INVESTIGATION INTO OPTIMAL AMIKACIN DOSING IN CHILDREN

by

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PREFACE

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

The research described in this thesis was carried out in the Department of Experimental and Clinical Pharmacology, University of Natal, Durban, under the supervision of Professor Julia Botha.

Statistical planning and analyses in this investigation were conducted in consultation with the Institute for Biostatistics of the Medical Research Council.

Papers and presentations arising from this study are as follows:

Publications:

1. Forsyth NB, Botha JH, Hadley GP.

   A comparison of two amikacin dosing regimens in paediatric surgical patients.

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2. Forsyth NB, Ramjee G, Botha JH, Hadley GP.

   Low molecular weight proteinuria in two amikacin dosing regimens.

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3. Forsyth NB, Botha JH.

Population pharmacokinetic parameters for amikacin in children using Nonlinear Mixed Effects Modelling.

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Congress Presentations (* denotes presenting author):

1. Forsyth NB*, Botha JH, Hadley GP.

Comparison of once versus twice daily dosing of amikacin in children. South African Pharmacology Society 28th Annual Congress. Cape Town, September 1994.

2. Forsyth NB*, Botha JH, Hadley GP.

Comparative toxicities of two amikacin dosing regimens in children.

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3. J Botha*, N Forsyth, M du Preez.

Population pharmacokinetic parameters for amikacin in South African children and neonates.

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ABSTRACT

Aminoglycoside antibacterial agents, such as amikacin, continue to play an important role in the treatment of Gram-negative infections. However, although extremely effective, they are not without potential adverse events, the most important of which being nephro- and ototoxicity. Research into factors thought to influence both the efficacy and toxicity, has challenged the rationale upon which these agents have classically been dosed. Various studies in adult patients have found that a new approach to dosing (use of single daily administration) has equal or greater efficacy or safety compared to the standard multiple daily dosing of these agents. Similar studies comparing regimens in children are few, and as yet no comparative investigation has been performed using amikacin in children (as a separate and distinct group). Additionally, in evaluating the impact of altering dose regimens, it is imperative that the documented age-related aminoglycoside pharmacokinetic alterations, be taken into account. Amikacin pharmacokinetic parameters (determined using traditional methods) have been previously published for various (usually small) groups of children. However, population parameters are not currently available for South African children. This study therefore aimed to investigate optimal amikacin dosing in children by studying: a) the comparative efficacy and toxicity of two dosing regimens, and b) the population pharmacokinetic parameters derived using one of the alternative approaches capable of utilising routine, sparse serum drug concentration time data.

This investigation was conducted in the paediatric surgical and burns wards of

King Edward VIII Hospital, Durban. Study patients (0.6-12 years) received amikacin either once daily (15mg/kg) or twice daily (7.5 mg/kg) by slow intravenous bolus. Concomitant medication was given as prescribed. Regimen efficacy (favourable, unfavourable or indeterminate outcome) was assessed by patient temperatures, clinical improvement and white cell counts. Clinical nephrotoxicity was evaluated by changes in serum creatinine, and renal tubular damage (investigated in a small subgroup of patients) was indicated by detection of urinary low molecular weight proteins. Ototoxicity (cochleotoxicity) was assessed by pure tone audiometry. Pertinent demographic and treatment details (amikacin concentration time data) were recorded for the population pharmacokinetic analysis. The Nonlinear Mixed Effects Model (NONMEM) programme was used to derive appropriate models describing clearance (CL) and volume of distribution (V), as well as mean values of these pharmacokinetic parameters for this population.

Fifty four patients were entered into the regimen assessment. Patients in the single daily regimen (n=27) had significantly greater ($p<0.05$) mean (SD) peak (±0.5 hour post-dose) serum amikacin levels (37.7 (6.9) mg/L) as well as cumulative dose (91.5 (26.5) mg/kg) and duration of therapy (5.7 (1.5) days) when compared with those of the twice daily group (19.5 (3.7) mg/L, 70.1 (26.1) mg/kg and 4.6 (1.6) days respectively). No statistically significant differences were found between the groups in terms of outcome (18/24 and 22/25 patients in the once and twice daily dosing groups had favourable outcomes; there were no unfavourable outcomes). Pure tone audiometry (evaluated post-therapy, in 20 patients from each dosing

regimen) revealed no statistically significant differences between the number of patients in the two groups with possible drug-related ototoxicity. None of the patients assessed (including an additional 14 patients with burn injury) developed clinical nephrotoxicity. Urinalysis was performed in 17 amikacin treated patients (9 and 8 from the once and twice daily dosing regimens respectively) and 9 control subjects. Low molecular weight proteinuria was absent in all of the latter patients except one, in whom pre-existing renal disease was suspected. Tubular dysfunction ascribed to possible drug effect was detected in similar numbers of patients in the two treatment groups (3 and 2 patients in the once and twice daily dosing groups respectively).

In the pharmacokinetic assessment (156 serum levels from 82 patients) using a one compartment model, the final models which best described the data were as follows :

CL (L/hr) = 0.271 x age(yrs) + 2.46 x body surface area(m^2),

V (L) = 7.34 x body surface area(m^2)

Other fixed effects tested, which did not render the data more probable, included serum creatinine measurements at the start of treatment, gender, presence of burn injury and drug regimen. Interpatient variation was 15% and 18% for CL and V respectively, with intrapatient variation or residual error of 10%. The weight adjusted population parameter estimates (95% Confidence Interval) for this group were CL = 0.180 (0.175,0.185) L/hr/kg and V = 0.293 (0.286, 0.300) L/kg, which are within the range of values published previously for other children of similar ages.

The findings of this investigation, consistent with those of other similar studies, indicate that daily amikacin administration (in combination with a β-lactam), to children with normal renal function, has similar efficacy to, and no greater toxicity than multiple daily dosing. However, the role, if any, of the significantly greater cumulative dose and duration of therapy in the daily dosing group is unknown. As uncertainty remains regarding the precise duration of certain post-exposure events (and hence, the ideal duration of the interdose interval), and with the rapid drug clearance in this group of patients, future *in vitro* and *in vivo* investigations may shed even further light on the optimal dosing approach in these patients.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND

Aminoglycosides, a group of antibacterials including agents such as gentamicin, netilmicin and amikacin, are widely used clinically. These drugs are extremely efficacious and are important in the treatment of serious, often life-threatening, systemic infections. They are considered to be bactericidal and act predominantly against Gram-negative bacilli, being most useful for the treatment of infections involving Enterobacteriaceae (in particular *E.coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp.) and *Pseudomonas aeruginosa*, although activity of the various agents can vary (Zaske et al 1986, Assael & Rusconi 1992, Gilbert 1995). Their action against Gram-positive bacteria is limited (Sande & Mandell 1991). Generally they are not used as first choice in treating infections due to Gram-positive cocci although when combined with a β-lactam they exhibit synergistic activity against staphylococcal and enterococcal organisms. Aminoglycosides are not used in the treatment of anaerobic bacteria as they have little activity against such microorganisms (see Section 1.2.1)(Ristuccia & Cunha 1985, Zaske et al 1986, Sande & Mandell 1991).

The aminoglycosides, being highly polar, are actively transported across the bacterial cell membrane. According to Jackson et al (1988) this transportation has 3 components:

1. Ionic binding phase - the drug attaches to complementary sites on the bacterial membrane. This is a passive process, being drug concentration dependent.

2. Energy Dependent Phase I (EDP I) - by chemical complexing, the drug enters the membrane with a mini zipper-like effect.
3. Energy Dependent Phase II (EDP II) - internalisation of the drug with release from the membrane into the cytoplasm of the organism.

Drug transport can be altered by pH, divalent cations and other factors (Ristuccia & Cunha 1985, Zaske et al 1986, Sande & Mandell 1991). Within the cell the agents bind irreversibly to the 30S and 50S bacterial ribosomal subunits. This blocks the recognition step in protein synthesis and causes misreading of the genetic code, resulting in nonfunctional protein accumulation within the cell and ultimately, cell death (Ristuccia & Cunha 1985, Zaske et al 1986).

Unfortunately, the great efficacy is part of a double-edged sword, as these agents are also known to be toxic. The two most common adverse drug effects being nephro- and ototoxicity. Other adverse reactions include neuromuscular blockade and hypersensitivity reactions which occur with relatively low frequency. Although nephrotoxicity is generally considered to be reversible, the proximal tubular cells affected being able to regenerate (Sande & Mandell 1991), ototoxicity can be irreversible (Gilbert 1995).

The ideal therapeutic objectives for an antibacterial agent would be:- rapid, total organism eradication, with complete safety for the patient. Bearing in mind the above limitations associated with aminoglycoside use, realistic therapeutic objectives would be:- to maximise efficacy and minimise toxicity. In order to attain

this, an understanding of the factors thought to influence efficacy and toxicity is necessary.

1.2 FACTORS INFLUENCING EFFICACY

Aminoglycoside efficacy is part of a dynamic relationship between host, drug, and bacterium, and as such, methods to measure or quantitate efficacy *in vitro* may not be truly representative of activity *in vivo*. Despite such limitations, recent investigations have characterised features playing a role in drug efficacy.

1.2.1 **Bacterial Features**

Several factors pertaining to bacteria influence drug effect. For example, the inherent susceptibility of the infective organism, minimum inhibitory concentration for the organism (MIC), doubling time and features of organism regrowth, may all play a role.

Lack of drug-organism interaction as a result of bacterial-specific features is seen with anaerobic bacteria. With aminoglycoside uptake into bacterial cells being an active oxygen-dependent process and with anaerobic bacteria, lacking an oxygen-utilising transport system, the agents are not transported intracellularly in these organisms. Anaerobic bacteria are thus routinely resistant to aminoglycosides (Zaske et al 1986).

1.2.2 **Drug-Bacterial Interactions**

Key determinants in efficacy relate to a variety of complex drug-bacterial interactions.

1.2.2.1 Peak Concentration Relationships

Literature refers widely to the importance of peak serum aminoglycoside concentration for efficacy. Aminoglycosides exhibit concentration dependent bactericidal activity (Vogelman & Craig 1986). A continual rise in overall magnitude and rate of bacterial killing has been observed with exposure to increasing aminoglycoside concentration (as graphically represented in the work of Vogelman & Craig (1986), with kill curves for *Pseudomonas aeruginosa* in the presence of tobramycin). In contrast to this, β-lactam antibiotics are thought to show little dependence on drug concentration, with high concentrations not increasing the rate of bactericidal activity.

Peak aminoglycoside concentration : MIC ratios have been considered to play an important role in antibacterial efficacy. Part of an *in vitro* study of Blaser et al (1987) investigated netilmicin and the importance of peak : MIC ratio for bactericidal activity. From their findings, generated using a model with changing drug concentrations and simulating infection in a neutropenic site, they suggest that the ratio of peak concentration to MIC may be an important parameter in the clinical use of aminoglycoside antibiotics.

In vivo studies include the work of Moore et al (1987), who retrospectively

investigated the influence of a variety of factors, on clinical response/no response. Several drug and/or bacterial features were found to be significantly associated with response, eg. maximal peak level and organism MIC. Response was strongly associated with either the maximal peak:MIC ratio ($p < 0.00001$) or the mean peak:MIC ratio ($p < 0.0001$). As it was possible that confounding clinical factors in the participating patients, might explain the associations found, further statistical analysis was used to examine the multivariate relationship among all the factors. It was found that a favourable underlying prognosis was the most important factor associated with clinical response to therapy, with the elevated maximal peak:MIC ratio being the next most significant factor. Their findings indicate that a high peak concentration relative to the MIC for the infecting organism is a major determinant of the clinical response to aminoglycoside therapy.

In an earlier study, Moore et al (1984a) retrospectively analysed the association of aminoglycoside levels with mortality from Gram-negative bacteraemia. They reported that of the patients who died, a significantly greater number had subtherapeutic early plasma levels. However, the relationship between patient survival and mean peak plasma levels was not significant. In addition to early therapeutic peak levels, severity of underlying illness, leukocyte count and patient temperatures were also associated with clinical outcome. They note that while their findings do not prove a cause-and-effect relationship, association between the early peak plasma level and mortality from bacteraemia is demonstrated, even with the influence of other factors taken into account.

Deziel-Evans et al (1986) retrospectively analysed the influence of 5 pharmacokinetic indices on therapeutic response. These included the ratio of steady-state peak serum concentration to MIC and time that the serum concentration remained above the MIC. All the indices significantly correlated with therapeutic response.

It must be noted however, that not all investigators have been able to demonstrate a relationship between either peak serum concentration or serum inhibitory activity and outcome (MacGowan & Reeves 1994). McCormack & Jewesson (1992) criticised the work of Moore et al (1984a,1987) as well as other studies relating peak concentration and outcome.

As expressed by MacGowan & Reeves (1994), while there is considerable evidence in patients receiving multiple daily aminoglycoside dosing, that serum concentrations and clinical response are related, (the majority of studies have shown the peak serum concentration to be related to efficacy), corresponding data for patients receiving once-daily regimens are not currently available.

1.2.2.2 Post Exposure Events

A phenomenon known as the **Post Antibiotic Effect (PAE)** exists with aminoglycosides (as well as with many other antibiotics). The PAE is a period of time after complete removal of the antimicrobial, during which there is no regrowth of the target organism (Zhanel et al 1991). As put by MacKenzie and Gould (1993), "The delayed regrowth of surviving bacteria following limited exposure to

an antimicrobial agent is referred to as the post-antibiotic effect". Knowledge of this phenomenon is incomplete, and the precise mechanisms by which antimicrobials induce PAE are unknown (Craig & Gudmundsson 1991). With aminoglycosides, the PAE may represent the time required for resynthesis of essential proteins (Craig & Gudmundsson 1991, MacKenzie & Gould 1993).

The duration of this effect is dependent on various factors eg. type of organism, size of inoculum, duration of exposure to, and concentration of, the antimicrobial agent (Craig & Vogelman 1987, Craig & Gudmundsson 1991, Zhanel et al 1991). According to Zhanel et al (1991), *in vitro* data document increasing PAE with increasing aminoglycoside peak concentrations. Fantin et al (1990) reported longer PAEs *in vivo* for mice with impaired renal function (ie longer duration of drug exposure) compared to those with normal renal function.

The presence of the PAE would be only a microbiological curiosity unless relevance to clinical situations could be shown. Its major impact would appear to be with dosing regimens (Fantin et al 1990, Craig & Gudmundsson 1991, Zhanel et al 1991). The presence of the PAE (as well as other post-exposure events) should permit serum and tissue antimicrobial concentrations to fall below the MIC for considerable time periods without compromising efficacy or permitting bacterial regrowth (Zhanel et al 1991, MacKenzie & Gould 1993). An allied effect, that of the Post Antibiotic Leucocyte Enhancement is discussed in Section 1.2.3. below.

In addition to the presence of the PAE, a further pharmacodynamic effect has

been documented by a group of Swedish researchers, that of the **Post Antibiotic Sub-MIC Effect (PASME)**(Odenholt-Tornqvist et al 1992, Cars & Odenholt-Tornqvist 1993, Löwdin et al 1993). Bacteria in the postantibiotic phase (following initial drug exposure), show delayed regrowth when exposed to subinhibitory antibacterial concentrations. In the *in vitro* study by Odenholt-Tornqvist et al (1992), PASME of 4 antibacterials (including amikacin) were investigated. Test (with PAE induced), and control (no prior antibiotic exposure) organisms were re-exposed to different low (subinhibitory) concentrations of the antibacterials. With regard to the amikacin experiments, it would appear from their results that bacterial growth suppression is extended beyond the duration of the PAE by the presence of low amikacin concentrations (greater duration of effect being evident by increases in concentration from 0.1 or 0.2 X MIC to 0.3 X MIC). They conclude that for antibiotic-bacterium combinations for which a PAE exists, a very long inhibition of bacterial growth can be achieved with sub-MICs after a suprainhibitory dose has been administered. This, they propose, may influence the dosing schedules of antibiotics.

The above investigation of the PASME *in vitro* would appear to mimic some of the pharmacokinetic scenarios occurring *in vivo*, as suprainhibitory concentrations will always be followed by subinhibitory concentrations in humans and experimental animal models. However, it would be foolhardy to transpose results obtained from *in vitro* experiments to *in vivo* situations. For example, in the abovementioned study by Odenholt-Tornqvist (1992), the initial PAE was induced by exposing the bacterial strains to 10 X MIC for 2 hours. In the *in vivo* situation, this initial

magnitude and extent of exposure would be unlikely.

Other authors have noted various effects caused by aminoglycosides at subinhibitory concentrations. Morris & Brown (1988) and Grimwood et al (1989), with use of subinhibitory concentrations of aminoglycosides, reported suppression of so-called virulence factors, in *Pseudomonas aeruginosa*.

The above concept of subinhibitory concentrations playing a role in continuing drug effect is further supported by the *in vitro* study of McLean et al (1994). They investigated the influence of trough gentamicin concentrations (Cmin) on *E. coli* previously exposed to the drug in a manner simulating bolus or infusion dosing. Their results showed that a Cmin threshold concentration was required for continuing bactericidal effect (arguing in favour of maintaining some trough concentration in patients).

In addition to those post-drug-exposure events mentioned above, other researchers have reported an alternative concept, that of **adaptive resistance** (Jackson et al 1988, Gilleland et al 1989, Daikos et al 1990). During an *in vitro* experiment, organisms were exposed to an initial drug dose (8 X MIC for 1 hour), and were then incubated in drug-free medium. Aliquots of the cultures removed at hourly intervals from this medium, were re-exposed to the test aminoglycoside. From 2 - 5 hours following initial drug exposure, organisms demonstrated increasing resistance to the drug, with maximum resistance reached after approximately 4 hours. Full susceptibility was recovered only after several hours

(6) had elapsed since the first exposure. According to the investigators (Daikos et al 1990) this so-called adaptive resistance is characterised by a temporary down-regulation of drug uptake during the EDP II phase. Among various other observations made by Daikos et al (1990), they note that the period of development and recession of adaptive resistance was incongruent with PAE (ie adaptive resistance was least immediately after bacteria were removed from the drug, whereas PAE was greatest at this stage, and adaptive resistance was greatest when growth of the exposed culture had resumed, which is generally considered to be the end of the PAE).

Barclay et al (1992) reported similar findings to that of Daikos et al (1990), and then went further, using a dynamic *in vitro* model (simulating exponential drug decay with half life of 2.5 hours) to mimic *in vivo* pharmacokinetics. The degree of adaptive resistance was greater and the duration longer with higher initial gentamicin concentrations. They also found that full recovery of organism susceptibility occurred at times in excess of 24 hours.

Results of an elaborate *in vivo* investigation in normal and neutropenic mice (Daikos et al 1991) show biphasic bactericidal activity and appear to concur with the above evidence for adaptive resistance, but raise further questions on the appropriateness of inter-dose intervals. Neutropenic mice receiving an aminoglycoside dose following heavy bacterial inoculation, did best when given a second dose 2 hours later (in the "prerefractory" period).

To complicate matters further, the practice of administration of concomitant antibiotics with the aminoglycosides, may suppress the emergence of adaptive resistance (Daikos et al 1990).

1.2.2.3 Concomitant Medication

As alluded to above, concomitant medication administration can play various roles in drug-bacterial interaction. For example, synergy between aminoglycosides and β-lactam antibiotics (penicillins and cephalosporins) has been shown *in vitro* and *in vivo*. A proposed mechanism for this is as follows: the cell wall damage effected by the β-lactam, allows the aminoglycoside more effective entry into the cell, to its site of action (Zaske et al 1986, Assael & Rusconi 1992, Kumana & Yuen 1994). With many regimens using such drug combinations and taking advantage of synergy, the extent to which one component directly influences the efficacy becomes less easy to distinguish.

Although efficacy can be enhanced by concomitant antibiotic use, it can also be compromised. A direct chemical interaction can occur between aminoglycosides and certain β-lactams. This can occur *in vitro* if the 2 substances are physically mixed together prior to administration. It is also possible *in vivo* in renally impaired patients who as a result of their impaired excretory mechanisms, may have prolonged high circulating levels of antibiotics which can interact (Ristuccia & Cunha 1985, Assael & Rusconi 1992). Amikacin is reputed to be the least inactivated of the aminoglycosides in this way (Ristuccia & Cunha 1985).

1.2.3 **Host Features**

Aminoglycoside efficacy can also be influenced by several host-specific features. For example the infection focus, if comprised of undrained pus, anaerobic conditions etc, may not permit the drug to work optimally. Another important host feature is the patient's immune status.

An *in vitro* study by McDonald et al (1981) reported that bacteria in the PAE phase are more susceptible to leukocyte phagocytosis, an effect termed **Post Antibiotic Leukocyte Enhancement (PALE)**. The enhanced susceptibility probably relates to perturbations of the bacterial cell wall. (Aminoglycosides have been shown to cause alterations in bacterial cell walls (Iida & Koike 1974)). The experiments were conducted for various antibacterial-organism combinations. The degree to which antibiotics sensitize bacteria to the activity of leukocytes varies both with the agent used as well as the strain of bacteria. Gentamicin had a marked effect which was possibly explained by the following:- rapid reduction in viable bacteria due to initial drug exposure resulted in increased ratio of leukocytes to viable organisms with ensuing enhanced phagocytosis.

By actively altering the immune status of experimental mice (inducing neutropenia by cyclophosphamide exposure), Fantin et al (1990) showed that *in vivo* PAEs were longer in normal mice than in neutropenic mice. This result they ascribed to probable *in vivo* relevance of PALE.

1.2.4 **Summary**

Factors thought to play a role in aminoglycoside efficacy are extremely complex, and represent a variety of poorly understood interactions, some of which are occurring simultaneously during therapy.

1.3 FACTORS INFLUENCING TOXICITY

Of the 2 most widely recognised adverse events of aminoglycoside use, insights into ototoxicity are less developed than those into nephrotoxicity, mainly because the site of the toxic lesion is less accessible (Mattie et al 1989).

1.3.1 **Mechanisms**

1.3.1.1 Nature and Mechanisms of Nephrotoxicity

The major pathway of aminoglycoside excretion is via glomerular filtration, with tubular secretion playing only a minor role. A portion of the filtered aminoglycoside (being cationic) binds to the anionic phospholipid receptors on the brush border cells of the proximal convoluted tubule. The drug gets incorporated into the cells by pinocytosis and accumulates in the lysosomes and other cellular components. The net reabsorption of aminoglycoside by the kidney results in high concentrations of the drug in the renal cortex. Via a series of hypothesised mechanisms impairment and damage of lysosomal function and integrity occur. Ultimately proximal cell damage and necrosis ensue (Rose 1987, Chan 1989, Appel 1990, Garrison et al 1990).

Early features of proximal tubular damage can be manifest as increased secretion of tubular cell enzymes (eg N-acetylglucosaminidase), of brush-border associated enzymes (eg alanine aminopeptidase), and of low molecular weight (LMW) proteins (eg β_2-microglobulin)(Zaske et al 1986). The latter are normally filtered at the glomerulus, and reabsorbed by the proximal tubular cells. Increased urinary excretion of such proteins could indicate proximal tubular dysfunction or saturation of tubular reabsorptive capacity (Wibell 1976).

Following this early tubular damage, glomerular filtration rate (GFR) may decline resulting in a rise in both blood urea nitrogen and serum creatinine. According to Assael & Rusconi (1992), the series of events leading from early tubular damage to the reduction in GFR needs clarification. Renal failure (typically nonoliguric) is also possible (Appel 1990).

1.3.1.2 Nature and Mechanisms of Ototoxicity

Aminoglycosides are known to cause cochlear and/or vestibular toxicity. The different agents within the group may show predominantly cochlear toxicity (eg. amikacin), whereas others are predominantly vestibulotoxic (eg. gentamicin) (Ristuccia & Cunha 1985, Moffat 1987). Toxicity may be bilateral or unilateral and may occur at variable lengths of time after initiation or discontinuation of aminoglycoside treatment (Zaske et al 1986).

With cochleotoxicity, degeneration of sensory cells in the organ of Corti occurs. The outer hair cells are generally more sensitive to the damage than the inner hair

cells. Outer hair cell degeneration occurs first at the basal turn of the cochlea, with progressive involvement of hair cells along the basilar membrane towards the apex (Shulman 1979, Moffat 1987, Matz 1993). Nerve fibres become affected only when the hair cells are missing; the effect is secondary to the hair cell degeneration (Shulman 1979, Matz 1993).

Cochlear dysfunction is generally manifest as a sensorineural hearing loss which may be preceded by a feeling of fullness in the ear, or tinnitus. The latter is usually high frequency and continuous (Moffat 1987). Similarly, the sensorineural hearing loss is initially also in the higher frequencies as a result of basal hair cell damage. As high frequency is outside the normal conversational hearing range, the patient may be unaware of the initial damage occurring. Garrison et al (1990), referring to other published literature, state that auditory toxicity is frequently reversible if detected in the early stages. If the insult progresses, hearing loss may then progress to involve speech (lower frequency) range and the patient may become profoundly and permanently deaf.

Vestibular toxicity is related to damage of sensory hair cells in the vestibular apparatus. Type I sensory cells are more sensitive to degeneration than Type II cells in the crista ampullaris, and degeneration begins in the central part and spreads peripherally. Degeneration of the crista ampullaris precedes that of the utricle and saccule (Moffat 1987). The clinical symptoms of vestibular toxicity include dizziness, vertigo, ataxia and/or nystagmus (Zaske et al 1986, Sande & Mandell 1991). The vestibulotoxicity may not become apparent immediately, since

the patients are often ill and confined to bed (Moffat 1987). The effects of the labyrinth impairment may be overcome by physiologic compensatory functions (eg vision), but this adaptation may not be sufficient if for example, the patient's occupation requires a high degree of co-ordination (Zaske et al 1986, Sande & Mandell 1991).

The pharmacokinetics of the drugs in the inner ear fluids (endolymph and perilymph) have been under investigation for several years. The kinetics of drug uptake and release, into and from the inner ear, are discussed further in Section 1.3.3.2. The mechanism of the distribution of the aminoglycosides within the cochlea is far from established (Moffat 1987).

According to Schacht (1993), the cellular and molecular mechanisms underlying the irreversible hair cell loss still remain speculative. Various biochemical mechanisms have been proposed to be involved in ototoxicity. These include drug interaction with polyphosphoinositides (Matz 1993), alterations of normal concentrations of ions in the labyrinthine fluids with impairment of electrical activity and nerve conduction (Sande & Mandell 1991), as well as the possibility of a toxic drug metabolite (Schacht 1993).

1.3.2 **Methods of Assessment**

Detection and understanding of aminoglycoside toxicity can be confounded by several factors such as variation in stringency and nature of criteria used for its

assessment, as well as the degree of specificity of the indicators used. Aminoglycoside-induced toxicity may be difficult to distinguish from other pathophysiological conditions concurrently present and manifesting with a similar profile. For example, hypotension and shock may progress to acute tubular necrosis if not successfully treated (Zaske et al 1986).

1.3.2.1 Nephrotoxicity Assessment

With the early stages of proximal tubular damage being manifest as enzymuria, LMW proteinuria etc, several studies have investigated these and other substances as markers of drug related toxicity (Schentag et al 1978, Schentag & Plaut 1980, Sethi & Diamond 1981, Gatell et al 1985, Ylitalo et al 1991). Early detection of drug induced nephrotoxicity is desirable, but rests heavily on the specificity of the indicators used as well as on correlation between their detection and the development of clinical nephrotoxicity.

These substances can be detected in urine in a number of conditions (Tomlinson 1992), and so are not specific to aminoglycoside damage (Garrison et al 1990). For example, Hemmingsen & Skaarup (1977) documented tubular type proteinuria in patients with fever. Patients with sepsis have also been reported as having increased fractional clearance for β_2-microglobulin, among other proteins (Richmond et al 1982). Proteinuria has also been detected in patients with burn injury (Yu et al 1983).

According to Trollfors et al (1984), increases in β_2-microglobulin cannot be used to predict clinical nephrotoxicity; but can be useful in comparing nephrotoxic potentials of different aminoglycosides (Trollfors 1985). Several authors have used the above and other indicators (eg. phospholipiduria) to compare the renal tubular toxicity of different aminoglycoside dosing regimens (Ibrahim et al 1990, Tulkens 1991, van der Auwera et al 1991, Skopnik et al 1992, Gonzalez et al 1993, Langhendries et al 1993, Ibrahim et al 1994).

For the most part, aminoglycoside nephrotoxicity is assessed by changes in serum creatinine (increases) or in calculated creatinine clearance (decreases). Such changes would not be anticipated until after several days of therapy, and maximum elevation of serum creatinine may even occur up to one week after stopping therapy (Zaske et al 1986). This method of detection relies strongly on the correlation between serum creatinine (or creatinine clearance) and the patient's ability to renally excrete aminoglycosides. A major limitation to this is that formation and elimination of creatinine (a degradation product of creatine, a component of skeletal muscle) depends on various factors and may vary from patient to patient and with differing disease states. Serum creatinine or creatinine clearance may be significantly influenced by changes in renal blood flow. For example, hypotension and shock may contribute to a significant decrease in renal blood flow, with subsequent decrease in GFR and increase in serum creatinine (Garrison et al 1990). Relying solely on serum creatinine as an accurate measure of renal function may therefore be misleading in certain circumstances. Bearing in mind its limitations, serial serum creatinine measurements are a practical,

convenient and inexpensive tool for detection of possible drug-related nephrotoxicity.

1.3.2.2 Ototoxicity Assessment

Various tests, performed by specialised personnel, can be used for the detection of ototoxicity. Tests of cochlear function comprise subjective methods (conventional and high frequency audiometry) and newer objective electrophysiologic techniques (eg.auditory brainstem response, electrocochleography, otoacoustic emission)(Heffernan et al 1979, Ruth & Lambert 1991, Martin et al 1994). Vestibular tests include electronystagmography (ENG) and caloric tests (Strome et al 1985).

A superficial summary of the basic principles of conventional audiometry is as follows: air conduction thresholds in the frequency range of 250 Hz to 8000 Hz, and bone conduction hearing thresholds in the range of 250 Hz to 4000 Hz are measured. The term "threshold" denotes the minimum level in decibels at which the tone is detected. Air conduction tones are delivered to the ear through ear phones, while bone conduction is obtained by placing a vibrator on the skull (usually mastoid process) with sufficient vibratory force to stimulate the ear (Dennis & Neely 1991). Differentiation of different types of hearing loss (eg conductive, sensorineural or mixed) is achieved by interpretation of the air and bone conduction thresholds recorded on the audiogram (Heffernan et al 1979).

High frequency audiometry testing, beyond 8000 Hz (8 kHz), appears to be a promising advance for patients who can actively respond to this method. There may be considerable technical difficulties in the clinical use and calibration of audiometers capable of operating in the frequency range 10 - 20 kHz (Moffat 1987). Both the above types of audiometry are subjective measures of cochlear function, as they require patient participation and response. They are not appropriate for use in the young child.

The objective tests for cochlear function have the advantage of not requiring patient response and active participation, making these methods useful for testing the young child. Despite several advantages, these methods are not without their limitations. For example, with auditory brainstem response method, where a series of stimuli is presented to the ear and scalp electrodes "map" the auditory pathway output, patient movement/agitation may interfere with the recording. Sedation may therefore be required (Strome et al 1985), which may not be beneficial to the sick child already receiving other medication. Furthermore, according to Campbell & Durrant (1993), these new tests eg. otoacoustic emission, are still evolving and their clinical utility is not yet determined.

Regarding vestibular assessment, ENG is the preferred method (Strome et al 1985). However, criticism has been levelled at the use of this method, and inconsistencies in performance and interpretation limit its clinical usefulness especially in the critically ill patient (Moffat 1987).

Additional considerations that need to be taken into account in the assessment of drug related ototoxicity include :-

1. The need for a (reliable) baseline test - this is often not feasible in the sick patient.
2. Limited attention span in children may impinge on audiometry test reliability.
3. Various disease states can produce sensorineural hearing losses or vestibular impairment (Strome et al 1985).
4. Tests can be costly and time consuming.

In the present study using amikacin (predominantly cochleotoxic), cochlear function was assessed using conventional audiometry as it was the only reliable method available in our institution. Several limitations, including that of equipment calibration, precluded audiometric testing beyond 8000 Hz.

1.3.3 **Factors Linked to Toxicity**

The identification of factors influencing the drug toxicity described above, is critical for prevention or limitation of these potential adverse events. A great number of risk factors pertaining to the patient or the therapy have been recorded as being linked to either nephro- or ototoxicity or both.

Difficulties encountered in establishing which factors are associated with toxicity include:

1. Assessment criteria: as mentioned previously, criteria are not standardised between studies which makes comparison difficult.

2. Small patient numbers : (particularly the number with toxic events) may limit the statistical power of the study. Fee (1980), in commenting on several parameters not showing significant association to ototoxicity stated: " I do not intend to imply that those parameters can or should be ignored regarding clinical usage of aminoglycosides. It is very possible that with an increased population of patients, these factors will emerge as significant".
3. Multiple concurrent variables : toxicity may be associated with multiple concurrent variables which makes assessment increasingly complex.
4. Study results are sometimes contradictory.

1.3.3.1 Patient Factors

Numerous patient factors including advanced age, prior renal dysfunction or impairment, liver disease, dehydration, hypotension, shock and bacteraemia have all been documented as being associated with aminoglycoside nephrotoxicity (De Broe et al 1986, Lerner et al 1986, Mattie et al 1989, Appel 1990).

Those factors thought to be associated with ototoxicity are harder to establish and there appears to be less consensus. Some of the above factors associated with nephrotoxicity, have been reported by some authors to be associated with ototoxicity, while other authors have not found an association (Moore et al 1984b, Lerner et al 1986, Gatell et al 1987). As ototoxicity has been associated with impaired renal function (Jackson & Arcieri 1971), factors increasing the risk of aminoglycoside renal damage should be recognised as potentially indirectly affecting more than just renal function. Other patient factors investigated regarding

aminoglycoside-induced ototoxicity include high temperature, haematocrit, underlying disease; again some authors find an association while others do not (Fee 1980, Moore et al 1984b, Gatell et al 1987). The possibility of a genetic predisposition to aminoglycoside induced hearing loss has also been raised (Schacht 1993).

1.3.3.2 Therapy Factors

Factors pertaining to therapy that have been associated with nephrotoxicity include:- the intrinsic nephrotoxic potential of the agent, total dose, total daily dose, duration of therapy, administration of other toxic drugs and previous exposure to aminoglycosides (De Broe et al 1986, Zaske et al 1986, Appel 1990). With regard to serum drug concentrations it is unclear whether initial high pre-dose concentrations precede nephrotoxicity, or are the result of it. However, in reference to the work of other researchers, MacGowan & Reeves (1994) state that high pre-dose concentrations occurring later in the course of therapy are linked to both tissue accumulation and nephrotoxicity. The association of peak serum levels to nephrotoxicity is unclear. According to Appel (1990) although both high trough and peak serum drug levels have been found to correlate with nephrotoxicity, the correlation is imprecise.

For a given aminoglycoside, the risk of nephrotoxicity increases as cortical concentration increases (De Broe et al 1986); any factor that increases renal uptake of aminoglycosides is a risk factor for nephrotoxicity (Mattie et al 1989). One of the factors influencing cortical concentration of aminoglycosides is drug

uptake kinetics. Giuliano et al (1986) demonstrated that cortical concentrations of gentamicin and netilmicin in rats reached a plateau level despite increasing serum concentrations, thus implying the presence of saturable cortical uptake. Cortical uptake of tobramycin was linear, whereas that of amikacin was mixed, being saturable at low concentrations and linear at high concentrations. Although these findings cannot be directly extrapolated to humans, the demonstration of saturable uptake mechanisms support the idea that a dosing regimen which facilitates a very low trough serum level following a transient high peak, would result in less renal uptake than a regimen with a sustained relatively constant serum drug level.

Dosing regimens have been found to influence the renal cortical accumulation of these agents. De Broe et al (1991) demonstrated *in vivo* that a single injection of amikacin resulted in significantly lower renal drug levels than did a continuous infusion or administration of the same dose over 3 injections. Furthermore, Bennett et al (1979) showed highly significant differences in serum creatinine increases between once, twice and thrice daily gentamicin dosing in rats, with the thrice daily group exhibiting the greatest increase.

As for nephrotoxicity, therapy factors that have been associated with ototoxicity include duration of therapy, total dose, previous aminoglycoside exposure, administration of other ototoxic drugs etc (Black et al 1976, Moore et al 1984b, Zaske et al 1986). Again, not all authors have reported the same associations (Lerner et al 1986, Gatell et al 1987).

Tran ba Huy et al (1986) studied the kinetics of entry and release of gentamicin in various organs, including the inner ear, in rats. They showed uptake into the inner ear tissues was dose-dependent up to a point of saturation. The release of drug from the inner ear was found to be extremely slow, subjecting the hair cells to prolonged drug exposure, and possibly accounting, in part, for the organ damage (Tran ba Huy et al 1986, Mattie et al 1989). Aran et al (1995) also document a slow phase (with prolonged half life) of drug release from the ear.

If back diffusion is facilitated when serum drug levels reach a trough (as suggested by Sande & Mandell (1991)), elevated trough levels might be anticipated to be associated with ototoxicity. Several authors have reported this association (Black et al 1976, Lerner et al 1986). High peak levels have also been associated (Black et al 1976), however, other workers have found no significant relationship between serum levels and ototoxicity (Fee 1980, Moore et al 1984b)

1.3.4 **Summary**

Aminoglycoside-induced nephro- and ototoxicity have been associated with various factors relating to the patient and/or to therapy. Such factors (which should not be considered comprehensive) need to be considered/managed as part of the approach to optimising clinical use of these agents.

1.4 DOSING REGIMENS

Aminoglycosides have classically been dosed according to multiple daily dosing

protocols eg. total daily dose divided into 3 equal doses given every 8 hours. This dosage practice was based on the following assumptions (Hustinx & Hoepelman 1993):

1) Plasma concentrations should be prevented from falling under the MIC for a given micro-organism in order to prevent its regrowth at the end of the dosage interval. The short elimination half life of aminoglycosides prompted the institution of twice and 3 times daily dosage regimens.
2) Optimal aminoglycoside bactericidal activity was achieved once a minimum bactericidal concentration was reached.
3) Toxicity of these agents occurs primarily in association with high peak plasma concentrations and a high total daily dose.

However, recent research (as mentioned in previous Sections) has indicated:

1) Aminoglycosides exhibit a concentration dependent bacterial killing rate.
2) Aminoglycosides inhibit growth of susceptible organisms for a variable period of time after plasma concentrations have fallen below the MIC. The duration of this so called postantibiotic effect (PAE) is related to aminoglycoside peak concentration and is longer *in vivo* than *in vitro*. It is related to the organism studied and increases in the presence of neutrophils.
3) Features such as "adaptive resistance" may imply benefit of an extended dosing interval.
4) Risk of toxicity is thought to be attenuated with single daily dosing. Saturable uptake mechanisms imply that high, infrequent, short-lived

aminoglycoside peak values would lead to less uptake and less nephrotoxicity than the protracted exposure to lower aminoglycoside concentrations typical of conventional dosage regimens.

These findings have lead to a series of clinical studies investigating the safety and efficacy of single versus multiple daily dosing of aminoglycosides (Hustinx & Hoepelman 1993).

1.4.1 **Single versus Multiple Daily Dosing**

To date, numerous clinical studies comparing single to multiple daily aminoglycoside dosing have been published, mainly for adult patients. In addition to various reviews, leading articles and commentaries regarding single daily dosing (Gilbert 1991, Hustinx & Hoepelman 1993, Barclay et al 1994, Bates & Nahata 1994, Rotschafer & Rybak 1994), several meta-analyses combining clinical comparative investigations, have also been published (Galløe et al 1995, Blaser & König 1995, Barza et al 1996, Hatala et al 1996, Munckhof et al 1996). Findings of these latter studies are summarised in Table I (pg 40). Overall, there appears to be no compromise in efficacy and no increase in toxicity when these agents are administered as single daily doses compared to conventional regimens.

Of the literature available regarding single daily aminoglycoside dosing, some studies involving adult patients also included children (Marik et al 1991b, IATCGEORTC 1993). However the efficacy and toxicity data available for children

given aminoglycosides in comparative studies (Shankar & Sharma 1987, Viganò et al 1992, Elhanan et al 1995) and as single daily doses (Principi et al 1977, Kafetzis et al 1991, Trujillo et al 1991, Viscoli et al 1991, Bouffet et al 1994) is relatively limited.

Of the above comparative studies considering only children, Viganò et al (1992) investigated netilmicin, while Shankar & Sharma (1987) and Elhanan et al (1995) investigated gentamicin. As yet no comparative investigation has been published for children (as a distinct group) given amikacin. Marik et al (1991b), and the IATCGEORTC group (1993), both investigated amikacin dosing regimens, but in both, children > 1 year of age were grouped with adults.

As the concentration-time-curve, which is influenced by patient pharmacokinetics, is important to the rationale for single daily dosing, a consideration of optimal amikacin dosing in this population group would need to take pharmacokinetics into account.

1.5 AMINOGLYCOSIDE PHARMACOKINETICS

Aminoglycosides, due to their polarity, are poorly absorbed from the gastro-intestinal tract and have to be administered parenterally for systemic effect. The distribution volume of these highly water soluble compounds closely approximates that of the extracellular fluid compartment (Zaske 1986). The major route of elimination is by the kidney via glomerular filtration, with large proportions of the

drug recovered unchanged in the urine (Zaske et al 1986).

Aminoglycosides demonstrate inter- as well as intra-patient variability in pharmacokinetics (Zaske 1986), resulting in variation in serum concentration time profiles, and uncertainty regarding the serum levels achieved after the administration of a particular dose in a particular patient.

Factors known to influence the disposition of aminoglycosides include disease states (eg. ascites, altered renal function, burns), fever. Age, with its associated changes in physiological process, plays an important role in altered pharmacokinetics for a variety of drugs, including the aminoglycosides (Milsap & Szefler 1986).

In adults, with aging, there is a decreasing proportion of total body water and/or muscle mass to the total body weight. Thus aminoglycoside volume of distribution will change in accordance with these changes in body water content. Additionally, in the elderly, as renal function decreases as part of aging, glomerular filtration rate and aminoglycoside clearance will also decrease (Zaske et al 1986). Neonates however, generally have larger extracellular fluid volumes, as a percentage of body weight, than older paediatric patients, and so have increased aminoglycoside volume of distribution (Milsap & Szefler 1986, Assael & Rusconi 1992). Drug half life is generally prolonged in newborn infants secondary to an increased volume of distribution and immature renal function (Zaske et al 1986).

In children, processes of organ growth, differentiation and maturation separate the infant and child from the adult, both in terms of physiology and pharmacology. In the first several years of life, growth and development are most rapid. Maturation continues at a slower pace throughout middle and later childhood. The liver and kidney reach maximum relative weight in the 1 to 2 year old child, the age when capacity for drug metabolism and elimination also tends to be greatest. Hepatic and renal function not only equals, but in some cases exceeds, normal adult function between 1 year of age and puberty (Kauffman 1992). Children generally have rapid aminoglycoside clearance and elimination rates (with short drug half life), although this can vary substantially among patients (Zaske et al 1986, Kauffman 1992).

Several investigations have been published to date, describing amikacin pharmacokinetics in children (Vogelstein et al 1977, Cleary et al 1979, Finkelstein & Hall 1979, Kafezis et al 1979, Kramer et al 1979, Lanao et al 1981, Yogev & Kolling 1981, Lanao et al 1982, Autret et al 1986, Grenier et al 1987, Kafetzis et al 1991, Kopcha et al 1991, Marik et al 1991a, Hendricks et al 1995). The pharmacokinetic parameters in these studies (which involved groups of children suffering a variety of disease states) have been determined using traditional methods.

1.5.1 **Traditional Approach to Pharmacokinetic Studies**

Traditional pharmacokinetic studies (also known as *standard two-stage* (S2S) studies) usually incorporate few subjects (eg 10 - 30), with serial sampling

performed (eg 20 plasma samples per patient). Study patients can be healthy volunteers or patients carefully selected to represent a particular aspect/disease state which is to be investigated. The dosage regimen is usually of simple design, sampling times are fixed and designed to reveal maximum information about each individual's pharmacokinetics (Sheiner & Beal 1980). In investigating a particular drug, a number of such studies may be performed in differing study populations allowing one to deduce how disease states or organ function may impact on the drug kinetics.

The experimental data generated can be analysed manually using basic pharmacokinetic equations (such as computing area under the concentration-time-curve, AUC) or can be analysed by various computer programmes using non-linear least squares regression (most common). Individual pharmacokinetic parameters (eg. clearance CL, and volume of distribution V) are calculated (*Stage 1*). These are meaned to give average population values for the respective parameters (*Stage 2*).

An extension of this is the *iterative two-stage* (I2S) method, which uses Bayesian forecasting and repeat iterations in its calculation of pharmacokinetic parameters (Jelliffe et al 1993). A further refinement of the S2S and the I2S methods is the *global two-stage* (G2S) method, in which the covariance and correlations between the parameters are also estimated (Jelliffe et al 1993).

The Traditional Approach has several advantages (Sheiner & Beal 1980):-

1. It is well known and reliable.
2. Studies are relatively quick to perform.
3. Data has little variability due to stringent methodology in the study design.
4. Statistical models are relatively simple.
5. Data analysis is generally rapid and inexpensive.
6. Standard computer programmes are available for data analysis.

There are, however, several disadvantages of this method (Sheiner et al 1977, Sheiner & Beal 1980):-

1. In certain groups, eg. the very young or the critically ill, the taking of serial blood samples may be neither appropriate nor ethical. Information may not be obtained for those whose kinetic disposition most needs to be understood. Furthermore with the number of subjects participating being relatively small, the parameter estimates derived may deviate substantially from true population values. The selected study group (eg. healthy volunteers, or the mildly ill) may not be truly representative of the population for whom the drug would most often be used.
2. Traditional studies can be costly, eg. assaying numerous samples.
3. As study conditions are strictly controlled, the chance discovery of other factors influencing kinetics is unlikely.

1.5.2 **Alternative Approaches**

With the limitations of the Traditional Approach, Alternative Methods eg. the Nonlinear Mixed Effects Model (NONMEM) (Sheiner et al 1977, Beal & Sheiner 1980), have been developed to utilise data generated during routine clinical management of sick patients. Where the Traditional Approach focused on the individual as the unit of analysis, the Alternative Approaches focus on the population as the unit of analysis (Sheiner & Beal 1980, Whiting et al 1986). The Alternative Approach is not focused on the parameter values as such; the focus is rather on how measurable physiological and pathophysiological features relate to these parameters.

In contrast to the Traditional Method, where several serum samples are obtained from few patients, the Alternative Methods can use sparse serum level data (ie few samples) from many patients. With relatively unstructured study design, data analysis becomes increasingly complicated and methods for analysis used in the Traditional Method are no longer applicable.

Several advantages are conferred by the Alternative Approach :-

1. Few ethical problems arise as few samples are required per patient, during the course of routine patient care. With the ability to utilise few serum level measurements per patient, the Alternative Approach is ideal for determination of pharmacokinetic parameters in paediatric patient populations.
2. The data is more likely to be representative of the population.

3. Costs are relatively low where samples comprise part of routine serum level monitoring, the cost being largely absorbed as part of patient care.
4. Data from a number of different sources can be combined eg. data from differing regimens or differing routes of administration can contribute to the analysis. The analysis can accommodate varying spectra of disease states found in the clinical setting in which the drug is used.
5. The approach, being inclusive of many types of patient, has the scope for possible detection of previously unknown influences on drug kinetics.

The disadvantages of the Alternative Approach include :-

1. Routine clinical data, as opposed to data obtained in strictly controlled experimental studies, may be less reliable. Accurate time ordered recordings of dose events, serum sampling and all pertinent patient features, are required and may be difficult to obtain in the normally busy clinical setting.
2. Sophisticated statistical computation is required.
3. The possibility of bias due to the effects of unknown concomitant variables that are correlated with included variables (Sheiner et al 1977).
4. Model mis-specification may lead to erroneous results (Sheiner & Beal 1980).

In addition to the Nonlinear Mixed Effects Model (NONMEM) system mentioned above, several other computer programmes have been designed for computation of population pharmacokinetic parameters using few data points per patient. These

are based on differing schools of thought eg. parametric versus nonparametric approaches. They include the Nonparametric Maximum Likelihood (NPML) method of Mallet and the Nonparametric Expectation Maximisation (NPEM) method of Schumitzky (Jelliffe et al 1993, Jelliffe et al 1994). Of these, NONMEM was the first true population modelling programme and is currently widely used.

As the computer packages for population pharmacokinetic analysis are sophisticated, training in their use is necessary. Output interpretation may also require the assistance of personnel with expertise. Accordingly, in the present study, the motivation and choice for selection of analytical technique was influenced by access to facilities (NONMEM is established in our institution), as well as there being local personnel with experience of the programme.

1.5.3 **The NONMEM system**

The NONMEM programme models fixed (measurable) effects in conjunction with random effects, to determine which effects significantly influence the pharmacokinetic parameters.

The dependence of pharmacokinetic parameters on patient-specific variables is of great interest. In the works of Sheiner et al (1977) and Sheiner & Beal (1983), the principles and mechanisms pertaining to NONMEM analysis are described. By way of a simplified summary (for illustrative purposes), for the jth individual, drug

clearance could be modelled by writing the following regression relation:

$$(1) \qquad CL_j = A + B.CL^{Cr}_j$$

where CL^{Cr}_j is the creatinine clearance for the jth individual, a quantity that can be measured/estimated. A and B are fixed effects or fixed constants which relate drug clearance to creatinine clearance; their values must be estimated from patient data.

Similarly, if the volume of distribution also depends on renal function, the following could be written :

$$(2) \qquad V_j = D + E.CL^{Cr}_j$$

where D and E are additional constants which must be estimated.

For any single individual, the above equations are approximate. If the jth individual represented the "typical" patient, then the "typical" value (or the population value) for clearance (TVCL) could be expressed as follows :

$$(3) \qquad TVCL = A + B.CL^{Cr}$$

However, each individual differs from the "typical" individual. The persistent "shift" of CL_j from the regression prediction of TVCL can be denoted by η_j^{CL}. Hence the clearance for the jth individual can be written as follows:

$$(4) \qquad CL_j = TVCL + \eta_j^{CL}$$

Although equation (1) explains some of the persistent difference of the jth patient from the average patient (eg. that difference due to a different creatinine clearance), the interindividual variability that cannot be accounted for by such, is thus accounted for by η_j^{CL}.

Similarly, for volume of distribution, if j represented the average "typical" individual, then V_j would be the "typical" (population) value for volume of distribution (TVV):

$$TVV = D + E.CL^{Cr} \quad (5)$$

But, as j differs from the "typical" individual, η_j^V denotes the persistent difference in V for the jth patient from that predicted by the regression equation for the "typical" individual:

$$V_j = TVV + \eta_j^V \quad (6)$$

The terms η_j^{CL} and η_j^V are assumed to be independent identically distributed random variables with zero mean and variances ω_{CL}^2 and ω_V^2 respectively.

Furthermore, additional uncertainty (eg. measurement error in drug assay) needs to be modelled. The statistical model used must deal with the errors intervening between true and observed drug concentrations:

$$C_{ij} = C_{mij} + \epsilon_{ij} \quad (7)$$

where C_{ij} is the ith observed concentration for the jth individual, C_{mij} is the ith concentration predicted by the model for the jth individual, and ϵ_{ij} (or residual intrasubject error) is an independent identically distributed statistical error with mean zero, and variance σ^2.

In the statistical models above, the inter-patient variability and the intra- patient variability (or residual error) are represented as homoscedastic (additive) error models. The variability may be modelled in various other ways, for example with

heteroscedastic (proportional) statistical error models :

$$CL_j = TVCL \times (1 + \eta_j^{CL}) \quad (8)$$

$$V_j = TVV \times (1 + \eta_j^{V})$$

$$C_{ij} = Cmij \times (1 + \epsilon_{ij})$$

In estimating the parameters, NONMEM uses an extension of nonlinear least squares, with linearization in respect to only the random terms η_j^{CL}, η_j^{V} and ϵ_{ij}. The method pools data from all individuals, but explicitly models and handles the complicated error structure arising from a proper accounting of the random effects. If all the random variables were normally distributed, the NONMEM method would be the maximum likelihood method applied to the linearized model (Sheiner & Beal 1983).

The NONMEM output comprises the following :

1. Minimum Objective Function (MOF); which is equal to minus twice the log-likelihood of the data and indicative of data fit.
2. Estimates for model parameters (A-E in the above equations).
3. Estimates for variance (ω^2) of the interindividual variation of clearance (η^{CL}) and volume of distribution (η^{V}).
4. Estimates for the variance (σ^2) of the residual random intraindividual error (ϵ).
5. Standard Errors for these estimates.

1.5.4 **NONMEM Studies of Aminoglycosides in Children**

Several population pharmacokinetic studies using NONMEM have been performed in neonates and young infants receiving aminoglycosides (Thomson et al 1983, Kelman et al 1984, Grasela et al 1985, Thomson et al 1988, Fattinger et al 1991, Jensen et al 1992, Weber et al 1993). However, studies incorporating children are few (Kelman et al 1984, Thomson et al 1995). The work of Kelman et al (1984) was with gentamicin, and that of Thomson et al (1995) with gentamicin and tobramycin. To date therefore, there are no published population pharmacokinetic analyses using Nonlinear Mixed Effects Modelling for amikacin in children.

AIMS OF STUDY

With little being known of comparative efficacy and toxicity of amikacin administered once versus twice daily to children, and with the population pharmacokinetic parameters for this agent, in South African children, having hitherto not been derived, this study aimed :

TO INVESTIGATE OPTIMAL AMIKACIN DOSING IN CHILDREN BY STUDYING:-

1. The comparative efficacy and toxicity of two dosing regimens,
2. The population pharmacokinetic parameters using the Nonlinear Mixed Effects Model.

TABLE I

Once versus multiple daily aminoglycoside dosing - summary of meta-analyses.

Authors	Publications included (n) *	Patient number	Efficacy	Ototoxicity	Nephrotoxicity
Galløe et al (1995)	16	> 1200	ND	ND	ND
† Blaser & König (1995)	23	3181	Clin = OD Bact = OD	ND	ND
Hatala et al (1996)	13	>1600	Mort = OD Bact = ND	OD	OD
Barza et al (1996)	21 (2)	3091	ND	ND	OD
Munckhof et al (1996)	19 (2)	2881	Clin = OD Bact = ND	ND	ND

* Between the meta analyses, 34 different publications were included

† Review, included as a meta-analysis (Prins & Büller 1996)

Number in parentheses indicates the number of studies with paediatric patients, as stated by the analysts.

ND = No significant difference found between the regimens, or regimens deemed equivalent

OD = Single daily regimen significantly better, or favoured

Clin = Clinical efficacy, Bact = Bacteriological efficacy, Mort = Mortality examination

CHAPTER 2

METHODOLOGY

2.1 SUBJECTS AND MEASUREMENTS.

Ethics Approval to conduct the prospective comparative investigation of 2 amikacin regimens was obtained from the University of Natal Ethics Committee. Further permission was granted to perform one extra serum creatinine measurement, additional to those made for the purpose of clinical investigation. This sample was taken post-therapy, if prior Informed Consent (parental/guardian) was obtained (consent form : see Appendix (a), pg 131).

2.1.1 **Patients.**

All patients aged 0.6 - 12 years inclusive who were admitted to the paediatric surgical and burn units of King Edward VIII Hospital, Durban, requiring an aminoglycoside for proven or strongly suspected Gram-negative infection were eligible for the study.

2.1.1.1 Exclusion Criteria

Patients were excluded according to the following:

Full Exclusion:

1. Known allergy to aminoglycosides
2. Known neuromuscular disease
3. Known hearing or vestibular impairment
4. Malignant neoplastic disease
5. Concomitant nephro- or ototoxic drug administration

Partial Exclusions:

Patients with the following conditions constituted partial exclusions for various sections of this investigation.

	Regimen comparison	Urinalysis	Kinetic analysis
Renal impairment	yes	yes	no
Sustained abnormal urea and electrolyte levels	yes	yes	no
Burn injury	specified	yes	no
Previous aminoglycoside therapy	yes	specified	no
Suspected urinary tract infection	no	yes	no

Yes = excluded

No = not excluded

Specified = inclusion specified in text

2.1.2 **Data Collection, Clinical and Laboratory Procedures**

In accordance with the above exclusion criteria, not all patients were eligible for inclusion in all parts of the study. Therefore the following measurements and recordings performed, do not necessarily apply to every patient:

a). Demographic Details

These included patients' personal details, gender, age, weight, and height.

b). Clinical Features

Full details of initial clinical and surgical findings, management instituted and daily progress were recorded. With regard to burn patients, percentage burn and time interval since injury were also noted.

c). Temperature

Continuous 4 hourly patient temperatures were transcribed from ward charts.

d). Cultures for Bacteriology

Specimens for culture were taken at the start of treatment from suspected foci of infection, as well as from blood. Whenever possible and/or indicated, follow-up cultures were performed. Culture and susceptibility testing were performed routinely by the hospital Department of Medical Microbiology. Results from these investigations were noted.

e). White Cell Counts

White Cell Counts (part of Full Blood Count), performed by the hospital Haematology Laboratory during routine patient care, were recorded before treatment, at the time of amikacin serum level determination and whenever clinically indicated.

f). Serum Creatinine

Serum creatinine levels were performed prior to starting treatment, at the time of serum drug level determination and after completion of amikacin therapy. Any other serum creatinine measurements performed as part of clinical monitoring of patient electrolyte

status were also recorded. Assays were performed by the hospital Department of Chemical Pathology.

g). Urinalysis

During the course of the investigation, assessment of low molecular weight (LMW) proteinuria was initiated in a group of non-burn patients without suspected urinary tract infection, to compare renal proximal tubular damage occurring in the 2 dosing regimens. A group of control patients (admitted for elective surgery, but not having received prior aminoglycoside therapy) were also recruited.

In these patients, in addition to the information recorded above, urine samples were obtained pre-operatively and/or pre-treatment, whenever possible. Samples were either free or from a catheter (where patients were catheterised as part of clinical management). Thereafter, timed samples were obtained where feasible, daily and at mid-morning, throughout therapy, care being taken to ensure any overnight collections had been voided. Where patients were catheterised, freshly collected urine was sampled. Sample pH was measured in the laboratory (Crison micropH 2000, pH meter) and with titrated additions of 1M NaOH, samples were rendered neutral or mildly alkaline where necessary. One to 2 drops sodium azide 0.05 % were added as preservative prior to freezing at -20 °C for batch analysis.

Urinalysis, to detect the band of LMW proteins (molecular weight below 40 kDa) by means of the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method, was performed by an investigator who was blinded to sample identity. Urine

samples containing < 3g/L total protein (concentrations determined by the biuret method) were processed undiluted. Those containing > 3g/L were diluted to a standard concentration of 100 - 300 mg/L prior to analysis ; 15 - 20 µg/ml protein was applied to the gel. A vertical discontinuous electrophoresis system was used. Urinary electrophoresis was performed on a gradient gel of 7.5% - 25% T acrylamide 4% C (constant) in presence of SDS for 1.5 hours with the following electrical settings, 85mA, 100V for half hour and 42mA, 200V for remainder of the run. The gels were fixed in 4% acetic acid : 40% methanol prior to staining with Coomassie blue. Technical details of the method are published elsewhere (Schiwara et al 1986).

Pure standards of albumin, beta-2-microglobulin and lysozyme (Sigma) together with other LMW markers (Biorad) underwent concurrent electrophoresis in order to identify specific proteins.

h). Audiology

Standard full conventional audiological assessment (using a Madsen OB 822 Clinical Audiometer and an American AE 105 Tympanometer) was performed by an investigator blinded to treatment regimens, on children (aged >4.5 years) well enough to undergo testing in the sound proof cabin. Results of tympanography, pure tone air and bone conduction (0.25 - 4 kHz), pure tone air conduction (6 - 8 KHz) and speech tests were recorded.

2.1.3 **Therapy and Serum Drug Levels**

Subjects were randomised to receive amikacin (AMIKIN^{R}, Bristol-Myers Squibb) either

15 mg/kg daily or 7.5 mg/kg twice daily as a slow intravenous bolus (over approximately 1–2 minutes). Doses were flushed in with ± 1.5 ml vacolitre solution. Care was taken to ensure that adequate flushing time was allowed prior to administration of concomitant therapy. Standard doses of concomitant medication were prescribed by the attending surgeon.

Close attention was paid to correct dose calculation, preparation and administration of all drugs. Exact amikacin dose administered, and times of both amikacin administration and serum drug level sampling were carefully documented. When the personal monitoring of dose measurement and administration was not possible (eg. night time dosing), an additional chart was attached to the patient file for completion by the nursing staff administering the drug.

Serum amikacin level measurements, performed approximately 48 hours after initiation or change of therapy, and whenever requested by the attending consultant, were assayed as part of routine patient care by the Department of Medical Microbiology, using Fluorescence Polarization ImmunoAssay (Abbott TDx, lowest measurable concentration distinguishable from zero with 95% confidence = 0.8mg/L, coefficient of variation < 5%). Initially sampling was performed according to standard practices, with a blood sample taken immediately prior to the next dose as the trough measurement, and a sample taken approximately 30 minutes after the completion of the bolus, as the peak. The timing of samples was subsequently modified, as during the course of the study it was noted that many of the trough serum levels (ie levels at 24 and 12 hours post-dose for the once and twice daily regimens respectively) were below the lower

reliable detectable limit of the assay. These measurements, although of diminished reliability (being below 0.8 mg/L) were still considered valuable for inclusion in the regimen assessment. However because of the possibly reduced reliability they could not be incorporated into the pharmacokinetic analysis. Consequently, two post-dose serum samples were taken, from which, for clinical purposes, the peak (level at 30 minutes) and trough (level prior to next dose) were extrapolated using the standard equation : $Cp = Cp_0.e^{-kt}$ (Rowland & Tozer 1989), where Cp is the plasma concentration (mg/L), Cp_0 is the plasma concentration at time zero (mg/L), k is the elimination rate constant (hr^{-1}) and t is time (hr). (See Appendix (b), pg 132, for example of calculation).

Doses were adjusted empirically where necessary, in consultation with the consultant, to achieve trough levels < 5 mg/L (Marik et al 1991b), and peak levels of 30 - 40 mg/L (Marik et al 1991b) and 15 - 30 mg/L (in accordance with the reference ranges used by the hospital) in the once and twice daily dosing groups respectively. Duration of antibiotic therapy was determined by the attending surgeon.

2.2. REGIMEN ASSESSMENT

2.2.1. **Assessment of Efficacy**

Ideally, to measure regimen efficacy, serial bacterial cultures would be necessary. However, as most patients were surgical patients, specimens for bacteriological culture were usually only available intra-operatively. Follow-up cultures were therefore not often possible and so regimen efficacy was assessed in terms of patient outcome.

Patients were considered to have a favourable outcome if they fulfilled all of the following 3 criteria :

1. Body temperature of ≤ 37.5 °C maintained for 48 hours.
2. Clinical improvement with resolution of signs and symptoms at the end of therapy.
3. Normal white cell count, or a decrease of 15 % or more, from beginning to end of therapy, provided results of these tests were available.

Failing this, patients were considered to have an unfavourable outcome unless they had such problems as, for example, nosocomial infection, surgical complication, or chest infection which could have accounted for not having achieved the criteria. In which case they were deemed to have an indeterminate outcome.

2.2.2 **Assessment of Toxicity**

Clinical nephrotoxicity

Serum creatinine measurements were used to indicate drug induced nephrotoxicity. Clinical nephrotoxicity was defined as a rise in serum creatinine of 45 µmol/L (≈0.5mg/dL) or more (Prins et al 1993) from initial, post rehydration values.

Renal Tubular Damage

Presence or absence of proteinuria in the 2 treatment groups was compared, in reference to that of the controls.

Ototoxicity

Patients with high frequency (6-8 KHz) hearing thresholds outside the normal 0 - 15 dB range for children (Strome et al 1985) were considered to have an abnormality that may have been drug-related. These patients were subsequently re-tested whenever possible to assess reversibility of the abnormality.

2.2.3 **Determination of Statistical Significance**

Statistical testing was carried out by the Biostatistician of the Medical Research Council, Durban. The Student's unpaired t-test was used to compare the regimens with respect to the continuous data. The subset of burn patients participating in the assessment of clinical nephrotoxicity were compared with the Wilcoxon 2-sample test. This test was also used in the urinalysis data to compare the 2 treatment groups, with the Kruskal-Wallis test employed for comparison of treated and control groups. Chi-squared test was used to compare the categorical data, and in the case of small cell sizes, the Fisher's exact test was used. The level of significance was $p < 0.05$.

2.3 POPULATION PHARMACOKINETIC ANALYSIS

Analysis of data to derive population pharmacokinetic parameters was performed using the Nonlinear Mixed Effects Model (NONMEM) computer programme (Version IV, Level 2.1, double precision)(Boeckmann et al 1994).

2.3.1 **Pharmacokinetic Model**

Although a 1 compartment model has been used to describe aminoglycoside

concentration-time data, a 3 compartment model may more accurately characterise their disposition (Zaske 1986, Zaske et al 1986). The characterisation of complex structural models was thought unlikely with the distribution of the data set, hence a 1 compartment model was initially selected. The ADVAN1 subroutine from the NONMEM-PREDPP-library was implemented, using the TRANS2 subroutine to reparameterise the model in terms of clearance(CL) and volume of distribution(V) (Boeckmann et al 1994). Two compartment model use (with ADVAN3 and TRANS4) was also attempted.

2.3.2 **Statistical Model**

Inter-subject variability in CL and V, and intra-subject variability or residual error, were first modelled with additive (homoscedastic) and then with proportional (heteroscedastic) statistical error models in the early model building phase (see Section 1.5.3). Statistical model selection (prior to covariate inclusion) was based on global assessment of goodness of fit (see Section 2.3.3.2). This was similarly evaluated at the completion of the model building phase, at which point the most important covariates had been included.

2.3.3 **Data Processing**

Examples of control files (NMTRAN records) constructed for data processing are shown in Appendix (m), pg 143 (early model) and (o), pg 145 (final model).

2.3.3.1 Model Building

To find the model which best described the data, a step-wise procedure was followed:

Step 1. Construction of simplest model (that assuming all individuals to be

identical, CL = θ_1, V = θ_2). Selection of most appropriate statistical error model.

Step 2. Determination of models best describing CL (with progressive evaluation of the contribution of fixed effects), with V = θ_2.

Step 3. Determination of models best describing V (with progressive evaluation of fixed effects), with CL = θ_1.

Step 4. Combination of successful intermediate models for CL and V.

Step 5. Verification of fixed effect and parameter inclusion by step-wise deletion from superior models.

Step 6. Rechallenge of superior models with important fixed effects.

Scatterplots were reviewed during the model building process to identify outliers. In particular:

1. PRED vs DV - plotting predicted serum concentration versus actual measured concentration.
2. WRES vs PRED - plotting the weighted residual (ie predicted - actual concentration / actual concentration) versus predicted concentration.

2.3.3.2 Model Evaluation

Successive models were evaluated and selected according to the following criteria (Boeckmann et al 1994):

1. Where models were considered to be restrictions of one another (ie a Full/Reduced Pair) the difference in minimum objective function (DOBF) was computed (the Likelihood Ratio Test). This difference is approximately χ^2

distributed with degrees of freedom equal to the number of parameters whose values are fixed in the reduced model. A DOBF of 7.9 associated with a p value of <0.005 (1 degree of freedom) was accepted as statistically significant. The model with the significantly smaller minimum objective function (MOF) was accepted as the better descriptor of the data.

2. Where models were not restrictions of one another, and where the number of free parameters were equivalent, global assessment of goodness of fit was considered (standard errors of the estimates, scatterplots, MOF etc).

3. Where models were not restrictions of one another, and where the number of free parameters differed, the Akaike Information Criteria (AIC) was used:

$$AIC = MOF_A - MOF_B + 2\,(p_A - p_B)$$

where p_A = number of free parameters in model A

and p_B = number of free parameters in model B.

If AIC > 0, model B was accepted as the superior, if AIC < 0, model A was accepted as the superior.

2.3.4. **Predictive Performance Testing**

In order to evaluate predictive performance of models, without the need for a further patient group, a process of "leave-one-out" cross validation was performed using the existing data (Efron & Tibshirani 1993). Principles of this were discussed with, and confirmed by, statisticians prior to commencing the process.

Cross validation uses part of the available data to fit the model and a different part to test it. "Leave-one-out" cross validation was performed as follows:

Considering the jth individual for example. The population model (derived using all the patients, n) was refitted leaving j out of the data set. Using the new parameter estimates so derived, CL and V for j were calculated. Using these values, predicted serum concentrations corresponding to times of actual serum concentrations in the jth individual were then computed. These were then compared to the actual values as follows:

Predicted - Actual serum concentration = Prediction Error

Model refitting was performed n times. Prediction errors for all the n individuals were thus obtained.

Model absolute bias (mean prediction error) and precision (mean squared prediction error) were calculated (Sheiner & Beal 1981). A one-sample t test (Altman 1991) was used to determine if mean prediction errors differed significantly from zero ($p<0.05$). In order to select the superior of 2 seemingly equivalent models, the relative precision and bias were also determined (Sheiner & Beal 1981).

To measure relative precision of 2 models (for trough as well as peak/2nd post-dose measurements), the following were calculated:

a) the difference in mean squared prediction error (Δmse),

$$\Delta mse = mse_1 - mse_2$$

where mse_1 is the mean squared prediction error for Model 1

and mse_2 is the mean squared prediction error for Model 2

Δmse is a measure of the magnitude of any difference in the precision between the 2 models.

b) the Standard Error of Δmse, and the respective 95% Confidence Intervals (CI).

If the Confidence Intervals derived, do not contain the value zero, the model with the smallest mean squared prediction error may be judged significantly more precise. If the Confidence Interval does include zero, the difference in mean squared prediction errors is not significant at the α level used to compute the Interval.

To measure relative bias, similar calculations to those above were performed:

a) difference in mean prediction error (Δme),

$$\Delta me = me_1 - me_2$$

where me_1 is the mean prediction error for Model 1

and me_2 is the mean prediction error for Model 2

Δme is a measure of the magnitude of any difference in bias between the 2 contending models.

b) the Standard Error of Δme, and the corresponding 95% CI.

As above, if the CI do not include the value zero, the model with the smaller mean prediction error may be judged significantly less biased.

CHAPTER 3

RESULTS

3.1 REGIMEN ASSESSMENT

3.1.1 **Demographic Details and Initial Clinical Parameters**

Fifty four patients were entered into the comparative investigation of dosing regimens. These comprised twenty seven subjects in each of the once daily and twice daily dosing regimens respectively (see Appendices (c) and (d), pgs 133-134, for full details). Groups were comparable regarding demographic details and clinical parameters at the start of therapy, as shown in Table II (pg 70).

3.1.2 **Disease States and Intervention**

The 2 groups were also similar in terms of the disease states represented, as shown in Figure 1 (pg 71). (Full details shown in Appendices (c) and (d), pgs 133-134). Those patients comprising the "other" category suffered degenerative leiomyopathy with colonectomy, prolapsed colostomy, pelvic and ischiorectal abscesses in the once daily dosing group, and pelvic inflammatory disease with urinary tract infection and psoas abscess, pelvic and psoas abscesses and retroperitoneal pelvic sepsis in the twice daily group. The groups were similar with respect to nature of intervention (as shown in Table III, pg 72). (Full details in Appendices (c) and (d), pgs 133-134). The number of patients who received pre-operative intravenous cephalosporin administration, and saline or saline/cephalosporin lavage intra-operatively, were approximately equally distributed between the two groups.

3.1.3 **Bacteriology**

Details of bacteriological investigations are shown in Table IVa and b (pg 73). Two patients in the once daily dosing group had initial positive blood cultures which resolved during treatment. The groups were otherwise similar regarding range of gram-negative organisms isolated from the positive cultures in both groups.

3.1.4 **Antibacterial Medication**

Details of amikacin treatment and concomitant antibacterial therapy administered to the two regimens are shown in Table V (pg 74). (Full details in Appendices (e) and (f), pgs 135-136). Mean daily amikacin doses were comparable for both groups, as were the mean trough serum levels. Mean serum amikacin levels were calculated using measured values (when standard pre-dose trough and post-dose peak measurements were made) or extrapolated values (from those patients for whom 2 post-dose measurements were obtained). Mean trough levels were considered to be below the stipulated lower sensitivity of the assay (0.8 mg/L). Mean peak serum levels, cumulative dose administered and duration of therapy were all significantly higher in the once daily regimen.

The majority of patients required broad antibacterial cover and received intravenous ampicillin and metronidazole in addition to amikacin. "Other" antibacterial agents included alternative β-lactams (eg. piperacillin, cephalosporin).

3.1.5 **Outcome Evaluation**

3.1.5.1 Patient Group

Of the 54 patients entered into the regimen comparison, 24 and 25 patients were evaluable for outcome assessment in the once and twice daily groups respectively, because the following were excluded : 2 patients in the once daily group, from whom amikacin resistant organisms were cultured ; 1 patient in each group who had only staphylococcal species isolated from the focus of infection ; and 1 regimen defaulter in the twice daily dosing group.

Demographic details, initial clinical features and antibiotic treatment details of the 2 groups assessed for regimen outcome were broadly similar to those listed in the Tables above. Three patients in the once daily group and 1 in the twice daily group had their concomitant antibiotic treatment changed from one β-lactam to another during the course of therapy.

3.1.5.2 Outcomes

According to the evaluation criteria stated in Section 2.2.1, 75% (18/24) and 88% (22/25) of patients from the once and twice daily dosing groups respectively had favourable outcomes. Indeterminate outcomes were recorded in 25% and 12% of patients respectively. There were no unfavourable outcomes. The differences in outcome were not statistically significant.

Of the patients with favourable outcomes, the mean (SD) time taken for body temperature to reach $\leq$ 37.5 °C, was 1.6 (1.4) days in the once daily group, and

1.8 (1.3) days in the twice daily group. Concurrent factors such as re-laparotomy, wound sepsis etc, which may have influenced outcome were similarly represented in both groups.

3.1.6 **Renal Evaluation**

3.1.6.1 Clinical Nephrotoxicity

Of the 54 patients entered into the study, 53 were evaluable for assessment of clinical nephrotoxicity. One patient from the twice daily regimen was excluded as serum creatinine data were not available. Details pertaining to the 2 groups are again similar to those shown in Tables II - V (pgs 70-74).

End-therapy serum creatinine values were determined 1.3 (1.3) days and 1.6 (1.1) days after stopping amikacin in the once and twice daily dosing groups. No patient developed nephrotoxicity on either of the treatment regimens. Mean serum creatinine values (shown in Table VI, pg 75) remained within normal range (Tietz 1983) for both groups.

3.1.6.2 Burn Patients

Fourteen additional patients with burn injuries (7 per dosing regimen) were evaluated for clinical nephrotoxicity. (Full details in Appendix (g), pg 137). The groups were comparable with respect to all parameters, including the number of patients receiving topical neomycin for facial burns. A statistically significant difference was only seen between the median peak serum amikacin concentrations of 35.2 mg/L and 15.6 mg/L respectively ($p = 0.0051$). Serum

creatinine values were within normal limits for all these patients during their monitoring period.

3.1.6.3 Tubular Damage

3.1.6.3.1 Patient Group

Nine patients receiving the once daily, and 8 receiving the twice daily regimens participated in the urinalysis. These patients (in the once and twice daily dosing groups respectively) had: appendicectomy (5 and 2), perforated/obstructed bowel (1 and 3), and worm bolus (3 and 2). One patient in the twice daily group was admitted with disembowelment. Nine control patients were studied. They were admitted for orchidopexy (6), inguinal hernia repair (2), and posterior sagittal ano-recto plasty (1). (Full details in Appendix (h), pg 138). Demographic details of the subjects are presented in Table VII (pg 76).

Regarding amikacin therapy, peak serum levels in the once and twice daily dosing groups were significantly different ($p=0.002$). The median (range) values were 35.9 (25.5 - 42.5) mg/L and 17.1 (13.1 - 25.6) mg/L respectively. Cumulative dose and duration of therapy were also significantly greater in the once daily group compared to the twice daily regimen ($p=0.003$, $p=0.005$ respectively). Median (range) cumulative doses were 82.5 (57.3-142.9) mg/kg and 52.5 (30.0-77.9) mg/kg, and duration of therapy was 5.0 (3.5-8.0) days and 3.5 (2.0-4.5) days in the once and twice daily groups respectively. There were no significant differences between the two treatment groups with respect to the median daily amikacin doses, the trough serum levels (below the stipulated limit in all patients), and the

concomitant use of β-lactam antibiotics and metronidazole. One patient on the twice daily dosing regimen received a single dose of gentamicin immediately preceding amikacin treatment. One patient in the control group received metronidazole and a β-lactam antibiotic.

3.1.6.3.2 Evaluation of Comparative Proteinuria

Of the 9 control patients, 2 had proteinuria detected. In the one patient, LMW proteins were recorded in the post-operative urine sample. This patient however also had concurrent high molecular weight (glomerular) proteinuria in both pre- and post-operative samples, indicating likely pre-existing renal disease (Brocklebank et al 1991). He was notified for referral to the renal clinic. The second patient (with a prior febrile episode) had albumin detected in only the post-operative sample. Post-surgical LMW proteinuria was otherwise entirely absent in the control population.

Of patients receiving amikacin, 4/9 and 5/8 from the once and twice daily groups respectively (including the one patient with the prior single gentamicin exposure), had normal urinalyses. Of the 5 patients with proteinuria in the once daily dosing group, 2 had this abnormality prior to treatment, thus precluding an assessment of drug effect. Details of the remaining 3, as well as the 3 patients with proteinuria from the twice daily dosing group, are shown in Table VIII (pg 77).

Mixed proteinuria was detected in 1 patient from the twice daily regimen who was also found to have underlying schistosomiasis. As proximal tubular dysfunction has

been documented in this condition (Cooppan et al 1987) the role of amikacin in this patient's proteinuria could not be determined. LMW proteinuria in 3 patients from the once daily regimen and 2 from the twice daily regimen was ascribed to possible drug effect. Of these 5 patients, 3 had maximum temperatures $\geq$ 38.0°C concurrent with, or during the 2 days preceding sampling. However, there were children from both treatment (n = 9) and control (n = 3) groups who had normal urinalyses despite maximum temperatures $\geq$ 38.0°C recorded at some stage during the 2 days preceding sampling.

Follow-up urine samples (1 - 2 per patient) were obtained in 4 of the 5 patients with the possible drug related proteinuria. One patient per dosing group had samples taken only 1 - 2 days post-therapy ; neither showed resolution of the proteinuria. The remaining 2 patients (1 per group) had samples taken at 6 - 8 days post-therapy ; the patient in the twice daily regimen showed resolution of the proteinuria at day 8. There was no evidence of clinical nephrotoxicity in any of the amikacin treated patients.

3.1.7 **Audiological Assessment**

3.1.7.1 Patient Group

Due to patients' conditions, pre-treatment audiology was not possible. Post-treatment audiology was performed in 20 patients in each dosing group, 3.7 (4.3) days and 2.8 (2.5) days respectively (not significantly different) after completion of amikacin treatment.

Details pertaining to these patients are represented in Table IX (pg 78). (Full details in Appendices (i) and (j), pgs 139-140). Mean cumulative dose and peak serum levels were significantly different between the dosing groups, being higher in the daily regimen.

3.1.7.2 Audiology Results

Figure 2. (pg 79) schematically illustrates the audiology results. Three and 11 patients in the 2 regimens demonstrated an abnormality of some kind ($p = 0.008$). Two patients in the once daily group had an exclusively high frequency hearing deficit on pure tone air conduction, the highest threshold recorded being 30 dB. In one of these the deficit was bilateral. Although the second audiogram on this patient showed partial resolution, further follow-up was not possible. Complete reversal of the problem was demonstrated in the patient with the unilateral deficit. One patient in the once daily group had a mixed high and low frequency deficit. As differentiation of the high frequency component by bone conduction was not possible (due to equipment limitations), a sensorineural and drug related deficit may have been present, but remained undetected.

In the twice daily group, two patients demonstrated exclusively low frequency hearing deficits, their high frequency thresholds being within normal limits. Five patients had a high frequency hearing deficit, the highest threshold recorded being 40dB. Of these, 3 had subsequently normal audiograms, 1 had an unresolved deficit at first follow-up, and in the last, no follow-up audiology was possible. One of the 5 patients had concurrent matching tinnitus which subsequently resolved.

The remaining 4 patients with abnormalities in the twice daily group had mixed high and low frequency deficits. Once again differentiation of the high frequency component was not possible and therefore a sensorineural and drug related deficit may have been present but remained undetected.

Test details of the 2 and 5 patients with high frequency deficits are summarised in Table X (pg 80). The deficits were not statistically significantly different between the two groups. Investigation into these 2 and 5 patients, showed nothing exceptional with regard to amikacin treatment details.

3.2 DERIVATION OF PHARMACOKINETIC PARAMETERS

3.2.1 **Demographic Details**

Eighty two patients were entered into the pharmacokinetic analysis (39 male, 43 female). Full details in Appendix (k), pg 141. Mean (SD) age, weight, height, body surface area (BSA) and serum creatinine (at the start of therapy) were 6.6 (3.1) years, 21.1 (7.4) kg, 113.8 (20.8) cm, 0.81 (0.21) m^2 and 47.9 (13.4) µmol/L respectively. BSA was calculated by the method of Haycock et al (1978):

$$BSA\ (m^2) = weight\ (kg)^{0.5378} \times height\ (cm)^{0.3964} \times 0.024265$$

Forty three patients received the daily dosing regimen.

Disease states represented were as follows:-

Appendicitis n = 32

Burn Injury n = 17

(Mean (SD)[Range] age = 4.7(3.1)[0.9 - 10] years; initial % burn estimate (range) = 10% - 63%; time interval since injury to treatment (range) = 1 - 67 days)

Perforation/obstruction of gastrointestinal tract	n = 11
Worm bolus	n = 10
Other conditions requiring aminoglycoside cover	n = 12

"Other conditions" included:- pelvic inflammatory disease with urinary tract infection and psoas abscess; prolapsed colostomy; pneumonias; MVA polytrauma; degenerative leiomyopathy with colonectomy; anal laceration; pelvic, ischiorectal and psoas abscesses.

3.2.2 **Sample Inclusion**

The pharmacokinetic analysis was performed using 156 serum level measurements (approximately 2 levels per patient), comprising 26 trough levels and 130 peak/2nd post-dose levels. (Serum trough level measurements below the sensitivity of the assay were not included in the data set. Four other levels were also excluded as they confirmed dose/administration irregularities eg. drip patency problem).

Figure 3. (pg 81) shows the number of serum levels obtained per patient during the course of therapy. No more than 2 levels were obtained per patient on any one day.

3.2.3 **Development of Regression Models**

Initially clearance and volume of distribution were assumed to be identical for all individuals (Model A : $CL = \theta_1$, $V = \theta_2$). An additive statistical error model (MOF =

731) was selected as superior to a proportional statistical error model (MOF = 812) by overall goodness of fit.

In the two compartment model, parameter estimates not only seemed unrealistic but were estimated with little confidence. Further model building was thus pursued with the one compartment model.

3.2.3.1 Building Regression Models for Clearance

Clearance was modelled first (keeping volume constant : $V = \theta_2$). The influence of the fixed effects of weight, age and BSA were investigated. Models incorporating each of these were constructed in a variety of ways so as to assess the fixed effect in linear (eg. $CL=\theta_1 WT$; $CL= \theta_1 WT+\theta_2$), power (eg. $CL= \theta_1 WT^{\theta 2}$) and exponential (eg. $CL = e^{\theta 1 WT}$) functions. Successive models were compared. Successful models for clearance incorporating single fixed effects were then evaluated against models constructed with more than one fixed effect represented (Table XI (pg 82)), with Models D and E being superior.

Addition to Models D and E, of a function incorporating serum creatinine values at the start of therapy, did not render the data more probable. Likewise, flags for gender, dosing regimen and burn injury (tested as additive and multiplicative functions) had no significant influence on these 2 good models for clearance. Thus, at this stage in the model development, Models D and E were considered equivalent.

3.2.3.2 Building Regression Models for Volume of Distribution

Regression models describing volume of distribution (keeping CL = θ_1) were then developed in a similar series to that for CL (above), successively testing weight, age and BSA. Successful intermediate models for V are shown in Table XII (pg 83).

Model F was the most successful intermediate model. Although it had a single fixed effect it was superior to a variety of other models containing more than one fixed effect, including Model I (DOBF = 4.9 ; not significant).

Serum creatinine, gender, dosing regimen and burn injury tested in Model F (method as described for CL above), did not render the data more probable.

3.2.3.3 Combining Regression Models for CL and V

Models D and E obtained for clearance, and Model F obtained for volume of distribution were next combined. The resultant models (J and K shown below) resulted in a large reduction in MOF:-

Model J :	$CL = \theta_1 AGE + \theta_3 BSA$	MOF = 529.157
	$V = \theta_2 BSA^{\theta 4}$	
Model K:	$CL = \theta_1 AGE + \theta_3 WT$	MOF = 532.263
	$V = \theta_2 BSA^{\theta 4}$	

The use of the additive statistical error model at the completion of the building phase, was rechecked.

3.2.3.4 Verification of Parameter Inclusion

Table XIII (pg 84) summarises the results of the step-wise deletion of parameters from Models J and K. Only the deletion of θ_4 (value close to unity) did not significantly worsen the fit of the data, hence the final models chosen were Models N and P:

Model N:	$CL = \theta_1 AGE + \theta_3 BSA$	MOF = 529.165
	$V = \theta_2 BSA$	
Model P:	$CL = \theta_1 AGE + \theta_3 WT$	MOF = 532.682
	$V = \theta_2 BSA$	

Influence of fixed effects gender and burn injury were once again tested (on both CL and V in the above 2 models); once again neither rendered the data more probable.

As Models N and P were essentially equivalent, their predictive performance was investigated to highlight any differences which might assist in selecting a single superior model.

3.2.4 **Outcome of Predictive Performance Testing**

The results of the absolute predictive performance testing are shown in Table XIV (pg 85). Neither model showed significant bias with respect to the Peak/2nd post-dose level predictions (ie mean prediction error was not significantly different to the hypothesised mean of zero; $p = 0.95$ and $p = 0.70$ for Models N and P

respectively). However, both models significantly underpredicted trough serum levels.

The relative performance of the models, one to the other, is shown in Table XV (pg 86). Of the 95% CI stated, only those for the relative bias of the Peak/2nd post-dose levels did not contain the value zero. Accordingly (see Section 2.3.4 above), the models may be judged significantly different for this one factor, and the superior is that with the lowest mean prediction error (ie. Model N).

3.2.5 **The Final Model and Population Pharmacokinetic Parameter Values**

Based on the findings of the relative predictive performance testing above, Model N was selected as the final model. Full parameter details for this model are shown in Table XVI (pg 87). (Where manual calculations were performed, the methods used can be seen in Appendix (I), pg 142).

Hence, with substitution, the final model may be written as follows :

$CL(L/hr) = 0.271 \times AGE(yrs) + 2.46 \times BSA(m^2)$

$V(L) = 7.34 \times BSA(m^2)$

Inter-individual variability was 15% and 18% for clearance and volume of distribution respectively, with intra-patient variability of 10% (or equivalent to a residual error of 2.02 mg/L expressed as a percentage of the average concentration of 19.8 mg/L).

Appendices (n) and (p), pgs 144,146, show the relationship between measured and predicted serum concentrations for Models A and N respectively. These demonstrate and confirm the improved data fit obtained with the final, compared to the simplest model.

Using substitution into the final model, individual parameter values were obtained for each patient. These were normalised for weight (to facilitate comparison with other publications). The mean (95% CI) of these values provides the following average parameter values:

CL = 0.180 (0.175, 0.185) L/hr/kg (≈3.0 ml/min/kg)

V = 0.293 (0.286, 0.300) L/kg

$T_{1/2}$ = 1.147 (1.104, 1.190) hr

k = 0.619 (0.599, 0.640) hr^{-1}

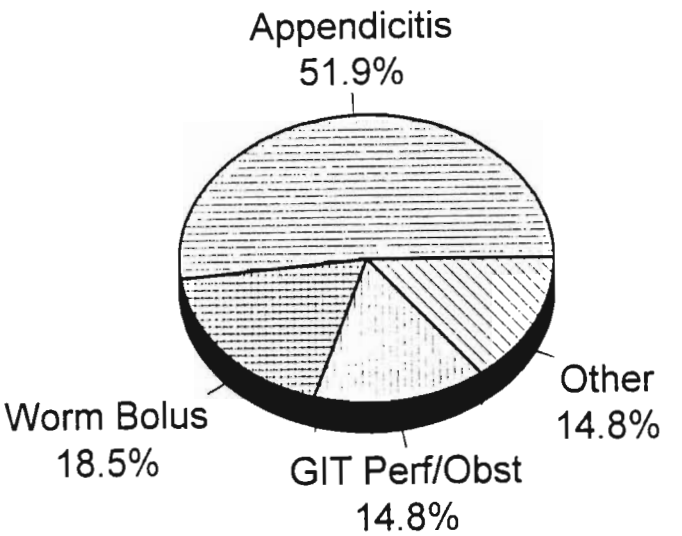
For the 17 burn patients, CL = 0.175 (0.170, 0.180) L/hr/kg (≈ 2.9ml/min/kg) and V = 0.313 (0.305, 0.322) L/kg.

TABLE II

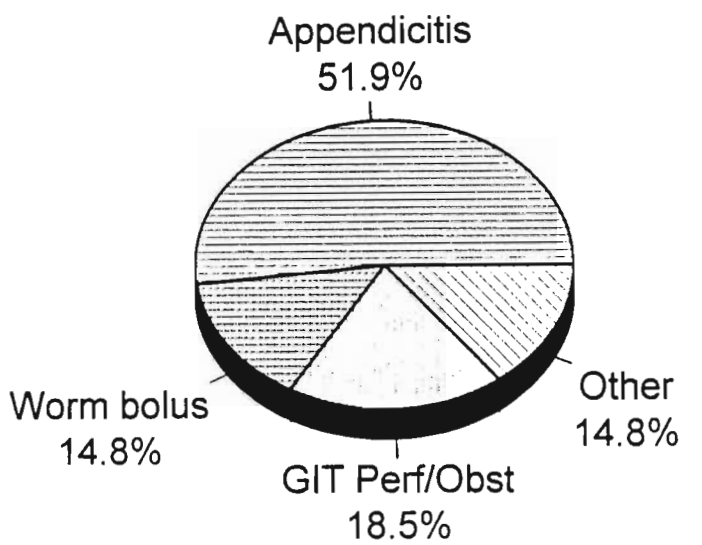
Demographic details and initial clinical parameters - patients included in regimen assessment. Mean (SD), or absolute patient numbers.

	Once Daily (n = 27)	Twice Daily (n = 27)
Demographic Details :		
Gender (M : F)	15 : 12	11 : 16
Age (yrs)	7.5 (3.2)	7.5 (2.4)
Weight (kg)	22.3 (7.1)	23.2 (7.1)
Height (cm)	117.7 (21.5)	120.8 (15.3)
Initial Clinical Features:		
Temperature (°C)	38.5 (0.9)	38.3 (0.9)
White Cell Count (10^9/L)	14.9 (5.6)(n = 26)	15.2 (7.9)(n = 26)
Serum Creatinine (µmol/L)	48.8 (14.5)(n = 22)	53.8 (13.0)(n = 24)

Serum Creatinine range for a child = 27 - 62 µmol/L. Tietz (1983).



Once Daily Regimen



Twice Daily Regimen

Fig.1. Disease States of Patients Participating in Regimen Assessment

TABLE III

Nature of intervention - patients included in regimen assessment. Values represent absolute patient numbers.

	Once Daily (n = 27)	Twice Daily (n = 27)
Non-surgical Management:	5	2
Surgical Intervention:	22	25
IV Cephalosporin prophylaxis	9	8
Saline Lavage	14	12
Cephalosporin/Saline Lavage	2	5

TABLE IVa

Initial bacteriological assessment - patients participating in regimen assessment.

	ONCE DAILY (n = 27)		TWICE DAILY (n = 27)	
	No. cultures performed	No. positive cultures	No. cultures performed	No. positive cultures
BLOOD	16	2 (2)	13	0
PUS	14	14 (13)	17	17 (15)
PERITONEAL FLUID	1	1 (1)	2	1
URINE	1	0	5	1 (1)

Number in parentheses indicates number of positive cultures in which Gram-negative organisms were cultured.

TABLE IVb

Gram-negative organisms isolated from positive cultures.

	ONCE DAILY	TWICE DAILY
Escherichia coli	13	18
Pseudomonas aeruginosa	3	0
Proteus species	2	1
Bacteroides species	0	1
Other	5	0
TOTAL	23	20
POLYMICROBIAL INFECTION	12	10

TABLE V

Antibacterial medication - patients included in regimen assessment. Mean (SD), or absolute patient numbers.

	Once Daily (n = 27)	Twice Daily (n = 27)
Amikacin Therapy:		
Dose (mg/kg/day)	15.1 (1.5)	14.9 (1.2)
Duration of therapy (days)*	5.7 (1.5)	4.6 (1.6)
Cumulative dose (mg/kg)†	91.5 (26.5)	70.1 (26.1)
Peak serum levels (mg/L)‡	37.7 (6.9)	19.5 (3.7)
Trough serum levels (mg/L)	0.4 (0.4)	0.4 (0.4)
Concomitant Therapy:		
Ampicillin + Metronidazole	23	24
Amoxycillin/clavulanic acid	2	2
Other	2	1

* $p = 0.019$, † $p = 0.004$, ‡ $p < 0.0001$

TABLE VI

Serum creatinine values - patients included in regimen assessment. Mean (SD).

SERUM CREATININE (µmol/L)	ONCE DAILY (n = 27)	TWICE DAILY (n = 26)
INITIAL	48.8 (14.5) (n=22)	53.8 (13.0) (n=24)
MID-THERAPY	40.6 (8.9)	40.9 (11.4) (n=25)
END-THERAPY	38.1 (8.9) (n=23)	39.2 (7.5) (n=18)

Serum Creatinine range for a child = 27 - 62 µmol/L. Tietz (1983).

TABLE VII

Patient demographics - patients participating in urinalysis. Values represent absolute patient numbers or medians with ranges in parentheses.

	Once Daily (n = 9)	Twice Daily (n = 8)	Controls (n = 9)
M : F	6 : 3	5 : 3	8 : 1
Age (yrs)	7.3 (1.5 - 10.0)	7.3 (5.5 - 10.5)	8.0 (1.3 - 12.5)
Weight (kg)	21.0 (9.8 - 34.0)	23.8 (20.0 - 30.0)	23.0 (13.9 - 29.9)

TABLE VIII

Summary of possible drug related proteinuria detected in the two treatment regimens. Values represent absolute patient numbers.

	ONCE DAILY DOSING (n = 9)	TWICE DAILY DOSING (n = 8)
Low Molecular Weight Proteinuria, plus Albuminuria	0	1 *†
Low Molecular Weight Proteinuria	3 ‡	2 ‡
Possible drug effect	3	2

* 1 patient with no pre-treatment sample

† 1 patient with Schistosomiasis

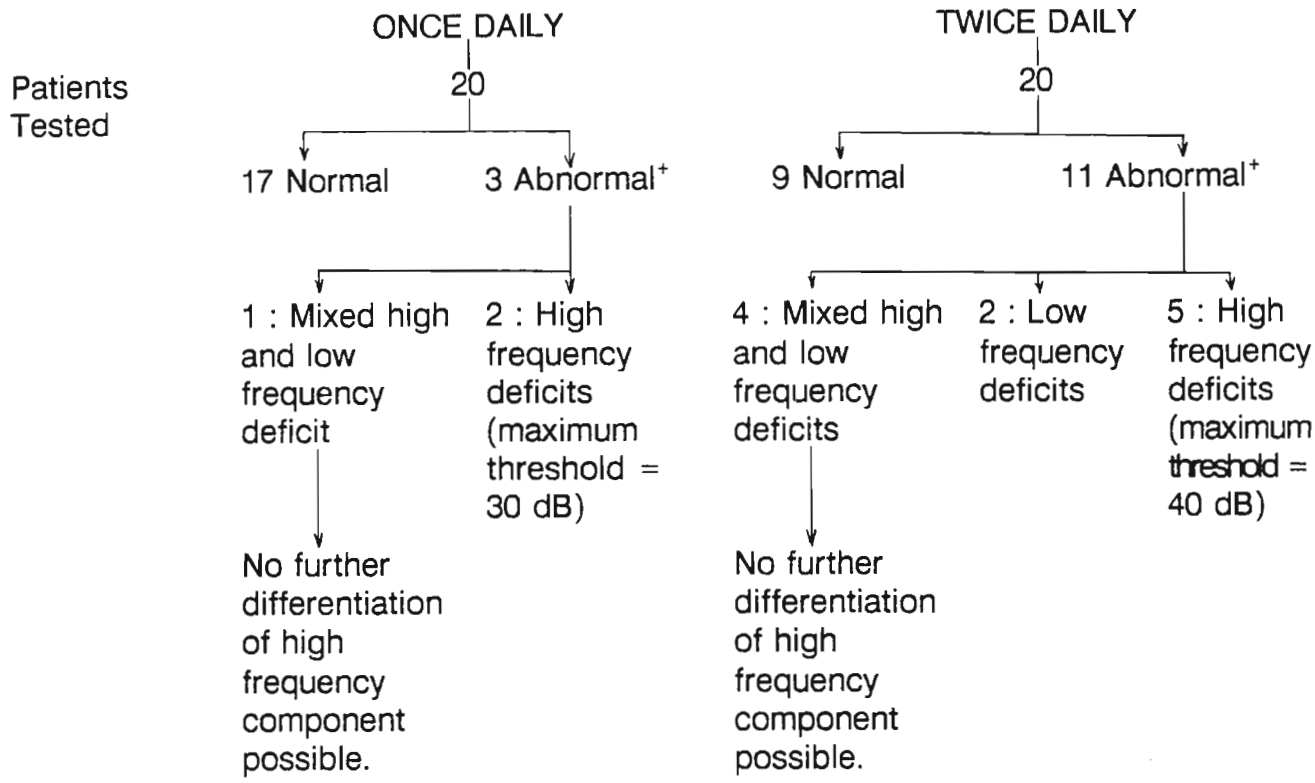
‡ 2 patients with no pre-treatment samples

TABLE IX

Patient details pertaining to those patients undergoing pure tone audiological assessment - patients included in regimen assessment. Mean (SD).

	ONCE DAILY (n = 20)	TWICE DAILY (n = 20)
Demographics:		
M:F	11 : 9	7 : 13
AGE (years)	8.8 (1.9)	7.9 (1.8)
WEIGHT (kg)	24.7 (4.0)	24.2 (7.3)
Amikacin Therapy Details:		
DOSE (mg/kg/day)	14.9 (1.6)	15.2 (1.2)
DURATION OF THERAPY (days)	5.5 (1.1)	4.8 (1.6)
CUMULATIVE DOSE (mg/kg) +	88.8 (19.9)	73.3 (27.1)
PEAK SERUM LEVEL (mg/L) •	37.6 (7.2)	19.9 (3.2)
TROUGH (mg/L)	0.4 (0.4)	0.4 (0.4)

+ $p < 0.05$, • $p < 0.0001$



+ total abnormalities were significantly different between the two groups, p = 0.008

Fig.2. Summary of audiological findings

TABLE X

Summary of high frequency hearing deficits - patients participating in the regimen assessment. Values represent absolute patient numbers.

	ONCE DAILY (n = 20)			TWICE DAILY (n = 20)		
	First Test	1st Recall	2nd Recall	First Test	1st Recall	2nd Recall
UNILATERAL DEFICIT	1	1	0	5*	3†	0†
BILATERAL DEFICIT	1	1	0†	0	-	-

* = 1 patient with tinnitus.

† = 1 patient not tested.

1st recall within 1 month of end of therapy,

2nd recall within 1 year.

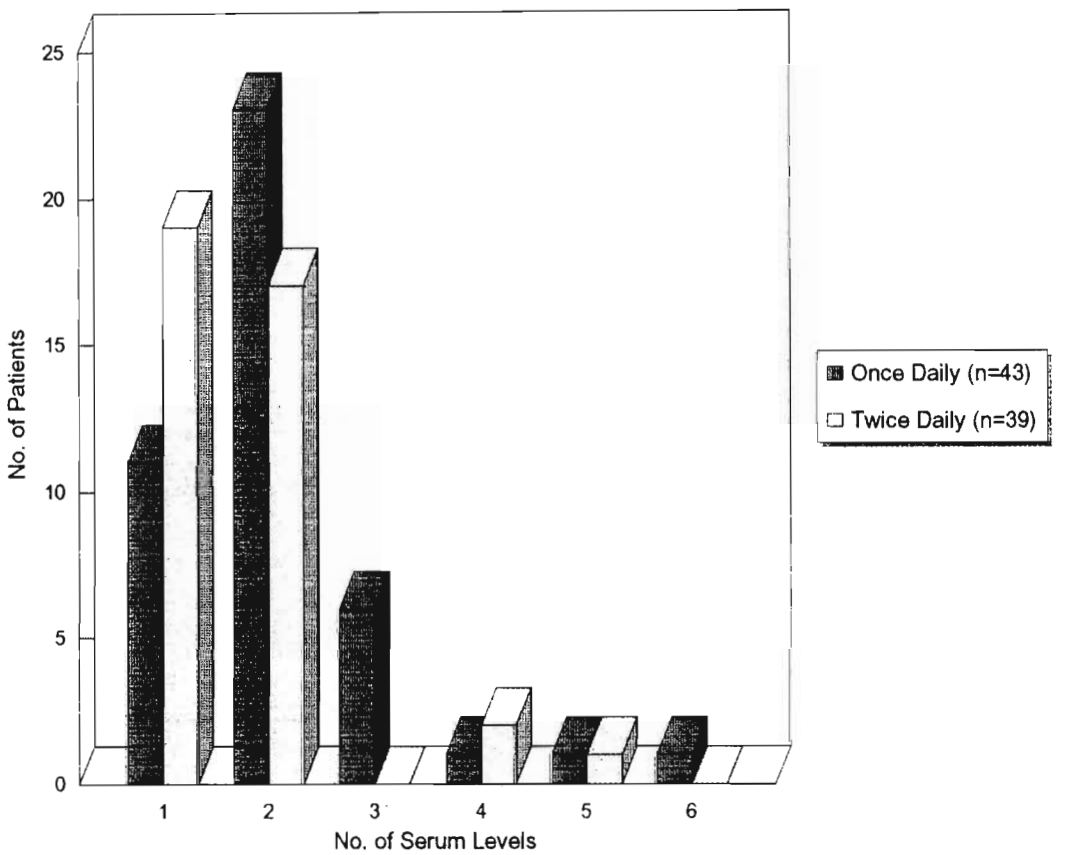


Fig.3. Histogram of Number of Serum Levels per Patient

TABLE XI

Successful intermediate models for clearance (additive statistical error). Model A included for reference.

Model		MOF	
A	$CL = \theta_1$ $V = \theta_2$	731.320	Simplest Model
B	$CL = \theta_1 AGE + \theta_3$ $V = \theta_2$	606.371	Single Fixed
C	$CL = \theta_1 AGE^{\theta 3}$ $V = \theta_2$	605.683	Effects Models
D	$CL = \theta_1 AGE + \theta_3 BSA$ $V = \theta_2$	601.231	Multiple Fixed
E	$CL = \theta_1 AGE + \theta_3 WT$ $V = \theta_2$	601.463	Effects Models

AGE = Patient age in years

WT = Patient weight in kg

BSA = Patient body surface area in m^2

TABLE XII

Successful intermediate models for volume of distribution (additive statistical error). Model A included for reference.

Model		MOF	
A	$CL = \theta_1$ $V = \theta_2$	731.320	Simplest Model
F	$CL = \theta_1$ $V = \theta_2 BSA^{\theta 3}$	604.347	
G	$CL = \theta_1$ $V = \theta_2 WT + \theta_3$	605.490	Single Fixed Effects Models
H	$CL = \theta_1$ $V = \theta_2 WT^{\theta 3}$	607.783	
I	$CL = \theta_1$ $V = \theta_2 BSA^{\theta 3} + \theta_4 WT^{\theta 5}$	599.446	Multiple Fixed Effects Model

AGE = Patient age in years

WT = Patient weight in kg

BSA = Patient body surface area in m^2

TABLE XIII

Results of step-wise parameter deletion.

MODEL	MOF	VERSUS MODEL	MOF	TEST	Select
Model J $CL = \theta_1 AGE + \theta_3 BSA$ $V = \theta_2 BSA^{\theta 4}$	529.157	Model L $CL = \theta_1 BSA$ $V = \theta_2 BSA^{\theta 3}$	543.614	DOBF = 14.5	J
		Model M $CL = \theta_1 AGE$ $V = \theta_2 BSA^{\theta 3}$	561.999	DOBF = 32.8	J
		Model N $CL = \theta_1 AGE + \theta_3 BSA$ $V = \theta_2 BSA$	529.165	AIC < 0	N
Model K $CL = \theta_1 AGE + \theta_3 WT$ $V = \theta_3 BSA^{\theta 4}$	532.263	Model M $CL = \theta_1 AGE$ $V = \theta_2 BSA^{\theta 3}$	561.999	DOBF = 29.7	K
		Model O $CL = \theta_1 WT$ $V = \theta_2 BSA^{\theta 3}$	544.590	DOBF = 12.3	K
		Model P $CL = \theta_1 AGE + \theta_3 WT$ $V = \theta_2 BSA$	532.682	AIC < 0	P

TABLE XIV

Predictive performance testing. (Absolute performance Models N and P).

	Model N	Model P
Troughs (n=26)		
Mean Prediction Error	-1.13 *	-1.12 *
(95% CI)	(-1.41,-0.85)	(-1.41,-0.84)
Mean Squared Prediction Error	1.75	1.74
(95% CI)	(0.29,3.21)	(0.27,3.20)
Peak/2nd post-dose level (n=130)		
Mean Prediction Error	-0.03	0.16
(95% CI)	(-0.86,0.81)	(-0.65,0.98)
Mean Squared Prediction Error	22.99	21.98
(95% CI)	(14.87,31.10)	(15.24,28.73)

* $p < 0.0001$

TABLE XV

Relative predictive performance testing. (Model N vs Model P).

	TROUGH LEVELS	PEAK/2ND POST-DOSE LEVELS
Relative Bias		
Δ Mean Prediction Error	-0.00885	-0.18769
95% CI	-0.0246,0.00695	-0.3495,-0.0258
Relative Precision		
Δ Mean Squared Prediction Error	0.0112	1.005
95% CI	-0.0077,0.0301	-1.681,3.691

TABLE XVI

Full parameter details Model N.

	Estimate	SEE	95% CI
θ_1	0.271	0.0733	0.125, 0.417
θ_2	7.34	0.186	6.97, 7.71
θ_3	2.46	0.572	1.32, 3.60
ω^2_{CL}	0.332	0.168	-0.002, 0.666
ω^2_V	1.20	0.334	0.535, 1.86
σ^2	4.08	1.07	1.95, 6.21

SEE is the Standard Error of the Estimate

95% CI is the 95% Confidence Interval (calculation shown in Appendix (I), pg 142)

CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 REGIMEN ASSESSMENT

4.1.1 **Regimen Efficacy**

The comparison of single versus twice daily amikacin dosing found no statistically significant difference in clinical outcome between the 2 treatment regimens. To assess efficacy in the clinical setting can be difficult. As mentioned in Section 2.2.1, the surgical nature of study patients unfortunately precluded full bacteriological evaluation. Assessment of outcome based entirely on clinical features, without full serial bacteriology, may be considered by some to be a rather gross measurement of efficacy. This criticism may be especially valid if one considers the numerous extraneous factors that can influence temperature and white cell counts (2 criteria used in the efficacy assessment) eg. chest infection. However, as this study was conducted in the clinical environment, and not in a controlled laboratory setting, the potential interference with efficacy assessment by such factors is unavoidable. Care was taken to as far as possible account for these factors, and so to a degree, improve the stringency of the evaluation.

It may be argued that inclusion of patients with conditions associated with low failure rates eg. perforated appendix in the young (Levison 1992), would not demonstrate differences in regimen efficacy. However, in the clinical setting in which this study was conducted, such conditions are often more serious than usual as a result of delayed presentation.

In analysing the findings of other workers, direct comparisons can be confounded

by differences in methodology, assessment criteria, population groups, drug administration and dosing etc. With regard to other studies of aminoglycoside regimens, 5 studies, comparing single to multiple daily dosing, have included children (Shankar & Sharma 1987, Marik et al 1991b, Viganò et al 1992, IATCGEORTC 1993, Elhanan et al 1995). In 2 of these studies (Marik et al 1991b, IATCGEORTC 1993) the adult groups incorporated children aged over 1 year, possibly obscuring the influence, if any, of their differing pharmacokinetic profile. Of all 5 studies, only that of Marik et al (1991b) documented a significantly higher cure rate with the single daily dosing regimen. This was in a subgroup of patients aged <1 year, as well as when patients of all ages were considered together. It would appear from their methodology however, that only the single daily dosing group received a loading dose. Bearing in mind that Moore et al (1984a) found early peak concentrations to be important in relation to clinical outcome (Section 1.2.2.1), one can only speculate as to the role, if any, that this loading dose played in the study results. The other 4 studies, like the present, found no statistically significant differences between the regimen efficacies.

Efficacy studies in children receiving a single daily amikacin regimen (not comparing to multiple dosing), include the work of Kafetzis et al (1991), Trujillo et al (1991) and Bouffet et al (1994). The first 2 studies found the regimen (in combination with other antibiotics when deemed appropriate) to be efficacious in the treatment of severe Gram-negative infections. In the third study (Bouffet et al 1994), an empiric dosing protocol including 15mg/kg daily amikacin infused over 30 minutes, was used in febrile episodes occurring in paediatric oncology patients.

In this study, although the treatment was successful in 92 % of episodes without regimen modification, treatment of documented Gram-negative sepsis had a significantly higher failure rate than that of Gram-positive sepsis (36 % vs 8 %). An additional non-comparative study, using single daily gentamicin dosing in children with urinary tract infections, reported the regimen to be efficacious with regard to urine sterilisation during therapy, but instances of infection recurrence during follow up were documented (Principi et al 1977).

Although the findings of the present comparative investigation are thus similar to others, the possible influence upon outcome of the significantly greater cumulative dose and duration of therapy in the once daily group, is not known. Furthermore, as with the other studies using concomitant medication, the contribution of the β-lactam to efficacy in the 2 groups is unclear. As aminoglycosides are used mainly in conjunction with other agents eg. β-lactams, the question of optimal aminoglycoside effectiveness ought to be rephrased to determine optimal efficacy of synergistic antibiotic combination therapy (Hustinx & Hoepelman 1993).

4.1.2 **Regimen Toxicity**

4.1.2.1 Nephrotoxicity

As described in Section 1.3.2.1, because its formation and elimination are dependent on various factors, serum creatinine has limitations as an indicator of drug induced renal toxicity (Garrison et al 1990). It is however, a practical tool in the clinical setting.

Clinical nephrotoxicity, as indicated by an increase in serum creatinine of 45µmol/L (≈0.5mg/dL) or more, was completely absent in the study children. This finding is consistent with the lower incidence of aminoglycoside-induced nephrotoxicity in children compared to adults (Viganò et al 1992), as well as the relatively short duration of therapy in both treatment groups. Drug-induced nephrotoxicity, in the children participating in the 5 comparative studies mentioned previously, was absent or low. Of these investigations Viganò et al (1992), with a sensitive definition (an increase in serum creatinine of only ≥ 0.3 mg/dL from baseline values), reported the highest number of nephrotoxic events. These were reversible and occurred in 2/70 and 2/74 children in the once and thrice daily netilmicin regimens respectively.

Similarly the 4 non-comparative studies found minimal or no nephrotoxicity (Principi et al 1977, Kafetzis et al 1991, Trujillo et al 1991, Bouffet et al 1994). A further non-comparative study by Viscoli et al (1991), involving a group of 13 children undergoing bone marrow transplantation, also found no renal problems in the absence of other potentially toxic drugs.

Although clinical nephrotoxicity was absent in the present study, the detection of low molecular weight proteinuria (without concurrent albumin or other high molecular weight proteins) in approximately 30% of amikacin treated children, may indicate aminoglycoside related tubular damage. Although the patient numbers were small (precluding statistical analysis) the incidence of tubular proteinuria, possibly related to amikacin therapy, was similar in the 2 treatment regimens.

Limitations to the urinalysis, in addition to the small patient numbers, include the lack of pre-treatment samples and uncertainty about the possible influence of other factors eg. fever. Although, fever and sepsis can affect low molecular, and other, proteinuria (Hemmingsen & Skaarup 1977, Richmond et al 1982) there were febrile patients (from some of whom infective organisms were cultured) in this study who had normal urinalyses. Unfortunately, as burn injury has been associated with tubular (as well as glomerular) proteinuria (Yu et al 1983), burn patients had to be excluded from the urinalysis and so regimen influences on tubular proteinuria in this group of patients could not be assessed.

Various other studies, using a variety of markers, have investigated renal tubular toxicity of single versus multiple daily aminoglycoside administration. In adult patients, a group of Belgian researchers have documented that daily dosing regimens result in less phospholipiduria than multiple daily dosing (Ibrahim et al 1990, van der Auwera et al 1991, Tulkens et al 1991), although the statistical significance in one study is unclear (van der Auwera et al 1991). These authors conclude that the daily regimens may be superior to multiple daily regimens in terms of their influence on phospholipid excretion. Other research in this field includes the work of Gonzalez et al (1993). They recorded a significantly greater elevation in urinary N-acetyl-beta-D-glucosaminidase in multiple daily, compared to single daily, gentamicin dosing regimens, while the rise in fractional excretion of β_2M was not significantly different between the two groups. In neonates, Skopnik et al (1992) found that urinary alanine aminopeptidase (a brush border associated enzyme), increased during and even after therapy in both single and twice daily

gentamicin treatment groups, with no significant difference between the groups. The Belgian researchers' work in neonates investigated the urinary excretion of various proteins, enzymes and total phospholipids in 2 amikacin treatment regimens. Although the former markers did not rise significantly in comparison to control patients, they found no statistically significant difference between the single and twice daily amikacin treatment groups with regard to any of the markers analysed (Langhendries et al 1993, Ibrahim et al 1994).

4.1.2.2 Ototoxicity

There was no statistically significant difference between the 2 regimens with respect to the numbers of patients demonstrating high frequency hearing losses which might be related to drug effect. Although this may indicate that the once daily dosing regimen was at least as safe as the twice daily regimen in this group of patients, the small patient numbers tested (20 in each treatment group) limits the statistical power of the comparison. The small sample size is a major limitation of the assessment.

Several other limitations (as alluded to in Section 1.3.2.2) need to be noted. As patients were too ill for pre-treatment (baseline) audiometry, the high frequency hearing deficits occurring post-therapy cannot, regrettably, be attributed with certainty to drug effect. Ideally ototoxic evaluations should be based on differences between pre- and post-treatment test results, with criteria standardised in all studies. In this study, the definitions used for the recording of possible hearing deficits (hearing threshold outside the normal 0 - 15 dB range for children), might

be considered by some to be rather stringent. In addition, several children were lost to formal evaluation as they were too young to participate in the pure tone audiometry testing. Another drawback of the test procedure used, was that bone conduction audiometry could not be performed reliably above 4000 Hz. This meant that certainty regarding the type of hearing loss at the higher frequencies could not be established. The timing of follow-up tests could also be criticised as it was not standardised. Repeat audiometry was performed whenever feasible. It is possible that deficits that might have occurred after a variable length of time following treatment discontinuation (as has been previously suggested (Zaske et al 1986)), would not have been detected with early post-treatment audiometry. Alternatively, late testing might have missed mild deficits which have the potential to reverse (reversibility of aminoglycoside-induced cochleotoxicity has been recorded (Fee 1980, Moore et al 1984b)). Elimination of these timing problems by serial screening of patients, with normal post-treatment audiograms on the first testing, was not practical.

Auditory function (using various methods) was tested in 3 of the 5 other published comparative studies, and in spite of variations in definition, there were few ototoxic events in the children (Viganò et al 1992, IATCGEORTC 1993, Elhanan et al 1995). In one of these studies, 2 of 20 children tested from the daily regimen demonstrated mild auditory impairment at 6 kHz frequency. However, these deficits, could not be attributed with certainty to the aminoglycoside as these patients were only tested post-therapy (Viganò et al 1992). In another of these studies (IATCGEORTC 1993) forty nine children, in whom the dosing regimen is

not specified, were evaluated. Of these, only 1 child, from the single daily regimen, had auditory toxicity. In the third investigation, 2/26 children of the daily regimen and 2/24 of the thrice daily regimen demonstrated ototoxicity, which was reversible where follow up was performed (Elhanan et al 1995).

The remaining 2 comparative studies found no evidence of ototoxicity on clinical grounds (Shankar & Sharma 1987, Marik et al 1991b).

In the noncomparative studies, ototoxicity was assessed by clinical evaluation in 2 studies (Trujillo et al 1991, Bouffet et al 1994) and by audiometry in 2 studies (Kafetzis et al 1991, Viscoli et al 1991). Overall, no ototoxicity was documented in the absence of other potentially ototoxic agents.

4.2 POPULATION PHARMACOKINETIC PARAMETERS

The most important determinants, in the final model derived to characterise amikacin pharmacokinetic parameters in this population of South African children, were found to be age and body surface area describing clearance (CL), and body surface area describing volume of distribution (V). Body surface area was calculated as a function of patient weight and height, both raised to a power. It could be argued that such models, requiring the additional computation of body surface area, would be less easy to apply in the clinical setting, compared to models describing CL and V in terms of, for example, weight. However, the model incorporating body surface area was still superior to simpler model combinations.

Additionally, body surface area is not an entirely foreign concept to the clinical situation (eg. its use in dose calculation for antineoplastic therapy) and so the implementation of models requiring its estimation would not be unrealistic. In a NONMEM investigation of gentamicin and tobramycin, Thomson et al (1995) found body surface area to be an important determinant describing V in cystic fibrosis patients. Weber et al (1993) and Jensen et al (1992), also using NONMEM, incorporated "size" (as a power, or scaled, function of weight) in their models derived for neonatal populations receiving gentamicin. According to Grenier et al (1987), in a traditional analysis, "the apparent volume of distribution, and consequently the loading dose necessary to obtain an effective and nontoxic plasma level, appears to be more precisely related to the body surface area than to the weight".

Of various NONMEM analyses of aminoglycosides, only that of Kelman et al (1984), using gentamicin, considered a subgroup of children with approximately similar ages to those of the present study. For 68 children (aged 6 months - 15 years, no mean (SD) given), they found the best of five models tested, to be:

CL = 0.19WT - 0.13AGE - 0.009CREAT (where CREAT is serum creatinine),
V = 0.28WT

In comparing the amikacin and gentamicin models, it is interesting to note that both include age and weight as fixed effects in describing CL, and weight in describing V (although in the amikacin models, weight was included as a power

function, as part of body surface area). The models contrast with regard to the importance of serum creatinine in describing CL, with it being incorporated in the gentamicin model, but not in the amikacin model. As patient serum creatinine values varied little outside normal ranges in this amikacin investigation it is not surprising that the inclusion of serum creatinine did not render the data more probable. This also highlights the important point that the model derived is not applicable for use in children with renal impairment.

The presence of burn injury did not significantly influence intermediate or final models describing CL or V in the present investigation. Burn injury initiates extensive alterations in body metabolism and fluid shifts (Finkelstein et al 1992) and has, not surprisingly, been reported to influence pharmacokinetic parameters (Zaske et al 1986). The role of percentage of surface area burnt, as well as time lapse since initial injury to start of treatment, may also be important considerations in aminoglycoside pharmacokinetics in burn patients. In assessing the influence of burn injury on pharmacokinetic parameters in children, it is important to note that pharmacokinetics in this group differ already from adults by virtue of the age factor (a distinction that does not always appear to have been made in the literature). There are seemingly few studies (generally with small patient numbers) of pharmacokinetics in burn patients, and fewer still incorporating only children. Glew et al (1976) investigated gentamicin in 18 burn children and 5 control patients. They found the burn patients to have significantly lower peak serum concentrations than nonburn patients. (Some caution should be exercised in interpretation of these results as peaks were drawn "within 2 hours" after dose

administration). Other findings include a lack of correlation between the extent of body surface area burned (range = 30% - 92%) and peak serum levels.

A study by Hollingsed et al (1993) involved both adult and paediatric burn patients. They did however quote pharmacokinetic parameters (for gentamicin) for the 2 age groups, separately. In the children (n=6, aged 1.3 - 7 years) therapy was initiated between 2 and 69 days postburn. Despite the limitations of their small sample size, the absence of age matched controls and the difficulties encountered in comparing studies with differing age distributions, they found a larger V and half life than that determined in the present study (for either the whole group, or for the burn patients alone).

Pharmacokinetic data specifically for burned children receiving amikacin is limited to the work of Kopcha et al (1991). Their study incorporated 38 children aged 3 months to 18 years, with mean age of 6.6 years and wide distribution about the mean (SD = 6.2 years). Their CL and V were both larger than the values in the present study. No significant correlation was found between the percentage total body surface area burnt (range = 11% - 87%) and V.

The lack of influence of burn injury found in the present study might be attributed to the fact that only approximately 21% of children included in the analysis were burn patients. Not all authors however have demonstrated significantly differing pharmacokinetic parameters between burn and nonburn patients. For example the present findings may be in keeping with those of Polk et al (1983) who found the

kinetic characteristics of tobramycin and gentamicin in severely burned adult patients (surface area burnt = 10% - 90%), to be similar to those reported for nonburn patients and normal volunteers. They state that the recommendation that burn patients require larger aminoglycoside dosages than patients without burns who have severe infections is not supported by their data. With the current paucity of pharmacokinetic studies of aminoglycosides in burn children, further investigations are required.

Although a 1 compartment model has been used to describe aminoglycoside concentration-time data, in certain circumstances, where a triphasic decay of serum concentration with time may be demonstrated, a 3 compartment model may more accurately characterise the data (Zaske 1986). In the present study, the underprediction of trough serum levels (as revealed during predictive performance testing) may point towards the existence of further compartments not taken into account by the use of a one compartment model. The attempt made during the course of the analysis to implement a two compartment model was unsuccessful. The lack of influence of dosing regimen as a covariate in the one compartment model might be considered as corroborating the use of this simpler pharmacokinetic model. Although the one compartment model has limitations, it appeared to adequately describe the data set.

The population pharmacokinetic parameters for amikacin in South African children, as derived by NONMEM, were as follows :

CL = 0.180 L/hr/kg or 3.0 ml/min/kg

V = 0.293 L/kg or 29% of body weight.

Pharmacokinetic analyses of amikacin in children, published by other South African researchers, (Marik et al 1991a, Hendricks et al 1995), have been based on the traditional method of parameter determination (as opposed to the population approach of the present study). In the former, pharmacokinetic parameters derived for children older than 1 year of age (number not stated) were included with those of the adult patients (Marik et al 1991a). The study by Hendricks et al (1995), was conducted specifically in kwashiorkor children (n=10, aged 1 - 4 years). The mean V (0.335 L/kg), extrapolated from their published data, is similar to that of the present study (0.324 L/kg) if only the patients aged 0.6-4 years (n = 25) are considered together. However, their mean CL and half life are smaller and greater respectively than those of the present investigation. According to Hendricks et al (1995), although a decreased glomerular filtration has been demonstrated in children with protein energy malnutrition (PEM), they state that their CL values do not reflect this. Relative to the CL value for similarly aged children in the present study however, it would appear that their values do concur with the reported alterations in renal function of such patients.

As discussed previously, comparison with other studies can be difficult, being confounded by a variety of factors. In spite of this, the values of CL and V derived in the study, are within the range of values derived by traditional methods and published for other groups of children of approximately similar ages (see Table

XVII below, pg 107).

Few of the studies shown in Table XVII (as well as of those for other age groups), have investigated the relationships of amikacin pharmacokinetic parameters to measurable physiological descriptors. When considering such relationships it is important to take cognisance of whether of not the parameters are weight adjusted eg. CL expressed as L/hr or L/hr/kg, as this may confound comparisons.

Cleary et al (1979) found that CL (ml/min/1.73m^2) and V (L/kg) did not vary significantly with respect to age, sex and body surface area, among other factors tested. Half life tended to increase with surface area but no significant correlation could be demonstrated. In contrast to this, Kopcha et al (1991), in burn patients, reported that both CL (L/hr/kg) and V (L/kg) correlated negatively with age, and half life was sex dependent. In both of these studies, the contribution of the older patients (up to 17 and 18 years of age respectively) to their findings is uncertain.

Patients with impaired renal function were incorporated in an investigation by Lanao et al (1981) and relationships were derived between pharmacokinetic parameters and creatinine clearance. In assessing amikacin disposition in various age groups, Lanao et al (1982) established a linear (positive) relationship between V (L) and patient weight. However, as the relationship was derived with the inclusion of a group of adult patients, it may not be truly representative for paediatric patients *per se*.

In this latter publication V (L/kg) and drug half life (hrs), when categorised for various age groups, are shown to decrease with increasing age, reaching minima in the 15-51 year old and the 3-11 year old age categories respectively (Lanao et al 1982). Table XVIII (pg 108) shows mean CL (L/hr/kg), V (L/kg) and half life (hrs) from the present study when categorised into various age groups. Also included are population values derived in a NONMEM analysis of data from a group of South African neonates receiving amikacin (Botha et al 1996), which concur with other published values for similarly aged patients. The findings show similar trends to those of Lanao et al (1982). A progressive decrease in mean V and half life is evident with increasing age (up to the study limit of 12 years). The findings of Table XVIII are also in accordance with the known influence of age on aminoglycoside pharmacokinetics (Section 1.5).

4.3 CONCLUSION

The pharmacokinetic evaluation in these children receiving amikacin confirms that children with normal renal function have a rapid aminoglycoside clearance. This manifests as a rapid post-dose fall in serum drug concentrations and, with dosing protocols as in the present study, low-to-undetectable trough serum levels could be expected. In contrast, neonates and adult patients, with comparatively slower drug clearance, are more likely to have unacceptably high trough serum levels with multiple daily dosing protocols. This could be limited in various ways. For example, use of single daily regimens would allow for a longer "washout" period. Dose individualisation based on serum drug level measurements could also be

implemented. Alternatively, customisation of initial dosing regimens using an appropriate population pharmacokinetic model could be initiated. In the present investigation in children with normal renal function, although pharmacokinetic models describing population clearance and volume of distribution were derived, the consistently acceptable serum trough concentrations achieved with either of the 2 empiric dosing regimens appears to limit the clinical usefulness of such models.

With regard to aminoglycoside toxicity, the current thinking regarding drug uptake kinetics into the organs susceptible to toxicity (Section 1.3.3.2), would infer that low trough serum levels are desirable, resulting in less drug uptake than regimens with higher circulating pre-dose levels. However, as drug uptake kinetics are not the only determinants of toxicity, it would possibly be unwise (although tempting) in the present study to attribute the absence of nephrotoxicity in, and the nonsignificant differences in ototoxicity between, the 2 treatment groups to the comparable low mean trough levels achieved with both dosing regimens.

Regarding efficacy, depending on the school of thought, very low trough concentrations and a relatively drug-free interval may be considered by some to be undesirable, by others to be necessary. It would not be inconceivable that during a prolonged interval where serum concentrations are negligible, bacterial regrowth could occur. McLean et al (1994), who reported that post-bolus, sustained trough concentrations (0.5 - 1.0 mg/L) are required for continued *in vitro* bactericidal effect (Section 1.2.2.2), conclude that their findings may represent a

limitation to once daily dosing in subjects with elevated clearance such as young patients. In a recent publication, McLean et al (1996) propose that in the young, and in other groups with a rapid renal clearance, aminoglycoside dose intervals of 8 or 12 hours may be appropriate. Rotshafer & Rybak (1994) suggest that consideration should be given to dosing intervals of 12 hours in hyperdynamic patients with life-threatening Gram-negative sepsis and short aminoglycoside serum half lives (< 2 hours).

In contrast to this, the concept of adaptive resistance (Section 1.2.2.2) implies a need for a certain interdose interval without measurable serum drug concentrations, and mitigates against repeat dosing when organisms may be quite resistant to the antibiotic. The crux of the issue lies in the precise duration of this effect. Some authors have found *in vitro* that full bacterial susceptibility is restored by 6-8 hours in drug free medium (Daikos et al 1990, Jackson et al 1988) in which case, according to MacKenzie & Gould (1993) a twice daily dosing regimen would probably be as effective as a single daily regimen if this effect is considered. However, others have reported times in excess of 24 hours *in vitro* for full recovery of bacterial susceptibility (Barclay et al 1992). The duration of this effect for various organisms *in vivo*, remains to be determined. The existence of the Post Antibiotic Effect and the Post Antibiotic Leukocyte Enhancement may support the use of larger doses at longer intervals, enabling continued antibacterial activity in the absence of drug.

Apart from uncertainty regarding the optimal duration of the interdose interval,

other questions that are raised include what are the desirable therapeutic levels and when should they be determined ? The upper limits of peak concentrations set previously for conventional therapy will inevitably be exceeded by once daily dosing regimens. Kumana & Yuen (1994) go so far as to suggest that the premise of keeping peaks below a so-called "toxic upper limit" is gradually being discarded. Critical trough levels are also under scrutiny. Furthermore, should novel timing of serum levels be adopted (eg. single serum level measurements at for example 8 hours post-dose, as proposed by Blaser et al (1994)) then again optimal target ranges will need to be re-addressed.

In the present study, in spite of the anticipated significantly larger (almost double in magnitude) peak serum levels in the single daily regimen, and in spite of the reported association of peak concentration and bactericidal activity, significant differences between the 2 regimens with regard to clinical outcome were not demonstrated.

From this investigation, in reference to other analyses in children, it would appear that a daily amikacin administration (in combination with a β-lactam) to children with normal renal function, has similar efficacy to, and no greater toxicity than, the classic multiple daily protocol. Although in the present study the relative costing of the 2 regimens (eg. cost of greater amount of drug administered in the single daily dosing group versus cost of greater number of consumables used in the twice daily dosing regimen) cannot be determined, various practical advantages conferred by the single daily regimen, such as reduced nursing time, may favourably dispose

one to this regimen. However, although the regimens are seemingly similar with regard to the end-point assessment, the possible contribution of the significantly larger cumulative dose and duration of therapy in the once daily dosing group, to the outcome, needs to be born in mind. Furthermore, the rapid drug elimination, by children with normal renal function, as found in the present study, raises the possibility that the daily regimen may not yet be the regimen best suited to children. Further investigations (with sufficiently large sample size), into various dose and/or interval combinations, are needed to clearly describe the dosing protocol which will afford optimal therapeutic use of this agent in this special population group.

TABLE XVII

Summary of amikacin pharmacokinetic parameters. Comparison of published values for children of approximately similar ages to those of the present study.

Ref	Mean(range)age	Disease (n)	CL	V
A	6.6 (0.6-12) yr	32 App, 17 Burn, 33 Other	0.180L/hr/kg 3.0ml/min/kg 135ml/min/1.73m^2	0.293 L/kg 29.3 % body weight
1	10.3 (4-16) yr	11 CF,4 Onc, 2 App,3 Other	2.49ml/kg/min 120ml/min/1.73m^2	32.1% body weight
2	(14mo-16.9 yr)	50 Onc	131ml/min/1.73m^2	0.26 L/kg
3	8.1 (1.3-16.9) yr	8 Onc	2.51ml/min/kg 109ml/min/1.73m^2	0.24 L/kg
4	6.6 (1.1-11) yr 5.9 (3-11) yr	10 RI 8 nonRI	1.14ml/min/kg 3.01ml/min/kg	0.37 L/kg 0.198 L/kg
5	11.5 (6-25) days 9.6 (4-18) mo 5.9 (3-11) yr	6 10 8	2.04ml/min/kg 2.99ml/min/kg 2.83ml/min/kg	0.429 L/kg 0.32 L/kg 0.201 L/kg
6	7.6 (3-15) yr 6.3 (1-12) yr	9 CF 4 nonCF	131ml/min/1.73m^2 157ml/min/1.73m^2	0.261 L/kg 0.264 L/kg
7	7.6 (3-15) yr 6.3 (1-12) yr	9 CF 4 nonCF	131ml/min/1.73m^2 155ml/min/1.73m^2	0.257 L/kg 0.265 L/kg

A = Present study, 1 = Vogelstein et al 1977, 2 = Cleary et al 1979,

3 = Kramer et al 1979, 4 = Lanao et al 1981, 5 = Lanao et al 1982,

6 = Autret et al 1986, 7 = Grenier et al 1987

App = Appendicitis, CF = Cystic Fibrosis, Onc = Oncology, RI = Renal Impaired.

TABLE XVIII

Population pharmacokinetic parameter values for amikacin for various age categories. Values represent mean (SD) for clearance (CL) L/hr/kg, volume of distribution (V) L/kg, and half life ($T_{1/2}$) hours.

	CL	V	$T_{1/2}$
Neonates (n = 56)	0.048* (0.010)	0.434* (0.070)	6.349 (0.767)
0.6 - 4 Years (n = 25)	0.163 (0.017)	0.324 (0.033)	1.388 (0.172)
5 - 8 Years (n = 34)	0.185 (0.019)	0.288 (0.020)	1.082 (0.063)
9 - 12 Years (n = 23)	0.190 (0.022)	0.267 (0.016)	0.982 (0.062)

* Botha et al 1996

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APPENDICES

LIST OF APPENDICES

ABBREVIATIONS USED IN THE APPENDICES

av : average value represented

BSA : Body surface area (m^2)

Cef Pro : Intravenous cephalosporin prophylaxis

- c'tax = cefotaxime
- c'man = cefamandole
- c'fox = cefoxitin
- c'rox = cefuroxime

Concom : Concomitant antibacterial therapy

- am = ampicillin + metronidazole
- au = amoxycillin/clavulanic acid
- cm = cephalosporin + metronidazole
- pm = piperacillin + metronidazole

Cumul. : Cumulative amikacin dose administered (mg/kg)

Disease(Dx) : Disease states

- app = appendicitis
- wb = worm bolus
- p/o = perforation/obstruction G.I.T.
- psarp = posterior sagittal ano-recto plasty
- orch = orchidopexy
- IH = inguinal hernia
- other - refer to text

Dose	:	daily amikacin dose (mg/kg/day)
Duration	:	Duration of amikacin therapy (days)
HT	:	Patient height (cm)
I.D.	:	Patient identification: OD = Single daily dosing regimen BD = Twice daily dosing regimen BOD = Burn patient, single daily dosing regimen BBD = Burn patient, twice daily dosing regimen
Lavage	:	Intra-operative lavage using either saline or cephalosporin/saline (c/sal) solution
Levels	:	Number of serum level measurements performed during course of therapy
NMID	:	Nonmem identification number
PEAK	:	Peak serum amikacin level (mg/L)
Post 1	:	1st post-dose serum amikacin level (mg/L)
Post 2	:	2nd post-dose serum amikacin level (mg/L)
Pre-dose	:	Pre-dose serum amikacin level (mg/L)
Reg	:	Regimen
SCR1	:	Initial serum creatinine (μmol/L)
SCR2	:	Serum creatinine at time of drug level determination (μmol/L)
SCR3	:	Serum creatinine at end of therapy (μmol/L)
Sx/Non	:	Sx = surgical intervention, Non = Non-surgical intervention
Temp1	:	Initial temperature (°C)

Test1	:	Results of 1st audiological assessment nad = no abnormality detected poss = mixed high and low frequency abnormality hf = high frequency abnormality nr = other abnormality
Time P	:	Time of peak serum drug level determination (hrs post-dose)
Time 1	:	Time of 1st post-dose serum drug level measurement (hrs)
Time 2	:	Time of 2nd post-dose serum drug level measurement (hrs)
TROUGH	:	Trough serum amikacin level (mg/L)
wcc1	:	Initial white cell count (10^9/L)
WT	:	Patient weight (kg)
*	:	Extrapolated values
#	:	Topical Neosporin^R applied to facial burns

APPENDIX (a). CONSENT FORM FOR SERUM CREATININE DETERMINATION.

E.8 INFORMED CONSENT FOR INCLUSION IN A CLINICAL TRIAL ¤

8.1 I, (Name) _____

hereby consent to the following Procedure and/or Treatment being conducted on myself or the person indicated in (iv) below

_____

8.2 I acknowledge that I have been informed by:

(Name) MRS NAN FORSYTH

concerning the possible advantages and possible adverse effects which may result from the abovementioned procedure and/or treatment and of the ways in which it is different from the conventional procedure and/or treatment

I, (Name) _____

hereby acknowledge that I understand and accept the "Information to Patients" leaflet handed to me in connection with this trial

8.3 I agree that the above procedure and/or treatment will be carried out and/or supervised by

(Name) DOCTOR ON DUTY

8.4 I acknowledge that I understand the contents of this form, including the information provided in "Information to Patients" leaflet and as the *SUBJECT/PARENT/GUARDIAN/OTHER (Specify) freely consent to the above procedure and/or treatment being conducted on:

(Name) _____

8.5 I am aware that I may withdraw my consent at any time without prejudice to further care.

Signed: _____ Date: _____
Subject/Parent/Guardian

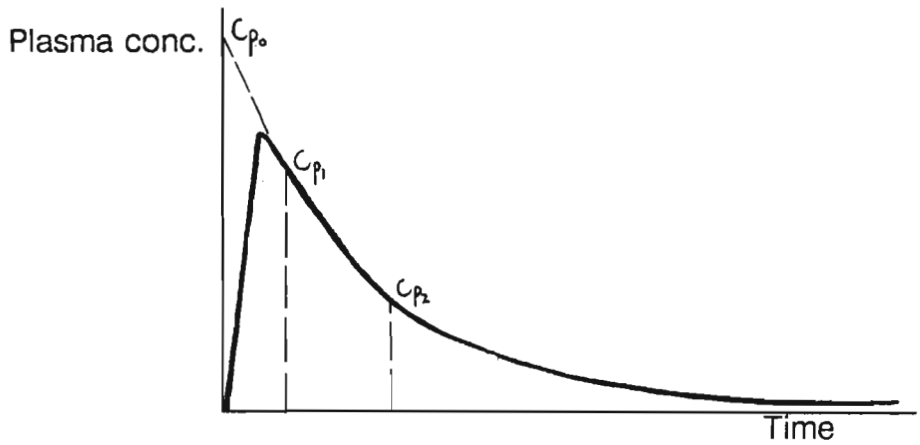
Signed: _____ Date: _____
Witness

Signed: _____ Date: _____
Informant

Signed: _____ Date: _____
Researcher

With the exception of the names and signatures in paragraphs 8.4 and 8.5 please provide the above information

APPENDIX (b). CALCULATION OF PLASMA CONCENTRATIONS.



eg. Dose administered at 10H10

Cp_1 = 31.0 mg/L at 10H58

Cp_2 = 14.0 mg/L at 12H22 (Cp_1 and Cp_2 included in pharmacokinetic analysis)

$k = (\ln [Cp_1/Cp_2])/t$

$k = (\ln [31.0/14.0])/1.4$

$k = 0.568\ hr^{-1}$

$Cp = Cp_o.e^{-kt}$

$31.0 = Cp_o.e^{-0.568x0.8}$

Cp_o = 48.8 mg/L

Calculated Peak ($Cp_{0.5}$) at 0.5 hours post-dose:

$Cp_{0.5} = 48.8.\ e^{-0.568x0.5}$

$Cp_{0.5}$ = 36.7 mg/L

Calculated Trough (Cp_{24}) at 24 hours post-dose:

$Cp_{24} = 48.8.\ e^{-0.568x24}$

Cp_{24} = < 0.1 mg/L ($Cp_{0.5}$ and Cp_{24} included in regimen assessment)

Appendix (c). Patient details (demographics, disease states, initial clinical parameters and intervention) Daily dosing regimen.

I.D.	NMID	SEX	AGE	WT	HT	Disease	Temp1	wcc1	SCR1	Sx/Non	Cef Pro	Lavage
OD-1	15	M	3.3	14.5	87	p/o	38.5	8.4	43	Sx	c'tax	
OD-2	16	F	7.0	19.0	114	wb	39.5	15.0	46	Non		
OD-3	17	M	4.6	20.0	108	other	38.0			Sx		
OD-4	18	F	12.0	29.4	141	app	39.5	6.9	93	Sx	c'man	saline
OD-5	19	F	1.3	7.1	68	p/o	39.0	6.2	40	Sx	c'man	
OD-6	20	F	7.9	19.4	115	wb	40.0	12.2	41	Non		
OD-7	21	M	11.0	20.5	119	other	37.2	5.2		Sx		
OD-8	22	F	9.8	27.5	136	app	39.0	13.9	51	Sx		saline
OD-9	24	M	7.7	22.9	122	app	39.2	19.4	54	Sx		saline
OD-10	25	F	0.6	10.2	74	other	37.0	16.6	33	Sx		
OD-11	27	F	8.0	28.0	129	p/o	37.4	10.4	57	Sx		saline
OD-12	29	F	9.5	27.6	135	app	38.0	13.7	46	Sx		saline
OD-13	30	M	11.1	28.9	139	app	38.2	10.1	57	Sx	c'tax	c/sal
OD-14	31	F	10.8	27.5	134	app	38.2	24.5	55	Sx		saline
OD-15	50	M	7.8	22.0	128	app	37.6	16.5	39	Sx		saline
OD-16	51	M	6.4	20.3	115	app	39.5	12.1	60	Sx		saline
OD-17	52	M	1.5	9.8	75	wb	37.2	13.2		Non		
OD-18	54	F	10.0	34.0	135	app	38.0	17.7	37	Sx		saline
OD-19	58	M	8.0	23.0	122	app	38.5	24.6		Sx		saline
OD-20	63	M	3.0	12.0	94	wb	38.5	16.8	42	Non		
OD-21	65	M	9.0	26.0	129	p/o	40.0	18.9		Sx	c'fox	c/sal
OD-22	66	F	9.0	26.0	124	app	39.5	27.8	80	Sx	c'fox	saline
OD-23	73	M	11.0	26.0	130	app	37.9	18.6	43	Sx	c'tax	saline
OD-24	74	M	10.7	34.8	152	other	38.0	16.1	42	Sx		
OD-25	75	M	8.2	24.9	126	app	39.9	10.9	42	Sx	c'fox	saline
OD-26	79	F	5.8	19.5	107	wb	38.0	14.8	36	Non		
OD-27	82	M	7.3	21.0	120	app	38.0	17.9	36	Sx	c'fox	saline

Appendix (d). Patient details (demographics, disease states, initial clinical parameters and intervention) Twice daily dosing regimen.

I.D.	NMID	SEX	AGE	WT	HT	Disease	Temp1	wcc1	SCR1	Sx/Non	Cef Pro	Lavage
BD-1	1	F	3.1	11.3	87	wb	39.0	11.1	40	Non		
BD-2	2	M	11.5	29.0	147	app	38.0	15.2		Sx		
BD-3	3	M	8.6	24.0	132	app	40.0	15.7	47	Sx	c'man	saline
BD-4	4	M	6.0	24.0	114	app	38.0	15.5	70	Sx	c'fox	saline
BD-5	5	F	11.3	49.8	153	other	38.0	16.8	49	Sx		c/sal
BD-6	6	F	9.0	23.7	126	app	39.5	25.5	66	Sx		saline
BD-7	7	F	5.7	21	112	other	37.2	5.7	69	Sx		saline
BD-8	8	M	3.7	16.0	97	p/o	38.5	17.1	41	Sx		
BD-9	9	F	8.0	29.5	127	other	38.7	26.8	46	Sx		perox
BD-10	10	M	7.3	22.5	123	app	39.0	7.1	62	Sx	c'fox	
BD-11	11	F	7.5	17.3	108	app	39.0	16.0	49	Sx	c'fox	
BD-12	12	M	9.3	24.5	128	app	37.0	14.0		Sx		c/sal
BD-13	13	F	3.2	16.2	96	other	37.0	14.0	37	Sx		saline
BD-14	14	M	6.0	22.0	125	p/o	37.8	10.7	56	Sx		saline
BD-15	32	M	9.0	29.2	132	app	39.5	10.4	62	Sx	c'tax	c/sal
BD-16	33	F	11.0	26.5	142	app	39.0	25.7	50	Sx		c/sal
BD-17	44	M	9.5	27.7	134	app	38.8		41	Sx	c'man	
BD-18	47	F	5.8	20.0	111	p/o	38.6	37.0	25	Sx		saline
BD-19	48	M	10.5	30.0	136	p/o	38.0	8.1	45	Sx		c/sal
BD-20	49	F	7.5	24.0	124	app	37.0	14.5	66	Sx		saline
BD-21	60	F	5.7	15.0	106	wb	37.0	5.1	59	Sx	c'rox	
BD-22	62	F	7.0	19.8	123	app	38.0	25.6		Sx	c'fox	
BD-23	64	F	7.1	23.5	113	wb	38.0	13.7	63	Non		
BD-24	69	F	10.0	20.7	118	app	38.5	10.7	53	Sx		saline
BD-25	71	F	6.3	19.0	115	app	38.3	20.8	83	Sx		saline
BD-26	77	M	5.5	20.6	114	wb	37.7	6.2	48	Sx		saline
BD-27	80	F	7.4	18.2	118	p/o	39.5	4.8	64	Sx		saline

Appendix (e). Antibacterial treatment details. Daily dosing regimen.

I.D.	Dose	Duration	Cumul.	Pre-dose	Post1	Time1	Post2	Time2	TROUGH	PEAK	Time P	Concom
OD-1	14.5	5.0	72.4	0.8	38.6	0.52			0.8	38.6	0.52	am
OD-2	14.7	6.0	88.4	1.2	41.5	0.55			1.2	41.5	0.55	am
OD-3	15.0	5.0	75.0	0.9	39.7	0.73			0.9	39.7	0.73	pm
OD-4	15.3	6.5	107.1	0.8	40.5	0.48			0.8	40.5	0.48	am
OD-5	15.5	4.0	62.0	0.0	28.3	0.57			0.0	28.3	0.57	am
OD-6	15.5	4.5	77.3	1.0	34.3	0.52			1.0	34.3	0.52	am
OD-7	av10.4	5.5	82.9	0.9	32.9	0.60			0.9	32.9	0.60	am
OD-8	av13.8	4.5	69.1	av0.5	av57.6	0.53			0.0	30.1	0.53	am
OD-9	av14.7	6.5	102.6	0.2	31.0	0.52			0.2	31.0	0.52	am
OD-10	14.7	4.5	73.5	0.6	47.9	0.50			0.6	47.9	0.50	am
OD-11	av16.0	5.0	96.0	1.1	30.3	0.50			1.1	30.3	0.50	am
OD-12	av15.3	5.5	92.0	av0.6	av42.7	av0.53			0.6	42.7	0.53	am
OD-13	av14.1	6.5	112.4	av0.5	av49.0	av0.51			0.5	49.0	0.51	am
OD-14	av14.4	4.5	71.8	0.0	33.8	0.52			0.0	33.8	0.52	am
OD-15	av14.9	5.0	74.3	0.4	36.7	0.57			0.4	36.7	0.57	au
OD-16	14.8	5.5	88.7	av0.8	av35.7	av0.53			0.8	35.7	0.53	am
OD-17	av17.9	8.0	142.9	av1.1	av32.3	av0.66			1.1	32.3	0.66	am
OD-18	14.7	4.0	58.8		34.8	0.55	4.4	3.95	*0.0	*35.9	*0.5	am
OD-19	av15.4	5.5	92.4		33.1	0.63	2.5	6.06	*0.0	*35.2	*0.5	am
OD-20	av16.5	5.0	82.5		35.1	0.57	4.6	3.90	*0.0	*36.6	*0.5	am
OD-21	av14.3	3.5	57.3		45.4	0.42	3.5	3.60	*0.0	*42.5	*0.5	am
OD-22	av16.1	7.5	128.8		32.5	0.83	20.5	1.53	*0.0	*40.2	*0.5	am
OD-23	av19.2	5.5	115.4		36.8	0.48	16.0	1.70	*0.0	*36.3	*0.5	au
OD-24	av15.5	10.5	171.0		av33.1	av0.78	av16.2	av1.15	*0.0	*40.0	*0.5	am
OD-25	15.1	6.0	90.4		39.5	0.62	9.5	3.44	*0.0	*42.0	*0.5	am
OD-26	av14.1	8.0	112.8		av24.0	av0.61	av12.4	av1.83	*0.0	*25.5	*0.5	cm
OD-27	14.3	5.0	71.4		28.3	0.60	7.6	2.65	*0.0	*30.1	*0.5	am

Appendix (f). Antibacterial treatment details. Twice daily dosing regimen.

I.D.	Dose	Duration	Cumul.	Pre-dose	Post1	Time1	Post2	Time2	TROUGH	PEAK	Time P	Concom
BD-1	14.2	3.0	42.5	0.0	13.1	0.77			0.0	13.1	0.77	am
BD-2	av13.2	3.0	39.7		18.6	0.65				18.6	0.65	am
BD-3	15.8	5.5	87.1	0.6	18.3	0.40			0.6	18.3	0.40	am
BD-4	av14.8	4.5	66.3	0.8	23.1	0.55			0.8	23.1	0.55	am
BD-5	15.0	3.5	52.7	0.7	28.0	0.58			0.7	28.0	0.58	am
BD-6	15.2	5.0	68.4	1.1	19.2	0.53			1.1	19.2	0.53	am
BD-7	av13.0	2.5	39.3	0.0	18.9	0.53			0.0	18.9	0.53	am
BD-8	av14.8	4.5	66.3	0.0	11.5	0.60			0.0	11.5	0.60	am
BD-9	av15.2	8.5	128.8	1.0	18.0	0.60			1.0	18.0	0.60	am
BD-10	av15.0	4.0	60.1	1.4	19.7	0.48			1.4	19.7	0.48	am
BD-11	av16.6	6.5	107.5	av0.7	av18.1	av0.59			0.7	18.1	0.59	am
BD-12	av14.0	7.0	97.7	0.3	21.9	0.50			0.3	21.9	0.50	am
BD-13	14.8	6.0	81.5	av0.4	25.6	0.58			0.4	25.6	0.58	am
BD-14	15.0	4.5	67.3	0.0	20.8	0.50			0.0	20.8	0.50	am
BD-15	15.4	6.5	92.5	0.0	21.6	0.53			0.0	21.6	0.53	am
BD-16	av15.4	4.5	77.4	0.0	18.7	0.52			0.0	18.7	0.52	am
BD-17	av14.2	3.5	56.7	0.0	16.2	0.48			0.0	16.2	0.48	am
BD-18	15.0	2.0	30.0	0.8	17.1	0.53			0.8	17.1	0.53	am
BD-19	av11.8	4.5	52.8	0.5	25.6	0.53			0.5	25.6	0.53	am
BD-20	av15.6	4.5	77.9	0.8	17.7	0.53			0.8	17.7	0.53	am
BD-21	14.6	4.0	58.7		18.6	0.55	1.6	4.85	*0.0	*19.1	*0.5	am
BD-22	15.2	3.5	53.0		18.3	0.57	0.7	4.49	*0.0	*19.4	*0.5	am
BD-23	15.3	2.0	30.6		21.6	0.65	7.9	1.75	*0.0	*24.7	*0.5	am
BD-24	17.4	6.0	104.3		11.8	1.00	5.8	1.93	*0.0	*17.4	*0.5	au
BD-25	14.7	5.0	73.7		15.2	0.68	6.0	1.86	*0.0	*17.5	*0.5	au
BD-26	14.6	4.0	58.3		16.0	0.57	7.2	1.64	*0.0	*16.8	*0.5	am
BD-27	av17.4	7.0	121.7		av19.7	av0.54	av8.3	av1.84	*0.0	*20.2	*0.5	am

Appendix (g). Burn patient details.

I.D.	Nonmem	SEX	AGE	WT	HT	Dose	Duration	Cumul.	Peak	Time P	Trough	SCR1	SCR2	SCR3
BOD-1#	34	F	4.8	16.0	102	av14.9	10.0	149.1	32.9	0.50	av0.6	43.0	43.0	34.0
BOD-2#	35	M	3.0	13.0	88	15.4	8.0	123.1	av30.7	av0.6	av0.7	41.0	43.0	36.0
BOD-3	55	M	2.7	12.0	96	av18.6	7.0	130.0	av37.0	av0.54	av0.0		42.0	
BOD-4	57	M	2.8	13.6	93	14.7	3.0	44.1	*36.1	*0.5	*0.0	29.0	30.0	24.0
BOD-5#	67	M	4.7	25.0	102	15.0	7.0	105.0	*42.6	*0.5	*0.0	47.0	54.0	37.0
BOD-6		F	1.6	14.0	86	15.0	4.0	60.0				52.0	37.0	
BOD-7	72	M	5.5	15.7	100	15.3	3.0	45.9	*34.2	*0.5	*0.0	47.0		49.0
BBD-1	36	F	0.9	7.2	68	av18.8	3.0	56.2	13.6	0.50	0.5		26	35
BBD-2		M	2.6	17.5	84	14.2	5.0	71.4			0.4	35	30	
BBD-3#	38	M	3.4	12.0	88	16.6	6.0	100.0	15.0	0.62	av0.5	29	35	44
BBD-4#	39	F	5.0	14.1	115	av17.2	9.0	154.6	14.5	0.48	0.0	38	54	58
BBD-5#	40	F	10.0	29.0	140	av13.0	9.0	117.2	19.2	0.48	0.6	43	58	59
BBD-6	45	F	8.5	24.0	120	15.0	2.5	37.5	16.2	0.87	4.4		29	39
BBD-7	70	F	4.0	17.0	98	15.3	4.0	61.2	24.4	0.50	*0.2	55	67	37

Patients with topical Neosporin applied to facial burns denoted by #

Appendix (h). Patients and control subjects undergoing urinalysis.

I.D.	Group	Sex	Age	WT	HT	Dose	Cumul.	Duration	PEAK	TROUGH
BD-17	BD	M	9.5	27.7	134	14.2	56.7	3.5	16.2	0.0
BD-18	BD	F	5.8	20.0	111	15.0	30.0	2.0	17.1	0.8
BD-19	BD	M	10.5	30.0	136	11.8	52.8	4.5	25.6	0.5
L-3	BD	M	8.5	24.8	124	14.9	52.2	3.5	13.1	0.0
BD-20	BD	F	7.5	24.0	124	15.6	77.9	4.5	17.7	0.8
L-16	BD	M	7.0	20.0	109	15.0	22.5	2.0		0.0
BD-23	BD	F	7.1	23.5	113	15.3	30.6	2.0	*24.7	*0.0
BD-26	BD	M	5.5	20.6	114	14.6	58.3	4.0	*16.8	*0.0
OD-15	OD	M	7.8	22.0	128	14.9	74.3	5.0	36.7	0.4
OD-16	OD	M	6.4	20.3	115	14.8	88.7	5.5	35.7	0.8
OD-17	OD	M	1.5	9.8	75	17.9	142.9	8.0	32.3	1.1
OD-18	OD	F	10.0	34.0	135	14.7	58.8	4.0	*35.9	*0.0
OD-20	OD	M	3.0	12.0	94	16.5	82.5	5.0	*36.6	*0.0
OD-21	OD	M	9.0	26.0	129	14.3	57.3	3.5	*42.5	*0.0
OD-22	OD	F	9.0	26.0	124	16.1	128.8	7.5	*40.2	*0.0
OD-26	OD	F	5.8	19.5	107	14.1	112.8	8.0	*25.5	*0.0
OD-27	OD	M	7.3	21.0	120	14.3	71.4	5.0	*30.1	*0.0
C-1		F	1.3	13.9	77					
C-2		M	12.5	23.0	126					
C-3		M	8.6	20.0	123					
C-4		M	7.8	20.0	110					
C-5		M	9.0	29.9	129					
C-6		M	8.5	25.0	132					
C-7		M	7.5	25.5	131					
C-8		M	4.5	20.5	113					
C-9		M	8.0	24.0	121					

Appendix (i). Patients undergoing audiology. Daily dosing regimen.

I.D.	SEX	AGE	WT	HT	Dose	Duration	Cumul.	PEAK	TROUGH	SCR1	SCR2	SCR3	Test1
OD-3	M	4.6	20.0	108	15.0	5.0	75.0	39.7	0.9		34		nad
OD-4	F	12.0	29.4	141	15.3	6.5	107.1	40.5	0.8	93	63		nad
OD-6	F	7.9	19.4	115	15.5	4.5	77.3	34.3	1.0	41	35	32	hf
OD-7	M	11.0	20.5	119	10.4	5.5	82.9	32.9	0.9		40	39	nad
OD-8	F	9.8	27.5	136	13.8	4.5	69.1	57.6	0.5	51	51	36	nad
OD-9	M	7.7	22.9	122	14.7	6.5	102.6	31.0	0.2	54	42		nad
OD-11	F	8.0	28.0	129	16.0	5.0	96.0	30.3	1.1	57	51	51	nad
OD-12	F	9.5	27.6	135	15.3	5.5	92.0	42.7	0.6	46	46	37	nad
OD-13	M	11.1	28.9	139	14.1	6.5	112.4	49.0	0.5	57	40	49	nad
OD-14	F	10.8	27.5	134	14.4	4.5	71.8	33.8	0.0	55	36	49	poss
OD-15	M	7.8	22.0	128	14.9	5.0	74.3	36.7	0.4	39	27	42	hf
OD-16	M	6.4	20.3	115	14.8	5.5	88.7	35.7	0.8	60	46	31	nad
OD-18	F	10.0	34.0	135	14.7	4.0	58.8	*35.9	*0.0	37	33	34	nad
OD-19	M	8.0	23.0	122	15.4	5.5	92.4	*35.2	*0.0		38	33	nad
OD-21	M	9.0	26.0	129	14.3	3.5	57.3	*42.5	*0.0		38	42	nad
OD-22	F	9.0	26.0	124	16.1	7.5	128.8	*40.2	*0.0	80	46	44	nad
OD-23	M	11.0	26.0	130	19.2	5.5	115.4	*36.3	*0.0	43	37	40	nad
OD-25	M	8.2	24.9	126	15.1	6.0	90.4	*42.0	*0.0	42	43	50	nad
OD-26	F	5.8	19.5	107	14.1	8.0	112.8	*25.5	*0.0	36	27	23	nad
OD-27	M	7.3	21.0	120	14.3	5.0	71.4	*30.1	*0.0	36	32	45	nad

Appendix (j). Patients undergoing audiology. Twice daily dosing regimen.

I.D.	SEX	AGE	WT	HT	Dose	Duration	Cumul.	PEAK	TROUGH	SCR1	SCR2	SCR3	Test1
BD-3	M	8.6	24.0	132	15.8	5.5	87.1	18.3	0.6	47	37		nad
BD-4	M	6.0	24.0	114	14.8	4.5	66.3	23.1	0.8	70	41	39	hf
BD-5	F	11.3	49.8	153	15.0	3.5	52.7	28.0	0.7	49	43		hf
BD-6	F	9.0	23.7	126	15.2	5.0	68.4	19.2	1.1	66	41		nr
BD-9	F	8.0	29.5	127	15.2	8.5	128.8	18.0	1.0	46	30	34	nad
BD-11	F	7.5	17.3	108	16.6	6.5	107.5	18.1	0.7	49	26	32	nad
BD-14	M	6.0	22.0	125	15.0	4.5	67.3	20.8	0.0	56	36		nad
BD-15	M	9.0	29.2	132	15.4	6.5	92.5	21.6	0.0	62	35	47	hf
BD-16	F	11.0	26.5	142	15.4	4.5	77.4	18.7	0.0	50	41	54	hf
BD-17	M	9.5	27.7	134	14.2	3.5	56.7	16.2	0.0	41	35		nad
BD-18	F	5.8	20.0	111	15.0	2.0	30.0	17.1	0.8	25	30		poss
BD-19	M	10.5	30.0	136	11.8	4.5	52.8	25.6	0.5	45	57	51	nad
BD-20	F	7.5	24.0	124	15.6	4.5	77.9	17.7	0.8	66	38	42	hf
BD-21	F	5.7	15.0	106	14.6	4.0	58.7	*19.1	*0.0	59	54	37	poss
BD-22	F	7.0	19.8	123	15.2	3.5	53.0	*19.4	*0.0		34	26	poss
BD-23	F	7.1	23.5	113	15.3	2.0	30.6	*24.7	*0.0	63	78	37	poss
BD-24	F	10.0	20.7	118	17.4	6.0	104.3	*17.4	*0.0	53	38	35	nr
BD-25	F	6.3	19.0	115	14.7	5.0	73.7	*17.5	*0.0	83	35	44	nad
BD-26	M	5.5	20.6	114	14.6	4.0	58.3	*16.8	*0.0	48	52	37	nad
BD-27	F	7.4	18.2	118	17.4	7.0	121.7	*20.0	*0.0	64	35	34	nad

Appendix (k). Information of all patients entered into NONMEM analysis

No.	ID	Sex	Age(yr)	Wt(kg)	Ht(cm)	BSA	Scr1	Levels	Reg	Dx
1	BD-1	F	3.1	11.3	87	0.525	40	1	BD	WB
2	BD-2	M	11.5	29.0	147	1.073		1	BD	APP
3	BD-3	M	8.6	24.0	132	0.929	47	1	BD	APP
4	BD-4	M	6.0	24.0	114	0.876	70	2	BD	APP
5	BD-5	F	11.3	49.8	153	1.458	49	1	BD	OTHER
6	BD-6	F	9.0	23.7	126	0.906	66	2	BD	APP
7	BD-7	F	5.7	21.0	112	0.810	69	1	BD	OTHER
8	BD-8	M	3.7	16.0	97	0.661	41	1	BD	P/O
9	BD-9	F	8.0	29.5	127	1.022	46	2	BD	OTHER
10	BD-10	M	7.3	22.5	123	0.872	62	2	BD	APP
11	BD-11	F	7.5	17.3	108	0.719	49	2	BD	APP
12	BD-12	M	9.3	24.5	128	0.928		1	BD	APP
13	BD-13	F	3.2	16.2	96	0.663	37	1	BD	OTHER
14	BD-14	M	6.0	22.0	125	0.867	56	1	BD	P/O
15	OD-1	M	3.3	14.5	87	0.600	43	2	OD	P/O
16	OD-2	F	7.0	19.0	114	0.773	46	2	OD	WB
17	OD-3	M	4.6	20.0	108	0.778		2	OD	OTHER
18	OD-4	F	12.0	29.4	141	1.063	93	2	OD	APP
19	OD-5	F	1.3	7.1	68	0.371	40	1	OD	P/O
20	OD-6	F	7.9	19.4	115	0.784	41	2	OD	WB
21	OD-7	M	11.0	20.5	119	0.819		2	OD	OTHER
22	OD-8	F	9.8	27.5	136	1.011	54	1	OD	APP
23	L-10	M	4.0	16.8	109	0.711	38	1	OD	P/O
24	OD-9	M	7.7	22.9	122	0.878	54	1	OD	APP
25	OD-10	F	0.6	10.2	74	0.466	33	1	OD	OTHER
26	L-11	M	2.6	16.0	90	0.642	34	1	OD	APP
27	OD-11	F	8.0	28.0	129	1.000	57	2	OD	P/O
28	L-12	F	7.3	20.0	112	0.789	29	2	OD	OTHER
29	OD-12	F	9.5	27.6	135	1.010	46	3	OD	APP
30	OD-13	M	11.1	28.9	139	1.047	57	3	OD	APP
31	OD-14	F	10.8	27.5	134	1.005	55	1	OD	APP
32	BD-15	M	9.0	29.2	132	1.032	62	1	BD	APP
33	BD-16	F	11.0	26.5	142	1.008	50	1	BD	APP
34	BOD-1	F	4.8	16.0	102	0.674	43	3	OD	BURN
35	BOD-2	M	3.0	13.0	88	0.569	41	3	OD	BURN
36	BBD-1	F	0.9	7.2	68	0.374		1	BD	BURN
37	L-13	M	1.8	13.9	77	0.559	45	4	BD	BURN
38	BBD-3	M	3.4	12.0	88	0.545	29	1	BD	BURN
39	BBD-4	F	5.0	14.1	115	0.661	38	1	BD	BURN
40	BBD-5	F	10.0	29.0	140	1.052	43	1	BD	BURN
41	L-1	F	8.0	30.0	125	1.025		1	OD	OTHER
42	L-2	M	6.0	15.4	110	0.681	39	1	OD	APP
43	L-3	M	8.5	24.8	124	0.922	35	1	BD	P/O
44	BD-17	M	9.5	27.7	134	1.009	41	1	BD	APP
45	BBD-6	F	8.5	24.0	120	0.894		2	BD	BURN
46	L-4	F	3.9	17.5	98	0.696	36	2	BD	OTHER
47	BD-18	F	5.8	20.0	111	0.786	25	2	BD	P/O
48	BD-19	M	10.5	30.0	136	1.060	45	1	BD	P/O
49	BD-20	F	7.5	24.0	124	0.906	66	2	BD	APP
50	OD-15	M	7.8	22.0	128	0.875	39	1	OD	APP
51	OD-16	M	6.4	20.3	115	0.804	60	3	OD	APP
52	OD-17	M	1.5	9.8	75	0.458		5	OD	WB
53	L-5	M	8.4	34.5	144	1.168	45	1	OD	APP
54	OD-18	F	10.0	34.0	135	1.130	37	2	OD	APP
55	BOD-3	M	2.7	12.0	96	0.564		2	OD	BURN
56	L-17	F	0.7	8.2	68	0.401	28	2	BD	OTHER
57	BOD-4	M	2.8	13.6	93	0.596	29	2	OD	BURN
58	OD-19	M	8.0	23.0	122	0.880		2	OD	APP
59	L-14	F	1.8	10.0	76	0.466	31	2	OD	BURN
60	BD-21	F	5.7	15.0	106	0.661	59	2	BD	WB
61	L-15	F	0.9	7.2	73	0.384	51	3	OD	BURN
62	BD-22	F	7.0	19.8	123	0.814		1	BD	APP
63	OD-20	M	3.0	12.0	94	0.559	42	2	OD	WB
64	BD-23	F	7.1	23.5	113	0.863	63	2	BD	WB
65	OD-21	M	9.0	26.0	129	0.961		2	OD	P/O
66	OD-22	F	9.0	26.0	124	0.946	80	2	OD	APP
67	BOD-5	M	4.7	25.0	102	0.857	47	2	OD	BURN
68	L-6	M	3.8	14.2	95	0.615	49	2	BD	WB
69	BD-24	F	10.0	20.7	118	0.820	53	2	BD	APP
70	BBD-7	F	4.0	17.0	98	0.686	55	5	BD	BURN
71	BD-25	F	6.3	19.0	115	0.775	83	2	BD	APP
72	BOD-7	M	5.5	15.7	100	0.662	47	2	OD	BURN
73	OD-23	M	11.0	26.0	130	0.964	43	2	OD	APP
74	OD-24	M	10.7	34.8	152	1.199	42	6	OD	OTHER
75	OD-25	M	8.2	24.9	126	0.930	42	2	OD	APP
76	L-7	F	9.8	30.0	137	1.063		2	OD	BURN
77	BD-26	M	5.5	20.6	114	0.807	48	2	BD	WB
78	L-8	F	5.5	23.0	126	0.891	59	2	BD	APP
79	OD-26	F	5.8	19.5	107	0.764	36	4	OD	WB
80	BD-27	F	7.4	18.2	118	0.765	64	4	BD	P/O
81	L-9	F	9.5	22.2	122	0.863		2	OD	BURN
82	OD-27	M	7.3	21.0	120	0.832	36	2	OD	APP

APPENDIX (I). Summary of manual calculations performed during NONMEM analysis.

1. Calculation of 95% Confidence Intervals from Standard Error of the Estimates:

Parameter estimate ± (SEE x 1.9897)

(where 1.9897 is the t value associated with 81 (n-1) degrees of freedom, at $p < 0.05$ significance)

2. Calculation of η^{CL} %, η^{V} % and ϵ %.

η^{CL} % = $(\omega^2_{CL})^{0.5}$ ÷ mean population estimate CL (L/hr) x 100 %

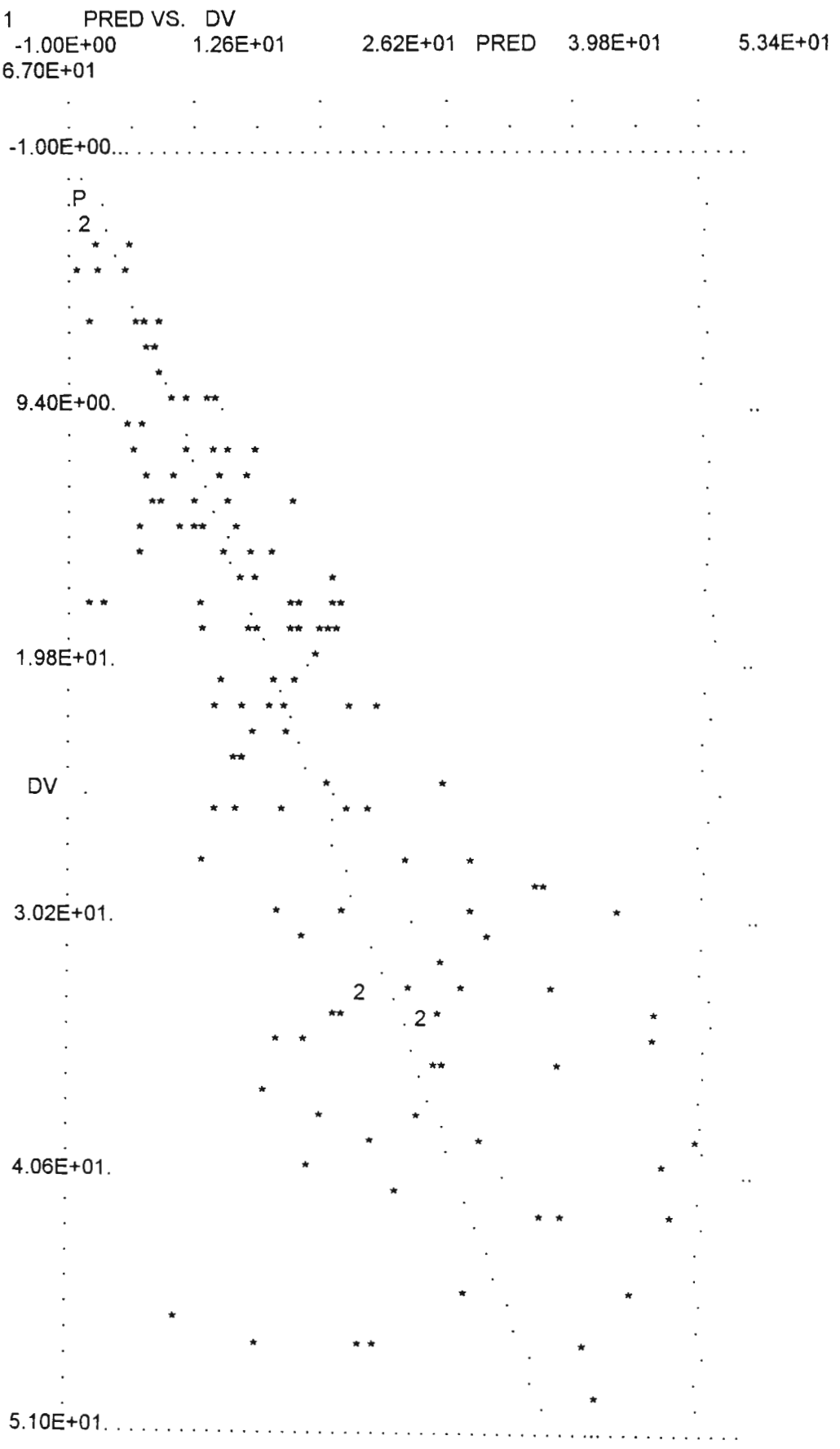
η^{V} % = $(\omega^2_{V})^{0.5}$ ÷ mean population estimate V (L) x 100 %

ϵ % = $(\sigma^2)^{0.5}$ ÷ average serum concentration (mg/L) x 100 %

APPENDIX (m). CONTROL FILE: MODEL A.

```
$PROB AMIKIN KID
$INPUT ID WT AGE HT SEX SCA SCB SCC TIME AMT EVID DV REG BURN
$DATA AMIKIDF2.DAT
$SUBROUTINES ADVAN1 TRANS2
$PK
 CL=THETA(1)+ETA(1)
 V =THETA(2)+ETA(2)
S1=V
$ERROR
Y=F+ERR(1)
$THETA (0.0001,,10.0)
$THETA (0.01,,100.0)
$OMEGA 0.64 9.0
$SIGMA 56.0
$EST MAXEVAL=3000
$COV
$TABLE ID AMT TIME PRED DV WRES
$SCAT PRED VS DV UNIT
$SCAT WRES VS PRED1NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)
DOUBLE PRECISION NONMEM    VERSION IV LEVEL 2.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER
```

APPENDIX (n). PRED vs DV: MODEL A.



APPENDIX (o). CONTROL FILE: MODEL N.

```
$PROB AMIKIN KID
$INPUT ID WT AGE HT SEX SCA SCB SCC TIME AMT EVID DV REG BURN
$DATA AMIKIDF2.DAT
$SUBROUTINES ADVAN1 TRANS2
$PK
 A=WT**0.5378
 B=HT**0.3964
 C=A*B*0.024265
 CL=THETA(1)*AGE+THETA(3)*C+ETA(1)
 V =THETA(2)*C+ETA(2)
S1=V
$ERROR
Y=F+ERR(1)
$THETA (0.001,,10.0)
$THETA (0.01,,100.0)
$THETA (0.001,,20.0)
$OMEGA 0.64 9.0
$SIGMA 56.0
$EST MAXEVAL=3000 POSTHOC
$COV
$TABLE ID AMT TIME PRED DV WRES
$TABLE ID CL V WT
$SCAT PRED VS DV UNIT
$SCAT WRES VS PRED1NONLINEAR MIXED EFFECTS MODEL PROGRAM (NONMEM)
DOUBLE PRECISION NONMEM    VERSION IV LEVEL 2.1
 DEVELOPED AND PROGRAMMED BY STUART BEAL AND LEWIS SHEINER
```

APPENDIX (p). PRED vs DV: MODEL N.