CHLORAL HYDRATE DISPOSITION IN CRITICALLY ILL PAEDIATRIC PATIENTS

A Thesis

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THIS THESIS IS DEDICATED TO MY FAMILY:

MOTHER AND FATHER

TRENT AND SUSAN

AND

KIMBERLEY

ABSTRACT

Chloral hydrate (CH), the oldest sedative-hypnotic used today, has largely been replaced in general medicine by newer drugs (benzodiazepines) but it still enjoys extensive use in paediatrics. However, few studies have investigated CH in that patient group. Chloral hydrate is rapidly metabolized to trichloroethanol (TCE), trichloroethanol-glucuronide (TCE-G) and trichloroacetic acid (TCA). The metabolites are excreted renally. Chloral hydrate is an irritant and has been linked to cardio-pulmonary and central nervous system toxicity in cases of intoxication. Drug interactions with CH have also been reported.

The objective of this study was to examine the disposition of CH within a critically ill paediatric patient population and to determine if routine use of CH has the potential to result in toxicity. The present investigation examined pharmacokinetic parameters of CH and its metabolites, plasma protein binding of TCA as a mechanism of the furosemide/CH drug interaction and the respective roles CH and TCE play in sedation.

Gas chromatography with electron capture detection was used to determine levels of CH and its metabolites in plasma and urine. The plasma protein binding of TCA was determined both *in vitro* and *ex vivo* using micropartition ultrafiltration.

Because of the paucity of information on routine dosages and dosing intervals in paediatric patients a small pilot study (n=7) was undertaken to determine if the potential for CH intoxication existed. The elimination of TCE, TCE-G and TCA were much slower in the study group than in adults. Trichloroacetic acid was especially persistent. Its plasma concentrations showed no signs of decline in four patients 14 days after the final CH administration. Dosing intervals were variable but, without exception, much shorter than the elimination half-life values ($t_{½}$) of the metabolites. In two cases TCE concentrations were in the toxic range (>100 µg/mL), however no signs of intoxication were noted.

In the single dose study, patients were from three age groups: preterm neonates (n=9), term neonates (n=8) and PICU patients (n=5). The clearance of CH was equivalent among the groups. TCE $t_{½}$ and area-under-the-curve (AUC) values were negatively correlated with age. The $t_{½}$ value for TCE in PICU patients was similar to that reported for adults, but in the less mature subjects it was approximately three (term) to four (preterm) times greater. Renal clearance for TCE was higher in PICU patients than in neonates. The AUC_{0-24} for TCA was also higher in PICU patients than in the neonatal groups but the renal clearance in the same time period was not significantly different.

Plasma protein binding of TCA was studied in vitro in adult, neonatal cord and neonatal exchange transfusion plasma. Trichloroacetic acid binding was highest in adult plasma followed by cord plasma and exchange transfusion plasma. Affinity (ka) appeared to be lower in the neonatal plasma. Furosemide concentrations of 2 and 20 μg/mL did not affect the plasma protein binding of TCA. Only the furosemide concentration which is well above that encountered clinically (200 μg/mL) resulted in significantly decreased binding. In neonates the decreased binding appeared to be due to decreased binding affinity for TCA. This could not be confirmed in adult plasma.

Ex vivo binding was lower in preterm samples than in the in vitro studies. Term neonates and adult samples more closely ressembled their respective in vitro data.

The sedative-hypnotic effects of CH have, in the past, been solely ascribed to TCE. However the $t_{½}$ of TCE is much longer than the dosing intervals normally used for CH. Therefore the relationship between plasma levels of TCE and the sedation status of infants was examined. A numerical scale was developed to assess the state of sedation and/or agitation of preverbal patients and used to quantitate the relative level of consciousness of infants following CH administration. There did not appear to be any correlation between the sedation scores and TCE plasma levels. However, when the sedation scores were plotted against CH

plasma concentrations a regular, cyclical pattern was detected. Levels of CH rather than TCE appeared to correlate with the sedation scores.

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LIST OF ABBREVIATIONS

ADH	Alcohol Dehydrogenase
AUC	Area-under-the-curve
CH	Chloral Hydrate
CT	Computerized Axial Tomography
C_{max}	Concentration maxima
CNS	Central nervous system
CPS	Compendium of Pharmaceuticals and Specialties
EC	Enzyme Commission International
ECD	Electron capture detector
EEG	Electroencephalograph
FFA	Free fatty acids
GC	Gas chromatograph
GFR	Glomerular filtration rate
GRAS	Generally Regarded As Safe
ID	Internal diameter
MADDS	Monoacetyl-diamino diphenylsulfone
NAD^+	Nicotinamide Adenine Dinucleotide (Oxidized)
NADH	Nicotinamide Adenine Dinucleotide (Reduced)
NICU	Neonatal Intensive Care Unit
PAH	Para-amino hippuric acid
PICU	Paediatric Intensive Care Unit
RUH	Royal University Hospital, Saskatoon
TCA	Trichloroacetic acid
TCE	Trichloroethanol
TCE-G	Trichloroethanol-glucuronide

TCP	Trichloropropane
$t_{1/2}$	Half-life
T_{max}	Time to reach maximum plasma concentration during study
Tm	Transport maxima

1.0 INTRODUCTION

Generally Regarded As Safe (GRAS) is a phrase which is very familiar to toxicologists. Simply put, GRAS is a list of compounds that have been in use for many years which have never been linked with toxicity and thus are considered safe. If a catalogue equivalent to the GRAS list existed in paediatric pharmacotherapy chloral hydrate would undoubtedly appear.

Chloral hydrate is the oldest sedative-hypnotic in use in medicine today. It has largely been replaced in medical practice by barbiturates, and more recently, by benzodiazepines. However in spite of the availability of newer drugs, chloral hydrate still enjoys substantial popularity in both paediatric and geriatric medicine. Unfortunately, studies of this medication in the patient populations where it is predominantly prescribed are lacking. With the exception of a few case reports and abstracts, little information is available about the disposition of chloral hydrate in neonates and older infants.

Drugs, even old ones, must be thoroughly investigated to ensure their safety and efficacy. Furthermore, it is now being widely recognized that these studies must be done in the patient populations and under conditions in which the drug will be used.

The GRAS list is not a static entity and compounds if found to be toxic upon further investigation are promptly reclassified. An effort must be made to make the same strides in the area of paediatric drug disposition. Tragedies such as "grey baby syndrome" illustrate very graphically what can occur if this is not done (Kacew & Lock, 1990).

The study of drugs in a critically ill paediatric population is, however, fraught with obstacles. Philosophically, the ethics of experimentation on non-consensual and in many cases pre-verbal patients is troublesome. The phrase, "Catch-22", coined by Joseph Heller in his classic novel of the same name is a more than apt description of the philosophical dilemma faced by scientists and clinicians in the field of paediatric research. Ethically is it better to abstain from paediatric research, conservatively use inadequately tested medications and risk causing unforseen effects (e.g. overdosing, underdosing, drug interactions) which could result in harm to the patients or is it more appropriate to expose a group of non-consensual human beings (i.e. infants) to the inherent risks of a controlled research study as well as the risks of the medication itself in order to gain information which could lead to more appropriate use of the drug in other patients in the future? Each side of this question could be argued at

length from a purely philosophical point of view but there are other considerations. The major one of these is that information gained by careful experimentation in infants cannot be duplicated with laboratory animals or adults. Therefore it seems clear that such experimentation is necessary. However, the invasiveness of all procedures should be kept to a minimum.

Difficulties are encountered when any clinically oriented research project is undertaken but these are compounded when a study is attempted with intensive care patients in the neonatal (0-31 days) and paediatric (>1 month) age groups. These patients have extremely limited blood volumes and often the collection of other fluids such as urine is problematic. Furthermore, the well being of the patient is paramount and must always take precedence over the scientific study. Therefore, it may be impossible to control details such as the concomitant administration of other medications.

The study presented in the following pages does not purport to be the single comprehensive treatise on the disposition of chloral hydrate in severely ill children. It is rather more like the portal through which total understanding is glimpsed but not quite attained. As Sir Ernest Rutherford once said:

> "It is not in the nature of things for any one man to make a sudden violent discovery; science goes step by step, and every man depends on the work of his predecessors..."

2.0 LITERATURE REVIEW

2.1 Chloral Hydrate

2.1.1 Background and Therapeutic Use

Chloral hydrate (2,2,2-trichloroethane-1,1-diol, Noctec®, CH; Figure 2.1) is a colourless crystalline substance which has a pungent but not acrid odour and a bitter taste (Fairbrother, 1973).

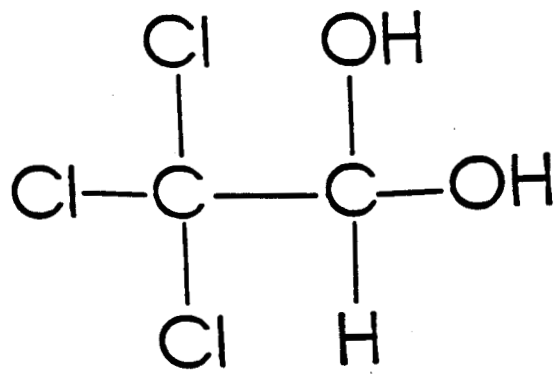

Figure 2.1 Chemical structure of chloral hydrate

It is the oldest synthetic sedative-hypnotic used in medicine today. The hypnotic effects of the drug were first described by Liebreich in 1869, some 37 years after its initial synthesis (Breimer, 1974; Hoskins, 1984). Only two years later the first reports of acute and chronic poisonings with CH began to be appear. In fact, CH was

described in 1910 as the most dangerous of all hypnotics (Hoskins, 1984). Nevertheless CH was used extensively for many years until it was largely supplanted by the barbiturates and more recently the benzodiazepines (Breimer, 1974; Miller & Greenblatt, 1979). In spite of the advent of these newer drugs, CH still remains a popular medication in both geriatric and paediatric medicine. The most common indication for CH in elderly patients is treatment of insomnia (Miller & Greenblatt, 1979). In paediatric medicine it is employed for a variety of purposes. Chloral hydrate is often used as a sedative in children undergoing non-invasive diagnostic procedures such as electroencephalography (EEG) or computerized axial tomography (CT) (Yeh, 1985), in paediatric dentistry (Duncan et al., 1983; Houpt et al., 1989) and in Neonatal Intensive Care Units (NICU) as a maintenance sedative in the management of agitation (Hartley et al., 1989).

The recommended dose for CH in adults is 500-1000 mg before bed for hypnosis or 250 mg three times daily for sedation (Gennaro, 1985; Krough, 1988). In children it has been suggested that a dose of 50 mg/kg (up to 1 g) is effective as an hypnotic (Gennaro, 1985; Krough, 1988) while others suggest 50-75 mg/kg is an appropriate dosage (Cole, 1984). While these dosages appear effective (Houpt et al., 1989; Anderson et al., 1990; Rumm et al., 1990), the drug does not have uniform efficacy in all patients

(Houpt et al., 1989). There may be a subgroup of patients with neurological disorders who are resistant to CH sedation (Rumm et al., 1990). Furthermore, a study of the anxiolytic capability of the drug revealed that only the highest recommended dose (75 mg/kg) provided "good" anxiolysis in children under the age of five while lower doses (25 and 50 mg/kg) were effective in older children (Saarnivaara et al., 1988). No standardized dosage recommendations for neonatal patients could be identified from literature reports although it appears that 25-50 mg/kg is often prescribed in this age group in a multiple dose regimen (Gershanik et al., 1981; Laptook & Rosenfeld, 1984; Hartley et al., 1989).

2.1.2 Pharmacokinetics

2.1.2.1 Biotransformation and Excretion

The metabolites resulting from the non-synthetic biotransformation of CH were first demonstrated to be trichloroethanol (TCE) and trichloroacetic acid (TCA) in 1948. A conjugation product (trichloroethanol-glucuronide; TCE-G) had been isolated and identified in 1882 (Butler, 1948). The metabolites appear rapidly in the plasma and reach concentrations which seem to indicate that only a small portion of CH is oxidized to TCA while most of the drug is reduced to TCE (Butler, 1948). Further studies in dogs and man demonstrated that the oxidative TCA metabolite

can be formed not only from the parent compound, CH, but also from TCE, although its formation from TCE was slower than from CH (Marshall & Owens, 1954). Although theoretically possible, a sulphate metabolite was not found (Owens & Marshall, 1955).

The rapid formation of TCE was at first attributed to the rapid reduction of CH by alcohol dehydrogenase (ADH; EC 1.1.1.1) (Friedman & Cooper, 1960). The results of more recent experiments supported this hypothesis (Sellers et al, 1973). In these studies Sellers and his co-workers (1973) showed that the combination of CH and ethanol led to an alteration of the metabolism of both substances. They hypothesized both substrates were metabolized through ADH but in opposite directions. In other words the oxidation of ethanol (which results in the formation of the co-factor NADH) was redox coupled to the reduction of CH (utilizing the NADH co-factor formed during ethanol oxidation). In the presence of ethanol, TCE is formed more rapidly than if CH is given alone (Sellers et al., 1972c). The reduction of CH via ADH was further confirmed by mass spectral studies. Co-administration of CH with deuterium labelled ethanol resulted in a high incorporation of deuterium into TCE. This is indicative of the well coupled redox reaction suggested by Sellers (Wong & Biemann, 1978). However, both of the above studies were carried out under non-physiological conditions. They were primarily designed to

investigate the observed interaction of ethanol and chloral hydrate and did not take into account that the presence of ethanol can alter the enzymology of the ADH system by artificially changing the levels of the pyridine nucleotides available as co-factors. In a normal physiological state, the amount of NAD far surpasses NADH in the liver and under these conditions the ability of the ADH to act as a reducing enzyme is severely curtailed (Shultz & Weiner, 1979). Alternatively, reduction of CH in the liver could be catalyzed by aldehyde reductase (EC 1.1.1.2) which employs NADPH as a co-factor. This hypothesis was tested by incubating CH in liver slice preparations in the presence and absence of known inhibitors of ADH and aldehyde reductase. The results of these investigations showed that inhibition of ADH caused a negligible inhibition of the TCE formation while even the partial inhibition of aldehyde reductase caused a 30% decrease in TCE formation. These data support the premise that aldehyde reductase, rather than ADH, is responsible for the reduction of CH. Furthermore, aldehyde reductase is a ubiquitous enzyme found throughout the body while ADH is primarily confined to the liver (Shultz & Weiner, 1979). Chloral hydrate is also reduced by an unspecified enzyme system in the red blood cell (Sellers et al., 1972c). Recent work has demonstrated that most CH reduction occurs at extra-hepatic sites (Hobara et al., 1987).

Small amounts of TCE are excreted unchanged in the urine (Sellers et al., 1972c, 1978; Breimer, 1974) but the majority is reported to be conjugated with glucuronic acid prior to excretion (Sellers et al., 1972c, 1978; Breimer, 1974). It was initially suggested, based on experiments in dogs, that the TCE-G was secreted and concentrated in the bile (Owens & Marshall, 1955). Because very little of this compound is found in the faeces (Garrett & Lambert, 1973), it is felt that bacterial deconjugation with subsequent reabsorption of the free TCE takes place (Sellers et al., 1978).

In addition to reductive biotransformation, CH is also oxidized to TCA (Butler, 1948; Marshall & Owens, 1954). This process, however, has received much less attention. The oxidation is catalyzed in the liver by a NAD-dependent aldehyde dehydrogenase (Cooper & Friedman, 1958). Since TCE can also be metabolized to TCA (Marshall & Owens, 1954), this has led to the speculation that CH acts as an intermediate in the reaction (Breimer, 1974). The formation of CH from TCE (i.e. TCE hydroxylation) was demonstrated by Ikeda (1980) in a 700 g supernatant prepared from rat liver. However, TCE hydroxylation activity was found to be very low in rat microsomes (Nakajima et al., 1990). Very recently it has been suggested there may be another enzyme system responsible for the biotransformation of CH (Hara et al., 1991). An

NAD(P) linked aldehyde dismutation reaction has been demonstrated in carbonyl reductase (EC 1.1.1.184) isolated from the lung tissue of guinea pigs. The enzyme irreversibly converted CH into TCA or TCE in the presence of reduced or oxidized co-factors. These results suggest that the enzyme oxidized the hydrated aldehyde (CH) to TCA with NAD(P) as the co-factor and reduced the non-hydrated substrate (chloraldehyde; chloral) to TCE with NAD(P)H as co-factor (Hara et al., 1991). A possible biotransformation scheme for CH is presented in Figure 2.2.

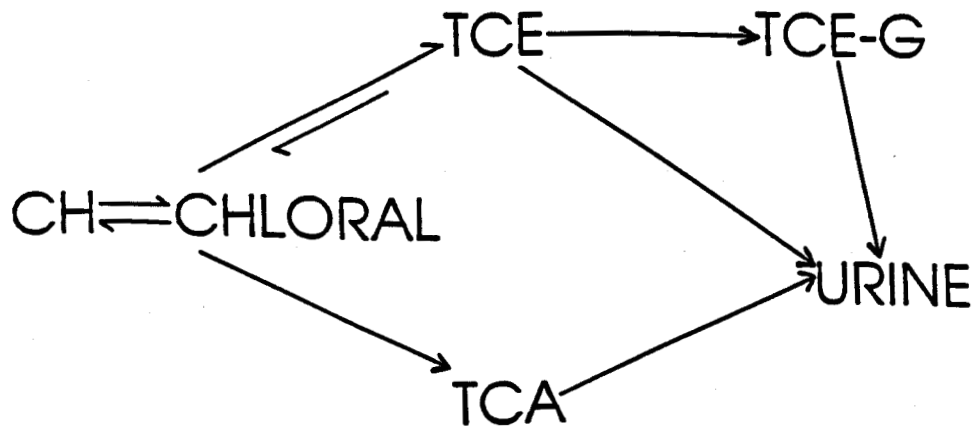

Figure 2.2 A possible metabolic scheme for CH

Regardless of the route of formation, TCA is eventually excreted in the urine (Paykoc & Powell, 1945;

Sellers et al., 1972c, 1978; Breimer, 1974). There is no suggestion of further metabolism before excretion.

The elimination half-life of CH has not been reported for human subjects but in the dog it appears to be only a few minutes (Garrett & Lambert, 1973). The half-life of TCE is between 8-12 hours in the adult (Breimer, 1974; Sellers et al., 1978) while that of the glucuronide is slightly shorter (6-8 hours) (Breimer, 1974). TCA has an extremely long half-life, between 67 (Sellers et al., 1972c) and 100 hours (Breimer, 1974). One report of an adult who had taken an overdose of CH calculated a half-life of 37 hours for TCE (Stalker et al., 1978). This value was virtually identical to that reported for a case of an overdose in a neonate (Laptook & Rosenfeld, 1984). In the latter age group considerable variation has been reported in TCE half-life values even with normal therapeutic doses (Gershanik et al., 1981).

2.1.2.2 Distribution and Protein Binding

Although specific data on the distribution of CH itself has not been reported, it is thought to be widely distributed in the body (Harvey, 1985). Trichloroethanol, the active metabolite, has a benzene:water partition of 3.5 (Butler, 1948) and thus is expected to have a large volume of distribution. In an overdose case, TCE was found in the blood, urine, brain and liver at levels of 55, 30, 91 and

200 µg/mL and µg/mg respectively (Poklis, 1973). The protein binding of this metabolite is in the range of 35-41% (Marshall & Owens, 1954; Sellers et al., 1978). Trichloroacetic acid on the other hand is a highly ionized compound at physiological pH and thus it would not be expected to diffuse through lipid membranes easily. Furthermore, TCA is reported to exhibit extensive plasma protein binding, as high as 95% (Sellers et al., 1978). However, other studies report plasma protein binding of about 88% for TCA at a concentration of 26 µg/mL and that binding decreases substantially as the level of TCA increases (Marshall & Owens, 1954).

2.1.3 Toxicology

2.1.3.1 Local Toxicity

Chloral hydrate is reported to be an irritant to the skin and mucous membranes and can cause gastrointestinal distress if taken undiluted or on an empty stomach (Harvey, 1985). The third most common complaint among hospitalized patients, prescribed CH was that of gastric upset (Miller & Greenblatt, 1979). Case reports detail other more severe incidents such as gastric necrosis (of the full thickness of the stomach wall) following an overdose of CH (Vellar et al., 1972). Autopsy following one extreme intoxication revealed that the entire stomach, part of the spleen and the distal two-thirds of the oesophagus acquired the

appearance of a histologically fixed tissue (Levine et al., 1985). A case of oesophageal stricture caused by an overdose of CH has also been reported (Gleich et al., 1967). In rats it was clearly demonstrated that CH can be ulcerogenic (Ogino et al., 1990).

2.1.3.2 Systemic Toxicity

The acute toxic dose of CH is thought to be 10 g (Harvey, 1985) although as little as 3 g has been known to be fatal (Baselt, 1982). There appears to be a wide interindividual variation in response.

Much of the toxicity data for CH is based on case reports, which for the most part describe situations of an overdose with the drug. The systemic toxicity caused by such intoxications falls into three main categories: central nervous system, cardiovascular and pulmonary effects. There have also been reports of dermatological reactions caused by CH administration (Miller et al., 1966) and a suggestion that CH or one of its metabolites may have carcinogenic potential (Smith, 1990). Hepatic toxicity has been alluded to but there appears to be little substantive evidence for or against this effect (Lambert et al., 1990).

The most widely discussed and debated toxic manifestation of CH is its ability to cause cardiac arrhythmias. Cardiac symptoms reported in case studies are summarized in Table 2.1. These cardiac symptoms are a

Table 2.1 Reports of CH related cardiac toxicity

DOSE	AGE	SEX	CARDIAC SYMPTOMS	REFERENCE
18 g	66 y	F	VT, VF	Gleich et al., 1967
1.5 g	28 mo	M	PVB	Nordenberg et al., 1971
15 g	29 y	F	VT	Marshall, 1977
10-20 g	62 y	F	VT	Marshall, 1977
≈30 g	39 y	F	PSVB, PVB	Gustafson et al., 1977
≈25 g	51 y	M	VT	Gustafson et al., 1977
≈20 g	21 y	F	VT	Gustafson et al., 1977
17.5 g	19 y	F	ST, PVC	Vaziri et al., 1977
38 g	38 y	F	PVC	Stalker et al., 1978
10 g	29 y	F	ST, VT, VEB	Brown & Cade, 1980
14 g	17 y	M	VT	Bowyer & Glasser, 1980
30 g	67 y	F	VT	Bowyer & Glasser, 1980
96 mg/kg/d	9 y	F	SVT	Hirsch & Zauder, 1986
1.4 g	30 mo	F	SVT	Hirsch & Zauder, 1986
≤ 500 mg/kg/d	10 mo	M	SVT	Hirsch & Zauder, 1986
283 mg/kg/d	5 mo	M	SVT	Hirsch & Zauder, 1986
?	42 y	M	VF, VT	Graham et al., 1988
?	25 y	F	ST, VEB, VT	Graham et al., 1988
≈15 g	26 y	F	ST, VEB, VT	Graham et al., 1988
?	25 y	F	ST, VEB, VT	Graham et al., 1988
?	20 y	F	ST, VEB	Graham et al., 1988
?	46 y	F	ST, VEB, VT	Graham et al., 1988
?	27 y	F	ST, VEB, VT	Graham et al., 1988
?	23 y	F	ST, VEB, VT	Graham et al., 1988
?	45 y	F	sinus rhythm; one VEB	Graham et al., 1988
?	28 y	F	ST, VT,torsade de pointes	Graham et al., 1988
?	42 y	M	ST, VEB	Graham et al., 1988

AF, atrial fibrillation; PSVB, Premature supraventricular beats; PVB, Premature ventricular beat; PVC, Premature ventricular contraction; ST, Sinus tachycardia; SVT, Supraventricular tachycardia; VEB, Ventricular ectopic beats; VF, Ventricular fibrillation; VPB, Ventricular premature beats; VT, Ventricular tachycardia

result of an overdose of CH. In therapeutic doses, CH has no harmful effects upon the heart in adult patients (Alstead, 1936). Even with overdoses, cardiac symptoms do not always occur (Wiseman & Hampel, 1978). However, based on a retrospective survey of poison control data over a three year period, Wiseman and Hampel postulated approximately 25% of CH intoxications will experience extrasystoles (Wiseman & Hampel, 1978). Currently two hypotheses have been proposed to explain CH-induced arrhythmias. First, CH and TCE may act by sensitizing the myocardium to the action of endogenous catecholamines (Brown & Cade, 1980). This theory is based on the structural similarity of CH or TCE to halothane (a known myocardium sensitizing agent) and the fact that in several cases the arrhythmias were rapidly abolished by beta-blockers. Alternatively, the mechanism could be a direct action of the drug or its metabolites resulting in an enhanced automaticity of supraventricular and ventricular pace-maker cells (Gustafson et al., 1977). Only one study has attempted to investigate the acute effects of CH on the heart (Trulson & Ulissey, 1987). This investigation reported CH can impair the metabolism of coronary vascular smooth muscle by attacking key cardiac enzymes. In this study, CH was administered intraperitoneally and no attempt was made to determine metabolite concentrations.

Severe respiratory depression can result from toxic

doses of CH (Harvey, 1985). However, studies in both neonates and laboratory animals indicate CH does not depress the central inspiratory drive (Hunt et al., 1982; Lees et al., 1982). In both of these studies, doses well in excess of the supposed toxic dose were administered to the laboratory animals with no effect. Nevertheless, at least two subsequent reports implicate CH as the causative agent in respiratory toxicity (Hershenson et al., 1984; Hartley et al., 1989). Both of these studies were in paediatric patients with underlying respiratory disease (obstructive sleep apnoea or bronchopulmonary dysplasia); the doses of CH administered were within commonly accepted guidelines. One study tested the hypothesis that the toxicity was mediated through selective depression of airway maintaining muscular contractions (Hershenson et al., 1984). They found, in rabbits and puppies, that CH can depress the activity of the genioglossus muscle starting within 10-20 minutes of drug administration and lasting for several hours thereafter.

In a study of over five thousand patients, who had received CH in the course of their stay in hospital, CNS depression was the most commonly reported category of adverse reaction (Miller & Greenblatt, 1979). In most cases, these effects were not life-threatening. However, in some cases, therapeutic doses of CH resulted in coma which resolved on cessation of the drug (Miller &

Greenblatt, 1979). Patients who suffered these life-threatening reactions had multiple disorders and were already severely compromised medically. A report implicates CH in development of toxic encephalopathy in a neonate prescribed the drug for the treatment of agitation to aid in mechanical ventilation. This infant received an excessive dose of the drug and had a TCE concentration of 72.8 mg/L five days following the last drug administration. The clinical findings included metabolic encephalopathy, anoxic-ischemic encephalopathy, intracranial haemorrhage and sepsis. Following cessation of the medication, neurological assessment improved until a normal status was achieved by day seven. The notable feature of this case was the delayed presentation of symptoms. The child displayed no ill effects until day four of treatment with CH (Laptook & Rosenfeld, 1984).

2.1.3.3 Drug Interactions

The ingestion of CH concurrently with ethanol is reported to produce two clinically important interactions. The first of these is a syndrome which appears indistinguishable from that caused by elevated levels of acetaldehyde: vasodilation, tachycardia, facial flushing, headache and hypotension. However, the most famous of the supposed drug interactions attributed to CH is the "knock out effect" which has been so revered in popular detective

novels as the "Mickey Finn". Interaction studies (Sellers et al., 1972a,c) indicate that after ingestion of both CH and ethanol the metabolism of each drug is altered. If ethanol is ingested half-an-hour after CH, the TCE levels are significantly higher and the peak blood ethanol concentration is achieved earlier than if each drug were taken separately (Sellers et al., 1972c). Chloral hydrate appears to enhance the CNS effects of the ethanol. Subjects perform less effectively on complex motor and auditory vigilance tasks than after taking either drug individually (Sellers et al., 1972a,c). In addition to the CNS effects, subjects experience vasodilation and, in some cases, tachycardia, facial flushing and headache (Sellers et al., 1972a). In light of the changes in heart rate and blood pressure, which are part of the vasodilation reaction, caution is advised in prescribing CH for patients with cardiovascular conditions. Alcohol should be avoided in these patients. Due to its effect on TCE ethanol enhances and prolongs the hypnotic effect of CH in man (Sellers et al., 1972c). However, in controlled studies, an immediate "knock-out effect" of the CH-alcohol combination has not been demonstrated (Sellers et al., 1972a).

The other significant drug interactions reported for CH are attributable to its TCA metabolite. The first of these interactions to be reported was the potentiation of

warfarin-induced hypoprothrombinemia (Sellers & Koch-Weser, 1970). During chronic warfarin therapy, TCA displaces warfarin from plasma proteins and can increase hypoprothrombinemia by 40-80% (Sellers & Koch-Weser, 1970). This effect, however, is transient and by the second week of therapy a new lower warfarin steady state is achieved (Sellers et al., 1972b). An interaction between furosemide and CH has also been reported (Malach & Berman, 1975; Dean et al., 1991). This interaction is characterized by diaphoresis, facial flushing, cardiovascular anomalies and uneasiness or agitation. A suggested mechanism is displacement of protein bound TCA by furosemide which in turn displaces thyroxin. The increase in free thyroxin could then account for the hypermetabolic state noted (Malach & Berman, 1975).

Finally, the interaction, of CH and bilirubin deserves mention. Two reports have been published which purport to have identified this interaction. In the first, a slight elevation of unconjugated bilirubin was noted in paediatric patients receiving multiple doses of CH (Reimche et al., 1989). In the second report, a retrospective analysis of medical records, CH was linked with elevated levels of conjugated bilirubin (Lambert et al., 1990). A recent protein binding study indicated that TCA competes with bilirubin for albumin binding, but the clinical relevance was questioned (Robertson & Onks, 1991). Clearly the

subject of the interaction between CH and bilirubin needs further investigation.

2.1.4 Analysis of Chloral Hydrate and its Metabolites

Two major analytical techniques have been used to quantitate CH and its metabolites; namely spectrophotometry and gas chromatography. In 1916, Fujiwara reported that halogenated hydrocarbons react with pyridine in the presence of alkali to form visible chromophores measurable quantitatively by spectrophotometry (Fairbrother, 1973). The analysis of CH and its metabolites using the Fujiwara procedure is complicated by its non-specificity. Trichloroethanol does not react directly with the Fujiwara reagent, but can be extracted from biological samples to react with dichromate and form TCA (Butler, 1948). Trichloroacetic acid, like CH, reacts with pyridine to form a chromophore. Chloral hydrate, but not TCA, decomposes at room temperature in the presence of strong alkali. Thus with a series of extractions and chemical manipulations it is possible to determine the levels of CH and its metabolites in biological materials. The use of a modified Fujiwara procedure for the quantitation of CH and/or its metabolites has been reported quite frequently and as late as 1980 (Paykoc & Powell, 1945; Marshall & Owens, 1954; Sellers et al., 1972c, 1978; McBay et al., 1980). These procedures however, are all relatively labour intensive,

insensitive and of questionable specificity. Thus, the emphasis on methods for the quantitation of CH and its metabolites has shifted to gas chromatography.

The development of chromatographic methods began in the mid 1960's. Initial attempts (Moss & Kenyon, 1964) had limited success since only CH and TCA could be separated and the sensitivity was no greater than previously reported spectrophotometric methods. Other procedures were limited to the analysis of CH and TCE (Jain et al., 1967; Wells & Cimbura, 1972). The first chromatographic method for CH and all of its metabolites was published by Garrett and Lambert in 1966 (Garrett & Lambert, 1973). Because TCA could not be resolved, it had to be determined indirectly by measuring the chloroform levels liberated following alkaline decarboxylation of TCA in the sample. This procedure involves several time-consuming extractions with diethyl ether. Because of the difficulty in chromatographically determining TCA levels, some investigators use GC-ECD for CH, TCE and TCE-G determinations and a modified Fujiwara procedure to quantitate TCA (Sellers et al., 1972c, 1978). Others use head space analysis for quantitation of CH and its metabolites (Breimer, 1974). Since this type of analysis requires that the analytes have low vapour pressure, it is necessary to convert CH to an aldehyde and to methylate the TCA prior to analysis. One of the greatest disadvantages of

this procedure, other than the specialized equipment required, is the volume of blood, at least three mL, required for each analysis.

Clearly none of the preceding procedures could be effectively utilized for the quantitation of CH or its metabolites in paediatric patients. However, a recent report describes a procedure that is not only simple but rapid, specific and sensitive (Gorecki et al., 1990). This method utilizes electron capture detection and a specialized gas chromatographic packing material initially developed for the separation of free fatty acids. Trichloroacetic acid is derivatized by methylation with diazomethane in this procedure. This method requires only 50 µL of plasma for the determination of CH and its unconjugated metabolites. A further 50 µL of plasma was required for determination of TCE-G concentrations by enzyme hydrolysis.

The analysis of the conjugated metabolite TCE-G by a direct GC method has been described (Ikeda et al., 1984), but is usually accomplished by enzymatically hydrolysing the glucuronide and determining total TCE. The concentration of TCE-G can then be calculated from the difference between the total TCE (post-hydrolysis) and the free TCE (pre-hydrolysis).

2.2 Pharmacokinetics in the Paediatric Patients

2.2.1 Absorption

In order to pass through biological membranes (i.e. lipid bilayers) a drug must be in a chemically unionized form. This depends both on the pKa of the drug and the pH of the surrounding milieu. The acid/base status in the adult gastrointestinal tract has been well characterized and is relatively constant if no complicating pathophysiological factors exist. However, in the neonate, the stomach pH varies widely. In normal full term infants, hydrochloric acid production is detectable shortly after birth (Grand et al., 1976; Besunder et al., 1988) and gastric acidity increases rapidly from about pH 5.6 at birth to pH 2.2 six hours later (Ebers et al., 1956). Except for a poorly understood and transient decrease at approximately one month, acid output increases progressively, until adult levels (i.e. lower level of normal adult values) are reached at three months of age (Agunod et al., 1969). In preterm newborns, acid production is initially lower than in full term infants but becomes equivalent by four weeks of age. The gestational maturity at birth appears to have no influence on this "catch-up" phenomenon (Hyman et al., 1985). The increase in acid output can be correlated with postnatal but not postconceptual age and thus is thought to be linked to postnatal extra-uterine stimuli (Hyman et al., 1985).

Most drug absorption, in infants as well as in adults, takes place in the small intestine because of its greater surface area, permeability and blood flow. As a result, gastric emptying and intestinal motility can affect absorption. In adults, the rate of gastric emptying for liquids approximates a first order process with an emptying half-time of 30 minutes (Rowland & Tozer, 1989). This process can be markedly affected by a variety of factors including posture, drugs and the presence of food. Neonatal gastric emptying can also be affected by food. Cavell (1979, 1981) noted that formula-fed infants (both preterm and term) had significantly slower gastric emptying rates than those fed human milk. In addition the majority of the infants displayed a biphasic emptying pattern. The reason for this phenomenon remains obscure but in all cases the rate of gastric emptying was significantly slower than in the adult. Adult values for gastric emptying rate are thought to be achieved by approximately 6-8 months of age, although this has not been demonstrated unequivocally (Besunder et al., 1988). Consequently, one would predict that, given equal doses and assuming equivalent first-pass effect, peak plasma drug levels in infants would occur later and be lower than in adults. Therefore, neonates dosed according to adult guidelines may not achieve therapeutic levels of a drug. Slower absorption may also explain why, in some cases, infants demonstrate a greater

tolerance to "toxic" doses of drugs.

Duodenal contractions are significantly reduced in premature infants (less than 29 weeks gestation) but in full term neonates are equal to those in fasting adults (Morriss et al., 1986). Overall, however, intestinal motility is still considerably slower and more erratic in newborns than in adults (Kearns & Reed, 1989). Absorption of a number of physicochemically unrelated substances with heterogeneous absorption mechanisms all demonstrate a decreased rate but unchanged extent of absorption in neonates as compared to older children. This apparently cannot be accounted for by gastric emptying and motility since the stomach emptying rate for the drug was equivalent in both study groups and metoclopramide treatment did not negate the age-dependent differences in absorption rate. Fundamental physiological differences may exist in the absorptive surface of the intestine of neonates which may account for their lower rates of absorption (Heimann, 1980). Unfortunately this aspect of absorption has not been thoroughly investigated.

Differences in enzymology of the neonatal gut, although not directly related to absorption, can play a major role in the bioavailability of certain drugs. These differences can arise from variation in gut colonization by intestinal flora (MacLeod & Radde, 1985) or from quantitative developmental differences in the naturally

occurring enzymes (Grand et al., 1976). One such increase in naturally occurring endogenous enzymes appears to be β-glucuronidase. Intestinal deconjugation of glucuronides is elevated in neonatal animal models (Kandall et al., 1973) and this could increase the availability of drugs since the unconjugated drug can be reabsorbed.

2.2.2 Distribution

Deposition of subcutaneous fat is most rapid in the third trimester of pregnancy. Therefore it is not surprising that in premature newborns a higher percentage of their total weight is comprised of lean body tissue than in full term infants. The percentage of body weight that is lean tissue decreases significantly with increased gestational age (Petersen et al., 1988). The percentage of body weight accounted for by total body water varies inversely with the amount of adipose tissue present. The fetus which has only about 1% fat, is 92% water by weight, 65% as extracellular fluid and 25% as intracellular fluid (Besunder et al., 1988). In infants born after full term gestation, total body water comprises 75% of body weight (80% in premature infants). This percentage decreases to adult levels by about three months of age (Edelman et al., 1952; Friis-Hansen, 1961, 1983). During maturation, the intracellular water gradually increases from 35% to 40% of body water while the extracellular fluid declines to the

adult level of 20% from an initial value of 40% (Friis-Hansen, 1983). In neonates, drugs which distribute into the total body water (i.e. polar and hydrophilic) will have a larger volume into which to distribute. Conversely drugs that are non-polar and lipophilic will have a smaller apparent volume of distribution in neonates due to their limited amounts of adipose tissue.

2.2.2.1 Protein binding

Until the 1960's, protein binding was not considered to be of major importance in drug therapy. It is now recognized as one of the major determinants of distribution, elimination and therapeutic or toxic action. A variety of classes of drugs have been examined for differences in protein binding between adults and neonates. In one study, 19 of the 20 drugs tested exhibited decreased protein binding in neonatal plasma over a wide range of concentrations (Kurz et al., 1977a). Similar findings have been reported for several other drugs (Taburet et al., 1982; Nau et al., 1984; Groce et al., 1985; Friel et al., 1987). Reduced protein binding can be attributed to either decreased binding affinity (i.e. weaker binding between drug and protein molecule) and/or decreased binding capacity (i.e. lower protein concentrations or fewer binding sites). Total protein and albumin concentrations are lower in cord (neonatal) blood than in adult blood and

the albumin levels appear to be proportional to gestational age (Ecobichon & Stephens, 1973; Wallace, 1976; Kurz et al., 1977b; Nau et al., 1984, Table 2.2). Thus it is expected that the binding of drugs in neonates is more

Table 2.2 Mean (± SD) concentrations of serum albumin, bilirubin and free fatty acids (FFA) in neonates and adults[1]

Serum source	Albumin (g/L)	Bilirubin (mg/100 mL)	FFA (μM)
Adult volunteers	40.2 ± 6.6 (12)	0.5 ± 0.2 (12)	555 ± 263 (24)
Neonate (Day 1)	29.2 ± 4.3 (9)	2.6 ± 0.9 (12)	1103 ± 515 (13)
Neonate (Day 2)	30.6 ± 4.6 (11)	4.5 ± 2.2 (12)	985 ± 323 (13)
Neonate (Day 3)	ND	7.7 ± 2.4 (13)	909 ± 215 (12)
Neonate (Day 4)	33.6 ± 4.3 (12)	6.4 ± 2.8 (12)	620 ± 199 (13)
Neonate (Day 6)	34.4 ± 3.8 (8)	5.9 ± 4.1 (10)	566 ± 85 (13)

[1] data from Nau et al., 1984
() numbers in parentheses indicate number of patients
ND not determined

capacity limited than in adults and while this is without doubt an important consideration, it cannot entirely account for all the noted differences. Adult and neonatal plasma should, if all the proteins are biochemically equivalent, bind drugs to the same extent if their protein

concentrations are the same. This is not the case (Kurz et al., 1977a) and thus it is assumed that either some intrinsic biochemical differences exist between neonatal and adult plasma proteins or that endogenous substances are present in the neonate which could alter binding affinity (Kurz et al., 1977a, b; Besunder et al., 1988).

Quantitatively the single most abundant binding protein in the plasma is albumin (Rowland & Tozer, 1989). Therefore much of the work in the area of plasma protein binding has been directed toward this molecule. Isoelectric focusing studies found fundamental differences between adult and neonatal albumin and demonstrated significant differences in the amino acid ratios (Wallace, 1977). There are also differences in the binding sites on the molecule. Two distinct binding sites have been identified on albumin: Site I and Site II (Sudlow et al., 1975). Studies with monoacetyl-diamino diphenylsulfone (MADDS; a deputy ligand for bilirubin) and diazepam indicated that these two compounds are independently bound consistent with two proposed binding sites (Brodersen, 1981). Binding affinity of neonatal albumin is significantly less than adult albumin for MADDS and warfarin but diazepam binds with equal affinity (Brodersen & Honoré, 1989). These findings may explain the decreased binding of bilirubin noted in isolated neonatal albumin (Alayoff et al., 1980).

Contrary to what would be expected from the aforementioned study (Brodersen & Honoré, 1989), the binding of diazepam is lower in neonates than in adults. The reduced binding capacity resulting from lower protein concentrations and the presence of endogenous compounds which can compete with the drug molecules contribute to the lower binding of diazepam. The two substances which may compete with drugs for protein binding are bilirubin and free fatty acids (Notarianni, 1990) which occur at much higher levels in neonatal blood than in adults (Nau et al., 1984)(Table 2.2). Increased levels of both bilirubin and free fatty acids have been correlated with higher free fractions of various drugs such as diazepam and phenytoin (Besunder et al., 1988).

Albumin is the most important plasma protein for binding acidic type drugs. Basic drugs, on the other hand, are preferentially bound to α-1 acid glycoproteins (Rowland & Tozer, 1989). Much less research has carried out with the latter, but once again, concentrations of these proteins are lower in neonatal plasma and thus drug binding capacity is decreased. Binding of propranolol and lidocaine are both correlated with levels of α-1 acid glycoprotein in neonatal plasma (Wood & Wood, 1981). α-fetal proteins (Notarianni, 1990) and globulins (Kurz et al., 1977a; Notarianni, 1990) may also be important for drug binding in neonatal plasma. More work is required for

a proper assessment of the relative significance of these proteins to binding of drugs in neonates.

In general, the plasma protein binding of drugs, both affinity and capacity, is reduced in infants.

2.2.3 Elimination

2.2.3.1 Biotransformation

The liver, because of its high enzyme density and blood flow, is recognized as the major site of biotransformation but other tissues such as the gut, lung, kidney, adrenal gland and skin may also be important (MacLeod & Radde, 1985). In the past, most metabolites were considered to be inactive but there are several examples of active (therapeutic or toxic) metabolic products being produced.

The rates of maturation of the various drug metabolizing systems vary amongst individuals and certainly amongst species. In addition, exposure *in utero* to inducing agents may alter the relative rates of development of various enzyme types. These factors make predictions of biotransformational maturity, based solely on postnatal age, very difficult (Stewart & Hampton, 1987). Nevertheless, three processes have been identified as being crucial to biotransformation: xenobiotic uptake into the cell, Phase I metabolism and Phase II metabolism (MacLeod & Radde, 1985; Besunder *et al.*, 1988).

In order for biotransformation to occur the drug molecule must be taken up by the metabolizing cell. An important determinant in the uptake process is ligandin, a protein found in liver, kidney and intestine, which is capable of binding endogenous substances (e.g. bilirubin) and xenobiotics (Besunder et al., 1988). The levels of this protein are low in the newborn, and adult levels are not attained until the infant is approximately 5 to 10 days of age (Reed & Besunder, 1989).

Phase I metabolism consists of a variety of non-synthetic processes: oxidation, reduction and hydrolysis. Ethical and methodological constraints have often made it necessary to extrapolate results from animal species. This is not an entirely acceptable situation since significant differences in biotransformation are known to exist between species and even between strains (Parke, 1984) however it is unavoidable and must be tolerated until more acceptable human data are available.

The cytochrome P-450 mixed function oxidases are quantitatively the most important of the oxidizing enzyme systems (Juchau, 1990). Oxidative capacity of neonates is markedly reduced at birth (50-70% of adult)(Stewart & Hampton, 1987; Reed & Besunder, 1989; Juchau, 1990) and this is reflected clinically by their limited ability to metabolize such drugs as diazepam and amobarbital (MacLeod & Radde, 1985). The P-450 mediated oxidative processes

increase to adult levels at variable rates, beginning shortly after parturition (Juchau, 1990). Cytochrome P-450 concentrations are thought to reach adult levels of activity by six months of age (Reed & Besunder, 1989). Other oxidative enzyme systems have been much less studied. There has been one report that alcohol dehydrogenase activity is present in fetal liver at 3-4% of adult activity. This activity appeared to increase in a linear fashion until the age of about five years when adult activity was reached (Pikkarainen & Raiha, 1967)

Studies of reduction and hydrolysis as biotransformational routes are rare in the neonate and little definitive information exists (Juchau, 1990). The development of blood esterase activity has been reported by Ecobichon and Stephen (1973). Pseudocholinesterase and arylesterase activities increased from 28 weeks gestation to one year at which point they reached adult levels. The rates of hydrolysis in both premature and full term infants were significantly lower than in older children and adults.

Phase II metabolism, increases the polarity of both endogenous (e.g. bilirubin, steroids) and exogenous (e.g. drugs) substances so they may be more easily excreted. Conjugation is catalyzed by a number of transferase enzymes, both membrane bound and soluble, which bind endogenous substrates (e.g. glucuronic acid, sulfate, glycine) to the available functional groups (e.g. hydroxyl,

carboxyl, amino or sulfhydryl). This takes place, for the most part, in the liver although transferase activity has been found in other tissues (Juchau, 1990, MacLeod & Radde, 1985). Glucuronidation is the most important of the phase II reactions in vertebrates because of the number of functional groups which it can act upon. Its synthetic capacity is not limited by a finite store of its endogenous substrate (glucuronic acid). Sulphation and glycine conjugation are much more limited in their activity (Dutton, 1978).

One of the most widely recognized facts about neonatal biotransformation is that glucuronidation is limited in newborns especially if they are premature. Compounds which are predominantly eliminated via glucuronidation have prolonged elimination half-lives in neonates (Cummings & Whitelaw, 1981; Besunder et al., 1988). Compounds such as acetaminophen, which are primarily glucuronidated in the adult, are eliminated via sulphation in the neonate (Levy et al., 1975; Miller,RP et al., 1976). From the little data available, sulphation in contrast to glucuronidation appears to function at an adult level from the time of birth (Juchau, 1990).

2.2.3.2 Renal Excretion

Drugs and their metabolites can be excreted from the body by a variety of routes (e.g. biliary, pulmonary)

however the majority are excreted by the kidney. Renal handling of drugs involves three mechanisms: glomerular filtration, tubular secretion and tubular reabsorption.

Renal blood flow in the fetus is low due to high vascular resistance in the kidney (MacLeod & Radde, 1985). After birth, this vascular resistance decreases and cardiac output increases resulting in an increase in blood flow to the kidneys (West et al., 1948). In newborns, the kidneys receive only 5-6% of the total cardiac output, while 15-25% reaches the kidneys in the adult (Hook & Bailie, 1979). Renal plasma flow in the first postnatal week is approximately 12 mL/min and increases to 140 mL/min by the end of the first year. If these data are normalized for body surface area, adult levels of renal perfusion may be attained by as early as 7-8 months of age (West et al., 1948). The increase in blood flow appears to be proportional to the development of the renal tubules (West et al., 1948) but the clinical implication of this in humans is unclear (Besunder et al., 1988).

Glomerular filtration is an energy independent process dependant on the hydrostatic pressure from cardiac output (Smith & Nelson, 1976). The rate of glomerular filtration (GFR) in neonates is well below that of adults even when normalized for body size (West et al., 1948; Guinard et al., 1975; Smith & Nelson, 1976; Arant, 1978; MacLeod & Radde, 1985). The GFR, as determined by creatinine

clearance at the time of birth, is linearly related to gestational age in neonates with a gestational age of 34 weeks or more. In infants between 28 and 34 weeks gestation, the GFR remains constant at approximately one mL/min (Arant, 1978). The reason for this phenomenon remains unclear although one possible explanation is that 34 weeks gestation is the approximate time in gestational development when glomerulogenesis is complete (Arant, 1978). Postnatally, the GFR increases until adult levels are reached at between 2.5 and 5 months of age (West et al., 1948). The increase in GFR is likely attributable to the previously mentioned changes in kidney perfusion (ie. increased cardiac output and decreased peripheral vascular resistance), an increased surface area for filtration and increased membrane pore size (Morselli et al., 1980; MacLeod & Radde, 1985; Besunder et al., 1988). Tubular functions of the kidney, both secretory and reabsorptive, are active processes and require energy in order to function (Smith & Nelson, 1976). Physiologically, active reabsorption is important for nutrients such as glucose which are virtually completely reabsorbed in the adult (Guyton, 1986). The transport maximum (Tm) for glucose is lower in neonates than in adults (Smith & Nelson, 1976) which results in significantly increased amounts of glucose in the urine of neonates (Arant, 1978). Tubular reabsorption of glucose is lowest in infants less than 34

weeks gestation and increases with gestational age (Arant, 1978). Tubular secretion mechanisms are also important physiologically. Two transport-limited pathways, one for secretion of organic acids and another for secretion of organic bases, have been identified. If the development of tubular secretion is judged by the ability to secrete para-amino hippuric acid (PAH), tubular secretion in the newborn infant is deficient (Smith & Nelson, 1976). The Tm for PAH increases approximately ten-fold in the first year of life. Adult levels of PAH tubular secretion (mL per m^2 surface area) are achieved at approximately 30 weeks of age (West et al., 1948). Several reasons for decreased tubular function due to immaturity have been suggested; e.g. the small size of the tubules, smaller mass of functioning tubules, reduced blood flow and immaturity of energy supplying processes (Besunder et al., 1988).

Generally, renal immaturity results in decreased clearance and increased half-lives of drugs that are primarily eliminated by the kidney.

2.3 Study Objectives

It is clear there is a scarcity of information about CH in the paediatric age group. Therefore, the study objective, in its most basic and broadest form was to investigate the disposition of CH within a critically ill paediatric patient population. It was obvious at the outset of the project that the scope of such an undertaking was well beyond that which could be accomplished in the course of a single thesis.

The specific objectives were:

1. To investigate if CH use for maintenance sedation in NICU and PICU patients has any potential toxicological implications;
2. To determine if the pharmacokinetics of CH differ between neonates and older infants;
3. To investigate the plasma protein binding of TCA in neonatal and adult plasma including the interaction of furosemide with TCA;
4. To investigate if plasma concentrations of TCE accepted as efficacious for sedation in adults are adequate for neonates.

3.0 EXPERIMENTAL

3.1 Preparation of Diazomethane

Diazomethane (for the methylation of TCA) was prepared using a commercial generator apparatus (Diazald Kit, Aldrich). All glass fittings were smooth and the procedure was carried out in a well ventilated fume hood behind a glass safety sash. A solution of Diazald® (N-methyl-N-nitroso-p-toluene sulfonamide; Aldrich), 4.3 g in 50 mL distilled diethyl ether (glass distilled; BDH), was added gradually through a dropping funnel to a heated (65° C) mixture containing 5 mL of ethanol (95%), 1 g of potassium hydroxide and 1.6 mL water. A further 10 mL of ether was then added and distillation was continued until the distilling ether was colourless. The yield of the reaction is approximately 50-60 mL of diazomethane. The resulting deep yellow ethereal solution of diazomethane was stored at -20° C in a tightly corked Ehrlenmeyer flask in an explosion proof freezer and used as required. Diazomethane was prepared approximately once per month to ensure optimum derivatizing capacity.

3.2 Gas Chromatographic System

Gas chromatography was performed on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni electron-

capture detector and a coiled glass column (2.44 m x 2 mm ID) packed with GP 10% SP 1200/1% H_3PO_4 on acid washed 80/100 mesh Chromsorb W. (Supelco). The column was conditioned in the following manner: 1) The analytical column was installed at the injector port of the GC; 2) A carrier gas flow of approximately 30 mL/min was established through the column and this was maintained for at least 30 minutes at ambient temperature; 3) The GC was then programmed to increase the column temperature at 2° C/min until a final temperature of 175° was attained; 4) The final temperature was maintained for 18-24 hours; 5) The column was cooled to ambient temperature and attached to the detector.

Analyses for chloral hydrate and its metabolites were carried out isothermally with injection port, column oven and detector temperatures of 160°, 115° and 300° C respectively. Argon-methane (95:5; Acklands) was used as a carrier gas at a flow rate of 12 mL/min.

A Shimadzu Model CR3A, CR5A or CR601A (Shimadzu) integrator-recorder was used for peak height determinations of the analytes.

3.3 Analysis of Biological Samples

3.3.1 Determination of Chloral Hydrate and its Unconjugated Metabolites

3.3.1.1 Handling and Storage of Plasma Samples

Blood samples (200-500 µL) were collected either from indwelling arterial lines or by "heel prick". The blood was immediately placed in a heparinized evacuated blood container (Venojet® or Vacutainer®) and put on ice in the refrigerator. Samples were transferred to the laboratory within one hour where the plasma was separated from the red blood cells by centrifugation (1000 x g; 10 minutes) and stored (capped 1.5 mL polypropylene centrifuge tubes at -70°C) until analyzed.

3.3.1.2 Handling and Storage of Urine Samples

Total urine voided was collected for 24 hour periods in the Pilot and Single Dose Studies. This was only possible from patients with catheters or urine collection bags in lieu of standard diapers. The urine was stored in brown glass bottles on ice during the 24 hour collection period. Total urine volume for the 24 hour period was determined with a volumetric cylinder and if necessary was adjusted according to nursing notes reporting spillage and volumes removed for clinical biochemistry tests. If

sufficient volume was available, two aliquots (≈ 5-10 mL) were stored at -70° C in scintillation vials (polypropylene; BDH) until analyzed.

3.3.1.3 Extraction of Samples

Plasma (50 µL) was added to a PTFE-lined screw-capped test tube (100 x 16 mm; Kimax, Canlab) along with 50 µL of an ethanol solution containing the internal standard (1,2,3-trichloropropane; 500 µg/mL), distilled deionized water (250 µL), sulfuric acid (250 µL; 3 M) and freshly distilled diethyl ether (5.0 mL). The tube was capped and mixed vortically for 20 minutes (Vibrax® mixer; Terochem) and centrifuged (5 min; 2000 g). An aliquot (2.0 mL) of the organic layer was transferred to another test tube (100 x 13 mm; Kimax, Canlab). For TCA analysis, 100 µL of diazomethane (diluted 1:6 with distilled diethyl ether) was added and the tube was mixed for approximately 10 seconds either by hand or vortically (Vortex®). After mixing, tubes were allowed to stand at room temperature for at least 10 minutes before analysis.

Aliquots (1 µL) of the ether extract were injected onto the GC column prior to diazomethane derivatization, for CH and TCE analysis, and after derivatization for TCA analysis.

3.3.1.4 Assay Calibration

Standard calibration curve samples were prepared at the time clinical samples became available and were stored in an identical manner to the clinical samples. New stock solutions of each analyte (CH, TCE, and TCA) were made each time a standard curve was prepared.

Standard curves were prepared by adding each compound to 9970 µL of drug free human plasma (Red Cross) (CH, TCE and TCA; 10 µL of 1.0 g/mL stock solutions prepared in absolute ethanol) to give a plasma concentration of 1000 µg/mL in a total volume of 10 mL of plasma. The stock plasma was then diluted serially with drug-free plasma to produce the required concentrations ranges of 0.25 µg/mL-200 µg/mL. Standard curves ranged from 2.5-200 µg/mL for TCA, 0.25-25.0 µg/mL for CH, and 0.25-200 µg/mL for TCE. Samples for the standard curve determination were extracted as previously outlined (3.3.1.2). Calibration curves were constructed by plotting the ratio of the peak height of each of the components (CH, TCE or TCA) to that of the internal standard against their respective concentration and analyzing the data with a logarithmic-logarithmic curve fitting procedure ($\ln y = \ln B + M \ln x$; Hewlett Packard 27S Scientific calculator).

Assay variability was assessed by a comparison of standard calibration curve parameters and/or individual peak height ratios.

3.3.2 Trichloroethanol-Glucuronide Analysis

3.3.2.1 Preparation of Sodium Acetate Buffer

A sodium acetate buffer (pH 5.2) was prepared by combining 1.05 mL of 2M acetic acid with 3.95 mL of 2M sodium acetate in a total volume of 100 mL. The acetic acid was prepared by diluting 11.55 mL of acetic acid with distilled deionized water to a total volume of 100 mL. The sodium acetate solution was prepared by dissolving 27.2 g of sodium acetate trihydrate in sufficient distilled deionized water to yield 100 mL.

Sodium acetate buffer, acetic acid and sodium acetate were stored at 4°C until required.

3.3.2.2 Incubation with β-glucuronidase

Helix pomatia β-glucuronidase (≈130,000 units/mL; Sigma) was used to hydrolyze trichloroethanol-glucuronide. Plasma samples (25-50 µL), acetate buffer (150 µL) and β-glucuronidase (50 µL) were combined in a culture tube (100 x 16 mm; Kimax). The tube was capped (lightly) and incubated at 37°C for six hours with gentle agitation in a shaking water bath (Dubnoff). Samples were diluted, if necessary, to ensure complete hydrolysis of the β-glucuronide linkage under the conditions outlined above.

3.4 Clinical Studies

Subjects for clinical studies were recruited from the patient population in the NICU of PICU. After the child was identified by a senior staff physician as a potential candidate (i.e. clinically stable, accessible blood supply/arterial line), patients were enrolled in the clinical studies if they were likely to be in hospital for the duration of the proposed study period and only if informed parental consent was obtained. Consent forms are included in Appendix B.

3.4.1 Pilot Study

To be eligible for the pilot study patients must have received at least five doses of CH within a 24 hour period. The dose of CH administered to the patients was not restricted.

Blood samples (≤200 μL) were drawn by nursing or phlebotomy personnel at 12 hours post dose and every 24 hours for the next 14 days or until the child was discharged from intensive care.

Urine samples were collected for the first 24 hour period following the last CH dose if the patient was catheterized or fitted with a urine collection bag.

3.4.2 Single Dose Study

The population for this study was limited to those children who, in the course of their hospitalization, would require sedation with a single dose of CH.

Blood samples (≤200 μL) were collected at 0 hour (pre-dose), 0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120 and 144 hours after administration of a single 50 mg/kg dose of CH.

Urine samples were collected for 24 hour periods following CH administration if the patient was catheterized or fitted with a urine collection bag.

3.4.3 Multiple Dose Study

Patients enrolled were expected to require a multiple dose regimen of CH in the course of their treatment. All patients enrolled in the study received CH 50 mg/kg q4-6 prn. However, no limitation was placed on the number of doses received for inclusion in this study.

Blood samples (≤200 μL) were collected immediately before the administration of each dose of CH and at 0.5 and 4.0 hours post dose. Following the final dose of the drug blood samples were collected when possible every 48 hours for six days. Opportunistic blood samples (i.e. taken when blood was being drawn for a clinical test) were also obtained during this time period.

3.4.3.1 Sedation/Agitation Scale

An adequate objective and non-invasive method for the assessment of the state of sedation and/or agitation in a preverbal patient could not be identified from the literature. Therefore, a scale was developed by members of the Perinatal Research Laboratory and Department of Pediatrics at Royal University Hospital (RUH), based upon the earlier work of Baker and Nisbet (1973) (Appendix C). The scale was used to assess the level of sedation or agitation of patients immediately prior to blood sampling or the administration of a subsequent dose of CH. It employed five subjective and objective observations (activity, heart rate, blood pressure, respiratory parameters and ventilator responses) which could be made easily and rapidly by the bedside nurse. Each observation or measurement was scored on a scale of 1-5 based on the established criteria. The overall sedation/agitation score was the summation of the five individual scores. A low score was indicative of a relatively sedated child while a high score denoted that the patient appeared agitated.

The variability between evaluators in assessing sedation scores using the scale was tested in a controlled study (i.e. study participants were unable to communicate during the assessment of the infants' sedation levels) in which five children were assessed on two separate occasions by six members of the intensive care unit medical and

nursing personnel.

3.5 Plasma Protein Binding of Trichloroacetic Acid

3.5.1 Ultrafiltration Procedure

A micropartition system (MPS-1; Amicon Corp.) consisting of a centrifuge and an anisotropic hydrophillic YMT ultrafiltration membrane was used to separate bound from free TCA. With this system plasma samples as small as 150-200 µL could be used for binding experiments. The binding apparatus was assembled by placing a dry YMT membrane (shiny side up) into the support base, placing the silicon rubber O ring on top of this and then affixing the sample reservoir on top of the O ring with the supplied plastic clips. The filtrate cup was then snapped onto the bottom of the support base (Figure 3.1). The plasma sample was introduced with a micropipettor by holding the apparatus at a 45 degree angle with the pipet held at a slight angle and the pipet tip touching the reservoir wall. Once the sample was introduced the reservoir was capped and the entire apparatus was placed into a fixed angle centrifuge rotor. Samples were spun at 2000 x g for 10 minutes. After centrifugation, the apparatus was carefully removed and the ultrafiltrate was removed from the filtrate cup using a pasteur pipet and placed in a 1.5 mL polypropylene centrifuge tube for storage at -70° C until GC analysis.

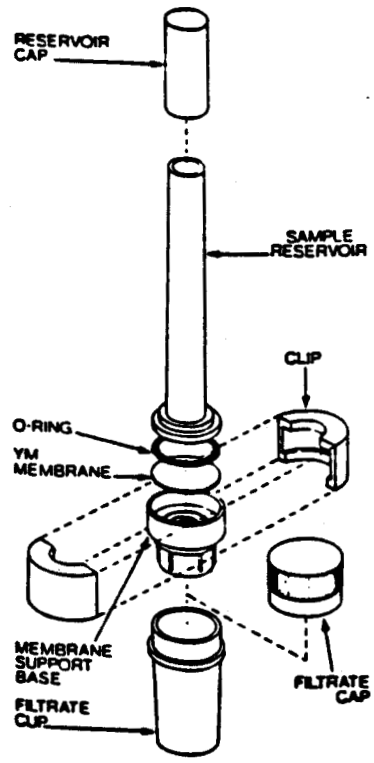

Figure 3.1 Ultrafiltration apparatus (Amicon)

All parts of the filtration apparatus were washed with a detergent solution (Decon®; BDH), rinsed several times with distilled water, air dried and then reused. The filtration membranes were used only once.

3.5.2 Method Optimization Procedure

In initial tests, the concentration of free TCA was determined in the ultrafiltrate after 5, 10 and 15 minutes of centrifugation. Non-specific binding of TCA to the binding apparatus was assessed using two experiments. In the first, aqueous TCA solutions were prepared and ultrafiltered in a normal manner. The TCA ultrafiltrate

concentration was compared to the pre-filtration sample concentration. In the second experiment, TCA solutions were prepared in plasma but the semi-permeable membrane was mechanically disrupted prior to ultrafiltration. Trichloroacetic acid concentrations in the resulting filtrate were compared to those in the non-filtered plasma samples. Time required for a ligand-macromolecule binding equilibrium to be established was assessed by comparing the peak height ratios of the ultrafiltrate (plasma water) obtained from plasma samples incubated at 37°C for one, two and three hours. The effect of freezing and thawing on binding characteristics was assessed by preparing samples in fresh plasma and determining the free fraction before freezing and after freezing and thawing. Fresh heparinized plasma and bag plasma (Red Cross) were evaluated to determine if the source of plasma affects TCA binding.

At least three replicates were used in all of these experiments. The concentrations of TCA used were 7.5, 75 and 300 µg/mL.

3.5.3 Sample Preparation

3.5.3.1 *In vitro* Studies

Plasma for *in vitro* protein binding was collected from three sources: a healthy adult volunteer, cord blood and neonatal exchange transfusions. These samples were collected as they became available and stored frozen in

borosilicate glass scintillation vials (20 mL; Canlab) until the binding samples were prepared. A series of samples (seven concentrations) containing TCA (5-200 µg/mL) were prepared by a serial dilution technique similar to that outlined for standard curves (3.3.1.4) with the exception that water, rather than ethanol, was used for the initial dissolution of the TCA.

Once prepared, the samples were placed in two mL borosilicate glass vials (Fisher) and stored frozen (-70° C) until the binding experiments were performed. For binding experiments, plasma samples were allowed to thaw at room temperature and then subsequently transferred to a 37° C walk-in incubator unit (Bally Engineering) where they were allowed to equilibrate for one hour with gentle mixing (multipurpose rotator; Scientific Industries Inc). Ultrafiltration of samples was done in triplicate using 150 µL of plasma. Centrifugation time was 10 min and the ultrafiltrate was stored at -70° C until analyzed by GC.

3.5.3.2 Ex vivo Studies

Plasma to determine binding ex vivo was collected from a healthy adult volunteer who had ingested 1000 mg of CH and from paediatric patients who received CH in the normal course of their treatment. The binding procedures were essentially identical to those outlined above. The volume of plasma available from paediatric patients was not

sufficient for triplicate analyses; whenever possible, duplicate determinations were performed.

3.5.4 Gas Chromatographic Analysis of Ultrafiltrate

3.5.4.1 Preparation of Standards and Samples

The preparation of standards and samples was identical to that reported previously (3.3.1.4). However, water, rather than drug-free human plasma, was used in the preparation of the calibration curves.

3.5.4.2 Gas Chromatographic Analysis

The analytical system used for the determination of TCA levels in the ultrafiltrate was identical to that used for analysis of TCA concentrations in plasma. However, the calibration curve range was extended 10 fold to 0.25 μg/mL. Only 50 μL of diazomethane was used for derivatization of TCA.

3.6 Data Analysis

3.6.1 Pharmacokinetics

The apparent first order elimination rate constant (k_d was estimated by log-linear least squares regression analysis using plasma concentrations obtained at the last three to four sampling times. Half-life ($t_{1/2}$) was calculated as $0.693/k_d$. The area under the plasma concentration-time curve (AUC) was calculated using the

linear trapezoidal rule (i.e. the sum of 0-$Cp_{(last)}$ and the tail of the graph ($Cp_{(last)}/k_d$)) for CH and TCE. In the case of TCA the AUC was calculated from 0-24 hours. Apparent oral clearance (Cl/F) of CH was calculated as D/AUC. Renal clearance was calculated by dividing the amount excreted in a given time interval by the plasma concentration at the midpoint of the time interval. The maximum plasma concentration of CH and its metabolites (C_{max}) and the time required to reach the maximum plasma concentration (t_{max}) were also recorded. In the Pilot Study these parameters represent the maximum concentration and the time at which it was achieved during the time period in which blood samples were collected.

In the <u>in vitro</u> protein binding experiments data was plotted according to the method of Rosenthal (1967) (Db/Df vs Db) for the estimation of affinity (-slope: Ka) and capacity (y-intercept: nKa[Pt]) of TCA binding.

3.6.2 Statistics

All results, unless otherwise indicated, are expressed as means ± standard deviation. For the purposes of statistical analysis, patients were grouped as: preterm infants, term infants and PICU patients (> one month postnatal age). The relationship between age group and half-life and age group and AUC for TCE were examined using the Spearman rank correlation test. The data from the

Single dose study was analyzed using oneway analysis of variance and Tukey's multiple range test. In the protein binding studies, multiple linear regression and multiple analysis of variance were employed to test hypotheses of linearity and parallelism of regression lines, respectively. A probability value (p) ≤ 0.05 was considered statistically significant. The Statistical Package for Social Sciences (SPSS) was used for all statistical analyses.

4.0 RESULTS

4.1 Analytical Methods

The analytical procedure used for the determination of CH and its metabolites was similar to that previously reported by our laboratory and since extraction efficiency and assay accuracy studies had been previously determined (Gorecki et al. 1990) these were not repeated.

4.1.1 Determination of CH, TCE and TCA in Plasma

Samples were injected before and after derivatization to ensure that derivatization did not produce any interfering peaks. There were no interfering peaks which co-eluted with the compounds of interest (Figure 4.1). All analytes were well resolved and had retention times of 1.63, 4.39, 6.48 and 9.48 minutes for CH, MTCA (methylated TCA: methyl trichloroacetate), TCP (trichloropropane: internal standard) and TCE respectively (Figure 4.2).

The ranges of the calibration curves utilized in this study were extended beyond those previously reported (Gorecki et al., 1990). Because of these new ranges and the nonlinear ECD response calibration curves were fitted with the previously described logarithmic-logarithmic curve fitting procedure (Section 3.3.1.4) rather than by simple least-squares linear regression.

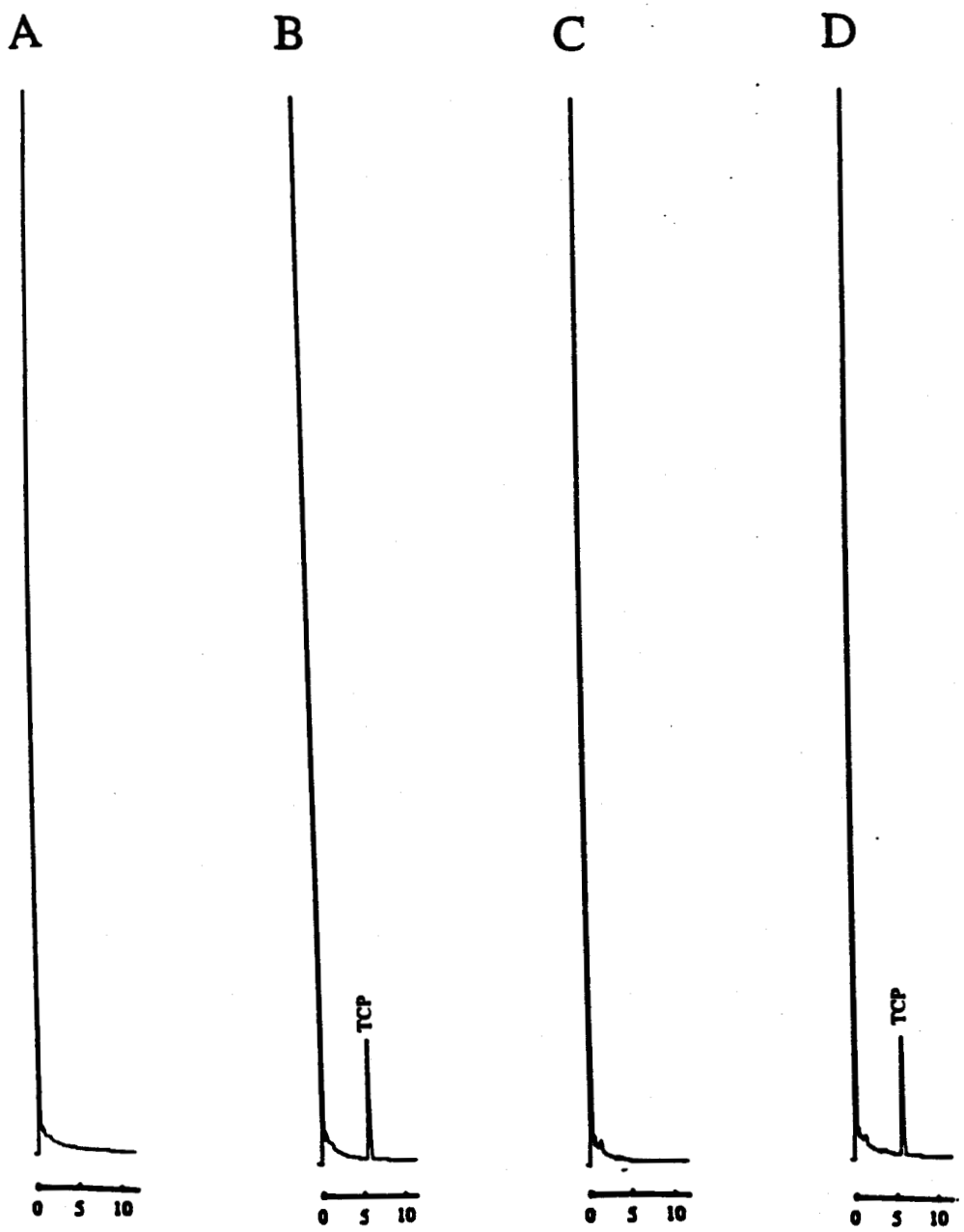

Figure 4.1 Chromatograms for extracts of A) blank plasma; B) blank plasma and 1,2,3-trichloropropane (TCP, internal standard); C) blank plasma derivatized; D) blank plasma derivatized and TCP

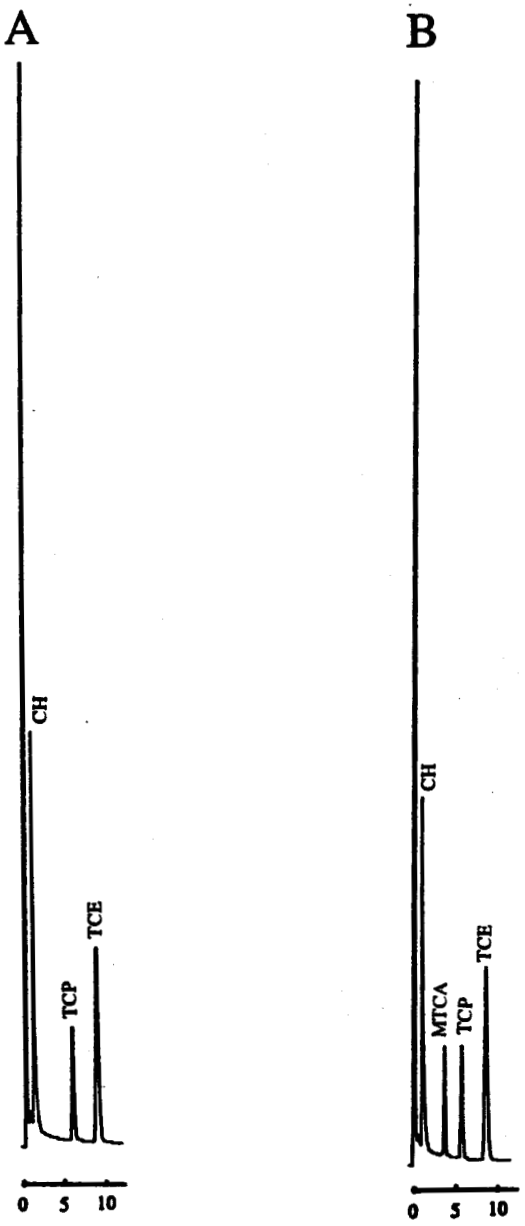

Figure 4.2 Chromatograms for extracts of A) plasma (25 µg/mL) not derivatized: CH=chloral hydrate, TCE= trichloroethanol; B) plasma (25 µg/mL) derivatized: MTCA=methyl trichloroacetate

4.1.2 Determination of CH, TCE and TCA in Urine

No interfering endogenous substances co-eluted with the analytes (Figure 4.3). The retention times were as reported for plasma analysis. Slopes and intercepts of calibration curves in urine and plasma were essentially the same.

4.1.3 Trichloroethanol-Glucuronide Analysis

The glucuronide linkage in TCE-G was hydrolyzed by incubating the sample with acetate buffer (pH 5.2) and β-glucuronidase at 37° C with gentle agitation. The incubation time used was a somewhat shorter than in previously published methods (Gorecki et al., 1990; Sellers et al., 1978). When ≥5000 Sigma units of β-glucuronidase were used there was no increase in the amount of TCE released beyond six hours of incubation time (Table 4.1). Routine analysis was therfore performed using six hours of incubation.

Two varieties of β-glucuronidase were tested: pure crystalline β-glucuronidase derived from bovine liver and β-glucuronidase from Helix pomatia. The differences in peak height ratio using bovine versus Helix pomatia β-glucuronidase were attributable to a systematic endogenous peak from the bovine preparation which co-eluted with TCE (Table 4.1). Because of this analytical problem with the bovine enzyme, only Helix pomatia β-glucuronidase was used

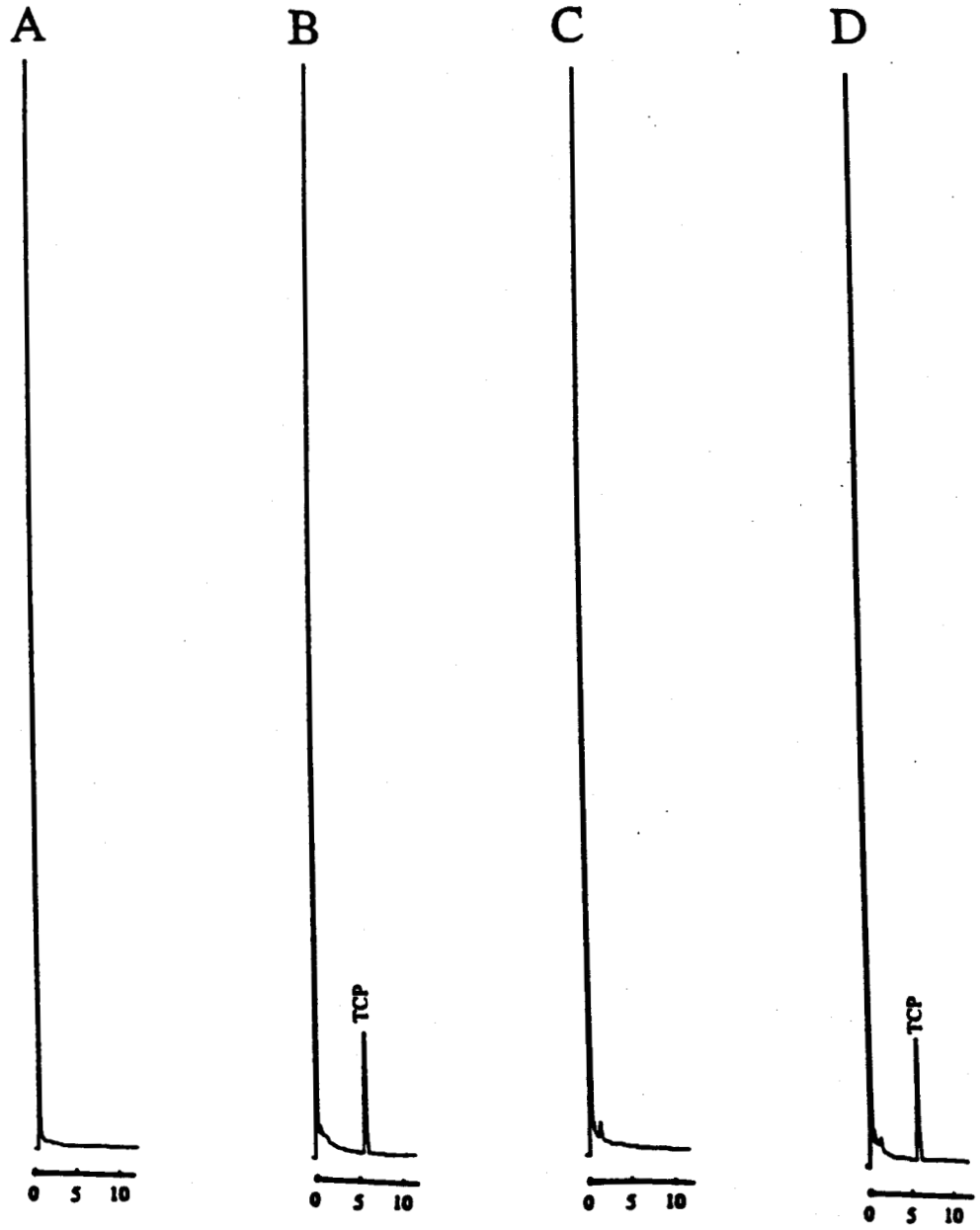

Figure 4.3 Chromatograms for extracts of A) blank urine; B) blank urine and 1,2,3-trichloropropane (TCP, internal standard); C) blank urine derivatized; D) blank urine and TCP

Table 4.1 Effect of incubation time on enzyme hydrolysis of glucuronide linkage of TCE-G

	PEAK HEIGHT RATIOS		
ENZYME	3 HOURS	6 HOURS	18 HOURS
HELIX	1.783 ± 0.117	2.033 ± 0.109	2.026 ± 0.047
BOVINE	2.273 ± 0.107	2.503 ± 0.236	2.528 ± 0.258
NONE	0.175 ± 0.080	0.246 ± 0.083	0.295 ± 0.246

HELIX= Helix pomatia β-glucuronidase (n=3)
BOVINE= Bovine β-glucuronidase (n=3)
NONE= No β-glucuronidase (n=3)

for subsequent experiments.

4.1.4 Analytical Assay Precision

The analytical procedure for the measurement of CH, TCE and TCA was reproducible over the range of their respective calibration curves. The intraday variations for peak height ratios of CH, TCE and TCA were less than 10, 4 and 3% (coefficients of variation), respectively (Table 4.2). Prolonged storage appeared to increase the variability slightly (Table 4.2).

Calibration curves for CH, TCE and TCA all had correlation coefficients of greater than 0.998. The interday variations in the slopes, intercepts or correlation coefficients of the curves were low (Table

Table 4.2 Intraday variability and stability data: Samples analyzed four months and one year after preparation

	PEAK HEIGHT RATIOS					
	AUGUST (4 months)			JANUARY (1 year)		
CONC	CH	TCE	TCA	CH	TCE	TCA
0.5	0.1083 ± 0.0108 (10.0%)	0.0423 ± 0.0028 (6.5%)	ND	0.1124 ± 0.0137 (12.2%)	0.0433 ± 0.0039 (8.9%)	ND
2.5	0.4236 ± 0.0526 (12.4%)	0.1908 ± 0.0043 (2.2%)	0.1398 ± 0.0055 (3.9%)	0.4660 ± 0.0595 (12.7%)	0.1961 ± 0.0052 (2.6%)	0.1366 ± 0.0035 (2.6%)
10.0	1.5897 ± 0.0944 (5.9%)	0.7060 ± 0.0182 (2.6%)	0.5287 ± 0.0105 (2.0%)	1.4215 ± 0.0820 (5.8)	0.6815 ± 0.0299 (4.4%)	0.4964 ± 0.0131 (2.6%)
50.0	8.8696 ± 0.7928 (8.9%)	3.0606 ± 0.0916 (3.0%)	2.4244 ± 0.0569 (2.4%)	8.9615 ± 1.2333 (13.8%)	2.9947 ± 0.0496 (1.7%)	2.3639 ± 0.0471 (2.0%)
200.0	ND	11.7902 ± 0.3437 (2.9%)	10.0022 ± 0.2783 (2.8%)	ND	11.6885 ± 0.5374 (4.6%)	9.8099 ± 0.5030 (5.1%)
MEAN CV	9.3%	3.4%	2.8%	11.1%	4.4%	3.1%

CONC, concentration µg/mL; CV, overall coefficient of variation; () individual coefficient of variation (n=5); ND, not determined

4.3).

Table 4.3 Reproducibility of calibration curves for CH, TCE and TCA

COMPOUND	MEAN	SD	N
CH			
slope	0.9673	0.0273	5
y-intercept	0.1567	0.0251	5
r	0.9987	0.0011	5
TCE			
slope	0.9478	0.0056	5
y-intercept	0.0832	0.0017	5
r	0.9998	0.0001	5
TCA			
slope	0.9836	0.0124	5
y-intercept	0.0656	0.0037	5
r	0.9995	0.0002	5

N = number of days; SD = standard deviation; r = correlation coefficient

4.1.5 Trichloroacetic Acid Plasma Protein Binding

Experiments to determine optimal conditions for protein binding studies indicated that changes in equilibration or centrifugation times did not alter the concentration of TCA in the ultrafiltrate. Likewise freeze/thaw and non-specific binding experiments demonstrated no effect on protein binding (Table 4.4). The

Table 4.4 Effect of experimental conditions on the protein binding (free fraction) of TCA in human plasma

	Trichloroacetic Acid Concentration		
Binding Conditions	**7.5 µg/mL**	**75.0 µg/mL**	**300.0 µg/mL**
Equilibration time	↔	↔	↔
Centrifugation time	↔	↔	↔
Freeze/Thaw	↔	↔	↔
Non-specific binding	↔	↔	↔
Bag vs. fresh plasma	↑	↑	↑

↔ = no change in free fraction concentration of TCA
↑ = increase in free fraction concentration of TCA

source of plasma had a major effect on extent of binding. Drug-free "bag plasma" from the Red Cross resulted in approximately twice as much free TCA when compared to fresh frozen heparinized plasma collected from a drug-free adult volunteer. Only fresh frozen heparinized plasma was used in subsequent in vitro experiments.

4.2 Pilot Study

The Pilot study included one term and four preterm infants in NICU and two patients in PICU who had received a normal therapeutic regimen of five or more doses of CH. Different dosing intervals and dosages were used for each patient (Table 4.5).

Plasma concentration versus time profiles of TCE and TCA for individual patients can be found in Appendix D. In two cases, the children had TCE levels above 100 µg/mL at 12 hours following the final drug administration. Chloral hydrate levels were below the level of quantitation and TCE-G levels, while detectable, were often erratic and thus did not allow for the calculation of elimination rate constants in all cases. Urine sampling proved problematic and appropriate collections were not available from all study participants. In some cases, collections were incomplete. In others, the length of the collection was correct (i.e. 24 hours) but the collection was not done over the same time interval as with the other patients. On

Table 4.5 Dosing regimens for CH pilot study

NEONATAL INTENSIVE CARE UNIT			
SUBJECT	DATE	NUMBER OF DOSES	DOSAGE (mg/kg)
NAG	02/03/89	3	25.0
	03/03/89	2	25.0
	04/03/89	1	25.2
NBG	02/03/89	2	22.5
	03/03/89	3	22.5
	04/03/89	1	22.5
NG	13/02/89	2	36.8
	14/02/89	1	36.8
		1	49.1
	15/02/89	3	49.1
AB	01/03/89	2	23.3
	02/03/89	3	23.3
	03/03/89	1	23.3
		2	29.1
BS	02/03/89	2	28.8
	03/03/89	3	28.8

PAEDIATRIC INTENSIVE CARE UNIT			
SUBJECT	DATE	NUMBER OF DOSES	DOSAGE (mg/kg)
DAD	02/02/89	2	26.4
		2	52.8
	03/02/89	2	52.8
FT	22/02/89	3	95.4
	23/02/89	1	95.4
		2	46.2
	24/02/89	1	46.2

other occasions, there were large unaccountable differences between nursing and laboratory estimates of urine volume. Finally, not all study participants were catheterized or fitted with urine collection bags and thus no collections were available.

The pharmacokinetic parameters for the pilot study are reported in Table 4.6. Since the sample size for this study was small, statistical analyses were not attempted. Concentration maxima (C_{max}) and time at which this is reached (t_{max}) were not used in the conventional manner in this study. The first blood sample was not drawn until 12 hours following the final administration of CH. Thus C_{max} reflects the highest concentration measured during the study period and t_{max} is the time at which it was achieved relative to the administration of the final dose of CH.

4.3 Single Dose Study

The single dose study was conducted in nine preterm and eight term infants in the NICU and five patients in PICU who had received single 50 mg/kg doses of CH.

Chloral hydrate, TCE and TCA in plasma were quantitated over the entire study period, but the levels of TCE-G were low and erratic so pharmacokinetic calculations were not attempted. Urine samples were collected when possible. Initially, collection of the total urine voided over the entire study period was attempted. This, however,

Table 4.6 Pharmacokinetic parameters for pilot study

PARAMETER	COMPOUND	PRETERM[1]	TERM	PICU[2]
$t_{½}$ (h)	TCE	43.98 ± 11.29	16.17	21.06 ± 3.44
	TCE-G	36.69 ± 20.77[2]	ND	19.65 ± 5.71
	TCA	147.79 ± 58.29[2]	121.72	ND
Cmax (µg/mL)	TCE	69.70 ± 31.50	19.82	84.54 ± 38.73
	TCE-G	33.08 ± 10.05	6.15	33.88 ± 21.23
	TCA	89.58 ± 37.95	74.52	520.96 ± 7.11
Tmax (h)	TCE	17.79 ± 6.03	12.90	12.45 ± 0.07
	TCE-G	17.21 ± 7.23	83.47	47.66 ± 49.72
	TCA	179.38 ± 120.43	58.97	108.33 ± 17.09
Cl_r (24 h; mL/h)	TCE	3.22 ± 2.95[3]	15.05	17.67
	TCE-G	11.58 ± 6.41[3]	348.29	ND
	TCA	0.46 ± 0.40[3]	1.02	1.06

1, n=4
2, n=2
3, n=3
ND = Not determined

proved impossible to accomplish for the same reasons as outlined in 4.2. The renal clearance of TCE and TCA were estimated in as many patients as possible for the 0-24 hour period following drug administration (Table 4.7). No significant difference was noted in renal clearance of TCA among the three age groups. Trichloroethanol, however, was cleared significantly faster by PICU patients that either of the NICU patient groups.

Half-life values for CH varied among the study patients. The majority had an apparent half-life of approximately 1 hour. In 2 patients the $t_{1/2}$ could not be calculated since the drug levels were below the lowest concentration quantitated in the standard curve. In several cases, CH appeared to display a "non-monoexponential" curve when its concentration was plotted against time (Figure 4.4A, Appendix E). In these cases the estimated terminal half-life was very long. The half-life for CH was significantly shorter in the preterm group as compared to the PICU group. However, if the patients exhibiting the non-monoexponential elimination were omitted from the analysis, no significant difference in CH half-life was noted among the groups.

Half-life and AUC values for TCE, the reportedly active hypnotic metabolite were significantly different in the three groups. A highly significant negative correlation was observed when age group was plotted against

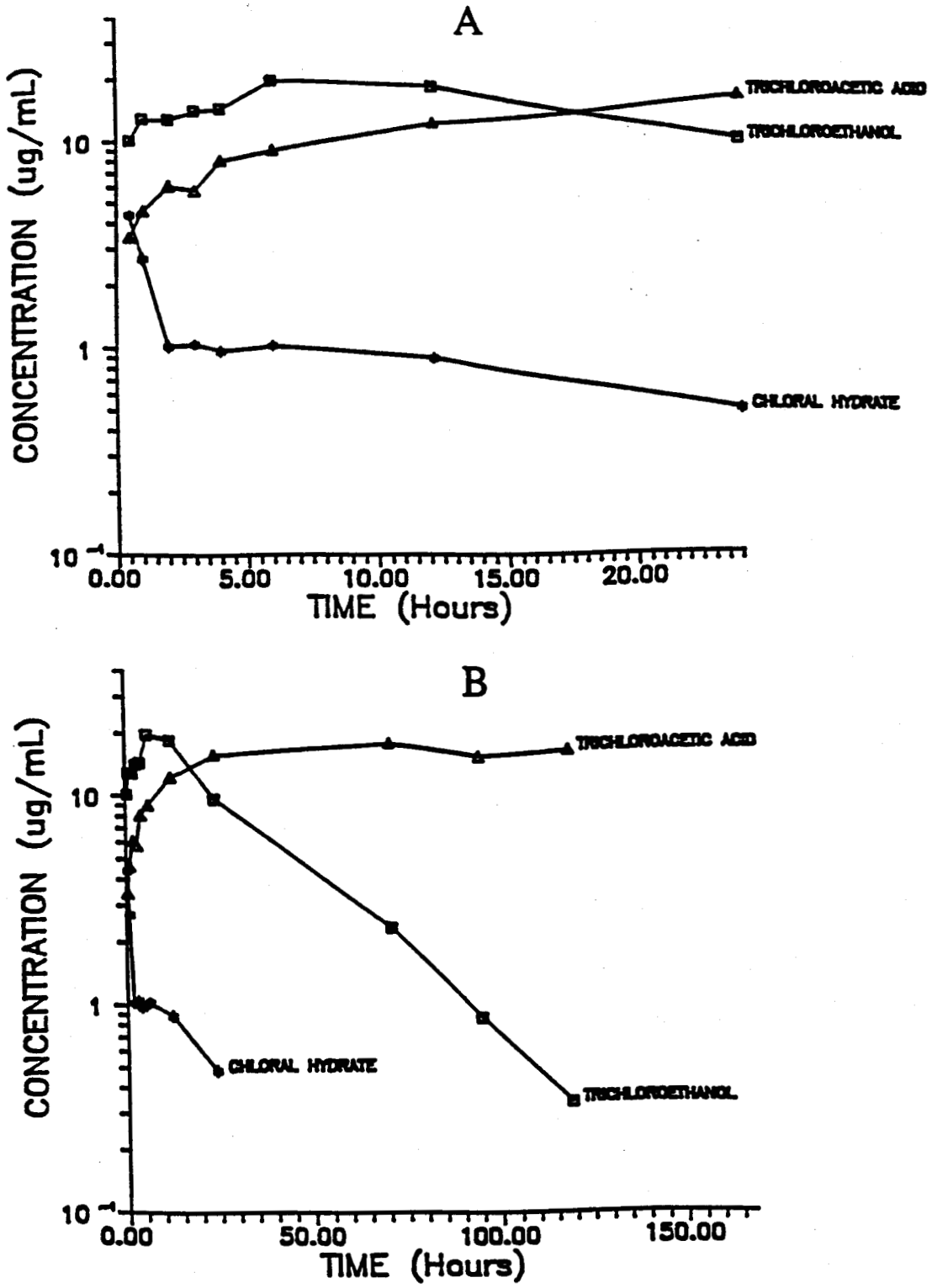

Figure 4.4 Semilogarithmic plot of CH, TCE and TCA in a neonatal subject (SB) over 24 (A) and 120 h (B) following a single oral dose of CH (50 mg/kg)

Table 4.7 Pharmacokinetic parameters after single dose CH

PARAMETER	COMPOUND	PRETERM	TERM	PICU
$t_{1/2}$ (h)	CH[1]	1.01 ± 0.97	3.01 ± 5.81	9.68 ± 7.73
	TCE[2]	39.82 ± 14.27	27.80 ± 21.32	9.67 ± 1.72
	TCA	ND	ND	ND
AUC $_{0-\infty}$ (mg/L/h)	CH	12.41 ± 4.91	11.97 ± 9.89	17.13 ± 9.35
	TCE[2]	2236.12 ± 848.89	1502.84 ± 1177.31	370.76 ± 165.30
	TCA[3]	435.17 ± 278.20	487.55 ± 354.51	1670.75 ± 708.19
Cl_t/F (L/h/kg)	CH	4.81 ± 2.28	5.66 ± 2.28	4.10 ± 2.94
Cmax (µg/mL)	CH	8.01 ± 5.12	6.23 ± 2.28	3.89 ± 2.87
	TCE	36.28 ± 10.65	28.23 ± 9.36	27.06 ± 10.36
	TCA[4]	30.02 ± 27.73	38.15 ± 31.76	113.77 ± 31.61
Tmax (h)	CH	0.64 ± 0.22	0.56 ± 0.16	0.71 ± 0.25
	TCE	5.02 ± 3.03	2.99 ± 2.17	2.23 ± 1.17
	TCA	41.93 ± 50.90	78.67 ± 33.26	68.37 ± 38.89
Cl_r (24 h; mL/h)[5]	TCE[4]	3.48 ± 1.65	4.76 ± 1.98	34.50 ± 20.37
	TCA	0.96 ± 0.90	0.31 ± 0.22	13.02 ± 15.73

1 statistically significant difference between preterm and PICU
2 statistically significant negative correlation with age
3 AUC calculated 0-24 hours; PICU statistically different from preterm and term neonates
4 statistically significant difference between preterm and PICU and term and PICU
5 preterm, n = 6; term n = 4; PICU, n = 4

half-life or AUC ($t_{½}$ correlation: -0.809; $p < 0.0005$; AUC: -0.764; $p < 0.0005$; Figure 4.5). There was also a

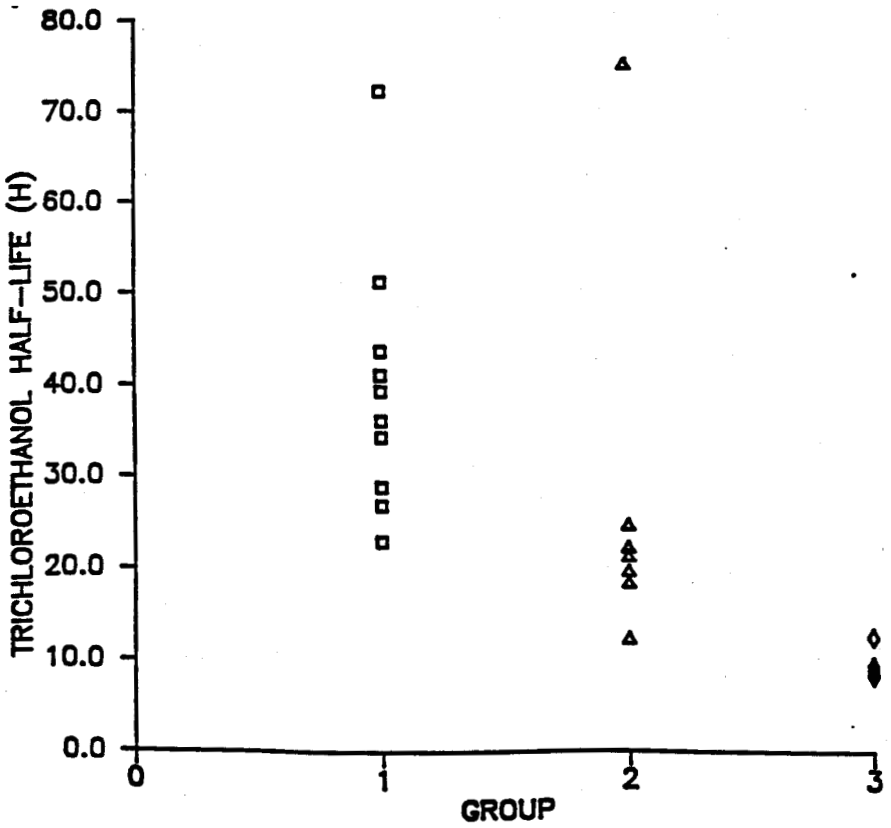

Figure 4.5 Scattergram of trichloroethanol half-life values in preterm (Group 1), term (Group 2) and PICU (Group 3) patients

statistically significant difference between the maximum concentrations attained during the study period and AUC_{0-24} of the other major metabolite, TCA. This difference was seen between infants in the preterm group and the PICU group and between the term infants and the children in PICU.

In all three study groups TCA levels rose to a plateau

and remained at this level for the duration of the study period (Figure 4.4B; Appendix E). With no decline in the metabolite levels it was impossible to calculate pharmacokinetic parameters such as $t_{½}$ or $AUC_{0-\infty}$. The AUC_{0-24} for TCA was higher in the PICU group than in the neonatal groups. The higher AUC could be due to increased metabolism to TCA over the 24-hour period and/or decreased clearance or volume of distribution in the older patients. One patient (NMD) in this group was an exception. A half-life of 28 hours could be calculated for TCA. No other significant differences between the age groups were observed in the pharmacokinetic parameters reported. In some individuals, the elimination of TCE appeared to have the characteristics of capacity-limited elimination. Baby APJ (Figure 4.6) was the best example of this phenomena but others (n=7) showed this characteristic to varying degrees (Appendix E).

4.4 Trichloroacetic Acid Plasma Protein Binding

4.4.1 In vitro Plasma Protein Binding

The in vitro studies were carried out with plasma collected from three sources: a healthy adult volunteer, five neonatal umbilical cords and three neonatal exchange transfusions. Trichloroacetic acid was found to be most highly bound in adult plasma (≈90-77%) followed by neonatal

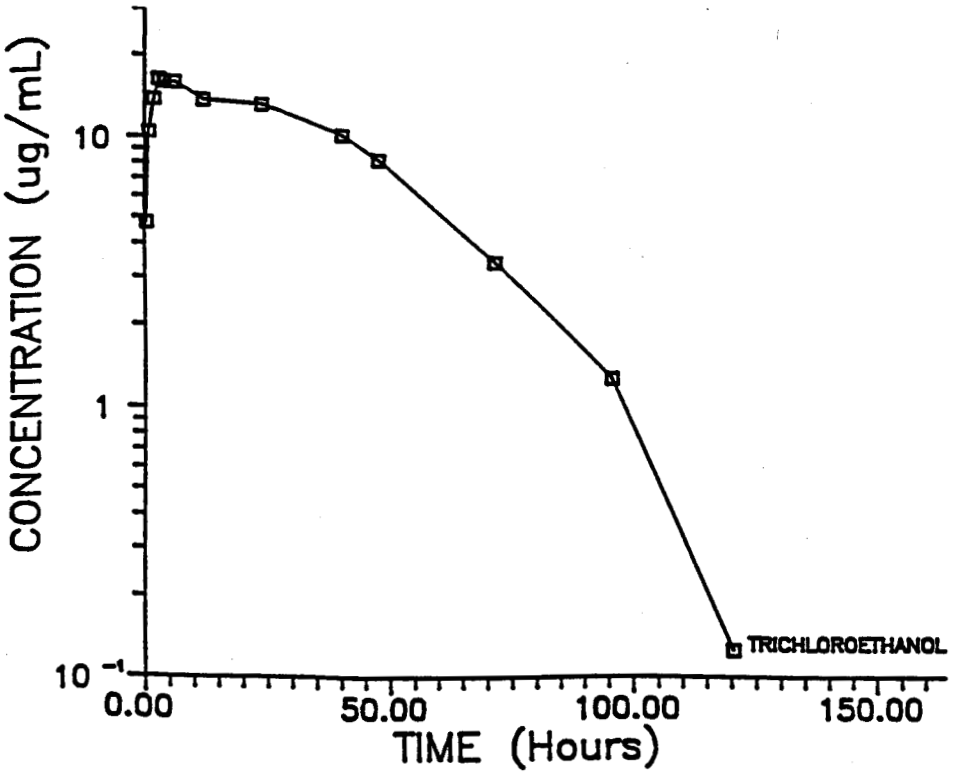

Figure 4.6 Semilogarithmic plot of the plasma concentration of TCE following a single oral dose of CH (50 mg/kg; APJ)

cord plasma (≈85-68%) and neonatal transfusion plasma (≈73-59%) (Table 4.8; Figure 4.7). Protein binding was also investigated in each of the plasma types at three furosemide concentrations: 2, 20 and 200 µg/mL. Only the highest concentration of furosemide appeared to influence the binding of TCA (Figures 4.8, 4.9 and 4.10; Table 4.8).

In an effort to gain further information regarding the relative differences in TCA binding between neonates and

Table 4.8 In vitro plasma protein binding of TCA; percent bound in adult and neonatal (cord and transfusion) plasma in the presence of furosemide

	FUROSEMIDE CONCENTRATION			
TCA (ug/mL)	0.0 ug/mL	2.0 ug/mL	20.0 ug/mL	200.0 ug/mL
Adult plasma				
5.0	89.7 ± 1.2	90.5 ± 2.5	88.3 ± 2.6	84.6 ± 0.9
10.0	87.4 ± 1.1	84.9 ± 0.5	89.6 ± 0.9	83.4 ± 0.4
25.0	86.8 ± 0.5	84.9 ± 0.5	85.6 ± 0.3	81.2 ± 0.9
50.0	85.0 ± 0.6	84.6 ± 0.2	84.5 ± 0.3	79.4 ± 0.4
100.0	81.2 ± 0.4	82.5 ± 0.8	82.2 ± 0.3	76.2 ± 0.5
150.0	79.7 ± 0.1	81.4 ± 0.3	80.4 ± 0.1	74.8 ± 0.2
200.0	77.2 ± 0.4	79.4 ± 0.6	77.4 ± 0.1	71.5 ± 0.4
Neonate plasma (cord)				
5.0	84.6 ± 2.1	78.3 ± 1.1	83.9 ± 3.4	71.6 ± 0.8
10.0	81.2 ± 0.3	77.9 ± 0.4	79.7 ± 1.0	70.6 ± 1.1
25.0	78.4 ± 0.3	77.2 ± 0.2	77.3 ± 0.5	68.7 ± 0.3
50.0	76.3 ± 0.4	77.9 ± 0.3	75.6 ± 0.3	67.3 ± 0.4
100.0	73.8 ± 0.2	76.5 ± 0.8	73.3 ± 0.3	67.5 ± 2.0
150.0	68.0 ± 0.9	69.6 ± 0.8	67.9 ± 0.6	60.1 ± 0.3
200.0	67.7 ± 1.1	67.2 ± 0.5	66.5 ± 0.2	61.2 ± 1.6
Neonate plasma (exchange trans)				
5.0	66.4 ± 0.4	69.1 ± 1.2	65.5 ± 0.9	52.5 ± 1.2
10.0	68.8 ± 0.7	66.2 ± 2.2	67.6 ± 0.5	57.5 ± 0.4
25.0	71.9 ± 0.06	72.8 ± 0.1	73.7 ± 1.6	62.5 ± 1.1
50.0	72.7 ± 0.8	72.2 ± 0.5	72.2 ± 0.9	62.5 ± 1.3
100.0	67.1 ± 0.7	69.5 ± 0.8	68.6 ± 0.4	58.0 ± 2.0
150.0	62.7 ± 0.2	59.4 ± 0.5	66.4 ± 0.8	55.5 ± 2.6
200.0	59.3 ± 2.4	61.3 ± 1.5	57.6 ± 1.5	51.7 ± 2.3

adults, an attempt was made to characterize the relevant binding parameters (i.e. capacity, affinity) of TCA. The method of Rosenthal (Rosenthal, 1967) was employed since binding was done in whole plasma, a heterogeneous protein mixture of unknown concentration. Individual Rosenthal plots may be found in Appendix F. In some of these plots (e.g. adult 0 μg/mL furosemide), two distinct binding sites (i.e. high affinity/low capacity and low affinity/high

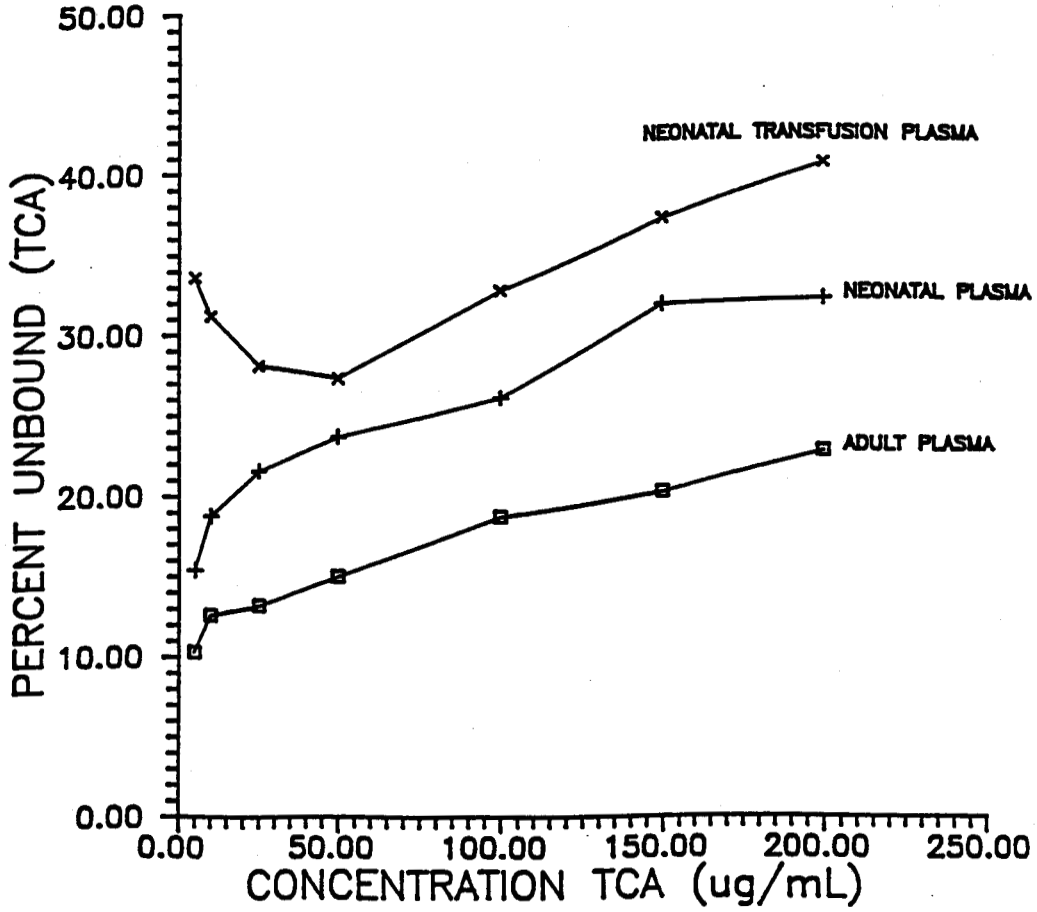

Figure 4.7 In vitro plasma protein binding of TCA in adult neonatal cord and neonatal transfusion plasma; percent unbound versus TCA concentration

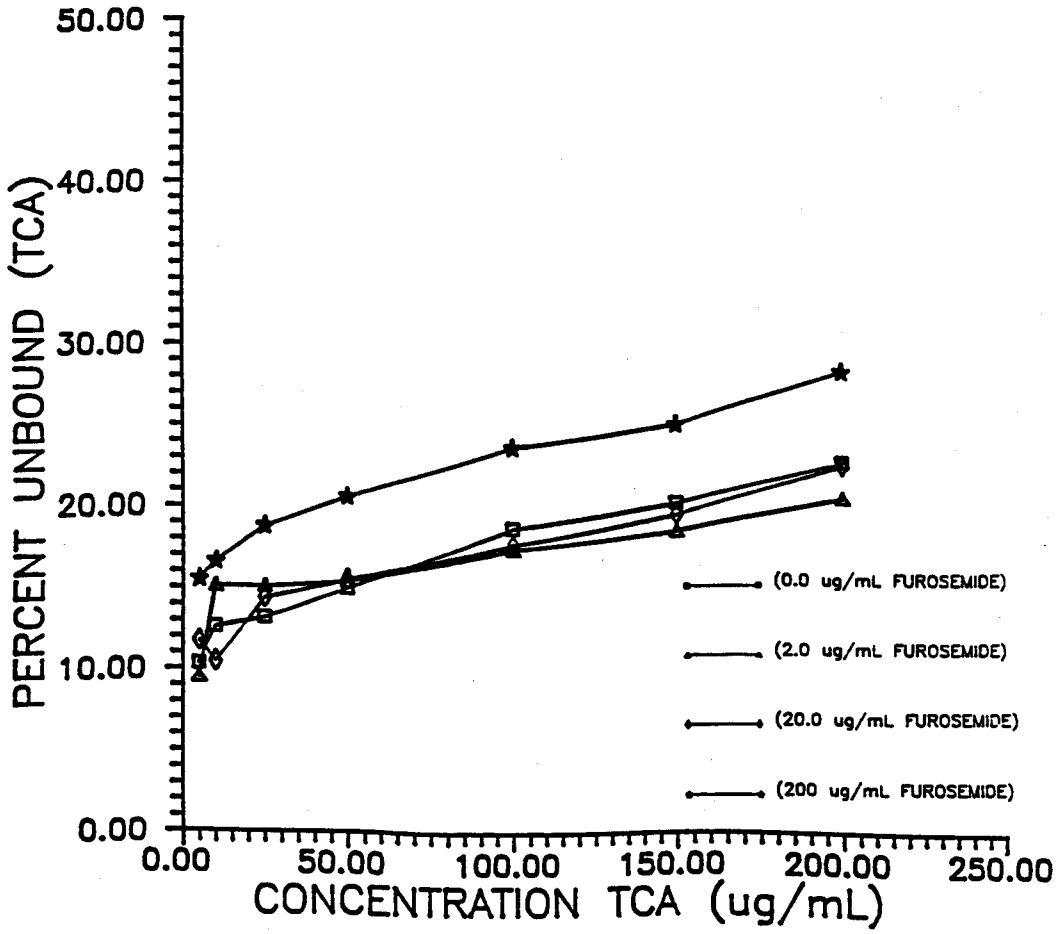

Figure 4.8 In vitro plasma protein binding of TCA and furosemide; percent unbound versus TCA concentration in adult plasma

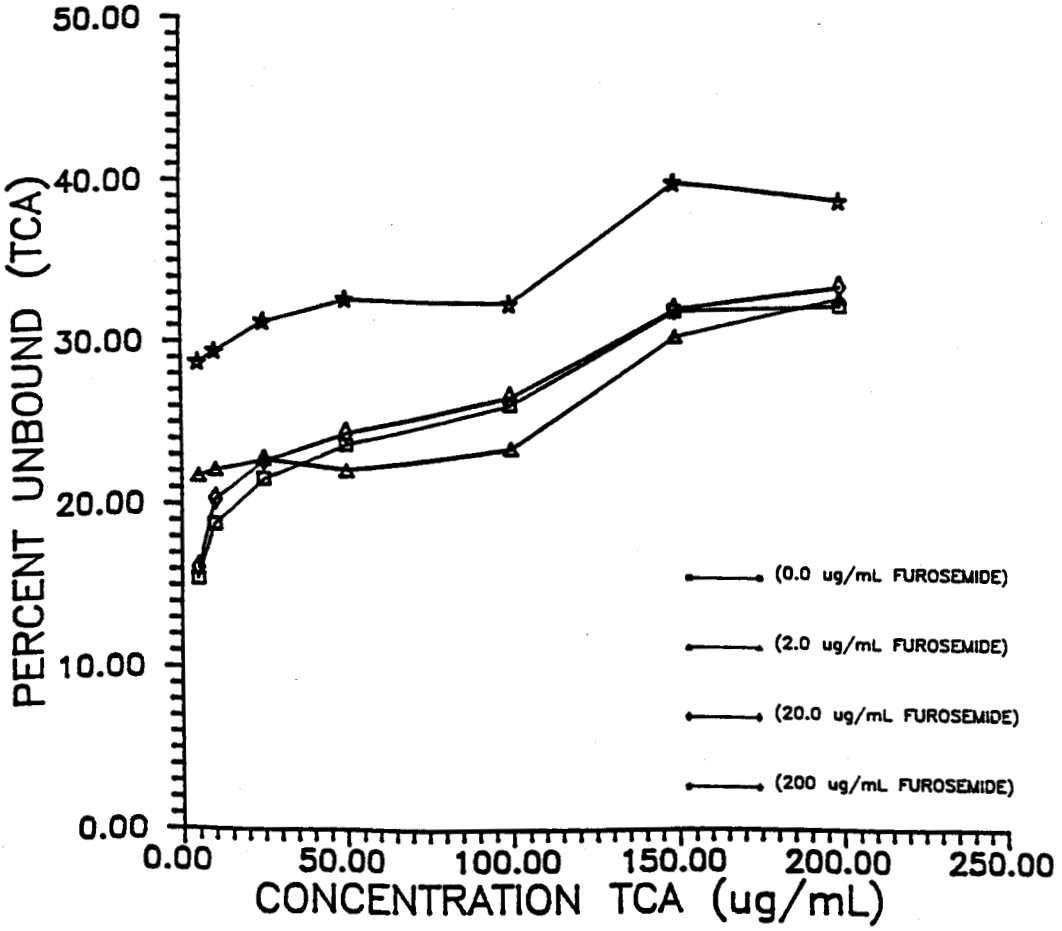

Figure 4.9 In vitro plasma protein binding of TCA and furosemide; percent unbound versus TCA concentration in neonatal (cord) plasma

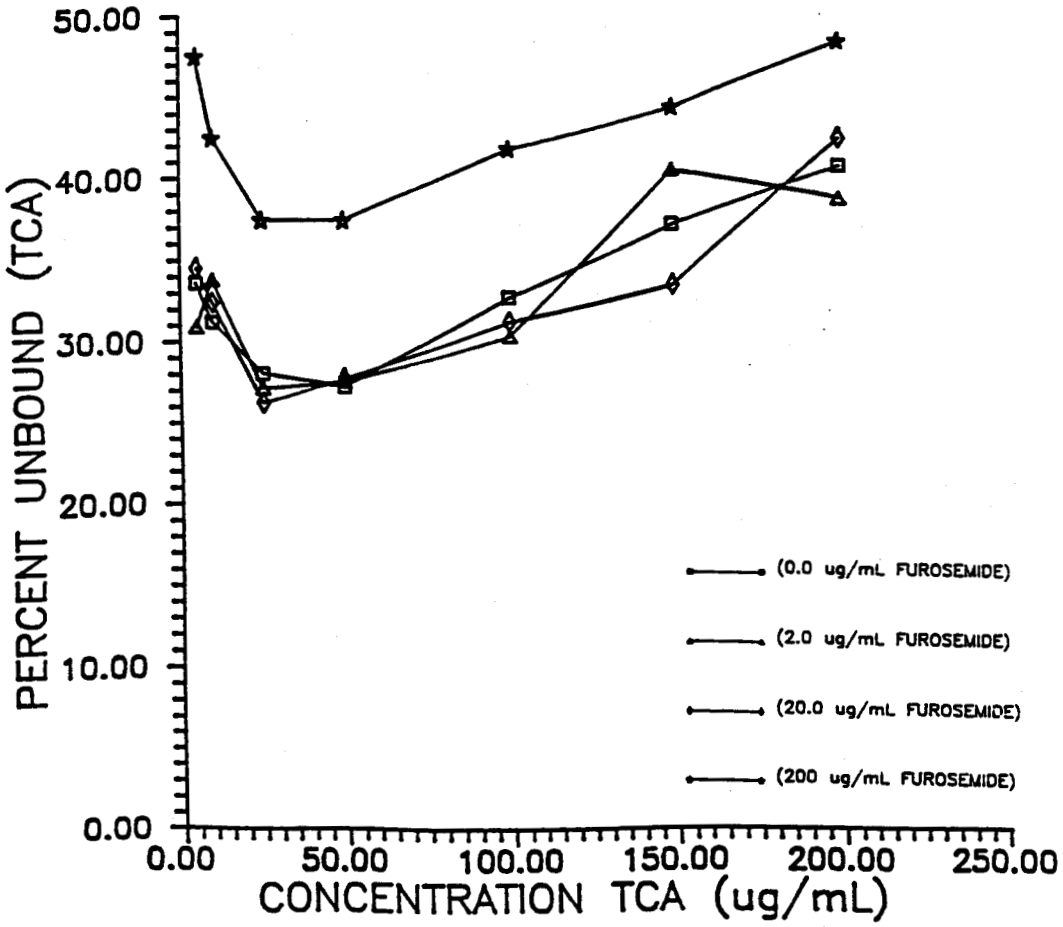

Figure 4.10 In vitro plasma protein binding of TCA and furosemide; percent unbound versus TCA concentration in neonatal (transfusion) plasma

capacity) appear to be present. Results of this nature require computerized curve fitting procedures to characterize of binding parameters. However, in this case, attempts to unambiguously fit binding curves would be extremely difficult due to the scarcity of data in the lower concentration ranges. Furthermore, not all of the Rosenthal plots were nonlinear. Binding for different types of plasma was compared by calculating the apparent binding parameters derived from the best characterized binding site; i.e. for concentrations from 25-200 μg/mL. The slopes and intercepts from the Rosenthal plots were estimated using least squares linear regression analysis (Table 4.9) since in this concentration range the curves were all linear ($p<0.0005$). The slope of the Rosenthal plot was less (i.e. higher affinity) for adult than for neonatal plasmas for all furosemide concentrations. There was, however, no difference in affinity for TCA between the two forms of neonatal plasma ($p=0.324$). Within each plasma source the addition of 200 μg/mL of furosemide resulted in a significant decrease in the affinity of the neonatal plasma to bind TCA. The other levels of furosemide had no demonstrable effect.

4.4.2 Ex vivo Plasma Protein Binding

When sufficient plasma was available, ex vivo protein binding was assessed in patient samples. Adequate plasma

Table 4.9 Apparent protein binding parameters of TCA in adult (AP), neonatal cord (NP) and neonatal exchange transfusion (TP) plasma

SAMPLE	Ka	n[Pt]	nKa[Pt]	Corr
AP	3.806E-3	1768.081	6.729	-0.9577
AP-F2	2.186E-3	2727.446	5.962	-0.9494
AP-F20	3.005E-3	2080.550	6.252	-0.9931
AP-F200	2.289E-3	1950.981	4.466	-0.9709
NP	2.272E-3	1675.849	3.807	-0.9607
NP-F2	2.158E-3	1803.527	3.891	-0.9135
NP-F20	2.136E-3	1697.634	3.626	-0.9784
NP-F200	1.057E-3	2187.047	2.313	-0.8326
TP	2.014E-3	1442.901	2.906	-0.9535
TP-F2	2.025E-3	1459.007	2.955	-0.9010
TP-F20	2.236E-3	1398.863	3.128	-0.9482
TP-F200	1.125E-3	1610.108	1.811	-0.8993

F2 = 2.0 µg/mL furosemide; F20 = 20.0 µg/mL furosemide; F200 = 200.0 µg/mL furosemide; Ka = affinity constant; n = number of binding sites; [Pt] = total protein concentration; corr = correlation coefficient

volumes for binding studies were available in 15 samples from 10 neonates in the preterm (TCA concentration range ≈ 13-160 µg/mL) group and in 11 samples from 4 neonates born at term (TCA concentration range ≈ 12-198 µg/mL). Although the binding was variable, the ex vivo samples from preterm neonates all demonstrated lower binding than found in vitro in cord plasma. The majority of the ex vivo samples also

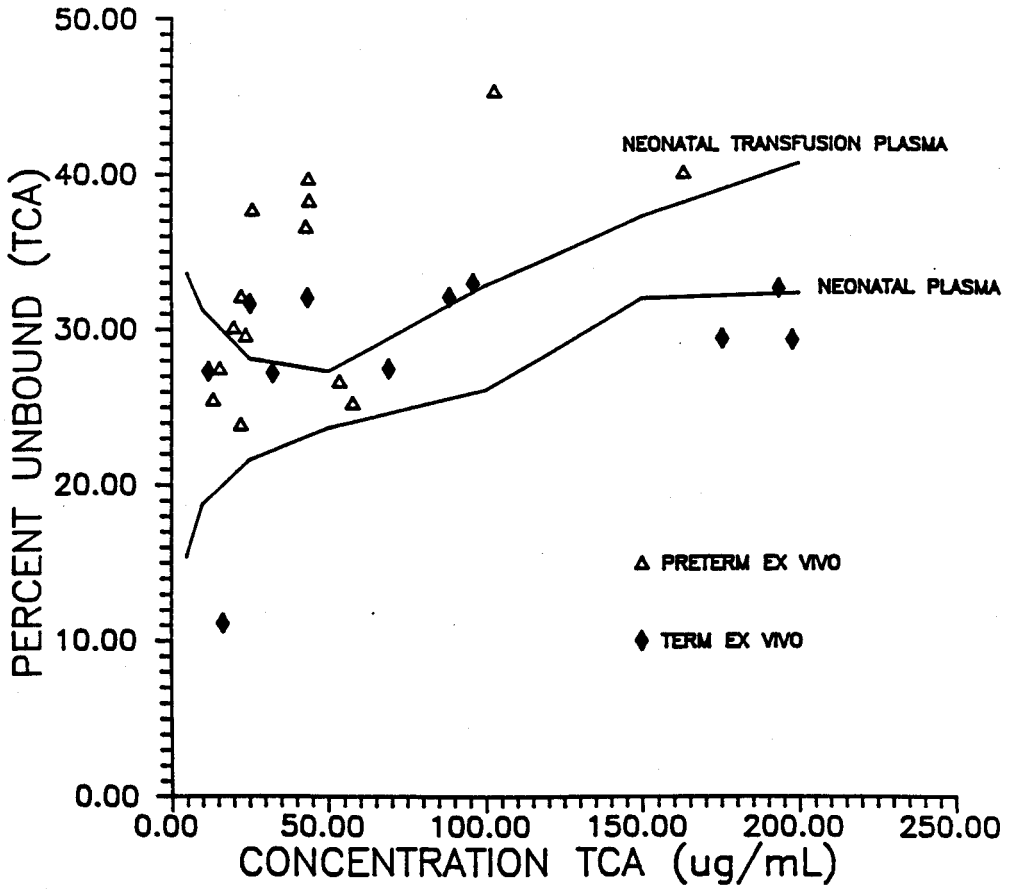

Figure 4.11 Comparison of <u>in vitro</u> (lines) and <u>ex vivo</u> (symbols) plasma protein binding studies in neonatal samples

showed lower binding than in exchange transfusion plasma (Figure 4.11). On the other hand, binding ex vivo in plasma samples from term infants did not appear to be either uniformly higher or lower than in vitro. Ex vivo binding in adult plasma samples was also measured, but over a narrower range of TCA concentrations (n=5; TCA concentration range ≈ 21-35 μg/mL). The ex vivo samples may have slightly lower binding, however, there was reasonable agreement with the in vitro results (Figure 4.12). Ex vivo studies using a wider range of TCA concentrations in the are needed before definitive in vitro/ex vivo comparisons can be made.

4.5 Multiple Dose Study

The study was conducted in 15 preterm infants, four term infants and three PICU patients who were prescribed 50 mg/kg of CH every four to six hours as necessary. In three cases (two preterm infants; one PICU patient) only a single dose of CH was administered. The remaining patients were given from two to 10 doses of CH. One infant (JPK) received five doses of CH prior to enrolment in the study.

The concentrations of CH, TCE, TCE-G and TCA in plasma were determined from blood samples collected immediately before the administration of each dose of CH (predose) and at 0.5 and four hours. If possible, after CH was discontinued, three additional blood samples were collected

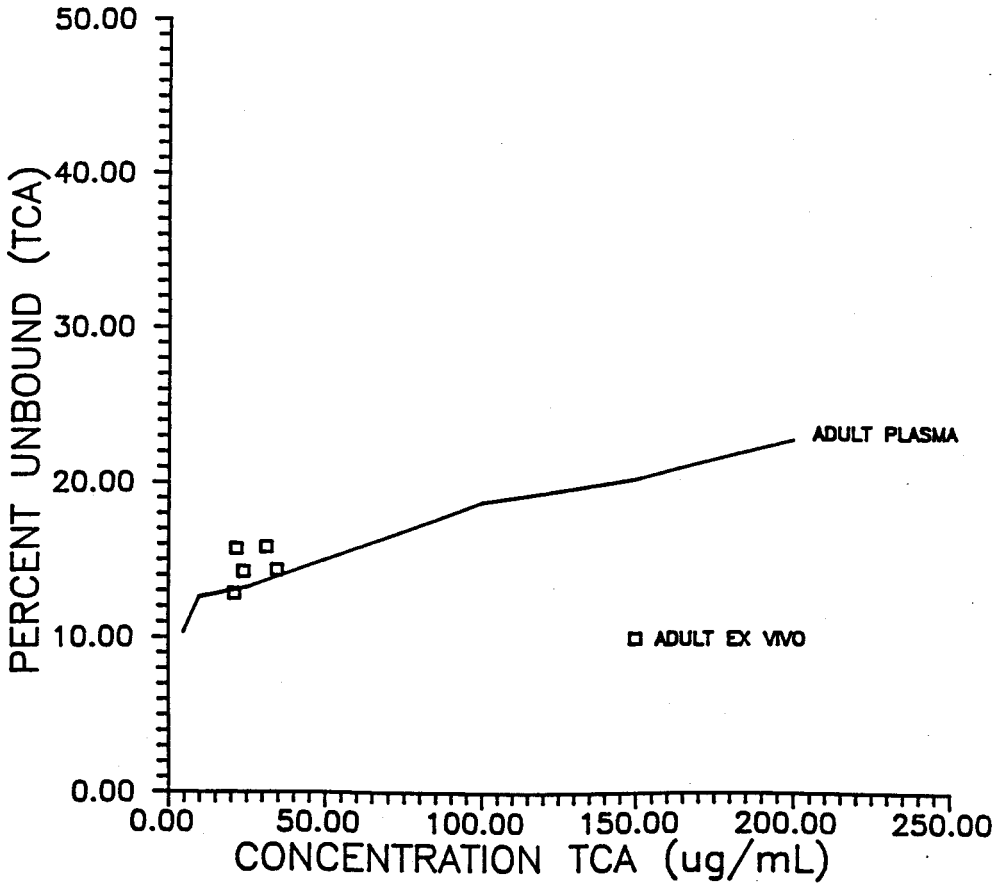

Figure 4.12 Comparison of in vitro (line) and ex vivo (symbols) plasma protein binding in adult samples

at 48 hour intervals. The latter blood samples allowed the calculation of $t_{1/2}$ values for TCE: preterm 41.3 ± 22.6 h; term 30.7 ± 12.2 h and PICU 13.7 ± 10.3 h. These values did not differ from those in the single dose study but should be viewed with some caution since they were estimated from a more limited number of data points. Semilogarithmic plots of individual patient plasma concentrations of TCE and TCA once again suggest evidence of nonlinearity in TCE elimination (Appendix G). These plots also indicate that TCE levels accumulate with multiple doses of CH. The calculation of pharmacokinetic parameters for TCE-G was difficult due to the low levels present in the plasma. However, it was possible to estimate the $t_{1/2}$ values in seven of the preterm infants. The $t_{1/2}$ of 42.5 ± 28.4 hours was comparable to that estimated in the Pilot study. As in the previous studies, the TCA metabolite concentration had not begun to decline by the end of the study period, therefore pharmacokinetic parameters could not be calculated. In three cases where six or more doses of CH were administered, TCE levels exceeded 100 µg/mL, a level suggested to be toxic (Gershanik et al., 1981) (Appendix G).

The focus of the multiple dose study was not purely pharmacokinetic in nature. Rather, it was designed to investigate whether or not a correlation between effect (i.e. sedation) and plasma concentrations of TCE, the

supposedly active hypnotic, could be demonstrated.

An scoring scale, which could monitor the sedative effect of CH administration over time in a non-invasive and convenient (for the nursing staff) manner, was developed (Department of Pediatrics, Perinatal Research Laboratory, RUH)(Appendix C). There were no demonstrable differences among evaluators in the assignment of sedation scores (Table 4.10). Although the intent of the study was to

Table 4.10 Interindividual variation in assessing sedation scores with sedation/agitation scale

DAY 1	ASSESSING INDIVIDUAL					
BABY	AN	SR	JR	SN 1	SN 2	RN
A	12	11	9	11	10	12
B	14	13	14	14	14	14
C	14	14	14	14	14	14
DAY 2	ASSESSING INDIVIDUAL					
BABY	AN	JR 1	JR 2	SN	BN	RN
D	17	16	16	16	17	16
E	8	9	10	9	9	9

AN = Attending neonatologist
SR = Senior resident physician
JR = Junior resident physician
SN = Senior nurse
BN = Beside nurse
RN = Research nurse

correlate this scale with electroencephalograph (EEG) tracings from infants in various stages of sedation/agitation, this was not done. Electroencephalograph's are not routinely performed in the NICU of RUH and an adequate number of calm and agitated children could not be identified over the 30 month period of the study. Whether the scale scores represent an

absolute measure of sedation remains unclear. However, relative to the use of CH within this study, the scale appears to indicate sedation and/or agitation in individual patients; i.e. when the score became elevated (indicating increased agitation) the child received another dose of the sedative (Appendix G). Chloral hydrate was administered every four to six hours as required for sedation. The decision to administer the CH was made independent of the score value.

Even though the scale was expressly designed for ease of use by the bedside nursing staff, the nature of intensive care nursing often interfered with the data collection. Twenty-two infants were enrolled in the study; one had no sedation score data recorded. Of the remaining 21, only nine infants had the required sedation scores for all of the time points. Three of these nine infants received only a single dose of CH. Therefore, only six infants could be classified as having complete multiple dose/pharmacodynamic data for this study. Nevertheless, enough data were available to provide a evidence for a somewhat unexpected result. Figure 4.13 shows representative plots of sedation score data versus CH and TCE plasma concentrations from an infant given a single dose of CH while Figure 4.14 illustrates similar plots for administration of multiple doses. The rest of the plots for which complete data was available may be found in

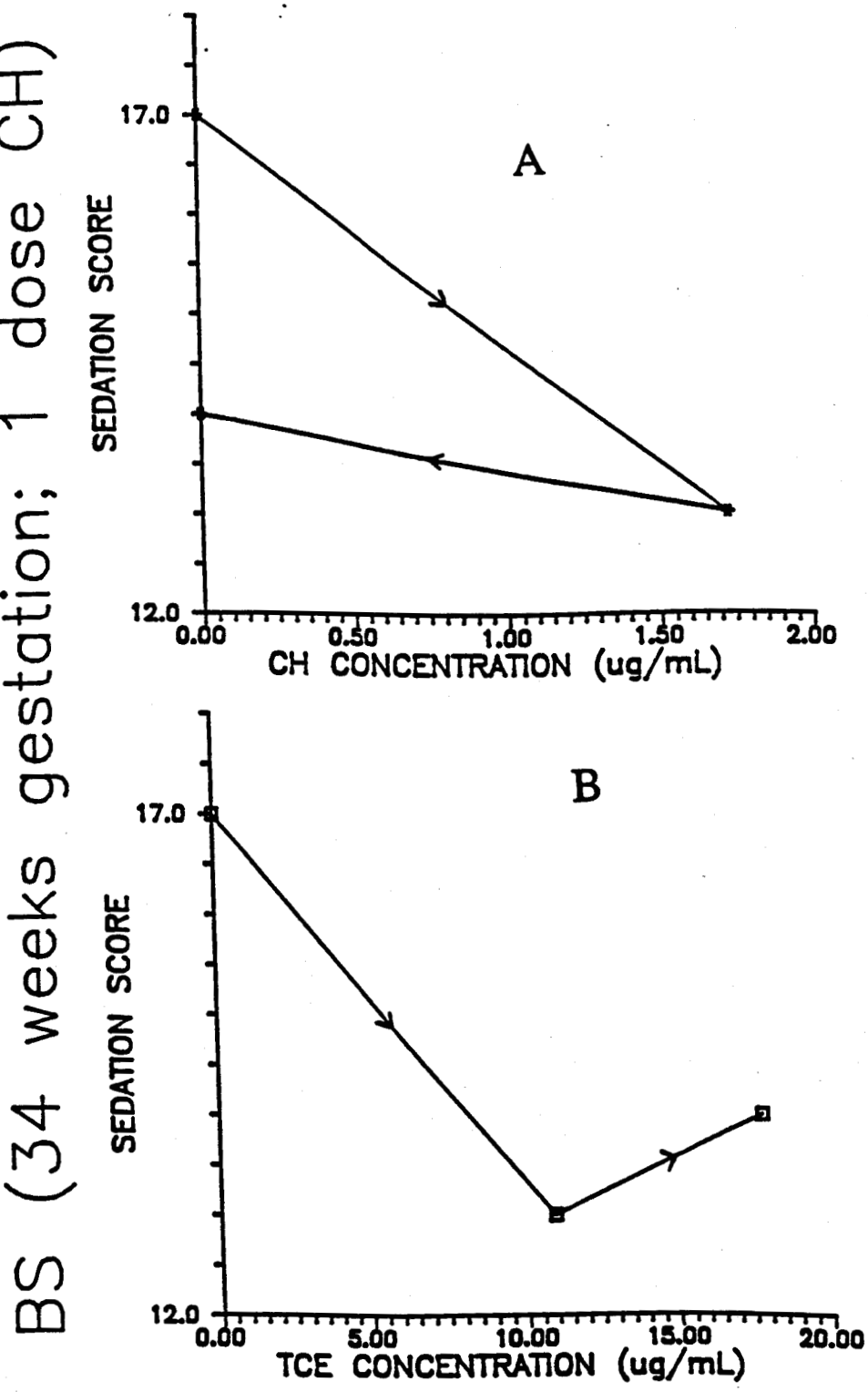

Figure 4.13 A) Sedation score versus CH concentration following a single dose of CH
B) Sedation score versus TCE concentration following a single dose of CH

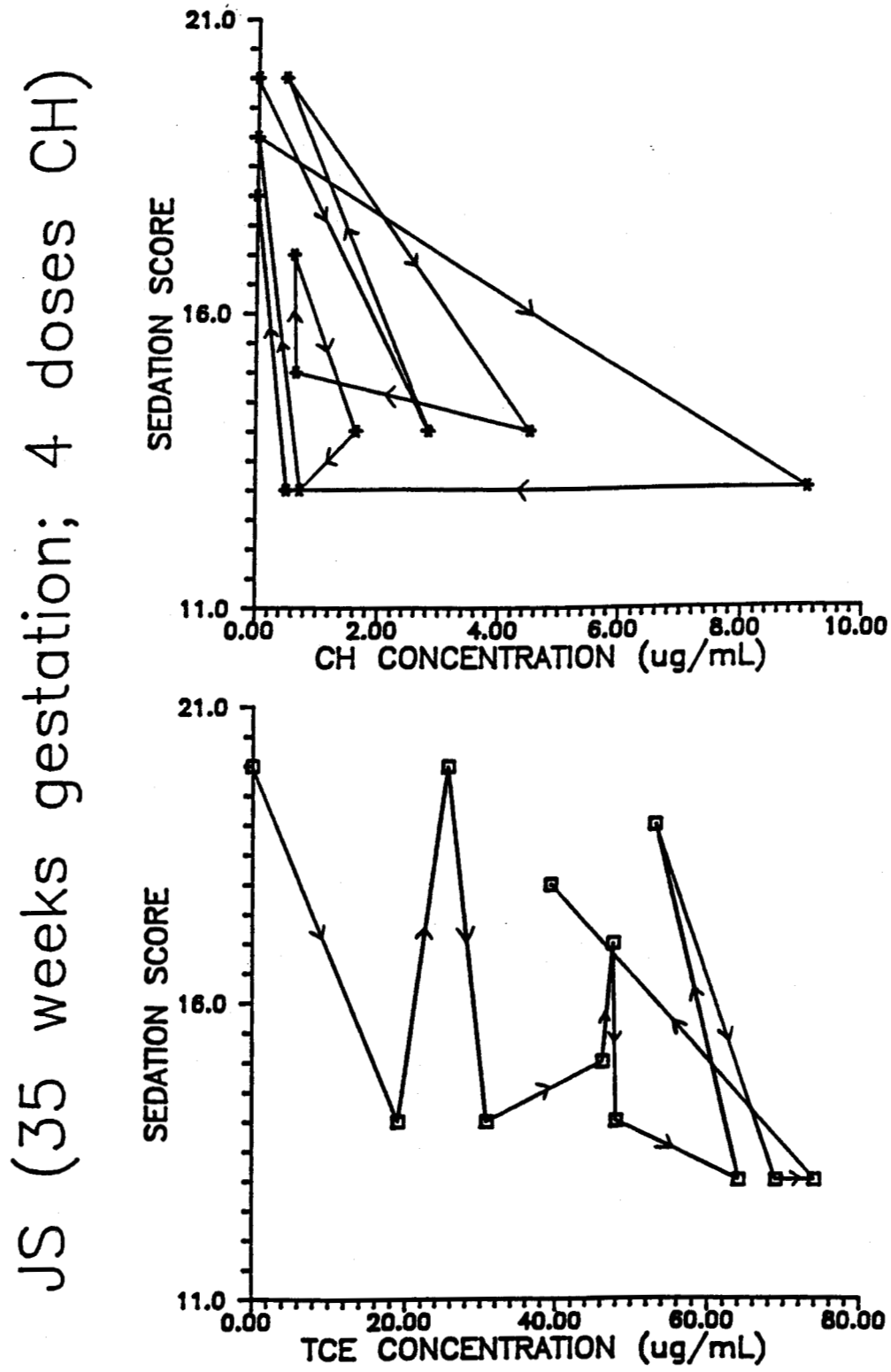

Figure 4.14 A) Sedation score versus CH concentration following multiple doses of CH
B) Sedation score versus TCE concentration following multiple doses of CH

Appendix G. It is evident from both the single and multiple dose graphs that the concentration of TCE rose (with minor fluctuations) following each administration of CH (Appendix G). However, in spite of the continual rise in TCE concentration additional administration of CH was necessary to restore sedation. On the other hand the pattern displayed in the CH plots show that the decrease in CH concentration in plasma appeared to be correlated with an increase in the infants' irritability (i.e. an increase in the sedation/agitation score). This relationship resulted in a "cyclic" CH plot. The sedation scores appeared to correlate better with the levels of CH in the plasma than with the levels of TCE.

5.0 DISCUSSION

5.1 Analytical Procedures

Various methods have been reported for measuring CH and metabolites in biological fluids including colourimetric procedures (Butler, 1948; Marshall,EK & Owens, 1954; McBay et al., 1980) and gas chromatography employing either flame ionization (Garrett & Lambert, 1966; Flanagan et al., 1978; Senft, 1985) or electron capture detection (Garrett & Lambert, 1966; Jain et al., 1967; Sellers et al., 1972c, 1978; Breimer, 1974; Berry, 1975; Van der Hoeven et al., 1979; Gershanik et al., 1981). Of the published procedures, few permit concurrent measurement of unchanged CH and its metabolites and none offer the required sensitivity for neonatal studies. The most sensitive of these methods could detect CH or TCE concentrations of 0.5 µg/mL (Jain et al., 1967; Wells & Cimbura, 1972; Breimer, 1974) and TCA concentrations of 0.1 µg/mL (Breimer, 1974). However, to achieve this sensitivity, 0.5-2.0 mL of biological fluid have to be extracted.

An important prerequisite for drug disposition studies in critically ill neonatal and paediatric patients is adequate analytical methodology. Analytical methods must be more sensitive than those developed for investigations

in adults because the volume and number of blood samples which can be obtained are smaller. Neonates have a smaller total blood volume (80-90 mL) (Geigy Scientific Tables, 1970) than adults. Also patients in intensive care are subjected to many clinical tests requiring blood samples which further deplete the limited blood supply. Therefore microassay techniques are often required, particularly when multiple blood samples are drawn over a relatively short period of time. The assay developed for this study required only 200 µL of blood: 50 µL of plasma for the determination of CH and its unconjugated metabolites and a further 50 µL for the analysis of TCE-G. Gas chromatography with electron capture detection was chosen because of its sensitivity to halogenated compounds.

Other than some minor changes, the analytical procedure was similar to that previously published (Gorecki et al., 1990). The concentration range of the standard curves were extended (0.25-200 µg/mL vs 3.91-25 µg/mL) in order to accommodate the range of analyte concentrations encountered during clinical studies. This modification was necessary since many of the samples were of such a volume that re-analysis was impossible if one or more of the analyte concentrations fell outside the range of the calibration curve. This created certain disadvantages. The linear dynamic range of ECD is quite narrow for CH and its metabolites (Fairbrother, 1973) and thus it was not

possible to fit these curves by least-squares linear regression. A number of curve fitting techniques were assessed. A logarithmic-logarithmic curve fitting procedure (Hewlett Packard 27S Scientific Calculator) proved to be reproducible over the total range of the standard curves.

Diazomethane was used to derivatize TCA in plasma. The use of diazomethane has the advantage of being inexpensive and complete derivatization is rapidly achieved. The most important advantage, however, is that it derivatizes TCA in an ether extract. Other methylating techniques require removal of ether prior to derivatization which could result in losses of the volatile analytes (i.e. CH, TCE, TCA). However, use of diazomethane resulted in unacceptable variability for CH response in the lowest concentration levels of the calibration curve. The reasons for this problem are not clear but two possibilities exist. Chloral hydrate can, under certain conditions, react with diazomethane to form unstable intermediates (Fairbrother, 1973). Alternatively, the ethereal diazomethane solution may contain endogenous compounds which interfere with low level analysis of CH. Therefore, samples were analyzed prior to (for CH concentrations) and after (for TCA concentrations) diazomethane derivatization. This modification did not increase the volume of neonatal plasma required for the analysis.

The procedure for the determination of total TCE was also modified slightly. A shorter incubation time was adequate for the hydrolysis of the glucuronide linkage (Gorecki et al. 1990, Sellers et al, 1978). Previous procedures were developed for adult plasma and/or urine samples which were larger in volume and had more TCE-G present. In the present study, very small volumes (50 μL) of plasma or urine were hydrolysed and therefore limited amounts of TCE-G were present. Complete hydrolysis was ensured by using excess β-glucuronidase (≥ 5000 units).

Although a sulphate metabolite of TCE has not been identified in adults (Owens & Marshall, 1955), compounds such as acetaminophen, which are primarily glucuronidated in adults are eliminated via sulphation in neonates (Levy et al., 1975; Miller, et al., 1976). Moreover, in infants, sulphoconjugation is more mature than glucuronidation (Kacew & Lock, 1990). To determine if a sulphate metabolite was present in neonates, two types of β-glucuronidase (Bovine and Helix pomatia) were used. The bovine enzyme has only β-glucuronidase activity while the Helix pomatia enzyme has both sulphatase and glucuronidase activity. Therefore, if both TCE-sulphate and TCE-glucuronide metabolites exist, the concentration of total TCE determined following hydrolysis with Helix pomatia should be greater than when the bovine enzyme was used. Unfortunately, an endogenous substance which interfered

with the quantitation of TCE was extracted from the bovine enzyme. Thus it was impossible to determine unequivocally if a sulphate metabolite was present.

5.2 Chloral Hydrate Disposition in Paediatric Patients

Chloral hydrate has been in continuous use as a sedative-hypnotic for over 120 years. With such a long history it would be reasonable to assume that a substantial amount of information should be available regarding its disposition. This unfortunately is not the case. In fact, few investigations of CH disposition exist (Breimer, 1974). Moreover, many of the older studies that do exist (Paykoc & Powell, 1945; Marshall,EK & Owens, 1954; Owens & Marshall, 1955) utilized colourimetric analytical methods which are less sensitive and specific than the chromatographic method used in the present study. Some pharmacokinetic studies were done in the 1970's in healthy adult volunteers (Sellers et al., 1972c, 1978; Breimer, 1974) but even these were somewhat limited in scope and in some cases (Sellers et al., 1972c, 1978) still relied on spectrophotometric methods for part of the analyses.

This lack of information in the adult population may be of little concern since CH has largely been replaced in these patients by barbiturates or benzodiazepines.

However, CH is still used extensively in paediatric patients. It is often used as a maintenance sedative in the management of agitation in the NICU (Hartley et al., 1989) and also in children undergoing non-invasive diagnostic (Yeh, 1985) or dental procedures (Duncan et al., 1983; Houpt et al., 1989). Even though CH enjoys widespread use, very little information is available about its disposition in critically ill neonates and children. At the outset of the present study, only two reports were available which attempted to address CH disposition in paediatric patients (Gershanik et al., 1981; Laptook & Rosenfeld, 1984). These investigations (an abstract and a case report) were limited in scope and dealt only with TCE. Gershanik et al. (1981) showed that considerable variation exists in the half-life values of TCE in neonates following normal therapeutic doses of CH. Three years later Laptook and Rosenfeld (1984) reported on CH induced toxicity in a neonate administered multiple doses of CH. They estimated the half-life of TCE in this patient to be approximately three to four times longer than reported in adults.

Paediatric dosing with CH is based on clinical judgement alone since no definitive studies have examined its disposition in this population. Neonates and infants dispose of drugs differently than adults (Besunder et al., 1988; Kacew & Lock, 1990) and no satisfactory literature data are available to judge the usefulness or potential

toxicity of CH in light of these differences. Furthermore in a critically ill paediatric population poly-pharmacy is unavoidable (Appendices D,E and G). The effects of multiple concomitantly administered drugs on CH disposition are difficult to assess. Nevertheless, the information gained through "patient" rather than "healthy volunteer" studies are more important from a clinical point of view since the drug is being studied under the conditions in which it is used.

Therefore, the disposition of CH in critically ill NICU and PICU patients was investigated with pilot, single dose and protein binding studies.

5.2.1 Pilot Study

In paediatrics, inadvertant overdoses are often the result of inappropriate dosing regimens coupled with a poor understanding of drug disposition. Therefore, a pilot study was used to investigate if CH use for maintenance sedation in NICU and PICU at RUH had the potential for toxicity and to serve as a basis for futher investigations. After patients were identified as being stable enough to permit participation in the study, only one limiting criterion was placed on patient enrolment; the patients had to have received ≥ five doses of CH. This allowed typical CH use at RUH to be observed and it also enabled initial estimates of pharmacokinetic parameters such as elimination

rate constants to be determined.

Statistical analyses were not performed since the pilot study had only a small number of patients (n=7). The first blood samples were collected 12 hours after the final CH administration therefore only TCE, TCE-G and TCA were quantitated. In some patients, traces of CH were present but concentrations were too low to quantify.

The dosing intervals and dosages were extremely variable (Table 4.5). Dosage recommendations for CH in infants are quite diverse ranging from 9 mg/kg (3 times per day) to 25 mg/kg as a sedative and from 50 mg/kg to 1 g (as a single dose) for hypnotic effects (Gennaro, 1985; Krough, 1988). Although it appears 25-50 mg/kg is often prescribed in a multiple dose regimen, (Gershanik et al., 1981; Laptook and Rosenfeld, 1984; Hartley et al.,1989) there is no mention of a recommended frequency for administration of the higher hypnotic doses in standard pharmaceutical dosing reference books (e.g. CPS, USP DI, Martindales). The dosages used in the pilot study were, for the most part, in the range recommended for hypnotic effects. Even with the wide range of dosages and dosing intervals, satisfactory clinical effects were observed in all infants but one. The latter patient (PICU) was withdrawn prematurely from the study and treated with pancuronium to aid mechanical ventilation when the CH proved ineffective as a hypnotic. The dosing regimens used at RUH were never as extreme in

either level or length of administration as those which reportedly led to toxicity (Laptook & Rosenfeld, 1984; Hartley et al., 1989). Nevertheless, in every case the dosing intervals were much shorter than the elimination half-life of TCE. Therefore, if continued, dosing regimens such as these could result in substantial accumulation of the metabolite believed to be associated with toxicity (TCE). Two patients achieved TCE concentrations above the reported "toxic" level (>100 μg/mL). However, there was no suggestion from the medical or nursing notes of any signs of toxicity which could be unequivocally associated with CH. All of these children were medically compromised and were supported by both mechanical and pharmacological means. It is conceivable that these other measures could "mask" the symptoms of toxicity produced by the high levels of TCE (e.g. respiratory depression).

Older children appeared to eliminate the TCE metabolite more rapidly than the preterm neonates (half-life values: ≈21 h vs. ≈44 h). The TCE-G metabolite was difficult to characterize in some patients because of its low plasma concentration. Nonetheless, a half-life was estimated in some of the patients (n=2) and was found to be similar to that for TCE (Table 4.6). The younger infants appeared to eliminate TCE-G more slowly than paediatric patients. The elimination half-life values for each of the metabolites were much longer than those reported in adults

(Breimer, 1974; Sellers et al., 1978). These findings are not surprising for a paediatric population. For many drugs elimination is slower because of the immaturity of biotransformation (e.g. glucuronidation) and renal excretion.

One of the most striking results noted in this pilot study was the persistence of TCA. An elimination rate constant and half-life (approximately 5-6 days) could be calculated in only three out of the seven patients. In the other four patients, even 14 days after the final dose of CH, no decline was noted in the concentration levels of TCA. The explanation for this finding is not readily apparent from any previously reported data. Trichloroacetic acid is formed directly from CH (Butler, 1948; Marshall & Owens, 1954) but it is also formed from the metabolism of TCE (Marshall & Owens, 1954) which may, in part, explain the slow rise to a plateau seen for TCA. It has been suggested that the slow elimination of TCA is the result of extensive plasma protein binding (i.e. ≈95%)(Sellers et al., 1978). This explanation seems less likely now since the plasma protein binding of TCA in this study was approximately 70%. TCA is an organic acid with a pKa of 0.7. Thus, at physiological pH, the metabolite exists in an ionized form which should be resistant to passive reabsorption in the renal tubules. Although active tubular reabsorption of TCA has not been documented, it is

possible that the continued high levels of TCA in plasma could result from active reabsorption of the compound in the kidney. Active reabsorption of organic acids is a carrier-mediated transport process known to exist in the renal tubules (Kosoglou & Vlasses, 1989). Uric acid is thought to be reabsorbed by an active transport system (Gibaldi, 1991). Alternatively, another plausible hypothesis to explain the persistence of TCA could be extensive binding to tissue proteins in, for example, the kidney or liver. Slow release of TCA into the plasma from these binding sites could result in the observed plateau in plasma concentration.

5.2.2 Single Dose Study

The single dose study was designed to determine the pharmacokinetic parameters of CH and its metabolites following a single 50 mg/kg dose in patients from NICU and PICU. In this study, the patient population (22 patients) was divided into three groups according to postconceptual age: preterm infants (n=9), term infants (n=8) and PICU patients (n=5).

Previous investigators have reported CH to be absent in adult plasma because of its rapid metabolism to TCE (Breimer, 1974; Sellers et al., 1978). Thus, it was

somewhat unexpected when CH was detected in all participants in the Single Dose Study. In all but two infants, concentrations of CH could be quantitated for several hours. The presence of CH in plasma cannot be solely attributed to immature neonatal drug disposition mechanisms; CH was also detectable for several hours in paediatric patients (4-156 months of age). The PICU patients, in some cases, were mature enough that their metabolic and excretory processes would be essentially equivalent to those in the adult. The infants in the present study were administered CH at a much higher dose than the adults in previous studies (50 mg/kg vs 15 mg/kg) (Breimer, 1974; Sellers et al., 1978) and therefore the plasma concentrations were higher. In all likelihood, CH was not detected in previous reports because CH was below the detection limit of the analytical methods.

In most cases, decline of CH plasma levels was mono-exponential. However, in some of the children, the semi-logarithmic plot of plasma levels with time was not a straight line, but seemed to include a distinct "distribution" phase. This non-monoexponential elimination was noted in at least one subject in each study group although it predominated in the paediatric group. Since CH has a benzene/water partition coefficient of 0.03 it appears unlikely that the drug readily distributes through lipid membranes into peripheral tissues (Butler, 1948).

The pattern observed may be indicative of a distribution phenomenon for CH which is not readily explained by the partition coefficient in a benzene/water system. Slow metabolism of TCE back to CH might also explain the non-monoexponential elimination. This has been speculated to occur in humans (Breimer, 1974) and has been demonstrated in animal studies (Ikeda, 1980). Another explanation may be formation of chloral from CH. The more lipophilic nature of chloral may allow storage in adipose tissues and thus the second phase could result from chloral's slow release from adipose tissue and subsequent elimination.

Trichloroethanol is reported to produce most, if not all of the sedative-hypnotic effect of CH therapy (Mackay & Cooper, 1962) and it is also the metabolite implicated in cases of toxicity (Hoskins, 1984). Previous reports have shown the half-life of this compound to be very long in critically ill neonates (mean=37.3 h; range=8.5-64 h) (Gershanik et al., 1981) and in a premature neonate inadvertently overdosed with CH (35.7 h) (Laptook & Rosenfeld, 1984). The first of these accounts noted there was great inter-individual variation in elimination half-life. Our study also demonstrated wide inter-individual variability and revealed that some of this variation may be attributed to patient maturity. The half-life of TCE was negatively correlated with age (correlation coefficient: -0.811; $p<0.0005$). Preterm neonatal half-life values (39.8

±14.3 hours) concurred with previous reports (Gershanik et al., 1981; Laptook & Rosenfeld, 1984) and were significantly longer than in either the full term neonates (27.8 ± 21.3 hours) or in the paediatric patients (9.7 ± 1.7 hours) ($p \leq 0.005$). The half-life for TCE in paediatric patients was comparable to that in the adult (approximately 8 hours)(Breimer, 1974; Sellers et al., 1978). The $AUC_{0-\infty}$ for TCE was also negatively correlated with age (correlation coefficient: -0.860; $p<0.0005$). The clearance of TCE could not be directly determined, however, based on AUC data, clearance probably increases with postconceptual age.

Collection of urine was complicated. Urine could only be collected from patients fitted with a urinary catheter or a urine collection bag and the decisions for the use of either of these devices were dictated by clinical rather than scientific necessity. Furthermore, collection of urine was sometimes started and stopped at clinically, not pharmacokinetically, appropriate times. For example, one 24 hour urine sample was collected was from 10-34 hours after the dose of CH not from 0-24 hours. Clearly, the loss of the first 10 hours of urine collection is significant if renal clearance is to be calculated. Due to the long elimination half-life values for the metabolites, the length of time that urine collection devices were in place was never adequate to allow for quantitative

collection of the total amount of metabolites excreted. Accurate determination of urine volume was complicated by losses of urine due to clinical tests and through leakage from the catheter or collection bag. Finally, there is some concern that infants do not empty their bladders completely when micturating (Osborne et al., 1978) so that even if the collections were done correctly the results from the non-catheterized children must be viewed with a certain amount of caution. Due to the lack of appropriate urinary data, metabolic clearance for TCE (or other metabolites) could not be calculated. Nevertheless, an estimate was made of the renal clearance of this metabolite over 0-24 hours in some patients (n=14). The renal clearance of TCE was significantly higher in the PICU group (34.5 ± 20.4 mL/h) than in the preterm (3.5 ± 1.7 mL/h) or the term group (4.8 ± 2.0 mL/h). This is expected because of the differences in maturity of the renal elimination mechanisms among these groups. The renal clearance of TCA was not different among the groups. The variability of the data and the small sample size could have contributed to the non-significant result.

Laptook and Rosenfeld (1984) suggested that the prolonged half-life of TCE in neonates may be due either to immaturity of hepatic metabolism or enzyme system saturation. They suggested that the latter was the more likely explanation since the half-life of the infant in

their case study closely resembled that of an adult overdose victim (Stalker et al., 1978). This may be a overly simplistic interpretation since enzyme system saturation and immaturity of hepatic metabolism are not necessarily mutually exclusive conditions. The patients in the current study received only a single dose of the drug and clearly did not achieve TCE concentrations which have been associated with overdose or toxicity. Half-life was negatively correlated with age, suggesting that the immaturity of paediatric elimination mechanisms (e.g. glucuronidation) may contribute to the longer half-life. Both glucuronyl transferase activity and glomerular filtration are diminished in immature subjects (Dutton, 1978; Kacew & Lock, 1990); both of these elimination mechanisms and others are known to increase with maturity (Besunder et al.,1988).

The results from our studies of CH have also produced qualitative evidence to support the hypothesis of a capacity-limited system being involved in the elimination of TCE. Plots of TCE plasma levels versus time from two patients showed the distinct downward convex patterns indicative of nonlinear pharmacokinetics. The nonlinearity of other plots was less apparent. The infants displaying these nonlinear elimination profiles were not limited to any single age or racial group (Appendix E). The mechanism responsible for the apparent nonlinear pharmacokinetics of

TCE is not known. Saturable binding to plasma proteins for a drug with low clearance and a large volume of distribution could result in elimination profiles such as those observed (Rowland & Tozer, 1989). However, since TCE is not highly plasma protein bound, this explanation seems unlikely. The most plausible explanation seems to be that the nonlinearity is the result of the saturation of one or more of the elimination pathways of TCE (Figure 2.2). Two of these pathways (oxidation to TCA and glucuronidation to TCE-G) are dependent on enzyme systems and thus could be capacity limited. Nonlinearity in patient (APJ) became apparent at a TCE concentration which is less than 20 µg/mL, well below the level (approximately 330 µg/mL) at which nonlinear TCE elimination in an adult was reported (Stalker et al., 1978). Nonlinearity may have serious clinical implications since slight changes in dosing regimens in compounds eliminated in this manner may result in disproportionately higher plasma levels than would have been predicted with a linear model. This characteristic of TCE is troublesome since CH is often prescribed in a multiple dose regimen for neonatal patients and as illustrated in the Pilot Study it is not unusual for dosing regimens to be altered during the course of therapy. If the dose given exceeds the individual's maximal rate of elimination, the pseudo-zero order elimination could lead to a more substantial and unpredictable accumulation of TCE

in some patients than in others. The differences in the ability to eliminate TCE might explain why reports of CH toxicity are somewhat sporadic.

The elimination of TCE likely depends on multiple elimination pathways, both linear and nonlinear. Elimination mechanisms in the paediatric age group are constantly and rapidly maturing but with considerable interindividual variability. The differential rate of maturation may explain the varied elimination profiles in subjects given the same dose of CH.

Trichloroacetic acid is generally acknowledged to have no pharmacological activity (Paykoc & Powell, 1945) but the metabolite remains in the body for an extraordinarily long time. The results following a single dose of CH mirrored those of the pilot study. The TCA plasma concentrations rose to a plateau and remained at this level for the duration of the study period (six days). Similar findings have been reported in animals (Butler, 1948). Since there was no decline in metabolite levels it was impossible to calculate pharmacokinetic parameters such as half-life or $AUC_{0-\infty}$. AUC_{0-24} was significantly higher in the PICU group (1670.8 ± 708.2 mg/L/h) when compared to either of the NICU groups (preterm: 435.2 ± 278.2 mg/L/h; term: 487.6 ± mg/L/h). The maximum concentration of TCA was also higher in the paediatric group (113.8 ± 31.6 µg/mL) than in either of the others (preterm: 30.0 ± 27.7 µg/mL; term: 38.2 ±

31.8 μg/mL)($p<0.05$). Possible explanations for these findings include: higher production of TCA (increased oxidative capacity), smaller volume of distribution for TCA or lower TCA clearance. It is known that the P-450 mediated oxidative processes reach adult levels by six months of age (Reed & Besunder, 1989) and these enzymes may play a role in the production of TCA (Breimer, 1974). Although other oxidative enzyme systems have been much less studied than cytochrome P-450 it seems likely that their activity is also increased in older infants. Neonates have a higher proportion of water in their bodies into which the polar TCA could distribute thus increasing the volume of distribution. Increased plasma protein binding in the older children could also contribute to a higher AUC. However, the apparent differences in plasma protein binding seen between neonates and adults do not appear to be of a great enough magnitude to account for the three-fold increased AUC in the PICU patients. Finally, a decreased clearance of TCA could result in the higher AUC. This last mechanism seems the least likely since no difference in renal clearance was noted among the age groups in the study and TCA is eliminated solely by renal excretion (Paykoc & Powell, 1945).

One PICU patient was exceptional in her elimination of TCA. A half-life of approximately 28 h for TCA was calculated. This is much shorter than in adults (≈67-100

h)(Paykoc & Powell, 1945; Breimer, 1974) and there appears to be no readily available explanation for this observation.

Trichloroacetic acid has many undesirable qualities which suggest that its presence should be viewed with some concern. It is a strong organic acid which is used routinely for the precipitation of proteins in the laboratory. It is highly bound to plasma proteins in adults (Sellers et al., 1978) which may be of concern because of its potential to displace acidic drugs from plasma proteins. Finally, the potential for accumulation with this compound is quite real and long term exposure to TCA has not been evaluated toxicologically.

5.2.3 Plasma Protein Binding

The protein binding characteristics of TCA in adult and in neonatal plasma was investigated in vitro and in vivo to determine if any significant differences exist. Other in vitro experiments with hyperbilirubinemic neonatal exchange transfusion plasma were conducted to determine if elevated levels of bilirubin alter TCA binding. A drug interaction between CH and furosemide, resulting from the displacement of TCA from plasma proteins by furosemide has been described (Malach & Berman, 1975). Because furosemide is used in combination with CH in both paediatric and adult

patients (Aranda et al., 1980; Malach & Berman, 1975), protein binding studies also investigated the possibility that furosemide can displace TCA.

Previous plasma protein binding studies of TCA have used either dialysis or ultrafiltration to separate bound from free TCA. In the present study, a micropartition ultrafiltration technique (Amicon) was used to accommodate the limited volumes of plasma available for the binding studies. Since this technique had never been employed before for TCA binding investigations, five aspects of the binding procedure which might affect the results were identified and investigated: ligand-macromolecule equilibration time; centrifugation time; effect of freezing and thawing plasma; non-specific TCA binding to apparatus; and source of plasma. Ligand-macromolecule equilibration was established rapidly. Incubation of plasma for one, two or three hours had no effect on TCA concentration in the ultrafiltrate. Protein binding results were similar whether samples were centrifuged for 5, 10 or 15 minutes. Protein concentration progressively increases during centrifugation as the ultrafiltrate is removed and may affect protein binding equilibrium. However, in these experiments, the only effect noted with increased centrifugation time was an increase in ultrafiltrate volume; the concentration of free TCA remained constant. Although Svensson et al. (1986) have speculated that

freezing and thawing might affect the binding capability of plasma proteins, the effects have not been thoroughly investigated. For TCA, there were no differences in the concentrations of TCA in the ultrafiltrate of fresh or frozen plasma. Non-specific binding was assessed in two ways. First, aqueous solutions of TCA were "filtered" normally and the concentrations of TCA in the ultrafiltrate compared to the non-filtered solution. Second, plasma samples were "filtered" through mechanically disrupted ultrafiltration membranes. No non-specific binding was detected; in both experiments, the concentrations of TCA were equivalent before and after ultrafiltration. The concentration of free TCA in the ultrafiltrate from Red Cross Plasma was twice that of fresh frozen heparinized plasma. The reasons for this difference may be the age of the Red Cross Plasma or the anticoagulants (i.e. citrate, oxalate) used. All subsequent binding studies were done with fresh frozen heparinized plasma.

Protein binding of TCA in adult plasma has been reported. However, it is difficult to compare these results with the current study because of differences in the methods used for protein binding determination. Marshall and Owens (1954) reported TCA binding of 88-71% at concentrations of 26-225 μg/mL. These studies were done with reconstituted lyophilized plasma dialyzed at refrigerator temperatures. In another study TCA binding in

oxalated plasma determined by ultrafiltration at 25°C over a concentration range 30-300 µg/mL varied from 94% to 66% (Sellers & Koch-Weser, 1973). In both of these studies the TCA concentrations were determined by a spectrophotometric rather than a chromatographic method. In the present study, in vitro plasma protein binding was measured in adult, umbilical cord and exchange transfusion plasma samples over a concentration range of 5-200 µg/mL. These concentrations approximated the range of TCA concentrations found in samples from the clinical studies. The most extensive binding was noted in adult plasma (≈90-77%) followed by cord (≈85-68%) and transfusion (≤72%) plasma. Rosenthal plots (Db/Df vs Db) (Appendix F) showed that there appeared to be two binding sites ("high affinity/low capacity" and "low affinity/high capacity") for TCA in the adult and cord plasma. In the exchange transfusion plasma only the "high capacity" site was discernable. Usually a computer curve fitting procedure is used to determine the binding parameters (affinity and capacity) for the two types of binding sites, however this was impractical because of the scarcity of data in the lower concentration ranges of the curve. Therefore, only binding parameters for the "low affinity/high capacity" site were estimated. This calculation at least allowed for a comparison of binding for the various types of plasma. The binding affinity (Ka) for TCA was higher in adult than in neonatal

plasma. The two varieties of neonatal plasma (cord and exchange transfusion) were not statistically different in binding affinity. Binding capacity appeared to be different among the plasma types but this could not be validated statistically since the binding plots had non-homogenous slopes and this violated one of the assumptions required for this statistical test. Differences in binding between neonatal and adult plasma are not unusual and have been demonstrated for a number of different drugs (Kurz et al., 1977a). The differences in the binding of TCA between neonatal and adult plasma are probably not due to a single factor. The total protein in adult plasma is 72-75 g/L of which the major protein, albumin, accounts for 44-45 g/L. Neonatal (cord) plasma has both lower concentrations of total protein (52-64 g/L) and albumin (31-38 g/L) than adult plasma (Wallace, 1976; Kurz et al., 1977a). Another reason for differences in binding is competition for available binding sites with endogenous substances. The two most important of these are bilirubin and free fatty acids. Both of these compounds are elevated in "normal" neonatal plasma (Nau et al., 1984; Wallace, 1976; Kurz et al., 1977a), and the present study shows the lowest percent binding in the plasma with the highest bilirubin concentration. The difference in Ka (affinity) noted between adult and neonatal plasma is likely the result of differences in neonatal and adult albumin (Wallace, 1977).

The two neonatal plasma types did not differ in affinity for TCA but did differ in overall binding. A major difference between these samples was the level of bilirubin present and thus it seems possible that the bilirubin acted as a competitive ligand for the plasma protein binding sites of TCA. This may also explain why only the "high capacity" site was demonstrable in the exchange transfusion plasma. The low capacity/high affinity sites may be occupied by bilirubin and were not available to bind TCA. This hypothesis is consistent with a recent report that TCA competes with bilirubin for binding sites but not to a clinically significant extent (Robertson and Onks, 1991).

Binding was also measured ex vivo in plasma samples obtained in the clinical studies. Binding determined ex vivo in plasma from preterm neonates was less extensive than cord plasma in vitro. The concentration of albumin in plasma increases with age and this could explain why the preterm samples bound TCA less extensively. The samples from patients who were of term gestational age were neither uniformly higher or lower in binding TCA as compared to cord or transfusion in vitro results. The variability in binding may be due to interindividual differences in the concentrations of endogenous competative ligands but this possibility was not specifically investigated. The adult ex vivo study was limited in scope since the available plasma samples covered a very small range of TCA

concentrations. However, in the range tested, binding ex vivo and in vitro were similar. This data was much less variable than in the neonatal studies since it was possible to do both in vitro and in vivo plasma protein binding studies in the same volunteer.

It has been noted that administration of furosemide to patients taking CH sometimes caused tachycardia, hypertension, flush and diaphoresis. These symptoms were suggestive of the presence of an excess of free thyroxin. Therefore the mechanism for the drug interaction between furosemide and CH was suggested to be due to competitive displacement of TCA by furosemide followed by a displacement of protein bound thyroxin by the free TCA (Malach & Berman, 1975). The effect of furosemide on TCA binding was determined with in vitro plasma protein binding studies at three concentrations (2.0, 20.0 and 200.0 µg/mL) of furosemide and seven concentrations of TCA. Furosemide concentrations of two or 20 µg/mL did not affect the plasma protein binding of TCA. Only the furosemide concentration well above that encountered clinically (200 µg/mL) resulted in significantly decreased TCA binding. In neonates, the decreased binding with furosemide appeared to be due to decreased binding affinity for TCA. In the adult plasma binding affinity was also altered by the addition of furosemide. However, in this plasma, the results appeared to be more variable and thus it could not be confirmed

statistically that the highest level of furosemide was implicated. These results suggest significant displacement of TCA by furosemide, at concentrations normally achieved in neonatal and adult plasma, does not occur and therefore is unlikely to explain the interaction between CH and furosemide.

Bilirubin, may displace TCA since TCA binding was lowest in the hyperbilirubinemic plasma. Furosemide is known to displace bilirubin (Shankaran & Poland, 1977). Therefore, furosemide, bilirubin and TCA may all be bound to the same binding sites on plasma proteins with the affinity for TCA less than for bilirubin or furosemide. Thus the addition of a high concentration of furosemide could displace the bilirubin and TCA from the high affinity site. This could not be confirmed in the present study since limited sample volumes did not permit determination of bilirubin and furosemide concentrations. The hypothesis does, however, seem to be consistent with the data presented and with the data in the literature.

5.3 Sedative-Hypnotic Response

The sedative-hypnotic effects following CH administration have been solely ascribed to its metabolite TCE for more than 40 years. This was concluded, in spite of evidence that CH had sedative properties (Mackay and

Cooper, 1962), because of the rapidity of CH metabolism to TCE (Marshall and Owens, 1954; Sellers et al., 1973; Breimer, 1974). This rapid metabolism made it impossible for previous investigators to detect CH and thus since no CH could be determined in plasma it was assumed that it must not have any significant hypnotic effect.

The relationship between drug and metabolite plasma levels to sedative-hypnotic response was examined in the multiple dose study.

The pharmacological effects of CH in paediatric patients could not be related, in a simple manner, to the plasma concentrations of TCE. The effective plasma concentration of TCE is accepted to be 4-7 μg/mL (Breimer, 1974). It is axiomatic that sedative-hypnotic type drugs exert their actions following entry into the central nervous system (CNS). Given its physicochemical characteristics, there should be no impediment for the passage of TCE into the CNS. Furthermore it has been established that the elimination of TCE is extremely slow in neonates. Therefore, it is reasonable to postulate that the plasma concentration of TCE reflects its level in the CNS. Based on the TCE concentrations measured in the present study, a single dose of CH administered to a neonatal patient should result in adequate sedation, in some cases, for several days. However, for the patients in this study it was necessary to administer CH in a multiple

dose regimen to maintain adequate sedation despite TCE levels which exceeded the "therapeutic" level 4-7 (μg/mL). Either a complex pharmacokinetic-pharmacodynamic model is necessary to explain TCE's effects or the therapeutic window for TCE is higher in neonates than in adults.

The most detailed and objective assessment of sleep is provided by EEG. However, this technique is expensive, time consuming and labour intensive (Leigh, 1989). Most investigators rely on subjective assessments which depend to some extent on a post-sleep questionnaire. Obviously this method is impractical in the preverbal patient. Therefore, sedation was assessed via second party observation with a numerical scoring scale. Scoring systems for assessing sedation in children (Barker & Nisbet, 1973) and neonates (Maloley et al., 1990) have been developed. The sedation/agitation scoring scale used in this study was based on the scoring system of Barker and Nisbet (1973) and consisted of five categories (activity, heart rate, blood pressure, respiratory parameters and ventilator responses) which were each scored between one and five indicating complete sedation and extreme agitation, respectively. Efforts were made to make the scale easily understandable and simple to use by minimizing the use of overly technical terminology. This explains the somewhat colourful and less than scientific descripters utilized (e.g. "rangy"). The subjective component in the

scale (e.g. activity level) lead to some concern that scores could be subject to interindividual variability of the assessor. This did not appear to be the case (Table 4.8). The correlation of the scoring scale with EEG data could not be done since not enough patients had EEG during the course of the study. The difficulties involved were largely due to the infrequent use of the EEG in NICU at the RUH. Nonetheless, the scores appeared to reflect the degree of sedation and the need for additional doses of CH (i.e. the score values increased indicating lower sedation prior to the administration of CH and decreased subsequent to the dose of CH).

The collection of the sedation/agitation score data presented problems. Only six of the 22 patients enrolled in the study had complete data recorded. The demands of critical care paediatric nursing often interfered with assessing and recording the scores. In other cases blood samples could not be obtained at the appropriate times. Finally, some patients were excluded from the study because they received only a single dose of CH. Nevertheless, the data available provided a somewhat unexpected result. The sedation/agitation scores appeared to be more related to the CH plasma concentrations rather than TCE concentration. The disposition of CH and TCE are fundamentally different. Chloral hydrate, when given every four to six hours as necessary, because of short elimination half-life does not

accumulate. On the other hand, the plasma concentrations of TCE rose to a higher level following each subsequent administration of CH. No concentration range of TCE appeared to result in a consistent sedation of the patients. No pattern could be seen when effect was plotted against concentration of TCE (Appendix G). However, when CH plasma concentrations were plotted against the sedation scores a qualitatively similar cyclical pattern emerged in all of the patients. The graph generated from data derived from an infant following a single dose of CH (Figure 4.7) shows a single cycle of this pattern quite plainly. The multiple dose patterns are then understandable as multiple single doses (Figure 4.8). A plausible and simple explanation for these results is that CH administration results in sedation and this sedation lightens as the CH is eliminated. Trichloroethanol however, is known to have sedative properties (Marshall & Owens, 1954) and cannot be totally discounted as an active metabolite.

Sedative properties of CH have been attributed to TCE for well over 40 years (Butler, 1948; Marshall & Owens, 1954). Investigators concluded that CH might have sedative-hypnotic qualities but since it was never detected in the plasma it was assumed not to play a significant clinical role. Based on the results of the present study, the sedative-hypnotic effects of TCE cannot necessarily be discounted, however, in paediatric patients CH may play a

much more important role than has previously been suggested. These findings agree with observations in mice 30 years ago. Mackay and Cooper (1962) presented convincing evidence for the activity of CH as a sedative-hypnotic. Following intravenous injection of CH (0.4 mg/g body weight), the righting reflex (a measure of consciousness) was lost when the mice had brain CH levels which were approximately 280 µg/g and TCE levels, 30 µg/g. In a subsequent experiment, TCE was administered intravenously in a dose which was ineffective as a sedative (0.04 mg/g) yet these animals developed levels of TCE in their brains which were almost three times higher (≈86 µg/g) than in the other experiment. They concluded that CH was a potent hypnotic, but of short duration since it is rapidly metabolized to TCE.

Both CH and TCE may play a role in sedation. This would explain why CH dosing intervals are not more consistent and why the effect versus concentration plots were not completely superimposable when identical doses of drug were given. The actual mechanism by which the two compounds cause sedation is unknown. However, it seems possible that CH is responsible for the immediate sedative effects while TCE is responsible for more long term effects in some patients. In many ways this could been seen as analogous to anaesthetic induction with a short acting barbiturate followed by maintenance with an inhaled agent.

It may also be possible that CH induces a deeper level of sedation than TCE and that the lighter sedation of TCE can be disturbed by external stimuli (e.g. nursing procedure) and thus CH is given again to re-establish the sedation following the stimuli.

6.0 Summary and Conclusions

Chloral hydrate, a venerable sedative-hypnotic, has not until this time, been studied in the critically ill pediatric population where it enjoys a great deal of its use. The present investigation was comprised of four major studies: pilot, single dose, multiple dose and plasma protein binding.

The pilot study was done to rapidly assess the validity of literature reports showing that multiple dosing regimens with CH can be toxic in paediatric patients. Thus a small study (five NICU patients and two PICU patients) was done to examine both CH dosing regimens and its elimination in critically ill neonates and older infants at RUH. The results of the pilot study indicated that the elimination of TCE, TCE-G and TCA were all much longer in neonatal patients than in the adult population. In addition, normal dosing procedures (i.e. every four to six hours as needed) may result in accumulation of TCE and TCA. This situation could result in patients achieving levels of TCE which are accepted as toxic. Two of the patients attained levels of this magnitude. However, there was no suggestion of the overt signs of toxicity which have been associated with CH intoxication in the past.

The single dose study was designed to determine the pharmacokinetic parameters of CH and its metabolites following a single 50 mg/kg dose of the drug in patients

from NICU and PICU. In this study the patient population (22 patients) was divided into three groups according to postconceptual age: preterm infants (31-37 weeks; n=9), term infants (38-42 weeks; n=8) and PICU patients (57-708 weeks; n=5). Chloral hydrate, contrary to what has been reported in the adult, was detectable for several hours after oral administration to patients in all three groups. The clearance of CH was equivalent among the groups. Trichloroethanol $t_{½}$ and area-under-the-curve (AUC) values were negatively correlated with age. The half-life value for TCE in the PICU group (9.7 h) was similar to that reported for the adult population, but in the less mature subjects it was approximately three (term: 27.8 h) to four (preterm: 39.8 h) times greater. Renal clearance for TCE was higher in PICU patients than in neonates. The AUC_{0-24} for TCA was also higher in PICU patients than in the neonatal groups but the renal clearance was not significantly different. In some individuals, the elimination of TCE appeared to have the characteristics of capacity-limited elimination.

A study was done to investigate the protein binding of TCA in adult and neonatal plasma and also to test the hypothesis that the interaction described between CH and furosemide might result from displacement of TCA by furosemide. Plasma was collected and pooled from three sources: healthy adults, umbilical cords and neonatal

exchange transfusions. Trichloroacetic acid was added to the plasma at seven different concentrations within the range observed in our previous studies (5-200 µg/mL). For each TCA concentration triplicate samples were prepared containing 0, 2, 20 and 200 µg/mL furosemide. Ultrafiltration was used to separate bound and free TCA. The free concentration of TCA was analyzed by a GC-ECD method. Apparent binding affinity constants (Ka) were estimated by the Rosenthal method. Trichloroacetic acid binding was highest in adult plasma (≈90-77%) followed by cord plasma (≈85-68%) and exchange transfusion plasma (≈72-59%). Affinity (Ka) appeared to be lower in neonatal plasma. Furosemide concentrations of 2 or 20 µg/mL did not affect the plasma protein binding of TCA. Only the furosemide concentration which is well above that encountered clinically (200 µg/mL) resulted in significantly decreased TCA binding. In neonates the decreased binding with furosemide appeared to be due to decreased binding affinity for TCA. This could not be confirmed in adult plasma. Some limited ex vivo studies were also done. The plasma samples from preterm neonates all demonstrated lower binding than found in vitro in cord plasma. The majority of the preterm ex vivo samples also had lower binding than in exchange transfusion plasma. The samples from the term infants did not appear to have either uniformly higher or lower binding than the in vitro plasma

experiments.

The sedative-hypnotic effects of CH have been solely ascribed to its metabolite TCE. Based on our studies of CH disposition in neonates, the half-life of TCE is much longer than the dosing intervals (4-6 h) normally used for the administration of CH. Therefore a study was undertaken to examine accumulation of TCE and the relationship between plasma levels of TCE and the sedation status of infants. A numerical scale was developed to assess the state of sedation and/or agitation in preverbal patients and used to quantitate the relative level of consciousness of the infant at 0 (predose), 0.5 and 4 h following each CH administration. Plasma samples obtained at the same times were analyzed for CH and TCE. Chloral hydrate was eliminated rapidly and did not accumulate with multiple dosing. However, elimination of TCE was slow in the neonates and accumulation of this metabolite was observed. There did not seem to be any correlation between sedation scores and TCE levels. However, when the sedation scores were plotted against CH plasma concentration a regular and cyclical pattern was detected in all patients for whom data was complete. Levels of CH rather than TCE appeared to correlate with sedation scores.

Several conclusions arise from the present investigation. The most obvious is that there appear to be developmental differences in the elimination of CH. The

elimination of TCE appears to be related to the age of the patient and is longer in less mature individuals. Furthermore, normal dosing regimens use time intervals which are variable but shorter than the elimination half-life values of TCE. Thus there is a real danger of TCE accumulation to levels which are potentially toxic. This danger is compounded in some patients who eliminate TCE in a nonlinear fashion. While there was never any indication of overt toxic reactions to TCE in the studies performed little is known of the mechanism of these toxicities. Therefore caution should be used if CH is to be administered to paediatric patients, especially the very young.

Trichloroacetic acid plasma protein binding was higher in adults than in neonates. However, this binding was not as high as previously reported and thus it seems unlikely that it alone can account for the long residency of TCA following CH administration. Our results further suggest significant displacement of TCA by furosemide, at concentrations normally achieved in neonatal and adult plasma does not occur and therefore is unlikely to explain the interaction between CH and furosemide.

Finally, levels of CH rather than TCE appeared to correlate with the sedation scores. This leads to the conclusion that TCE is not solely responsible for the hypno-sedative actions of CH. In the neonatal population

studied it appears that CH itself may play a major role in the efficacy of this drug.

7.0 Further Research

The first modern studies investigating CH were conducted in the late 1940's and 1950's. Research interest intensified once again in the 1970's and now in the 1990's the time appears to be right again for further investigations into this oldest of sedatives.

Further research is warranted in several areas. One of the major questions which has never been adequately investigated is the reason for the extremely long persistence of TCA in plasma. A possible explanation for this phenomenon may be tissue binding of the compound. This hypothesis cannot be tested in human subjects but could be assessed through animal experiments. Animals, gavaged with CH could be killed and the TCA could be quantified in tissues collected. The tissues which would likely be of most interest for TCA binding would be the high perfusion organs such as the liver and kidneys although other organs could be examined as well.

The elimination of TCE is much slower in immature individuals than in adults and, in some cases, appears to be nonlinear. This aspect of CH disposition has the potential to have very serious consequences from a clinical point of view since slow elimination could lead to accumulation of potentially toxic metabolites. Once again the use of animal models would be necessary to investigate the mechanism responsible for the prolongation of TCE

elimination. One possibility which should be explored is role that glucuronidation plays in the overall elimination of TCE. Glucuronidation activity is known to be lower in immature individuals and this could be the elimination pathway which is responsible for the apparently nonlinear kinetics observed in some paediatric patients. A possible animal model for studies of this type is the Gunn rat. This strain of rat is glucuronyl transferase deficient and thus may be a good model for the limited glucuronidation found in neonates. Chloral hydrate could be administered to both Gunn rats and Wistar rats (normal glucuronidation) at several doses and the pharmacokinetics could be followed through serial blood samples and urine collections. The availability of the microassay GC-ECD analysis method make these studies possible. Moreover, the distribution of CH and its metabolites could be studied since the analytes could be quantitated in various organs. This study could potentially provide information on one mechanism for the altered kinetics seen in infants. A clinical study might also be considered to assess if adults with Gilbert's syndrome eliminate TCE differently than the general population.

It may also be possible, with animal studies, to artificially produce decreased renal function through chemical means (uranyl nitrate) and thus test the hypothesis that the kinetics of TCE elimination in neonates

are due to their limited renal capacity.

Unconjugated bilirubin is elevated in neonates taking CH (Reimche et al., 1988). However, TCA does not appear to displace bilirubin from plasma proteins in a clinically relevant amount (Robertson & Onks, 1991). Competition for glucuronidation between bilirubin and TCE has been suggested to cause the elevation of unconjugated bilirubin (Reimche et al., 1988). No experimental evidence is available to support this hypothesis. The amount of bilirubin glucuronide in bile could be assessed in rats by cannulating the bile ducts and analyzing the bile produced over a specific period of time. Once the normal amount of conjugated bilirubin has been established parallel experiments could be done in animals treated with CH. A decrease in the amount of conjugated bilirubin would then suggest that TCE is competing with bilirubin for glucuronidation.

The suspected toxic effects due to CH or its metabolites have never been adequately studied. Studies could be instituted to determine if cardiotoxicity is indeed one of the toxic effects of CH administration. Isolated organ preparations may be useful to determine the mechanistic details of CH and its metabolites singly and in combination on the heart. It would be useful to do these studies in mature and neonatal hearts to determine if there are important differences due to maturity.

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APPENDIX A

APPARATUS AND EQUIPMENT

MATERIALS AND SUPPLIES

APPARATUS AND EQUIPMENT

Accumet Model 805 MP pH meter (Fisher Scientific Ltd., Toronto, ON)

Centrifree MPS-1 Micropartition Apparatus (Amicon Canada Ltd., Oakville, ON)

Coiled glass column 6' x 1/4", 2 mm id (Supelco Canada, Ltd., Oakville, ON)

Diazald Kit: diazomethane generator (Aldrich Chemical Co. Ltd., Milwaukee, Wisconsin)

Dispensette Automatic Solvent dispenser (Brinkmann Instrument Co., Division of Sybron Corporation, Brinkmann Instruments Ltd., Rexdale, ON)

Dubnoff Metabolic Shaking Incubator (GCA Precision Scientific, Chicago, Illinois)

Dynac centrifuge (Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NJ)

Environmental Room (Bally Engineering Structures of Canada, Brockville, ON)

Hamilton 10 µl glass gas chromatography syringes (Supelco Canada, Ltd., Oakville, ON)

IEC HN-II centrifuge (Damon/IEC Division)

IKA Vibrax VXR (Janke and Kunkel GMBH and Co, IKA-Labortechik, Stafen, West Germany)

Mettler balance Model AE 163 (Greifensee, Zurich)

Microcentrifuge tube storage racks (Mandel Scientific, Scientific, Guelph, ON)

Multipurpose rotator, Model 151 (Scientific Industries Inc., Bohemia, New York)

Pipet Filler (Canlab Scientific Products, Mississauga, ON) Scientific Products, Mississauga, ON)

Pipetman variable capacity microliter pipettors, 2-20, 10-100, 20-200, 200-1000 µl capacity (Gilson International, Mandel Scientific, Guelph, ON)

Shimadzu Model CR3A Integrator (Shimadzu Corporation, Kyoto, Japan)

Shimadzu Model C-R5A Integrator (Shimadzu Corporation, Kyoto, Japan)

Shimadzu Model CR601 Integrator (Shimadzu Corporation, Kyoto, Japan)

Varian Model 3700 Gas Chromatograph equipped with ECD (Varian Instrument Group, Walnut Creek, California)

Vortex-Genie (Scientific Industries Inc., Bohemia, New York)

MATERIALS AND SUPPLIES

Amicon YMT Membranes (14 mm, Amicon Canada Ltd., Oakville, ON)

Argon-methane (95:5) purified compressed gas (Acklands, Saskatoon)

β-glucuronidase (helix pomatia) (Sigma, St. Louis, MO)

β-glucuronidase (bovine) (Sigma, St. Louis, MO),

Chloral Hydrate (Squibb Pharmaceutical, Montreal, Quebec)

Diethyl ether, glass distilled (BDH Chemicals, Toronto, ON)

Ethanol, 95% (BDH Chemicals, Toronto, ON)

Furosemide, Lot no. RW-1192 (Smith Klein and French, Medical Department, Montreal, Quebec)

Gas Chromatography column packing: GP 10% SP 1200/1% H_3PO_4 on 80/100 Chromosorb W, AW (Supelco Canada, Ltd., Oakville, ON)

Glacial Acetic Acid (BDH Chemicals, Toronto, ON)

Glass culture tubes (16 x 100 mm and 13 x 100 mm; BDH Chemicals, Toronto, ON)

Glass pipettes (10 ml, disposable) (Kimble, Canlab Scientific Products, Mississauga, ON)

Graphite ferrules (1/4", Supelco Canada, Ltd., Oakville, ON)

Methanol, spectrophotometric grade, (BDH Chemicals, Toronto, ON)

Methyl trichloroacetate (Aldrich Chemical Co., Milwaukee, Wisconsin)

Micropipette tips (disposable) (Mandel Scientific, Guelph, ON)

Moisture trap (GBMT-100-2, 1/8", Chromatographic Specialties, Brockville, ON)

Oxygen trap, (OT-3-D, Chromatographic Specialties, Brockville, ON)

Pasteur pipettes (Canlab Scientific Products, Mississauga, ON)

Screw cap vials, borosilicate glass(15 x 45 mm; Kimax, Fisher Scientific, Toronto, ON)

Sodium acetate ($3H_2O$) (BDH Chemicals, Toronto, ON)

Sulfuric acid, concentrated, (BDH Chemicals, Toronto, ON)

Thermogreen LB-2 GC Septa (9.5 mm, Supelco Canada, Ltd., Oakville, ON)

Trichloroacetic acid (Fluka Chemicals, Terochem Laboratories Ltd., Edmonton, AB)

Trichloroethanol (Aldrich Chemical Co., Milwaukee, Wisconsin)

APPENDIX B

HUMAN ETHICS CONSENT FORM

PILOT STUDY PATIENT CONSENT FORM

SINGLE DOSE STUDY PATIENT CONSENT FORM

MULTIPLE DOSE STUDY PATIENT CONSENT FORM

HUMAN ETHICS COMMITTEE CONSENT

UNIVERSITY ADVISORY COMMITTEE ON ETHICS IN HUMAN EXPERIMENTATION

Name and E.C. File #: Dr. K. Sankaran 83-25 September 23, 1988

Your project entitled: Disposition of Chloral Hydrate in Neonates and Infants.

has been approved by the Committee.

1. Therefore you are free to proceed with the project subject to the following conditions:

Approved.

2. Please submit the revisions requested above to the Director of Research Services, Room 50, Murray Building.

3. Any significant changes of your protocol should be reported to the Director of Research Services for Committee consideration in advance of its implementation.

Sincerely,

ROLAND MUIR
Director of Research Services
University of Saskatchewan

Roland Muir

for Dr. E.A. McKenna, Chairman
University Advisory Committee on
Ethics in Human Experimentation

PILOT STUDY

CHLORAL HYDRATE STUDY CONSENT

NAME:_____ I (we)_____

bearing the relationship of _____ to this child,

hereby allow Dr. K. Sankaran, Dr. K.W. Hindmarsh and their

associates to include my child in the following study.

_____ has explained the purpose of this study and

and the contents of this consent form to me (us).

PURPOSE:

Your son/daughter may require some sedation during his/her hospital stay. One of the medications commonly used for this purpose is Chloral Hydrate. We know that it is an excellent sedative but are still lacking information on it's breakdown and elimination from the body.

This study is designed to collect that information. We would require two very small samples of blood on the day the medication is stopped. This would be followed by one sample per day for two weeks. These samples will be obtained at the time of regularly scheduled blood work whenever possible so as not to cause any unnecessary discomfort to your child. There may be occasions when this is not possible. In these instances, a heel or finger prick would be sufficient to collect the tiny sample.

Information collected may be used by Dr. Sankaran and Dr. Hindmarsh in their studies of Chloral Hydrate. Confidentiality is assured as no names or personal information will be released.

Refusal to participate in this study will not adversely affect your baby's care in any way. If you have any questions or require further information, please contact Dr. K. Sankaran at 966-8131, Dr. K.W. Hindmarsh at 966-6337 or any of the research nurses at 966-8138.

I agree to permit my child's participation in the study as outlined above. I understand that I have the right to withdraw that permission at any time.

Signature _____ Date _____

Signature _____ Date _____

Witness _____ Date _____

SINGLE DOSE STUDY

CHLORAL HYDRATE STUDY CONSENT

NAME:_____ I (we)_____

bearing the relationship of _____ to this child,

hereby allow Dr. K. Sankaran, Dr. K.W. Hindmarsh and their

associates to include my child in the following study.

_____ has explained the purpose of this study and

and the contents of this consent form to me (us).

PURPOSE:

Your son/daughter may require some sedation during his/her hospital stay. One of the medications commonly used for this purpose is Chloral Hydrate. We know that it is an excellent sedative but are still lacking information on it's breakdown and elimination from the body.

This study is designed to collect that information. Your child would receive one dose of Chloral Hydrate the next time he/she requires sedation. Seven small samples of blood would be taken on the day the medication is given. This would be followed by one sample per day for 6 days. No more than 6-8 ml (about 1/4 of an ounce) will be taken in total over the entire study period. These samples will usually be from the arterial catheter already in place in your child. There may be occasions when this is not possible. In these instances, a heel or finger prick would be sufficient to collect the tiny sample. We will make every effort to time these with other blood work your child requires.

Information collected may be used by Dr. Sankaran and Dr. Hindmarsh in their studies of Chloral Hydrate. Confidentiality is assured as no names or personal information will be released.

Refusal to participate in this study will not adversely affect your baby's care in any way. If you have any questions or require further information, please contact Dr. K. Sankaran at 966-8131, Dr. K.W. Hindmarsh at 966-6337 or any of the research nurses at 966-8138.

I agree to permit my child's participation in the study as outlined above. I understand that I have the right to withdraw that permission at any time.

Signature _____ Date _____

Witness _____ Date _____

MULTIPLE DOSE STUDY

CHLORAL HYDRATE STUDY CONSENT

NAME:_____ I (we)_____
bearing the relationship of _____ to this child, hereby allow Dr. K. Sankaran, Dr. K.W. Hindmarsh and their associates to include my child in the following study. _____ has explained the purpose of this study and and the contents of this consent form to me (us).

PURPOSE:

Your son/daughter may require some sedation during his/her hospital stay. One of the medications commonly used for this purpose is Chloral Hydrate. We know that it is an excellent sedative but are still lacking information on it's mechanisms, breakdown and elimination in the body of a child. No untoward effects are expected with the use of this drug.

This study is designed to look at the effectiveness and the elimination of Chloral Hydrate in babies and children. Your child would receive Chloral Hydrate each time he/she requires sedation. A small sample of blood would be taken before the medication is given. This would be followed by post-dose blood samples at 30 minutes and 4 hours after the Chloral Hydrate is given. Each sample is less than 0.5 cc and in some cases, the 4 hour post dose sample may also be used as the pre dose sample, decreasing the total number of blood samples required.

The blood samples will usually be taken from the arterial catheter already in place in your child. There may be occasions when this is not possible. In these instances, a heel or finger prick would be sufficient to collect the tiny sample. We will make every effort to time these with other blood work your child requires.

Information collected may be used by Dr. Sankaran and Dr. Hindmarsh in their studies of Chloral Hydrate. Confidentiality is assured as no names or personal information will be released.

Refusal to participate in this study will not adversely affect your baby's care in any way. If you have any questions or require further information, please contact Dr. K. Sankaran at 966-8131, Dr. K.W. Hindmarsh at 966-6337 or any of the research nurses at 966-8138.

I agree to permit my child's participation in the study as outlined above. I understand that I have the right to withdraw that permission at any time.

Signature _____ Date _____

Witness _____ Date _____

APPENDIX C

SEDATION/AGITATION SCORING SCALE

CHLORAL HYDRATE STUDY - SEDATION/AGITATION SCALE

Please note: 1) Normal for Age Values and Percentile Charts are Given on the Reverse of This Form.
2) Please wait at least 15 minutes beyond any invasive or painful procedure before assessments are made.

	1 (Out Cold)	2 (Sedated)	3 (Comfortable)	4 (Sl. Agitated)	5 (Rangy)
ACTIVITY *Do this assessment last	- sleeping, eyes closed - does not move in response to firm touch	- sleeping, eyes closed - moves in response to firm touch or sound	- awake, eyes open - moving spontaneously - quiet periods	- grimacing and/or crying - eyes open or closed - active but can be settled	- crying and/or thrashing - eyes open or closed - very active - unable to settle
HEART RATE	- cardiac arrhythmias thought to be due to over sedation	- decreased from mean for age to within 5th-25th percentile	- normal for age - 25th to 75th percentile	- increased beyond 75th percentile but no higher than 95th from mean for age	- increased beyond 95th from mean for age
BLOOD PRESSURE - Systolic	- hypotensive requiring treatment i.e., - dopamine - FFP push	- B.P. below normal for age	- B.P. normal for age	- B.P. above normal for age	- hypertensive requiring treatment
RESPIRATORY PARAMETERS	- incr. PaCO2 and/or decr. SaO2 on unchanged FiO2 and/or apneic episodes with diminished activity in past 15 min	- change in pulse oximeter O2 saturation by at least 5% either way and/or decr. spont. resp. rate in past 15 min with decr. activity	- blood gases unchanged or improved or not clinically required in last two hours	- change in pulse oximeter O2 sat. by at least 5% either way and/or incr. spont. resp. rate in past 15 min. with increased activity	- change in PaCO2 either way and/or decr. SaO2 on unchanged FiO2 with excessive activity in past 15 min
VENTILATOR CHANGES/RESPONSES	- incr. vent. rate by 10 breaths per minute or more and/or incr. FiO2 25% or more in past 15 min. with decr. activity	- incr. vent. rate < 10 breaths per min and/or incr. FiO2 < 25% in past 15 min with decr. activity	- no changes required	- incr. vent. rate < 10 breaths per min and/or incr. FiO2 < 25% in past 15 min with increased activity	- incr. vent. rate by 10 breaths per minute or more or incr. FiO2 25% or more in past 30 min. with incr. activity

Date														
Time														
Score														

NORMAL VALUES FOR HEART RATE AND BLOOD PRESSURE ASSESSMENTS

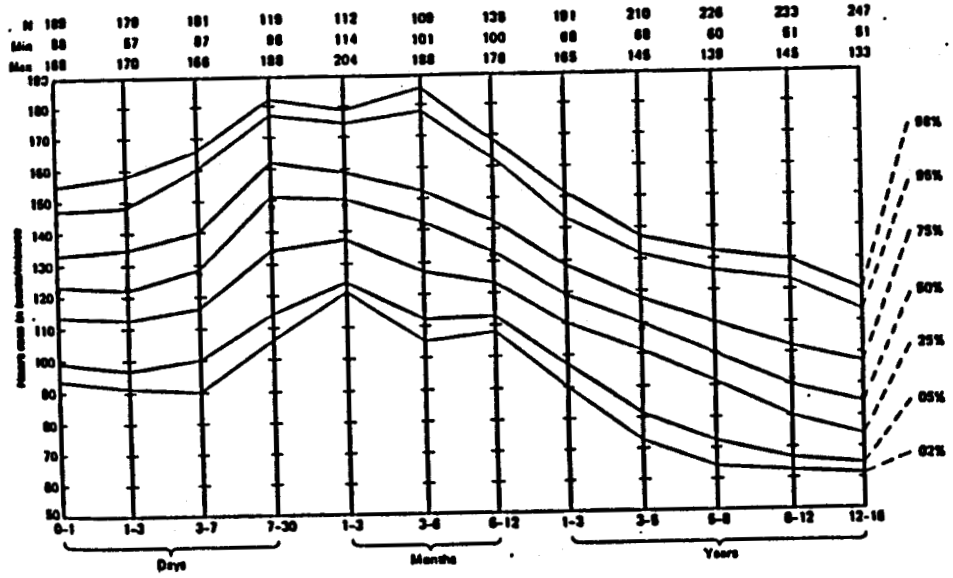


Fig. 1. Heart rate vs. age (⊖ = mean)

Heart rates in the 5% to 95% range for age will be considered normal.

Please note:

Premature Babies may exhibit grimacing while still in a sleep state. A "2" may be scored for activity if the baby is grimacing but otherwise appears to be sleeping.

If a child is on a dopamine drip, score "1" for Blood Pressure even if the B.P. is currently within the normal range.

A score of "1" for heart rate generally refers to bradycardia or tachycardia thought to be due to over sedation.

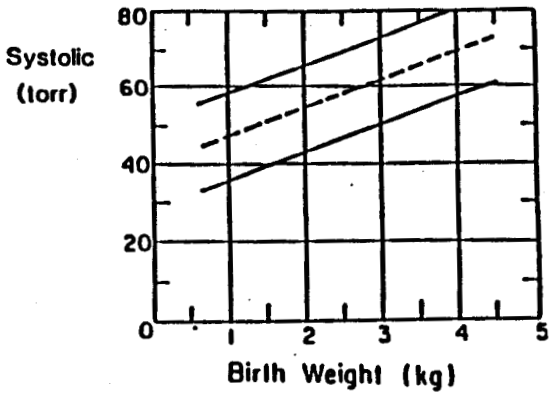


Table 21–4. Normal blood pressure for various ages (mm Hg).*

Ages	Mean Systolic ± 2 SD	Mean Diastolic ± 2 SD
1 month	80 ± 16	46 ± 16
6 months to 1 year	89 ± 28	60 ± 10†
1 year	96 ± 30	66 ± 25†
2 years	99 ± 25	64 ± 25†
3 years	100 ± 25	67 ± 23†
4 years	99 ± 20	65 ± 20†
5–6 years	94 ± 14	55 ± 9
6–7 years	100 ± 15	56 ± 8
7–8 years	102 ± 15	56 ± 8
8–9 years	105 ± 16	57 ± 9
9–10 years	107 ± 16	57 ± 9
10–11 years	111 ± 17	58 ± 10
11–12 years	113 ± 18	59 ± 10
12–13 years	115 ± 18	59 ± 10
13–14 years	118 ± 19	60 ± 10

* Reproduced, with permission, from Nadas A: *Pediatric Cardiology*, 2nd ed. Saunders, 1963.
† In this study the point of muffling was taken as the diastolic pressure.

APPENDIX D

PILOT STUDY PATIENT CLINICAL DATA

SEMILOGARITHMIC CONCENTRATION TIME PLOTS TCE AND TCA

Clinical data from patients enrolled in pilot study

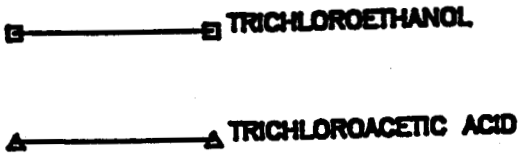
SUBJECT	AGE		SEX	RACE	WEIGHT (kg)	DIAGNOSIS	OTHER MEDICATIONS
	GEST	POST					
NAG	30	3 d	F	W	1.63	RDS	AMIN, AMP, CAFF, ERYTH, GENT, MAA, MORP, PANC, PHEN, TOL, VIT E, VIT K
NBG	30	3 d	F	W	1.11	RDS	AMIN, AMP, DEX, FUR, GENT, IND, MAA, MORP, PANC, PHEN, VIT D, VIT E, VIT K
NG	31	2 d	F	W	1.63	RDS	AMIN, AMP, CEF, GENT, HCT, HEP, HYDR, IND, MAA, NYS, PANC, PHEN, SPI, VIT D, VIT E, VIT K
AB	33	4 d	M	W	1.72	RDS	AMIN, AMP, CAFF, ERYTH, GENT, IND, MAA, MORP, PHEN, POLYVISOL, TOB, VIT D, VIT E, VIT K,
BS	T	5 d	M	N	3.12	GASTRO-SCHESIS	ACET, AMP, ERYTH, FUR, GENT, MAA, PANC, PETH, PHEN, VIT E, VIT K
DAD		5 mo	M	N	7.58	RSV	ACET, AMIN, AMOX, CEFU, FE, PHENY, SAL, DIAZ, MORP, SUC, RIBA, PANC
FT		14mo	M	W	6.5	BPD	ATRO, CEFU, CLOX, DIAZ, DIG, FUR, GENT, ISO, LIDO, MAA, MORP, NYS, PANC, SPI, SAL, SUC

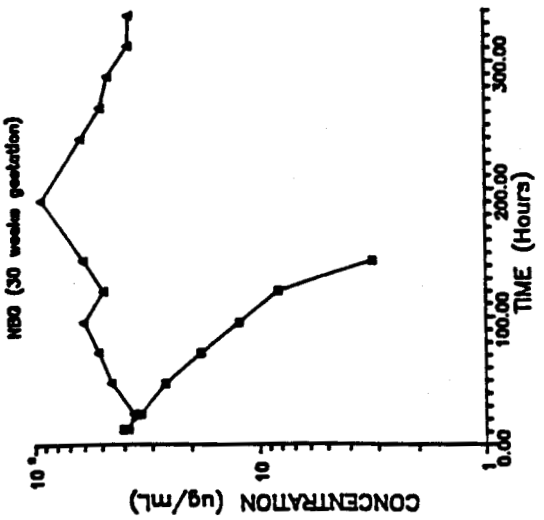
Race: N= Native North American; W= White

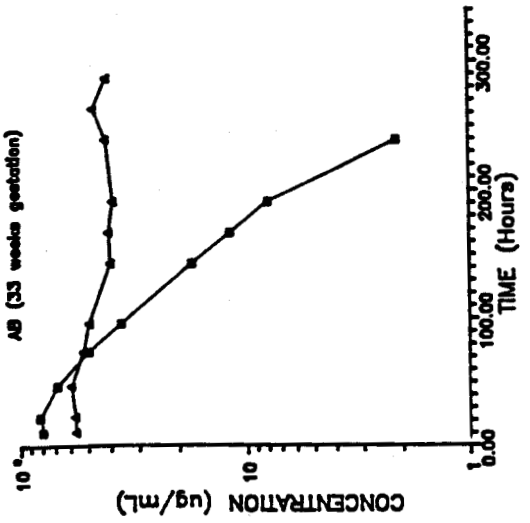
Diagnosis: RDS= Repiratory Distress Syndrome; RSV= Respiratory Syncitial Virus; BPD= Bronchopulmonary Dysplasia

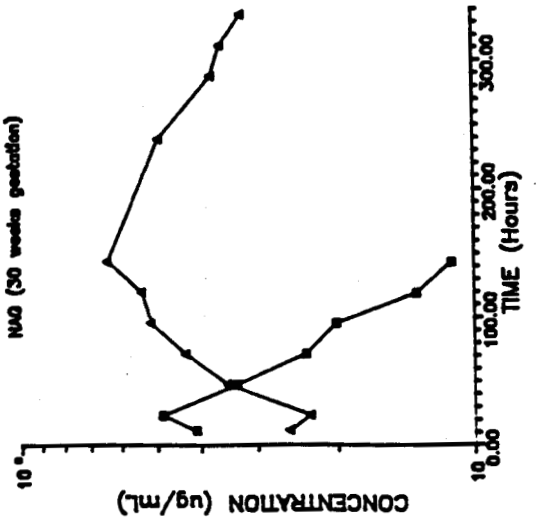
Medications: ACET= Acetaminophen; AMIN= Aminophylline; AMP= Ampicillin; AMOX= Amoxicillin; ATRO= Atropine; CAFF= Caffeine; CEF= Cefotaxime; CEFU= Cefuroxime; CLOX= Cloxacillin; DEX= Dexamethasone; DIAZ= Diazepam; DIG= Digoxin; ERYTH= Erythromycin; FE= Iron; FUR= Furosemide; GENT= Gentamicin; HCT= Hydrochlorothiazide; HEP= Heparin; HYDR= Hydralazine; IND= Indomethacin; ISO= Isoproterenol; LIDO= Lidocaine; MAA= Maalox (Alumimum hydroxide, magnesium hydroxide); MORP= Morphine; NYS= Nystatin; PANC= Pancuronium; PETH= Pethidine; PHEN= Phenobarbital; PHENY= Phenytoin; POLY= Polysporin; RIBA= Ribavirin; SAL= Salbutamol; SPI= Spironolactone; SUC= Sucralfate; TOB= Tobramycin; TOL= Tolazoline; VIT D= Vitamin D; VIT E= Vitamin E; VIT K= Vitamin K

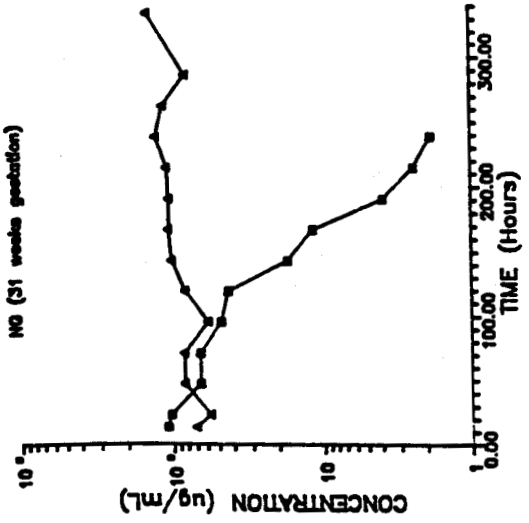
SEMILOGARITHMIC CONCENTRATION TIME PLOTS; TCE AND TCA

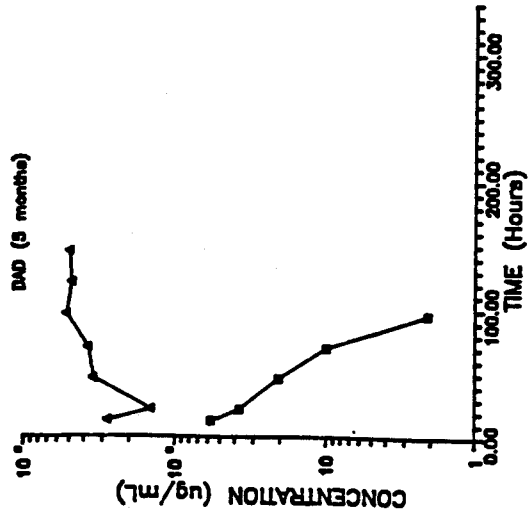


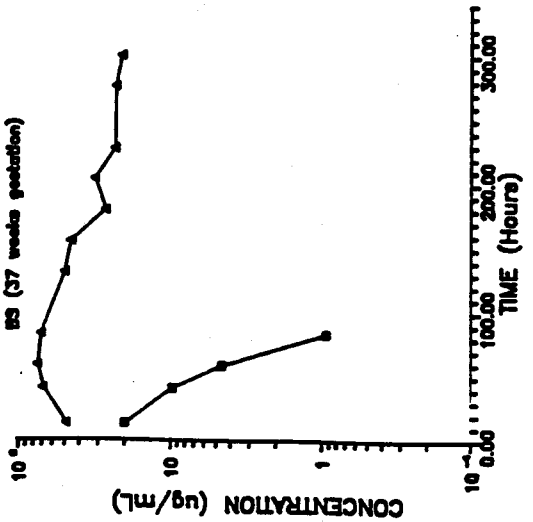

NBO (30 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
100.00
200.00
300.00

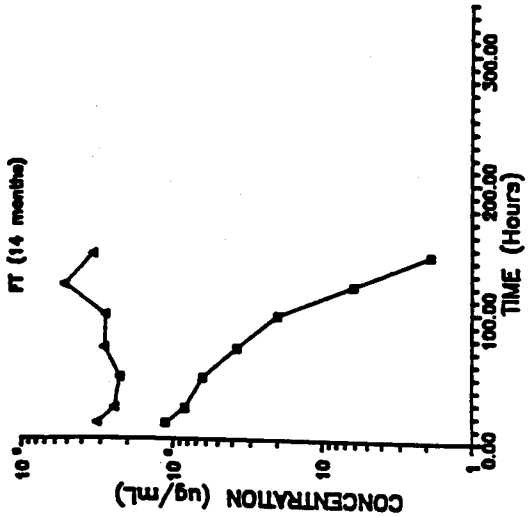

AB (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
100.00
200.00
300.00


NAG (30 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
100.00
200.00
300.00


NG (31 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
100.00
200.00
300.00


DAD (3 months)
CONCENTRATION (ug/mL)
TIME (Hours)


BS (37 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)


FT (14 months)
CONCENTRATION (ug/mL)
TIME (Hours)

APPENDIX E

SINGLE DOSES STUDY PATIENT CLINICAL DATA

SEMILOGARITHMIC CONCENTRATION TIME PLOTS

Clinical data from patients enrolled in single dose study

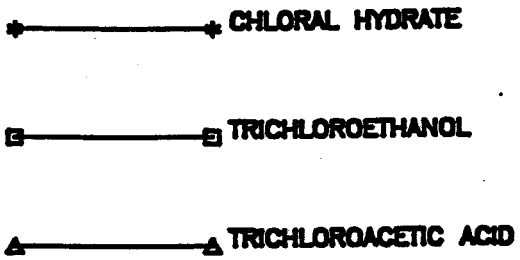
SUBJECT	AGE		SEX	RACE	WEIGHT (kg)	DIAGNOSIS	OTHER MEDICATIONS
	GEST	POST					
BB	31	2 d	M	W	1.58	RDS	AMIN, AMP, GENT, MID, PHEN, SUC, VIT D, VIT E, VIT, K
YB	32	3 d	M	W	1.88	RDS	AMIN, AMP, EXO, FENT, GENT, IND, SUC, VIT D, VIT E, VIT K
GB	33	2 d	M	W	1.97	RDS, PDA	AMIN, AMP, DEX, ERYTH, EXO, GENT, IND, VIT E, VIT K, SUC,
JB	33	2 d	M	W	2.22	RDS	AMIN, AMP, ERYTH, EXO, GENT, IND, MORP, PHEN, POLYVISOL, VIT E, VIT K
UB	33	2 d	M	W	2.10	RDS	AMP, GENT, MID, PHEN, SUC, VIT D, VIT E, VIT K
BBA	34	4 d	M	W	2.04	RDS	AMP, CEF, CLOX, SUC, VIT D, VIT E, VIT K
FB	34	2 d	M	W	2.08	RDS	AMP, ERYTH, EXO, FENT, GENT, SUC, TRIVISOL, VIT E, VIT K
BH	35	3 d	M	W	1.73	GS	AMP, CEF, FENT, GENT, PHEN, VIT D, VIT K
MG	36	2 d	F	W	2.05	RDS	AMIN, AMP, EXO, GENT, IND, SUC, VIT D, VIT E, VIT K
LB	37	6 d	M	W	3.63	AS	AMP, CAP, CEFA, CLOX, DIG, ERYTH, FENT, FUR, GENT, MORP, NYS, PANC, PHEN, POLYVISOL, RAN, SUC, VIT K
HB	38	2 d	M	W	3.86	ASPHYXIA	AMP, FENT, GENT, GLUCA, IND, LOR, PEN G, PHEN, PHENY, SUC, VIT E
YAL	38	3 d	F	O	2.80	DH	AMP, CEF, DA, FENT, GENT, MET, MID, PANC, PETH, VAN
PBB	39	1 d	M	N	3.63	RD	AMP, FENT, GENT, SUC
JN	T	24 d	M	W	3.95	HLH	CEF, CLOX, DA, DEX, DIG, FENT, FUR, MID, MORP, NYS, PHEN, SPI, SUC, VIT K
YC	T	9 d	F	N	4.52	CHF	ALB, AMP, BIC, DIAZ, FUR, GENT, NYS, POLYVISOL, SPI, SUC
SB	T	9 d	M	N	3.75	ASPHYXIA	ACET, AMP, CEF, ERYTH, GENT, GLUCA, MET, SUC, VAN, VIT K
APJ	T	3 d	M	N	2.77	MEC. ASP.	AMP, AMR, CEF, DIG, EPI, FUR, GENT, PANC, PETH, PHEN, SUC, VIT K
SN		4 mo	F	N	3.52	BILATERAL PNEUMONIA	ACET, CEF, CEFU, CHLOR, CLOX, HCT, MORP, PANC, PHEN, PHENY, SPI, SUC
CR		11 mo	M	W	7.20	TRACHEAL MALACIA	ACET, CEF, CEFA, CHLOR, DIAZ, FE, FENT, KCL, MID, PANC, POLY, PROM
TCJ		12 mo	M	W	8.00	TE FISTULA, ESOPHAGEAL STRICTURE	ACET, CEF, CLOX, DEX, FENT, MID, MORP, NYS, PANC, PHEN, SAL, SUC
BD		68.5 mo	M	W	22.10	ASD CLOSURE	ACET, CEFU, FENT, KCL, MAN, MID, PROT, SUC
NMD		156 mo	F	W	36.00	TETRALOGY OF FALLOT	BIC, CEFU, CEPH, DA, DES, FENT, FUR, MAN, MID, SUC

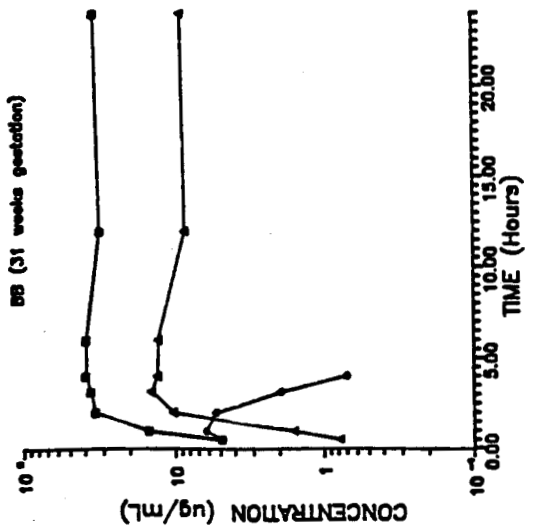
Race: N= Native North American; O= Oriental; W= White

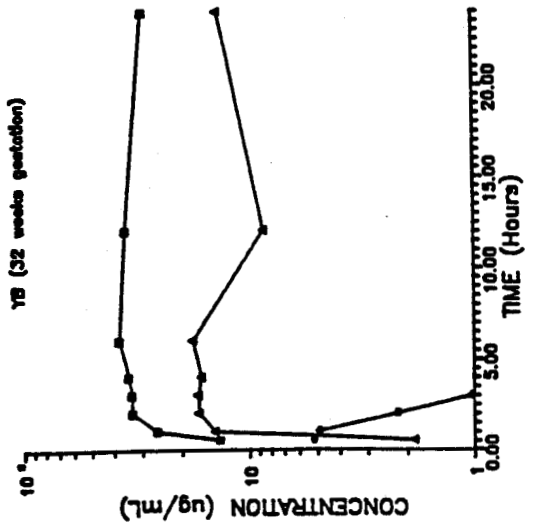
Diagnosis: RDS= Repiratory Distress Syndrome; PDA= Patent Ductus Arteriosus; MEC. ASP.= Meconium Aspiration; GS= Gastroschesis; AS= Aortic stenosis; DH= Diaphragmatic hernia; RD= Respiratory Distress (aetiology unknown); HLH= Hypoplastic Left Heart Syndrome

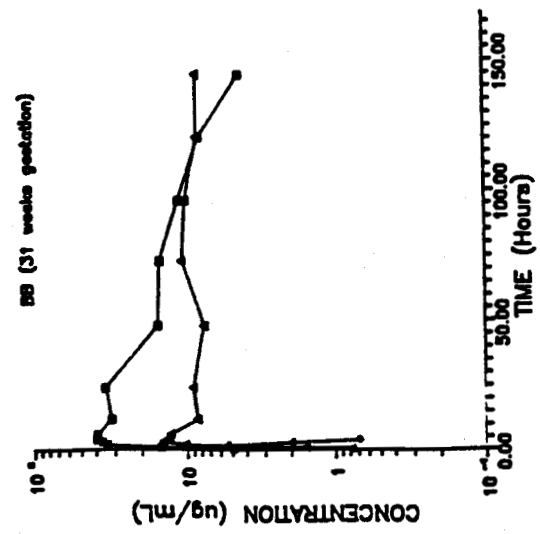
Medications: ACET= Acetaminophen; ALB= Albumin; AMIN= Aminophylline; AMP= Ampicillin; AMR= Amrinone; BIC= Sodium Bicarbonate; CAP= Captopril; CEF= Cefotaxime; CEFA= Cefazolin; CEFU= Cefuroxime; CEPH= Cephalexin; CHLOR= Chlorpromazine; CLOX= Cloxacillin; DA= Dopamine; DES= Desmopresin; DEX= Dexamethasone; DIAZ= Diazepam; DIG= Digoxin; EPI= Epinephrine; ERYTH= Erythromycin; EXO= Exosurf (artificial surfactant); FE= Iron; FENT= Fentanyl; FUR= Furosemide; GENT= Gentamicin; GLUCA= Glucagon; GLY= Glycine; HCT= Hydrochlorothiazide; IND= Indomethacin; LOR= Lorazepam; MAN= Mannitol; MET= Metronidazole; MID= Midazolam; MORP= Morphine; NYS= Nystatin; PANC= Pancuronium; PEN G= Penicillin G; PETH= Pethidine; PHEN= Phenobarbital; PHENY= Phenytoin; POLY= Polysporin; PROM= Promethazine; RAN= Ranitidine; SAL= Salbutamol; SPI= Spironolactone; SUC= Sucralfate; VAN= Vancomycin; VIT D= Vitamin D; VIT E= Vitamin E; VIT K= Vitamin K

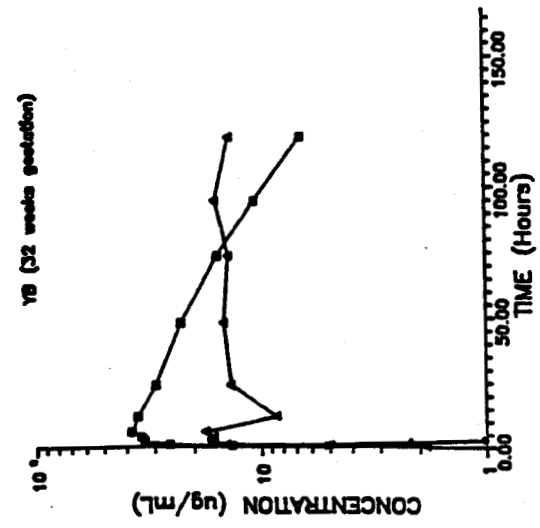
SEMILOGARITHMIC CONCENTRATION TIME PLOTS

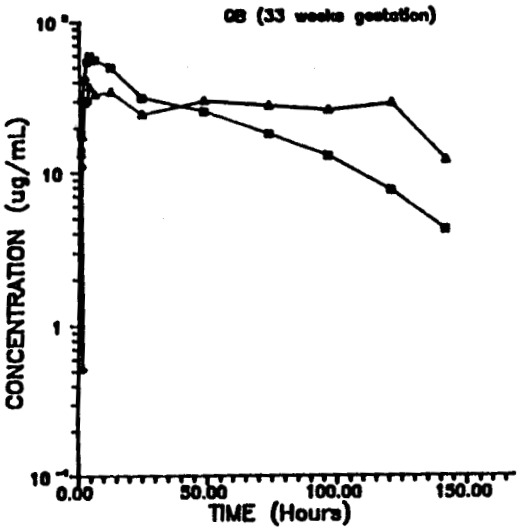


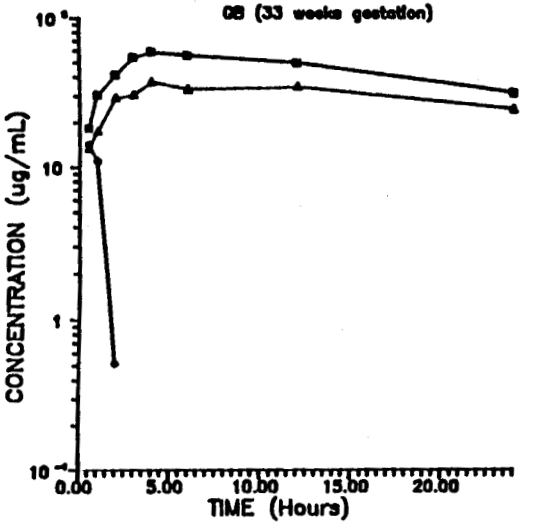

BB (31 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

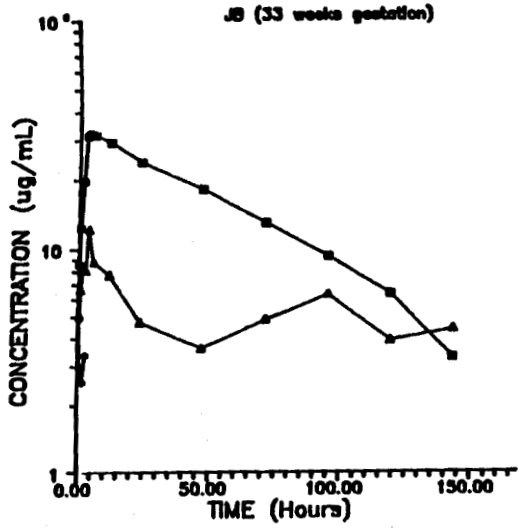

YB (32 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

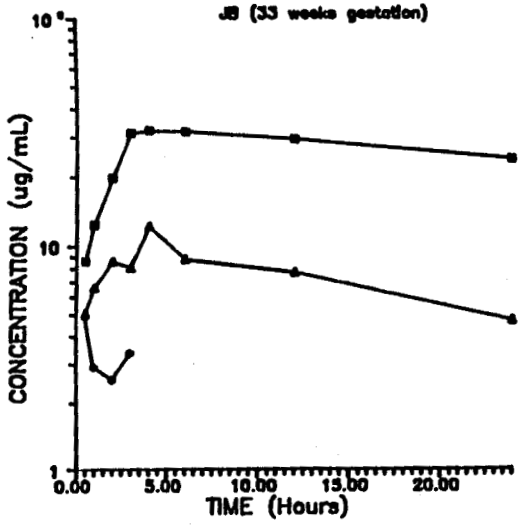

BB (31 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

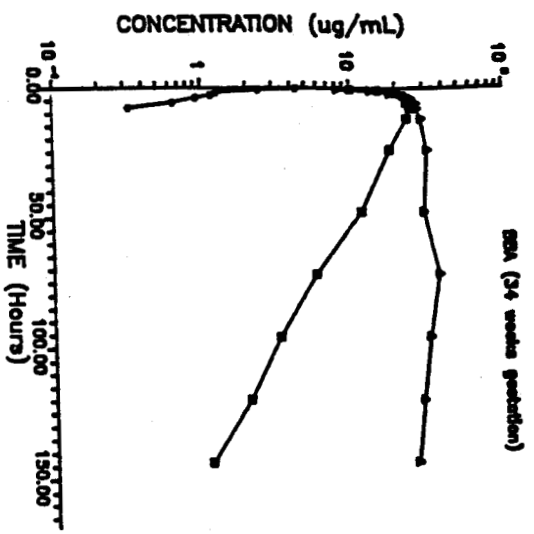

YB (32 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

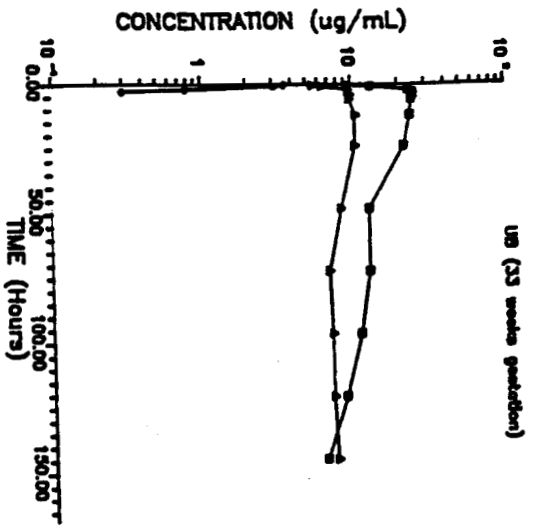

GB (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

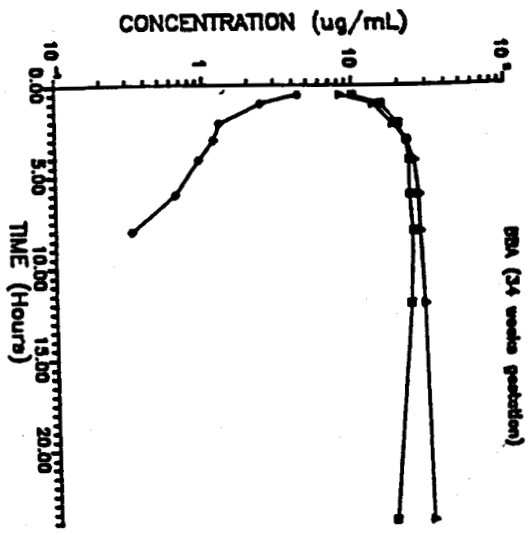

GB (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

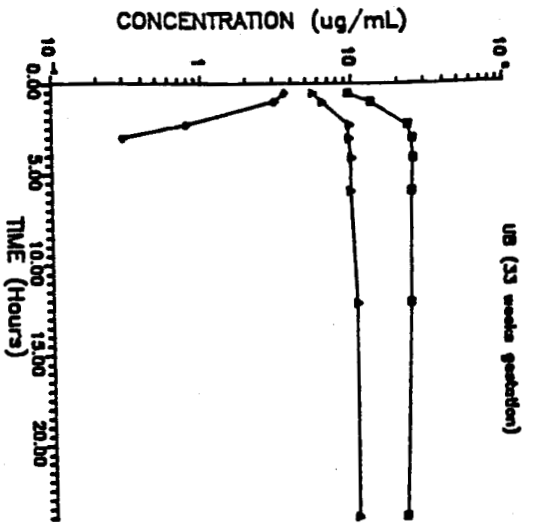

JB (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

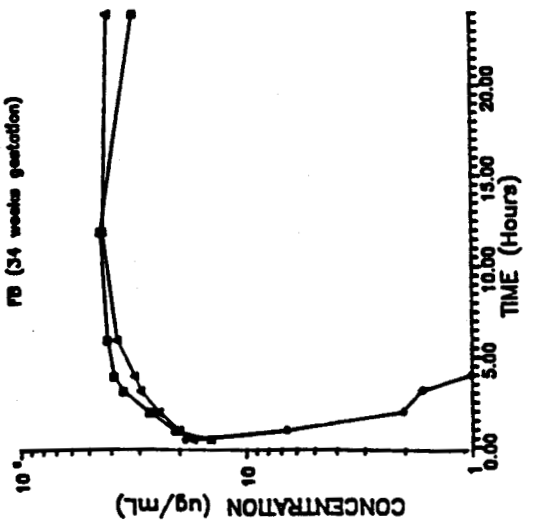

JB (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

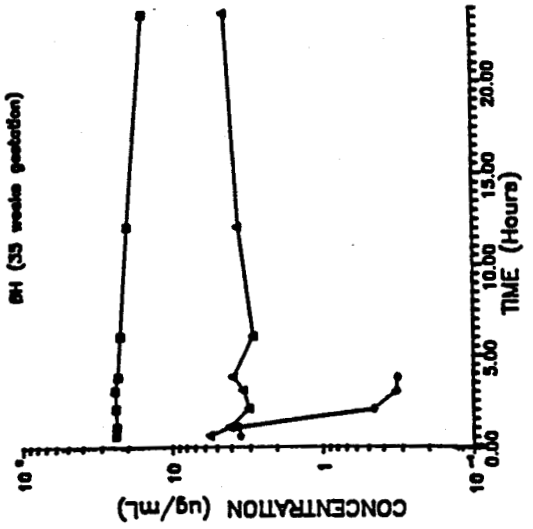

CONCENTRATION (ug/mL)
TIME (Hours)
SBA (34 weeks gestation)

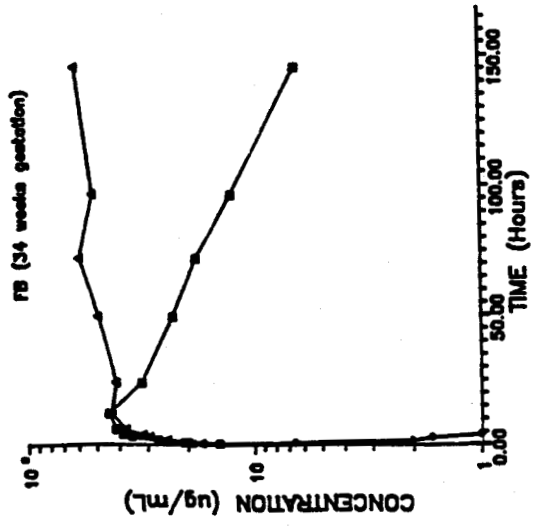

CONCENTRATION (ug/mL)
TIME (Hours)
UB (33 weeks gestation)

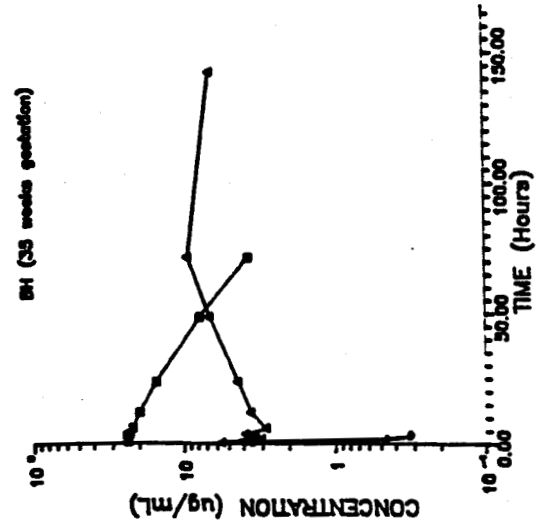

CONCENTRATION (ug/mL)
TIME (Hours)
SBA (34 weeks gestation)

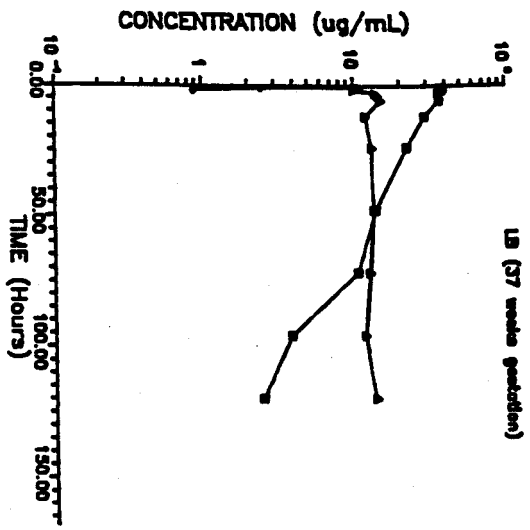

CONCENTRATION (ug/mL)
TIME (Hours)
UB (33 weeks gestation)

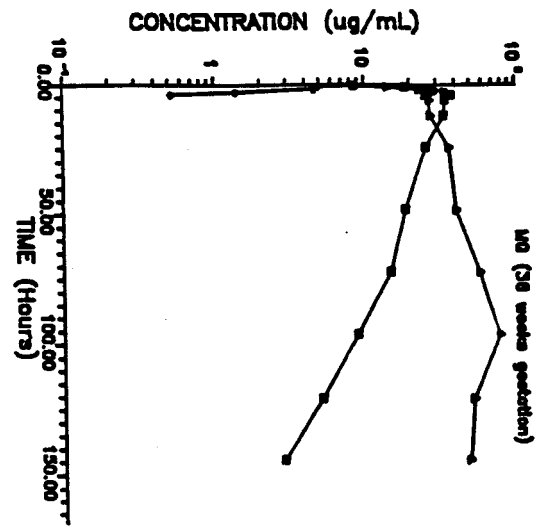

FB (34 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

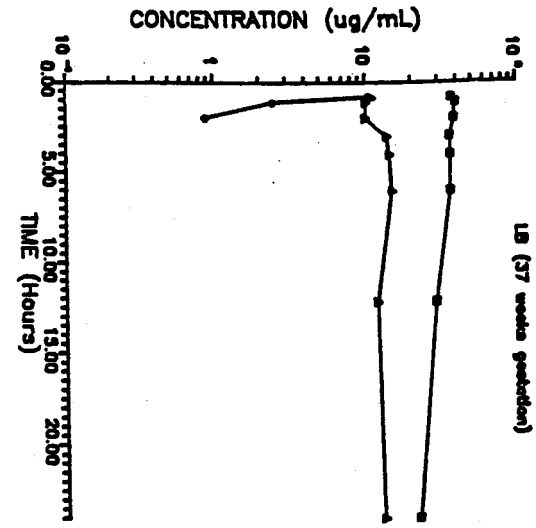

BH (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

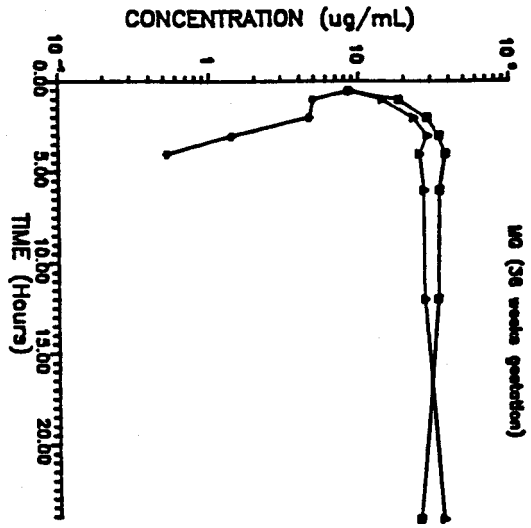

FB (34 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

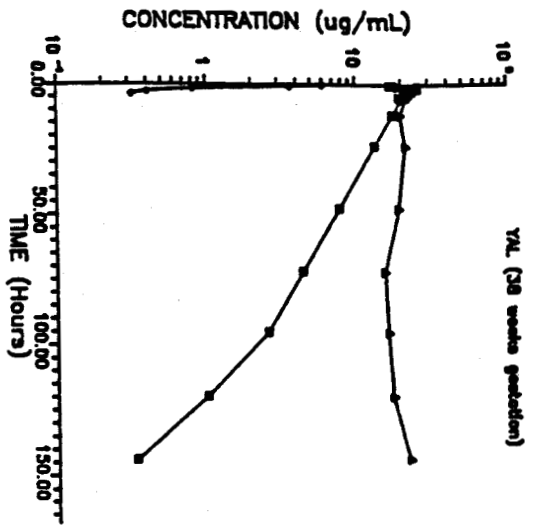

BH (33 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)

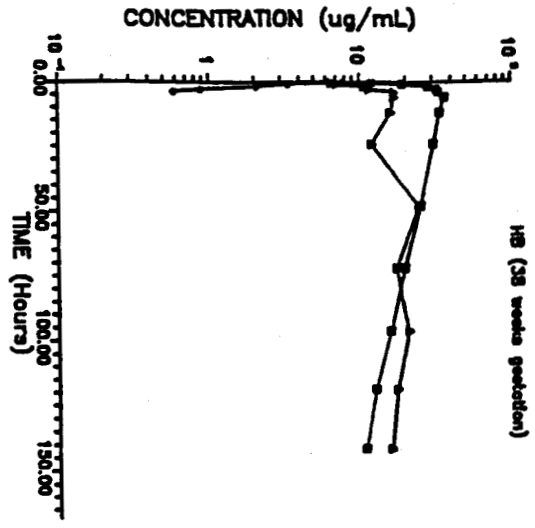

CONCENTRATION (ug/mL)
TIME (Hours)
LB (37 weeks gestation)

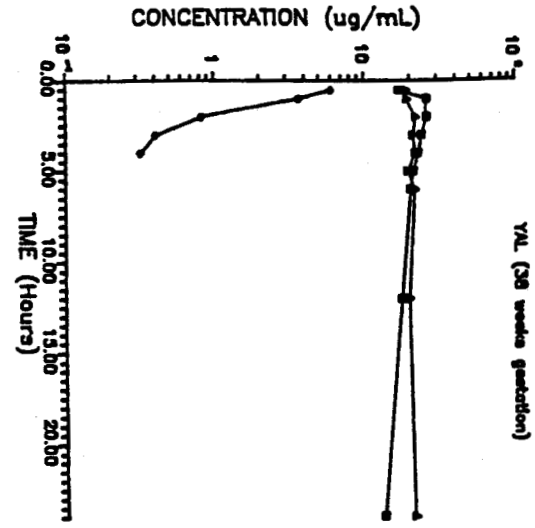

CONCENTRATION (ug/mL)
TIME (Hours)
MO (38 weeks gestation)

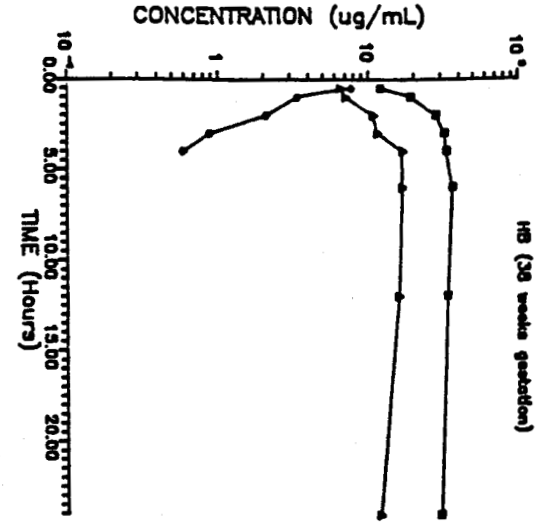

CONCENTRATION (ug/mL)
TIME (Hours)
LB (37 weeks gestation)

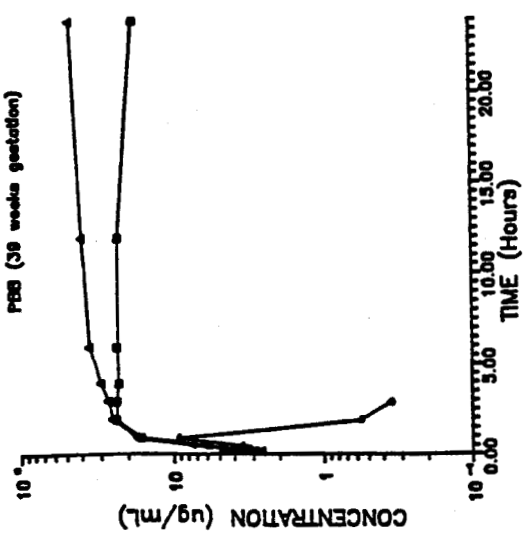

CONCENTRATION (ug/mL)
TIME (Hours)
MO (38 weeks gestation)

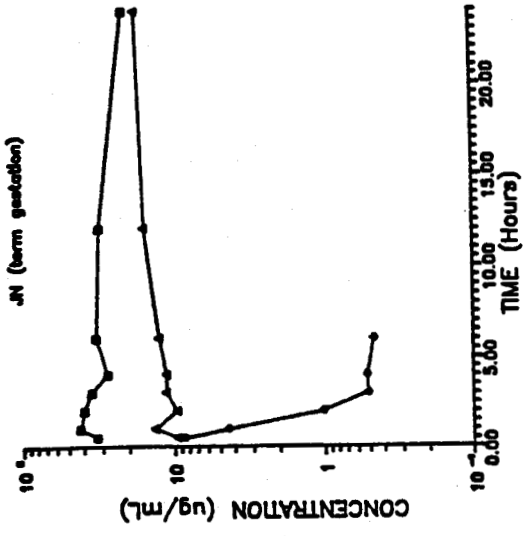

CONCENTRATION (ug/mL)
10⁻¹
1
10
10¹
0.00
50.00
100.00
150.00
TIME (Hours)
YAL (38 weeks gestation)

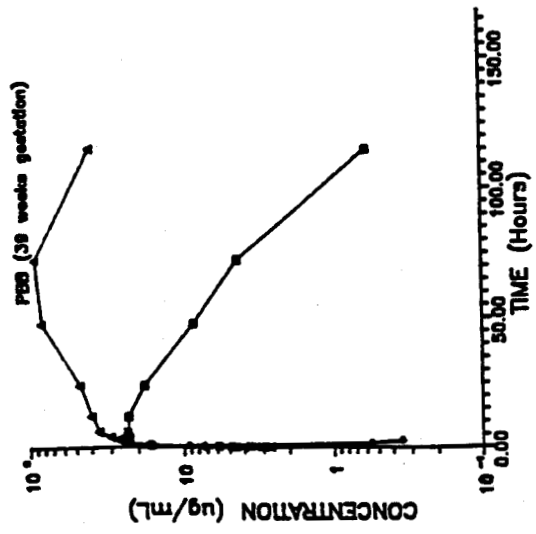

CONCENTRATION (ug/mL)
10⁻¹
1
10
10¹
0.00
50.00
100.00
150.00
TIME (Hours)
HB (38 weeks gestation)

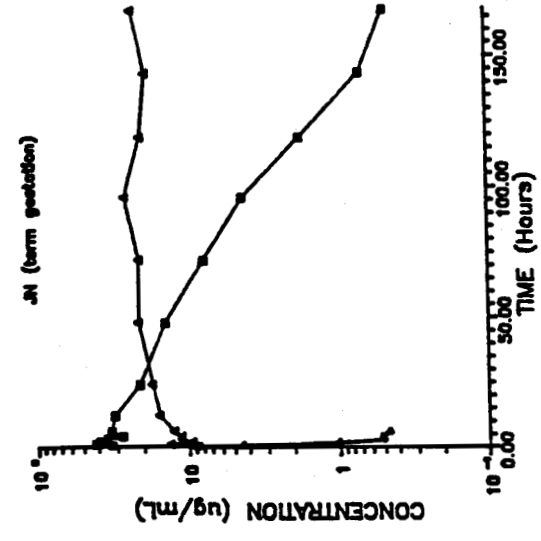

CONCENTRATION (ug/mL)
10⁻¹
1
10
10¹
0.00
5.00
10.00
15.00
20.00
TIME (Hours)
YAL (38 weeks gestation)

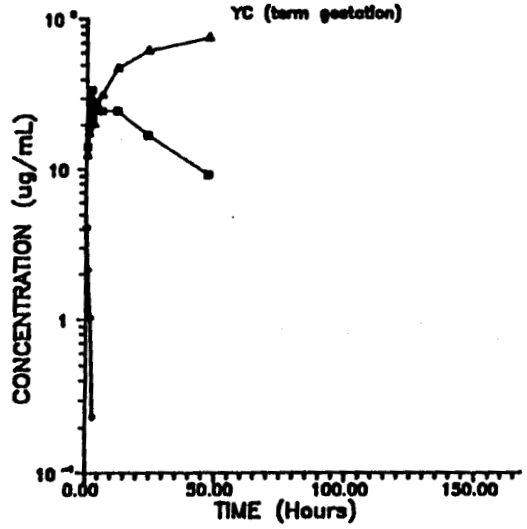

CONCENTRATION (ug/mL)
10⁻¹
1
10
10¹
0.00
5.00
10.00
15.00
20.00
TIME (Hours)
HB (38 weeks gestation)

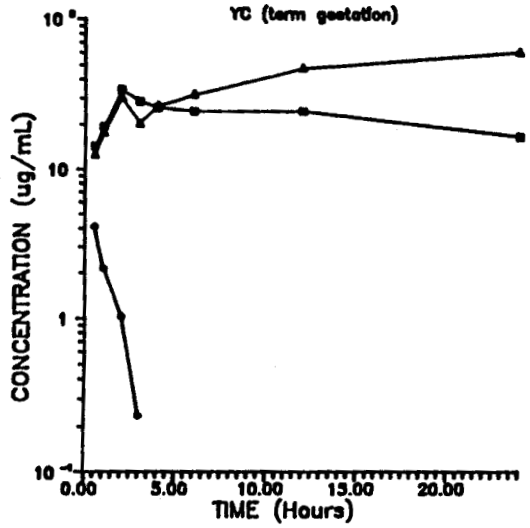

PBB (38 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

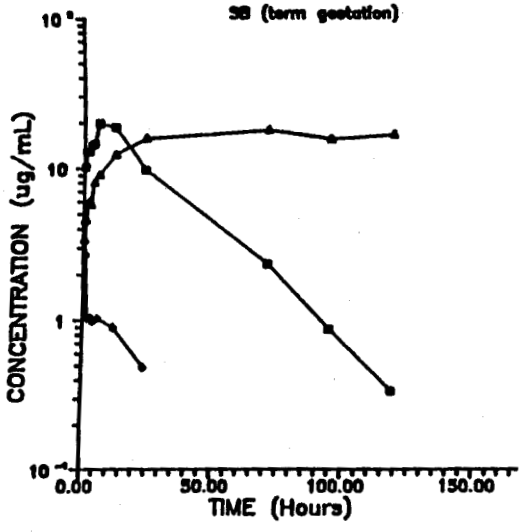

JN (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

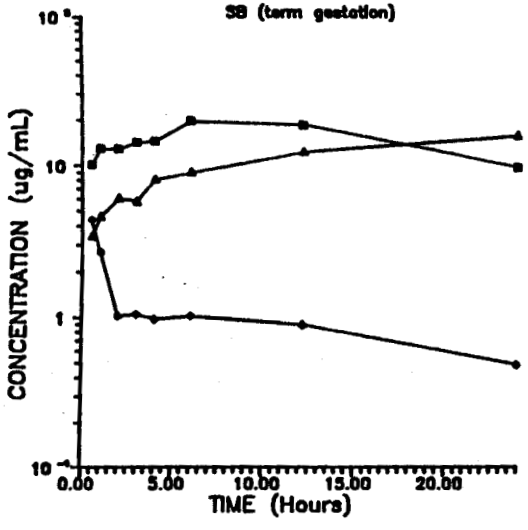

PBB (38 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

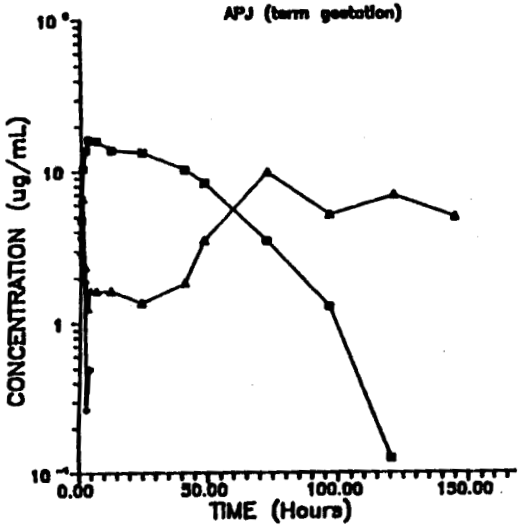

JN (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

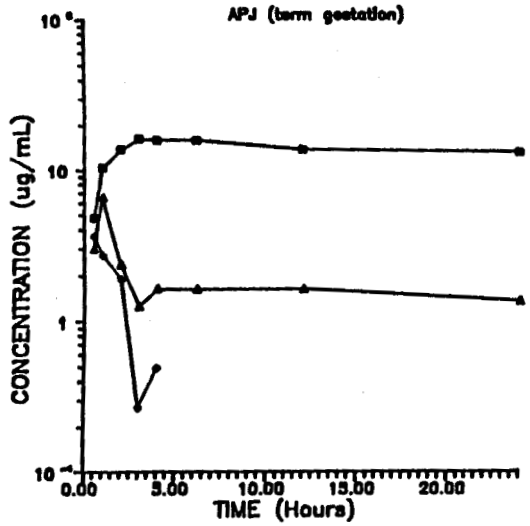

YC (term gestation)
CONCENTRATION (ug/mL)
10 °
10
1
10 -1
0.00
50.00
100.00
150.00
TIME (Hours)

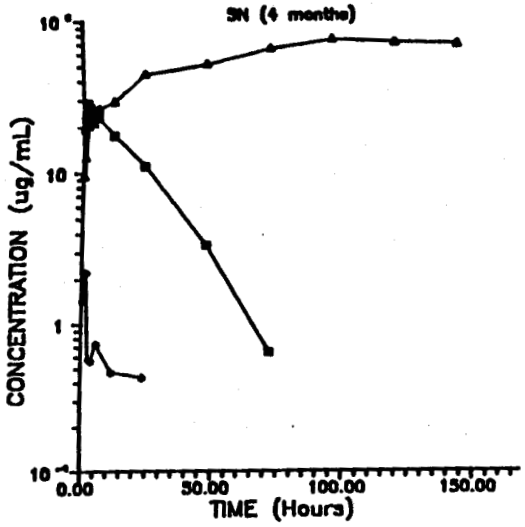

YC (term gestation)
CONCENTRATION (ug/mL)
10 °
10
1
10 -1
0.00
5.00
10.00
15.00
20.00
TIME (Hours)

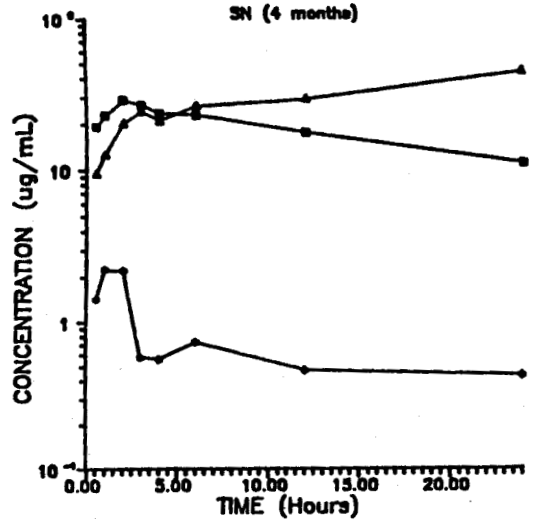

SB (term gestation)
CONCENTRATION (ug/mL)
10 °
10
1
10 -1
0.00
50.00
100.00
150.00
TIME (Hours)

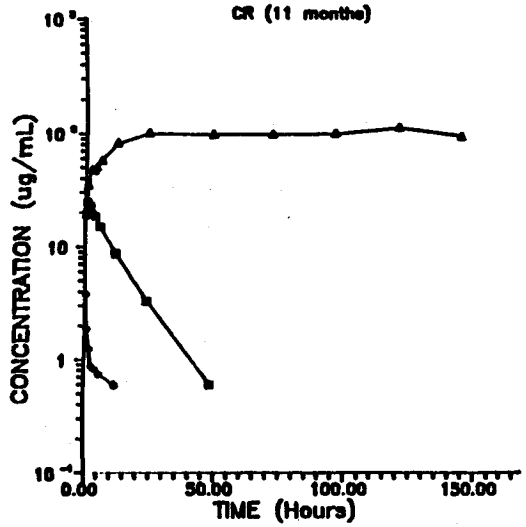

SB (term gestation)
CONCENTRATION (ug/mL)
10 °
10
1
10 -1
0.00
5.00
10.00
15.00
20.00
TIME (Hours)

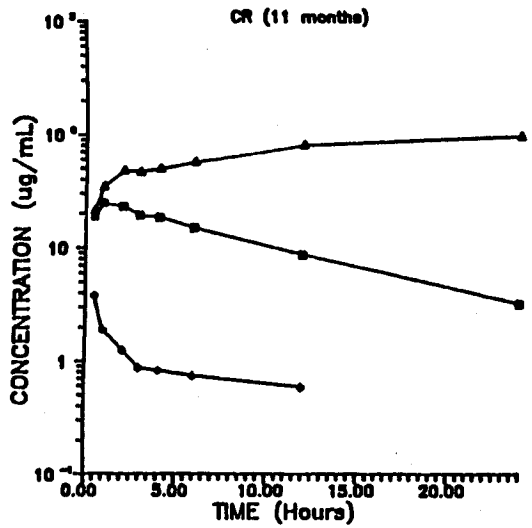

APJ (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

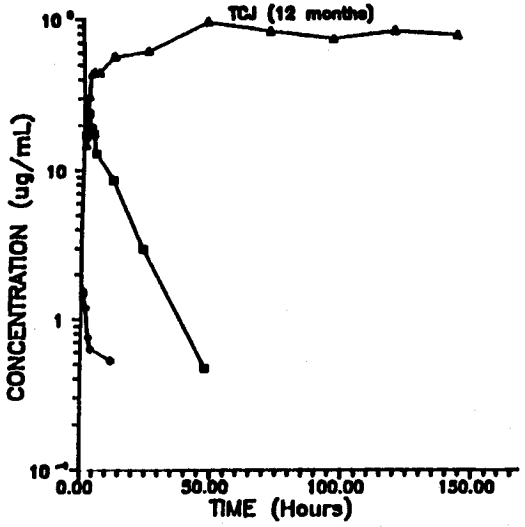

APJ (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

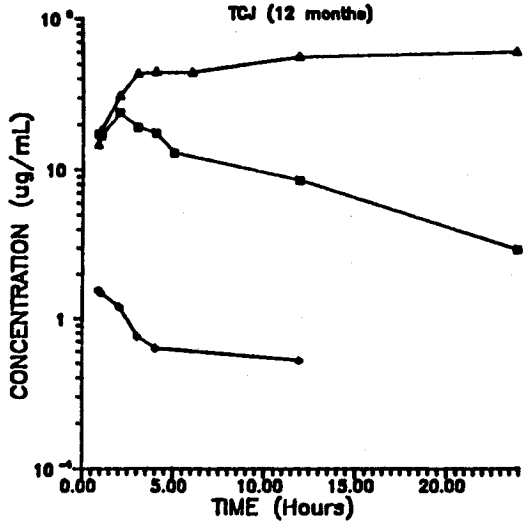

SN (4 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

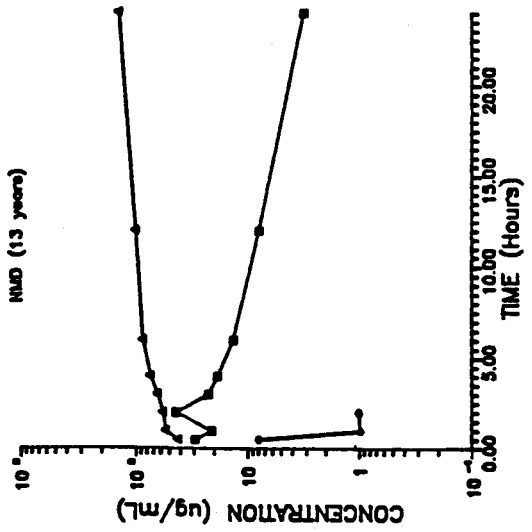

SN (4 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

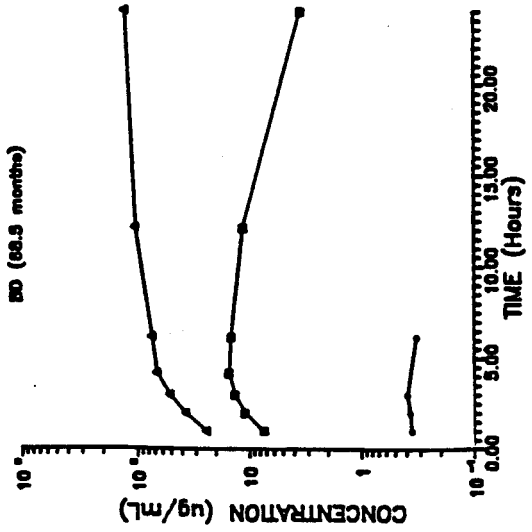

CR (11 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00


CR (11 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00


TCJ (12 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

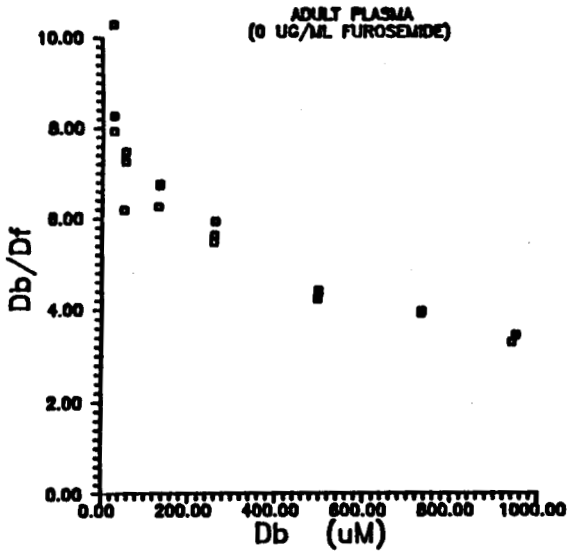

TCJ (12 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

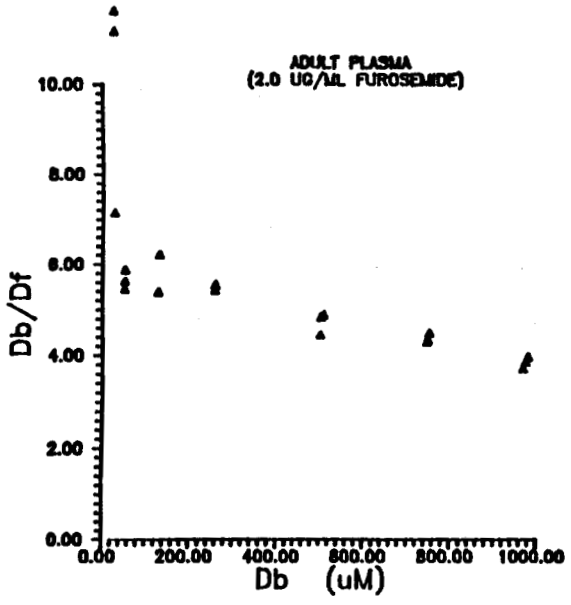

NMD (13 years)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

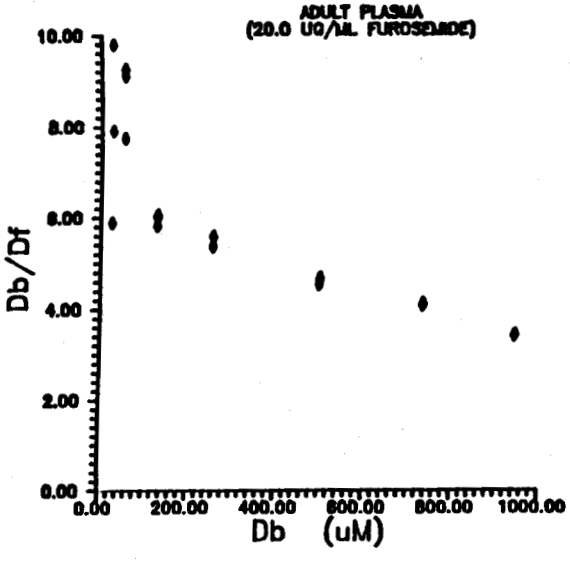

BD (60.5 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
5.00
10.00
15.00
20.00

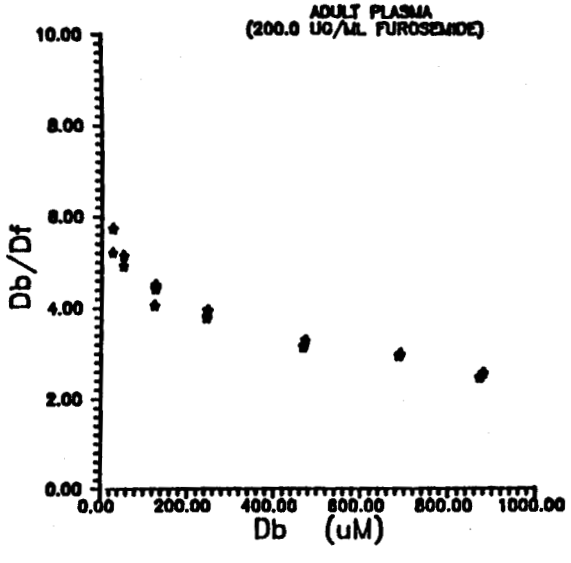
APPENDIX F

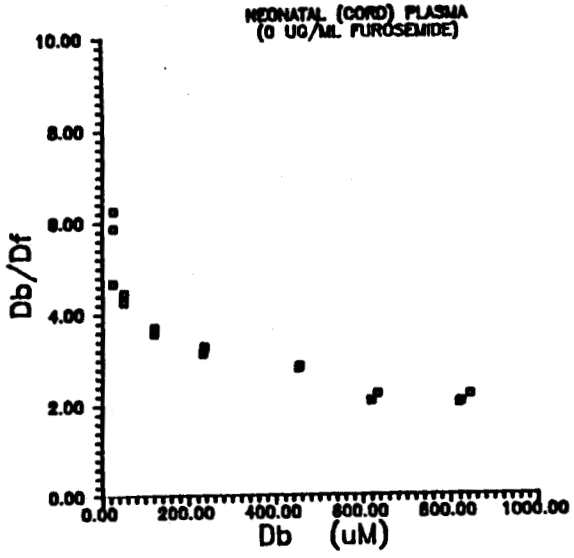
ROSENTHAL PLOTS

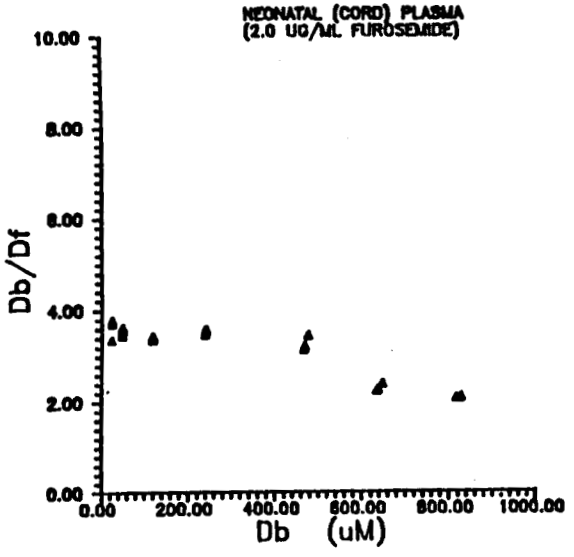

ADULT PLASMA
(0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

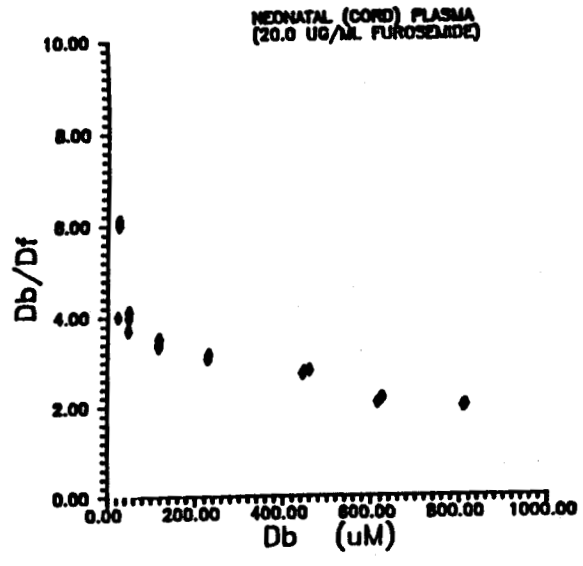

ADULT PLASMA
(2.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

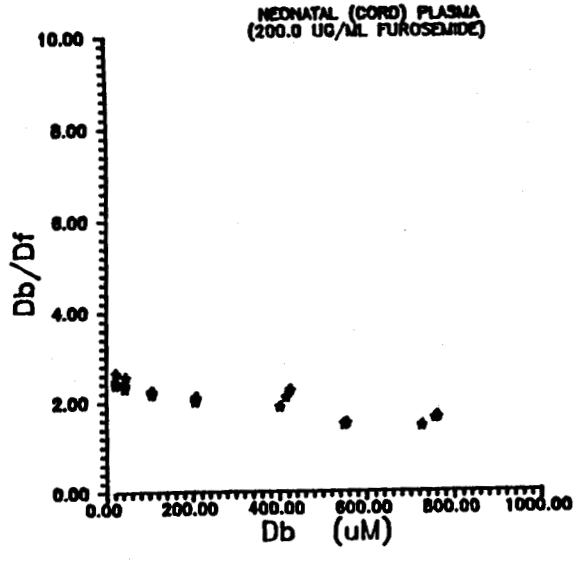

ADULT PLASMA
(20.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

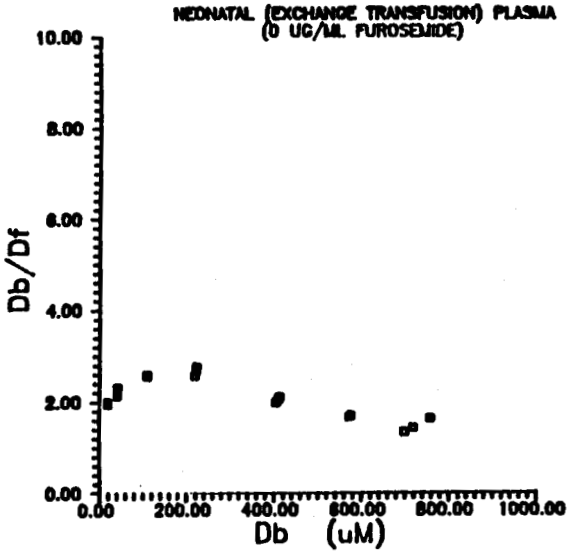

ADULT PLASMA
(200.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

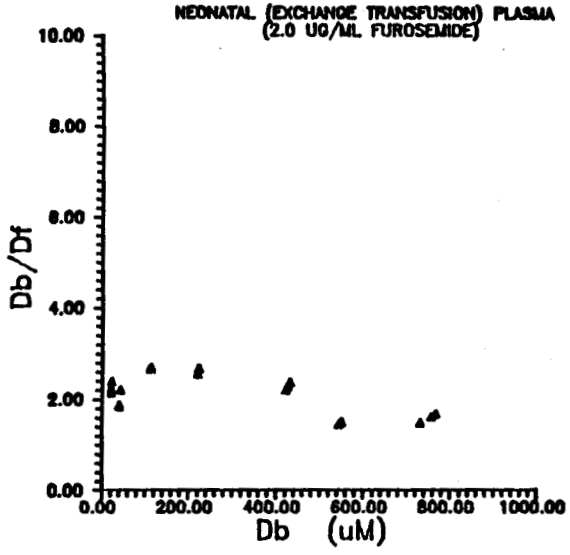

NEONATAL (CORD) PLASMA
(0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

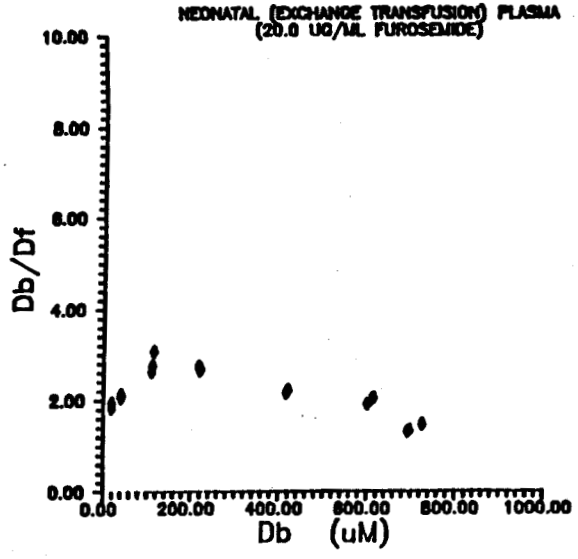

NEONATAL (CORD) PLASMA
(2.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

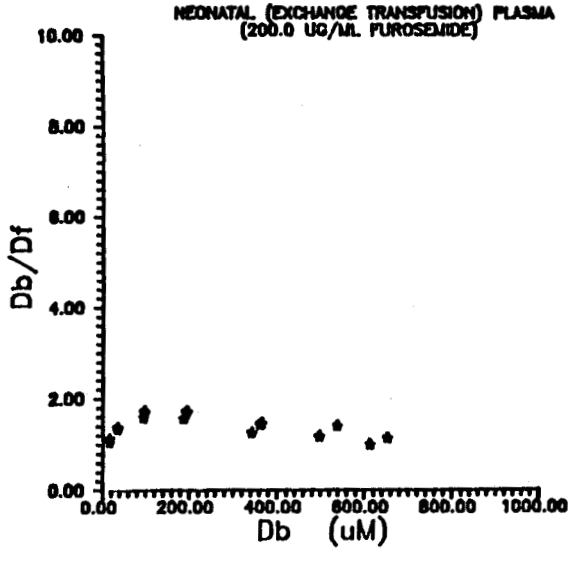

NEONATAL (CORD) PLASMA
(20.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)


NEONATAL (CORD) PLASMA
(200.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)


NEONATAL (EXCHANGE TRANSFUSION) PLASMA
(0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)


NEONATAL (EXCHANGE TRANSFUSION) PLASMA
(2.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)


NEONATAL (EXCHANGE TRANSFUSION) PLASMA
(20.0 UG/ML FUROSEMIDE)
Db/Df
10.00
8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)


NEONATAL (EXCHANGE TRANSFUSION) PLASMA
(200.0 UG/ML FUROSEMIDE)
Db/Df
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8.00
6.00
4.00
2.00
0.00
0.00
200.00
400.00
600.00
800.00
1000.00
Db (uM)

APPENDIX G

MULTIPLE DOSE STUDY PATIENT CLINICAL DATA

SEMILOGARITHMIC CONCENTRATION TIME PLOTS

CH ADMINISTRATION AND SEDATION/AGITATION SCORES

SEDATION SCORE VERSUS CH AND TCE CONCENTRATION PLOTS

Clinical data from patients enrolled in multiple dose study

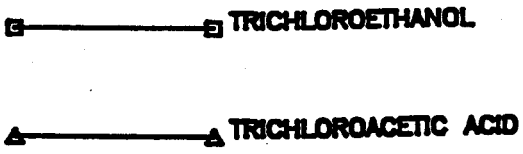
SUBJECT	AGE		SEX	RACE	WEIGHT (kg)	DIAGNOSIS	OTHER MEDICATIONS
	GEST	POST					
PS	24	6 d	M	N	0.769	RDS/PDA	AMP,BRET,CLOX,EXO,FENT,GENT, IND,MORP,PANC,SUC,VIT D,VIT E,VIT K
BBM	29	1 d	M	W	1.61	RDS	AMIN,AMP,DEX,EXO,GENT,IND,SUC, VIT E,
BW	30	3d	M	N	1.56	RDS/sepsis	AMIN,AMP,CEF,EXO,FENT,FUR,GENT, GLY,NYS,PANC,SUC,TOL,VAN,
ABB	30	2d	M	W	1.26	RDS	AMIN,AMP,GENT,SUC
HBB	32	0d	M	W	2.45	RDS	AMP,GENT,SUC,SUR
DJ	32	1d	M	W	1.58	RDS	AMP,GENT,SUC,VIT E
WDR	33	0d	F	W	2.24	RDS	AMP,EXO,FENT,GENT,SUC,VIT E,VIT K
JBG	33	1d	F	B	2.42	RDS	AMP,EXO,FENT,GENT,VIT E, VIT K
BBS	34	1d	M	N	2.12	RDS	AMIN,AMP,EXO,FENT,GENT,SUC
BS	34	5d	F	W	2.06	RDS	AMP,FENT,GENT,SUC,VIT K
BGJ	35	0d	F	W	3.10	RDS	AMP,EXO,FENT,GENT,SUC,TOL
JS	35	3d	M	W	2.96	RDS	AMP,FENT,GENT,SUC
RN	35	1d	F	W	2.33	RDS	AMP,EXO,FENT,GENT,SUC
BGC	35	1d	F	W	2.48	RDS	AMP,FENT,GENT,IND,SUC
CB	37	1d	M	W	2.94	RDS	AMP,FENT,GENT,SUC,SUR
SBB	37	6d	N	N	3.03	MEC. ASP.	AMP,CEF,FENT,FUR,GENT,PANC,SUC
KBG	41	0d	F	W	4.30	MEC. ASP.	AMP,CLOX,GENT,POLY,SUC,VIT E
TB	T	0d	M	W	3.01	MEC. ASP.	AMP,FENT,GENT,SUC
WJ	T	0d	M	N	4.42		AMIN,AMP,CEF,FENT,PHEN,SUC
BBH	32	1 mo	M	W	2.52	RSV	AMP,ATRO,GENT,RIBA,TRI-VI-SOL
JLM		4 mo	F	N	5.52	RSV	ACET,AMP,DIAZ,HCT,MORP,RIBA, SAL,SPI
JPK		18mo	M	W	10.48	TRACHEITIS	ACET,ATRO,CEF,CLOX,DIAZ,EPI,FE, FENT,GLY,MID,MORP,SUC

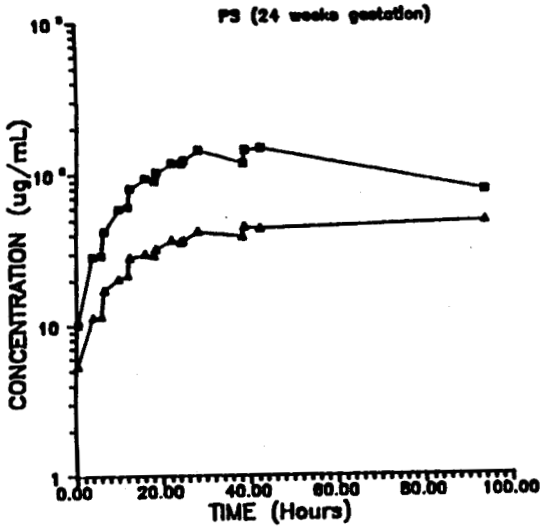
Race: B= Black; N= Native North American; W= White

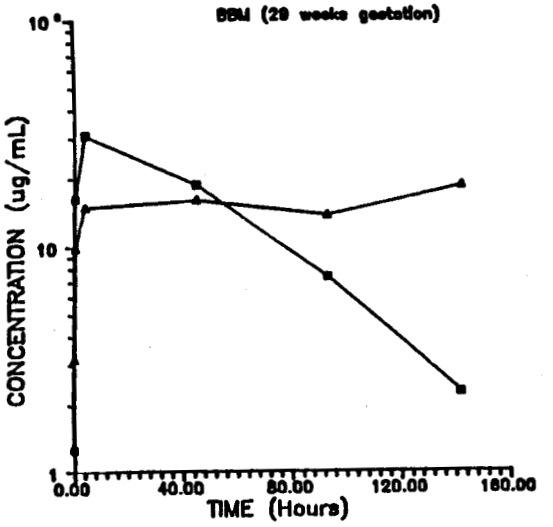
Diagnosis: RDS= Repiratory Distress Syndrome; PDA= Patent Ductus Arteriosus; MEC. ASP.= Meconium Aspiration; RSV= Respiratory Syncitial Virus

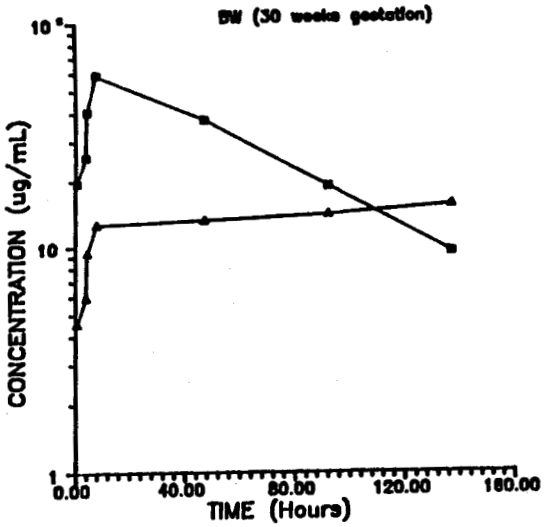
Medications: ACET= Acetaminophen; AMIN= Aminophylline; AMP= Ampicillin; ATRO= Atropine; BRET= Bretyllium; CEF= Cefotaxime; CLOX= Cloxacillin; DEX= Dexamethasone; DIAZ= Diazepam; EPI= Epinephrine; EXO= Exosurf (artificial surfactant); FE= Iron; FENT= Fentanyl; FUR= Furosemide; GENT= Gentamicin; GLY= Glycine; HCT= Hydrochlorothiazide; IND= Indomethacin; KAY= Kayexalate (Sodium polystyrene resin); MID= Midazolam; MORP= Morphine; NYS= Nystatin; PANC= Pancuronium; PHEN= Phenobarbital; POLY= Polysporin; RIBA= Ribavirin; SAL= Salbutamol; SPI= Spironolactone; SUC= Sucralfate; SUR= Survanta (surfactant); TOL= Tolazoline; VAN= Vancomycin; VIT D= Vitamin D; VIT E= Vitamin E; VIT K= Vitamin K

SEMILOGARITHMIC CONCENTRATION TIME PLOTS




PS (24 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
20.00
40.00
60.00
80.00
100.00


BBM (29 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
40.00
80.00
120.00
160.00


BW (30 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
40.00
80.00
120.00
160.00

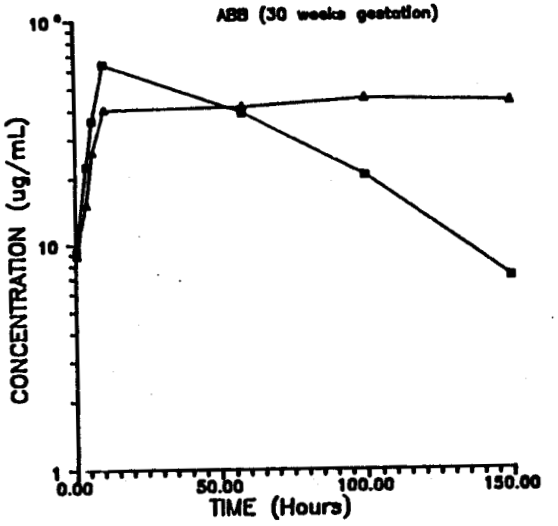
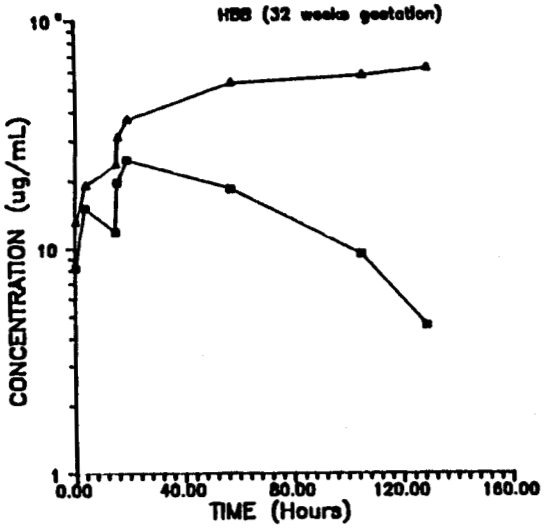
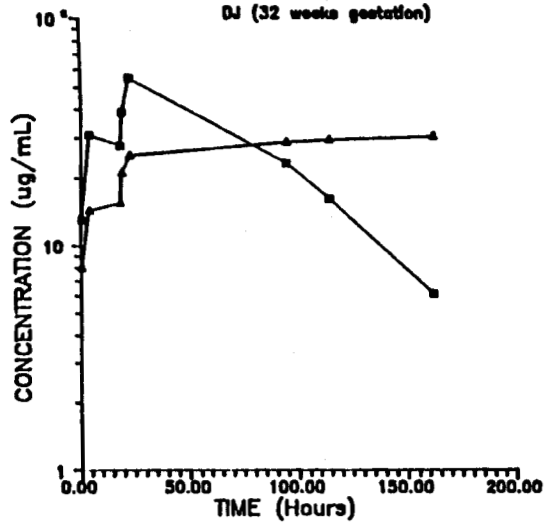
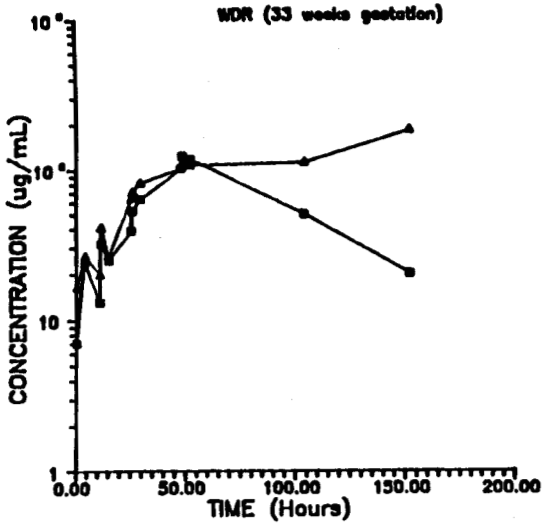
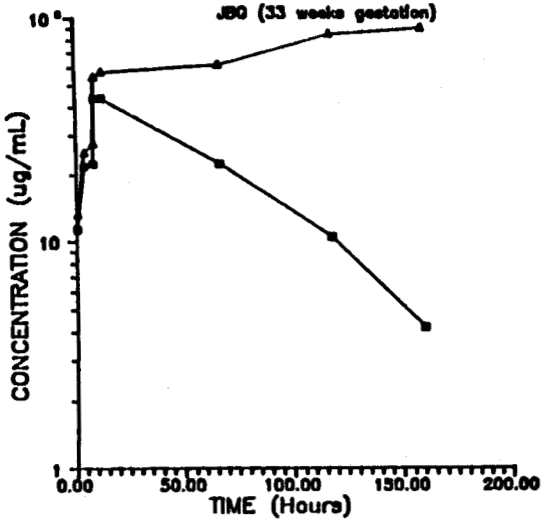


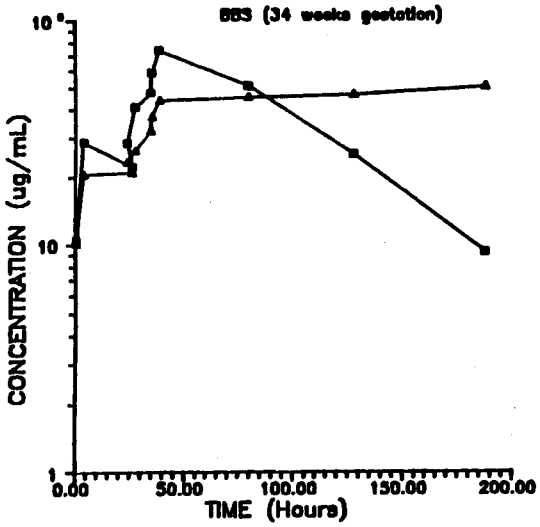
ABB (30 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

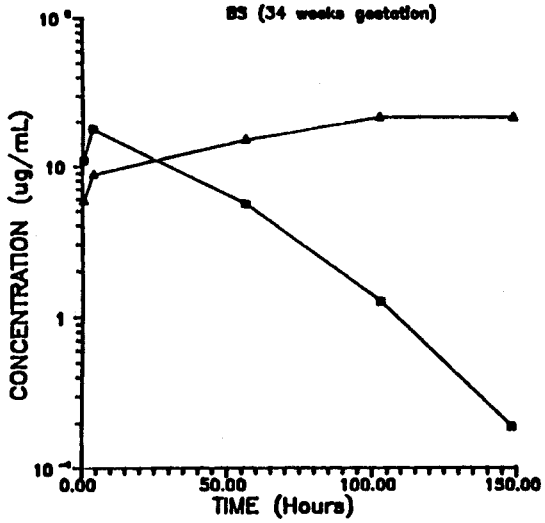

HBB (32 weeks gestation)
CONCENTRATION (ug/mL)
10
1
0.00
40.00
80.00
120.00
160.00
TIME (Hours)

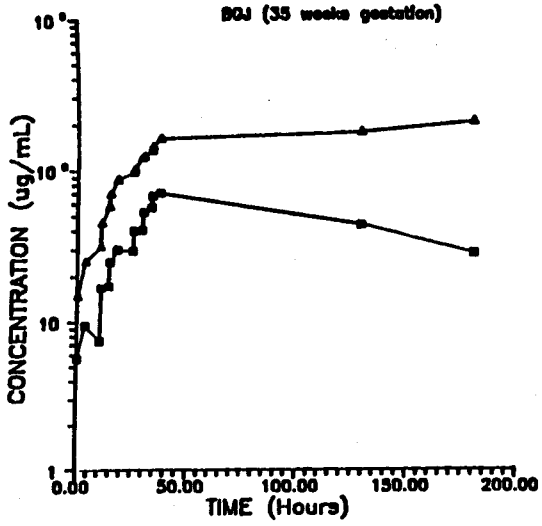

DJ (32 weeks gestation)
CONCENTRATION (ug/mL)
10
1
0.00
50.00
100.00
150.00
200.00
TIME (Hours)

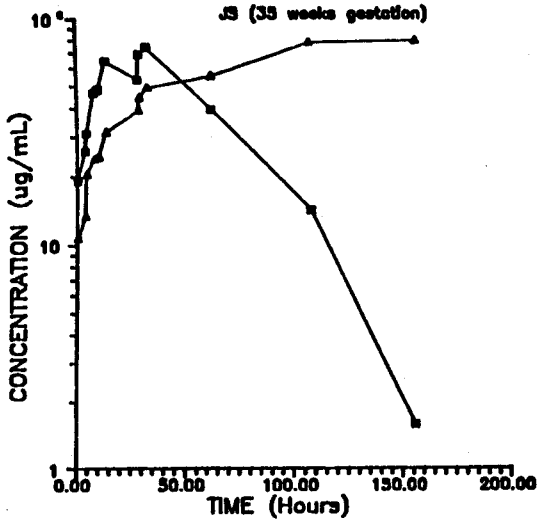

WDR (33 weeks gestation)
CONCENTRATION (ug/mL)
10
1
0.00
50.00
100.00
150.00
200.00
TIME (Hours)

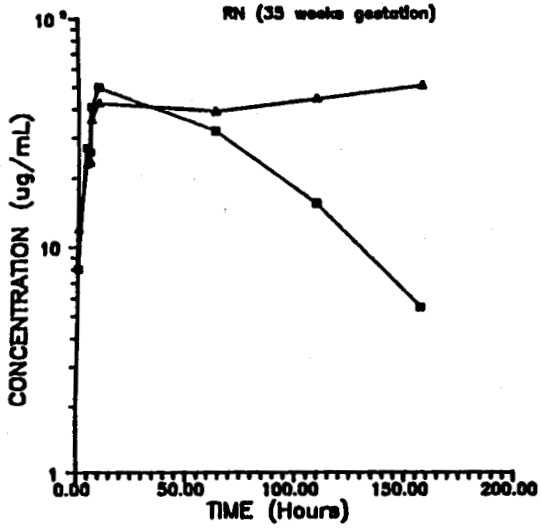

JBO (33 weeks gestation)
CONCENTRATION (ug/mL)
10
1
0.00
50.00
100.00
150.00
200.00
TIME (Hours)

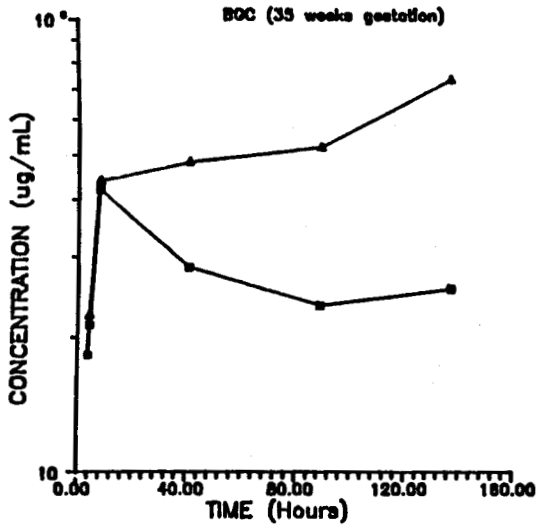

BBS (34 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00
200.00

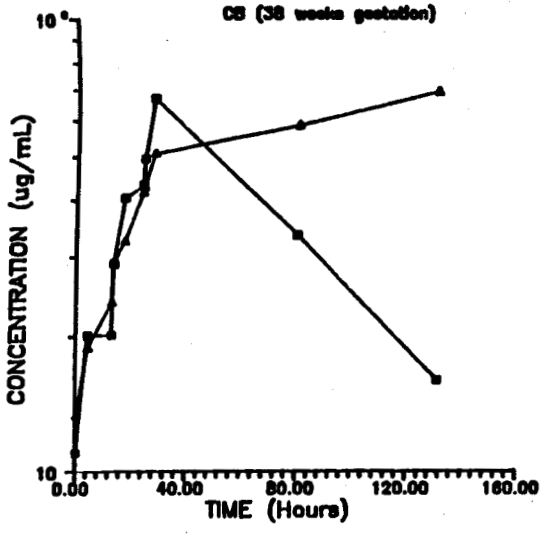

BS (34 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00

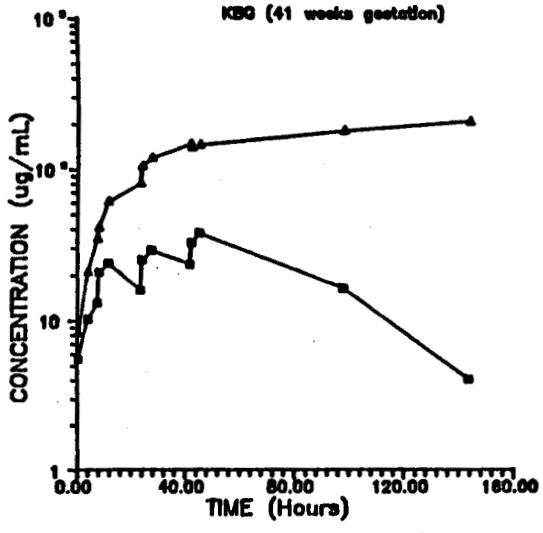

BGJ (35 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00
200.00

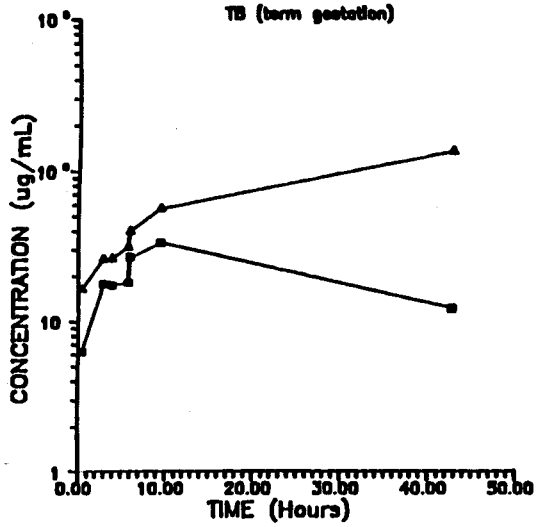

JS (35 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
50.00
100.00
150.00
200.00

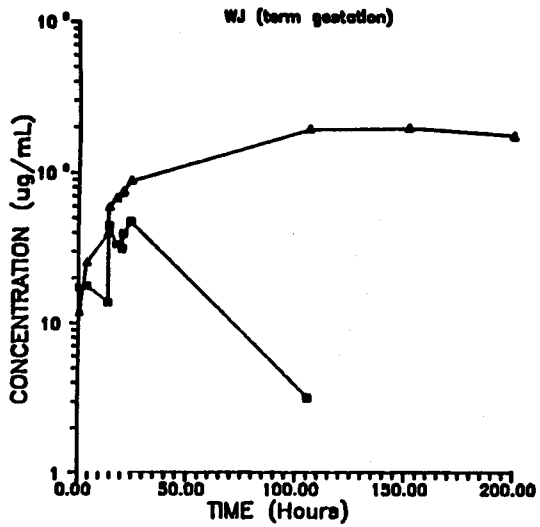

RN (35 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
10
1
0.00
50.00
100.00
150.00
200.00

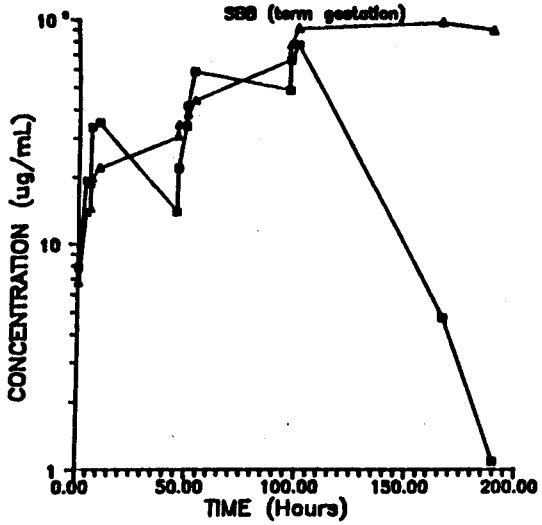

BOC (35 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
10
0.00
40.00
80.00
120.00
160.00

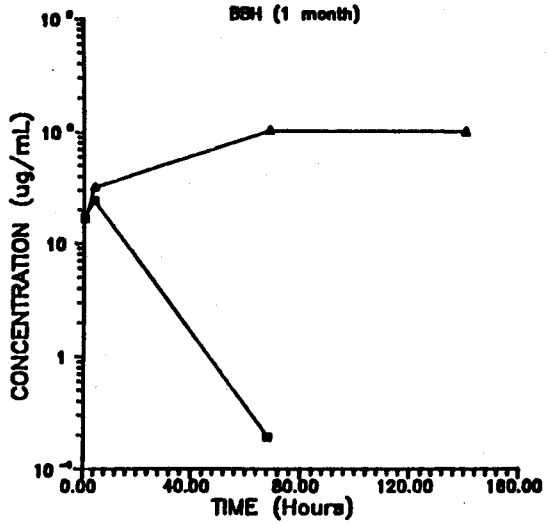

CS (38 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
10
0.00
40.00
80.00
120.00
160.00

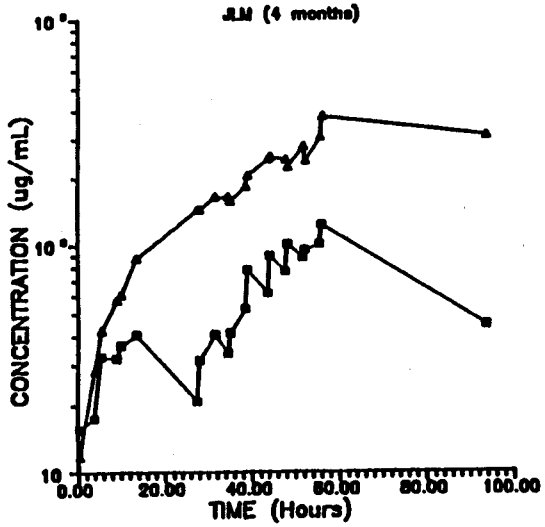

KBG (41 weeks gestation)
CONCENTRATION (ug/mL)
TIME (Hours)
10
1
0.00
40.00
80.00
120.00
160.00

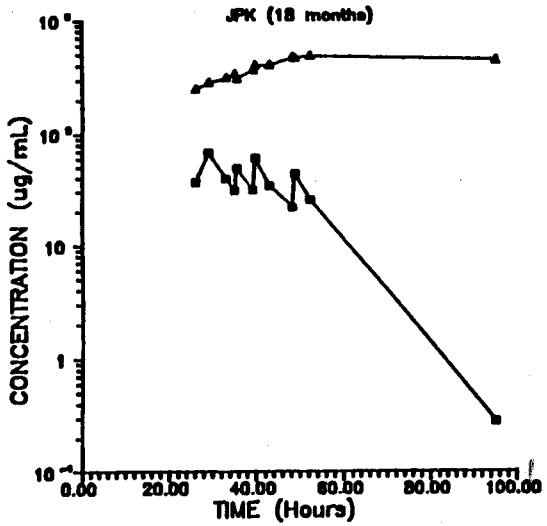

TB (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)


WJ (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)


SBB (term gestation)
CONCENTRATION (ug/mL)
TIME (Hours)


BBH (1 month)
CONCENTRATION (ug/mL)
TIME (Hours)


JLM (4 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
20.00
40.00
60.00
80.00
100.00


JPK (18 months)
CONCENTRATION (ug/mL)
TIME (Hours)
0.00
20.00
40.00
60.00
80.00
100.00

CH ADMINISTRATION AND SEDATION/AGITATION SCORE DATA

(CH)= administration of chloral hydrate

Relationship of CH administration to sedation score in individual patients

SUBJECT	DATE	TIME	SCORE
PS	18/09/90	1030 (CH)	17
		1100	16
		1430	16
		1630 (CH)	17
		1710	16
		2030	16
		2230 (CH)	15
		2300	13
	19/09/90	0230	16
		0430 (CH)	15
		0500	13
		0830	?
		1035 (CH)	16
		1105	16
		1430	?
	20/09/90	0042 (CH)	?
		0110	?
		0440	?
BBM	13/03/91	1115 (CH)	19
		1145	14
		1515	16
BW	21/11/90	1430 (CH)	18
		1510	15
		1830 (CH)	15
		1900	15
		2230	?
ABB	16/03/91	0440 (CH)	20
		0510	18
		0840	15
		1020 (CH)	20
		1050	17
		1500	15
HBB	29/10/90	1055 (CH)	19
		1125	13
		1500	15
	30/10/90	0205 (CH)	16
		0235	16
		0600	14
DJ	02/08/91	1415 (CH)	16
		1445	16
		1815	14
	03/08/91	0830 (CH)	19
		0910	10
		1245	13

SUBJECT	DATE	TIME	SCORE
WDR	25/02/91	2350 (CH)	18
	26/02/91	0015	?
		0400	?
		1100 (CH)	18
		1130	14
		1500	13
	27/02/91	0115 (CH)	22
		0145	14
		0515	14
		1000 (CH)	22
		1400 (CH)	?
		2350	21
	28/02/91	0000 (CH)	?
		0030	13
		0400	13
		0810	17
JBG	23/07/91	1625 (CH)	13
		1700	14
		2025	10
	24/07/91	0030	16
		0045 (CH)	?
		0115	10
		0445	15
BBS	11/02/91	1225 (CH)	15
		1255	11
		1630	14
	12/02/91	1150 (CH)	16
		1220	?
		1600	?
		2315 (CH)	18
		2345	?
	13/02/91	0315	11
BS	11/11/90	0420 (CH)	17
		0450	13
		0800	14
JS	27/02/91	2100 (CH)	20
		2130	14
	28/02/91	0100 (CH)	20
		0130	14
		0500	15
		0630 (CH)	17
		0700	14
		1030	13
	01/03/91	0120 (CH)	19
		0200	13
		0530	13
	02/03/91	1040	18

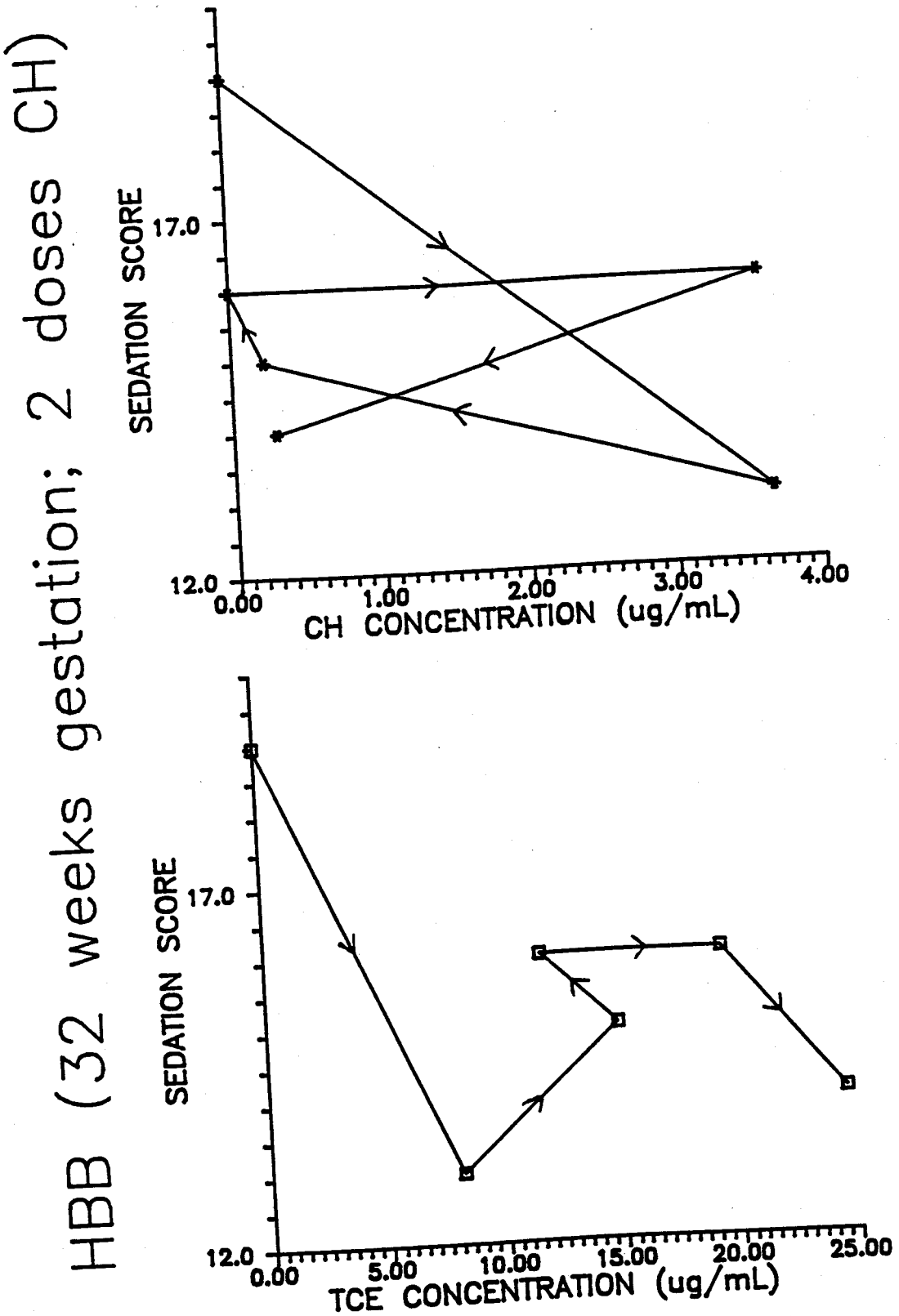
SUBJECT	DATE	TIME	SCORE
BGJ	22/02/91	2210 (CH)	?
		2240	19
	23/02/91	0210	16
		0905 (CH)	17
		0935	16
		1300	18
		1310 (CH)	?
		1340	14
		1700	14
	24/02/91	0000	17
		0007 (CH)	?
		0037	15
		0407	16
		0430 (CH)	?
		0500	14
		0830 (CH)	19
		0900	?
		1230	14
RN	07/10/91	1900 (CH)	17
		1930	?
		2300	14
	08/10/91	0030 (CH)	16
		0100	14
		0430	?
BGC	07/10/91	1515 (CH)	?
		1545	?
		1920 (CH)	?
		1945	?
		2315	?
CB	28/08/91	2310 (CH)	21
		2340	13
	29/08/91	0350	13
		1240 (CH)	19
		1310	?
		1700	?
		2330 (CH)	21
	30/08/91	0000	11
		0330	13
WJ	17/07/91	0100 (CH)	17
		0130	14
		0500	14
		1450 (CH)	17
		1520	14
		1845	14
		2115 (CH)	17
		2145	15
		0115	14
	19/07/91	0220 (CH)	?
		0250	?

SUBJECT	DATE	TIME	SCORE
KBG	09/11/90	1100 (CH)	17
		1130	16
		1500	14
		1830 (CH)	18
		1900	14
		2230	?
	10/11/90	1015 (CH)	12
		1415	16
	11/11/90	0430 (CH)	17
		0500	11
		0755	?
TB	07/03/91	1715 (CH)	17
		1745	14
		2015	?
		2115	14
		2250 (CH)	19
		2320	13
	08/03/91	0250	14
SBB	15/08/91	1515 (CH)	13
		1545	12
		1915	12
		2100 (CH)	14
		2130	12
	16/08/91	0100	12
	17/08/91	1245	?
		1315	?
		1645	?
		1715	?
		2045	?
	18/08/91	1540 (CH)	?
	19/08/91	0640 (CH)	?
		1615 (CH)	19
		1645	?
		2015	15
BBH	06/02/91	1555 (CH)	12
		1625	12
		2000	13

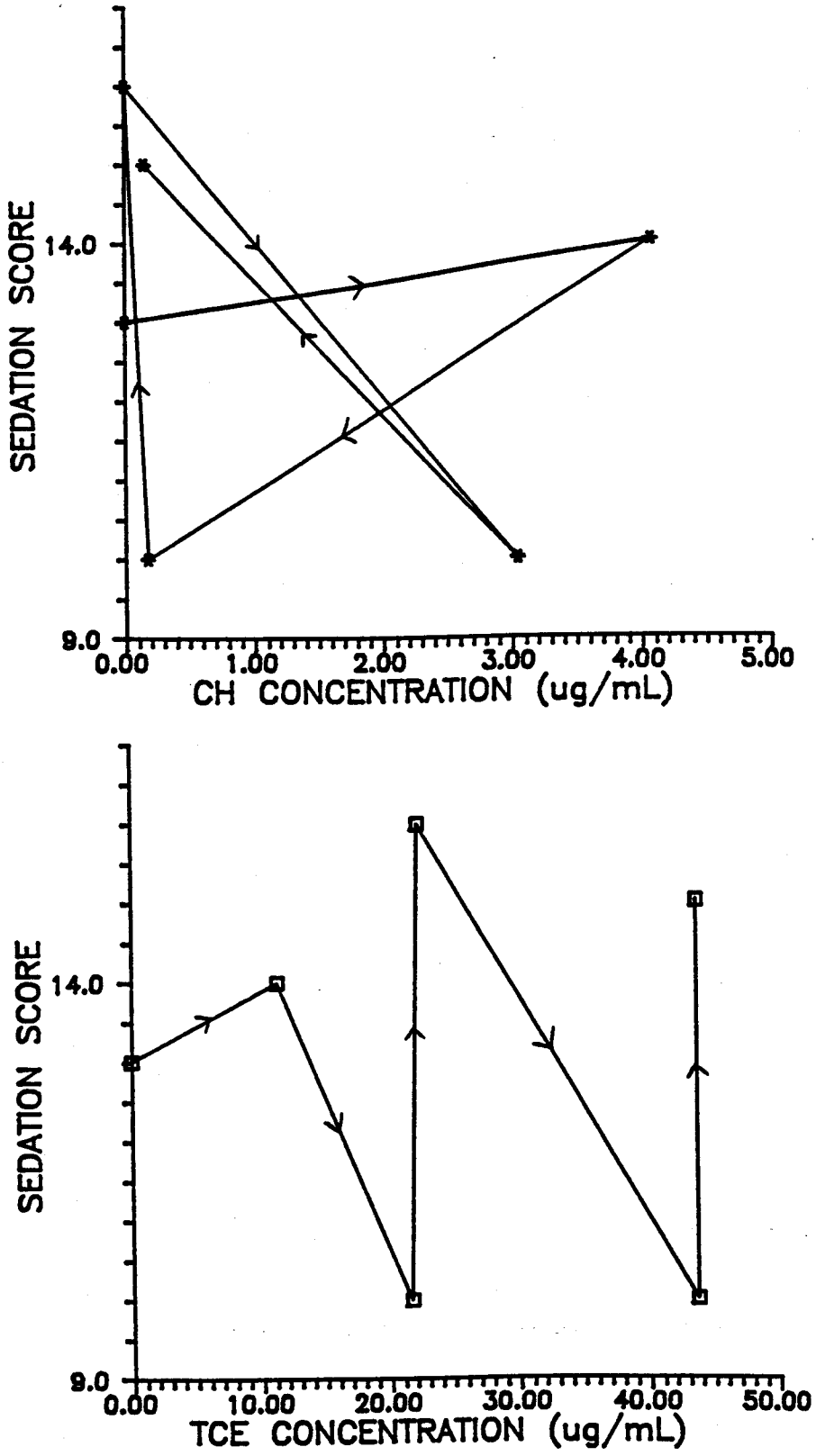
SUBJECT	DATE	TIME	SCORE
JLM	29/01/91	1330 (CH)	15
		1400	13
		1730	12
		1830 (CH)	?
		1900	14
		2230	12
		2300 (CH)	14
		2330	14
	30/01/91	0300	16
		1700 (CH)	12
		1730	14
		2100	13
	31/01/91	0000 (CH)	14
		0030	14
		0400 (CH)	13
		0430	13
		0915	13
		0940	13
		1310 (CH)	14
		1340	14
		1710 (CH)	14
		1740	11
		2100 (CH)	?

SUBJECT	DATE	TIME	SCORE
JPK	15/10/90	0855 (CH)	?
		1410 (CH)	?
		2010 (CH)	?
	16/10/90	0000 (CH)	?
		0600 (CH)	?
		1100	?
		1400 (CH)	16
		1430	?
		1800	?
		2000 (CH)	18
		2030	?
		2400 (CH)	?
	17/10/90	0015	12
		0045	?
		0400	?
		0920 (CH)	17
		0950	14
		1320	16

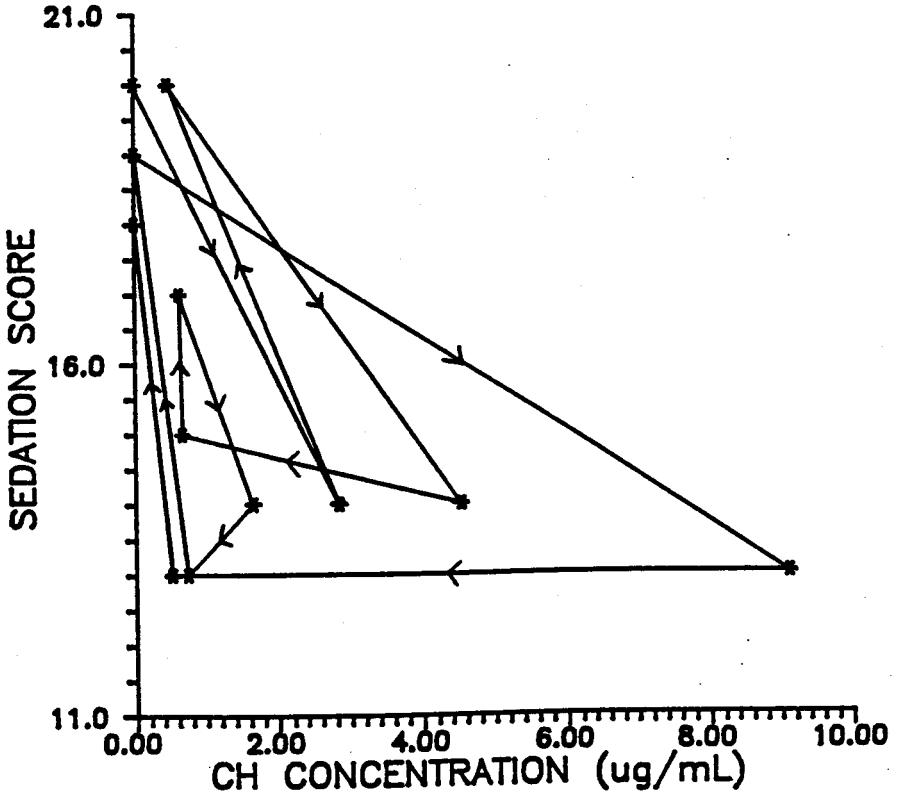
SEDATION SCORE VERSUS CH AND TCE CONCENTRATION PLOTS

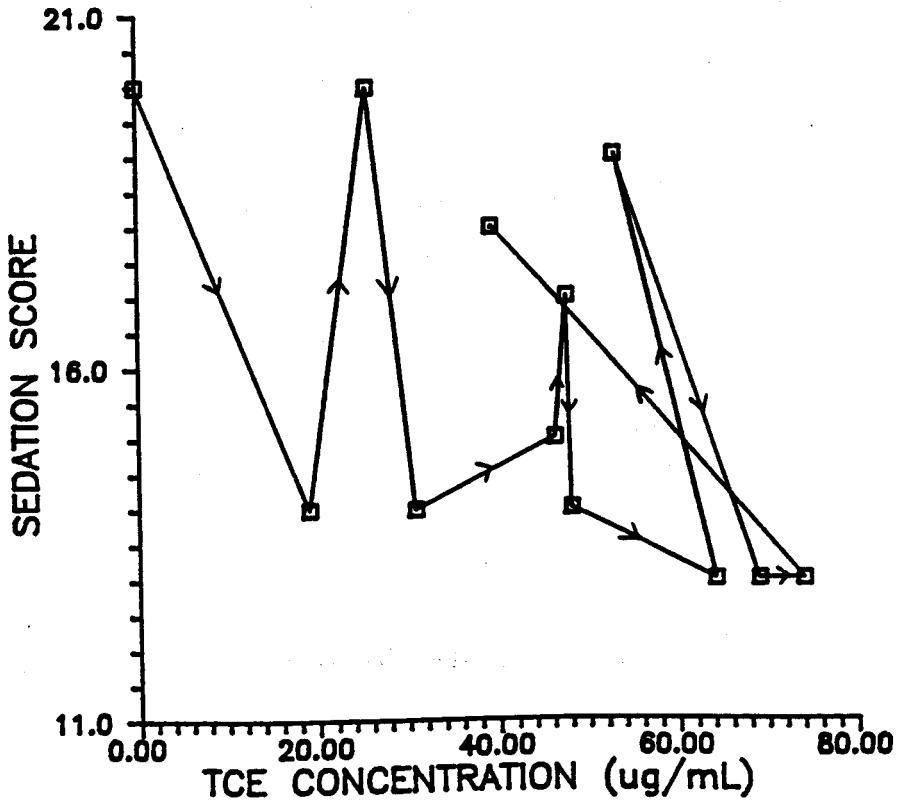

HBB (32 weeks gestation; 2 doses CH)
SEDATION SCORE
17.0
12.0
0.00
1.00
2.00
3.00
4.00
CH CONCENTRATION (ug/mL)
SEDATION SCORE
17.0
12.0
0.00
5.00
10.00
15.00
20.00
25.00
TCE CONCENTRATION (ug/mL)

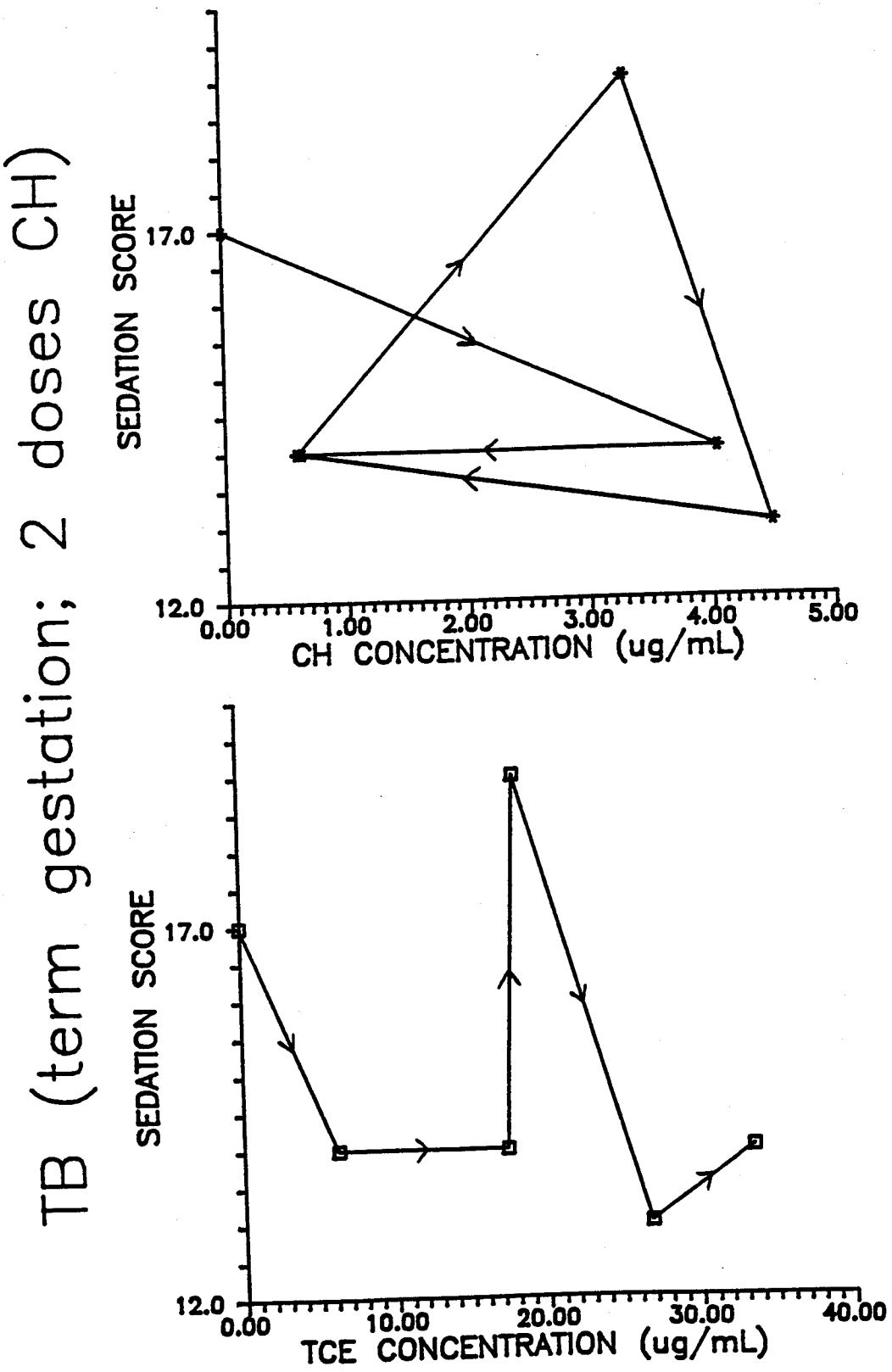
JBG (33 weeks gestation; 2 doses CH)



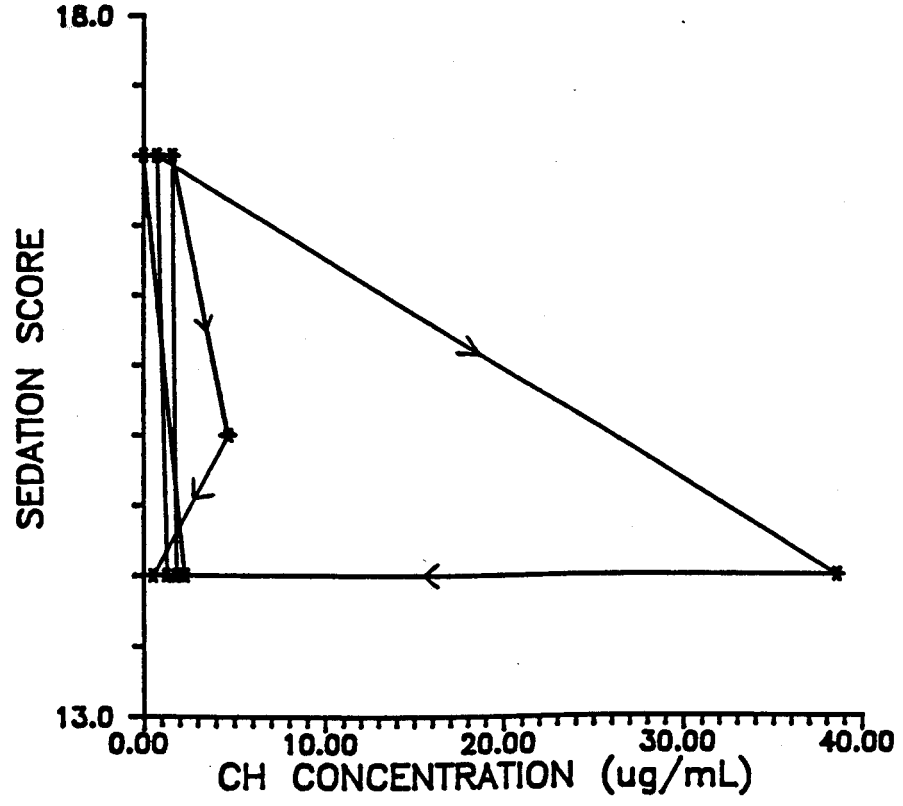
JS (35 weeks gestation; 4 doses CH)

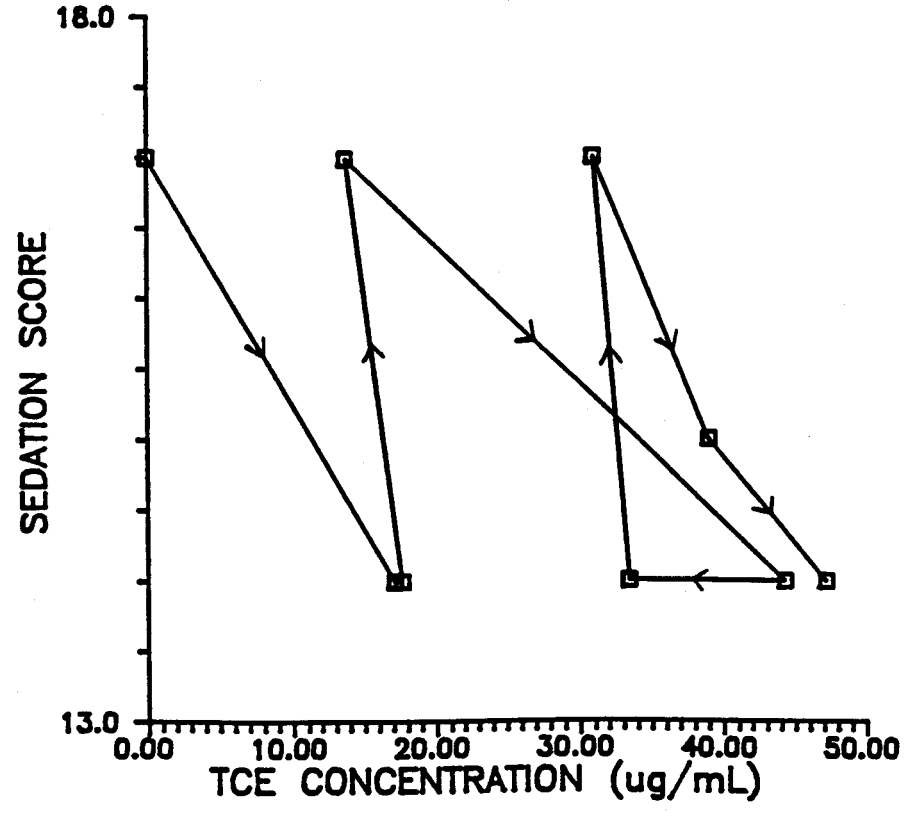





TB (term gestation; 2 doses CH)
SEDATION SCORE
17.0
12.0
0.00
1.00
2.00
3.00
4.00
5.00
CH CONCENTRATION (ug/mL)
SEDATION SCORE
17.0
12.0
0.00
10.00
20.00
30.00
40.00
TCE CONCENTRATION (ug/mL)

WJ (term gestation; 3 doses CH)





DJ (32 weeks gestation; 2 doses CH)