
Empiric Therapy - Sepsis	23 (65.7)	11 (36.7)	34 (52.3)
Coagulase Negative Staphylococcal Sepsis	8 (22.9)	14 (46.7)	22 (33.8)
Necrotizing Enterocolitis	1 (2.9)	3 (10.0)	4 (6.2)
Empiric Therapy - Necrotizing Enterocolitis	2 (5.7)	2 (6.7)	4 (6.2)
Other	1 (2.9)	0 (0)	1 (1.5)
<b>Clinical Presentation at the Initiation of Each Course</b>			
Chronic Lung Disease	26 (74.3)	13 (43.3)	39 (60.0)
Coagulase Negative Staphylococcal Sepsis	8 (22.9)	14 (46.7)	22 (33.8)
Dopamine <sup>a</sup>	1 (2.9)	10 (33.3)	11 (16.9)
Indomethacin <sup>a</sup>	1 (2.9)	7 (23.3)	8 (12.3)
Necrotizing Enterocolitis	1 (2.9)	3 (10.0)	4 (6.2)
<b>Number of Serum Drug Concentration Determinations</b>	<b>160</b>	<b>139</b>	<b>299</b>
Number of Peak or Trough Concentration Determinations	90	92	182
Number of Intradose Concentration Determinations	70	47	117
Number of Pre-Dose 3 Concentrations	35	29	64
Number of Post-Dose 3 Concentrations	35	18	53

<sup>a</sup> Pharmacotherapy within 72 hours of serum concentration determination.



**Figure 30. Distribution of Gestational and Post-Conceptional Age by Groups.** Gestational age distribution reflects the age at birth of the 65 patients. Post-conceptional age reflects the age at birth plus the post-natal age from the time of birth to the initiation of each course (n = 65) of vancomycin therapy.



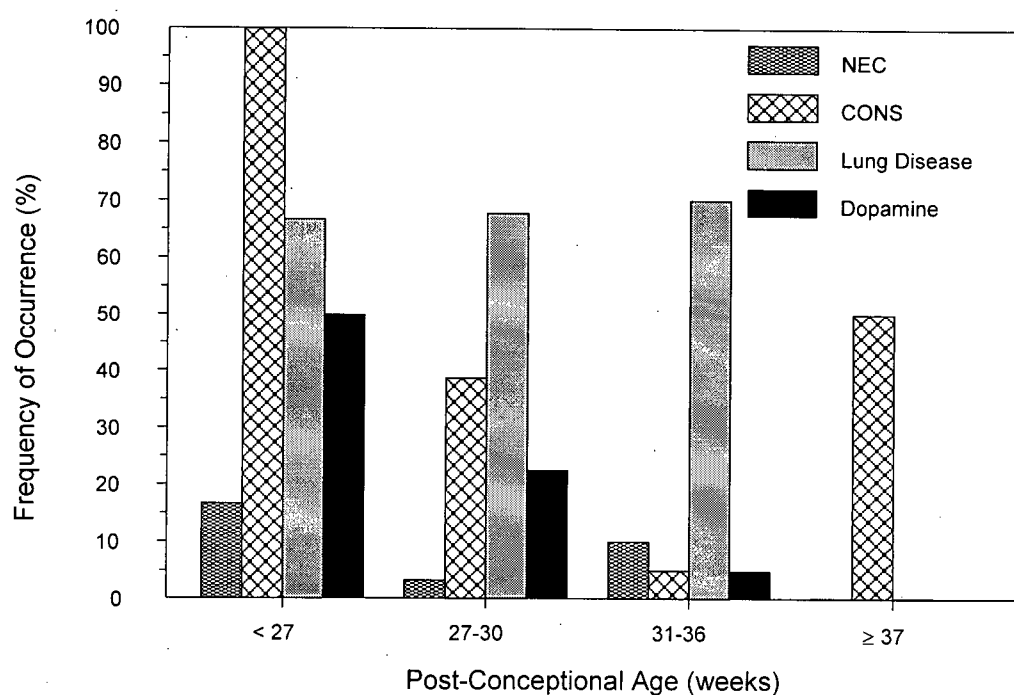
**Figure 31. Distribution of Patient Weight among the Post-Conceptional Age Groups at the Initiation of Each Course of Vancomycin Therapy.** Vancomycin courses numbered 65 in 65 patients. The Box-Whisker plots illustrate the median weights, the 25<sup>th</sup> to the 75<sup>th</sup> percentiles (Box), the 5<sup>th</sup> to the 95<sup>th</sup> percentiles (Whisker), and all data points (•).

conceptional age and weight at the initiation of each vancomycin course were  $31.6 (\pm 4.8)$  weeks and  $1.3 (\pm 0.7)$  kg, respectively. Figure 31 demonstrates a dynamic pattern of increasing weight and apparent variability with increasing post-conceptional age, similar to that of the original model building sample (Figure 5). The frequencies of medical diagnoses and pharmacotherapy are illustrated in Figure 32. In this regard, the incidence of chronic lung disease remained high throughout the preterm period, dopamine therapy declined with maturation, and Coagulase Negative Staphylococcal infection was highest in the youngest patients, but common among all age groups. The distributions of vancomycin peak, trough, and intradose concentrations are illustrated in Figure 33, as are those from the 10 patients later identified as outliers (Section 3.1.2). The mean peak and trough concentrations were within the target ranges of 25 - 40 mg/L and 5 - 10 mg/L, respectively, with considerable variability.

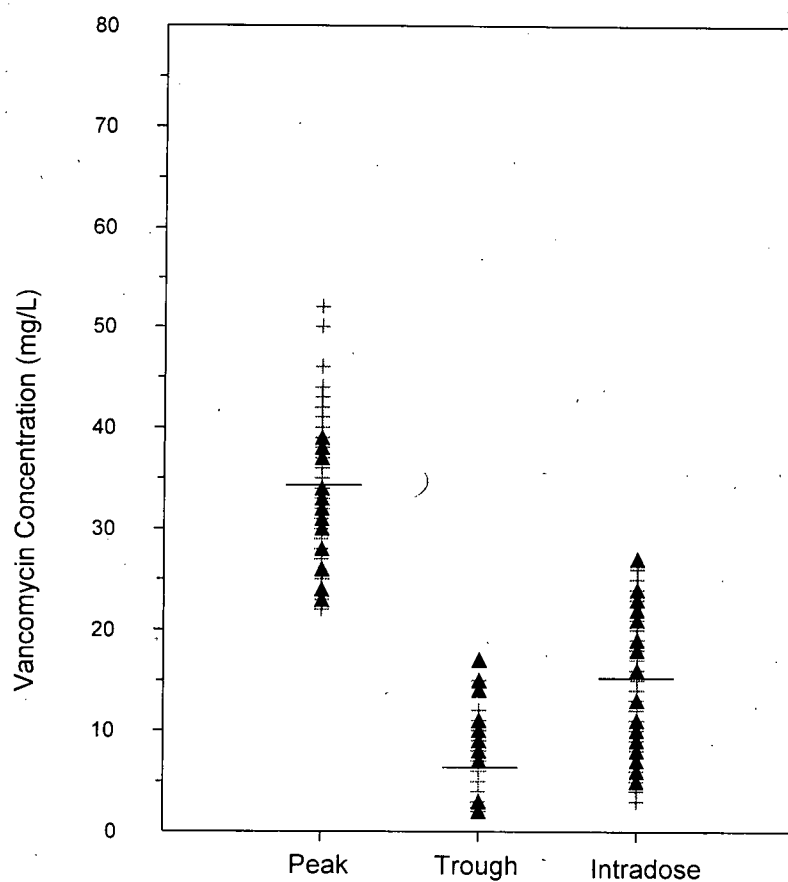
### **3.2.2. Comparison of One- and Two-Compartment Models for Bayesian Forecasting**

Figures 34 and 35 depict the error associated with Bayesian predictions of vancomycin peak and trough concentrations, respectively. These predictions were generated from patient-specific data with measured feedback concentrations supplied to the optimal one- (1h, Table 11) and two- (2h, Table 8) compartment models with the benefit of Bayesian estimation. Together, the feedback concentrations and appropriate population prior estimates implemented in a NONMEM Bayesian algorithm permitted the computation of case-specific predictions of vancomycin concentrations.

The results for all cases (Figure 34A) indicate that the relative mean error (accuracy) of two-compartment predictions of peak concentrations based upon pre-third dose, trough only, and post-third dose feedback were 1%, 2%, and 1%, respectively (Figure 34A). For one-compartment predictions of peak concentrations using only trough feedback demonstrated similar relative mean error (2%). In this regard, the two-compartment predictions exhibited lower mean error using pre- and post-third dose concentration feedback; however, both models were similar when only trough feedback was provided. The relative mean absolute error (precision) of two-compartment predictions based upon trough only feedback was 6% and was similar for both models regardless of feedback.



**Figure 32. Distribution of Clinical Diagnoses and Concurrent Pharmacotherapy by Post-Conceptual Age Groups.** Illustrates the frequency of Necrotizing Enterocolitis (NEC), Coagulase Negative Staphylococcal Sepsis (CONS) and Chronic Lung Disease (Lung Disease) clinical diagnoses and concurrent Dopamine pharmacotherapy at the initiation of each course (n = 65) of vancomycin therapy.

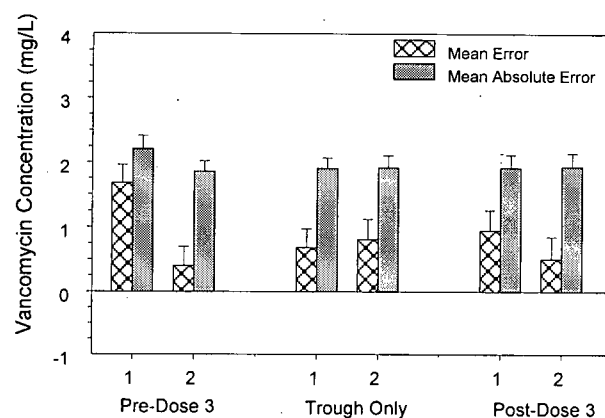


**Figure 33. Distribution of Measured Vancomycin Concentrations.** Peak, trough, and intradose interval serum vancomycin concentrations were analyzed from 65 patients prescribed 65 courses of therapy. Vancomycin concentrations from all patients included in the Bayesian analyses (+) and those identified as outliers (▲) (Section 3.1.2) are presented with mean ( $\pm$  sd) peak, trough, and intradose interval concentrations of  $35 (\pm 7)$  mg/L,  $7 (\pm 4)$  mg/L, and  $15 (\pm 7)$  mg/L, respectively.

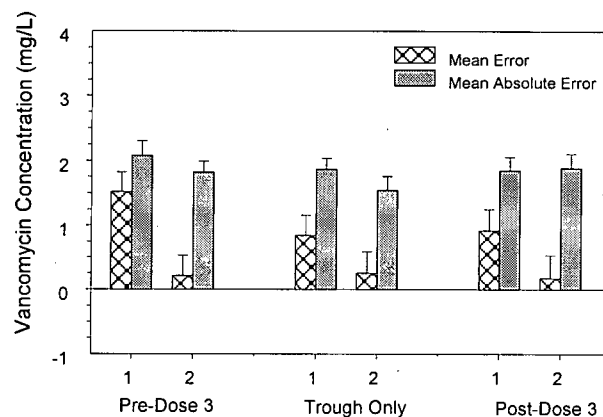
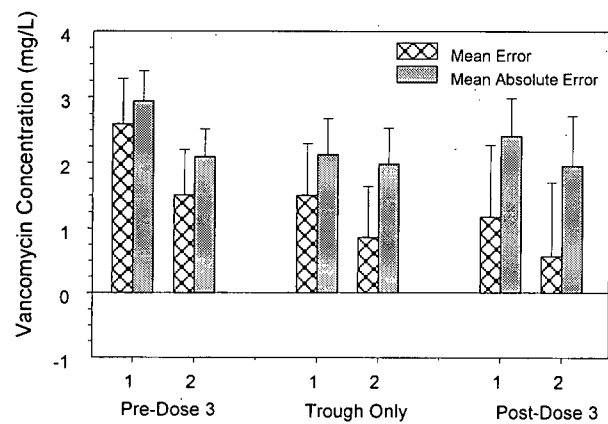


**Figure 34. Error Associated with Bayesian Predictions of Vancomycin Peak Concentrations.** ME ( $\pm$  se) and MAE ( $\pm$  se) of peak predictions of vancomycin concentrations based upon one- (1h) and two-compartment (2h) models and indicated feedback were evaluated in all cases (A), mean ( $\pm$  sd) peak concentration was 35 ( $\pm$  6) mg/L; cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) peak concentration was 36 ( $\pm$  6) mg/L; and cases  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) peak concentration was 28 ( $\pm$  6) mg/L.

(A) Prediction Error in All Cases (n = 55)



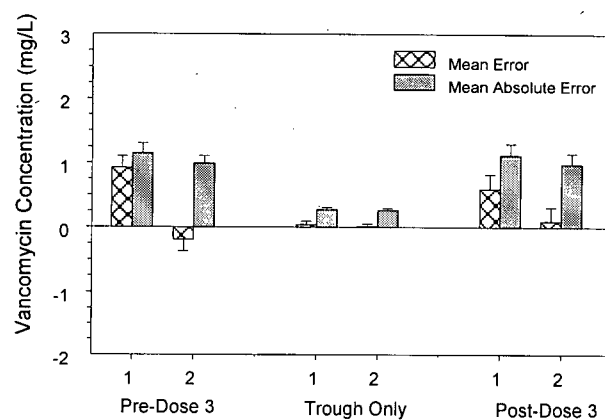
(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 47)

(C) Prediction Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 8)

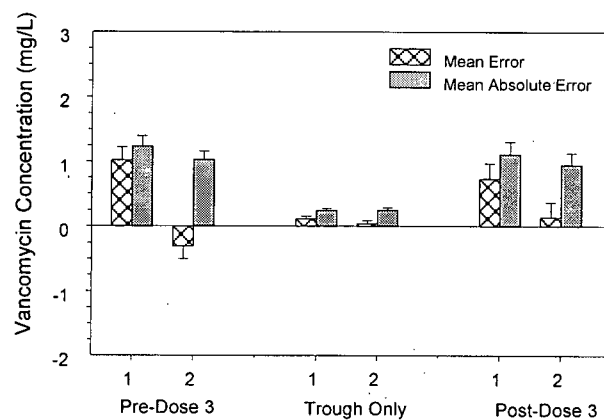
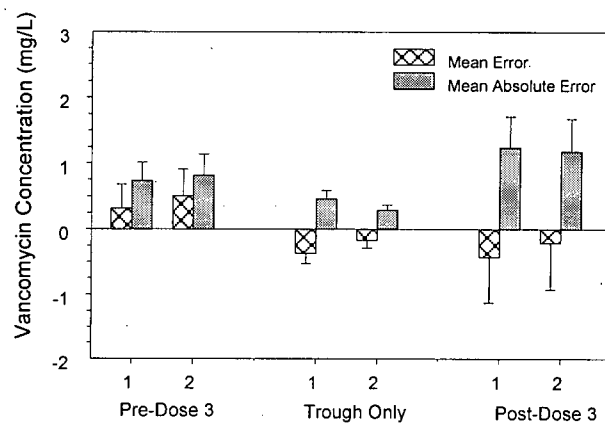
**Figure 35. Error Associated with Bayesian Predictions of Vancomycin Trough Concentrations.**

ME ( $\pm$  se) and MAE ( $\pm$  se) of trough predictions of vancomycin concentrations based upon one- (1h) and two-compartment (2h) models and indicated feedback were evaluated in all cases (A), mean ( $\pm$  sd) trough concentration was 6 ( $\pm$  3) mg/L; cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) trough concentration was 6 ( $\pm$  3) mg/L; and  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) trough concentration was 4 ( $\pm$  2) mg/L.

(A) Prediction Error in All Cases (n = 55)



(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 47)

(C) Prediction Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 8)

In those cases < 36 weeks post-conceptual age the lower mean error associated with the two-compartment model and similarity in mean absolute error between one- and two-compartment models can be discerned from Figure 34B. The mean error of two-compartment predictions based upon pre-third dose, trough only, and post-third dose feedback each represented <1% of the measured peak concentration. In contrast, the relative mean error associated with one-compartment predictions represented >2% of the peak concentration for each feedback sample.

In those cases  $\geq$  36 weeks post-conceptual age (Figure 34C), the results indicate that the relative mean error of the two-compartment predictions of peak concentrations was 5%, 3%, and 4% based upon pre-third dose, trough only, and post-third dose feedback, respectively. Whereas, one-compartment predictions using pre-third dose, trough only, and post-third dose feedback represented 9%, 5%, and 4% of the measured peak concentration. In this relatively small group, the data demonstrated the superior accuracy and to a lesser extent, precision, of the two-compartment model with various feedback concentrations. Overall, the tendency of the superior predictive performance of the two-compartment model in the < 36 week post-conceptual age group is consistent with the evidence suggesting that the two-compartment model better specifies the pharmacokinetic behavior of vancomycin in this age group.

In agreement with the foregoing results, the two-compartment predictions of trough concentrations generally demonstrated lower mean error compared to one-compartment predictions; however, both models exhibited similar mean absolute error (Figure 35). From the results for all cases (Figure 36A), the relative mean error of two-compartment predictions of trough concentrations based upon pre-third dose, trough only, and post-third dose feedback concentrations was 3%, <1%, and 2%, respectively. For one-compartment predictions of measured trough concentrations using only trough feedback demonstrated similar relative mean error (<1%). Trough concentrations were used to generate Bayesian predictions of trough (ie. predictor predicting itself) to illustrate the limit of the predictive performance of the Bayesian method.

Again, in cases < 36 weeks post-conceptual age, the trend favoring the two-compartment model with respect to error is apparent (Figure 34B). In this group, the mean error of two-compartment predictions represented 5% and 2% of measured trough concentrations based upon pre- and post-third dose feedback, respectively; whereas, the relative mean error of

one-compartment predictions of trough concentrations was 17% and 12% using the same feedback concentrations.

Similarly, in the small group of cases  $\geq 36$  weeks post-conceptual age, the two-compartment model generally exhibited lower mean error with no obvious pattern in mean absolute error (Figure 35C). However, the relative mean error on the one-compartment predictions of trough concentrations based upon pre-third dose feedback was lower (8%) than for the two-compartment (13%).

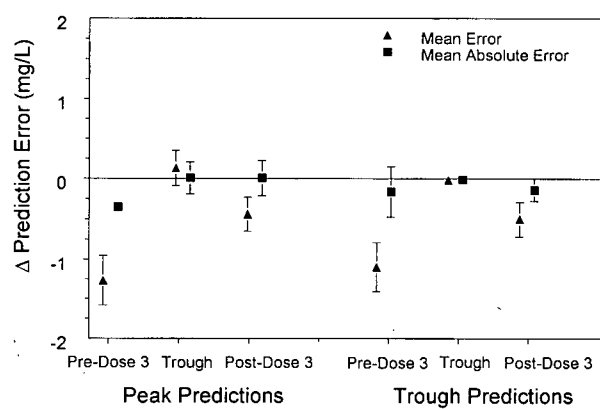
To illustrate the range of differences in Bayesian prediction error between one- and two-compartment models, the 95% confidence intervals were constructed around these differences and are presented in Figure 36. The trend for all cases (Figure 36A) favored the two-compartment predictions of peak and trough concentrations with respect to pre- and post-third dose concentration feedback, as the respective confidence intervals failed to cross zero. The mean absolute error between models and among feedback samples was similar for peak and trough predictions with some improved precision of two-compartment peak predictions using the pre-third dose feedback. Similarly, in cases  $<36$  weeks post-conceptual age, the observed differences in prediction error favored the two-compartment model as depicted by the confidence intervals that failed to include zero (Figure 36B). In those cases  $\geq 36$  weeks post-conceptual age, the confidence intervals demonstrated a trend toward superior predictive performance of the two-compartment model (Figure 36C). Although the sample size was small, this evidence supports the validation analyses results suggesting that the one-compartment model may approximate the pharmacokinetic behavior in neonates  $\geq 36$  weeks post-conceptual age.

### **3.2.3. Error Associated with Predictions of Follow-Up Concentrations**

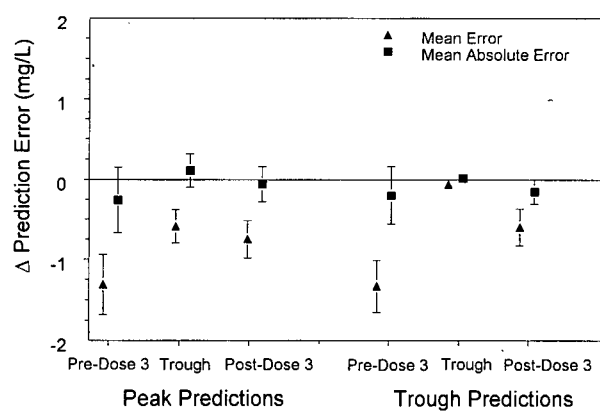
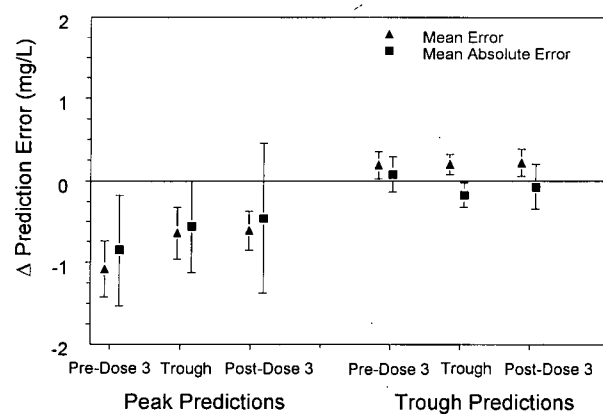
A number of patients required a dosage adjustment based upon measured vancomycin concentrations and their clinical condition. The patients in whom follow-up concentrations were ordered around the third dose of the revised regimen allowed the opportunity to evaluate the predictive performance of the Bayesian method in predicting future vancomycin concentrations. These predictions were generated from patient-specific data with feedback concentrations supplied to the optimal two-compartment (2h, Table 8) model with the benefit of Bayesian estimation. As the two-compartment model was observed to be superior to the one-compartment model (Figures 34, 35, and 36) in generating Bayesian predictions of vancomycin concentrations

**Figure 36. Confidence Interval (95%) Constructed Around the Difference Between Two- and One-Compartment Bayesian Predictions.** Mean difference (two-compartment error minus one-compartment error) and confidence interval of predictions of peak and trough vancomycin concentrations are depicted in all cases (A), and cases  $< 36$  (B) and  $\geq 36$  (C) weeks post-conceptual age. The two-compartment model was favored for all cases in which the confidence interval did not include zero, except for the mean error associated with pre-dose 3 predictions of trough concentrations in cases  $\geq 36$  weeks post-conceptual age (C).

(A) Difference in Prediction Error in All Cases (n = 55)



(B) Difference in Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 47)

(C) Difference in Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 8)



within a course of therapy, only the two-compartment model was used in the follow-up analyses. Together, the feedback concentrations obtained during the initial dosage regimen, and appropriate population prior estimates implemented in a NONMEM Bayesian algorithm allowed the computation of case-specific predictions of vancomycin concentrations.

### 3.2.3.1. *Comparison of Bayesian and Sawchuk-Zaske (1976) Methods*

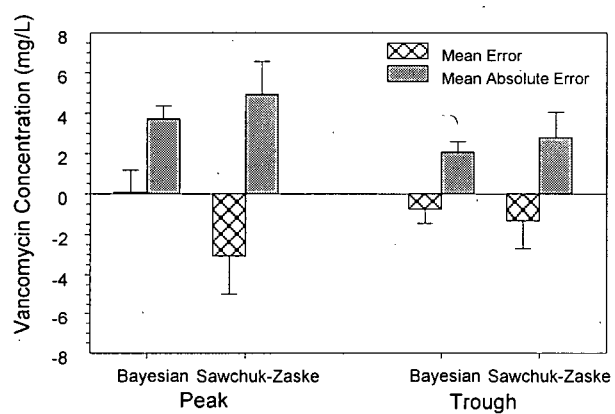
The Bayesian method using both peak and trough feedback concentrations from the initial dosage regimen as feedback was compared to the standard Sawchuk-Zaske (1976) approach that also requires peak and trough concentrations (Section 2.2.6.2). The results for all cases (Figure 37A) indicate that the relative mean error (accuracy) of Bayesian predictions of follow-up peak and trough concentrations were <1% and 11%, respectively; whereas, the mean error of Sawchuk-Zaske (1976) derived predictions represented 9% and 19% of follow-up peak and trough concentrations, respectively. To this end, the Bayesian method demonstrated a notably lower mean error and somewhat reduced mean absolute error than the Sawchuk-Zaske (1976) approach. In cases < 36 weeks post-conceptual age, a similar pattern was observed (Figure 37B). Whereby, the mean error associated with the Bayesian predictions represented 3% and 21% of follow-up peak and trough concentrations, and the relative mean error of Sawchuk-Zaske (1976) predictions of follow-up peak and trough concentrations were 15% and 34%, respectively. Although both the Bayesian and Sawchuk-Zaske (1976) predictions were similar in cases  $\geq 36$  weeks post-conceptual age (Figure 37C), identification of trends in the data is complicated by the small sample size ( $n = 5$ ).

To illustrate the range of differences in prediction error between Bayesian and Sawchuk-Zaske (1976) methods, in this small patient sample, the 95% confidence intervals were constructed around these differences and are depicted in Figure 38. The trend for all cases (Figure 38A) and the pattern presented in cases < 36 weeks post-conceptual age (Figure 38B) demonstrated superior accuracy of the Bayesian predictions of follow-up peak concentrations, as the confidence interval failed to include zero. Since the number of cases  $\geq 36$  weeks post-conceptual age was small this limited the ability to adequately assess the predictive performance of both methods (Figure 38C).

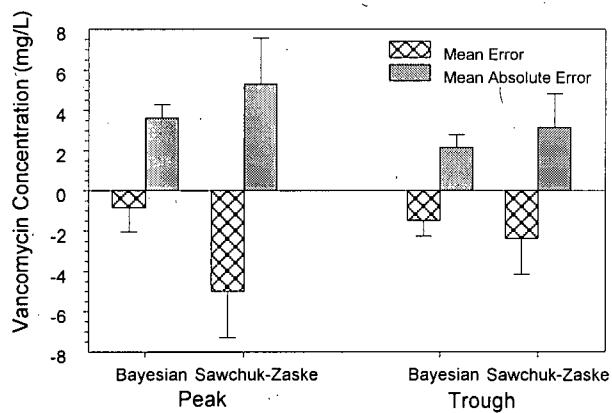
**Figure 37. Error Associated with Predictions of Vancomycin Follow-Up Peak and Trough**

**Concentrations.** ME ( $\pm$  se) and MAE ( $\pm$  se) of predictions of follow-up vancomycin concentrations based upon the Bayesian and Sawchuk-Zaske (1976) methods were evaluated in all cases (A), mean ( $\pm$  sd) follow-up peak and trough concentrations were 34 ( $\pm$  6) mg/L and 7 ( $\pm$  3) mg/L, respectively. For cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) follow-up peak and trough concentrations were 34 ( $\pm$  6) mg/L and 7 ( $\pm$  3) mg/L, respectively. In cases  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) follow-up peak and trough concentrations were 31 ( $\pm$  6) mg/L and 8 ( $\pm$  4) mg/L, respectively. Bayesian (two-compartment model, 2h) and Sawchuk-Zaske (1976) predictions were based upon the routine peak and trough concentrations obtained from the previous dosage regimen.

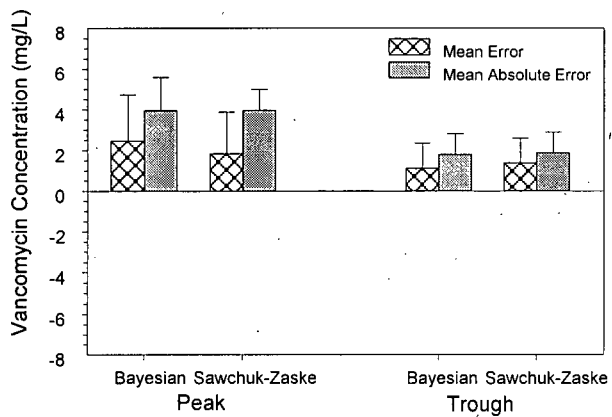
(A) Prediction Error in All Cases (n = 16)



(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 11)

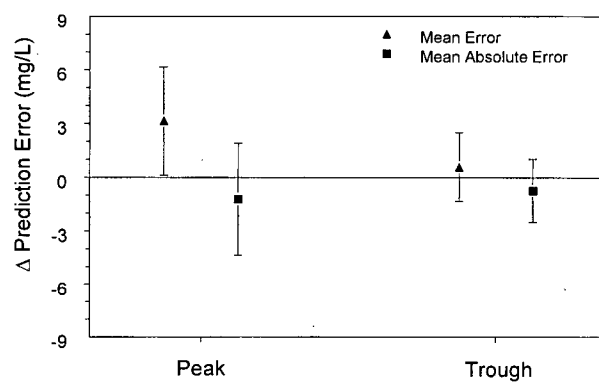


(C) Prediction Error in Cases ≥ 36 weeks Post-Conceptional Age (n = 5)

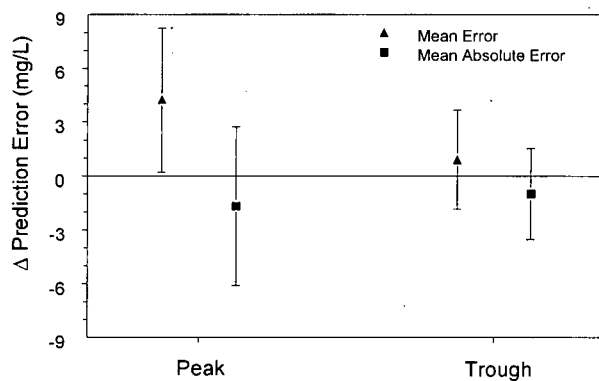
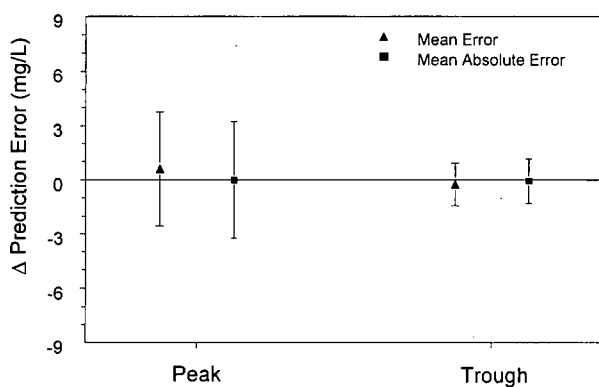


**Figure 38. Confidence Interval (95%) Constructed Around the Difference Between a Bayesian and Sawchuk-Zaske (1976) Method.** Mean difference (Bayesian error minus Sawchuk-Zaske error) and confidence interval of predictions of follow-up peak and trough vancomycin concentrations are depicted in all cases (A), and cases  $< 36$  (B) and  $\geq 36$  (C) weeks post-conceptional age. Bayesian (two-compartment model, 2h) and Sawchuk-Zaske predictions were based upon the routine peak and trough concentrations obtained from the previous dosage regimen. The Bayesian method was favored for both cases in which the confidence interval did not include zero.

(A) Difference in Prediction Error in All Cases (n = 16)



(B) Difference in Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 11)

(C) Difference in Prediction Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 5)

### 3.2.3.2. *Comparison of Single- and Two-Sample Bayesian Feedback*

To explore the potential of single- and two-concentration feedback using Bayesian forecasting for predicting future peak and trough concentrations, individual and combined (peak and trough) concentrations from the initial dosage regimen were used to predict follow-up concentrations after a dosage adjustment. The results for all cases (Figure 39A) indicated that the mean error using pre-third dose, trough only, and post-third dose single sample feedback represented <3% of peak concentrations. Similarly, the relative mean error associated with two-sample (peak and trough) predictions of follow-up peak concentrations was <1%. The relative mean absolute error associated with peak concentration predictions using trough only feedback was 12% and similar to that using two-sample (peak and trough) feedback.

In cases < 36 weeks post-conceptual age, the pattern of follow-up peak prediction error is illustrated in Figure 39B. The relative mean error associated with follow-up peak concentration predictions based upon pre-third dose, trough only, and post-third dose single sample feedback were 6%, <1%, and 6%, respectively. The relative mean error of two-sample (peak and trough) predictions represented 3% of measured peak concentrations. A tendency to overestimate follow-up peak predictions was observed, though the relative mean error remained small. Further, in this group, the trough concentration alone demonstrated a lower mean error than the combined (peak and trough) feedback.

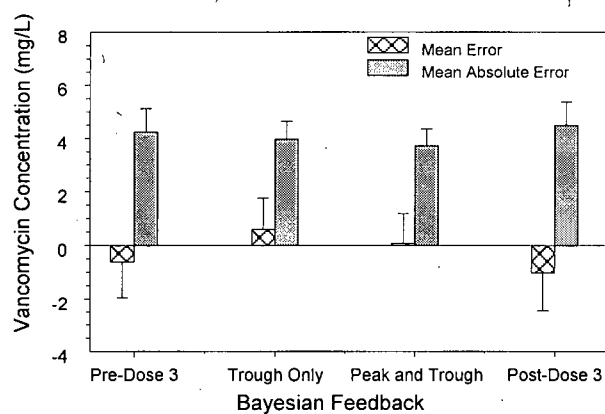
In cases  $\geq 36$  weeks post-conceptual age, the mean error of single- and two-sample predictions of follow-up peak concentrations was similar, although that reported from the post-third dose feedback was notably lower (Figure 39C). The results indicated that the relative mean error associated with follow-up peak concentration predictions based upon pre-third dose, trough only, peak and trough, and post-third dose feedback was 11%, 8%, 8%, and 4%, respectively. All feedback samples were similar with respect to mean absolute error of follow-up peak predictions.

Figure 40 illustrates the error associated with Bayesian predictions of follow-up trough concentrations. In agreement with the foregoing results, feedback using trough only and combined (peak and trough) concentrations was associated with lower mean error (Figure 40A). The results for all cases (Figure 40A) indicated that the relative mean error of follow-up trough concentration predictions using pre-third dose, trough only, and post-third dose single sample

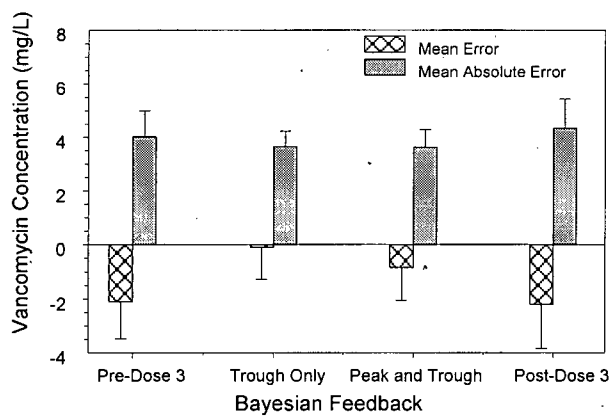
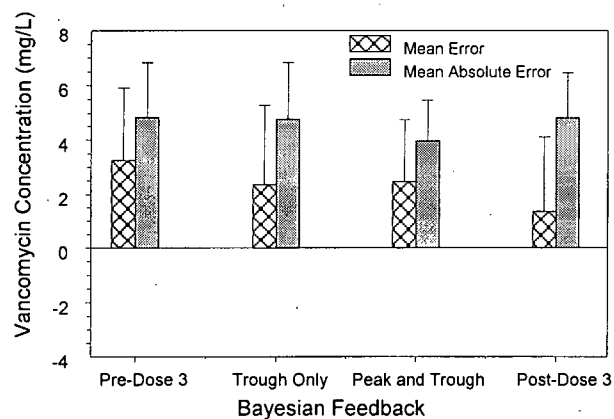
**Figure 39. Error Associated with Predictions of Vancomycin Follow-Up Peak Concentrations.**

ME ( $\pm$  se) and MAE ( $\pm$  se) of predictions of follow-up peak vancomycin concentrations based upon a two-compartment (model 2h) Bayesian method with indicated feedback from the previous dosage regimen were evaluated in all cases (A), mean ( $\pm$  sd) peak concentration was 34 ( $\pm$  6) mg/L; cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) peak concentration was 34 ( $\pm$  6) mg/L; and cases  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) peak concentration was 31 ( $\pm$  6) mg/L.

(A) Prediction Error in All Cases (n = 16)



(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 11)

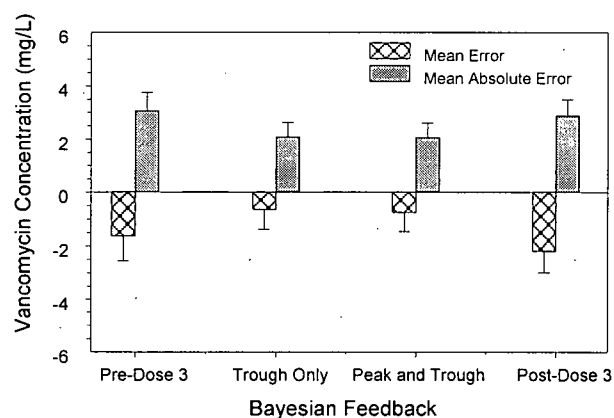
(C) Prediction Error in Cases  $\geq 36$  weeks Post-Conceptional Age (n = 5)



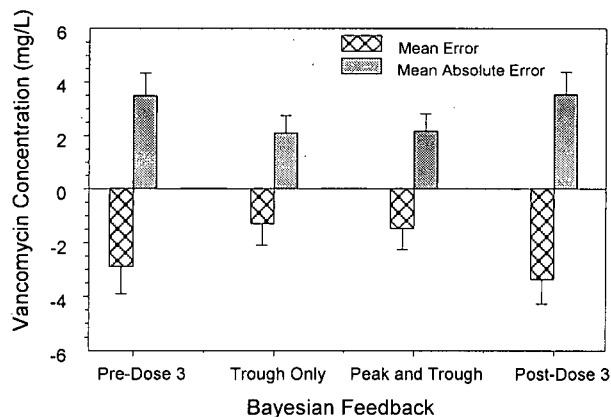
**Figure 40. Error Associated with Predictions of Vancomycin Follow-Up Trough Concentrations.**

ME ( $\pm$  se) and MAE ( $\pm$  se) of predictions of follow-up trough vancomycin concentrations based upon a two-compartment (2h) Bayesian method with indicated feedback from the previous dosage regimen were evaluated in all cases (A), mean ( $\pm$  sd) trough concentration was 7 ( $\pm$  3) mg/L; cases < 36 weeks post-conceptual age (B), mean ( $\pm$  sd) trough concentration was 7 ( $\pm$  3) mg/L; and  $\geq$  36 (C) weeks post-conceptual age, mean ( $\pm$  sd) trough concentration was 8 ( $\pm$  4) mg/L.

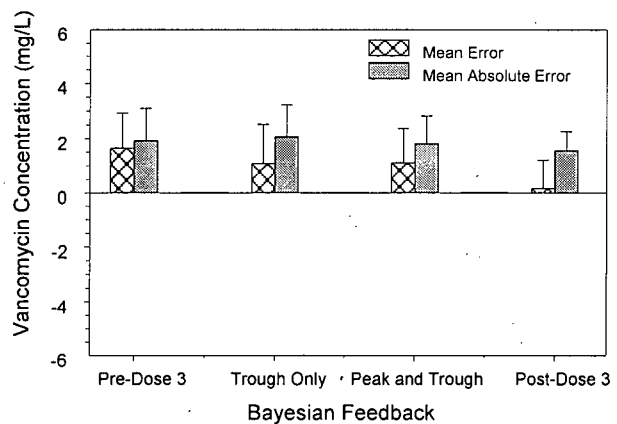
(A) Prediction Error in All Cases (n = 16)



(B) Prediction Error in Cases &lt; 36 weeks Post-Conceptional Age (n = 11)



(C) Prediction Error in Cases ≥ 36 weeks Post-Conceptional Age (n = 5)



feedback was 23%, 9%, and 31%, respectively. The mean error using two-sample (peak and trough) feedback represented 11% of the measured follow-up trough concentration. Again, a tendency to overestimate trough concentrations was observed, though trough concentration alone and combined feedback demonstrated similar accuracy.

In cases  $< 36$  weeks (Figure 40B) and  $\geq 36$  weeks (Figure 40C) post-conceptional age, the pattern is consistent, except the single, post-third dose feedback concentration exhibited a lower mean error in the  $\geq 36$  weeks post-conceptional age group. All approaches provided similar estimates of mean absolute error. Collectively, the data suggest that single samples supplied to a Bayesian method have the potential to adequately predict follow-up peak concentrations. Further, the results indicate that single, trough samples applied in a Bayesian algorithm may provide clinically acceptable predictions of both follow-up peak and trough concentrations.

## **DISCUSSION**

### **4.1 POPULATION PHARMACOKINETIC MODELING**

#### **4.1.1. Review of Demographic Characteristics**

In the general Canadian population, an IMR of 5.5 per 1000 live births is expected (Joseph, 2000). Based upon the SCN total admission demographics during the present study period, an IMR of 72.4 per 1000 live births was reported. This 13-fold increase can largely be explained by the observation that approximately 44% of all deaths, 32.4 per 1000 live births, were related to admissions < 28 weeks gestation; furthermore, 80% of all deaths were associated with preterm births (data not shown). As indicated previously, approximately 75% to 85% of all neonatal deaths of normally formed infants are related to preterm delivery in the United States (Chescheir and Hansen, 1999).

During the present study period, survival of births among all SCN admissions was 50% at 22 weeks GA, 25% at 23 weeks GA, 66% at 24 weeks, 74% at 25 weeks GA, and > 85% at 26 and 27 weeks (data not shown). While there were limited data for neonates < 23 weeks gestational age (data not shown), the remaining survival rates were consistent with those previously reported for a NICU population (Lorenz, 2000). Overall, 72% of all admissions to the SCN were related to preterm delivery: over 29% were born at < 31 weeks gestation, 38% were born between 31 and 37 weeks gestation, and 33% were  $\geq$  37 weeks of gestation (data not shown). Together, these data demonstrate that the general population from which the study cohort was derived was representative of a population typically receiving treatment in a NICU.

The 625 neonates (25% of admissions) prescribed vancomycin during the conduct of this investigation exhibited essentially similar characteristics. Any differences between the general SCN population and those prescribed vancomycin were explained by the prevalence of complications of the newborn requiring primary or secondary vancomycin therapy, including RDS, PDA, CLD, and infectious diseases that are more prevalent amongst the most premature neonates (Kliegman, 1998). In this regard, over 90% of neonates prescribed vancomycin had experienced preterm delivery, 68% were born at < 31 weeks and 21% were born between 31 and 37 weeks of gestation (data not shown). In this sample, males were treated more frequently than females. A male gender-linked factor related to thymus function or immunoglobulin synthesis has

been postulated to explain the preponderance of males among those neonates with neonatal sepsis (Polin and St. Geme, 1992). Expectedly, the length of hospitalization was 2.5-fold higher for patients prescribed vancomycin than the SCN general admission population.

The population-based analysis from 185 patients and 628 serum vancomycin concentrations that was implemented in the present investigation can be compared to that of Seay *et al* (1994), who evaluated data from 192 patients with 520 serum concentrations. The population approach was also implemented by Grimsley and Thompson (1999) and de Hoog *et al* (2000), although much smaller groups of 115 and 59 patients, respectively, were enrolled. The present investigation included the largest validation group of 65 patients, with 332 serum vancomycin concentrations, reported to date. The sample sizes of the validation groups ranged from 22 – 30 patients in the other population-based analyses (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000). As vancomycin population pharmacokinetics were also characterized from the combined, model development and validation, cohort, the present investigation is the largest population-based analysis of vancomycin in neonates.

The central tendency measurements of GA, PNA and weight at the start of vancomycin therapy for this investigation compared favorably with the previous population studies (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000). Although the covariate data collected for each patient in the present study were essentially similar to those obtained by Seay *et al* (1994); notably, data for the incidence of RDS, CLD, infection, indomethacin and dopamine therapy, preterm birth, and Apgar score distribution were either not collected or reported by the investigators. In this regard, direct comparisons of clinical presentation and management were not possible. However, it may be postulated that, given the general adoption of surfactant therapy for the treatment of RDS in the last decade, the present investigation is more representative of the immaturity and co-morbidities currently managed in the NICU. Further, the incidences of RDS, CLD, and indomethacin pharmacotherapy for the treatment of PDA for the model development (Table 6) and validation cohorts (Table 13) were in agreement with the prevalence of these diagnoses in the general NICU population (Kliegman, 1998).

The distribution of the five-minute Apgar scores, predominance of the male gender and incidence of confirmed infection reported by Grimsley and Thompson (1999) were in agreement with those obtained in the present study (Sections 3.1.1, 3.1.4, and 3.1.6); however, these investigators did not report data for RDS, CLD or concurrent indomethacin pharmacotherapy.

Only PCA, GA and weight at the start of vancomycin therapy were tested as potential covariates by de Hoog *et al* (2000); accordingly comparisons between patient samples are not possible. Given the paucity of data available from the preceding population-based analyses (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000), differences in the derived models from the present investigation may be attributed to inherent differences in the patient populations and methodology in model building.

#### **4.1.2. Model Development**

In the present investigation, when the unadjusted base models were compared, the two-compartment model appeared superior; however, both one- and two-compartment pharmacokinetic models were systematically developed and evaluated. At all stages, the one-compartment model appeared inferior to the two-compartment model. In contrast, Seay *et al* (1994) and Grimsley and Thompson (1999) only conducted model building with their best, two-compartment, model. Additionally, those covariate factors determined to be significant were assumed by these authors to apply to the one-compartment model and final comparisons were made. As covariates assigned to volumes of distribution are often dependent on the assumed compartmental model, this latter strategy may lead to spurious results. The systematic, iterative process of model building was not implemented by de Hoog *et al* (2000); rather, only a one-compartment structural model was developed though no rationale was provided. Further, as both one- and two-compartment models had been used to describe vancomycin pharmacokinetic parameters in neonates (Schiabbe *et al*, 1986; James *et al*, 1987; Reed *et al*, 1987; Leonard *et al*, 1989; Asbury *et al*, 1993; McDougal *et al*, 1995; Seay *et al*, 1994; Grimsley and Thomson, 1999; de Hoog *et al*, 2000), a definitive compartmental model had not been established and accordingly, both structural models should have been examined.

##### **4.1.2.1. Two-Compartment Model**

Age (GA, PNA, and PCA) and body weight are related to maturational changes in neonates, and many studies have identified these factors as linear influences on vancomycin pharmacokinetics (Schiabbe *et al*, 1986; Reed *et al*, 1987; Asbury *et al*, 1993; McDougal *et al*, 1995; Seay *et al*, 1994; Grimsley and Thomson, 1999; de Hoog *et al*, 2000). Creatinine clearance has been identified as an influence on vancomycin clearance in a limited number of

studies (James *et al*, 1987; Grimsley and Thomson, 1999). Only, Seay *et al* (1994) identified dopamine exposure to be a significant covariate associated with a reduced vancomycin clearance in their final model.

In the present investigation, weight and PCA yielded a significant reduction in the MOF when included as single covariates on vancomycin clearance (Figures 9 and 11; Table 7), and this is consistent with the findings of several studies (Schiabbe *et al*, 1986; Reed *et al*, 1987). When added to the unadjusted, base model, a mathematical power function best described the association between clearance and weight. Comparable power functions (0.78 – 1.36) have been used to describe the effect of weight on clearance in population analyses of gentamicin in neonates (Jensen *et al*, 1992; Weber *et al*, 1993). The value (0.78) of Weber *et al* (1993) was essentially similar to the point estimate (0.839) obtained in the present study; hence, a two-fold increase in patient weight resulted in a 79% increase in clearance (Table 8). Both Seay *et al* (1994) and Grimsley and Thompson (1999) utilized linear weight models in their respective analyses. Grimsley and Thompson (1999) reported that the relationship between clearance and weight appeared linear; however, upon inspection of their scatterplots, the scarcity of data beyond 2.50 kg makes interpretation difficult. Based on the limited data presented by de Hoog *et al* (2000), it is assumed, though not explicitly stated, that the authors utilized a linear weight model as well. In general, Seay *et al* (1994), Grimsley and Thompson (1999), and de Hoog *et al* (2000) did not indicate the various mathematical functions tested to elucidate the relationships between pharmacokinetic parameters and continuous variables.

In the present investigation, the potential relationships between GA, PNA, and PCA and vancomycin clearance were examined. PCA, relative to term (PCA/40) gestation, was optimally modeled as a power function and produced the greatest reduction in the MOF when included in the clearance term (Table 7). Similar clearance models have described one-compartment gentamicin disposition in neonates (Weber *et al*, 1993) and two-compartment netilmicin pharmacokinetics (Fattinger *et al*, 1994). The application of PCA as a function of term birth is supported by physiological evidence suggesting that nephrogenesis continues until 36 weeks of gestation (Kearns, 2000) and the GFR for full term neonates ranges from 2 – 4 mL/min, in contrast to 1 mL/min for preterm births (Besunder *et al*, 1988). Importantly, the GFR increase after birth appears to be dependent upon PCA and not PNA (Besunder *et al*, 1988). Conversely, de Hoog *et al* (1999) reported that their individual estimates of clearance did not correlate with

either GA or PCA. Inspection of their PCA distribution data suggests that representation across a full range of PCA groups would have been sufficient to discern an effect of age. However, details of the population analysis methods used were not provided by the authors; hence, their failure to identify a maturational effect on vancomycin clearance cannot be interpreted. Seay *et al.* (1994) incorporated GA into their final model as a dichotomous variable, in response to a reported bimodal distribution in the data with a break at 32 weeks. This technique permitted the assessment of a maturational effect relating time from conception to birth, but failed to partition differences in clearance due to maturation after birth (PNA). Grimsley and Thompson (1999) reported that the addition of PCA to a clearance term containing weight and serum creatinine offered no advantage. In their model, clearance was a function of  $1/\text{serum creatinine}$ ; therefore, patients with impaired renal function demonstrated a lower vancomycin clearance. In these patients serum creatinine may be a marker for a maturational effect, normally modeled by PCA, as extremely premature neonates can exhibit higher serum creatinine concentrations that may still be considered within the normal range (Kim and Emma, 1998). Moreover, the persistence of maternal creatinine in the newborn may influence measurements in the newborn (Grimsley and Thompson, 1999). Based upon visual inspection of the data, if the model had been developed with PCA prior to inclusion of creatinine, the resultant model may have included PCA without creatinine.

In the present investigation, univariate analysis was used to reduce the initial list of patient factors that might have individually affected vancomycin pharmacokinetics. Thorough, systematic covariate screening was not implemented by de Hoog *et al.* (1999); therefore, the strength of population-based analysis was not exploited, which underscores the limitations of their report. In the present study, the incidence of dopamine pharmacotherapy within 72 hours of serum vancomycin concentration was 9.5% in the model development cohort (Table 6), thereby permitting the identification of this factor for inclusion in NONMEM analyses. In the affected cases, dopamine exposure produced a significant reduction in the MOF, and was associated with a 34% reduction in vancomycin clearance (Figure 15, Table 8). Similarly, Seay *et al.* (1994) incorporated dopamine exposure into their final model, and they observed a reduction of 54% in vancomycin clearance, regardless of GA group. Dopamine is commonly used for its pressor effect, although it also exhibits  $\alpha$ - and  $\beta$ -adrenergic activity to increase cardiac output (Kliegman, 1998; Polin and Spitzer, 1998). Despite the fact that the mean dopamine dose of



7.5 mg/kg/min administered to patients in the present investigation was consistent with the dosage guidelines to increase urinary output, dopamine exposure was associated with a decrease in vancomycin clearance. Dopamine may have been prescribed for the treatment of systemic hypotension and thus, dopamine exposure may represent a marker for cardiovascular dysfunction or hemodynamic instability, resulting in decreased drug elimination.

Patient weight significantly influenced the central volume of distribution when modeled as a linear function, in the present study (Figure 10, Table 7). Similarly, the other population-based analyses of vancomycin included a linear weight function on the central of volume of distribution terms for two-compartment models (Seay *et al*, 1994; Grimsley and Thompson, 1999) and volume of distribution for the one-compartment model (de Hoog *et al*, 2000). Consistent with the findings of Grimsley and Thompson (1999), the addition of PCA to the central volume term of the present investigation offered no advantage.

The inclusion of dopamine exposure on the peripheral volume of distribution parameter produced a significant reduction in the MOF during model building (Figure 12, Table 7), but dopamine was removed during backwards elimination from the revised, full model (Figure 15, Table 7). The weight-normalized peripheral volume appeared to demonstrate greater variability, with larger values in patients < 36 weeks PCA (Figure 15); however, the inclusion of weight in the model did not result in a significant change in the MOF or predicted concentrations and residuals. In contrast, Seay *et al* (1994) incorporated patient weight in their final two-compartment model. The authors did not indicate the continuous and dichotomous variables assessed in the peripheral volume term. Moreover, clinical covariates of RDS and CLD were not collected or evaluated. In the present investigation, the covariate CLD, which reflects a diagnosis of BPD and/or apnea of prematurity, was included in the peripheral volume term and produced a significant reduction in the MOF (Figure 12, Table 7). In the presence of CLD (62% of cases), a 276% increase in the peripheral volume was observed (Table 8). The diagnosis of CLD may be a marker for the dynamic changes in body composition that occur between preterm and term neonates (Friis-Hansen, 1971). Total body water, as a percentage of total body weight, has been estimated to be 85% and 78% in preterm and term neonates, respectively (Friis-Hansen, 1971). Also, the extracellular fluid volume approximates 65% of body weight in preterm neonates and 50% in term neonates. In CLD, the interstitium may be altered by fibrosis and cellular hyperplasia; interstitial fluid clearance is disrupted, resulting in pulmonary edema and

fluid retention (Parad and Berger, 1998). The large and highly variable peripheral volume of distribution observed in this study may be a reflection of these maturational changes, alone, or combined with the ongoing clinical presentation and management.

Nine cases (3.6%) from the population modeling dataset were identified as outliers (Figure 13) and removed, as their clinical presentation was not consistent with the remaining patient sample. These cases included: death within 24 hours of serum vancomycin concentration measurement, renal failure with serum creatinine  $> 150 \mu\text{mol/L}$  and blood urea nitrogen  $> 10 \text{ mmol/L}$ , congestive heart failure with or without congenital heart defects, and hydrops fetalis. The highly variable renal function and fluid balance were atypical and compromised the analyses. Similarly, a patient demonstrating a high serum creatinine concentration secondary to hypoxia was excluded from the analyses by Grimsley and Thompson (1999). A patient population reflecting a larger sample of these underrepresented patients might permit the development of an appropriate comprehensive model describing the inherent differences in vancomycin pharmacokinetic behavior.

In the present investigation, interpatient variability in clearance was 27% (Table 8), essentially similar to that reported by Grimsley and Thompson (1999) and lower than the 31% and 36% reported by de Hoog *et al* (2000) and Seay *et al* (1994), respectively. Interpatient variability in central volume (11%) and peripheral volume (11%) (Table 8) were notably less than those observed in the other population-based analyses, which ranged from 18 – 54% (Seay *et al*, 1994; Grimsley and Thompson, 1999; de Hoog *et al*, 2000). The standard deviation of residual variability was 0.5 mg/L in the present study, this compares to 3.8 mg/L and 4.5 mg/L observed by Seay *et al* (1994) and Grimsley and Thompson (1999), respectively.

Seay *et al* (1994) were the first to report elimination half-lives longer than those previously observed for vancomycin in neonates. In the present investigation, elimination half-lives approximated those of Seay *et al* (1994); however, continuous distribution of PCA was evident (Figure 4), rather than a bimodal distribution of  $< \text{or } \geq 32$  weeks GA. The prolonged elimination half-life of 32.8 hours for those  $< 27$  PCA, decreased slightly over the range of 27 – 36 weeks PCA, and this was followed by a dramatic reduction in neonates  $\geq 37$  weeks PCA to 10.0 hours (Table 9). This supports the conclusion that extremely premature neonates, particularly those with concomitant CLD and dopamine pharmacotherapy, may not achieve steady-state vancomycin concentrations using the standard sampling strategy for therapeutic drug

monitoring, as frequently assumed. Consequently, for the youngest of patients, therapeutic drug monitoring would be required at day 7 or following the fifth dose (given q36 h) of therapy. In the present study, the distribution half-life remained longer ( $> 4$  hours) across all PCA groups (Table 9) than those reported by Seay *et al* (1994). Further, distribution equilibrium would not be achieved until approximately 27 hours have elapsed. Terminal half-lives estimated with the use of the standard two-stage approach based upon the assumption of a one-compartment model using standard peak and trough sampling around the third dose of therapy are likely erroneously short.

The weight-normalized central volume remained constant across all age groups; whereas, peripheral volume decreased appreciably with increasing PCA (Table 9). Surprisingly, the peripheral volume of distribution represented 50% of the volume of distribution at steady-state in the youngest patients, but only 9% in the oldest patients. This suggested that a one-compartment model may be a close approximation of vancomycin pharmacokinetics in neonates  $\geq 37$  weeks PCA. This leads to the expectation that the two-compartment model reflects a better description of vancomycin pharmacokinetics in premature neonates whereas, a one-compartment model may be a close approximation of vancomycin disposition in neonates  $\geq 37$  weeks PCA.

Vancomycin also appears to be best described by a two-compartment model in children (Schaad *et al*, 1980; Lamarre *et al*, 2000; Wrishko *et al*, 2000). Therefore, based on the current observations, it may be postulated that a transition from a two-compartment to a one-compartment and back to a two-compartment model may best explain the pharmacokinetics of vancomycin in neonates, infants, and children, respectively. The present study, together with other population-based analyses (Seay *et al*, 1994; Grimsley and Thompson, 1999), demonstrated that the two-compartment model is superior to the one-compartment model; however, similar findings of the appropriateness of the one-compartment model for neonates  $\geq 37$  weeks have not been reported. In order to support these observations, further data are required from term births through the first year of life.

#### 4.1.2.2. One-Compartment Model

As with the two-compartment model building, an iterative process was implemented to generate a one-compartment model of vancomycin disposition in the present study. Again, patient weight was best modeled as a power function to describe the association between

clearance and weight (Table 10). The point estimate associated with patient weight in the clearance term resulted in a 73% increase in clearance with a doubling of patient weight (Table 11). Patient weight was modeled as a linear function on volume of distribution, as in the two-compartment model (Table 10). Based on the limited data presented by de Hoog *et al* (2000), it is assumed that the authors utilized linear weight models on clearance and volume of distribution for their one-compartment model. Consistent with the previous description of two-compartment model building results, in the present study PCA relative to term gestation was optimally modeled as power function in the clearance term (Table 10). In contrast to the two-compartment model, dopamine exposure did not produce a significant reduction in the MOF when included in the clearance term. However, CLD (62% of cases) produced significant reductions in MOF both in both clearance and volume of distribution terms, when modeled as power functions (Table 10). In this regard, CLD was associated with a 27% increase in clearance and a 9% reduction in volume of distribution (Table 11). Accordingly, the assumptions of Seay *et al* (1994) and Grimsley and Thompson (1999) leading to the incorporation of covariates developed in an initial compartmental model into another structural model without appropriate screening are not supported.

From the one-compartment model in the present study, the coefficients of variation with respect to interindividual variability in clearance and volume of distribution were 26%, and 9%, respectively (Table 11). These values were lower than those reported by de Hoog *et al* (2000), who reported that interindividual variability for clearance and volume of distribution were 31% and 25%, respectively. The standard deviation of residual variability was 1.0 mg/L, in this study.

In the present study, the half-lives were notably longer for patients < 27 weeks PCA through 30 weeks PCA; however, they were considerably less than the elimination half-life estimates generated in the refined two-compartment model (Table 12), which likely reflects the long distribution half-life. Beyond 31 weeks PCA, the half-life of vancomycin is consistent with that reported by de Hoog *et al* (2000), based upon a one-compartment population-based analysis, and that reported from two-stage analyses (Rodvold *et al*, 1997). Based upon the present data and those reported by Seay *et al* (1994), the elimination half-life of vancomycin is much longer than the one-compartment results suggest; therefore, patients are not at steady-state at the time of routine therapeutic monitoring. Based upon the limited analyses presented by de Hoog *et al* (2000), the authors proposed a standard dose of 10 mg/kg every eight hours regardless of age or

renal function. Given the two-compartment elimination half-lives reported in the present investigation (Table 9), the vancomycin dosing regimen proposed by de Hoog *et al* (2000) would result in spurious measurements of peak and trough concentrations based upon standard therapeutic drug monitoring sampling around the third dose (24 hours). In this regard, steady-state concentrations would not be achieved until the 21<sup>st</sup> dose for neonates <27 weeks PCA, 20 doses for those 27 – 30 weeks, 16 doses for those 31 – 36 weeks, and 6 doses for those  $\geq 37$  weeks PCA. In the present study, the weight-normalized volume of distribution remained constant across all PCA groups, but were lower in the youngest patients than in the two-compartment model.

To test the appropriateness of the two-compartment model, validation analyses were completed in a naïve cohort of patients. The predictive performance of peak, trough, and intradose interval predictions based upon one- and two-compartment models were assessed (Section 4.1.2.4).

#### 4.1.2.3. *Combined Two-Compartment Model*

As previously described, an iterative process was developed to generate unadjusted, full and final one- and two-compartment models for the combined dataset of 1028 observations from 250 patients and 357 courses of vancomycin therapy. At all stages, the one-compartment model appeared inferior to the two-compartment model. The final two-compartment model compiled from the complete dataset was identical to that reported in model development, with one exception. Dopamine continued to be an important factor in the clearance term; however, indomethacin was also included as a covariate (Table 15). Exposure to indomethacin (mean dose = 0.1 mg/kg/day) was associated with a 16% lower clearance (Table 16). This observed association may be attributed to the larger sample size, as 10% of the combined group had received indomethacin therapy within 72 hours of serum vancomycin concentration determination whereas, only 8% of those patients enrolled in the original model building component had received indomethacin. Previously, anecdotal evidence suggested that indomethacin may alter vancomycin clearance by decreasing urine output (Spivey *et al*, 1986); however, other population-based analyses have failed to identify this covariate. The most probable cause for this omission relates to the increased incidence of PDA and therefore, indomethacin administration due to increased admissions of extremely premature neonates since

1994. Importantly, neither Grimsley and Thompson (1999) nor de Hoog *et al* (2000) collected data related to indomethacin exposure. Like dopamine, indomethacin exposure may directly reduce vancomycin clearance, or may be a surrogate marker for impaired cardiac function that, in turn, decreases vancomycin elimination. The coefficients of variation with respect to interindividual variability in clearance, central volume, and peripheral volume were 25%, 8%, and 75%, respectively (Table 16). The standard deviation of residual variability was 0.7 mg/L. The mean pharmacokinetic estimates were consistent with the trends observed in the original model building (Table 17). Based upon a decreasing peripheral volume relative to volume of distribution at steady-state, a two-compartment model best described vancomycin disposition in premature neonates whereas; a one-compartment model may sufficiently represent the pharmacokinetic behavior in term neonates.

#### 4.1.2.4. *Validation Analyses*

The purpose of external validation is to examine the precision and accuracy of predicted concentrations generated by pharmacokinetic models (Sheiner and Beal, 1981). In the present investigation, the original final one- and two-compartment models were evaluated with data from a separate cohort of 65 patients with 400 observations. Model values ( $\theta$ ,  $\eta$ ,  $\varepsilon$ ) were fixed and the population predictions of vancomycin concentrations were generated without the benefit of feedback concentrations (Section 2.1.9.1).

As all components of the present investigation were observational and prospective, patient characteristics were similar throughout. Consistent with the model development patient sample, males comprised the majority of the cohort and the GA and Apgar score assessment at admission were similar (Sections 3.1.1 and 3.1.4). The indications for vancomycin therapy were consistent with the model building sample. Similarly, the central tendency measures of PCA and PNA at the start of vancomycin therapy from both patient cohorts were in agreement. The percent of patients receiving indomethacin therapy in the validation cohort (10.5%) was greater than that in the model building sample (7.9%). This was likely a consequence of the adoption of indomethacin prophylaxis for the prevention of PDA in extremely premature neonates (Schmidt *et al*, 2001). The present patient sample reflects the largest cohort reported for validation of a population pharmacokinetic model to date.

Seay *et al* (1994) evaluated the predictive performance of their one- and two-compartment models in 30 patients, most (67%) of whom were < 32 weeks GA and comparable to the patient demographics reported in the present investigation (Figure 18). Recall, though, that Seay *et al* (1994) incorporated GA into their models as a dichotomous variable; whereas, PCA relative to term birth was modeled as a power function in the description of vancomycin clearance in the present study. Moreover, the general process of model development for one- and two-compartment models was implemented in the present investigation; however, Seay *et al* (1994) assumed that the covariates applied to the best, two-compartment, model were applicable to the one-compartment model.

In the present investigation, the two-compartment model was superior for describing vancomycin pharmacokinetics during model development. Nevertheless, given the complexity of using two-compartment models for therapeutic drug monitoring in the clinical setting both one- and two-compartment models were evaluated in the validation analyses. Relative performance was characterized by determining the differences between population predictions, without feedback, and measured concentrations. Overall, comparison of one- and two-compartment models suggested that there was little advantage in using the more complex approach for the prediction of peak concentrations. However, the two-compartment predictions represented 2% and 1% of trough and intradose concentrations, respectively, and these were more accurate than those of the one-compartment model (Figure 22). In support of the model development findings suggesting that a two-compartment model best described vancomycin pharmacokinetics in neonates < 36 weeks PCA; the two-compartment model exhibited lower mean error for all concentrations evaluated than the one-compartment in this age group (Figure 22). Additionally, one- and two-compartment predictions of peak and trough concentrations were similar in neonates  $\geq$  36 weeks PCA, but one-compartment predictions of intradose concentrations were more accurate (Figure 22). These findings are consistent with the hypothesis that a one-compartment model may approximate vancomycin pharmacokinetics in neonates  $\geq$  36 weeks PCA.

Clearly, the validation cohort demonstrated the predictive ability of the population models. Similar to the observations of Seay *et al* (1994), the mean prediction error and absolute error were small for both peak and trough concentrations using either the one- or two-compartment model. These data support the potential use of either the one- or two-compartment

model in the clinical setting to establish appropriate dosing guidelines that would result in a majority of peak and trough concentrations in the target range. Importantly, as Seay *et al* (1994) did not examine the effect of CLD on vancomycin disposition and their patient sample likely was not representative of current medical care in the NICU, the relative applicability of their one- and two-compartment models in the current environment would require further investigation.

Rather than implementing a conventional validation analysis to assess the appropriateness of pharmacokinetic models, Grimsley and Thompson (1999) and de Hoog *et al* (2000) developed vancomycin dosing guidelines based upon their respective population-based models, and they prospectively evaluated the number of patients within the target peak and trough concentrations after standard therapeutic drug monitoring. Grimsley and Thompson (1999) advocated dosing guidelines based upon a one-compartment model, dependent upon patient weight and serum creatinine. Based upon the MOF, their two-compartment model provided a better fit to the data; however, the authors elected to use a less complex one-compartment model. These authors assessed their dosing guidelines in 25 patients, for whom demographic characteristics were not reported. They reported that 72% and 86% of the initial trough and peak concentrations, respectively, were within the target ranges. However, based upon the estimated elimination half-lives from the present study and Seay *et al* (1994), the majority of patients were likely not at steady-state. Generally, the superiority of a better specified model is confirmed by validation analyses. The assumption of Grimsley and Thompson (1999) that the less complex, one-compartment model adequately described their patient population would only be supported by implementation of two-compartment derived dosing guidelines with appropriate evaluations.

Like that of Grimsley and Thompson (1999), the primary objective of de Hoog *et al* (2000) was to develop neonatal vancomycin dosing guidelines based upon a population model. The latter authors developed a one-compartment model, without the benefit of detailed covariate screening, from data of 115 neonates. Based upon the estimated volume of distribution (0.43 L/kg), clearance (0.057 L/h/kg) and half-life (6.0 hours) several simulated dose and dose interval combinations were developed. The application of a vancomycin regimen of 10 mg/kg every eight hours was prospectively tested in 22 patients. The demographic characteristics of these patients were essentially similar to their model building cohort and to those presented in the present investigation. The authors reported that 95.5% of second dose trough concentrations were in the desired target range. However, trough concentrations prior to the fifth dose were



considerably higher than those before the second dose. Peak concentrations were measured only after the fifth dose, of which 86.4% were in the target range. Based upon the accumulation index observed following the fifth dose, the prospective data do not support a half-life of 6 hours in this group of patients. Clearly, steady-state concentrations were not achieved by 30 hours; hence, it is likely that some patients would exceed the desired range of trough concentrations and be at risk for toxicity. Consequently, a more comprehensive population-based analysis is warranted to develop more appropriate dosing and therapeutic drug monitoring guidelines. In this regard, the data from the present investigation would permit the development of vancomycin dosing guidelines that would not only be age and weight appropriate, but would also reflect the effects of concomitant medication and medical diagnoses. Dosing guidelines based upon the one- and two-compartment models reported in the present investigation should be systematically generated and evaluated to confirm the best model for appropriate PCA groups.

## **4.2. BAYESIAN FORECASTING**

Bayesian forecasting alters prior estimates of multiple parameters based on one or more measured serum concentrations as feedback (Sheiner *et al*, 1979). Forecasting individual serum concentrations includes: formulating a model for the patient system that links dosage, time, and observable features; initiating the model for the individual patient; and adjusting the model accounting for observed patient responses (Sheiner *et al*, 1979). Parameter means and variances, as well as and intra-individual variance obtained by application of NONMEM, are ideally suited for the development of a Bayesian regression algorithm for optimization of therapy (Ludden 1988). The coupling of NONMEM and Bayesian forecasting, resulting in true model-based, goal-oriented drug therapy, permits achievement of carefully selected targets that are individualized for each patient's perceived need for the drug (Jelliffe *et al*, 1998). The expectation is that the better specified population model would result in superior predictive performance of the individualized (Bayesian) predictions of initial and subsequent serum concentrations following any dose adjustments.

### **4.2.1 One- and Two-Compartment Predictions**

Previously, application of a Bayesian forecasting method using feedback concentrations to modify initial vancomycin population-based parameters estimates had only been reported for

neonates and infants by Rodvold *et al* (1995). These investigators applied a method of Bayesian estimation and linear regression to retrospective data from 29 neonates to develop a one-compartment population model for use in Bayesian forecasting. The model they developed was weight-normalized for volume of distribution and standardized to creatinine clearance on vancomycin clearance. The predictive performance of naïve estimates and Bayesian predictions of vancomycin peak and trough concentrations were then evaluated in a prospective sample of 18 patients. Inspection of mean data, suggested that the model building sample demonstrated similar demographic characteristics in terms of weight, GA, PNA, and gender bias to those in the present study (Section 3.2.1). Initial peak and trough concentrations obtained by standard sampling around the third dose of therapy were both applied as feedback concentrations to the one-compartment model to generate predictions of subsequent peak and trough concentrations (Rodvold *et al*, 1995). Given the method of data collection, those patients enrolled in the Bayesian evaluation were older (PNA) than those used to construct the structural model, due either to longer duration of therapy or multiple courses of vancomycin therapy. Moreover, the patient sample used for Bayesian forecasting was older, in terms of GA and PNA, with increased weights, than those reported in the present investigation. The authors observed that the Bayesian method did not perform better than naïve estimates at forecasting future concentrations ( $> 30$  days), this likely reflects the fact that the population model used was not a thorough population-based model with explanatory covariates. In addition the model did not account for the dynamic changes in the neonatal population.

In the present investigation, the predictive performance of Bayesian forecasting was evaluated from one course of vancomycin therapy in 65 patients. As all components of the present investigation were prospective, patient characteristics were similar throughout (Sections 3.1.1, 3.1.4, and 3.2.1). Pre-third dose, trough only, and post-third dose vancomycin concentrations were sequentially supplied as feedback observations in the revised, final, one- and two-compartment models to obtain case-specific predictions of vancomycin peak concentrations (Section 2.2.5.1). Similarly, pre-third dose, peak only, and post-third dose vancomycin concentrations were sequentially applied as feedback in the revised, final one- and two-compartment models to obtain Bayesian predictions of trough concentrations (Section 2.2.5.1). Predictions based on Bayesian estimates were presented in a NONMEM generated output. Overall, comparison of one- and two-compartment models suggested that the two-compartment

predictions of initial peak concentrations demonstrated better accuracy using pre- and post-third dose feedback (Figure 34). However, both models were similar when trough only feedback was provided (Figure 34). The precision was similar for both models. In support of the model development and conventional validation findings suggesting that a two-compartment model best describes vancomycin pharmacokinetics in neonates < 36 weeks PCA, lower mean error associated with two-compartment peak predictions using pre-third dose, trough only, and post-third dose feedback was observed (Figure 34). In the small subset of patients  $\geq 36$  weeks PCA, the data did not support conclusive use of the less complex, one-compartment model, to generate Bayesian predictions (Figure 34). Consistent with the previously described findings, the trend favoring the two-compartment Bayesian predictions in neonates < 36 weeks PCA was observed with respect to initial trough concentration predictions (Figure 35). To investigate possible superiority, 95% confidence intervals were constructed around the differences in prediction error between the two models. The two-compartment model demonstrated superiority over the one-compartment model in the prediction of initial peak and trough concentrations, regardless of feedback concentrations in neonates < 36 weeks PCA (Figure 36). Likely, the overall benefit of a two-compartment model was restrained by the possibility of improved performance of an one-compartment model in neonates  $\geq 36$  weeks PCA. Rodvold *et al* (1995) only used a one-compartment model, and therefore a direct comparison between their results and those in the present investigation is not possible.

Similar to the results reported by Rodvold *et al* (1995), Bayesian forecasting in the present study demonstrated improved predictive performance compared to population-based parameter estimates, alone. Moreover, Rodvold *et al* (1995) failed to discern any superiority of Bayesian forecasting compared to naïve predictions when the feedback concentrations were obtained after 30 days from the initial set of peak and trough concentrations. Likely, this finding was the consequence of a mis-specified model that did not adequately describe changing vancomycin disposition in a dynamic population. Population modeling (NONMEM) permits the development of a comprehensive, representative model that includes covariates that change in a dynamic patient group (neonates). As validation of a population model can be explored with and without concentration feedback, Bayesian forecasting itself serves as method of validation. In the present study, the accuracy of Bayesian predictions of peak and trough concentrations and the apparent superiority of the two-compartment model particularly, in those neonates < 36

weeks PCA (Figure 36), provide further validation of the derived two-compartment model for describing vancomycin pharmacokinetics in this population.

Importantly, the full benefit of Bayesian forecasting in neonates was not realized in the investigation of Rodvold *et al* (1995). In this regard, the advantage of implementing single concentration sampling strategies with complex models was not implemented. In contrast, the present investigation provided a means to assess the utility of single vancomycin samples in combination with comprehensive population-based models, which has not been reported in neonates to date. The prediction errors based upon single sample feedback appear to be clinically acceptable (Figures 34 and 35) and support the potential use of the two-compartment model in therapeutic drug monitoring using Bayesian forecasting for neonates < 36 weeks PCA, where appropriate. The present study, therefore, provides evidence that the invasiveness (number of samples) of vancomycin serum concentration monitoring may be minimized in neonates when conducted with Bayesian forecasting using parameter estimates derived from an appropriate population-based model.

#### **4.2.2 Follow-Up Bayesian Predictions**

##### *4.2.2.1. Comparison to Standard Individualization of Therapy*

The most common method of therapeutic monitoring of vancomycin has been to measure peak and trough concentrations at what has been presumed to be steady-state, to individualize the dose to achieve target concentrations, based upon a one-compartment model according to the method of Sawchuk and Zaske (1976). In neonates, to minimize the effect of incomplete distribution on the calculation of vancomycin pharmacokinetic parameters, peak serum concentrations have been obtained one-hour following the completion of a one-hour infusion of the third dose (James *et al*, 1987; Lisby-Sutch and Nahata, 1988; Asbury *et al*, 1993; McDougal *et al*, 1995). These guidelines are based upon the relatively short distribution and elimination half-lives reported by Schaad *et al* (1980) suggesting that distribution is complete and steady-state achieved by these sampling times. Trough concentrations are obtained 30 minutes prior to the third dose. However, the population-based two-compartment model developed in the present study provides estimates of the distribution half-life that are, on average, 67-fold longer than those suggested by Schaad *et al* (1980), but are similar to those of Seay *et al* (1994).

In the present study, the predictive performance of the Bayesian method was also evaluated in patients from whom follow-up third dose peak and trough vancomycin concentrations were measured following a dosage adjustment. In order to compare a Bayesian method with the standard monitoring protocol, both peak and trough vancomycin concentrations obtained from the initial dosing regimen were supplied as feedback observations in the revised, final two-compartment model to obtain individual predictions of the follow-up peak and trough concentrations (Section 2.2.5.2). The two-compartment model was selected, as it appeared to best describe vancomycin disposition in neonates through model development and validation analyses. The Bayesian method demonstrated a notably lower mean error and somewhat reduced mean absolute error than the Sawchuk-Zaske approach (Figure 37). In cases < 36 weeks PCA, a similar pattern was observed. The small sample size of neonates  $\geq$  36 weeks PCA did not support discernment of an appreciable difference between methods (Figure 38). Furthermore, the predictive performance of the Sawchuk and Zaske (1976) method was likely enhanced because all dosage adjustments leading to follow-up concentration measurements were made based upon the standard protocol implementing Sawchuk and Zaske (1976) assumptions. A larger sample of older neonates with follow-up concentration measurements is required to clearly establish the magnitude of the difference between Bayesian and Sawchuk and Zaske (1976) methods, as similar comparisons have not been reported in the literature.

#### 4.2.2.2. *Comparison of Single- and Two-Point Sampling*

To explore the potential of single- and two-concentration feedback using Bayesian forecasting for predicting future peak and trough concentrations, individual (pre-third dose, peak, trough, post-third-dose) and combined (peak and trough) vancomycin concentrations obtained from the initial dosing regimen were supplied as feedback observations in the revised, final two-compartment model (Section 2.2.5.2). Collectively, the data suggested that single samples supplied to a Bayesian method have the potential to adequately predict follow-up peak concentrations (Figure 39). Further, the results indicated that single, trough samples applied in a Bayesian algorithm may provide clinically acceptable predictions of both follow-up peak (Figure 39) and trough concentrations (Figure 40). This method of application would support those proponents of monitoring trough concentrations only (Wilhelm and Estes, 1999). According to the pharmacokinetic parameters presented in the model development of this investigation, the

current trough concentrations are not representative of steady-state concentrations, therefore the ability to supply these concentrations to a Bayesian routine is particularly advantageous for adequate therapy. Moreover, a single sampling strategy would alleviate the necessity for aggressive, invasive blood sampling in extremely premature neonates with inherently low blood volume.

## SUMMARY AND CONCLUSIONS

### 5.1. SUMMARY

- The present investigation represents the largest population-based analysis of vancomycin pharmacokinetics in neonates requiring intensive care, reported to date.
- The two-compartment NONMEM model was superior to the one-compartment model, particularly in neonates < 36 weeks PCA. The evidence suggests that a one-compartment model may be adequate to describe of vancomycin disposition in neonates  $\geq 37$  weeks PCA.
- A power function best described the association between clearance and weight; whereby, a doubling of patient weight was associated with in a 79% increase in clearance. Similarly, clearance increased with increasing PCA (relative to term gestation), when modeled as a power function. In contrast, dopamine exposure within 72 hours of vancomycin serum concentration determination was associated with a 34% decline in vancomycin clearance.
- In the final, combined two-compartment model indomethacin exposure within 72 hours of serum vancomycin concentration measurement was associated with a 16% reduction in vancomycin clearance.
- Patient weight was modeled as a linear function on the central volume of distribution; however, this covariate did not affect peripheral volume of distribution. The presence of chronic lung disease was associated with a 276% increase in the peripheral volume, but offered no advantage when added to either central volume or clearance.
- The population mean elimination half-life estimated in the two-compartment model (25.3 hours) was greater than that for the one-compartment model (4.8 hours). This suggests that standard vancomycin serum concentration monitoring around the third dose of therapy would usually not represent steady-state concentrations, as is frequently assumed. Further, sampling of peak concentrations at 60 minutes following a 60-minute infusion of

vancomycin would not reflect post-distributional peak concentrations based upon the estimated average distribution half-life of 4.8 hours.

- Implementation of the derived one- and two-compartment models in a Bayesian method indicated that the better specified, two-compartment model generated more accurate Bayesian predictions of peak and trough concentrations in neonates < 36 weeks PCA.
- While the data were limited, they do suggest that Bayesian forecasting using the derived two-compartment model may be more accurate and precise than the standard method of Sawchuk and Zaske (1976) in predicting follow-up vancomycin concentrations after a dosage adjustment, particularly in neonates < 36 weeks PCA.
- Single, trough samples used as feedback in a Bayesian method with the derived two-compartment model provided relatively accurate and precise estimates of initial and follow-up vancomycin peak concentrations.

## 5.2. CONCLUSIONS

A two-compartment provides a better description of vancomycin pharmacokinetics than does a one-compartment model in neonates requiring intensive care. When combined with an appropriate population-based model, Bayesian forecasting offers greater utility and flexibility than standard therapeutic drug monitoring in this population. In particular, single trough sample, when applied in a Bayesian method, can minimize the invasiveness of concentration monitoring and provide clinically acceptable predictions of current and future vancomycin concentrations in neonates using parameter estimates from the best specified model.

The development of new dosing and therapeutic concentration monitoring guidelines based upon the combined, two-compartment model of vancomycin pharmacokinetics in neonates requiring intensive care can be realized from the present data. Moreover, integration of the derived population model with Bayesian forecasting using only single residual or trough samples as feedback could be implemented in current NICU settings, which would reduce the need for blood procurement for vancomycin concentration monitoring.



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## **APPENDIX 1**

### **Reference Fetal and Postnatal Growth**

Smoothed Percentiles of Birth Weight (g) for Gestational Age: US 1991 Single Births to Resident Mothers

Mean Daily Growth Rates (g/kg/day) in Appropriate for Gestational Age Infants



**Smoothed Percentiles of Birth Weight (g) for Gestational Age: US 1991 Single Births to Resident Mothers.**

Gestational Age (weeks)	Both Genders				
	10 <sup>th</sup> Boys Percentile	10 <sup>th</sup> Girls Percentile	10 <sup>th</sup> Percentile	50 <sup>th</sup> Percentile	90 <sup>th</sup> Percentile
20	270	256	275	412	772
21	328	310	314	433	790
22	388	368	376	496	826
23	446	426	440	582	882
24	504	480	498	674	977
25	570	535	558	779	1138
26	644	592	625	899	1362
27	728	662	702	1035	1635
28	828	760	798	1196	1977
29	956	889	925	1394	2361
30	1117	1047	1085	1637	2710
31	1308	1234	1278	1918	2986
32	1521	1447	1495	2203	3200
33	1751	1675	1725	2458	3370
34	1985	1901	1950	2667	3502
35	2205	2109	2159	2831	3596
36	2407	2300	2354	2974	3668
37	2596	2484	2541	3117	3755
38	2769	2657	2714	3263	3867
39	2908	2796	2852	3400	3980
40	2986	2872	2929	3495	4060
41	3007	2891	2948	3527	4094
42	2998	2884	2935	3522	4098
43	2977	2868	2907	3505	4096
44	2963	2853	2885	3491	4096

Data from Ehrenkranz RA. Growth Outcomes of very low-birth weight infants in the newborn intensive care unit. Clinics in Perinatology 2000; 27: 325-343.

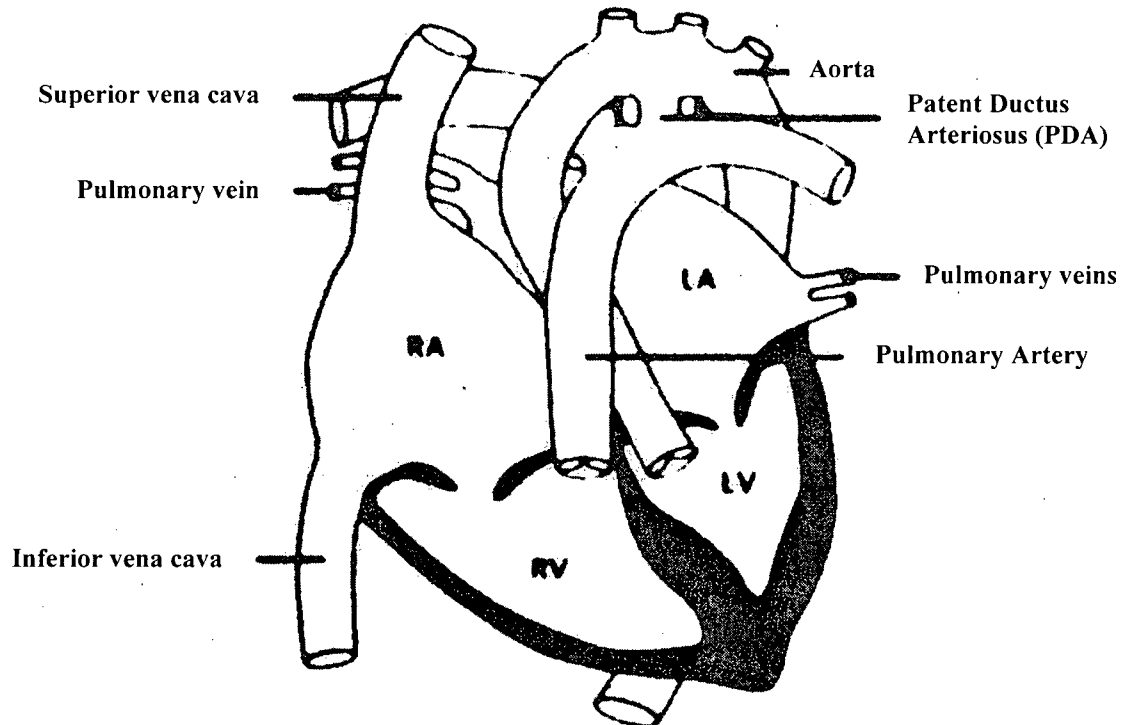
**Mean Daily Growth Rates (g/kg/day) in Appropriate for Gestational Age Infants.**

Postnatal Age (weeks)	Gestation (weeks)				
	< 25	26	27	28	29
1	-18.3	-20.2	-15.8	-14.8	-12.3
2	10.6	28.8	12.8	11.2	10.5
3	12.3	12	11.4	13.6	14.5
4	14.1	15.5	17.1	16.3	14.2
5	14.4	14.2	15.4	15.8	18.1
6	14	14.7	17.2	15.8	16.7
7	17.6	15.7	16.5	16.4	15.7
8	17.5	15.7	18.2	15.8	
9	17.5	15	14	13.5	
10	16	16.7	11.9		
11	16	14.5			
12	14.4	13.9			
13	15				
14	12				

Data from Ehrenkranz RA. Growth Outcomes of very low-birth weight infants in the newborn intensive care unit. Clinics in Perinatology 2000; 27: 325-343.

## APPENDIX 2

Patent Ductus Arteriosus (Adapted from Kirsten, 1996).



**APPENDIX 3****Spectrum of Vancomycin Activity**

**Spectrum of Vancomycin Activity** (Adapted from Milliken, 1988; Wilhelm and Estes, 1999).

ORGANISM	STRAIN	MIC <sub>50</sub> (mg/L)
<i>Staphylococcus aureus</i>	734	≤ 1.0
Methicillin-sensitive	616	0.25 - 1.0
	29	1.6
	20	0.25
Methicillin-resistant	537	0.5 - 2.0
	60	1.0
	14	1.6
<i>Staphylococcus epidermidis</i>	488	≤ 2.0
	73	1.0
	19	3.2
Methicillin-sensitive	554	1.0 - 8.0
Methicillin-resistant	172	1.6 - 6.3
<i>Streptococcus pneumoniae</i>	7	≤ 1.0
	217	0.04 - 1.0
Multiply drug-resistant	6	0.25
Relatively penicillin-resistant	10	0.5
Penicillin-resistant	7	0.4 - 0.8
	4	0.25
	7	≤ 0.5
<i>Streptococcus pyogenes</i>	265	0.03 - 5.0
	18	0.5
	10	0.25
Group B Streptococcus	335	0.06 - 1.6
	18	0.5
	10	0.5
<i>Streptococcus bovis</i>	32	≤ 2.0
	96	0.5 - 0.6
Enterococcus	927	≤ 4.0
	673	1.6 - 8.0
	98	1.6
	97	1.0
<i>Streptococcus viridans</i>	118	0.6 - 1.0
	21	≤ 1.0
Penicillin-resistant	10	0.5
Diphtheroids	137	0.04 - 0.8
<i>Listeria monocytogenes</i>	3	≤ 1.0
	33	2.5 - 5.0
	10	1.0
<i>Clostridium difficile</i>	227	0.8 - 2.0
	5	1.6
	111	≤ 1.0
<i>Clostridium perfringens</i>	49	0.4 - 0.8
	3	3.2
	50	0.3
<i>Neisseria sp.</i>		0.5 - 5.0
<i>Corynebacterium sp.</i> <sup>7</sup>		0.5
<i>Bacillus anthracis</i>		0.5 - 5.0

## APPENDIX 4

### Differential Diagnoses of Neonatal Sepsis and Necrotizing Enterocolitis

#### Clinical Symptoms of Neonatal Sepsis (Adapted from Polin and St. Geme, 1992).

SYSTEM	PRESENTATION
Temperature instability	Hypothermia; hyperthermia
Behavioral changes	Lethargy; irritability; change in tone
Skin	Poor peripheral perfusion; cyanosis; mottling; pallor; petechiae; rashes; sclerema; jaundice
Feeding difficulties	Feeding intolerance; emesis; diarrhea; abdominal distension with or without bowel loops
Cardiopulmonary	Tachypnea; respiratory distress; apnea especially within the first 24 hours or new onset; tachycardia; hypotension
Metabolic	Hypoglycemia; hyperglycemia; metabolic acidosis

#### Clinical Symptoms of Neonatal Necrotizing Enterocolitis (Adapted from Faix and Adams, 1994).

SYSTEMIC	GASTROINTESTINAL	LABORATORY
Temperature instability	Abdominal distension	Acidosis
Apnea	Abdominal tenderness	Neutropenia
Bradycardia	Abdominal wall induration	Neutrophilia
Poor perfusion	Emesis	Thrombocytopenia
Lethargy	Gastric residuals	Coagulopathy
Irritability	Feeding intolerance	Anemia
Hemorrhage	Ascites	Hypoproteinemia
Respiratory distress	Right lower quadrant mass	Electrolyte imbalances
	Diarrhea	
	Occult fecal blood	
	Mucous-containing stool	
	Blue-black abdominal wall	

**APPENDIX 5****Vancomycin Pharmacokinetics in Adults**

# Vancomycin Pharmacokinetics in Adults.

Reference	Patient Type	n	Age (years)	Model	Cl <sub>cr</sub> <sup>a</sup> (mL/min)	Cl <sup>a</sup> (L/h/kg)	t <sub>1/2</sub> α <sup>a</sup> (hours)	t <sub>1/2</sub> β <sup>a</sup> (hours)	t <sub>1/2</sub> γ <sup>a</sup> (hours)	Vd <sub>ss</sub> <sup>a</sup> (L/kg)
Krogstad <i>et al</i> , 1980	Adults	4	36.3	3	120	0.07	0.12	1.02	8.9	0.92
Blouin <i>et al</i> , 1982	Adults	4	27.0	3	138.3	0.07	0.11	0.97	4.79	0.39
Rotschafer <i>et al</i> , 1982	Adults	13	40.2	2	184.1	0.08		0.50	5.6	0.62
	Adults	11	43.9	2	85.2	0.06		0.50	6.1	0.54
Brown <i>et al</i> , 1983	Adults	6	36.0	2	153.7	0.15			2.6	0.48
Cutler <i>et al</i> , 1984	Elderly	6	68.5	3	97.7	0.05	0.13	1.92	12.1	0.76
Garaud <i>et al</i> , 1984	Critical	5		2	82.4	0.05		7.8	12.1	0.58
	Critical	5		2	29.4	0.03		18.3		0.30
Matzke <i>et al</i> , 1984	Adults	7	46.5	1	87.6	0.06			9.1	0.72

(continued)

<sup>a</sup> Values expressed as means.

Abbreviations: Cl<sub>cr</sub>=creatinine clearance; Cl=total vancomycin clearance; t<sub>1/2</sub>α=initial distribution phase half-life; t<sub>1/2</sub>β=second distribution phase half-life; t<sub>1/2</sub>γ=elimination phase half-life; V<sub>c</sub>=volume of the central compartment; Vd<sub>ss</sub>=volume of distribution at steady-state; Burn=thermal injury; IVDA=intravenous drug abuser; VDRF=various degrees of renal function; ESRD=end-stage renal disease.



# Vancomycin Pharmacokinetics in Adults (concluded).

Reference	Patient Type	n	Age (years)	Model	Cl <sub>cr</sub> <sup>a</sup> (mL/min)	Cl <sup>a</sup> (L/h/kg)	t <sub>1/2</sub> α <sup>a</sup> (hours)	t <sub>1/2</sub> β <sup>a</sup> (hours)	t <sub>1/2</sub> γ <sup>a</sup> (hours)	Vd <sub>ss</sub> <sup>a</sup> (L/kg)
Brater <i>et al</i> , 1986	Burn	10	41.0	1	105.0	0.09				0.62
Healy <i>et al</i> , 1987	Adults	11	24.7	3	110.0	0.08	0.23	1.6	8.1	0.92
Comstock <i>et al</i> , 1988	ESRD	10	37.7	3	3.6	0.003	0.15	3.6	156	0.71
Garrelts and Peterie, 1988	Burn Control	9 8	27.8 28.1	1 1	131.0 117.0	0.09 0.07			3.8 5.2	0.51 0.50
Golper <i>et al</i> , 1998	Adults	9	27.5	2	110.0	0.09		0.62	5.7	0.89
Rodvold <i>et al</i> , 1988	VDRF	10 14 13	46.3 49.5 61.6	2 2 2	93.4 51.0 23.9	0.06 0.04 0.02		0.4 0.49 0.51	5.2 10.5 19.9	0.50 0.59 0.64
Rybak <i>et al</i> , 1988	Burn IVDA VDRF	10 14 13	36.4 36.0 38.0	1 1 1	111.0 85.5 68.3	0.11 0.07 0.05				0.59 0.56 0.52
Tan <i>et al</i> , 1990	ESRD	6	59.3	3	3.9	0.005	0.42	2.9	142.6	1.07

<sup>a</sup> Values expressed as means.

Abbreviations: Cl<sub>cr</sub>=creatinine clearance; Cl=total vancomycin clearance; t<sub>1/2</sub>α=initial distribution phase half-life; t<sub>1/2</sub>β=second distribution phase half-life; t<sub>1/2</sub>γ=elimination phase half-life; Vc=volume of the central compartment; Vd<sub>ss</sub>=volume of distribution at steady-state; Burn=thermal injury; IVDA=intravenous drug abuser; VDRF=various degrees of renal function; ESRD=end-stage renal disease.

## **APPENDIX 6**

### **Certificates of Ethical Approval**

Clinical and Behavioral Sciences Research Ethics Boards (UBC) – Certificate of Approval for Continuing Review

British Columbia's Children's Hospital Research Review Committee – Certificate of Approval

Clinical Screening Committee for Research Involving Human Subjects (UBC) – Certificate of Approval

**APPENDIX 7**  
**Nursing Instructions**

**APPENDIX 8****Data Collection Form**



[illegible]

3. Budesonide: Y or N. If Y, enter last dose and date

[illegible]

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Parameter	1. Research level 1a	2. Research level 1b	3. Pre 3rd dose level	4. Post 3rd dose level	5. Research level 2	6. Pre 6th dose level	7. Post 6th dose level
start time last dose							
stop time last dose							
admin. date							
admin. time							
level date							
level time							
RL: HR since LD							
level (mg/L)							

Pharmaco-kinetic Data	PK 3rd dose	PK 6th dose
KE		
Weight (kg)		
half-life (h)		
Vd (ABS) L		
Vd (Adj) L/kg		
Cl (ABS) L/h		
Cl (Adj) (L/kg/h)		

## APPENDIX 9

## Variable Definitions for the NONMEM Pharmacokinetic Dataset Listed Alphabetically

Variable Name	Variable Description
AMT	reserved PREDPP data label for amount of vancomycin given (mg)
CLD	chronic lung disease
CP	measured serum vancomycin concentration
DAT1	record date (day month year)
DOP	dopamine pharmacotherapy within 72 hours of vancomycin concentration determination
DOSE	amount of vancomycin given (mg)
DROP	permits DAT1 to be omitted from the NM-TRAN data set by the data processor and thereby eliminates non-numeric values
DV	dependent variable = measured serum vancomycin concentration
EVID	NONMEM required field for event identification (0=observation, 1=dose, 2=other, 3=reset, 4=reset-dose)
IND	indomethacin pharmacotherapy within 72 hours of vancomycin concentration determination
PCA	post-conceptional age (weeks)
RATE	reserved PREDPP data label for duration of infusion, calculated as Dose / Rate (h)
TIME	reserved PREDPP data label for record time (hh:mm)
WT	patient weight (kg)

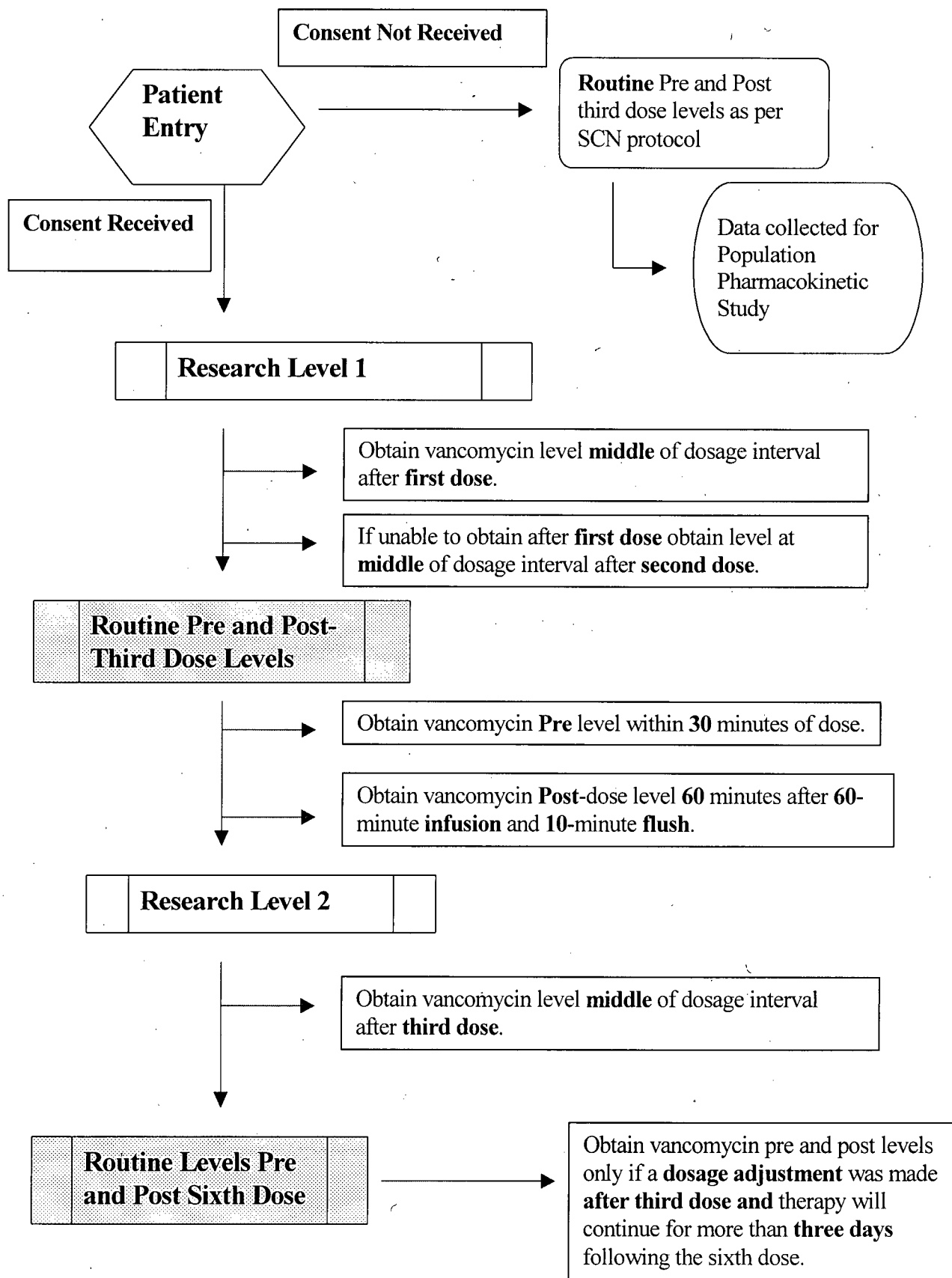


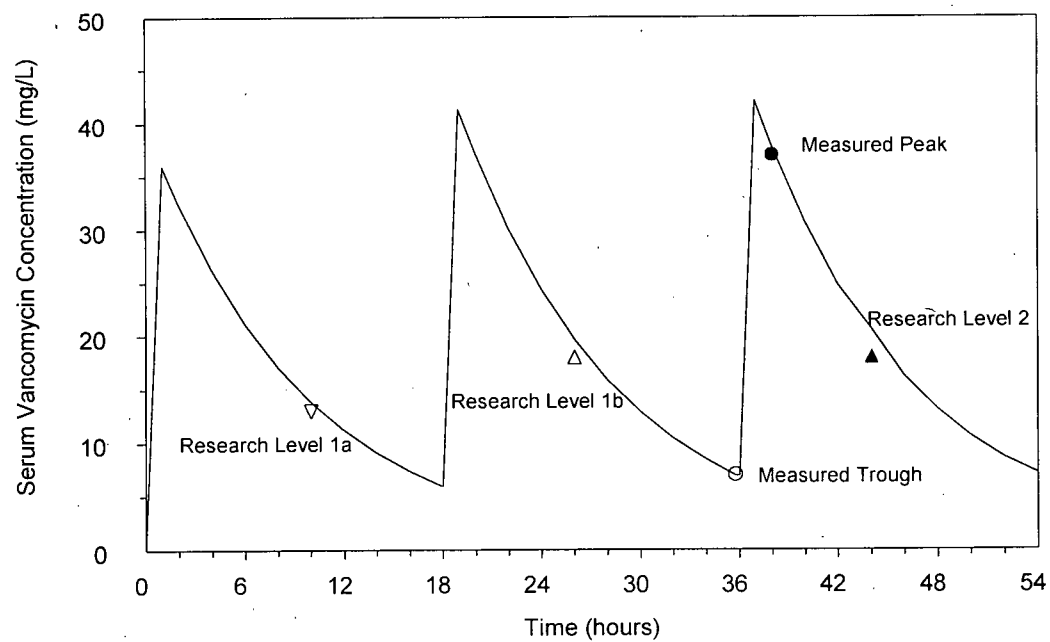
## APPENDIX 10

### Midinterval Vancomycin Blood Sample Collection Times

Flowchart for Sample Collection

Illustration of Sample Collection



**Midinterval Vancomycin Sampling Times.**

## **APPENDIX 11**

### **Patient Consent Form**



Children's Hospital



BC WOMEN'S

# PATIENT CONSENT FORM

## Can a Bayesian forecasting technique predict vancomycin dosage requirements in premature neonates, using a single serum concentration?

Investigators: Dr. Marc Levine, Department of Pharmacy/University of British Columbia;  
822-5027, 875-2059

Mr. Al McDougal, Department of Pharmacy/Special Care Nursery;  
875-2059, pager 41-01177

Dr. Emily Ling, Department of Pediatrics/Special Care Nursery, 875-3258

Ms. Rebecca Wrishko, Department of Pharmacy/University of British Columbia;  
875-2059, pager 41-02235

### BACKGROUND AND PURPOSE OF THE STUDY

An antibiotic called vancomycin has been ordered for your baby because of a possible infection. This antibiotic is effective against a group of bacteria referred to as gram positive, especially one called Staphylococcus epidermidis. It is routine to check patient's blood levels of vancomycin to ensure they are adequate and to reduce the risk of side effects. Small blood samples (0.5 mL or one tenth of a teaspoon) are usually drawn in pairs, just before the third dose of vancomycin and again about one hour after the dose has been given. The results of these tests are used to adjust the vancomycin dose to provide optimal antibiotic treatment. The purpose of this study is to determine whether single blood samples, taken earlier than usual in treatment, can predict the vancomycin dose that babies need as well as the current practice of using two samples.

### STUDY PROCEDURES

Vancomycin will be administered to your baby through a vein (intravenously) and the routine pair of blood samples will be taken before and after the third dose and one or more times later, if necessary, to guide dose adjustments. These blood samples are taken either by making a small prick on the heel of one of your baby's feet or through a tube, if one is already in place in one of your baby's blood vessels. These procedures are done for all babies receiving vancomycin therapy. If you agree to have your baby participate in this study, one extra blood sample will need to be taken after the first or second dose of vancomycin and another extra sample will need to be taken after the third or fourth dose of vancomycin. Other than these extra blood samples, the vancomycin treatment of your baby will be identical to what is routinely done.

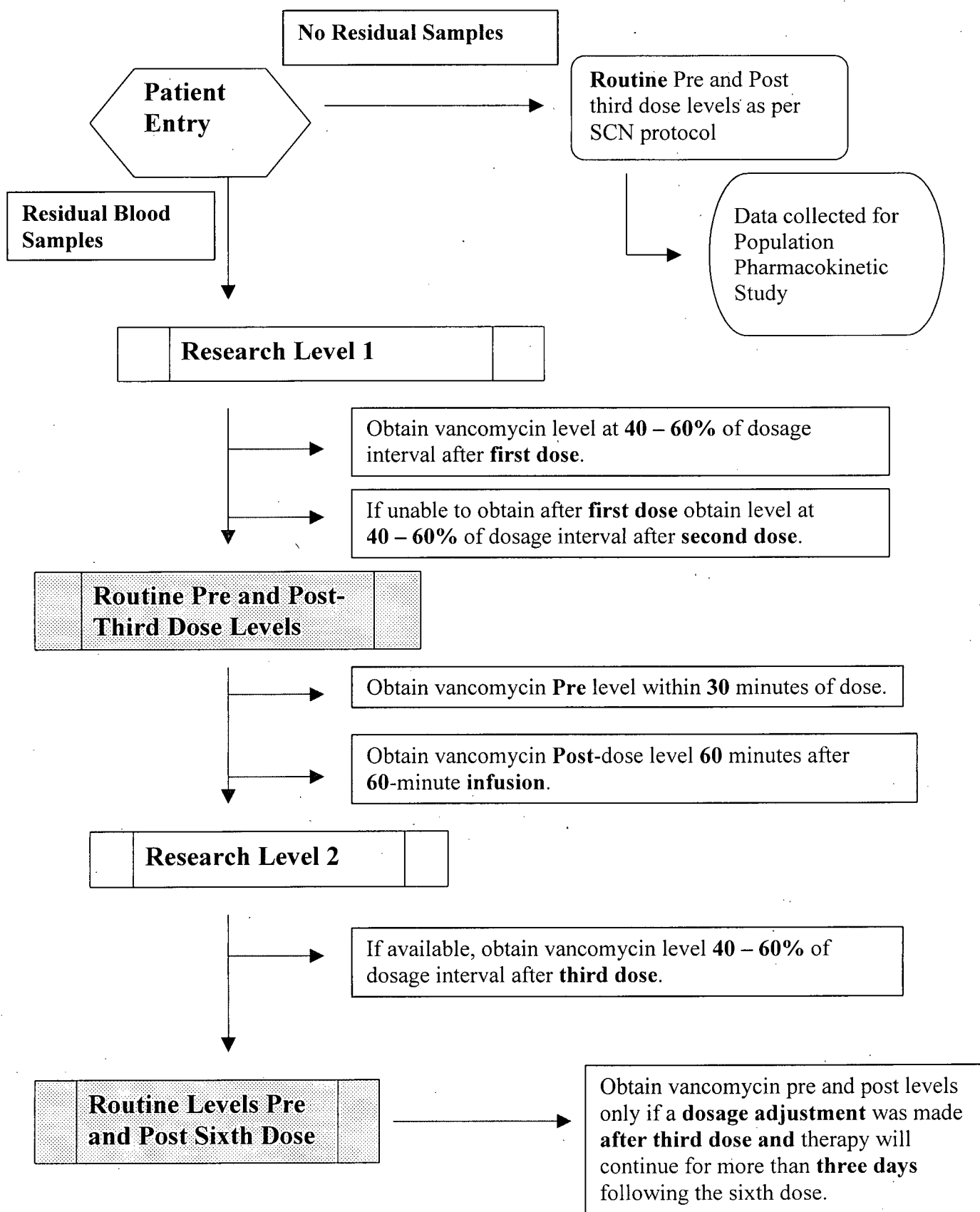
BC WOMEN'S 4500 Oak Street, Vancouver, B.C. V6H 3N1 Telephone: (604) 875-2424 Fax: (604) 875-2289  
B.C.'S CHILDREN'S HOSPITAL 4480 Oak Street, Vancouver, B.C. V6H 3V4 Telephone: (604) 875-2345 Fax: (604) 875-2292  
*Teaching Facilities Associated with the University of British Columbia*

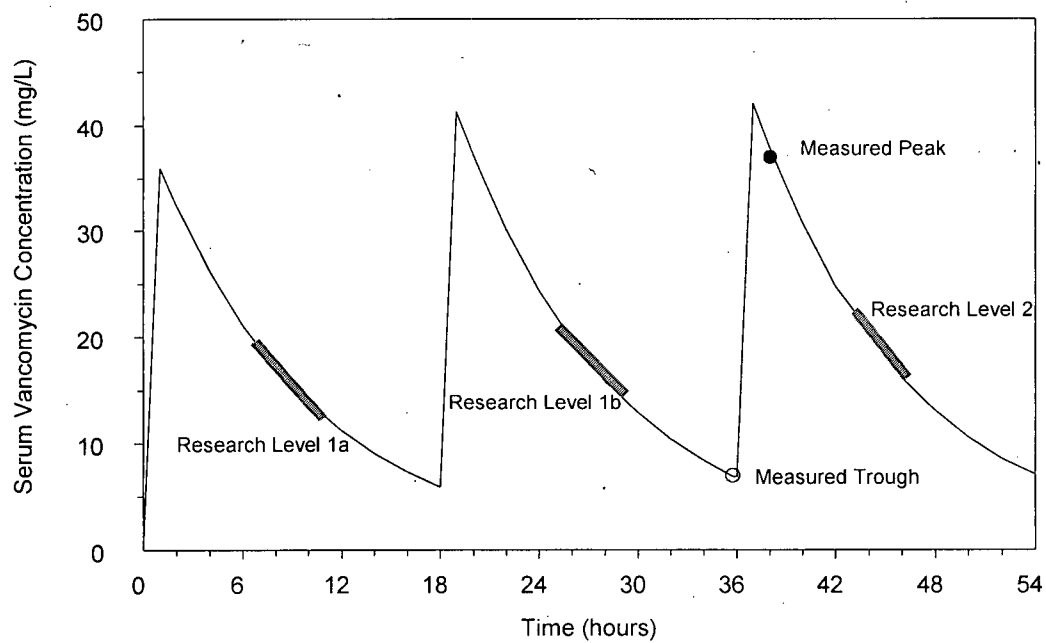
## **APPENDIX 12**

### **Residual Vancomycin Blood Sample Collection Times**

Flowchart for Sample Collection

Illustration of Vancomycin Concentrations Quantified from Residual Samples



**Vancomycin Concentrations Quantified from Residual Samples.**



**APPENDIX 13****NONMEM Two-Compartment Model Building Control Records**

Model 2a

Model 2b

Model 2c

Model 2d

Model 2e

Model 2f

Model 2g

Model 2h

\$PROBLEM NEONATAL POPULATION COHORT; **Model 2a**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT

\$DATA DPOP2.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data Normalized for WT with Exponential Eta and Epsilon and Posthoc

\$PK

TVCL=THETA(1) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(2) ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(3) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(4) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,1) ; lower and initial estimates of cl  
 (0,1) ; lower and initial estimates of v1  
 (0,0.5) ; lower and initial estimates of v2  
 (0,1) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop2f.tbl

\$PROBLEM NEONATAL POPULATION COHORT; **Model 2b**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT

\$DATA DPOP2.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data Normalized for WT on cl with Exponential Eta and Epsilon and Posthoc

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2)) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(3) ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(4) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(5) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
 (0,1) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimates of v1  
 (0,2) ; lower and initial estimates of v2  
 (0,1) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 .NOPRINT ONEHEADER FILE=tpop2h4.tbl

\$PROBLEM NEONATAL POPULATION COHORT; **Model 2c**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT

\$DATA DPOP2.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data Normalized for WT on cl and v1 with Exponential Eta and Epsilon and Posthoc

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2)) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(3)\*WT ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(4) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(5) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship  
 K12=Q/V1  
 K21=Q/V2  
 S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
 (0,1) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimates of v1  
 (0,2) ; lower and initial estimates of v2  
 (0,0.5) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop2hi.tbl

\$PROBLEM NEONATAL POPULATION COHORT; **Model 2d**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA

\$DATA DPOP7.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data Normalized for WT and PCA on cl and WT on v1 with Exponential Eta and Epsilon and Posthoc

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2))\*((PCA/40)\*\*THETA(3)) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(4)\*WT ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(5) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(6) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
 (0,1) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimate of theta 3  
 (0,0.5) ; lower and initial estimates of v1  
 (0,2) ; lower and initial estimates of v2  
 (0,0.5) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop8i.tbl

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 2e

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  DOP  CLD

$DATA  DPOP22.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data Normalized for WT and PCA with DOP and CLD with Exponential Eta and
Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))*((PCA/40)**THETA(3))*(THETA(4)**DOP)  ;
typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(5)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(6)*(THETA(7)**DOP)*(1+THETA(8)**CLD)  ;  typical peripheral
volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(9)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimate of theta 2
        (0,0.5)  ;  lower and initial estimate of theta 3
        (0,2)  ;  lower and initial estimate of theta 4
        (0,0.5)  ;  lower and initial estimates of v1
        (0,0.5)  ;  lower and initial estimates of v2
        (0,0.5)  ;  lower and initial estimates of theta 7
        (0,0.5)  ;  lower and initial estimates of theta 8
        (0,0.5)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop11c.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 2f

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  DOP  CLD

$DATA  DPOP41.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data Normalized for WT and PCA with DOP and CLD with Exponential Eta and
Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))*((PCA/40)**THETA(3))*(THETA(4)**DOP)  ;
typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(5)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(6)*(THETA(7)**DOP)*(1+THETA(8)**CLD)  ;  typical peripheral
volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(9)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimate of theta 2
        (0,2)  ;  lower and initial estimate of theta 3
        (0,0.5)  ;  lower and initial estimate of theta 4
        (0,0.5)  ;  lower and initial estimates of v1
        (0,0.5)  ;  lower and initial estimates of v2
        (0,1)  ;  lower and initial estimates of theta 7
        (0,3)  ;  lower and initial estimates of theta 8
        (0,0.5)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  ETA1  TVV1  V1  ETA2
        TVV2  V2  ETA3  NOPRINT  ONEHEADER  FILE=tpop14n.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 2g

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  DOP  CLD

$DATA  DPOP45.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data Normalized for WT and PCA with DOP and CLD with Different Eta and
Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))*((PCA/40)**THETA(3))*(THETA(4)**DOP)  ;
typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(5)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(6)*(THETA(7)**DOP)*(1+THETA(8)**CLD)  ;  typical peripheral
volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(9)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimate of theta 2
        (0,2)  ;  lower and initial estimate of theta 3
        (0,0.5)  ;  lower and initial estimate of theta 4
        (0,0.5)  ;  lower and initial estimates of v1
        (0,0.5)  ;  lower and initial estimates of v2
        (0,1)  ;  lower and initial estimates of theta 7
        (0,3)  ;  lower and initial estimates of theta 8
        (0,0.5)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.000001  ;  twenty percent cv of eta

$SIGMA  0.04  0.04  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))+EPS(2)  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  ETA1  TVV1  V1  ETA2
        TVV2  V2  ETA3  NOPRINT  ONEHEADER  FILE=tpop16k.tbl

```



\$PROBLEM NEONATAL POPULATION COHORT; **Model 2h**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA DOP CLD

\$DATA DPOP45.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data Normalized for WT and PCA with DOP and CLD with Different Eta and Epsilon and Posthoc

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2))\*((PCA/40)\*\*THETA(3))\*(THETA(4)\*\*DOP) ;  
typical clearance

CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability

TVV1=THETA(5)\*WT ; typical central volume

V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability

TVV2=THETA(6)\*(1+THETA(7)\*\*CLD) ; typical peripheral volume

V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability

Q=THETA(8) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
(0,1) ; lower and initial estimate of theta 2  
(0,2) ; lower and initial estimate of theta 3  
(0,0.5) ; lower and initial estimate of theta 4  
(0,0.5) ; lower and initial estimates of v1  
(0,2) ; lower and initial estimates of v2  
(0,1) ; lower and initial estimates of theta 7  
(0,0.5) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.000001 ; twenty percent cv of eta

\$SIGMA 0.04 0.04 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1))+EPS(2) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL ETA1 TVV1 V1 ETA2  
TVV2 V2 ETA3 NOPRINT ONEHEADER FILE=tpop17b.tbl

**APPENDIX 14****NONMEM One-Compartment Model Building Control Records**

Model 1a

Model 1b

Model 1c

Model 1d

Model 1e

Model 1f

Model 1g

Model 1h

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1a

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT

$DATA  DPOP2.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and No Reset

$PK
  TVCL=THETA(1)  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(2)  ;  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,1)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimates of v

$OMEGA  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV  V
        NOPRINT  ONEHEADER  FILE=tpop1d.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1b

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT

$DATA  DPOP2.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with power on WT with Exponential Eta and Epsilon

$PK
  TVCL=THETA(1)*(WT**THETA(2))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(3);  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
        (0,2)  ;  lower and initial estimates of theta 2
        (0,2)  ;  lower and initial estimates of v

$OMEGA  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$error
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV  V
        NOPRINT  ONEHEADER  FILE=tpoplk.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1c

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT

$DATA  DPOP2.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with power on WT with Exponential Eta and Epsilon

$PK
  TVCL=THETA(1)*(WT**THETA(2))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(3)*WT  ;  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
        (0,2)  ;  lower and initial estimates of v
        (0,0.5)  ;  lower and initial estimates of theta 4

$OMEGA  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV  V
        NOPRINT  ONEHEADER  FILE=tpoplr.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1d

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA

$DATA  DPOP7.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and No Reset linear factored pca

$PK
  TVCL=THETA(1)*(WT**THETA(2))+((PCA/40)*THETA(3))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(4)*WT  ;  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
         (0,2)  ;  lower and initial estimates of theta 2
         (0,0.5)  ;  lower and initial estimates of theta 3
         (0,0.5)  ;  lower and initial estimates of v

$OMEGA  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV  V
        NOPRINT  ONEHEADER  FILE=tpoplt2.tbl

```

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1e

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  CLD

$DATA  DPOP25.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with lung disease with Exponential Eta and Epsilon and No Reset linear
factored pca

$PK
  TVCL=THETA(1)*(WT**THETA(2))*(THETA(3)**CLD)*((PCA/40)**THETA(4))  ;
typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(5)*WT*(THETA(6)**CLD)  ;  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
        (0,2)  ;  lower and initial estimates of theta 2
        (0,0.5)  ;  lower and initial estimates of theta 3
        (0,0.5)  ;  lower and initial estimates of theta 4
        (0,0.5)  ;  lower and initial estimates of v
        (0,0.5)  ;  lower and initial estimates of theta 6

$OMEGA  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV  V
        NOPRINT  ONEHEADER  FILE=tpop12g.tbl

```

\$PROBLEM NEONATAL POPULATION COHORT; **Model 1f**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA CLD

\$DATA DPOP53.TXT

\$SUBROUTINES ADVAN1 TRANS2; One Compartment Linear Model for Population Data with lung disease with Exponential Eta and Epsilon and No Reset linear factored pca

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2))\*(THETA(3)\*\*CLD)\*((PCA/40)\*\*THETA(4)) ;  
typical clearance

CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability

TVV=THETA(5)\*WT\*(THETA(6)\*\*CLD) ; typical volume of distribution

V=TVV\*EXP(ETA(2)) ; interindividual volume variability

K=CL/V ; reparameterization relationship

S1=V ; scale for central compartment

\$THETA (0,0.5) ; lower and initial estimates of cl  
(0,1) ; lower and initial estimates of theta 2  
(0,2) ; lower and initial estimates of theta 3  
(0,2) ; lower and initial estimates of theta 4  
(0,0.5) ; lower and initial estimates of v  
(0,2) ; lower and initial estimates of theta 6

\$OMEGA 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL ETA1 TVV V ETA2  
NOPRINT ONEHEADER FILE=tpopl3r.tbl



\$PROBLEM NEONATAL POPULATION COHORT; **Model 1g**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA CLD

\$DATA DPOP56.TXT

\$SUBROUTINES ADVAN1 TRANS2; One Compartment Linear Model for Population Data with lung disease with Exponential Eta and Epsilon and No Reset linear factored pca

\$PK

TVCL=THETA(1)\*(WT\*\*THETA(2))\*(THETA(3)\*\*CLD)\*((PCA/40)\*\*THETA(4)) ;  
typical clearance

CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability

TVV=THETA(5)\*WT\*(THETA(6)\*\*CLD) ; typical volume of distribution

V=TVV\*EXP(ETA(2)) ; interindividual volume variability

K=CL/V ; reparameterization relationship

S1=V ; scale for central compartment

\$THETA (0,0.5) ; lower and initial estimates of cl  
(0,1) ; lower and initial estimates of theta 2  
(0,2) ; lower and initial estimates of theta 3  
(0,2) ; lower and initial estimates of theta 4  
(0,0.5) ; lower and initial estimates of v  
(0,2) ; lower and initial estimates of theta 6

\$OMEGA 0.04 0.004 ; twenty percent cv of eta

\$SIGMA 0.01 0.1 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1))+EPS(2) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL ETA1 TVV V ETA2  
NOPRINT ONEHEADER FILE=tpopl5h.tbl

```

$PROBLEM  NEONATAL POPULATION COHORT;  Model 1h

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  CLD

$DATA  DPOP56.TXT

$SUBROUTINES  ADVAN1 TRANS2;  One Compartment Linear Model for Population
Data with lung disease with Exponential Eta and Epsilon and No Reset linear
factored pca

$PK
  TVCL=THETA(1)*(WT**THETA(2))*(THETA(3)**CLD)*((PCA/40)**THETA(4))  ;
typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV=THETA(5)*WT*(THETA(6)**CLD)  ;  typical volume of distribution
  V=TVV*EXP(ETA(2))  ;  interindividual volume variability

  K=CL/V  ;  reparameterization relationship
  S1=V  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimates of theta 2
        (0,2)  ;  lower and initial estimates of theta 3
        (0,2)  ;  lower and initial estimates of theta 4
        (0,0.5)  ;  lower and initial estimates of v
        (0,2)  ;  lower and initial estimates of theta 6

$OMEGA  0.04  0.004  ;  twenty percent cv of eta

$SIGMA  0.01  0.1  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))+EPS(2)  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  ETA1  TVV  V  ETA2
        NOPRINT  ONEHEADER  FILE=tpop15h.tbl

```

**APPENDIX 15****NONMEM Two-Compartment Combined Model Building Control Records**

Model c2a

Model c2b

Model c2c

Model c2d

Model c2e

Model c2f

Model c2g

Model c2h

```

$PROBLEM  NEONATAL COMBINED COHORT;  Model c2a

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA

$DATA  DPOP86.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data Exponential Eta and Epsilon and Posthoc

$PK
  TVCL=THETA(1)  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(2)  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(3)  ;  typical peripheral volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(4)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,1)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimates of v1
        (0,0.5)  ;  lower and initial estimates of v2
        (0,1)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop33a.tbl

```

```

$PROBLEM  NEONATAL COMBINED COHORT;  Model c2b

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA

$DATA  DPOP86.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(3)  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(4)  ;  typical peripheral volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(5)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
         (0,2)  ;  lower and initial estimate of theta 2
         (0,2)  ;  lower and initial estimates of v1
         (0,2)  ;  lower and initial estimates of v2
         (0,2)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop33c.tbl

```

```

$PROBLEM  NEONATAL COMBINED COHORT;  Model c2c

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA

$DATA  DPOP86.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(3)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(4)  ;  typical peripheral volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(5)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
         (0,2)  ;  lower and initial estimate of theta 2
         (0,0.5)  ;  lower and initial estimates of v1
         (0,1)  ;  lower and initial estimates of v2
         (0,0.05)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop33j.tbl

```

```

$PROBLEM  NEONATAL COMBINED COHORT;  Model c2d

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA

$DATA  DPOP86.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and Posthoc

$PK
  TVCL=THETA(1)*(WT**THETA(2))*((PCA/40)**THETA(3))  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(4)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(5)  ;  typical peripheral volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(6)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.05)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimate of theta 2
        (0,0.5)  ;  lower and initial estimate of theta 3
        (0,0.5)  ;  lower and initial estimates of v1
        (0,2)  ;  lower and initial estimates of v2
        (0,0.05)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop33m.tbl

```

```

$PROBLEM  NEONATAL COMBINED COHORT;  Model c2e

$INPUT  ID  DAT1=DROP  TIME  DOSE=AMT  RATE  CP=DV  WT  PCA  IND  DOP  CLD

$DATA  DPOP84.TXT

$SUBROUTINES  ADVAN3 TRANS4;  Two Compartment Linear Model for Population
Data with Exponential Eta and Epsilon and Posthoc

$PK
  A=THETA(1)*(WT**THETA(2))*((PCA/40)**THETA(3))
  TVCL=A*(THETA(4)**IND)*(THETA(5)**DOP)  ;  typical clearance
  CL=TVCL*EXP(ETA(1))  ;  interindividual clearance variability
  TVV1=THETA(6)*WT  ;  typical central volume
  V1=TVV1*EXP(ETA(2))  ;  interindividual central volume variability
  TVV2=THETA(7)*(THETA(8)**DOP)*(THETA(9)**CLD)  ;  typical peripheral volume
  V2=TVV2*EXP(ETA(3))  ;  interindividual volume variability
  Q=THETA(10)  ;  typical intercompartmental clearance

  K=CL/V1  ;  reparameterization relationship
  K12=Q/V1
  K21=Q/V2
  S1=V1  ;  scale for central compartment

$THETA  (0,0.5)  ;  lower and initial estimates of cl
        (0,1)  ;  lower and initial estimate of theta 2
        (0,0.5)  ;  lower and initial estimate of theta 3
        (0,0.5)  ;  lower and initial estimate of theta 4
        (0,0.5)  ;  lower and initial estimate of theta 5
        (0,0.5)  ;  lower and initial estimates of v1
        (0,2)  ;  lower and initial estimates of v2
        (0,2)  ;  lower and initial estimates of theta 8
        (0,1)  ;  lower and initial estimates of theta 9
        (0,0.5)  ;  lower and initial estimates of q

$OMEGA  0.04  0.04  0.04  ;  twenty percent cv of eta

$SIGMA  0.02  ;  ten percent cv of epsilon

$ERROR
  Y=F*EXP(EPS(1))  ;  exponential error term for residual error

$ESTIMATION  MAXEVAL=5000  SIGDIGITS=4  POSTHOC

$COVARIANCE

$TABLE  ID  TIME  DV  TVCL  CL  TVV1  V1  TVV2  V2
        NOPRINT  ONEHEADER  FILE=tpop341.tbl

```



\$PROBLEM NEONATAL COMBINED COHORT; **Model c2f**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA IND DOP CLD

\$DATA DPOP83.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data with Exponential Eta and Epsilon and Posthoc

\$PK

A=THETA(1)\*(WT\*\*THETA(2))\*((PCA/40)\*\*THETA(3))  
 TVCL=A\*(THETA(4)\*\*IND)\*(THETA(5)\*\*DOP) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(6)\*WT ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(7)\*(THETA(8)\*\*DOP)\*(THETA(9)\*\*CLD) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(10) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship  
 K12=Q/V1  
 K21=Q/V2  
 S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of c1  
 (0,2) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimate of theta 3  
 (0,1) ; lower and initial estimate of theta 4  
 (0,1) ; lower and initial estimate of theta 5  
 (0,0.5) ; lower and initial estimates of v1  
 (0,0.5) ; lower and initial estimates of v2  
 (0,0.5) ; lower and initial estimates of theta 8  
 (0,2) ; lower and initial estimates of theta 9  
 (0,0.05) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.02 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1)) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop34m.tbl

\$PROBLEM NEONATAL COMBINED COHORT; **Model c2g**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA IND DOP CLD

\$DATA DPOP83.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data with Posthoc

\$PK

A=THETA(1)\*(WT\*\*THETA(2))\*((PCA/40)\*\*THETA(3))  
 TVCL=A\*(THETA(4)\*\*IND)\*(THETA(5)\*\*DOP) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(6)\*WT ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(7)\*(THETA(8)\*\*DOP)\*(THETA(9)\*\*CLD) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(10) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
 (0,2) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimate of theta 3  
 (0,1) ; lower and initial estimate of theta 4  
 (0,1) ; lower and initial estimate of theta 5  
 (0,0.5) ; lower and initial estimates of v1  
 (0,0.5) ; lower and initial estimates of v2  
 (0,0.5) ; lower and initial estimates of theta 8  
 (0,2) ; lower and initial estimates of theta 9  
 (0,0.05) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.5 1 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1))+EPS(2) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop35a.tbl

\$PROBLEM NEONATAL COMBINED COHORT; **Model c2h**

\$INPUT ID DAT1=DROP TIME DOSE=AMT RATE CP=DV WT PCA IND DOP CLD

\$DATA DPOP83.TXT

\$SUBROUTINES ADVAN3 TRANS4; Two Compartment Linear Model for Population Data with Posthoc

\$PK

A=THETA(1)\*(WT\*\*THETA(2))\*((PCA/40)\*\*THETA(3))  
 TVCL=A\*(THETA(4)\*\*IND)\*(THETA(5)\*\*DOP) ; typical clearance  
 CL=TVCL\*EXP(ETA(1)) ; interindividual clearance variability  
 TVV1=THETA(6)\*WT ; typical central volume  
 V1=TVV1\*EXP(ETA(2)) ; interindividual central volume variability  
 TVV2=THETA(7)\*(THETA(8)\*\*CLD) ; typical peripheral volume  
 V2=TVV2\*EXP(ETA(3)) ; interindividual volume variability  
 Q=THETA(9) ; typical intercompartmental clearance

K=CL/V1 ; reparameterization relationship

K12=Q/V1

K21=Q/V2

S1=V1 ; scale for central compartment

\$THETA (0,0.05) ; lower and initial estimates of cl  
 (0,2) ; lower and initial estimate of theta 2  
 (0,0.5) ; lower and initial estimate of theta 3  
 (0,1) ; lower and initial estimate of theta 4  
 (0,1) ; lower and initial estimate of theta 5  
 (0,0.5) ; lower and initial estimates of v1  
 (0,1) ; lower and initial estimates of v2  
 (0,1) ; lower and initial estimates of theta 8  
 (0,0.05) ; lower and initial estimates of q

\$OMEGA 0.04 0.04 0.04 ; twenty percent cv of eta

\$SIGMA 0.5 1 ; ten percent cv of epsilon

\$ERROR

Y=F\*EXP(EPS(1))+EPS(2) ; exponential error term for residual error

\$ESTIMATION MAXEVAL=5000 SIGDIGITS=4 POSTHOC

\$COVARIANCE

\$TABLE ID TIME DV TVCL CL TVV1 V1 TVV2 V2  
 NOPRINT ONEHEADER FILE=tpop35e.tbl